Plant assemblages to phytomanage metal (Cu and Zn/Pb/Cd)-contaminated soils, rhizofiltrate Cu-contaminated water, and deliver usable biomass for the bioeconomy

Nadège Oustrière

THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE SCIENCES ET ENVIRONNEMENTS

Écologie évolutive, fonctionnelle et des communautés

Nadège OUSTRIÈRE

Assemblages végétaux pour phytomanager des sols contaminés en métaux (Cu et Zn/Pb/Cd), rhizofiltrer de l'eau contaminée en Cu et fournir des biomasses à la Bioéconomie

Plant assemblages to phytomanage metal (Cu and Zn/Pb/Cd)-contaminated soils, rhizofiltrate Cu-contaminated water, and deliver usable biomass for the Bioeconomy

Sous la direction de : Michel Mench

Soutenue le 05 Décembre 2016.

Devant la commission d'examen formée de :

M. ALARD, Didier	Professeur, Université de Bordeaux, France	Président du jury
M. VANGRONSVELD, Jaco	Professeur, Universiteit Hasselt, Belgique	Rapporteur
M. DOUAY, Francis	Professeur, LGCgE, ISA, Lille, France	Rapporteur
Mme. GRISON, Claude	Professeur, Université Montpellier II, France	Examinateur
M. CHALOT, Michel	Professeur, Université Franche-Comté, France	Examinateur
Mme. BERT, Valérie	Ingénieur de Recherche et Etude, INERIS, France	Examinateur
Mme. CADIERE, Frédérique	Ingénieur, ADEME, Angers, France	Invitée
M. MENCH, Michel	Directeur de recherche, INRA-Univ. Bordeaux	Directeur de thèse

Titre: Assemblages végétaux pour phytomanager des sols contaminés en métaux (Cu et Zn/Pb/Cd), rhizofiltrer de l'eau contaminée en Cu et fournir des biomasses à la Bioéconomie

Résumé:

Le phytomanagement de matrices contaminées en métaux couple leur réhabilitation écologique avec la production de biomasses végétales pour la bioéconomie. Un front de science est d'identifier des assemblages végétaux et d'optimiser leur production, aidée ou non par l'ajout d'amendements. Le phytomanagement de deux sols, l'un contaminé en Cu, l'autre en Cd, Pb et Zn, a été testé en conditions contrôlées. L'emploi conjoint de biochar et de grenaille d'acier diminue la phytotoxicité des 2 sols. En pots, sur 2 ans, cette combinaison d'amendements séquestre du carbone, diminue la phytotoxicité du sol contaminé en Cu et produit une biomasse d'*Arundo donax* L. et de *Populus nigra* L. non contaminée, utilisable par le secteur de l'énergie. Ces modalités de culture et d'amendement ont été installées pour un suivi à long terme sur le site contaminé en Cu. Parallèlement, en microcosmes, parmi 4 macrophytes utilisées en zone humide construite (CW) pour décontaminer des matrices aqueuses (i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L. et *Phalaris arundinacea* L.), *A. donax* est la mieux adaptée pour fournir des racines à forte concentration en Cu utilisables pour produire un écocatalyseur riche en Cu. Le phytomanagement d'un effluent de bouillie bordelaise (EB, 69 µM Cu) par *A. donax* a été testé en CW pilote. Il est décontaminé en 48h, sa concentration en Cu respectant la réglementation du rejet d'effluent en réseau d'assainissement. Cependant, après un cycle de circulation, la concentration en Cu des racines d'*A. donax* (623 ± 140 mg Cu kg^{-1}) est inférieure aux besoins de l'éco-catalyse, et le cycle serait à répéter pour atteindre les 1000 mg Cu kg^{-1} requis.

Mots clés: Biochar, bioéconomie, biomasse, canne de Provence, écocatalyse, macrophytes, métal, oxydes de fer, peuplier, phytomanagement, phytotechologies, racine, rhizofiltration, stabilisation in situ, ultrastructure, zone humide construite.

INRA / Université de bordeaux

BIOGECO – UMR 1202 – Equipe

Diversité et Fonctionnement des Communautés

Bat B2, Allée Geoffroy Saint-Hilaire, CS50023, 33615 PESSAC cedex- France

Title: Plant assemblages to phytomanage metal (Cu and Zn/Pb/Cd)-contaminated soils, rhizofiltrate Cu-contaminated water, and deliver usable biomass for the Bioeconomy

Abstract:

The phytomanagement of metal-contaminated matrices (soils and water) combines their ecological remediation and the production of non-food crops for the bioeconomy. One science frontier is to identify plant assemblage and to optimize their biomass production, aided or not by amendment addition and cultural practices. A Cu-contaminated soil and a Cd/Pb/Zn-contaminated one were phytomanaged in controlled conditions. The combination of biochar and iron grit reduced the phytotoxicity in both soils. In a 2-year pot experiment, this amendment combination decreased the phytotoxicity of the Cu-contaminated soil, enhanced soil C sequestration and produced an uncontaminated biomass of *Arundo donax* L. and *Populus nigra* L. adapted for bioenergy production. These combinations of culture and amendment are tested in field trial at the Cu-contaminated site. In parallel, in microcosm experiment, out of 4 macrophytes commonly used in constructed wetlands (CW) to clean up aqueous matrices (i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L. and *Phalaris arundinacea* L.), *A. donax* was the best adapted to produce a high Cu-rich root mat potentially usable as Cu-ecocatalyst. Clean up of a Bordeaux mixture effluent (BME, 69 µM Cu) by *A. donax* was tested in a pilot-scale CW. The BME was decontaminated in 48 hours, its Cu concentration being in compliance for indirect discharge of chemical industry effluents. However, after one BME circulation cycle, root Cu concentration of *A. donax* roots (623 ± 140 mg kg^{-1}) was lower than threshold value for Cu-ecocatalysts (1000 mg kg^{-1}) and successive treatments must be repeated to achieve required Cu concentration.

Keywords: Biochar, bioeconomy, biomass, constructed wetland, ecocatalysis, Giant reed, in situ stabilization, iron oxides, macrophytes, metal, phytomanagement, phytotechologies, poplar, rhizofiltration, root, ultrastructure.

INRA / Université de bordeaux
BIOGECO – UMR 1202 – Equipe
Diversité et Fonctionnement des Communautés

Bat B2, Allée Geoffroy Saint-Hilaire, CS50023, 33615 PESSAC cedex- France

Remerciements

Je souhaite sincèrement remercier mon directeur de thèse le Dr Michel MENCH pour m'avoir accueillie d'abord en tant qu'étudiante de Master, puis pour m'avoir fait confiance, en me proposant ce travail de thèse. Merci de m'avoir guidée et formée, avec patience et persévérance, tout au long de ces années. Au-delà du travail de recherche, je vous remercie d'avoir partagé avec moi votre passion pour les voyages et de m'avoir fait visiter le monde, aussi bien l'ouest (Canada), que l'est (Japon), le sud (Turquie) et le Nord (Danemark) … Merci également pour m'avoir initiée aux bases de l'œnologie. Au début de ma thèse, vous m'aviez dit : 'aucun de mes « thésards » ne terminera sa thèse sans apprécier le vin'… me voilà fin prête à soutenir.

La deuxième personne que je tiens à remercier du fond du cœur est le Dr Lilian MARCHAND. Pour toi non plus, ça n'a pas dû être facile de m'épauler durant toutes ces années! Merci pour tes conseils toujours judicieux, ta patience, ton optimisme constant et un grand merci pour m'avoir toujours encouragée et soutenue dans les moments difficiles. Je te remercierai bien par des boîtes de Haribo, mais pour t'éviter des carries, je m'abstiendrai! Et faute de quoi, j'espère bien qu'à mon tour, je pourrai te rendre l'aide que tu m'as apportée.

J'aimerais vivement remercier le Dr Matthieu BRAVIN de m'avoir fait confiance et confié un poste au sein de son équipe au CIRAD de la Réunion. Cette année forte enrichissante entre mon stage de MASTER et ma thèse a conforté l'idée de poursuivre dans la recherche.

Je suis très reconnaissante aux Professeurs Jaco VANGRONSVELD et Francis DOUAY pour avoir accepté d'être rapporteurs de ce travail. Je remercie aussi les membres du jury, le Pr. Didier ALARD, le Pr. Michel CHALOT, le Pr. Claude GRISON, le Dr. Valérie BERT, et Mme Frédérique CADIERE, qui m'ont fait l'honneur de juger ce travail.

Je remercie les financeurs de ce projet : l'ANR programme CD2i (Chimie Durable – Industries, Innovation) avec le projet PHYTOCHEM, le programme FP7 de l'Union Européenne avec le projet européen GREENLAND et surtout l'ADEME (contrat de thèse n° 2013-ID5081) qui m'ont permis de débuter mon travail de thèse en janvier 2014 au sein de l'UMR BIOGECO. Je souhaite particulièrement remercier Mme Frédérique CADIERE, ingénieur ADEME, pour avoir suivi avec intérêt l'évolution de mes travaux. Je la remercie pour ses suggestions et sa présence dans le jury en tant qu'invitée.

Mes remerciements vont aussi aux directeur et directeur-adjoint de l'UMR BIOGECO (UMR1202), M. Rémi PETIT et M. Didier ALARD, et au directeur de l'équipe «Diversité et Fonctionnement des Communautés», M. Emmanuel CORCKET, pour leur accueil. J'aimerai également remercier l'Ecole Doctorale Sciences et Environnements de l'Université de Bordeaux ainsi que l'association Francaise d'étude des sols pour avoir financer ma participation à la «11th International Phytotechnologies Conference » en 2014 à Heraklion, Grèce.

Merci aux formidables stagiaires qui ont su contribuer avec brio aux expériences réalisées, tout en assurant une excellente ambiance de travail. Sans vous, j'en serais encore aux expériences de labo et de terrain, merci William GALLAND, Eli ROULET, Lunel GABON, Gabriel ROSETTE, Myriam GARROUJ et Camille ROUSSET. Un merci aussi à Stéphanie POINY-TOPLAN, même si tu as été présente avant ma thèse je ne t'oublie pas.

Je tiens à remercier les équipes des différents laboratoires qui m'ont chaleureusement accueillie en particulier : le Pr. Alexander LUX, le Dr. Mareck VACULIK, le Dr. Michal MARTIKA et toute l'équipe de l'université de Bratislava qui m'a enseigné l'utilisation du microscope à epifluorescence. Ils ont toujours été à l'écoute de mes demandes. Je remercie le Dr Jacques BRISON, pour m'avoir accueillie une semaine au sein de son laboratoire au conservatoire botanique de Montréal et le Dr Michaël MOTELICA pour m'avoir permis de réaliser des mesures de carbones organiques solubles au sein de son laboratoire à l'université d'Orléans.

Je n'oublie pas de remercier tout le personnel du laboratoire BIOGECO qui a, de près ou de loin, participé à ce travail, pour son soutien, ses remarques et ses conseils avisés qui m'ont permis de progresser dans mes recherches. Un gros merci aussi pour tous les échanges récréatifs autour de cafés ou pause déj'. Je ne pourrai pas citer tout le monde, de crainte d'oublier certains noms, mais sachez que j'ai passé de supers années en votre compagnie.

J'ai eu la chance de passer trois années à proximité de mes trois merveilleux colocataires de bureaux, j'ai nommé les fantastiques Max LARTER, le « British », Nastasia MERCERON, la « Lot et Garonnaise » et Marion WALBOTT, la « Landaise ». Je citerai aussi mes voisins de bureau au top : Elsa, Hafiz, Marius, Thomas. Je vous remercie pour tous les bons moments passés ensemble, les discussions, les pauses thé/café/glaces, ces moments de complicité me manqueront énormément ! Au-delà du bureau, la team burger restera toujours dans mon cœur : Cécile, Marie, Nastasia, Pauline, Anthony, Emeric, Max, Paul. Vous « gérez du pâté », ma thèse aurait été tellement moins drôle sans vous!

Un super merci à tous 'les petits choux' de grenoblois, qui ont accepté de relire ma biblio, j'ai toujours su que je pouvais compter sur vous, vous êtes et avez toujours été au TOP : Alex, Gwen, Mathilde, Romain, Fafa, Titinou, Mariquette et la sous team grenoblois/bordelais Claire, Lucile et Max.

Je n'oublierai pas mes colocs actuels ou passés. Merci de m'avoir écoutée et soutenue durant toutes ces années. Merci à Clémence, Laura, Aaron, Nico, Sam, Simo, Yves-Marie et à tous les autres. Une pensée particulière à Sylvain qui a encore plus que les autres dû me supporter. Et un merci à mes petits bordelais adorés : Caroline, Guillemette, Reyes, Anthony, Jp, Lili, Rémi et Sacha.

Enfin, je réserve une mention particulière à ma famille, vous avez toujours cru en moi, des exposés de CE2 à la relecture de la thèse, vous avez toujours pu m'aider. Merci d'avoir su être là et m'avoir soutenu dans les moments les plus durs. Je vous remercie de tout mon cœur, pour ça et pour tout le reste!

A tous, MERCI.

Merci aux organismes et aux projets suivants pour leur participation à cette thèse

Synthèse des travaux en Français

En 2016, en France, 6405 sites industriels étaient considérés comme pollués (12% étant traités et libres de toutes restrictions), alors qu'ils étaient 4318 en 2012 (Basol, 2016). De plus en plus de sites sont reconnus comme contaminés par les éléments traces (ET, en sciences du vivant : métaux et non-métaux, essentiels ou non aux fonctions biologiques, et dont les concentrations fréquentes dans les organismes n'excèdent pas 100 mg kg^{-1} masse sèche, MS, Adriano et al., 2001), cyanures, autres composés minéraux, etc., et/ou des xénobiotiques organiques, e.g. hydrocarbures aromatiques polycycliques (HAP) ou aliphatiques, polychlorobiphényles (PCB), solvants halogénés, produits pharmaceutiques ou phytosanitaires, produits cosmétiques, etc. à cause d'activités anthropogéniques présentes ou passées. En parallèle, des utilisations novatrices et diversifiées des biomasses végétales, se développent pour remplacer des énergies fossiles par des énergies renouvelables ou bien fournir des matières premières à la bioéconomie (i.e. bio-fuels, bioplastiques, éco-matériaux). Dans un contexte de population mondiale en constante augmentation, le changement de l'utilisation des terres agricoles pour produire de la biomasse à usage non-alimentaire n'est pas une option économiquement et politiquement soutenable, face aux conflits potentiels. Une partie du besoin en terres cultivables pourrait être satisfaite par la production de biomasse sur des sites marginaux et contaminés. L'utilisation des terres marginales pour la production de biomasses valorisables a favorisé le développement des techniques de remédiation de sols contaminés. Dans le contexte du développement durable, le concept de phytomanagement couple la réhabilitation écologique de matrices contaminées par des options de remédiations douces (GRO, utilisant les plantes (phyto-), les champignons (myco-) ou les méthodes basées sur la microbiologie avec ou sans amendements (minéraux, organiques ou chimiques) pour réduire le transfert des contaminants vers les récepteurs biologiques) avec la production d'une biomasse valorisable, offrant un profit financier (Cundy et al., 2013). Les fronts de sciences concernent les assemblages végétaux au regard des filières d'utilisation (biomasse produite, composition, qualité, gestion, etc.), les microorganismes associés, les fonctions écologiques rétablies et les services écosytémiques associés, les cycles de vie, etc. Aussi cette thèse a pour but l'acquisition de connaissances sur ce sujet (**Partie 1**), afin de contribuer au développement de nouvelles méthodes et d'options de phytomanagement sur matrices sols (**Partie 2**) et eau (**Partie 3**) contaminées par les métal(loid)es avec pour finalité la production de biomasses à forte valeur ajoutée utilisables par les procédés de la bio-économie.

Cette problématique est l'un des thèmes principaux développés par l'ADEME "Impact de la pollution des sols et évaluation environnementale de la gestion des déchets " (Agence de l'Environnement et de la Maîtrise de l'Énergie), les projets européens GREENLAND (FP7-KBBE-266124, Options de remédiation douce pour des sols contaminés en éléments traces, http://www.greenland-project.eu/, 2010-2014), Intense (Era-net facce surplus), PhytoSUDOE (Interreg SUDOE) et l'Agence Nationale de

la Recherche [PHYTOCHEM ANR-13-CDII-0005-01], http://www.axelera.org/, 2014-2017) qui ont soutenu ce projet.

Peut-on produire une biomasse capable d'intéger les chaines de valorisation locales, via l'implantation d'options de phytomanagement sur matrices contaminées en metaloïdes ?

L'une des principales contaminations auxquelles fait face l'Aquitaine est celle en Cu. L'application sur le long terme de fongicides à base de Cu a contribué à l'augmentation locale du Cu total dans les sols (e.g. vignobles, vergers, site de préservation du bois), avec des concentrations pouvant excéder les valeurs de références du fonds pédogéochimique. La migration diffuse du Cu et l'érosion des sols ont aussi contribué à la contamination en Cu des eaux de surface dans l'estuaire de la Gironde (se superposant aux contaminations industrielles historiques telles que les rejets dans le bassin versant, e.g. Riou mort à Decazeville).

Organisation des travaux et des résultats

Nos travaux portent sur la mise en oeuvre d'options de phytomanagement pour des matrices environnementales contaminées principalement en Cu et en Cd, Pb et Zn. La production de biomasse valorisable sur ces matrices contaminées a été orientée selon 2 axes majeurs:

(1) La production de biomasses végétales aux concentrations fréquentes en ET pouvant intégrer les filières de valorisation de la biomasse (i.e. biofuels, bioplastiques, éco-matériaux) (**Partie 2**), et (2) la production de biomasse végétale avec des concentrations élevées en ET essentiels (niveau d'oxydation inhabituel, nouvelles espèces chimiques associées, effet de synergie) pour la nouvelle voie de valorisation de l'éco-catalyse (**Partie 3**). Les problématiques principales sont (1) l'évaluation de l'adéquation des espèces végétales (et microorganismes associés) et/ou d'amendement des sols pour la remédiation de matrices contaminées, (2) la sélection d'espèces adaptées (composition, qualité, rendement, résilience, etc.) au besoin de la bio-économie, et (3) l'intégration de ces connaissances pour des options de phytomanagement à échelle locale, par exemple pour résoudre des problèmes de contamination auxquels fait face la région d'Aquitaine.

La première partie correspond à l'introduction générale. Elle produit un état de l'art sur le phytomanagement des sols et eaux contaminés (**Partie 1**).

Partie 2: Parmi les amendements à disposition pour la remediation de sols contaminés, les biochars sont des résidus carbonisés, riches en carbone, produit par la pyrolyse de biomasse à température élevée et sous faible teneur en oxygène. L'intérêt porté aux biochars s'est développé à la suite de discussions entre les communautés politiques et scientifiques. De ces échanges a émergé l'idée qu'augmenter la séquestration du carbone dans les sols agricoles, par ajout de biochars, permettrait de compenser l'augmentation annuelle de CO_2 dans l'atmosphère et de résoudre partiellement le problème du réchauffement climatique. De plus, les biochars ont pour effets potentiels l'amélioration de la qualité des sols et l'immobilisation des ET et de certains xénobiotiques organiques (dont des organochlorés). Ils peuvent être utilisés en tant qu'amendement pour la stabilisation in situ. L'utilisation des biochars pour « stabiliser in situ » la contamination des sols tout en produisant une biomasse avec une concentration en ET dans la gamme de valeurs fréquentes apparait comme une GRO. En Aquitaine, la plateforme de phytoremédiation de St. Médard d'Eyrans est un site de préservation du bois contaminé en Cu suite à l'utilisation sur le long terme de sels contenant Cu comme agent fongicide et insecticide et le subséquent lessivage du bois traités. Plusieurs GRO ont été testées sur ce site (e.g. stabilisation in situ avec compost, laitiers sidérurgiques, dolomie plus compost, compost et grenaille d'acier, etc., Bes et Mench, 2008; Lagomarsino et al., 2011; Kolbas et al., 2011) qui ont contribué à stabiliser efficacement la contamination au Cu, et à diminuer sa biodisponibilité environnementale. Cependant, pour être en phase avec la décision politique d'augmenter la séquestration du carbone dans les sols, une étude a été réalisée sur l'effet de l'ajout de biochars sur ce site.

Chapitre 1: Cette expérience en pot vise à évaluer l'effet à court-terme (3 mois) de l'ajout de biochars issu de biomasse d'origine animale ou végétale, (i.e. fientes de poulet ou copeaux d'écorce de pin), de compost et de grenaille d'acier, utilisés seuls ou en combinaison, sur ce sol contaminé. Une hypothèse est : quelles sont les conséquences du matériau d'origine sur l'efficacité recherchée. Un biotest normalisé effectué avec des haricots (*Phaseolus vulgaris* L.) utilisé en tant que bioindicateur a permis d'évaluer les effets des amendements sur la phytotoxicité du sol. Cette étude de cas a montré combien des précautions sont nécessaires avant d'utiliser du biochar dans un sol contaminé en Cu (chaque biochar étant un cas d'étude selon son procédé de fabrication). A court terme, le Cu peut se complexer avec la matière organique dissoute (DOM), entrainant le lessivage du Cu hors de la zone racinaire et favoriser potentiellement sa migration dans l'environnement. Le choix de biochars et d'amendement adapté permet cependant de juguler cet effet. L'amendement le plus efficace, i.e. l'ajout de biochars dérivés de copeaux d'écorce de pin, en combinaison avec de la grenaille d'acier dans le sol contaminé en Cu, fait diminuer significativement le Cu libre et total dans la solution du sol et la concentration en Cu dans les parties aériennes des plantes, et a de fait conduit à une moindre phytotoxicité du sol. Cette étude a été publiée (Science of the Total Environment, 566–567. pp 146–153, 2016), et s'intitule: "*Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site*".

La stabilisation in situ de sols mono-contaminés (e.g. contaminé en Cu) avec du biochar est parfois plus simple que pour les sols poly-contaminés (e.g. contamination en Cd, Pb et Zn) puisque l'effet de chaulage du biochar, ou des réactions de surface avec sa matrice carbonée, permettent l'immobilisation de certains cations divalents mais peuvent favoriser la mobilité d'autres éléments (e.g. As). Dans une seconde étude, nous avons vérifié si les amendements réduisant la phytotoxicité du sol contaminé en Cu permettaient aussi la remédiation d'un sol poly-contaminé en Cd, Pb et Zn.

Chapitre 2: La seconde expérience en pot a eu pour but d'évaluer l'effet de l'ajout de biochar (i.e. dérivé de copaux d'écorce de pin), seul et en combinaison avec du compost ou de la grenaille d'acier, pour stabiliser la contamination en Cd, Pb, et Zn d'un sol agricole. L'ajout de biochar dérivé de copeaux d'écorce de pin en combinaison avec de la grenaille d'acier a fait diminuer les concentrations en Cd, Pb et Zn dans la solution du sol et les parties aériennes de la plante test (haricot) jusqu'à la gamme de valeurs fréquentes dans les parties aériennes des haricots. Ce travail a été publiée (Environmental Science and Pollution Research, 24. pp 7468–7481, 2017), sous le titre "*Wood derived-biochar combined with compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil".* Cependant, dans ces 2 cas (**Chapitre 1 et 2**), cette combinaison d'amendement n'a pas favorisé la production de biomasse des haricots.

Ces études ont été réalisées sur le court terme (une période de réaction de 3 mois suivis par 2 semaines de bio-essai), or au fil du temps, il est nécessaire de considérer les changements potentiels de phytotoxicité des sols amendés en biochar. C'est pourquoi une troisième expérience en pot a été réalisée.

Chapitre 3: L'étude a eu pour but, d'évaluer à moyen terme (2 ans) l'effet de l'ajout de biochars, seuls ou en combinaison avec de la grenaille d'acier sur la phytotoxicité du sol contaminé en Cu. Les coûts associés aux options de phytomanagement utilisant la grenaille d'acier en combinaison avec le biochar sont relativement élevés. De fait, la culture de biomasse végétale à croissance rapide et valorisable à un coût attractif est nécessaire afin d'assurer un retour financier permettant d'assumer en partie ces coûts de remédiation. Les peupliers (*Populus niga* L.*)* et la canne de Provence (*Arundo donax* L.) sont 2 cultures pouvant être utilisées pour stabiliser la contamination tout en fournissant une biomasse valorisable par la bioéconomie. Elles peuvent être utilisées dans les secteurs suivants: (1) le secteur de l'énergie, (2) la production de biochar, (3) la chimie biosourcée pour la production de molécules (plates-formes) chimiques (dont celles à haute valeur ajoutée), (4) des bio-produits dérivés de biomasse lignocellulosique, (5) les écomatériaux et (6) l'industrie du papier. C'est pourquoi, nous avions aussi pour but de tester la capacité des 2 espèces à produire un fort rendement de biomasse sur sol contaminé au Cu. Les résultats montrent la difficulté de stabiliser la contamination au Cu sur le long terme en utilisant un biochar (mais pour mémoire cela dépend du type de biochar utilisé). Les amendements et les réactions avec les composants des sols évoluent au fil du temps, et certaines interactions biotiques et abiotiques peuvent remobiliser les contaminants sorbés. L'effet sur le long terme du biochar, avec et sans grenaille d'acier, dépend de l'espèce végétale cultivée. La culture d'*A. donax* augmente la concentration de DOM dans la solution du sol, réduisant sa concentration en Cu^{2+} mais élevant en parallèle la concentration totale en Cu. La culture de *P. nigra* accroit la concentration en Cu^{2+} de la solution du sol. Contrairement au peuplier, la canne de Provence favoriserait le lessivage de Cu total en dehors de la zone racinaire. L'ajout de grenaille d'acier en combinaison avec du biochar, dans ce sol contaminé en Cu, a contré l'augmentation des concentrations en Cu total et Cu libre pour les 2 espèces cultivées, en comparaison avec l'ajout de biochar seul. Ce procédé a aussi diminué la concentration en Cu dans les parties aériennes des peupliers et des cannes de Provence jusqu'à atteindre les valeurs fréquentes en Cu dans les parties aériennes de plantes. Cependant, il n'a pas augmenté la production de biomasses racinaires et aériennes pour les 2 espèces. Ce travail a été publiée (Science of the Total Environment, 579. pp 620–627, 2017), et s'intitule: "*Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site*".

La fiabilité des études en pot pour prédire la croissance et la performance des plantes sur le terrain est souvent imprécise. En effet, leur environnement diffère fréquemment des conditions sur le terrain, souvent à cause de l'effet de bord et des conditions climatiques ou de pression de ravageurs. Suite aux expérimentations en pot, il est recommandé de tester les modalités d'options de phytomanagement en parcelles, sur le terrain. Cette étape permettra de détecter les défaillances potentielles de ces options dues aux changements sur le long terme, tels que le vieillissement des phases ajoutées au sol, la variabilité climatique interannuelle, les attaques parasitaires, le dépôt et l'accumulation de litière, la remise en solution de matières organiques solubles, les changements dans les communautés animales et végétales (Kidd et al., 2015).

Informations additionnelles: Suite à ces études, des parcelles amendées en biochar, seul ou en combinaison avec du compost, ont été établies directement sur le site de traitement du bois. Elles serviront à évaluer la production durable de biomasse de peuplier et de cannes de Provence ainsi que le cycle de vie d'une telle option de phytomanagement.

Ce travail montre que la combinaison de biochar avec la grenaille d'acier ou du compost peut être utilisée comme amendement pour la stabilisation in situ du sol contaminé en Cu, sur un site de préservation du bois (**Chapitre 1 et 3**) ainsi que celle d'un sol agricole poly-contaminé en Cd, Pb et Zn à proximité d'une usine métallurgique (Pb/Zn) (**Chapitre 2**). Dans l'ensemble des expériences, les parties aériennes des plantes ont une concentration en Cu, Cd, Pb ou Zn correspondant à la gamme de valeurs fréquentes dans les parties aériennes. Cependant, dans ces 3 premiers chapitres, aucun des traitements étudiés n'a été capable d'augmenter la production de biomasse végétale en comparaison au sol non-traité. Une fertilisation complémentaire est sans doute à évaluer. Les résultats de ces 3 études proviennent d'expériences en pot. Ils ont été utiles pour évaluer la fiabilité des stratégies de stabilisation in situ. Cependant, ils ne nous permettent pas encore de répondre à la question suivante: « La stabilisation in situ par l'ajout de biochars, est-elle une option durable pour produire des biomasses aux valeurs fréquentes tout en remédiant la contamination dans les sols? ».

Durant l'été 2016, l'Aquitaine a fait face à une sècheresse ayant affecté la croissance des plantes sur nos expériences de terrain. Cette expérience de stabilisation in situ sur parcelle doit être poursuivie en irriguant régulièrement pour permettre l'installation des jeunes plantes (**Informations additionnelles**).

Partie 3 : Le second problème relatif à l'utilisation de pesticides à base de Cu en Aquitaine est la production en quantité importante d'effluents de bouillie bordelaise (EB) générés lors des traitements des vignobles (i.e. 2 500 000 L an^{-1}) (Maille, 2004). La diffusion de résidus dilués d'EB sur le terrain, autorisée par l'article L. 253-1 du Code rural, contribue à l'augmentation locale du Cu total dans les sols. Cependant, pour éviter cet épandage non nécessaire, l'une des options est de « rhizofiltrer » cet effluent en zones humides construites (CW). Ces CW sont des systèmes écotechnologiques de traitement d'effluents dans lesquels le dense réseau de racines et de rhizomes des plantes aquatiques, habitat de nombreuses communautés microbiennes, vient former un filtre naturel. Dans ce système, la « rhizofiltration » des effluents contaminés en ET pourrait produire une biomasse racinaire à forte concentration en métaux ou non-métaux pouvant en général intégrer la voie de valorisation de l'éco-catalyse. L'éco-catalyse se base sur l'utilisation d'espèces métalliques (niveau d'oxydation inhabituel, nouvelles espèces chimiques associées, effet de synergie) provenant de plantes afin d'obtenir des réactions chimiques organiques pour la synthèse de molécules à forte valeur ajoutée (e.g. les industries pharmaceutiques, cosmétiques, et agrochimiques) (Clavé et al., 2016). Ce nouveau champ de recherche conduit à l'étude d'éco-catalyseurs à base de Cu provenant de biomasse végétale à concentration élevée en Cu (i.e. ≥1000 mg kg^{-1} MS pour atteindre les prérequis de la production d'éco- catalyseurs). Dans un tel système, les racines (et parties aériennes de façon moindre) des macrophytes produites pourraient atteindre la concentration en Cu requise pour la production d'éco-catalyseurs.

Chapitre 4 : La première étude visait à <u>évaluer la capacité de 4 macrophytes</u> (i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L., et *Phalaris arundinacea* L.) couramment utilisés en CW <u>à produire un tapis racinaire riche en Cu</u>. Ces 4 macrophytes ont été évalués sur un gradient en Cu (i.e. solution nutritive contaminée au Cu: 0.08, 2, 10, 20 et 40 µM Cu, $CuSO_4.5H_2O$) dans des conditions contrôlées en batch pendant 2 mois. Parmi les macrophytes testées, seulement *I. pseudacorus* et *A. donax* ont produit un tapis racinaire potentiellement utilisable en tant qu'éco-catalyseurs. Les racines ont respectivement atteint jusqu'à 1099 et 3512 mg Cu kg^{-1} après exposition à 40 µM Cu. *Iris pseudacorus* a aussi produit une biomasse aérienne importante aux concentrations en Cu dans la gamme basse des valeurs fréquentes. Cette biomasse peut intégrer une voie de valorisation ordinaire telle que le secteur de l'énergie. Dans la gamme d'exposition 10-40 µM Cu, la concentration de Cu dans les parties aériennes d'*A. donax* n'était pas suffisante pour son utilisation en tant que éco-catalyseurs (< 1000 mg

Cu kg^{-1} MS) tout en étant au-dessus des valeurs fréquentes en Cu (i.e. 42-175 mg Cu kg^{-1}). Si elle ne peut pas intégrer les voies de valorisation ordinaire, cette biomasse pourrait être compostée et utilisée en tant que fertilisant pour les sols ayant une déficience en Cu (cas des podzols des Landes). La pyrolyse d'une telle biomasse est une autre option. Dans cette étude, nous avons utilisé de jeunes plantules (âgées de 7 mois) avec une faible biomasse pouvant favoriser les fortes concentrations en Cu dans les parties aériennes. Selon Brisson et Chazarenc (2009), utiliser une plante mature est primordial pour évaluer correctement le bénéfice de traitement d'une espèce végétale en CW. Par conséquent, les concentrations en Cu mesurées dans nos plantes âgées de 7 mois sont probablement surestimées en comparaison à celles de plantes matures. Ce travail est présenté dans le chapitre 4 et s'intitule "*Potential of macrophyte roots for producing Cu-ecocatalysts*".

Chapitre 5: Suite à ces résultats, nous avons créé un CW-pilote pour conjointement rhizofiltrer l'EB et faciliter le management de la biomasse produite dans le système. Nous avons sélectionné *A. donax* plutôt que *I. pseudacorus* pour être planté dans le CW-pilote afin de maximiser l'accumulation du Cu dans les racines. En effet, *A. donax* présentait des concentrations en Cu supérieures à celle de *I. pseudacorus* pour une même exposition au cuivre. Comparées à nos jeunes plantules, les plantes matures de notre CW ont une biomasse plus importante. Une plus faible concentration en Cu à la fois dans les racines et les parties aériennes devrait découler de la dilution du Cu dans cette biomasse. Nous avons fait le pari que les concentrations foliaires en Cu des plantes d'*A. donax* (âge > 26 mois) tomberaient dans la gamme des concentrations en Cu fréquentes et permettraient à cette biomasse d'intégrer les voies de valorisation communes (e.g. Secteur de l'énergie: bioéthanol, biofuel, combustion; Fertilisants potentiels: compost, biochar; Bio-produits : matériaux de constructions et composés à base de fibre végétale). Le CW consistait en 6 unités (800L chacune), 3 unités étant plantées avec *A. donax* (*Ad*) et 3 autres unités restant sans plante (Ctrl). L'EB avait une concentration de 69 µM Cu (e.g. limite de solubilité). Nous avons évalué la capacité d'*A. donax* en hydroponie dans le CW-pilote à (1) rhizofiltrer le Cu de l'effluent, (2) fournir une biomasse racinaire riche en Cu utilisable par l'éco-catalyse, et (3) fournir une biomasse aérienne non contaminée. Le traitement du Cu dans l'effluent a été réalisé en 48 heures dans les 2 types d'unités (i.e. 93% dans l'unité plantée et 80% dans l'unité non-plantée) mais les concentrations en Cu dans l'EB ont atteint le seuil de rejet d'effluents en réseau d'assainissement collectif (i.e. 0.5 mg Cu L^{-1}), après 48 heures pour les unités *Ad* et 21 jours pour les unités Ctrl. Durant les 30 jours d'exposition, la biomasse aérienne et racinaire a augmenté respectivement de 200 g et 700 g par unités de CW. A T_{30}, les concentrations en chlorophylles et caroténoïdes ont significativement diminué, de même que les concentrations foliaires en Fe, indiquant que la synthèse de chlorophylles et caroténoïde a été affectée par l'excès de Cu. Comme nous l'attendions, les parties aériennes de la Canne de Provence ont des concentrations en Cu dans la gamme des valeurs fréquentes des parties aériennes (8 mg Cu kg^{-1} DW). Néanmoins, la concentration en Cu dans les racines a seulement atteint 62% (i.e. 623 mg Cu kg^{-1} DW) des valeurs requises pour une utilisation en écocatalyse. D'autres cycles de traitement seraient à répéter pour potentiellement atteindre au moins 1000 mg Cu kg^{-1} DW dans les racines. Ce travail a été accepté avec révision mineure dans Ecological Engineering et s'intitule "*Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using Arundo donax L. coupled with potential Cu-ecocatalyst production*".

Même si nos CW pouvaient (1) produire une biomasse racinaire avec des concentrations en Cu >1000 mg Cu kg^{-1} DW, (2) produire une biomasse aérienne avec de faibles concentrations pouvant intégrer les voies de valorisation fréquente, et (3) être capable de traiter l'EB, leurs mise en œuvre dans les exploitations viticoles est loin d'être réaliste de nos jours. Le domaine de l'éco-catalyse a émergé dans les années 2000; le management, le transport et la conversion des biomasses ont besoin d'être développés. Du point de vue de l'environnement, ce CW-pilote pourrait générer des bénéfices non-négligeables pour préserver des fonctions écologiques du sol et des services écosystémiques (éviter l'augmentation du Cu total dans les sols et du lessivage du Cu et donc protéger la biodiversité). Jusqu'à maintenant aucun système ne permettait de traiter facilement un tel effluent contaminé au Cu.

Preamble

In 2016, 6405 industrial sites were referenced in France as polluted (12% being remediated and free of restricted uses), compared with 4318 sites in 2012 (Basol, 2016). More and more sites are considered as contaminated by trace element (TE, essential and non-essential metal(loid)s with common concentrations in plant shoots below 100 mg kg^{-1} dry weight, DW, Adriano et al 2001), cyanides, etc., and/or organic compounds, e.g. polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyl (PCB), etc. because of past and present anthropogenic activities. In parallel, innovative and diversified uses of plant biomass for industrial processings related to sustainable development are emerging. Plant biomass is considered as a suitable renewable energy source to replace fossil fuels or to provide raw materials and biomaterials for industrial processes (i.e. bio-economy: biofuels, bioplastics, eco-materials). Faced with the challenge of a constant growing worldwide population, arable land-use change for the production of non-food biomass is not an option economically and politically viable. Some of this land requirement could be met by biomass production on marginal and contaminated sites. The valorization of marginal land creates paradigm shifts in remediation techniques as it reduces the cost associated with phytomanagement options. In the context of sustainable development, this concept pairs the ecological rehabilitation of contaminated matrices by Gentle Remediation Options (GRO, using plant (phyto-), fungal (myco-) or microbiologically-based methods, with or without chemical additives, for reducing contaminant transfer to local receptors by in situ stabilisation or extraction of contaminants), with the production of valuable biomass resulting in a financial return (Cundy et al., 2013). Therefore this PhD aimed at acquiring new knowledge (**Part 1**), and developing novel methods for implementing phytomanagement options on metal(loid)-contaminated matrices, both soil (**Part 2)** and water (**Part 3)**, with a special focus on the production of valuable biomass usable in bio-economy processes.

This issue is one of the core theme "Soil pollution impact, environmental assessment of waste management" developed by ADEME (French Agency for Environment and Energy Management), the European project GREENLAND (FP7-KBBE-266124, Gentle remediation options for Trace element-contaminated soils, http://www.greenland-project.eu/, 2010-2014) and the French National Research Agency [PHYTOCHEM ANR-13-CDII-0005-01], http://www.axelera.org/, 2014-2017) funders of this project., in relation with the INTENSE and PhytoSUDOE European projects.

Can we produce a biomass able to integrate local biomass processing chains, through the implementation of phytomanagement options on metal(loid)-contaminated matrices?

In Aquitaine, France, one of the major contamination issue that we face is Cu-contamination. Long-lasting application of Cu-based fungicide contributes to locally increase total soil Cu (e.g. vineyards, orchards, wood preservation site) above the guideline values in topsoils while diffuse migration of Cu and soil erosion result also in Cu contamination of surface waters in the Gironde estuary. Thus, this PhD addressed mainly the implementation of phytomanagement options on Cu contaminated environmental matrices. The production of valuable biomass on contaminated matrix was orientated along two major axes:

(1) The production of plant biomass with metal(loid) concentrations within common ranges able to integrate local processing chains for biomass recovery (i.e. biofuels, bioplastics, eco-materials) (**Part 2**), and (2) the production of plant biomass with high essential metal(loid) concentrations (unusual oxidation levels, new associated chemical species, effects of synergy) for the new Eco-catalysis processing chains (**Part 3**). The main issues are (1) to assess the adequacy of plant species and/or soil amendments for remediating contaminated matrices, (2) to select relevant species tailored to the needs of bio-economy, and (3) to integrate this knowledge in phytomanagement options locally to solve real scale issue encountered in the Aquitaine region.

Part 1: This first part is the general introduction of this manuscript. It is a literature review of the phytomanagement of contaminated soils and water.

Part 2: Biochars are carbon-rich carbonized residues produced by waste biomass pyrolysis under high temperatures and mid to low oxygen. Recently, sparked interest in biochar started with discussion of politician together with scientists which arrived with the idea that boosting soil carbon sequestration in agricultural soils by adding biochar to offset annual increases in atmospheric CO_2 may partly solve the climate change issue. In addition, biochar as amendment for in situ stabilization option has gained attention due to its numerous ability for soil quality improvement but also for its capacity to immobilize TE. Using biochar to "in situ stabilize" soil contamination while producing a biomass with metal(loid) concentrations within the common ranges is a potential GRO. More specifically, in Aquitaine, the "phytoremediation platform" of St. Médard d'Eyrans is a wood preservation site contaminated by Cu due to long time use of Cu-based salts and washings of treated wood. Several GRO have been already tested at this site (e.g. in situ stabilization with compost, Linz–Donawitz slag, dolomitic limestone with compost, compost with iron grit, etc…, Bes and Mench, 2008; Lagomarsino et al., 2011; Kolbas et al., 2011) and efficiently stabilized and decreased the Cu bioavailability. However, to fit in the political decision of increasing carbon sequestration, we also assessed the effect of adding biochar, compost and iron grit, alone and in combination, in this Cu contaminated soil, in a short term (3 months) pot experiment.

Chapter 1: A biotest realized with dwarf beans (*Phaseolus vulgaris* L.) used as bioindicator allowed to evaluate the effect of *in situ* stabilization option on soil phytotoxicity and to discuss the relevance of such soil amendments. This work was published in Science of the Total Environment (566–567. pp

146–153, 2016) and entitled "*Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site*".

Chapter 2: For confirming the efficacy of soil amendments to stabilize a poly-contaminated soil, a second study aimed at assessing the combined effect of amending a Pb, Zn and Cd-contaminated soil with biochar and either compost or iron grit, again in a short term (3 months) pot experiment. This work was published in Environmental Science and Pollution Research (24. pp 7468–7481, 2017) and entitled "*Wood derived-biochar combined with compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil*".

Chapter 3: Both studies allowed to set up a third pot experiment which aimed (1) at evaluating the mid-term effect (2 years) of amending the Cu-contaminated soil with biochar, alone or in combination with iron grit, on soil phytotoxicity, and (2) the feasibility of growing poplars and Giant reeds (*Arundo donax* L.) as relevant species for biomass production in such in situ stabilized Cu-contaminated soil. This work was published in Science of the Total Environment (579. pp 620–627, 2017) and entitle "*Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site*".

Additional information: Based on these studies and on a literature review, field plots with biochar, alone or in combination with compost, have been implemented on a Cu-contaminated soil at a wood preservation site and are currently investigated after being planted with poplars and Giant reeds.

Part 3: In Aquitaine, France, a second issue related to Cu-based salts is the significant amounts of Bordeaux mixture effluent (BME) generated by the treatments of vineyards. Spreading of residual diluted BME on the field, authorized by the Article L. 253-1 of the rural Code, contributes to locally increase total soil Cu. However, to avoid such unnecessary spreading, one option is to "rhizofiltrate" this effluent in constructed wetlands (CW). These CW are ecotechnological wastewater treatment systems where aquatic vegetation forms filters by their dense interwoven roots and rhizomes. In such system, the "rhizofiltration" of metal(loid) contaminated effluent may produce a root biomass with high metal(loid) concentrations which generally cannot integrate regular recovering processing chains but may be adapted to the investigation field of Eco-catalysis. Eco-catalysis is based on the use of metal species originating from plants to fine organic chemical reactions for the synthesis of molecules with high added value (e.g. pharmaceuticals, cosmetics, and agrochemicals).

Chapter 4: The first study consisted in a batch experiment (2 months) and aimed at evaluating the capacity of 4 macrophytes (i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L., and *Phalaris arundinacea* L.) frequently used in CWs to produce Cu-rich root and rhizome mats. Among the 4 macrophytes, *A. donax* produced a shoot biomass with Cu concentration within the low common Cu range and root biomass with high Cu concentration exceeding 1000 mg Cu kg^{-1} DW when exposed to 10-40 µM Cu, which makes this plant species a relevant candidate for the need of Eco-catalysis. This work is presented in the chapter 4 and is entitled: "*Potential of macrophyte roots for producing Cu-ecocatalysts*".

Chapter 5: Out of these results we set a pilot-scale CW planted with *A. donax* which aimed at assessing the ability of this plant to jointly (1) rhizofiltrate Cu from the Bordeaux mixture effluent and (2) provide a Cu-rich root mat for ecocatalysis. This work was accepted with minor revision in Ecological engineering and entitled "*Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using Arundo donax L. coupled with potential Cu-ecocatalyst production*".

Table of contents

List of figures

Part. 1. Introduction

1. Trace element contamination in the environment

2. Phytomanagement of contaminated matrices

Part. 2.

Chapter. 1. Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site

Chapter. 2. Wood derived-biochar combined with either compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil

Chapter. 3. Mid-term stabilization of Cu and biomass production of *Arundo donax* L. and *Populus nigra* L. after adding wood-derived biochar, alone and in combination with iron grit, into a contaminated soil from a wood preservation site

Additional information - Phytomanagement of a Cu-contaminated soil at a wood preservation site using Giant reed, poplar, and biochars, alone and combined with compost: in situ assessment of soil phytotoxicity with *Vicia faba* L.

Part. 3.

Chapter. 4. Potential of macrophyte roots for producing Cu-ecocatalysts

Chapter. 5. Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using *Arundo donax* L. coupled with potential Cu-ecocatalyst production

Part. 5. General discussion

List of Tables

Part. 1. Introduction

2. Phytomanagement of contaminated matrices

Part. 2.

Chapter. 1. Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site

Chapter. 2. Wood derived-biochar combined with either compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil

Chapter. 3. Mid-term stabilization of Cu and biomass production of *Arundo donax* L. and *Populus nigra* L. after adding wood-derived biochar, alone and in combination with iron grit, into a contaminated soil from a wood preservation site

Additional information - Phytomanagement of a Cu-contaminated soil at a wood preservation site using Giant reed, poplar, and biochars, alone and combined with compost: in situ assessment of soil phytotoxicity with *Vicia faba* L.

Part. 3.

Chapter. 4. Potential of macrophyte roots for producing Cu-ecocatalysts

Chapter. 5. Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using *Arundo donax* L. coupled with potential Cu-ecocatalyst production

List of abbreviations

AB:	Poultry manure
AHA:	Arabidopsis H^+ ATPases
ANOVA:	Analysis of variance
APX:	Ascorbate peroxidase
ATX1:	Specific chaperones
AsA:	Ascorbic acid
BME:	Bordeaux mixture effluent
CAT:	Catalase
CCA:	Chromated copper arsenate
CEC:	Cation-exchange capacity
Chl:	Chlorophyll
CM:	Culture medium
COPT:	Cu transporters
Ctd:	Carotenoids
CSD1:	Superoxide dismutase
CW:	Constructed wetland
Cys:	Cysteine
Df:	Degree of freedom
DOC:	Dissolve organic carbon
DOM:	Dissolved organic matter
DW:	Dry wreight
EC:	Electrical conductivity
Eh:	Redox potential
EU:	European Union
Fvalue:	Fisher value
Fv/Fm:	Maximum efficiency of Photosystem II
GHG:	Greenhouse gases
Glu:	Glutamine
Gly:	Glycine
GPX:	Glutathione peroxidase
GR:	Glutathione reductase
GRO:	Gentle remediation options
GS:	Glutathione synthetase
GSSG con:	Glutathione conjugates
GSH:	Glutathione
HMA:	Heavy Metal ATPases
HNS:	Hoagland Nutrient Solution
HRT:	Hydraulic retention time
LMW:	Low molecular weight
NSCC:	Non-selective cationic channels
MDHA:	Monodehydroascorbate
Me:	Metal

Means sq:	Mean of squares
MT:	Metallothioneins
NA:	Nicotianamine
P:	Pvalue
PAH:	Polycyclic aromatic hydrocarbons
PB:	Pine bark
PCA:	Principal component analysis
PIN1:	Pin-formed 1
POD:	Peroxidase
PCD:	Programmed Cell Death
PSII:	Photosystem II
qN:	Photochemical quenching
OM:	Organic matter
ROS:	Reactive oxygen species
SOD:	Superoxide dismutase
SOM:	Soluble organic matter
SRC:	Short rotation coppices
SPW:	Soil pore water
TE:	Trace element
VOC:	Volatile organic compound
WHC:	Water holding capacity
XAFS:	X-ray absorption fine structure spectroscopy
Y(II):	Real efficiency of PSII
YSL:	Yellow Stripe-Like
ZIP:	Zinc/iron-regulated transporter like protein
γ-ECS:	γ-glutamylcysteine synthetase

Table of contents – Part 1

Part. 1. Introduction

1. Trace element contamination in the environment

Anthropogenic activities, e.g. metal(loid) mining and smelting, uses of fertilizers, waste and sewage sludge management, pesticides, wastewater irrigation, coal and fuel combustion residues, car traffic, and atmospheric depositions from industrial and urban activities, are sources of chronic contamination by trace elements (TE, essential and non-essential metal(loid)s with common concentrations in plant shoots below 100 mg kg^{-1} dry weight, DW, Adriano et al., 2001) and organic contaminants, e.g. polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Wuana and Okieimen, 2011) (Fig. 1). The number of sites referenced as polluted is increasing in France (i.e. 6405 in 2016 vs 4318 in 2012; Basol, 2016). Among trace elements, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn are common in terrestrial ecosystems (EC, 2013). The legacies of long term industrial, urban and agricultural activities may result in soil concentrations exceeding the pedogeochemical background values (Baize, 1997; Villanneau et al., 2008; INDIQUASOL, 2016). Due to the ubiquitous nature and non-biodegradability of TE, they accumulate in soils and in turn, polluted soils may become sources of diffuse contamination, through erosion, runoff, leaching and percolation. Some TE may migrate to aquatic ecosystems and contaminate surface and ground water (Salpeteur and Angel, 2010). Contaminants may damage several soil functions, generate exposure pathways and pollutant linkages, enter via plants in the food chain, affect the biological receptors and become a serious threat for the environment (fauna and flora) and sometimes the human health (Maldeveanu, 2014).

In this review, attention will be paid on (1) Cu- and (2) Cd/Pb/Zn-contamination which are both mainly studied in this PhD thesis.

1.1. Historical Cu contamination in Aquitaine

In France, Cu concentration in topsoils varies from 3 mg Cu kg^{-1} to 40.2 mg Cu kg^{-1} (Baize, 1997; El Hadri al., 2012; Fig. 1). Several anthropogenic activities, e.g. organic wastes used as soil amendment (manure, pig slurries, and sewage sludges), Cu-based fungicides and wood preservatives, and emissions from metallurgical industry, can result in Cu-contaminated soils (Villanneau et al., 2008; Cestonaro do Amaral et al., 2014; Basias, 2015; Huang et al., 2016). More specifically, since the 18th Century, in Aquitaine, Cu-based salts are profusely used in vineyards, orchards, some cash crops (e.g. tomato, potato tubers) and wood preservation as pesticides to control insects and fungi (Karjalainen et al., 2009). Among Cu-based salts, Bordeaux mixture (BM, $Ca(OH)_2 + CuSO_4$) is widely used in vineyards, but the range of Cu-based compounds authorized in France is larger: Cu oxychloride, cupric oxides or hydroxide and Cu tallate (Mackie et al., 2012). Long-lasting application of Cu-based salts and wood washings frequently contribute to locally increase total soil Cu (i.e. > 1000 mg Cu kg^{-1} above the inquiry threshold values in topsoils (RMQS: 40.2 mg Cu kg^{-1} DW; El Hadri al., 2012). Moreover, diffuse migration and soil erosion result in Cu contamination of surface waters in the Gironde estuary (i.e. 0.2-1.3 µg L^{-1}; IFREMER, 2014) above the upper critical threshold value defined by the French Water Agency for freshwater quality (0.1 µg L^{-1} Cu; SEQ EAU, 2003). This may threat flora and fauna biodiversity, e.g. abundance and species composition of earthworms, marine organisms such as mollusca arthropoda (Baker et al., 2014), bacteria, fungi (Lagomarsino et al., 2011; Qiu et al., 2013; Mackie et al., 2015; Taylor and Walker, 2016), and algae communities (Rocha et al., 2016) and inhibit activity of hydrocarbon-degrading microorganisms thus impairing C and N cycles (Mackie et al., 2015). Over 35 mg Cu kg^{-1} soil, the soil may be exposed to an anthropogenic contamination, and an investigation is highly recommended (Baize, 1997; Saby et al., 2011). To illustrate the soil Cu contamination in Aquitaine, we investigated the case study of a wood preservation site at St-Médard d'Eyrans, France, Cu-contaminated (i.e. 65-2600 mg Cu kg^{-1}) due to long term use of Cu-based salts such as $CuSO_4$ and chromated copper arsenate (CCA) combined with washings of treated wood (Mench and Bes, 2009). On top of that, filling and rinsing the tanks of crop sprayers containing Cu-based salts generate significant amounts of Cu-contaminated effluents. Based on the 150.000 ha of Aquitaine vineyards, the total volume of Bordeaux mixture effluents (BME) is estimated at 2.500.000 L (Maille, 2004). Spreading of these diluted BME on the French fields, authorized by the Article L. 253-1 of the rural Code, contributes to locally increase total soil Cu. To avoid such unnecessary spreading, the management of such BME needs to be studied.

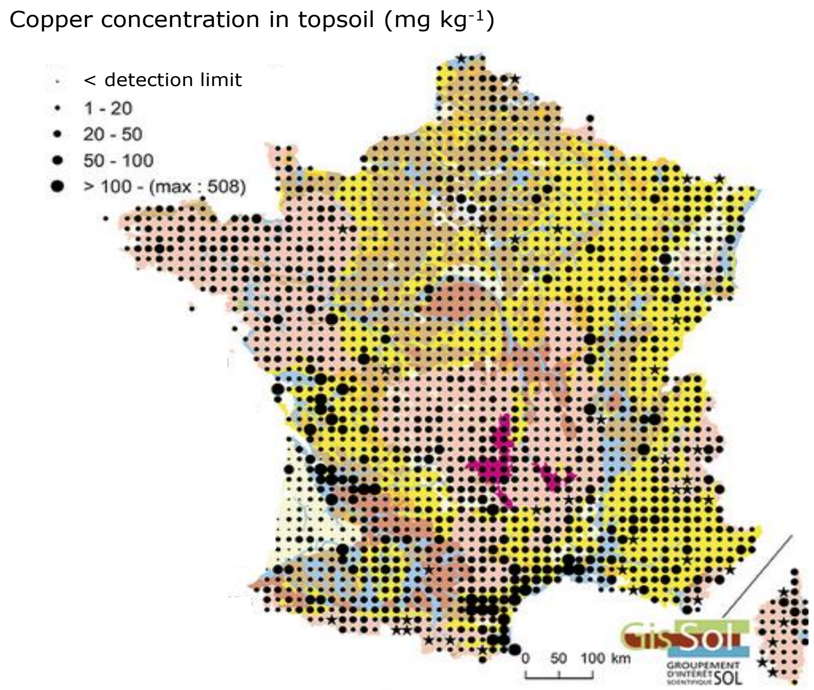


Fig. 1. Topsoil Cu concentration in France (mg kg^{-1}) (adapted from GIS sol, 2009)

1.2. Poly-contamination with Cd, Pb and Zn: the Arnoldstein case study

It is rare to find a site contaminated by only one element (i.e. mono-contaminated), usually soils are poly-contaminated. The cross contamination of Cd, Pb and Zn is quite common in contaminated soils. Some cases studies in France are ecosystems nearby MetalEurope, Mortagne du Nord, Bazoche, etc. (Villanneau et al., 2008; Mench et al., 2009; Nsanganwimana et al., 2016); other sites in EU are listed in Kidd et al., (2015). It results from mining, emissions from metallurgical industry, traffic and waste deposit at dumpsite (Bert et al., 2012; Janssen et al., 2015; Johnson et al., 2016; Fatemitalab et al., 2016). Remediating a poly-contaminated soil is more complex than a mono-contaminated soil, as elements interact with their environment in different way. Remediation options for contaminated site must be tailored to various type of cross contamination. As a case study, contamination in the Arnoldstein area, Carinthia, Austria has been studied, notably in the EU Greenland project (Kidd et al., 2015). Several hundred years of smelting and processing of mining ores have caused widespread pollution of field areas surrounding the industrial site of Arnoldstein (Asami, 1988). The Pb smelting started in 1495, followed in the 1950's by an increasing production of zinc, cadmium, and germanium. The Arnoldstein smelter closed in 1992 but the surrounding soils used for housing (playgrounds), horticulture, forestry, and alpine grassland agriculture with pastures and feed production are now contaminated by Pb (i.e. 1300 mg Pb kg^{-1}), Cd (i.e. 9 mg Cd kg^{-1}) and Zn (i.e. 1110 mg Zn kg^{-1}) and, to a lesser extent, Cu and As (Friesl et al., 2006; Friesl et al., 2009). This contamination resulted in: high Pb and Cd concentrations in arthropods species sampled near the smelter (Rabitsch, 1995), high Pb-concentration in blood and teeth of inhabitants living nearby the smelter (Kasperowski, 1993), high metal(loid) concentrations (mg kg^{-1}) in *Zea mays* L. shoots, i.e. Pb 54, Zn 286, and Cd 2.73 (Friesl et al., 2006), leading to orders restricting agricultural and horticultural land utilization by the Austrian Federal Environmental Agency (Kasperowski, 1993; Rabitsch, 1995).

1.3. Bioavailability in the Cu- and Cd/Pb/Zn-contaminated soils

Metal(loid) excess in a contaminated site generates different exposure pathways for a biological receptor, directly through dermal contact, breathing or eating contaminated soil particles and drinking contaminated water. Indirect exposure pathways may come from metal(loid) uptake by plants growing on contaminated land, through foliar and/or root exposures, and contaminants transfer through the food chain.

<u>*Caution for readers*</u>: Because this part was not the core theme of this PhD thesis, it was voluntarily kept short, to avoid making the manuscript more cumbersome but updated review references are included all along to inform readers about the undetailed parts.

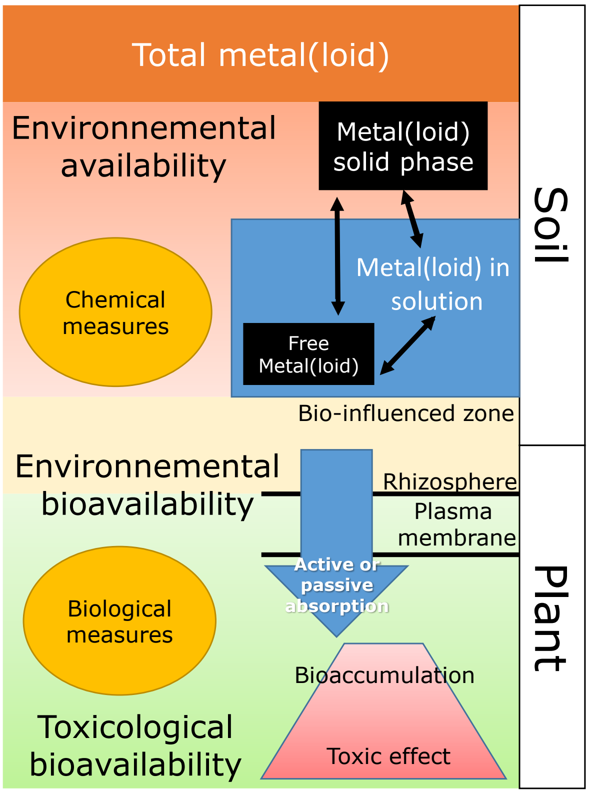


Fig. 2. Bioavailability of metal(loid)s (adapted from Bravin et al., 2008)

1.3.1. Environmental availability

It is recognized that pollutant linkages related to metal(loid) uptake by roots do not dependent only on total soil contaminants. Several processes obscure the relationship between soil total metal(loid) concentrations and its availability in the soil (Smolders et al., 2009). Environmental availability is the pool of contaminant in the environment that can potentially be taken up by the organism (Fig. 2) (Harmsen, 2007). This fraction of total metal(loid) concentration depends on the chemical speciation of the metal(loid) and the soil physico-chemical and biological properties. They govern the partition (mobility) of the metal(loid) between the soil or sediment solid and liquid phase (Carrillo-González et al., 2006). In the soil, metal(loid) forms are: (1) inert as atoms in minerals, (2) sorbed in organic matter, clay or colloids (3) precipitated or co-precipitated with oxyhydroxides, phosphates and carbonates, (4) free ions and (5) complexed but dissolved in the soil solution (Fig. 3) (Violante et al., 2010). The environmental availability of metal(loid)s depends on their location into the soil fractions and sorption strength to various inorganic and organic soil compounds (Maiz et al., 2000).

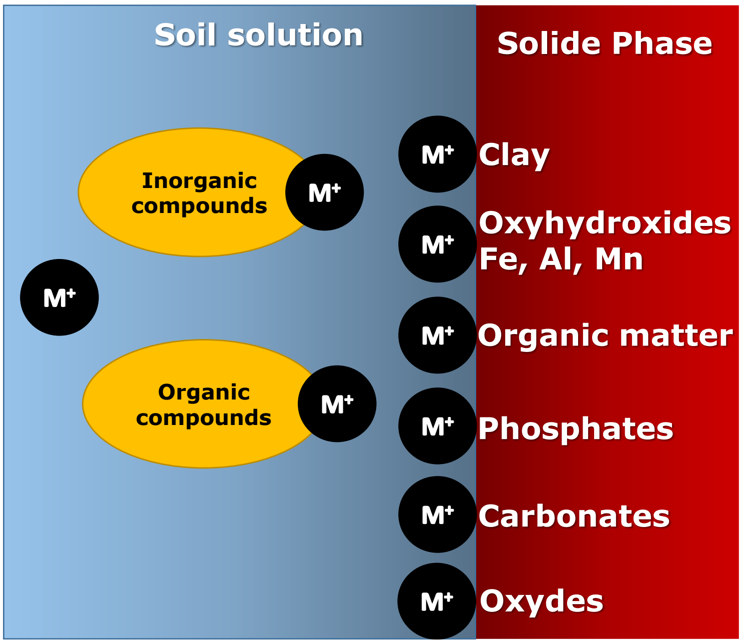


Fig. 3. Metal(loid)s speciation in the soil. M = Metal (adapted from Bravin et al., 2008)

Three main mechanisms control metal(loid) environmental availability: specific adsorption (e.g. complexation reaction with hydroxyl groups and organic functional groups at the soil surface), nonspecific adsorption (e.g. electrostatic with soil charges) and precipitation or (co)precipitation (Fig. 4) (Manceau et al., 2002).

Factors which influence metal(loid) mobility in the soil are : its total soil concentration (cumulating both anthropogenic and geochemical sources), physico-chemical soil properties (e.g. pH, redox potential, temperature, and water holding capacity), soil compounds (e.g. organic matter (OM), dissolved OM (DOM), clay and oxyhydroxide content) and the microbial activity (Baize et al., 1997; Carrillo-González et al., 2006). The effects of these parameters on TE mobility are described in Violante et al., (2010). Stable forms in the residual fraction are unlikely to be rapidly released in the soil solution, while the soluble fractions, exchangeable and chelated species are more mobile and therefore more available for root uptake. Therefore, a contaminated site may display high total metal(loid) concentrations in the soil but with a low environmental availability resulting in minimal exposure and impact for the biological receptors (Smolders et al., 2012; Marchand et al., 2015). Copper in moderately and highly organic contaminated soils is present in less mobile and bioavailable forms, whereas in mineral soils, the labile fraction is often higher (Mench and Bes, 2009). The studied wood preservation site (at St Médard d'Eyrans) is characterised by unfavourable soil properties, e.g. lack of structure with low OM content, low nutrient availability and acidic pH. In this soil, Cu excess enhanced the Cu concentration in the labile pool of the soil (i.e. environmental availability) (Mench and Bes, 2009; Bes et al., 2010; Hattab et al., 2014; Thaler and Humar, 2014). At the Arnoldstein site, high total soil Cd and Zn associated with acidic soil pH resulted in a relatively high environmental availability for both metals (Friesl et al., 2006). However, at pH 6, Pb may form hydroxide and oxide precipitates, e.g.

$Pb_3(OH)_4^{2+}$, $Pb_6O(OH)_6^{4+}$ and PbO, controlling soil Pb solubility and leading to low environmental Pb availability (Hale et al., 2012). Moreover the rhizosphere including the root system and its associated microorganisms interacts with its environment, resulting in changes in physico-chemical soil properties (i.e. exsorption of H^+/HCO_3^- to control soil pH, root exudates, etc.) (Kidd et al., 2009). All these parameters influence the chemical speciation and forms of metal(loid)s in the soil, facilitating or preventing metal(loid) sorption by roots and creating a bio-influenced zone (Fig. 2).

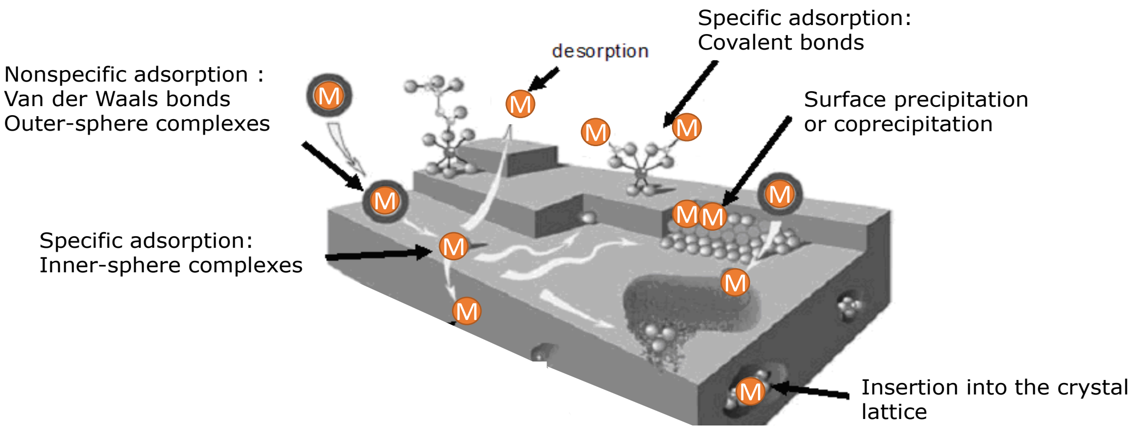


Fig. 4. Interactions of metal(loid)s at the solid-liquid interface (adapted from Manceau et al., 2002) M: metal(loid)

1.3.2. Environmental bioavailability

Toxicity of a contaminant for a biological receptor will depend on its environmental bioavailability, which is in the case of root exposure to TE, the metal(loid) flux through the root surface of a plant for a defined exposure duration. Metal(loid)s are absorbed by plant roots as free ions or chemical species in soluble forms dissolved in the soil solution. As essential metal ions, Zn and Cu have specific transporters to enter into the root cell (crossing the plasma membrane). Zinc is taken up by roots under the form Zn^{2+} by a group of transporters belonging to the Zn- and Fe-regulated transporter protein ZIP family (Fig. 5 and 6) (Zinc/Iron-Regulated Transporter Like Protein, Gupta et al., 2016). Copper is taken up by roots under the form Cu^+ by the COPT1 to COPT7 and ZIP transporters (Fig. 5, 6 and 7) (Yamasaki et al., 2009; Penarrubia et al., 2010; Zhang et al., 2015; Bashir et al., 2016). As non-essential metal ions, Cd and Pb have non-specific transport mechanisms to cross the plasma membrane. Both metals enter root cells under the form Cd^{2+} and Pb^{2+} by using uptake mechanisms for essential cations (e.g. Fe^{2+}, Zn^{2+}, Cu^{2+} and Ca^{2+}) exposing a low substrate specificity (Clemens, 2001 and 2006). Transport mechanisms of Cu and Cd, Pb and Zn to cross the plasma membrane are described in Marchand (2012) and Janssen (2015), respectively.

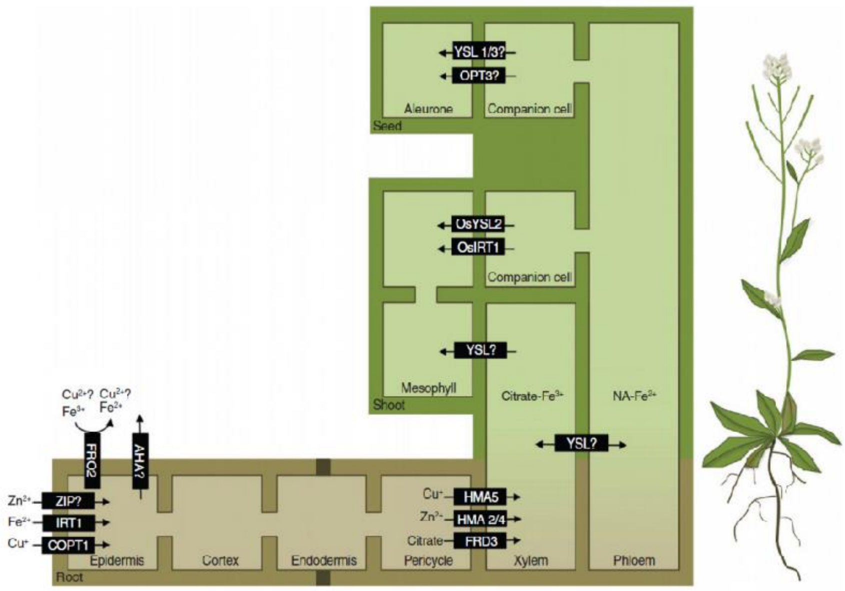


Fig. 5. Intercellular metal transport in dicots (Palmer and Guerinot, 2009). Fe, Zn and Cu are taken up into the symplast by transporters in the epidermis. Reduction of Fe and possibly Cu by FRO2 and acidification of the soil by Arabidopsis H+ ATPases (AHA) contribute to increased metal uptake. Metals can then travel through the symplastic space to the vasculature, by passing the waxy Casparian strip on the endodermis. Transport into the xylem is still not fully characterized but is thought to involve members of the Heavy Metal ATPases (HMA) family and the citrate effluxer FRD3. In the xylem, metals are carried to the shoot through the transpiration stream where they are unloaded into the shoot, most likely by a member of the YSL family. YSLs transporters may also translocate metals to the phloem, where they can then be delivered to the seed. The dark brown boxes represent the Casparian strip.

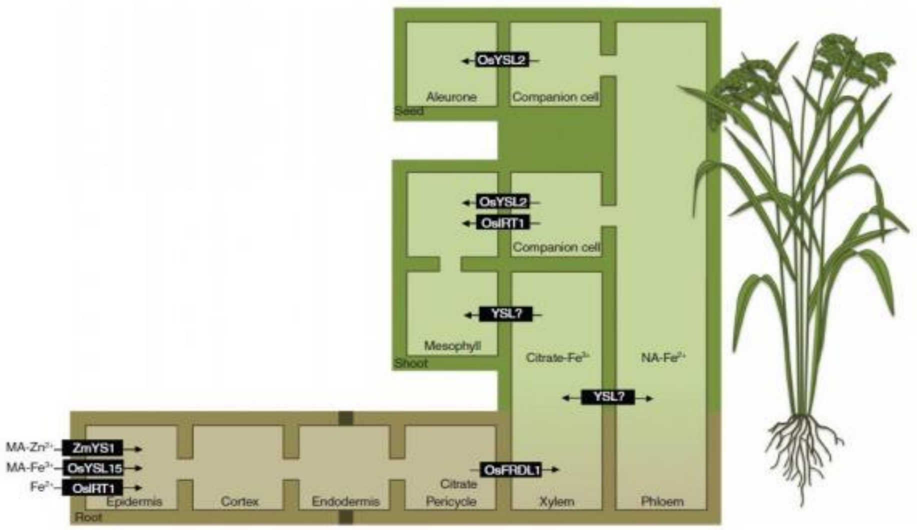


Fig. 6. Intercellular metal transport in monocots (Palmer and Guerinot, 2009). Fe and Zn are taken up as phytosiderophore chelates by Yellow Stripe-Like (YSL) transporters in the epidermis. Fe can also be taken up by OsIRT1. Metals move through the symplastic space to the vasculature, bypassing the waxy Casparian strip on the endodermis. The citrate effluxer FRDL1 is important for loading of citrate into the xylem and subsequent Fe transport into the xylem in citrate-Fe^{3+} form. Fe is transported to the shoot through the transpiration stream. The dark brown boxes represent the Casparian strip.

Once uptake by the roots, metal(loid)s are redistributed through more or less specific transporters to maintain homeostasis and adequate physiological functions of the plant (Fig. 5, 6, 7) and the cell (Fig. 8) (Palmer and Guerinot, 2009). The transport of Cu and Cd, Pb and Zn into the plant will not be discussed here, but is reviewed in Page and Feller (2015). For Cu, its affinity for the root apoplast results in preferential Cu accumulation in roots (Marschner, 1995). The ratio of total Cu concentrations between roots and shoot vary wildly ranging from 2.5 to 166 depending on the level of plant exposure to Cu (Santibañez et al., 2008).

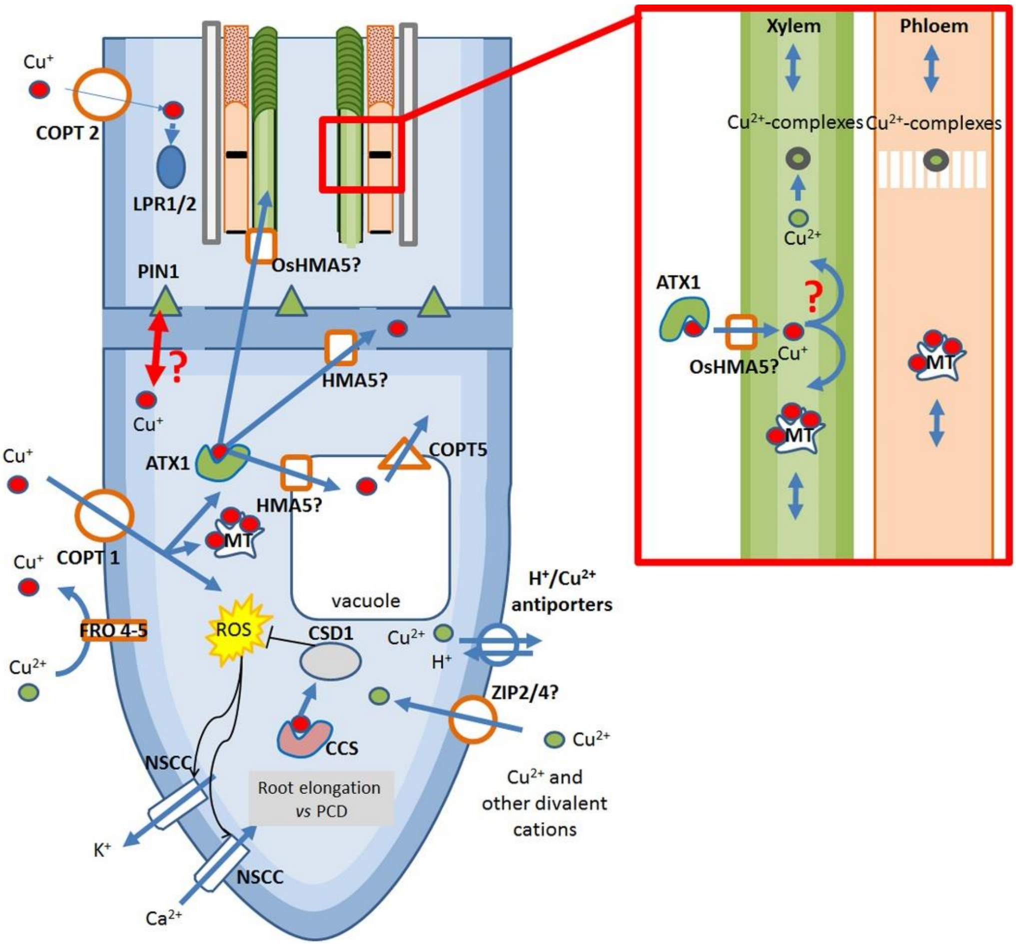


Fig. 7. Overview of the Cu-transport system occurring at the root tip of dicots (Printz et al., 2016). Copper is taken up in the roots in its reduced form Cu^{+} by COPT proteins, highly selective Cu-transporters. Alternative, but still controversial, Cu uptake system may be non-selective ZIP proteins whereas Cu^{2+}-efflux is mediated by H^{+}/Cu^{2+} antiporters. In the cytosol, free Cu^{+} induces the generation of ROS thereby opening non-selective cationic channels (NSCC) allowing the entry of Ca^{2+} and inducing root growth. Cu may further modulate root growth through interaction with auxin efflux carrier pin-formed 1 (PIN1) and with the multicopper oxidases LPR1/2 (low phosphate response multicopper oxidase). In case excessive Cu enters the roots, the massive generation of ROS activates also the efflux of K^{+} through NSCC, causing activation of Programmed Cell Death (PCD). ROS can be quenched by the activity of cytosolic Cu/Zn superoxide dismutase (CSD1) which acquires Cu from Cu-chaperones to Cu/Zn superoxide dismutase (CCS). To prevent ROS generation, Cu^{+} is usually chelated by intracellular metallothioneins (MT) or specific chaperones (ATX1). Cu-transport to extracellular compartments in mediated by HMA5 proteins (i.e. Oryza sativa heavy metal ATPase 5/Arabidopsis thaliana heavy metal ATPase 5). In the phloem and in the xylem, Cu in transported in Cu(II)-complexes or Cu(I)-MT complexes.

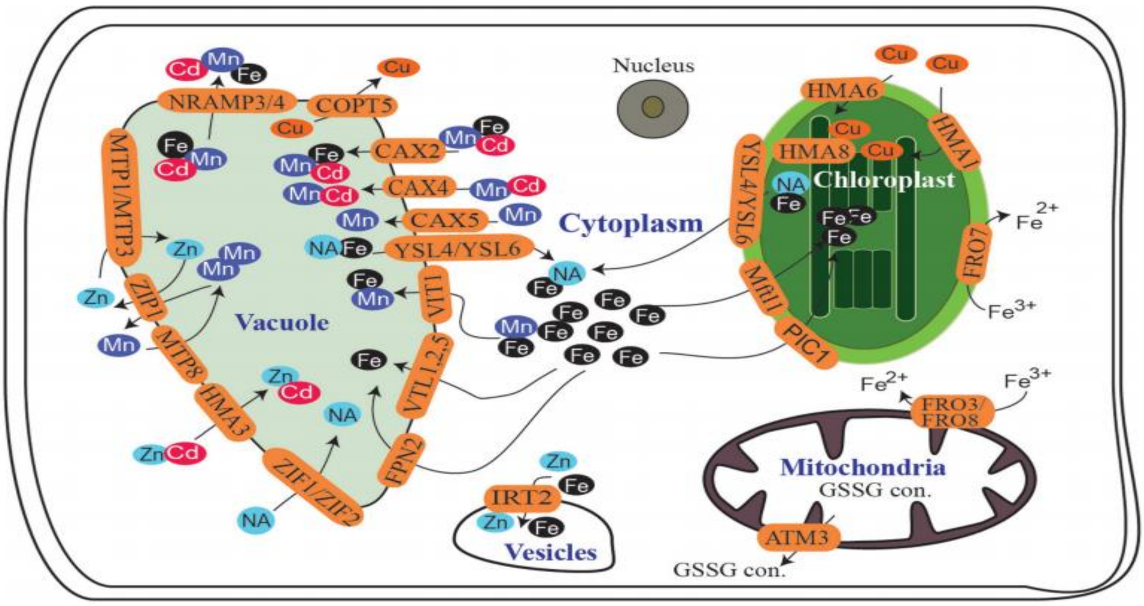


Fig. 8. Summary of subcellular metal transport in *Arabidopsis thalliana* (Bashir et al., 2016). Proteins participating in Fe, Mn, Cu, or Zn transport into or out of different cellular organelles are shown. NA, nicotianamine; GSSG con., glutathione conjugates.

1.3.3. Toxicological availability

To limit Cu, Cd, Pb and Zn phytotoxicity, plants can manage a cascade of physiological processes to maintain the cellular homeostasis by (1) controlling metal(loid) uptake, (2) stimulating the activity of enzymes involved, directly or not, in ROS removal, e.g. superoxide dismutases (SOD), gaïacol-peroxidases (POD) and catalase (CAT), and the production of antioxidants (Glutathione (GSH) and ascorbic acid (AsA)), and (3) producing metallochaperones for metal detoxification or various ligands for metal(loid) binding, e.g. nicotianamine, phytochelatins and metallothioneins (Fig. 7, 9) (Andres-Colas et al., 2010; Leitenmaier and Küpper, 2013; Han et al., 2016). In addition, Cu-tolerant plants have efficient root cellular mechanisms to exclude Cu from the shoots (i.e. improved efflux Cu pumping at the plasma membrane, Cu compartmentation in the root vacuole, Yruela, 2009). The process of metal(loid) mechanisms of plants tolerance is well described in Singh et al., (2016).

The activation of these physiological processes is used in ecotoxicology for setting lower and upper critical threshold values, based on biomarkers, for environmental availability and metal(loid) concentrations in plant parts (Jouili et al., 2011). In most cases, changes in the (1) activity of ascorbate peroxidase (APX), CAT, SOD and glutathione peroxidase (GPX) or (2) contents of their metabolites, may serve as biomarkers for metal(loid) exposure since their activity present a dose-effect relationship with increasing metal(loid) exposure (Mocquot et al., 1996; Thounaojam et al., 2014). Enzymes are highly sensitive to oxidative stress in plants (Cuypers et al., 2000). As the network of defense mechanisms is complex and various biomarkers behave differently, the determination of total antioxidant status of plant parts may give an indication of their tolerance vs. metal exposure (Ghorbanli et al., 2011).

Over the upper critical threshold values, metal(loid)s in excess will induce firstly reversible, and thereafter irreversible, toxic effects in the plant as the exposure increased. The accumulation of a metal(loid) in plants before it induces toxic effect will depend on its toxicological availability (Fig. 2). In contrast to Cd and Pb, Zn and Cu are essential micronutrients for living organisms. Copper is a transition metal and exists under two redox forms (Cu^+ and Cu^{2+}) due to its faculty to exchange electrons from its d orbital (Palmer and Guerinot, 2009). It acts as cofactor for a variety of enzymes such as superoxide dismutase, cytochrome c, oxidase and plastocyanin in many physiological processes of plants, e.g. photosynthesis, mitochondrial respiration, oxidative stress responses, and transduction of ethylene signal, because it is able to exist in multiple oxidation states in vivo (Printz et al., 2016; Aguirre and Pilon, 2016). Zinc is involved in (1) cell division in higher organisms, (2) growth and reproduction, (3) functionality of a variety of enzymes, (4) the stabilization of DNA and gene expression, and (5) the immune system (Lin et al., 2012; Gupta et al., 2016). However, Cu and Zn excess may induce symptoms of toxicity at exposure higher than the cellular Cu / Zn homeostasis (i.e. 3 - 20 mg kg^{-1} mg Cu kg^{-1} DW and 10 - 150 mg kg^{-1} mg Zn kg^{-1} DW) (Tremel-Schaub and Feix, 2005).

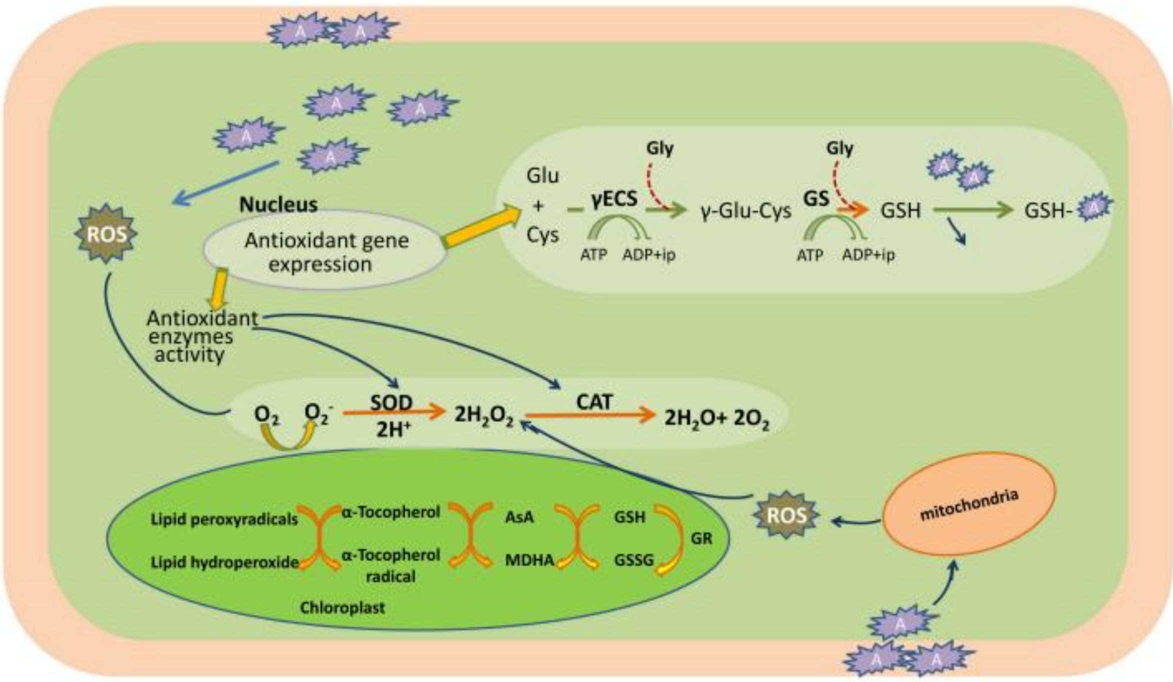


Fig. 9. Metal(loid)s induced-oxidative stress, tolerance, and detoxification mechanisms in the plant cell (Singh et al., 2016). AsA, ascorbic acid; CAT, catalase; Cys, cysteine; γ-ECS, γ-glutamylcysteine synthetase; Glu, glutamine; Gly, glycine; GR, glutathione reductase; GS, glutathione synthetase; GSH, glutathione (reduced); GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDHA, monodehydroascorbate; O_2, oxygen molecule; O^-_2, superoxide radicals; ROS, reactive oxygen species; SOD, superoxide dismutase; A, metal(loid).

1.3.4. Copper, Cd, Pb and Zn phytotoxicity

Excess of Cu, Cd, Pb and Zn can cause damages to plants at various organization levels. At the cellular level, Cu, Cd, Pb and Zn toxicity may result from: (1) DNA damages induced by binding the nucleophilic centres in nucleic acids, (2) inactivation of enzyme activities and disturbed protein structure (e.g. chlorophyll biosynthesis) due to metal(loid) binding with sulfhydryl (-SH) and carboxyl groups in proteins (-COOH), (3) induction of macronutrient (Ca, Mg, P and K) and micronutrient (Mn, Fe, etc.) deficiencies due to nonspecific ion uptake, (4) impaired cell transport processes due to metal(loid) substitutions in metallochaperones, and (5) oxidative damages (Fig. 10) (Arora et al., 2002; Sharma and Dubey, 2005). For a detailed review of mechanisms of metal(loid) toxicity in plants see Küpper and Andresen (2016). Copper, excess induce oxidative damages by inducing, directly or indirectly, Reactive Oxygen Species (ROS) such as superoxide radicals (O_2^-), hydroxyl radicals (HO•) and hydrogen peroxide (H_2O_2) formation through Fenton and Haber-Weiss Reaction. Cadmium, Pb and Zn only induce oxidative stress indirectly, as Cd^{2+}, Zn^{2+} and Pb^{2+} are redox-inert metals, not able to induce ROS production through a Fenton-like reaction (Smeets et al., 2005). Oxidative stress (increase ROS concentrations in cells) is indirectly induced by (1) reducing the pool of antioxidant GSH, (2) disruption of the electron transport chain or (3) activating Ca-dependent systems affecting Fe mediated processes (Noctor et al., 2014). These free radicals may cause severe damage to cells, e.g. polyunsaturated lipid peroxidation, changes in DNA, proteins and small molecules, damages in the photosynthetic apparatus decreasing the photosynthesis efficiency and biomass production (Hego et al., 2016). Cuypers et al., (2016) have reviewed the relationships between metals and oxidative stress in plants. At the plant level, Cu, Cd, Pb and Zn excess are harmful to most plants by inducing very broad range of toxic effects such as chlorosis, necrosis and leaf epinasty, inhibition of germination, decrease in plant growth, and in root and shoot yields (Clemens, 2001; Kuepper et al., 2009). The effect of Cu excess on growth and physiology of important food crops are reviewed in Adrees et al., (2015).

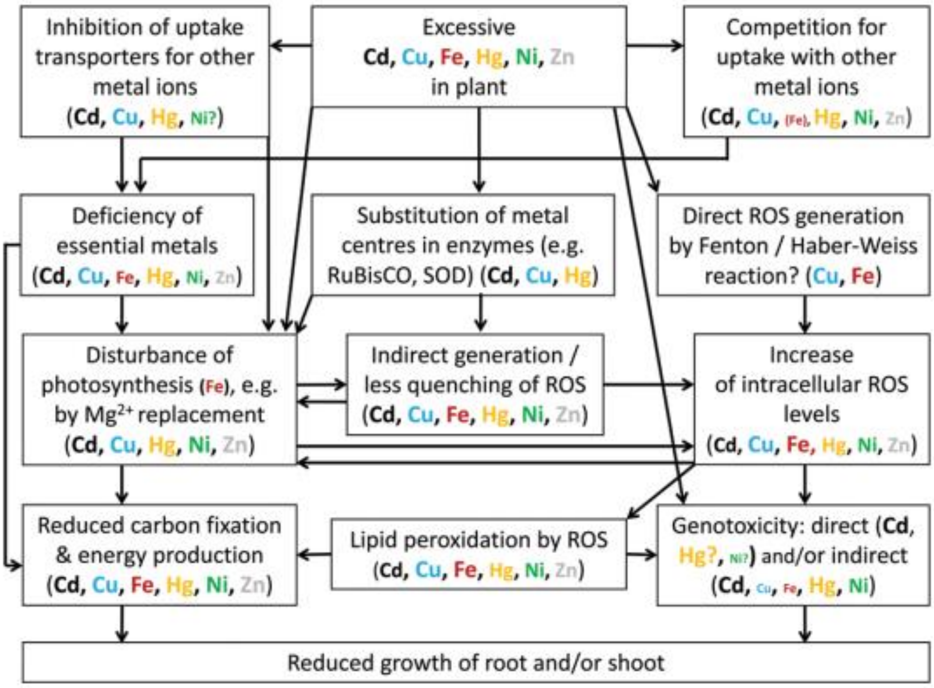


Fig. 10. Mechanisms (including interactions) of metal(loid) toxicity in plants (Küpper and Andresen, 2016). The different sizes of the elemental symbols refer to a more or less pronounced effect of that metal.

1.4. Remediation options of contaminated matrices

Soil and wetland pollution was identified by the European Commission as a major threat to European environment [COM (2002) 0176 final]. In France, since 1977, a legislation on soil pollution "regulation of risk prevention and protection of the environment" obliges industrial enterprises, upon cessation of activities, to secure (art. 34-1 II) and rehabilitate (art. 34-1 III) their contaminated sites (article 34-1, law N°77-1133 of 21/09/1977) this law was reinforced by the circular of 08/02/2007, edited by the Ministry of Ecology, Sustainable Development and Energy (http://www.developpement-durable.gouv.fr/Circulaire-du-8-fevrier-2007,19383.html). These contaminated soils can be managed by multiple rehabilitation techniques. Depending on the selected option, the fate of the contamination will be different, (1) remove from the matrix, (2) stabilize in the matrix and (3) degraded (only for organic contaminants). There are 11 types of techniques recommended for remediating contaminated soils: (1) in situ chemical oxidation, (2) biodegradation, (3) bio-immobilization (4) bioleaching, (5) thermal desorption, (6) phytoextraction, (7) phytostabilisation, (8) physico-chemical stabilization, (9) washing by chemicals and surfactants, (10) physical or physico-chemical sorting, and (11) extraction under reduced pressure and multiphase extraction. All are described in the guide "Treatability of polluted soil, guidelines for the selection of techniques and evaluating their performance" and "Phytotechnologies applied to contaminated land" (ADEME, 2008, 2012; see ITRC, 2006 for a guide in English). The selection of a rehabilitation technique rather than another will be based on several criteria: (1) the physico-chemical nature of the contaminant(s) and the soil, (2) concentration, depth and age of the contamination, (3) size, location and maneuverability of the site to treat, (4) climate, (5) site management e.g. need in fertilization and irrigation, (6) cost estimation and stakeholder engagement (Mulligan et al., 2001; Cundy et al., 2013, Kidd et al., 2015).

For large scale, diffuse contaminations, it is often relevant to use gentle remediation options (GRO), notably based on phytotechnologies (e.g. phytoextraction, phytostabilisation, and biodegradation) rather than conventional 'hard' techniques" (in situ chemical oxidation, thermal desorption, etc.). Conventional techniques are generally costly and harmful for the environment because they either remove the contaminated topsoil from the site or change its properties substantially affecting the composition, biodiversity and ecological functions of the soil (Cundy et al., 2015). Moreover, the soil decontamination or the alleviation of pollutant linkages at a site must not be the only purpose of a remediation technique. Remediation options have to be sustainable (CL.AIRE, 2011). Current international debate in "sustainable" remediation is focused on how sustainability benefits can be maximized and how environmental, economic and social negative consequences can be avoided or limited. In broad terms concepts of sustainable remediation are based on the achievement of net benefits overall across a range of environmental, economic and social concerns that are judged to be representative of sustainability (Cundy et al., 2013). The land end use of the site is now a necessity to meet societal and economic expectation imposed by the UE policy. Since Robinson et al., (2009), the paradigm of remediation of contaminated matrices has evolved into a more comprehensive concept 'the phytomanagement'. Phytomanagement methods encompass the remediation and the valorization of contaminated matrices.

2. Phytomanagement of contaminated matrices

Phytomanagement uses ecological engineering methods (including ecological processes and functions related to gentle remediation options) to rehabilitate potential ecosystem services of contaminated matrices including the production of usable biomass for the production of renewable energy or provide feedstock for the circular bioeconomy. Bioeconomy is defined by the European Union (EU) as the sustainable production and conversion of biomass for food, health, energy, and industrial products. Plant biomass is a term for all organic plant materials (McKendry, 2002). It is consider as a sustainable source to produce renewable energy, replace fossil fuels and provide value-added bioproducts for industrial process (i.e. biofuels, bioplastics, eco-materials, etc.). Above all, the interest for energy crops increased particularly due to its potential use as a carbon-neutral, clean and eco-friendly source of renewable energy (Pandey et al., 2016). The development of renewable energy and reduction of greenhouse gas (GHG) emissions are now established priorities within EU policy. In 2009, the EU has established a directive to reduce global warming by limiting GHG emissions (EU Directive 2009/28/EC). It intended to increase (1) the production of renewable energy sources and (2) the biofuel proportion in the road transportation sector by at least 10% in each member State by 2020 (Gomes, 2012). In the context of energetic and environmental crises, the development of alternative energy sources state the limited space of arable land and highlight the conflict over the use of land and resources (Grandjean, 2007). Using arable land for non-food crops is not an option economically and politically viable in the context of a rapidly increasing population (Rowe et al., 2009). Instead, a sustainable practice could be to use crops to remediate metal(loid) contaminated soils or treat wastewater in constructed wetlands (CW) without claiming further arable land [COM (2006) 34 final Official Journal C 67 –March 18, 2006]. The biomass produced by phytomanagement on marginal lands can be economically valorized and contribute to important environmental co-benefit by achieving economic, social and environmental sustainability (Witters et al., 2012). According to Jiang et al., (2015), combining land remediation with post-process biomass to energy conversion or high value bio-products is an option to enhance the financial viability of gentle remediation options.

Gentle Remediation Options use plant (phyto-), fungal (myco-) or microbiologically-based methods, with or without chemical additives, for reducing contaminant transfer to local receptors by in situ stabilization or extraction of contaminants (Cundy et al., 2015). These technologies are designed to stimulate the ecological functions, reduce pollutant linkages related to contamination of soils or aqueous matrices (e.g. immobilization, mobilization or degradation of contaminants) and propose a path towards rehabilitated ecosystems (Vangronsveld et al., 2009; Mench et al., 2010). Moreover, they may allow the remediation of a range of ecological functions and ecosystem services, which improve: water quality, habitat for microbial and animal communities, C sequestration, biogeochemical cycles of elements and organic matter, primary biomass production, etc. (Bardos et al., 2011; Gomes, 2012; Andersson-Sköld, 2014). Among GRO, two main processes can be distinguished, remediation through: (1) stabilization of contaminant(s) in the soil, and (2) extraction of contaminant(s) from the soil or aqueous matrix (rhizofiltration).

2.1. Stabilization of contaminant by Gentle Remediation Options

Out of gentle options for stabilizing contaminants in soils, **in situ stabilization** of contaminants through mineral and organic soil amendments aims at (1) improving soil biophysical and chemical properties (e.g. organic matter and nutrient contents), and (2) immobilizing metal(loid)s in the solid phase through various reactions, i.e. sorption, precipitation, complexation, ion exchange and redox process, thereby decreasing their mobility and environmental bioavailability (Kumpiene et al., 2008). Sometimes, a plant cover may be implemented in addition to the in situ stabilization option. These plants do no influence the metal(loid) labile pools but prevent the dispersal of polluted dusts through wind and water erosion from formerly bare and sparsely vegetated sites; this technic is called **phytoexclusion**.

Phytostabilisation decreases the environmental availability and bioavailability of pollutants in the contaminated matrix by using plants and their associated microorganisms. The selected plants must (1) accumulate contaminants in their roots (and rhizomes) without a significant root-to-shoot transfer (excluder phenotype), (2) help to either sorb, precipitate or complex the contaminant(s) in the rhizosphere through the rhizodeposition and by facilitating microorganism action, (3) decrease the metal(loid) leaching through an increase of evapotranspiration and (4) limit wind erosion, water runoff, migration to the deeper soil layers by the installation of a dense vegetation cover (Vangronsveld et al., 2009; Mench et al., 2010). The combination of phytostabilisation and in situ stabilization is named **aided-phytostabilization**. In this case, plants, associated microorganisms and amendments stabilize the soil contaminants (Kidd et al., 2015).

Whatever the form of the stabilization option, the goal is to (1) limit pollutant linkages, (2) decrease contaminant exposure inducing potential detrimental effects on living organisms, and (3) restore the cascade of biological processes and functions which in turn promotes ecosystem services (Mench et al., 2010; Bolan et al., 2014; Kidd et al., 2015). Moreover, the plants cultivated in such soils may provide an uncontaminated biomass usable for the production of renewable energy or provide feedstock for the circular bioeconomy.

2.1.1. Amendment for stabilization of contaminants

The amendment selection generally depends on soil pH and metal(loid)s contamination, nutrient and OM contents, and sorbing phases such as Fe, Mn and Al hydr(oxides) and clays in the soil (Kumpiene et al., 2008). The most effective amendments (or their derived compounds after reaction in the soil) must (1) either have or create a large surface reactive area, (2) have fast but long lasting action, (3) be low cost and commercially available, (4) easy to use without negative effect on the soil and plants, (5) be safe for workers, and (6) in compliance with legislation (Bolan et al., 2014). Amendments used to stabilize metal(loid)-contaminated soil include : liming agents including basic slags, phosphates, Fe/Mn (oxy)hydroxides, clay and organic materials, natural and synthetic zeolites, cyclonic and fly ashes, red muds, and iron grit (Ruttens et al., 2006).

2.1.2. Inorganic amendments

Several inorganic soil amendments such as clays, liming materials, phosphate minerals and Fe-, Mn-, Al oxides were reported as effective for immobilizing Cd, Cu, Pb and Zn (Kumpiene et al., 2008).

Inputs of **phosphate compounds** (H_3PO_4, triple calcium phosphate, hydroxyapatite, and phosphate rocks) influence Cd, Cu, Pb and Zn in the soils through various processes: (1) their direct adsorption/substitution by phosphate compounds, (2) P anion-induced adsorption, (3) precipitation with phosphates in solution (Bolan et al., 2014). Immobilization of Cd, Pb and Zn by phosphate compounds were reported as efficient (Castaldi et al., 2005; Gray et al., 2006). In a field study, apatite (phosphates) efficiently stabilized and decreased the bioavailability of Pb, Zn, Cu and Cd in the Pb/Zn-contaminated soils of Arnoldstein (Tica et al., 2011). In a pot experiment, addition of two phosphate compounds (i.e., ammonium phosphate, triple super phosphates) increased the soil pore water Cu concentration, whereas Thomas basic slags decreased it, in the Cu-contamination soils from the wood preservations site (Bes and Mench, 2008). Phosphate compounds should be used with caution as their addition may lead to soil acidification, increasing the mobility of some element such as arsenates (Mench et al., 2005).

Input of **alkaline materials** such as red mud, basic slags, cyclonic and fly ashes, and lime may improve soil acidity and in the same time enhance sorption of Cd, Cu, Pb and Zn by reducing the H^+ concentration and increasing negatively charged sites. Dolomitic limestone (0.2% w/w) in combination with compost (5% w/w) or calcium oxides (0.1% w/w) have proved to decrease Cu concentration in the soil pore water at a wood preservation site (Bes and Mench, 2008; Kolbas et al., 2011). In a field trial at the Arnoldstein site, red mud was effective after five years for immobilizing Cd, Pb and Zn (1M NH_4NO_3-extractable fractions reduced up to 99%) and to limit contaminant uptake by barley (*Hordeum vulgare*. L., *spp. distichon*, Friesl et al., 2009).

Clay materials such as vermiculite may allowed metal(loid) sorption on the clay layers, especially by covalent binding with the hydroxyl groups or by ion exchange (Kumpiene et al., 2008). This process is pH dependent; alkaline pH, cation exchange capacity (CEC) is high and the hydroxyl groups retain metal(loid) including As, Cu, Zn and Pb. At acid pH, CEC decreases and the mobility of metal(loid) may increase and they become more available for the root uptake. In a pot experiment, siderite bearing material reduced extractable (NH_4NO_3) Zn and Pb concentrations and maize uptake in the Cd, Pb and Zn contaminated Arnoldstein soil (Touceda-González et al., 2015). In a pot experiment, the Cr and Mo concentrations in the soil pore water of a polycontaminated-technosol amended with vermiculite (5% w/w) combined with compost (5%) were respectively 6 and 2 time lower than in the untreated soil (Oustriere et al., 2016b).

Fe, Mn and Al hydr(oxides): iron grit (e.g. Fe(0) 97% and Mn 3%) corrodes once in the soil to form newly Fe/Mn/Al oxi(hydro)xides, which can sorb Cd, Cu, Pb and Zn (Tiberg et al., 2016). Such oxides may reduce the available fraction of metal(loid)s, notably in the root zone, and thus lower the pollutant linkages associated with their leaching, ecotoxicity, plant uptake and human exposure (Komárek et al., 2013). Gravel sludge is a fine-grained waste product of the gravel industry that mainly consists in SiO_2, Al_2O_3, and Fe_2O_3, (i.e. 40–65%, 3–7% and 5–12%, respectively). In the Arnoldstein site, gravel sludge stabilized the Cd, Pb and Zn contamination over five years (1M NH_4NO_3-extractable) mostly due to iron oxides immobilizing mechanism and pH increase (Friesl et al., 2009). In a pot experiment, iron grit (1% w/w) was the most effective amendments to decrease the soil pore water Cu concentration at the wood preservation site (Bes and Mench, 2008).

2.1.3. Organic amendments

Input of organic amendments such as **compost**, sewage sludge, biosolid, paper mill waste and peat may (1) form immobilized complexes between organic ligands and metal(loid)s mainly Cu, Pb and As, (2) improve soil texture and structure, (3) promote microbial community functioning, (4) stimulate OM cycle and humification process, notably if the soil mesofauna is present, (5) increase nutrient status and water retention and (6) change soil pH (Kumpiene et al., 2008). Their effects on metal(loid) bioavailability depend on the OM type, its microbial degradability (i.e. C/N ratio), the salt content, soil pH and Eh, soil type, and metal(loid)s of concern (Hattab et al., 2014). However, a risk assessment of the amended soils in pot experiment is usually required before the application of OM directly in the contaminated soil, as OM input enhance DOM concentration which may increase Cu, Pb and As mobility (Beesley et al., 2011).

In addition, **biochar** as amendment for in situ stabilization option has gained attention due to its ability for improving soil quality, its capacity to decrease the mobility of some metal(loid)s, its low degradability and its ability to boost soil carbon sequestration on the long term. Further attention of biochar will be given in the next paragraph.

2.1.4. The origin of biochar involvement: Climate change – Waste management – Energy production – Soil improvement.

Biochar – Climate change: The increasing greenhouse gas (GHGs) emissions in line with global climate change are challenging the world to find new and better ways to deal with such global environmental issues. Solutions are need to support world's increasing needs for energy while reducing GHGs (Lee et al., 2010). Among GHGs, CO_2 is one of the major anthropogenic greenhouse gases (76%) mainly attributed to fossil fuel combustion, followed by methane (CH_4 - 16%) attributed to agriculture and fossil fuel use and nitrous oxide (N_2O - 9%), mainly from agriculture (IPCC, 2007). Therefore, to quench climate change and reverse the global warming trajectory, a program of mitigation measures to reduce CO_2 emission was needed (Wang et al., 2010). Two options may allow to rebalance the global carbon cycle (Fig. 11) (1) improving carbon sequestration into the soil as it may also benefits in terms of soil quality (Lal et al., 2008), and (2) producing energy crops to decrease fossil fuel use (see Introduction 2.2.). According to the Intergovernmental Panel on Climate Change (IPCC, 2007) and the 4 per 1000 initiative (4per1000, 2016) a change in this trend is possible.

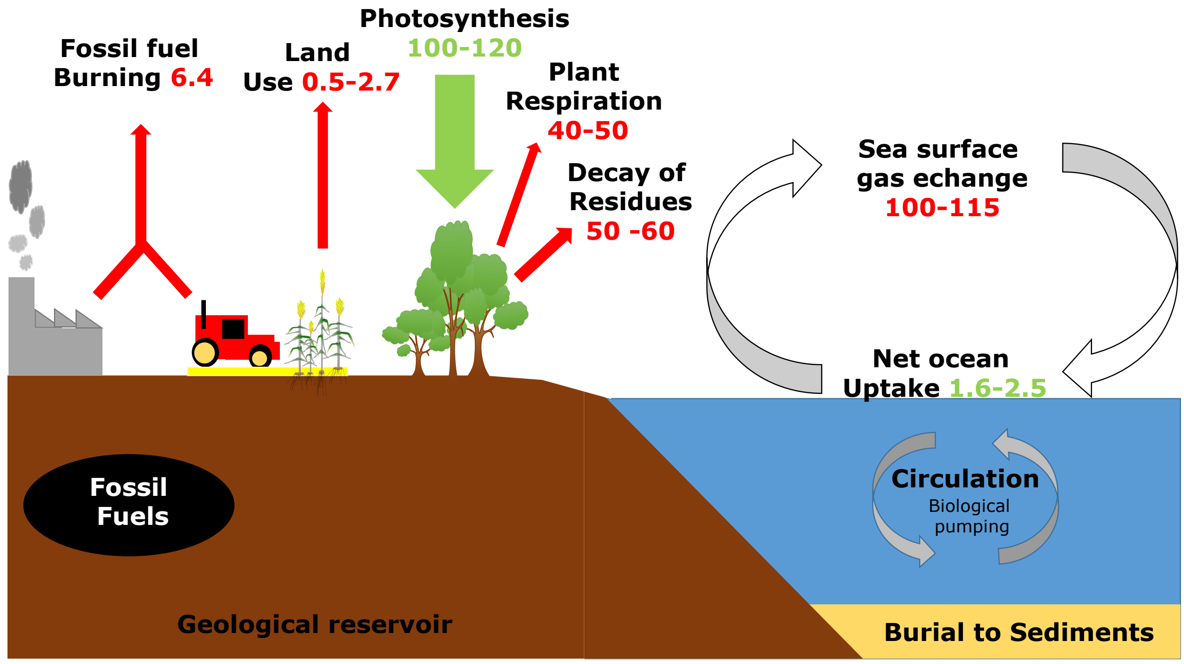


Fig. 11. Sources and sinks of CO_2 - The global carbon cycle (adapted from Steiner, 2006)

Among organic amendments, which can be used to sequester carbon in soils, biologically derived charcoal (biochar) could be a promising candidate (Lehmann et al., 2006; Laird, 2008; Ahmad et al., 2014). Biochars are carbon-rich carbonized residues produced by waste biomass pyrolysis under high temperatures and mid to low oxygen (Lehmann, 2007) or under nitrogen atmosphere (Bach et al., 2016). In an oxygen depleted atmosphere, the burned biomass forms structures that are much more resistant than the original feedstock (Fig. 12).

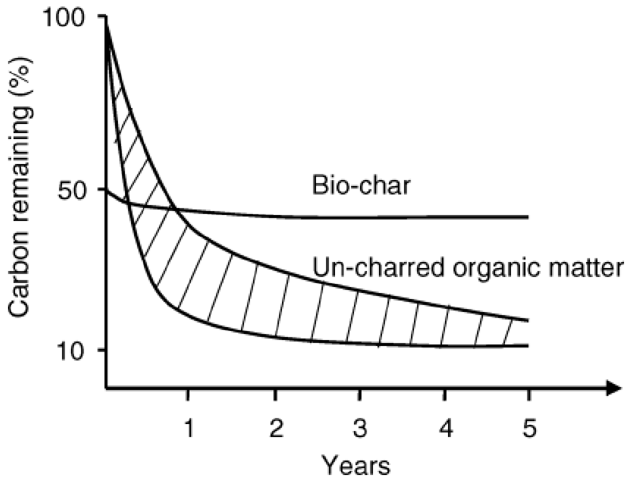


Fig. 12. Stability of biochar C and biomass C in soil (adapted from Lehmann et al., 2006)

This stability against decomposition is attributed to the high proportion of C content, arranged as aromatic compounds (characterized by rings of 6 C linked together without oxygen or hydrogen). When added as a soil amendment, the condensed aromatic nature of biochar makes it more resistant to decomposition and more stable in the environment (Amonette and Joseph, 2009). Consequently, the three main building blocks of biomass undergo transformation with increasing pyrolysis temperature: the content of cellulose and hemicellulose is reduced while lignin structure increases. This phenomenon, associated with biomass loss increases the soil residence time of the biochar C, making biochar a viable option to sequester C in soils (Baldock and Smernik, 2002; Brassard et al., 2016).

Biochar – Waste management & Energy production: The source materials for biochar production are usually organic residues from agriculture, e.g. field and process residues and animal manures, forestry production, e.g. wood residues, and industrial wastes, e.g. paper sludges and biosolids (Jones and Healey, 2010; Sohi et al., 2010; Beesley et al., 2011). Biochars are one of the components created during pyrolysis of crops for energy production, making the biochar systems 'carbon-negative': In the natural carbon cycle (1) plants use CO_2 from the atmosphere to produce biomass, then (2) plants die and decay, emitting carbon dioxide in the atmosphere - making the natural cycle of '**carbon neutral**'. In a Biochar system, (1) plants use CO_2 from the atmosphere to produce biomass, (2) the plant-captured carbon is used to produce energy (i.e. biogas, see Introduction 2.2.), and (3) or/and stabilized as biochar and amended in the soil - making the biochar systems '**carbon-negative**' (Fig. 13).

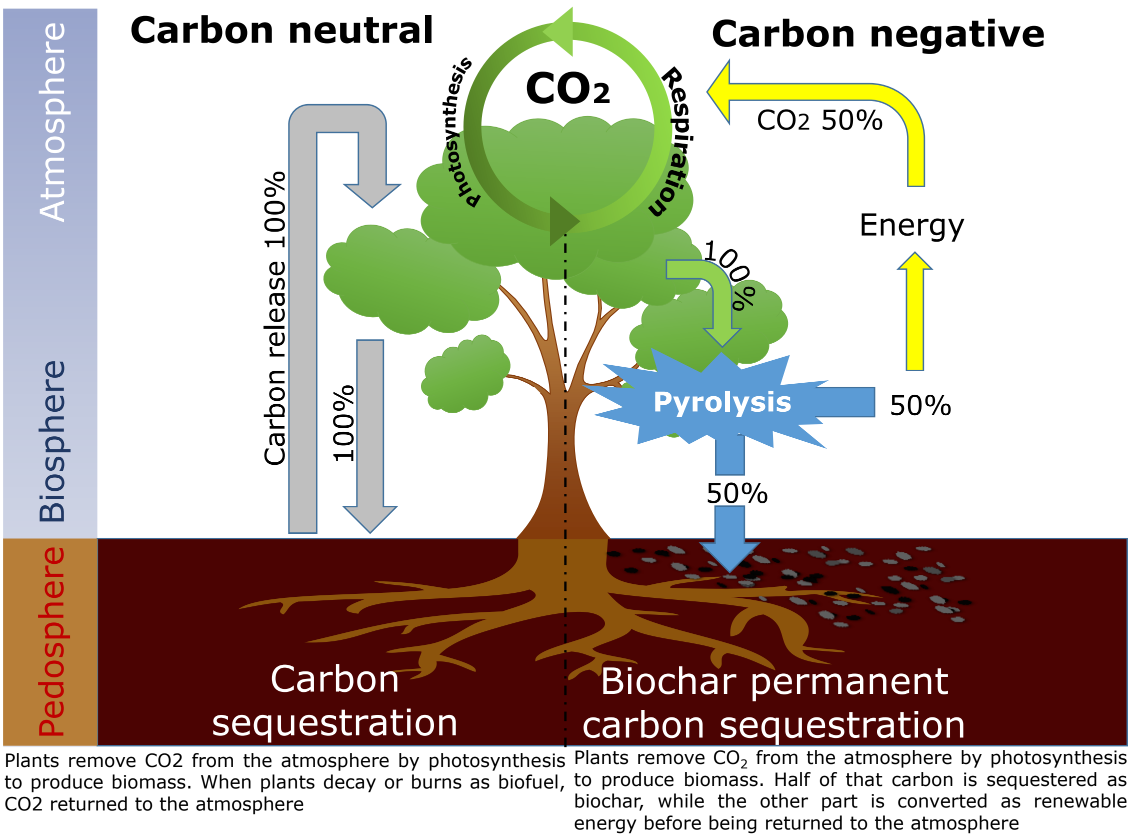


Fig. 13. The carbon cycle versus the biochar cycle (adapted from Glaser et al., 2009)

Biochar – Soil improvement: Land application of biochar-like amendments is not a new concept. Much of our knowledge on biochar were essentially based on studies of biochar-like derives from "terra preta" soils in the Amazonian Dark Earths basin (Lehmann et al., 2003). This name comes from the large amount of residues from biomass burning-derived C stocks remaining in the soil hundreds of years after they were abandoned. These deliberate soil applications by Amerindian populations altered soil physical and chemical properties and led to an improvement of crop production (Lehmann et al, 2006). Its physical and chemical properties depend on the (1) feedstock origin and composition, (2) pyrolysis condition, (3) potential additive such as Fe oxides and (4) postproduction conditions as application rates (Rees et al., 2014). Biochars are a relevant large-scale soil amendment to supply a range of agronomic benefits such as improving physical, chemical and biological properties of soils (Lehmann and Joseph, 2009). It can ameliorate soil fertility, plant growth, root proliferation and crop yields (Sohi et al., 2010; Kookana et al., 2011). Amending soils with biochar significantly affects essential soil properties such as increasing CEC (Rondon et al., 2007), pH (Novak et al., 2009), and retention of water and nutrients for plant uptake (Karhu et al., 2011; Gaskin et al., 2010), prevents nutrient losses with run-off and leaching (Mizuta et al., 2004), improves microbial soil habitats, and decreases bulk density (Sohi et al., 2010).

Biochar – Trace elements: In addition to agronomic benefits, biochar may immobilize few metal(loid)s in soils (Fig. 14) (Luo et al., 2014). Interactions between biochar and metal(loid)s are complex and the possible mechanisms are (1) formation of metal(loid) (hydr)oxides, (2) precipitation or co-precipitation with carbonates or phosphates, (3) electrostatic attraction with negatively charged surfaces on soil particles activated by the increase of pH (cation exchange), (4) sorptive interactions between metallic cations and aromatic π electronic systems from C=C bounds of biochars, (5) specific metal(loid)-ligand complexation involving surface functional groups of biochars (in particular oxygen, phosphorus, sulfur, and nitrogen functional groups) that may or may not involve cation exchange (Uchimiya et al., 2010a). Every sorption mechanisms are more or less expressed depending on the proportion of organic to carbonized fractions comprising the sorbent, soil properties and competition between metal(loid)s for sorption (Uchimiya et al., 2011). An increased number of studies reports the biochar ability to immobilize Cu, Cd, Pb and Zn and to reduce the phytotoxicity of metal(loid)s-contaminated soils (Park et al., 2011; Luo et al., 2014). Biochar made from oak, ash, sycamore and birch was the more effective treatment at reducing pore water Cu concentrations of a Cu mine in Cheshire, UK (Karami et al., 2011). Negatively-charged biochar surfaces sorb Cu, improve water supply, and ameliorate Cu toxicity in sandy soils (Buss et al., 2012). The Cu leaching was reduced following biochar addition in a $Cu(NO_3)_2$–spiked soil (Bakshi et al., 2014). Water soluble Cu was decreased by hardwood-derived biochar at a gasworks site in Brighton, UK (Gomez-Eyles et al., 2011). Addition of a bamboo and rice straw biochar resulted in 97 % reductions of extractable Cu in a polycontaminated paddy field (Yang et al., 2016). Several experiments explained the decrease in Cd, Pb and Zn mobility and phytoavailability in biochar-amended soils by increase in soil pH and CEC, adsorption of metal(loid)-complexing DOM and electrostatic interaction between the positively charged metal(loid) ions and negative charges associated with delocalized π-electrons on aromatic structures of biochar (Beesley and Marmiroli, 2011; Karami et al., 2011): e.g. sugar cane biochar, Cd/Pb/Zn, *Mucuna aterrima* (Piper & Tracy) Holland, Zn-contaminated mine soil (Puga et al., 2015a); bamboo and rice straw biochars, Cd/Cu/Pb/Zn, *Sedum plumbizincicola* L. (Lu et al., 2014); poplar derived-biochar; Cd/Pb/Zn, *Lolium multiflorum* Lam from the Arnoldstein soil (Karer et al., 2015).

However, potential changes in the phytotoxicity of biochar-amended soils over time must be considered. Long-term efficiency of soil amendments is pivotal to preserve stability of metal(loid) immobilization in contaminated soils and to avoid their leaching out of the root zone soils but long term effect of biochar on contaminated soils remain poorly documented. Based on the literature, long term field studies of biomass production on biochar amended-contaminated soils are few. In a field study, 2 years after amendment, grain yields and biomass of wheat were not affected by both rate 10 and 40 t ha^{-1} of wheat straw-derived biochar addition in a Cd-contaminated paddy soil but biochar reduced Cd concentration in wheat grains (Cui et al., 2012). Germination of grass (mix of *Festuca rubra* L. and *Lolium perenne* L.) on site failed in a 3-year field experiment on a contaminated (Ni/Zn) soil amended with broad leaf hardwood-derived biochar (Shen et al., 2016). Two years after amendment, poplar-derived biochar enriched with compost and nitrogen reduced the labile metal fraction of the Cd, Pb and Zn-contaminated soil from Arnoldstein area and allowed the production of an uncontaminated Miscanthus biomass suitable for energy crop production (Puschenreiter et al., 2016).

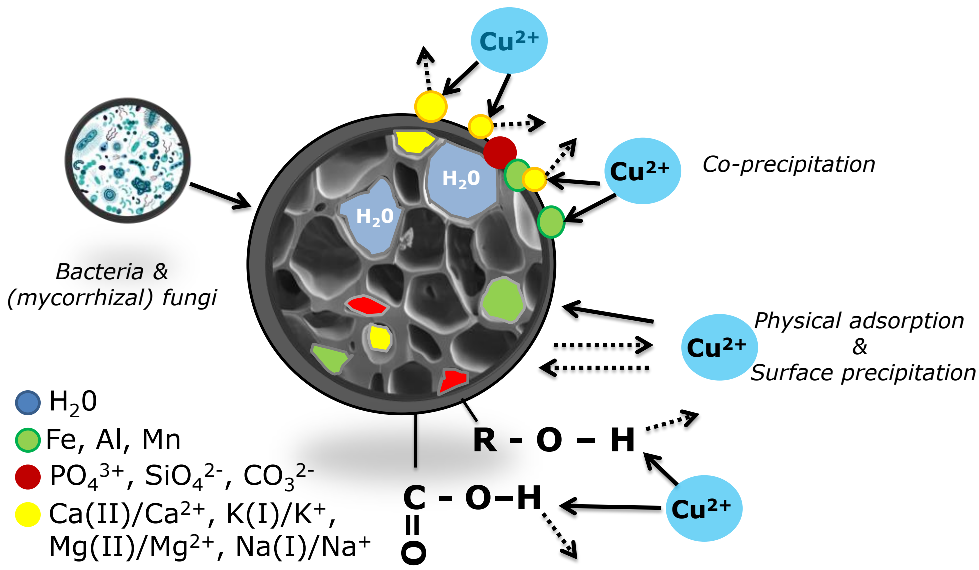


Fig. 14. Postulated mechanisms of the interactions of biochar with Cu^{2+} (adapted from Ahmad et al., 2014)

Negative impacts of biochars: Conversely some negative effects of biochars were reported (Buss et al., 2015). During the pyrolysis process, **polycyclic aromatic hydrocarbons** (PAHs), **dioxins**, **furans** and **volatile organic compounds** (VOC, e.g. low molecular weight, organic acids, alcohols, ketones and phenols, Buss et al., 2015) are likely formed. Moreover, conservative metal(loid)s accumulate in the residual material. Usually, bioavailable PAH, VOC and metal(loid) concentrations in biochars are sufficiently low for not being considered as a threat to plants and the environment (Singh et al., 2010; Hale et al., 2012) but at high concentrations they are suspected to induce acute toxicity to various organisms (Rogovska et al., 2012; Oleszczuk et al., 2013).

Increase soil salinity and electrical conductivity (EC) were reported in biochar-amended soils (Oustriere et al., 2016a), depending on biochar type, and salinity is a limiting factor to plant growth rate, crop yield, plant vigor and seed germination (Munns and Tester, 2008). High soil pore water EC values can cause slight to severe damages to salt sensitive plants (Negim et al., 2012). The soil pore water of saline soils is composed of a range of dissolved salts, such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3, each contributing to salinity stress (Tavakkoli et al., 2010).

Increase of DOM concentration in the soil pore water (SPW) following biochar addition was reported in numerous articles, leading to increase mobility of certain elements such as Cu and Pb: Uchimiya et al., (2013) reported Cu mobilization after inactivated plant biochar addition in a sandy soil induced by either metal(loid) ion-coordinating organic fractions or competition between DOM and metal(loid) ions for the sorption sites of biochar and soil components. Hardwood-derived biochar can enhance 30 times Cu concentration in the SPW due to increased dissolved organic carbon (DOC) in a soil contaminated by As, Cu, Zn and Cd, Kidsgrove, Staffordshire, UK (Beesley et al., 2010). A hardwood biochar and a compost, alone and in combination, as soil amendment did not reduce the DTPA-extractable Cu fraction in a vineyard Cu-contaminated soil but influenced the microbial community composition (Mackie et al., 2015). In degraded soils only amended with biochars, metal(loid) solubility (i.e. As, Cu, and Pb) may increase by co-mobilisation with DOM.

Increase in soil pH due to biochar addition may enhance the immobilization of elements but increase the mobility of others: Biochar amendment reduced the extractability and bioavailability of Cd, Zn and Pb in a soil contaminated by atmospheric depositions (Houben et al., 2013a,b), but root-induced acidification of the rhizosphere counteracted the liming effect of biochar and, in turn, suppressed short-term metal(loid) immobilization (Houben and Sonnet, 2015). *Miscanthus*-derived biochar increased soil pH of a contaminated sewage field and reduced Zn and Cd concentrations in the soil solution whereas those of Pb and Cu increased due to soluble complexes with DOM (Wagner and Kaupenjohann, 2015).

Combining biochar with other amendments: According to Beesley and Marmiroli (2011), to avoid negative biochar effect, one option is to combine amendments such as compost and iron oxides with biochar instead than using biochar alone. Biochar–compost blends can increase total C, N and P in soils and stabilize soil aggregates, as well as stimulate microorganisms (Sizmur et al., 2011; Schulz et al., 2013). Biochar combined with iron grit could reduce the water-soluble soil fraction of metal(loid)s and thus the pollutant linkages associated with their leaching and ecotoxicity (Wagner and Kaupenjohann, 2015). Compost and biochar mixtures can promote Cu sorption and reduce plant Cu content compared to biochar singly (Sizmur et al., 2011; Borchard et al., 2012). Combining pine bark chip-derived biochar (2.5% w/w) and compost (5% w/w) allowed the highest reductions in leachable Cu and plant yield (sunflower) improvements at the wood preservation site (Jones et al., 2016). Biochar–compost mixtures were more effective to decrease water-extractable Cd, Pb and Zn concentrations in contaminated soils than biochar alone (Beesley et al., 2010). Compost and biochar with *Brassica juncea* (L.) Czern. reduced slightly the Cu, Ni and Pb mobility in a mine soil as compared to biochar alone (Rodríguez-Vila et al., 2014). However, the potential gains of adding biochar with either compost or iron grit as compared to biochar alone in metal(loid)-contaminated soils remain poorly documented at field scale.

2.2. Uncontaminated biomass for bioenergy and bioproducts from contaminated sites stabilized by Gentle Remediation Options

Selecting plant species for the phytomanagement of contaminated sites stabilized with GRO is tricky as the selected species need to be (1) adapted for the need of processing chain, (2) tolerant to soil contamination (Gomes, 2012) and (3) ideally display few of these characteristics : have an excluder phenotype regarding metal(loid)s (i.e. phytostabilisation), be perennial, fast growth and biomass production, large and deep root system to explore large soil volumes, be tolerant to biotic and abiotic stress such as drought or pest, non-invasive, and ease of management and harvesting (Vangronsveld et al., 2009; Mench et al., 2010; Kidd et al., 2015). There are multitude of plant species that can be used for the phytomanagement of contaminated soils as well as obtaining useful end products such as bioethanol, biodiesel, fiber, wood, charcoal, alkaloids, bioplastics etc. during the clean-up process (Tripathi et al., 2016). Optimizing agronomic practices are essential for the successful and sustainable phytomanagement of contaminated sites (Fig. 15).

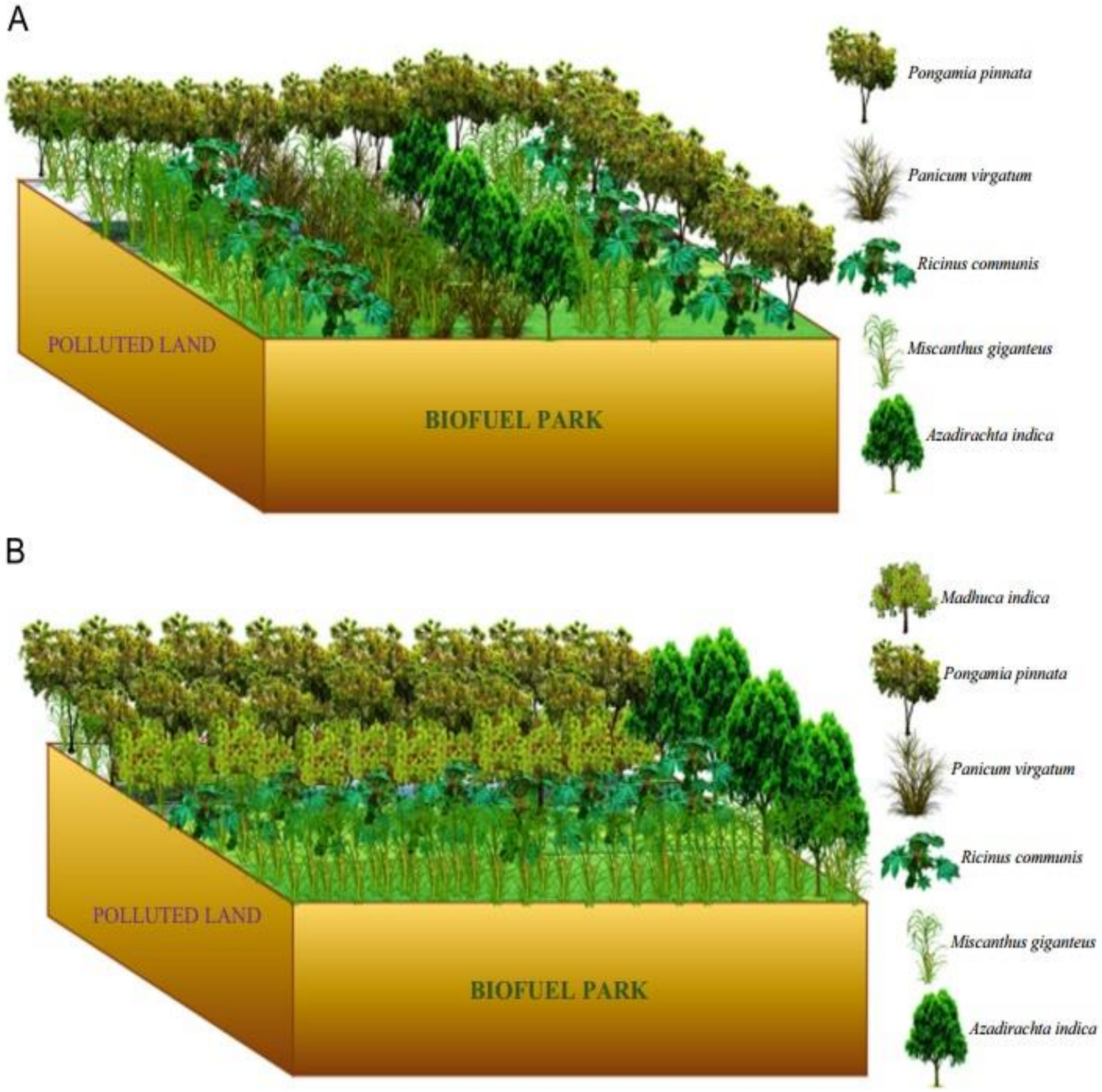


Fig. 15. A and B represent two kinds of intensive biofuel plantation models based on multiple biofuel crops (Tripathi et al., 2016). In both cases, the biofuel trees like *Madhuca indica*, *Pongamia pinnata* and *Azardirachta indica* are intercropped with *Ricinus communis* (shrub) and grasses like *Miscanthus giganteus* and *Panicum virgatum*. Since the gestation periods of trees are longer, the economic returns from such biofuel parks can be maintained by using shrubs and fast growing species like Miscanthus, switchgrass etc. The tree species are recommended for the periphery as they can prevent the mobility of pollutants to the neighboring non-polluted system.

There are two main types of conversion for plant biomass: the bioenergy production, in which the biomass will be used directly as heat or processed into gases or liquids (e.g. ethanol, biodiesel) (Fig. 16), and the production of value-added bioproducts (e.g. timber, paper pulp, bioplastic, dye, pharmaceuticals, etc.) (Coates, 2005; Rockwood et al., 2006; Evangelou et al., 2015). (Fig. 17).

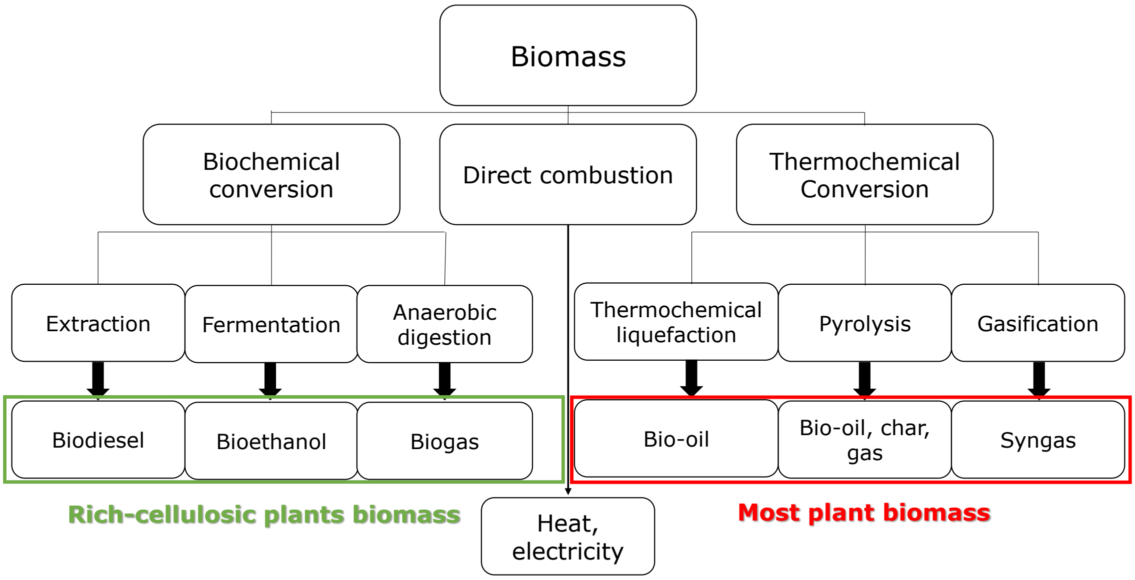


Fig. 16. General conversion routes of biomass for bioenergy production (adapted from Seshadri et al., 2015).

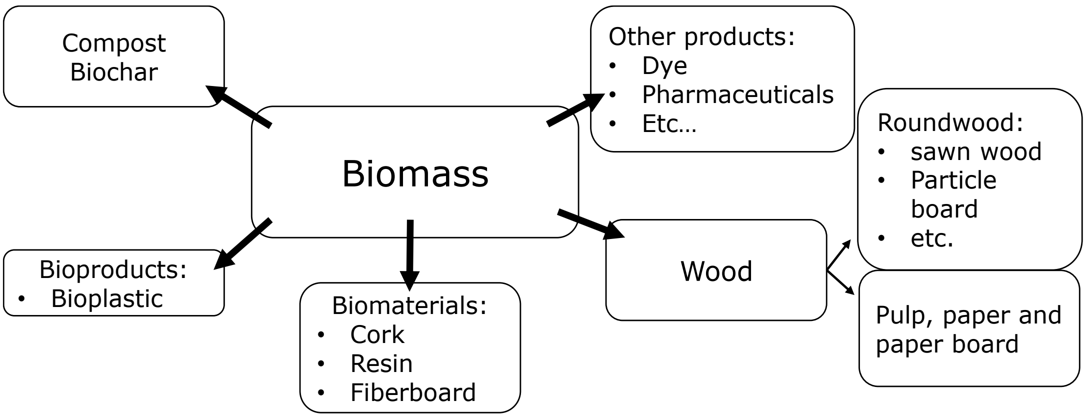


Fig. 17. Biomass-processing opportunities related to the phytomanagement coupled with phytostabilisation of metal(loid)-contaminated soils (adapted from Favas et al., 2015)

Selected plant species for phytomanagement options must be adapted to the conversion process and the form in which the biomass is required. According to Ahmann and Dorgan., (2007), the orientation of a plant biomass towards a processing chain more than another will depend on its characteristics and composition: (1) cellulose, hemicellulose, lignin composition, (2) triglycerides concentration and composition, (3) fiber content, (4) molecules of various interests (i.e. pharmaceutical, dye, anti-oxidant, etc.). Different plant organs from a same plant species may display different characteristics and often integrate different processing chain.

2.2.1. Energy crops for bioenergy production

Energy crops are plants that are cultivated specially for the need of energy production (Pandey et al., 2016). Among them, few species were intensely studied for their ability of producing a valuable biomass while performing sustainable GRO (Fig. 18).

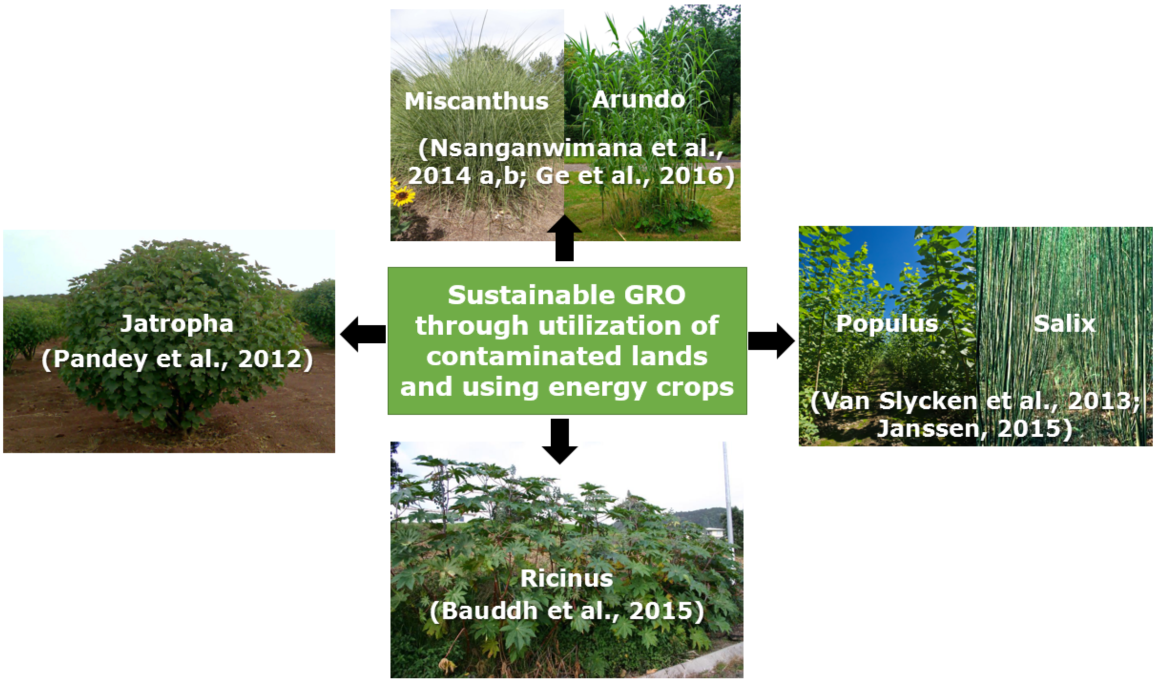


Fig. 18. Towards sustainable phytomanagement through the utilization of contaminated lands and deployment of energy crops (adapted from Pandey et al., 2016).

The type of biomass required for bio-energy production is mainly determined by the energy conversion process and the form in which the energy is required (McKendry, 2002). Considerable efforts have been made to identify and diversify suitable energy crops species for the valorization of contaminated soils. Among plant species proposed for commercial energy farming: woody species, grasses/herbaceous plants, starch and sugar crops may be suitable for biomass production on marginal land. Energy crops are composed of annuals and perennials species (non-exhaustive list): (1) the annuals include sorghum (*Sorghum bicolor* L. Moench), kenaf (*Hibiscus cannabinus* L.), rapeseed (*Brassica napus* L.), *Ricinus communis* L., sunflower (*Helianthus annuus* L.), (2) the perennial include cardoon (*Cynara cardunculus*), reeds (*Arundo donax* L.), Miscanthus (*Miscanthus giganteus*), switchgrass (*Panicum virgatum*), canary reed grass (*Phalaris arundinacea*) and woody species such as willows (*Salix sp*.), poplars (*Populus sp*.), eucalyptus (*Eucalyptus camalduensis* Dehnh.), and black locust (*Robinia pseudoacacia*) (Gomes, 2012).

2.2.2. The case study of two grassy energy crops *Miscanthus* sp and *Arundo donax* L.

Miscanthus sp. is a C_4 perennial grass (Poaceae family) which produces a high yield biomass (e.g. may be harvested twice in a year). It originates from southeast Africa and subtropical Asia. Among *Miscanthus* sp. *Miscanthus sinensis*, *M. sacchariflorus*, *M. floridulus*, and *M. x giganteus* are the most used cultivars (Pidlisnyuk et al., 2014). It tolerates a wild range of environmental conditions such as : coastal lands, stream sides, waste lands, milling sites, damping sites, foothills and mountain slopes up to the 3500 m (Sun et al., 2010). *Miscanthus* sp. are tolerant to metal(loid) excess: As, Sb and Pb (Wanat et al., 2013), Cd, Pb and Zn (Nsanganwimana et al., 2016), Cr, Cu, Ni, and Al (Pandey et al., 2016) and generally accumulate the contaminant in the belowground biomass (Nsanganwimana et al., 2014b). The uncontaminated aboveground biomass may integrate regular processing chain: its rich lignocellulosic biomass is suitable for the production of bioethanol or for heat production with wood and coal (Collura et al., 2006; Han et al., 2011). It may also provide raw materials for several industries (i.e. pulp and fiber production, animal litter, fiber-boards, building blocks and composite particleboards) (Park et al., 2012).

Due to its fast growth and cellulose content Giant reed (*A. donax*) is considered as one of the most promising energy crops for marginal lands. This rhizomatous grass (Poaceae family) is native from the Mediterranean basin and Eastern Asia. It was introduced in temperate and hot zones worldwide and it is now considered as naturalized species considering its wide distribution (Nsanganvimana et al., 2014). Giant reed is claimed as one of the fastest growing grass (Palmer et al., 2014) with high photosynthetic rate and able to reach a maximal stem height between 8–14 m. Under optimal growth conditions, it can produce 20–38 t DW ha^{-1} $year^{-1}$ of shoot biomass or 4-10 cm day^{-1} depending on the location (Bonanno et al., 2012; Alshaal et al., 2015). This C3 grass is able to grow in marginal land, can adapt to a large range of hostile ecological conditions such as extreme salinity and pH, and tolerates severe and prolonged drought (Alshaal et al., 2013a). Giant reed is also tolerant to metal(loid) excess: Zn, Cr (Kausar et al., 2012; Li et al., 2014), Cd and Ni (Papazoglou et al., 2005), Cu (Elhawat et al., 2015), and As (Mirza et al., 2010 and 2011) and accumulates metal(loid)s mainly in its roots and rhizomes (Nsanganvimana et al., 2014). Arundo uncontaminated aboveground biomass is suitable for the production of solid biofuels for direct combustion and co-combustion (Hoffmann et al., 2010), gasification and pyrolysis (Ghetti et al., 1996), and for anaerobic digestion to produce biogas, or even submitted to alcoholic fermentation for bioethanol production (Pilu et al., 2012). Its culms represent an important source of cellulose for the production of paper pulp (Fiorentino et al., 2013). Its residual lignin content can be used for production of wooden build materials such as lignin-based resin coatings and composite and plant fiber/plastic composites (see Nsanganvimana et al., 2014 for a review).

2.2.3. The case study of two woody species *Populus* sp. and *Salix* sp.

Short rotation coppices (SRC) use fast growing woody species characterized by fast juvenile growth (i.e. in 2-5 year cycles for poplars and willows) for high biomass production in short amount of time and space (up to 7.7 t $ha^{-1}year^{-1}$ with *Salix sp*.; Mola-Yudego et al., 2015). Several energy crops for SRC have been tested for phytomanaging contaminated soils (Lucas-Borja et al., 2011; Kidd et al., 2015; Janssen et al., 2015; Gonsalvesh et al., 2016), the cellulosic biomass being used to produce heat and electricity by direct combustion or transformed by pyrolysis and gasification into biofuels and biochars (Bridgwater, 2006; Nsanganwimana et al., 2014a). Among energy crops, poplar (*Populus spp.*) and willows (*Salix sp*.) SRC are the most often used species to phytomanage metal(loid)-contaminated sites (Evangelou et al., 2012; Kidd et al., 2015; Mola-Yudego et al., 2015). Both species can grow from temperate to tropical region and have marked adaptability to various soils and climates (Pandey et al., 2016). They are also tolerant to metal(loid) excess: Cd (Robinson et al., 2000), Cr (Sebastiani et al., 2004 for poplar; Yu et al., 2010 for willow), Cu (Bes, 2008), Se (Pilon-Smits et al., 1998 for poplar; Yu and Gu, 2008 for willow) and poly-contaminated Cd, Pb, Zn (Delplanque et al., 2013; Chen et al., 2015; Janssen et al., 2015). Poplar and willow mainly accumulate Cu and Pb in their roots, Cd and Zn mainly in their shoots but with low contaminant accumulation in the wood (Laureysens et al., 2004; Borghi et al., 2008; Chen et al., 2012; Wang et al., 2014; Enell et al., 2016; Qasim et al., 2016). Their high cellulose as well as moderate amount of lignin and hemicellulose in wood make them suitable candidates for bio-ethanol production (Zamora et al., 2013; Stolarski et al., 2015). The higher heating value of poplar, less ash content, specific element ratios and lignin, cellulose and hemicellulose proportion in its wood make it promising candidate for thermal energy production by biomass combustion (Pandey et al., 2016).

2.3. Extraction of contaminants in the contaminated matrix by Gentle Remediation Options

Phytoextraction extracts pollutants from contaminated soils by using 2 types of metal(loid)-accumulating plants: (1) hyperaccumulators, able to uptake and tolerate high concentration of one or two (rarely more) metal(loid)s in shoots: i.e. (in mg kg^{-1} DW) 100 for Cd, Se and Tl, 300 for Cu, Co and Cr, 1000 for Ni, As, and Pb, 3000 for Zn, and 10,000 for Mn, van der Ent et al., 2013); and (2) accumulators high biomass yielding plants, which display a secondary accumulator phenotype (relatively high shoot concentrations of metal(loid)s as compared to common ranges but lower than threshold values for hyperaccumulators) but produce a higher aerial biomass than hyperaccumulators (Table 1) (Vangronsveld et al., 2009; Kidd et al., 2015). Metal(loid)s are translocated and stored in the shoots which may be then harvested and valorized (see Introduction 2.4.1.). Phytoextraction techniques can be improved by incorporating organic and inorganic soil amendments, which enable plants to uptake higher contaminant amounts when availability of metal(loid)s in the soils is insufficient for the active root absorption (Mahar et al., 2015). Potential chelating agents are organic and mineral acids, elemental sulfur and ammonium fertilizers. The application of chelating (chelate forming) agents, for instance, synthetic aminopolycarboxylic acid ethylenediamine-tetraacetic acid (EDTA), is efficient to enhance Cu, Cd, Pb, Zn and Ni uptake, particularly Pb (Mahar et al., 2016) but few disadvantages were reported while using EDTA (1) adverse effects on the soil microorganisms, (2) increase in leaching of metal and possible contamination of ground water, and (3) slow decomposition of the synthetic organic acids (Evangelou et al., 2007). To overcome these problems, some rapid decomposing natural organic acids are considered as alternatives to synthetic chelating agents i.e. ethylene diaminedisuccinic (EDDS) acids and nitrilotriacetic (NTA) (Evangelou et al., 2007).

Rhizofiltration extracts pollutants from contaminated aqueous matrices by using roots and rhizomes, notably those of macrophytes and some terrestrial plants. This phytotechnology exploits the ability of root systems to sorb and/or uptake the contaminant and store it in the belowground biomass (Marchand et al., 2010; Wu et al., 2015a). The metal(loid) fraction usually transferred to the shoot of macrophytes is estimated at <2% (Marchand et al., 2010). This method is more often used in constructed wetlands even if it also used in natural wetlands. Constructed wetlands (CW) are artificially created wetland in which the effluent flow in planted ponds is either on the surface, under the surface, vertical, horizontal, or mixed (Fig. 19. Nivala et al., 2013; Wu et al., 2015a). Successful rhizofiltration of Cu-contaminated effluent with macrophytes planted in constructed wetlands were reported (Guittonny-Philippe et al., 2014; Marchand et al., 2014a; Newete and Byrne, 2016).

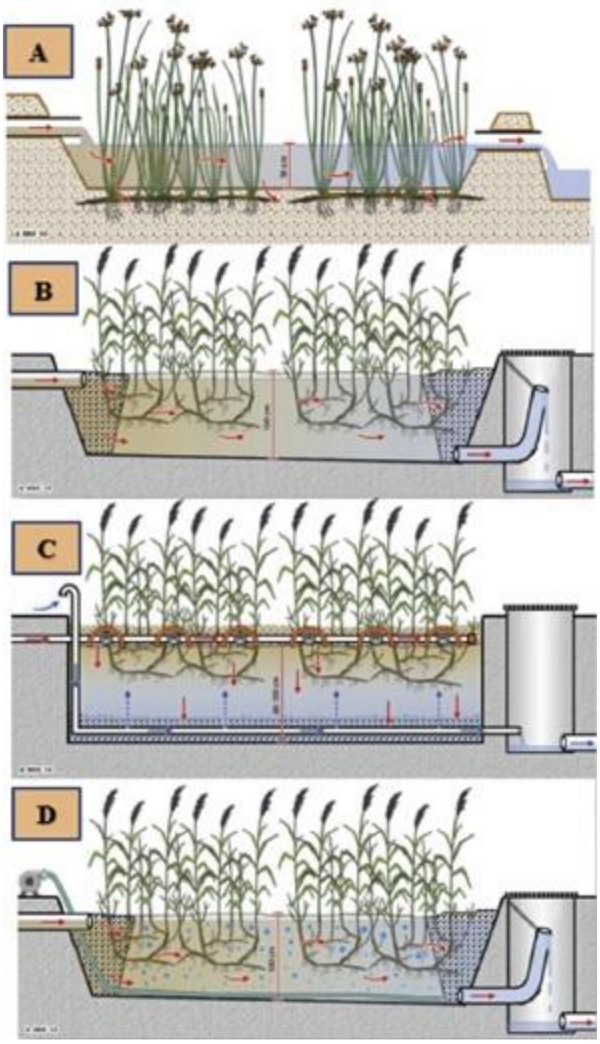


Fig. 19**.** Effluent flow in CWs: (A) in surface (B) horizontal in subsurface (C) vertical (D) mixed system

A successful rhizofiltration will depend on (1) the macrophyte species planted in the CW system, (2) the effluent residence time in the CW, (3) the physicochemical conditions of the effluent, such as pH and condition redox, (4) microorganism communities (ecto and endophytes, fungi) associated with the plants (Marchand et al., 2010; Gilbert et al., 2012; Nivala et al., 2013). The selected plant species must display one or more of these characteristics (1) be tolerant to high metal(loid)-concentrations, (2) have a rapid growth and a (relatively) high biomass yield, and (3) accumulate contaminants in their root without transferring to its shoot (excluder phenotype) (Bonanno and Lo Giudice, 2010; Nsanganwimana et al., 2014a).

Many macrophytes were tested to rhizofiltrate metal(loid) contaminated effluents, each species reaching different levels of effluent purification. *Phragmites australis* (Cav.) Trin. ex Steud is commonly used species in CW (Sun et al., 2013; Rocha et al., 2014; Huguenot et al., 2015; Kumari and Tripathi, 2015).

It removed 98% of Cu in a stormwater bioretention systems (Rycewicz-Borecki et al., 2016). *Phragmites australis* and *Phalaris arundinacea* L. removed 90 and 82% respectively of Cu in a CW contaminated by a $CuSO_4$ solution (Marchand et al., 2014). *Phalaris arundinacea* showed similar Cu removal capacity as *P. australis* (Vymazal et al., 2007; Maine et al., 2009). *Arundo donax* L., *Iris pseudacorus* L., and *Cyperus eragrostis* Lam., are less commonly used in CW but their tolerance to high metal(loid)-concentrations, including Cu makes them relevant candidates for their use in CW (Soda et al., 2012; Idris et al., 2012; Yadav et al., 2012; Bonanno, 2013; Sun et al., 2013; Guittonny-Philippe et al., 2015). In Aquitaine, *A. donax* L., *C. eragrostis* Lam., *I. pseudacorus* L., *P. arundinacea,* and *P. australis* are common macrophyte species tolerant to high concentration of metal(loid)s (Marchand et al., 2014). Rhizofiltration of Cu contaminated effluent in CW would (1) treat contaminated effluents and (2) promote biomass production which can be valorized (see Introduction 2.5.).

Table. 1. Case studies assessing phytoextraction with hyperaccumulator or accumulators in Cu, Cd, Pb and Zn contaminated soils (adapted from Kidd et al., 2015)

Species	**Genotype selection criteria**	**Study type**	**Soil characteristics**	**Metal(loid)s mg kg-1**	**References**
Amaranthus hypochondriacus	Cd hyperaccumulator	F	Metal-contaminated soil affected by long term irrigation with local metal-contaminated water	Cd/Pb/Zn (4.52/721/96.74)	Li et al., 2016b
Anthyllis vulneraria	Zn-hyperaccumulator	F	mine site	Cd/Pb/Zn	Grison et al., 2015b
S. smithiana Willd. (Salix caprea × Salix viminalis (BOKU 03 CZ-001))	Metal accumulator	G	7 metal(loid)-contaminated soils	Cd/Pb/Zn	Puschenreiter et al. 2013
Populus tremula	Rapid growth, tolerance to various climatic conditions and high adaptability to a wide range of soils and presence of TEs	G	Metal(loid) -contaminated soil affected by former smelter; Calcaric Cambisol; pH 7.2; organic C 27.3 g kg-1 and total N 2.1 g kg-1	Cd/Pb/Zn (24.3/3560/1960)	Langer et al. 2012
Salix caprea, S. purpurea, S. fragilis, Populus tremula, P. nigra, Betula pendula	Metal accumulators	F	Cd/Zn-contaminated soils across Central Europe	Cd/Zn	Unterbrunner et al.2007
Salix caprea, S. fragilis, S. × smithiana (S. caprea × S. viminalis), S. × dasyclados (S. caprea × S. cinerea × S. viminalis)	Metal-accumulators	G	Agricultural soils affected by former Zn/Pb smelter; loamy sand; pH 6.5-7.5	Cd/Zn (4.0-13.4/490-955)	Wieshammer et al.2007
Helianthus annuus L.	Metal-accumulators	F	Cu-contaminated soil affected by copper based salt for wood preservation	Cu (163-1170)	Kolbas et al. 2011

Study type: F, field experiment; G, greenhouse based

2.4 Processing chain for metal(loid)-rich biomass

Biomass cultivated on metal(loid)-contaminated soils may display metal(loid) concentrations above the common values in plants, therefore the management and disposal of such plant biomass is of concern. When plants have undergone significant metal(loid) uptake, valorization of biomass involves some new aspects related to harvest, procedure and final product reutilization (Dilks et al., 2016). Such metal(loid)-enriched biomass must be used or disposed safely. This technical issue is not completely solved yet.

2.4.1. Energy production processing chains adapted for metal(loid)-rich biomass

For metal(loid)-rich plant biomass, the safer solution for energy production is the combustion or pyro-gasification of biomass for energy production (Lievens et al., 2008). When treating a metal-rich plant feedstock by pyrolysis, the objective is to maximize the metal content in the char and minimize it in the liquid and gaseous phases, thus preserving the high value of the newly generated products. Controlled variables such as temperature, heating rate and vapor residence time must ideally be adjusted accordingly to the feedstock initial characteristics (Dilks et al., 2016). Such processes operate at high temperatures (> 850°C) at which metal(loid)s are more easily volatized or accumulate in residual waste (Stals et al., 2010). For a Cd, Cu, Pb and Zn-rich biomass (birch and sunflower) obtained by phytomanaging contaminated soils, an acute temperature adjustment for fast pyrolysis allowed the production of bio-oil free of metal(loid)s with an accumulation of the contaminants in the ash/char fraction (Lievens et al., 2008). Pyrolysis temperature is pivotal for the transfer of metals into the volatile pyrolysis products. For this study, at a temperature of 450°C, almost all metal(loid)s were accumulated in the char/ash residue. Even though enriched in metal(loid)s, the char/ash residues produced during the pyrolysis process may be in turn valorized (see Introduction 2.1.4) (Gonsalvesh et al., 2016). Shoots of tobacco used for phytomanaging soils contaminated mainly with either Cu or Cd/Zn were converted by pyrolysis and subsequent physical activation by steam into activated carbons. These activated carbons were enriched in metal(loid)s but their leachability was limited, which indicates that those products may be safely applied as adsorption media (Gonsalvesh et al., 2016). After such process, metal(loid) recovery from fly ash may be performed using hydrometallurgical routes (Fiorentino et al., 2013). Combustion of metal(loid)-rich biomass may also be processed in boilers (i.e. collective or industrial) equipped with efficient air cleaning systems (i.e. multi-cyclones, electrostatic precipitator, baghouse) (Chalot et al., 2012; Delplanque et al., 2013). Contaminants in the remaining ashes influence their potential use. Depending on their metal(loid) concentrations, these ashes may not be considered as potential fertilizers for agricultural soils and must be disposed as hazardous waste. However, if the ashes contained acceptable metal(loid) concentrations, they can be recycled for cultivable grounds (Bonanno et al., 2013). Trace elements accumulated in plant parts during the phytomanagement and remediation of a marginal land may influence the activity of microorganisms that participate in the conversion process (Cao et al., 2015). In certain conditions, minor quantities of certain metal(loid)s such as Ni, Co, Mn and Fe are found to enhance biogas potential stimulating the bacterial activities, but in most cases metal(loid) toxicity slowdown the microbial-driven conversion of lignin cellulose to ethanol and biopolymers and these options are usually set aside (Srujana, 2015). Copper, As and Pb may induce an inhibitory effect on bio-conversion due to their toxic effect on the microbes. Copper, Pb, Cr(VI) and Zn inactivate enzymes during bio-gasification (Selling et al., 2008). Therefore, when contaminant-enriched crops are used for energy purposes, their impact on conversion efficiency as well as the energy should be considered.

2.4.2. Specific processing chains for metal(loid)-rich biomass

Few additional processing chains may valorize the metal(loid)-rich biomass by considering the metal(loid)s as a potential profit and not a threat:

- The "Phytomining" for the production and commercial exploitation of metal(loid)s (Ni, Zn, Co, etc.) extracted by hyperaccumulators.

- The “Biofortification” for the production of biofortified products used as dietary supplements.

- The "Ecocatalysis" for the production of metal(loid) species with unusual characteristics able to catalyze fine organic chemical reactions.

Phytomining is based on the ability of plants to accumulate high concentrations of metal(loid) (Ni, Co, Zn, Se, Tl, Cd, and Cu) in their aerial parts (Tibbett, 2015). The first studies about phytomining date back to the 90s with the first patent in 1988 (Nicks & Chambers, 1995; Robinson et al., 1997; Chaney et al., 1988). In this process; shoots are harvested to extract the economically valuable metal(loid). This processing chain depends on the labile fractions of metal(loid) in soils, plant uptake, biomass yield and particularly the metal(loid) price at the world market (Robinson et al., 2015). Nickel phytomining was proven to be economically feasible in the USA (Chaney et al., 2007). Also in Europe (Albania) successful field experiments using a Ni hyperaccumulator (*Alyssum murale*) were reported (Bani et al., 2007). Phytomining is, currently, only suitable for metals (such as nickel), with high market value (i.e. 9.7 \$ kg^{-1} Ni vs 5.2 \$ kg^{-1} Cu).

Biofortification is an agricultural process that increases the uptake and accumulation of specific nutrients (Rouached, 2013). According to WHO, enrichment or fortification of foods is defined as "the deliberate adding of one or more micronutrients as dietary supplements, to increase the intake of these micronutrients, correct or prevent a dietary deficiency and provide a health benefit” (CX/NFSDU 13/35/10). Nowadays, the Codex General Principles for the Addition of Essential Nutrients to Foods (CAC / GL 09-1987) do not contain any directive directly related to these micronutrient additions through bio-fortification of crops by conventional breeding or agricultural practices. More than 60% of the world population is supposed to be deficient in Fe, 30% in Zn and/or I and 15% in Se (Rawat et al., 2013). In addition, Ca, Mg, and Cu deficiencies are common in many developed and developing countries (White and Broadley, 2009). The jointly use of phytoremediation and biofortification technologies could both produce food enriched in one or more essential elements (Fe, Zn, I, Se, Ca, Mg and Cu) and treat contaminated soils (Wu et al., 2015b). This option has been mainly used for the Se-enrichment of food. Bañuelos and Mayland (2000) and Bañuelos et al., (2010) found that a *Brassica* *juncea* Se-enriched by phytoextraction (2 ± 0.5 µg Se g^{-1} DW) can be used safely as dietary supplement in animal rations.

Ecocatalysis is a process based on the novel use of metal(loid) chemical species originating from plant biomasses with high metal(loid) concentrations. Such biomasses produced metal(loid)-ligand complexes used as "Lewis acids" to catalyze fine organic chemical reactions (Fig. 19). The originality of eco-catalysts compared to conventional catalysts comes from their unusual oxidation levels, new associated chemical species and synergic effect of the eco-catalyst mix (Clavé et al, 2016 a). These catalytic mix allow the formation of carbon-carbon bonds, modification of one or more electrophilic elements in functional group and catalyze reactions of oxido-reduction. They can (1) simultaneously interact with several chemical functions of the same compound, limiting the chemo-selectivity (the reactivity order with various functional groups of a molecule), (2) preferentially orientate the catalytic reaction in some reactive sites rather on another (region-selectivity), and (3) orientate the catalytic reaction toward a reaction product rather than another (stereo-selectivity) (Escande, 2014). This catalytic mix allows to discover new reactions or ameliorate already existing one, sometimes resulting in different products from those obtained through conventional catalysts (Fig. 20). This catalytic mix allowed the synthesis of molecules with high added value: pharmaceuticals (e.g. anticancer and antiviral agents), cosmetics, agrochemicals (e.g. green pesticides) and textiles. Metal(loid)s such as Rhodium, Palladium, Nickel, Zinc, Copper, Manganese, Molybdenum and Cobalt are involved in these catalytic reactions.

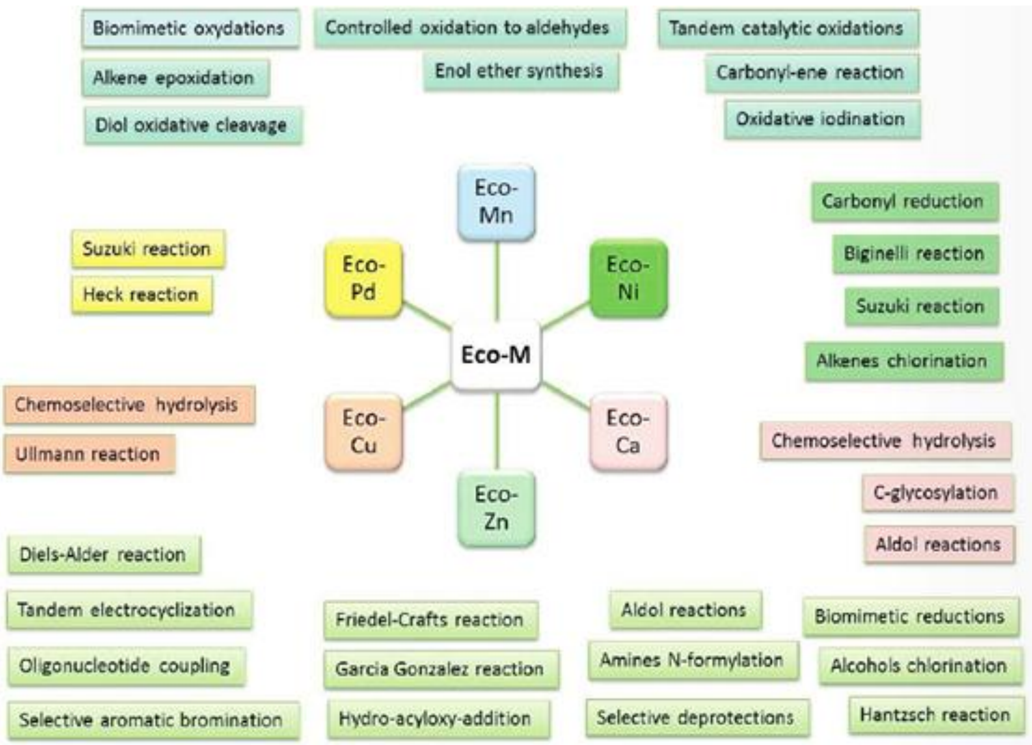


Fig. 20. Organic synthesis by eco-catalysts (Grison et al., 2015a)

2.5. The search for new Cu-eco-catalysts

New ecocatalysts are needed to increase the number of potential catalyze reactions and among metal(loid)s, Cu is of interest (Clavé et al., 2016 a). Cu-based catalysts are sustainable and cost-competitive catalyzers for the high yield production of next-generation biorefinery components (Yuan et al., 2013). High Cu concentrations (i.e. ≥1000 mg kg-1 DW) are needed to meet the requirement for biocatalysis (Clavé et al., 2016 b). Such high shoot Cu concentration is unusual in plants. The criteria for Cu hyperaccumulation is 300 mg kg^{-1} shoot DW, only 46 species can meet this requirement (van der Ent et al., 2013) and the majority of these Cu-hyperaccumulators are originated from Congo (with at least 32 species; Reeves and Baker, 2000), China or Sri Lanka (van der Ent et al., 2013). However, in a study where intensive washing method was employed to clean these hyperaccumulator shoots, authors found that shoot concentrations rarely reached the threshold limit of hyperaccumulation and that most of the samples previously analyzed were contaminated by dust containing Cu (Faucon et al., 2007). Obtaining a biomass displaying shoot Cu concentration required for biocatalysis is a challenge, especially if the use of local plants species is desired. One solution for high Cu concentrations is to use macrophyte plant species such as *Eleocharis acicularis*. Concentrations up to 575 mg Cu kg^{-1} DW in the shoots were measured for *E. acicularis* exposed 15 days to 1 mg Cu L^{-1} (Ha et al., 2011). Concentrations up to 25 000 mg Cu kg^{-1} DW were reached in the roots of *Typha sp.* exposed to 2.52 µM Cu (Valipour et al., 2014) and 2610 mg Cu kg $^{-1}$ DW in the roots of *Cyperus alternifolius* L. exposed to 16.5 µM Cu (Cheng et al., 2002). In the roots of *Iris pseudoacorus* L. exposed to 0.7 µM Cu, Cu concentrations peaked at 1430 ± 170 mg Cu kg $^{-1}$ DW (Sun et al., 2013). Due to their high ability to tolerate high metal(loid) concentrations and to remove contaminants such as metal(loid)s mainly by immobilization in the rhizosphere and storage in the belowground biomass, macrophytes are able to rhizofiltrate contaminated effluents (Marchand et al., 2010; Wu et al., 2015a). In parallel, significant amounts of Bordeaux mixture effluents (BME) occurred in Aquitaine region, notably by filling and rinsing the tanks of crop sprayers. Based on Aquitaine vineyard area (150 000 ha), the average tank volume (600 L of BME at 1 500 g Cu ha^{-1} for 3 ha) of crop sprayers, residual volume per tank (5 L) and an average of 10 treatments per year, the total volume of BME would be 2 500 000 L (Maille, 2004). Treatment of these effluents through conventional physical, chemical, and biological techniques are usually costly and generally operators are not able to fully afford them. They prefer to spread such BME on the field, as authorized by the Article L. 253-1 of the rural Code in France. Cu-contaminated effluents were successfully clean-up in CW (Guittonny-Philippe et al., 2014; Marchand et al., 2014; Newete and Byrne, 2016). To avoid such unnecessary spreading, rhizofiltration of BME in CW planted with macrophytes may be an efficient and cost-effective solution (Marchand et al., 2010; Wu et al., 2015a). Based on these observations, macrophytes could simultaneously rhizofiltrate Cu-contaminated effluents and provide a belowground biomass with high Cu concentration for the eco-catalysis process.

2.6. Limitation of phytomanagement options

Although there is a great need and lots of benefits of producing usable biomass for renewable energy or provide feedstock for the circular bioeconomy, some constraints are related with their cultivation: (1) energy crops are mostly cultivated as monospecific culture which is not the best option to restore biodiversity in contaminated soils, (2) to maximize biomass yield increases in nutrient and pesticide supply are often needed, (3) most of the cultivated energy crops due to their high rate of evapotranspiration (i.e. *Arundo donax*, *Miscanthus*) required an important amount of water (Pandey et al., 2016). Moreover, despite the growing interest in GRO techniques, some limitations must be considered: (1) the treatment of contaminated soils is limited to the area/volume prospected by the roots and concerns only the bioavailable pool of contaminants taken up by the plants, which implies the decontamination of only a fraction of the total soil metal(loid)s (Kidd et al., 2015) (thus in countries with a legistation based on total soil TE instead of specific risk assessment, it would be a bottleneck), (2) stabilization techniques can be implemented on a large scale, however, the long term behavior of metal(loid)s may remain uncertain, (3) one phytoextraction benefit is to clean up the matrix by extracting metal(loid)s, but this process is generally slow if the legislation is based on the total soil metal(loid)s, as the labile metal(loid)s pool is mostly concern (on such basis it is somewhat much faster than expected, Herzig et al., 2014). In general, the physical and/or chemical infertility of certain soils prevents or limits the use of these techniques.

Table of contents – Part 2

Part. 2. Biomass production with (essential) metal(loid) concentrations within the common ranges usable by local biomass processing chains.

2.1. Risk assessment

2.1.1. Chapter 1 - Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site.

Published in Science of the Total Environment, in 2016
Doi: 10.1016/j.scitotenv.2016.05.091

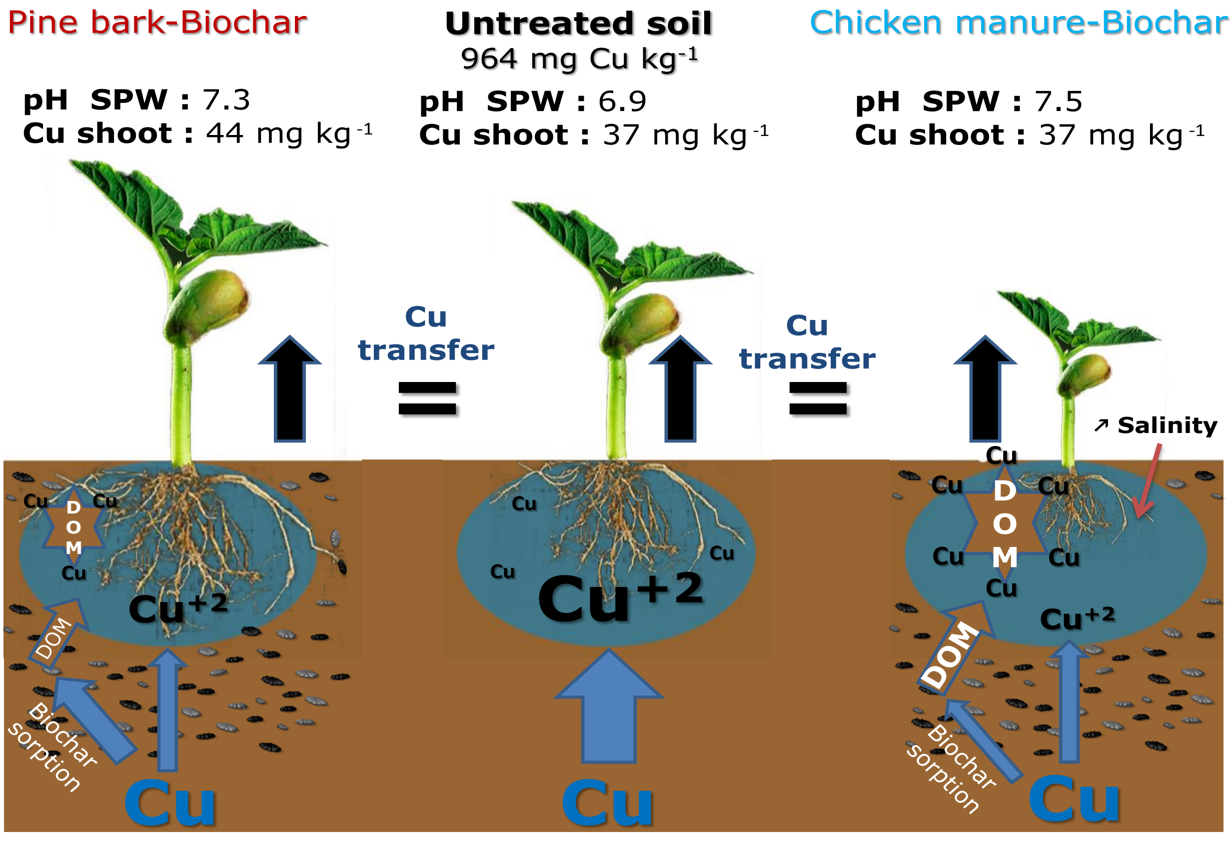


Fig. A. Graphical abstract – Chapter 1.

Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site

Nadège Oustriere[1,2], Lilian Marchand[1,2], Joseph William Galland[1,2], Lunel Gabbon[1,2], Nathalie Lottier[3], Mikael Motelica[3], Michel Mench[1,2]

[1]UMR BIOGECO INRA 1202, DIVERSITY AND FUNCTIONING OF COMMUNITIES, UNIVERSITY OF BORDEAUX, BÂT. B2, ALLÉE GEOFFROY ST-HILAIRE, CS50023, F-33615 PESSAC CEDEX, FRANCE.

[2]INRA, UMR BIOGECO INRA 1202, 69 ROUTE D'ARCACHON, 33610 CESTAS, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; mench@bordeaux.inra.fr; lunelgabon@gmail.com; galland.william@outlook.fr

[3]ISTO UMR 7327-CNRS, UNIVERSITY OF ORLÉANS, CAMPUS GÉOSCIENCES, 1A, RUE DE LA FEROLLERIE, 45071 ORLÉANS CEDEX 2, FRANCE.

E-mails: nathalie.lottier@univ-orleans.fr; mikael.motelica@univ-orleans.fr

Abstract

Two biochars, a greenwaste compost and iron grit were used, alone and in combination, as amendment to improve soil properties and in situ stabilize Cu in a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site. The pot experiment consisted in 9 soil treatments (% w/w): untreated Cu-contaminated soil (Unt); Unt soil amended respectively with compost (5%, C), iron grit (1%, Z), pine bark-derived biochar (1%, PB), poultry-manure-derived biochar (1%, AB), PB or AB + C (5%, PBC and ABC), and PB or AB + Z (1%, PBZ and ABZ). After a 3-month reaction period, the soil pore water (SPW) was sampled in potted soils and dwarf beans were grown for a 2-week period. In the SPW, all amendments decreased the Cu^{2+} concentration, but total Cu concentration increased in all AB-amended soils due to high dissolved organic matter (DOM) concentration. No treatment improved root and shoot DW yields, which even decreased in the ABC and ABZ treatments. The PBZ treatment decreased total Cu concentration in the SPW while reducing the gap with common values for root and shoot yields of dwarf bean plants. A field trial is underway before any recommendation for the PB-based treatments.

Keywords: copper contamination, in situ stabilization, *Phaseolus vulgaris* L., soil remediation, soil pore water.

1. Introduction

Since the 18th Century, Cu-based salts are used as wood preservatives to control insects and fungi (Karjalainen et al., 2009). Long time use of Cu-based salts such as $CuSO_4$ and chromated copper arsenate (CCA) combined with washings of treated wood often results in soil Cu contamination (Bes and Mench, 2008). Copper in moderately and highly organic contaminated soils is present in less mobile and bioavailable forms, whereas in mineral soils, the labile fraction is often higher (Mench and Bes, 2009). Copper excess in topsoils can enhance its concentration in the labile soil pool (environmental availability) and in tissues of biological receptors (bioavailability) (Bolan et al., 2014). This can force plants to adapt their cellular Cu homeostasis (Ravet and Pilon, 2013), impact their growth (Cuypers et al., 2000; Bes et al., 2013; Kolbas et al, 2015) and select Cu-tolerant plant species and populations (Hego et al., 2014). It can also decrease soil biodiversity, e.g. abundance and species composition of earthworm, bacteria and fungi communities (Lagomarsino et al., 2011; Qiu et al., 2013; Mackie et al., 2015), and inhibit activity of hydrocarbon-degrading microorganisms thus impairing C and N cycles (Mackie et al., 2015). In addition, wood preservation sites are often characterised by unfavourable soil properties, e.g. lack of structure with low organic matter (OM) content, low nutrient availability and acidic pH (Mench and Bes, 2009; Bes et al., 2010; Hattab et al., 2014; Thaler and Humar, 2014).

In situ stabilization of contaminants through mineral and organic soil amendments is one of the feasible gentle remediation options (GRO) which can be implemented at contaminated sites (Kidd et al., 2015). This option aims at (1) improving soil biophysical and chemical properties such as OM and nutrient contents (2) reducing the pollutant linkages (here the combination of a source-pathway-receptor, Cundy et al., 2015) and (3) restoring the cascade of biological processes and functions which in turn promotes ecosystem services (Mench et al., 2010; Bolan et al., 2014). The soil amendments can lead to immobilize Cu in the solid phase through various reactions, i.e. sorption, precipitation, complexation, ion exchange and redox process, thereby decreasing Cu environmental (bio) availability (Kumpiene et al., 2008).

Biochars (biologically derived charcoal) are carbon-rich carbonized residues produced by waste biomass pyrolysis (thermal treatment) under high temperatures (450 to 650°C) and mid to low oxygen (Lehmann, 2007; Ahmad et al., 2014). Amending soils with biochar has gained attention due to its ability to (1) improve soil fertility and plant yields (2) resist to chemical/biological degradation and thus serve as a long-term storage of carbon, (3) increase cation exchange capacity (CEC), pH, and water and nutrient retention, and (4) promote microbial communities able to degrade xenobiotics (Zhang et al., 2013). In addition, an increased number of studies reports the biochar ability to immobilize trace elements (TE), including Cu, and to reduce the phytotoxicity of TE-contaminated soils (Park et al., 2011; Luo et al., 2014). Biochar effects on Cu-contaminated soils depend on many parameters such as biochar properties (i.e. raw material, initial carbon content, pyrolysis temperature and sorption capacity) and characteristics of soils to be remediated (e.g. pH, soil texture, and OM content) (Park et al., 2011). Biochar made from oak, ash, sycamore and birch was the more effective treatment at reducing pore water Cu concentrations of a Cu mine in Cheshire, UK (Karami et al., 2011). Negatively-charged biochar surfaces sorb Cu, improve water supply, and ameliorate Cu toxicity in sandy soils (Buss et al., 2012). The Cu leaching was reduced following biochar addition in a $Cu(NO_3)_2$–spiked soil (Bakshi et al., 2014). Water soluble Cu was decreased by hardwood-derived biochar at a gasworks site in Brighton, UK (Gomez-Eyles et al., 2011). Addition of a bamboo and rice straw biochar resulted in 97 % reductions of extractable Cu in a polycontaminated paddy field (Yang et al., 2016).

Conversely negative effects of biochar are reported (Buss et al., 2015). During the pyrolysis process, polycyclic aromatic hydrocarbons (PAHs), dioxins and furans are likely formed and conservative TE accumulate in the residual material. Usually, bioavailable PAH and TE concentrations in biochars are sufficiently low for not being considered as a threat to plants and the environment (Singh et al., 2010; Hale et al., 2012). However, depending on their concentrations, PAHs and TE are suspected to induce acute toxicity to various organisms (Rogovska et al., 2012; Oleszczuk et al., 2013). Uchimiya et al., (2013) reported Cu mobilization after inactivated plant biochar addition in a sandy soil induced by either metal ion-coordinating organic fractions or competition between dissolved organic matter (DOM) and metal ions for the sorption sites of biochar and soil components. Hardwood-derived biochar can enhance 30 times the Cu concentration in the soil pore water (SPW) due to increased dissolved organic carbon (DOC) in a soil contaminated by As, Cu, Zn and Cd, Kidsgrove, Staffordshire, UK (Beesley et al., 2010). A hardwood biochar and a compost, alone and in combination, as soil amendment did not reduce the DTPA-extractable Cu fraction in a vineyard Cu contaminated soil but influenced the microbial community composition (Mackie et al., 2015). In degraded soils only amended with biochars, TE (i.e. As, Cu, and Pb) solubility can increase by co-mobilisation with DOM. According to Beesley and Marmiroli, (2011), the combination of amendments such as compost and iron oxides with biochar may be more suitable than biochar alone for the remediation and revegetation of contaminated land as it may promote TE immobilization and buffer nutrient depletion.

Compost inputs into depleted Cu-contaminated soil can (1) improve soil texture and structure, (2) promote microbial community functioning, (3) stimulate OM cycle and humification process, notably if the soil mesofauna is present or can colonize, (4) form immobilized complexes between humic acids and Cu, (5) increase nutrient status and water retention, and (6) change soil pH and CEC (Kumpiene et al., 2008). Its effects on Cu environmental bioavailability depend on the composted material, its microbial degradability (i.e. C/N ratio), the salt content, soil pH and Eh, and soil type (Beesley et al., 2010). Biochar–compost blends can increase total carbon, nitrogen and phosphorus in soils and stabilize soil aggregates, as well as stimulate microorganisms (Sizmur et al., 2011; Schulz et al., 2013). Compost and biochar mixtures can promote Cu sorption and reduce plant Cu content compared to biochar alone (Sizmur et al., 2011; Borchard et al., 2012).

Iron grit mainly consists of zerovalent Fe(0) (e.g. 97%) and some impurities (e.g. Mn 3%). Once in the soil, iron grit corrodes to form newly Fe/Mn oxi(hydro)xides, which can sorb TE such as Cu (Tiberg et al., 2016). Such oxides can reduce the available fraction of metal(loid)s, notably in the root zone, and thus lower the pollutant linkages associated with their leaching, ecotoxicity, plant uptake and human exposure (Komárek et al., 2013). Single use of iron grit is usually insufficient to remediate Cu-contaminated soils. Its combination with other amendments, mainly organic, is recommended to enhance plant growth on these soils (Bes and Mench, 2008).

Information is scarce on the combined effect of amending a Cu-contaminated soil from a wood preservation site with biochar and either compost or iron grit. This study aimed at assessing the potential benefits and drawbacks posed by adding biochar derived from either animal or plant feedstocks, i.e. poultry manure and pine bark chips, compost and iron grit, alone and in combination, in a Cu-contaminated soil from a wood preservation site. The novelty of this study was to quantify the influence of these treatments on (1) Cu and nutrient mobility, sorption and/or leaching; (2) dwarf bean (*Phaseolus vulgaris* L.) yield; and (3) Cu concentration and mineral mass in the aerial bean parts, with two biochars produced from raw materials inducing large differences in their elemental composition.

2. Material and Methods

2.1. Soil, biochars, compost and iron grit

The Cu-contaminated topsoil (Unt, 0-25cm, Fluvisol – Eutric Gleysols, World Reference Base for soil resources) was randomly collected (100 kg) with an unpainted stainless steel spade at the sub-site P1-3 of a wood preservation site (10 ha) located at Saint-Médard d'Eyrans, Gironde, SW France (44°43.353′ N, 000°30.938′ W, Mench and Bes, 2009). Its texture is sandy, i.e. 85.8 % sand, 5.9 % clay, and 8.3 % silt, with 1.3 % OM, C/N 16, neutral pH (7 ± 0.09) and a low CEC (2.5 cmol kg^{-1}). As compared to background TE values for French sandy soil, it is mainly contaminated by Cu, but not significantly by As and Cr (Table 1).

Table. 1. Main soil properties

Parameter	Mean ± SD	Background levels [A]	Risk assessment - remediation needed [C]
pH	7 ± 0.09	6.60	
CEC	2.5 ± 0.2		
P_2O_5 (g kg^{-1}, Olsen extractable)	0.03 ± 0.002		
Organic matter (g kg^{-1})	13 ± 0.4		
Organic C (g kg^{-1})	8 ± 0.3	14.5	
Total N (g kg^{-1})	0.5 ± 0.01		
C/N	16 ± 0.2	10.02	
Texture (g kg^{-1})			
Sand	858	≥ 650	
Silt	83	≤ 350	
Clay	59	≤ 180	
Total TE (mg kg^{-1})			
Cr	21 ± 0.7	14–40	250
Cu	964 ± 20	3.2–8.4	100
Ni	5 ± 0.3	4.2–14.5	100
Zn	37 ± 1.6	17–48	500
As	7 ± 0.4	1–25 [B]	30
PAHs (mg kg^{-1})			
Fluoranthene	1.9	0.4	10–100
Indeno[1,2,3-cd]pyrene	0.95	0.01–0.015	1
Benzo[g,h,i]perylene	0.8		1–10
Benzo[b]fluoranthene	0.8	0.1	1

[A]. Frequent total concentrations in French sandy topsoils (Baize, 1997; Baize, 2000; Villanneau et al., 2008, INDIQUASOL, 2016).

[B]. Frequent total As concentrations for all French soil types (Bes and Mench, 2008).

[C]. Québec guidelines for risk assessment and remediation - Land Protection and Rehabilitation Regulation (2016).

Two commercial biochars (Florentaise, Saint-Mars-du-Désert, France) were tested, one was produced using pine bark (PB) and the other one using chicken manure (AB) via pyrolysis at 420°C for 180 s. Soluble salts were not removed from biochars (Table. 2). The compost (C) made from green wastes (9–12 months) was purchased from Gonzales frères, Martignas sur Jalle, France. Zerovalent iron grit (Z, GH120, particle size <0.1 mm) was obtained from Wheelabrator Allevard, France (Bes and Mench, 2008). Elemental composition, carbon content and PAH concentrations of biochars and compost were determined at the INRA Laboratoire d'Analyses des Sols (LAS, Arras, France) with standard methods (INRA LAS, 2011) (Table. 2).

Table. 2. Composition of soil amendments

	AB	PB	C	French upper critical thresholds for organic amendments [A]	Z	EBC [B]	IBI [C]
Ph	10.3	9.89	7.53			-	-
CEC (cmol kg^{-1})	12.3	1.23	26.7			-	-
Major elements (%DW)							
H	0.8	0.8	-			-	-
N	3.4	0.3	0.69			-	-
S	0.9	0.03	-			-	-
Cl	1.6	0.01	-			-	-
C	48.9	90.3	10.9			>50%	Class 1: ≥60% Class 2: [30% - 60%] Class 3: [10% - 30]
Nutrients (g kg^{-1})							
Ca	53.9	18.6	22.5			-	-
K	102	13.8	5.4			-	-
Mg	23.5	29.9	1.9			-	-
Na	9.8	0.389	-			-	-
P	37.7	0.105	0.374			-	-
Elements (mg kg^{-1})							
Al	1790	1650	12700		600	-	-
As	0.57	0.461	4.47	18	70	-	12-100
Cd	<0.5	<0.5	<0.5	3	0.03	1	1.4-39
Cr	183	45.9	21	300	3 500	80	64-1200
Cu	362	230	85.8	120	1 010	100	63-1500
Mn	-	-	-		7 710	-	-
Fe	6320	4470	6830		973 000	-	-
Hg	<0.1	<0.1	<0.1	2		1	1-17
Ni	75.1	28.3	7.98	60	739	30	47-600
Pb	<2	2.61	51.5	180	20	120	70-500
Zn	1100	50.5	174	600	104	400	200-7000
PAHs (mg kg^{-1})							
Sum of 16 US EPA PAHs [D]	19.8	163	<DL			4	6-300
Fluoranthene	< 0.1	7.48	< 0.1	4		-	-
Benzo(b)fluoranthene	< 0.1	0.149	< 0.1	2.5		-	-
Benzo(a)pyrene	< 0.1	< 0.2	< 0.3	1.5		-	-
Naphtalene	18.8	101	<0.2			-	-

[A.] French upper critical thresholds for organic amendments (NF U 44 051, Dec. 2010).

[B.] Following Switzerland's Chemical Risk Reduction Act (ChemRRV) on recycling fertilizers.

[C.] Range of Maximum Allowed Thresholds reflects different soil tolerance levels for these elements in compost, biosolids, or soils established by regulatory bodies in the US, Canada, EU and Australia (See Appendix 3 of the IBI Biochar Standards for further information).

[D.] Corresponds to the PAHs threshold defined in the Swiss Chemical Risk Reduction Act (Chem RRV).

2.2. Soil treatments

The Unt soil was air-dried, sieved to 5 mm, and manually homogenized. Biochars were crushed, sieved to 2 mm and manually homogenized before mixing with the soil. Each biochar was trialed in a pot experiment, alone and in combination (% w/w) with compost and iron grit (in triplicate) to consist 9 treatments:

(1) untreated Cu-contaminated soil (Unt)
(2) Unt soil + 5 % Compost (C),
(3) Unt soil + 1% Z (Z),
(4) Unt soil + 1% AB (AB),
(5) Unt soil + 1% AB + 5% C (ABC),
(6) Unt soil + 1% AB + 1% Z (ABZ),
(7) Unt soil + 1% PB (PB),
(8) Unt soil + 1% PB + 5% C (PBC),
(9) Unt soil + 1% PB + 1% Z (PBZ).

Soil treatments were homogenized by rotation in a plastic flask, potted in plastic pots (1 kg, 11 cm x 11cm x 11cm, 1.3 L), and placed under controlled conditions in a greenhouse.

2.3. Plant testing

All potted soils were weekly watered (70% of water holding capacity, WHC) with deionized water for a 3-month period (March-June 2014), allowing soil amendment to react with the contaminants and microbial communities to develop. In June, soil phytotoxicity was assessed using the PlantTox biotest adapted from the ISO 11269-2 standard protocol (ISO 2012; Ruttens et al., 2006). Dwarf beans (*P. vulgaris* L., cv. Skipper, Vilmorin) were sown (4 seeds/pot, 3 pots per treatment) in all pots, and cultivated during 15 days in controlled conditions (16 /8 h light/darkness; 65 ± 5% relative humidity; 25± 2°C) in a greenhouse. Potted soils were daily watered (deionized water) to maintain a 65% WHC rate. After 15 days, the shoots and roots were harvested, washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields.

2.4. Soil pore water and plant analysis

For each pot, dried shoots of the four plants were pooled, ground (< 1 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) in digests was determined by ICP-MS (Thermo X series 200, INRA USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. All element concentrations in plant parts are expressed in mg or g DW kg^{-1}. The shoot Cu removal was calculated as follows: Cu (µg $plant^{-1}$) = Shoot DW yield (g $plant^{-1}$) x shoot Cu concentration (µg g^{-1} DW).

The soil pore water (SPW) was collected on day 14 of the phytotoxicity test in all potted soils (two times 10mL) using Rhizon MOM moister samplers placed in March 2014 (Eijkelkamp Agrisearch Equipment, The Netherlands) and samples kept at 4°C prior to their analysis. The pH, electrical conductivity (EC), and Cu^{2+} concentration in the SPW samples were determined using electrodes (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34, Tetracon 325 WTW, and Cupric ion electrode, Fischer Bioblock, USA), respectively. Aluminum, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn were analyzed by ICP-OES (Varian Liberty 200, INRA USRAVE laboratory, Villenave d'Ornon, France). Dissolved organic carbon (DOC) was measured in SPW collected with Rhizon samplers. Analyses were performed with a Shimadzu© TOC 5000A carbon analyzer. The accuracy of the instrument was checked by performing calibration with a standard reference solution of potassium hydrogen phthalate (KHP) at a concentration of 1000 mg Carbon L^{-1}. Four repeated measurements of DOC were performed for each SPW solution.

2.5. Statistical analysis

Influence of soil treatments on SPW parameters, shoot DW yields and shoot ionome of dwarf beans were tested using one-way analysis of variance (ANOVAs). Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests. Differences were considered statistically significant at $p<0.05$. When element concentrations were below the detection limits in the UNT soil, influence of soil treatments were not statistically tested. Principal component analysis (PCA) was conducted for total Cu, Cu^{2+} and DOC concentrations in SPW and shoot and root DW yields. Only these parameters were chosen to respect the ratio of 1/6 between studied (5) and predictor (36) variables. In a second PCA (Supplemental material 1), Ca, P, Na and K were added to the first PCA to show the influence of these variables on soil treatments. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

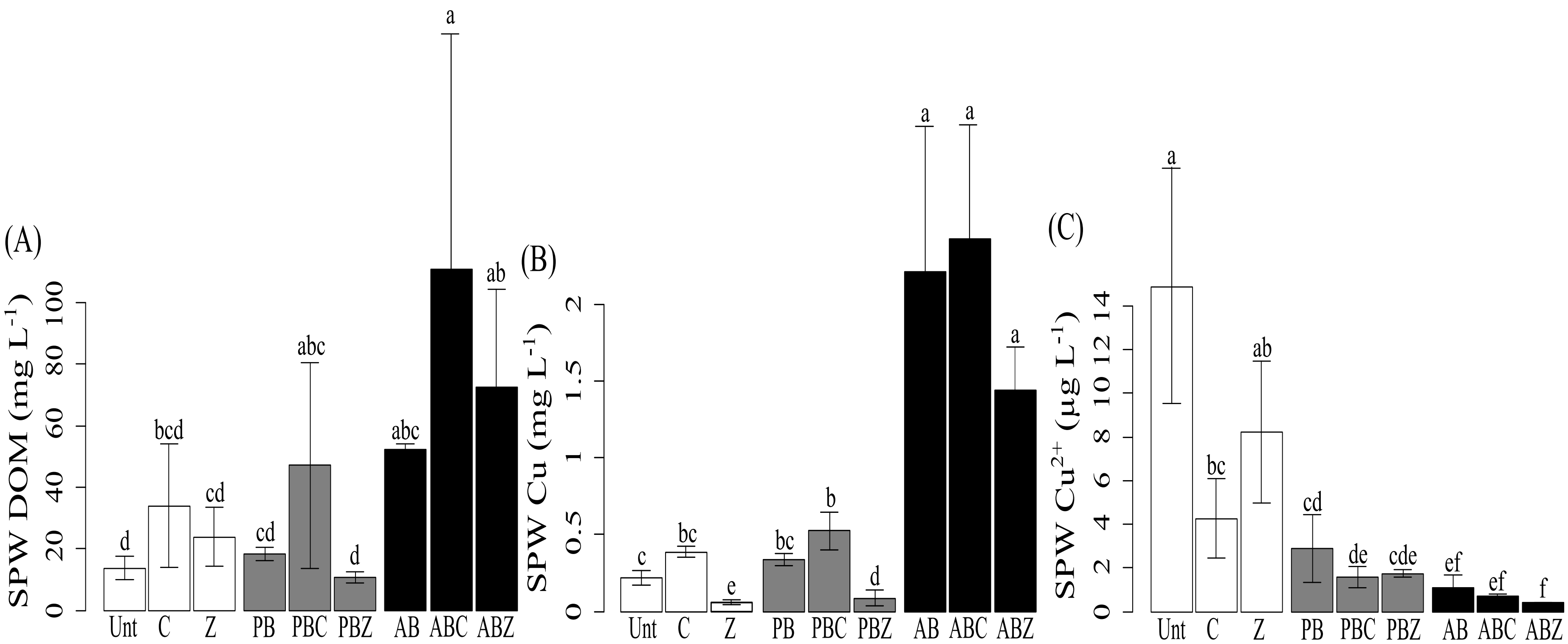


Fig. 1. (A) DOM, (B) total Cu and (C) Cu^{2+} concentration in the soil pore water samples; Cu-contaminated soil (Unt, white), amended with compost (C) or iron grit (Z); Cu-contaminated soil amended with plant-derived biochar (PB, grey) and animal-derived biochar (AB, black), singly and in combination with compost (PBC and ABC) or iron grit (PBZ and ABZ). Mean values per treatment (n=3 / n=6 for Unt, C and Z). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

Table. 3. Physico-chemical parameters of soil pore waters

Treatments		($\mu S\ cm^{-1}$)	Nutrients ($mg\ L^{-1}$)					
	pH	**EC**	**Ca**	**Fe**	**K**	**Mg**	**Na**	**P**
Without biochar (n=6)								
Unt	6.9 ± 0.1 e	1024 ± 117 bc	143 ± 39 ab	< 0.02	14 ± 5 d	5 ± 2 cd	12 ± 3 c	< 0.2
C	7.1 ± 0.09 de	1285 ± 129 b	185 ± 29 a	< 0.02	35 ± 8 **c**	15 ± 3 **b**	23 ± 2 **b**	0.4 ± 0.1
Z	7.0 ± 0.2 e	1063 ± 208 bc	144 ± 40 ab	< 0.02	15 ± 8 d	5 ± 1 cd	13 ± 5 c	< 0.2
Animal derived biochar (n=3)								
AB	7.5 ± 0.2 **abc**	3450 ± 612 **a**	119 ± 18 bc	0.07 ± 0.03	633 ± 171 **a**	49 ± 14 **a**	125 ± 25 **a**	9 ± 4
ABC	7.5 ± 0.1 **abc**	3720 ± 322 **a**	132 ± 12 abc	0.1 ± 0.03	938 ± 177 **a**	49 ± 7 **a**	183 ± 45 **a**	11 ± 0.6
ABZ	7.7 ± 0.1 **a**	4733 ± 440 **a**	96 ± 16 bcd	0.08 ± 0.07	719 ± 144 **a**	47 ± 14 **a**	117 ± 25 **a**	5 ± 4
Plant derived biochar (n=3)								
PB	7.3 ± 0.08 **cd**	483 ± 51 d	59 ± 5 d	< 0.02	43 ± 11 **bc**	3 ± 0.3 d	13 ± 2 c	0.3 ± 0.05
PBC	7.4 ± 0.01 **bc**	754 ± 115 c	74 ± 7 cd	< 0.02	71 ± 11 **b**	8 ± 2 bc	20 ± 5 bc	0.6 ± 0.2
PBZ	7.6 ± 0.05 **ab**	477 ± 110 d	46 ± 20 d	< 0.02	53 ± 16 **bc**	3 ± 1 d	17± 6 bc	< 0.2
Common values in sandy soil (unpublished data)	-	-	143 ± 66	0.05 ± 0.01	27 ± 44	21 ± 11	63 ± 12	± 2

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). Mean values followed by letters in bold are significantly higher as compared to the Unt soil.

3. Results

3.1. Soil pore water

3.1.1. pH, EC and DOM (Table 3, Fig. 1A)

The SPW pH of the Unt soil was neutral. Both biochars significantly increased the SPW pH. Combining Z with either AB or PB resulted in the highest SPW pH values. The SPW EC significantly increased in all AB-amended soils, in the 3450 (AB) – 4733 (ABZ) range ($\mu S\ cm^{-1}$) as compared to the Unt soil. Conversely, the SPW EC significantly decreased in all PB-amended soils, with lowest values in the PB and PBZ soils. The DOM values significantly peaked in the ABC and ABZ soils, i.e. ($mg\ L^{-1}$) 111 ± 75 (ABC) and 73 ± 32 (ABZ), relative to the Unt soil (24 ± 10), while the DOM concentration decreased in the Z and PBZ soils albeit not significantly (i.e. 10 ± 2 and 14 ± 2, respectively).

3.1.2. Total Cu and Cu^{2+} concentrations in the soil pore water (Fig. 1. B and C)

Total Cu concentration in the SPW was 4 to 10 folds higher in AB-amended soils, alone and in combination with either C or Z, than in the Unt soil (i.e. 251 ± 30 $\mu g\ L^{-1}$). Conversely, adding PB into the Unt soil did not change total Cu concentration in the SPW (336 ± 39 $\mu g\ L^{-1}$). The Z addition, alone and in combination with PB, however resulted in the lowest SPW Cu concentration (i.e. 86 ± 50 and 29 ± 22 $\mu g\ L^{-1}$ respectively).

Compost and biochars, alone and in combination, significantly reduced the SPW Cu^{2+} concentration. It ranged ($\mu g\ L^{-1}$) from 15 ± 5 (Unt) to 0.05 ± 0.01 (ABZ), in the decreasing order (% as compared to Unt): Unt ≥ Z (-46%) ≥ C (-73%) ≥ PB (-81%) ≥ PBZ (-89%) = PBC (-89%) ≥ AB (-93%) ≥ ABC (-95%) ≥ ABZ (-97%) (Fig. 1C). Combining either compost or iron grit with biochars was as efficient as biochar alone to reduce the SPW Cu^{2+} concentration.

3.1.3. Nutrient concentrations in the soil pore water (Table 3)

The compost addition significantly increased the K, Mg, Na and P concentrations in the SPW. The Z addition did not change the nutrient concentrations in the SPW. The SPW K concentration was promoted in all PB-amended soils. The SPW Ca, K, Mg, Na and P concentrations significantly increased in all AB-amended soils.

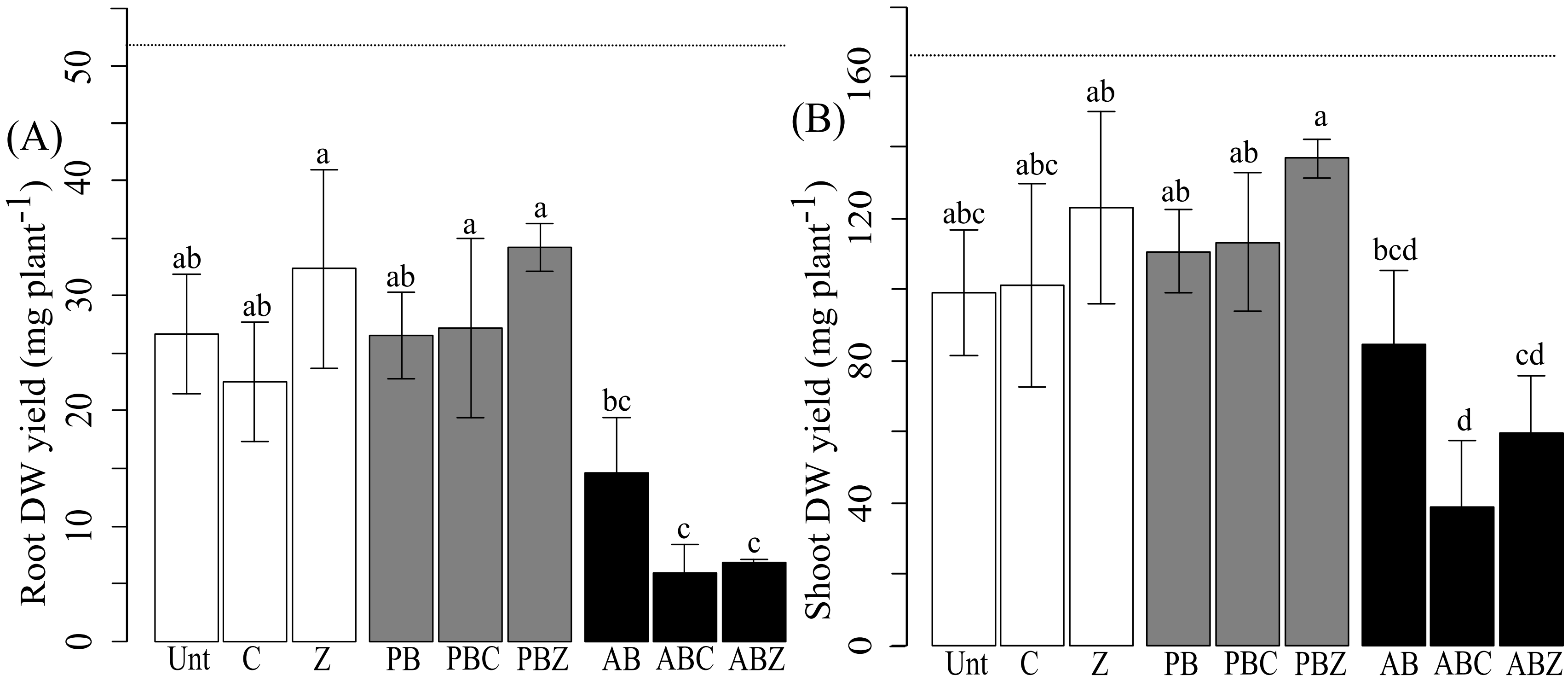


Fig. 2. (A) Root and (B) shoot DW yields of dwarf bean (in g DW plant^{-1}) after the 15-day growth period. Cu-contaminated soil (Unt, white), amended with compost (C) or iron grit (Z); Cu-contaminated soil amended with plant-derived biochar (PB, grey) and animal-derived biochar (AB, black), alone and in combination with compost (PBC and ABC) or iron grit (PBZ and ABZ). The dotted lines represent common values of root and shoot DW yields of dwarf beans cultivated in an uncontaminated sandy soil (Marchand et al., 2015). Mean values per treatment (n=3 / n=6 for Unt, C and Z). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

Table. 4. Shoot ionome and shoot Cu removal of dwarf beans

Treatment		Copper		Shoot nutrient concentrations (g kg^{-1})					
		Shoot Cu concentration (mg kg^{-1} DW)	Shoot Cu removal (µg plant^{-1})	**Ca**	**Fe**	**K**	**Mg**	**Na**	**P**
Without biochar	(n=6)								
Unt		37 ± 3 ab	3.9 ± 0.4 ab	13 ± 4 bc	0.12 ± 0.04 ab	19 ± 3 c	1.6 ± 0.2 c	0.26 ± 0.1 b	3.8 ± 0.7 c
C		44 ± 3 a	3.8± 1.6 ab	23 ± 2 **a**	0.11 ± 0.01 ab	44 ± 7 **b**	3.3 ± 0.7 **a**	0.36 ± 0.1 ab	4.4 ± 1 c
Z		38 ± 2 ab	4.3 ± 1.3 ab	20 ± 5 ab	0.11 ± 0.03 ab	24 ± 5 c	1.9 ± 0.2 bc	0.33 ± 0.2 b	3.5 ± 0.6 c
Animal-derived biochar	(n=3)								
AB		37 ± 3 ab	3.1 ± 0.5 bc	7 ± 1 cd	0.12 ± 0.04 ab	116 ± 6 **a**	3.0 ± 0.1 **a**	0.72 ± 0.3 **a**	8.4 ± 0.6 **a**
ABC		37 ± 6 ab	3.2 ± 2 bc	8 ± 1 cd	0.19 ± 0.06 a	114 ± 4 **a**	3.1 ± 0.4 **a**	0.66 ± 0.2 **a**	8.4 ± 0.8 **a**
ABZ		30 ± 4 b	1.6 ± 0.7 c	3 ± 2 d	0.20 ± 0.1 a	110 ± 5 **a**	2.0 ± 0.6 bc	0.68 ± 0.01 **a**	6.7 ± 2 **ab**
Plant-derived biochar	(n=3)								
PB		44 ± 5 a	4.8 ± 0.2 ab	10 ± 1 cd	0.09 ± 0.02 b	25 ± 4 c	1.7 ± 0.1 c	0.26 ± 0.09 b	4.4 ± 0.2 c
PBC		49 ± 10 a	5.4 ± 0.1 a	17 ± 1 ab	0.13 ± 0.04 ab	52 ± 7 **b**	2.8 ± 0.3 **ab**	0.45 ± 0.02 ab	5.0 ± 0.2 bc
PBZ		37 ± 4 ab	5.0 ± 0.7 a	17 ± 3 ab	0.11 ± 0.02 ab	53 ± **b**	2.1 ± 0.2 **ab**	0.32 ± 0.09 b	3.7 ± 0.8 c
Common values	*	3 – 20	-	1 – 50	0.02 – 0.3	20 – 50	1.5 – 3.5	-	1.6 – 6.0
Common values in dwarf beans	**	6.2 ± 0.7	-	11 – 21	0.143	19 – 32	2.5 – 3	-	4.8 – 5.7

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). Mean values followed by letters in bold are significantly higher as compared to the Unt soil. *(Tremel-Schaub and Feix, 2005) **(Negim et al., 2012, Bes and Mench, 2008).

3.2. Phytotoxicity assessment

3.2.1. Plant growth parameters (Fig. 2. A and B)

The root DW yield was significantly reduced for the AB soil (mg plant^{-1}) from 27 ± 5 to 15 ± 5. The ABC and ABZ soils led to the lowest root production. Root DW yield was unchanged in other amended soils. The shoot DW yield was significantly decreased for the ABC and ABZ soils, whereas other treatments had insignificant impact.

3.2.2. Shoot ionome (Table 4; Fig. 2B)

Shoot Cu concentration of dwarf beans varied between 30 (ABZ) and 49 mg kg^{-1} (PBC), but its mean values for all-amended soils did not differ from that of the Unt plants. Shoot Cu concentration was significantly lower for the ABZ plants relative to the C, PB and PBC plants. The shoot Cu removal ranged (µg plant^{-1}) from 1.6 (ABZ) to 5 (PB, PBC and PBZ). Shoot Cu removals increased for the PB, PBC and C treatments albeit not significantly, remained unchanged for the AB, ABC and PBZ treatments, but significantly decreased for the ABC one as compared to the Unt plants, mainly due the lower shoot biomass. The C addition significantly increased the shoot Ca, K and Mg concentrations (i.e. 23, 44 and 3 g kg^{-1}, respectively). The Z addition did not significantly influence the shoot nutrient concentrations. The SPW nutrient concentrations were enhanced in the AB-amended soils (Table 3) whereas shoot DW yields decreased for these treatments. Consequently, shoot K, Na, and P concentrations significantly increased for the AB, ABC and ABZ plants, except shoot Ca concentration of the ABZ plants which was reduced (Table 4, Supplemental material 1). For the PB-amended soils, only shoot K concentration was significantly promoted in the PBC and PBZ plants, up to 52 g kg^{-1} as compared to both the Unt and PB plants.

3.2.3. PCA on the SPW composition and plant growth parameters (Fig. 3)

The X canonical weights of the Principal Component Analysis (PCA) accounting for total Cu, Cu^{2+} and DOM concentrations in the SPW and the shoot and root DW yields explained 80% of the total variance. The first axis (54%) was characterized by SPW Cu and DOM concentrations and the biomass production. The second axis (26%) was driven by SPW Cu^{2+} and DOM concentrations. The first axis opposed all AB-amended soils, with high SPW DOM and Cu concentrations, low root and shoot DW yields and low SPW Cu^{2+} concentration, to all other treatments. The second axis opposed the Unt soil, with high SPW Cu^{2+} concentration and low SPW DOM concentration, to other soils, and notably the PBC and PBZ soils.

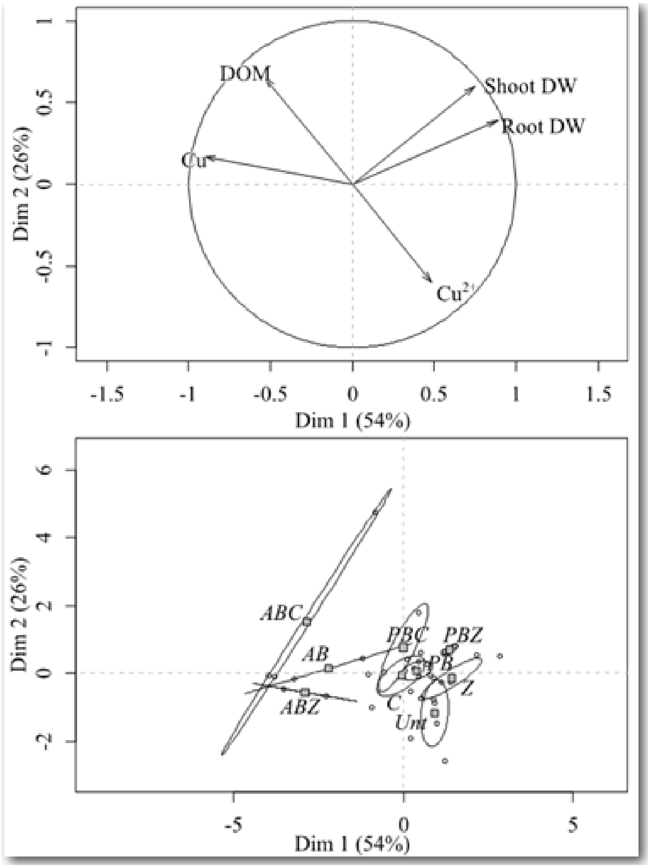


Fig. 3. Principal Component Analysis (PCA) on total Cu, Cu^{2+} and DOM concentrations in the soil pore water and shoot and root DW yield of dwarf beans.

4. Discussion

Besides their ability to supply a range of agronomic benefits and to contribute to carbon sequestration, biochars used as soil amendment have attracted much interest to stabilize metals, e.g. Cu, in contaminated soils. Here, decreased SPW Cu^{2+} concentrations after soil amendment were associated with increased soil pH and SPW P and DOM concentrations and decreased SPW Ca concentrations (Table 3, Fig. 1). Influence of these parameters is successively discussed.

Soil pH

Biochar incorporation into Cu-contaminated soils can increase soil pH and extractable Cu concentrations (Yang et al., 2016). Here, pH raised in all biochar-amended soils, ABZ and PBZ causing the highest increase (Table 3). Increase in SPW pH may (1) result in Cu^{2+} hydrolysis to form $Cu(OH)^{+}$ and precipitate Cu as hydroxides ($Cu(OH)_2$) thus decreasing SPW Cu^{2+} concentrations (Bakshi et al., 2014), and (2) increase biochar negatively-charged surface area, resulting in greater Cu affinity to biochar surface. Based on X-ray absorption fine structure spectroscopy (XAFS), Cu^{2+} sorption onto biochar is pH dependent (Ippolito et al., 2012; Ahmad et al., 2014). Biochars contain various active functional groups, e.g. carboxyls, phenols, hydroxyls, carbonyls, and quinines, which can form inner sphere complexes with Cu^{2+}, resulting in decreased SPW Cu^{2+} concentrations (Bakshi et al., 2014).

Phosphates and carbonates

Precipitation with phosphates and carbonates may contribute to decrease SPW Cu^{2+} concentrations in the soils amended by the compost, biochars and their combination (Fig. 1). The AB biochar contains higher Ca and P concentrations than the PB biochar and compost (Table 2). Albeit not statistically tested due to some values below the detection limit, the SPW P concentration slightly increased in the C, PB and PBC soils, but highly increased in all AB-amended soils (Table 3). SPW Ca concentration decreased in all PB-based treatments. Increase in SPW P concentration opposed to reduced SPW Cu^{2+} concentrations which suggested Cu^{2+} reaction with phosphates as expected by Rajapaksha et al., (2015) (Table 3, Fig. 1C, Supplemental material 1). Phosphate and carbonate-Cu complexes may also sorb on the surface of biochar and Fe and Al oxy-hydroxides (Ahmad et al., 2014). In the PB-amended soils, decrease in SPW Ca concentration (Table 3) may reflect retention of Cu^{2+} on biochar surface via complexation and surface precipitation with Ca^{2+} (Chintala et al., 2014; Bakshi et al., 2014). Decrease of SPW Cu^{2+} concentrations was expected in the ABZ and PBZ soils as compared to single biochar addition to mirror the potential sorption of free Cu^{2+} ions or phosphate and carbonate-Cu complexes on Fe/Mn oxy-hydroxides (Bes and Mench, 2008; Kumpiene et al., 2011; Tiberg et al., 2015), but this trend was not strong enough to be significant (Fig. 1C).

Organic compounds

Generally, biochars pyrolyzed under 500°C have high DOC content (Ahmad et al., 2014). Associations between Cu and DOC were reported (Bernal et al., 2009). High DOC content could facilitate the formation of soluble Cu complexes with dissolved organic compounds (Beesley et al., 2010; Park et al., 2011; Karami et al., 2011). Reaction of SPW DOM with Cu may modify Cu solubility, chemical species, and resupply from soil bearing phases (Ashworth and Alloway, 2007; Hattab et al., 2014). Here, AB addition (pyrolyzed at 420°C) into the untreated soil promoted the SPW DOM concentration and, likely due to subsequent Cu complexation, reduced the SPW Cu^{2+} concentration (Fig. 3, Supplemental material 2). The combination of compost and biochar can promote both soluble and insoluble Cu complexes with organic compounds, reducing free Cu^{2+} concentration but increasing total Cu concentration in the SPW (Fig. 1 and 3). Competition can however occur between DOM and Cu^{2+} for retention on biochar surfaces (Beesley et al., 2014). The DOM can block the biochar pores preventing Cu sorption (Bolan et al., 2010; Cao et al., 2011). In the AB soil, SPW DOM concentration is 4 and 3 times higher than in the Unt and PB soils, respectively (Fig. 1A). For the same pyrolysis temperature, a lignin-rich biomass (i.e. pine bark chips) will release less DOM than a biomass with low contents of phenolic and lignin materials (e.g. 5.9% in poultry manure) (Uchimiya et al., 2013). In addition, compost amendment may stimulate the degradation of more recalcitrant biochar fractions in the PBC and ABC soils. In these treatments, fulvic acids might bind Cu and increase its mobility from the soil solid phase to the soil pore water, but here this potential effect was not strong enough to be significant in the three C-amended soils (Fig. 1B).

Ionic strength and other elements

The AB biochar contains nutrients (i.e. K, Mg, Na, and P, Table 2), and SPW K, Mg, Na and P concentrations peaked in all AB-amended soils. These concentrations are 23, 2 and 1.8 fold higher in the AB soil than in an uncontaminated sandy soil (Table 3). They are all anti-correlated to SPW Cu (R^2: 0.86, 0.85, 0.89 and 0.92, respectively, Table 3). High nutrient concentrations, notably for Na, can break up soil aggregates, increase bulk density making the soil more compact and decrease total porosity, thereby hampering soil aeration (Tavakkoli et al., 2010; Kargar et al., 2015). In the AB soil, potential dispersion of soil aggregates may release DOM-Cu complexes, increase SPW Cu concentration and promote Cu leaching out of the root zone.

Plants

Frequently, biochar addition into Cu-contaminated soils, alone and in combination with compost, can increase plant yields and reduce shoot Cu concentration (Beesley et al., 2011). Forestry-residue biochar was able to sorb Cu and decrease phytotoxicity for quinoa (*Chenopodium quinoa* Willd.) in a sandy Cu-spiked soil (Buss et al., 2012). Strong Cu binding to biochar reduces its bioavailability for maize (Namgay et al., 2010). Here, root and shoot DW yields of the dwarf beans grown in the Unt soil were 2 fold lower than their values in an uncontaminated sandy soil, i.e. 52 ± 19 and 186 ± 25 mg DW plant^{-1}, using the same plant testing (Marchand et al., 2015); none of the tested treatments has enabled the dwarf beans to reach these values (Fig. 2). The lack of beneficial effect of PB biochar on bean growth (Fig. 2) agreed with several previous studies: the biomass produced by *Brassica juncea* L. was unchanged in a Cu mine soil amended with different percentages of compost and biochar mixture (Rodríguez-Vila et al., 2014). Biochar, compost and their combination did not influence biomass production of cover crop plants in a Cu-contaminated vineyard (Mackie et al., 2015). Negative effect of biochar on plant yield as for the AB biochar is less frequent (Fig. 2). Nutrient deficiencies (i.e. Ca, Fe, K, Mg, and P) can be ruled out as their concentrations in bean shoots were for all treatments within the ranges of common values (Tremel-Schaub and Feix, 2005, Table 4). Several hypotheses might explain the reduced bean growth in all AB-amended soils: (1) higher exposure to PAH and volatile organic compounds (VOC, e.g. low molecular weight, organic acids, alcohols, ketones and phenols, Buss et al., 2015), (2) higher salinity and (3) higher SPW Cu concentration. Total PAH concentration was 5 and 25 fold higher in the AB and PB biochars than the threshold value defined by the European Biochar Certificate V4.8 (European Biochar, 2015, Table 2). However PAH concentration was 5 fold higher in the PB biochar than in the AB one, whereas bean biomass was lower for the AB soil (Fig. 2). The co-occurrence of VOCs able to cause a phytotoxicity must be assessed (Buss et al., 2015). The SPW EC values in all AB-amended soils (i.e. 3450 ± 612 µS cm^{-1}, Table 3) exceeded the range for saline soils (i.e. 2200 µS cm^{-1}, Mendez and Maier, 2008). Both Na and Cl concentrations peaked in the AB biochar, and K and Ca in a lesser extent (Table 2). Salinity can limit plant growth rate, crop yield, plant vigor and seed germination (Munns and Tester, 2008) as its excess induces water stress, nutritional disorders (e.g. [K+]/[Na+] ratio), and ion toxicity, and most bean cultivars are salt-sensitive crops (Assimakopoulou et al., 2015). The SPW Cu concentration increased in the PBC and AB-amended soils (Fig. 1), whereas these soil amendments reduced the SPW Cu^{2+} concentration potentially available for root uptake. Unexpectedly, shoot Cu concentrations of dwarf beans (mg kg^{-1}) were statistically similar in all treatments, ranging from 30 ± 4 (ABZ) to 49 ± 10 (PBC) (Table 4). They exceeded common values in plants (3 - 20 mg kg^{-1}, Tremel-Schaub and Feix, 2005), notably primary leaves of dwarf beans (5.7 - 7.0 mg kg^{-1}, Mench et al. 1996) and were slightly above the upper critical foliar Cu concentration for dwarf beans (15–30 mg Cu kg^{-1}, MacNicol and Beckett, 1985). Shoot Cu concentrations between 40 and 60 mg Cu kg^{-1} reduce shoot growth, inhibit leaf expansion and increase lipid peroxidation of dwarf beans (Cuypers et al., 2000). Total Cu concentration in the SPW was 4 to 10 fold higher in the AB-amended soils, alone and in combination with either C or Z, than in the Unt soil. In these treatments, soluble Cu was likely bound to DOM as the Cu^{2+} concentrations were the lowest (Fig. 1). High molecular weight DOM-Cu complexes are not easily uptake by plant roots (Pandey et al., 2000), but low molecular weight (LMW) ligands (less than 1 kDa) such as organic acids could increase the Cu phytoavailability (Inaba and Takenaka, 2005). Copper content in lettuce sprouts, roots and leaves increases with DOM fractions <1 kDa, resulting in short roots and causing high phytotoxicity (Wang et al., 2010). Potential contributions of LMW DOM fractions in the soil pore water to phytotoxicity experienced by bean plants on AB-amended soils need further investigations.

Using biochar, compost and iron grit to reclaim the Cu-contaminated soil from this wood preservation site

The AB biochar, alone or in combination with C or Z, is not suitable to decrease Cu solubility and phytotoxicity. It decreased SPW Cu^{2+} concentration but increased SPW Cu and Na concentrations and EC, resulting in a lower plant growth. One recommendation may be to wash the AB biochar to remove salt and DOM fraction in excess before its use. Pyrolyzing the poultry manures at higher temperatures is a second option. This may reduce DOM fraction and increase the biochar absorptivity to Cu and several ionic nutrients. Increase in pyrolysis temperature from 450 to 550°C decreased DOC concentration by more than one order of magnitude (Uchimiya et al., 2013). The PB biochar highly decreased SPW Cu^{2+} concentration, potentially reducing root exposure to Cu. However it did not have much effect on Cu solubility since total Cu concentration in the SPW remained similar to its value in the Unt soil. Such Cu complexes may be leached from the root zone. The addition of Z with PB helped to decrease the SPW Cu concentration while reducing the gap between bean root and shoot yields produced on the PBZ-amended soil (i.e. 34 and 136 mg plant^{-1}) and their common values (Marchand et al., 2015; Fig. 2). Cationic metal species such as Cu^{2+} or DOM-Cu complex have high affinity for Fe oxides and can sorb on the newly formed Fe and Mn oxy-hydroxides provided by Z addition (Kumpiene et al., 2008; Komárek et al., 2013). However the costs associated with such option using Fe(0) are relatively high and the additional reduction in SPW Cu^{2+} concentration was not significant (Fig. 1). The estimated costs would reach 24,000 € ha^{-1} for experiments using Fe(0) (Hanauer et al., 2011) and 3000 € ha^{-1} when a cheaper Fe material (steelwork cheroot) is used (Komárek et al., 2013). In situ remediation option using stabilized nanoparticles may be a promising option (Liu et al., 2015). The economic feasibility of in situ stabilization with Fe(0) is influencing its implementation at large-scale. However, the culture of fast growing plant biomass for the bio-economy on such contaminated site could provide additional economical value (Vangronsveld et al., 2009). Adding compost in combination with biochar slightly increased nutrient supply, but it did not promote plant growth (Fig. 2), outlining the need to pay attention on the compost type and fertilization.

5. Conclusion

The incorporation of AB and PB biochars, alone and in combination with either compost or iron grit into the Cu-contaminated soil has the potential to reduce free Cu ions in the soil pore water. This positive effect was mainly attributed to Cu reaction with DOM after the biochar and compost amendments. The newly formed Fe/Mn (hydr)oxides after iron grit corroded did not bring an additional reduction of the SPW Cu^{2+} concentration in this short-term period. Even though the pyrolysis temperature was similar, the lignin-rich PB biochar (derived from pine bark chips) released less DOM than the lignin-poor biochar (derived from poultry manure). Total Cu concentration in the soil pore water peaked for the AB-amended soils, due to its high DOM concentration, which may explain decreased root and shoot DW yields of dwarf beans and promote Cu leaching from the root zone. High EC values and Na and Cl concentrations in the soil pore water of the AB-amended soils may contribute to impact the plant growth. Compost combined with the AB biochar resulted in the lowest shoot DW yield. Dwarf bean biomass did not change in the PB-amended soils as compared to the untreated soil. The PBZ combination slightly enabled to reduce the SPW Cu concentration relative to PB alone, but the additional Z cost can impair this option. To better assess PB and PBZ as soil amendments, a 2-year pot experiment was carried out with *Arundo donax* L. and *Populus nigra* L. before to implement field plots. Data on plant biomasses and environmental bioavailability of soil Cu are pending.

6. Acknowledgements

This work was supported by ADEME (French Agency for the Environment and Energy) (PhD grant of N. Oustrière) and the French National Research Agency (ANR C2ii, program PHYTOCHEM). The

UMR Biogeco is a member of the INRA Ecotoxicologist network, ECOTOX and the ERA-Net FACCE SURPLUS (INTENSE project: Intensify production, transform biomass to energy and novel goods and protect soils in Europe; http://faccesurplus.org/research-projects/intense/).

7. References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere. 99, 19–33. doi:10.1016/j.chemosphere.2013.10.071

Ashworth, D.J., Alloway, B.J., 2007. Complexation of copper by sewage sludge-derived dissolved organic matter: effects on soil sorption behaviour and plant uptake. Water Air Soil Pollut. 182, 187–196. doi:10.1007/s11270-006-9331-7

Assimakopoulou, A., Salmas, I., Nifakos, K., Kalogeropoulos, P., 2015. Effect of salt stress on three green bean (*Phaseolus vulgaris* L.) cultivars. Not. Bot. Horti. Agrobo. 43(1), 113–118. doi:10.15835/nbha4319905

Baize, D., 1997. Un Point sur les teneurs totales en éléments traces métalliques dans les sols. First ed. Paris, France: INRA Éditions.

Baize, D., 2000. Guide des analyses en pédologie. Second ed. Paris, France : INRA Éditions.

Bakshi, S., He, Z.L., Harris, W.G., 2014. Biochar amendment affects leaching potential of copper and nutrient release behavior in contaminated sandy soils. J. Environ. Qual. 43, 1894–1902. doi:10.2134/jeq2014.05.0213.

Beesley, L., Moreno-Jimenez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287. doi:10.1016/j.envpol.2010.02.003

Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480. doi:10.1016/j.envpol.2010.10.016

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159, 3269–3282. doi:10.1016/j.envpol.2011.07.023

Beesley, L., Inneh, O.S., Norton, G.J., Moreno-Jimenez, E., Pardo, T., Clemente, R., Dawson, J.J.C., 2014. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. Environ. Pollut. 186, 195–202. doi:10.1016/j.envpol.2013.11.026

Bernal, MP., Clemente, R., Walker, DJ., 2009. Interactions of heavy metals with soil organic matter in relation to phytoremediation. In: Phytoremediation: the Green Salvation of the World. Research Signpost. Kerala, India: Navarro-Aviño, J.P., 109–129.

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization, Environ. Pollut.156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bes, C., Mench, M., Aulen, M., Gaste, H., Taberly, J., 2010. Spatial variation of plant communities and shoot Cu concentrations of plant species at a timber treatment site. Plant Soil. 330, 267–280. doi:10.1007/s11104-009-0198-4

Bes, C., Jaunatre, R., Mench, M., 2013. Seed bank of Cu-contaminated topsoils at a wood preservation site: impacts of copper and compost on seed germination. Environ. Monit. Assess. 185, 2039–2053. doi:10.1007/s10661-012-2686-x

Boisson, J., Mench, M., Sappin-Didier, V., Solda, P., Vangronsveld, J., 1998. Short-term in situ immobilization of Cd and Ni by beringite and steel shots application to long-term sludged plots. Agronomie. 18, 347–359. doi:10.1051/agro:19980502

Bolan, N.S., Adriano, D., Senesi, N., Kunhikrishnan, A., James, T., McDowell, R., 2010. Dissolved organic carbon: biogeochemistry, dynamics and agro-environmental significance in soils. Adv. Agron. 110, 1–67.

Bolan, N.S, Kunhikrishnan, A., Thangarajana, R., Kumpiene, J., Parke, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metalloid.-contaminated soils – To mobilize or to immobilize? J. Hazard. Mater. 266, 141–166. doi:10.1016/j.jhazmat.2013.12.018

Borchard, N., Prost, K., Kautz, T., Moeller, A., Siemens, J., 2012. Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. Eur. J. Soil Sci. 63, 399–409. doi:10.1111/j.1365-2389.2012.01446.x

Buss, W., Kammann, C., Koyro, H.W., 2012. Biochar reduces copper toxicity in *Chenopodium quinoa* Willd. in a sandy soil. J. Environ. Qual. 41, 1157–1165. doi:10.2134/jeq2011.0022

Buss, W., Mašek, O., Graham, M., Wüst, D., 2015. Inherent organic compounds in biochar- Their content, composition and potential toxic effects. J. Environ. Manag. 156, 150–157. doi:10.1016/j.jenvman.2015.03.035

Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45, 4884–4889. doi:10.1021/es103752u

Chintala, R., Schumacher, T.E., McDonald, L.M., Clay, D.E., Malo, D.D., Papiernik, S.K., Clay, S.A., Julson, J.L., 2014. Phosphorus sorption and availability from biochars and soil/biochar mixtures. CLEAN: Soil, Air. Water. 42, 626–634. doi:10.1002/clen.201300089

Cundy, A., Bardos, P., Puschenreiter, M., Witters, N., Mench, M., Bert, V., Friesl-Hanl, W., Muller, I., Weyens, N., Vangronsveld, J., 2015. Developing effective decision support for the application of "gentle" remediation options: The GREENLAND project. Remediation Journal. 25, 101–114. doi:10.1002/rem.21435

Cuypers, A., Vangronsveld, J., Clijsters, H., 2000. Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of *Phaseolus vulgaris* seedlings during the early stages of metal assimilation. Physiol. Plant. 110, 512–517. doi:10.1111/j.1399-3054.2000.1100413.x

European Biochar. Available online at http://www.european-biochar.org/en/ebc-ibi (verified on October 10th, 2016)

Gomez-Eyles, J.L., Sizmur, T., Collins, C.D., Hodson, M.E., 2011. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environ. Pollut. 159, 616–622. doi:10.1016/j.envpol.2010.09.037

Hale, B., Evans, L., Lambert, R., 2012. Effects of cement or lime on Cd, Co, Cu, Ni, Pb, Sb and Zn mobility in field-contaminated and aged soils. J. Hazard. Mater. 199–200, 119–127. doi:10.1016/j.jhazmat.2011.10.065.

Hanauer, T., Felix-Henningsen, P., Steffens, D., Kalandadze, B., Navrozashvili, L., Urushadze, T., 2011. In situ stabilization of metals (Cu, Cd, and Zn) in contaminated soils in the region of Bolnisi, Georgia. Plant Soil. 341, 193–208. doi:10.1007/s11104-010-0634-5

Hattab, N., Motelica-Heino, M., Bourrat, X., Mench, M., 2014. Mobility and phytoavailability of Cu, Cr, Zn, and As in a contaminated soil at a wood preservation site after 4 years of aided phytostabilisation. Environ. Sci. Pollut. Res. 21, 10307–10319. doi:10.1007/s11356-014-2938-0

Hego, E., Bes, C., Bedon, F., Palagi, P.M., Chaumeil, P., Barré, A., Claverol, S., Dupuy, J.W., Bonneu, M., Lalanne, C., Plomion, C., Mench, M., 2015. Differential accumulation of soluble proteins in roots of metallicolous and nonmetallicolous populations of *Agrostis capillaris* L. exposed to Cu. Proteomics. 14(15), 1746–1758. doi:10.1002/pmic.201300168

Inaba, S, Takenaka, C., 2005. Effects of dissolved organic matter on toxicity and bioavailability for lettuce sprouts. Environ. Int. 31(4), 603–608. doi:10.1016/j.envint.2004.10.017

INDIQUASOL. 2016. Indicator of soil quality data base. INRA, Orleans. Available online at http://acklins.orleans.inra.fr/geoindiquasol/index.php (verified on October 10th, 2016)

Ippolito, J.A., Strawn, D.G., Scheckel, K.G., Novak, J.M., Ahmedena, M., Niandou, M.A.S., 2012. Macroscopic and molecular investigations of copper sorption by a steam-activated biochar. J. Environ. Qual. 41, 150–156. doi:10.2134/jeq2011.0113

Karami, N., Clemente, R., Moreno-Jimenez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J. Hazard. Mater. 191, 41–8. doi:10.1016/j.jhazmat.2011.04.025

Kargar, M., Clark, O.G., Hendershot, W.H., Jutras, P., Prasher, S.O., 2015. Immobilization of trace metals in contaminated urban soil amended with compost and biochar. Water Air Soil Pollut. 226, 191. doi:10.1007/s11270-015-2450-2

Karjalainen, A.M., Kilpi-Koski, J., Vaisanen, A.O., Penttinen, S., Van Gestel, C.A.M., Penttinen, O.P., 2009. Ecological risks of an old wood impregnation mill: application of the Triad approach. Integr. Enviro. Assess. Manage. 5, 379–389. doi:10.1897/IEAM_2008-072.1

Kidd, P., Mench, M., Álvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J., Puschenreiter, M., 2015. Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int. J. Phytorem. 17, 1005–1037. doi:10.1080/15226514.2014.1003788

Kolbas, A., Kidd, P., Guinberteau, J., Jaunatre, R., Herzig, R., Mench, M., 2015. Endophytic bacteria take the challenge to improve Cu phytoextraction by sunflower. Environ. Sci. Pollut. Res. Int. 7, 5370–5382. doi:10.1007/s11356-014-4006-1

Komárek, M., Vanek, A., Ettler, V., 2013. Chemical stabilization of metals and arsenic in contaminated soils using oxides. A review. Environ. Pollut. 172, 9–22. doi:10.1016/j.envpol.2012.07.045

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste. Manage. 28, 215–225. doi:10.1016/j.wasman.2006.12.012

Kumpiene, J., Mench, M., Bes, C.M., Fitts, J.P., 2011. Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environ. Pollut. 159, 1536–1542. doi:10.1016/j.envpol.2011.03.005

Lagomarsino, A., Mench, M., Marabottini, R., Pignataro, A., Grego, S., Renella, G., Stazi, S.R., 2011. Copper distribution and hydrolase activities in a contaminated soil amended with dolomitic limestone and compost. Ecotox. Environ. Safe. 74 (7), 2013–2019.

Lehmann, J., 2007. A handful of carbon. Nature. 447, 143–144. doi:10.1038/447143a

Liu, W., Tian, S., Zhao, X., Xie, W., Gong, Y., Zhao, D., 2015. Application of stabilized nanoparticles for in situ remediation of metal-contaminated soil and groundwater: a critical review. Curr. Pollution. Rep. 1, 280–291. doi:10.1007/s40726-015-0017-x

Luo, F., Song, J., Xia, W., Dong, M., Chen, M., Soudek, P., 2014. Characterization of contaminants and evaluation of the suitability for land application of maize and sludge biochars. Environ. Sci. Pollut. Res. 21, 8707–8717. doi:10.1007/s11356-014-2797-8

Mackie, K.A., Marhan, S., Ditterich, F., Schmidt, H.P., Kandeler, E., 2015. The effects of biochar and compost amendments on copper immobilization and soil microorganisms in a temperate vineyard. Agr. Ecosyst. Environ. 201, 58–69. doi:10.1016/j.agee.2014.12.001

MacNicol, R.D., Beckett, P.H.T., 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil. 85, 107–129.

Marchand, L., Quintela Sabaris, C., Desjardins, D., Oustrière, N., Pesme, E., Butin, D., Wicart, G., Mench, M., 2015. Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons. Environ. Sci. Pollut. Res. 23(4), 3120–3135. doi:10.1007/s11356-015-4984-7

Mench, M., Coussin, F., Mocquot, B., Bussière, S., Prunet, T., Lagriffoul, A., 1996. Evaluation des apports d'un biotest végétal et de la spéciation des éléments traces à la mise au point d'une batterie de tests d'écotoxicologie terrestre applicables aux sols et aux déchets. Final report no. 4930029, INRA, Unité d'Agronomie, Bordeaux—ADEME, Recherche, Milieux et Impact, Paris, France.

Mench, M., Bes, C., 2009. Assessment of ecotoxicity of topsoils from a wood treatment site. Pedosphere. 19(2), 143–155. doi:10.1016/S1002-0160(09)60104-1

Mench, M, Lepp., N., Bert, V., Schwitzguébel, J.P., Gawronski, S.W., Schröder, P., Vangronsveld, J., 2010. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J. Soils Sediments. 16, 876–900. doi:10.1007/s11368-010-0190-x

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Bio/Technol. 7, 47–59. doi:10.1007/s11157-007-9125-4

Munns, R., 2008. Tester M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681. doi:10.1146/annurev.arplant.59.032607.092911

Namgay, T., Singh, B., Singh, B.P., 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize *Zea mays* L. Aust. J. Soil Res. 48, 638–647. doi:10.1071/SR10049

Oleszczuk, P., Josko, I., Kusmierz, M., 2013. Biochar properties regarding to contaminants content and ecotoxicological assessment. J. Hazard. Mater. 260, 375–82. doi:10.1016/j.jhazmat.2013.05.044

Pandey, A.K., Pandey, S.D., Misra, V., 2000. Stability constants of metal-humic complexes and its role in environmental detoxification. Ecotoxicol. Environ. Saf. 47, 195–200. doi:10.1006/eesa.2000.1947

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil. 348, 439–451. doi:10.1007/s11104-011-0948-y

Québec. 2016. Land Protection and Rehabilitation Regulation. Environment Quality Act. Gouvernement du Québec. Available online at: http://www2.publicationsduquebec.gouv.qc.ca/dynamicSearch/telecharge.php?type=3&file=/Q_2/Q2R37_A.htm (verified on October 10th, 2016)

Qiu, H., Vijver, M.G., He, E., Peijnenburg, W.J., 2013. Predicting copper toxicity to different earthworm species using a multicomponent Freundlich model. Environ. Sci. Technol. 47, 4796–4803. doi:10.1021/es305240n.

Rajapaksha, A.U., Ahmad, M., Vithanage, M., Kim, K.R., Chang, J.Y., Lee, S.S., Ok, Y.S., 2015. The role of biochar, natural iron oxides, and nanomaterials as soil amendments for immobilizing metals in shooting range soil. Environ. Geochem. Health. 37, 931–942. doi:10.1007/s10653-015-9694-z.

Ravet, K., Pilon, M., 2013. Copper and iron homeostasis in plants: the challenges of oxidative stress. Antioxid. Redox. Signal. 19 (9), 919–932. doi:10.1089/ars.2012.5084.

Rodríguez-Vila, A., Covelo, E.F., Forján, R., Asensio, V., 2014. Phytoremediating a copper mine soil with *Brassica juncea* L., compost and biochar. Environ. Sci. Pollut. Res. 21, 11293–11304. doi:10.1007/s11356- 014-2993–6

Rogovska, N., Laird, D., Cruse, R.M., Trabue, S., Heaton, E., 2012. Germination tests for assessing biochar quality. J. Environ. Qual. 41, 1014-1022. doi:10.2134/jeq2011.0103

Ruttens, A., Mench, M., Colpaert, J.V., Boisson, J., Carleer, R., Vangronsveld, J., 2006. Phytostabilization of a metal contaminated sandy soil. I: Influence of compost and/or inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of metals. Environ. Pollut. 144, 524–532. doi:10.1016/j.envpol.2006.01.021

Schulz, H., Dunst, G., Glaser, B., 2013. Positive effects of composted biochar on plant growth and soil fertility. Agron. Sust. Dev. 33, 817–827. doi:10.1007/s13593-013-0150-0

Singh, B., Singh, B.P., Cowie, A.L., 2010. Characterisation and evaluation of biochars for their application as a soil amendment. Aust. J. Soil Res. 48, 516–525. doi:10.1071/SR10058

Sizmur, T., Wingate, J., Hutchings, T., Hodson, M.E., 2011. *Lumbricus terrestris* L. does not impact on the remediation efficiency of compost and biochar amendments. Pedobiologia. 54 Suppl., S211 S216. doi:10.1016/j.pedobi.2011.08.008

Tavakkoli, E., Rengasamy, P., McDonald, G.K., 2010. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J. Exp. Bot. 61(15), 4449–4459. doi:10.1093/jxb/erq251

Thaler, N., Humar, M., 2014. Copper leaching from copper-ethanolamine treated wood: Comparison of field test studies and laboratory standard procedures. BioResources. 9(2), 3038−3051.

Tiberg, C., Kumpiene, J., Gustafsson, J.P., Marsz, A., Persson, I., Mench, M., Kleja, D.B., 2016. Immobilization of Cu and As in two contaminated soils with zerovalent iron – Long-term performance and mechanisms. Appl. Geochem. 67, 144–152. doi:10.1016/j.apgeochem.2016.02.009

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie. Angers, and EDP Sciences, Les Ulis.

Uchimiya, M., Ohno, T., He, Z., 2013. Pyrolysis temperature-dependent release of dissolved organic carbon from plant, manure, and biorefinery wastes. J. Anal. Appl. Pyrol. 104, 84–94. doi:10.1016/j.jaap.2013.09.003

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., Van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765–794. doi:10.1007/ s11356-009-0213-6

Villanneau, E., Perry-Giraud, C., Saby, N., Jolivet, C., Marot, F., Maton, D., Floch-Barneaud, A., Antoni, V., Arrouays, D., 2008. Détection de valeurs anomaliques d'éléments traces métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. Etude et Gestion des Sols. 15(3), 183–200.

Wang, X., Chen, X., Liu, S., Ge, X., 2010. Effect of molecular weight of dissolved organic matter on toxicity and bioavailability of copper to lettuce. J. Environ.Sci. 22(12), 1960–1965. doi:10.1016/S1001-0742(09)60346-6

Yang, X., Liu, J., McGrouther, K., Ye, Z., Huang, H., Lu, K., Guo, X., He, L., Lin, X., Che, L., Ye, Z., Wang H., 2016. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. Environ. Sci. Pollut. Res. 23(2), 974–984. doi:10.1007/s11356-015-4233-0

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei. J., Huang, H., 2013. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ. Sci. Pollut. Res. 20, 8472–8483. doi:10.1007/s11356-013-1659-0

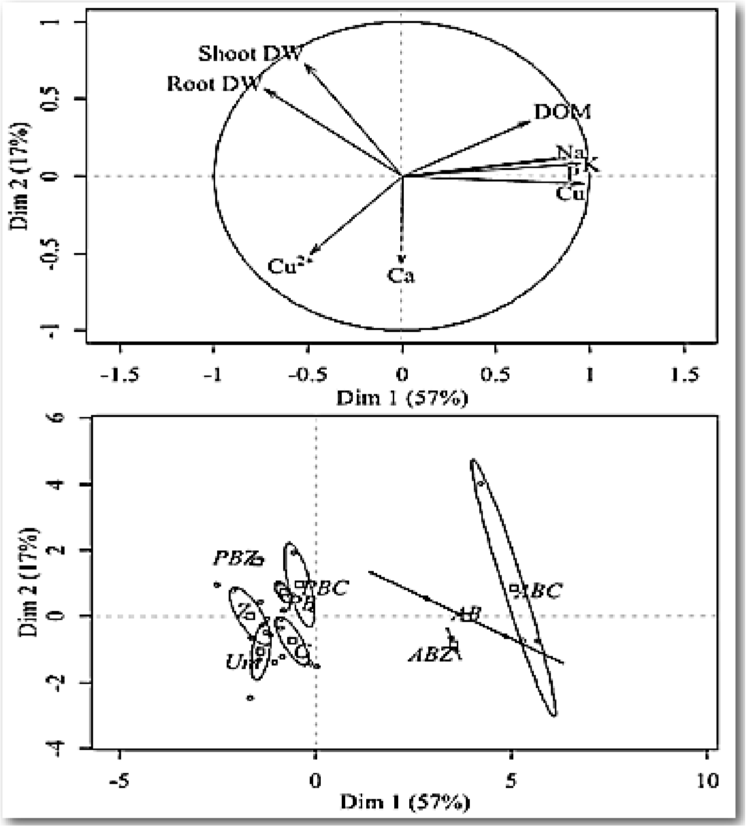


Fig. Supplemental material 1. Principal Component Analysis (PCA) on total Cu, Cu^{2+}, Ca, P, Na, K and DOM concentrations in the soil pore water and shoot and root DW yields of dwarf beans.

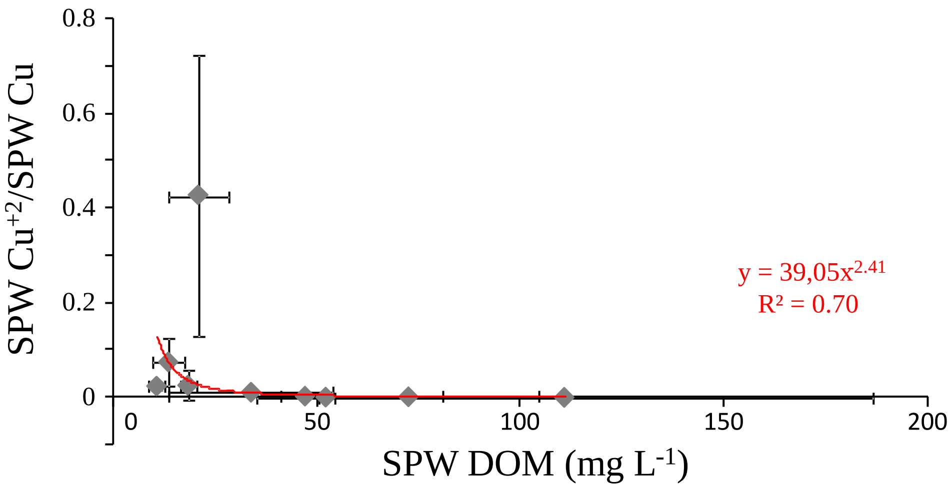


Fig. Supplemental material 2. Relationships between the Cu^{2+} concentration/Cu concentration ratio in the SPW and the SPW DOM concentration (mg L^{-1}). The trend line of the power function and the correlation coefficient R^2 were donated by the Excel software.

Part. 2. Biomass production with (essential) metal(loid) concentrations within the common ranges usable by local biomass processing chains.

2.1. Risk assessment

2.1.1. Chapter 2 **-** Wood derived-biochar combined with either compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil.

Published in Environmental Science and Pollution Research, in 2017

Doi: 10.1007/s11356-017-8361-6

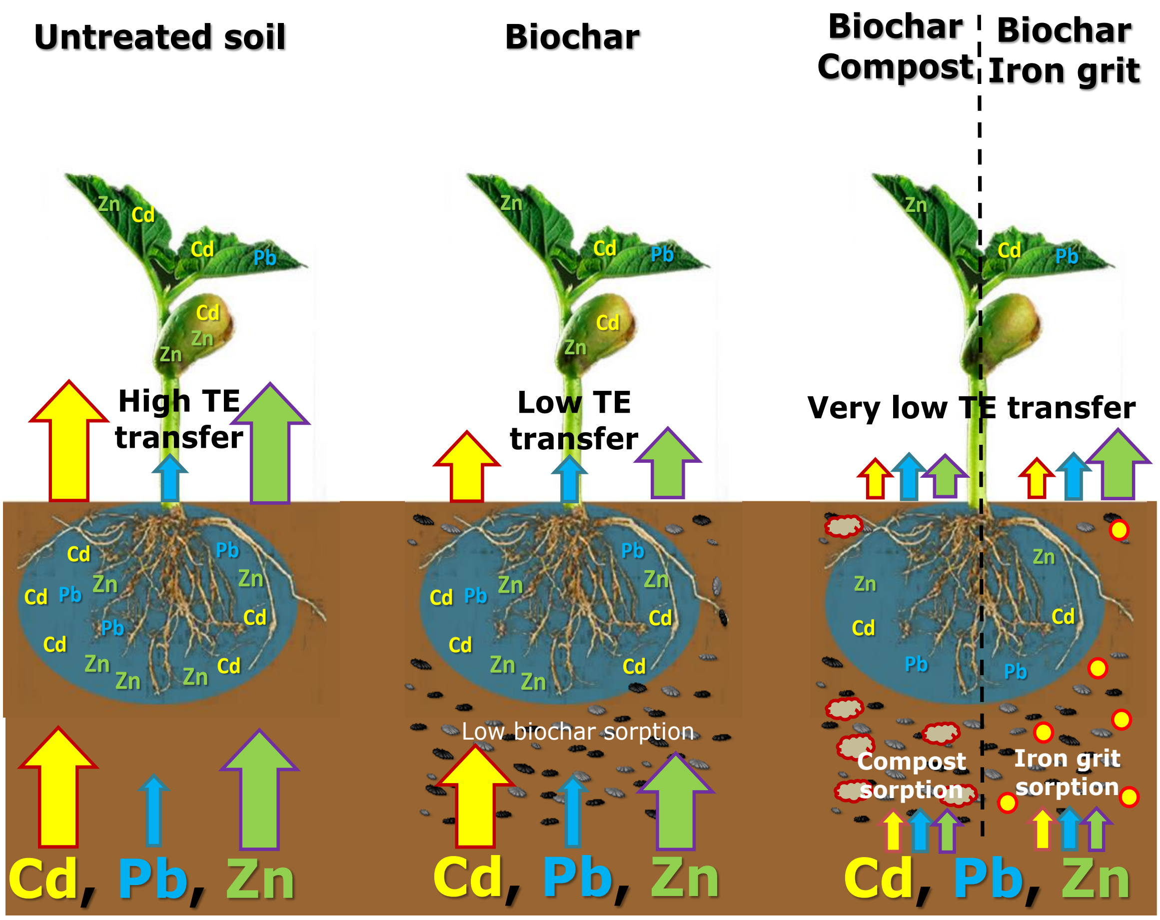


Fig. B. Graphical abstract – Chapter 2

Wood derived-biochar combined with either compost or iron grit for in situ stabilization of Cd, Pb and Zn in a contaminated soil

Nadège Oustriere[1], Lilian Marchand[1], Gabriel Rosette[1], Wolfgang Friesl-Hanl[2], Michel Mench[1]

[1]BIOGECO, INRA, UNIV. BORDEAUX, 33615 PESSAC, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; mench@bordeaux.inra.fr;

[2] ENERGY DEPARTMENT, AIT AUSTRIAN INSTITUTE OF TECHNOLOGY GMBH, KONRAD-LORENZ-STRAßE 24, 3430 TULLN, AUSTRIA.

E-mails: Wolfgang.Friesl-Hanl@ait.ac.at;

Abstract

In situ stabilization of Cd, Pb and Zn in an Austrian agricultural soil contaminated by atmospheric depositions from a smelter plant was assessed with a pine bark chip-derived biochar, alone and in combination with either compost or iron grit. Biochar amendment was also trialed in an uncontaminated soil to detect any detrimental effect. The pot experiment consisted in 10 soil treatments (% w/w): untreated contaminated soil (Unt); Unt soil amended with biochar alone (1%: B1; 2.5%: B2.5) and in combination: B1 and B2.5 + 5% compost (B1C and B2.5C), B1 and B2.5 + 1% iron grit (B1Z and B2.5Z); uncontaminated soil (Ctrl); Ctrl soil amended with 1% or 2.5% biochar (CtrlB1, CtrlB2.5). After a 3-month reaction period, the soil pore water (SPW) was sampled in potted soils and dwarf beans were grown for a 2-week period. The SPW Cd, Pb and Zn concentrations decreased in all amended-contaminated soils. The biochar effects increased with its addition rate and its combination with either compost or iron grit. Shoot Cd and Zn removals by beans were reduced and shoot Cd, Pb and Zn concentrations decreased to common values in all amended soils except the B1 soil. Decreases in the SPW Cd/Pb/Zn concentrations did not improve the root and shoot yields of plants as compared to the Ctrl soil.

Keywords: Metal, *Phaseolus vulgaris* L., Phytomanagement, Soil contamination, Soil pore water.

1. Introduction

Several hundred years of smelting and processing of mining ores have caused widespread pollution of field areas around the industrial site of Arnoldstein in Carinthia, Austria (Asami 1988), where the Zn/Cd/Ga smelter closed in 1992. The surrounding soils used for housing (playgrounds), horticulture, forestry, and alpine grassland agriculture with pastures and feed production are contaminated by Pb, Cd and Zn and, to a lesser extent, Cu and As (Friesl et al., 2006; Friesl et al., 2009). Such soil contamination by trace elements (TE) generated detrimental effects on the ecosystems with TE transfer from the soil to the environment. Although Cu and Zn concentrations were under homeostatic control, high Pb and Cd concentrations were measured in arthropods species (Rabitsch, 1995). High Pb-concentration occurred in blood and teeth of inhabitants living nearby the smelter (Kasperowski, 1993). Metal concentrations (mg kg^{-1}) were high in *Zea mays* L. shoot, i.e. Pb 54, Zn 286, and Cd 2.73 (Friesl et al., 2006). Based on the Austrian Federal Environmental Agency, the restoration of such contaminated soils was needed (Kasperowski, 1993, Rabitsch, 1995).

To phytomanage agricultural TE-contaminated soils, in situ stabilization of the labile TE pools in excess in the soil combined with the use of TE-excluder cultivars of high yielding crops is one of the gentle remediation options (GRO, Kidd et al., 2015). It relies on soil amendments to (1) improve substrate bio-physicochemical properties (e.g. organic matter and nutrient contents), (2) immobilize TE in the solid phases preventing water-soluble TE migration from the root zone, (3) limit exposure to contaminants and detrimental effects on living organisms, (4) promote safe crops and other vegetation covers and (5) stimulate ecological restoration of soil processes and functions in line with ecosystem services (Mench et al., 2010; Bolan et al., 2014). Soil amendments can lead to immobilize Pb, Cd and Zn in the solid phase by one or more of the following processes, i.e. sorption, precipitation, complexation, ion exchange and redox process, thereby decreasing their mobility and bioavailability (Kumpiene et al., 2008). Input of conventional organic matter (OM) such as compost, paper mill waste and sewage sludges can (1) form immobilized complexes between organic ligands and metals, (2) improve soil texture and structure, (3) increase nutrient status and water retention and (4) change soil pH. Inorganic soil amendments such as clays, liming materials, phosphate minerals and Fe-, Mn-, Al oxides are effective for immobilizing Cd, Pb and Zn (Kumpiene et al., 2008). Newly Fe/Mn (hydr)oxides formed after iron grit (Fe(0)) corroded in the soil can reduce the available fraction of metal(loid)s and lower the risks associated with their leaching and ecotoxicity (Komarek et al., 2013; Tiberg et al., 2016).

Several GRO have been tested in the Pb/Zn-contaminated soils of Arnoldstein. Apatite and a commercial mixture of dolomite, diatomite, smectite basaltic tuff, bentonite, alginate and zeolite (Slovakite) efficiently stabilized and decreased the bioavailability of Pb, Zn, Cu and Cd (Tica et al., 2011). This amendment also improved microbial activities and the functional status of the contaminated soil. In a field trial, gravel sludge and red mud were effective after five years for immobilizing Cd, Pb and Zn (1M NH_4NO_3-extractable fractions reduced up to 99%) and to limit contaminant uptake by barley (*Hordeum vulgare*. L., spp. *distichon*, Friesl et al., 2009). Gravel sludge and siderite bearing material reduced extractable (NH_4NO_3) Zn and Pb concentrations and maize uptake (Touceda-González et al., 2015).

Biochar amendment is another option for *in situ* stabilization of TE-contaminated soils (Beesley and Marmiroli, 2011; Beesley et al., 2011; Oustriere et al., 2016a). Biochar is the solid product derived from waste biomass pyrolysis, under mid to low oxygen supply and high temperatures (Lehmann 2007, Ahmad et al., 2014). Amending soils with biochar has gained attention as : (1) it replenishes C stocks and improves long term C sequestration in soil (Sohi et al., 2010; Atkinson et al., 2010, Kookana et al., 2011); (2) it can increase soil fertility, plant growth and root proliferation by improving soil structure, porosity and physico-chemical properties, nutrient and water availability, and microbial communities able to degrade xenobiotics (Rizwan et al., 2016); and (3) it can reduce leaching and phytoavailability of TE in contaminated soils (Park et al., 2011). However all these potential gains depend on the biochar quality (Oustriere et al., 2016a).

Several experiments explained the decrease in Cd, Pb and Zn mobility and phytoavailability in biochar-amended soils by increase in soil pH and CEC, adsorption of metal-complexing DOM and electrostatic interactions between the positively charged metal ions and negative charges associated with delocalized π-electrons on aromatic structures of biochar (Beesley and Marmiroli, 2011; Uchimiya et al., 2010a; Karami et al., 2011): e.g. sugar cane biochar, Cd/Pb/Zn, *Mucuna aterrima* (Piper & Tracy) Holland, Zn-contaminated mine soil (Puga et al., 2015a); bamboo and rice straw biochars, Cd/Cu/Pb/Zn, *Sedum plumbizincicola* L. (Lu et al., 2014). Biochar amendment reduced the extractability and bioavailability of Cd, Zn and Pb in a soil contaminated by atmospheric depositions (Houben et al., 2013a,b), but root-induced acidification of the rhizosphere counteracted the liming effect of biochar and, in turn, suppressed short-term metal immobilization (Houben and Sonnet, 2015). *Miscanthus*-derived biochar increased soil pH of a contaminated sewage field and reduced Zn and Cd concentrations in the soil solution whereas those of Pb and Cu increased due to soluble complexes with dissolved organic matter (DOM) (Wagner and Kaupenjohann, 2015). In the Arnoldstein soil, addition of poplar derived-biochar decreased the (NH_4NO_3) extractable fraction of Pb, Zn and Cd but metal concentrations in the shoots of *Lolium multiflorum* Lam. 1779 did not decrease (Karer et al., 2015).

According to Beesley and Marmiroli (2011), the combination of compost and iron oxides with biochar may be more suitable than biochar alone to promote TE immobilization and buffer nutrient depletion in contaminated soils. Compost combined with biochar may improve total soil C, N and P, stabilize soil aggregates and stimulate microorganisms (Beesley et al., 2010; Sizmur et al., 2011; Schulz et al., 2013; Rodríguez-Vila et al., 2015). Depending on ligand types, pH, surface properties of the oxides and ligand/metal(loid) ratio, Fe/Mn/Al (hydr)oxides can promote metal(loid) adsorption (Komarek et al., 2013; Tiberg et al., 2016). Biochar combined with iron grit could reduce the water-soluble soil fraction of metals and thus the pollutant linkages associated with their leaching and ecotoxicity (Wagner and Kaupenjohann, 2015). The potential gains of adding biochar with either compost or iron grit as compared to biochar alone in metal-contaminated soils remain poorly documented. This pot experiment aimed at assessing the efficiency of a biochar derived from pine bark chips, alone and in combination with either compost or iron grit, to stabilize Cd, Pb and Zn in an agricultural, contaminated soil from the Arnoldstein area. Metals in the soil pore water and soil phytotoxicity on dwarf beans (*Phaseolus vulgaris* L.), reported as root and shoot dry weight (DW) yields and Pb, Cd and Zn uptake by plants, were determined.

2. Material and Methods

2.1. Soils and amendments

The soil was sampled nearby (300 m) the former metal smelter located at Arnoldstein, Carinthia, Austria (latitude: 46°33′13.74″; longitude: 13°41′23.70″, Table 1). This smelting activity goes back to 500 years and the site has continuously experienced Cd, Zn, and Pb (and in a lesser extent Cu and As) atmospheric depositions. Topsoil (Unt, 0-15cm, Leptosol, 100 kg) was collected from grassland at the sub-site ARN-D (Touceda-González et al., 2015), air-dried for 2 weeks at ambient temperature, sieved to 5 mm, and manually homogenized. Its texture is sandy, i.e. 43% sand, 22% clay, and 35% silt, with 3.5 % organic C and slightly acidic pH (i.e. 6 Table 1). Based on guideline values for agricultural production (Austrian Standard S 2088-2), it is mainly contaminated by Pb, Zn and Cd, and in a lesser extent by Cu and As (Table 1). An uncontaminated sandy topsoil (Ctrl, pH 7.9, 0-20 cm) was collected in a kitchen garden, Gradignan, Gironde, France. The biochar (B) was a commercial product (Florentaise, Saint-Mars-du-Désert, France; pyrolysis: 180 s at 420°C) derived from pine bark chips. It was crushed, sieved to 2 mm and manually homogenized. In our experiments, soluble salts were not removed from the biochar. Commercial grade compost (C), made of green wastes for 9 to 12 months, was obtained from Gonzales frères, Martignas sur Jalle, France. Zerovalent iron grit (Z, GH120, particle size <0.1 mm) was obtained from Wheelabrator Allevard, France (Bes and Mench, 2008). Amended soils were thoroughly homogenized in large plastic containers and individually prepared prior to use. Elemental composition, carbon content and PAH concentrations of biochar and compost were determined at the INRA Laboratoire d'Analyses des Sols (LAS, Arras, France) with standard methods (INRA LAS, 2011) (Table 2).

Table. 1. Main soil properties

Parameter	Arnoldstein soil	Ctrl Soil	Background levels [A]
pH	6	7.9	6.6
CEC	12	16.1	
Total $CaCO_3$ (g kg^{-1})	<1	-	
Organic matter (g kg^{-1})	60	4	
Organic C (g kg^{-1})	35	-	14.5
Total N (g kg^{-1})	3.53	2.9	
C/N	10	14	10.02
P_2O_5 (g kg^{-1})	0.021	-	
Texture (g kg^{-1})			
Sand	858	665	≥ 650
Silt	83	155	≤ 350
Clay	59	180	≤ 180
Nutrients (g kg^{-1})			
Ca	0.042	0.104	
Mg	0.0659	0.012	
Na	0.0731	-	
K	0.162	0.1879	
Fe	0.424	0.069	
Mn	0.171	0.214	
Total TE (mg kg^{-1})			
Cr	71	8.3	
Cu	96	22	60
Ni	46	6.6	-
Zn	1110	75	300
Pb	1300	24.4	100
Cd	9	0.27	1
Mo	2.9	1.43	-
As	47	6.6	1–25 [B]
PAHs (mg kg^{-1})			
Naphtalene	<0.0204	-	
Acenaphtene	<0.0102	-	
Fluorene	<0.0051	-	
Phenanthrene	0.0173	-	
Anthracene	<0.0051	-	
Fluoranthene	0.0204	-	
Pyrene	0.0143	-	
Benzo(a)anthracene	<0.0102	-	
Chrysene	<0.051	-	
Benzo(a)fluoranthene	0.0133	-	
Benzo(k)fluoranthene	<0.0051	-	
Benzo(k)pyrene	<0.0102	-	
Dibenzo(ah)anthracene	<0.0204	-	
Benzo(ghi)perylene	<0.051	-	
Indeno(123 cd)pyrene	<0.0102	-	
Acenaphtylene	<0.0306	-	

[A.] According to frequent total concentrations in French sandy topsoils and Austrian Standard S 2088-2 (Baize, 1997; Baize, 2000; Villanneau et al., 2008, INDIQUASOL, 2016).
[B.] Frequent total As concentrations for all French soil types (Bes and Mench, 2008).

Table. 2. Composition of soil conditioners. In bold, values exceeding the European Biochar Certificate (EBC) V4.8 thresholds

	Biochar (B)	**EBC [A]**	**IBI [B]**	**Compost (C)**	**Iron grit (Z)**
pH	9.89	-	-	7.53	
CEC (cmol kg^{-1})	1.23	-	-	26.7	
Major elements (%DW)					
H	0.8	-	-	-	
Total N	0.3	-	-	0.69	
S	0.03	-	-	-	
Cl	0.01	-	-	-	
Organic C	90.3	>50%		10.9	
Nutrients (g kg^{-1})					
Ca	18.6	-	-	22.5	
K	13.8	-	-	5.4	
Mg	29.9	-	-	1.9	
Na	0.389	-	-	-	
P	0.105	-	-	0.374	
Elements (mg kg^{-1})					
Al	1650	-	-	12700	600
As	0.461	-	12-100	4.47	70
Cd	<0.5	1	1.4-39	<0.5	0.03
Cr	45.9	80	64-1200	21	3500
Cu	230	100	63-1500	85.8	1010
Mn	-	-	-	-	7710
Fe	4470	-	-	6830	973000
Hg	<0.1	1	1-17	<0.1	
Ni	28.3	30	47-600	7.98	739
Pb	2.61	120	70-500	51.5	20
Zn	50.5	400	200-7000	174	104
PAHs (mg kg^{-1})					
Sum of 16 US EPA PAHs [C]	163	4	6-300	<DL	
Fluoranthene	7.48	-	-	<0.1	
Benzo(b)fluoranthene	0.149	-	-	<0.1	
Benzo(a)pyrene	<0.2	-	-	<0.3	
Naphtalene	101	-	-	<0.2	

[A.] Following Switzerland's Chemical Risk Reduction Act (ChemRRV) on recycling fertilizers.

[B.] Range of Maximum Allowed Thresholds reflects different soil tolerance levels for these elements in compost, biosolids, or soils established by regulatory bodies in the US, Canada, EU and Australia (See Appendix 3 of the IBI Biochar Standards for further information).

[C.] Corresponds to the PAH threshold defined in the Swiss Chemical Risk Reduction Act (ChemRRV).

2.2. Experimental set up

The contaminated soil was mixed by rotation in a plastic flask with 1% or 2.5% (w/w) of biochar, alone and in combination with either compost or iron grit. The uncontaminated soil was similarly mixed only with 1% or 2.5% (w/w) of biochar. Therefore, the pot experiment included ten treatments:

(1) untreated contaminated soil (Unt),
(2) Unt soil + 1% B (B1),
(3) Unt soil + 1% B + 5% C (B1C),
(4) Unt soil + 1% B + 1% Z (B1Z),
(5) Unt soil + 2.5% B (B2.5),
(6) Unt soil + 2.5% B + 5% C (B2.5C),
(7) Unt soil + 2.5% B + 1% Z (B2.5Z),
(8) uncontaminated soil (Ctrl),
(9) Ctrl soil + 1% B (CtrlB1),
(10) Ctrl soil + 2.5% B (CtrlB2.5).

Untreated and amended soils were potted in plastic pots (1 kg, 11 cm x 11cm x 11cm, 1.3 L, in triplicates), and randomly placed in a greenhouse under controlled conditions. One Rhizon MOM moister samplers (Eijkelkamp Agrisearch Equipment, The Netherlands) was inserted with a 45° angle into each potted soil. Before sowing, amendments were allowed to react for a 3-month period with the soils, pots being manually maintained five times a week at 65% of the water holding capacity (WHC) with deionized water.

The plant test was adapted from Ruttens et al., (2006) (modified protocol from ISO 11269-2, ISO 2012). In June, four seeds of dwarf beans (P. vulgaris L. cv. Skipper, Vilmorin) were sown in all pots. Plants were cultivated during 15 days with controlled conditions (16 /8 h light/darkness; 65 ± 5% relative humidity; 25± 2°C) in the greenhouse. Potted soils were daily watered (deionized water) to maintain a 65% WHC rate. After 2 weeks, the shoots and roots were harvested, washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields.

2.3. Plant and soil pore water analysis

For each pot, dried shoots of the four plants were pooled, ground (< 1 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, As, B, Cd, Ca, Cr, Co, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, K, Na and Zn) in digests was determined by ICP-MS (Thermo X series 200, INRA USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. All element concentrations in plant parts are expressed in mg or g DW kg^{-1}. The shoot metal (Me) removal was calculated as follows, using the mean shoot value of the four plants: Me (µg $plant^{-1}$) = Shoot DW yield (g $plant^{-1}$) x shoot Me concentration (µg g^{-1} DW).

The soil pore water (SPW) was collected after plant harvest (day 14) in all potted soils (two times 10mL with a 3-day interval) using the Rhizon samplers and samples kept at 4°C prior to their analysis. The pH (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34), redox potential (Eh) and electrical conductivity (EC) (Tetracon 325 WTW), and Cu^{2+} concentration (Cupric ion electrode, Fischer Bioblock, USA) of SPW samples were measured, and their element composition (same elements as for plant ionome) analyzed by ICP-MS (Thermo X series 200) or ICP-AES (Varian Liberty 200) at the INRA USRAVE laboratory, Villenave-d'Ornon, France.

2.4. Statistical analysis

Influence of soil treatments on SPW parameters, shoot DW yields, shoot ionome and element removals of plants were tested using one-way analysis of variance (ANOVAs). Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests. Differences were considered statistically significant at $p<0.05$. When element concentrations were below the detection limits in the UNT samples, influence of soil treatments were not statistically tested. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results and Discussion

3.1. Soil pore water

3.1.1. EC, pH and nutrient concentrations (Table 3)

Except for the B1 treatment, the SPW EC significantly decreased in all amended soils, with lowest values in the B2.5Z and B2.5 soils. These lower EC values likely arose from (co-)precipitation or sorption of divalent cations on biochar and compost surface, as suggested by the significant decrease of SPW Ca and Mg concentrations in the B2.5 and B2.5Z soils (i.e. 2 and 3 fold as compared to the Unt soil). In previous studies, decrease in leachate concentrations of divalent cations was attributed to the increased CEC of biochar-amended soils (Lehmann et al., 2003; Ding et al., 2010; Bakshi et al., 2014). In contrast, the SPW concentrations of K and Na increased with the biochar and compost loading rate. The SPW K concentrations were 3 - 4 fold higher in amended soils than in the Unt soil. The SPW Na concentration was slightly enhanced in all amended soils albeit not significantly. Gain in K+, Na+ and NH4+ concentrations following biochar addition to contaminated sandy soils was previously reported (Bakshi et al., 2014). The SPW P concentration was below the detection limit (<0.2 mg L-1) in all treatments. The SPW pH increased significantly from 6.2 (Unt) to 6.9 (B2.5C) in the increasing order: Unt < B1 = B1Z < B2.5Z = B2.5 ≤ B1C = B2.5C. Such increase was likely due to the biochar alkalinity (Table 2). Biochar combined with compost led to the highest SPW pH value, in line with Beesley et al. (2010, 2014).

3.1.2. As, B and Mo concentrations (Table 3)

Increase in soil pH after biochar and compost addition was correlated with enhanced SPW As, B and Mo concentrations in the B1C, B2.5 and B2.5C soils, i.e. from 0.8 (Unt) to 1.8 (B2.5C) µg As L-1, 42 (Unt) to 56 (B1C) µg B L-1 and <0.4 (Unt) to 1.3 (B2.5C) µg Mo L-1, these elements forming oxyanions. However, these concentrations remained low compared to their values in the Ctrl soil. In the Arnoldstein soil, increased As concentration in the labile pool correlated with increasing soil pH following red mud and triple superphosphate amendment (Friesl et al., 2004, 2006). Beesley et al., (2011) found also an enhanced As mobility with increasing soil pH after biochar amendment. Authors postulated that other oxyanions would behave similarly. Riedel et al., (2015) reported a higher release of U, W and Mo oxyanions in soil leachate of biochar-amended soils with increasing pH. Biochar combined with Z, despite increased soil pH did not enhance the SPW As, B and Mo concentrations. The As, B and Mo oxyanions have high affinity for Fe (hydr)oxides and may have been sorbed by the newly-formed Fe and Mn oxy-hydroxides after iron grit corroded in the Z-amended soils (Kumpiene et al., 2008; Komárek et al., 2013).

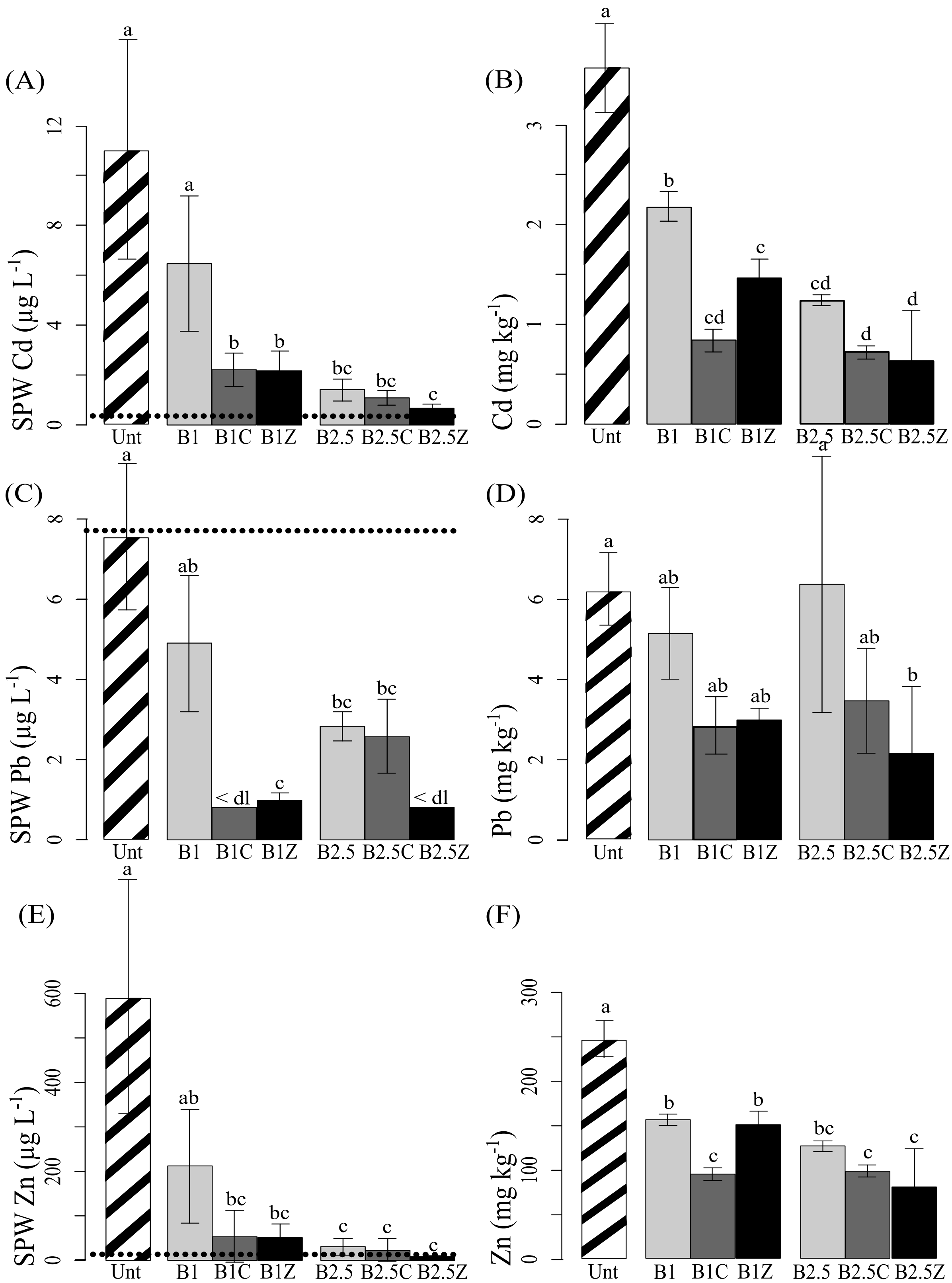


Fig. 1. (A, B) Cd,(C, D) Pb, and (E, F) Zn concentrations (µg L^{-1}) in the soil pore water (A, C, E) and in the shoots of dwarf beans (mg Kg^{-1}) (B, D, F) after the 15-day growth period in the Arnoldstein contaminated soil (Unt, black hatch), amended with 1% or 2.5% of biochar, alone (light grey) or in combination with either compost (dark grey) or iron grit (black). Dashed lines indicate the SPW element concentration in the control soil. Mean values per treatment (n=3; n=6 for Unt). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

Table. 3. Chemical composition of the soil pore water

Sample			Elements (µg L^{-1})									Nutrients (mg L^{-1})					
	pH	EC (µS cm^{-1})	Al	As	B	Cr	Co	Cu	Fe	Mn	Mo	Ni	Ca	K	Mg	P	Na
Unt	6.2±0.1d	1251±179a	< 50	0.8±0.1cd	42±4bc	<0.2	<0.2	<8	<20	68±39	<0.4	4±1a	126±49a	3±0.4d	21±8a	<0.2	5±1a
B1	6.4±0.01c	886±117ab	< 50	1.1±0.2bc	55±10ab	<0.2	<0.2	<8	<20	<20	<0.4	3±1**b**	118±41ab	12±4**bc**	21±7a	<0.2	8±2a
B1C	6.7±0.04**ab**	843±24**b**	< 50	1.6±0.1**a**	56±4**a**	<0.2	<0.2	<8	<20	<20	0.6±0.1	2±0**bc**	100±15abc	15±2**abc**	16±3ab	<0.2	7±2a
B1Z	6.4±0.05**c**	816±166**b**	< 50	0.6±0.2d	39±2c	<0.2	<0.2	<8	<20	<20	<0.4	3±0**b**	72±16abc	10±1cd	13±3ab	<0.2	6±0.2a
B2.5	6.7±0.1**b**	544±40**b**	< 50	1.4±0.3**ab**	45±2abc	<0.2	<0.2	<8	<20	<20	0.8±0.4	1±0**c**	43±7**c**	18±3**ab**	8±1b	<0.2	6±0.3a
B2.5C	6.9±0.03**a**	856±223**b**	< 50	1.8±0.1**a**	54±5ab	<0.2	<0.2	<8	<20	<20	1.3±0.3	1±0**c**	69±15abc	20±3**a**	11±2ab	<0.2	7±1a
B2.5Z	6.6±0.05**b**	611±156**b**	< 50	0.6±0.1d	43±4bc	<0.2	<0.2	<8	<20	<20	<0.4	2±0**bc**	50±23**bc**	18±5**ab**	9±4b	<0.2	7±1a
Ctrl	7.6 ± 0.1	832 ± 58	< 50	10 ± 5.6	20 ± 10	<0.2	<0.2	48 ± 11	50 ± 10	40 ± 20	24 ± 12	3 ± 1	143±66	27±44	21±11	5±2	63±12

References	As	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Oustrière et al., 2016	10- 16	0.3 - 6	3- 1517	<8 - 91	<20 - 181	<20 –348	124 -3450	7 - 22	0.4 – 3	<7 - 21
Marchand et al., 2014	4.4 - 24	-	0.7 – 2	8 - 31	-	< 20	1 - 63	3 - 16	< 0.8 – 2.8	<7 - 39
Di Bonito, 2005	7-34	-	0.5 - 18	63 - 241	-	26	0.038 - 9	10 - 1041	1 – 3.7	5 - 1151
Beesley et al., 2014	200 - 1500	-	-	10 - 1150	-	-	-	-	3 – 97	150 - 7500
Moreno-Jimenez et al., 2011	1 - 2901	-	-	2 - 1190	-	-	-	-	1 - 495	6 - 6470

Mean value ± SD for each treatment (n=3 / Unt n=6). Values with different letters differ significantly (one way ANOVA, p-value <0.05). Values in bold indicated significant differences compared to the Unt soil in a column.

3.1.3. Cd, Pb and Zn concentrations (Fig. 1A, C & E)

The SPW Cd, Pb and Zn concentrations dropped in all amended soils, ranging (µg L^{-1}) from 12 to 0.7 for Cd, 7.4 to <0.8 (dl) for Pb and 600 to 7.6 for Zn in the Unt and B2.5Z soils, respectively. These decreases after biochar addition agreed with previous studies: incorporation of sugar cane straw-derived biochar decreased the DTPA-extractable concentrations of Cd, Pb and Zn in a Zn mining soil, Vazante (Minas Gerais, Brazil, Puga et al., 2015a,b). A sewage sludge-derived biochar reduced the 0.1M $CaCl_2$-extractable Cu, Ni, Zn, Cd and Pb concentrations in a sandy Mediterranean agricultural Cambisol (Méndez et al., 2012). Here, reductions of SPW Cd, Pb and Zn concentrations were not significant for 1% biochar addition but decreases became significant for the C and Z combinations at both application rates of biochar. Decrease in 0.01M $CaCl_2$-extractable Cd, Zn and Pb concentrations with increasing concentrations of *Miscanthus*-derived biochar (1, 5, and 10%) was reported for a metal-contaminated soil nearby Zn and Pb smelters (Houben et al., 2013a,b). Regarding additional benefit of compost on metal sorption in biochar-amended soils, incorporation of hardwood-derived biochar combined with greenwaste compost into a multi-contaminated soil decreased more Cd, Pb and Zn concentrations in the SPW than biochar alone (Beesley et al., 2014). Compost combined with biochar reduced more the Cu, Ni, Pb and Zn mobility in a contaminated mine soil than biochar alone (Rodríguez-Vila et al., 2015).

Interactions between biochar-amended soils and metals are complex and the possible mechanisms are: (1) electrostatic interactions with negatively charged surfaces on soil particles activated by the pH increase, (2) specific metal-ligand complexation involving surface functional groups of biochars (in particular O, P, S, and N functional groups) that may or not involve cation exchange, and (3) sorptive interactions between cations and aromatic π electronic systems from C=C bounds of biochars (Uchimiya et al., 2010b; Zhang et al., 2013).

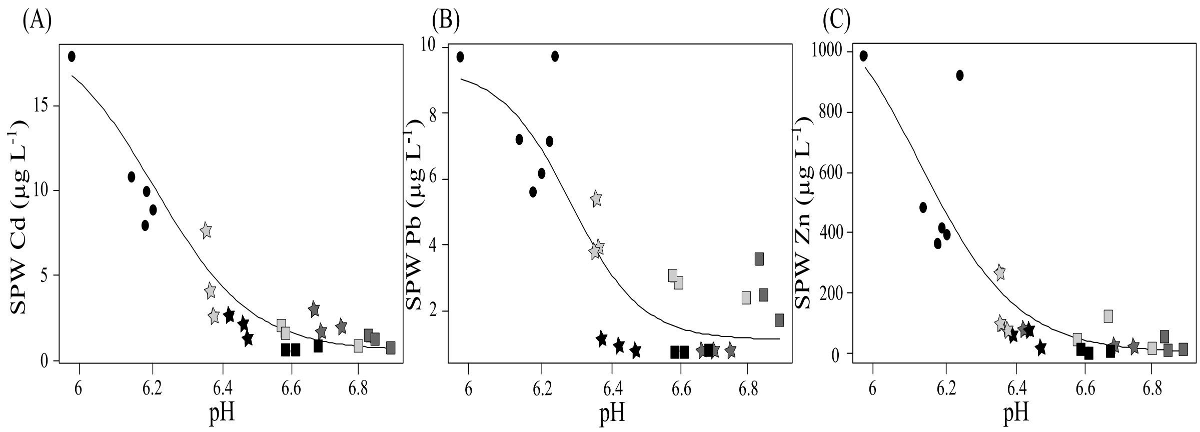


Fig. 2. Relationships between (A) Cd, (B) Pb and (C) Zn concentrations (µg L^{-1}) measured in the Arnoldstein contaminated soil (Unt, black dots), amended with 1% (stars) or 2.5% (squares) of biochar, alone (light grey) or in combination with either compost (dark grey) or iron grit (black) and pH in the soil pore water. The trend line power were donated by the R software.

Despite high total soil Pb (Table 1), the SPW Pb concentrations remained low (i.e. 7.4 µg L^{-1}, Unt) in all treatments (Fig. 1C), below the Ctrl value (i.e. 7.6 µg L^{-1}) and in the low concentration range reported in the literature (Table 3). Above pH 6, Pb may form hydroxide and oxide precipitates, e.g. $Pb_3(OH)_4^{2+}$, $Pb_6O(OH)_6^{4+}$ and PbO, controlling soil Pb solubility (Hale et al., 2012). Chelation by organic matter (OM), sorption on Fe, Al, and Mn (hydr)oxides or precipitation of metal hydroxides can immobilize Pb in the soil (Bolan et al., 2014). For this Arnoldstein soil, Friesl et al. (2006, 2009) reported increased soil pH can promote Pb retention in the soil solid phase thus decreasing NH_4NO_3-extractable Pb. Here, the SPW Pb concentration decreased as SPW pH raised up to 6.7 (Fig. 2B) and with the biochar addition rate (Fig. 1C). A part of water-soluble Pb may precipitate as metal oxy(hydr)oxides or form soluble complexes with the dissolved organic matter (DOM) provided by the biochar and compost addition into the Unt soil (Beesley et al., 2014) as Pb can strongly associate with oxygen-containing functional groups of DOM (Kargar et al., 2015; Wagner and Kaupenjohann, 2015). Such Pb oxy(hydr)oxides and Pb-DOM complexes can be retained on biochar surface (Zhang et al., 2013). The SPW Pb concentration slightly increased in the B2.5C treatments (i.e. 2.6 µg Pb L^{-1}) compared to the B1C treatments (i.e. <0.8 µg Pb L^{-1}, Fig. 1C). Formation of DOM increases when pH is higher than 5.5 (Bravin et al., 2012). Due to increase in SPW pH and high OM input by biochar (2.5%) and compost (5%), organic complexes might increasingly dissolve which may lead to a competition between DOM and Pb hydroxides or DOM-Pb complexes for retention on biochar surfaces (Beesley et al., 2014). The DOM may cloak the biochar pores preventing the sorption of elements (Bolan et al., 2010; Cao et al., 2011). This effect may depend on the soil ability to retain DOM, which is important to preserve the long term efficiency of amendments and avoid Pb leaching out of the root zone. The lower Pb mobility in soils amended by biochar (2.5%) plus iron grit compared to biochar (2.5%), alone or with compost, may be attributed to the sorption of inorganic Pb- or DOM-Pb complexes by newly formed Fe and Mn oxyhydroxides (Kumpiene et al., 2008). A strong Pb sorption onto ferrihydrite has been reported in the Arnoldstein soil (Friesl et al., 2006).

High SPW Cd and Zn concentrations (Fig. 1) reflected their total concentrations in the Unt soil (Table 1). Their values exceeded those of the Ctrl soil in all treatments except for the SPW Zn concentration in the B2.5Z soil. In the amended soils except B1, values were in the low range as compared to the literature (Table 3). Potential mechanisms for explaining decreased SPW Cd and Zn concentrations in the amended soils are: surface complexation of Cd and Zn on biochar functional groups in line with increase in soil pH, co-precipitation, inner sphere complexation of metals (Cd, Zn) and trace elements exchange with Ca^{2+} and Mg^{2+} (Chen et al., 2007; Sohi et al., 2010; Uchimiya et al., 2010b; Zhang et al., 2013; Mohamed et al., 2015). Surface complexation of Cd/Zn through –OH groups or delocalized π electrons of biochars was considered as a minor contribution (<25%, Xu et al., 2013) The liming effect of biochar and compost addition was suggested (R^2: 0.79 and 0.78 respectively, Fig. 2A and 2C), confirming Beesley et al. (2010). In the Arnoldstein soil, decreases in water-soluble and/or exchangeable Cd/Zn fractions were partly attributed to pH increase after addition of Slovakite and apatite (Tica et al., 2011), synthetic zeolite and ferrihydrite-bearing amendments (Friesl et al., 2006), poplar derived-biochar, gravel sludge with siderite-bearing material and lime (Karer et al., 2015). Increase in SPW pH may result in Cd and Zn hydrolysis species ($CdOH^+$ and $ZnOH^+$), which may precipitate as hydroxides ($Cd(OH)_2$), and $Zn(OH)_2$) (Melo et al., 2016). Here, such mechanisms may occur to a limited extent as the soil pH was below 7 and such species are mainly formed at alkaline pH (Uchimiya et al., 2010b). Friesl et al. (2006) found a Cd and Pb immobilization in the Arnoldstein soil after gravel sludge with ferrihydrite addition due to their chemisorption onto the Fe oxides. Accordingly, additional decrease of SPW Cd, Pb and Zn concentrations was expected in the B1Z and B2.5Z soils as compared to biochar alone to mirror the potential metal sorption on Fe/Mn oxyhydroxides (Kumpiene et al., 2011; Komarek et al., 2013) but this was only validated for Cd in B1Z and Pb in B2.5Z (Fig.1A and E).

3.1.4. Al, Cr, Co, Cu, Fe, Mn and Ni concentrations (Table 3)

The SPW concentrations (µg L^{-1}) decreased from 68 (Unt) to <20 in all amended soils for Mn and from 4 (Unt) to 1 (B2.5 and B2.5C) for Ni, in line with increased in SPW pH. In all soils, SPW Al, Cr, Co, Cu and Fe concentrations were below their detection limit of <0.05, <0.2, <0.2, <8 and <20 µg L^{-1}, respectively. All concentrations of these elements were similar or below the values in the Ctrl soil.

3.2. Plants

3.2.1. Plant growth parameters (Fig. 3)

Root and shoot DW yields of the Unt plants were lower as compared to the Ctrl plants albeit not significantly. The amendments did not significantly influence the plant yield, despite they reduced bean exposure to Cd and Zn (Fig. 1). Root DW yield decreased in the biochar-amended Ctrl soil albeit not significantly. The stimulation lack on biomass production after amendment of Cd, Pb and Zn-contaminated soils was previously reported regardless the type of plants, biochar and application rate: straw-derived biochar (1.5 - 5% w/w) did not increase the shoot DW yield of *M. aterrima* in a Cd/Pb/Zn-contaminated mining soil (Puga et al., 2015a,b); wood biochar (0.5 – 1.5%) did not influence the DW yield of maize plants, in a (As, Cd, Cu, Pb, and Zn)-spiked soil (Namgay et al., 2010); wheat chaff or oil mallee plant-derived biochar (0.5 and 5 %) did not improve the growth of emergent wetland species *Juncus subsecundus* N.A.Wakef. in a Cd-contaminated soil (Zhang et al., 2013). *Miscanthus*-derived biochar amendment (1, 5, and 10 %) did not promote DW yield of Italian ryegrass (*L. multiflorum*) grown in a soil contaminated nearby Zn and Pb smelters (Houben et al., 2013a,b).

Table. 4. Shoot ionome of dwarf beans

Treatments								Nutrients (g k^{-1})					
	Al	**Br**	**Cr**	**Co**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Ca**	**K**	**Mg**	**P**	**Na**
Unt	42±16a	23±2ab	0.4±0.1a	0.07±0.02a	15±1a	136±13a	60±11ab	2±0.2b	2±0.1ab	34±3a	28±2c	6±0.4a	4±0.4a	0.22±0.06bc
B1	46±12a	20±2b	0.5±0.2a	0.08±0.03a	12±1**b**	138±14a	51±3abc	2±0.3b	1.6±0.2ab	29±1ab	40±4**b**	5±0.2**b**	3±0.2a	0.2±0.08bc
B1C	44±23a	18±0b	0.4±0.03a	0.07±0.01a	13±0.4ab	143±25a	45±2**c**	3±0.2b	1.1±0.3b	30±1ab	47±2**b**	5±0.05**b**	3±0.1a	0.16±0.02c
B1Z	24±3a	21±2ab	0.4±0.1a	0.06±0.01a	13±1ab	141±30a	46±6abc	2±1b	2.6±0.6a	28±1**b**	40±2**b**	5±0.2**b**	3±1a	0.35±0.04ab
B2.5	33±15a	20±1b	0.6±0.2a	0.1±0.03a	14±2ab	195±84a	64±1a	3±1b	2.4±0.7a	27±1**bc**	49±4**ab**	5±0.1**b**	3±0.4a	0.29±0.06bc
B2.5C	48±35a	22±2ab	0.4±0.1a	0.1±0.03a	15±1a	151±33a	56±5abc	6±1**a**	1.5±0.3ab	29±4ab	58±2**a**	5±0.5**b**	4±0.2a	0.49±0.13**a**
B2.5Z	32±7a	25±2a	0.5±0.03a	0.07±0.03a	14±0.1ab	128±35a	42±6**c**	3±2b	1.9±0.7ab	21±4**c**	46±8**b**	4±1**c**	4±1a	0.24±0.06bc
Common values*	-	0-5	-	0-3	-	3-20	20-300	50-500	0.1-2	0.1–6	1–50	20–50	1.5–3.5	1.6–6.0
Average values in stressed plants**	-	-	-	5–30	-	20–100		300–500	60-1575$^{\$}$	10-100	-	-	-	-

Mean values ± SD per treatment (n=3). Values with different letters differ significantly (one way ANOVA, p-value<0.05). Values in bold differed from the Unt values; * Tremel-Schaub and Feix (2005), ** Kabata-Pendias and Pendias (1984).

3.2.2. Plant nutrients (Table 4)

The SPW K and Na concentrations increased in all biochar-amended soils (Tab. 3). Consequently, shoot Na concentrations significantly increased for the B1C and B2.5C plants, while shoot K concentration significantly increased in all amended soils, being in the upper range of common values in plants (i.e. 20–50 g K kg^{-1} DW). Such relationship was less evident for Ca, Mg and P. The SPW Ca and Mg concentrations decreased in the biochar-amended soils, especially in the B2.5 and B2.5Z treatments (Tab. 3). Consequently, shoot Ca concentrations significantly decreased for the B1Z, B2.5 and B2.5Z plants and shoot Mg concentration significantly fell in plants from all amended soils, but all values remained in the common ranges for shoots (i.e. 1–50 g Ca and 1.5–3.5 g Mg kg^{-1} DW). Antagonistic and synergistic effects may alter plant ionome, as well as DW yield and development stage (Wagner and Kaupenjohann, 2014). Increase of SPW and shoot K concentrations due to amendment addition (Tab. 3 and 4) may contribute to decrease shoot Ca and Mg concentrations (Jakobsen, 1993). The shoot P concentration did not differ across treatments and its values remained in the common range for shoots (i.e. 1.6–6.0 g P kg^{-1} DW). Combination of compost or iron grit with biochar did not have an additional effect on shoot nutrient concentrations as compared to biochar alone.

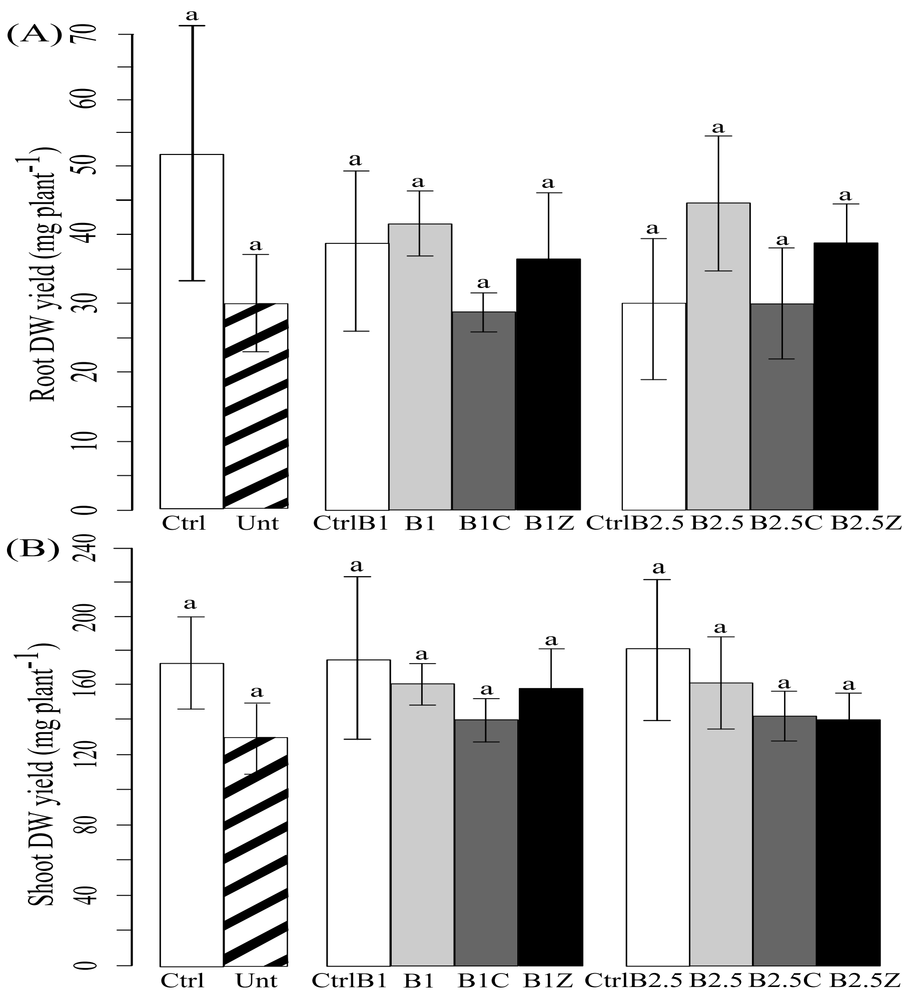


Fig. 3. (A) root and (B) shoot DW yields of dwarf bean (mg DW plant-1) after the 15-day growth period in the control soil (white, Ctrl), amended with 1% (CtrlB1) or 2.5% (CtrlB2.5) of biochar or in the Arnoldstein-contaminated soil (Unt, black hatch), amended with 1% or 2.5% of biochar, alone (light grey) or in combination with either compost (dark grey) or iron grit (black). Mean values per treatment (n=3; n=6 for Unt). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

3.2.3. Shoot As, B and Mo concentrations (Table 4)

The SPW As, B and Mo concentrations increased in the B1C, B2.5 and B2.5C treatments (Tab. 3). Changes in shoot As, B and Mo concentrations across treatments however were mostly insignificant. Only shoot Mo concentration in the B2.5C plants was significantly higher than in the Unt plants, exceeding the common range for shoots but remaining below Mo concentrations in stressed plants (Tremel-Schaub and Feix, 2005).

3.2.4. Shoot Pb concentrations and removals (Fig. 1 D, Tab. 4 and 5)

Shoot Pb concentration did not significantly change after soil amendment except a decrease for the B2.5Z plants (Fig. 1 D). Shoot and SPW Pb concentrations were correlated in our soil series (Fig. 1 C). For all plants, the shoot Pb concentrations were relatively close to or in their common range (Tab. 4, Tremel-Schaub and Feix, 2005). In the B2.5 soil, the shoot Pb concentration is higher, albeit not significantly, than in the Unt soil, this trend reflecting increase in SPW Pb concentration (Fig. 1 C). The shoot Pb removal (µg Pb plant^{-1}) was only significantly lower in the B1C (i.e. 0.4) and B2.5Z (i.e. 0.3) treatments as compared to the Unt plants (i.e. 0.9).

Table. 5. Shoot element removals by the dwarf beans (µg plant^{-1})

	Unt	**B1**	**B1C**	**B1Z**	**B2.5**	**B2.5C**	**B2.5Z**
Cd	0.5±0.04a	0.3±0.05**b**	0.09±0.02**de**	0.23±0.02**c**	0.2±0.03**cd**	0.12±0.02**de**	0.08±0.07**e**
Pb	0.9±0.2ab	0.8±0.2abc	0.4±0.1bc	0.5±0.1abc	1±0.3a	0.5±0.2abc	0.3±0.2**c**
Zn	34±4a	25±3ab	13±1**c**	24±6**b**	20±3**bc**	14±2**c**	11±6**c**

Mean values ± SD per treatment (n=3). Values (in a line) with different letters differ significantly (one way ANOVA, p-value<0.05). Values in bold differed from the Unt values.

3.2.3. Shoot Cd and Zn concentrations and removals (Fig. 1 B & F, Tab. 4 and 5)

All treatments significantly decreased the shoot Cd and Zn concentrations (mg kg^{-1}) by up to 83% and 66%, (i.e. Cd: from 3.7 ± 0.08 to 0.6 ± 0.5 - Zn: from 245 ± 9 to 80 ± 43). Shoot Cd concentrations varied (mg kg^{-1}) between 3.5 ± 0.3 (Unt) and 0.6 ± 0.5 (B2.5Z) in the decreasing order: Unt > B1 > B1Z > B2.5 = B1C ≥ B2.5C = B2.5Z, this reduction being enhanced by the biochar addition rate and the combination with C or Z. For the Unt, B1, BIZ and B2.5 plants, shoot Cd concentration exceeded its common values (Tab. 4, Tremel-Schaub and Feix, 2005) and the maximum permitted concentration (MPC) in forage (0.5 - 1 mg kg^{-1}, Tremel-Schaub and Feix, 2005). For the B1C, B2.5C and B2.5Z plants, shoot Cd concentration was in its common range. Shoot Cd removal peaked for the Unt plants (i.e. 0.5 µg Cd plant^{-1}), being 2-fold and 6-fold higher as compared to the B1 and B1C plants, respectively (Tab. 5). Shoot Zn concentration (mg kg^{-1}) significantly dropped for all amended soils from 238 ± 20 (Unt) to 80 ± 43 (B2.5Z), in the decreasing order: Unt > B1 = B1Z ≥ B2.5 ≥ B2.5C = B1C = B2.5Z. Except for the B1 plants, all values were in the common range (10 – 150 mg kg^{-1}, Tremel-

Schaub and Feix, 2005). Shoot Zn removal peaked for the Unt plants (i.e. 34 µg Zn plant^{-1}) and significantly decreased in all amended soils except for the B1 plants (Tab. 5).

Decreased in SPW and shoot Cd/Zn concentrations were correlated (R^2: 0.81 and 0.71, respectively; Supplemental material 1) (Fig. 1 A & E). This confirmed previous reports on biochar-amended soils and amendment testing in the Arnoldstein soil (Friesl et al., 2006; Bolan et al., 2003; Beesley et al., 2010; Zhang et al., 2013; Kargar et al., 2015). *Miscanthus*-derived biochar (at 10%) reduced the shoot Cd, Zn and Pb concentrations of *Brassica napus* L. by -71%, -87% and -92% (Houben et al., 2013a). Bamboo and rice straw-derived biochar (1% and 5%) decreased shoot Cd, Cu, Pb and Zn concentrations of *S. plumbizincicola* in a Cd, Cu, Pb and Zn-contaminated soil nearby a Cu smelter (Lu et al., 2014). Straw-derived biochar (1.5%, 3.0% and 5.0%) added to a Cd, Pb and Zn-contaminated mining site decreased the shoot Cd, Pb and Zn concentrations of *M. aterrima* by -56%, -50% and -54%, respectively (Puga et al., 2015a,b). Authors mentioned that decreased shoot Cd and Zn concentrations may reflect metal immobilization through sorption by biochar amendment. Here, as decrease in SPW metal concentrations globally did not significantly influence the root and shoot DW yields (Fig. 3), a potential 'dilution effect' in the shoot biomass would be insignificant (Park et al., 2013).

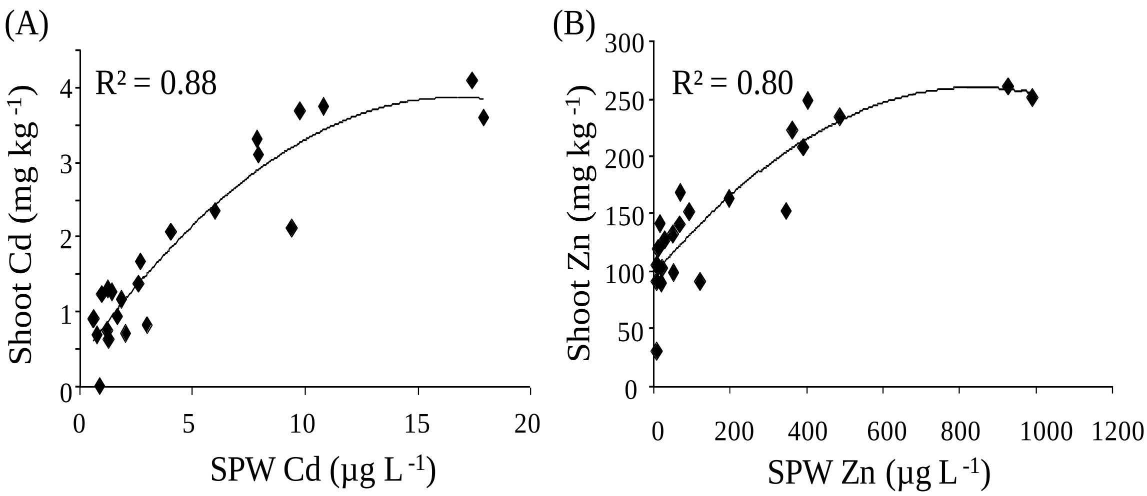


Fig. 4. Relationships between shoot (a) Cd and (b) Zn concentrations (mg kg^{-1}) and SPW (a) Ca and (b) Zn concentrations (µg L^{-1}). The trend line power and the correlation coefficient R^2 were donated by the Excel software.

3.3. Practical implications

Except for 1% biochar alone, all treatments reduced the SPW Cd, Pb and Zn concentrations to reach their low value ranges as compared to the literature. Similarly, shoot Cd, Pb and Zn concentrations decreased in all treatments, except for 1% B. Biochar addition rate of 2.5% or a combination with either compost or iron grit was necessary to stabilize such metals in this Arnoldstein soil. Likely due to the Pb affinity for DOM, the SPW Pb concentration increased in the B2.5 and B2.5C soils; accordingly shoot Pb concentration increased in the B2.5C plants as compared to the B1C. Only the B1C, B2.5C and

B2.5Z plants had shoot metal concentration in the common ranges for Cd, Pb and Zn. Influence of these three treatments to stabilize these metals in this Arnoldstein soil must be long term investigated, the biochar combination with compost being less costly albeit its lasting effect is questionable as compost OM would decay and it slightly promoted the As concentration in the soil pore water (Tab. 3). At short-term, the biomass production of dwarf bean in the uncontaminated Ctrl and Unt soils was statistically similar, albeit influence on soil biota was not determined. No tested amendment improved the root and shoot DW yield as compared to the Ctrl soil, but N fertilization may be necessary in the biochar-amended soils. After this option appraisal, plots for testing the best combination can be implemented and compare with gravel sludge, siderite and red muds, notably with non-food crops useable for the bio-economy.

4. Conclusion

Pine bark chip-derived biochar combined with either compost or iron grit efficiently stabilized the labile Cd, Pb and Zn pools in the Arnoldstein soil contaminated by atmospheric depositions. This lowered the potential metal leaching out of the root zone to proximal waters and dispersion through the environment. Decrease in Cd/Pb/Zn mobility was improved by the biochar loading rate and the combination with either compost or iron grit. This positive effect was mainly attributed to increase in soil pH, (co-)precipitation and various sorption mechanisms with the biochar surface. Consequently, dwarf bean exposure to Cd and Zn was reduced and shoot Cd, Pb and Zn concentrations were in the common ranges for plants grown in all amended soils except for 1% biochar alone. However decrease in Cd, Zn, and Pb concentrations in the soil pore water did not improve the root and shoot DW yields of dwarf bean.

5. Acknowledgement

This work was supported by ADEME (French Agency for the Environment and Energy) (PhD grant of N. Oustrière) and the French National Research Agency (ANR C2ii, program PHYTOCHEM). The UMR Biogeco is a member of the INRA Ecotoxicologist network, ECOTOX.

6. References

Ahmad, M., Rajapaksha, A.U., Lim, J.E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S.S., Ok, Y.S., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere. 99, 19–33. doi:10.1016/j.chemosphere.2013.10.071

Amonette, J.E., Jospeh, S., 2009, Characteristics of Biochar: Microchemical Properties. In: Lehmann J., Joseph S. eds.: Biochar for Environmental Management Science and Technology. Earthscan, London, 33–43.

Asami, T., 1988. Soil pollution by metals from mining and smelting activities. In W. Salomon & U. Forstner Eds.., Chemistry and biology of solid waste: Dredged material and mine tailings 144–169. Berlin, Germany: Springer-Verlag.

Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil. 337, 1–18. doi:10.1007/s11104-010-0464-5

Baize, D., 1997. Un Point sur les Teneurs Totales en Eléments Traces Métalliques dans les Sols. First ed. Paris, France: INRA Éditions.

Baize, D., 2000. Guide des analyses en pédologie. Second ed. Paris, France : INRA Éditions. 257 p.

Bakshi, S., He, Z.L., Harris, W.G., 2014. Biochar amendment affects leaching potential of copper and nutrient release behavior in contaminated sandy soils. J. Environ. Qual. 43, 1894–1902. doi:10.2134/jeq2014.05.0213

Beesley, L., Moreno-Jimenezn, E., Gomez-Eylesn, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287. doi:10.1016/j.envpol.2010.02.003

Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480. doi:10.1016/j.envpol.2010.10.016

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159, 3269–3282. doi:10.1016/j.envpol.2011.07.023

Beesley, L., Inneh, O.S., Norton, G.J., Moreno-Jimenez, E., Pardo, T., Clemente, R., Dawson, J.J.C., 2014. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. Environ. Pollut. 186, 195–202. doi:10.1016/j.envpol.2013.11.026

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilisation. Environ. Pollut. 156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bolan, N.S., Adriano, D.C., Mani, S., Khan, A.R., 2003. Adsorption, complexation and phytoavailability of copper as influenced by organic manure. Environ. Toxicol. Chem. 22, 450–456. doi:10.1002/etc.5620220228

Bolan, N.S., Adriano, D., Senesi, N., Kunhikrishnan, A., James, T., McDowell, R., 2010. Dissolved organic carbon: biogeochemistry, dynamics and agro-environmental significance in soils. Adv. Agron. 110, 1–67

Bolan, N.S., Kunhikrishnan, A., Thangarajana, R., Kumpiene, J., Parke, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metalloid.-contaminated soils – To mobilize or to immobilize? J. Hazard. Mater. 266, 141–166. doi:10.1016/j.jhazmat.2013.12.018

Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. Geochim. Cosmochim. Acta, 84, 256–268

Cao, X.D., Wahbi, A., Ma, L.N., Li, B., Yang, Y.L., 2009. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. J. Hazard. Mater. 164, 555–564. doi:10.1016/j.jhazmat.2008.08.034

Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45, 4884–4889. doi:10.1021/es103752u

Chen, J., Zhu, D., Sun, C., 2007. Effect of heavy metals on the sorption of hydrophobic organic compounds to wood charcoal. Environ. Sci. Technol. 41, 2536–3541. doi:10.1021/es062113+

Di Bonito, M., 2005. Trace elements in soil pore water: A comparison of sampling methods, University of Nottingham, United Kingdom.

Ding, Y., Liu, Y.X., Wu, W.X., Shi, D.Z., Yang, M., Zhong, Z.K., 2010. Evaluation of biochar effects on nitrogen retention and leaching in multilayered soil columns. Water Air Soil Pollut. 213, 47–55. doi:10.1007/ s11270-010-0366-4

Friesl, W., Horak, O., Wenzel, W.W., 2004. Immobilization of heavy metals in soils by the application of bauxite residues: pot experiments under field conditions. J. Plant. Nutr. Soil Sci. 167, 54–59.

Friesl, W., Friedl, J., Platzer, K., Horak, O., Gerzabek, M.H., 2006. Remediation of contaminated soils in the vicinity of a former Pb/Zn smelter in Austria: batch, pot, and field experiments. Environ. Pollut. 1441, 40–50. doi:10.1016/j.envpol.2006.01.012

Friesl-Hanl, W., Platzer, K., Horak, O., Gerzabek, M.H., 2009. Immobilising of Cd, Pb, and Zn contaminated arable soils close to a former Pb/Zn smelter: a field study in Austria over 5 years. Environ. Geochem. Health. 31, 581–594. doi 10.1007/s10653-009-9256-3

Hale, B., Evans, L., Lambert, R., 2012. Effects of cement or lime on Cd, Co, Cu, Ni, Pb, Sb and Zn mobility in field-contaminated and aged soils. J. Hazard. Mater. 199–200, 119–127. doi:10.1016/j.jhazmat.2011.10.065

Houben, D., Evrard, L., Sonnet, P., 2013a. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere. 92, 1450–1457. doi:10.1016/j.chemosphere.2013.03.055

Houben, D., Evrard, L., Sonnet, P., 2013b. Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed Brassica napus L., Biomass. Bioenerg. 57, 196–204. doi:10.1016/j.biombioe.2013.07.019

Houben, D., Sonnet, P., 2015. Impact of biochar and root-induced changes on metal dynamics in the rhizosphere of Agrostis capillaris and Lupinus albus. Chemosphere. 139, 644–651. doi:10.1016/j.chemosphere.2014.12.036

Jakobsen, S.T., 1993. Interaction between plant nutrients III. Antagonism between potassium, magnesium and calcium. Acta Agriculturae Scandinavica Section B: Soil and Plant Science 43, 1–5. doi:10.1080/09064719309410223

Karami, N., Clemente, R., Moreno-Jimenez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J. Hazard. Mater. 191, 41–8. doi:10.1016/j.jhazmat.2011.04.025

Karer, J., Wawra, A., Zehetner, F., Dunst, G., Wagner, M., Pavel, P.B., Puschenreiter, M., Friesl-Hanl, W., Soja, G. 2015. Effects of biochars and compost mixtures and inorganic additives on immobilisation of heavy metals in contaminated soils. Water. Air. Soil. Pollut. 226, 342. doi:10.1007/s11270-015-2584-2

Kargar, M., Clark, O.G., Hendershot, W.H., Jutras, P., Prasher, S.O., 2015. Immobilization of trace metals in contaminated urban soil amended with compost and biochar. Water Air Soil Pollut. 226, 191. doi: 10.1007/s11270-015-2450-2

Kasperowski, E., 1993. Schwermetalle in Böden im Raum Arnoldstein. Monographien Bd. 33, Umweltbundesamt UBA, Wien, Austria.

Kidd, P., Mench, M., Álvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J., Puschenreiter, M., 2015. Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int. J. Phytorem. 17, 1005–1037. doi:10.1080/15226514.2014.1003788

Komárek, M., Vanek, A., Ettler, V., 2013. Chemical stabilization of metals and arsenic in contaminated soils using oxides. A review. Environ. Pollut. 172, 9–22. doi:10.1016/j.envpol.2012.07.045

Kookana, R.S., Sarmah, A.K., Van Zwieten, L., Krull, E., Singh, B., 2011. Chapter three—Biochar application to soil: agronomic and environmental benefits and unintended consequences. Adv Agron. 112, 103–143

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste. Manage. 28, 215–225. doi:10.1016/j.wasman.2006.12.012

Kumpiene, J., Mench, M., Bes, C., Fitts, J.P., 2011. Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environ. Pollut. 159, 1536–1542. doi:10.1016/j.envpol.2011.03.005

Lehmann, J., Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil. 249, 343–357

Lehmann, J., 2007. A handful of carbon. Nature. 447, 143–144. doi:10.1038/447143a

Lehmann, J., 2009. Biochar for environmental management: science and technology. Earthscan, London and Sterling, VA USA.

Lu, K., Yang, X., Shen, J., Robinson, B., Huang, H., Liu, D., Bolan, N., Pei, J., Wang, H., 2014. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to Sedum plumbizincicola. Agric. Ecosyst. Environ. 191, 124–132. doi:10.1016/j.agee.2014.04.010

Marchand, L., Nsanganwimana, F., Cook, B., Vystavna, Y., Huneau, F., Lecoustumer, P., Lamy, J.B., Oustrière, N., Mench, M., 2014b. Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors. Ecological Indicators. 46, 425–437. doi:10.1016/j.ecolind.2014.07.011

Melo, L.C.A., Puga, A.P., Coscione, A.R., Beesley, L., Abreu, C.A., Camargo, O.A., 2016. Sorption and desorption of cadmium and zinc in two tropical soils amended with sugarcane-straw-derived biochar. J. Soils Sediments. 16, 226–234. doi:10.1007/s11368-015-1199-y

Mench, M., Lepp, N., Bert, V., Schwitzguébel, J.P., Gawronski, S.W., Schröder, P., Vangronsveld, J., 2010. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J. Soils Sediments. 16, 876–900. doi:10.1007/s11368-010-0190-x

Méndez, A., Gómez, A., Paz-Ferreiro, J., Gascó, G., 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere. 89, 1354–1359. doi:10.1016/j.chemosphere.2012.05.092

Mohamed, I., Zhang, G., Li, Z., Liu, Y., Chen, F., Dai, K., 2015. Ecological restoration of an acidic Cd contaminated soil using bamboo biochar application. Ecol. Eng. 84, 67–76. doi:10.1016/j.ecoleng.2015.07.009

Moreno-Jiménez, E., Beesley, L., Lepp, N.W., Dickinson, N.M., Hartley, W., Clemente, R., 2011. Field sampling of soil pore water to evaluate trace element mobility and associated environmental risk, Environ. Pollut. 159, 3078–3085. doi: 10.1016/j.envpol.2011.04.004

Namgay, T., Singh, B., Singh, B.P., 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize Zea mays L. Aust. J. Soil Res. 48, 638–647. doi:10.1071/SR10049

Oustriere, N., Marchand, L., Bouchardon, J.L., Faure, O., Moutte, J., Mench, M., 2016a. Aided phytostabilization of a trace element-contaminated technosol developed on steel mill wastes. J. Hazard. Mater. 320, 458–468. doi:10.1016/j.jhazmat.2016.08.048

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi, T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil. 348, 439–451. doi:10.1007/s11104-011-0948-y

Park, J.H., Choppala, G., Lee, S.J., Bolan, N., Chung, J.W., Edraki, M., 2013. Comparative sorption of Pb and Cd by biochars and its implication for metal immobilization in soils. Water Air Soil Pollut. 224, 1711. doi:10.1007/s11270-013-1711-1

Puga, A.P., Abreu, C.A., Melo, L.C.A., Beesley, L., 2015a. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. J. Environ. Manage. 159, 86–93. doi:10.1016/j.jenvman.2015.05.036

Puga, A.P., Abreu, C.A., Melo, L.C.A., Paz-Ferreiro, J., Beesley, L., 2015b. Cadmium, lead, and zinc mobility and plant uptake in a mine soil amended with sugarcane straw. Environ. Sci. Pollut. Res. 22, 17606–17614. doi:10.1007/s11356-015-4977-6

Rabitsch, W.B., 1995. Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. I. Environ. Pollut. 90, 221–237.

Riedel, T., Hennessy, P., Iden, S.C., Koschinsky, A., 2015. Leaching of soil-derived major and trace elements in an arable topsoil after the addition of biochar. Eur. J. Soil Sci. 66, 823–834 doi:10.1111/ejss.12256

Rizwan, M., Ali, S., Farooq Qayyum, M., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., Ok, Y.S., 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environ. Sci. Pollut. Res. 23, 2230–2248. doi:10.1007/s11356-015-5697-7

Rodríguez-Vila, A., Asensio, V., Forján, R., Covelo, E.F., 2015. Chemical fractionation of Cu, Ni, Pb and Zn in a mine soil amended with compost and biochar and vegetated with Brassica juncea L. J. Geochem. Explor. 158, 74–81. doi:10.1016/j.gexplo.2015.07.005

Ruttens, A., Mench, M., Colpaert, J.V., Boisson, J., Carleer, R., Vangronsveld, J., 2006. Phytostabilization of a metal contaminated sandy soil. I: Influence of compost and/or inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of metals. Environ. Pollut. 144, 524–532. doi:10.1016/j.envpol.2006.01.021

Schulz, H., Dunst, G., Glaser, B., 2013. Positive effects of composted biochar on plant growth and soil fertility. Agron. Sust. Dev. 33, 817–827. doi:10.1007/s13593-013-0150-0

Sizmur, T., Wingate, J., Hutchings, T., Hodson, M.E., 2011. Lumbricus terrestris L. does not impact on the remediation efficiency of compost and biochar amendments. Pedobiologia. 54 Suppl., S211 S216. doi:10.1016/j.pedobi.2011.08.008

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Adv. Agron. 105, 47–82. doi:10.1016/S0065-211310.05002-9

Tiberg, C., Kumpiene, J., Gustafsson, J.P., Marsz, A., Persson, I., Mench, M., Kleja, D.B., 2016. Immobilization of Cu and As in two contaminated soils with zerovalent iron – Long-term performance and mechanisms. Appl. Geochem. 67, 144–152. doi:10.1016/j.apgeochem.2016.02.009

Tica, D., Udovic, M., Lestan, D., 2011. Immobilization of potentially toxic metals using different soil amendments. Chemosphere. 85, 577–583.

Touceda-González, M., Brader, G., Antonielli, L., Balakrishnan Ravindran, V., Waldner, G., Friesl-Hanl, W., Corretto, E., Campisano, A., Pancher, M., Sessitsch, A., 2015.. Combined amendment of immobilizers and the plant growth-promoting strain Burkholderia phytofirmans PsJN favours plant growth and reduces heavy metal uptake. Soil Biol. Biochem. 91, 140–150. doi:10.1016/j.soilbio.2015.08.038

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers, and EDP Sciences, Les Ulis.

Uchimiya, M., Lima, I.M., Klasson, K.T., Wartelle, L.H., 2010a. Contaminant immobilization and release by biochar soil amendment: Roles of natural organic matter. Chemosphere. 80, 935–940. doi:10.1016/j.chemosphere.2010.05.020

Uchimiya, M., Lima, I.M., Klasson, K.T., Chang, S., Wartelle, L.H., Rodgers, J.E., 2010b. Immobilization of heavy metal ions CuII, CdII, NiII, and PbII. by broiler litter-derived biochars in water and soil. J. Agric. Food Chem. 58, 5538–5544. doi:10.1021/jf9044217

Wagner, A., Kaupenjhann, M., 2014. Suitability of biochars pyro- and hydrochars. for metal immobilization on former sewage-field soils. Eur. J. Soil Sci. 65, 139–148. doi:10.1111/ejss.12090

Wagner, A., Kaupenjhann, M., 2015. Biochar addition enhanced growth of *Dactylis glomerata* L. and immobilized Zn and Cd but mobilized Cu and Pb on a former sewage field soil. Eur. J. Soil Sci. 66, 505–515. doi:10.1111/ejss.12246

Xu, X., Cai, X., Zhao, L., Wang, H., Yu, H., Gao, B., 2013. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. Environ. Sci. Pollut Res. 201, 358–368. doi:10.1007/s11356-012-0873-5

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei, J., Huang, H., 2013. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ. Sci. Pollut. Res. 20, 8472–8483. doi:10.1007/s11356-013-1659-0

Part. 2. Biomass production with (essential) metal(loid) concentrations within the common ranges usable by local biomass processing chains.

2.2. Option appraisal

2.2.1. Chapter 3 - Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site

Published in Science of the Total Environment, in 2017

Doi: 10.1016/j.scitotenv.2016.11.048

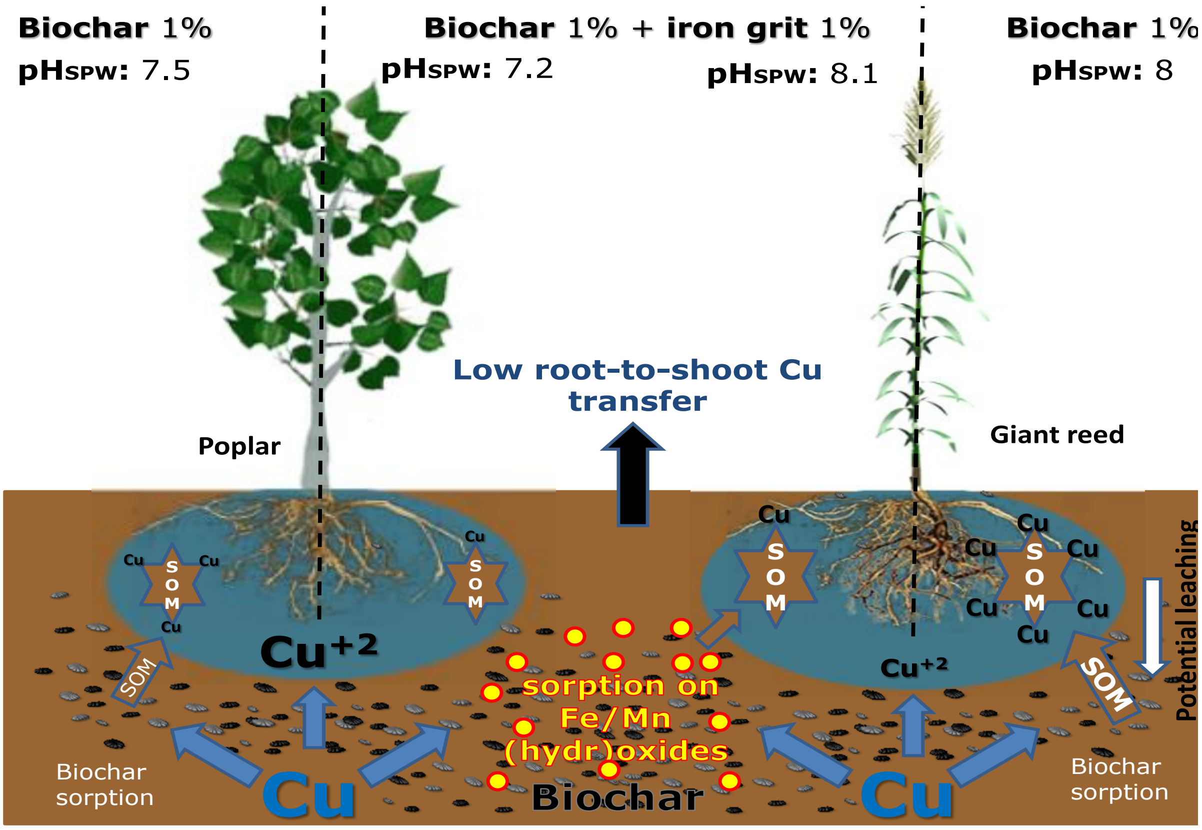


Fig. C. Graphical abstract – Chapter 3

Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site

Nadège Oustriere[1], Lilian Marchand[1], Nathalie Lottier[2], Mikael Motelica[2], Michel Mench[1]

[1]BIOGECO, INRA, UNIV. BORDEAUX, 33615 PESSAC, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; mench@bordeaux.inra.fr;

[2]ISTO UMR 7327-CNRS, UNIVERSITY OF ORLÉANS, CAMPUS GÉOSCIENCES, 1A, RUE DE LA FEROLLERIE, 45071 ORLÉANS CEDEX 2, FRANCE.

E-mails: nathalie.lottier@univ-orleans.fr; mikael.motelica@univ-orleans.fr

Abstract

A 2-year pot experiment was carried out to examine the aging effect of biochar (B), alone or combined with iron grit (Z), on Cu stabilization and plant growth in a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site. The experiment consisted in 3 soil treatments, either planted with Arundo donax L. (Ad) or Populus nigra L. (Pn): (1) untreated Cu-contaminated soil (Ad, Pn); (2) Unt + 1% (w/w) B (AdB, PnB), and (3) Unt + 1% B + 1% Z (AdBZ, PnBZ). After 22 months, the soil pore water (SPW) was sampled and roots and shoots were harvested. The SPW compositions at 3 and 22 months were compared, showing that the SPW Cu^{2+} concentration increased again in the PnB and PnBZ soils. Cultivation of A. donax enhanced the dissolved organic matter concentration in the SPW, which decreased its Cu^{2+} concentration but promoted its total Cu concentration in the Ad and AdB soils. Adding Z with B reduced both SPW Cu^{2+} and Cu concentrations in the pots cultivated by A. donax and P. nigra as compared to B alone. The B and BZ treatments did not enhance root and shoot yields of both plant species as compared to the Unt soil but their shoot Cu concentrations were in the range of common values.

Keywords: *Arundo donax* L., *Populus nigra* L., Soil pore water, Copper contamination, In situ stabilization, Phytomanagement

1. Introduction

Soil Cu contamination at wood preservation sites frequently resulted from long-term use of Cu-based salts as wood preservatives and wood washings (Bes and Mench, 2008; Oustriere et al., 2016a). The functioning of ecosystems associated with such Cu-contaminated soils is generally affected, due to soil ecotoxicity and Cu dispersion through natural agencies (Bakshi et al., 2014).

Out of gentle options for remediating Cu-contaminated soils, *in situ* stabilization is an alternative to physico-chemical methods. It aims at reducing the pollutant linkages by managing the contaminant pathways to protect the biological receptors (Cundy et al., 2015). It relies on incorporation of amendments into the soil to immobilize metal(loid)s in the stable solid phase by sorption, precipitation, complexation, ion exchange or redox process, thereby decreasing their mobility and bioavailability (Kumpiene et al., 2008; Bolan et al., 2014). Amending soils with biochar can (1) increase the cation exchange capacity (CEC), pH, and retention of water and nutrients, (2) improve microbial soil habitats and (3) immobilize contaminants (Zhang et al., 2013). Several studies have assessed the biochar effectiveness for in situ stabilization of Cu-contaminated soils (Beesley and Marmiroli, 2011; Beesley et al., 2011). As suggested by Beesley and Marmiroli (2011), combining amendments with biochar may be more efficient than biochar alone for the remediation and revegetation of contaminated soils. Iron grit mainly consists of zerovalent Fe(0) and Mn and rapidly corrodes into the soil to form newly Fe/Mn oxi(hydro)xides. Such (hydro)xides can sorb metal(loid)s and reduce their (bio)availability (Kumpiene et al., 2011; Tiberg et al., 2016). In a previous pot experiment, a pine bark-derived biochar (1% w/w) decreased Cu^{2+} concentration in the soil pore water (SPW) of a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site (Oustriere et al., 2016a). Adding this biochar with iron grit (1% w/w) decreased both total Cu and Cu^{2+} concentrations in the SPW. However biochar alone and in combination did not promote the growth of dwarf bean (*Phaseolus vulgaris* L.). Moreover this study was limited to a 3-month reaction period followed by a 2-week plant testing. Potential changes in the phytotoxicity of biochar-amended soils over time must be considered. Long-term efficiency of soil amendments is pivotal to sustain metal(loid) immobilization in contaminated soils and to avoid their leaching out of the root zone.

Few long-term studies reported an increased immobilization of trace elements (TE) over time in biochar-amended soils (Cd, Pb: Bian et al., 2014; Ni, Zn: Shen et al., 2016). Such long-term effects on TE immobilization however depend on biochar and soil types. After 3 years, hardwood-derived biochar gradually reduced the ($CaCl_2$)-extractable Cd and Cu concentrations and uptake by rice, while these values did not change in the soil amended by corn-straw-derived biochar (Li et al., 2016a). Eucalyptus wood-derived-biochar aged for 12 months into two (Cd/As)-spiked soils increased Cd and As sorption in the Inceptisol whereas it decreased As sorption in the Oxisol (Nagodavithane et al., 2014). The sorption capacity of aged biochar may depend on (1) metal(loid)s, (2) SPW pH, and (3) soil properties such as organic matter (OM) content and oxidation process through both abiotic and biotic mechanisms (Nagodavithane et al., 2014).

Among energy crops, poplar (*Populus* sp.,) and Giant reed (*Arundo donax* L.) used in short rotation coppices (SRC) have been tested for producing valuable feedstock for bioenergy productions on contaminated soils (Lucas-Borja et al., 2011; Evangelou et al., 2012; Kidd et al., 2015; Mola-Yudego et al., 2015; Gonsalvesh et al., 2016). The lignocellulosic biomass of such plants can be either used to produce heat and electricity by direct combustion, transformed by pyrolysis and gasification into biofuels and biochars or used for producing derived bioproducts notably platform chemicals (Bridgwater, 2006; Nsanganwimana et al., 2014a). Poplar is a fast growing tree, producing large yields, tolerant to high TE exposures, with low contaminant accumulation in the wood (Cd/Zn are mainly accumulated in the leaves) and having high energy potential (Calfapietra et al., 2010). *Arundo donax* is a productive species, highly stress resilient (e.g. cold, drought, salinity, and extreme pH) (Mantineo et al., 2009), tolerant to high TE exposure (Elhawat et al., 2015), usable by many conversion chains, e.g. energy sector, litter, pulp and building materials (Scordia et al., 2012; Nsanganwimana et al., 2014a).

In situ stabilization combined with either *Populus nigra* L. or *A. donax* cultivation is a potential option to produce a valuable biomass while stabilizing Cu-contaminated soils at wood preservation sites. Therefore, we investigated the long-term aging effect of biochar, alone and combined with iron grit, in such Cu-contaminated soil cultivated with both plant species through (1) Cu and nutrient concentrations in the soil pore water, (2) biomass production of *P. nigra* and *A. donax*, and (3) shoot Cu concentrations and removals.

2. Material and Methods

2.1. Soil, amendments and soil treatments

The wood preservation site (about 10 ha, Saint-Médard d'Eyrans, Gironde, SW France; 44°43.353′ N, 000°30.938′ W) has been used for over a century, with various Cu-based salts (mainly Cu-sulphate based on the duration and amount used) as wood preservatives (Mench and Bes, 2009). Topsoil (Unt, 0-25cm, Fluvisol - Eutric Gleysol, World Reference Base for soil resources) was collected (100 kg) at the P1-3 sub-site. This sandy soil (85.8 % sand, 5.9 % clay, and 8.3 % silt; 1.3% OM; C/N 16), with a low CEC, is mainly contaminated by Cu (Tab. 1, Oustriere et al., 2016a), which largely exceeds its median and upper whisker background values in French sandy soils, but its total soil As and Cr are at background levels (Tab. 1, Baize, 2000; Villanneau et al., 2008). Soil was air-dried and homogenized after sieving through a 5 mm mesh.

Table. 1. Main physico-chemical soil parameters

Parameter	Mean ± SD	Background levels [A]
pH	7 ± 0.09	6.60
CEC	2.5 ± 0.2	
P_2O_5 (g kg^{-1})	0.03 ± 0.002	
Organic matter (g kg^{-1})	13 ± 0.4	
Organic C (g kg^{-1})	8 ± 0.3	14.5
Total N (g kg^{-1})	0.5 ± 0.01	
C/N	16 ± 0.2	10.02
Texture (g kg^{-1})		
Sand	858	≥ 650
Silt	83	≤ 350
Clay	59	≤ 180
Total TE (mg kg^{-1})		
Cr	21 ± 0.7	14–40
Cu	964 ± 20	3.2–8.4
Ni	5 ± 0.3	4.2–14.5
Zn	37 ± 1.6	17–48
As	7 ± 0.4	1–25 [B]

[A]. Frequent total concentrations in French sandy topsoils (Baize, 2000; Villanneau et al., 2008).
[B]. Frequent total As concentrations for all French soil types (Bes and Mench, 2008).

Two treatments, a pine bark-derived biochar (B, pyrolysis of 420°C for 180 s; not desalted; Florentaise, Saint-Mars-du-Désert, France) alone and combined with zerovalent iron grit (BZ), were trialed. Biochar was crushed, sieved at 2 mm and manually homogenized. Elemental composition, carbon (C) content and polycyclic aromatic hydrocarbon (PAH) concentrations were determined at the INRA Laboratoire d'Analyses des Sols (LAS, Arras, France) with standard methods (INRA LAS, 2011) (Table 2). Zerovalent iron grit (Z, GH120, particle size <0.1 mm) was obtained from Wheelabrator Allevard, France (Bes and Mench, 2008). Amended soils were thoroughly homogenized in large plastic containers and individually prepared prior to use.

The pot experiment consisted in 3 soil treatments: (1) untreated Cu-contaminated soil (Unt); (%, w/w) (2) Unt + 1% B (B), (3) Unt + 1% B + 1% Z (BZ). Each treatment was made in 10 replicates. Soils (3 kg) were potted (5L, plastic pots) and placed in a greenhouse. Potted soils, with a bottom cup to avoid any leaching, were watered and weekly maintained to 70% of water holding capacity (WHC, 10% of air-dried soil) with deionized water, and allowed to react for one month in May 2014. Thereafter, plant were cultivated from June 2014 to Mars 2016.

2.2. Plant testing

Stem cuttings of *P. nigra* and Giant reed (*A. donax*) (roughly 20 cm long) were collected in May 2013. Poplars were sampled on 4-year old trees growing at this wood preservation site. Giant reed plants were sampled from natural stand along a drainage ditch, San Remo, Italy, and cultivated since 2012 in a greenhouse. Stem cuttings were rooted in individual pots (9*8*9 cm^3) on perlite imbibed with a quarter-strength Hoagland nutrient solution (HNS, Marchand et al., 2014) for one year in a greenhouse. For each treatment, one standardized plant of either *P. nigra* or *A. donax* was transplanted in potted soils (five replicates) and cultivated during 22 months from June 2014 to March 2016 in a greenhouse. The experiment consisted in 6 treatments:

(1) untreated Cu-contaminated soil planted with P. nigra (Pn),
(2) B-amended soil planted with P. nigra (PnB),
(3) BZ-amended soil planted with P. nigra (PnBZ),
(4) untreated Cu-contaminated soil planted with A. donax (Ad),
(5) B-amended soil planted with A. donax (AdB),
(6) BZ-amended soil planted with A. donax (AdBZ).

Pots were arranged in a fully randomized block and maintained at 70% of WHC using deionized water without loss from drainage. Hoagland nutrient solution (250 mL) was applied each month in all pots to avoid nutrient deficiencies. Before the beginning of the 2015 growing season, in March, dry shoots of *A. donax* were harvested (Cut 1), 1 cm above the soil surface. In March 2016, the shoots and roots of

P. nigra (Cut 1) and *A. donax* (Cut 2) were harvested. All harvested biomass were washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields.

Table. 2. Composition of soil amendments. In bold, values exceeding the European Biochar Certificate V4.8 thresholds

	B	Z	EBC [A]	IBI [B]	French upper critical thresholds for organic amendments [C]
pH	9.89		-	-	
CEC (cmol kg^{-1})	1.23		-	-	
Major elements (%DW)					
H	0.8		-	-	
N	0.3		-	-	
S	0.03		-	-	
Cl	0.01		-	-	
C	90.3		>50 %	Class 1: ≥60% Class 2: [30% - 60%] Class 3: [10% - 30]	
Nutrients (g kg^{-1})					
Ca	18.6		-	-	
K	13.8		-	-	
Mg	29.9		-	-	
Na	0.389		-	-	
P	0.105		-	-	
Elements (mg kg^{-1})					
Al	1650	600	-	-	
As	0.461	70	-	12-100	18
Cd	<0.5	0.03	1	1.4-39	3
Cr	45.9	3 500	80	64-1200	300
Cu	230	1 010	100	63-1500	120
Mn	-	7 710	-	-	
Fe	4470	973 000	-	-	
Hg	<0.1		1	1-17	2
Ni	28.3	739	30	47-600	60
Pb	2.61	20	120	70-500	180
Zn	50.5	104	400	200-7000	600
PAHs (mg kg^{-1})					
Sum of 16 US EPA PAHs [D]	163		4	6-300	
Fluoranthene	7.48		-	-	4
Benzo(b)fluoranthene	0.149		-	-	2.5
Benzo(a)pyrene	< 0.2		-	-	1.5
Naphtalene	101		-	-	

[A.] Following Switzerland's Chemical Risk Reduction Act (ChemRRV) on recycling fertilizers
[B.] Range of Maximum Allowed Thresholds reflects different soil tolerance levels for these elements in compost, biosolids, or soils established by regulatory bodies in the US, Canada, EU and Australia (See Appendix 3 of the IBI Biochar Standards for further information).
[C.] French upper critical thresholds for organic amendments (NF U 44 051, Dec. 2010).
[D.] Corresponds to the PAHs threshold defined in the Swiss Chemical Risk Reduction Act (Chem RRV).

2.3. Soil pore water and plant analysis

Dried shoots were ground (< 1.0 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW pot^{-1}) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3 and 2mL 30% (v/v) H_2O_2 not stabilized by phosphates. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) in digests was determined by ICP-MS (Thermo X series 200) at the INRA USRAVE laboratory, Villenave-d'Ornon, France. All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. Shoot Cu removal was calculated as: Cu (µg $plant^{-1}$) = shoot DW yield (g $plant^{-1}$) x shoot Cu concentration (µg g^{-1} DW).

23 months after the soil amendment, the soil pore water (SPW) was collected in all pots just before the harvest of *P. nigra* and *A. donax* (March 2016, three times 10 mL) using Rhizon MOM moister samplers (Eijkelkamp Agrisearch Equipment, The Netherlands) placed in January 2016 and samples kept at 4°C prior to their analysis. The pH, electrical conductivity (EC), and Cu^{2+} concentration in the SPW samples were determined using electrodes (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34, Tetracon 325 WTW, and Cupric ion electrode, Fischer Bioblock, USA), respectively. Aluminum, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn were analyzed by ICP-OES (Varian Liberty 200). Dissolved organic carbon (DOC) in SPW samples was analyzed with a Shimadzu© TOC 5000A carbon analyzer. The measurement accuracy was checked by performing calibration with a standard reference solution of potassium hydrogen phthalate (KHP) at a concentration of 1000 mg C L^{-1}. Four DOC measures were performed for each SPW solution. The SPW composition was compared to previous values reported by Oustriere et al., (2016a) for the same unplanted soil treatments 3 months after soil amendment.

2.4. Statistical analysis

Influence of soil treatments on SPW parameters, shoot DW yields, shoot ionome and element removals were tested using one-way analysis of variance (ANOVAs). Shoot DW yields and shoot ionome of both plants were tested separately. Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests. Differences were considered statistically significant at $p<0.05$. When element concentrations were below the detection limits in the Unt samples, influence of soil treatments were not statistically tested. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results

3.1. Soil pore water

3.1.1. pH, EC and nutrient concentrations (Table 3)

The Unt soil has a neutral SPW pH (Table 3). At month 3, biochar alone (B) and combined with iron grit (BZ) significantly increased the SPW pH (i.e. 7.3 ± 0.08 and 7.6 ± 0.05, respectively) and decreased the SPW EC (µS cm^{-1}) albeit not significantly (i.e. 483 ± 51 and 477 ± 110 respectively). At month 22 with *A. donax*, the SPW pH and EC rose significantly in all soil treatments from 6.9 ± 0.1 (Unt) to 8.1 ± 0.2 (*Ad*BZ) for the pH and from 1024 ± 117 (Unt) to 2037 ± 284 (*Ad*BZ) for the EC. In the *Pn* and *Pn*BZ treatments, the SPW pH did not differ from that of the Unt soil whereas it remained higher in the *Pn*B soil (i.e. 7.5 ± 0.06). The SPW EC increased in the *Pn*B soil (Month 22) as compared to the B soil (Month 3). The SPW Ca concentration decreased in the B and BZ soils at month 3 and in all planted soils at month 22 as compared with the Unt soil. The SPW Fe and P concentrations remained below their detection limit, except for P in the B soil. At month 3, the SPW K concentration increased in the B and BZ soils but it decreased at month 22 for both plant species in all soils, and the SPW Mg concentration as well. The SPW Na concentration was significantly lower in the soils planted with *A. donax* as compared with the Unt soil.

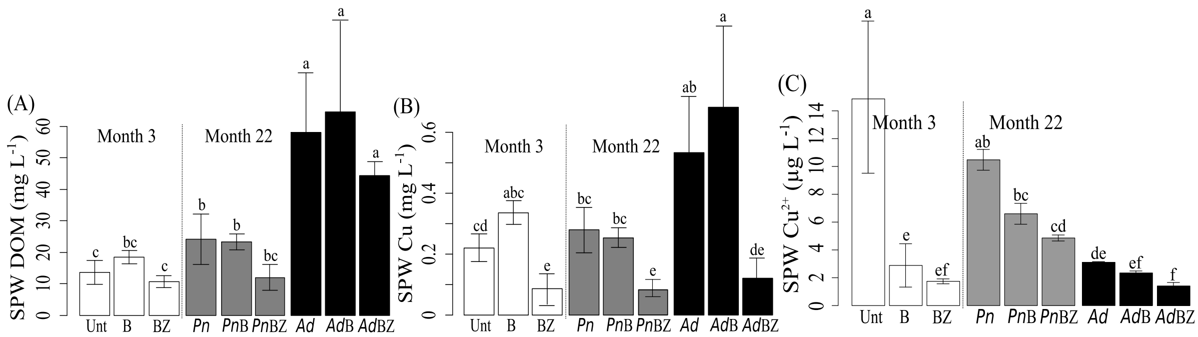


Fig. 1. (A) DOM, (B) total Cu and (C) Cu^{2+} concentrations in the soil pore water; Cu contaminated soil (Unt, white), amended with either biochar (B) or biochar plus iron grit (BZ) in the Cu contaminated soil cultivated with *Populus nigra* (Pn, grey) or *Arundo donax* (Ad, black), alone and amended with either biochar (PnB and AdB) or biochar plus iron grit (PnBZ and AdBZ). Mean values per treatment (n = 5, Unt: n = 6). Values with different letters differ significantly (one way ANOVA, p-value). <0.05).

Table. 3. Comparison of physico-chemical parameters of soil pore waters, 3 and 22 months after soil amendment

Treatments			(µS cm^{-1})	Nutrients (mg L^{-1})					
		pH	EC	Ca	Fe	K	Mg	Na	P
SPW: Month 3									
Unt	(n=6)	6.9 ± 0.1 f	1024 ± 117 cd	143 ± 39 a	< 0.02	14 ± 5 b	5 ± 2 a	12 ± 3 ab	< 0.2
B	(n=3)	7.3 ± 0.08 def	483 ± 51 d	59 ± 5 **b**	< 0.02	43 ± 11 **a**	3 ± 0.3 a	13 ± 2 a	0.3 ± 0.05
BZ	(n=3)	7.6 ± 0.05 **bcd**	477 ± 110 d	46 ± 20 **b**	< 0.02	53 ± 16 **a**	3 ± 1 ab	17± 6 a	< 0.2
SPW: Month 22									
Pn	(n=5)	7.1 ± 0.1 ef	890 ± 171 cd	2.5 ± 0.5 **c**	< 0.02	4.1 ± 0.5 **c**	1.1 ± 0.2 **c**	9.7 ± 1.9 abc	< 0.2
PnB	(n=5)	7.5 ± 0.06 **cde**	1299 ± 118 bc	3.4 ± 0.3 **c**	< 0.02	6.3 ± 0.8 bc	1.4 ± 0.1 **bc**	12.9 ± 1.1 a	< 0.2
*Pn*BZ	(n=5)	7.2 ± 0.1 def	755 ± 231 cd	2.1 ± 0.6 **c**	< 0.02	4 ± 1.1 **c**	1.1 ± 0.2 **c**	5.7 ± 2.8 bcd	< 0.2
Ad	(n=5)	7.9 ± 0.2 **abc**	1737 ± 300 **ab**	< 0.5	< 0.02	1 ± 0.3 **d**	0.8 ± 0.4 **c**	< 0.5	< 0.2
AdB	(n=5)	8.0 ± 0.3 **ab**	1819 ± 529 **ab**	1.1 ± 0.5 **c**	< 0.02	1 ± 0.5 **de**	1.3 ± 0.2 **bc**	3.8 ± 3.9 **cd**	< 0.2
*Ad*BZ	(n=5)	8.1 ± 0.2 **a**	2037 ± 284 **a**	0.8 ± 1.1 **c**	< 0.02	0.5 ± 0.3 **e**	1.1 ± 0.6 **c**	1 ± 1.5 **d**	< 0.2
Common values in sandy soil (Oustriere et al., 2016)		-	-	143 ± 66	0.05 ± 0.01	27 ± 44	21 ± 11	63 ± 12	5 ± 2

SPW: soil pore water; Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value b 0.05). Mean values followed by letters in bold are significantly different as compared to the Unt soil.

3.1.2. DOM, Cu and Cu^{2+} (Fig. 1. A, B and C)

The DOM concentration in the SPW peaked in all soils cultivated with *A. donax,* being 3 - 5 fold higher than in the Unt soil (Fig. 1. A, B and C). In the *Pn* and *Pn*B soils, the DOM increased significantly, i.e. (mg L^{-1}) 24 ± 7 (*Pn*) and 23 ± 2 (*Pn*B) relative to the Unt soil (13 ± 4). Total Cu concentration in the SPW (mg L^{-1}) peaked in the *Ad* and *Ad*B soils (i.e. 0.53 ± 0.18 and 0.68 ± 0.26, respectively) as compared to the Unt soil (i.e. 0.22 ± 0.05). Conversely, adding Z into the Unt soil significantly decreased the SPW total Cu concentration only in the *Ad*BZ soil relative to *Ad* and *Ad*B. All treatments, except the *Pn* soil, significantly decreased the SPW Cu^{2+} concentration as compared to the Unt soil (i.e. 14.9 ± 5.3).

3.2. Plants

3.2.1. Plant growth parameters (Fig. 2 A and B)

At month 22, the root and shoot DW yields of *P. nigra* and *A. donax* for the B-and BZ-amended soils did not differ from those for the Unt soil (Fig. 2 A and B). The root DW yields (mg DW $plant^{-1}$) ranged from 17 ± 11 to 29 ± 9 for *P. nigra* and from 57 ± 12 to 84 ± 14 for *A. donax* whereas the shoot DW yields (mg DW $plant^{-1}$) range from 19 ± 7 to 25 ± 11 for *P. nigra* and from 22 ± 4 to 26 ± 3 for *A. donax.* For *A. donax*, the shoot DW yield produced at month 10 (Cut 1) was lower than that harvested at month 22 (Cut 2).

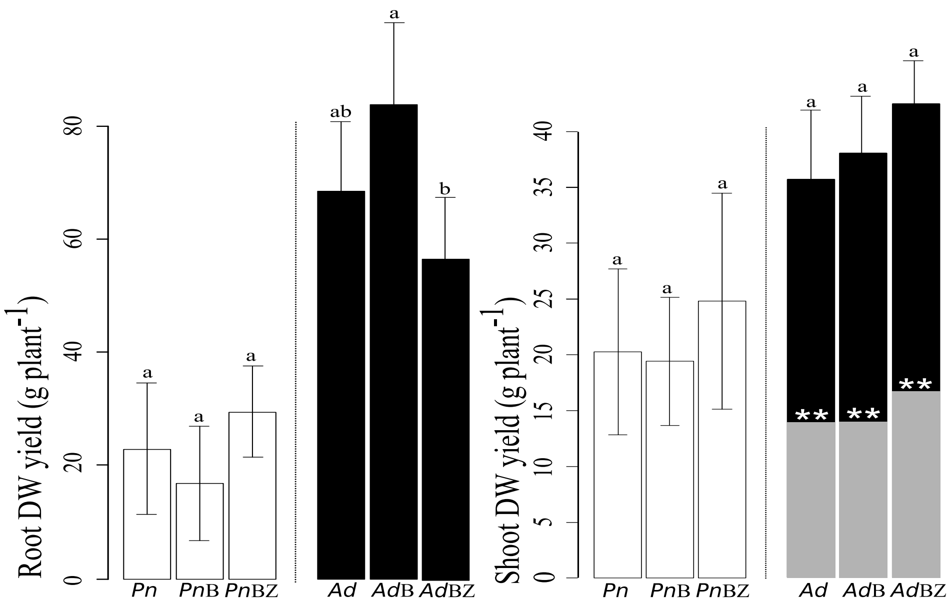


Fig. 2. (A) Root and (B) shoot DW yields of *P. nigra* (*Pn*, white, month 22) and *A. donax* (*Ad*, grey, and black) (mg DW $plant^{-1}$) at month 22 in the Cu-contaminated soil (Unt) and in the soil amended with either biochar (*Pn*B and *Ad*B) or biochar plus iron grit (*Pn*BZ and *Ad*BZ). The grey bare correspond to the biomass at month 10 (Cut 1). Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

Table. 4. Shoot ionome and shoot Cu removal of *P. nigra* and *A. donax* at month 22 (n = 5)

Treatment	Cu		Shoot nutrient concentrations ($g\ kg^{-1}$)						Shoot metal concentrations ($mg\ kg^{-1}$)	
	Shoot Cu concentration ($mg\ kg^{-1}$ DW)	Shoot Cu removal ($\mu g\ plant^{-1}$)	Ca	Fe	K	Mg	Na	P	Zn	Mn
Pn	5.1 ± 0.7 a	2.6 ± 0.4 a	5.3 ± 1.1 a	0.019 ± 0.005 a	5.8 ± 0.3 a	0.87 ± 0.21 a	1.06 ± 0.28 a	1.24 ± 0.48 a	25 ± 6 a	8 ± 2 a
PnB	4.3 ± 0.4 ab	2.2 ± 0.2 ab	5.1 ± 0.9 a	0.015 ± 0.002 a	6.6 ± 1.1 a	0.89 ± 0.12 a	0.68 ± 0.29 ab	1.2 ± 0.16 a	25 ± 8 a	10.4 ± 4 a
*Pn*BZ	4.0 ± 0.5 **b**	2.0 ± 0.2 **b**	4.8 ± 0.9 a	0.016 ± 0.003 a	6.5 ± 1 a	0.84 ± 0.12 a	0.47 ± 0.15 **b**	1.24 ± 0.19 a	32 ± 6 a	9.8 ± 2 a
Ad	4.4 ± 0.7 **b**	2.2 ± 0.4 **b**	2.8 ± 1.6 a	0.015 ± 0.003 a	6.1 ± 1.2 a	1.49 ± 0.63 a	0.19 ± 0.05 a	0.16 ± 0.03 a	6 ± 4 a	13 ± 7 b
AdB	7.0 ± 2 a	3.5 ± 1.2 a	3.2 ± 0.8 a	0.016 ± 0.004 a	8.3 ± 1.4 a	1.68 ± 0.43 a	0.25 ± 0.1 a	0.25 ± 0.06 a	9 ± 5 a	14 ± 6 b
*Ad*BZ	5.2 ± 1 ab	2.6 ± 0.5 ab	3.2 ± 0.7 a	0.021 ± 0.007 a	8.7 ± 2.6 a	1.77 ± 0.28 a	0.2 ± 0.09 a	0.19 ± 0.03 a	7 ± 5 a	32 ± 2 a
Common values *	3 – 20	-	1 – 50	0.02 – 0.3	20 – 50	1.5 – 3.5	-	1.6 – 6.0		

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). * (Tremel-Schaub and Feix, 2005).

3.2.2. Shoot ionome (Table 4)

Shoot Cu concentration of *P. nigra* was significantly lower for the *Pn*BZ plants relative to the *Pn* plants (Table 4). For *A. donax*, the shoot Cu concentration was significantly higher for the *Ad*B plants relative to the *Ad* plants. Shoot Cu removal slightly dropped for the *Pn*BZ plants relative to the *Pn* plants and significantly increased for the *Ad*B plants as compared to the *Ad* ones, mainly due to changes in shoot Cu concentration. Shoot Ca, Fe, K, Mg, Na, P, Zn and Mn concentrations of poplar and Giant reed were globally similar on the amended and untreated soils, except two significant cases: shoot Na concentration was lower for the *Pn*BZ plants relative to the *Pn* ones, and shoot Mn concentration higher for the *Ad*BZ plants as compared to *Ad* plants. For *A. donax*, shoot element concentrations for the Cut 1 were globally higher than those for the Cut 2. Only shoot Na and Mg concentrations were higher for the *Ad* and *AdBZ* plants of Cut 2 relative to plants harvested in Cut 1 (Supplemental material 1.).

4. Discussion

Due to biogeochemical reactions between soil phases, amendments, and biological organisms, the behavior of chemical elements (e.g. Cu) in amended soils may change over time, which is referred as "aging" (Kookana, 2010). Plant rhizodeposition and associated microorganisms may influence soil Cu speciation, especially when cultivated in a long-time basis (Merino et al., 2015). Here, we discuss the aging effect on the efficiency of biochar, alone and combined with iron grit, and how plants and amendments may influence Cu (im)mobilization and crop productivity for this Cu-contaminated soil from a wood preservation site.

4.1. Soil pore water

4.1.1. Composition at month 3

At short-term (3 months), the B amendment decreased Cu^{2+} concentration while the BZ combination reduced both Cu^{2+} and Cu concentrations in the soil pore water (Oustriere et al., 2016a). In the B soils, the decreased SPW Cu^{2+} concentrations were associated with increased soil pH and SPW P and DOM concentrations and decreased SPW Ca concentrations. Based on X-ray absorption fine structure spectroscopy (XAFS), Cu^{2+} sorption onto biochar is pH dependent (Ippolito et al., 2012). Free Cu^{2+} may have (1) form inner sphere complexes with the active functional groups of biochar surface in line with SPW pH, (2) precipitate with phosphates and carbonates based on biochar composition (Tab. 2), and (3) form soluble Cu complexes with DOM increasing total Cu concentration in the SPW. Adding Z in combination with B increase soil pH. In the BZ treatment, free Cu^{2+} ions may primarily be sorbed on Fe/Mn oxy-hydroxides, potentially by bidentate inner-sphere complexes with Fe (hydr)oxides (Oustriere et al., 2016a; Tiberg et al., 2016).

4.1.2. Composition at month 22

Poplar and aging effect poorly affected pH, DOM and total Cu concentrations in the soil pore water (Tab. 3; Fig. 1), which agreed with previous studies: pH and total Cu in the SPW did not change over 36 months in a poly-contaminated soil at Aznalcázar, South West of Spain, cultivated with *Populus alba* L. (Ciadamidaro et al., 2013); pH and total Cu in the SPW were similar after 5 years in the untreated and compost-amended field plots cultivated with *Populus trichocarpa* x *deltoides* cv. Beaupré at the P7 sub-site (soil contamination with Cu and PAHs) of this wood preservation site (Mench, unpublished data). Some studies on biochars supported the lack of aging effect on pH and DOM and total Cu concentrations in the soil pore water: in a PAH-contaminated soil, soil pH remained steady after artificial chemical, biological, and physical aging of corn stover residues-derived biochar (Hale et al., 2011). The soil pH was kept between 7.9 and 8.1 over a 3-year period in a (Ni/Zn)-contaminated soil, amended by broadleaf hardwood-derived biochar, Castleford, UK, (Shen et al., 2016). After 3 years, SOM and ($CaCl_2$)-extractable Cu concentration were stable in a (Cu/Cd)-contaminated soil from a former Cu mine, amended by corn-straw-derived biochar (Li et al., 2016a). At month 22, the SPW Cu^{2+} concentration increased in the *Pn*B and *Pn*BZ soils as compared to the B and BZ ones (at month 3) (Fig. 2). This might be explained by: (1) less potential precipitation with phosphates in line with lower SPW P concentration in the *Pn* soil than in the B one, and (2) decrease of the sorption capacity of biochar over the time (Martin et al., 2012). Rozada et al. (2008) suggested that Cu^{2+} sorption on biochar surface was easily reversible. When biochar aged, the proportion of oxygen-containing acidic functional groups (e.g., COO^{-}, COH and OH) increases on the biochar surface increased, while carboxyl groups slightly decreased (Nguyen and Lehmann, 2009; Guo et al., 2014), contributing to the negative charge of the biochar. Their association with cations (i.e. Ca, K and Mg), in line with the low SPW cation concentrations (Tab. 3), and strong affinity for water and DOM may decrease the sorption capacity of biochar for free Cu^{2+} (Li et al., 2016a). In addition, gradual coating and interactions with organic and OM and inorganic phases (Sorrenti et al., 2016) and microbial colonization may decrease the reactivity of the biochar surface. After 4 years, aging increased biochar skeletal density and reduced the water imbibition rate within fragments as a consequence of partial pore clogging (Sorrenti et al., 2016). The cation exchange capacity (CEC) and adsorption capacity of Cu(II) on the aged biochar were smaller than those of new biochar in a 300 day incubation time pot experiment (Guo et al., 2014). Here, aged biochar particles after cultivation could be further retrieved from the amended soils to assess their composition and reactivity and evidence the main mechanisms.

Enhanced SPW pH and DOM concentration in all amended soils planted with *A. donax* (Tab. 3; Fig. 1) agreed with an increase in soil pH (5–9 %) after a 3-month cultivation of *A. donax* on red mud-contaminated soil (Alshaal et al., 2013b). Change in SPW pH may partly result from root activity, imbalance uptake of anions (e.g. NO_3^-) vs. cations increasing the rhizosphere pH in the basal root zone

(Hinsinger et al., 2003; Bravin et al., 2009; Qasim et al., 2016). Calcium uptake by plant, in line with low SPW Ca concentration (Tab. 3), may initiate a further cation desorption from the solid phase, promote H^+ sorption and increase soil pH. In addition, increased SPW pH may be related to the higher SPW DOM concentration (Fig. 1; Zhang et al., 2014), which was previously reported in soils cultivated with *A. donax* (Riffaldi et al., 2010). DOM may originate from decayed plant litter, microbial and root depositions, and hydrolysis of insoluble SOM (Haynes, 2005). No leachate from plant litter was produced here as shoots were harvested, but *A. donax* formed a dense mat of roots and rhizomes in the soil (Fig. 2). Giant reed rhizodeposition can highly contribute to SOM available for microbial activity and the DOM pool (Cattaneo et al., 2014; Nsanganwimana et al., 2014a; Monti et al., 2016). Enhanced microbial activity may stimulate both the degradation of SOM and production of microbial DOM (Kiikkilä et al., 2012; Hagedorn et al., 2015). In *A. donax* cultivated soil, pH increased between 7.9 ± 0.2 (*Ad*) and 8.1 ± 0.2 (*Ad*BZ) as compared to the Unt soil (i.e. 6.9 ± 0.1), which may promote DOM solubilization (Fang et al., 2016). At month 22, the SPW Cu concentration increased in the *Ad* and *Ad*B soils as compared to the B and BZ ones (at month 3) (Fig. 2). Enhanced DOM concentration may form soluble complexes with Cu and increase its SPW concentration (Beesley et al., 2010; Karami et al., 2011). Here, Giant reed-derived-DOM may act as competitive ligands preventing Cu retention on biochar surfaces (Beesley et al., 2014). In parallel, with ageing, negative charges on biochar surface may form direct or indirect surface complexes with soil components. In addition, coating of DOM may gradually mask sorptive surfaces, thus limiting Cu sorption (Pignatello et al., 2006).

In contrast, the SPW Cu^{2+} and Cu concentrations in the *Ad*BZ and *Pn*BZ soils were lower than in the untreated and biochar-amended-soils cultivated with *A. donax* and *P. nigra*, albeit not significantly for Cu^{2+} as compared to *Ad*B and *Pn*B. The SPW Cu^{2+} and Cu concentrations in the *Ad*BZ and *Pn*BZ soils at month 22 were as low as in the BZ soil at month 3 after soil amendment, albeit not significantly for Cu^{2+} in the *Ad*BZ soil. Cationic metal species such as Cu^{2+} or DOM-Cu complex have high affinity for Fe oxides and can sorb on the newly formed Fe and Mn oxy-hydroxides after Z corrosion in the soil (Kumpiene et al., 2008; Komárek et al., 2013). The sorption of Cu on Fe and Mn oxy-hydroxides is pH-dependent with stronger sorption at high pH. The additional liming effect of biochar can favor the net negative surface charge of Fe and Mn oxy-hydroxides and enhance Cu sorption. Tiberg et al. (2016) investigated Cu-contaminated soils collected in untreated and Z-treated plots at our sampling sub-site after 6 years: the bound Cu was primarily associated to SOM in the Unt soil whereas with Z addition and increased soil pH, Cu sorption shifted towards metal (hydr)oxides. Based on the Cu-ferrihydrite EXAFS spectrum, Cu was found to primarily bind as inner-sphere bidentate complexes with iron (hydr)oxide (Tiberg et al., 2016).

4.2. Plants

At month 3, this biochar alone and combined with iron grit did not promote the root and shoot yields of dwarf beans (Oustriere et al., 2016a). At month 22, this result was similar for *P. nigra* and *A. donax* (Fig. 2) and agreed with some studies: root and shoot biomass produced by *Lolium multiflorum* Lam. was unchanged in a (Cd, Zn, and Pb)-contaminated soil amended with 1% miscanthus-derived-biochar after 28 days and 56 days (Houben et al., 2013a,b). After 2 years, grain yields and biomass of wheat were not affected by both rate 10 and 40 t ha^{-1} of wheat straw-derived biochar addition in a Cd contaminated paddy soil (Cui et al., 2012). Germination of grass (mix of *Festuca rubra* L. and *Lolium perenne* L.) on site failed in a 3-year field experiment on a contaminated (Ni/Zn) soil amended with broad leaf hardwood-derived biochar (Shen et al., 2016). Here, despite HSN supply, Ca, Fe, K, Mg and P sub-deficiencies might be suggested as their concentrations in *P. nigra* and *A. donax* shoots were below or in the low ranges as compared to common values (Tremel-Schaub and Feix, 2005, Table 4). At month 22, Ca, Fe, K, Mg and P concentrations in the SPW were low as compared to common values in sandy soil (Table 3), likely due to (1) reaction with soil and amendment phases (Cheng et al., 2014), and (2) root uptake. Nutrient concentrations in *A. donax* shoot even decreased between the Cut 1 and the Cut 2 (Supplemental material 1). It may reflect a dilution effect as shoot DW yield increased. The shoot Cu concentrations of *P. nigra* and *A. donax* (mg kg^{-1}) were similar across the treatments, ranging from 4.0 ± 0.5 (*Pn*BZ) to 7.0 ± 2 (*Ad*BZ) (Table 4), and in the range of common values (3 - 20 mg kg^{-1}, Tremel-Schaub and Feix, 2005). Stem and foliar Cu concentrations (mg kg^{-1}) reached 3.21 and 7.17 for *A. donax* on a poly-contaminated urban stream (Bonanno et al., 2013). Shoot Cu concentration ranged between 7 and 17 mg kg^{-1} in *P. alba* grown for 3 years in a Cu-contaminated soil (Ciadamidaro et al., 2013). Giant reed and poplars are Cu excluders accumulating Cu in their roots, which may explain their low shoot Cu concentration (Kabata-Pendias, 2001; Bonanno et al., 2013; Ciadamidaro et al., 2013; Elhawat et al., 2014).

4.3. Practical implication

Effect of plant species: Giant reed was more efficient than *P. nigra* to decrease Cu^{2+} exposure in its rhizosphere but increased total Cu concentration in the soil pore water, which may potentially enhance Cu leaching from the root zone (Fig. 1). After 2 years, *P. nigra* did not contribute to Cu immobilization (Fig. 1). Without soil amendment, both plant species were unable to stabilize Cu in excess.

Effect of soil amendment: combining iron grit with biochar promoted Cu stabilization but not the growth of *A. donax* and *P. nigra*. An additional fertilization may be required. Long-term field plots must investigate the sustainability of Cu stabilization, biomass production of non-food crops and the life cycling of such phytomanagement option.

5. Conclusion

After a 22-month reaction period, the SPW Cu^{2+} concentration increased again in the B and BZ soils cultivated with *P. nigra* as compared to its value at month 3. Cultivation of *A. donax* incremented the DOM concentration in the soil pore water. Such high SPW DOM concentration in the Giant reed-planted soils, which may induce the formation of Cu-DOM complexes, matched with a decrease of Cu^{2+} concentration but increased total Cu concentration in the soil pore water. Iron grit with biochar was more effective to stabilize soil Cu than biochar alone with both *A. donax* and *P. nigra* cultivation. Biochar alone and combined with iron grit did not promote the root and shoot yields of *A. donax* and *P. nigra* as compared to the untreated soil but the shoot Cu concentrations were in the common ranges for both plants.

6. References

Alshaal, T., Domokos-Szabolcsy, É., Márton, L., Czakó, M., Kátai, J., Balogh, P., Elhawat, N., El-Ramady, H., Fari, M., 2013b. Phytoremediation of bauxite-derived red mud by giant reed. Environ. Chem. Lett. 11, 295–302. doi:10.1007/s10311-013-0406-6

Bakshi, S., He, Z.L., Harris, W.G., 2014. Biochar amendment affects leaching potential of copper and nutrient release behavior in contaminated sandy soils. J. Environ. Qual. 43, 1894–1902. doi:10.2134/jeq2014.05.0213

Baize, D., 2000. Guide des analyses en pédologie. Second ed. Paris, France : INRA Éditions; 257

Beesley, L., Moreno-Jimenez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287. doi:10.1016/j.envpol.2010.02.003

Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480. doi:10.1016/j.envpol.2010.10.016

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159, 3269–3282. doi:10.1016/j.envpol.2011.07.023

Beesley, L., Inneh, O.S., Norton, G.J., Moreno-Jimenez, E., Pardo, T., Clemente, R., Dawson, J.J.C., 2014. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. Environ. Pollut. 186, 195–202. doi:10.1016/j.envpol.2013.11.026

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization. Environ. Pollut. 156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bian, R., Joseph, S., Cui, L., Pan, G., Li, L., Liu, X., Zhang, A., Rutlidg, H., Wong, S., Chia, C., Marjo, C., Gong, B., Munroe, P., Donne, S., 2014. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. J. Hazard. Mater. 272, 121–128. doi:10.1016/j.jhazmat.2014.03.017

Bolan, N.S., Adriano, D.C., Mahimairaja, S., 2014. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Crit. Rev. Environ. Sci. Technol. 34, 291–338. doi:10.1080/10643380490434128

Bonanno, G., Cirelli, G.L., Toscano, A., Lo Giudice, R., Pavone, P., 2013. Heavy metal content in ash of energy crops growing in sewage-contaminated natural wetlands: Potential applications in agriculture and forestry? Sci. Total Environ. 452–453, 349–354. doi:10.1016/j.scitotenv.2013.02.048

Bravin, M.N., Marti A.L., Clairotte M., Hinsinger P., 2009. Rhizosphere alkalisation — a major driver of copper bioavailability over a broad pH range in an acidic, copper-contaminated soil. Plant Soil. 318, 257–268. doi:10.1007/s11104-008-9835-6

Bridgwater, T., 2006. Biomass for energy. J. Sci. Food. Agric. 86, 1755–1768. doi:10.1002/jsfa.2605

Calfapietra, C., Gielen, B., Karnosky, D., Ceulemans, R., Scarascia Mugnozza, G., 2010. Response and potential of agroforestry crops under global change. Environ. Pollut. 158, 1095–104. doi:10.1016/j.envpol.2009.09.008

Cattaneo, F., Barbanti, L., Gioacchini, P., Ciavatta, C., Marzadori, C., 2014. 13C abundance shows effective soil carbon sequestration in Miscanthus and giant reed compared to arable crops under Mediterranean climate. Biol. Fertil. Soils. 50, 1121–1128. doi:10.1007/s00374-014-0931-x

Cheng, C.H., Lin, T.P., Lehmann, J., Fang, L.J., Yang, Y.W., Menyailo, O.V., Chang, K.H., Lai, J.S., 2014. Sorption properties for black carbon (wood char) after long term exposure in soils. Org. Geochem. 70, 53–61. doi:10.1016/j.orggeochem.2014.02.013

Ciadamidaro, L., Madejón, E., Puschenreiter, M., Madejón, P., 2013. Growth of Populus alba and its influence on soil trace element availability. Sci. Total Environ. 454–455, 337–347. doi:10.1016/j.scitotenv.2013.03.032

Cundy, A., Bardos, P., Puschenreiter, M., Witters, N., Mench, M., Bert, V., Friesl-Hanl, W., Muller, I., Weyens, N., Vangronsveld, J., 2015. Developing effective decision support for the application of “gentle” remediation options: The GREENLAND project. Remediation Journal 25, 101–114. doi:10.1002/rem.21435

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, E., El-Ramady., H, Márton, L., Czakó, M., Kátai, J., Balogh, P., Sztrik, A., Molnár, M., Popp, J., Fári, MG., 2014. Phytoaccumulation potentials of two biotechnologically propagated ecotypes of Arundo donax in copper-contaminated synthetic wastewater. Environ. Sci. Pollut. Res. 21, 7773–7780. doi:10.1007/s11356-014-2736-8

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, E., El-Ramady, H., Antal, G., Márton, L., Czakó, M., Balogh, P., Fári, M.G., 2015. Copper uptake efficiency and its distribution within bioenergy grass giant reed. Bull. Environ. Contam. Toxicol. 95, 452–458. doi:10.1007/s00128-015-1622-5

Evangelou, M.W.H., Deram, A., Gogos, A., Studer, B., Schulin, R., 2012. Assessment of suitability of tree species for the production of biomass on trace element contaminated soils. J. Hazard. Mater. 209–210, 233–239. doi:10.1016/j.jhazmat.2012.01.008

Fang, W., Wie, Y., Liu, J., 2016. Comparative characterization of sewage sludge compost and soil: Heavy metal leaching characteristics. J. Hazard. Mater. 310, 1–10. doi:10.1016/j.jhazmat.2016.02.025

Gomes, H., 2012. Phytoremediation for bioenergy: challenges and opportunities. Environ. Technol. 1(1), 59–66. doi:10.1080/09593330.2012.696715

Gonsalvesh, L., Yperman, J., Carleer, R., Mench, M., Herzig, R., 2016. Vangronsveld J. Valorisation of heavy metals enriched tobacco biomass through slow pyrolysis and steam activation. J. Chem. Technol. Biotechnol. 91, 1585–1595. doi:10.1002/jctb.4889

Hagedorn, F., Bruderhofer, N., Ferrari, A., Niklaus, P.A., 2015. Tracking litter-derived dissolved organic matter along a soil chronosequence using 14C imaging: Biodegradation, physico-chemical retention or preferential flow? Soil. Biol. Biochem. 88, 333–343. doi:10.1016/j.soilbio.2015.06.014

Hale, S., Hanley, K., Lehmann, J., Zimmerman, A.R., Cornelissen, G., 2011. Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar. Environ. Sci. Technol. 45, 10445–10453. doi:10.1021/es202970x

Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Adv. Agron. 85, 221–268. doi:10.1016/S0065-2113(04)85005-3

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil. 248, 43–59. doi:10.1023/A:1022371130939

Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere. 92, 1450–1457. doi:10.1016/j.chemosphere.2013.03.055

Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants. Third edition. Boca Raton, Florida: CRC Press, Inc.

Karami, N., Clemente, R., Moreno-Jimenez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J. Hazard. Mater. 191, 41–8. doi:10.1016/j.jhazmat.2011.04.025

Kidd, P., Mench, M., Álvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J., Puschenreiter, M., 2015. Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int. J. Phytorem. 17, 1005–1037. doi:10.1080/15226514.2014.1003788

Kiikkilä, O , Kitunen, V , Spetz, P , Smolander, A., 2012. Characterization of dissolved organic matter in decomposing Norway spruce and silver birch litter. Eur. J. Soil Sci. 63, 476–486. doi:10.1111/j.1365-2389.2012.01457.x

Kookana, R.S., 2010. The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. Aust. J. Soil Res. 48, 627–637. doi:10.1071/SR10007

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste. Manage. 28, 215–225. doi:10.1016/j.wasman.2006.12.012

Kumpiene, J., Mench, M., Bes, C.M., Fitts, J.P., 2011. Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environ. Pollut. 159, 1536–1542. doi:10.1016/j.envpol.2011.03.005

Li, H., Ye, X., Geng, Z., Zhou, H., Guo, X., Zhang, Y., Zhao, H., Wang, G., 2016a. The influence of biochar type on long-term stabilization for Cd and Cu in contaminated paddy soils. J. Hazard. Mater. 304, 40–48. doi:10.1016/j.jhazmat.2015.10.048

Lucas-Borja, M.E., Wic-Baena, C., Moreno, J.L., Dadi, T., García, C., Andrés-Abellá, M., 2011. Microbial activity in soil under fast-growing Paulownia (Paulownia elongata×fortunei) plantations in Mediterranean areas. Appl. Soil. Ecol. 51, 42–51. doi:10.1016/j.apsoil.2011.08.011

Mantineo, M., D'agosta, G.M., Copani, V., Patanè, C., Cosentino, S.L., 2009. Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. Field Crop. Res. 114(2), 204–213. doi:10.1016/j.fcr.2009.07.020

Marchand, L., Nsanganwimana, F., Gonnelli, C., Colzi, I., Fletcher, T., Oustriere, N., Kolbas, A., Kidd, P., Bordas, F., Newell, P., Alvarenga, P., Lamy, J.B., Deletic, A., Mench, M., 2014a. Intra-specific variability versus constitutive-like tolerance to face Cu exposure in populations of six rooted macrophytes. Environ. Pollut. 193, 205–215. doi:10.1016/j.envpol.2014.07.001

Martin, S.M., Kookana, R.S., Van Zwieten, L., Krull, E., 2012. Marked changes in herbicide sorption–desorption upon ageing of biochars in soil. J. Hazard. Mater. 231–232, 70–78. doi:10.1016/j.jhazmat.2012.06.040

Mench, M., Bes, C., 2009. Assessment of ecotoxicity of topsoils from a wood treatment site. Pedosphere. 19(2), 143–155. doi:10.1016/S1002-0160(09)60104-1

Merino, C., Nannipieri, P., Matus, F., 2015. Soil carbon controlled by plant, microorganism and mineralogy interactions. J. Soil Sci. Plant Nutr. 15(2), 321–332.

Monti, A., Zegada-Lizarazu, W., 2016. Sixteen-year biomass yield and soil carbon storage of giant reed (Arundo donax L.) grown under variable nitrogen fertilization rates. Bioenerg. Res. 9, 248–256. doi:10.1007/s12155-015-9685-z

Nagodavithane, C.L., Singh, B., Fang, Y., 2014. Effect of ageing on surface charge characteristics and adsorption behaviour of cadmium and arsenate in two contrasting soils amended with biochar. Soil Research. 52, 155–163. doi:10.1071/SR13187

Nguyen, B.T., Lehmann, J., 2009. Black carbon decomposition under varying water regimes. Org. Geochem. 40, 846–853. doi:10.1016/j.orggeochem.2009.05.004

Nsanganwimana, F., Marchand, L., Douay, F., Mench, M., 2014a. Arundo donax L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock, A review. Int. J. Phytorem. 16, 982-1017. doi:10.1080/15226514.2013.810580

Nsanganwimana, F., Pourrut, B., Mench, M., Douay, F., 2014b. Suitability of Miscanthus species for managing inorganic and organic contaminated land and restoring ecosystem services. A review. J. Environ. Manage. 143, 123–134. doi:10.1016/j.jenvman.2014.04.027

Oustriere, N., Marchand, L., Galland, W., Gabon, L., Lottier, N., Motelica, M., Mench, M., 2016a. Influence of biochars, compost and iron grit, singly and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Sci. Total Environ. 566–567, 816–825. doi:10.1016/j.scitotenv.2016.05.091

Pignatello, J.J., Kwon, S., Lu, Y,. 2006. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): attenuation of surface activity by humic and fulvic acids. Environ. Sci. Technol. 40, 7757–7763. doi:10.1021/es061307m

Qasim, B., Motelica-Heino, M., Bourgerie, S., Gauthier, A., Morabito, D., 2016. Rhizosphere effects of Populus euramericana Dorskamp on the mobility of Zn, Pb and Cd in contaminated technosols. J. Soils Sediments. 16, 811–820. doi:10.1007/s11368-015-1270-8

Riffaldi, R., Saviozzi, A., Cardelli, R., Bulleri, F., Angelini, L., 2010. Comparison of soil organic-matter characteristics under the energy crop giant reed, cropping sequence and natural grass. Commun. Soil Sci. Plan. Nut. 41, 173–180. doi:10.1080/00103620903426972

Rozada, F., Otero,. M., Morán,. A., García, A.I., 2008. Adsorption of heavy metals onto sewage sludge-derived materials. Bioresour. Technol. 99, 6332–6338. doi:10.1016/j.biortech.2007.12.015

Rowe, R.L., Street, N.R., Taylor, G., 2009. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. Renewable Sustainable Energy Rev. 13, 271–290.

Scordia, D., Cosentino, S.L., Lee, J.W., Jeffries, T.W., 2012. Bioconversion of giant reed (Arundo donax L.) hemicelluloses hydrolysate to ethanol by Scheffersomyces stipitis CBS6054. Biomass Bioenerg. 39, 296–305. doi:10.1016/j.biombioe.2012.01.023

Shen, Z., Md Som, A., Wang, F., Jin, F., McMillan, O., Al-Tabbaa, A., 2016. Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. Sci. Total Environ. 542, 771–776. doi:10.1016/j.scitotenv.2015.10.057

Tiberg, C., Kumpiene, J., Gustafsson, J.P., Marsz, A., Persson, I., Mench, M., Kleja, D.B., 2016. Immobilization of Cu and As in two contaminated soils with zerovalent iron – Long-term performance and mechanisms. Appl. Geochem. 67, 144–152. doi:10.1016/j.apgeochem.2016.02.009

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers: EDP Sciences, Les Ulis. pp.106.

Vigil, M., Marey-Pérez, M.F., Martinez Huerta, G., Álvarez Cabal, V., 2015. Is phytoremediation without biomass valorization sustainable?—Comparative LCA of landfilling vs. anaerobic co-digestion. Sci. Total Environ. 505, 844–850. doi:10.1016/j.scitotenv.2014.10.047

Villanneau, E., Perry-Giraud, C., Saby, N., Jolivet, C., Marot, F., Maton, D., Floch-Barneaud, A., Antoni, V., Arrouays, D., 2008. Détection de valeurs anomaliques d'éléments traces métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. Etude et Gestion des Sols. 15(3), 183–200.

Witters, N., Mendelsohn, R.O., Slycken, S.V., Weyens, N., Schreurs, E., Meers, E., Tack, F., Carleer, R., 2012. Vangronsveld. Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass. Bioenerg. 39, 454-469. doi:10.1016/j.biombioe.2011.08.016

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei. J., Huang, H., 2013. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ. Sci. Pollut. Res. 20, 8472–8483. doi:10.1007/s11356-013-1659-0

Zhang, S., Li, T., Zhang, X., Yu, H., Zheng, Z., Wang, Y., Hao, X., Pu, Y., 2014. Changes in pH, dissolved organic matter and Cd species in the rhizosphere soils of Cd phytostabilizer Athyrium wardii (Hook.) Makino involved in Cd tolerance and accumulation. Environ. Sci. Pollut. Res. 21, 4605–4613. doi:10.1007/s11356-013-2421-3

Table. Supplemental material 1. Shoot ionome and shoot Cu removal of *A. donax* at month 10 (Cut 1, n = 5)

Treatment	Cu		Shoot nutrient concentrations ($g\ kg^{-1}$)						Shoot metal concentrations ($mg\ kg^{-1}$)	
	Shoot Cu concentration ($mg\ kg^{-1}$ DW)	Shoot Cu removal ($\mu g\ plant^{-1}$)	Ca	Fe	K	Mg	Na	P	Zn	Mn
Ad	7.6 ± 2.3 a	0.11 ± 0.04 a	5.8 ± 3.3 a	0.023 ± 0.005 a	12 ± 3 a	1.3 ± 0.7 a	0.18 ± 0.07 a	0.48 ± 0.14 a	13 ± 4 a	17 ± 11 a
AdB	6.8 ± 1.8 a	0.09 ± 0.01 a	7 ± 1.2 a	0.036 ± 0.013 a	14 ± 3 a	1.8 ± 0.4 a	0.28 ± 0.17 a	0.46 ± 0.18 a	13 ± 7 a	16 ± 11 a
AdBZ	8.2 ± 2.2 a	0.14 ± 0.05 a	4.1 ± 1.7 a	0.027 ± 0.003 a	14 ± 2 a	1.3 ± 0.4 a	0.12 ± 0.04 a	0.47 ± 0.08 a	20 ± 8 a	37 ± 16 a
Common values *	3 – 20	-	1 – 50	0.02 – 0.3	20 – 50	1.5 – 3.5	-	1.6 – 6.0		

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). *(Tremel-Schaub and Feix, 2005).

Part. 2. Biomass production with (essential) metal(loid) concentrations within the common ranges usable by local biomass processing chains

2.3. Implementation of the remediation strategy in situ

2.3.1 Additional information - Phytomanagement of a Cu-contaminated soil at a wood preservation site using Giant reed, poplar, and biochars, alone and combined with compost: in situ assessment of soil phytotoxicity with *Vicia faba* L.

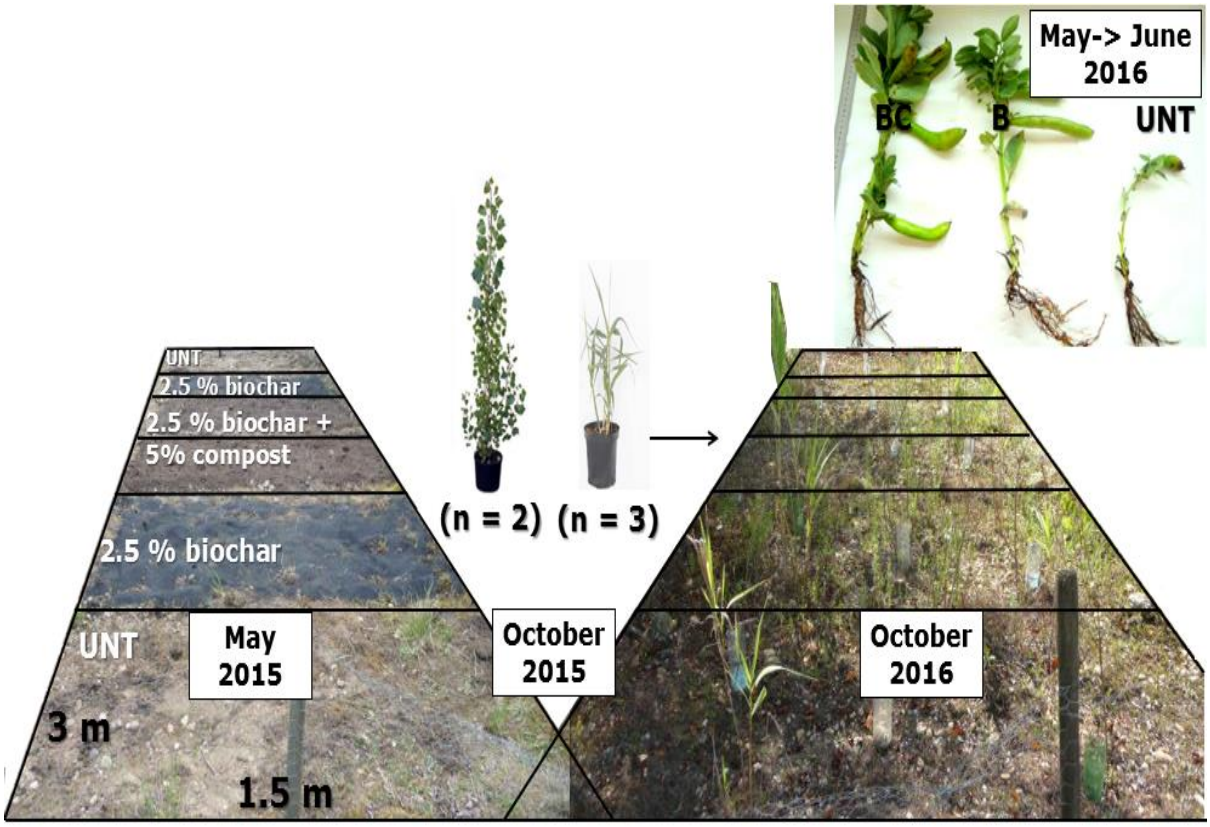


Fig. D. Graphical abstract – Additional information

Phytomanagement of a Cu-contaminated soil at a wood preservation site using Giant reed, poplar, and biochars, alone and combined with compost: in situ assessment of soil phytotoxicity with Vicia faba L.

Nadège Oustriere[1], Lilian Marchand[1], Michel Mench[1]

(1) UMR BIOGECO INRA 1202, DIVERSITY AND FUNCTIONING OF COMMUNITIES, UNIV. BORDEAUX, BÂT. B2, ALLÉE GEOFFROY ST-HILAIRE, CS50023, F-33615 PESSAC CEDEX, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; mench@bordeaux.inra.fr;

Abstract

A biochar was used, alone and in combination with greenwaste compost, as amendment to improve soil properties and in situ stabilize Cu in a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site. The pot experiment consisted in 3 soil treatments planted with *Arundo donax* L. and *Populus nigra* L.: (% w/w): untreated Cu-contaminated soil (Unt); Unt soil amended with biochar alone (2.5%, B) or in combination with compost (5%, BC). After a 5-month growth period, the maximal shoot height of *A. donax* and *P. nigra* was unchanged in both amended soils as compared to the Unt soil. Conversely root and shoot DW yields of *Vicia faba* significantly increased in the Cu-contaminated soil amended with biochars alone or in combination with compost. The shoot Cu concentration in *V. faba* from the B soils only slightly exceeded its common values in plants. This experiment highlighted how the biochar, alone and in combination with compost, is able to promote the production of a biomass on site with low shoot Cu concentration. Further researches are needed on this Cu-contaminated site and new measures of plants growth will be repeated next year.

Keywords: Giant reed, Poplar, Copper contamination, In situ experiment, Phytomanagement.

1. Introduction

Soil Cu contamination at wood preservation sites frequently resulted from long term use of Cu-based salts as wood preservatives and wood washings (Bes and Mench, 2008; Oustriere et al., 2016a). Copper excess in topsoils can enhance its concentration in the labile soil pool and in tissues of biological receptors (Bakshi et al., 2014). *In situ* stabilization is the incorporation of amendments into the soil to immobilize contaminants. It aims to reduce horizontal and vertical migration of contaminants by reducing their labile pool, limiting exposure of rhizosphere organisms, and promoting a plant cover (Kidd et al., 2015). It can be combined with the culture of metal-excluder plants and their associated microorganisms to improve soil stabilization (i.e. aided phytostabilization). Giant reed (*Arundo donax* L.) and Poplar (*Populus nigra* L.) are both Cu excluders, which may help to stabilize soil Cu contamination. Biochars are carbon-rich carbonized residues produced by waste biomass pyrolysis under high temperatures and mid to low oxygen (Lehmann, 2007). As suggested by Beesley and Marmiroli (2011), combining amendments with biochar may be more efficient than biochar alone for the remediation and revegetation of contaminated soils. In previous pot experiments, decrease in Cu exposure following biochar and compost amendment were reported in a Cu-contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site (Bes and Mench, 2008): a poplar wood-derived biochar (2.5% w/w) in combination of compost (5%) decreased Cu concentration in the soil pore water (SPW) and highly improved root and shoot yields of sunflower (*Helianthus annuus* L.) (Jones et al., 2016). Moreover, in a 3-month pot experiment, pine bark and poultry manure-derived biochars (1% w/w) in addition with compost (5%) can decrease soil pore water (SPW) Cu^{2+} concentration (Oustriere et al., 2016a). In a 2 year pot experiment, the Cu-contaminated soil amended with pine bark-biochar and cultivated with either Giant reed (*Arundo donax* L.) or poplar (*Populus nigra* L.) also displayed a decreased SPW Cu^{2+} concentration (Oustriere et al., 2016b). The reliability of pot studies for predicting results at the field scale is not always accurate, as pot environments differ from field conditions, mainly through the edge effect, changes in climatic conditions, and establishment of roots in the subsoil. Results obtained in pot experiment could hardly be extrapolated directly to the field to evaluate whether the combination of biochar and compost cultivated with Giant reed and poplar is a relevant option for reducing soil phytotoxicity and soil phytomanagement. Implementing small-scale field plots may allow to detect potential failures of selected phytomanagement options due to long-term changes, such as the ageing of the amendments added to the soil, inter-annual climate variability, pest attacks, deposition and accumulation of litter, release of soluble organic matter, and changes in animal and plant communities (Kidd et al., 2015). Therefore, to investigate the in situ effect of biochar, alone and combined with compost, we have implemented on a Cu-contaminated soil, at a wood preservation site, six field plots cultivated with Giant reed and poplar. As an in situ plant testing, changes in soil phytotoxicity was assessed with broad bean (*Vicia faba* L.) by determining its root and shoot dry weight (DW) yields and Cu uptake in aerial plant parts.

2. Material and Methods

2.1. Soil, biochars and compost

The wood preservation site (about 10 ha, Saint-Médard d'Eyrans, Gironde, SW France; 44°43.353′ N, 000°30.938′ W) has been used for over a century, with various Cu-based salts (mainly Cu-sulphate based on the duration and amount used) as wood preservatives (Mench and Bes, 2009). Its texture is sandy, i.e. 85.8 % sand, 5.9 % clay, and 8.3 % silt, with 1.3 % OM, C/N 16, neutral pH (7 ± 0.09) and a low CEC (2.5 cmol kg^{-1}). It is mainly contaminated by Cu (Tab. 1, Oustriere et al., 2016a), which largely exceeds its median and upper whisker background values in French sandy soils, but its total soil As and Cr are at background levels (Table 1, Baize, 2000; Villanneau et al., 2008). The commercial biochars (Florentaise, Saint-Mars-du-Désert, France) was produced using pine bark via pyrolysis at 420°C for 170 s. Soluble salts were not removed from biochars (Table 2). The compost (C) made from green wastes (9–12 months) was purchased from Gonzales frères, Martignas sur Jalle, France. Elemental composition, carbon content and PAH concentrations of biochars and compost were determined at the INRA Laboratoire d'Analyses des Sols (LAS, Arras, France) with standard methods (INRA LAS, 2011) (Table 2).

Table. 1. Main soil properties

Parameter	Mean ± SD	Background levels [A]	Risk assessment - remediation needed [C]
pH	7 ± 0.09	6.60	
CEC	2.5 ± 0.2		
P_2O_5 (g kg^{-1}, Olsen extractable)	0.03 ± 0.002		
Organic matter (g kg^{-1})	13 ± 0.4		
Organic C (g kg^{-1})	8 ± 0.3	14.5	
Total N (g kg^{-1})	0.5 ± 0.01		
C/N	16 ± 0.2	10.02	
Texture (g kg^{-1})			
Sand	858	≥ 650	
Silt	83	≤ 350	
Clay	59	≤ 180	
Total TE (mg kg^{-1})			
Cr	21 ± 0.7	14–40	250
Cu	964 ± 20	3.2–8.4	100
Ni	5 ± 0.3	4.2–14.5	100
Zn	37 ± 1.6	17–48	500
As	7 ± 0.4	1–25 [B]	30
PAHs (mg kg^{-1})			
Fluoranthene	1.9	0.4	10–100
Indeno[1,2,3-cd]pyrene	0.95	0.01–0.015	1
Benzo[g,h,i]perylene	0.8		1–10
Benzo[b]fluoranthene	0.8	0.1	1

[A] Frequent total concentrations in French sandy topsoils (Baize, 1997; Baize, 2000; Villanneau et al., 2008, INDIQUASOL, 2016).

[B] Frequent total As concentrations for all French soil types (Bes and Mench, 2008).

[C] Québec guidelines for risk assessment and remediation - Land Protection and Rehabilitation Regulation (2016).

Table. 2. Composition of soil conditioners. In bold, values exceeding the European Biochar Certificate (EBC) V4.8 thresholds

	Biochar (B)	EBC [A]	IBI [B]	Compost (C)
pH	9.89	-	-	7.53
CEC (cmol kg^{-1})	1.23	-	-	26.7
Major elements (% DW)				
H	0.8	-	-	-
Total N	0.3	-	-	0.69
S	0.03	-	-	-
Cl	0.01	-	-	-
Organic C	90.3	>50%		10.9
Nutrients (g kg^{-1})				
Ca	18.6	-	-	22.5
K	13.8	-	-	5.4
Mg	29.9	-	-	1.9
Na	0.389	-	-	-
P	0.105	-	-	0.374
Elements (mg kg^{-1})				
Al	1650	-	-	12700
As	0.461	-	12-100	4.47
Cd	<0.5	1	1.4-39	<0.5
Cr	45.9	80	64-1200	21
Cu	230	100	63-1500	85.8
Mn	-	-	-	-
Fe	4470	-	-	6830
Hg	<0.1	1	1-17	<0.1
Ni	28.3	30	47-600	7.98
Pb	2.61	120	70-500	51.5
Zn	50.5	400	200-7000	174
PAHs (mg kg^{-1})				
Sum of 16 US EPA PAHs [C]	163	4	6-300	<DL
Fluoranthene	7.48	-	-	<0.1
Benzo(b)fluoranthene	0.149	-	-	<0.1
Benzo(a)pyrene	<0.2	-	-	<0.3
Naphtalene	101	-	-	<0.2

[A.] following Switzerland's Chemical Risk Reduction Act (ChemRRV) on recycling fertilizers.

[B.] Range of Maximum Allowed Thresholds reflects different soil tolerance levels for these elements in compost, biosolids, or soils established by regulatory bodies in the US, Canada, EU and Australia (See Appendix 3 of the IBI Biochar Standards for further information).

[C.] Corresponds to the PAH threshold defined in the Swiss Chemical Risk Reduction Act (ChemRRV).

2.2. Field trial

In May 2015, field plots (3 m x 1.5 m; n = 2 plots per treatment) were implemented at the P1-3 sub-site (nearby the plant nursery) of the wood preservation site (Bes et al., 2009). The Cu-contaminated soil was either un-amended (Unt), amended by 2.5% biochar (B) or 2.5% B + 5% compost (BC). Amendments were carefully incorporated into the 0–0.25 m soil layer by a shallow ploughing using a broad-tined fork. In June 2015, topsoil samples were collected from each plot and individually potted (3kg). 1-year-old seedlings of *P. nigra* (3 replicates) and *A. donax* (2 replicates) were individually planted for all soil treatments (30 pots) and placed in a greenhouse with controlled conditions (16 /8 h light/darkness; 65 ± 5% relative humidity; 25± 2°C) for acclimatization. They were then transplanted to the field in October 2015. Maximum shoot height was measured for both plant species. In May 2016 (year 1), changes in soil phytotoxicity were assessed. Broad beans (*Vicia faba* L., Vilmorin) were sown (5 seeds/plot) in all plots, and cultivated during 2 months in field conditions without irrigation. In parallel, broad beans were sown in a similar uncontaminated Fluviosol in a kitchen garden, Gradignan, France and used as an uncontaminated control (Ctrl). At the end of June 2016, after 2 months, the shoots and roots of *V. faba* were harvested, washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields.

2.3. Broad bean analysis

For each plot, dried shoots of the five *V. faba* plants were ground (< 1 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) in digests was determined by ICP-MS (Thermo X series 200, INRA USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. All element concentrations in plant parts are expressed in mg or g DW kg^{-1}. The shoot Cu removal was calculated as follows: Cu (µg $plant^{-1}$) = Shoot DW yield (g $plant^{-1}$) x shoot Cu concentration (µg g^{-1} DW).

2.4. Statistical analysis

Influence of soil treatments on the maximal shoot height of *A. donax* and *P. nigra*, shoot DW yields and shoot ionome of *V. faba* was tested using one-way analysis of variance (ANOVAs). Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests.

Differences were considered statistically significant at $p<0.05$. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results and Discussion

3.1. Maximal shoot height of *A. donax* and *P. nigra* at month 5 after transplantation

After a 5-month growth period, the maximal shoot height of *A. donax* and *P. nigra* was unchanged in both amended soils as compared to the Unt soil. The lack of beneficial effect of biochar and compost on *A. donax* and *P. nigra* growth (Fig. 1) agreed with Rodríguez-Vila et al., (2014), Mackie et al., (2015) and Oustrière et al., (2016b) who found unchanged biomass production of *Brassica juncea* L, grass crops and *Phaseolus vulgaris* L. respectively, in Cu-contaminated soils amended with compost and biochar. In contrast, Jones et al., (2016) found an improvement of sunflower (*Helianthus annuus* L.) root and shoot yields for the same Cu-contaminated soil also amended with 2.5% biochar and 5% compost. This discrepancy may be due to biochar quality (the compost used in both studies was the same). In Jones et al., (2016), it was a hardwood derived-biochar pyrolysed at 525°C. In our study, biochar was made from pine bark chips pyrolysed at 420°C. As reported by Jamieson et al., (2013), the dissolved organic matter (DOM) released by a biochar depends on the raw material and the pyrolysis conditions. Increase in pyrolysis temperature from 450 to 550°C decreased DOC concentration by more than one order of magnitude (Uchimiya and Bannon, 2013). Increase in DOM concentration, in the BC soil can increase Cu concentration in the soil pore water, enhance soil phytotoxicity and decrease plant growth (Oustriere et al., 2016a). Moreover, *A. donax* and *P. nigra* cultivation in the Cu-contaminated soil may enhance DOM concentration in the soil pore water as reported by (Oustriere et al., 2017) reinforcing the Cu phytotoxicity instead of decreasing Cu leaching and enhancing Cu stabilization in the soil.

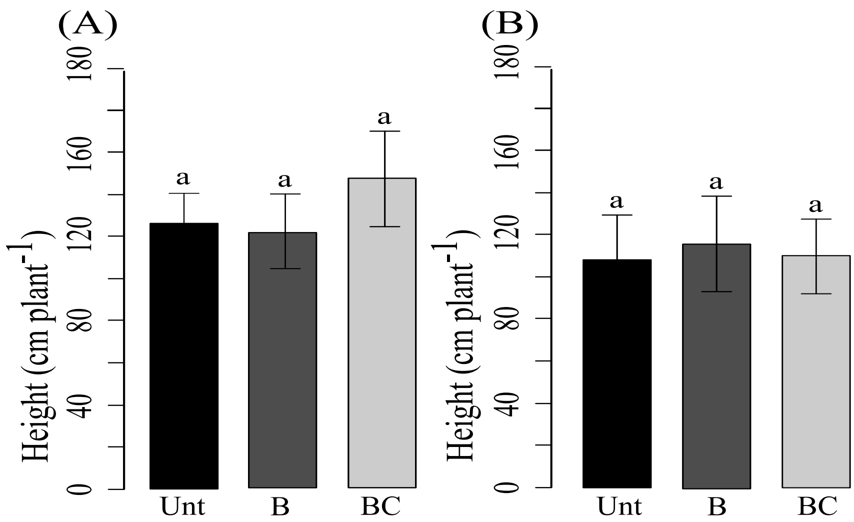


Fig. 1. Maximal shoot height of *Arundo donax* (A) and *Populus nigra* (B) (cm plant^{-1}) after the 5-month growth period. Untreated Cu-contaminated soil (Unt, black); biochar-amended soil (B, dark grey), and biochar + compost-amended soil (BC, light grey). Mean values per treatment (n= 6 *P. nigra* / n=4 *A. donax*). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

3.2. Soil phytotoxicity on *V. faba* one year after soil amendment

Frequently, biochar addition into Cu-contaminated soils, alone and in combination with compost, can increase plant yields and reduce shoot Cu concentration (Beesley et al., 2011). Here, root and shoot DW yields of *V. faba* significantly increased in the Cu-contaminated soil amended with biochars alone or in combination with compost (Fig. 2 A and B). The root DW yields of *V. faba* in the Ctrl soil and the BC-amended soil were statistically similar. Similarly, the shoot Cu concentration of *V. faba* decreased in both amended soils (Fig. 3 A and B). It ranged (in mg kg^{-1}) from 163 ± 94 (Unt) to 24 ± 16 (B). Biochar alone was more efficient than its combination with compost to decrease shoot Cu concentration of *V. faba*. However, due to the high shoot DW yield, Cu removal was significantly higher in the BC-amended soil than in the B soil. These results confirmed a decreased phytotoxicity for quinoa (*Chenopodium quinoa* Willd.) in a sandy Cu-spiked soil (Buss et al., 2012) and a reduced Cu bioavailability in maize (Namgay et al., 2010) due to Cu sorption by the biochar. Here, shoot Cu concentration in *V. faba* from the B soils slightly exceeded its common values in plants (3 - 20 mg Cu kg^{-1}, Tremel-Schaub and Feix, 2005) and for the BC soil was in the low range of Cu-stressed plants (20–100 mg Cu kg^{-1}, Kabata-Pendias and Pendias, 1984). In the BC and B soils, *V. faba* displayed a marked decrease in root growth as compared to the Ctrl soil, correlated with the increase in shoot Cu concentration. Nutrient deficiencies (i.e. Ca, Fe, K, Mg, and P) can be ruled out for this decrease as their concentrations in *V. faba* shoots were for all biochar amended soils within the ranges of common values (Tremel-Schaub and Feix, 2005, Table 1). However this agreed with Saadani et al., (2016) reporting that *V. faba* accumulates Cu primarily in the roots due to a low root-to-shoot Cu translocation. Increase in root Cu concentration of *V. faba* (i.e. 185 mg Cu kg^{-1}) may result from the differential hormetic dose responses of the antioxidant systems, HSP70, oxidatively modified proteins, and proteolytic activities in the roots which lead to a decrease root biomass (Xu et al., 2015).

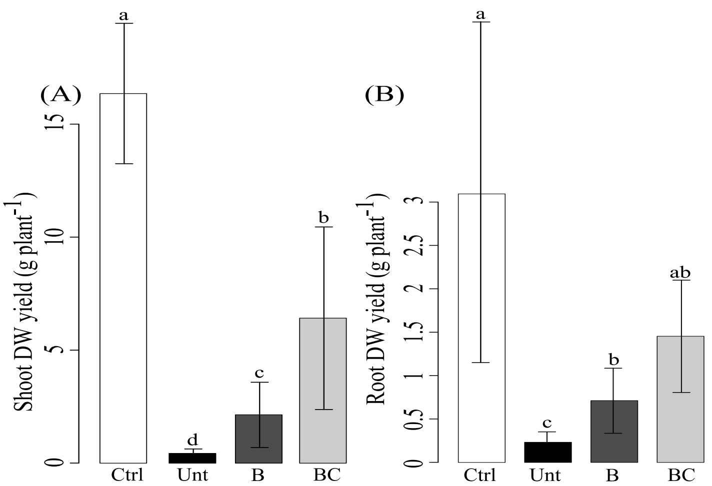


Fig. 2. (A) Shoot and (B) root DW yields of *Vicia faba* (in g DW $plant^{-1}$) after the 2-month growth period. Cu-contaminated soil (Unt, black), amended with biochar (B, dark grey) or biochar + compost (BC, light grey), and uncontaminated soil (Ctrl, white). Mean values per treatment (n= 10 / n=5 for Ctrl). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

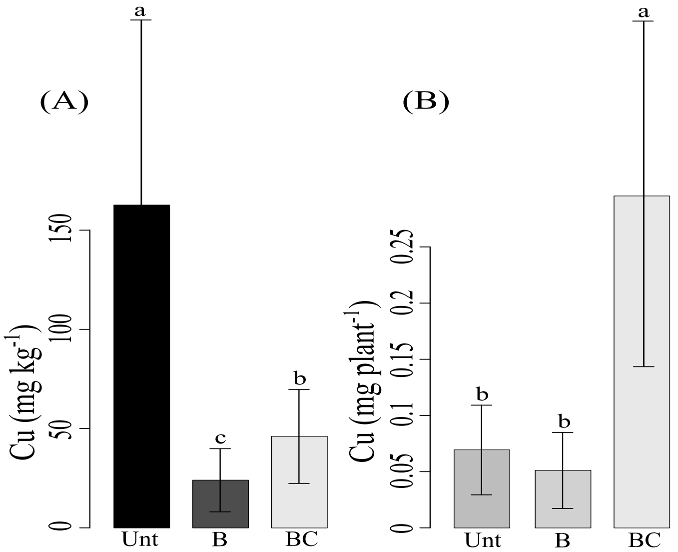


Fig. 3. (A) Cu concentration (in mg kg^{-1}) and (B) Cu removal (in mg plant^{-1}) in the shoots of *Vicia faba* after the 2-month growth period. Cu-contaminated soil (Unt, black), amended with biochar (B, dark grey) or biochar + compost (BC, light grey). Mean values per treatment (n= 10). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

Table. 3. Shoot ionome of *Vicia faba*

Treatment	Shoot nutrient concentrations (g kg^{-1})						Shoot metal concentrations (mg kg^{-1})	
	Ca	Fe	K	Mg	Na	P	Mn	Zn
Unt	13 ± 11 a	0.4 ± 0.3 a	14 ± 11 b	2 ± 1 b	0.6 ± 0.5 b	3 ± 2 a	93 ± 78 a	64 ± 31 a
B	22 ± 16 a	0.2 ± 0.08 b	19 ± 12 ab	4 ± 2 ab	1.2 ± 0.8 **a**	3 ± 2 a	59 ± 39 a	48 ± 17 b
BC	11 ± 3 a	0.3 ± 0.1 ab	29 ± 7 **a**	5 ± 2 **a**	1.3 ± 0.4 ab	4 ± 1 a	36 ± 11 a	53 ± 13 ab
Common values *	1 – 50	0.02 – 0.3	20 – 50	1.5 – 3.5	-	1.6 – 6.0	50 – 500	10 – 150

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). Mean values followed by letters in bold are significantly higher as compared to the Unt soil. * (Tremel-Schaub and Feix, 2005)

4. Conclusion and perspectives

The incorporation of biochar (2.5% w/w), alone or in combination with compost (5% w/w), has the potential to increase root and shoot DW yields of *V. faba* as compared to the Unt soil, but only the shoot yield was similar to that for plants grown in the uncontaminated soil. The harvested shoots of the B and BC plants displayed shoot Cu concentration slightly above its common range. This field experiment highlighted how the pine bark-derived biochar, alone and in combination with compost, is able to enhance root and shoot DW yields and reduce the shoot Cu concentration. However, in month 5, *A. donax* and *P. nigra* did no display any difference between the amended and untreated soils.

Furthermore, as we wanted to minimize the management practice on site, we decided to irrigate the plants only when necessary along the year. In August the industrial site in which the platform is based was closed and plant irrigation was impossible. Moreover, in summer 2016, Aquitaine faced a severe drought and the plants did not either grow this year and even some of them died. This emphasized the importance of irrigation for soil revegetation. During their first years young plants must be irrigated until their root system grown enough to empower them in their water supply. Further researches are needed on this Cu-contaminated site and new measures of plants growth will be repeated next year.

5. References

Baize, D., 1997. Un Point sur les Teneurs Totales en Eléments Traces Métalliques dans les Sols. First ed. Paris, France:INRA Éditions.

Baize, D., Guide des analyses en pédologie. 2000. Second ed. Paris, France : INRA Éditions. 257 p.

Bakshi, S., He, Z.L., Harris W.G., 2014. Biochar amendment affects leaching potential of copper and nutrient release behavior in contaminated sandy soils. J. Environ. Qual. 43, 1894–1902. doi:10.2134/jeq2014.05.0213

Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480. doi:10.1016/j.envpol.2010.10.016

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization. Environ. Pollut. 156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Buss, W., Kammann, C., Koyro, H.W., 2012. Biochar reduces copper toxicity in *Chenopodium quinoa* Willd. in a sandy soil. J. Environ. Qual. 41, 1157–1165. doi:10.2134/jeq2011.0022

INDIQUASOL., 2016. Indicator of soil quality data base. INRA, Orleans. Available online at http://acklins.orleans.inra.fr/geoindiquasol/index.php

Jamieson, T., Sager, E., Guégue, C., 2014. Characterization of biochar-derived dissolved organic matter using UV–visible absorption and excitation–emission fluorescence spectroscopies. Chemosphere. 103, 197–204. doi:10.1016/j.chemosphere.2013.11.066

Jones, S., Bardos, R.P., Kidd, P., Mench, M., de Lei, j.F., Hutchings, T., Cundy, A., Joyce, C., Soja, G., Friesl-Hanl, W., Herzig, R., Menger, P., 2016. Biochar and compost amendments enhance copper immobilisation and support plant growth in contaminated soils. J. Environ. Manag. 171, 101–112. doi:171;101:112.10.1016/j.jenvman.2016.01.024

Kabata-Pendias, A., Pendias, H., 1984. Trace Elements in Soils and Plants, CRC Press, Boca Raton, Florida.

Kidd, P., Mench, M., Álvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Jansseng, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J., Puschenreiter, M., 2015. Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int. J. Phytorem. 17, 1005–1037. doi:10.1080/15226514.2014.1003788

Lehmann, J., 2007. A handful of carbon. Nature. 447, 143–144. doi:10.1038/447143a

Mackie, K.A., Marhan, S., Ditterich, F., Schmidt, H.P., Kandeler, E., 2015. The effects of biochar and compost amendments on copper immobilization and soil microorganisms in a temperate vineyard. Agr. Ecosyst. Environ. 201, 58–69. doi:10.1016/j.agee.2014.12.001

Namgay, T., Singh, B., Singh, B.P., 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.). Aust. J. Soil Res. 48, 638–647. doi:10.1071/SR10049

Oustrière, N., Marchand, L., Galland, W., Gabon, L., Lottier, N., Motelica, M., Mench, M., 2016a. Influence of biochars, compost and iron grit, singly and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Science of the Total Environment. 566–567, 816–825. doi:10.1016/j.scitotenv.2016.05.091

Oustrière, N., Marchand, L., Lottier, N., Motelica, M., Mench, M., 2017. Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site.Science of the Total Environment. Science of the Total Environment. 579, 620–627. doi: 10.1016/j.scitotenv.2016.11.048

Rodríguez-Vila, A., Covelo, E.F., Forján, R., Asensio, V., 2014. Phytoremediating a copper mine soil with *Brassica juncea* L., compost and biochar. Environ. Sci. Pollut. Res. 21, 11293–11304. doi:10.1007/s11356-014-2993–6

Saadani, O., Fatnassi, I.C., Chiboub, M., Abdelkrim, S., Barhoumi, F., Jebara, M., Jebara, S.H., 2016. In situ phytostabilisation capacity of three legumes and their associated Plant Growth Promoting Bacteria (PGPBs) in mine tailings of northern Tunisia. Ecotox. Environ. Safe. 130, 263–269. doi:10.1016/j.ecoenv.2016.04.032

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers: EDP Sciences, Les Ulis. p 106.

Uchimiya, M., Bannon, D.I.., 2013a. Solubility of lead and copper in biochar-amended small arms range soils: Influence of soil organic carbon and pH. J. Agric. Food Chem. 61, 7679−7688. doi:10.1021/jf401481x

Villanneau, E., Perry-Giraud, C., Saby, N., Jolivet, C., Marot, F., Maton, D., Floch-Barneaud, A., Antoni, V., Arrouays, D., 2008. Détection de valeurs anomaliques d'éléments traces métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. Etude et Gestion des Sols. 15(3), 183–200.

Xu, X., Huang, Z., Wang, C., Zhong, L., Tian, Y., Li, D., Zhang, G., Shi, J., 2015. Toxicological effects, mechanisms, and implied toxicity thresholds in the roots of *Vicia faba* L. seedlings grown in copper-contaminated soil. Environ. Sci. Pollut. Res. 22, 13858–13869. doi:10.1007/s11356-015-5073-7

Part. 2. Biomass production with (essential) metal(loid) concentrations within the common ranges usable by local biomass processing chains

2.4. Summary

<u>*Reminder*</u>: The European Union has established a directive to increase the production of renewable energy sources and the biofuel proportion by at least 10% by 2020, aiming at reducing global warming and gradually displacing fossil fuels. Using arable land for non-food crops is not an economically and politically acceptable option, but valorizing marginal land (i.e. idle land, land contaminated by metal(loid)s) may be one. In Aquitaine, Bordeaux mixture effluent (BM, $Ca(OH)_2 + CuSO_4$) is the most Cu-based fungicide used in vineyards, orchards, and some cash crops. Cu-based salts were long-time used as wood preservatives to control insects and fungi. At wood preservation site, their long time use combined with washings of treated wood often results in soil Cu contamination. "In situ stabilization" may be implemented at such sites to promote the production of a biomass with metal(loid) concentrations within the common ranges. Biochars are carbon-rich carbonized residues produced by waste biomass pyrolysis under high temperatures and mid to low oxygen. Amending soils with biochar can improve soil quality (i.e. CEC, pH, nutrient stock) while contributing to TE immobilization. The combination of biochar with other amendments such as compost and iron oxides may also be a suitable option to increase TE immobilization while buffering nutrient depletion in metal(loid)-contaminated soils, thus limiting soil phytotoxicity. However, before implementing a remediation option in the large scale, advanced management procedures for contaminated land based on several phases must be followed: (1) risk assessment, (2) option appraisal, and (3) feasibility and implementation of a remediation strategy.

A pot experiment aimed at assessing the potential benefits and drawbacks posed by adding biochar derived from either animal or plant feedstocks, i.e. poultry manure and pine bark chips, compost and iron grit, alone and in combination, in a Cu-contaminated soil from a wood preservation site (**Part 2, Chapter 1**). A biotest realized with dwarf beans (*Phaseolus vulgaris* L.) used as bioindicator allowed to evaluate the effect of the amendment(s) on soil phytotoxicity. This case study showed how tricky it is to use biochar in a Cu-contaminated soil, as at short-term DOM-Cu complexes may increase Cu leaching out of the root zone, thus potentially increasing the Cu-related pollutant linkages. The choice of appropriate biochar and combinations of amendments may counteract this effect. The most efficient amendment, i.e. incorporation of pine bark-derived biochar in combination with iron grit into the Cu-contaminated soil, allowed the highest decreases of free and total Cu in the soil pore water and shoot Cu concentration, leading to the lowest phytotoxicity.

In situ stabilization of mono-contaminated soil (i.e. Cu-contaminated) with biochar is somewhat easier than for poly-contaminated soils (e.g. Cd, Pb and Zn-contamination) as the liming effect of biochar may decrease the mobility of some metals while mobilizing some others (e.g. As). In a second study we wanted to assess whether the relevant amendment to decrease the phytotoxicity of the Cu-contaminated soil was also effective in a Cd, Pb and Zn poly-contaminated soil. A second pot experiment aimed at investigating the efficiency of a biochar derived from pine bark chips, alone and in combination with either compost or iron grit, to stabilize Cd, Pb and Zn in an agricultural, contaminated soil from the Arnoldstein area (**Part 2, Chapter 2**). Once again, the incorporation of pine bark-derived biochar in combination with iron grit into this soil allowed the highest decrease of Cd, Pb and Zn concentrations in the soil pore water, while reducing shoot Cd, Pb and Zn concentrations to fit the range of common values for plants. In both studies (**Chapters 1 and 2**) incorporation of pine bark-derived biochar in combination with iron grit did not promote the growth of dwarf bean (*Phaseolus vulgaris* L.). These studies were however implemented on the short-term (i.e. a 3-month reaction period followed by a 2-week plant testing) and potential changes in the phytotoxicity of biochar-amended soils need to be considered over time.

Thus, in a third study (**Part 2, Chapter 3**), we evaluated the mid-term effect of amending the Cu-contaminated soil with biochar, alone or in combination with iron grit on soil phytotoxicity during 2 years. As the costs associated with the phytomanagement option using iron grit in combination with biochar are relatively high, the culture of fast growing plant biomass is needed to provide additional economical value and partly reduce the remediation costs. Poplar (*Populus nigra L*) and Giant reed (*Arundo donax* .L) are two energy crops which can be used to phytomanage contaminated soils. Potential uses for their biomasses are multiple: (1) energy sector, (2) biochar production, (3) biosourced (bio)chemistry including high-value platform chemicals and biocatalysis, (4) lignocellulosic derived bioproducts and (5) cellulosic and paper industry, (6) pesticides (only for *Arundo donax*). Thus, we also aimed at evaluating the feasibility of growing poplars and giant reeds as relevant species for biomass production in such in situ stabilized Cu-contaminated soil. This case study shows how tricky it is to use biochar in a long-term basis, as amendments and soil components evolve over the time and some biotic and abiotic processes may remobilize the sorbed contaminant. The long-term effect of biochar amendment, with and without iron grit, depended on the cultivated plant species. Cultivation of *A. donax* enhanced the dissolved organic matter (DOM) concentration in the SPW, which decreased its Cu^{2+} concentration but promoted its total Cu concentration. Cultivation of *P. nigra* increased the SPW Cu^{2+} concentration. In contrast to poplar, giant reed promoted the potential leaching of Cu out of the root-zone. Adding iron grit with biochar into the Cu-contaminated soil counteracted the mobilization of total Cu and Cu^{2+} concentrations in the pots cultivated by *A. donax* and *P. nigra* as compared to biochar alone. It also reduced the shoot Cu concentrations of poplar and giant reed to fit the range of common Cu values for plant shoots. However it did not enhance the root and shoot yields of both species.

The reliability of pot studies for predicting the growth and performance of plants in the field is often inaccurate, because pot environments differ from field conditions, mainly through the edge effect. Following pot experiments, it is thus commonly preconized to test the selected phytomanagement option directly on site in field plots. This step will allow to detect potential failures of phytomanagement option due to long-term changes, such as the aging of amendments added to the soil, inter-annual climate variability, pest attacks, deposition and accumulation of litter, release of soluble organic matter, changes in animal and plant communities (Kidd et al., 2015). Based on these studies and on literature review, field plots amended with biochar, alone or in combination with compost, have been implemented at the Cu-contaminated site used for wood preservation for assessing the sustainable biomass production of poplar and Giant reed and the life cycling of such phytomanagement option. These investigations are reported in (**Additional information**). In summer 2016, we faced a drought in Aquitaine and the plant growth was affected. This in situ stabilization experiment must be continued next year with regular irrigation of young plants. Failure of the implementation of the in situ stabilization strategy shows why it is important to follow the management procedures for contaminated land.

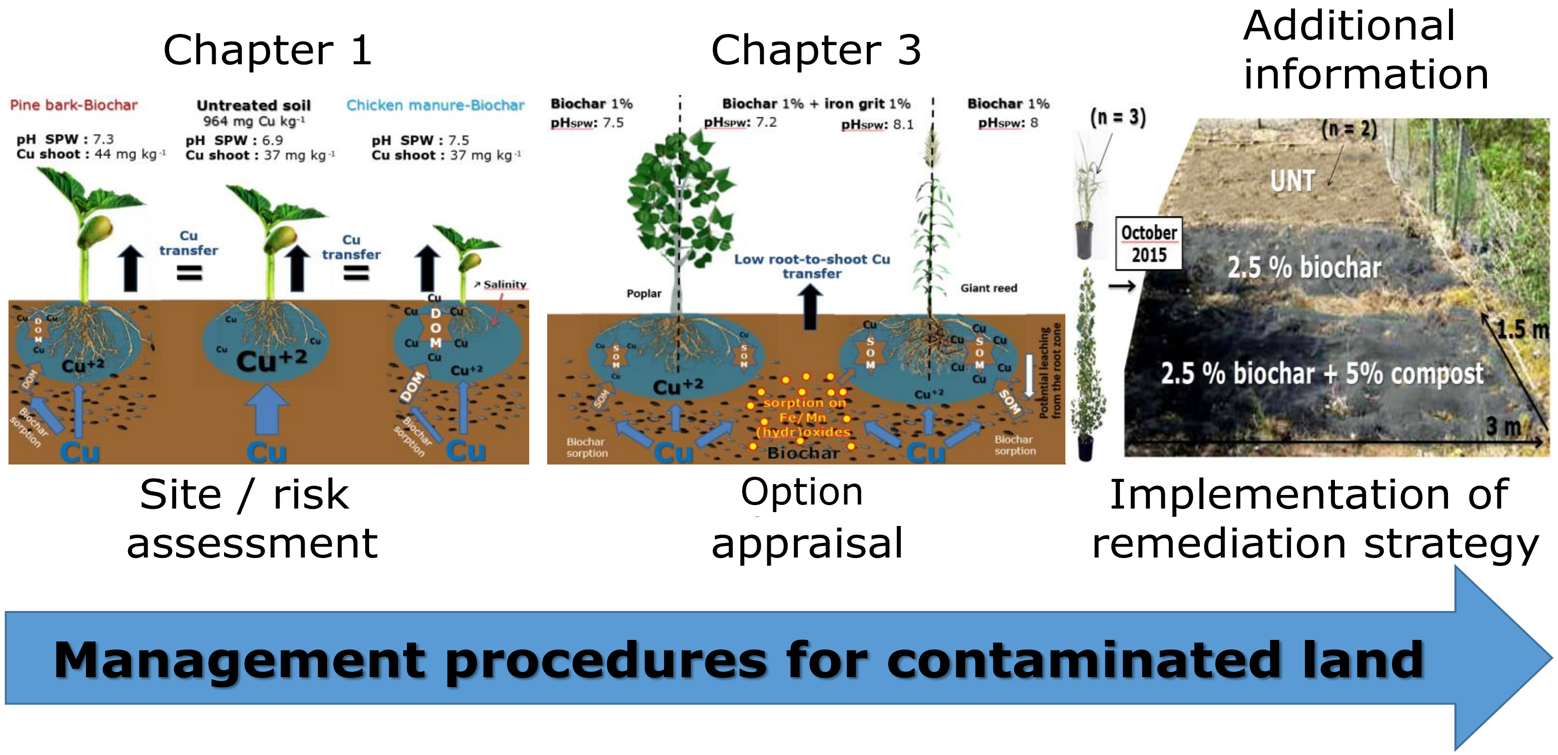

Chapter 1
Chapter 3
Additional information
Pine bark-Biochar
Untreated soil
964 mg Cu kg-1
Chicken manure-Biochar
pH SPW : 7.3
Cu shoot : 44 mg kg-1
pH SPW : 6.9
Cu shoot : 37 mg kg-1
pH SPW : 7.5
Cu shoot : 37 mg kg-1
Cu transfer
Cu transfer
Salinity
Cu+2
Biochar sorption
DOM
Biochar 1%
pHSPW: 7.5
Biochar 1% + iron grit 1%
pHSPW: 7.2
pHSPW: 8.1
Biochar 1%
pHSPW: 8
Poplar
Giant reed
Low root-to-shoot Cu transfer
sorption on Fe/Mn (hydr)oxides
Biochar
SOM
Potential leaching from the root zone
(n = 3)
(n = 2)
October 2015
UNT
2.5 % biochar
2.5 % biochar + 5% compost
1.5 m
3 m
Site / risk assessment
Option appraisal
Implementation of remediation strategy
Management procedures for contaminated land

Table of contents - Part 3

Part. 3. Biomass production with high metal(loid) concentrations able to integrate recovery Eco-catalysis processing chains

3.1. Option appraisal

3.1.1. Chapter 4 - Potential of macrophyte roots for producing Cu-ecocatalysts

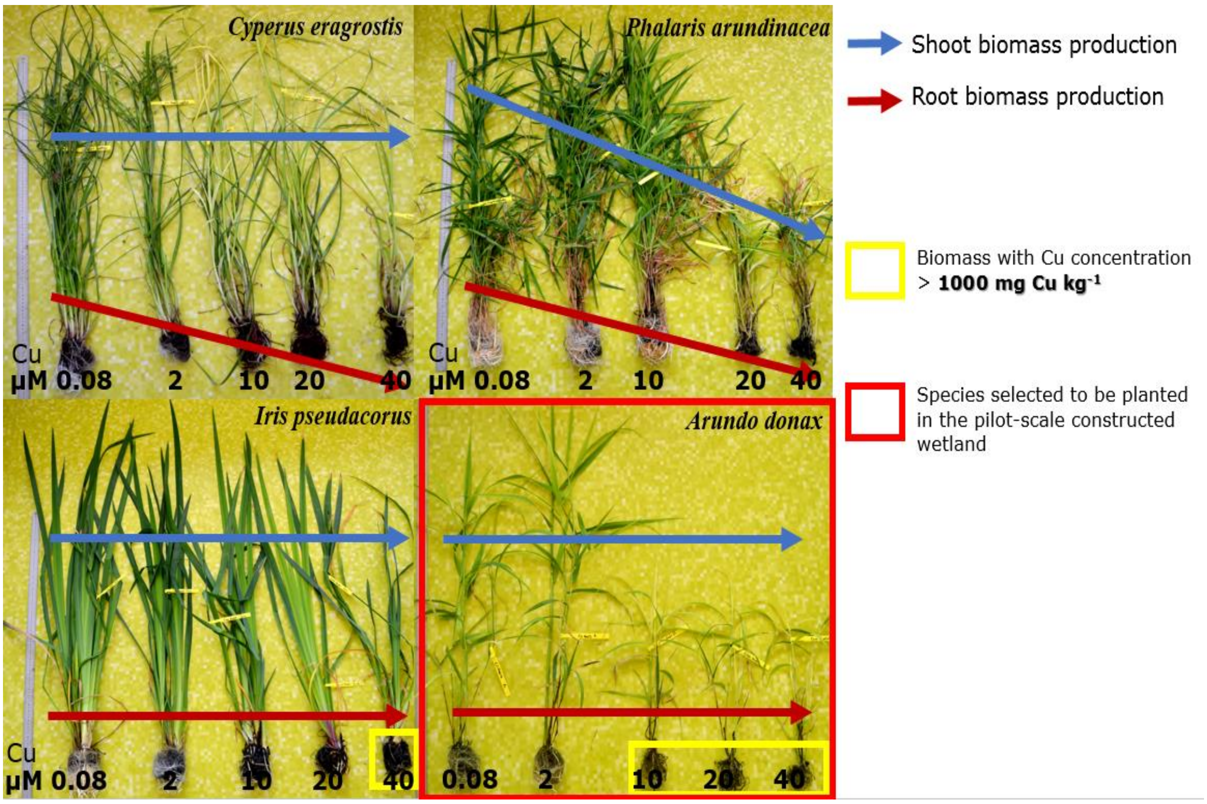


Fig. E. Graphical abstract – Chapter 4

Potential of macrophyte roots for producing Cu-ecocatalysts

Nadège Oustriere[1*], Lilian Marchand[1], Eli Roulet[1], Camille Rousset[1], Francois Bordas[2], Michel Mench[1]

[1]BIOGECO, INRA, UNIV. BORDEAUX, 33615 PESSAC, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; elicas@hotmail.fr; cam.rousset@laposte.net; mench@bordeaux.inra.fr;

[2] GRESE, UNIV. LIMOGES, 123 AVENUE ALBERT THOMAS, FR-87060, LIMOGES, FRANCE.

E-mails: francois.bordas@unilim.fr

Abstract

The ability of *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L., and *Phalaris arundinacea* L. to produce Cu-rich plant biomass (so-called Cu-ecocatalyst) along a Cu concentration gradient (0.08, 2, 10, 20 and 40 µM Cu) was assessed in batch conditions. Copper exposure affected the root DW yield of *C. eragrostis* in the 2 - 40 µM Cu range. This plant species was not relevant for producing Cu-ecocatalyst. *Phalaris arundinacea* produced root and shoot biomasses with concentration lower than 1000 mg Cu kg^{-1} DW in the 0.08 – 40 µM Cu range and was not relevant for producing Cu-ecocatalyst. *Iris pseudacorus* produced a high shoot biomass with Cu concentration within the common values and a root biomass with high Cu concentration. This shoot biomass can integrate regular recovering processing chains such as the energy sector. *Arundo donax* displayed low root and shoot biomasses with high Cu concentrations. Root Cu concentration exceeded 1000 mg Cu kg^{-1} DW at 40 µM Cu for *I. pseudacorus* and over 10 µM Cu for *A. donax*, their roots being relevant for producing Cu-ecocatalysts.

Keywords: Biocatalysis, Biomass valorization, Bioresource, Rhizofiltration, Phytoremediation, Phytomanagement.

1. Introduction

In Aquitaine, France, Bordeaux mixture (BM, Ca $(OH)_2$ + $CuSO_4$) is frequently used as Cu-based fungicide (Mackie et al., 2012). Filling and rinsing the tanks of BM crop sprayers generate significant effluent amounts. Based on the 150.000 ha of Aquitaine vineyards, the total volume of BM effluents is estimated at 2.500.000 L (Maille, 2004). The spreading of these diluted BM effluents on the field is authorized by the Article L. 253-1 of the rural Code. Its long-lasting application in Bordeaux vineyards contributes to locally increase total soil Cu (i.e. > 1000 mg Cu kg^{-1}) above the inquiry threshold values in topsoils (RMQS : 40.2 mg Cu kg^{-1} DW, El Hadri al., 2012). Diffuse migration of Cu and soil erosion result in Cu contamination of surface waters in the Gironde estuary (i.e. 0.2-1.3 µg L^{-1}; IFREMER, 2014) above the upper critical threshold value defined by the French Water Agency for freshwater quality (0.1 µg L^{-1} Cu; SEQ EAU, 2003). This may threat flora and fauna biodiversity, e.g. abundance and species composition of marine organisms such as mollusca and arthropoda (Baker et al., 2014), bacteria, fungi, (Taylor and Walker, 2016), and algae communities (Rocha et al., 2016). Rhizofiltration, notably in constructed wetlands (CW), is a green way for treating contaminated wastewaters and effluents (Marchand et al., 2010; Wu et al., 2015a). The management of BM effluents in CW may be an efficient and cost-effective solution to prevent ecosystem exposure to Cu excess. Constructed wetlands planted with macrophytes were successfully implemented, aiming at purifying Cu-contaminated effluents (Guittonny-Philippe et al., 2014; Marchand et al., 2014c; Newete and Byrne, 2016), mainly by immobilization in the rhizosphere and storage in the belowground biomass (Marchand et al., 2010; Istenič et al., 2012; Bonanno, 2012; Ben Salem et al., 2014). More specifically hydroponic root mats are ecotechnological wastewater treatment systems where aquatic vegetation forms buoyant filters by their dense interwoven roots and rhizomes, sometimes supported by rafts or other floating materials (Chen et al., 2016). In mesocosm tanks with plant root mats (*Carex virgate* (Boott) Cheeseman, *Cyperus ustulatus* A. Rich., *Juncus edgariae* L.A.S. Johnson & K.L.Wilson, and *Schoenoplectus. tabernaemontani* (C.C. Gmel.) Palla) used to treat artificial stormwater contaminated in Cu and Zn, achieved removal rates were 3.8–6.4 mg m^{-2} day^{-1} for Cu and 25–88 mg m^{-2} day^{-1} for Zn (Tanner and Headley, 2008). The biomass produced in such system display both root and shoot Cu concentrations above the common Cu values in plants (3 – 20 mg Cu kg^{-1}, Tremel-Schaub and Feix, 2005) which cannot integrate regular recovering processing chains.

Biocatalysis, based on the novel use of metal species originating from plant biomasses with high metal(loid) concentrations (unusual oxidation levels, new associated chemical species, and effects of synergy) is an emerging technique (Clavé et al., 2016a). Such biomasses produced metal-ligand complexes used as "Lewis acids" to catalyze fine organic chemical reactions for the synthesis of molecules with high added value: pharmaceuticals (e.g. anticancer and antiviral agents), cosmetics, agrochemicals (e.g. green pesticides) and textiles (Escande et al., 2015). New ecocatalysts are needed to increase the number of potential catalyze reactions and among metal(loid)s, Cu is of interest (Clavé et al., 2016a). Cu-based catalysts are sustainable and cost-competitive catalyzers for the high yield production of next-generation biorefinery components (Yuan et al., 2013). High Cu concentrations (i.e. ≥1000 mg kg^{-1} DW) are needed to meet the requirement for biocatalysis (Clavé et al., 2016b). Such high shoot Cu concentration is unusual in plants. Excluders are plants that accumulate metal(loid)s in their roots while avoiding translocation to their shoots. Such plant species often showed high root Cu concentration. Out of the excluder list, some macrophytes reached Cu concentration ≥1000 mg kg^{-1} root DW (Newete and Byrne, 2016). Concentrations up to 25000 mg Cu kg^{-1} DW were reached in the roots of *Typha* sp. exposed to a concentration of 2.52 µM Cu (Valipour et al., 2014) and 2610 mg Cu kg^{-1} DW in the roots of *Cyperus alternifolius* L. exposed to a concentration of 16.5 µM Cu (Cheng et al., 2002). In the roots of *Iris pseudoacorus* L. exposed to a concentration of 0.7 µM Cu, Cu concentrations peaked at 1430 ± 170 mg Cu kg^{-1} DW (Sun et al., 2013). Based on the ability of macrophytes to store metal(loid) in their belowground biomass, their use to clean Cu-contaminated effluents may provide both interwoven root and rhizome mats with high Cu concentration, potentially usable as Cu-ecocatalysts.

This study aimed at investigating the capacity of macrophytes frequently used in CWs to produce Cu-rich root and rhizome mats. Four macrophytes, i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *I. pseudacorus,* and *Phalaris arundinacea* L. were assessed along a Cu concentration gradient in controlled batch conditions. Copper concentration and accumulation in the roots and shoots of macrophytes as well as root and shoot dry weight (DW) yields and chlorophyll fluorescence parameters were determined.

2. Material and Methods

2.1. Plants

Plant samplings of *A. donax, C. eragrostis, I. pseudacorus,* and *P. arundinacea* were realized in October 2014. Based on Marchand et al., (2014c), the root DW yield of *I. pseudacorus* and *P. arundinacea* (sampled on-sites with soil Cu concentration in the 2.9-205 mg Cu kg^{-1} range) exposed to a Cu gradient (0.8- 25 µM) depended on the population established at the sampling site. For maximizing their biomass production, we collected our macrophytes at the sites of the Bordeaux region allowing the highest root biomass production at high Cu exposure. *Iris pseudacorus* was sampled at the "Sanguinet site", a riverbank sandy soil with acid soil pH of the uncontaminated (i.e. 3.3 mg Cu kg^{-1}) Sanguinet Lake (Landes, France, 44°30'20''N; 1°08'01''E). *Phalaris arundinacea* was sampled nearby the "Lagnet site", a drainage ditch with neutral soil pH located in the vineyards of Saint-Emilion (Gironde, France, 44°54'54''N; 0°08'23''W), annually treated with the Bordeaux mixture (i.e. 27 mg Cu kg^{-1}). *Cyperus eragrostis* was sampled in the "Jalle d'Eysines 2" a riverbank with neutral soil pH along the Jalle d'Eysines River (Eysines , France, 44°53'36''N; 00°40'40''W) impacted by runoff from adjacent organic farms and located 1 km downstream from a water treatment plant (i.e. 33 mg Cu kg^{-1}). *Arundo donax* was sampled from a drainage ditch, San Remo, Italy, and cultivated since 2011 in a greenhouse (soil pH and Cu concentration unavailable for this site). For each plant species, 30-40 samples of rhizomes (*I. pseudacorus*), young plants (*C. eragrostis*), and bud-bearing stems (10-20 cm) (*P. arundinacea* and *A. donax*) were collected and rooted in individual pots (9*8*9 cm^3) on perlite imbibed with a quarter-strength Hoagland Nutrient Solution (1/4HNS, Marchand et al., 2014c) for 6 months in a greenhouse, Centre INRA-Bordeaux Aquitaine, Villenave d'Ornon, France. Culture medium (CM) was renewed every month to avoid anoxia and nutrient depletion. In March 2015, 25 standardized plants (with similar stem and root size or volume) of either *I. pseudacorus*, *P. arundinacea*, *C. eragrostis* and *A. donax* were isolated, transplanted individually in plastic bottle (1.5L) filled with 1L of 1/4HNS and grown for 1 month.

2.2. Plant exposure to Cu

In April 2015, just before the Cu exposure, macrophyte roots were blackened with activated plant coal (concentration: 1.5%, Marchand et al., 2014c). 1L of 1/4HNS was spiked with Cu ($CuSO_4.5H_2O$) to achieve five Cu concentrations: 0.08, 2, 10, 20 and 40 µM Cu (five replicates concentration^{-1}) and consist 6 series (Table 1). In the series, 25 plants (5 Cu concentrations X 5 replicates) of either *I. pseudacorus*, *P. arundinacea*, *C. eragrostis*, or *A. donax* were individually placed in the Cu-spiked solutions. All plants were randomly placed in the greenhouse and cultivated for 2 months from April to May 2015 (16 /8 h light/darkness; 65 ± 5% relative humidity; 25± 5°C). All CM were changed every six days to maintain Cu concentrations and avoid nutrient depletion and anoxia.

Table. 1. Physico-chemical parameters of the culture medium along the Cu gradient at T_0 (n=14, day 0, solution replacement) and T_6 (n=8, day 6 after solution replacement)

Total Cu added (µM)	pH	(mV) Eh	Cu^{2+} (µg L^{-1}) Measured **Cu**	Modelled **Cu**
T_0				
0.08	7.6 ± 0.3a	259 ± 43a	1.1 ± 1.2d	-
2	7.4 ± 0.2ab	248 ± 29a	1.5 ± 1.7cd	3.5
10	7.4 ± 0.1ab	262 ± 26a	9 ± 8abc	14
20	7.3 ± 0.1abc	282 ± 38a	16 ± 9a	17
40	7.3 ± 0.2 abc	285 ± 37a	19 ± 10a	19
T_6				
0.08	7.0 ± 0.3d	272 ± 16a	1.2 ± 1.6d	-
2	7.0 ± 0.2d	254 ± 24a	0.6 ± 0.7d	9
10	6.9 ± 0.2d	258 ± 24a	1.2 ± 2.2d	39
20	7.1 ± 0.2cd	265 ± 25a	2.0 ± 1.7bcd	29
40	7.2± 0.1bcd	268 ± 19a	9 ± 7ab	18

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05).

2.3. Solution and plant analysis

The pH (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34), redox potential (Eh) and Cu^{2+} concentration (Cupric ion electrode, Fischer Bioblock, USA) were randomly measured for all series (n=2 for each Cu concentration, Table 1) after (T_0, day 0) and before (T_6, day 6) changing the growing solutions. Comparatively, potential Cu speciation in the solutions at T_0 and T_6 was computed using the MINEQL+4.6 software (Table 1, Marchand et al., 2014c). After 2 months of Cu exposure, chlorophyll fluorescence parameter: maximum efficiency of Photosystem II (PSII) (Fv/Fm ratio), real efficiency of PSII [Y(II)] and non photochemical quenching (qN), were measured for all plants using a portable modulated fluorometer (Pam-2500 Waltz, Germany) (Marchand et al., 2016). Then, roots and shoots were harvested. The black and white parts of roots formed respectively before (black-stained) and after Cu exposure were separated. Root and shoot samples were washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60°C to constant weight for 72h and then weighed for determining the shoot and root DW yields. For all plants, dried white roots and shoots were ground (< 1 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW) were wet-digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, P, K and Na) in digests was determined by ICP-MS (Thermo X series 200, INRA USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. All element concentrations in plant parts are expressed in mg or g

DW kg^{-1}. For white roots and shoots, Cu removal was calculated as follows: Cu (mg $plant^{-1}$) = DW yield (g $plant^{-1}$) x Cu concentration (mg kg^{-1} DW).

2.4. Statistical analysis

Influence of Cu exposure on Cu concentration and accumulation in roots and shoots as well as root and shoot DW yields were tested using one-way analysis of variance (ANOVAs) for each plant species. Crossed effects of both Cu exposure and plant species on root and shoot Cu concentrations and accumulations and root and shoot DW yields were analyzed using a two-way ANOVA. Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests. For the two-way ANOVA, there was a crossed effect for all plant parameters studied, except the root DW yield, and a HSD test was performed on these plant parameters. A post Hoc Tukey HSD test was performed for the root DW yield. Differences were considered statistically significant at $p<0.05$. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results

3.1. Physico-chemical parameters of the solution culture

At T_0 and T_6, the pH in the CM was neutral. It did not change across the Cu gradient but is significantly different between T_0 and T_6 (Table 1). At T_0 and T_6, the Eh was slightly oxidative and did not significantly change across the Cu gradient. At T_0, the Cu^{2+} concentration in the CM, being potentially available for root uptake, significantly increased along the Cu gradient, which was consistent with the modeled Cu^{2+} concentrations and the increasing total Cu concentration in the spiked solutions. At T_6, Cu^{2+} concentration in the CM decreased as compared to T_0 for all Cu levels except at 40µM Cu, reaching a 0.6-2 µg L^{-1} range. This decrease in Cu^{2+} concentration may be due to Cu sorption on the batch surface, chemical and biological reactions, notably root and microbe uptake and complex formation with ligands from the rhizodeposition (Marchand et al., 2014c). After a 6-day exposure, the model predicted much higher Cu^{2+} concentrations than the measured ones.

3.2. Root and shoot DW yields

The shoot DW yield (g $plant^{-1}$) of *C. eragrostis* did not significantly change across the Cu gradient, ranging from 8 ± 4.6 at 40 µM Cu to 16 ± 4.7 at 0.08 µM Cu (Fig. 1A, Table 2). Conversely, its root DW yield was 5 time lower when exposed to 2 µM Cu as compared to 0.08 µM Cu and roots stopped to growth at 40 µM Cu. For *I. pseudacorus*, the shoot and root DW yields (g $plant^{-1}$) remained similar across the Cu gradient, varying from 15 ± 8 at 20 µM Cu to 26 ± 9 at 0.08 µM Cu and from 0.9 ± 0.2 at 40 µM Cu to 1.9 ± 1.2 at 0.08 µM Cu, respectively (Fig. 2A, Table 2). For *P. arundinacea,* the shoot and root DW yields significantly decreased when plants were exposed to ≥ 20 µM Cu as compared with 0.08 µM Cu (i.e. 6 ± 3 and 25 ± 3 for the shoots and 2 ± 0.6 and 0.6 ± 0.2 for the roots, respectively) (Fig. 3A, Table 2). The shoot DW yield of *A. donax* was significantly higher at 2 µM Cu than at 0.08 µM Cu, highlighting a hormesis effect as defined by Calabrese and Blain (2009) but did not significantly differ in the 10 - 40 µM Cu range. The root DW yield of *A. donax* was only significantly lower at 40µM Cu as compared to 0.08 µM Cu (Fig. 4A, Table 2).

At 40 µM Cu, the shoot biomass of tested macrophytes (g $plant^{-1}$) ranked in the increasing order: *A. donax* (2.8 ± 1.2 b) < *C. eragrostis* (8 ± 4.6 b) < *P. arundinacea* (9.6 ± 4.1 ab) < *I. pseudacorus* (17 ± 6 a); for the roots, the increasing order was: *C. eragrostis,* (0 ± 0 b) < *A. donax* (0.13 ± 0.11 b), < *I. pseudacorus* (0.8 ± 0.12 a) < *P. arundinacea* (1.0 ± 0.5 a) (Fig. 5A, Table 3).

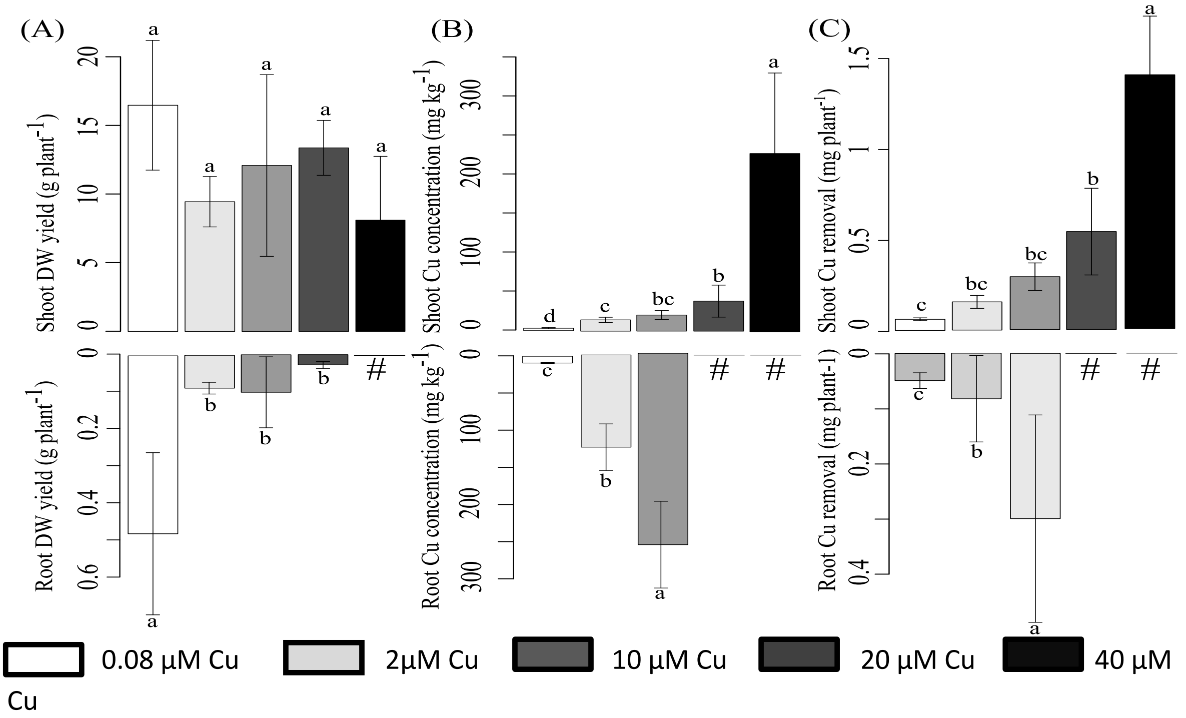


Fig. 1. *Cyperus eragrostis*: (A) shoot and root DW yields (g DW plant^{-1}), (B) Cu concentration (mg kg^{-1}) in and (C) Cu removal (mg plant^{-1}) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value<0.05). # These plants did not produce either new roots or enough biomass to be wet-digested.

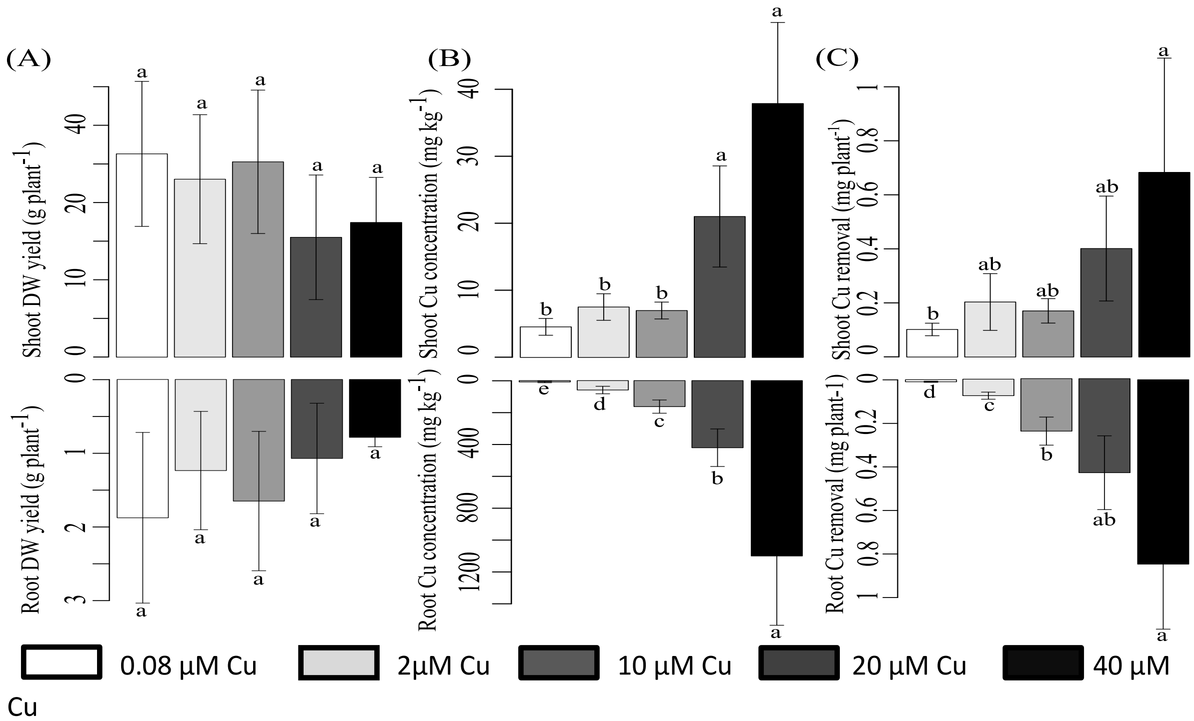


Fig. 2. *Iris pseudacorus*: (A) shoot and root DW yields (g DW plant^{-1}), (B) Cu concentration (mg kg^{-1}) in and (C) Cu removal (mg plant^{-1}) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

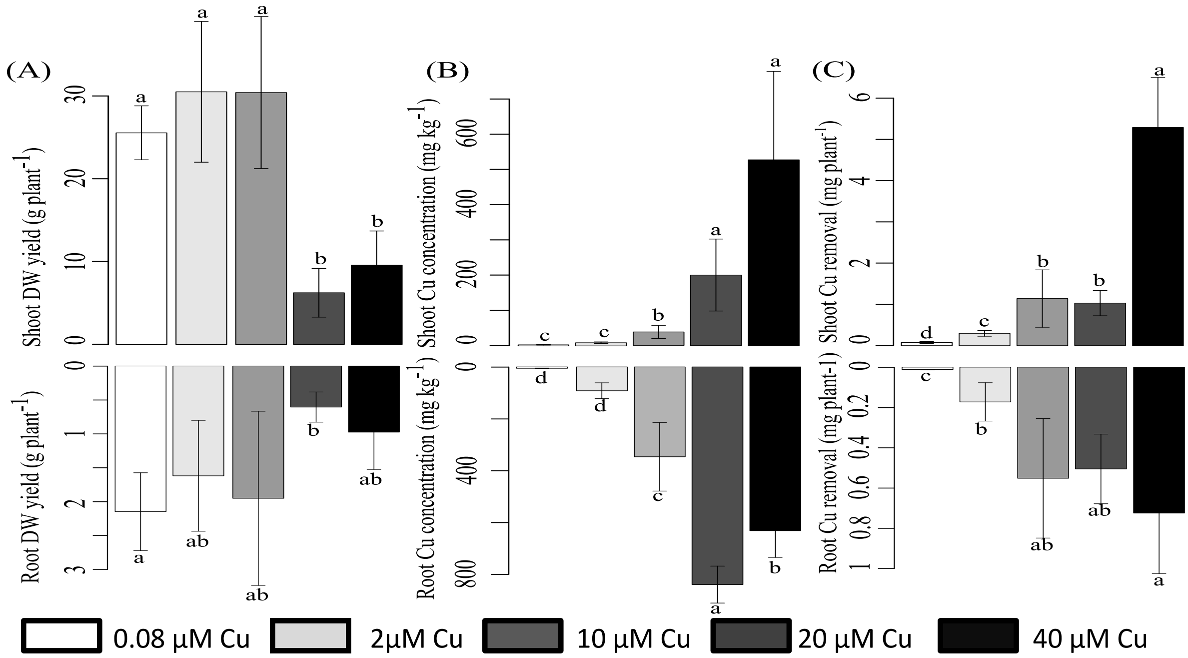


Fig. 3. *Phalaris arundinacea*: (A) shoot and root DW yields (g DW plant^{-1}), (B) Cu concentration (mg kg^{-1}) in and (C) Cu removal (mg plant^{-1}) by the shoots and roots after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

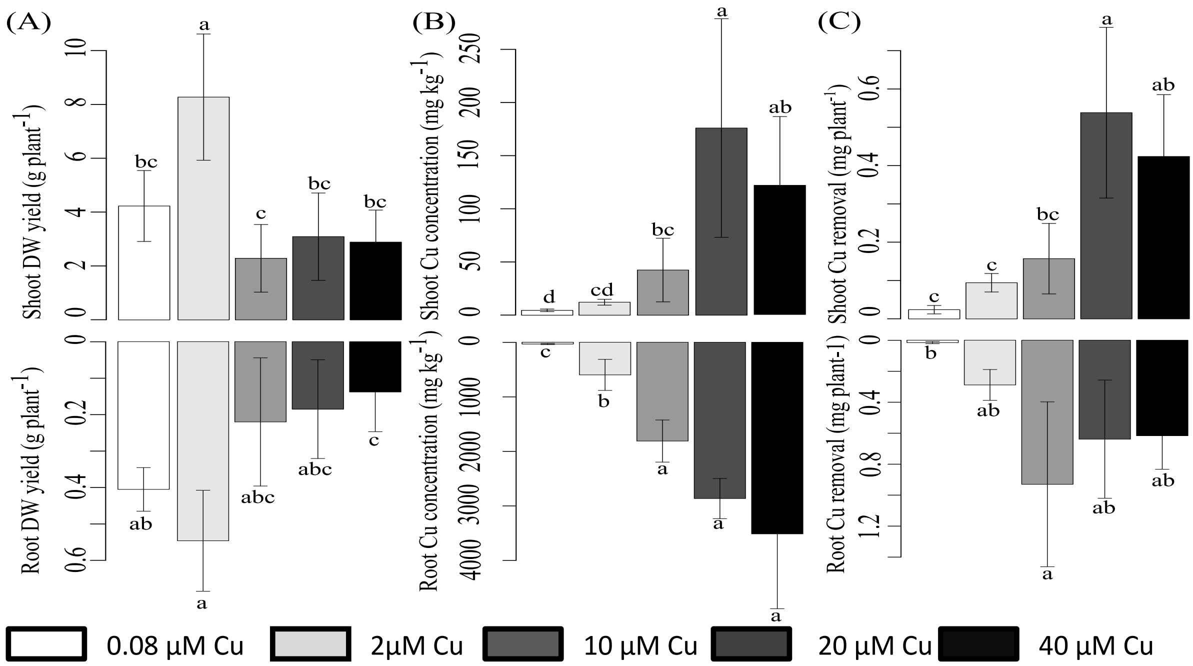


Fig. 4. *Arundo donax*: (A) shoot and root DW yields (g DW plant^{-1}), (B) Cu concentrations (mg kg^{-1}) in and (C) Cu removal (mg plant^{-1}) by the shoots and roots of *A. donax* after a 2 month-exposure to the Cu gradient. Mean values per treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value<0.05).

3.3. Root and shoot Cu concentrations

Copper concentrations (mg Cu kg^{-1}) in *C. eragrostis* significantly increased with the Cu gradient ranging from 3.4 ± 0.8 at 0.08 µM Cu to 246 ± 111 at 40 µM Cu for the shoots and from 9 ± 0.7 at 0.08 µM Cu to 256 ± 58 at 10 µM Cu for the roots (Fig. 1B, Table 2). The shoot Cu concentration of *I. pseudacorus* increased at 20 µM Cu and peaked at 40 µM Cu whereas its root Cu concentration linearly increased with the Cu gradient ranging from 9 ± 0.7 at 0.08 µM Cu to 1099 ± 434 at 40 µM Cu (Fig. 2B, Table 2). For *P. arundinacea*, both shoot and root Cu concentrations significantly increase at 10 µM Cu and shoot Cu concentration peaked at 40 µM Cu (i.e. 838 ± 71) (Fig. 3B, Table 2). Shoot Cu concentration of *A. donax* significantly raised at 10 µM Cu and culminated at 20 µM Cu (i.e. 175 ± 103) while root Cu concentration increased linearly with Cu exposure albeit not significantly in the 10 - 40 µM Cu range (i.e. 1809 ± 386 and 3512 ± 1372) (Fig. 4.B, Table 2).

At 40 µM Cu, shoot Cu concentration (mg Cu kg^{-1} DW) ranked in the increasing order: *I. pseudacorus* (38 ± 12 b) < *A. donax* (121 ± 64 b) < *C. eragrostis* (247 ± 111 ab) < *P. arundinacea* (527 ± 251 a). Root Cu concentration varied in the increasing order: *P. arundinacea* (631 ± 103 b) < *I. pseudacorus* (1099± 434 b) < *A. donax* (3512 ± 1372 a) (Fig. 5B, Table 3).

3.4. Root and shoot Cu removals

Shoot Cu removal (µg Cu $plant^{-1}$) of *C. eragrostis* started significantly to increase at 20 µM Cu (Fig. 1C, Table 2) and its root Cu removal linearly increased with Cu exposure from 4.9 ± 1.4 at 0.08 µM Cu to 30 ± 19 at 10 µM Cu. For *I. pseudacorus*, shoot Cu removal was only significantly higher 40µM Cu (i.e. 682 ± 422) as compared to 0.08 µM Cu (i.e. 102 ± 23) (Fig. 2C, Table 2). Its root Cu removal linearly increased across with Cu exposure and peaked up to 846 ± 298 at 40 µM Cu. For *P. arundinacea*, Cu removal increased from 75 ± 22 at 0.08 µM Cu to 5286 ± 1210 at 40 µM Cu for the shoots and from 11 ± 1.7 at 0.08 µM Cu to 723 ± 300 at 40 µM Cu for the roots (Fig. 3C, Table 2). Shoot Cu removal (µg Cu $plant^{-1}$) of *A. donax* started significantly to raise at 20 µM Cu (Fig. 4C, Table 2) and its root Cu removal plateaued at 2 µM Cu.

At 40 µM Cu, shoot Cu removal (mg Cu $plant^{-1}$) ranked in the increasing order: *A. donax* (424 ± 161 b) = *I. pseudacorus* (683 ± 423 b) = *C. eragrostis* (1394 ± 322 b) < *P. arundinacea* (5286 ± 1210 a) whereas the ranking for root Cu removal (mg Cu $plant^{-1}$) was: *A. donax* (616 ± 216 a) = *P. arundinacea* (724 ± 300 a) = *I. pseudacorus* (846 ± 298 a) (Fig. 5C, Table 3).

Table. 2. Letter of the results from the two-way analysis of variance (ANOVA) investigating the combined effects of Cu concentration in the culture medium and the plant species on plant parameters.

Plant species	**Shoot DW yield**	**Root DW yield**	**Shoot Cu concentration**	**Root Cu concentration**	**Shoot Cu removal**	**Root Cu removal**
A. donax						
0.08	fg	cd	d	e	e	e
2	fg	bcd	d	de	e	bcde
10	g	cd	cd	bc	de	a
20	fg	cd	bcd	ab	bcde	abc
40	g	d	bcd	a	cde	abcd
C. eragrostis						
0.08	bcdef	cd	d	e	e	e
2	efg	d	d	e	de	e
10	defg	d	d	de	cde	de
20	cdefg	d	cd	ND	bcde	ND
40	fg	d	b	ND	b	ND
I. pseudacorus						
0.08	abc	ab	d	e	e	e
2	abcde	abcd	d	e	de	cde
10	abcd	abc	d	e	de	bcde
20	cdefg	abcd	d	de	cde	abcde
40	abcdef	abcd	cd	cd	bcde	a
P. arundinacea						
0.08	abcd	a	d	e	e	e
2	a	abc	d	e	cde	cde
10	ab	ab	cd	de	bc	abcd
20	fg	bcd	bc	cde	bcd	abcde
40	efg	abcd	a	de	a	ab

Values with different letters differ in column significantly (two way ANOVA, p-value <0.05).
ND: not determined due to no root growth

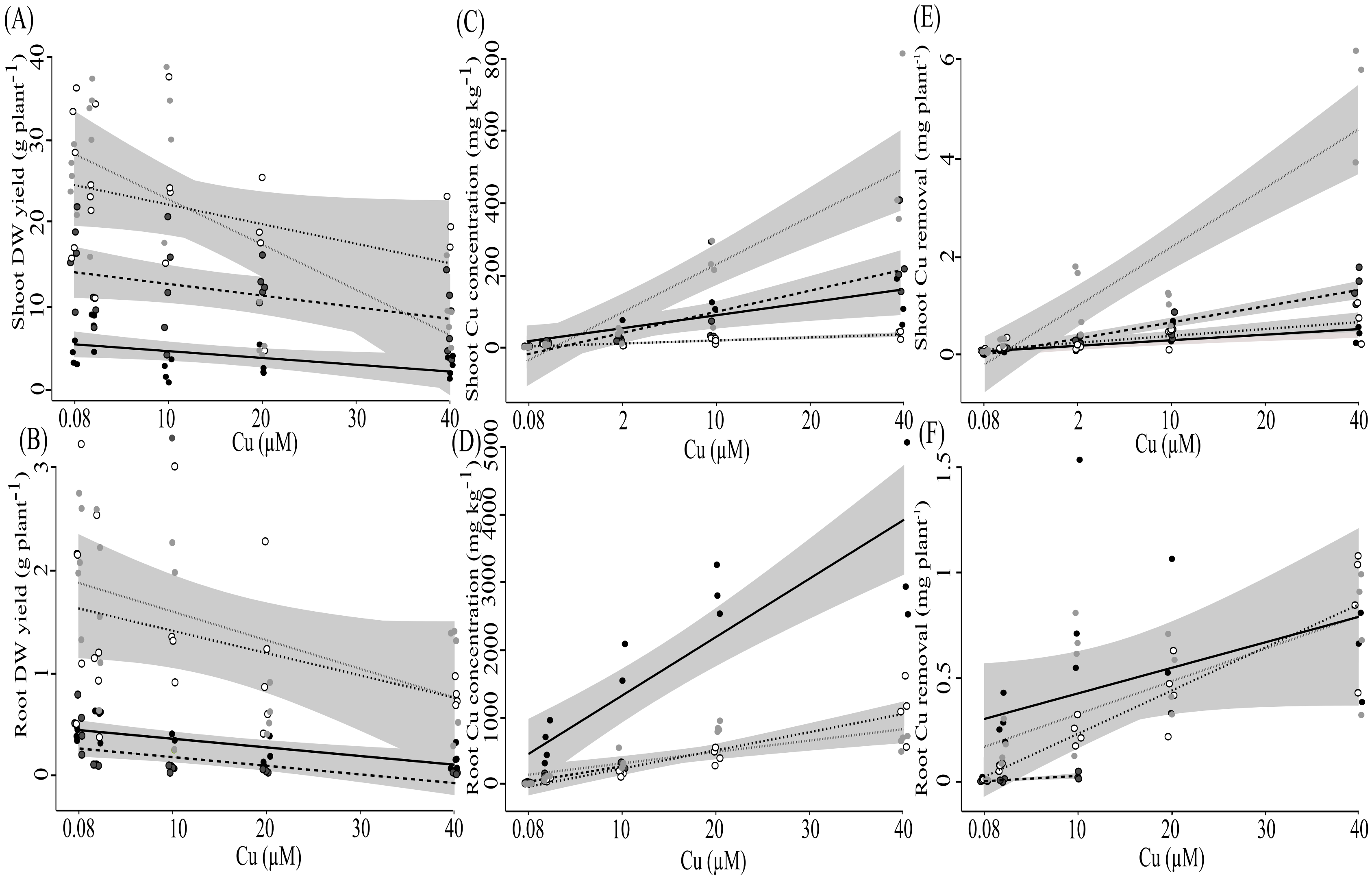


Fig. 5. ANCOVA plots for (A) shoot and (B) root DW yields, (C) shoot and (D) root Cu concentrations, and (E) shoot and (F) Cu removals (µg L^{-1}): *Cyperus eragrostis*: (⬤ and dash line), *Iris pseudacorus* (○ and dotted line), *Phalaris arundinacea* (● and close dotted line) and *Arundo donax* alone (• and black line) after 2 month-exposure to the Cu gradient.

Table. 3. Results of the ANOCA investigating the effects of Cu exposure (0.08, 2, 10, 20 and 40 µM Cu), the plant species (*A. donax, C. eragrostis, I. pseudacorus,* and *P. arundinacea*), and their interactions on plant parameters

Parameters	**Df**	**Mean sq**	**Fvalue**	**p(>F)**	**P**
Shoot DW yields					
Cu concentration	5	7885	1576.9	68.459	< 2e-16 ***
Plant species	4	1204	300.9	13.063	1.04e-08 ***
Cu concentration X Plant species	20	2019	100.9	4.381	2.50e-07 ***
Residuals	108	2488	23		
Root DW yields					
Cu concentration	5	39.59	7.918	34.019	< 2e-16 ***
Plant species	4	5.66	1.415	6.079	0.000192 ***
Cu concentration X Plant species	20	6.81	0.341	1.463	0.110505
Residuals	106	24.67	0.233		
Shoot Cu concentrations					
Cu concentration	5	212155	42431	9.625	2.84e-07 ***
Plant species	4	898152	224538	50.934	< 2e-16 ***
Cu concentration X Plant species	20	490457	24523	5.563	1.02e-08 ***
Residuals	83	365898	4408		
Root Cu concentrations					
Cu concentration	5	60185855	12037171	72.41	< 2e-16 ***
Plant species	4	81413948	20353487	122.43	< 2e-16 ***
Cu concentration X Plant species	18	36284438	2015802	12.13	7.31e-16 ***
Residuals	78	12966935	166243		
Shoot Cu removal					
Cu concentration	5	18957856	3791571	46.04	<2e-16 ***
Plant species	4	27634043	6908511	83.89	<2e-16 ***
Cu concentration X Plant species	20	39230766	1961538	23.82	<2e-16 ***
Residuals	82	6752617	82349		
Root Cu removal					
Cu concentration	5	2330377	466075	12.578	5.44e-09 ***
Plant species	4	7346277	1836569	49.563	< 2e-16 ***
Cu concentration X Plant species	18	1795410	99745	2.692	0.00135 **
Residuals	79	2927337	37055		

Significance levels: '***' 0.001'**' 0.01'*' 0.05'.' 0.1''1; Df: Degree of freedom, p: pvalue,
F: Fisher value, Means sq: Mean of square

4. Discussion

Plant species display various strategies to quench Cu toxicity, most having an excluder phenotype, with Cu accumulation in their belowground biomass and a low root-to-shoot transfer, and few others accumulating also Cu in their shoots (Marchand et al., 2010). Here, root and shoot Cu concentrations increased for the four macrophytes along the Cu gradient, but at all Cu levels Cu concentration was higher in the roots than in the shoots, with various TF patterns suggesting different physiological responses to Cu excess (Supplemental material. 4).

There is ample evidence that high Cu excess may causes damages in the photosynthetic apparatus which may decrease the photosynthesis efficiency (Hego et al., 2016). However, despite high Cu exposure and root Cu concentrations (Fig. 5B), all measured chlorophyll fluorescence parameters did not differ in the 0.08 – 40 µM Cu range for the four studied macrophytes (Supplemental material. 3). The maximum efficiency of photosystem II (Fv/Fm) is accepted as an indicator of photo-inhibition. The real efficiency of Photosystem II (Y(II)) is measuring photochemistry related to electron transport and thus to photosynthesis and non-photochemical quenching (qN) assesses changes in heat dissipation (Caldelas et al., 2012). Here, Fv/Fm, Y(II) and qN values were optimal for the four macrophytes. To limit Cu phytotoxicity, plants can manage a cascade of physiological processes to maintain the cellular homeostasis (1) Cu binding by ligands, i.e. nicotianamine, phytochelatins and metallothioneins) and (2) Cu compartmentation in the shoot vacuole (Yruela, 2005; Leitenmaier and Küpper, 2013). Several mechanisms were reported in macrophytes. In *Iris halophila* seedlings, oxidative stress induced by exposure to Pb, Cd, Zn, and Cu-contaminated tailings triggered the accumulation in the root of superoxide dismutase (SOD), peroxidase (POD), glutathione (GSH) and ascorbate (AsA) to keep the overall balance between ROS production and enzyme levels (Han et al., 2016). Exposures at 50 and 100 µM Cu induced the accumulation of metallothioneins in the leaves and roots of *Iris lacteal* (Gu et al., 2015). *Iris pseudacorus* response to stress tolerance include low lipid peroxidation, increased proline and malondialdehyde concentrations, and increased peroxidase, catalase, superoxide dismutase, and ascorbate peroxidase activities (Zhang et al., 2007; Qiu and Huang, 2008; Zhou et al., 2010). In *A. donax* transcripts, homologous to genes involved in metal stress responses through chelation and transport were identified, e.g. ATP-phosphoribosyl transferase, ROS scavenging (Ascorbate Peroxidase), the rate-limiting step in histidine biosynthesis, Phytochelatin Synthase, and metallothioneins (Sablok et al., 2014). However, to detoxify and sequester high metal amount, plants need to spend energy which leaves less resources for reproduction, growth and other processes (Audet and Charest, 2008; Maestri et al., 2010). Increase in plant maintenance cost may be indicated by decrease in biomass production. Biomass production of macrophytes responded to Cu exposure from no effect to severe inhibition along the Cu gradient (Fig. 1-4). Root and shoot DW yields of *P. arundinacea* decreased over 10 µM Cu, being 2.2-fold and 2.7-fold lower at 40 µM Cu than at 0.08 µM Cu, respectively (Fig. 3A). These decreases were correlated with increasing root Cu concentration (linear relationship, R^2: 0.57 and 0.75, respectively) confirming that *P. arundinacea* was affected by Cu excess (Marchand et al., 2014c). The shoot DW yield of *C. eragrostis* and both roots and shoots of *I. pseudacorus* showed moderate and insignificant differences across the Cu gradient, after the 2 month-Cu exposure (Fig. 1A and 2A) but *C. eragrostis* displayed a marked decrease in root growth as Cu exposure rose (Fig. 1A), correlated with the increase in root Cu concentration (exponential relationship, R^2: 0.81). Similarly, *Cyperus alternifolius* Linn. exposed 15 days to poly-contaminated wastewater from electroplating (i.e. 0.7 µM Cu) showed the most marked decrease in root length even though it produced a high leaf biomass (Sun et al., 2013). One explanation was that only roots were submerged and in direct contact with the contaminant resulting firstly, on this short-term exposure, in subsequent toxicity in roots. Conversely, the shoot DW yield of *A. donax,* did not decrease (Fig. 4A) and its root DW yield was significantly lower only at 40 µM Cu (Fig. 4A). This agreed with Elhawat et al., (2014) reporting that shoot and root biomass of *A. donax* slightly decreased at 42.5 µM Cu in a spiked-nutrient solution. Weak changes in root and shoot DW yields of *I. pseudacorus* and *A. donax* along the Cu gradient matched with their Cu tolerance in this Cu range (Marchand et al., 2014c; Nsanganwimana et al., 2014a).

As noted by Ouzounidou et al., (1995), Cu-tolerant plants have frequently lower Cu concentration in their shoots than the sensitive plants. In addition to efficient detoxification mechanisms, such Cu-tolerant plants must have efficient root cellular mechanisms to exclude Cu from the shoots (i.e. improved efflux Cu pumping at the plasma membrane, Cu compartmentation in the root vacuole, Yruela, 2009). The shoot Cu concentrations of *I. pseudacorus* was relatively low and constant along the Cu gradient (Fig. 2B) and only slightly exceeded the common range for plants (3 – 20 mg Cu kg^{-1}, Tremel-Schaub and Feix, 2005). This disagreed with high shoot Cu concentrations for *I. pseudacorus* (Sun et al., 2013) and *Iris ensata* (Usman et al., 2012) (Table 4). Given the high root Cu concentration, shoot Cu concentration of *A. donax* was quite low at all Cu exposures ranging from 4.5 to 176 mg Cu kg^{-1} DW (Fig. 4B). Root Cu concentration over 1000 mg Cu kg^{-1} DW were measured at 40 µM Cu for *I. pseudacorus* and in the 10-40 µM Cu range for *A. donax.* Both species had low TF, from 0.16 to 0.01 for *A. donax* and 0.63 to 0.04 for *I. pseudacorus* (Supplemental material. 4) which classified them as excluders (Baker, 1981). This agreed with the general pattern of macrophytes accumulating higher Cu concentrations in their roots than in their shoots (Vymazal et al., 2009; Marchand et al., 2010). Although, this pattern is widely accepted for *I. pseudacorus* (Mungur et al., 1997; Sun et al., 2013), the ability of *A. donax* to accumulate such root Cu concentration is quite unusual (Table 4). Here, *I. pseudacorus* and *A. donax* accumulated Cu mainly in their root and rhizome, until the belowground biomass became saturated and then transfer Cu excess to the shoots (Mavrogianopoulos et al., 2002).

Species	Organ	Concentration	Conditions	Exposure Concentration	Time	Reference

Table. 4. Frequent Cu concentrations in plant parts of emerged, rooted macrophytes potentially usable for Cu rhizofiltration

Species	Organ	Concentration	Conditions	Exposure Concentration	Time	Reference
Cyperus alternifolius						
	Leaves	7 ± 0.3				
	Shoots	8 ± 0.1				
	Rhizomes	309 ± 16.3				
	Main roots	2610 ± 380				
	Lateral roots	15600 ± 238	Constructed wetland	16.5 µM Cu	5 months	Cheng et al., 2002
Cyperus alternifolius						
	Shoots	670 ± 70			15 days	
	Roots	1310 ± 10	Hydroponic experiment	0.7 µM Cu		Sun et al., 2013
Cyperus alternifolius						
	Shoots	1000	Constructed wetland	2.14 µM Cu	3 months	Soda et al., 2012
Cyperus alternifolius						
	Roots	386				
	Stem	70	Constructed wetland			
	Leaves	79		0.16 µM Cu	1 month	Adav et al., 2012
Cyperus rotundus						
	Roots	689				
	Shoots	78				
	Leaves	134				
	Flowers	85	Plant sampling	1756 mg kg^{-1} (Soil)	-	Ashraf et al., 2011
Cyperus involucratus						
	Leaves	112 ± 14		9902 ± 1 089 mg kg^{-1} (Soil)	-	
	Shoots	112 ± 23	Plant sampling	1114 ± 342 mg kg^{-1} (Soil)	-	Křĭbek et al., 2011
Iris pseudoacorus						
	Shoots	5.8 ± 0.5	Plant sampling	7 ± 0 .2 µg L^{-1} (Water) 6 ± 0.5 mg kg^{-1} (Sediment)	-	Samecka-Cymerman et al., 2001
Iris pseudoacorus						
	Shoots	430 ± 30				
	Roots	1430 ± 170	Hydroponic experiment	0.7 µM Cu	15 days	Sun et al., 2013
Iris ensata						
	Shoots	257 ± 4.8				
	Roots	263 ± 16	Plant sampling	27–120 mg kg^{-1} (Soil) 0.4 ± 0.01 mg kg^{-1} (Sediment)	-	Usman et al., 2012

Species	Organ	Concentration	Conditions	Exposure Concentration	Time	Reference
Phalaris arundinacea						
	Shoots	7.0 ± 0.6	Plant sampling	198 ± 12 µg L-1 (Water) 0.4 ± 0.01 mg kg-1 (Sediment)	-	Samecka-Cymerman et al., 2001
Phalaris arundinacea						
	Shoots	1.6 - 9.7	Plant sampling	7.4 - 31 µg L^{-1} (Water)	2-4 years	Vymazal et al., 2007
Phalaris arundinacea						
	Stem Leaf sheath Leaf blade	5.9 - 6.34 4.11 - 7.33 5.99 - 8.22	Field experiment	-	2-8 years	Pahkala and Pihala, 2000
Phalaris arundinacea						
	Leaves Rhizomes	6 – 10 10 – 20	Plant sampling	2.6 - 15 mg kg^{-1} (Sediment)	-	Parzych et al., 2015
Phalaris arundinacea						
	Leaves	0.56 - 12	Plant sampling	1.93 - 8.09 µg L^{-1} (Water) 1.35 - 247 mg kg^{-1} (Sediment)	-	Łojko et al., 2015
Phalaris arundinacea						
	Roots and Rhizomes Stems Leaves	3.83 - 24 0.89 - 5.61 3.59 - 8.74	Plant sampling	0.54 - 6.50 µg L^{-1} (Water) 2.71 - 42.5 mg kg^{-1} (Sediment)	-	Polechońska and Klink, 2014
Arundo donax						
	Shoots Roots	600 630	Hydroponic experiment	0.7 µM Cu	15 days	Sun et al., 2013
Arundo donax						
	Roots Stems Leaves	7.42 ± 0.88 1.88 ± 0.15 1.13 ± 0.13	Plant sampling	22.7 ± 3.45 µg L^{-1} (Water) 115 ± 17.6 mg kg^{-1} (Sediment)	-	Bonanno, 2013

Cyperus eragrostis displayed relatively high Cu concentration in roots (i.e. 246 ± 111 mg Cu kg^{-1} DW at 10 µM) and shoot (i.e. 256 ± 58 mg Cu kg^{-1} DW at 40µM) (Fig. 1C). Higher Cu concentrations in both roots and shoots were published for other *Cyperus* sp. (Table 4). More than 30% of Cu supplied during these experiments were accumulated in *C. alternifolius*, suggesting its potential for phytoextracting Cu from polluted soils (Cheng et al., 2002; Soda et al., 2012; Sun et al., 2013) (Table 4). Similarly, at 40 µM Cu, high root concentration was measured in *P. arundinacea* (i.e. 631 ±103 mg Cu kg^{-1} DW). Its shoot Cu concentration exceeded the threshold value for Cu hyperaccumulator (i.e.

300 mg Cu kg^{-1} DW; van der Ent et al., 2013) (Fig 3B). To our knowledge, such results was not previously reported as *P. arundinacea* was exposed to lower Cu concentration (Table 4). However, for both species, this coincided with a strong decrease in biomass production (especially for *C. eragrostis* roots), indicating that plants were affected by Cu excess. For *P. arundinacea,* TF raised with increasing Cu exposure from 0.11 ± 0.06 to 0.86 ± 0.37 (Supplemental material. 4). This plant response showed a Cu dilution in the whole plant biomass as storage capacities in roots were exceeded.

Practical implication: The biomass production of *C. eragrostis*, especially the roots, was affected by Cu excess and its shoot and root concentrations did not meet the requirement for Cu-ecocatalysts (> 1000 mg Cu kg^{-1} ; Clavé et al., 2016b), confirming Sun et al., (2013). The use of *Cyperus alternifolius* may be an alternative since it displays higher Cu concentration than *C. eragrostis* (Table 4). At 20 – 40 µM Cu, the biomass production of *P. arundinacea* was impacted by Cu phytotoxicity. Even though, its root and shoot Cu concentrations were relatively high, it was insufficient for their use as Cu-ecocatalysts. Plant inoculation with endophytic bacteria may be an option to promote root Cu concentration of *P. arundinacea* and better fit Cu-ecocatalyst requirements. Enhanced root Cu accumulation, Cu translocation, biomass production, nutrient availability, and plant Cu tolerance were reported in plants inoculated with endophytic bacteria (Weyens et al., 2009; Ma et al., 2011). At 40 µM Cu, *I. pseudacorus* produced high root and shoot biomasses with low shoot Cu concentration but high root Cu concentration (i.e. 1099 mg Cu kg^{-1}) just meeting the requirement for Cu-ecocatalysts. As its shoot Cu concentration was within the common Cu values in plants (3 – 20 mg Cu kg^{-1}, Tremel-Schaub and Feix, 2005), potential uses of this shoot cellulosic biomass are multiple: (1) energy sector (i.e. biofuel, bioethanol) (2) derived bioproducts, and (3) cellulosic and paper industry (Nsanganwimana et al., 2014a; Evangelou et al., 2014; Vigil et al., 2015). In the 10-40 µM Cu range, root Cu concentration for *A. donax* met the requirement for Cu-ecocatalysts. Such Cu-rich biomass may be used to catalyze fine organic chemical reactions to synthesize molecules with high added value: pharmaceuticals (e.g. anticancer and antiviral agents), cosmetics, agrochemicals (e.g. green pesticides) and textiles (Escande et al, 2015). Conversely, Cu concentration in *A. donax* shoots was insufficient for their use as Cu-ecocatalysts and may be too high to be merged with other plant biomass, even though they may be used to fertilize Cu-deficient soils and substrates. One option may be to limit Cu root-to-shoot transfer by adding silicon in the culture medium. Silicon is deposited in the endodermal tissue in the roots of some plant species and may at least partially block the apoplastic bypass flow across the roots and restrain the apoplastic transport of Cu, thus limiting Cu root-to-shoot transfer (Zargar et al., 2010; Khandekar and Leisner, 2011; Caldelas et al., 2012). Such uncontaminated biomass could integrate local processing chains (e.g. Energy sector: bioethanol, biofuels, combustion; potential fertilizers: compost, biochar, litter; bioproducts: construction of building materials and plant fiber/plastic composites, Alshaal et al., 2013a).

5. Conclusion

The four macrophytes achieved different biomass yields, Cu concentrations and Cu removals on this 0.08-40µM Cu gradient. *Iris pseudacorus* and *A. donax* can deliver root mats potentially usable as Cu-ecocatalyst if the effluent concentration reaches 40 µM Cu and 10 µM Cu, respectively. As shoot biomass of *I. pseudacorus* displayed common Cu concentration even at 40 µM Cu, it can be merged with other biomasses in processing chains for the Bioeconomy. *Phalaris arundinacea* produced root and shoot biomasses with Cu concentration lower than 1000 mg Cu kg^{-1} DW in the 0.08 – 40 µM Cu range and was not relevant to harvest potential Cu-ecocatalyst. *Cyperus eragrostis* with low root and shoot DW yields and low Cu concentration in both plant parts was also not appropriate to produce Cu-ecocatalyst. One further research will be to long term assess *Iris pseudacorus* and *A. donax* in large vats with Cu-contaminated effluents from the cleaning of crop sprayer tanks.

6. Acknowledgements

This work was supported by ADEME (French Agency for the Environment and Energy, PhD grant of N. Oustrière), the French National Research Agency (PHYTOCHEM ANR-13-CDII-0005-01) and the ERA-Net FACCE SURPLUS (project INTENSE; http://faccesurplus.org/research-projects/intense/). The UMR Biogeco is a member of the INRA Ecotoxicologist network, ECOTOX.

7. References

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M.K., Bharwana, S.A., 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environ. Sci. Pollut. Res. 22, 8148–8162. doi:10.1007/s11356-015-4496-5

Alshaal, T., Domokos-Szabolcsy, E., Marton, L., Czako, M., Katai, J., Balogh, P., Elhawat, N., El-Ramady, H., Gerocs, A., Fari, M., 2013a. Restoring soil ecosystems and biomass production of *Arundo donax* L. under microbial communities-depleted soil. Bioenerg. Res. 7, 268–278. doi:10.1007/s12155-013-9369-5

Anjum, N.A., Adam, V., Kizek, R., Duarte, A.C., Pereira, E., Iqbal, M., Lukatkin, A.S., Ahmad, I., 2015. Nanoscale copper in the soil–plant system–toxicity and underlying potential mechanisms. Environ. Res. 138, 306–325. doi:10.1016/j.envres.2015.02.019

Audet, P., Charest, C., 2008. Allocation plasticity and plant-metal partitioning: metaanalytical perspectives in phytoremediation. Environ. Pollut. 156, 290–296.

Baker, A.J.M., 1981. Accumulators and excluders strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654.

Baker, T.J., Tyler, C.R., Galloway, T.S., 2014. Impacts of metal and metal oxide nanoparticles on marine organisms. Environ. Pollut. 186, 257−271. doi:10.1016/j.envpol.2013.11.014

Basias, 2015. Inventaire historique de sites industriels et activités de service. Available at : http://basias.brgm.fr/ (verified on October 20th, 2016)

Ben Salem., Z., Laffray, X., Ashoour, A., Ayadi, H., Aleya, L., 2014. Metal accumulation and distribution in the organs of Reeds and Cattails in a constructed treatment wetland (Etueffont, France). Ecol. Eng. 64, 1–17. doi:10.1016/j.ecoleng.2013.12.027

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization, Environ. Pollut. 156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bonanno, G., Lo Giudice, R., 2010. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. Ecol. Indic. 10, 639–645. doi:10.1016/j.ecolind.2009.11.002

Bonanno, G., 2012. *Arundo donax* as a potential biomonitor of trace element contamination in water and sediment. Ecotox. Environ. Safe. 80, 20–27. doi:10.1016/j.ecoenv.2012.02.005

Bonanno, G., 2013. Comparative performance of trace element bioaccumulation and biomonitoring in the plant species *Typha domingensis*, *Phragmites australis* and *Arundo donax*, Ecotox. Environ. Safe. 97, 124–130. doi:10.1016/j.ecoenv.2013.07.017

Calabrese, E.J., Blain, R.B., 2009. Hormesis and plant biology. Environ. Pollut. 157, 42–48. doi:10.1016/j.envpol.2008.07.028

Caldelas, C., Bort, J., Febrero, A., 2012. Ultrastructure and subcellular distribution of Cr in *Iris pseudacorus* L. using TEM and X-ray microanalysis. Cell Biol. Toxicol. 28, 57–68. doi:10.1007/s10565-011-9205-7

Chalot, M., Blaudez, D., Rogaume, Y., Provent, A.S., Pascual, C., 2012. Fate of trace elements during the combustion of phytoremediation wood. Environ. Sci. Technol. 46, 13361–13369. doi:10.1021/es3017478

Cheng, S., Grosse, W., Thoennessen, M., 2002. Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Ecol. Eng. 18(3), 35–38. doi:10.1016/S0925-8574(01)00091-X

Chen, Z., Cuervo, D.P., Müller, J.A., Wiessner, A., Köser, H., Vymazal, J., Kästner, M., Kuschk, P., 2016. Hydroponic root mats for wastewater treatment—a review. Environ Sci Pollut Res. 23, 15911–15928. doi:10.1007/s11356-016-6801-3

Ciadamidaro, L., Madejón, E., Puschenreiter, M., Madejón, P., 2013. Growth of *Populus alba* and its influence on soil trace element availability. Sci. Total. Environ. 454–455, 337–347. doi:10.1016/j.scitotenv.2013.03.032

Clavé, G., Garel, C., Poullain, C., Renard, B.L., Olszewski, T.K., Lange, B., Shutcha, M., Faucon, M.P., Grison, C., 2016a. Ullmann reaction through ecocatalysis: insights from bioresource and synthetic potential. RSC Adv. 6, 59550–59564. doi:10.1039/C6RA08664K

Clavé, G., Garoux, L., Boulanger, C., Hesemann, P., Grison. C., 2016b. Ecological recycling of a bio-based catalyst for Cu click reaction: a new strategy for a greener sustainable catalysis. Chemistry Select. 6, 1–8. doi:10.1002/slct.201600430

Collin, B., Doelsch, E., Keller, C., Cazevieille, P., Tella, M., Chaurand, P., Panfili, F., Hazemann, J.L., Meunier, J.D., 2014. Evidence of sulfur-bound reduced copper in bamboo exposed to high silicon and copper concentrations. Environ. Pollut. 187, 22–30. doi:10.1016/j.envpol.2013.12.024

Delplanque, M., Collet, S., Del Gratta, F., Schnuriger, B., Gaucher, R., Robinson, B., Bert, V., 2013. Combustion of Salix used for phytoextraction: The fate of metals and viability of the processes. Biomass Bioenergy. 49, 160–170. doi:10.1016/j.biombioe.2012.12.026

El hadri, H., Chery, P., Jalabert, S., Lee, A., Potin-Gautier, M., Lespes, G., 2012. Assessment of diffuse contamination of agricultural soil by copper in Aquitaine region by using French national databases. Sci. Total. Environ. 441, 239–247. doi:10.1016/j.scitotenv.2012.09.070

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, E., El-Ramady, H., Márton, L., Czakó, M., Kátai, J., Balogh, P., Sztrik, A., Molnár, M., Popp, J., Fári, M.G., 2014. Phytoaccumulation potentials of two biotechnologically propagated ecotypes of *Arundo donax* in copper-contaminated synthetic wastewater. Environ. Sci. Pollut. Res. 21, 7773–7780. doi:10.1007/s11356-014-2736-8

Escande, V., Velati, A., Garel, C., Renard, B.L., Petit, E., Grison, C., 2015. Phytoextracted mining wastes for ecocatalysis: Eco-Mn®, an efficient and eco-friendly plant-based catalyst for reductive amination of ketones. Green chem. 17, 2188–2199. doi:10.1039/C4GC02193B

Evangelou, M.W.H., Deram, A., Ugolini, F., Abiven, S., Schulin, R., 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. J. Environ. Manage. 146, 100–106. doi:10.1016/j.jenvman.2014.07.046

Garbisu, C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 77, 229–236. doi:10.1016/S0960-8524(00)00108-5

Gomes, H., 2012. Phytoremediation for bioenergy: challenges and opportunities. Environ. Technol. Rev. 1(1), 59–66. doi:10.1080/09593330.2012.696715

Gu, C.H., Liu, L.Q., Deng, Y.M., Zhu, X.D., Huang, S.Z., Lu, X.Q., 2015. The heterologous expression of the *Iris lactea* var. *Chinensis* type 2 metallothionein IlMT2b gene rnhances copper tolerance in *Arabidopsis thaliana*. Bull. Environ. Contam. Toxicol. 94, 247–253. doi:10.1007/s00128-014-1444-x

Guittonny-Philippe, A., Masotti, V., Höhener, P., Boudenne, J.L., Viglione, J., Laffont-Schwob, I., 2014. Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: A review to overcome obstacles and suggest potential solutions. Environ. Int. 64, 1–16. doi:10.1016/j.envint.2013.11.016

Han, Y., Zhang, L., Yang, Y., Yuan, H., Zhao, J., Gu, J., Huang, S., 2016. Pb uptake and toxicity to *Iris halophila* tested on Pb mine tailing materials. Environ. Pollut. 214, 510–516. doi:10.1016/j.envpol.2016.04.048

Hego, E., Vilain, S., Barré, A., Claverol, S., Dupuy, J.W., Lalanne, C., Bonneu, M., Plomion, C., Mench, M., 2016. Copper stress-induced changes in leaf soluble proteome of Cu-sensitive and tolerant Agrostis capillaris L. populations. Proteomics. 16(9), 1386–97. doi:10.1002/pmic.201500083

Huguenot, D., Bois, P., Cornu, J.Y., Jezequel, K., Lollier, M., Lebeau, T., 2015. Remediation of sediment and water contaminated by copper in small-scaled constructed wetlands: effect of bioaugmentation and phytoextraction. Environ. Sci. Pollut. Res. 22, 721–732. doi:10.1007/s11356-014-3406-6

Idris, S.M., Jones, P.L., Salzman, S.A., Croatto, G., Allinson, G., 2012. Evaluation of the giant reed (*Arundo donax*) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. Environ. Sci. Pollut. Res. 19, 1159−1170. doi:10.1007/s11356-011-0642-x

IFREMER., 2014. Qualité du Milieu Marin Littoral Bulletin de la surveillance 2013, 2014. Institut Français de Recherche pour l'Exploitation de la Mer, Available at : http://envlit.ifremer.fr/content/download/81930/580311/version/2/file/bull_ar_2014.pdf (Verified on August 10th, 2016)

Istenič, D., Arias, C.A., Vollertsen, J., Nielsen, A.H., Wium Andersen, T., Hvitved-Jacobsen, T., Brix, H., Improved urban stormwater treatment and pollutant removal pathways in amended wet detention ponds. J. Environ. Sci. Health. Part A. 47, 1466–1477. doi:10.1080/10934529.2012.673306

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. CRC Press, Lewis Publishers, Boca Raton, Florida, USA.

Khandekar, S., Leisner, S., 2011. Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress, J. Plant Physiol. 168, 699–705. doi:10.1016/j.jplph.2010.09.009

Kolbas, A., Marchand, L., Herzig, R., Nehnevajova, E., Mench, M., 2013. Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. Plant Soil. 376, 377–397. doi:10.1007/ s11104-013-1974-8

Kolbas, A., Kidd, P., Guinberteau, J., Jaunatre, R., Herzig, R., Mench, M., 2015. Endophytic bacteria take the challenge to improve Cu phytoextraction by sunflower. Environ. Sci. Pollut. Res. Int. 7, 5370–5382. doi:10.1007/s11356-014-4006-1

Kumari, M., Tripathi, B.D., 2015. Efficiency of *Phragmites australis* and *Typha latifolia* for heavy metal removal from wastewater, Ecotox. Environ. Safe. 112, 80–86. doi:10.1016/j.ecoenv.2014.10.034

Leitenmaier, B., Küpper, H., 2013. Compartmentation and complexation of metals in hyperaccumulator plants. Front. Plant Sci. 4, 374. doi:10.3389/fpls.2013.00374

Lequeux, H., Hermans, C., Lutts, S., Verbruggen, N., 2010. Response to copper excess in *Arabidopsis thaliana*: impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48, 673–682. doi:10.1016/j.plaphy.2010.05.005

Lievens, C., Yperman, J., Vangronsveld, J., Carleer, R., 2008. Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis: part I. Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. Fuel. 87, 1894–1905. doi:10.1016/j.fuel.2007.10.021

Liang, Y.C., Sun, W.C., Zhu, Y.G., Christie, P., 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ. Pollut. 147, 422–428. doi:10.1016/j.envpol.2006.06.008

Lukáčová Kuliková, Z., Lux, A., 2010. Silicon influence on maize, *Zea mays* L., hybrids exposed to cadmium treatment. Bull. Environ. Contam. Toxicol. 85, 243–250. doi:10.1007/s00128-010-0046-5

Ma, Y., Rajkumar, M., Freitas, H., 2009. Improvement of plant growth and nickel uptake by nickel resistant-plant growth promoting bacteria. J. Hazard. Mater. 166, 1154–61. doi:10.1016/j.jhazmat.2008.12.018

Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotech. Adv. 29, 248–258. doi:10.1016/j.biotechadv.2010.12.001

Mackie, K.A., Müller, T., Kandeler, E., 2012. Remediation of copper in vineyards - A mini review, Environ. Pollut. 167, 16–26. doi:10.1016/j.envpol.2012.03.023

Maestri, E., Marmiroli, M., Visioli, G., Marmiroli, N., 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ. Exp. Bot. 68, 1–13.

Maille, E., 2004. Enquete phytosanitaire Gironde – Dordogne. Available at : http://www.itab.asso.fr/downloads/actes/actes_jt_viti_04_avignon_mini.pdf (Verified on August 10th, 2016)

Maine, M.A., Sune, N., Hadad, H., Sanchez, G., Bonetto, C., 2009. Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J. Environ. Manage. 90, 355–363. doi:10.1016/j.jenvman.2007.10.004

Marchand, L., Mench, M., Jacob, D.L., Otte, M.L., 2010. Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ. Pollut. 158, 3447–3461. doi:10.1016/j.envpol.2010.08.018

Marchand, L., Nsanganwimana, F., Oustriere, N., Grebenshchykova, Z., Lizama-Allende, K., Mench, M., 2014b. Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*. Ecol. Eng. 64, 291–300. doi:10.1016/j.ecoleng.2013.12.017

Marchand, L., Nsanganwimana, F., Cook, B., Vystavna, Y., Huneau, F., Lecoustumer, P., Lamy, J.B., Oustrière, N., Mench, M., 2014c. Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors. Ecological Indicators. 46, 425–437. doi:10.1016/j.ecolind.2014.07.011

Marchand, L., Pelosi, C., González-Centeno, M.R., Maillard, A., Ourry, A., Galland, W., Teissedre, P.L., Bessoule, J.J., Mongrand, S., Morvan-Bertrand, A., Zhang, Q., Grosbellet, C., Bert, V., Oustriere, N., Mench, M., Brunel-Muguet, S., 2016. Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere. 156, 150–162.

Marschner, H., 2011. Mineral nutrition of higher plants, 3rd edn. Academic, London.

Mavrogianopoulos, G., Vogli, V., Kyritsis, S., 2002. Use of wastewater as a nutrient solution in a closed gravel hydroponic culture of *Arundo donax*. Biores. Technol. 82, 103–107. doi:10.1016/S0960-8524(01)00180-8

Mungur, A.S., Shutes. R.B.E., Revitt, D.M., House, M.A., 1997. An assessment of metal removal by a laboratory scale wetland. Water Sci. Technol. 35, 125–133. doi:10.1016/S0273-1223(97)00061-9

Newete, S.W., Byrne, M., 2016. The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. Environ. Sci. Pollut. Res. 23, 10630–10643. doi:10.1007/s11356-016-6329-6

Nsanganwimana, F., Marchand, L., Douay, F., Mench, M., 2014a. *Arundo donax* L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock, A review. Int. J. Phytorem. 16, 982–1017. doi:10.1080/15226514.2013.810580

Ouzounidou, G., Ciamporova, M., Moustakas, M. 1995. Responses of maize (*Zea mays* L.) plants to copper stress – I. Growth, mineral content and ultrastructure of roots. Environ. Exp. Bot. 35, 167–176.

Qiu, S., Huang, S., 2008. Study on growth and Cd accumulation of root system of *Iris pseudacorus* seedling under Cd stress. J. Plant Res. Environ. 17, 33–38.

Qualité du Milieu Marin Littoral Bulletin de la surveillance 2013, 2014. Institut Français de Recherche pour l'Exploitation de la Mer, Available at: http://envlit.ifremer.fr/content/download/81930/580311/version/2/file/bull_ar_2014.pdf (Verified on August 10th, 2016)

Reed, M.L.E., Glick, B.R., 2005. Growth of canola (*Brassica napus*) in the presence of plant growth promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Canadian Journal of Microbiology. 51, 1061–1069.

Rocha, A.C.S., Almeida, C.M.R., Basto, M.C.P., Vasconcelos, M.T.S.D., 2014. Antioxidant response of *Phragmites australis* to Cu and Cd contamination. Ecotox. Environ. Safe. 109, 152–160. doi:10.1016/j.ecoenv.2014.06.027

Rocha, G.S., Lombardi, A.T., Melão, M.G.G., 2016. Influence of phosphorus on copper toxicity to *Selenastrum gracile* (Reinsch) Korshikov. Ecotox. Environ. Safe. 128, 30–35. doi:10.1016/j.ecoenv.2016.02.007

Rossini Oliva, S., Mingorance, M.D., Leidi, E.O., 2011. Effects of silicon on copper toxicity in Erica and evalensis Cabezudo and Rivera: a potential species to remediate contaminated soils, J. Environ. Monitor. 13, 591–596. doi:10.1039/c0em00549e

Rycewicz-Borecki, M., McLean, J.E., Dupont, R.R., 2016. Bioaccumulation of copper, lead, and zinc in six macrophyte species grown in simulated stormwater bioretention systems. J. Environ. Manage. 166, 267–275. doi:10.1016/j.jenvman.2015.10.019

Sablok, G., Fu, Y., Bobbio, V., Laura, M., Rotino, G.L., Bagnaresi, P., Allavena, A., Velikova, V., Viola, R., Loreto, F., Li, M., Varotto, C., 2014. Fuelling genetic and metabolic exploration of C3 bioenergy crops through the first reference transcriptome of *Arundo donax* L. Plant Biotech. J. 12, 554–567. doi:10.1111/pbi.12159

Schulz, B., Boyle, C., 2006. What are endophytes? In Microbial Root Endophytes. Edited by Schulz, B.J.E., Boyle, C.J.C., Sieber TN. 1-13.

SEQ EAU, 2003. Agence de l'eau Rhône Méditerranée Corse, Available at: ……….... http://sierm.eaurmc.fr/eaux-superficielles/fichiers-telechargeables/grilles-seqeau-v2.pdf (In French). (Verified on August 10th, 2016)

Shaheen, S., Mahmood, Q., Pervez, A., Mirza, N., Bhatti, Z.A., Hayat, T., Meryem, S.S., Ullah, F., 2016. Chromium uptake by giant reed under rhizobacterial inhibition. Int. J. Environ. Sci. Technol. doi:10.1007/s13762-016-0996-1

Shi, X., Zhang, C., Wang, H., Zhang, F., 2005. Effect of Si on the distribution of Cd in rice seedlings. Plant Soil. 272, 53–60. doi:10.1007/s11104-004-3920-2

Soda, S., Hamada, T., Yamaoka, Y., Ikea, M., Nakazato, H., Saeki, Y., Kasamatsu, T., Sakurai, Y., 2012. Constructed wetlands for advanced treatment of wastewater with a complex matrix from a metal processing plant: Bioconcentration and translocation factors of various metals in *Acorus gramineus* and *Cyperus alternifolius*. Ecol. Eng. 39, 63–70. doi:10.1016/j.ecoleng.2011.11.014

Stals, M., Thijssen, E., Vangronsveld, J., Carleer, R., Schreurs, S., Yperman, J., 2010. Flash pyrolysis of heavy metal contaminated biomass from phytoremediation: influence of temperature, entrained flow and wood/leaves blended pyrolysis on the behaviour of heavy metals. J. Anal. Appl. Pyrolysis. 87, 1–7. doi:10.1016/j.jaap.2009.09.003

Sun, H., Wang, Z., Gao, P., Liu, P., 2013. Selection of aquatic plants for phytoremediation of heavy metal in electroplate wastewater. Acta Physiol. Plant. 35, 355–364. doi:10.1007/s11738-012-1078-8

Tanner, C.C., Headley, T.R., 2011. Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. Ecol. Eng. 37, 474–486. doi:10.1016/j.ecoleng.2010.12.012

Taylor, A.A., Walker, S.L., 2016. Effects of copper particles on a model septic system's function and microbial community. Water Research. 91, 350–360. doi:10.1016/j.watres.2016.01.014

Tibbett, M., 2015. Mining in ecologically sensitive landscape, CSIRO publishing, Clayton South.

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie, Angers: EDP Sciences, Les Ulis, p 106.

Usman, A.R.A., Lee, S.S., Awad, Y.M., Lim, K.J., Yang, J.E., Ok, Y.S., 2012. Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites, Korea. Chemosphere. 87, 872–878. doi:10.1016/j.chemosphere.2012.01.028

Valipour, A., Hamnabard, N., Woo, K.S., Ahn, Y.H., 2014. Performance of high-rate constructed phytoremediation process with attached growth for domestic wastewater treatment: effect of high TDS and Cu. J. Environ. Manage. 145, 1–8. doi:10.1016/j.jenvman.2014.06.009

Van der Ent, A., Baker, A.J.M, Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362, 319–334. doi:10.1007/s11104-012-1287-3

Vaculík, M., Landberg, T., Greger, M., Luxová, M., Stoláriková, M., Lux, A., 2012. Silicon modifies root anatomy, and uptake and subcellular distribution of cadmium in young maize plants, Ann. Bot, 110, 433–43. doi:10.1093/aob/mcs039

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., Van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765–794. doi:10.1007/s11356-009-0213-6

Vigil, M., Marey-Pérez, M.F., Martinez Huerta, G., Álvarez Cabal, V., 2015. Is phytoremediation without biomass valorization sustainable? Comparative LCA of landfilling vs. anaerobic co-digestion. Sci. Total. Environ. 505, 844–850. doi:10.1016/j.scitotenv.2014.10.047

Vymazal, J., Švehla, J., Kröpfelová, L., Chrastný, V., 2007. Trace metals in *Phragmites australis* and *Phalaris arundinacea* growing in constructed and natural wetlands. Sci. Total. Environ. 380, 154–162. doi:10.1016/j.scitotenv.2007.01.057

Vymazal, J., Kröpfelová, L., Švehla, J., Chrastný, V., Štíchovíá, J., 2009. Trace elements in *Phragmites australis* growing in constructed wetlands for treatment of municipal wastewater. Ecol. Eng. 35, 303–309. doi:10.1016/j.ecoleng.2008.04.007

Vymazal, J., 2016. Concentration is not enough to evaluate accumulation of heavy metals and nutrients in plants. Sci. Total. Environ. 544, 495–498. doi:10.1016/j.scitotenv.2015.12.011

Weyens, N., Van der Lelie, D., Taghavi, S., Vangronsveld, J., 2009. Phytoremediation: plant-endophyte partnerships take the challenge. Current Opinion in Biotechnology. 20, 248–254. doi:10.1016/j.copbio.2009.02.012

Wu, S., Wallace, S., Brix, H., Kuschk, P., Kirui, W.K., Masi, F., Dong, R., 2015a. Treatment of industrial effluents in constructed wetlands: challenges, operational strategies and overall performance. Environ. Pollut. 201, 107–120. doi:10.1016/j.envpol.2015.03.006

Yadav, A.K., Abbassi, R., Kumar, N., Satya, S., Sreekrishnan, T.R., Mishra, B.K., 2012. The removal of heavy metals in wetland microcosms: Effects of bed depth, plant species, and metal mobility. Chem. Eng. J. 211–212, 501–507. doi:10.1016/j.cej.2012.09.039

Ye, Z.H., Baker, A.J.M., Wong, M.H., Willis, A.J., 2003. Copper tolerance, uptake and accumulation by *Phragmites australis*. Chemosphere. 50, 795–800. doi:10.1016/S0045-6535(02)00221-7

Yruela, I., 2009. Copper in plants: acquisition, transport and interactions. Funct. Plant Biol. 36, 409–430.

Yuan, J., Li, S.S., Yu, L., Liu, Y.M., Cao, Y., He, H.Y., Fan, K.N., 2013. Copper-based catalysts for the efficient conversion of carbohydrate biomass into γ-valerolactone in the absence of externally added hydrogen. Energy Environ. Sci. 6, 3308–3313. doi:10.1039/c3ee40857d

Zargar, S.M., Nazir, M., Agrawal, G.K., Kim, D.W., Rakwal, R., 2010. Silicon in plant tolerance against environmental stressors: towards crop improvement using omics approaches. Curr Prot. 7, 135–143. doi:10.2174/157016410791330507

Zhang, X.B., Liu, P., Yang, Y.S., Chen W.R., 2007. Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. J. Environ. Sci. 19, 902–9.

Zhou, Y.Q., Huang, S.Z., Yu, S.L., Gu, J.G., Zhao, J.Z., Han, Y.L., Fu, J.J., 2010. The physiological response and sub-cellular localization of lead and cadmium in *Iris pseudacorus* L. Ecotoxicol. 19, 69–76.

Table. Supplemental material 1. Shoot ionome of macrophytes (mg kg^{-1}) after the 2 month-exposure to the Cu gradient. Mean values per treatment (n=4). In bold, values lower than common values in plants

Cu exposure (µM)	Ca	Fe	K	Mg	Na	P
A. donax						
0.08	4.3 ± 1.4	0.04 ± 0.01	38 ± 10	3 ± 1	0.6 ± 0.62	3.5 ± 1.2
2	3.7 ± 0.2	0.04 ± 0.01	39 ± 9	2.8 ± 0.3	0.1 ± 0.02	3.2 ± 0.8
10	4.9 ± 1.9	0.05 ± 0.02	47 ± 8	3.1 ± 0.7	0.4 ± 0.19	4 ± 1.1
20	6.3 ± 1.6	0.07 ± 0.02	41 ± 4	3.1 ± 0.8	0.4 ± 0.08	3 ± 0
40	5.5 ± 1.3	0.04 ± 0.01	32 ± 3	2.7 ± 0.7	0.5 ± 0.14	2.2 ± 0.4
C. eragrostis						
0.08	4.4 ± 0.5	0.05 ± 0.01	31 ± 5	2.6 ± 0.3	5.2 ± 1.07	2.7 ± 0.3
2	4.6 ± 0.6	0.04 ± 0.01	41 ± 10	3 ± 0.5	3 ± 0.13	3.8 ± 0.6
10	3.6 ± 0.1	0.03 ± 0.01	38 ± 8	2.4 ± 0.2	3.2 ± 1.4	2.8 ± 0.6
20	2.7 ± 0.9	0.08 ± 0.11	32 ± 7	2.1 ± 0.5	2.4 ± 0.88	2.3 ± 0.4
40	3.2 ± 0.5	0.06 ± 0.03	32 ± 6	2 ± 0.2	1.7 ± 0.29	2.2 ± 0.3
I. pseudacorus						
0.08	14 ± 3.4	0.05 ± 0.02	28 ± 8	4.5 ± 1.4	1.9 ± 0.87	2.9 ± 0.8
2	13 ± 1	0.04 ± 0	26 ± 7	3.9 ± 0.5	2.4 ± 0.81	2.5 ± 0.4
10	12 ± 3.5	0.03 ± 0.01	33 ± 12	4 ± 0.8	2.3 ± 0.96	2.3 ± 0.3
20	10 ± 1.7	0.03 ± 0.01	30 ± 3	3.1 ± 0.5	1.8 ± 0.58	1.9 ± 0.5
40	9.9 ± 2	0.04 ± 0.01	27 ± 4	3.2 ± 0.6	2 ± 0.77	1.5 ± 0.2
P. arundinacea						
0.08	4.5 ± 1.2	0.06 ± 0	22 ± 3	2.2 ± 0.5	0.4 ± 0.06	1.9 ± 0.3
2	3.3 ± 1.3	0.03 ± 0.01	17 ± 5	1.7 ± 0.6	0.3 ± 0.09	1.3 ± 0.4
10	5.7 ± 0.9	0.05 ± 0.02	23 ± 7	2.9 ± 0.6	0.8 ± 0.29	1.8 ± 0.7
20	6.9 ± 0.9	0.14 ± 0.08	37 ± 5	3.8 ± 0.6	1.2 ± 0.59	3.4 ± 0.4
40	3.4 ± 1.1	0.11 ± 0.06	21 ± 4	1.7 ± 0.5	0.6 ± 0.05	1.5 ± 0.4
Common values*	**1 – 50**	**0.02 – 0.3**	**20 – 50**	**1.5 – 3.5**	**-**	**1.6 – 6.0**

Mean value ± SD for each treatment (n=4).

Table. Supplemental material 2. Root ionome of macrophytes (mg kg^{-1}) after the 2 month-exposure to the Cu gradient. Mean values per treatment (n=4).

Cu Exposure (μM)	**Ca**	**Fe**	**K**	**Mg**	**Na**	**P**
A. donax						
0.08	2.4 ± 0.3	0.5 ± 0.15	49 ± 3	3 ± 0.5	1.6 ± 0.5	4.9 ± 0.8
2	2.9 ± 0.5	0.7 ± 0.3	40 ± 10	3.3 ± 0.4	1.3 ± 0.8	4.1 ± 0.8
10	2.7 ± 0.5	1 ± 0.51	36 ± 9	2.3 ± 0.6	0.4 ± 0	2.9 ± 0.6
20	1.6 ± 0.1	0.4 ± 0.11	37 ± 4	1.5 ± 0.1	0.3 ± 0	1.9 ± 0.4
40	1.7 ± 0.2	0.7 ± 0.36	33 ± 2	1.3 ± 0.2	0.3 ± 0	1.9 ± 0.5
C. eragrostis						
0.08	3.9 ± 0.3	0.7 ± 0.24	29 ± 2	3.9 ± 0.3	3.3 ± 0.4	2.9 ± 0.3
2	3.7 ± 0.9	0.8 ± 0.35	30 ± 4	3.1 ± 0.5	3.2 ± 0	3.3 ± 0.5
10	2.7 ± 0.2	0.5 ± 0.21	29 ± 2	3.3 ± 0.8	3.3 ± 1.4	3 ± 0.4
20	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND
I. pseudacorus						
0.08	4.8 ± 1.8	0.4 ± 0.25	33 ± 22	2.6 ± 0.9	7 ± 1.1	3.1 ± 1.5
2	5.2 ± 1	0.3 ± 0.08	37 ± 10	2.8 ± 0.8	7 ± 1.4	3.3 ± 0.6
10	5.3 ± 0.5	0.2 ± 0.1	26 ± 4	1.6 ± 0.1	5.6 ± 1	2.4 ± 0.3
20	4.5 ± 0.5	0.2 ± 0.06	16 ± 4	1.3 ± 0.2	4.5 ± 1.1	2.5 ± 0.8
40	4.3 ± 0.4	0.3 ± 0.04	17 ± 5	1.3 ± 0.1	4.1 ± 1	2.7 ± 0.4
P. arundinacea						
0.08	2.4 ± 0.4	0.4 ± 0.03	19 ± 4	1.3 ± 0.2	4.2 ± 1.8	3.1 ± 0.4
2	3.2 ± 0.4	0.3 ± 0.04	15 ± 2	1.4 ± 0.2	3.7 ± 1.1	2.7 ± 0.3
10	2.5 ± 0.4	0.3 ± 0.18	22 ± 5	1.1 ± 0.1	3 ± 0.9	3.6 ± 0.8
20	1.4 ± 0.3	0.3 ± 0.08	26 ± 1	0.8 ± 0.1	1.6 ± 0.1	5.3 ± 0.4
40	1.1 ± 0.1	0.2 ± 0.09	20 ± 2	0.7 ± 0	1.2 ± 0.1	3.5 ± 0.6

Mean value ± SD for each treatment (n=4). ND: not determined due to no root growth

Table. Supplemental material 3. Response surface for the maximum efficiency of PSII (Fv/Fm), real efficiency of PSII (Y(II)), and non-photochemical quenching (qN), in the macrophyte leaves after the 2 month-exposure to the Cu gradient. Mean values per treatment (n=5).

Plant species Cu exposure (µM Cu)	Fv/Fm	Y(II)	qN
A. donax			
0.08	0.85 ± 0.009 a	0.24 ± 0.1 a	0.39 ± 0.1 a
10	0.78 ± 0.03 a	0.37 ± 0.1 a	0.58 ± 0.2 a
40	0.51 ± 0.3 a	0.29 ± 0.2 a	0.64 ± 0.2 a
C. eragrostis			
0.08	0.82 ± 0.03 a*	0.37 ± 0.1 a	0.46 ± 0.1 a
10	0.8 ± 0.05 a*	0.52 ± 0.02 a	0.53 ± 0.1 a
40	0.77 ± 0.03 a*	0.51 ± 0.05 a	0.54 ± 0.1 a
I. pseudacorus			
0.08	0.83 ± 0.04 a*	0.18 ± 0.04 a	0.32 ± 0.3 a
10	0.8 ± 0.05 a*	0.35 ± 0.2 a	0.43 ± 0.1 a
40	0.82 ± 0.02 a*	0.38 ± 0.2 a	0.5 ± 0.1 a
P. arundinacea			
0.08	0.8 ± 0.004 a	0.22 ± 0.05 a	0.72 ± 0.2 a
10	0.77 ± 0.07 a	0.33 ± 0.1 a	0.62 ± 0.1 a
40	0.67 ± 0.1 a	0.37 ± 0.09 a	0.6 ± 0.1 a

Mean value ± SD for each treatment (n=5). Values with different letters differ significantly (one way ANOVA, p-value <0.05). * Wilcoxon pairwise tests.

Table. Supplemental material 4. Root and shoot Cu concentrations of macrophytes (mg kg^{-1}) and transfer factor after the 2 month-exposure to the Cu gradient. Mean values per treatment (n=4).

Cu exposure (µM)	**Shoot Cu concentration**	**Root Cu concentration**	**Transfer Factor (TF)**
A. donax			
0.08	4 ± 1	31 ± 10	0.16 ± 0.07 a
2	12 ± 3	597 ± 284	0.02 ± 0.01 c
10	42 ± 30	1810 ± 386	0.01 ± 0 c
20	176 ± 103	2864 ± 368	0.06 ± 0.03 b
40	121 ± 65	3512 ± 1372	0.03 ± 0.01 bc
C. eragrostis			
0.08	3 ± 1	9 ± 1	0.38 ± 0.07 a
2	15 ± 4	123 ± 31	0.13 ± 0.07 b
10	22 ± 6	256 ± 58	0.08 ± 0.01 b
20	41 ± 22	ND	ND
40	247 ± 111	ND	ND
I. pseudacorus			
0.08	5 ± 1	8 ± 5	0.63 ± 0.19 a
2	7 ± 2	59 ± 24	0.15 ± 0.1 b
10	7 ± 1	163 ± 42	0.04 ± 0.01 b
20	21 ± 8	420 ± 118	0.06 ± 0.03 b
40	38 ± 12	1099 ± 434	0.05 ± 0.03 b
P. arundinacea			
0.08	3 ± 1	5 ± 1	0.59 ± 0.18 ab
2	9 ± 2	91 ± 30	0.11 ± 0.06 bc
10	38 ± 19	346 ± 132	0.11 ± 0.06 c
20	200 ± 102	839 ± 71	0.24 ± 0.12 c
40	527 ± 251	631 ± 103	0.86 ± 0.37 a

Mean value ± SD for each treatment (n=4). Values with different letters differ significantly (one way ANOVA, p-value <0.05). ND: not determined due to no root growth

Part. 3. Biomass production with high metal(loid) concentrations able to integrate Eco-catalysis processing chains

3.2. Implementation of the remediation strategy in situ

3.2.1. Chapter 5 - Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using *Arundo donax* L. coupled with potential Cu-ecocatalyst production

Accepted with revisions in Ecological engineering

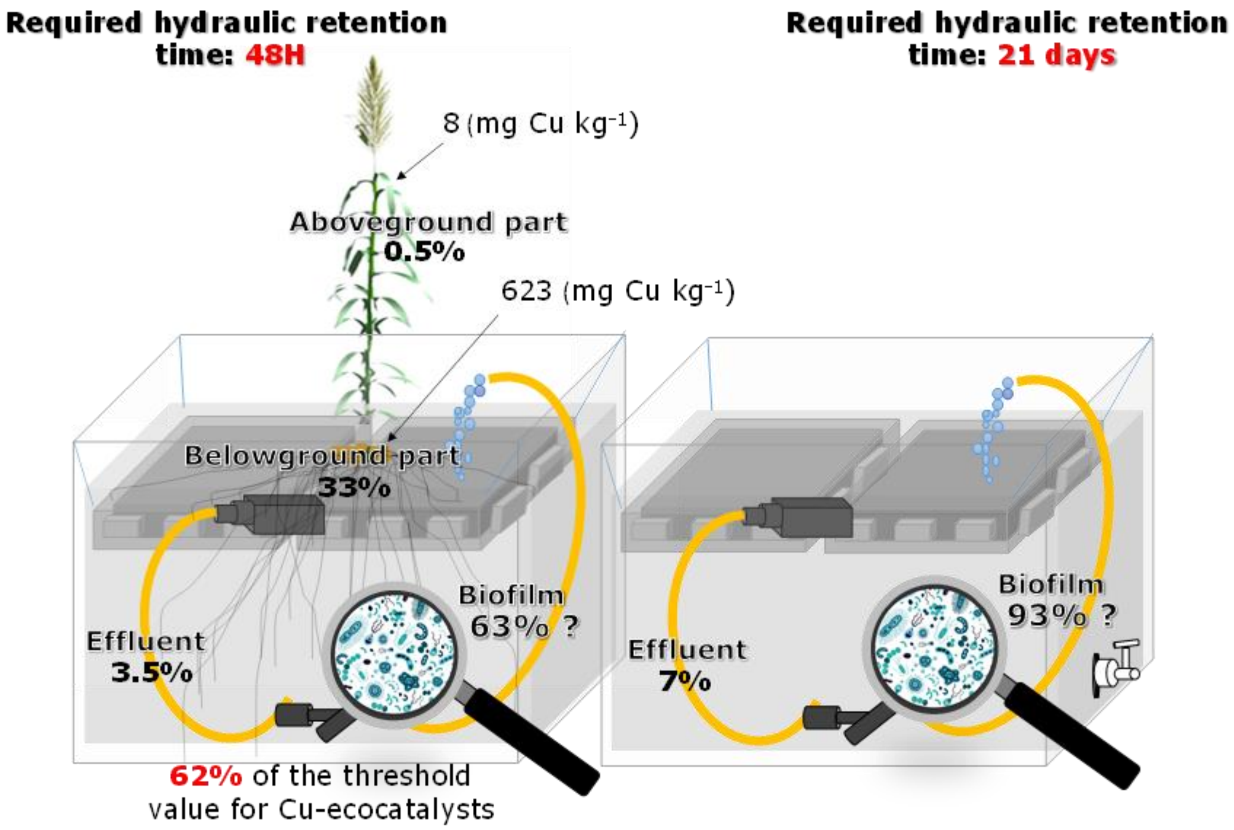


Fig. F. Graphical abstract – Chapter 5

Rhizofiltration of a Bordeaux mixture effluent in pilot-scale constructed wetland using Arundo donax L. coupled with potential Cu-ecocatalyst production

Nadège Oustriere[1], Lilian Marchand[1], Eli Roulet[1], Michel Mench[1]

[1]BIOGECO, INRA, UNIV. BORDEAUX, 33615 PESSAC, FRANCE.

E-mails: oustriere.nadege@gmail.com; marchand.lilian@gmail.com; roulet.eli@gmail.com; mench@bordeaux.inra.fr;

Abstract

Rinsing tanks of crop sprayers produces significant volumes of Cu-rich Bordeaux mixture effluents (BME) that can be treated by rhizofiltration in constructed wetlands (CWs). A pilot-scale CW (6 × 600 L) was developed to jointly rhizofiltrate such BME, produce Cu-rich root mat for ecocatalysis and provide usable shoot biomass with low Cu concentration. Three CW units were unplanted control (Ctrl) while three others were planted with *Arundo donax* L. (*Ad*) in floating racks. The rhizofiltration was carried out during 30 days in the early growing season. Total Cu concentration in the BME was 4.4 mg Cu L^{-1}. Copper removal peaked within the 48 first hours after Bordeaux mixture addition in the *Ad* and Ctrl units (i.e. 92 and 81% respectively). The BME Cu concentration met the requirement for indirect discharge of chemical industry effluents (i.e. 0.5 mg Cu L^{-1}) at T_{48h} (0.4 ± 0.2) and T_{21days} (0.4 ± 0.1) for the *Ad* and Ctrl units, respectively. At day 30, in the *Ad* units, Cu concentration remaining in the water and distributed between *A. donax* roots, shoots was respectively 3.5, 33 and 0.5% of the initial Cu input. In the Ctrl units, Cu remaining in water was low (7%) and Cu removal (93%) could be partly explained by its immobilization in the Cu-rich biofilm (i.e. 207210 ± 18516 mg Cu kg^{-1}) coating the vat wall. Foliar chlorophyll (i.e. a, b and total) and carotenoid contents decreased at day 30 but root and shoot dry weight (DW) yields increased by 23% and 47% per *Ad* unit, respectively. The shoot Cu concentration remained in the common range (i.e. 3-20 mg Cu kg^{-1}) while the root Cu concentration reached 623 ± 140 mg kg^{-1} allowing 786 mg Cu removal by the root mat. Higher Cu concentration in BME or subsequent repetitions of treatment cycle must be tested to achieve at least 1000 mg Cu kg^{-1} DW in roots (threshold value for Cu-ecocatalyst) whereas the biofilm role must deserve more attention.

Keywords: Bioeconomy, biofilm, biomass, biosourced biochemistry, giant reed, phytoremediation

1. Introduction

Several anthropogenic activities, e.g. mining and acid mine drainages, waste landfilling, pig slurries, Cu electroplating process, use of Cu-based fungicides and wood preservation, can result in Cu-contaminated effluents (Bes and Mench, 2008; Jiang et al., 2012; Guigue et al., 2013; Cestonaro do Amaral et al., 2014; Basias, 2015; Huang et al., 2016). In such effluents, Cu concentration often does not meet the requirement for direct discharge into lotic water systems. In Europe, the regulation guideline for indirect discharge of chemical industry effluents is 0.5 mg Cu L^{-1} (JORF, 2014). Treatment of these effluents through conventional physical, chemical, and biological techniques are usually costly and sometimes operators are not able to fully afford it (Wu et al., 2015a). Their release into aquatic environment may threat flora and fauna biodiversity, e.g. abundance and species composition as well as functionality of marine organisms such as mollusca and arthropoda (Baker et al., 2014), bacteria, fungi (Taylor and Walker, 2016), and algae communities (Rocha et al., 2016a). Contaminant bioaccumulation in plant and animal communities, with further consequences in the food chain and on ecological functions can also be of concern (Rocha et al., 2016b). To prevent ecosystem exposure to Cu excess, one affordable option is to either collect and purify leachates or directly treat *in situ* contaminated effluents in constructed wetlands (CWs) (Babcsányi et al., 2014; Wu et al., 2015a). Bordeaux mixture (BM, $Ca(OH)_2 + CuSO_4$) is the most Cu-based fungicide used in vineyards, orchards, and some cash crops (e.g. tomato, potato tubers) (Mackie et al., 2012). Its long term application in Aquitaine (France) led to soil Cu contamination (i.e. > 1000 mg Cu kg^{-1}, Mackie et al., 2012). Significant amounts of Bordeaux mixture effluent (BME) occurred in Aquitaine region, notably by filling and rinsing the tanks of crop sprayers. Based on Aquitaine vineyard area (150.000 ha), the average tank volume (600 L of BME at 1500 g Cu ha^{-1} for 3 ha) of crop sprayers, residual volume per tank (5 L) and an average of 10 treatments per year, the total volume of BME would be 2.500.000 L (Maille, 2004). Spreading of these diluted BME on the field, authorized by the Article L. 253-1 of the rural Code, contributes to locally increase total soil Cu. To avoid such unnecessary spreading, the management of BME in CWs is an option. Such CW planted with macrophytes are an efficient and cost-effective solution for treating trace element-contaminated wastewaters and effluents (Marchand et al., 2010; Wu et al., 2015a). Cu-contaminated effluents were successfully clean-up in CWs (Guittonny-Philippe et al., 2014; Marchand et al., 2014b; Newete and Byrne, 2016).

Among the growing list of plant species used in CWs, Arundo donax L. is an emerging and promising ones. This rhizomatous grass (Poaceae family) is native from the Mediterranean basin and Eastern Asia. It was introduced in temperate and hot zones worldwide and it is now considered as naturalized species considering its wide distribution (Nsanganvimana et al., 2014). This characteristic makes its use as suitable species in CWs in southern European regions, albeit its invasiveness is a matter of debate (Bonanno et al., 2012). Arundo donax is tolerant to TE excess: Zn, Cr (Kausar et al., 2012; Li et al., 2014), Cd and Ni (Papazoglou et al., 2005), Cu (Elhawat et al., 2015), and As (Mirza et al., 2010 and 2011). In addition, it displays many attractive characteristics for producing biomass, which can be potentially converted by several processes (Nsanganvimana et al., 2014). It can be cultivated as energy crop for bioethanol production, direct combustion and other thermal transformations (Williams et al., 2009; Pilu et al., 2012; Scordia et al., 2012) but also meets requirements for paper pulp production, construction of wooden build materials (see Nsanganvimana et al., 2014 for a review; Elhawat et al., 2015; Luka et al., 2015). Despite these advantages, few studies referenced the ability of A. donax to phytoremediate contaminated matrices (Vymazal, 2011; Mirza et al., 2011; Bonanno, 2012; Yang et al., 2012; Li et al., 2014; Alshaal et al., 2013a) and only some concern the rhizofiltration of contaminated effluents (Mirza et al., 2010; Bonanno, 2013; Elhawat et al., 2014, 2015; Shaheen et al., 2016). In CWs, A. donax removes contaminants such as trace elements (TE) mainly by immobilization in the rhizosphere and storage in the belowground biomass (Sun et al., 2013). Based on this property, its use to rhizofiltrate Cu-contaminated effluents could provide both a belowground biomass with high Cu concentration and a shoot biomass with a common shoot Cu concentration. This shoot biomass may integrate the local processing chains (e.g. Energy sector: bioethanol, biofuels, combustion; Potential fertilizers: compost, biochar, litter; Bioproducts: construction of building materials and plant fiber/plastic composites, Alshaal et al., 2013a) whereas the Cu-rich belowground biomass may be used in biosourced (bio)chemistry as Cu-ecocatalyst (Escande et al., 2014). Ecocatalysis is based on the plant ability to produce plant-borne metal species usable as key reactants to catalyze fine organic chemical reaction for the production of biorenewable transportation fuels, industrial chemicals and pharmaceuticals (Clavé et al., 2016a). Copper-based catalysts are promising candidates, as they are sustainable and cost-competitive catalyzers for the high yield production of next-generation biorefinery components (Yuan et al., 2013).

This study aimed at investigating the ability of A. donax in free-surface water, pilot-scale CW units with recirculation of a Cu-contaminated BME to (1) rhizofiltrate Cu from the effluent, (2) provide a Cu-rich belowground biomass potentially usable as Cu-ecocatalyst, and (3) an uncontaminated shoot biomass.

2. Material and Methods

2.1. Pilot-scale constructed wetland

A pilot-scale CW was installed in January 2015 in a greenhouse (44°47'20.6"N 0°34'48.1"W, INRA Centre Bordeaux-Aquitaine, Villenave-d'Ornon, France). This CW consisted in six 800 L plastic vats with the top removed (width 800 cm × length 1200 cm × height 1180 cm, Denios, Nassandres, France) and a water column deep of 900 cm. All units contained a submerged pump (AquaMax *Eco premium 4000*, Oase; flow: 4000 L h^{-1}) for water recirculation in a closed-loop process. Three out of the 6 units contained each 2 floating racks in polypropylene linked together with plastic rope (60 cm x 40 cm x 7.5cm; Ref. 12.3556.01; Axess-industries, Strasbourg, France) for supporting plant collars (see graphical abstract).

2.2. Plants

Arundo donax was sampled from a drainage ditch, San Remo, Italy, and cultivated by clonal multiplication since 2011 in a greenhouse, Centre INRA-Bordeaux Aquitaine, Villenave d'Ornon, France. In January 2014, bud-bearing stems of *A. donax* (n=100) were sampled and rooted individually for 6 months (January-to-June 2014) on perlite imbibed with a quarter-strength (1/4) Hoagland Nutrient Solution (HNS) (Marchand et al., 2014b) in plastic pots placed in polyethylene vats (60 cm × 40 cm × 15cm). Growing medium was changed every month to avoid anoxia and nutrient depletion. In June, 50 standardized seedlings were individually transferred into plastic pot (3L) filled with compost (Gonzales frères, Martignas sur Jalle, France) and cultivated during 8 months (June-2014 to January-2015) in a greenhouse. In January 2015, plants were collected and roots carefully washed with tap water to remove the compost. In three CW units, 12 plants per unit were placed on the floating racks and fixed at the collar by a plastic rope. For convenience, we will refer throughout the text to (*Ad*) for the units planted with *A. donax* and to (Ctrl) for the unplanted units. All CW units were filled with 600 L of 1/8 HNS prepared with tap water (Supplemental material. 1) (Marchand et al., 2014b). Floating racks were fixed 10 cm above the water surface using plastic rope. Plants were cultivated during 14 months (January-2015 to March-2016) in this culture medium up to 26 month old. Every month 50 L of 1/8 HNS were added per unit to avoid nutrient depletion. Tap water was twice a week added into the CW to maintain the water level. In November 2015 ($T_{november-2015}$), at the end of the growing season, all the dried shoots were harvested (i.e. 4.6 ± 0.6 kg DW $unit^{-1}$). At this time, *A. donax* roots had totally colonized the water volume.

2.3. Sampling and analysis

The rhizofiltration experiment was carried out in the early growing season (March 2016). The effluent retention time (HRT) in the CWs tested during this experiment was 30 days. In week 9, one week before the experiment, CW units were emptied and then refilled with 600 L of 1/8 HNS prepared with tap water in order to homogenize the six units. In week 10, Bordeaux mixture (MACC 80 jardin Geolia, 20% Cu, Weldom, Clermont, France) was added at 4.4 mg Cu L^{-1} corresponding to the BM solubility limit (roughly 105 µM Cu) into the growing medium for mimicking a BME. After the BM addition, the water level was not maintained during the 30 day-experiment to avoid a dilution of initial Cu input (final volume : 550 ± 10 L *Ad* and 580 ± 5 L *Ctrl*). Floating racks were maintained 10 cm above the water surface. Before contamination of the CW with BM (T_{BC}) and immediately after BM addition (T_0), water samples (50 mL) were collected using plastic cups in all CW units. The water samples were collected at the outlet water flows of the pump. Samplings were repeated 1, 3, 5 and 48 hours after BM addition and then every 3 days during 1 month. Physico-chemical parameters, i.e. pH, redox potential (Eh), electrical conductivity (EC) and Cu^{2+} concentration, were determined in all water samples using electrodes (Hanna instruments, pH 210, combined electrode Ag/AgCl – 34 and Cupric ion electrode, Fischer Bioblock, USA, respectively). Concentrations of Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn were determined at $T_{november\text{-}2015}$, T_{BC} (just before contamination), T_0, T_{1h}, $T_{48h,}$ T_{7days}, T_{14days}, T_{21days}, and T_{30days} by ICP-OES (Varian Liberty 200, INRA USRAVE laboratory, Villenave d'Ornon, France). The Cu removal rate from the effluent was calculated as follows: Cu Removal rate (%) $= 100 - \frac{[Cu]_T}{[Cu]_{T0}} \times 100$ with [Cu] in mg Cu L^{-1}. At T_{BC} and T_{30days}, total chlorophyll (*Chl*Tot), including chlorophyll a (*Chl*a) and b (*Chl*b), and total carotenoids (*Ctd*) in the 3rd leaf starting from the stem top (2 x 0.5 cm^2 sampled per leaf from each side of the midrib, one third from the tip) were extracted with N,N-dimethylformamide for three plants per units and their foliar contents computed from measurements at 470, 647 and 664.5 nm of the extracts (spectrophotometer CARY 100 Scan, Lagriffoul et al., 1998). At T_{30days}, water evaporation in the Ctrl units and evapotranspiration in the *Ad* units were determined by measuring the difference of water level between the end and start of the experiment. At T_{BC} and T_{30days}, the maximum stem length of all plants in each unit was measured for determining the dry shoot biomass per unit, using a linear biometric relationship (maximum stem length vs. shoot dry weight (DW) biomass) beforehand calibrated with *A. donax* stem samples (R^2: 94, Supplemental material. 2). In the same way, at T_0 and T_{30days}, the volume of the belowground system (roots and rhizomes, referred thereafter as root volume for convenience) in each unit was measured as Volume $_{root}$ = Volume $_{water + root}$ - Volume $_{water}$, with Volume $_{water}$ measured after lifting the racks with a pulley system, for determining the dry belowground biomass per unit, using a regression (root volume vs. root DW biomass) beforehand calibrated with *A. donax* root samples (R^2: 99, Supplemental material. 3). At T_{BC} and T_{30days}, for each *Ad* unit, two stems, 10 leaves and 50 g roots were harvested randomly among the *A. donax* plants, washed twice with deionized water, blotted with filter paper, placed in paper bags, oven dried at 60°C to constant weight for 72h, and ground (< 1 mm particle size, Retsch MM200). Rhizomes were not sampled to maintain the plant cover for subsequent treatment cycles. Weighed aliquots (0.5 g DW) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5mL supra-pure 14M HNO_3, 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1 mL MilliQ water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (i.e. Cu, Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn) in digests was determined by ICP-MS (Thermo X series 200, INRA

USRAVE laboratory, Villenave d'Ornon, France). All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. All element concentrations in plant parts are expressed in mg or g DW kg^{-1}. The Cu removal by the shoots ($Cu_{shootT30}$) and the roots ($Cu_{rootT30}$) of *A. donax* was calculated as follows: Cu (µg per CW unit) = DW yield (g of either shoots or roots per CW unit) x Cu concentration (µg g^{-1} DW in either shoots or roots). Total Cu removal per CW unit was computed as $Cu_{shootT30}$ + $Cu_{rootT30}$.

At T_{30days}, in the *Ad* units, the initial Cu input (Cu_{T0}) was divided between Cu amount remaining in the water ($Cu_{waterT30}$), Cu amounts in *A. donax* shoots and roots ($Cu_{shootT30}$ and $Cu_{rootT30}$) and Cu amount located in other compartments ($Cu_{othersT30}$). The $Cu_{othersT30}$ ($Cu_{\mathbf{othersT30}}$) value was estimated through a Cu mass balance (mg) in the *Ad* units and calculated as follows:

($Cu_{othersT30}$) = (Cu_{T0}) – ($Cu_{waterT30}$) – ($Cu_{shootT30}$) – ($Cu_{rootT30}$), with Cu_{T0} (mg) = $[Cu]_{T0}$ x 600 L (i.e. $[Cu]_{T0}$ = 4.4 mg Cu L^{-1}); $Cu_{waterT30}$ = $[Cu]_{T30}$ x (600 (L) – Evapotranspiration (L));

At T_{30days}, in the Ctrl units, Cu_{T0} was distributed between $Cu_{waterT30}$ and $Cu_{othersT30}$. The $Cu_{othersT30}$ value was assessed through a Cu mass balance (mg) in the Ctrl units as follows: ($Cu_{othersT30}$) = (Cu_{T0}) – ($Cu_{waterT30}$) ; $Cu_{waterT30}$ = $[Cu]_{T30}$ x (600 (L) – Evaporation (L)).

2.4. Biofilm complementary analysis

Three months after T_{30days}, after determining the mineral composition of water and plant parts and performing a preliminary Cu mass balance, most of the biofilm coating the vat wall was collected in the Ctrl units (but it was not possible to totally reap it), oven dried at 60°C to constant weight for 72h and weighted. Ionome (i.e. Cu, Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn) in biofilm-digests (in triplicate) was determined by ICP-MS following the protocol for plant samples. The biofilm DW yield in the Ctrl units was estimated as follows: Biofilm DW yield (g of biofilm per CW unit) = $Cu_{biofilm}$ (µg per CW unit) ÷ Cu concentration (µg g^{-1} DW in biofilm) with $Cu_{biofilm}$ = $Cu_{othersT30}$.

2.5. Statistical analysis

Shoot and root DW yields, shoot and root Cu concentrations, and ChlTot, Chla, Chlb, Chla/Chlb ratio, and Ctd concentrations of A. donax at TBC and T30days were tested using a Student test (T.test). Normality and homoscedasticity were met for all tests. Differences were considered statistically significant at p<0.05. Changes in physico-chemical parameters and total Cu and Cu2+ concentrations of water depending on elapsed time after the addition of Bordeaux mixture (0-30 days), the treatments (CW units either unplanted or planted with A. donax), and their interaction were analyzed for both units using an ANCOVA. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results

3.1. Water physico-chemical parameters

3.1.1. pH, Eh and EC (Table 1, Fig. 1)

The water pH ranged from 8.4 to 9.0 (*Ad*) and 8.5 to 9.1 (Ctrl). It significantly differed between both treatments ($p<0.05$) but it did not depend on the exposure duration. The water Eh significantly increased in both treatments after the BM addition all along the 30-day experiment ($p<0.05$) and was significantly higher in the Ctrl units than in the *Ad* ones ($p<0.0001$): it ranged (mV) from 213 to 272 (Ctrl) and 230 to 281 (*Ad*). Conversely, the water EC (µS cm^{-1}) was significantly higher in the *Ad* units ($p< 0.0001$) than in the Ctrl ones: it increased with the exposure time from 445 (T_{1h}) to 512 (T_{30days}) and from 440 (T_{1h}) to 456 (T_{30days}) in the *Ad* and Ctrl units, respectively ($p<0.002$).

Table. 1. Results of the ANCOVA investigating the effects of elapsed time after the addition of Bordeaux mixture (0-30 days), the treatment (units either unplanted or planted with A. donax), and their interaction on water parameters.

Parameters	Df	Mean sq	Fvalue	p(>F)	P
pH					
Time	1	0.0009	0.000898	0.029	0.9
Treatment	1	0.1	0.138017	4.4612	0.04 *
Time X Treatment	1	0.0514	0.051401	1.6615	0.2
Residuals	92	2.84623	0.030937		
Eh					
Time	1	1846	1846	5.4494	0.02 *
Treatment	1	5452.6	5452.6	16.0965	0.0001 ***
Time X Treatment	1	340.3	340.3	1.0046	0.3
Residuals	92	31164.6	338.7		
EC					
Time	1	9979	9978.7	9.8372	0.002 **
Treatment	1	18568	18568.2	18.3049	4.6×10^{-5} ***
Time X Treatment	1	1651	1650.9	1.6275	0.2
Residuals	92	92309	1014.4		
Cu^{2+}					
Time	1	143.331	143.331	88.7017	6.9 x 10-15 ***
Treatment	1	6.529	6.529	4.0405	0.05
Time X Treatment	1	0.009	0.009	0.0053	0.9
Residuals	92	138.966	1.616		
Cu					
Time	1	161.848	161.848	21.2044	4.5×10^{-5} ***
Treatment	1	1.026	1.026	0.1344	0.7
Time X Treatment	1	0.005	0.005	0.0006	1.0
Residuals	38	290.045	7.633		

Significance levels: '***' 0.001'**' 0.01'*' 0.05'.' 0.1''1; Df: Degree of freedom, p: pvalue, Fvalue: Fisher value, Means sq: Mean of squares.

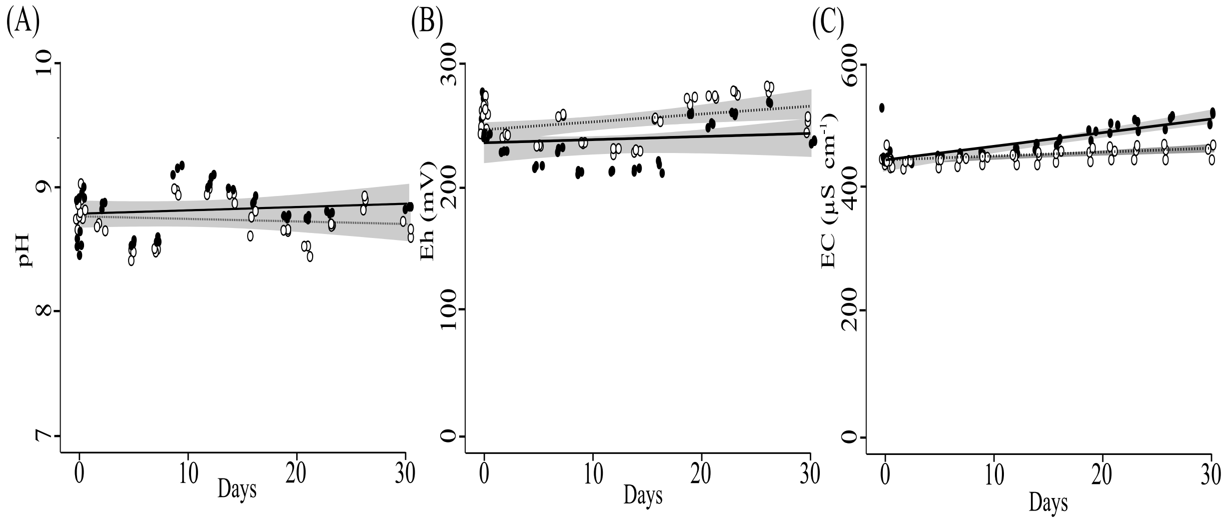


Fig. 1. ANCOVA plot for (A) pH, (B) Eh, (C) EC in the water of CW units either planted with *A. donax* (●, black line) or unplanted (○, dotted line) during the 30-day exposure to the Bordeaux mixture effluent (n=3 per treatment). The trend line power and shaded areas were donated by the ggplot library of the R software. They represent model predictions and corresponding standard error (95%).

3.1.2. Total Cu and Cu2+ concentrations and Cu removal (Table 1 and 2, Fig. 2)

The water Cu^{2+} concentration (µg L^{-1}) decreased for both treatments with the exposure time ($p< 0.0001$) from 6 to 0.5 (*Ad*) and 6 to 0.6 (Ctrl) but it did not differ between the treatments ($p=0.05$). At T_{48h}, the Cu removal rate was almost complete, reaching 92% and 81% in the *Ad* and Ctrl units, respectively. After 48h of exposure, the Cu removal rate slightly increased over the 28 left days reaching 97% and 93% in the *Ad* and Ctrl units, respectively. The residual Cu concentration (mg L^{-1}) decreased for both treatments with the exposure time ($p< 0.0001$), but did not differ between both treatments ($p>0.05$).

Table. 2. Total Cu concentration (mg L^{-1}) and Cu^{2+} concentration in water (µg L^{-1}), and Cu removal rate (%) in the CW units either unplanted or planted with *A. donax*, during the 30-day experiment (n=3 per treatment).

Time (day)	**Cu (mg L^{-1})**	**Removal rate (%)**	**Cu^{2+} (µg L^{-1})**
A. donax			
0	4.4 ± 0	-	6 ± 3
2	0.4 ± 0.2	92	2 ± 0.3
7	0.2 ± 0.03	96	0.5 ± 0.2
14	0.1 ± 0.01	98	0.5 ± 0.2
21	0.1 ± 0.04	98	0.9 ± 0.2
30	0.1 ± 0.03	98	0.5 ± 0.1
Unplanted			
0	4.4 ± 0	-	6 ± 2
2	0.8 ± 0.8	81	3 ± 1
7	1.1 ± 0.3	75	2 ± 0.7
14	0.6 ± 0.2	86	1.1 ± 0.3
21	0.4 ± 0.1	91	1.7 ± 0.6
30	0.3 ± 0.1	93	0.6 ± 0.4

Mean value ± SD for each treatment.

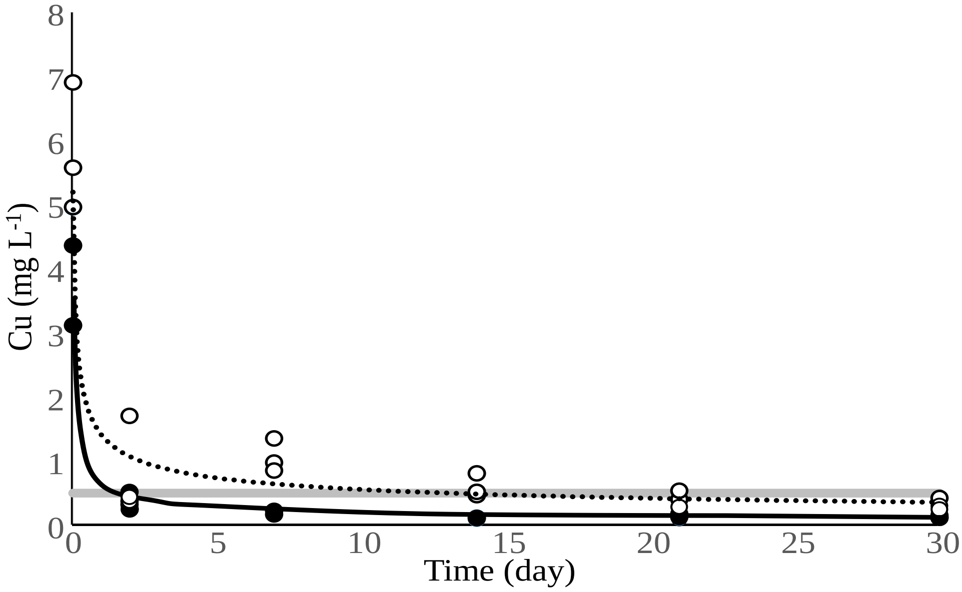


Fig. 2. Cu concentration (mg L-1) in the water of CW units either planted with A. donax (●, black line) or unplanted (○, dotted line) during the 30-day exposure to the Bordeaux mixture effluent (n=3 per treatment) and guideline for indirect discharge of chemical industry effluents (grey line). The trend line of the power function were donated by the Excel software.

3.2. Plant parameters (Table 3 and 4)

During the 30-day exposure, the root and shoot DW yields significantly increased by 23% and 47% in average per *Ad* unit, respectively. At T_0, shoot Cu concentration was 6.2 ± 1.3 mg Cu kg^{-1}. This value did not significantly change at the end of the experiment (i.e. 8 ± 2.5 at T_{30days}). Conversely, root Cu concentration was 14.6 mg Cu kg^{-1} at T_0 and 623 ± 140 mg Cu kg^{-1} at T_{30days} being 43 times higher than at T_0. Consequently, root Cu removal was 66 times higher than shoot Cu removal. Between T_0 and T_{30days}, *Chl*Tot decreased from 303 to 71 mg m^{-2}, and *Chl* a and *Chl* b significantly dropped (4.7 and 3.3 folds) more than carotenoids (2.2 folds). The *Chl* a/*Chl* b ratio was also lower at T_{30days} than at T_0.

Table. 3. Shoot and root DW yields (kg), shoot and root Cu concentrations (mg kg^{-1}), and shoot, root and total Cu removals (mg Cu) of *A. donax* before (T_{BC}) and 30 days (T_{30}) after the addition of Bordeaux mixture in the CW units (n=3 per treatment).

Time	Shoot DW yield (kg)	Root DW yield (kg)	Shoot Cu concentration (mg kg^{-1})	Root Cu concentration (mg kg^{-1})	Shoot Cu removal (mg Cu)	Root Cu removal (mg Cu)	Total Cu removal (mg Cu $units^{-1}$)
T_{BC}	0.8 ± 0.1 b	1 ± 0.2 a	6.2 ± 1.3 a	14.6 ± 1.6 a	-	-	-
T_{30}	1.5 ± 0.03 a	1.3 ± 0.01 a	8 ± 2.5 a	623 ± 140 b	12 ± 3.9	786 ± 179	798 ± 181

Mean value ± SD for each treatment. Values with different letters in a column differ significantly (T.Test, p-value <0.05).

Table. 4. Total chlorophyll, chlorophyll *a (*Chl *a)*, chlorophyll *b (*Chl *b)*, carotenoid densities (mg m^{-2}) and ratio of chlorophyll *a* and *b* in *A. donax* 3rd-leaves (from the stem top) before (T_{BC}) and 30 days (T_{30}) after the addition of Bordeaux mixture in the CW units (n=9 per treatment).

Time	Total chlorophyll (mg m^{-2})	Chl *a* (mg m^{-2})	Chl *b* (mg m^{-2})	Chl *a* vs. Chl *b*	Carotenoids (mg m^{-2})
T_{BC}	303 ± 156 a	226 ± 124 a	77 ± 32 a	3 ± 0.5 a	32 ± 17 a
T_{30}	71 ± 23 b	48 ± 17 b	23 ± 6 b	2 ± 0.3 b	14 ± 2 b

Mean value ± SD for each treatment. Values with different letters in a column differ significantly (T.Test, p-value <0.05).

3.3. Cu mass balance (Fig. 3)

At T_{30days}, in the *Ad* units, a layer of senescent biomass was noticed on the vat bottom and only a thin blue layer was visible on the plastic walls. Conversely, in the Ctrl units, a visible blue biofilm was coating the vat walls without a biomass layer on the vat bottom. At T_{30days}, Cu in the *Ad* units was distributed between the roots (33%) and shoots (0.5%), Cu remaining in the water (3.5%) and 64% in other compartments likely including rhizomes, decaying plant-borne organic matter (OM) on the vat bottom, and biofilm on vat walls. In the Ctrl units, Cu was distributed between Cu remaining in the water (7%) and 93% in other compartments (e.g. biofilms, sorption on CW plastic walls). Three months after T_{30days}, based on the Cu concentration in the biofilm coating the vat wall (i.e. 207210 ± 18516 mg Cu kg^{-1}), we estimated that around 11.4 g of biofilm per vat (18 mg L^{-1}) would be necessary to explain the 93% Cu removal in the unplanted CW units.

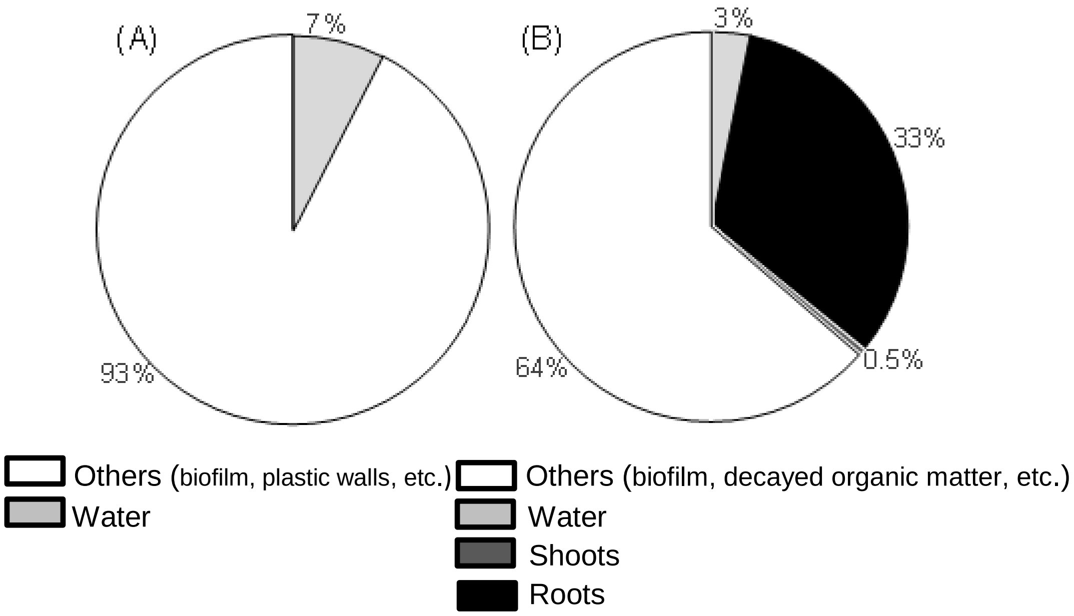


Fig. 3. Copper partitioning (%) in the CW unplanted (A) or planted with *A. donax* (B) after the 30-day exposure to the Bordeaux mixture effluent (n=3 per treatment).

4. Discussion

At T_{30days}, soluble Cu corresponded respectively to 3% and 7% of the initial Cu amount in the planted and the unplanted CW units. Although the removal rates of total Cu and free Cu^{2+} were slightly higher in the *Ad* units along the experiment, the Ctrl units also displayed an high and fast Cu removal pattern. The experiment lasted one month but Cu removal rate quickly reached 92% in the *Ad* and 81% in the Ctrl units within the 48 first hours after BM addition in both units (Table 1). This fast Cu removal in unplanted CW units agreed with several previous studies: Cu removal rate in unplanted CW units was higher (i.e. 40-49 %) than in a *Juncus articulatus* L. and *Phragmites australis* (Cav.) Trin. ex Steud planted CW units (i.e. 7-35 %) in a closed-loop pilot-scale CW filled with a 2.5 µM Cu ($CuSO_4$) spiked solution (Marchand et al., 2014b). Similarly, *Typha angustifolia* L. and *P. australis* planted units in a mesocosm-scale horizontal CW (both free water surface and subsurface flow) filled with wastewater from primary settler were less efficient than the unplanted ones regarding Al, As, Fe, Mn, Pb and Zn removals (Pedescoll et al., 2015). These authors postulated that the substrate (i.e. a mix of gravels and perlite and gravels, respectively) was involved in TE removal mainly by chemical (ad)sorption. Here, the Ctrl units did not contain any substrate thus other compartments such as biofilm on vat walls would be involved in Cu removal.

4.1. Copper removal by *A. donax*

In the *Ad* units, 33% of the Cu input were likely trapped into and onto the giant reed root mat through two mechanisms (Fig. 2): (1) it provided Cu sorption sites and (2) it directly absorbed and stored Cu. The slightly oxidative environment in the units (i.e. 230 - 281 mV) favored Fe/Mn oxyhydroxide precipitation onto the root surface, leading to the formation of a Fe/Mn plaque providing Cu binding sites and contributing to decrease dissolved Cu concentration (Table 1) (Mantovi et al., 2003; Otte et al., 2004; Kissoon et al., 2010). *Arundo donax* can also uptake and store large TE amounts in its roots: *A. donax* exposed 15 days to poly-contaminated wastewater from electroplating (i.e. 0.7 µM Cu) showed root Cu concentration of 630 mg Cu kg^{-1} DW (Sun et al., 2013). Giant reed exposed 2 months to a Cu-contaminated nutrient solution (i.e. 40 µM Cu) showed root Cu concentration of 3512 ± 1372 mg Cu kg^{-1} DW. Conversely, at T_{30days} the shoot Cu concentrations of *A. donax* was within the common Cu values in aerial plant parts (3 – 20 mg Cu kg^{-1}, Tremel-Schaub and Feix, 2005). This disagreed with Sun et al. (2013) who found higher shoot Cu concentrations for *A. donax*. However in both studies, authors used young seedlings with low root and shoot biomass while here, plants were 26 month-old, with a voluminous root mat. This highlights the influence of using a mature CW system for correctly assessing the relative treatment benefits of this plant species (Brisson and Chazarenc, 2009). Higher Cu concentration in roots than in shoots of *A. donax* was explained by Cu accumulation mainly in roots and rhizomes until this belowground biomass became saturated and then Cu transfer to the shoots increased (Mavrogianopoulos et al., 2002; Elhawat et al., 2015). Here, only 3 % of Cu input was located in the giant reed shoots, 10 times lower than in the roots (Table 3). This confirmed giant reed as a Cu-excluder (Elhawat et al., 2014, 2015).

4.2. Copper removal by periphyton

At T_{30days}, in the Ctrl and *Ad* units, respectively 93% and 64% of the initial Cu amount was located in other compartments than the residual BME or/and the roots and shoots of *A. donax* (see above, Fig. 3). Since no substrate was used in our CWs, this Cu amount was expected to be mostly bound with the periphyton (also called *biofilm*). The vicinity of the support matrix, stems and roots are preferred environments for many micro-organisms to attach and to degrade pollutants in the phytoremediation process (Valipour et al., 2014). In the *Ad* units, a Cu precipitate was visible only during the first 2 days of the experiment then a thin blue layer was visible on the CW walls. In the Ctrl units, a Cu precipitate was observed during the first 2 weeks of the experiment, fading with time and gradually replaced by a blue layer on the plastic walls leading to the formation of a blue biofilm coating the vat walls (i.e. 207210 ± 18516 mg Cu kg^{-1}). Biofilms are key players for Cu removal in CWs (Tanner and Headley, 2011; Valipour et al., 2009, Marchand et al., 2014b). Such biofilms contribute to TE removal from contaminated effluents thought complexation and ion exchange (Choi et al., 2009; Sheng et al., 2010; Han and Tao, 2014). The extracellular polymeric substance of biofilms contains various active functional groups, e.g. carboxyls, phenols, hydroxyls, carbonyls, groups containing phosphorus and thiols (Sheng et al., 2010). In the Ctrl and *Ad* units, free Cu^{2+} may have been gradually partly sorbed by the extracellular polymeric substances of the biofilm. Few studies supported this hypothesis: Gill et al. (2014) reported that biofilm sorption contributed to 50% of Cu removal in CWs filled with wood leachate. Sekomo et al. (2012) found a sorption of 19% in the resident biofilm from a Cu-contaminated effluent. In a CW spiked with Cu, 14 bacterial strains were isolated from the surface of the support matrix, and 9 from the plant root surface, but microbial populations were reduced above the detection limit at 30 mg Cu L^{-1} (Valipour et al. 2014). Here we estimated that around 11.4 g of biofilm per vat would be necessary to explain the 93% Cu removal in the unplanted CW units.

4.3. Copper sorption by the decayed OM and bound to dissolved OM

Decayed OM derived from plants can sorb Cu and may contribute to explain the deficit in Cu mass balance in the *Ad* units (i.e. 64%, Fig. 3). In these units, a small layer of decaying OM was noticed on the vat bottom at T_{30days}, but it was not possible to collect it without drying the vats and affecting the future plant growth.

Differences between total Cu and Cu^{2+} concentrations in water were important at all sampling times (Tab. 1): e.g. by difference, Cu complexed in solution with organic and inorganic ligands would be 99.5% and 99.8% at T_{30} in the *Ad* and Ctrl units. Dissolved OM (DOM) is a key player for Cu speciation in CWs mainly through the formation of Cu-DOM complexes (Grybos et al., 2007; Ashworth and Alloway, 2007; Guigue et al., 2013). The DOM fractions are multiple: particulates (e.g. bacteria), colloids (e.g. peptidoglycanes, fulvic acids, hydroxyl-acids and acidic peptides, basic peptides, carbohydrates, etc. Chen et al., 2003; Du et al., 2014; Yao et al., 2016) and they provide functional groups for Cu ion exchange, e.g. carboxyls, phenols, hydroxyls, carbonyls, amines and thiols (Gill et al., 2014). Lower Cu^{2+} concentration in water of the *Ad* units as compared to the Ctrl units on the 2-21 day period (Tab. 1) may be due to additional Cu-chelating compounds from the rhizosphere microbes and root exudates, e.g. mugineic acids, histidine, citrate, etc.. Water Fe concentration (likely Fe(III) inorganic and organic complexes) was relatively low and decreased during the experiment, i.e. between 80 and <20 µg Fe L^{-1} in both *Ad* and Ctrl units. In response to such low Fe availability, grassy species such as giant reed may have produced root-borne ligands such as phytosiderophores (e.g. mugineic acids derived from nicotianamine) to promote Fe uptake and indirectly bind Cu in solution (Tsednee et al., 2012, Schenkeveld et al., 2014). In *A. donax* transcripts, homologous to genes involved in transporters for metal ions complexed with nicotianamine (YELLOW STRIPE1-LIKEgenes) or citrate (FERRIC REDUCTASE DEFECTIVE 3) were identified (Sablok et al., 2014). The aggregation and/or sedimentation of Cu-DOM complexes (Guigue et al., 2013; El Bishlawi et al., 2015) may contribute to decrease total Cu concentration in water of the *Ad* and Ctrl units with elapsed time (Tab. 1).

4.4. Copper removal by precipitation of mineral phases

Here, water pH values ranged from 8.4 to 9.0 (*Ad*) and 8.5 to 9.1 (Ctrl) and macrophytes and water recirculation promoted an oxidizing environment (Arroyo et al., 2010). In such conditions, Cu^{2+} may hydrolyze to form $Cu(OH)^{+}_{(aq)}$, $Cu(OH)_{2(aq)}$, $Cu_2(OH)_2{}^{2+}_{(aq)}$, or may react with anions including Cl^{-}, $CO_3{}^{2-}$ and $PO_4{}^{3-}$ to form $CuCl^{+}$, $CuCO_3$, and $CuHPO_4$ in aqueous solution (Powell et al. 2007; Reddy and DeLaune, 2008; Komarek et al., 2009). Alkaline and oxidizing conditions can also favor the negative charge of Fe (oxyhydr)oxides and allow their potential coprecipitation with Cu, which matched with decreasing water Fe concentration during the 30 days of experiment in the *Ad* and Ctrl units (Supplemental material 4) (Kadlec and Wallace, 2009; Marchand et al., 2010). Precipitation with phosphates, carbonates and nitrates to form $Cu_3(PO_4)_2$, $Cu(CO_3)OH^-$, $Cu(CO_3)_2^{2+}$, and $Cu_2(OH)_3NO_3$ may also lead to decrease the Cu concentration in the BME (Powell et al. 2007). In both *Ad* and Ctrl units, the decreased water P concentration along the experiment may reflect the formation of Cu-phosphate complex (Supplemental material 4).

4.5. Removal rate Required hydraulic retention time

At T_{48h} and T_{21days} for the *Ad* and Ctrl units respectively, total Cu concentration in the BME (i.e. 0.4 ± 0.2 (*Ad*) and 0.4 ± 0.1 mg Cu L^{-1} (Ctrl), Table. 1) met the requirement for indirect discharge of chemical industry effluents (i.e. 0.5 mg Cu L^{-1}; JORF, 2014) but still exceeded the ecotoxicological recommendations for lotic water systems of the French Water Agency (freshwater quality: 0.1 µg Cu L^{-1}; SEQ EAU, 2003) in both CW units. After a 48h-hydraulic retention time (HRT), total Cu and free Cu^{2+} concentrations were respectively twice and 1.5 higher in the Ctrl units (0.8 mg L^{-1}) than in the *Ad* ones. Thus, the CW units planted with *A. donax* were more efficient for removing Cu with a short HRT step. Such results agreed with a higher Cu reduction at the outlet in CW units planted with *T. latifolia* than in the unplanted ones after a 17h HRT (Valipour et al., 2014). The required retention time in both *Ad* and Ctrl units would be here respectively of 2 and 21 days.

4.6. Plants: Cu ecotoxicological availability

In plants, Cu excess may induce oxidative stress. This redox metal may be involved in Haber–Weiss and/or Fenton reactions forming hydroxyl radicals (HO•) (Arora et al., 2002). This free radical is a reactive oxygen species (ROS) which may cause severe damage to cells, e.g. polyunsaturated lipid peroxidation, changes in DNA, proteins and small molecules, damages in the photosynthetic apparatus decreasing the photosynthesis efficiency and biomass production (Mocquot et al., 1996; Hego et al., 2016). During the 30 day-experiment, giant reed plants were exposed from 4.4 (T_0) to 0.1 (T_{30days}) mg Cu L^{-1} and respectively produced in average 300 g and 700 g of root and shoot DW yields per CW unit (Table 3). Shoot biomass was doubled, whereas the belowground biomass increased by 16%. Similarly, shoot and root biomass of *A. donax* only slightly decreased when exposed to a 42.5 µM Cu spiked-nutrient solution (Elhawat et al., 2014). High Cu exposures (up to 422 µM Cu) did not show any adverse effects on the biomass of *A. donax* plants (Elhawat et al., 2015). This lack of negative impact on biomass production despite Cu removal from the water matrix may be explained by a range of detoxification mechanisms that *A. donax* likely set when exposed to Cu excess. In *A. donax* transcripts, homologous to genes involved in metal stress responses through chelation and transport were identified, e.g. ATP-phosphoribosyl transferase (the rate-limiting step in histidine biosynthesis), Ascorbate Peroxidase (ROS scavenging), phytochelatin synthase, and metallothioneins (Sablok et al 2014). However, to detoxify and limit adverse effects on biomass production, plants need to spend energy which leaves less resources for other processes (Audet and Charest, 2008; Maestri et al., 2010). Here, at T_{30days}, both chlorophyll and carotenoid concentrations significantly decreased (Table 4). Chlorophyll degradation due to Cu excess is a common response in macrophytes (Prasad et al. 2001; Srivastava et al. 2006). Such decreases in chlorophyll concentration may be explained by (1) degradation and/or modification of chloroplasts induced by Cu excess (see Adrees et al. 2015 for a review), (2) decrease in chlorophyll synthesis due to foliar Mg sub-deficiency, and (3) inhibition of enzymes such as carbonic anhydrase due to foliar Zn sub-deficiency (Prasad et al. 2001; Monferrán et al. 2009). In our study, despite HSN supply, giant reed shoots (stem + leaves) displayed Fe, K, Mg, P and Mn sub-deficiencies as their concentrations were below or in the low ranges for common values (Tremel-Schaub and Feix, 2005, Supplemental material 5). At T_{30days}, Fe, K, P and Mn concentrations in the effluent were low (Supplemental material 4). Nutrient concentrations in *A. donax* leaves and roots even gradually decreased between $T_{November\text{-}2015}$, T_0 and T_{30days} except for K and Zn in both roots and shoots and Fe in the roots. This result may reflect (1) a lack in nutrient availability likely due to precipitation and/or sorption on bearing phases (e.g. decayed OM, biofilm), (2) nutrient dilution in giant reed biomass, (3) poor plant nutrition due to Cu excess as a nonspecific ion uptake and/or Cu alterations of membrane permeability, and (4) disorder in cellular metabolism (Palmer and Guerinot, 2009). One recommendation to limit this Cu phytotoxicity could be to increase the nutrient supply to enhance giant reed nutrition.

4.7. Plants: as valuable biomass

At T_{30days}, shoot Cu concentration (i.e. 8 ± 2.5 mg Cu kg^{-1} tab. 3) was in its common range (3 - 20 mg kg^{-1}, Tremel-Schaub and Feix, 2005). At the end of the growing season, these dried shoots can be harvested, their potential uses being multiple: (1) energy sector (i.e. biofuel, bioethanol, Mohd Idris et al., 2012), (2) cellulosic derived bioproducts, (3) paper industry and (4) biosourced biochemistry such as production of γ-valerolactone (Nsanganwimana et al., 2014a; Evangelou et al., 2014; Vigil et al., 2015). Giant reed shoots could substitute hardwoods suitable in kraft pulp mills processing chain without major equipment changes (Lewis and Jackson 2002). Since the European Union has established the European Directive 2009/28/EC to increase the production of renewable energy sources and the biofuel proportion by at least 10% in each Member State by 2020 (Gomes, 2012), processing of giant reed shoots produced by phytotechnologies may be a suitable option (Mohd Idris et al., 2012). At T_{30days} after only one treatment cycle, root Cu concentration of giant reed (i.e. 623 ± 140 mg Cu kg^{-1} table 3) did no fulfill the requirements for Cu-ecocatalysts (i.e. Cu concentration > 1000 mg kg^{-1} DW, Escande, 2014). Some options could both increase root Cu concentration of giant reed to fit Cu-ecocatalyst requirements and improve giant reed Cu tolerance to Cu excess: (1) in batch, plant inoculation with endophytic bacteria can promote root Cu accumulation and may enhance biomass production, nutrient availability, and plant Cu tolerance (Weyens et al., 2009; Ma et al., 2011); and (2) silicon addition to the effluent could increase plant tolerance, plant growth and Cu accumulation in the belowground biomass (Zargar et al. 2010; Khandekar and Leisner, 2011; Caldelas et al., 2012). In a previous 2-month batch experiment, root Cu concentration of *A. donax* plants exposed to a 10 µM Cu -spiked solution (i.e. 1809 ± 286 mg Cu kg^{-1}) was lower than in those exposed to a 10 µM Cu+ 1 µM Si (i.e. 2059 ± 679 mg Cu kg^{-1}) and in the roots of *A. donax* inoculated with endophytic bacteria (EB) and exposed to 10 µM Cu (i.e. 2278 ± 1095 mg Cu kg^{-1}). Moreover, EB-inoculated plants produced higher root and shoot biomass at high Cu exposure than non-inoculated plants. This option may help *A. donax* to fulfill the requirement for a Cu-ecocatalyst processing chain.

5. Conclusion

The Cu concentration in the BME met the requirement for indirect discharge of chemical industry effluents at T_{48h} and T_{21} for the *Ad* and Ctrl units, respectively. Copper removal was faster and more effective in the *Ad* than in the Ctrl units. In the planted CW units, Cu was distributed between the roots (33%) and shoots (0.5%) of *A. donax*, Cu remaining in the water (3.5%) and other compartments such as the biofilm on vat walls and the senescent organic matter layer on the vat bottom (64%). Although devoid of substrate, Cu remaining in the water of unplanted CW units was low (7%), and Cu would be partly sorbed by the visible light blue Cu-rich biofilm coating the vat walls (i.e. 207 210 ± 18 516 mg Cu kg^{-1}). At T_{30days}, foliar chlorophyll and carotenoid concentrations significantly decreased, as well as foliar Fe concentration, indicating that their synthesis was affected by Cu excess while shoot and root biomass increased. After one 30-day recirculation of water, shoots of giant reed displayed common Cu concentration whereas root Cu concentration reached 62% of the threshold value for Cu-ecocatalysts.

6. Acknowledgements

This work was supported by ADEME (French Agency for the Environment and Energy, PhD grant of N. Oustrière), the French National Research Agency (PHYTOCHEM ANR-13-CDII-0005-01) and the ERA-Net FACCE SURPLUS (project INTENSE; http://faccesurplus.org/research-projects/intense/). The UMR Biogeco is a member of the INRA Ecotoxicologist network, ECOTOX.

7. References

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M.K., Bharwana, S.A., 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environ. Sci. Pollut. Res. 22, 8148–8162. doi:10.1007/s11356-015-4496-5

Alshaal, T., Domokos-Szabolcsy, E., Marton, L., Czako, M., Katai, J., Balogh, P., Elhawat, N., El-Ramady, H., Gerocs, A., Fari, M., 2013. Restoring soil ecosystems and biomass production of *Arundo donax* L. under microbial communities-depleted soil. Bioenerg. Res. 7, 268–278. doi:10.1007/s12155-013-9369-5

Alshaal, T., Elhawat, N., Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., El-Ramady, H., Fári, M.G., 2015. Giant reed (*Arundo donax* L.): a green technology for clean environment. In: Ansari, A.A., Gill, S.S., Gill, R., Lanza, G.R., Newman, L. (Eds.), Phytoremediation: Management of Environmental Contaminants, vol. 1. Springer International Publishing, Switzerland, pp. 3–20

Arora, A., Sairam, R.K., Srivastava, G.C., 2002. Oxidative stress and antioxidative system in plants. Curr. Sci. 10, 1227–1238.

Arroyo, P., Ansola, G., De Luis, E., 2010. Effectiveness of a full-scale constructed wetland for the removal of metals from domestic wastewater. Water Air Soil Pollut. 210, 473–481. doi:10.1007/s11270-009-0272-9

Ashworth, D.J., Alloway, B.J., 2007. Complexation of copper by sewage sludge-derived dissolved organic matter: effects on soil sorption behaviour and plant uptake. Water Air Soil Pollut. 182, 187–196. doi:10.1007/s11270-006-9331-7

Babcsányi, I., Imfeld, G., Granet, M., Chabaux, F., 2014. Copper stable isotopes to trace copper behavior in wetland systems. Environ. Sci. Technol. 48, 5520−5529. doi:10.1021/es405688v

Baker, T.J., Tyler, C.R., Galloway, T.S., 2014. Impacts of metal and metal oxide nanoparticles on marine organisms. Environmental Pollution. 186, 257−271. doi:10.1016/j.envpol.2013.11.014

Basias, 2015. Inventaire historique de sites industriels et activités de service. http://basias.brgm.fr/ (verified on August 10th, 2016)

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization, Environ. Pollut.156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bonanno, G., 2012. *Arundo donax* as a potential biomonitor of trace element contamination in water and sediment. Ecotox. Environ. Safe. 80, 20–27. doi:10.1016/j.ecoenv.2012.02.005

Bonanno, G., 2013. Comparative performance of trace element bioaccumulation and biomonitoring in the plant species *Typha domingensis*, *Phragmites australis* and *Arundo donax*. Ecotox. Environ. Safe. 97, 124–130. doi:10.1016/j.ecoenv.2013.07.017

Brisson, J., Chazarenc, F., 2009. Maximizing pollutant removal in constructed wetlands: should we pay more attention to macrophyte species selection? Sci. Total. Environ. 407, 3923–3930.

Caldelas, C., Bort, J., Febrero, A., 2012. Ultrastructure and subcellular distribution of Cr in *Iris pseudacorus* L. using TEM and X-ray microanalysis. Cell Biol. Toxicol. 28, 57–68. doi:10.1007/s10565-011-9205-7

Calero, S., Segura, M., Rojo, C., Rodrigo, M.A., 2015. Shifts in plankton assemblages promoted by free water surface constructed wetlands and their implications in eutrophication remediation. Ecol. Eng. 74, 385–393. doi:10.1016/j.ecoleng.2014.11.003

Cestonaro do Amaral, A., Kunz, A., Radis Steinmetz, R.L., Justi, K.C., 2014. Zinc and copper distribution in swine wastewater treated by anaerobic digestion. J. Environ. Manag. 141, 132–137. doi:10.1016/j.jenvman.2014.03.021

Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environ. Sci. Technol. 37, 5701–5710. doi:10.1021/es034354c

Choi, A., Wang, S., Lee, M., 2009. Biosorption of cadmium, copper, and lead ions from aqueous solutions by *Ralstonia sp*. and *Bacillus sp*. isolated from diesel and heavy metal contaminated soil. Geosci. J. 13, 331–341. doi:10.1007/s12303-009-0031-3

Du, X., Xu, Z., Li, J., Zheng, L., 2014. Characterization and removal of dissolved organic matter in a vertical flow constructed wetland. Ecol. Eng. 73, 610–615. doi:10.1016/j.ecoleng.2014.09.098

Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., Tack, F.M., 2009. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review. Sci. Total Environ. 2009, 407(13), 3972−3985. doi:10.1016/j.scitotenv.2008.07.025

El Bishlawi, H., Jaffe, P.R., 2015. Characterization of dissolved organic matter from a restored urban marsh and its role in the mobilization of trace metals. Chemosphere. 127, 144–151. doi:10.1016/j.chemosphere.2014.12.080

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, É., El-Ramady, H., Márton, L., Czakó, M., Kátai, J., Balogh, P., Sztrik, A., Molnár, M., Popp, J., Fári, M.G., 2014 Phytoaccumulation potentials of two biotechnologically propagated ecotypes of *Arundo donax* in copper-contaminated synthetic wastewater. Environ. Sci. Pollut. Res. 21, 7773–7780. doi:10.1007/s11356-014-2736-8

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, E., El-Ramady, H., Antal, G., Marton, L., Czako, M., Balogh, P., Fari, M., 2015. Copper uptake efficiency and its distribution within bioenergy grass giant reed, Bull. Environ. Contam. Toxicol. 95, 452–458. doi:10.1007/s00128-015-1622-5

Escande, V., Olszewski, T.K., Grison, C., 2014. Preparation of ecological catalysts derived from Zn hyperaccumulating plants and their catalytic activity in Diels–Alder reaction. C. R. Chimie. 17, 731–737. doi:10.1016/j.crci.2013.09.009

Escande, V. 2014. De la phytoextraction à l'Écocatalyse : Valorisation de la biomasse chargée en éléments métalliques pour la conception de nouveaux catalyseurs et leur utilisation en synthèse organique durable, Thèse de Doctorat, Ecole Doctorale Systèmes intégrés en Biologie, Agronomie, Géosciences, Hydrosciences et Environnement, Écosystèmes et Sciences Agronomiques, Université Montpellier 2, Montpellier, 31-40 p,

Evangelou, M.W.H., Deram, A., Ugolini, F., Abiven, S., Schulin, R., 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. J. Environ. Manage. 146, 100–106. doi:10.1016/j.jenvman.2014.07.046

Gill, L.W., Ring, P., Higgins, N.M.P., Johnston, P.M. 2014. Accumulation of heavy metals in a constructed wetland treating road runoff. Ecol. Eng. 70, 133–139. doi:10.1016/j.ecoleng.2014.03.056

Gomes, H., 2012. Phytoremediation for bioenergy: challenges and opportunities. Environ. Technol. Rev. 1(1), 59–66. doi:10.1080/09593330.2012.696715

Grybos, M., Davranche, M., Gruau, G., Petitjean, P., 2007. Is trace metal release in wetland soils controlled by organic matter mobility or Fe-oxyhydroxides reduction? J. Colloid Interface Sci. 314, 490–501. doi:10.1016/j.jcis.2007.04.062

Guigue, J., Mathieu, O., Lévêque, J., Denimal, S., Steinmann, M., Milloux, M.J., Grisey, H., 2013. Dynamics of copper and zinc sedimentation in a lagooning system receiving landfill leachate. Waste Manage. 33, 2287–2295. doi:10.1016/j.wasman.2013.06.004

Guittonny-Philippe, A., Masotti, V., Höhener, P., Boudenne, J.L., Viglione, J., Laffont-Schwob, I., 2014. Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: A review to overcome obstacles and suggest potential solutions. Environ. Int. 64, 1–16. doi: 10.1016/j.envint.2013.11.016

Han, J., Tao, W., 2014. Treatment performance and copper removal mechanisms of a vegetated submerged bed receiving leachate from ACQ-treated lumber. Ecol. Eng. 70, 162–168. doi:10.1016/j.ecoleng.2014.05.005

Huang, X., Xu, Y., Shan, C., Li, X., Zhang, W., Pan, B., 2016. Coupled Cu(II)-EDTA degradation and Cu(II) removal from acidic wastewater by ozonation: Performance, products and pathways. Chemical Eng. J. 299, 23–29. doi:10.1016/j.cej.2016.04.044

JORF, 2014. Arrêté du 17 juin 2014 modifiant l'arrêté du 2 février 1998 relatif aux prélèvements et à la consommation d'eau ainsi qu'aux émissions de toute nature des ICPE soumises à autorisation. JORF, 0154 (5 juillet 2014), 11149, texte 9. Available at https://www.legifrance.gouv.fr/eli/arrete/2014/6/17/DEVP1327393A/jo

Jiang, Z.F., Huang, S.Z., Han, Y.L., Zhao, J.Z., Fu, J.J., 2012. Physiological response of Cu and Cu mine tailing remediation of *Paulownia fortunei* (Seem) Hemsl. Ecotoxicology, 21, 759–767. doi:10.1007/s10646-011-0836-5

Kadlec, R., Wallace, S., 2008. Treatment Wetlands. CRC Press, Boca Raton, Florida.

Kanoun-Boule, M., Vicente, J.A.F., Nabais, C., Prasad, M.N.V., Freitas, H., 2009. Ecophysiological tolerance of duckweeds exposed to copper. Aquat. Toxicol. 91, 1–9. doi:10.1016/j.aquatox.2008.09.009

Kausar, S., Mahmood, Q., Raja, I.A., Khan, A., Sultan, S., Gilani, M.A., Shujaat, S., 2012. Potential of *Arundo donax* to treat chromium contamination, Ecol. Eng. 42, 256–259. doi:10.1016/j.ecoleng.2012.02.019

Khandekar, S., Leisner, S., 2011. Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress, J. Plant Physiol. 168, 699–705. doi: 10.1016/j.jplph.2010.09.009

Kissoon, L.T., Jacob, D.L., Otte, M.L., 2010. Multi-element accumulation near Rumex crispus roots under wetland and dryland conditions. Environ. Pollut. 158, 1834–1841. doi:10.1016/j.envpol.2009.11.001

Komárek, M., Vaněk, A., Chrastný, V., Száková, J., Kubová, K., Drahota, P., Balík. J., 2009. Retention of copper originating from different fungicides in contrasting soil types. J. Hazard. Mater. 166, 1395–1402. doi:10.1016/j.jhazmat.2008.12.061

Lagriffoul, A., Mocquot, B., Mench, M., Vangronsveld, J., 1998. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Plant Soil. 200, 241–250. doi:10.1023/a:1004346905592

Lewis, M., Jackson, M., 2002. Nalgrass: a nonwood fiber source suitable for existing US pulp mills. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. ASHS, Alexandria, pp 371–376

Li, C., Xiao, B., Wang, Q.H., Yao, S.H., Wu, J.Y., 2014. Phytoremediation of Zn- and Cr-contaminated soil using two promising energy grasses, Water Air Soil Pollut. 225:2027. doi:10.1007/s11270-014-2027-5

Luca, C., Pilu, R., Tambone, F., Scaglia, B., Adani, F., 2015. New energy crop giant cane (*Arundo donax* L.) can substitute traditional energy crops increasing biogas yield and reducing costs. Bioresource Technol. 191, 197–204. doi:10.1016/j.biortech.2015.05.015

Ma, Y., Rajkumar, M., Freitas, H., 2009. Improvement of plant growth and nickel uptake by nickel resistant-plant growth promoting bacteria. J. Hazard. Mater. 166, 1154–61. doi:10.1016/j.jhazmat.2008.12.018

Mackie, K.A., Müller, T., Kandeler, E., 2012. Remediation of copper in vineyards - A mini review, Environ. Pollut. 167, 16–26. doi:10.1016/j.envpol.2012.03.023

Maille, E., 2004. Enquete phytosanitaire Gironde – Dordogne. http://www.itab.asso.fr/downloads/actes/actes_jt_viti_04_avignon_mini.pdf (Verified on August 10th, 2016)

Mantovi, P., Bonazzi, G., Maestri, E., Marmiroli, N., 2003. Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. Plant Soil. 250, 249–257. doi:10.1023/A:1022848131043

Marchand, L., Mench, M., Jacob, D.L., Otte, M.L., 2010. Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ. Pollut. 158, 3447–3461. doi:10.1016/j.envpol.2010.08.018

Marchand, L., Nsanganwimana, F., Oustriere, N., Grebenshchykova, Z., Lizama-Allende, K., Mench, M., 2014b. Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*. Ecol. Eng. 64, 291–300. doi:10.1016/j.ecoleng.2013.12.017

Mirza, N., Mahmood, Q., Pervez, A., Ahmad, R., Farooq, R., Shah, M.M., Azim, M.R., 2010. Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater, Biores. Technol. 101, 5815–5819. doi:10.1016/j.biortech.2010.03.012

Mirza, N., Pervez, A., Mahmood, Q., Shah, M.M., Shafqat, M.N., 2011. Ecological restoration of arsenic contaminated soil by *Arundo donax* L, Ecol. Eng. 37, 1949–1956. doi:10.1016/j.ecoleng.2011.07.006

Mocquot, B., Vangronsveld, J., Clijsters, H., Mench, M., 1996. Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil. 182, 287–300.

Mohd Idris, S., Jones, P.J., Salzman, S.A., Croatto, G., Allinson, G., 2012. Evaluation of the giant reed (*Arundo donax*) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. Environ. Sci. Pollut. Res. 19, 1159–1170. doi:10.1007/s11356-011-0642-x

Monferrán, M.V., Agudo, J.A.S, Pignata, M.L., Wunderlin, D.A., 2009. Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte *Potamogeton pusillus*. Environ. Pollut. 157, 2570–2576. doi:10.1016/j.envpol.2009.02.034

Newete, S.W., Byrne, M., 2016. The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. Environ. Sci. Pollut. Res. 23, 10630–10643. doi:10.1007/s11356-016-6329-6

Nsanganwimana, F., Marchand, L., Douay, F., Mench, M., 2014a. *Arundo donax* L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock, A review. Int. J. Phytoremediation, 16, 982–1017. doi:10.1080/15226514.2013.810580

Otte, M.L., Matthews, D.J., Jacob, D.L., Moran, B.M., Baker, A.J.M., 2004. Biogeochemistry of metals in the rhizosphere of wetland plants - an explanation for “innate” metal tolerance? Wetl. Ecosyst. Asia: Funct. Manag. 1, 87–94. doi:10.1016/B978-044451691-6/50009-0

Palmer, C.M., Guerinot, M.L., 2009. Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat. Chem. Biol. 5, 333–340. doi:10.1038/nchembio.166

Palmer, I.E., Gehl, R.J., Ranney, T.G., Touchell, D., George, N., 2014. Biomass yield, nitrogen response, and nutrient uptake of perennial bioenergy grasses in North Carolina. Biomass Bioenerg. 63, 218–228. doi:10.1016/j. biombioe.2014.02.016

Papazoglou, E.G., Karantounias, G.A., Vemmos, S.N., Bouranis, D.L., 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. Environ. Int. 31, 243–249. doi:10.1016/j.envint.2004.09.022

Pedescoll, A., Sidrach-Cardona, R., Hijosa-Valsero, M., Bécares, E., 2015. Design parameters affecting metals removal in horizontal constructed wetlands for domestic wastewater treatment, Ecol. Eng. 80, 92–99. doi:10.1016/j.ecoleng.2014.10.035

Pilu, R., Bucci, A., Badone, FC., Landoni, M., 2012. Giant reed (*Arundo donax* L.): A weed plant or a promising energy plant? Afr. J. Biotechnol. 11, 9163–9174. doi:10.5897/AJB11.4182

Powell, K.J. Brown, P.L., Byrne, R.H., Gajda, T., Hefter, G., Sjöberg, S., Wanner, H., 2007. Chemical speciation of environmentally significant metals with inorganic ligands part 2: the Cu^{2+}, OH^{-}, Cl^{-}, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-} systems. Pure Appl. Chem., 79(5), 895–950. doi:10.1351/pac200779050895

Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M., Strazalka, K., 2001. Physiological responses of *Lemna trisulca* L. (duckweed) to cadmium and copper bioaccumulation. Plant Sci. 161, 881–889. doi:10.1016/S0168-9452(01)00478-2

Reddy, K.R., DeLaune, R.D., 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press, Boca Raton, Florida, USA, pp. 774

Rocha, G.S., Lombardi, A.T., Melão, M.G.G., 2016a. Influence of phosphorus on copper toxicity to *Selenastrum gracile* (Reinsch) Korshikov. Ecotox. Environ. Safe. 128, 30–35. doi:10.1016/j.ecoenv.2016.02.007

Rocha, G.S., Tonietto, A.E., Lombardi, A.T., Melão, M.G.G., 2016b Effect of copper contaminated food on the life cycle and secondary production of *Daphnia laevis*. Ecotox. Environ. Safe. 133, 235–242. doi: 10.1016/j.ecoenv.2016.07.011

Rossini Oliva, S., Mingorance, M.D., Leidi, E.O., 2011. Effects of silicon on copper toxicity in Erica andevalensis Cabezudo and Rivera: a potential species to remediate contaminated soils, J. Environ. Monitor. 13, 591–596. doi:10.1039/c0em00549e

Sablok, G., Fu, Y., Bobbio, V., Laura, M., Rotino, G.L., Bagnaresi, P., Allavena, A., Velikova, V., Viola, R., Loreto, F., Li, M., Varotto, C., 2014. Fuelling genetic and metabolic exploration of C3 bioenergy crops through the first reference transcriptome of *Arundo donax* L. Plant Biotech. J. 12, 554–567. doi: 10.1111/pbi.12159

Schenkeveld, W.D.C., Oburger, E., Gruber, B., Schindlegger, Y., Hann, S., Puschenreiter, M., Kraemer, S.M., 2014. Metal mobilization from soils by phytosiderophores - experiment and equilibrium modeling, Plant Soil. 383, 59–71. doi: 10.1007/s11104-014-2128-3

Scordia, D., Cosentino, SL., Lee, J.W., Jeffries, T.W., 2012. Bioconversion of giant reed (*Arundo donax* L.) hemicellulose hydrolysate to ethanol by *Scheffersomyces stipites* CBS6054. Biomass. Bioenerg. 39, 296–305. doi:10.1016/j.biombioe.2012.01.023

Sekomo, C.B., Rousseau, D.P.L., Saleh, S.A., Lens, P.N.L., 2012. Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. Ecol. Eng. 44, 102–110. doi:10.1016/j.ecoleng.2012.03.003

Shaheen, S., Mahmood, Q., Pervez, A., Mirza, N., Bhatti, Z.A., Hayat, T., Meryem, S.S., Ullah, F., 2016 Chromium uptake by giant reed under rhizobacterial inhibition. Int. J. Environ. Sci. Technol. 13(6), 1581–1590. doi:10.1007/s13762-016-0996-1

Sheng, G.P., Yu, H.Q., Li, X.Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv. 28, 882–894. doi:10.1016/j.biotechadv.2010.08.001

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Gupta, D.K., 2006. Copper induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. Aquat. Toxicol. 80, 405–415. doi:10.1016/j.aquatox.2006.10.006

Sun, H., Wang, Z., Gao, P., Liu, P., 2013. Selection of aquatic plants for phytoremediation of heavy metal in electroplate wastewater. Acta. Physiol. Plant. 35, 355–364. doi:10.1007/s11738-012-1078-8

Tanner, C.C., Headley, T.R., 2011. Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. Ecol. Eng. 37, 474–486. doi:10.1016/j.ecoleng.2010.12.012

Taylor, A.A., Walker, S.L., 2016. Effects of copper particles on a model septic system's function and microbial community. Water Research. 91, 350–360. doi:10.1016/j.watres.2016.01.014

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie. Angers: EDP Sciences, Les Ulis 106

Tsednee, M., Mak, Y.W., Chen, Y.R., Yeh, K.C., 2012. A sensitive LC-EST-Q-TOF-MS method reveals novel phytosiderophores and phytosiderophore–iron complexes in barley. New Phytol. 195, 951–961. doi:10.1111/j.1469-8137.2012.04206.x

Valipour, A., Raman, V.K., Ghole, V.S., 2009. A new approach in wetland systems for domestic wastewater treatment using *Phragmites sp*. Ecol. Eng. 35, 1797–1803. doi:10.1016/j.ecoleng.2009.08.004

Vigil, M., Marey-Pérez, M.F., Martinez Huerta, G., Álvarez Cabal, V., 2015. Is phytoremediation without biomass valorization sustainable?—Comparative LCA of landfilling vs. anaerobic co-digestion. Sci. Total. Environ. 505, 844–850. doi:10.1016/j.scitotenv.2014.10.047

Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia. 674, 133–156. doi:10.1007/s10750-011-0738-9

Weyens, N., van der Lelie, D., Taghavi, S., Vangronsveld, J., 2009. Phytoremediation: plant-endophyte partnerships take the challenge. Current Opinion in Biotechnology. 20, 248–254. doi: 10.1016/j.copbio.2009.02.012

Williams, C.M.J., Biswas, T.K., Black, I.D., Marton, L., Czako, M., Harris, P.L., Pollock, R., Heading, S., Virtue, J.G., 2009. Use of poor quality water to produce high biomass yields of giant reed (*Arundo donax*) on marginal lands for biofuel or pulp/paper. Acta Horticulturae. 806, 595–602. doi:10.17660/ActaHortic.2009.806.74

Wu, S., Wallace, S., Brix, H., Kuschk, P., Kirui, W.K., Masi, F., Dong, R., 2015a. Treatment of industrial effluents in constructed wetlands: challenges, operational strategies and overall performance. Environ. Pollut. 201, 107–120. doi:10.1016/j.envpol.2015.03.006

Yang, M., Xiao, X.Y., Miao, X.F., Guo, Z.H., Wang, F.Y., 2012. Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead, Trans. Nonferrous Met. Soc. China, 22, 1462–1469. doi:10.1016/S1003-6326(11)61342-3

Yao, Y., Li, Y.Z., Guo, X.J., Huang, T., Gao, P.P., Zhang, Y.P., Yuan, F. 2016. Changes and characteristics of dissolved organic matter in a constructed wetland system using fluorescence spectroscopy. Environ. Sci. Pollut. Res. 23, 12237–12245. doi:10.1007/s11356-016-6435-5

Yuan, J., Li, S.S., Yu, L., Liu, Y.M., Cao, Y., He, H.Y., Fan, K.N., 2013. Copper-based catalysts for the efficient conversion of carbohydrate biomass into γ-valerolactone in the absence of externally added hydrogen. Energy Environ. Sci. 6, 3308–3313. doi:10.1039/c3ee40857d

Zargar, S.M., Nazir, M., Agrawal, G.K., Kim, D.W., Rakwal, R., 2010. Silicon in plant tolerance against environmental stressors: towards crop improvement using omics approaches. Curr. Prot. 7, 135–43. doi:10.2174/157016410791330507

Table. Supplemental material 1. Main physico-chemical parameters of the tap water used to prepared the BME

Parameters	Value
pH	7.3
EC (µS cm^{-1})	430
Total organic carbon (mg L^{-1})	0.6
CO_3^{-2} (mg L^{-1})	< 6
HCO_3^- (mg L^{-1})	207
NO_2^- (mg L^{-1})	< 0.02
NO_3^- (mg L^{-1})	0.3
SO_4^{2-} (mg L^{-1})	34
NH_4^+ (mg L^{-1})	0.1
Cl (mg L^{-1})	11
F (mg L^{-1})	2
Ca (mg L^{-1})	25
K (mg L^{-1})	5
Mg (mg L^{-1})	16
Na (mg L^{-1})	30
P (mg L^{-1})	< 0.02
Cd (µg L^{-1})	< 1
Fe (µg L^{-1})	185
Mn (µg L^{-1})	< 5
Ni (µg L^{-1})	< 5

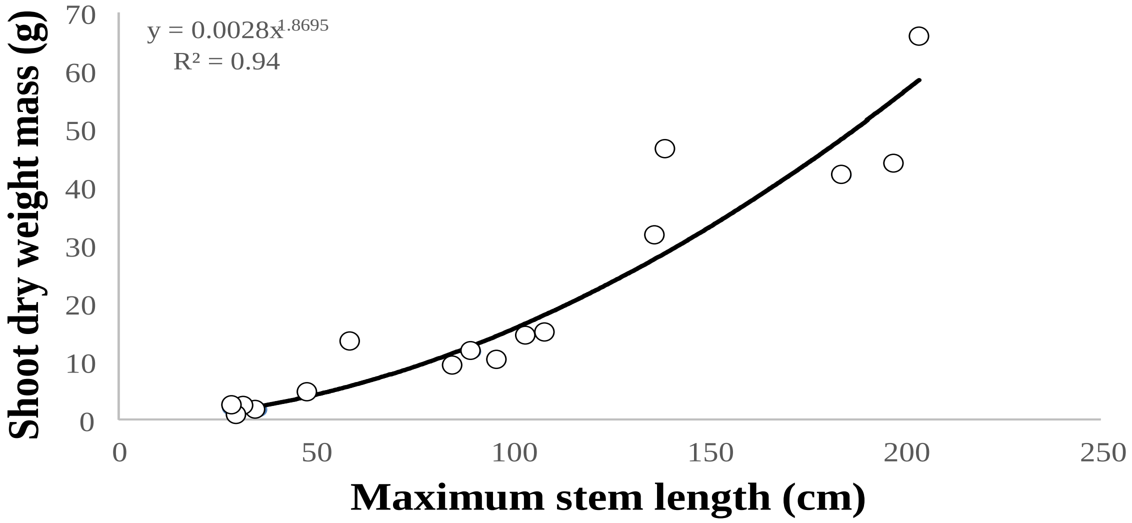


Fig. Supplemental material 2. Relationship between the maximum stem length (cm) and the shoot dry weight mass (g plant^{-1}) of *A. donax*. The trend line of the power function and the correlation coefficient R^2 were donated by the Excel software.

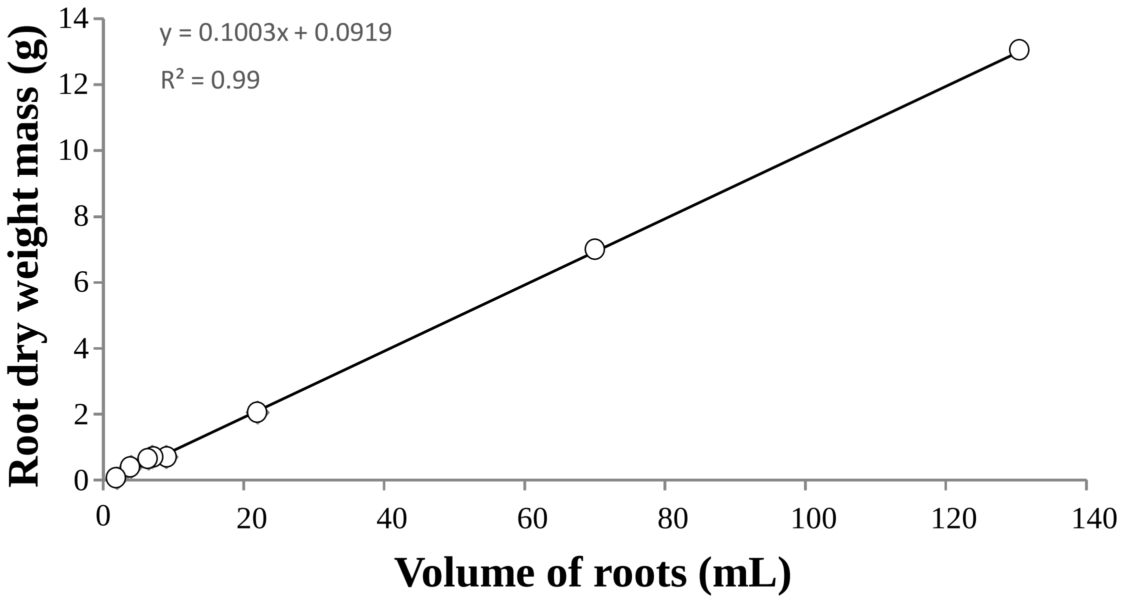


Fig. Supplemental material 3. Relationship between the volume of the belowground plant parts (mL) and the root dry weight mass (g plant^{-1}) of *A. donax*. The trend line of the linear regression and the correlation coefficient R^2 were donated by the Excel software.

Table. Supplemental material 4. Nutrient concentrations (mg L^{-1}) in the water of CW units either planted with *A. donax* or unplanted during the 30-day experiment (n=3).

Time (day)	Ca	Fe	K	Mg	Na	P	Mn	Zn
A. donax								
0	35.1 ± 1	0.1 ± 0.08	0.96 (n=2)	19.1 ± 1	43 ± 0.5	0.04 ± 0.02	<0.02	0.02 ± 0.004
2	32.2 ± 1	0.02*	0.7 ± 0.6	17.7 ± 1	**	<0.02	<0.02	< 0.007
7	32.7 ± 0.4	0.02*	1.5 ± 1.1	17.6 ± 0.1	**	<0.02	<0.02	< 0.007
14	34.0 ± 1	<0.02	0.6 ± 0.1	18.7 ± 0.3	47 ± 1	<0.02	<0.02	< 0.007
21	36.2 ± 2	<0.02	1 ± 1	19.8 ± 1	51 ± 2.5	<0.02	<0.02	< 0.007
30	38.4 ± 2	0.02 ± 0.001	0.5 ± 0.2	20.9 ± 1	55 ± 3	<0.02	<0.02	< 0.007
Unplanted								
0	33.3 ± 1.1	0.05 ± 0.02	7.1 ± 0.2	18 ± 0.6	38 ± 1.2	0.02 ± 0.01	<0.02	0.04 ± 0.005
2	31.3 ± 1.9	0.03*	6 ± 0.6	16.9 ± 1.1	37 ± 4.1	<0.02	<0.02	0.02 ± 0.001
7	31.3 ± 0.3	0.03*	6.8 ± 0.3	16.5 ± 0.2	**	<0.02	<0.02	0.02 ± 0.01
14	34.4 ± 1.4	<0.02	7.3 ± 0.7	18.6 ± 0.8	41 ± 1.8	<0.02	<0.02	0.02 ± 0.007
21	34.6 ± 2.1	<0.02	7.1 ± 0.5	18.8 ± 1.1	41 ± 2.1	<0.02	<0.02	0.02 ± 0.003
30	33.9 ± 2.4	<0.02	6.8 ± 0.8	18.5 ± 1.4	40 ± 3.3	<0.02	<0.02	0.02 ± 0.005

Mean value ± SD for each treatment (n=3). * values below the detection limit were removed (n=1)
** outliers values.

Table. Supplemental material 5. Stem, leaf and root ionomes (mg kg^{-1} DW) of *A. donax*, after 11 months of cultivation in the units ($T_{novembre\ 2015}$) before the addition of Bordeaux mixture (T_{BC}) in the CW units and after the 30-day exposure (T_{30}). In bold, values below the common concentrations in plants.

	Ca	**Fe**	**K**	**Mg**	**Na**	**P**	**Mn**	**Zn**
$T_{november-2015}$								
Leaf	8949 ± 1294	64 ± 8	18674 ± 2963	7067 ± 153	59 ± 15	1307 ± 238	30 ± 14	28 ± 4
Stem	778 ± 177	40 ± 10	11196 ± 8436	1945 ± 260	18 ± 4	531 ± 334	15 ± 5	41 ± 15
Roots	3430 ± 123	80 ± 16	7748 ± 403	4902 ± 956	21± 7	900 ± 69	50 ± 28	28 ± 5
T_{BC}								
Leaf	5555 ± 2245	43 ± 6	20848 ± 2291	4049 ± 1619	39 ± 10	**1273** ± 191	**13** ± 6	34 ± 11
Stem	**601** ± 399	**16** ± 9	**17581** ± 3719	**688** ± 359	21 ± 6	**800** ± 41	**4.8** (n=1)	14 ± 4
Roots	1855 ± 916	91 ± 33	23586 ± 12845	2993 ± 1291	19 ± 10	1095 ± 235	5.7 ± 0.6	32 ± 5
T_{30}								
Leaf	3001 ± 885	25 ± 3	20472 ± 4004	2358 ± 661	20 ± 4	**1406** ± 243	**5.4** ± 1	19 ± 5
Stem	1031 ± 250	**14** ± 3	**18870** ± 7411	**1499** ± 212	24 ± 8	**1174** ± 709	< 0.02	23 ± 5
Roots	1355 ± 77	90 ± 37	19469 ± 2712	2220 ± 62	13 ± 11	726 ± 115	5.4 ± 0.4	32 ± 4
Common shoot concentrations*	1000–50000	20 - 300	20000–50000	1500–3500	-	1600–6000	50 - 500	10-150
Arundo donax shoots**	4300 ± 1400	40 ± 10	3800 ± 1000	3000 ± 1000	-	3500 ± 1200	-	-

Mean value ± SD for each treatment (n=3). * (Tremel-Schaub and Feix, 2005), ** Personal data.

Part. 3. Biomass production with high metal(loid) concentrations able to integrate the Eco-catalysis processing chains

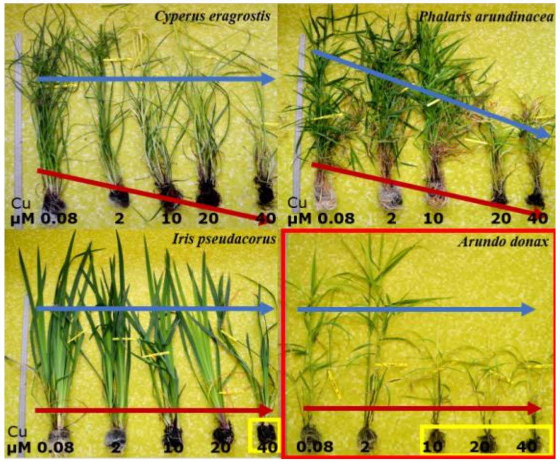
3.3. Summary

Reminder: Biocatalysis is based on the use of metal species originating from plant biomass with high metal(loid) concentrations (e.g. unusual oxidation levels, new associated chemical species, and effects of synergy) (Clavé et al., 2016a). In this context, a novel research area investigating Cu-based catalysts produced from biomass with high Cu concentration (i.e. ≥1000 mg kg^{-1} DW needed to meet the requirement for eco-catalyst production) is emerging. In Aquitaine, France, the Bordeaux mixture used in vineyards generates significant amounts of Cu contaminated effluents (BME, i.e.: 2.500.000 L $year^{-1}$, Maille, 2004). Rhizofiltration in constructed wetlands (CW) is one option for managing such effluents. In parallel, roots (and in a lesser extent shoots) produced in CWs may reach the Cu concentrations required for Cu eco-catalyst production.

The first study of this part aimed at evaluating the capacity of 4 macrophytes (i.e. *Arundo donax* L., *Cyperus eragrostis* Lam., *Iris pseudacorus* L., and *Phalaris arundinacea* L.) commonly used in CWs for producing Cu-rich root mats (**Part 3, Chapter 4**). These 4 macrophytes were assessed on a Cu gradient (i.e. Cu-spiked nutrient solution: 0.08, 2, 10, 20 and 40 µM Cu, $CuSO_4.5H_2O$) in controlled batch conditions during 2 months. Among them, only *I. pseudacorus* and *A. donax* delivered root mats potentially usable as Cu-ecocatalyst since they respectively peaked up to 1099 and 3512 mg Cu kg^{-1} when exposed at 40 µM Cu. *Iris pseudacorus* also produced the highest shoot biomass but with Cu concentration within the low common Cu range. Therefore, such biomass can integrate regular processing chains such as the energy sector (Chapter 4). In the 10-40 µM Cu range, shoot Cu concentrations of *A. donax* were not sufficient for its use as Cu-ecocatalysts (< 1000 mg Cu kg^{-1} DW) but at the same time above the common shoot Cu concentrations (i.e. 42-175 mg Cu kg^{-1}). This biomass may be considered as contaminated wastes and cannot integrate regular processing chains. However it may be composted and used to fertilize Cu-deficient soils as podzols and commercial peat substrates. Its pyrolyzation is another option. In this study, we used young seedlings (7-month-old) with low shoot biomass which may enhance shoot Cu concentration. According to Brisson and Chazarenc (2009), using a mature plant is essential for correctly assessing the relative treatment benefits of a plant species. Copper concentrations measured in our 7-month-old plants would probably be overestimated as compared to mature plants.

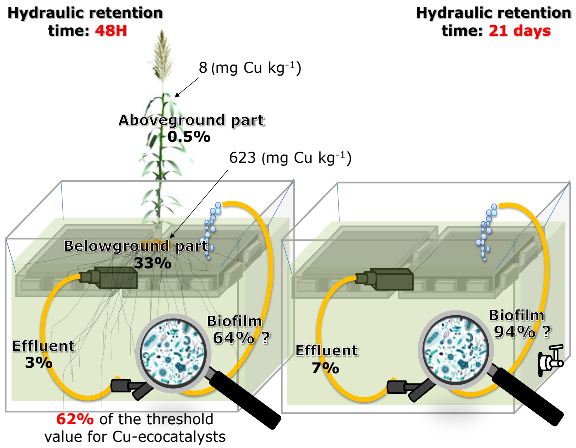
In a second time (**Part 3, Chapter 5**), a pilot-scale CW was developed to jointly rhizofiltrate BME while easily managing the biomass produced in the system. We selected *A. donax* over *I. pseudacorus* to be planted in the pilot-scale CW for maximizing the Cu accumulation in the roots, as *A. donax* displayed higher Cu concentration than *I. pseudacorus* at the same Cu exposure. Compared to our young seedlings, the mature plants in the CW system designed to rhizofiltrate BME displayed higher plant biomass which could dilute Cu concentration leading to lower Cu concentration in both roots and shoots. In this way, we made the bet that foliar Cu concentrations of mature *A. donax* (>26 months) would fall in the common Cu range thus allowing this biomass to integrate various processing chains (e.g. Energy sector: bioethanol, biofuels, combustion; Potential fertilizers: compost, biochar, litter; Bioproducts: construction of building materials and plant fiber/plastic composites). The pilot CW consisted in 6 units (800 L each), 3 units being planted with *A. donax* and three others remaining unplanted (Ctrl). The BME has a Cu concentration of roughly 69 µM Cu (e.g. solubility limit). We investigated the ability of *A. donax* in free-surface water, pilot-scale CW units with recirculation of a Cu-contaminated BME to (1) rhizofiltrate Cu from the effluent, (2) provide a Cu-rich belowground biomass potentially usable as Cu-ecocatalyst, and (3) an uncontaminated shoot biomass. Copper removal peaked within the 48 first hours after BME addition in both units (i.e. 92% in the planted and 81% in the unplanted CW units) but the BME Cu concentration met the requirement for indirect discharge of chemical industry effluents (i.e. 0.5 mg Cu L^{-1}) at T_{48h} for the planted CW units and only at T_{21days} for the unplanted CW units. During the 30-day exposure, the root and shoot DW yields increased by 200 g and 700 g per CW unit, respectively. At day 30, foliar chlorophyll and carotenoid concentrations significantly decreased, as well as foliar Fe concentrations, indicating that chlorophyll and carotenoid synthesis was affected by Cu excess. As we expected, giant reed shoots finally displayed Cu concentrations in the common range for plant shoots (8 mg Cu kg DW) whereas root Cu concentration reached only 62% (i.e. 623 mg Cu kg^{-1} DW) of the threshold value for Cu-ecocatalysts. Subsequent repetitions of treatment cycle must be tested to potentially achieve at least 1000 mg Cu kg^{-1} DW in roots.

Chapter 4



Option appraisal

Chapter 5



Feasibility of the rhizofiltration strategy

Further researches

In situ implementation

Management procedures for contaminated land

Table of contents – Part 4

Part. 4. General discussion

4.1. General discussion (Part. 1.)

Reminder: The European Union has established a directive to gradually displace fossil fuels by biofuel produced from biomass. Using arable land for non-food crops is not an option but land requirement could be met by biomass production on marginal land including contaminated sites. In parallel, biochars used as amendment is one option considered by policymakers as a sustainable solution to boost carbon sequestration in agricultural soils and offset annual increases in atmospheric CO_2. In addition, biochar has a strong environmental positive impacts on soil quality (i.e. CEC, pH, nutrient stock) and has the potential ability to immobilize TE. Using biochar to "in situ stabilize" soil contamination while producing a biomass with metal(loid) concentrations within the common ranges was investigated.

Can we produce a biomass with metal(loid) concentrations within the common ranges, able to integrate local biomass processing chains, through the implementation of phytomanagement options on metal(loid)-contaminated matrices?

This was the first issue of this PhD. To answer, we chose to study the ability of biochar, used alone or in combination with compost and iron grit, to in situ stabilize contaminants in a Cu-contaminated soil from a wood preservation site (**Chapter 1**) and in a Cd, Pb and Zn poly-contaminated soil nearby a Pb/Zn smelter (**Chapter 2**). A short term pot experiment, a 2-week biotest realized with dwarf beans (*Phaseolus vulgaris* L.) used as bioindicator (preceded by a 3-month reaction time between soils and amendments), allowed to evaluate the amendment effect on soil phytotoxicity. A first step towards a successful phytomanagement option was to select relevant amendments able to reduce pollutant linkages. Based on these studies, we were able to select appropriate biochar and combination of amendments for a sustainable stabilization of metals in the soil in pot experiment. In all our experiments, biochar alone was not sufficiently efficient to decrease the pollutant linkages. However, the combination of lignin-rich biochar with iron grit or compost leaded to long-term metal immobilization in the soil and to the production of a shoot biomass with TE values in the common ranges. Results and reflections that lead us to this conclusion are discussed thereafter:

One of the main parameters which affect metal(loid) mobility and prevent metal(loid) stabilization into our biochar-amended soils was the release of DOM from biochar. It is well established that DOM may increase metal(loid) mobility by (1) competing with metal(loid)s for sorption sites onto bearing phases and (2) forming soluble organo-metallic complexes with metal(loid)s (Uchimiya et al., 2013). As reported by Jamieson et al., (2014), the DOM released by biochar is closely related to the origin of the raw material and to the pyrolysis conditions of biochar. In the chapter 1, two biochars were compared, one made from pine bark chips and the other one from poultry manure. They were both pyrolysed at

420°C. In general, for the same pyrolysis temperature, biochar contains a higher C content when derived from plant biomass (e.g. lignocellulosic compounds) than when derived from animal biomass/waste (Uchimiya et al., 2013). As expected, in the chapter 1, the poultry manure-derived biochar (1% w/w) (e.g. low contents of phenolic and lignin materials) released more DOM than the pine bark chip-derived biochar (1% w/w) (e.g. lignin-rich biomass) and, likely due to subsequent Cu complexation, promoted soluble DOM-Cu complexes, reducing free Cu^{2+} concentration but increasing total Cu concentration in the SPW (Chapter 1, Fig. 1). Further investigations could characterize such Cu-DOM complexes. Overall, at short term, biochars which produce high DOM amount do not play their role of carbon sequestration in soils.

In addition, biochar toxicity due to high salinity, PAH and volatile organic compounds may affect the development of plant growth. Negative impact of poultry manure-derived biochar on plant yield was evidenced (Chapter 1, Fig. 2). Marchand et al., (2016) reported a decrease (-12%) of seed yield for rapeseed grown in a Cd/Cu/Pb/Zn-contaminated technosol amended by the same poultry manure-derived biochar. Authors suggested this adverse effect was probably due to (1) higher soluble Cu, Ni, Zn, Cd, and Mo concentrations in the biochar-amended soil and (2) high PAH exposure. Such phytotoxic effects of biochar through PAH and supply of volatile and biologically active compounds are reported (Spokas et al., 2010; Kookana et al., 2011; Biederman and Harpole, 2013).

Washing the biochar to partly remove their soluble ash fraction (notably Na and Cl) may be an option to face these problems. Washing the biochar may (1) remove the water soluble components such as DOM resulting from the production process of biochar, (2) remove salt and/or potential contaminants in excess, (3) enhance the formation of water-stable macro-aggregates and improve soil aggregation, and (4) free some sorption sites of biochar blocked by DOM compounds (George et al., 2012; Klasson et al., 2014).

A second parameter affecting metal(loid) mobility in biochar-amended soils was the liming effect of biochar. In the chapter 2, pine bark chip-derived biochar was selected for incorporation into the Cd/Pb/Zn-contaminated soil over the poultry manure-derived biochar as we wanted to limit the DOM release from biochar. Its incorporation was at two application rates, 1% and 2.5% (w/w). Increase in soil pH is one parameter affecting metal(loid)s in contaminated soils amended by biochar (Bakshi et al., 2014) : the liming effect of biochar resulted in (1) hydroxide and oxide precipitates of metal(loid)s, and (2) activation of the electrostatic interactions between negatively charged surfaces of biochar and metal(loid)s. In the chapter 2, the negatively-charged surface area of biochar bound Cd, Pb and Zn but released As, B and Mo in the SPW (Chapter 2, Tab. 3). These increases in As, B and Mo concentrations were higher at 2.5% than 1% biochar addition. Such mobilization of TE through liming effects of biochar are reported (Houben and Sonnet, 2015; Wagner and Kaupenjohann, 2015). Authors reported that oxyanions (i.e. U, W and Mo) would behave similarly following an increase in soil pH after biochar addition (Riedel et al., 2015).

The combination of biochar with other amendments may be an option to diversify the characteristics of bearing phases, maximizing the retention of TE in the soil and avoiding the potential negative liming effect of biochar. The combination of amendments such as compost and iron oxides with biochar may (1) increase the number of sorption site thus increasing TE immobilization or sorption of DOM and soluble organic complexes and (2) buffer nutrient depletion (Beesley and Marmiroli, 2011).

Most of research papers about combining amendments with biochar into metal(loid)-contaminated soils are related to the use of biochar with compost. They highlight the positive effect of adding compost in combination with biochar: improvement of TE immobilization in contaminated soils, increase in total soil C, N and P, enhanced stabilization of soil aggregates and stimulation of microorganisms (Beesley et al., 2010; Sizmur et al., 2011; Rodríguez-Vila et al., 2015; Jones et al., 2016). Few studies reported a higher TE mobilization when soils were amended by both biochar and compost, especially for TE with strong affinity for OM such as Cu, Pb and As (Moreno-Jiménez et al., 2016; Forján et al., 2016).

Responses of metal(loid) mobility in contaminated soil amended with biochar and compost are very variable : in the Chapter 1, adding compost (5% w/w) in combination with biochar (1% w/w) in the Cu-contaminated soil slightly increased nutrient supply but did not have a significant effect neither on DOM, Cu^{2+}, total Cu concentration in the SPW, nor the plant growth (Chapter 1, Fig. 1). For the same Cu contaminated soil, combining pine bark chip-derived biochar (2.5% w/w) and compost (5% w/w) allowed the highest reductions in leachable Cu and plant yield (sunflower) improvements (Jones et al., 2016). In the chapter 2, compost added with the pine bark chip-derived biochar in the Cd/Pb/Zn-contaminated soil decreased the SPW Cd and Pb concentrations and the shoot Cd and Zn concentration of dwarf bean as compared with biochar alone albeit not significantly at the 2.5% biochar addition rate (Chapter 2. Fig. 1). In the same Cd/Pb/Zn-contaminated soil, addition of biochar and compost significantly increased the shoot Cu and Pb concentrations of *Lolium multiflorum* (Karer et al., 2015). These increases would be due to high dissolved organic carbon found in the biochar/compost amended soil which may have promoted the formation of soluble organometallic complexes. In overall, compost amendment may stimulate the degradation of more recalcitrant biochar fractions and impaired its role in carbon sequestration.

Conversely, incorporation of pine bark-derived biochar in combination with iron grit into Cu and Cd/Pb/Zn-contaminated soils allowed the highest decreased of metal(loid) concentrations in the soil pore water and plant shoots, leading to the lowest soil phytotoxicity. Cationic metal species and soluble organo-metallic complexes have high affinity for Fe/Mn oxides and can sorb on the newly formed Fe and Mn oxy-hydroxides after iron grit corroded in the amended soil (Tiberg et al., 2016). In both soils, addition of iron grit did not result in any adverse effect and always decreased the soil phytotoxicity as compared to biochar alone. Incorporation of pine bark-derived biochar with iron grit could be a relevant combination to decrease soil phytotoxicity if the costs associated with such option were not relatively high. As estimated by Hanauer et al., (2011), the costs would reach 24,000€ ha^{-1} for experiments using Fe(0). The long-term culture of high yielding plants on such contaminated site could partly cover the additional costs and meet the requirement of biomass production for biofuel production.

Amendments and soil components evolve over the time; moreover, cultivation of plants and their associated microorganism may change the physico-chemical soil properties and some biotic and abiotic processes may remobilize the sorbed contaminants (Kidd et al., 2015). In the biochar and biochar plus iron grit amended soils, the cultivation of poplars for 2 years did not have any additional effect on total Cu concentration in the SPW as compared to the results obtained in chapter 1 but increased again the SPW Cu^{2+} concentration (Chapter 3. Fig. 1). Cultivation of *A. donax* enhanced the SPW DOM concentration after a 2-year cultivation in the unamended soil and in the soil amended with biochar alone. This indicates that *A. donax* would not be the best species choice, as the enhanced DOM concentration decreased the Cu^{2+} one but promoted the total Cu concentration in the SPW. However, the combination of iron grit with biochar into the Cu-contaminated soil decreased total Cu and Cu^{2+} concentrations in the SPW of pots cultivated by *A. donax* and *P. nigra* through a shifted sorption of Cu towards metal (hydr)oxides rather than DOM. It also reduced the shoot Cu concentrations of poplar and giant reed to fit the range of common Cu values for plant shoots. In overall, *P. nigra* would be more relevant than *A. donax*, as in the soil amended with both iron grit and biochar, the DOM concentration was similar to that in the Unt soil, which indicated a stable sequestration of carbon in the soil, over the 2 year experiment.

The reliability of pot studies for predicting the growth and performance of plants in the field is often inaccurate, because pot environments differ from field conditions, mainly through the edge effect, changes in climatic conditions, and establishment of roots in the subsoil (Kidd et al., 2015). The results obtained in pot experiment could hardly be extrapolated directly to the field to evaluate whether the production of poplar and giant reed biomass is sufficient to provide enough economical value to counter costs associated with such option or not. A rule for a successful implementation of in situ stabilisation strategy is to follow the management procedures for contaminated land: (1) risk assessment, (2) option appraisal, and (3) feasibility and implementation of a remediation strategy (Mench et al., 2010). Following pot experiments, from chapter 1 and 2 (risk assessment) and from chapter 3 (option appraisal), it is thus commonly preconized to test in field plots the selected phytomanagement option directly on site (feasibility and implementation of a remediation strategy) for several years and in situ long term assessment (but not to directly implement the phytomanagement option on all the cluster areas of the site).

In order to address the question: Are we able to phytomanage a metal(loid)-contaminated site to produce a biomass with metal(loid) concentrations within the common ranges by using biochar, the in situ strategy was implemented on the Cu-contaminated soil from the wood preservation site. This step may allow to detect potential failures of phytoremediation option due to long-term changes, such as the aging of the amendments added to the soil, inter-annual climate variability, pest attacks, deposition and accumulation of litter, release of soluble organic matter, and changes in animal and plant communities (Kidd et al., 2015).

Based on a study from the European project HOMBRE (Holistic Management of Brownfield Regeneration), Jones et al. (2016) found that the combined addition of 5% of compost and 2.5% biochar allowed the greatest reductions in leachable Cu and highest plant yield (sunflower) improvements in the Cu contaminated soil from the wood preservation site. We implemented our field study based on these results, before to obtain the results of my previous pot experiments (to be consistent with the planning of this PhD). Field plots were implemented with biochar (2.5% w/w), alone or in combination with compost (5% w/w), on the Cu-contaminated soil at the wood preservation site and were investigated with poplars and Giant reeds.

Poplars and Giant reeds were planted in situ in October 2015, as we wanted to minimize the management practice on site, we decided to not irrigate the plants along the year. In summer 2016, Aquitaine faced a severe drought and the plants did not grow. This result are in agreement with Bes (2008), who planted trees in 2005 at this Cu-contaminated site and also decided to not irrigate them (i.e; *Amorpha fruticosa, Populus nigra, Salix caprea* and *S. viminalis*). The trees did not survived the summer. The next year, the same experiment was repeated with a regular irrigation which allowed to increase the survival rate and growth of the trees. This is also in agreement with Kolbas, (2012) who was forced to water tobacco plants throughout the growing season, notably from April to end of August, for maintaining the plants alive. This emphasized the importance of irrigation for soil re-vegetation. During their first years young plants must be irrigated until their root system grown enough to empower them in their water supply.

A phytotoxicity test was also performed, using broad bean (*Vicia faba* L.) in the field plots with no biochar, and biochar (2.5% w/w), alone or in combination with compost (5% w/w), and an uncontaminated kitchen garden. Both biochar treatments increased the root and shoot DW yields as compared to the unamended soil (Additional information, Fig. 2). The shoot Cu concentrations of all broad bean plants were slightly above the range of common shoot Cu values. This experiment highlighted how the pine-bark-derived biochar, alone and in combination with compost, is able to produce a biomass on site with low shoot Cu concentration. However, it is also important to keep in mind that abiotic conditions such as drought, or pest, in addition to soil Cu contamination, must be taken into account in phytomanagement option as they may threaten their success.

4.2. Conclusions and perspectives (Part. 1.)

The chapter 1 gave us the opportunity to underline how biochars react depending on their properties and led to different metal(loid) mobilization in the soil. The remobilization of Cu in this study due to biochar application is not an isolated example. In the past, several studies indicated a metal(loid) remobilization due to DOM released from biochar (Riedel et al., 2015; Hartley et al., 2009; Kloss et al., 2014). Among metal(loid)s, those with strong affinity for OM such as Cu and Pb or competing with DOM (e.g. Arsenicals) would be more subject to be remobilized from bearing phases. Enhanced DOM concentration is one of the pivotal problem in biochar amended soils. In further experiments, we must analyze the composition of DOM released in our experiment to determine its origin and composition (i.e. released from biochar compounds, decayed plant litter, microbial and root depositions, and hydrolysis of insoluble SOM). Fluorescence regional integration (FRI) could help us to provide information about the source and characterize the composition of DOM (Yao et al., 2016). Moreover, a high pyrolysis temperature generally produces biochars with a high level of stable C (Singh et al., 2010). Increase in pyrolysis temperature from 420 to 520°C would decreased DOC concentration from our biochar (Uchimiya and Bannon, 2013).

A general guideline for the in situ stabilization of metal(loid) contaminated soil with biochar would be to select biochar pyrolysised at higher temperature for limiting DOM release and the subsequent remobilization of metal(loid)s. A second guideline must be to wash the biochar before using it. To our knowledge, only few studies washed biochar as a pre-treatment and none of them concern metal(loid)-contaminated soil. To examine whether or not washing biochar is a relevant pre-treatment, the short term pot experiment from chapter 1 and 2 must be repeated to compare the effect of washed and un-washed biochar on metal(loid) mobility, soil phytotoxicity and DOM release (George et al., 201; Klasson et al., 2014).

The Chapter 2 points out how important it is to consider all the TE composing a contaminated soil before selecting a phytomanagement option. For biochar, its liming effect may immobilize some metals but increase the mobility of other elements (including metal(loid)s in oxyanion forms). Over all, the chapters 1 and 2 highlighted how important it is to combine biochar addition with other amendments in order to diversify the characteristics of bearing phases and maximizing the retention of TE in the soil. However, as a general guideline, compost should not be considered as a standard amendment to add in combination with biochar as it is currently done in research papers. The response of metal(loid) mobility in contaminated soil amended with biochar and compost could be variable depending on the amendment quality and application rate, TE contamination and local soil conditions. Moreover it may evolve over the time. The mechanisms governing sorption/desorption of contaminants from biochar(s) over the time are not well understood. In a further experiment, one step forward must be to characterize amended soil samples over different period of time to detect any changes in their composition using elemental analysis, SEM (electron microscopy system), BET (surface area measurement), FTIR (to quantify the functional groups and aromatic carbon groups) and XRF (to determine the elemental composition of the samples) as performed by Abdel-Fattah et al. (2015).

This work has shown that combination of biochar and iron grit can be used as relevant amendment for the in situ stabilization of a Cu-contaminated soil from a wood preservation site (**Chapter 1 and 3**) and in a Cd/Pb/Zn-contaminated soil nearby a Pb/Zn smelter (**Chapter 2**). In all experiments, plants displayed shoot Cu, Cd, Pb and Zn concentrations in the range of common values. However in the three first chapters, no treatment was able to increase the plant yield as compared to the untreated soil. Additional mineral fertilization (NPK-fertilizer) must be considered even though this option will increase the phytomanagement cost and will not facilitate maintenance operation. Inoculating the plants with endophytic bacteria could be a second option. Improved Cu tolerance and biomass production were previously reported in plants inoculated with endophytic bacteria depending on the extent of Cu exposure (Weyens et al., 2009; Kolbas et al., 2014). Moreover, in further experiment additional amendment in combination with biochar may be tested to avoid the cost associated with the use of Fe(0).

The results from these three chapters came from pot experiments. They were useful to evaluate the reliability of in situ stabilization strategies but did not allow to draw conclusions on whether in situ stabilization using biochar is a relevant option to produce biomass with metal(loid) concentrations within the common ranges. Base on the literature, long term case-studies of biomass production on biochar amended contaminated soil were reported only 4 times: in a pot experiment, root and shoot biomass produced by *Lolium multiflorum* Lam. was unchanged in a (Cd, Zn, and Pb)-contaminated soil amended with 1% miscanthus-derived-biochar after 28 days and 56 days, but biochar amendment reduced the extractability and bioavailability of Cd, Zn and Pb (Houben et al., 2013a,b). In a field study, 2 years after amendment, grain yields and biomass of wheat were not affected by both rate 10 and 40 t ha^{-1} of wheat straw-derived biochar addition in a Cd contaminated paddy soil but biochar reduced Cd concentration in wheat grains (Cui et al., 2012). Germination of grass (mix of *Festuca rubra* L. and *Lolium perenne* L.) on site failed in a 3-year field experiment on a contaminated (Ni/Zn) soil amended with broad leaf hardwood-derived biochar (Shen et al., 2016). Two years after amendment, poplar-derived biochar enriched with compost and nitrogen reduced the labile metal fraction of the Cd, Pb and Zn-contaminated soil from Arnoldstein area and allowed the production of an uncontaminated Miscanthus biomass suitable for energy crop production (Puschenreiter et al., 2016).

Once again these diversified results based on a low number of studies do not clearly evidence whether in situ stabilization using biochar is a relevant sustainable option to produce biomass with metal(loid) concentrations within the common ranges. In our site, the in situ stabilization option implemented generated limited results because of drought and the experiment needs to be continue with regular irrigation of young plants to let us draw conclusions about this question. One other option could be to select plants with higher drought resistance as the tobacco mutant lines which showed outstanding performance in drought resistance, in shoot DW yield, and shoot metal (Cd, Zn, and Cu) removals on contaminated soils at the Biogeco site (Kolbas, 2012). Thereafter, biomass productivity and its sustainability over the years need to be assessed and we also must ensure that this biomass must integrate regular processing chains.

4.3. General discussion (Part. 2.)

<u>*Reminder*</u>: In Aquitaine, France, a second faced issue with Cu-contamination is the significant amounts of Bordeaux mixture effluent (BME) generated by vineyard protection against powdery mildew. Spreading of these diluted BME on the field contributes to locally increase total soil Cu. Rhizofiltration in constructed wetlands (CW) is one of the ways to manage such effluents. The "rhizofiltration" of BME may produce both root (and in a lesser extent shoots) biomass with high Cu concentrations. In parallel, biocatalysis is based on the use of metal species originating from plant biomass with high metal(loid) concentrations. Among metals, Cu-ecocatalysts are of interest. The use of "rhizofiltration" of BME to produce a biomass with high Cu concentrations adapted to Eco-catalysis was investigated.

Can we produce a biomass with metal species originating from plant biomasses with high metal(loid) concentrations through the implementation of phytomanagement option on metal(loid)-contaminated matrices?

This was the second issue of this PhD. To answer this question, we chose to study the ability of 4 macrophytes frequently used in CW to produce Cu-rich root and rhizome mats (**Chapter 4**). Accordingly we developed a pilot-scale CW planted with *Arundo donax* L. to jointly rhizofiltrate BME while producing a Cu-rich root mat for ecocatalysis (**Chapter 5**).

Before to implement a pilot-scale CW able to treat BME, we wanted to ensure that the selected plant species to be cultivated in the CW may display a root biomass with Cu concentration >1000 mg Cu kg^{-1} DW (threshold value for Cu-ecocatalysts production) and shoot Cu concentration within the common range (which can integrate regular processing chains). Out of macrophytes generally used in CWs, four were selected for their ability to accumulate high root Cu concentration and/or tolerate high Cu exposure and produce an abundant biomass. Two out of these 4 plant species, *A. donax* and *Iris pseudacorus* L., met the requirement to be cultivated in such CW and only *A. donax* was chosen to be implemented in our pilot-scale CW. Results and reflections that lead us to this conclusion are discussed thereafter:

In the chapter 4, *A. donax*, *Cyperus eragrostis* Lam., *Iris pseudacorus* L., and *Phalaris arundinacea* L. were exposed to a Cu concentration gradient (0.08, 2, 10, 20 and 40 µM Cu) in batch conditions during 2 months. We found that *C. eragrostis* and *P. arundinacea* were not relevant species to produce Cu-ecocatalyst as they were clearly too sensitive to Cu exposure and did not meet the requirement for Cu-ecocatalysts. For *P. arundinacea*, we expected a higher shoot biomass production at high Cu exposure, as the sampled population was supposed Cu tolerant according to Marchand et al. (2014a). In this study, authors found a consistent root biomass production of *P. arundinacea* in the 0.08 – 25 µM Cu range. We might assume that we did not sample exactly the same population. For *C. eragrostis*, we also expected a higher Cu tolerance and root Cu concentration, as high Cu concentrations were previously measured in other *Cyperus sp.*, for a lower exposure *(i.e. Cyperus alternifolius*: 1310 mg kg^{-1}, Sun et al., 2013). These results emphasized the need to deepen the research on intra-specific and inter-specific variability of Cu tolerance.

Conversely, *I. pseudacorus* and *A. donax* were both Cu tolerant and delivered root mats potentially usable as Cu-ecocatalyst (Chapter 4, Fig. 2 and 3). *Iris pseudacorus* displayed abundant shoot biomass with low Cu concentration usable by regular processing chains. In the Chapter 4, shoot Cu concentrations of *A. donax* were not sufficient for its use as Cu-ecocatalysts but too high to be manage in regular processing chains.

Although Brisson and Chazarenc (2009) expressed their concerns about how extrapolating results obtained from young plant seedlings may misleading the prediction of treatment benefits of a plant species in mature CW system, still a lot of studies use young plant seedlings in their experiment (usually to fit in the timing imposed by their project funder). In your study, the plants were 7-month-old, still immature and with a low shoot and root biomass compared to mature plants. Thus we assumed that the Cu concentrations measured in our 7-month-old plants would probably be overestimated compared to mature plants (according to dilution in the biomass). For a similar Cu exposure, *A. donax* displayed higher Cu concentration in its roots than *I. pseudacorus*. In the pilot-scale CW, we wanted to maximize the Cu accumulation in the roots, thus we retained *A. donax* over *I. pseudacorus*. In this way, we made the bet that root Cu concentrations of mature *A. donax* (26-month-old) will still meet the requirement of Cu concentration for Cu-ecocatalysts and foliar Cu concentrations of mature *A. donax* would fall in the common Cu range.

It is however reported that *P. australis* may take up to 3 years before reaching maturity (Vymazal and Krőpfelová, 2005). We assumed that it took roughly the same time for *A. donax* to reach maturity. When we perform the experiment to rhizofiltrate the BME in the pilot-scale CW planted with *A. donax,* the plants were more than 2-year-old (26-month-old) and the mesocosms were almost mature. As we expected, after 30 days in the CW, giant reed shoots finally displayed Cu concentrations in the common range for shoots, but root Cu concentration reached only 62% of the threshold value for Cu-ecocatalysts (Chapter 5, Table 3). The large difference of Cu concentration accumulated in the roots of young and mature *A. donax,* independently of the Cu exposure emphasized the need to use mature plants for the prediction of treatment benefits of a plant species.

In chapter 5, planted CW units were compared to unplanted CW units. In both cases, Cu removal peaked within the 48 first hours after BME addition. These results reopens the debate about the role of macrophytes in CW (Chazarenc and Brisson, 2009; Marchand et al., 2010). In general, authors postulated that the substrate was involved in TE removal mainly by chemical (ad)sorption. However, here our unplanted CW units did not contain any substrate and we suggested that other compartments such as decayed OM and biofilm would be involved in Cu removal. The measures of Cu concentration in the biofilm (i.e. 207 210 ± 18 516 mg Cu kg^{-1}) coating the vat wall, showed us that only 11.4 g of biofilm per vat would be need to explain the Cu removal in the unplanted CW units. This magnitude is in adequacy with the biofilm quantity that we estimated (sampling all the biofilm was not possible). These results highlights the role of biofilm involved in Cu removal. Such immobilization of TE through biofilms in CW are reported (Tanner and Headley, 2011; Valipour et al., 2009, Marchand et al., 2014b).

However, this experiment also showed that the plants in the CW allowed the reduction of hydraulic retention time (HRT) for BME to meet the requirement for indirect discharge of chemical industry effluents (i.e. 0.5 mg Cu L^{-1}). In planted CW the HRT was 48h while in the unplanted CW units it was 21-days (Chapter 5, Fig. 2). This gain in HRT highlights the role of plant macrophytes in such system. This results agreed with a higher Cu reduction at the outlet in CW units planted with *T. latifolia* than in the unplanted ones after a 17h HRT (Valipour et al., 2014). However, the removal rate of contaminated-effluent in CW depends on the changes in rhizosphere conditions largely related to seasonality. These variations are due to the change of the inherent growth momentum in the plant, as well as changes of bioavailability and in water (Hardej & Ozimek. 2002). Bragato et al., (2006) stated that to maximize the purification of effluents, harvesting the aerial parts of macrophytes should be carried out when the concentrations of TE are the most important in the plants, that is to say the late fall, after plant senescence.

4.4. Conclusions and perspectives (Part. 2.)

The chapter 4 gave us the opportunity to underline how interspecific variability results in variable Cu accumulation in root and shoot of macrophytes. To enlarging the macrophytes list able to produce Cu-rich root and rhizome mats further research are needed. Using mature plants is a prerequisite to predict correctly the treatment benefits of a plant species. It is important to keep in mind that plant resistance to transplantation is an important factor, in addition to Cu tolerance or Cu accumulation, to be consider when selecting a plant species. At the beginning of the batch experiment (chapter 4) seven macrophyte species were sampled and three of them, *Spartina anglica, Phragmites australis* and *Typha latifolia* did not resist the stress of transplantation, standardization and growth in culture medium.

In the chapter 5, root Cu concentration of *Arundo donax* did not meet the requirement for Cu-ecocatalyst. Once again, the inoculation of *A. donax* with endophytic bacteria could improve root Cu accumulation and plant Cu tolerance (Weyens et al., 2009; Ma et al., 2011). In an unpublished study following the same protocol as chapter 4, we found that inoculation of *A. donax* by crude seed extracts of Cu-tolerant *Agrostis capillaris* containing endophytic bacteria led to higher Cu accumulation in the roots than without inoculation (i.e. 5059 ± 334 and 3512 ± 1372 mg Cu kg^{-1} respectively at 40µM Cu). To examine whether or not inoculation of *A. donax* with endophytic bacteria is a relevant solution to meet the requirement for Cu-ecocatalyst, the treatment of BME must be repeated with inoculated *A. donax*.

Inter-seasonal variability may result in different Cu accumulation in the roots and in the shoots along the year, therefore, we need to repeat this experiment at different period of the year, in order to ensure that the root Cu concentration requirement for eco-catalysts is still met. Another way to achieve at least 1000 mg Cu kg^{-1} DW in *A. donax* root must be to repeat several times the treatment cycle but we must ensure that our plants of *A. donax* are able to stand such repeated treatments. It could be interesting to identify and understand the potential Cu tolerance mechanisms in *A. donax*. One step further must be to perform few techniques on our samples to investigate the distribution and speciation of Cu into the plant parts: laboratory chemical micro-X-ray fluorescence spectroscopy (µ-XRF) and scanning electron microscopy combined with energy dispersive X-ray analysis (SEM-EDX) may be assessed to locate Cu in the roots. Synchrotron based X-ray absorption spectroscopy may also be used to (1) investigate the oxidation states of Cu in different plant parts (Cu K-edge X-ray absorption near-edge spectroscopy-XANES), (2) analyze the various ligands involved in metal complexation in various plant parts (X-ray absorption fine structure spectroscopy - EXAFS) (Collin et al., 2014). The study of molecular determinants via the analysis of transcripts may be a second option (Hego et al., 2016).

The Chapter 5 points out how biofilm in a key player in Cu removal in CW. One way to better understand the mechanisms which guide Cu removal in CW would be to characterize and quantify the microbial communities in the CW (even though we started the experiment with tap water). Characterization of microbial communities at the end of the treatment cycle would help to determine the microbial strains involved in Cu removal. It may be accomplished by agarose gel electrophoresis of 16S (bacteria) and 18S (fungal) ribosomal rRNA genes (Zhang et al., 2016).

Although the pilot-scale CW would (1) produce a root biomass with Cu concentration >1000 mg Cu kg^{-1} DW, (2) produce a shoot biomass with low Cu concentration able to integrate regular processing chain, and (3) be able to treat BME, their implantation at vineries are far from realistic nowadays. The eco-catalyst field only boomed recently, the management, the rooting and conversion of this biomass still need to be developed. Regarding the environment, this pilot-scale CW may generate none negligible benefits to restore few ecosystem services (avoid to increase total Cu soil and Cu leaching, thus protecting the biodiversity); until now no system allowed to easily manage such Cu-contaminated effluents.

4.5. General conclusion

This work showed the ability of phytomanagement options to limit soil contamination by a relevant management of contaminated-effluents (**Part 3**), to decrease pollutants linkages (**Part 2**), and to provide a valuable biomass (**Part 2 and 3**) (Fig. 1). Our phytomanagement options decrease the **exposure pathways** of contaminants (i.e. environmental availability, environmental bioavailability) but also provide profits for the environment such as the restauration of **ecological functions** (i.e. water and soil quality, cycle of elements such as carbon, increase in plant and microorganism biodiversity), their associated ecosystem services, and an **economical valorization** of the marginal land (Fig. 2). However, these profits still need to be quantified. Besides general perspectives, several complementary researches could be initiated to answer several questions raised during this work:

Ecological functions and associated ecosystem services (Fig. 2)

Carbon cycle (Fig. 3): **Carbon storage** in the soil is one of the main goal of biochar use (Ahmad et al., 2014). Long-term cultivation of plants in contaminated soils amended with biochar may influence carbon soil storage and needs to be studied. The SOCLE project (2015-2018, coordinated by the CIRAD unit "Biomass, wood, energy and bioproduct"), tries to understand and characterize carbon storage mechanisms in soils and aims to set a method to quantify and evaluate the impact of agricultural practice on carbon soil storage. The 4per1000 initiative promotes soil carbon sequestration to accelerate the restoration of soils in order to reverse global warming. A collaboration within these project and initiative can be an option.

Biodiversity: In situ stabilization decreases soil phytotoxicity and facilitates implementation of a plant cover as well as animals (i.e. crickets, snails, ants, lizards, rabbits, granivorous and carnivorous birds: based on site observations at the St-Médard d'Eyrans site). **Listing insect species** in the untreated and amended Cu-contaminated plots needs to be completed (Sinchuk, 2012). It could be interesting to evaluate the transfer of contaminants in these insects through the food chains and networks. Researches in this direction has already been initiated (Garrouj et al., 2016). In the BIOGECO UMR, Dr. Castagneyrol et al. study the insect pests and could help us with this project.

Water quality: The implementation of a CW to treat BME instead to release them on the edge of vineyards, limits unnecessary spreading of this Cu-based fungicide (Bordeaux mixture) on soils and thus decreases run off and percolation of contaminant (Cu) leading finally to improve water quality. To evaluate the relevance of CW-pilot installation in wineries, one long-term project could be to compare the water quality around vineyards with and without the management of CW-pilot. The research issue of the "National research institute of technologies and science for the environment and agriculture"

(IRSTEA-Bordeaux) aim to evaluate the **water quality**. This project could be done in collaboration with this institute.

Economical aspects (Fig. 2)

Increase biomass yield: Assemblage of plants with **endophytic bacteria** is often a successful method to improve plant yield. One option to improve our field experiment could be to inoculate our (mycorrhizal) plants species (i.e. poplar and Giant reed). One possible collaboration to select relevant endophytes could be with the team of the Centre for Environmental Sciences, directed by Prof. Jaco Vangronsveld in Hasselt, Belgium. They successfully increased the yield of inoculated poplars cultivated in a Cd, Pb, Zn contaminated soil. This work is notably reported in the PhD thesis of Dr. Jolien Janssen.

Processing chains: Phytomanagement of our contaminated matrices provides usable biomass, especially the constructed wetland pilot. However, these biomasses were inadequate for eco-catalysis. To **pyrolyse** our Cu-rich *Arundo donax* biomass (623 ± 140 mg kg^{-1}) to (1) produce bioenergy and (2) produce enriched Cu-biochar is an option. This biochar could be then applied in Cu-deficient soil such as podzols. Again in the Centre for Environmental Sciences, Hasselt, Gonsalvesh et al., (2016) and Lievens et al., (2008) studied the pyrolysis of metal(loid)-rich biomass. They found an accumulation of contaminant in the biochar fraction. We could benefit from their experiment to valorize our Cu-rich biomass.

Life cycle assessment: The biomass produced by phytomanagement on contaminated sites can be economically valorized but also contributes to important environmental co-benefit by achieving economic, social and environmental sustainability. One long-term project must be to perform a **Life Cycle Analysis** (LCA) in our amended Cu-contaminated plots planted with *A. donax* and *P. nigra*. A collaboration with Dr. Witters from the Centre for Environmental Sciences, Hasselt, could be an option, as she successfully performed a LCA to examine the energy production and CO_2 abatement of energy crops cultivated in a poly-contaminated soil.

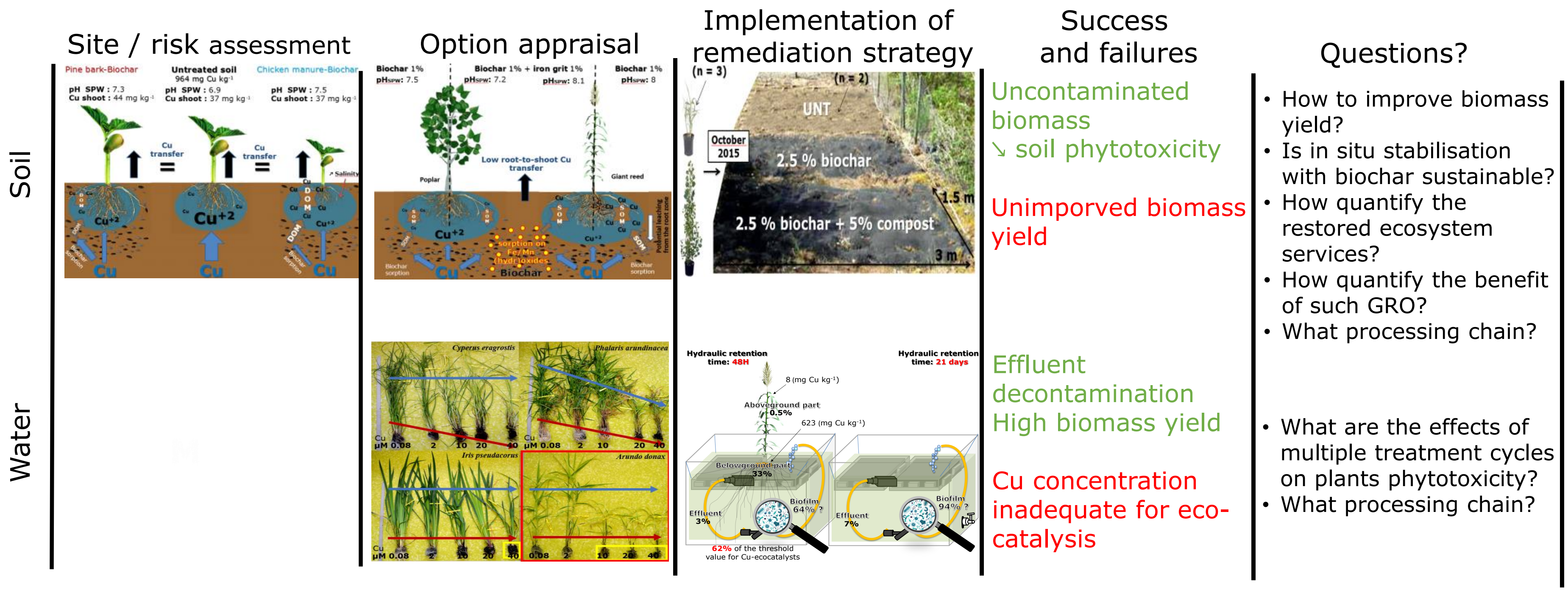


Fig. 1. Success and failures in both parts of this PhD thesis

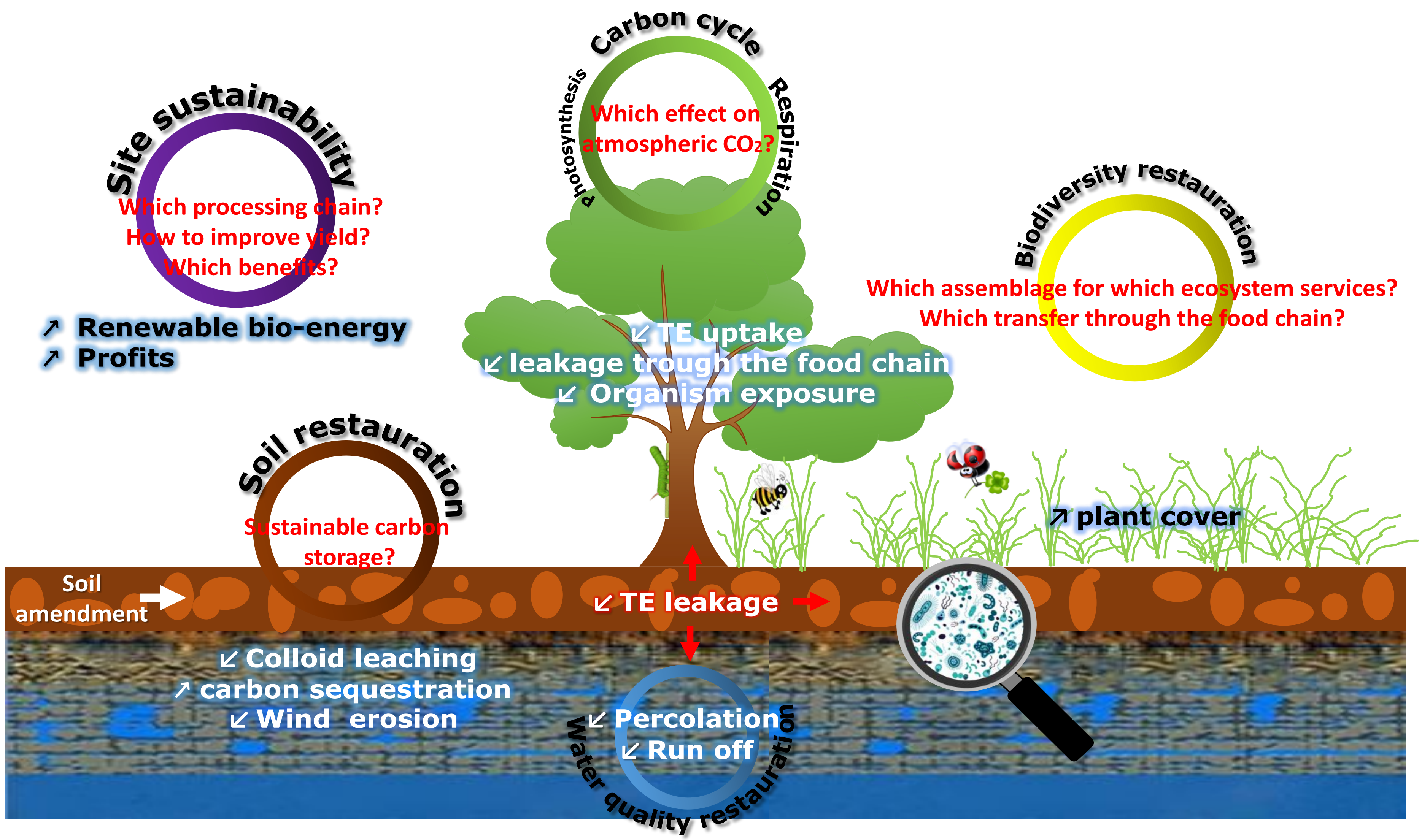


Fig. 2.Global benefits of phytomanagement options

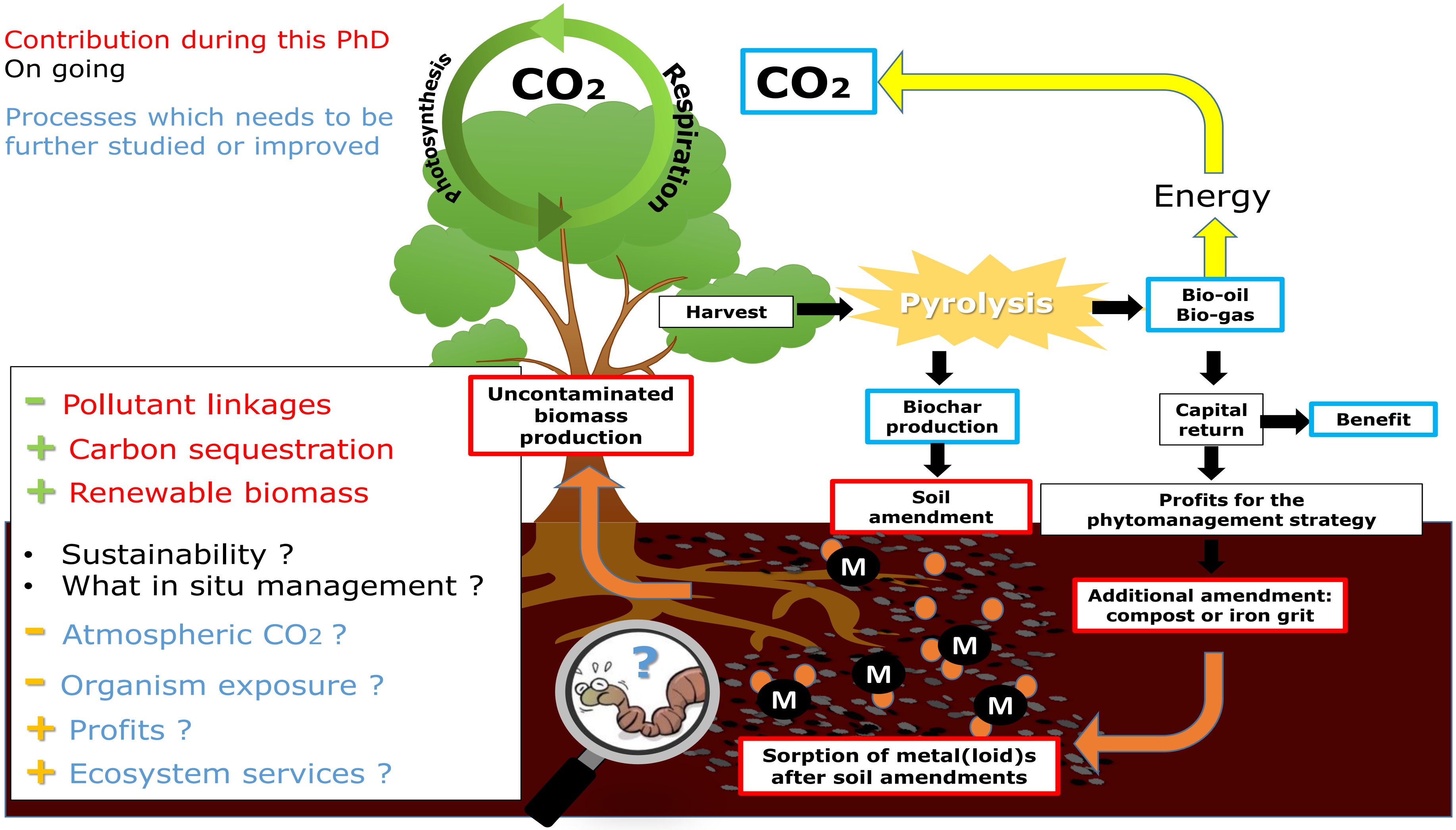


Fig. 3. Carbon cycle improved by biochar and biomass production in contaminated soils. M=metal(loid)s

References

4per1000., 2016. La séquestration du carbone dans les sols pour la sécurité alimentaire et le climat. Available at: http://4p1000.org/comprendre (Verified on August 10th, 2016)

Abdel-Fattah, T.M., Mahmoud, M.E., Ahmed, S.M., Huff, M.D., Lee, J.W., Kumar, S., 2015. Biochar from woody biomass for removing metal contaminants and carbon sequestration. J. Ind. Eng.Chem. 22, 103–109. doi:10.1016/j.jiec.2014.06.030

ADEME, 2008. Guide ADEME, traitabilité des sols pollués: Guide méthodologique pour la sélection des techniques et l'évaluation de leurs performances. Available at: http://www.ademe.fr/sites/default/files/assets/documents/traitabilite-sols-pollues-guide-methodologique-evaluation-2008-rapport-final-3.pdf (Verified on August 10th, 2016)

ADEME, 2012. Guide ADEME, les phytotechnologies appliquées aux sites et sols pollués. Available at: http://www.ineris.fr/centredoc/phytotechnologies-ademe-2013-1463054029.pdf (Verified on August 10th, 2016)

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M.K., Bharwana, S.A., 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environ. Sci. Pollut. Res. 22, 8148–8162. doi:10.1007/s11356-015-4496-5

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, second ed., Springer, New York.

Aguirre, G., Pilon, M., 2016. Copper delivery to chloroplast proteins and its regulation. Front. Plant Sci. 6, 1250. doi:10.3389/fpls.2015.01250

Ahmann, D., Dorgan, J.R., 2007. Bioengineering for pollution prevention through development of biobased energy and materials state of the science report. Rapport US EPA. Golden, Colorado.

Alshaal, T., Domokos-Szabolcsy, E., Marton, L., Czako, M., Katai, J., Balogh, P., Elhawat, N., El-Ramady, H., Gerocs, A., Fari, M., 2013a. Restoring soil ecosystems and biomass production of *Arundo donax* L. under microbial communities-depleted soil. Bioenerg. Res. 7, 268–278. doi:10.1007/s12155-013-9369-5

Alshaal, T., Domokos-Szabolcsy, É., Márton, L., Czakó, M., Kátai, J., Balogh, P., Elhawat, N., El-Ramady, H., Fari, M., 2013b. Phytoremediation of bauxite-derived red mud by giant reed. Environ. Chem. Lett. 11, 295-302. doi:10.1007/s10311-013-0406-6

Alshaal, T., Elhawat, N., Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., El-Ramady, H., Fári, M.G., 2015. Giant reed (*Arundo donax* L.): a green technology for clean environment. In: Ansari, A.A., Gill, S.S., Gill, R., Lanza, G.R., Newman, L. (Eds.), Phytoremediation: Management of Environmental Contaminants, vol. 1. Springer International Publishing, Switzerland, pp. 3–20.

Amonette, J.E., Jospeh, S., 2009, Characteristics of Biochar: Microchemical Properties. In: Lehmann J., Joseph S. eds.: Biochar for Environmental Management Science and Technology. Earthscan, London, 33–43.

Andersson-Sköld, Y., Bardos, P., Chalot, M., Bert, V., Crutu, G., Phanthavongsa, P., Delplanque, M., Track, T., Cundy, A.B., 2014. Developing and validating a practical decision support tool (DST) for biomass selection on marginal land. J. Environ. Manag. 145, 113–121.

Andres-Colas, N., Perea-Garcia, A., Puig, S., Penaarubia, L., 2010. Deregulated copper transport affects *Arabidopsis* development especially in the absence of environmental cycles. Plant Physiol. 153, 170–184.

Anjum, N.A., Adam, V., Kizek, R., Duarte, A.C., Pereira, E., Iqbal, M., Lukatkin, A.S., Ahmad, I., 2015. Nanoscale copper in the soil–plant system–toxicity and underlying potential mechanisms. Environ. Res. 138, 306–325. doi:10.1016/j.envres.2015.02.019

Arora, A., Sairam, R.K., Srivastava, G.C., 2002. Oxidative stress and antioxidative system in plants. Curr. Sci. 10, 1227–1238.

Arroyo, P., Ansola, G., De Luis, E., 2010. Effectiveness of a full-scale constructed wetland for the removal of metals from domestic wastewater. Water Air Soil Pollut. 210, 473–481. doi:10.1007/s11270-009-0272-9

Asami, T., 1988. Soil pollution by metals from mining and smelting activities. In: Salomon, W., Forstner, U., Eds. Chemistry and biology of solid waste: Dredged material and mine tailings. 144–169 Berlin, Germany: Springer-Verlag.

Ashworth, D.J., Alloway, B.J., 2007. Complexation of copper by sewage sludge-derived dissolved organic matter: effects on soil sorption behaviour and plant uptake. Water Air Soil Pollut. 182, 187–196. doi:10.1007/s11270-006-9331-7

Assimakopoulou, A., Salmas, I., Nifako,s K., Kalogeropoulos, P., 2015. Effect of salt stress on three green bean (*Phaseolus vulgaris* L.) cultivars. Not Bot Horti Agrobo 43(1), 113–118. doi:10.15835/nbha4319905

Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil. 337, 1–18. doi:10.1007/s11104-010-0464-5

Audet, P., Charest, C., 2008. Allocation plasticity and plant-metal partitioning: metaanalytical perspectives in phytoremediation. Environ. Pollut. 156, 290–296.

Babcsányi, I., Imfeld, G., Granet, M., Chabaux, F., 2014. Copper stable isotopes to trace copper behavior in wetland systems. Environ. Sci. Technol. 48, 5520−5529. doi:10.1021/es405688v

Bach, Q.V., Chen, W.H., Chu, Y.S., Skreiberg, Ø., 2016. Predictions of biochar yield and elemental composition during torrefaction of forest residues. Bioresour. Technol. 215, 239–246. doi:10.1016/j.biortech.2016.04.009

Baize, D., 1997. Un Point sur les Teneurs Totales en Eléments Traces Métalliques dans les Sols. First ed. Paris, France: INRA Éditions.

Baize, D., 2000. Guide des analyses en pédologie. Second ed. Paris, France : INRA Éditions. 257 p.

Baker, A.J.M., 1981. Accumulators and excluders strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654.

Baker, T.J., Tyler, C.R., Galloway, T.S., 2014. Impacts of metal and metal oxide nanoparticles on marine organisms. Environ. Pollut. 186, 257−271. doi:10.1016/j.envpol.2013.11.014

Bakshi, S., He, Z.L., Harris, W.G., 2014. Biochar amendment affects leaching potential of copper and nutrient release behavior in contaminated sandy soils. J. Environ. Qual. 43, 1894–1902. doi:10.2134/jeq2014.05.0213

Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. Org. Geochem. 34 (9), 1093–1109.

Bani, A., Echevarria, G., Sulçe, S., Morel, J.L., Mullai, A., 2007. In-situ phytoextraction of Ni by a native population of *Alyssum murale* on a ultramafic site (Albania). Plant Soil. 293, 79–89.

Bañuelos, G.S., Mayland, H.F., 2000. Absorption and distribution of selenium in animals consuming canola grown for selenium phytoremediation. Ecotox. Environ. Safe. 46, 322–328.

Bañuelos, G.S., Robinson, J., daRoche, J., 2010. Developing selenium-enriched animal feed and biofuel from canola planted for managing Se-laden drainage waters in the Westside of central California. I. Int. J. Phytorem.12, 243–253.

Bardos, R., Bone, B., Andersson-Sköld, Y., Suer, P., Track, T., Wagelmans, M., 2011. Crop-based systems for sustainable risk-based land management for economically marginal damaged land. J. Environ. Cleanup Costs Technol. Tech. 21, 11–33.

Bashir, K., Rasheed, S., Kobayashi, T., Seki, M., Nishizawa, N.K., 2016. Regulating subcellular metal homeostasis: The key to crop improvement. Front. Plant Sci. 7(1192). doi:10.3389/fpls.2016.01192

Basias, 2015. Inventaire historique de sites industriels et activités de service. http://basias.brgm.fr/ (verified on October 20th, 2016)

BASOL., 2016. Ministère de l'Écologie, du Développement Durable, des Transports et du Logement. Available online at: http://basol.environnement.gouv.fr/accueil.php. (Verified on August 10th, 2016)

Beesley, L., Moreno-Jimenez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ. Pollut. 158, 2282–2287. doi:10.1016/j.envpol.2010.02.003

Beesley, L., Marmiroli, M., 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ. Pollut. 159, 474–480. doi:10.1016/j.envpol.2010.10.016

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., Harris, E., Robinson, B., Sizmur, T., 2011. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environ. Pollut. 159, 3269–3282. doi:10.1016/j.envpol.2011.07.023

Beesley, L., Inneh., O.S., Norton, G.J., Moreno-Jimenez, E., Pardo, T., Clemente, R., Dawson, J.J.C., 2014. Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil. Environ. Pollut. 186, 195–202. doi:10.1016/j.envpol.2013.11.026

Ben Salem, Z., Laffray, X., Ashoour, A., Ayadi, H., Aleya, L., 2014. Metal accumulation and distribution in the organs of Reeds and Cattails in a constructed treatment wetland (Etueffont, France). Ecol. Eng. 64, 1–17. doi:10.1016/j.ecoleng.2013.12.027

Bert, V., Lors, C., Ponge, J.F., Caron, L., Biaz, A., Dazy, M., Masfaraud, J.F., 2012. Metal immobilization and soil amendment efficiency at a contaminated sediment landfill site: a field study focusing on plants, springtails, and bacteria. Environ. Pollut. 169, 1–11. doi:10.1016/j.envpol.2012.04.021

Bernal, M.P,. Clemente, R,. Walker, D.J., 2009. Interactions of heavy metals with soil organic matter in relation to phytoremediation. In: Phytoremediation: the Green Salvation of the World. Research Signpost. Kerala, India: Navarro-Aviño, J.P., 109–129.

Bes, C., 2008. Phytoremédiation des sols d'un site de traitement du bois contaminés par le cuivre. PhD thesis, Université de Bordeaux, Talence.

Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilization, Environ. Pollut.156, 1128–1138. doi:10.1016/j.envpol.2008.04.006

Bes, C,. Mench, M,. Aulen, M,. Gaste, H,. Taberly, J., 2010. Spatial variation of plant communities and shoot Cu concentrations of plant species at a timber treatment site. Plant Soil. 330, 267–280. doi:10.1007/s11104-009-0198-4

Bes, C., Jaunatre, R., Mench, M., 2013. Seed bank of Cu-contaminated topsoils at a wood preservation site: impacts of copper and compost on seed germination. Environ. Monit. Assess. 185, 2039–2053. doi:10.1007/s10661-012-2686-x

Bian, R., Joseph, S., Cui, L., Pan, G., Li, L., Liu, X., Zhang, A., Rutlidg, H., Wong, S., Chia, C., Marjo, C., Gong, B., Munroe, P., Donne, S., 2014. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. J. Hazard. Mater. 272, 121–128. doi:10.1016/j.jhazmat.2014.03.017

Biederman, L.A., Harpole, S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy. 5, 202–214. doi:10.1111/gcbb.12037

Boisson, J., Mench, M., Sappin-Didier, V., Solda, P., Vangronsveld, J., 1998. Short-term in situ immobilization of Cd and Ni by beringite and steel shots application to long-term sludged plots. Agronomie. 18, 347–359. doi:10.1051/agro:19980502

Bolan, N.S., Adriano, D.C., Mani, S., Khan, A.R., 2003. Adsorption, complexation and phytoavailability of copper as influenced by organic manure. Environ. Toxicol. Chem. 22, 450–456. doi:10.1002/etc.5620220228

Bolan, N.S., Adriano, D.C., Mahimairaja, S., 2004. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Crit. Rev. Environ. Sci. Technol. 34, 291–338. doi:1.1080/10643380490434128

Bolan, N.S., Adriano, D., Senesi, N., Kunhikrishnan, A., James, T., McDowell, R., 2010. Dissolved organic carbon: biogeochemistry, dynamics and agro-environmental significance in soils. Adv. Agron. 110, 1–67.

Bolan, N.S, Kunhikrishnan, A., Thangarajana, R., Kumpiene, J., Parke, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metalloid.-contaminated soils – To mobilize or to immobilize? J. Hazard. Mater. 266, 141–166. Doi:10.1016/j.jhazmat.2013.12.018

Bonanno, G., Lo Giudice, R., 2010. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. Ecol. Indic. 10, 639–645. doi:10.1016/j.ecolind.2009.11.002

Bonanno, G., 2012. *Arundo donax* as a potential biomonitor of trace element contamination in water and sediment. Ecotox. Environ. Safe. 80, 20–27. doi:10.1016/j.ecoenv.2012.02.005

Bonanno, G., 2013. Comparative performance of trace element bioaccumulation and biomonitoring in the plant species *Typha domingensis*, *Phragmites australis* and *Arundo donax*. Ecotox. Environ. Safe. 97, 124–130. doi:10.1016/j.ecoenv.2013.07.017

Bonanno, G., Cirelli, G.L., Toscano, A., Lo Giudice, R., Pavone, P., 2013. Heavy metal content in ash of energy crops growing in sewage-contaminated natural wetlands: Potential applications in agriculture and forestry? Sci. Total Environ. 452–453, 349–354. doi:10.1016/j.scitotenv.2013.02.048

Borghi, M., Tognetti, R., Monteforti, G., Sebastiani, L., 2008. Responses of two poplar species (*Populus alba and Populus x Canadensis*) to high copper concentrations. Environ. Exp. Botany. 62, 290–299. doi:10.1016/j.envexpbot.2007.10.001

Bragato, C., Brix, H., Malagoli, M., 2006. Accumulation of nutrients and heavy metals in *Phragmites australis* (Cav.) Trin. ex Steudel and *Bolboschoenus maritimus* (L.) Palla in a constructed wetland of the Venice lagoon watershed, Environ. Pollut., 144, 967–975.

Brassard, P., Godbout, S., Raghavan, V., 2016. Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. J. Environ. Manag. 181, 484–497. doi:10.1016/j.jenvman.2016.06.063

Bravin., 2008. Processus rhizospheriques déterminant la biodisponibilité du cuivre pour le blé dur cultive en sols à antécédent viticole. PhD thesis, Montpellier SupAgro. Montpellier.

Bravin, M.N., Marti A.L., Clairotte M., Hinsinger P., 2009. Rhizosphere alkalisation — a major driver of copper bioavailability over a broad pH range in an acidic, copper-contaminated soil. Plant Soil. 318, 257–268. doi:10.1007/s11104-008-9835-6

Bravin, M.N., Garnier, C., Lenoble, V., Gérard, F., Dudal, Y., Hinsinger, P., 2012. Root-induced changes in pH and dissolved organic matter binding capacity affect copper dynamic speciation in the rhizosphere. Geochim. Cosmochim. Acta, 84, 256–268.

Bridgwater, T., 2006. Biomass for energy. J. Sci. Food. Agric. 86, 1755–1768. doi:10.1002/jsfa.2605

Brisson, J., Chazarenc, F., 2009. Maximizing pollutant removal in constructed wetlands: should we pay more attention to macrophyte species selection? Sci. Total. Environ. 407, 3923–3930.

Borchard, N., Prost, K., Kautz, T., Moeller, A., Siemens, J., 2012. Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. Eur. J. Soil Sci. 63, 399–409. doi:10.1111/j.1365-2389.2012.01446.x

Buss, W., Kammann, C., Koyro, H.W., 2012. Biochar reduces copper toxicity in *Chenopodium quinoa* Willd. in a sandy soil. J. Environ. Qual. 41,1157–1165. doi:10.2134/jeq2011.0022

Buss, W., Mašek, O., Graham, M., Wüst, D., 2015. Inherent organic compounds in biochar- Their content, composition and potential toxic effects. J. Environ. Manag. 156, 150–157. doi:10.1016/j.jenvman.2015.03.035

Calabrese, E.J., Blain, R.B., 2009. Hormesis and plant biology. Environ. Pollut. 157, 42–48. doi:10.1016/j.envpol.2008.07.028

Caldelas, C., Bort, J., Febrero, A., 2012. Ultrastructure and subcellular distribution of Cr in *Iris pseudacorus* L. using TEM and X-ray microanalysis. Cell Biol. Toxicol. 28, 57–68. doi:10.1007/s10565-011-9205-7

Calero, S., Segura, M., Rojo, C., Rodrigo, M.A., 2015. Shifts in plankton assemblages promoted by free water surface constructed wetlands and their implications in eutrophication remediation. Ecol. Eng. 74, 385–393. doi:10.1016/j.ecoleng.2014.11.003

Calfapietra, C., Gielen, B., Karnosky, D., Ceulemans, R., Scarascia Mugnozza, G., 2010. Response and potential of agroforestry crops under global change. Environ. Pollut. 158, 1095–104. doi:10.1016/j.envpol.2009.09.008

Cao, X.D., Wahbi, A., Ma, L.N., Li, B., Yang, Y.L., 2009. Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. J. Hazard. Mater. 164, 555–564. doi:10.1016/j.jhazmat.2008.08.034

Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol. 45, 4884–4889. doi:10.1021/es103752u

Cao, Z., Wang, S., Wang, T., Chang, Z., Shen, Z., Chen, Y., 2015. Using contaminated plants involved in phytoremediation for anaerobic digestion. Int. J. Phytoremediation. 17(1–6), 201–207. doi:10.1080/15226514.2013.876967

Carrillo-Gonzalez, R., Simunek, J., Sauve, S., 2006. Mechanisms and pathways of trace element mobility in soils. Adv. Agr. 91, 130–131.

Castaldi, P., Santona, L., Melis, P., 2005. Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. Chemosphere. 60, 365–371.

Cattaneo, F., Barbanti, L., Gioacchini, P., Ciavatta, C., Marzadori, C., 2014. ^{13}C abundance shows effective soil carbon sequestration in Miscanthus and giant reed compared to arable crops under Mediterranean climate. Biol. Fertil. Soils. 50, 1121–1128. doi:10.1007/s00374-014-0931-x

Cestonaro do Amaral, A., Kunz, A., Radis Steinmetz, R.L., Justi, K.C., 2014. Zinc and copper distribution in swine wastewater treated by anaerobic digestion. J. Environ. Manag. 141, 132–137. doi:10.1016/j.jenvman.2014.03.021

Chalot, M., Blaudez, D., Rogaume, Y., Provent, A.S., Pascual, C., 2012. Fate of trace elements during the combustion of phytoremediation wood. Environ. Sci. Technol. 46, 13361–13369. doi:10.1021/es3017478

Chaney, R.L., Angle, J.S., Baker, A.J.M., Li, J.M., 1998. Method for phytomining of nickel, cobalt, and other metal from soil. US Patent #. 5, 711–784.

Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual. 36, 1592–1443.

Chen, G.C., Liu, Z., Zhang, J., Owens, G., 2012. Phytoaccumulation of copper in willow seedlings under different hydrological regimes. Ecological Engineering. 44, 285–289. doi:10.1016/j.ecoleng.2012.04.018

Chen, J., Zhu, D., Sun, C., 2007. Effect of heavy metals on the sorption of hydrophobic organic compounds to wood charcoal. Environ. Sci. Technol. 41, 2536–3541. doi:10.1021/es062113+

Chen, L., Hu, X., Yang, W., Xu, Z., Zhang, D., Gao, S., 2015. The effects of arbuscular mycorrhizal fungi on sex-specific responses to Pb pollution in *Populus cathayana*. Ecotoxicol. Environ. Saf. 113, 460–468.

Chen, Z., Cuervo, D.P., Müller, J.A., Wiessner, A., Köser, H., Vymazal, J., Kästner, M., Kuschk, P., 2016. Hydroponic root mats for wastewater treatment—a review. Environ. Sci. Pollut. Res. 23, 15911–15928. doi:10.1007/s11356-016-6801-3

Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environ. Sci. Technol. 37, 5701–5710. doi:10.1021/es034354c

Cheng, C.H., Lin, T.P., Lehmann, J., Fang, L.J., Yang, Y.W., Menyailo, O.V., Chang, K.H., Lai, J.S., 2014. Sorption properties for black carbon (wood char) after long term exposure in soils. Org. Geochem. 70, 53–61. doi:10.1016/j.orggeochem.2014.02.013

Cheng, S., Grosse, W., Thoennessen, M., 2002. Efficiency of constructed wetlands in decontamination of water polluted by heavy metals. Ecol. Eng. 18(3), 35–38. doi:10.1016/S0925-8574(01)00091-X

Chintala, R., Schumacher, T.E., McDonald, L.M., Clay, D.E., Malo, D.D., Papiernik, S.K., Clay, S.A., Julson, J.L., 2014. Phosphorus sorption and availability from biochars and soil/biochar mixtures. CLEAN: Soil, Air. Water. 42, 626–634. doi:10.1002/clen.201300089

Choi, A., Wang, S., Lee, M., 2009. Biosorption of cadmium, copper, and lead ions from aqueous solutions by *Ralstonia sp*. and *Bacillus sp*. isolated from diesel and heavy metal contaminated soil. Geosci. J. 13, 331–341. doi:10.1007/s12303-009-0031-3

Ciadamidaro. L., Madejón. E., Puschenreiter. M., Madejón. P., 2013. Growth of *Populus alba* and its influence on soil trace element availability. Sci. Total. Environ. 454–455, 337–347. doi:10.1016/j.scitotenv.2013.03.032

CL AIRE., 2011. The SuRF-UK Indicator set for sustainable remediation assessment. London, UK. ISBN 978-1-905046-1292-5. Available at: www.claire.co.uk/surfuk (Verified on August 10th, 2016)

Clavé, G., Garoux, L., Boulanger, C., Hesemann, P., Grison, C., 2016a. Ecological recycling of a bio-based catalyst for Cu click reaction: a new strategy for a greener sustainable catalysis. Chemistry Select. 6, 1–8. doi:10.1002/slct.201600430

Clavé, G., Garel, C., Poullain, C., Renard, B.L., Olszewski, T.K., Lange, B., Shutcha, M., Faucon, M.P., Grison, C., 2016b. Ullmann reaction through ecocatalysis: insights from bioresource and synthetic potential. RSC Adv. 6, 59550–59564. doi:10.1039/C6RA08664K

Clemens, S., 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta. 212(4), 475–486.

Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie. 88(11), 1707–1719. doi:10.1016/j.biochi.2006.07.003

Coates, W., 2005. Tree species selection for a mine tailings bioremediation project in Peru. Biomass Bioenerg. 28, 418–423.

Collin, B., Doelsch, E., Keller, C., Cazevieille, P., Tella, M., Chaurand, P., Panfili, F., Hazemann, J.L., Meunier, J.D., 2014. Evidence of sulfur-bound reduced copper in bamboo exposed to high silicon and copper concentrations. Environ. Pollut. 187, 22–30. doi:10.1016/j.envpol.2013.12.024

Collura, S., Azambre, B., Finqueneisel, G., Zimny, T., Weber, J.V., 2006. *Miscanthus x giganteusstraw* and pellets as sustainable fuels. Combustion and emission tests. Environ. Chem. Lett. 4, 75–8.

Cui, L.Q., Pan, G.X., Li, L.Q., Yan, J.L., Zhang, A., Bian, R.J., Chang, A., 2012. The reduction of wheat Cd uptake in contaminated soil via biochar amendment: A two-year field experiment. Bioresources. 7, 5666–5676.

Cundy, A.B., Bardos, R.P., Church, A., Puschenreiter, M., Friesl-Hanl, W., Muller, I., Neu, S., Mench, M., Witters, N., Vangronsveld, J., 2013. Developing principles of sustainability and stakeholder engagement for "gentle" remediation approaches: The European context. J. Environ. Manag. 129, 283–291.

Cundy, A., Bardos, P., Puschenreiter, M., Witters, N., Mench, M., Bert, V., Friesl-Hanl, W., Muller, I., Weyens, N., Vangronsveld, J., 2015. Developing effective decision support for the application of "gentle" remediation options: The GREENLAND project. Remediation Journal 25. 101–114. doi:10.1002/rem.21435

Cuypers, A., Vangronsveld, J., Clijsters, H., 2000. Biphasic effect of copper on the ascorbate-glutathione pathway in primary leaves of *Phaseolus vulgaris* seedlings during the early stages of metal assimilation. Physiol. Plant. 110, 512–517. doi:10.1111/j.1399-3054.2000.1100413.x

Cuypers, A., Hendrix, S., Amaral dos Reis, R., De Smet, S., Deckers, J., Gielen, H., Jozefczak, M., Loix, C., Vercampt, H., Vangronsveld, J., Keunen, E., 2016. Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Front. Plant Sci. 7, 470. doi:10.3389/fpls.2016.00470

Delplanque, M., Collet, S., Del Gratta, F., Schnuriger B., Gaucher, R., Robinson, B., Bert, V., 2013. Combustion of Salix used for phytoextraction: The fate of metals and viability of the processes. Biomass Bioenerg. 49, 160–170. doi:10.1016/j.biombioe.2012.12.026

Di Bonito, M., 2005. Trace Elements in Soil Pore Water: A Comparison of Sampling Methods, University of Nottingham, United Kingdom.

Dilks, R.T., Monette, F., Glaus, M., 2016. The major parameters on biomass pyrolysis for hyperaccumulative plants - A review. Chemosphere. 146, 385–395. doi:10.1016/j.chemosphere.2015.12.0620045-6535

Ding, Y., Liu, Y.X., Wu, W.X., Shi, D.Z., Yang, M., Zhong, Z.K., 2010. Evaluation of biochar effects on nitrogen retention and leaching in multilayered soil columns. Water Air Soil Pollut. 213, 47–55. doi:10.1007/ s11270-010-0366-4

Du, X., Xu, Z., Li, J., Zheng, L., 2014. Characterization and removal of dissolved organic matter in a vertical flow constructed wetland. Ecol. Eng. 73, 610–615. doi:10.1016/j.ecoleng.2014.09.098

Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., Tack, F.M., 2009. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review. Sci. Total Environ. 2009, 407(13), 3972−3985. doi:10.1016/j.scitotenv.2008.07.025

EC-European Commision., 2013. Soil contamination: impacts on human health. In: European Commision DG Environment (Eds.), Science for the environment policy in-depth report. Science Communication Unit. University of the West England, Bristol, pp. 29.

El Bishlawi, H., Jaffe, P.R., 2015. Characterization of dissolved organic matter from a restored urban marsh and its role in the mobilization of trace metals. Chemosphere. 127, 144–151. doi:10.1016/j.chemosphere.2014.12.080

El Hadri, H., Chery, P., Jalabert, S., Lee, A., Potin-Gautier, M., Lespes, G., 2012. Assessment of diffuse contamination of agricultural soil by copper in Aquitaine region by using French national databases. Sci. Total. Environ. 441, 239–247. doi:10.1016/j.scitotenv.2012.09.070

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, É., El-Ramady, H., Márton, L., Czakó, M., Kátai, J., Balogh, P., Sztrik, A., Molnár, M., Popp, J., Fári, M.G., 2014. Phytoaccumulation potentials of two biotechnologically propagated ecotypes of *Arundo donax* in copper-contaminated synthetic wastewater. Environ. Sci. Pollut. Res. 21, 7773–7780. doi:10.1007/s11356-014-2736-8

Elhawat, N., Alshaal, T., Domokos-Szabolcsy, E., El-Ramady, H., Antal, G., Marton, L., Czako, M., Balogh, P., Fari, M., 2015. Copper uptake efficiency and its distribution within bioenergy grass giant reed, Bull. Environ. Contam. Toxicol. 95, 452–458. doi:10.1007/s00128-015-1622-5

Enell, A., Andersson-Sköld, Y., Vestin, J., Wagelmans, M., 2016. Risk management and regeneration of brownfields using bioenergy crops. J. Soils Sediments. 16, 987–1000. doi:10.1007/s11368-015-1264-6

Escande, V., 2014. De la phytoextraction à l'Écocatalyse: Valorisation de la biomasse chargée en éléments métalliques pour la conception de nouveaux catalyseurs et leur utilisation en synthèse organique durable, PhD thesis, Université Montpellier 2, Montpellier, pp.31-40.

Escande, V., Olszewski, T.K., Grison, C., 2014. Preparation of ecological catalysts derived from Zn hyperaccumulating plants and their catalytic activity in Diels–Alder reaction. C. R. Chimie. 17, 731–737. doi:10.1016/j.crci.2013.09.009

Escande, V., Velati, A., Garel, C., Renard, B.L., Petit, E., Grison, C., 2015. Phytoextracted mining wastes for ecocatalysis: Eco-Mn®, an efficient and eco-friendly plant-based catalyst for reductive amination of ketones. Green chem. 17, 2188–2199. doi:10.1039/C4GC02193B

European Biochar at http://www.european-biochar.org/en/ebc-ibi (verified on January 04th, 2016)

Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere. 68, 989–1003.

Evangelou, M.W.H., Deram, A., Gogos, A., Studer, B., Schulin, R., 2012. Assessment of suitability of tree species for the production of biomass on trace element contaminated soils. J. Hazard. Mater. 209–210, 233–239. doi:10.1016/j.jhazmat.2012.01.008

Evangelou, M.W.H., Deram, A., Ugolini, F., Abiven, S., Schulin, R., 2014. Soil application of biochar produced from biomass grown on trace element contaminated land. J. Environ. Manage. 146, 100–106. doi:10.1016/j.jenvman.2014.07.046

Evangelou, M.W.H., Fellet, G., Ji, R., Schulin, R., 2015. Phytoremediation and biochar application as an amendment. In Ansari, A.A, Gill, S.S, Gill, R., Lanza G R, Newman L (eds.) Phytoremediation. Management of Environmental Contaminants. Volume 1. Springer International Publishing, Cham. pp. 253–263.

Fang, W., Wie, Y., Liu, J., 2016. Comparative characterization of sewage sludge compost and soil: Heavy metal leaching characteristics. J. Hazard. Mater. 310, 1–10. doi:10.1016/j.jhazmat.2016.02.025

Fatemitalab, R., Zare, R., Kardar, S., 2016. Assessment of cadmium, zinc and lead contamination in leaf and root of four various species. Int. J. Environ. Sci. Techn. 13(5), 1229–1234. doi:10.1007/s13762-015-0931-x

Faucon, M.P., Ngoy Shutcha, M., Meerts, P., 2007. Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil, Plant Soil. 301, 29–36.

Favas, P.J.C., Pratas, J., Chaturvedi, R., Paul, M.S., Prasad, M.N.V., 2015. Tree crops on abandoned mines for environmental remediation and industrial feedstock. In: Prasad, M.N.V., (Eds.), Bioremediation and bioeconomy, Amsterdam. pp 250.

Fiorentino, N., Fagnano, M., Adamo, P., Impagliazzo, A., Mori, M., Pepe, O., Ventorino, V., Zoina, A., 2013. Assisted phytoextraction of heavy metals: compost and Trichoderma effects on giant reed (*Arundo donax* L.) uptake and soil N-cycle microflora. Ital. J. Agron.. doi:10.4081/ija.2013.e29

Forján, R., Asensio, V., Rodríguez-Vila, A., Covelo, E.F., 2016. Contributions of a compost-biochar mixture to the metal sorption capacity of a mine tailing. Environ. Sci. Pollut. Res. 23, 2595–2602. doi:10.1007/s11356-015-5489-0

Friesl, W., Horak, O., Wenzel, W.W., 2004. Immobilization of heavy metals in soils by the application of bauxite residues: pot experiments under field conditions. J. Plant Nutr. Soil Sci. 167, 54–59.

Friesl, W., Friedl, J., Platzer, K., Horak, O., Gerzabek, M.H., 2006.. Remediation of contaminated soils in the vicinity of a former Pb/Zn smelter in Austria: batch, pot, and field experiments. Environ. Pollut. 1441, 40–50. doi:10.1016/j.envpol.2006.01.012

Friesl-Hanl, W., Platzer, K., Horak, O., Gerzabek, M.H., 2009. Immobilising of Cd, Pb, and Zn contaminated arable soils close to a former Pb/Zn smelter: a field study in Austria over 5 years. Environ. Geochem. Health. 31, 581–594. Doi:10.1007/s10653-009-9256-3

Garbisu, C., Alkorta, I., 2001. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 77, 229–236. doi:10.1016/S0960-8524(00)00108-5

Gaskin, J.W., Speir, R.A., Harris, K., Das, K.C., Lee, R.D., Morris, L.A., Fisher, D.S., 2010. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. Agron. J. 102, 623–633.

Ge, X., Xu, F., Vasco-Correa, J., Li, Y., 2016. Giant reed: A competitive energy crop in comparison with miscanthus. Renew. Sust. Energ. Rev. 54, 350–362. doi:10.1016/j.rser.2015.10.0101364-0321/

George, C., Wagner, M., Kücke, M., Rillig, M.C., 2012. Divergent consequences of hydrochar in the plant–soil system: Arbuscular mycorrhiza, nodulation, plant growth and soil aggregation effects. Appl. Soil Ecol. 59, 68–72. doi:10.1016/j.apsoil.2012.02.021

Ghetti, P., Ricca, L., Angelini, L., 1996. Thermal analysis of biomass and corresponding pyrolysis products. Fuel. 75, 565–573.

Ghorbanli, M., Sateei, A., Savadkohi, S.N., 2011. Effect of various concentrations of copper on antioxidant enzymes activity and phenolic compounds content in leave and root of *Plantago major* L. Iran. J Med.Aromat. Plant. 26, 544–554.

Gilbert, N., Fulthorpe, R., Kirkwood, A.E., 2012. Microbial diversity, tolerance and biodegradation potential of urban wetlands with different input regimes. Canadian J. Microbiol. 58, 887–892.

Gill, L.W., Ring, P., Higgins, N.M.P., Johnston, P.M. 2014. Accumulation of heavy metals in a constructed wetland treating road runoff. Ecol. Eng. 70, 133–139. doi:10.1016/j.ecoleng.2014.03.056

GIS sol., 2009. Nouvelle collecte nationale d'analyses d'éléments traces métalliques - Statistiques par région agricole. Available at http://www.gissol.fr/donnees/cartes/les-teneurs-en-cuivre-total-des-horizons-de-surface-0-30-cm-des-sols-de-france-2394 (Verified on August 10th, 2016)

Glaser, B., Parr, M., Braun, C., Kopolo, G., 2009. Biochar is carbon negative. Nature Geoscience. 2(2). doi:10.1038/ngeo395

Gomes, H., 2012. Phytoremediation for bioenergy: challenges and opportunities. Environ. Technol. Rev. 1(1), 59–66. doi:10.1080/09593330.2012.696715

Gomez-Eyles, J.L., Sizmur, T., Collins, C.D., Hodson, M.E., 2011. Effects of biochar and the earthworm *Eisenia fetida* on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements. Environ. Pollut. 159, 616–622. doi:10.1016/j.envpol.2010.09.037

Gonsalvesh, L., Yperman, J., Carleer, R., Mench, M., Herzig, R., 2016. Vangronsveld J. Valorisation of heavy metals enriched tobacco biomass through slow pyrolysis and steam activation. J. Chem. Technol. Biotechnol. 91, 1585–1595. doi:10.1002/jctb.4889

Gray, C., Dunham, S., Dennis, P., Zhao, F., McGrath, S., 2006. Field evaluation of in situ remediation of a heavy metal contaminated soil using lime and red-mud. Environ. Pollut. 142, 530–539.

Grandjean, A., 2007. Où produire les énergies renouvelables. Concurrences d'usages et pression foncière. Dossier du colloque de l'ADEF, Paris.

Grison, C., Esclandre, V., Olszewski, T.K, 2015a. Ecocatalysis: A new approch toward bioeconomy. In: Prasad, M.N.V., (Eds.), Bioremediation and bioeconomy, Amsterdam. pp. 660.

Grison, C.M., Mazel, M., Sellini, A., Escande, V., Biton, J., Grison, C., 2015b. The leguminous species *Anthyllis vulneraria* as a Zn-hyperaccumulator and eco-Zn catalyst resources. Environ. Sci. Pollut. Res. 22, 5667–5676. doi:10.1007/s11356-014-3605-1

Grybos, M., Davranche, M., Gruau, G., Petitjean, P., 2007. Is trace metal release in wetland soils controlled by organic matter mobility or Fe-oxyhydroxides reduction? J. Colloid Interface Sci. 314, 490–501. doi:10.1016/j.jcis.2007.04.062

Gu, C.H., Liu, L.Q., Deng, Y.M., Zhu, X.D., Huang, S.Z., Lu, X.Q., 2015. The heterologous expression of the *Iris lactea* var. *Chinensis* type 2 metallothionein IlMT2b gene rnhances copper tolerance in *Arabidopsis thaliana*. Bull. Environ. Contam. Toxicol. 94, 247–253. doi:10.1007/s00128-014-1444-x

Guigue, J., Mathieu, O., Lévêque, J., Denimal, S., Steinmann, M., Milloux, M.J., Grisey, H., 2013. Dynamics of copper and zinc sedimentation in a lagooning system receiving landfill leachate. Waste Manage. 33, 2287–2295. doi:10.1016/j.wasman.2013.06.004

Guittonny-Philippe, A., Masotti, V., Höhener, P., Boudenne, J.L., Viglione, J., Laffont-Schwob, I., 2014. Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: A review to overcome obstacles and suggest potential solutions. Environ. Int. 64, 1–16. doi: 10.1016/j.envint.2013.11.016

Gupta, N., Ram, H., Kumar, B., 2016. Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. Rev. environ. sci bio-tech.. 15(1), 89–109. doi:10.1007/s11157-016-9390-1

Ha, N.T.H., Sakakibara, M., Sano, S., 2011. Accumulation of Indium and other heavy metals by *Eleocharis acicularis*: An option for phytoremediation and phytomining. Bioresour. Technol. 102, 2228–2234.

Hagedorn, F., Bruderhofer, N., Ferrari, A., Niklaus, P.A., 2015. Tracking litter-derived dissolved organic matter along a soil chronosequence using ^{14}C imaging: Biodegradation, physico-chemical retention or preferential flow? Soil. Biol. Biochem. 88, 333–343. doi:10.1016/j.soilbio.2015.06.014

Hale, S., Hanley, K., Lehmann, J., Zimmerman, A.R., Cornelissen, G., 2011. Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar. Environ. Sci. Technol. 45, 10445–10453. doi:10.1021/es202970x

Hale, B., Evans, L., Lambert, R., 2012. Effects of cement or lime on Cd, Co, Cu, Ni, Pb, Sb and Zn mobility in field-contaminated and aged soils. J. Hazard. Mater. 199-200, 119-127. doi:10.1016/j.jhazmat.2011.10.065

Han, M., Choi, G.W., Kim, Y., Koo, B.C., 2011. Bioethanol production by *Miscanthus* as a lignocellulosis biomass: focus on high efficient conversion to glucose and ethanol. Bio. Resour. 6, 1939–1953.

Han, J., Tao, W., 2014. Treatment performance and copper removal mechanisms of a vegetated submerged bed receiving leachate from ACQ-treated lumber. Ecol. Eng. 70, 162–168. doi:10.1016/j.ecoleng.2014.05.005

Han, Y., Zhang, L., Yang, Y., Yuan, H., Zhao, J., Gu, J., Huang, S., 2016. Pb uptake and toxicity to *Iris halophila* tested on Pb mine tailing materials. Environ. Pollut. 214, 510–516. doi:10.1016/j.envpol.2016.04.048

Hanauer, T., Felix-Henningsen, P., Steffens, D., Kalandadze, B., Navrozashvili, L., Urushadze, T., 2011. In situ stabilization of metals (Cu, Cd, and Zn) in contaminated soils in the region of Bolnisi, Georgia. Plant Soil. 341, 193–208. doi:10.1007/s11104-010-0634-5

Hardej, M., Ozimek, T., 2002. The effect of sewage sludge flooding on growth and morphometric parameters of *Phragmites australis* (Cav.) Trin. ex Steudel, Ecol. Engin., 18, 343–350.

Harmsen, J., 2007. Measuring bioavailability: from a scientific approach to standard methods. J. Environ. Qual. 36, 1420–1428.

Hartley, W., Dickinson, N.M., Riby, P., Lepp, N.W., 2009. Arsenic mobility in brownfield soils amended with green waste compost or biochar and planted with *Miscanthus*. Environ. Pollut. 157, 2654–2662. doi:10.1016/j.envpol.2009.05.011

Hattab, N., Motelica-Heino, M., Bourrat, X., Mench, M., 2014. Mobility and phytoavailability of Cu, Cr, Zn, and As in a contaminated soil at a wood preservation site after 4 years of aided phytostabilisation. Environ. Sci. Pollut. Res. 21, 10307–10319. doi:10.1007/s11356-014-2938-0

Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: An overview. Adv. Agron. 85, 221–268. doi:10.1016/S0065-2113(04)85005-3

Hego, E., Bes, C., Bedon, F., Palagi, P.M., Chaumeil, .P, Barré, A., Claverol, S., Dupuy, J.W., Bonneu, M., Lalanne, C., Plomion, C., Mench, M., 2015. Differential accumulation of soluble proteins in roots of metallicolous and nonmetallicolous populations of *Agrostis capillaris* L. exposed to Cu. Proteomics. 14(15), 1746–1758. doi:10.1002/pmic.201300168

Hego, E., Vilain, S., Barré, A., Claverol, S., Dupuy, J.W., Lalanne, C., Bonneu, M., Plomion, C., Mench, M., 2016. Copper stress-induced changes in leaf soluble proteome of Cu-sensitive and tolerant Agrostis capillaris L. populations. Proteomics. 16(9), 1386–97. doi:10.1002/pmic.201500083

Herzig, R., Nehnevajova, E., Pfistner, C., Schwitzguébel, J.P., Ricci, A., Keller, C., 2014. Feasibility of labile Zn phytoextraction using enhanced tobacco and sunflower: results of five- and one-year field-scale experiments in Switzerland. Int. J. Phytorem. 16(7–8), 735–754.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil. 248, 43–59. doi:10.1023/A:1022371130939

Hoffmann, G., Schingnitz, D., Schnapke, A., Bilitewski, B., 2010. Reduction of CO_2-emissions by using biomass in combustion and digestion plants. Waste Manag. 30, 893–901. doi :10.1016/j.wasman.2009.12.001

Houben, D., Evrard, L., Sonnet, P., 2013a. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere. 92, 1450–1457. doi:10.1016/j.chemosphere.2013.03.055

Houben, D., Evrard, L., Sonnet, P., 2013b. Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed *Brassica napus* L.. Biomass. Bioenerg. 57, 196–204. doi:10.1016/j.biombioe.2013.07.019

Houben, D., Sonnet, P., 2015. Impact of biochar and root-induced changes on metal dynamics in the rhizosphere of *Agrostis capillaris* and *Lupinus albus*. Chemosphere. 139, 644–651. doi:10.1016/j.chemosphere.2014.12.036

Huang, X., Xu, Y., Shan, C., Li, X., Zhang, W., Pan, B., 2016. Coupled Cu(II)-EDTA degradation and Cu(II) removal from acidic wastewater by ozonation: Performance, products and pathways. Chemical Eng. J. 299, 23–29. doi:10.1016/j.cej.2016.04.044

Hrynkiewicz, K., Baum, C., 2012. The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils, in Malik A, Grohmann E, (eds.), Environmental Protection Strategies for Sustainable Development, Strategies for Sustainability, New York, 35–64. doi:10.1007/978-94-007-1591-2_2

Huguenot, D., Bois, P., Cornu, J.Y., Jezequel, K., Lollier, M., Lebeau, T., 2015. Remediation of sediment and water contaminated by copper in small-scaled constructed wetlands: effect of bioaugmentation and phytoextraction. Environ. Sci. Pollut. Res. 22, 721–732. doi:10.1007/s11356-014-3406-6

Idris, S.M., Jones, P.L., Salzman, S.A., Croatto, G., Allinson, G., 2012. Evaluation of the giant reed (*Arundo donax*) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. Environ. Sci. Pollut. Res. 19, 1159−1170. doi:10.1007/s11356-011-0642-x

IFREMER., 2014. Qualité du Milieu Marin Littoral Bulletin de la surveillance 2013, 2014. Institut Français de Recherche pour l'Exploitation de la Mer, Available at : http://envlit.ifremer.fr/content/download/81930/580311/version/2/file/bull_ar_2014.pdf

Inaba, S, Takenaka, C., 2005. Effects of dissolved organic matter on toxicity and bioavailability for lettuce sprouts. Environ. Int. 31(4), 603–608. doi:10.1016/j.envint.2004.10.017

IPCC., 2007. Synthesis report. In: Fourth assessment report: Climate change. Cambridge, UK: Cambridge University Press.

INDIQUASOL. 2016. Indicator of soil quality data base. INRA, Orleans. Available online at http://acklins.orleans.inra.fr/geoindiquasol/index.php

ITRC, 2006. Planning and promoting ecological land reuse of remediated sites, Washington. Available online at: https://frtr.gov/costperformance/pdf/remediation/ITRC-Ecological_Land_Reuse.pdf (Verified on August 10th, 2016)

Ippolito, J.A., Strawn, D.G., Scheckel, K.G., Novak, J.M., Ahmedena, M., Niandou, M.A.S., 2012. Macroscopic and molecular investigations of copper sorption by a steam-activated biochar. J. Environ. Qual. 41, 150–156. doi:10.2134/jeq2011.0113

Istenič, D., Arias, C.A., Vollertsen, J., Nielsen, A.H., Wium Andersen, T., Hvitved-Jacobsen, T., Brix, H., 2012. Improved urban stormwater treatment and pollutant removal pathways in amended wet detention ponds. J. Environ. Sci. Health., Part A. 47, 1466–1477. doi:10.1080/10934529.2012.673306

Jakobsen, S.T., 1993. Interaction between plant nutrients III. Antagonism between potassium, magnesium and calcium. Acta Agriculturae Scandinavica Section B: Soil and Plant Science. 43**,** 1–5. doi:10.1080/09064719309410223

Jamieson, T., Sager, E., Guégue, C., 2014. Characterization of biochar-derived dissolved organic matter using UV–visible absorption and excitation–emission fluorescence spectroscopies. Chemosphere. 103, 197–204. doi:10.1016/j.chemosphere.2013.11.066

Janssen, J., 2015. Metal-phytoextraction: the potential of high biomass crops and improving efficiency using short rotation coppice (SRC) of willow. PhD thesis, Hasselt University, Hasselt, Belgium.

Jiang, Z.F., Huang, S.Z., Han, Y.L., Zhao, J.Z., Fu, J.J., 2012. Physiological response of Cu and Cu mine tailing remediation of Paulownia fortunei (Seem) Hemsl. Ecotoxicol. 21, 759–767. doi:10.1007/s10646-011-0836-5

Jiang, J., Xu, R., 2013. Application of crop straw derived biochars to CuII. contaminated Ultisol: Evaluating role of alkali and organic functional groups in CuII. immobilization. Bioresour. Technol. 133, 537–545. doi:10.1016/j.biortech.2013.01.161

Jiang, Y., Lei, M., Duan, L., Longhurst, P., 2015. Integrating phytoremediation with biomass valorisation and critical element recovery: A UK contaminated land perspective. Biomass Bioenerg. 83, 328–339.

Johnson, A.W., Gutiérrez, M., Gouzie, D., McAliley, L., 2016. State of remediation and metal toxicity in the Tri-State Mining District, USA. Chemosphere. 144, 1132–1141. doi:10.1016/j.chemosphere.2015.09.080

Jones, D.L., Healey, J.R., 2010. Organic amendments for remediation: putting waste to good use. Elem. 6, 369–374.

Jones, S., Bardos, R.P., Kidd, P., Mench, M., de Lei,j F., Hutchings, T., Cundy, A., Joyce, C., Soja, G., Friesl-Hanl, W., Herzig, R., Menger, P., 2016. Biochar and compost amendments enhance copper immobilisation and support plant growth in contaminated soils. J. Environ. Manag. 171, 101–112. doi:171;101:112.10.1016/j.jenvman.2016.01.024

Jouili, H., Bouazizi, H., El Ferjani, E., 2011. Plant peroxidases: biomarkers of metallic stress. Acta Physiol. Plant. 33, 2075–2082.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. CRC Press, Lewis Publishers, Boca Raton, Florida, USA.

Kadlec, R., Wallace, S., 2008. Treatment Wetlands. CRC Press, Boca Raton, Florida.

Kanoun-Boule, M., Vicente, J.A.F., Nabais, C., Prasad, M.N.V., Freitas, H., 2009. Ecophysiological tolerance of duckweeds exposed to copper. Aquat. Toxicol. 91, 1–9. doi:10.1016/j.aquatox.2008.09.009

Karami, N., Clemente, R., Moreno-Jimenez, E., Lepp, N.W., Beesley, L., 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J. Hazard. Mater. 191, 41–8. doi:10.1016/j.jhazmat.2011.04.025

Karer, J., Wawra, A., Zehetner, F., Dunst, G., Wagner, M., Pavel, P.B., Puschenreiter, M., Friesl-Hanl, W., Soja, G. 2015. Effects of biochars and compost mixtures and inorganic additives on immobilisation of heavy metals in contaminated soils. Water. Air. Soil. Pollut. 226, 342. doi:10.1007/s11270-015-2584-2

Kargar, M., Clark, O.G., Hendershot, W.H., Jutras, P., Prasher, S.O., 2015. Immobilization of trace metals in contaminated urban soil amended with compost and biochar. Water Air Soil Pollut. 226, 191. doi:10.1007/s11270-015-2450-2

Karhu, K., Mattila, T., Bergstrom, I., Regina, K., 2011. Biochar addition to agricultural soil increased CH(4) uptake and water holding capacity results from a short term pilot field study. Agric. Ecosyst. Environ. 140, 309–313.

Karjalainen, A.M., Kilpi-Koski, J., Vaisanen, A.O., Penttinen, S., Van Gestel, C.A.M., Penttinen, O.P., 2009. Ecological risks of an old wood impregnation mill: application of the Triad approach. Integr. Enviro. Assess. Manage. 5, 379–389. doi:10.1897/IEAM_2008-072.1

Kasperowski, E.. 1993. Schwermetalle in Böden im Raum Arnoldstein. Monographien Bd. 33, Umweltbundesamt UBA, Wien, Austria.

Kausar, S., Mahmood, Q., Raja, I.A., Khan, A., Sultan, S., Gilani, M.A., Shujaat, S., 2012. Potential of *Arundo donax* to treat chromium contamination, Ecol. Eng. 42, 256–259. doi:10.1016/j.ecoleng.2012.02.019

Khandekar, S., Leisner, S., 2011. Soluble silicon modulates expression of Arabidopsis thaliana genes involved in copper stress. J. Plant Physiol. 168, 699–705. doi:10.1016/j.jplph.2010.09.009

Kidd, P., Barcelob, J., Bernalc, M., Navari-Izzo, F., Poschenrieder, C., Shileve, S., Clemente, R., Monterrosof, C., 2009. Trace element behavior at the root–soil interface: Implications in phytoremediation. Environ. Exp. Botany. 67, 243–259.

Kidd, P., Mench, M., Álvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J.O., Kolbas, A., Müller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J., Puschenreiter, M., 2015. Agronomic practices for improving gentle remediation of trace element-contaminated soils. Int. J. Phytorem. 17, 1005–1037. doi:10.1080/15226514.2014.1003788

Kiikkilä, O , Kitunen, V , Spetz, P , Smolander, A., 2012. Characterization of dissolved organic matter in decomposing Norway spruce and silver birch litter. Eur. J. Soil Sci. 63, 476–486. doi:10.1111/j.1365-2389.2012.01457.x

Kissoon, L.T., Jacob, D.L., Otte, M.L., 2010. Multi-element accumulation near Rumex crispus roots under wetland and dryland conditions. Environ. Pollut. 158, 1834–1841. doi:10.1016/j.envpol.2009.11.001

Klasson, K.T., Boihem Jr., L.L., Uchimiya, M., Lima, I.M. Influence of biochar pyrolysis temperature and post-treatment on the uptake of mercury from flue gas. Fuel Process. Technol. 123, 27–33. doi:10.1016/j.fuproc.2014.01.034

Kloss, S., Zehetner, F., Wimmer, B., Buecker, J., Rempt, F., Soja, G., 2014. Biochar application to temperate soils: effects on soil fertility and crop growth under greenhouse conditions. J. Plant. Nutr. Soil. Sci. 177, 3–15.

Kolbas, A., Mench, M., Herzig, R., Nehnevajova, E., Bes, C.M., 2011. Copper phytoextraction in tandem with oilseed production using commercial cultivars and mutant lines of sunflower. Int. J. Phytorem. 13(S1), 55–76.

Kolbas, A., Marchand, L., Herzig, R., Nehnevajova, E., Mench, M., 2013. Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. Plant Soil. 376, 377–397. doi:10.1007/ s11104-013-1974-8

Kolbas, A., Kidd, P., Guinberteau, J., Jaunatre, R., Herzig, R., Mench, M., 2015. Endophytic bacteria take the challenge to improve Cu phytoextraction by sunflower. Environ. Sci. Pollut. Res. Int. 7, 5370-5382. doi:10.1007/s11356-014-4006-1

Komárek, M., Vaněk, A., Chrastný, V., Száková, J., Kubová, K., Drahota, P., Balík. J., 2009. Retention of copper originating from different fungicides in contrasting soil types. J. Hazard. Mater. 166, 1395–1402. doi:10.1016/j.jhazmat.2008.12.061

Komárek, M., Vanek, A., Ettler, V., 2013. Chemical stabilization of metals and arsenic in contaminated soils using oxides. A review. Environ. Pollut. 172, 9–22. doi:10.1016/j.envpol.2012.07.045

Kookana, R.S., 2010. The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. Aust. J. Soil Res. 48, 627–637. doi:10.1071/SR10007

Kookana R.S., Sarmah, A.K., Van Zwieten, L., Krull, E., Singh, B., 2011. Chapter three—Biochar application to soil: agronomic and environmental benefits and unintended consequences. Adv Agron. 112, 103–143.

Kuepper, H., Goetz, B., Mijovilovich, A., Kuepper, F.C., Meyer-Klaucke, W., 2009. Complexation and toxicity of copper in higher plants. I. Characterization of copper accumulation, speciation, and toxicity in *Crassula helmsii* as a new copper accumulator. Plant Physiol. 151, 702–714.

Kumari, M., Tripathi, B.D., 2015. Efficiency of *Phragmites australis* and *Typha latifolia* for heavy metal removal from wastewater, Ecotox. Environ. Safe. 112, 80–86. doi:10.1016/j.ecoenv.2014.10.034

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. Waste. Manage. 28, 215–225. doi:10.1016/j.wasman.2006.12.012

Kumpiene, J., Mench, M., Bes, C.M., Fitts, J.P., 2011. Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environ. Pollut. 159, 1536–1542. doi:10.1016/j.envpol.2011.03.005

Küpper, H., Andresen, E., 2016. Mechanisms of metal toxicity in plants. Metallomics, 8(3), 269–85. doi:10.1039/c5mt00244c

Laghari, M., Hu, Z., Mirjat, M.S., Xiao B., Tagar, A.A., Hu M., 2015. Fast pyrolysis biochar from sawdust improves quality of desert soils and enhances plant growth. J. Sci. Food. Agric. doi:10.1002/jsfa.7082

Lagriffoul, A., Mocquot, B., Mench, M., Vangronsveld, J., 1998. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Plant Soil. 200, 241–250. doi:10.1023/a:1004346905592

Lagomarsino, A., Mench, M., Marabottini, R., Pignataro, A., Grego, S., Renella, G., Stazi, S.R., 2011. Copper distribution and hydrolase activities in a contaminated soil amended with dolomitic limestone and compost. Ecotoxicol. Environ. Saf. 74 (7), 2013-2019.

Laird, D.A., 2008. The charcoal vision: A win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water auality. Agronomy J. 100, 178–181.

Lal, R., 2008. Crop residues as soil amendments and feedstock for bioethanol production. Waste Manag. 28, 747–758.

Langer, I., Santner, J., Krpata, D., Fitz, W.J., Wenze, W.W., Schweiger, P., 2012. Ectomycorrhizal impact on Zn accumulation of *Populus tremula* L. grown in metalliferous soil with increasing levels of Zn concentration. Plant Soil. 355, 283–297.

Laureysens, I., Blust, R., De Temmerman, L., Lemmens, C., Ceulemans, R., 2004. Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environ. Pollut. 131, 485–494. doi:10.1016/j.envpol.2004.02.009

Lazaridis, N.K., Peleka, E.N., Karapantsios, T.D., Matis, K.A., 2004. Copper removal from effluents by various separation techniques. Hydrometallurgy. 74, 149–156. doi:10.1016/j.hydromet.2004.03.003

Lee, J.W., Kidder, M., Evans, B.R., Paik, S., Buchanan, A.C., Garten, C.T., Brown, R.C., 2010. Characterization of biochars produced from cornstovers for soil amendment. Environ. Sci. technol. 44, 7970–7974.

Lehmann, J., Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil. 249, 343–357.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Biochar sequestration in terrestrial ecosystems – a review. Mitigation Adaptat. Strateg. Glob. Chang. 11, 403–427.

Lehmann, J., 2007. A handful of carbon. Nature. 447, 143–144. doi:10.1038/447143a

Lehmann, J., 2009. Biochar for environmental management: science and technology. Earthscan, London and Sterling, VA USA.

Leitenmaier, B., Küpper, H., 2013. Compartmentation and complexation of metals in hyperaccumulator plants. Front. Plant Sci. 4, 374. doi:10.3389/fpls.2013.00374

Lequeux, H., Hermans, C., Lutts, S., Verbruggen, N., 2010. Response to copper excess in *Arabidopsis thaliana:* impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48, 673–682. doi:10.1016/j.plaphy.2010.05.005

Lewis, M., Jackson, M., 2002. Nalgrass: a nonwood fiber source suitable for existing US pulp mills. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. ASHS, Alexandria, pp 371–376.

Li, C., Xiao, B., Wang, Q.H., Yao, S.H., Wu, J.Y., 2014. Phytoremediation of Zn- and Cr-contaminated soil using two promising energy grasses, Water Air Soil Pollut. 225, 2027. doi:10.1007/s11270-014-2027-5

Li, H., Ye, X., Geng, Z., Zhou, H., Guo, X., Zhang, Y., Zhao, H., Wang, G., 2016a. The influence of biochar type on long-term stabilization for Cd and Cu in contaminated paddy soils. J. Hazard. Mater. 304, 40–48. doi:10.1016/j.jhazmat.2015.10.048

Li, N., Guo, B., Li, H., Fu, Q., Feng, R., Ding, Y., 2016b. Effects of double harvesting on heavy metal uptake by six forage species and the potential for phytoextraction in field. Pedosphere. 26(5), 717–724. doi:10.1016/S1002-0160(15)60082-0

Liang, Y., Cao, X., Zhao, L., Arellano, E., 2014. Biochar- and phosphate-induced immobilization of heavy metals in contaminated soil and water: implication on simultaneous remediation of contaminated soil and groundwater. Environ. Sci. Pollut. Res. 21, 4665–4674. doi:10.1007/s11356-013-2423-1

Liang, Y.C., Sun, W.C., Zhu, Y.G., Christie, P., 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ. Pollut. 147, 422–428. doi:10.1016/j.envpol.2006.06.008

Lievens, C., Yperman, J., Vangronsveld, J., Carleer, R., 2008. Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis: part I. Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. Fuel. 87, 1894–1905. doi:10.1016/j.fuel.2007.10.021

Lin, Y., Aarts, M.G.M., 2012. The molecular mechanism of zinc and cadmium stress response in plants. Cell. Mol. Life Sci. 69, 3187–3206. doi:10.1007/s00018-012-1089-z

Liu, W., Tian, S., Zhao, X., Xie, W., Gong, Y., Zhao, D., 2015. Application of stabilized nanoparticles for in situ remediation of metal-contaminated soil and groundwater: a critical review. Curr. Pollution. Rep. 1, 280–291. doi:10.1007/s40726-015-0017-x

Lu, K., Yang, X., Shen, J., Robinson, B., Huang, H., Liu, D., Bolan, N., Pei, J., Wang, H., 2014. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to *Sedum plumbizincicola.* Agric. Ecosyst. Environ. 191, 124–132. doi:10.1016/j.agee.2014.04.010

Luca, C., Pilu, R., Tambone, F., Scaglia, B., Adani, F., 2015. New energy crop giant cane (*Arundo donax* L.) can substitute traditional energy crops increasing biogas yield and reducing costs. Bioresour. Technol. 191, 197–204. doi:10.1016/j.biortech.2015.05.015

Lucas-Borja, M.E., Wic-Baena, C., Moreno, J.L., Dadi, T., García, C., Andrés-Abellá, M., 2011. Microbial activity in soil under fast-growing Paulownia (*Paulownia elongata×fortunei*) plantations in Mediterranean areas. Appl. Soil. Ecol. 51, 42–51. doi:10.1016/j.apsoil.2011.08.011

Lukáčová Kuliková, Z., Lux, A., 2010. Silicon influence on maize, *Zea mays* L., hybrids exposed to cadmium treatment. Bull. Environ. Contam. Toxicol. 85, 243–250. doi:10.1007/s00128-010-0046-5

Luo, F., Song, J., Xia, W., Dong, M., Chen, M., Soudek, P., 2014. Characterization of contaminants and evaluation of the suitability for land application of maize and sludge biochars. Environ. Sci. Pollut. Res. 21, 8707–8717. doi:10.1007/s11356-014-2797-8

Lutts, S., Lefevre, I., Delperee, C., Kivits, S., Dechamps, C., Robledo, A., Correal, E., 2004. Heavy metal accumulation by the halophyte species Mediterranean saltbush. J. Environ. Qual. 33, 1271–1279. doi:10.2134/jeq2004.1271

Ma, Y., Rajkumar, M., Freitas, H., 2009. Improvement of plant growth and nickel uptake by nickel resistant-plant growth promoting bacteria. J. Hazard. Mater. 166, 1154–61. doi:10.1016/j.jhazmat.2008.12.018

Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotech. Adv. 29, 248–258. doi:10.1016/j.biotechadv.2010.12.001

Mackie, K.A., Müller, T., Kandeler, E., 2012. Remediation of copper in vineyards - A mini review, Environ. Pollut. 167, 16–26. doi:10.1016/j.envpol.2012.03.023

Mackie, K.A., Marhan, S., Ditterich, F., Schmidt, H.P., Kandeler, E., 2015. The effects of biochar and compost amendments on copper immobilization and soil microorganisms in a temperate vineyard. Agr Ecosyst. Environ. 201, 58–69. doi:10.1016/j.agee.2014.12.001

MacNicol, R.D., Beckett, P.H.T., 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil. 85, 107–129.

Maestri, E., Marmiroli, M., Visioli, G., Marmiroli, N., 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ. Exp. Bot. 68, 1–13.

Mahar, A., Ping Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R., Zhang, Z., 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. Ecotox. Environ. Safe. 126, 111–121. doi:10.1016/j.ecoenv.2015.12.0230147-6513/

Maille, E., 2004. Enquete phytosanitaire Gironde – Dordogne. http://www.itab.asso.fr/downloads/actes/actes_jt_viti_04_avignon_mini.pdf (Verified on August 10th, 2016)

Maine, M.A., Sune, N., Hadad, H., Sanchez, G., Bonetto, C., 2009. Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J. Environ. Manage. 90, 355–363. doi:10.1016/j.jenvman.2007.10.004

Maiz, I., Arambarri, I., Garcia, R., Millan, E., 2000. Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. Environ. Poll. 110, 3–9.

Maldeveanu, A.M., 2014. Assessment of soil pollution with heavy metals in Romania, in: Hernandez-Soriano, M.C. (Eds.), Environmental risk assessment of soil contamination. In Tech. pp. 285–305.

Manceau, A., Marcus, M.A., Tamure, N., 2002. Quantitative speciation of heavy metals in soils and sediments by synchrotron X-ray techniques. Rev. Mineral. Geochem. 49, 341–428.

Mantineo, M., D'agosta, G.M., Copani, V., Patanè, C., Cosentino, S.L., 2009. Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. Field Crop. Res. 114(2), 204–213. doi:10.1016/j.fcr.2009.07.020

Mantovi, P., Bonazzi, G., Maestri, E., Marmiroli, N., 2003. Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. Plant Soil. 250, 249–257. doi:10.1023/A:1022848131043

Marchand, L., Mench, M., Jacob, D.L., Otte, M.L., 2010. Metal and metalloid removal in constructed wetlands, with emphasis on the importance of plants and standardized measurements: a review. Environ. Pollut. 158, 3447–3461. doi:10.1016/j.envpol.2010.08.018

Marchand., 2012. Phytoremédiation en zones humides construites d'eaux contaminées en cuivre. PhD thesis. Université de Bordeaux, Talence.

Marchand, L., Nsanganwimana, F., Gonnelli, C., Colzi, I., Fletcher, T., Oustriere, N., Kolbas, A., Kidd, P., Bordas, F., Newell, P., Alvarenga, P., Lamy, J.B., Deletic, A., Mench, M., 2014a. Intra-specific variability versus constitutive-like tolerance to face Cu exposure in populations of six rooted macrophytes. Environ. Pollut. 193, 205–215. doi:10.1016/j.envpol.2014.07.001

Marchand, L., Nsanganwimana, F., Oustriere, N., Grebenshchykova, Z., Lizama-Allende, K., Mench, M., 2014b. Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*. Ecol. Eng. 64, 291–300. doi:10.1016/j.ecoleng.2013.12.017

Marchand, L., Nsanganwimana, F., Cook, B., Vystavna, Y., Huneau, F., Lecoustumer, P., Lamy, JB., Oustrière, N., Mench, M., 2014c. Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors. Ecol. Indic. 46, 425–437. doi:10.1016/j.ecolind.2014.07.011

Marchand, L., Quintela Sabaris, C., Desjardins, D., Oustrière, N., Pesme, E., Butin, D., Wicart, G., Mench, M., 2015. Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons. Environ. Sci. Pollut. Res. doi:10.1007/s11356-015-4984-7

Marchand, L., Pelosi, C., González-Centeno, M.R., Maillard, A., Ourry, A., Galland, W., Teissedre, P.L., Bessoule, J.J., Mongrand, S., Morvan-Bertrand, A., Zhang, Q., Grosbellet, C., Bert, V., Oustriere, N., Mench, M., Brunel-Muguet, S., 2016. Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere. 156, 150–162.

Marschner, H., 2011. Mineral nutrition of higher plants, 3rd edn. Academic, London.

Martin, S.M., Kookana, R.S., Van Zwieten, L., Krull, E., 2012. Marked changes in herbicide sorption–desorption upon ageing of biochars in soil. J. Hazard. Mater. 231–232, 70–78. doi:10.1016/j.jhazmat.2012.06.040.

Mavrogianopoulos, G., Vogli, V., Kyritsis, S., 2002. Use of wastewater as a nutrient solution in a closed gravel hydroponic culture of *Arundo donax*. Biores. Technol. 82, 103–107. doi:10.1016/S0960-8524(01)00180-8

McKendry, P., 2002. Energy production from biomass (part 1): overview of biomass. Bioresour. Technol. 83, 37–46.

Melo, L.C.A., Puga, A.P., Coscione, A.R., Beesley, L., Abreu, C.A., Camargo, O.A., 2016. Sorption and desorption of cadmium and zinc in two tropical soils amended with sugarcane-straw-derived biochar. J. Soils Sediments. 16, 226–234. doi:10.1007/s11368-015-1199-y

Mench, M., Coussin, F., Mocquot, B., Bussière, S., Prunet, T., Lagriffoul, A., 1996. Evaluation des apports d'un biotest végétal et de la spéciation des éléments traces à la mise au point d'une batterie de tests d'écotoxicologie terrestre applicables aux sols et aux déchets. Final report no. 4930029, INRA, Unité d'Agronomie, Bordeaux—ADEME, Recherche, Milieux et Impact, Paris, France.

Mench, M., Bes, C., 2009. Assessment of ecotoxicity of topsoils from a wood treatment site. Pedosphere. 19(2), 143–155. doi:10.1016/S1002-0160(09)60104-1

Mench, M., Chartier, S., Girardi, S., Solda, P., Van Oort, F., Baize, D., 2009. Exposition de végétaux aux éléments-traces, évaluation et gestion des risques pour la sûreté des aliments d'origine végétale – Exemple de la zone agricole de Mortagne-du-Nord. In Contaminations métalliques des agrosystèmes et ecosystème péri-industriels. Cambier, P., Schvartz, C., van Oort ; F., (eds.), Collection Update Sciences & Technologies, Editions Quae, Versailles, France. ISBN 978-2-7592-0275-1. p. 85–116.

Mench. M., Lepp, N., Bert, V., Schwitzguébel, J.P., Gawronski, S.W., Schröder, P., Vangronsveld, J., 2010. Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J. Soils Sediments. 16, 876–900. doi:10.1007/s11368-010-0190-x

Méndez, A., Gómez, A., Paz-Ferreiro, J., Gascó, G., 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere 89, 1354–9. doi:10.1016/j.chemosphere.2012.05.092

Merino, C., Nannipieri, P., Matus, F., 2015. Soil carbon controlled by plant, microorganism and mineralogy interactions. J. Soil Sci. Plant Nutr. 15(2), 321–332.

Mirza, N., Mahmood, Q., Pervez, A., Ahmad, R., Farooq, R., Shah, M.M., Azim, M.R., 2010. Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater, Biores. Technol. 101, 5815–5819. doi:10.1016/j.biortech.2010.03.012

Mirza, N., Pervez, A., Mahmood, Q., Shah, M.M., Shafqat, M.N., 2011. Ecological restoration of arsenic contaminated soil by Arundo donax L, Ecol. Eng. 37, 1949–1956. doi:10.1016/j.ecoleng.2011.07.006

Mizuta, K., Matsumoto, T., Hatate, Y., Nishihara, K., Nakanishi, T., 2004. Removal of nitrate-nitrogen from drinking water using bamboo powder charcoal. Bioresour. Technol. 95, 255–257.

Mocquot, B., Vangronsveld, J., Clijsters, H., Mench, M., 1996. Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil. 182, 287–300.

Mohamed, I., Zhang, G., Li, Z., Liu, Y., Chen, F., Dai, K., 2015. Ecological restoration of an acidic Cd contaminated soil using bamboo biochar application. Ecol. Eng. 84, 67–76. doi:10.1016/j.ecoleng.2015.07.009

Mohd Idris, S., Jones, P.J., Salzman, S.A., Croatto, G., Allinson, G., 2012. Evaluation of the giant reed (*Arundo donax*) in horizontal subsurface flow wetlands for the treatment of recirculating aquaculture system effluent. Environ. Sci. Pollut. Res. 19, 1159–1170. doi:10.1007/s11356-011-0642-x

Mola-Yudego, B., Díaz-Yáñez, O., Dimitriou, I., 2015. How much yield should we expect from fast-growing plantations for energy? Divergences between experiments and commercial willow plantations. Bioenerg. Res. 8(4). doi:10.1007/s12155-015-9630-1

Monferrán, M.V., Agudo, J.A.S, Pignata, M.L., Wunderlin, D.A., 2009. Copper-induced response of physiological parameters and antioxidant enzymes in the aquatic macrophyte Potamogeton pusillus. Environ. Pollut. 157, 2570–2576. doi:10.1016/j.envpol.2009.02.034

Monti, A., Zegada-Lizarazu, W., 2016. Sixteen-year biomass yield and soil carbon storage of giant reed (*Arundo donax* L.) grown under variable nitrogen fertilization rates. Bioenerg. Res. 9, 248–256. doi:10.1007/s12155-015-9685-z

Moreno-Jiménez, E., Beesley, L., Lepp, N.W., Dickinson, N.M., Hartley, W., Clemente, R., 2011. Field sampling of soil pore water to evaluate trace element mobility and associated environmental risk, Environ. Pollut. 159, 3078–3085. doi: 10.1016/j.envpol.2011.04.004

Moreno-Jiméne, E., Fernández, J.M., Puschenreiter, M., Williams, P.N., Plaza, C., 2016. Availability and transfer to grain of As, Cd, Cu, Ni, Pb and Zn in a barley agri-system: Impact of biochar, organic and mineral fertilizers. Agriculture, Ecosystems and Environment. 219, 171–178. doi:10.1016/j.agee.2015.12.001.

Mulligan, C., Yong, R., Gibbs, B., 2001. Remediation technologies for metal contaminated soils and groundwater: an evaluation. Eng. Geol. 60(1), 193–207.

Mungur, A.S., Shutes. R.B.E., Revitt, D.M., House, M.A., 1997. An assessment of metal removal by a laboratory scale wetland. Water Sci. Technol. 35, 125–133. doi:10.1016/S0273-1223(97)00061-9

Nagodavithane, C.L., Singh, B., Fang, Y., 2014. Effect of ageing on surface charge characteristics and adsorption behaviour of cadmium and arsenate in two contrasting soils amended with biochar. Soil Research. 52, 155–163. doi:10.1071/SR13187

Namgay, T., Singh, B., Singh, B.P., 2010. Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize *Zea mays* L. Aust. J. Soil Res. 48, 638–647. doi:10.1071/SR10049

Negim, O., Mench, M., Bes, C., Motelica-Heino, M., Amin, F., Huneau, F., Le Coustumer, P., 2012. In situ stabilization of trace metals in a copper contaminated soil using P-spiked Linz-Donawitz slag. Environ. Sci. Pollut. Res. 19, 847–857.

Newete, S.W., Byrne, M., 2016. The capacity of aquatic macrophytes for phytoremediation and their disposal with specific reference to water hyacinth. Environ. Sci. Pollut. Res. 23, 10630–10643. doi:10.1007/s11356-016-6329-6

Nguyen, B.T., Lehmann, J., 2009. Black carbon decomposition under varying water regimes. Org. Geochem. 40, 846–853. doi:10.1016/j.orggeochem.2009.05.004

Nivala, J., Knowles, P., Dotro, G., Garcia, J., Wallace, S., 2012. Clogging in subsurface flow treatment wetlands: measurement, modeling and management. Water Res. 46 (6), 1625–1640.

Noctor, G., Lelarge, C., Mhamdi, A., 2015. The metabolomics of oxidative stress. Phytochemistry. 112, 33–53. doi:10.1016/j.phytochem.2014.09.002

Novak, J., Busscher, W., Laird, D., Ahmedna, M., Watts, D., Niandou, M., 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Sci. 174, 105–120.

Nsanganwimana, F., Marchand, L., Douay, F., Mench, M., 2014a. *Arundo donax* L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock, A review. Int. J. Phytorem. 16, 982–1017. doi:10.1080/15226514.2013.810580

Nsanganwimana, F., Pourrut, B., Mench, M., Douay, F., 2014b. Suitability of *Miscanthus* species for managing inorganic and organic contaminated land and restoring ecosystem services. A review. J. Environ. Manage. 143, 123–134. doi:10.1016/j.jenvman.2014.04.027

Nsanganwimana, F., Waterlot, C., Louvel, B., Pourrut, B., Douay, F., 2016. Metal, nutrient and biomass accumulation during the growing cycle of *Miscanthus* established on metal-contaminated soils. J. Plant Nutr. Soil Sci. 179, 257–269. doi:10.1002/jpln.201500163

Oleszczuk, P., Josko, I., Kusmierz, M., 2013. Biochar properties regarding to contaminants content and ecotoxicological assessment. J. Hazard. Mater. 260, 375–82. doi:10.1016/j.jhazmat.2013.05.044

Otte, M.L., Matthews, D.J., Jacob, D.L., Moran, B.M., Baker, A.J.M., 2004. Biogeochemistry of metals in the rhizosphere of wetland plants - an explanation for “innate” metal tolerance? Wetl. Ecosyst. Asia: Funct. Manag. 1, 87–94. doi:10.1016/B978-044451691-6/50009-0

Oustriere, N., Marchand, L., Galland, W., Gabon, L., Lottier, N., Motelica, M., Mench, M., 2016a. Influence of biochars, compost and iron grit, singly and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Sci. Total Environ. 566–567, 816–825. doi:10.1016/j.scitotenv.2016.05.091

Oustriere, N. Marchand, L., Bouchardon, J.L., Faure, O., Moutte, J., Mench, M., 2016b. Aided phytostabilization of a trace element-contaminated technosol developed on steel mill wastes. J. Hazard. Mater. 320, 458–468. doi:10.1016/j.jhazmat.2016.08.048

Oustrière, N., Marchand, L., Lottier, N., Motelica, M., Mench, M., 2017. Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site. Sci. Total Environ. 579, 620–627. doi: 10.1016/j.scitotenv.2016.11.048

Ouzounidou, G., Ciamporova, M., Moustakas, M. 1995. Responses of maize (*Zea mays* L.) plants to copper stress – I. Growth, mineral content and ultrastructure of roots. Environ. Exp. Bot. 35, 167–176.

Ozgur, R., Uzilday, B., Sekmen, A.H., Turkan, I., 2013. Reactive oxygen species regulation and antioxidant defence in halophytes. Funct. Plant Biol. 40, 832–847. doi:10.1071/FP12389

Page, V., Feller, U., 2015. Heavy metals in crop plants: Transport and redistribution processes on the whole plant level. Agronomy. 5, 447–463. doi:10.3390/agronomy5030447

Palmer, C.M., Guerinot, M.L., 2009. Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat. Chem. Biol. 5, 333–340. doi:10.1038/nchembio.166

Palmer, I.E., Gehl, R.J., Ranney, T.G., Touchell, D., George, N., 2014. Biomass yield, nitrogen response, and nutrient uptake of perennial bioenergy grasses in North Carolina. Biomass Bioenerg. 63, 218–228. doi:10.1016/j. biombioe.2014.02.016

Pandey, V.C., Bajpai, O., Singh, N., 2016. Energy crops in sustainable phytoremediation. Renew. Sust. Energy. Reviews. 54, 58–73. doi:10.1016/j.rser.2015.09.078

Papazoglou, E.G., Karantounias, G.A., Vemmos, S.N., Bouranis, D.L., 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. Environ. Int. 31, 243–249. doi:10.1016/j.envint.2004.09.022

Pandey, A. K., Pandey, S. D., Misr,a V., 2000. Stability constants of metal-humic complexes and its role in environmental detoxification. Ecotoxicol. Environ. Saf. 47, 195–200. doi:10.1006/eesa.2000.1947

Pandey, V.C., Singh, K., Singh, J.S., Kumar, A., Singh, B., Singh, R.P., 2012. *Jatropha curcas*: A potential biofuel plant for sustainable environmental development. Renew. Sust. Energ. Rev. 16, 2870–2883. doi:10.1016/j.rser.2012.02.004

Pandey, V.C., Bajpai, O., Singh, N., 2016. Energy crops in sustainable phytoremediation. Renew. Sust. Energ. Rev. 54, 58–73. doi:10.1016/j.rser.2015.09.078

Park,.H.J., Choppala, G.K., Bolan, N.S., Chung, J.W., Chuasavathi T., 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348, 439–451. doi:10.1007/s11104-011-0948-y

Park, H.J., Oh, S.W., Wen, M.Y., 2012. Manufacture and properties of *Miscanthus* wood particle composite boards. J. Wood. Sci. 58, 459–64.

Park, H.J., Choppala, G., Lee, S.J., Bolan, N., Chung, J.W., Edraki, M., 2013. Comparative sorption of Pb and Cd by biochars and its implication for metal immobilization in soils. Water Air Soil Pollut. 224, 1711. doi:10.1007/s11270-013-1711-1

Pedescoll, A., Sidrach-Cardona, R., Hijosa-Valsero, M., Bécares, E., 2015. Design parameters affecting metals removal in horizontal constructed wetlands for domestic wastewater treatment, Ecol. Eng. 80, 92–99. doi:10.1016/j.ecoleng.2014.10.035

Peñarrubia, L., Andrés-Colás, N., Moreno, J., Sergi, P., 2010. Regulation of copper transport in Arabidopsis thaliana: a biochemical oscillator? J. Biol. Inorg. Chem. 15(29). doi:10.1007/s00775-009-0591-8

Pidlisnyuk, V., Stefanovska, T., Lewis, E.E., Erickson, L.E., Davis, L.C., 2014. *Miscanthus* as a productive biofuel crop for phytoremediation. Crit. Rev. Plant. Sci. 33 (1), 1–19.

Pignatello, J.J., Kwon, S., Lu, Y,. 2006. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): attenuation of surface activity by humic and fulvic acids. Environ. Sci. Technol. 40, 7757–7763. doi:10.1021/es061307m

Pilon-Smits, E.A.H., De Souza, M.P., Lytle, C.M., Shang, C., Lugo, T., Terry, N., 1998. Selenium volatilization and assimilation by hybrid poplar (*Populus tremula alba*). J. Exp. Bot. 49, 1889–1892.

Pilu, R., Bucci, A., Badone, FC., Landoni, M., 2012. Giant reed (*Arundo donax* L.): A weed plant or a promising energy plant? Afr. J. Biotechnol. 11, 9163–9174. doi:10.5897/AJB11.4182

Powell, K.J. Brown, P.L., Byrne, R.H., Gajda, T., Hefter, G., Sjöberg, S., Wanner, H., 2007. Chemical speciation of environmentally significant metals with inorganic ligands part 2: the Cu^{2+}, OH^{-}, Cl^{-}, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-} systems. Pure Appl. Chem. 79(5), 895–950. doi:10.1351/pac200779050895

Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M., Strazalka, K., 2001. Physiological responses of *Lemna trisulca* L. (duckweed) to cadmium and copper bioaccumulation. Plant Sci. 161, 881–889. doi:10.1016/S0168-9452(01)00478-2

Puga, A.P, Abreu, C.A, Melo, L.C.A., Beesley, L., 2015a. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. J. Environ. Manage. 159, 86–93. doi:10.1016/j.jenvman.2015.05.036

Puga, A.P., Abreu, C.A., Melo, L.C.A., Paz-Ferreiro, J., Beesley, L., 2015b. Cadmium, lead, and zinc mobility and plant uptake in a mine soil amended with sugarcane straw. Environ. Sci. Pollut. Res. 22, 17606–17614. doi:10.1007/s11356-015-4977-6

Puschenreiter, M., Wittstock, F., Friesl-Hanl, W., Wenzenl WW., 2013. Predictability of the Zn and Cd phytoextraction efficiency of a *Salix smithiana* clone by DGT and conventional bioavailability assays. Plant Soil. 369, 531–541.

Puschenreiter, M., Friesl-Hanl, W., Dunst, G., Wagner, M., Zehetner, F., Karer, J., Wawra, A., Fessl, J., Hoefer, C., Soja, G., 2016. Immobilization of metals using biochar: results from a 2-year greenhouse and field experiment. 13th International Phytotechnologies Conference. Sept 24 – 26, Hangzhou, China.

Qasim, B., Motelica-Heino, M., Bourgerie, S., Gauthier, A., Morabito, D., 2016. Rhizosphere effects of *Populus euramericana* Dorskamp on the mobility of Zn, Pb and Cd in contaminated technosols. J. Soils Sediments. 16, 811–820. doi:10.1007/s11368-015-1270-8

Qiu, H., Vijver, M.G., He, E., Peijnenburg, W.J., 2013. Predicting copper toxicity to different earthworm species using a multicomponent Freundlich model. Environ. Sci. Technol. 47, 4796−4803. doi:10.1021/es305240n.

Qiu, S., Huang, S., 2008. Study on growth and Cd accumulation of root system of *Iris pseudacorus* seedling under Cd stress. J. Plant Res. Environ. 17, 33–38.

Qualité du Milieu Marin Littoral Bulletin de la surveillance 2013, 2014. Institut Français de Recherche pour l'Exploitation de la Mer, Available online at: http://envlit.ifremer.fr/content/download/81930/580311/version/2/file/bull_ar_2014.pdf

Québec. 2016. Land Protection and Rehabilitation Regulation. Environment Quality Act. Gouvernement du Québec. Available online at: http://www2.publicationsduquebec.gouv.qc.ca/dynamicSearch/telecharge.php?type=3&file=/Q_2/Q2R37_A.htm

Rabitsch, W.B., 1995. Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. I. Environ. Pollut. 90, 221–237.

Rajapaksha, A.U., Ahmad, M., Vithanage, M., Kim, K.R., Chang, J.Y., Lee, S.S., Ok, Y.S., 2015. The role of biochar, natural iron oxides, and nanomaterials as soil amendments for immobilizing metals in shooting range soil. Environ. Geochem. Health. 37, 931–942. doi:10.1007/s10653-015-9694-z.

Ravet, K., Pilon, M., 2013. Copper and iron homeostasis in plants: the challenges of oxidative stress. Antioxid. Redox. Signal. 19 (9), 919-932. doi:10.1089/ars.2012.5084.

Rawat, N., Neelam, K., Tiwari, V.K., Dhaliwa, H.S., 2013. Biofortification of cereals to overcome hidden hunger. Plant Breeding. 132, 437–445. doi:10.1111/pbr.12040

Reddy, K.R., DeLaune, R.D., 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press, Boca Raton, Florida, USA, pp. 774

Reed, M.L.E., Glick, B.R., 2005. Growth of canola (*Brassica napus*) in the presence of plant growth promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Canadian Journal of Microbiology. 51, 1061–1069.

Rees, F., Simonnot, M.O., Morel, J.L., 2014. Short-term effects of biochar on soil heavy metal mobility are controlled by intra-particle diffusion and soil pH increase. Eur. J. Soil Sci. 65, 149–161.

Reeves, R.D., Baker, A.J.M., 2000. Metal-accumulating plants. In: Raskin, I., Ensleyn B.D., editors. Phytoremdiation of toxic metals: using plants to clean up the environment, New York: John Wiley & Sons. pp. 193–229.

Riedel, T., Hennessy, P., Iden, S.C., Koschinsky, A., 2015. Leaching of soil-derived major and trace elements in an arable topsoil after the addition of biochar. Eur. J. Soil Sci. 66, 823–834. doi: 10.1111/ejss.12256

Riffaldi, R., Saviozzi, A., Cardelli, R., Bulleri, F., Angelini, L., 2010. Comparison of soil organic-matter characteristics under the energy crop giant reed, cropping sequence and natural grass. Commun. Soil Sci. Plan. 41, 173–180. doi:10.1080/00103620903426972

Rizwan, M., Ali, S., Farooq Qayyum, M., Ibrahim, M., Zia-ur-Rehman, M., Abbas, T., Ok, Y.S., 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environ. Sci. Pollut. Res. 23, 2230–2248. doi:10.1007/s11356-015-5697-7

Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H., Gregg, P.E.H., 1997. The potential of the high biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. J.Geochem. Explor. 60, 115–128.

Robinson, B.H., Mills, T.M., Petit, D., Fung, L.E., Green, S.R., Clothier, B.E., 2000. Natural and induced cadmium-accumulation in poplar and willow: implications for phytoremediation. Plant Soil. 227, 301–306.

Robinson, B.H., Banuelos, G., Conesa, H.M., Evangelou, M.W.H., Schulin, R., 2009. The phytomanagement of trace elements in soil. Crit. Rev. Plant Sci. 28, 240–266.

Robinson, B.H., Anderson, C.W.N., Dickinson, N.M., 2015. Phytoextraction: Where’s the action? J.Geochem. Explor. 151, 34–40. doi:10.1016/j.gexplo.2015.01.001

Rocha, A.C.S., Almeida, C.M.R., Basto, M.C.P., Vasconcelos, M.T.S.D., 2014. Antioxidant response of *Phragmites australis* to Cu and Cd contamination. Ecotox. Environ. Safe. 109, 152–160. doi:10.1016/j.ecoenv.2014.06.027

Rocha, G.S., Lombardi, A.T., Melão, M.G.G., 2016. Influence of phosphorus on copper toxicity to *Selenastrum gracile* (Reinsch) Korshikov. Ecotox. Environ. Safe. 128, 30–35. doi:10.1016/j.ecoenv.2016.02.007

Rockwood, D.L., Carter, D.R., Langholtza, M.H., Strickerb, J.A., 2006. Eucalyptus and Populus short rotation woody crops for phosphate mined lands in Florida USA. Biomass Bioenerg. 30, 728–734. doi:10.1016/j.biombioe.2005.08.007

Rodríguez-Vila, A., Covelo, E.F., Forján, R., Asensio, V., 2014. Phytoremediating a copper mine soil with Brassica juncea L., compost and biochar. Environ. Sci. Pollut. Res. 21, 11293–11304. doi:10.1007/s11356- 014-2993–6

Rodríguez-Vila, A., Asensio, V., Forján, R., Covelo, E.F., 2015. Chemical fractionation of Cu, Ni, Pb and Zn in a mine soil amended with compost and biochar and vegetated with *Brassica juncea* L. J. Geochem. Explor. 158, 74–81. doi:10.1016/j.gexplo.2015.07.005

Rogovska, N., Laird, D., Cruse, R.M., Trabue, S., Heaton, E., 2012. Germination tests for assessing biochar quality. J. Environ. Qual. 41, 1014–1022. doi:10.2134/jeq2011.0103.

Rondon, M.A., Lehmann, J., Ramírez, J., Hurtado, M., 2007. Biological nitrogenfixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol. Fertil. Soils. 43, 699–708.

Rossini Oliva, S., Mingorance, M.D., Leidi, E.O., 2011. Effects of silicon on copper toxicity in *Erica andevalensis* Cabezudo and Rivera: a potential species to remediate contaminated soils. J. Environ. Monitor. 13, 591–596. doi:10.1039/c0em00549e

Rouached, H., 2013. Recent developments in plant zinc homeostasis and the path toward improved biofortification and phytoremediation programs. Plant Signal. Behav. 8(22681).

Rowe, R.L., Street, N.R., Taylor, G., 2009. Identifying potential environmental impacts of large-scale deployment of dedicated bioenergy crops in the UK. Renew. Sust. Energ. Rev.13, 271–290.

Rozada, F., Otero,. M., Morán,. A., García, A.I., 2008. Adsorption of heavy metals onto sewage sludge-derived materials. Bioresour. Technol. 99, 6332–6338. doi:10.1016/j.biortech.2007.12.015

Ruttens, A., Mench, M., Colpaert, J.V., Boisson, J., Carleer, R., Vangronsveld, J., 2006. Phytostabilization of a metal contaminated sandy soil. I: Influence of compost and/or inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of metals. Environ. Pollut. 144, 524–532. doi:10.1016/j.envpol.2006.01.021

Rycewicz-Borecki, M., McLean, J.E., Dupont, R.R., 2016. Bioaccumulation of copper, lead, and zinc in six macrophyte species grown in simulated stormwater bioretention systems. J. Environ. Manage. 166, 267–275. doi:10.1016/j.jenvman.2015.10.019

Saadani, O., Fatnassi, I.C., Chiboub, M., Abdelkrim, S., Barhoumi, F., Jebara, M., Jebara, S.H., 2016. In situ phytostabilisation capacity of three legumes and their associated Plant Growth Promoting Bacteria (PGPBs) in mine tailings of northern Tunisia. Ecotox. Environ. Safe. 130, 263–269. doi:10.1016/j.ecoenv.2016.04.032

Sablok, G., Fu, Y., Bobbio, V., Laura, M., Rotino, G.L., Bagnaresi, P., Allavena, A., Velikova, V., Viola, R., Loreto, F., Li, M., Varotto, C., 2014. Fuelling genetic and metabolic exploration of C3 bioenergy crops through the first reference transcriptome of *Arundo donax* L. Plant Biotech. J. 12, 554–567. doi:10.1111/pbi.12159

Saby, N.P.A., Marchant, B.P., Lark, R.M., Jolivet, C.C., Arrouays, D., 2011. Robust geostatistical prediction of trace elements across France. Geoderma, 162(3), 303–311.

Salpeteur, I., Angel, J.M., 2010. Valeurs de références pour les teneurs en éléments traces dans les eaux de rivières et les sédiments, obtenues en France dans le cadre du nouvel atlas géochimique Européen (FOREGS). Environ Risque et Santé. 9, 121–35.

Santibanez, C., Verdugo, C., Ginocchio, R., 008 .Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Sci. Total Environ. 395, 1–10.

Schenkeveld, W.D.C., Oburger, E., Gruber, B., Schindlegger, Y., Hann, S., Puschenreiter, M., Kraemer, S.M., 2014. Metal mobilization from soils by phytosiderophores - experiment and equilibrium modeling. Plant Soil. 383, 59–71.

Schulz, B., Boyle, C., 2006. What are endophytes? In Microbial Root Endophytes. Edited by Schulz, B.J.E., Boyle, C.J.C., Sieber TN. 1-13.

Schulz, H., Dunst, G., Glaser, B., 2013. Positive effects of composted biochar on plant growth and soil fertility. Agron. Sust. Dev. 33, 817–827. doi:10.1007/s13593-013-0150-0

Scordia, D., Cosentino, SL., Lee, J.W., Jeffries, T.W., 2012. Bioconversion of giant reed (*Arundo donax* L.) hemicellulose hydrolysate to ethanol by *Scheffersomyces stipites* CBS6054. Biomass. Bioenerg. 39, 296–305. doi:10.1016/j.biombioe.2012.01.023

Sebastiani, L, Scebba, F., Tognetti, R., 2004. Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoids x maximowiczii*) and I-214 (*P. x euramericana*) exposed to industrial waste. Environ. Exp. Bot. 52, 79–88.

Sekomo, C.B., Rousseau, D.P.L., Saleh, S.A., Lens, P.N.L., 2012. Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. Ecol. Eng. 44, 102–110. doi:10.1016/j.ecoleng.2012.03.003

Selling, R., Håkansson, T., Björnsson, L., 2008. wo-stage anaerobic digestion enables heavy metal removal. Water Sci Technol. 57(4), 553–558. doi:10.2166/wst.2008.054

SEQ EAU, 2003. Agence de l'eau Rhône Méditerranée Corse, Available online at: http://sierm.eaurmc.fr/eaux-superficielles/fichiers-telechargeables/grilles-seqeau-v2.pdf (In French).

Shaheen, S., Mahmood, Q., Pervez, A., Mirza, N., Bhatti, Z.A., Hayat, T., Meryem, S.S., Ullah, F., 2016. Chromium uptake by giant reed under rhizobacterial inhibition. Int. J. Environ. Sci. Technol. 13(6), 1581–1590. doi:10.1007/s13762-016-0996-1

Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Braz. J. Plant Physiol. 17(1), 35–52.

Shen, Z., Md Som, A., Wang, F., Jin, F., McMillan, O., Al-Tabbaa, A., 2016. Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. Sci. Total Environ. 542, 771–776. doi:10.1016/j.scitotenv.2015.10.057

Sheng, G.P., Yu, H.Q., Li, X.Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv. 28, 882–894. doi:10.1016/j.biotechadv.2010.08.001

Shi, X., Zhang, C., Wang, H., Zhang, F., 2005. Effect of Si on the distribution of Cd in rice seedlings. Plant Soil. 272, 53–60. doi:10.1007/s11104-004-3920-2

Singh, B., Singh, B.P., Cowie, A.L., 2010. Characterisation and evaluation of biochars for their application as a soil amendment. Aust. J. Soil Res. 48, 516–525. doi:10.1071/SR10058

Singh, S., Parihar, P., Singh, R., Singh V.P., Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6(1143). doi:10.3389/fpls.2015.01143

Sizmur, T., Wingate, J., Hutchings, T., Hodson, M.E., 2011. *Lumbricus terrestris* L. does not impact on the remediation efficiency of compost and biochar amendments. Pedobiologia. 54 Suppl., S211 S216. doi:10.1016/j.pedobi.2011.08.008

Smeets, K., Cuypers, A., Lambrechts, A., Semane, B., Hoet, P., Van Laere, A., Vangronsveld, J., 2005. Induction of oxidative stress and antioxidative mechanisms in *Phaseolus vulgaris* after Cd application. Plant Physiol. Biochem. 43(5), 437–444.

Smolders, E., Oorts, K., van Sprang, P., Schoeters, I., Janssen, C.R., McGrath, S.P., McLaughlin, M. J., 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination: using calibrated bioavailability model to set ecological soil standards. Environ. Toxicol. Chem. 28, 1633–1642.

Smolders, E., Oorts, K., Lombi, E., Schoeters, I., Ma, Y., Zrna, S., McLaughlin, M.J., 2012. The availability of copper in soils historically amended with sewage sludge, manure, and compost. J. Environ. Qual. 41, 1–9. doi:10.2134/jeq2011.0317

Soda, S., Hamada, T., Yamaoka, Y., Ikea, M., Nakazato, H., Saeki, Y., Kasamatsu, T., Sakurai, Y., 2012. Constructed wetlands for advanced treatment of wastewater with a complex matrix from a metal processing plant: Bioconcentration and translocation factors of various metals in *Acorus gramineus* and *Cyperus alternifolius*. Ecol. Eng. 39, 63–70. doi:10.1016/j.ecoleng.2011.11.014

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Adv. Agron. 105, 47–82. doi:10.1016/S0065-211310.05002-9

Spokas, K.A., Baker, J.M., Reicosky, D.C., 2010. Ethylene: potential key for biochar amendment impacts. Plant. Soil. 333, 443–452. doi:10.1007/s11104-010-0359-5

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Gupta, D.K., 2006. Copper induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. Aquat. Toxicol. 80, 405–415. doi:10.1016/j.aquatox.2006.10.006

Srujana, K., 2015. Bioenergy from phytoremediated phytomass of aquatic plants via gasification. In: Prasad, M.N.V., (Eds.), Bioremediation and bioeconomy, Amsterdam. pp. 250.

Stals, M., Thijssen, E., Vangronsveld, J., Carleer, R., Schreurs, S., Yperman, J., 2010. Flash pyrolysis of heavy metal contaminated biomass from phytoremediation: influence of temperature, entrained flow and wood/leaves blended pyrolysis on the behaviour of heavy metals. J. Anal. Appl. Pyrolysis. 87, 1–7. doi:10.1016/j.jaap.2009.09.003

Steiner, 2006. Biochar balance carbon and restore soil fertility. Available at: http://www.biochar.org/joomla/index.php?option=com_content&task=view&id=67&Itemid=7&limit=1&limitstart=4 (Verified on October 2nd, 2016)

Stolarski, M.J., Krzyzaniak, M., Łuczynski, M., Ałuski, D., Szczukowski, S., Tworkowski, J., Gołaszewski, J., 2015. Lignocellulosic biomass from short rotation woody crops as a feedstock for second-generation bioethanol production. Ind. Crops Prod. 75, 66–75. doi:10.1016/j.indcrop.2015.04.025

Sun, H., Wang, Z., Gao, P., Liu, P., 2013. Selection of aquatic plants for phytoremediation of heavy metal in electroplate wastewater. Acta. Physiol. Plant. 35, 355–364. doi:10.1007/s11738-012-1078-8

Tang, J., Li, X., Luo, Y., Li G., Khan, S., 2016. Spectroscopic characterization of dissolved organic matter derived from different biochars and their polycyclic aromatic hydrocarbons (PAHs) binding affinity. Chemosphere. 152, 399-406. doi:10.1016/j.chemosphere.2016.03.0160045-6535/

Tanner, C.C., Headley, T.R., 2011. Components of floating emergent macrophyte treatment wetlands influencing removal of stormwater pollutants. Ecol. Eng. 37, 474–486. doi:10.1016/j.ecoleng.2010.12.012

Tavakkoli, E., Rengasamy, P., McDonald, G.K., 2010. High concentrations of Na^+ and $Cl^−$ ions in soil solution have simultaneous detrimental effects on growth of *faba* bean under salinity stress. J. Exp. Bot. 61(15), 4449–4459. doi:10.1093/jxb/erq251

Taylor, A.A., Walker, S.L., 2016. Effects of copper particles on a model septic system's function and microbial community. Water Research. 91, 350–360. doi:10.1016/j.watres.2016.01.01

Thaler, N., Humar, M., 2014. Copper leaching from copper-ethanolamine treated wood: Comparison of field test studies and laboratory standard procedures. BioResources. 9(2), 3038−3051.

Tibbett, M., 2015. Mining in ecologically sensitive landscape, CSIRO publishing, Clayton South.

Tiberg, C., Kumpiene, J., Gustafsson, J.P., Marsz, A., Persson, I., Mench, M., Kleja, D.B., 2016. Immobilization of Cu and As in two contaminated soils with zerovalent iron – Long-term performance and mechanisms. Appl. Geochem. 67, 144–152. doi:10.1016/j.apgeochem.2016.02.009

Tica, D., Udovic, M., Lestan, D., 2011. Immobilization of potentially toxic metals using different soil amendments. Chemosphere. 85, 577–583.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., 2012. Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol. Biochem. 53, 33–39.

Touceda-González, M., Brader, G., Antonielli, L., Balakrishnan Ravindran, V., Waldner, G., Friesl-Hanl, W., Corretto, E., Campisano, A., Pancher, M., Sessitsch, A., 2015. Combined amendment of immobilizers and the plant growth-promoting strain Burkholderia phytofirmans PsJN favours plant growth and reduces heavy metal uptake. Soil Biol. Biochem. 91, 140–150. doi:10.1016/j.soilbio.2015.08.038

Tremel-Schaub, A., Feix, I., 2005. Contamination des Sols - Transferts des Sols vers les Plantes, Agence de l'Environnement et de la Maîtrise de l'Énergie. Angers, and EDP Sciences, Les Ulis.

Tripathi, V., Edrisi, S.A., Abhilash, P.C., 2016. Towards the coupling of phytoremediation with bioenergy production. Renewable and Sustainable Energy Reviews. 57. doi:1386–1389.10.1016/j.rser.2015.12.1161364-0321/

Tsednee, M., Mak, Y.W., Chen, Y.R., Yeh, K.C., 2012. A sensitive LC-EST-Q-TOF-MS method reveals novel phytosiderophores and phytosiderophore–iron complexes in barley. New Phytol. 195, 951–961. doi:10.1111/j.1469-8137.2012.04206.x

Uchimiya, M., Lima, I.M., Klasson, K.T., Wartelle, L.H., 2010a. Contaminant immobilization and release by biochar soil amendment: Roles of natural organic matter. Chemosphere. 80, 935–940. doi:10.1016/j.chemosphere.2010.05.020

Uchimiya, M., Lima, I.M., Klasson, K.T., Chang, S., Wartelle, L.H., Rodgers, J.E., 2010b. Immobilization of heavy metal ions CuII, CdII, NiII, and PbII. by broiler litter-derived biochars in water and soil. J. Agric. Food Chem. 58, 5538–5544. doi:10.1021/jf9044217

Uchimiya, M., Klasson, K.T., Wartelle, L.H., Lima, I.M., 2011. Influence of soil properties on heavy metal sequestration by biochar amendment: 1. Copper sorption isotherms and the release of cations. Chemosphere. 82, 1431–1437.

Uchimiya, M., Bannon, D.I., 2013. Solubility of lead and copper in biochar-amended small arms range soils: Influence of soil organic carbon and pH. J. Agric. Food Chem. 61, 7679−7688. doi:10.1021/jf401481x

Uchimiya, M., Ohno, T., He, Z., 2013. Pyrolysis temperature-dependent release of dissolved organic carbon from plant, manure, and biorefinery wastes. J. Anal. Appl. Pyrol. 104, 84–94. doi:10.101/j.jaap.2013.09.003.

Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustos, P., Zupan, M., Wenzel, W.W., 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environ Pollut. 148, 107–114.

Usman, A.R.A., Lee, S.S., Awad, Y.M., Lim, K.J., Yang, J.E., Ok, Y.S., 2012. Soil pollution assessment and identification of hyperaccumulating plants in chromated copper arsenate (CCA) contaminated sites, Korea. Chemosphere. 87, 872–878. doi:10.1016/j.chemosphere.2012.01.028

Vaculík, M., Landberg, T., Greger, M., Luxová, M., Stoláriková, M., Lux, A., 2012. Silicon modifies root anatomy, and uptake and subcellular distribution of cadmium in young maize plants, Ann. Bot, 110, 433–43. doi:10.1093/aob/mcs039

Valipour, A., Raman, V.K., Ghole, V.S., 2009. A new approach in wetland systems for domestic wastewater treatment using *Phragmites sp*. Ecol. Eng. 35, 1797–1803. doi:10.1016/j.ecoleng.2009.08.004

Valipour, A., Hamnabard, N., Woo, K.S., Ahn, Y.H., 2014. Performance of high-rate constructed phytoremediation process with attached growth for domestic wastewater treatment: effect of high TDS and Cu. J. Environ. Manage. 145, 1–8. doi:10.1016/j.jenvman.2014.06.009

Van der Ent, A., Baker, A.J.M, Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362, 319–334. doi:10.1007/s11104-012-1287-3

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., Thewys, T., Vassilev, A., Meers, E., Nehnevajova, E., Van der Lelie, D., Mench, M., 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ. Sci. Pollut. Res. 16, 765–794. doi:10.1007/s11356-009-0213-6

Van Slycken, S., Witters, N., Meiresonne, L., Meers, E., Ruttens, A., VanPeteghem, P., Weyens, N., Tack, F.M.G., Vangronsveld, J., 2013. Field evaluation of willow under short rotation coppice for phytomanagement of metal-polluted agricultural soils. Int. J. Phytorem. 15, 677–689.

Vigil, M., Marey-Pérez, M.F., Martinez Huerta, G., Álvarez Cabal, V., 2015. Is phytoremediation without biomass valorization sustainable?—Comparative LCA of landfilling vs. anaerobic co-digestion. Sci. Total. Environ. 505, 844–850. doi:10.1016/j.scitotenv.2014.10.047

Villanneau, E., Perry-Giraud, C., Saby, N., Jolivet, C., Marot, F., Maton, D., Floch-Barneaud, A., Antoni, V., Arrouays, D., 2008. Détection de valeurs anomaliques d'éléments traces métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. Etude et Gestion des Sols. 15(3), 183–200.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A.G., Pigna, M., 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. J. Soil Sci. Plant. Nutr. 10(3), 268–292.

Vymazal, J., Švehla, J., Kröpfelová, L., Chrastný, V., 2007. Trace metals in *Phragmites australis* and *Phalaris arundinacea* growing in constructed and natural wetlands. Sci. Total. Environ. 380, 154–162. doi:10.1016/j.scitotenv.2007.01.057

Vymazal, J., Kröpfelová, L., Švehla, J., Chrastný, V., Štíchovíá, J., 2009. Trace elements in *Phragmites australis* growing in constructed wetlands for treatment of municipal wastewater. Ecol. Eng. 35, 303–309. doi:10.1016/j.ecoleng.2008.04.007

Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia. 674, 133–156. doi:10.1007/s10750-011-0738-9

Vymazal, J., 2016. Concentration is not enough to evaluate accumulation of heavy metals and nutrients in plants. Sci. Total. Environ. 544, 495–498. doi:10.1016/j.scitotenv.2015.12.011

Wagner, A., Kaupenjhann, M., 2014. Suitability of biochars pyro- and hydrochars. for metal immobilization on former sewage-field soils. Eur. J. Soil Sci. 65, 139–148. doi:10.1111/ejss.12090

Wagner, A., Kaupenjhann, M., 2015. Biochar addition enhanced growth of *Dactylis glomerata* L. and immobilized Zn and Cd but mobilized Cu and Pb on a former sewage field soil. Eur. J. Soil Sci. 66, 505–515. doi: 10.1111/ejss.12246

Wanat, N., Austruy, A., Joussein, E., Soubrand, M., Hitmi, A., Gauthier-Moussard, C., Lenain, J.F., Vernay, P., Munch, J.C., Pichon, M., 2013. Potentials of *Miscanthus x giganteus* grown on highly contaminated technosols. J. Geochem. Explor. 126–127, 78–84.

Wang, S., Xiang Shi, X., Sun, H., Chen, Y., Pan, H., Yang, X., Rafiq, T., 2004. Variations in metal tolerance and accumulation in three hydroponically cultivated varieties of *Salix integra* treated with lead. 9(9). doi:10.1371/journal.pone.0108568

Wang, X., Chen, X., Liu, S., Ge, X., 2010. Effect of molecular weight of dissolved organic matter on toxicity and bioavailability of copper to lettuce. J. Environ.Sci. 22(12), 1960–1965. doi:10.1016/S1001-0742(09)60346-6

Weyens, N., van der Lelie, D., Taghavi, S., Vangronsveld, J., 2009. Phytoremediation: plant-endophyte partnerships take the challenge. Curr. Opin. Biotech. 20, 248–254. doi: 10.1016/j.copbio.2009.02.012

Wieshammer, G., Unterbrunner, R., Garcia, T.B., Zivkovic, M.F., Puschenreiter, M., Wenzel, W.W., 2007. Phytoextraction of Cd and Zn from agricultural soils by *Salix sp* and intercropping of *Salix caprea* and *Arabidopsis halleri*. Plant Soil. 298, 255–264.

Witters, N., Mendelsohn, R.O., Slycken, S.V., Weyens, N., Schreurs, E., Meers, E., Tack, F., Carleer, R., Vangronsveld, J., 2012. Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass. Bioenerg. 39, 454–469. doi:10.1016/j.biombioe.2011.08.016

White, P.J., Broadley, M.R., 2009. Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182, 49–84.

Wu, S., Wallace, S., Brix, H., Kuschk, P., Kirui, W.K., Masi, F., Dong, R., 2015a. Treatment of industrial effluents in constructed wetlands: challenges, operational strategies and overall performance. Environ. Pollut. 201, 107–120. doi:10.1016/j.envpol.2015.03.006

Wu, Z., Bañuelos, G.S., Lin, Z.Q., Liu, Y., Yuan, L., Yin, X., 2015b. Biofortification and phytoremediation of selenium in China. Front. Plant Sci. 6(136). doi:0.3389/fpls.2015.00136

Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Int. Sch. Res. Netw. Ecol. 1–20. doi:10.5402/2011/402647

Yadav, A.K., Abbassi, R., Kumar, N., Satya, S., Sreekrishnan, T.R., Mishra, B.K., 2012. The removal of heavy metals in wetland microcosms: Effects of bed depth, plant species, and metal mobility. Chem. Eng. J. 211–212, 501–507. doi:10.1016/j.cej.2012.09.039

Yamasaki, H., Hayashi, M., Fukazawa, M., Kobayashi, Y., Shikanai, T., 2009. SQUAMOSA promoter binding protein-like7 is a central regulator for copper homeostasis in Arabidopsis. Plant Cell. 21, 347–361.

trivalent chromium by hybrid willows. Ecotoxicol. 19, 61–68. doi:10.1007/s10646-009-0386-2

Yu, X.Z., Gu, J.D., 2008. Differences in uptake and translocation of selenite and selenite by the weeping willow and hybrid willow. Environ. Sci. Pollut. Res. 15, 499–508. doi:10.1007/s11356-008-0036-x

Yu, X.Z., Peng, X.Y., Xing, L.Q., 2010. Effect of temperature on phytoextraction of hexavalent and

Xu, X., Cai, X., Zhao, L., Wang, H., Yu H., Gao, B., 2013. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. Environ. Sci. Pollut Res. 201, 358–368. Doi:10.1007/s11356-012-0873-5

Xu, X., Huang, Z., Wang, C., Zhong, L., Tian, Y., Li, D., Zhang, G., Shi, J., 2015. Toxicological effects, mechanisms, and implied toxicity thresholds in the roots of *Vicia faba* L. seedlings grown in copper-contaminated soil. Environ. Sci. Pollut. Res. 22, 13858–13869. doi:10.1007/s11356-015-5073-7

Yang, M., Xiao, X.Y., Miao, X.F., Guo, Z.H., Wang, F.Y., 2012. Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead, Trans. Nonferrous Met. Soc. China. 22, 1462–1469. doi:10.1016/S1003-6326(11)61342-3

Yang, X., Liu, J., McGrouther, K., Ye, Z., Huang, H., Lu, K., Guo, X., He, L., Lin, X., Che, L., Ye, Z., Wang H., 2016. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. Environ. Sci. Pollut. Res. 23(2), 974–984. doi:10.1007/s11356-015-4233-0

Yao, Y., Li, Y.Z., Guo, X.J., Huang, T., Gao, P.P., Zhang, Y.P., Yuan, F. 2016. Changes and characteristics of dissolved organic matter in a constructed wetland system using fluorescence spectroscopy. Environ. Sci. Pollut. Res. 23, 12237–12245. doi:10.1007/s11356-016-6435-5

Ye, Z.H., Baker, A.J.M., Wong, M.H., Willis, A.J., 2003. Copper tolerance, uptake and accumulation by Phragmites australis. Chemosphere. 50, 795–800. doi:10.1016/S0045-6535(02)00221-7

Yruela, I., 2009. Copper in plants: acquisition, transport and interactions. Funct. Plant Biol. 36, 409–430.

Yuan, J., Li, S.S., Yu, L., Liu, Y.M., Cao, Y., He, H.Y., Fan, K.N., 2013. Copper-based catalysts for the efficient conversion of carbohydrate biomass into valerolactone in the absence of externally added hydrogen. Energy Environ. Sci. 6, 3308–3313. doi:10.1039/c3ee40857d

Zamora, D.S., Wyatt, G.J., Apostol, K.G., Tschirner, U., 2013. Biomass yield, energy values, and chemical composition of hybrid poplars in short rotation woody crop production and native perennial grasses in Minnesota, USA. Biomass Bioenerg. 49, 222–230.

Zargar, S.M., Nazir, M., Agrawal, G.K., Kim, D.W., Rakwal, R., 2010. Silicon in plant tolerance against environmental stressors: towards crop improvement using omics approaches. Curr. Prot. 7, 135–43. doi:10.2174/157016410791330507

Zhang, D., Luo, J., Ping Lee, Z.M., Gersberg, R.M., Liu, Y., Tan, S.K., Jern Ng, W., 2016. Characterization of microbial communities in wetland mesocosms receiving caffeine-enriched wastewater. Environ. Sci. Pollut. Res. 23, 14526–14539. doi:10.1007/s11356-016-6586-4

Zhang, H., Yang, J., Wang, W., Li, D., Hu, X., Wang, H., Wei, M., Liu, Q., Wang, Z., Li, C., 2015. Genome-wide identification and expression profiling of the copper transporter gene family in *Populus trichocarpa*. Plant Physiol. Biochem. 97, 451–460. doi:10.1016/j.plaphy.2015.10.035

Zhang, S., Li, T., Zhang, X., Yu, H., Zheng, Z., Wang, Y., Hao, X., Pu, Y., 2014. Changes in pH, dissolved organic matter and Cd species in the rhizosphere soils of Cd phytostabilizer *Athyrium wardii* (Hook.) Makino involved in Cd tolerance and accumulation. Environ. Sci. Pollut. Res. 21, 4605–4613. doi:10.1007/s11356-013-2421-3

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., Bolan, N.S., Pei. J., Huang, H., 2013. Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ. Sci. Pollut. Res. 20, 8472–8483. doi:10.1007/s11356-013-1659-0

Zhang, X.B., Liu, P., Yang, Y.S., Chen W.R., 2007. Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes. J. Environ. Sci. 19, 902–909.

Zhou, Y.Q., Huang, S.Z., Yu, S.L., Gu, J.G., Zhao, J.Z., Han, Y.L., Fu, J.J., 2010. The physiological response and sub-cellular localization of lead and cadmium in *Iris pseudacorus* L. Ecotoxicol. 19, 69–76.

Annexes

Publications

Ecological Engineering 64 (2014) 291–300

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*

Lilian Marchand[a,*], Florien Nsanganwimana[a,b], Nadège Oustrière[a], Zhanna Grebenshchykova[a], Katherine Lizama-Allende[c], Michel Mench[a]

[a] UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bât. B2, Avenue des Facultés, 33405 Talence, France
[b] Equipe Sols et Environnement, Laboratoire Génie Civil et géo-Environnement, Lille Nord de France, EA 4515, Groupe ISA, 48 boulevard Vauban, 59046 Lille cedex, France
[c] Departamento de Ingeniería Civil,Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Avda. Blanco Encalada 2002, 8370449 Santiago, Chile

ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 6 November 2013
Accepted 19 December 2013
Available online 28 January 2014

Keywords:
Biofilm
Constructed wetland
Decontamination
Macrophyte
Phytoremediation

ABSTRACT

A bio-rack system was developed for treating Cu-contaminated freshwaters. Each pilot constructed wetland (CW, 110 dm^3) contained 15 perforated vertical pipes filled with a mixture of gravel (diorite; 80%) and perlite (20%) and assembled as a rack. The whole experimental device consisted of 12 CW planted either with *Phragmites australis*, *Phalaris arundinacea* or *Juncus articulatus*, and unplanted as control (in triplicates). All plants were sampled at a Cu-contaminated site. The CWs were filled with a mix of freshwater (30%) from the Jalle d'Eysines River (Bordeaux, France) and tap water (70%). Water was spiked with Cu (2.5 μM, 158.5 $\mu g L^{-1}$). Three CW batches were carried out, i.e. in early spring (March, S#1), beginning of the growing season (May, S#2), and peak growing season (June, S#3). The S#3 water was initially acidified to pH 6. For all batches, water was recirculated in the CW during 14 days. Physico-chemical parameters (pH, electrical conductivity, redox potential, BOD_5 and Cu^{2+} concentrations) were measured every three days. Water pH of both S#1 and #2 ranged between 7.8 and 8.5 for all treatments during the experiment. Initial and final total Cu concentrations were analysed for all CWs and batches. Relative Treatment Efficiency Index (RTEI) indicated the plant effect compared to the unplanted CW. Free Cu^{2+} removal was <10% for all S#1 treatments (RTEI ranged between 0 and −1) whereas it increased to 77% (RTEI = 0.1) in S#2 for *P. arundinacea*. In acidic conditions (S#3), Cu^{2+} removal was 99% for all treatments (RTEI = 0). For S#1 and S#2, highest total Cu removal occurred in CW planted with *P. arundinacea* (respectively 52% and 68%, RTEI = 0.1 and 0.2). For S#3, total Cu removal peaked up to 90% in the unplanted CW. The RTEI values suggested no beneficial effect of macrophytes on Cu removal at short term. Conversely, the CW planted with *J. articulatus* generally displayed a lower efficiency. The lowest value for total Cu concentration in water after the 14-day period was 13 $\mu g L^{-1}$ in S#3 unplanted and planted with *P. arundinacea*. The role of the biofilm as a key-player of Cu removal in such bio-racks is discussed.

1. Introduction

1.1. Constructed wetlands (CW)

Water quality issues are a major challenge faced by mankind in the 21st Century (Corcoran et al., 2010). Treatment of municipal wastewater streams aims at eliminating nutrients, pathogenic microbes, persistent organic pollutants, xenobiotics derived from the pharmaceutical industry and trace elements (TE) from wastewater streams (Schwartzenbach et al., 2010). In industrialized countries, connection to municipal wastewater treatment plants ranges from 50% to 95%, whereas more than 80% of the municipal wastewaters in low-income countries are discharged without any treatment, polluting rivers, lakes, and coastal sea areas (UNESCO, 2009). As a consequence, pollutants accumulate in aquatic ecosystems in surface waters, groundwater, substrates and plants (Aksoy et al., 2005; Demirezen et al., 2007; Lizama et al., 2011, 2012). In Bordeaux area (France), Cu is one of the major contaminant since soluble formulations of Cu-sulphate and chromated Cu-arsenate (CCA)-type C are used as treatment agents against insects and

* Corresponding author at: UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bât. B2, Avenue des Facultés, 33405 Talence, France. Tel.: +33 05 40 00 31 14; fax: +33 05 40 00 36 57.
E-mail address: marchand.lilian@gmail.com (L. Marchand).

0925-8574/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ecoleng.2013.12.017

Science of the Total Environment 496 (2014) 510–522

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Selecting chemical and ecotoxicological test batteries for risk assessment of trace element-contaminated soils (phyto)managed by gentle remediation options (GRO)

Jurate Kumpiene [a,*], Valérie Bert [b], Ioannis Dimitriou [c], Jan Eriksson [d], Wolfgang Friesl-Hanl [e], Rafal Galazka [f], Rolf Herzig [g], Jolien Janssen [h], Petra Kidd [i], Michel Mench [j], Ingo Müller [k], Silke Neu [k], Nadège Oustriere [j], Markus Puschenreiter [l], Giancarlo Renella [m], Pierre-Hervé Roumier [j], Grzegorz Siebielec [f], Jaco Vangronsveld [h], Nicolas Manier [n]

[a] Luleå University of Technology, Waste Science & Technology, SE-97187 Luleå, Sweden
[b] INERIS, Technologies and Sustainable and Clean Processes, Parc Technologique Alata, BP2, 60550 Verneuil en Halatte, France
[c] Swedish University of Agriculture Sciences, Department of Crop Production Ecology, SE-750 07 Uppsala, Sweden
[d] Swedish University of Agriculture Sciences, Department of Soil and Environment, SE-750 07 Uppsala, Sweden
[e] AIT Austrian Institute of Technology GmbH, Health and Environment Department, 3430 Tulln, Austria
[f] Institute of Soil Science and Plant Cultivation - State Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland
[g] Phytotech Foundation and AGB, Quartiergasse 12, 3013 Bern, Switzerland
[h] Hasselt University, Centre for Environmental Sciences, Agoralaan Building D, B-3590 Diepenbeek, Belgium
[i] Instituto de Investigaciones Agrobiológicas de Galicia (IIAG), Consejo Superior de Investigaciones Científicas (CSIC), Santiago de Compostela 15706, Spain
[j] INRA, UMR1202 BIOGECO, F-33610 Cestas, France and Univ. Bordeaux, BIOGECO, UMR 1202, F-33600 Pessac, France
[k] Saxon State Office for Environment, Agriculture and Geology, Pillnitzer Platz 3, 01326 Dresden Pillnitz, Germany
[l] University of Natural Resources and Life Sciences Vienna — BOKU, Department of Forest and Soil Sciences, 3430 Tulln, Austria
[m] University of Florence, Department of Agrifood Production and Environmental Sciences, P.le delle Cascine 28, I-50144 Florence, Italy
[n] INERIS, Expertise and Assays in Ecotoxicology, Parc Technologique Alata, BP2, 60550 Verneuil en Halatte, France

HIGHLIGHTS

- NH_4NO_3-extractable trace elements correlated best with ecotoxicological responses.
- Dwarf beans and stress enzymes were most responsive to the soil treatments.
- Extractable trace elements decreased more in stabilized than in phytoextracted soils.

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 21 May 2014
Accepted 29 June 2014
Available online xxxx

Editor: Filip M.G. Tack

Keywords:
Ecotoxicity
Metal
Nematode
Plant
Single extraction
Worm avoidance

ABSTRACT

During the past decades a number of field trials with gentle remediation options (GRO) have been established on trace element (TE) contaminated sites throughout Europe. Each research group selects different methods to assess the remediation success making it difficult to compare efficacy between various sites and treatments. This study aimed at selecting a minimum risk assessment battery combining chemical and ecotoxicological assays for assessing and comparing the effectiveness of GRO implemented in seven European case studies. Two test batteries were pre-selected; a chemical one for quantifying TE exposure in untreated soils and GRO-managed soils and a biological one for characterizing soil functionality and ecotoxicity. Soil samples from field studies representing one of the main GROs (phytoextraction in Belgium, Sweden, Germany and Switzerland, aided phytoextraction in France, and aided phytostabilization or in situ stabilization/phytoexclusion in Poland, France and Austria) were collected and assessed using the selected test batteries. The best correlations were obtained between NH_4NO_3-extractable, followed by $NaNO_3$-extractable TE and the ecotoxicological responses. Biometrical parameters and biomarkers of dwarf beans were the most responsive indicators for the soil treatments and changes in soil TE exposures. Plant growth was inhibited at the higher extractable TE concentrations, while plant stress enzyme activities

* Corresponding author. Tel.: +46 920493020; fax: +46 920492818.
E-mail address: jurate.kumpiene@ltu.se (J. Kumpiene).

http://dx.doi.org/10.1016/j.scitotenv.2014.06.130

Ecological Indicators 46 (2014) 425–437

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors

L. Marchand[a,*], F. Nsanganwimana[a], B.J. Cook[b], Y. Vystavna[c,d], F. Huneau[e,f], P. Le Coustumer[c], J.B. Lamy[a], N. Oustrière[a], M. Mench[a]

[a] *Université de Bordeaux, UMR 1202 BIOGECO INRA, Bât. B2, Avenue des facultés, F-33405 Talence, France; INRA UMR 1202 BIOGECO, 69 route d'Arcachon, 33610, Cestas, France*
[b] *Minnesota State University, Department of Biological Sciences, Mankato, MN 56001, USA*
[c] *Université de Bordeaux, EA4592 Géoressources & Environnement, ENSEGID, 1 allée F. Daguin, F-33607 Pessac, France*
[d] *O.M. Beketov National University of Urban Economy at Kharkiv, Department of Environmental Engineering and Management, vul. Revolutsii 12, Kharkiv 61002, Ukraine*
[e] *Université de Corse Pascal Paoli, Faculté des Sciences et Techniques, Laboratoire d'Hydrogéologie, Campus Grimaldi, BP 52, F-20250 Corte, France*
[f] *CNRS, UMR 6134, SPE, F-20250 Corte, France*

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 4 July 2014
Accepted 6 July 2014

Keywords:
Ranunculus acris L.
Phragmites australis (Cav.) Trin. Ex Steud.
Carex riparia Ehrh.
Lythrum salicaria L.
Iris pseudacorus L.
Juncus effusus L.
Phalaris arundinacea L.

ABSTRACT

Biomonitoring complements the physico-chemical analysis of environmental matrices, accounting for the subtle biological changes in organisms affected by exogenous chemicals. Here, the relationships between the concentrations of trace elements (TE) in the soil, soil-pore water and leaves of seven rooted macrophytes (*Ranunculus acris* L., *Phragmites australis* (Cav.) Trin. Ex Steud., *Carex riparia* Ehrh., *Lythrum salicaria* L., *Iris pseudacorus* L., *Juncus effusus* L., and *Phalaris arundinacea* L.) were investigated along an urban river – the Jalle d'Eysines River, France – with increasing TE contamination in riverbank soils, from its source to its confluence with the Garonne River. Copper, Zn, Cd, Cr, Pb, Ni, Mo and As were considered. Macrophytes were sampled in June 2011, at the peak of the growing season. For five species, a canonical correspondence analysis (CCA) was used to assess correlations between foliar TE concentrations and total TE concentrations in the soil. Along the Jalle d'Eysines River, *P. australis* and *P. arundinacea* are relevant biomonitors for soil Mo contamination. *P. australis* and *C. riparia* biomonitor soil Cd contamination, while *R. acris* is a relevant biomonitor of soil Ni contamination. Copper and Mo concentrations in the soil-pore water are monitored by, respectively *P. arundinacea* and *P. australis*.

1. Introduction

The integrity of many wetlands is affected by anthropogenic activities (Schwartzenbach et al., 2010). Consequently some wetlands are major sinks of Trace Elements (TE—here essential and non-essential metal(loid)s with common concentrations in plant shoots below 100 mg kg^{-1} dry weight, DW, Adriano, 2001). Trace elements may accumulate in surface waters, groundwaters, soils, and macrophytes (Marchand et al., 2011; Bonanno, 2011, 2012, 2013; Ladislas et al., 2012; Basile et al., 2012). Wetland soils commonly contain 20–50% of water, and the chemistry of their soil solution can significantly differ from both the soil matrix and the standing water above them (Burbridge et al., 2012). In such conditions, root uptake, translocation to aerial parts, and foliar concentrations of TE depend on plant species, intra-specific variability and growing season but also on abiotic and biotic factors of both the soil matrix and the soil-pore water (Bragato et al., 2006; Baldantoni et al., 2009; Du Laing et al., 2009a,b; Kidd et al., 2009; Teuchies et al., 2013; Juckers and Watmough, 2014). Rooted macrophytes, not floating plants, with dense fibrous root systems, large surface areas, and/or well developed rhizome tissues have a high capacity for metal(loid) accumulation expressed as a mineral mass (Cardwell et al., 2002; Bonanno and Lo Giudice, 2010; Nuñez et al., 2011; Bonanno, 2011, 2012). Root anatomy such as lignin and suberin deposition, sclerenchymatous fibres with thick secondary walls and densely packed cells in the outer layers of cortex commonly confer TE exclusion ability in rooted macrophytes

* Corresponding author. Tel.: +33 540003114.
E-mail addresses: marchand.lilian@gmail.com (L. Marchand), mench@bordeaux.inra.fr (M. Mench).

http://dx.doi.org/10.1016/j.ecolind.2014.07.011
1470-160X/

Environmental Pollution 193 (2014) 205–215

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Root biomass production in populations of six rooted macrophytes in response to Cu exposure: Intra-specific variability versus constitutive-like tolerance

L. Marchand [a, b, *], F. Nsanganwimana [a, b], J.B. Lamy [a, b, c], C. Quintela-Sabaris [d], C. Gonnelli [e], I. Colzi [e], T. Fletcher [f, g], N. Oustrière [a, b], A. Kolbas [a, b, h], P. Kidd [i], F. Bordas [j], P. Newell [k], P. Alvarenga [l], A. Deletic [e], M. Mench [a, b]

[a] INRA, UMR 1202 BIOGECO, 69 route d'Arcachon, FR-33612, Cestas cedex, France
[b] University of Bordeaux 1, UMR 1202 BIOGECO, Bât B2, Avenue des facultés, FR-33405, Talence, France
[c] Ifremer, SG2M, LGPMM, Avenue Mus de Loup, F-17390, La Tremblade, France
[d] Departamento de Biología Vegetal y Ecología, Facultad de Ciencia y Tecnología, Universidad del País Vasco/EHU, 48080, Bilbao, Spain
[e] Dipartimento di Biologia, Laboratorio di Ecologia e Fisiologia Vegetale, Università degli Studi di Firenze, via Micheli 1, IT-50121, Firenze, Italy
[f] Department of Civil Engineering, Monash University, Room 118, Building 60, Clayton Campus, Clayton Victoria, 3168, Melbourne, Australia
[g] Melbourne School of Land & Environment, The University of Melbourne, 500 Yarra Boulevard, Burnley, 3121 and 221 Bouverie St, Parkville, Vic, 3010, Australia
[h] Brest State University named after A.S. Pushkin, 21, Boulevard of Cosmonauts, 224016, Brest, Belarus
[i] Instituto de Investigaciones Agrobiológicas de Galicia, Consejo Superior de Investigaciones Científicas (CSIC), Santiago de Compostela, Spain
[j] GRESE, Université de Limoges, 123 Avenue Albert Thomas, FR-87060, Limoges, France
[k] Department of Environment and Conservation, Contaminated Sites Branch, Locked Bag 104, Bentley, DC, 6983, Australia
[l] Departamento de Tecnologias e Ciências Aplicadas, Escola Superior Agrária - Instituto Politécnico de Beja, Rua Pedro Soares - Campus do IPB, Apartado 6155, PT-7801-295, Beja, Portugal

A R T I C L E I N F O

Article history:
Received 19 November 2013
Received in revised form
18 May 2014
Accepted 1 July 2014
Available online

Keywords:
Copper tolerance
Phytoremediation
Root biomass
Trace element
Wetland

A B S T R A C T

Intra-specific variability of root biomass production (RP) of six rooted macrophytes, i.e. *Juncus effusus*, *Phragmites australis*, *Schoenoplectus lacustris*, *Typha latifolia*, *Phalaris arundinacea*, and *Iris pseudacorus* grown from clones, in response to Cu exposure was investigated. Root biomass production varied widely for all these macrophytes in control conditions (0.08 μM) according to the sampling site. Root biomass production of *T. latifolia* and *I. pseudacorus* in the 2.5–25 μM Cu range depended on the sampling location but not on the Cu dose in the growth medium. For *P. australis*, *J. effusus*, *S. lacustris*, and *P. arundinacea*, an intra-specific variability of RP depending on both the sampling location and the Cu-dose was evidenced. This intra-specific variability of RP depending on the sampling location and of Cu-tolerance for these last four species suggests that Cu constitutive tolerance for all rooted macrophytes is not a species-wide trait but it exhibits variability for some species.

1. Introduction

Aquatic ecosystems are used, directly and indirectly, as recipients of potentially toxic effluents and wastes from domestic, agricultural and industrial activities (Demirezen et al., 2007; Peng et al., 2008). Copper is one of the Trace Elements (TE) potentially toxic in excess, which may migrate in dissolved and solid forms from urban areas and (agro)ecosystems to surface waters, groundwater and wetland substrates, and its excess may accumulate in living organisms (Kamal et al., 2004; van der Ent et al., 2013). Copper acts as a cofactor in many processes in plants, e.g. respiration, photosynthesis, scavenging of oxidative stress, perception of

Abbreviations: AMD, Acid Mine Drainage; CCA, Chromated Copper Arsenate; CW, Constructed wetland; HNS, Hoagland Nutrient Solution; MetE, methyltetrahydropteroyltriglutamatehomocysteine methyltransferase; MT, Metallothionein; PC, Phytochelatin; RB, Retarding Basin; ROL, Radial oxygen loss; ROS, Reactive Oxygen Species; SAMS, S-adenosylmethionine synthase; SOD, Superoxide dismutase; TE, Trace Element; TIM, Triosephosphate isomerase cytosolic; WTP, Wastewater Treatment Plant.

* Corresponding author.
E-mail address: marchand.lilian@gmail.com (L. Marchand).

http://dx.doi.org/10.1016/j.envpol.2014.07.001

Environ Sci Pollut Res (2016) 23:3120–3135
DOI 10.1007/s11356-015-4984-7

Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons

Lilian Marchand[1,2,3] · Celestino-Quintela Sabaris[4] · Dominic Desjardins[5] · Nadège Oustrière[1,2] · Eric Pesme[3] · Damien Butin[3] · Gaetan Wicart[3] · Michel Mench[1,2]

Received: 1 May 2015 / Accepted: 29 June 2015 / Published online: 16 July 2015

Abstract *Medicago sativa* was cultivated at a former harbor facility near Bordeaux (France) to phytomanage a soil contaminated by trace elements (TE) and polycyclic aromatic hydrocarbons (PAH). In parallel, a biotest with *Phaseolus vulgaris* was carried out on potted soils from 18 sub-sites to assess their phytotoxicity. Total soil TE and PAH concentrations, TE concentrations in the soil pore water, the foliar ionome of *M. sativa* (at the end of the first growth season) and of *Populus nigra* growing in situ, the root and shoot biomass and the foliar ionome of *P. vulgaris* were determined. Despite high total soil TE, soluble TE concentrations were generally low, mainly due to alkaline soil pH (7.8–8.6). Shoot dry weight (DW) yield and foliar ionome of *P. vulgaris* did not reflect the soil contamination, but its root DW yield decreased at highest soil TE and/or PAH concentrations. Foliar ionomes of *M. sativa* and *P. nigra* growing in situ were generally similar to the ones at uncontaminated sites. *M. sativa* contributed to bioavailable TE stripping by shoot removal (in g ha^{-1} $harvest^{-1}$): As 0.9, Cd 0.3, Cr 0.4, Cu 16.1, Ni 2.6, Pb 4, and Zn 134. After 1 year, 72 plant species were identified in the plant community across three subsets: (I) plant community developed on bare soil sowed with *M. sativa*; (II) plant community developed in unharvested plots dominated by grasses; and (III) plant community developed on unsowed bare soil. The shoot DW yield (in mg ha^{-1} $harvest^{-1}$) varied from 1.1 (subset I) to 6.9 (subset II). For subset III, the specific richness was the lowest in plots with the highest phytotoxicity for *P. vulgaris*.

Keywords Ecological restoration · Gentle remediation option · *Medicago sativa* · Plant community · Phytoremediation

Responsible editor: Elena Maestri

Highlight *Medicago sativa* well developed on an alkaline soil highly contaminated by trace elements and polycyclic aromatic hydrocarbons at a former harbor dock and displayed low trace element concentrations in its shoots. *Phaseolus vulgaris* was a relevant bioindicator of soil phytotoxicity.

Electronic supplementary material The online version of this article (doi:10.1007/s11356-015-4984-7) contains supplementary material, which is available to authorized users.

✉ Lilian Marchand
lilian.marchand@hotmail.fr

1 INRA, UMR 1202 BIOGECO, 69 route d'Arcachon, FR-33612 Cestas cedex, France

2 University of Bordeaux, UMR 1202 BIOGECO, Bat B2, Allée Geoffroy St-Hilaire, CS50023, FR-33615 Pessac cedex, France

3 Mairie de BORDEAUX, Service Aménagements Paysagers, Direction des Parcs, des Jardins et des Rives, 77 Boulevard Alfred Daney, 33000 Bordeaux, France

4 Departamento Biología Vegetal y Ecología, Facultad de Ciencia y Tecnología, Universidad del País Vasco/EHU, 48080 Bilbao, Spain

5 Institut de Recherche en Biologie Végétale (IRBV), Université de Montréal–Jardin Botanique de Montréal, 4101 Rue Sherbrooke, Est Montréal, QC H1X 2B2, Canada

Chemosphere 156 (2016) 150–162

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol

Lilian Marchand [a, b, *], Céline Pelosi [c, d], María Reyes González-Centeno [e], Anne Maillard [f, g, h], Alain Ourry [f, g, h], William Galland [b], Pierre-Louis Teissedre [e], Jean-Jacques Bessoule [i], Sébastien Mongrand [i], Annette Morvan-Bertrand [f, g, h], Qinzhong Zhang [j], Claire Grosbellet [k], Valérie Bert [l], Nadège Oustrière [a, b], Michel Mench [a, b], Sophie Brunel-Muguet [f, g, h, **]

[a] INRA, UMR 1202 BIOGECO, 69 Route d'Arcachon, FR-33612, Cestas cedex, France
[b] Université de Bordeaux, UMR 1202 BIOGECO, Bât B2, Allée G. St-Hilaire, CS50023, FR-33615, Pessac cedex, France
[c] INRA, UMR 1402 ECOSYS, F-78026, Versailles cedex, France
[d] AgroParisTech, UMR 1402 ECOSYS, F-78850, Thiverval-Grignon, France
[e] Université de Bordeaux, Institut des Sciences de la Vigne et du Vin, Unité de recherche Oenologie, EA 4577, USC 1366 INRA, IPB, 210, chemin de Leysotte, CS 50008, 33882, Villenave d'Ornon cedex, France
[f] INRA, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, CS14032, 14032, Caen Cedex 5, France
[g] Normandie Université, 14032, Caen, France
[h] UNICAEN, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, CS14032, 14032, Caen Cedex 5, France
[i] INRA, UMR 5200 CNRS-Université Bordeaux, Laboratoire de Biogenèse Membranaire, 71, avenue Edouard Bourlaux, 33883, Villenave-d'Ornon Cedex, France
[j] Key Laboratory of Agricultural Environment, Ministry of Agriculture, Sino-Australian Joint Laboratory for Sustainable Agro-Ecosystems, Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
[k] Florentaise, La grande Gâcherie, 44850, Saint Mars du Désert, France
[l] INERIS, Technologies and Sustainable and Clean Processes, Parc Technologique Alata, BP2, 60550, Verneuil en Halatte, France

HIGHLIGHTS

- Biochar incorporation into a contaminated technosol did not promote Cd, Cu and Zn phytoextraction by rapeseed.
- It increased the As, Cd, Cu, Mo, Ni, Pb and Zn solubility and their potential leaching.
- It did not impact the rapeseed yield, except a slight decrease in seed biomass, nor the seed nutritional quality.
- Rapeseed cultivation allowed stripping of the phytoavailable technosol Zn and Cd fractions.

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form
26 April 2016

ABSTRACT

Background and aims: Rapeseed (*Brassica napus* L.) is a Cd/Zn-accumulator whereas soil conditioners such as biochars may immobilize trace elements. These potentially complementary soil remediation options were trialed, singly and in combination, in a pot experiment with a metal(loid)-contaminated technosol.

Abbreviations: AA, Amino Acid; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; B, (Poultry Manure Derived) Biochar; DM, Dry Matter; DMA, Dimethylarsonic; DW, Dry Weight; EC, Electrical Conductivity; EU, European Union; F soil, Soil from the Phytosed scale 1- platform; FA, Fatty Acid; FAO, Food and Agriculture Organization; FAMES, Fatty Acid Methyl Esters; F-B, F soil amended with biochar; F-BR, F soil amended with biochar and cultivated with rapeseed; F-R, F soil cultivated with rapeseed; FRAP, Ferric Reducing Antioxidant Power; FW, Fresh Weight; GC FID, Gas Chromatography with Flame Ionization Detection; GRO, gentle remediation options; GS, Growth Stage; IBI, International Biochar Initiative; ICP AES, Inductively Coupled Plasma Atomic Emission Spectroscopy; ICP MS, Inductively Coupled Plasma Mass Spectrophotometry; ICP OES, Inductively Coupled Plasma Optical Emission Spectrometry; INRA, French National Institute for Agricultural Research; ISO, International Organization for Standardization; MMA, Monomethylarsonic; PAH, Polycyclic Aromatic Hydrocarbons; RFOs, Raffinose Family Oligosaccharides; SA, Synaptic Acid; TE, Trace Element; TDW, Total Dry Weight; TSW, Thousand-Seed Weight; VDLUFA, Association of German Agricultural Analytic and Research Institutes; WHC, Water Holding Capacity; WSS, Water Soluble Sugar.

* Corresponding author. INRA, UMR 1202 BIOGECO, 69 Route d'Arcachon, FR-33612, Cestas cedex, France.
** Corresponding author. INRA, UMR 950 Ecophysiologie Végétale, Agronomie et nutritions N, C, S, Esplanade de la Paix, CS14032, 14032, Caen Cedex 5, France.

http://dx.doi.org/10.1016/j.chemosphere.2016.04.129

Science of the Total Environment 566–567 (2016) 816–825

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site

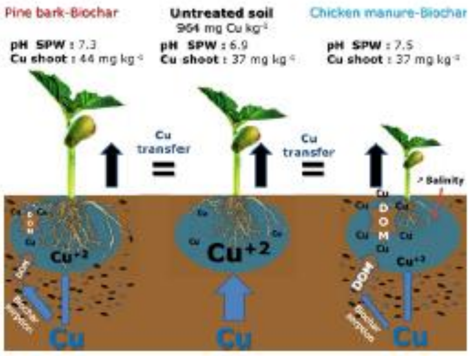
Nadège Oustriere [a,b,*], Lilian Marchand [a,b], William Galland [a,b], Lunel Gabbon [a,b], Nathalie Lottier [c], Mikael Motelica [c], Michel Mench [a,b]

[a] UMR BIOGECO INRA 1202, Diversity and Functioning of Communities, University of Bordeaux, Bât. B2, allée Geoffroy St-Hilaire, CS50023, F-33615 Pessac cedex, France
[b] INRA, UMR BIOGECO INRA 1202, 69 Route d'Arcachon, 33610 Cestas, France
[c] ISTO UMR 7327-CNRS, University of Orléans, campus géosciences, 1A, rue de la férollerie, 45071 Orléans cedex 2, France

HIGHLIGHTS

- The contaminated soil displayed high soluble Cu concentration.
- All tested amendments decreased the Cu^{2+} concentration in the soil pore water.
- Poultry manure-derived biochar increased Cu concentration in the soil pore water.
- Pine bark-derived biochar mixed with iron grit decreased Cu in the soil pore water.
- None of the tested amendments has significantly improved plant yields.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 10 May 2016
Accepted 13 May 2016
Available online xxxx

Editor: F.M. Tack

Keywords:
Copper contamination
In situ stabilization
Phaseolus vulgaris L.

ABSTRACT

Two biochars, a green waste compost and iron grit were used, alone and in combination, as amendment to improve soil properties and in situ stabilize Cu in a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site. The pot experiment consisted in 9 soil treatments (% w/w): untreated Cu-contaminated soil (Unt); Unt soil amended respectively with compost (5%, C), iron grit (1%, Z), pine bark-derived biochar (1%, PB), poultry-manure-derived biochar (1%, AB), PB or AB + C (5%, PBC and ABC), and PB or AB + Z (1%, PBZ and ABZ). After a 3-month reaction period, the soil pore water (SPW) was sampled in potted soils and dwarf beans were grown for a 2-week period. In the SPW, all amendments decreased the Cu^{2+} concentration, but total Cu concentration increased in all AB-amended soils due to high dissolved organic matter (DOM) concentration. No treatment improved root and shoot DW yields, which even decreased in the ABC and ABZ treatments. The PBZ treatment decreased total Cu concentration in the SPW while reducing the gap with common values for root and shoot yields of dwarf bean plants. A field trial is underway before any recommendation for the PB-based treatments.

* Corresponding author at: UMR BIOGECO INRA 1202, Diversity and Functionality of Communities, University of Bordeaux, Bât. B2, allée Geoffroy St-Hilaire, CS50023, F-33615 Pessac cedex, France.
E-mail addresses: oustriere.nadege@gmail.com (N. Oustriere), marchand.lilian@gmail.com (L. Marchand), galland.william@outlook.fr (W. Galland), lunelgabon@gmail.com (L. Gabbon), nathalie.lottier@univ-orleans.fr (N. Lottier), mikael.motelica@univ-orleans.fr (M. Motelica), mench@bordeaux.inra.fr (M. Mench).

http://dx.doi.org/10.1016/j.scitotenv.2016.05.091
0048-9697/

Journal of Hazardous Materials 320 (2016) 458–468

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Aided phytostabilization of a trace element-contaminated technosol developed on steel mill wastes

Nadège Oustriere[a,*], Lilian Marchand[a], Jean Luc Bouchardon[b], Olivier Faure[b,c], Jacques Moutte[b], Michel Mench[a]

[a] BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France
[b] ENSM-SE Centre SPIN, 158 cours Fauriel, 42023 St Etienne, France
[c] Université Jean Monnet, 23 rue Dr. Paul Michelon, 42100 St Etienne, France

HIGHLIGHTS

- A technosol developed on steel mill wastes displayed high soluble Cr and Mo levels.
- Input of *ramial chipped wood* and composted sewage sludge reduced soluble Cr in soil.
- *Festuca pratensis* best developed on the composted sewage sludge-amended soil.
- Its shoot Cr, Ni and Mo concentrations were lowest for the compost-amended soil.
- Shoot Mo concentration exceeded the maximum permitted concentration in forage.

ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 15 June 2016
Accepted 18 August 2016
Available online 20 August 2016

Keywords:
Chromium
Composted sewage sludge
Festuca pratensis L.
Molybdenum
Nickel

ABSTRACT

Aided phytostabilization of a barren, alkaline metal(loid)-contaminated technosol developed on steel mill wastes, with high soluble Cr and Mo concentrations, was assessed in a pot experiment using (1) Ni/Cd-tolerant populations of *Festuca pratensis* Huds., *Holcus lanatus* L., and *Plantago lanceolata* L. sowed in mixed stand and (2) six soil treatments: untreated soil (UNT), ramial chipped wood (RCW, 500 m^3 ha^{-1}), composted sewage sludge (CSS, 120 t DW ha^{-1}), UNT soil amended with compost (5% w/w) and either vermiculite (5%, VOM) or iron grit (1%, OMZ), and an uncontaminated soil (CTRL). In the CSS soil, pH and soluble Cr decreased whereas soluble Cu, K, Fe, Mn, Mg, Ni and P increased. The RCW treatment enhanced soluble Fe, Mn, and Mg concentrations. After 15 weeks, shoot DW yield and shoot Cd, Cu, Fe, Mn, Mo, Zn, and Mg removals peaked for *F. pratensis* grown on the CSS soil, with lowest shoot Cr, Ni and Mo concentrations. *Holcus lanatus* only grew on the CTRL, UNT, and CSS soils and *P. lanceolata* on the CTRL soil. Best treatment, *F. pratensis* grown on the CSS soil, led to a dense grass cover but its shoot Mo concentration exceeded the maximum permitted concentration in forage.

1. Introduction

Soil contaminations by trace elements (TE), here metal(loid)s with common concentrations in living organisms below 100 mg kg^{-1} [1], are often legacies of long term industrial activities [2]. Several smelting activities are located in eastern France [3]. In particular, a large steel mill (30 ha) located at Chateauneuf, nearby Rive-de-Gier (Loire, France) has produced and dumped a huge amount (100 000 m^3) of foundry wastes, slags, fire-bricks, and other by-products such as more or less hydrated lime in an internal landfill [4]. A technosol, i.e. with properties and pedogenesis dominated by artificial or transported materials [5], has developed on these old on-site tailings [6]. Its total TE concentrations exceed the background values for French sandy soils [7,8]. Such TE-contaminated technosols may pose a danger to human health and the environment [9]. For the Chateauneuf tailings, windblown dust, TE leaching beneath the technosol, and topsoil ecotoxicity were evidenced and this diffuse contamination may generate pollutant linkages nearby [4,6].

* Corresponding author at: UMR BIOGECO INRA 1202, Diversity and Functionality of Communities, Univ. Bordeaux, Bât. B2, Allée Geoffroy St-Hilaire, CS50023, F-33615 Pessac Cedex, France.
E-mail addresses: oustriere.nadege@gmail.com (N. Oustriere), marchand.lilian@gmail.com (L. Marchand), bouchardon@emse.fr (J.L. Bouchardon), ofaure@emse.fr (O. Faure), moutte@emse.fr (J. Moutte), mench@bordeaux.inra.fr (M. Mench).

http://dx.doi.org/10.1016/j.jhazmat.2016.08.048
0304-3894/

Science of the Total Environment 579 (2017) 620–627

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site

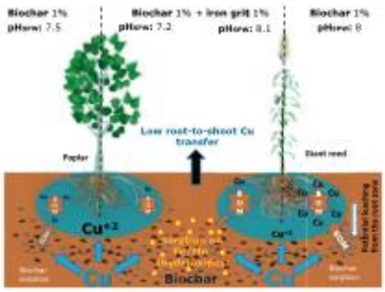
Nadège Oustriere [a,*], Lilian Marchand [a], Nathalie Lottier [b], Mikael Motelica [b], Michel Mench [a]

[a] BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France
[b] ISTO UMR 7327-CNRS, University of Orléans, Campus Géosciences, 1A, rue de la ferollerie, 45071 Orléans cedex 2, France

HIGHLIGHTS

- Biochar with and without iron grit was added in a Cu-contaminated soil.
- Soil pore water: biochar with iron grit reduced its Cu^{2+} and Cu contents in year 2
- Both amendments did not improve Giant reed and poplar growth.
- Shoot Cu concentration of poplar and Giant reed reached common values.
- Giant reed can increase the potential Cu leaching out of the root-zone.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 August 2016
Received in revised form 2 November 2016
Accepted 8 November 2016
Available online 23 November 2016

Editor: Ajit Sarmah

Keywords:
Arundo donax L.
Populus nigra L.
Soil pore water
Copper contamination
In situ stabilization
Phytomanagement

ABSTRACT

A 2-year pot experiment was carried out to examine the aging effect of biochar (B), alone or combined with iron grit (Z), on Cu stabilization and plant growth in a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site. The experiment consisted in 3 soil treatments, either planted with *Arundo donax* L. (*Ad*) or *Populus nigra* L. (*Pn*): (1) untreated Cu-contaminated soil (*Ad*, *Pn*); (2) Unt + 1% (w/w) B (*Ad*B, *Pn*B), and (3) Unt + 1% B + 1% Z (*Ad*BZ, *Pn*BZ). After 22 months, the soil pore water (SPW) was sampled and roots and shoots were harvested. The SPW compositions at 3 and 22 months were compared, showing that the SPW Cu^{2+} concentration increased again in the *Pn*B and *Pn*BZ soils. Cultivation of *A. donax* enhanced the dissolved organic matter concentration in the SPW, which decreased its Cu^{2+} concentration but promoted its total Cu concentration in the *Ad* and *Ad*B soils. Adding Z with B reduced both SPW Cu^{2+} and Cu concentrations in the pots cultivated by *A. donax* and *P. nigra* as compared to B alone. The B and BZ treatments did not enhance root and shoot yields of both plant species as compared to the Unt soil but their shoot Cu concentrations were in the range of common values.

1. Introduction

Soil Cu contamination at wood preservation sites frequently resulted from long-term use of Cu-based salts as wood preservatives and wood washings (Bes and Mench, 2008; Oustriere et al., 2016a). The

* Corresponding author.
E-mail addresses: oustriere.nadege@gmail.com (N. Oustriere), marchand.lilian@gmail.com (L. Marchand), nathalie.lottier@univ-orleans.fr (N. Lottier), mikael.motelica@univ-orleans.fr (M. Motelica), michel.mench@inra.fr (M. Mench).

http://dx.doi.org/10.1016/j.scitotenv.2016.11.048

Environ Sci Pollut Res
DOI 10.1007/s11356-017-8361-6

RESEARCH ARTICLE

Wood-derived-biochar combined with compost or iron grit for in situ stabilization of Cd, Pb, and Zn in a contaminated soil

Nadège Oustriere[1] · Lilian Marchand[1] · Gabriel Rosette[1] · Wolfgang Friesl-Hanl[2] · Michel Mench[1]

Received: 4 July 2016 / Accepted: 2 January 2017

Abstract In situ stabilization of Cd, Pb, and Zn in an Austrian agricultural soil contaminated by atmospheric depositions from a smelter plant was assessed with a pine bark chip-derived biochar, alone and in combination with either compost or iron grit. Biochar amendment was also trialed in an uncontaminated soil to detect any detrimental effect. The pot experiment consisted in ten soil treatments (% *w/w*): untreated contaminated soil (Unt); Unt soil amended with biochar alone (1%: B1; 2.5%: B2.5) and in combination: B1 and B2.5 + 5% compost (B1C and B2.5C), B1 and B2.5 + 1% iron grit (B1Z and B2.5Z); uncontaminated soil (Ctrl); Ctrl soil amended with 1 or 2.5% biochar (CtrlB1, CtrlB2.5). After a 3-month reaction period, the soil pore water (SPW) was sampled in potted soils and dwarf beans were grown for a 2-week period. The SPW Cd, Pb, and Zn concentrations decreased in all amended-contaminated soils. The biochar effects increased with its addition rate and its combination with either compost or iron grit. Shoot Cd and Zn removals by beans were reduced and shoot Cd, Pb, and Zn concentrations decreased to common values in all amended soils except the B1 soil. Decreases in the SPW Cd/Pb/Zn concentrations did not improve the root and shoot yields of plants as compared to the Ctrl soil.

Keywords Metal · *Phaseolus vulgaris* L. · Phytomanagement · Soil contamination · Soil pore water

Responsible editor: Hailong Wang

Electronic supplementary material The online version of this article (doi:10.1007/s11356-017-8361-6) contains supplementary material, which is available to authorized users.

✉ Nadège Oustriere
oustriere.nadege@gmail.com

Lilian Marchand
marchand.lilian@gmail.com

Wolfgang Friesl-Hanl
Wolfgang.Friesl-Hanl@ait.ac.at

Michel Mench
mench@inra.fr

1 BIOGECO, INRA, University of Bordeaux, 33615 Pessac cedex, France

2 Energy Department, AIT Austrian Institute of Technology GmbH, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria

Published online: 22 January 2017

Introduction

Several hundred years of smelting and processing of mining ores have caused widespread pollution of field areas around the industrial site of Arnoldstein in Carinthia, Austria (Asami 1988), where the Zn/Cd/Ge smelter closed in 1992. The surrounding soils used for housing (playgrounds), horticulture, forestry, and alpine grassland agriculture with pastures and feed production are contaminated by Pb, Cd, and Zn and, to a lesser extent, Cu and As (Friesl et al. 2006; Friesl et al. 2009). Such soil contamination by trace elements (TE) generated detrimental effects on the ecosystems with TE transfer from the soil to the environment. Although Cu and Zn concentrations were under homeostatic control, high Pb and Cd concentrations were measured in arthropods species (Rabitsch 1995). High Pb concentration occurred in blood and teeth of inhabitants living nearby the smelter (Kasperowski 1993). Metal concentrations (mg kg^{-1}) were high in *Zea mays* L. shoot, i.e., Pb 54, Zn 286, and Cd 2.73 (Friesl et al. 2006). Based on the Austrian Federal Environmental Agency, the restoration of such contaminated soils was needed (Kasperowski 1993; Rabitsch 1995).

Posters

Aided phytostabilisation of a Cr, Mo and Ni-contaminated technosol

Nadège Oustriere[1*], Michel Mench[1], Lilian Marchand[1], Jean Luc Bouchardon[2], Olivier Faure[2b]

[1]UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bât. B2, Avenue des Facultés, 33405 Talence, France, and INRA, 69 Route d'Arcachon, 33610 Cestas, France.

[2]ENSM-SE centre SPIN, 158 cours Fauriel 42023 St Etienne/ [2b] Université Jean Monnet, 23 rue Dr. Paul Michelon 42100 St Etienne, France

Corresponding author : Oustriere.nadege@gmail.com

Rational and Objective

Aided phytostabilisation : use of excluder plants, associated microorganisms, and soil conditioners to minimize pollutant linkages, as metal(loid) transfer in the environmental compartments and into the food chain. [1].

◄ At Rive de Gier, an aided phytostabilisation program (PHYSAFIMM) is implemented on metal(loid)-contaminated tailings (30 ha) of a steel smelter [2]

This phytoremediation option should :

- Restore a vegetation cover
- Reduce labile soil Cr, Ni and Mo concentrations
- Decrease exposure pathways.

➤ Aim: Assessment of the efficiency of Ni/Cd-tolerant plants and soil conditioners for performing aided phytostabilisation on a metal (Cr, Ni, and Mo)-contaminated technosol

Materiel & Methods

Ni/Cd-tolerant ecotypes :
Festuca pratensis, Holcus lanatus, Plantago lanceolata ▼

Soil conditioners :

- Ramial chipped wood (RCW)
- Composted sewage sludge (CSS)
- Vermiculite + Organic matter (VOM)
- Iron grit + Organic matter (OMZ)
- Uncontaminated control soil (CTRL)

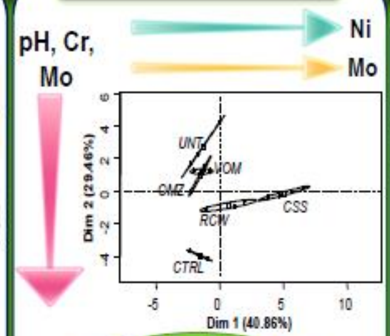
Shoot ionome and Soil Pore Water characteristics :

- Element concentrations (shoots and SPW)
- pH & electrical conductivity of soil pore water

Functional traits :

- Shoot dry weight (DW) yields

SPW characteristic



Biomass & shoot ionome

After 15 weeks, *F. pratensis* developed well on all soil treatments while *H. lanatus* and *P. lanceolata* only grew on the CTRL, UNT, CSS and CTRL soil treatments and with a low growth rate.

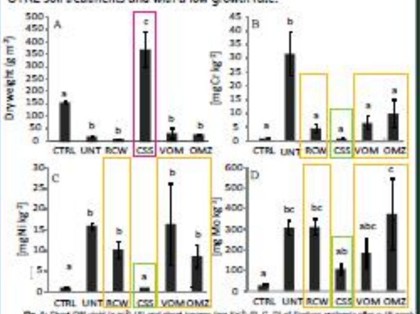


Fig. 1: Shoot DW yield (g m⁻²) (A) and shoot ionome (mg Kg⁻¹) (B, C, D) of *Festuca pratensis* after a 15-week growth period. Mean values per treatment (n=3).

CSS treatment :
↗ Shoot DW yield of *F. pratensis*
↘ Shoot Cr, Ni and Mo concentrations

RCW, VOM & OMZ treatment :
↘ Shoot Cr concentrations

Take-Home Message

Combination (*F. pratensis* / CSS)

\+
- Nutrient inputs
- ↘ Shoot Ni, Cr, Mo concentrations
- High recovery rate at field scale

\-
- High soluble Ni and Mo concentrations

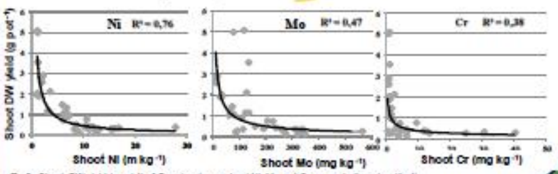
well adapted to the aided phytostabilisation of the Rive-de-Gier technosol

Dilution effect

Trace element concentrations in shoots

BIOMASS

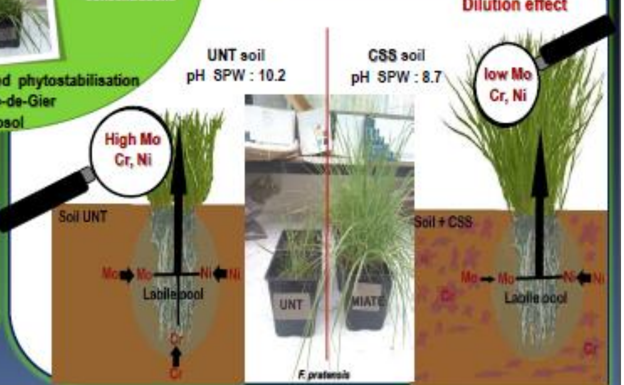
CSS treatment :
↗ Ni, Mo exposure
↘ Shoot Cr, Ni and Mo concentrations
↗ Shoot DW yield of *F. pratensis*
→ **Dilution effect**



▲Fig.2 : Shoot DW yield (g pot⁻¹) of *F. pratensis* vs. shoot Ni, Mo and Cr concentrations (mg Kg⁻¹)

UNT vs. CSS

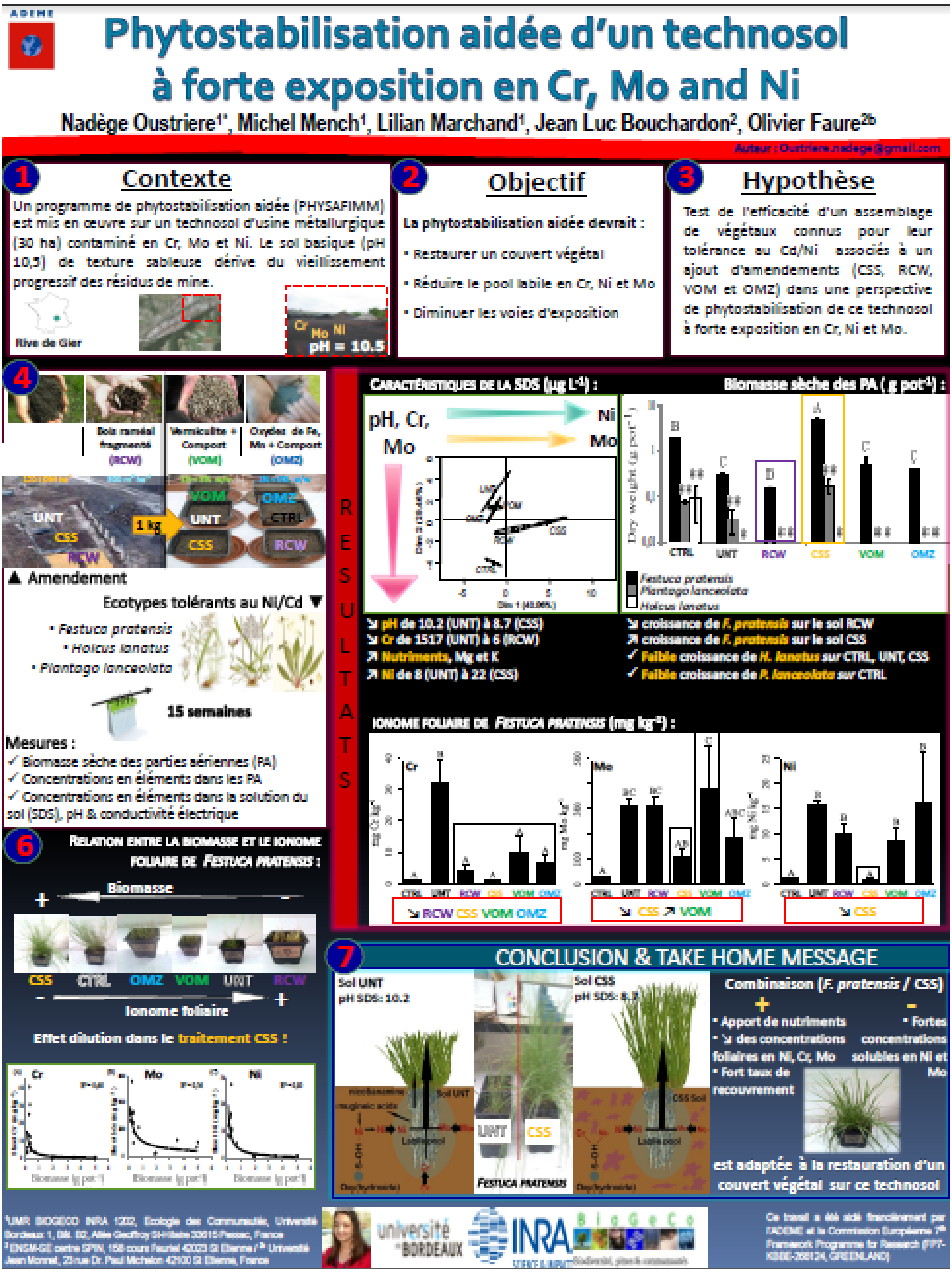
Dilution effect



[1] Kumpiene J., Mench M., Bes C., Fitts J. (2011) Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environmental Pollution 159 : 1536-1542

[2] Bouchardon, J.-L., Crosaz, Y., Faure, O., Gallice, F., Lespagnol, G. and Mench, M. (2011) Phytostabilization of metallurgical landfills: combined effects of soil amendment and plant assemblage (Physafimm project). 11th Int. Conf. Biogeochemistry Trace Elements, Firenze, Italy. Part. II, pp. 127-128.

This work was financially supported by ADEME (Physafimm) and the European Commission under the 7th Framework Programme for Research (FP7-KBBE-266124, GREENLAND)


ADEME
Phytostabilisation aidée d'un technosol à forte exposition en Cr, Mo and Ni
Nadège Oustriere1*, Michel Mench1, Lilian Marchand1, Jean Luc Bouchardon2, Olivier Faure2b
Auteur : Oustriere.nadege@gmail.com
1 Contexte
Un programme de phytostabilisation aidée (PHYSAFIMM) est mis en œuvre sur un technosol d'usine métallurgique (30 ha) contaminé en Cr, Mo et Ni. Le sol basique (pH 10,5) de texture sableuse dérive du vieillissement progressif des résidus de mine.
Rive de Gier
Cr Mo Ni
pH = 10.5
2 Objectif
La phytostabilisation aidée devrait :
• Restaurer un couvert végétal
• Réduire le pool labile en Cr, Ni et Mo
• Diminuer les voies d'exposition
3 Hypothèse
Test de l'efficacité d'un assemblage de végétaux connus pour leur tolérance au Cd/Ni associés à un ajout d'amendements (CSS, RCW, VOM et OMZ) dans une perspective de phytostabilisation de ce technosol à forte exposition en Cr, Ni et Mo.
4
Bois raméal fragmenté (RCW)
Vermiculite + Compost (VOM)
Oxydes de Fe, Mn + Compost (OMZ)
UNT
CSS
RCW
1 kg
VOM
OMZ
CTRL
▲ Amendement
Ecotypes tolérants au Ni/Cd ▼
• Festuca pratensis
• Holcus lanatus
• Plantago lanceolata
15 semaines
Mesures :
✓ Biomasse sèche des parties aériennes (PA)
✓ Concentrations en éléments dans les PA
✓ Concentrations en éléments dans la solution du sol (SDS), pH & conductivité électrique
RESULTATS
Caractéristiques de la SDS (µg L-1) :
pH, Cr, Mo
Ni
Mo
Dim 1 (43.08%)
Biomasse sèche des PA (g pot-1) :
Dry weight (g pot-1)
CTRL
UNT
RCW
CSS
VOM
OMZ
Festuca pratensis
Plantago lanceolata
Holcus lanatus
↘ pH de 10.2 (UNT) à 8.7 (CSS)
↘ Cr de 1517 (UNT) à 6 (RCW)
↗ Nutriments, Mg et K
↗ Ni de 8 (UNT) à 22 (CSS)
↘ croissance de F. pratensis sur le sol RCW
↗ croissance de F. pratensis sur le sol CSS
✓ Faible croissance de H. lanatus sur CTRL, UNT, CSS
✓ Faible croissance de P. lanceolata sur CTRL
Ionome foliaire de Festuca pratensis (mg kg-1) :
Cr
Mo
Ni
↘ RCW CSS VOM OMZ
↘ CSS ↗ VOM
↘ CSS
6 Relation entre la biomasse et le ionome foliaire de Festuca pratensis :
Biomasse
CSS CTRL OMZ VOM UNT RCW
Ionome foliaire
Effet dilution dans le traitement CSS !
Cr
Mo
Ni
Biomasse (g pot-1)
7 CONCLUSION & TAKE HOME MESSAGE
Sol UNT
pH SDS: 10.2
Sol CSS
pH SDS: 8.7
UNT
CSS
Festuca pratensis
Combinaison (F. pratensis / CSS)
+
• Apport de nutriments
• ↘ des concentrations foliaires en Ni, Cr, Mo
• Fort taux de recouvrement
-
• Fortes concentrations solubles en Ni et Mo
est adaptée à la restauration d'un couvert végétal sur ce technosol
1UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bât. B2, Allée Geoffroy St-Hilaire 33615 Pessac, France
2 ENSM-SE centre SPIN, 158 cours Fauriel 42023 St Etienne / 2b Université Jean Monnet, 23 rue Dr. Paul Michelon 42100 St Etienne, France
université BORDEAUX
INRA
BioGeCo
Ce travail a été aidé financièrement par l'ADEME et la Commission Européenne 7th Framework Programme for Research (FP7-KBBE-266124, GREENLAND)

Oral communications


BioGeCo
université de BORDEAUX
ADEME
GREENLAND
INRA SCIENCE & IMPACT
Phytomanagement of a metal-contaminated technosol
Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O.
International Congress on the Phytoremediation of Polluted Soils, Vigo July 29-30, 2014


BioGeCo
université de BORDEAUX
ADEME
GREENLAND
INRA SCIENCE & IMPACT
Aided phytostabilisation of a Cr, Mo and Ni-contaminated technosol
Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O.
11th International Phytotechnologies Conference, Heraklion, Crete, Greece, Sept 30 – Oct 3, 2014
oustriere.nadege@gmail.com


BioGeCo
université de BORDEAUX
ADEME
GREENLAND
INRA
SCIENCE & IMPACT
Évaluation de la phytotoxicité d'un sol contaminé en Cu géré par stabilisation in situ :
Le cas des biochars
Nadège Oustrière, Lilian Marchand, Joseph William Galland, Lunel Gabbon, Michel Mench
Biogeco_Biochar_veg2.5_comp0_jrent
REP1
oustriere.nadege@gmail.com
5ème Séminaire d'Ecotoxicologie de l'INRA,
Biarritz 25 au 27 Novembre 2014


BioGeCo
université de BORDEAUX
ADEME
GREENLAND
INRA
SCIENCE & IMPACT
Phytotoxicity assessment of a Cu-contaminated soil managed by in situ stabilisation
Nadège Oustrière, Lilian Marchand, Joseph William Galland, Lunel Gabbon, Michel Mench
REP1
13th ICOBTE, July 12-16, 2015, Fukuoka, Japon

Peer-reviewed publications

Published

Oustrière N, Marchand L, Friesl-Hanl W, Mench M. **2017.** Wood-derived-biochar combined with compost or iron grit for in situ stabilization of Cd, Pb, and Zn in a contaminated soil. Environmental Science and Pollution Research. (DOI: 10.1007/s11356-017-8361-6) **IF 2.8**

Oustrière N, Marchand L, Lottier N, Motelica M, Mench M. **2017.** Long-term Cu stabilization and biomass yields of Giant reed and poplar after adding a biochar, alone or with iron grit, into a contaminated soil from a wood preservation site. Science of the Total Environment. 579, 620–627 (DOI: 10.1016/j.scitotenv.2016.11.048) **IF 4.0**

Oustrière N, Marchand L, Bouchardon JL, Faure O, Moutte J, Mench M. **2016**. Aided phytostabilization of a trace element contaminated technosol developed on steel mill wastes. Journal of Hazardous Materials. 320, 458–468 (DOI: 10.1016/j.jhazmat.2016.08.048). **IF 4.8**

Oustrière N, Marchand L, Galland W, Gabon L, Lottier N, Motelica M, Mench M. **2016**. Influence of biochars, compost and iron grit, singly and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Science of the Total Environment. 566–567, 816–825 (DOI:10.1016/j.scitotenv.2016.05.091) IF **4.0**

Marchand L, Pelosi C, Gonzalez-Centeno R, Maillard A, Ourry A, Galland W, Teissedre PL, Bessoule JJ, Mongrand S, Morvan-Bertrand A, Zhang Q, Grosbellet C, Bert V, **Oustriere N**, Mench M, Brunel-Muguet S. **2016.** Trace element bioavailability, yield and seed quality of rapeseed (*Brassica napus* L.) modulated by biochar incorporation into a contaminated technosol. Chemosphere 156, 150-162 (DOI:10.1016/j.chemosphere.2016.04.129) **IF 3.3**

Marchand L, Sabaris CQ, Desjardins D, **Oustrière N**, Pesme E, Butin D, Wicart G, Mench M. **2015**. Plant responses to a phytomanaged urban technosol contaminated by trace elements and polycyclic aromatic hydrocarbons. Environmental Science and Pollution Research 23, 3120-3135 (DOI: 10.1007/s11356-015-4984-7) IF **2.8**

Marchand L, Nsangawimana F, Gonnelli C, Colzi I, Fletcher T, **Oustrière N**, Kolbas A, Kidd P, Bordas F, Newell P, Alvarenga P, Lamy JB, Deletic A, Mench M. **2014**. Intra-specific variability versus constitutive-like tolerance to face Cu exposure in populations of six rooted macrophytes. Environmental Pollution 193, 205-215 (DOI: 10.1016/j.envpol.2014.07.001) IF **3.9**

Kumpiene J, Bert V, Dimitriou I, Eriksson J, Friesl-Hani W, Galazka K, Herzig R, Janssen J, Kidd P, Mench M, Muller I, Neu S, **Oustriere N**, Puschenreiter M, Renella G, Roumier PH, Siebielec G, Vangronsveld J, Manier N. **2014**. Selecting chemical and ecotoxicological test batteries for risk assessment of trace element-contaminated soils (phyto)managed by gentle remediation options (GRO). Science of the total environment. 496, 510-522 (DOI: 10.1016/j.scitoteny.2014.06.130) **IF 4.1**

Marchand L, Nsanganwimana F, Cook B, Vystavna Y, Huneau F, Lecoustumer P, Lamy JB, **Oustrière N**, Mench M. **2014**. Trace element transfer from soil to leaves of macrophytes along the Jalle d'Eysines River, France and their potential use as contamination biomonitors. Ecological Indicators 46, 425–437 (DOI: 10.1016/j.ecolind.2014.07.011) **IF 3.2**

Marchand L, Nsanganwimana F, **Oustrière N**, Grebenshchykova Z, Lizama-Allende K, Mench M. **2014**. Copper removal from water using a bio-rack system either unplanted or planted with *Phragmites australis*, *Juncus articulatus* and *Phalaris arundinacea*. Ecological Engineering 64, 291-300 (DOI: 10.1016/j.ecoleng.2013.12.017) **IF 3.0**

Accepted with revisions

Oustrière N, Marchand L, Roulet E, Mench M. **2017.** Rhizofiltration of a Bordeaux mixture effluent in pilotscale constructed wetland using *Arundo donax* L. coupled with potential Cu-ecocatalyst production. Accepted with minor revisions

Oral communications

Oustrière N., Marchand L., Rousset C., Roulet E., Mench M. Copper removals from Bordeaux mixture effluents using *Arundo donax* root mat while producing recoverable biomass. Plant-based solutions for environmental solutions: from lab to field. 13th International International Phytotechnologies Conference, Sept 26– 29, **2016** • HangZhou, China.

Oustrière N., Marchand L., Galland J.W., Gabbon L., Lottier N., Motelica M., Mench M. Pot testing of biochars, compost and iron grit, alone and in combination, in a copper-contaminated soil from a wood preservation site. 5th International Conference on Soil Pollution and Remediation, Sept 24 – 26, **2016** • HangZhou, China

Oustrière N., Marchand L., Galland J.W., Gabbon L., Mench M. Phytotoxicity assessment of a Cu-contaminated soil managed by in situ stabilization. 13th International Conference on the Biogeochemistry of Trace Elements. July 12 – 16, **2015** • Fukuoka, Japon.

Oustrière N., Marchand L., Galland J.W., Gabbon L., Mench M. Évaluation de la phytotoxicité d'un sol contaminé en Cu géré par stabilisation in situ : Le cas des biochars. 5th Seminar of INRA Ecotoxicology. Nov 25 – 27, **2014** • Biarritz, France.

Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O. Phytomanagement of a metal-contaminated technosol. International Congress on the Phytoremediation of Polluted Soils. July 29 – 30, **2014** • Vigo, Spain.

Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O. Aided phytostabilisation of a Cr, Mo and Ni-contaminated technosol. 11th International Phytotechnologies Conference. Sept 30 – Oct 3, **2014** • Heraklion, Crete.

Posters

Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O. Phytostabilisation aidée d'un technosol à forte exposition en Cr, Mo and Ni. 3rd national research meeting on contaminated land of ADEME. Nov 18 – 19, **2014** • Paris, France.

Oustrière N., Mench M., Marchand L., Bouchardon J.L., Faure O. Aided phytostabilisation of a Cr, Mo and Ni-contaminated technosol. COST ACTION FA 0905: Mineral improved crop production for healthy food and feed, March 17 – 20, **2014** • Antalya, Turkey. **Prize for the best second poster.**

Oustrière N., Lahbib-Burchard T., Doelsch E., Feder F., Wassenaar T.,Bravin M. Predictive modelling of the long-term accumulation of trace metals in tropical soils amended with organic wastes – field trial validation. 15th international conference of the Network on Recycling of Agricultural, Municipal and Industrial Residues in Agriculture (RAMIRAN). June 3 – 5, **2013** • Versailles, France.

Reviewer for:

"ESPR : Environmental Science and Pollution Research"

"EMAS : Environmental Monitoring and Assessment"

Grants and awards:

ICOBTE- Student Travel Award, **2015** • Fukuoka, Japon

Demolon-Student Travel Award, **2014** • Heraklion, Crete

COST0905- Second best poster, **2014** • Antalya, Turkey

Doctoral School-Student Travel Award, **2014**

Relevant volunteer activities:

Representative of Bordeaux University PhD student 2014-2015

Member of board of directors of 2AD-ADEME association 2014-2016

Editor of the 2AD-ADEME Newsletter 2014-2016