Heavy metal mobility in contaminated soils as affected by plants, amendments and biochar

Implications for phytostabilization

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Thèse présentée en vue de l'obtention du grade de
Docteur en sciences agronomiques et ingénierie biologique

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Louvain-la-Neuve, Janvier 2013

A mon Papy

A ceux qui gardent leur pessimisme pour des jours meilleurs

« Enfin, rentré chez lui, la tête pleine, il éteint sa lampe et longuement,
avant de s'endormir, il se plaît à compter ses images.
Dociles, elles renaissent au gré du souvenir.
Chacune d'elles qui s'agite en éveille une autre, et sans cesse leur troupe
phosphorescente s'accroît de nouvelles venues, comme des perdrix
poursuivies et divisées tout le jour chantent le soir,
à l'abri du danger, et se rappellent au creux des sillons. »

Extrait du Chasseur d'Images, Jules Renard

Remerciements

« *Mesdames et messieurs, veuillez rejoindre vos sièges et attacher vos ceintures de sécurité, nous allons atterrir …* »

Diantre ! Il ne me reste que peu de temps. Ce vol de quatre ans est sur le point de se terminer. Toutefois, avant de revenir les pieds sur Terre, je ne pourrais clore ce fabuleux voyage sans remercier toutes les personnes qui m'ont soutenu et m'ont permis de réaliser et vivre mes passions.

Tout d'abord, je remercie sincèrement *Philippe Sonnet*, commandant de bord de ce long-courrier. Vous m'avez offert l'opportunité de réaliser ce long voyage dans des conditions irréprochables. Votre disponibilité, votre écoute, votre motivation et vos précieux conseils sont autant d'atouts sur lesquels j'ai pu compter. Votre enthousiasme sans frontière et votre chaleur humaine ont constitué un cadre épanouissant et prolifique à la réalisation de mes recherches, à l'éveil de ma curiosité et à l'enrichissement de mon bagage en Sciences. A l'écriture de ces remerciements, j'ai enfin compris pourquoi vous étiez de temps à autres quelque peu tête en l'air (quoi de plus normal pour un commandant de bord …).

Un si long périple n'aurait pu aboutir sans la présence de membres d'équipage de haut vol.

C'est pourquoi, je tiens à faire part de ma profonde reconnaissance aux membres de mon Jury de thèse, Messieurs *Joseph Dufey*, *Stanley Lutts*, *Erik Smolders* et *Christophe Schwartz*. Merci pour vos commentaires, vos conseils et le temps que vous m'avez consacré. Merci aussi pour nos échanges lors de discussions plus informelles.

Je remercie également *Bruno Delvaux* qui, lors de la réalisation de mon mémoire, m'a transmis sa passion pour la Science du Sol et m'a ensuite ouvert les portes de son laboratoire.

L'expertise et la qualité du laboratoire de Science du Sol n'a d'égal que l'estime que j'ai pour ses membres. Merci à *Anne Iserentant* ou Madame-je-cours-partout-je-suis-pressée-ça-n'arrête-pas. Merci à *Claudine Givron*, dame d'un certain âge (pour ne pas dire d'un âge certain) mais avec qui, finalement, les discussions tiennent encore bien la route. Merci à *Patrick*

Populaire. Patrick, pas troc, patraque ou Pat Gyver et ses histoires rocambolesques qui agrémentaient les mises au point fastidieuses de nos dispositifs expérimentaux. Merci à *André Lannoye*, vieux briscard toujours à l'affut d'une bonne blague à deux balles (« Pan, pan »). Merci à *Françoise Hauwen* qui veille, avec tant d'efficacité, au bon fonctionnement quotidien du laboratoire. Merci pour ta disponibilité, ton dévouement et ton sourire.

Je n'aurais pu réaliser les analyses isotopiques sans l'enthousiasme de *Sophie Opfergelt* et de *Nadine Mattielli*. Merci à vous de m'avoir offert l'opportunité d'aborder cette technique. Merci aussi à *Wendy Debouge* et sa joie de vivre pour m'avoir superbement encadré en salle blanche. Merci à *Ivan Petrov* pour sa sympathie et son aide lors des analyses au MC-ICP-MS. Merci également à Eléonore Couder pour son précieux coup de main.

J'ai également pu bénéficier de l'aide de *Laurence Ryelandt* et de *Marty* pour la réalisation d'analyses au SEM-EDS. Merci à vous d'avoir consacré une partie de votre temps à m'initier à cet univers inconnu.

Je remercie enfin *Cindy* pour le temps qu'elle a consacré à relire et corriger l'anglais plutôt Voltairien que Shakespearien de certaines parties de ma thèse. Comme j'ai coutume d'écrire à la fin de mes articles: « Cynthia Rozewicz is gratefully acknowledged for proofreading the manuscript as a native English speaker».

Un si long vol n'est supportable que si les passagers sont d'agréable compagnie. Merci, chers collègues, *Paul Ayris, Isabelle Caignet, Jean-Thomas Cornelis, Eléonore Couder, Thomas Delfosse, Pierre Delmelle, Bruno Delvaux, Joseph Dufey, Séverine Delstanche, Océane Derzelle, Marie Detienne, Claudine Givron, Fanny Hamels, Brieuc Hardy, Françoise Hauwen, Anne Iserentant,* Mère *Lucie Kablan, André Lannoye, Elena Maters, Sébastien Mauro, Céline Meunier, Séverin Nijimbere, Sophie Opfergelt, Benoît Pereira, Patrick Populaire, Arnaud Schneider, Philippe Sonnet, Hugues Titeux, Koffi Tomety-Mensah, Florence Trum, Aubry Vandeuren* et *Marie-Liesse Vermeire* (tchuti, quelle longue liste… j'implore le pardon de celui que j'ai oublié). Que ce soit lors de nos pauses café, de nos missions de terrains, de nos verres en terrasse ou de congrès, j'ai pris beaucoup de plaisir à vous côtoyer.

J'aimerais adresser des remerciements particuliers à mes deux principales ~~mijoles de~~ collègues de bureau. Merci *Eléonore* pour m'avoir donné l'envie

de continuer dans la recherche à l'issue de mon mémoire que tu avais parfaitement encadré. Merci pour le partage de ta vision des choses et pour nos moments de « Vous voulez un potin ? ». Merci *Florence*, ou Madame-peu-m'importe-je-suis-toujours-de-bonne-humeur, pour la fantaisie que tu apportais dès l'ouverture de la porte de notre bureau.

Je remercie également plus particulièrement *Jean-Thomas Cornélis*, JTC, Jean-Trois Chimay, et blanches SVP ! Nos fructueuses discussions scientifiques entamées lors de la première débouchaient toujours sur des élucubrations idéologiques à la deuxième. A la troisième, nous refaisions le monde. Que de bons moments passés ~~pendant~~ après les journées de boulot. Je tiens également à remercier *Hugues*. Tes explications (en deux temps) étaient toujours prodigieuses. Là où je m'arrachais les cheveux, toi, tu te les coupais en quatre (dans le sens de la longueur diraient les mauvaises langues …).

Je tiens également à exprimer un merci particulier à *Joseph Dufey* qui m'a emmené avec tant d'enthousiasme à la découverte des aires de fauldes (ma vision des champs labourés est inconsciemment différente dorénavant) et des Bons Cousins Charbonniers. Je remercie ces derniers pour leur accueil à bras ouverts et l'initiation à leur folklore. Merci particulier aussi à *Brieuc* pour cette découverte partagée ainsi que pour les folles nuits passées à l'Euromotel perdu au milieu d'un zoning industriel de Bari…

Trois passagers sont venus s'asseoir à mes côtés pour me prêter main forte entre deux escales. Merci aux trois mémorants que j'ai eu la chance d'encadrer. Merci à *Jonathan Pircar*, le moteur à diesel, à *Guillaume Tricot*, le zen, et à *Laurent Evrard*, le perfectionniste.

Enfin, à quelques rangées de sièges plus éloignées se trouvent toutes les personnes que j'ai pu rencontrer lors d'instants furtifs. En particulier, mon passage en SOLS m'aura permis de m'investir dans la défense des intérêts des chercheurs, notamment via la création de l'ACELI. Je souhaite dès lors une excellente continuation à tous les membres de cette association.

J'exprime également mes sincères remerciements au FNRS pour le mandat FRIA et le soutien financier qui m'a été octroyé, véritable carte d'embarquement pour la réalisation de mes recherches dans les meilleures conditions.

Je ne pense pas me méprendre scientifiquement en affirmant que sans combustible, un avion est condamné à rester à l'aéroport. Chers *amis*, vous m'avez sans cesse permis de faire le plein lors de nos nombreuses et indispensables escales. Soirées Koh, « simple » verre, mariage, naissance et autres ~~murges~~ joyeusetés festives, avec ou sans prétexte, ont rythmé ma vie extra-professionnelle. Merci à tous ceux qui se reconnaitront.

Reste enfin l'élément le plus indispensable. Merci à ma *famille*, vous êtes mes ailes. Sans vous, sans votre soutien inconditionnel, je n'aurais pu prendre mon envol et arriver au bout cette longue traversée.

Et puis, merci *Céline*. Pour ta patience, ta spontanéité, ta joie de vivre communicative. Merci pour le ciel salvateur de tes yeux. Lorsque j'entrais en zone de turbulences, m'y plonger me faisait découvrir de meilleurs horizons.

Boutonville, le 17 décembre 2012

Content

Chapter 1

Introduction

1. Heavy metal soil contamination

1.1. A worldwide issue

Bioreactor at the interface between the lithosphere, atmosphere, hydrosphere and biosphere, the soil is a dynamic, living resource whose condition is vital to both the production of food, energy and fiber and to global balance and ecosystem function; or in essence, to the sustainability of life on Earth (Doran et al., 1996). For instance, soil serves as a substrate supporting plant growth, as a nutrient reservoir, and as the site for many biological processes involved in decomposition and recycling of plant and animal products. Soils influence air quality through interactions with the atmosphere and act as a storage and purification medium for water (Wienhold et al., 2004). Although soils' knowledge dates to the earliest known practices of agricultural (about 11,000 BP), humans have always had an intimate relation with the soil (Brevik and Hartemink, 2010). The importance of soils to humankind is documented by the many ancient civilizations, some of which vanished or collapsed because mismanagement destroyed the soils on which they depended (McNeill and Winiwarter, 2004; Wienhold et al., 2004; Minami, 2009). According to Diamond (2005), soil degradation is one of the eight environmental problems which have historically contributed to the collapse of past societies and one of the twelve problems facing mankind today. However, although the principles of soil conservation have been known for centuries and recognition of the dangers of soil degradation has prompted national soil conservation programs in many countries, inventories of soil productive capacity indicate human-induced degradation on nearly 40% of the world's agricultural land as a result of soil erosion, atmospheric pollution, extensive soil cultivation, over-grazing, land clearing, salinization, and desertification (Doran, 2002).

In particular, while the growth of agriculture and industry has improved living standards for much of the world's population, this growth has created, at the same time, environmental problems in many part of the world by generating large inputs of contaminants to soils. As a result, contaminated soils represent nowadays an important issue with clear consequences on the three dimensions of sustainability, i.e. the environmental, the economic and the social one (Fernández et al., 2006). The environmental perspective covers not only terrestrial communities, but also aquatic ecosystems exposed to drainage and run-off processes. Regarding the economic point of view, in addition to the cost of prevention, control and remediation, the historically contaminated sites represent a burden from the past, which affects the possibilities for current development. And finally, considering the social dimension, the regulation of soils has a main difference with other environmental compartments, as it has to be taken into account that most soils are privately owned (Fernández et al., 2006).

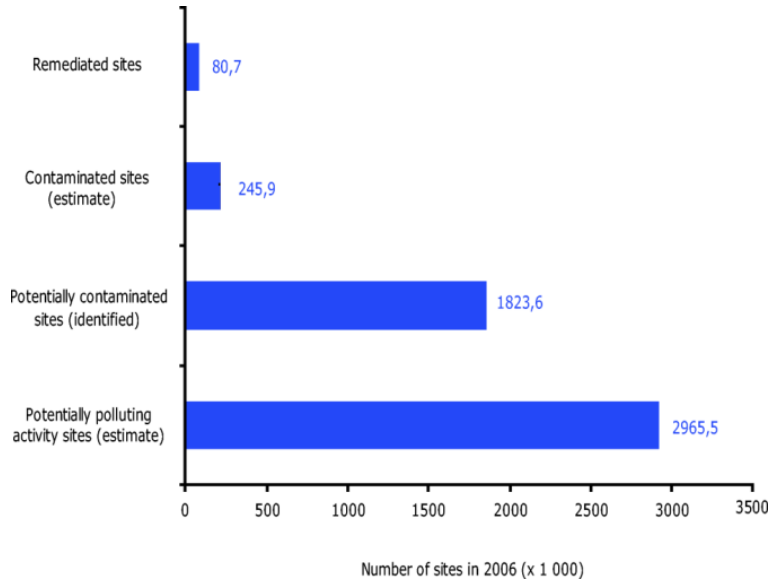


Fig. 1.1 Overview of progress in the management of contaminated sites in Europe (European Environment Agency, 2007).

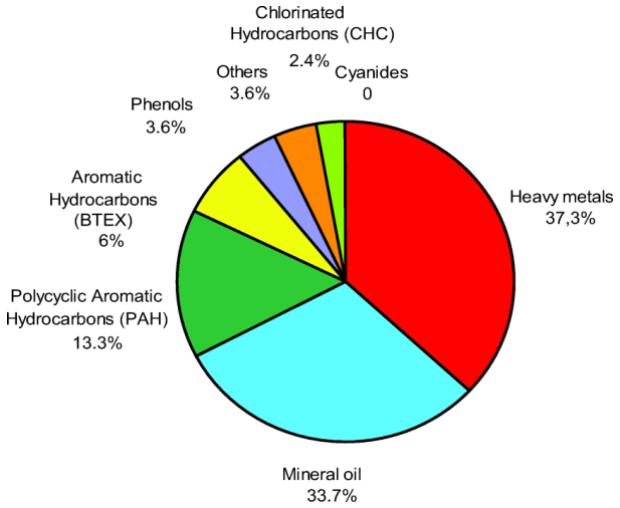


Fig. 1.2 Overview of contaminants affecting soil and groundwater in Europe (European Environment Agency, 2007).

In the European Union, it is estimated that there may be up to three million potentially contaminated soils (European Environment Agency, 2007). Around 80,700 sites have been cleaned up over the last 30 years in the countries where data on remediation are available while at least 250,000 polluted sites require urgent attention (Fig. 1.1). According to the European Environment Agency (2007), although the range of contaminants found in the investigated sites varies from country to country, overall estimates identify heavy metals as the main soil contaminants in Europe. Other contaminants include mineral oil, polycyclic aromatic hydrocarbon (PAH), aromatic hydrocarbons (BTEX), phenols and chlorinated hydrocarbons (CHC) (Fig. 1.2). The heavy metals essentially become contaminants in the soil environments because (i) their rates of generation via man-made cycles are more rapid relative to natural ones, (ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the concentrations of the metals in discarded products are relatively high compared to those in the receiving environment, and (iv) the chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable (D'Amore et al., 2005). Metal-bearing solids at contaminated sites can originate from a wide variety of anthropogenic sources in the form of metal mine tailings, disposal of high metal wastes in improperly protected landfills, leaded gasoline and

lead-based paints, land application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition (Alloway, 1995; Wuana and Okieimen, 2011).

1.2. Distribution and chemical speciation of heavy metals in the soil

As soils consist of heterogeneous mixture of different organic and organomineral substances, clay minerals, oxides of iron, manganese and aluminum and other solid components as well as of a variety of soluble substances, the binding mechanisms for heavy metals in soils are manifold and vary with the composition of the soils, the soil reaction and redox conditions (Fig. 1.3) (Bruemmer et al., 1986). Thus, a metal may form different species according to whether it is bound to various soil compounds, reacting surfaces and external or internal binding sites with different bonding energy. It is generally recognized that information about the physicochemical forms of the elements is required for understanding their environmental behavior, i.e. their mobility, pathways and bioavailability (Tack and Verloo, 1995). A chemical species is a specific form of an element defined on the basis of its isotopic composition, electronic or oxidation state, and/or complex or molecular structure (Templeton et al., 2000). The distribution of heavy metals between the various chemical species in soil solid or solution phase is referred to as speciation (Templeton et al., 2000). Broadly, categories of chemical species may be defined as follows (Tack, 2010):

- Soil solution-phase species:
    - free ion;
    - inorganic complex;
    - organic complex;
    - bound to suspended colloids (clay, organic matter, sesquioxides).

- Soil solid phase species:
    - exchangeably bound to charged surfaces;

- complexed with or occluded within organic matter;
- adsorbed or occluded in carbonates;
- as precipitate (carbonates, phosphates, sulfides);
- as structural components in minerals.

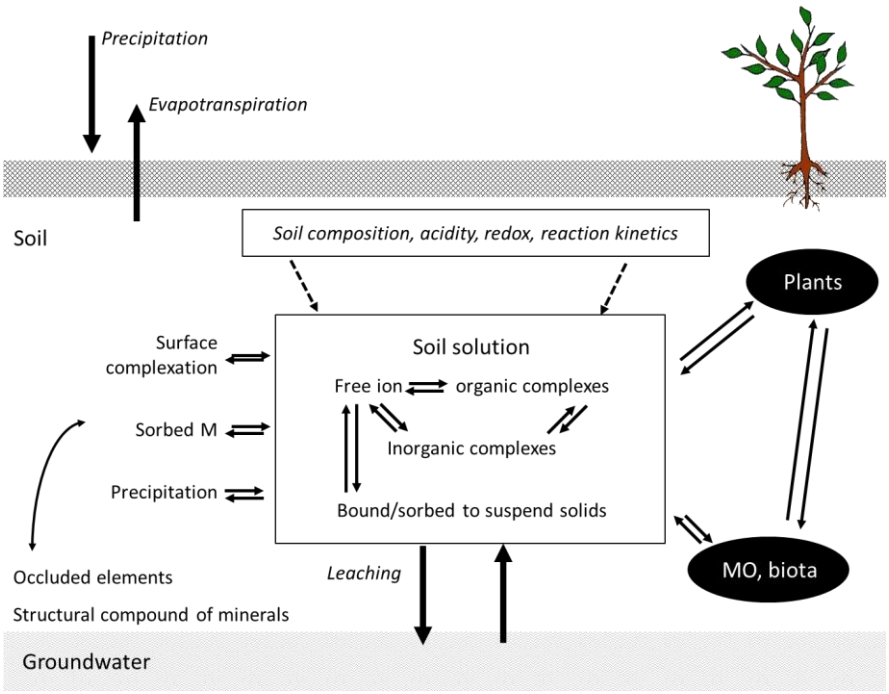


Fig. 1.3 Schematic representation of various pools of trace elements in the soil system. The soil solution is the central gateway through which various froms of elements interact with the soil solid phase and soil biological activity (after Bruemmer et al., 1986 and Tack, 2010)

Mobility of heavy metal in soil environments is greatly affected by sorption-desorption reactions, which are the predominant factors that control the bioavailability of metals (Scheidegger and Sparks, 1996; Sauvé et al., 2000; Basta et al., 2005; Violante et al., 2008). Sorption is a general term that denotes the loss of a solute from aqueous solution to a contiguous solid phase (Sparks et al., 1999). The soil components responsible for heavy metal sorption include soil humic substances, phyllosilicates, carbonates, and variable-charge minerals (hydroxydes and oxyhydroxydes of Fe, Al, Mn and Ti, short-range-ordered aluminoslicates such as allophones and imogolites, and phylosilicates coated by OH-Al or OH-Fe species), but they differ greatly in their sorption capacities, in their cation- and anion-

exchange capacities, and in the binding energies of their sorption sites (Sparks, 2003; Violante et al., 2008). The sorption mechanisms are various and involve both physical and chemical processes (Fig. 1.4) (Sparks, 2003). These mechanisms include (Alloway, 2005):

1. Metal exchange in which ions are attracted to oppositely charged sites on the solid surfaces. Metals in a diffuse ion association or in an outer sphere complex are surrounded by waters of hydration and are not directly bonded to the soil surface. These ions accumulate at the interface of the charged surfaces in response to electrostatic forces. These reactions are rapid and reversible with only a weak dependence on the electron configuration of the surface group and the adsorbed ion (Sparks, 2003). These two metal-surface interactions (i.e., diffuse ion and outer sphere complex) contribute to nonspecific adsorption (Bradl, 2004).
2. Specific adsorption in which metals are held by ligand exchange in the form of ionic or covalent bonds. This reaction occurs when no water molecule is present between an ion and a functional group on a solid surface and is also termed inner-sphere complexation (Tack, 2010). Specific adsorption brings about strong and irreversible binding of heavy metal ions with organic and variable charge minerals (McBride, 1994; Bradl, 2004).
3. Co-precipitation in which metal ions are precipitated on surface simultaneously with other inorganic compounds, such as iron, aluminum, and manganese oxides, or calcium carbonate.
4. Insoluble precipitates of metals on surfaces, including the formation of insoluble carbonates, sulfides, phosphates and hydroxides. There is often a continuum between surface complexation (adsorption) and surface precipitation. This continuum depends on several factors: (i) ratio of the number of surface sites vs the number of metal ions in solution; (ii) the strength of the metal oxide bond; and (iii) the degree to which the bulk solution is undersaturated with respect to the metal hydroxide precipitate. At low surface coverages, surface complexation (e.g., outer- and inner-sphere adsorption) tends to dominate. As surface

coverage increases, nucleation occurs and results in the formation of distinct entities or aggregates on the surface. As surface loadings increase further, surface precipitation becomes the dominant mechanism (Sparks, 2003) (Fig. 1.5).

5. Organic complexation with solid-state organic ligands. Humic substances with reactive groups, which include hydroxyl, phenoxyl, and carboxyl groups, form coordination complexes with metals.

6. Fixation or absorption, which involves the diffusion of an aqueous metal species into the solid phase (Sposito, 1989). Like surface precipitation or co-precipitation, absorption is three-dimensional in nature. Heavy metals that are specifically adsorbed onto clay minerals and metal oxides may diffuse into the lattice structures of these minerals. The metals become fixed into the pore spaces of the mineral structure (solid-state diffusion) (Bradl, 2004). This aging process can be irreversible and, thereby, cause a permanent decrease in metal availability (McLaughlin, 2001; Ma et al., 2006).

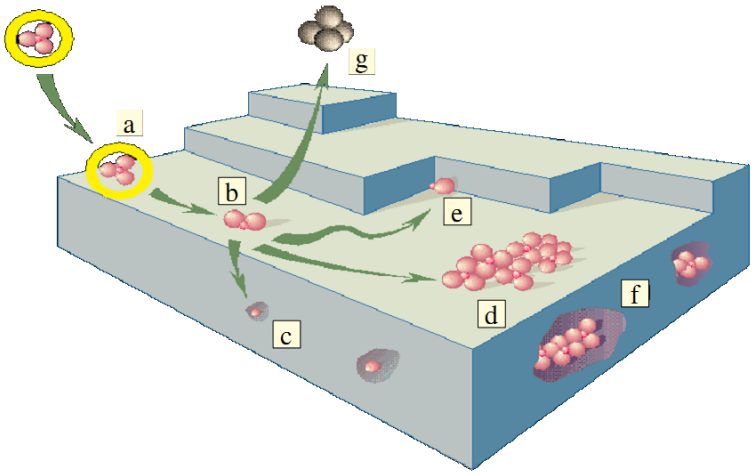


Fig. 1.4 Various mechanisms of sorption of an ion at the mineral/water interface: adsorption of an ion via formation of an outer-sphere complex (a); loss of hydration water and formation of an inner-sphere complex (b); lattice diffusion and isomorphic substitution within the mineral lattice (c); rapid lateral diffusion and formation either of a surface polymer (d), or adsorption on a ledge (which maximizes the number of bonds to the atom) (e). Upon particle growth, surface polymers end up embedded in the lattice structure (f); finally, the adsorbed ion can diffuse back in solution, either as a result of dynamic equilibrium or as a product of surface redox reactions (g) (Sparks, 2003).

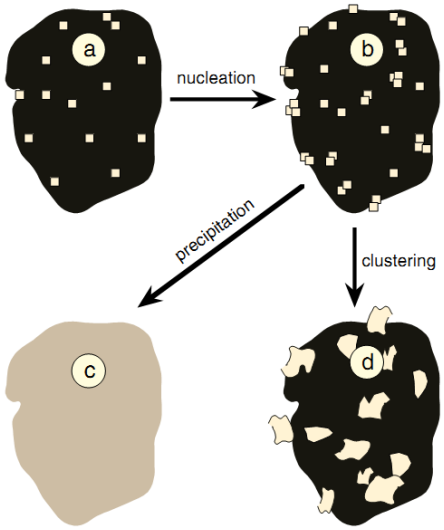


Fig. 1.5 An illustration of metal ion sorption reactions on (hydr)oxide. (a) At low surface coverage, isolated site binding (adsorption) is the dominant sorption mechanism; (b) with increased metal loading, hydroxide nucleation begins. Further increases in metal loadings results in (c) surface precipitation or (d) surface clusters (Sparks, 2003).

The factors controlling adsorption and desorption of metals in soils include (Alloway, 2005):

1. The properties, speciation, and concentration of specific metals.
2. The composition of the soil, especially the relative abundance of clay minerals of different types, and total contents of iron, aluminum and manganese oxides, free calcium carbonate, and organic matter.
3. Soil physicochemical conditions, which include pH, redox status, and concentration of other cations and anions.

2. Biogeochemistry of metals at the soil-root interface

The rhizosphere, a concept which was put forward for the first time by Hiltner (1904), is the volume of soil subjected to the direct influence of root activity (Darrah, 1993; Hinsinger, 1998). In this unique environment, biogeochemical processes are now largely recognized as different with respect to those occurring in the bulk or plant-free soil (Marschner and Römheld, 1996). For decades, the bulk of investigations of chemical elements in the rhizosphere had focused on the macronutrients N, P, and K, and the essential micronutrient Fe, whereas comparably little work was done on the impact of root-induced processes on the solubility, speciation, bioavailability, and mobility (including leaching) of heavy metals in the rootzones of natural and managed ecosystems (Wenzel et al., 2011). The understanding of heavy metal behavior in the rhizosphere is however essential not only because it has a considerable effect on the use of plants for contaminated soil restoration (so-called phytoremediation) but also because rhizosphere is a focal point of entry of heavy metals into the food chain. The major root-induced changes and their effects on the metal mobility and bioavailability are illustrated at Fig. 1.6 and briefly reviewed below. It is important to keep in mind that the rhizosphere is not a fixed environment, but an environment which evolves continuously in space and time. Indeed, many root functions involved in biochemical, chemical and physical processes occurring in the rhizosphere are not evenly distributed along the roots. In addition, a portion of the soil, once it becomes part of the rhizosphere, is exposed to a sequence of events because of the temporal dynamics of root growth (Hinsinger et al., 2005).

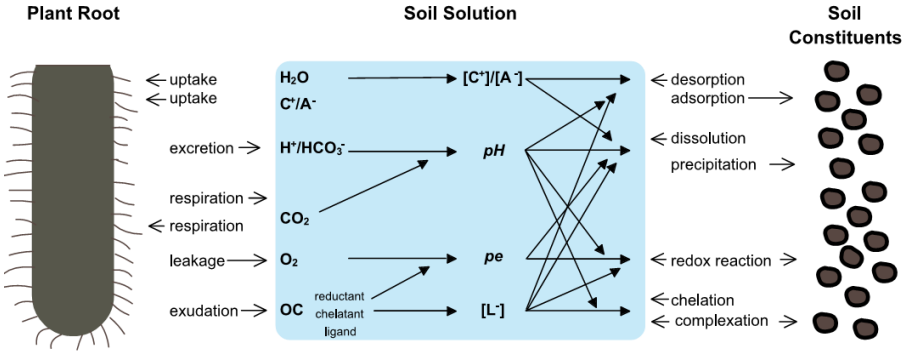


Fig. 1.6 Schematic representation of the rhizosphere, showing the various exudates and how they can influence abiotic factors and mechanisms in the soil-solution interface. Legends: OC = organic carbon; C^+ = cation; A^- = anion; L^- = ligand; pe = redox potential (Modified from Hinsinger, 2001; Adriano et al., 2004).

2.1. Effects of root-induced changes in soil water content

Water uptake by plant roots induces convective transport of solutes toward the root. Depending on how this flux of solutes matches with its uptake by the roots from the soil solution, solutes will either accumulate or be depleted in the rhizosphere (Lorenz et al., 1994; Marschner, 1995). Moreover, whenever a decrease in the concentration of heavy metal occurs, the direct consequence will be a diffusion of the trace element toward the root along the created gradient. According to Le Chatelier's principle (Mass Action Law), a further consequence will be the shift in the various reaction equilibria involving this metal, in order to replenish the soil solution in the rhizosphere (Hinsinger, 2001).

There is no consensus in the literature with respect to the soluble metal content of the rhizosphere. For instance, Lorenz et al. (1997) showed that the soil was generally depleted in water-extractable Cd and Zn after the growth of raddish (*Raphanus sativus* cv. Crystal Ball) as a consequence of the uptake of metals as well as the reduction of the ionic strength and the subsequent changes in the redistribution of metals on exchange sites. Similarly, Knight et al. (1997) reported a Zn depletion in the rhizosphere solution of *Thlaspi* (now *Noccaea*) *caerulescens*. Conversely, Séguin et al. (2004) found an almost systematic increase in water-soluble Al, Cd, Cu, Fe, Mn, Ni, Pb and Zn in the rhizosphere of different tree species. Likewise,

Courchesne et al. (2001) showed a higher $BaCl_2$-exchangeable Al, Fe, Mn, Cu, Zn content in the rhizosphere of forest soils.

2.2. Effects of root-induced changes in pH

Changes of pH in the rhizosphere have been extensively demonstrated and their origins have been reviewed in several publications (Nye, 1981; Marschner, 1995; Hinsinger et al., 2003). The major process responsible for the pH variation in the rhizosphere is the release of H^+ or OH^-/ HCO_3^- due to the unequal uptake of cations and anions by plant roots (Haynes, 1990; Hinsinger et al., 2003). The release is a necessary consequence of the regulation of cellular electrical charge and pH which is maintained within a narrow range of about 7.3 in the cell cytoplasm (Marschner, 1995). When a plant takes up more cations than anions, protons are released from the cytoplasm to the apoplasm to maintain electrical neutrality; these protons are then released into the rhizosphere contributing to its acidification. Conversely, when anion uptake into the cells exceeds cation uptake, the excess negative charge is balanced by the release of hydroxyl or bicarbonate (the former is usually rapidly carbonated by respired CO_2), leading to alkalization of the rhizosphere (Gregory, 2006). The contribution of organic acids released by roots into the rhizosphere is most often considered negligible (Hinsinger and Courchesne, 2008). At the near-neutral pH of the plant cytoplasm, organic acids are almost entirely dissociated from their protons. Consequently, they are released as organic anions rather than as acids (Hinsinger, 2001). The release of organic anions may nevertheless contribute to rhizosphere acidification by inducing the release of protons, or sometimes cations such as K^+ (Murphy et al., 1999), to compensate the release of net negative charge they represent (Ryan et al., 2001) but, in general their effect on pH is small relative to the effect of the inorganic cation/anion balance of the plant (Hinsinger et al., 2003). Soil microorganisms can also be involved in the production of massive amounts of organic acids and even inorganic acids (e.g., nitric acids in nitrifiers) but their contribution to pH gradients has seldom been quantified (Hinsinger and Courchesne, 2008). Finally, although poorly documented, it has been reported that the respiration of roots and microorganisms and the

subsequent buildup of pCO_2 can significantly affect the pH in the rhizosphere (Hinsinger et al., 2003). Given the pK_1 value of carbonic acid (6.36), this process is expected to have a negligible effect in most acidic soils and, conversely, a great effect in neutral to calcareous soils.

According to Solís-Dominguez et al. (2012), screening the ability of plants to maintain an elevated pH is a key point to take into consideration for the long-term successful outcome for phytostabilization in pyritic tailings amended with alkalizing agents (e.g., lime or organic matter). Indeed, root-induced chemical changes in pH can dramatically alter dynamic metal speciation in the rhizosphere. For instance, Bravin et al. (2009) recently showed that Cu depletion in the rhizosphere of durum wheat (*Triticum turgidum durum* L.) grown in a strongly acidic soil was almost entirely driven by the root-induced alkalization while the contribution of root uptake had little impact. Similarly, Cornu et al. (2007) and Chaignon et al. (2009) evidenced that tomato and rapeseed, respectively, were able to significantly alkalize their rhizosphere and thereby reduce Cu bioavailability to plants grown in acidic Cu-contaminated soils. On the contrary, Loosemore et al. (2004) found that root-induced acidification in the rhizosphere of tobacco was so high that it counteracted the effect of lime on the Zn bioavailability alleviation. In pot experiments using *Brassica juncea*, Wu et al. (2003) observed a slight decrease of pH of about 0.5 units within the first 10 days which was followed by a more pronounced decrease from 10 days to 20 days. From this result, the authors concluded that rhizosphere acidification could be one of the important mechanisms by which *Brassica juncea* mobilizes nutrients and heavy metals. Using planar pH optodes, Blossfeld et al. (2010) observed an alkalization of the rhizosphere of both alpine pennycress (*Noccaea caerulescens* (J. Presl & C. Presl) F.K. Mey) (Fig. 1.7a) and ryegrass (*Lolium perenne* L.) and a concomitant decrease of the Cd availability compared to the bulk soil. Conversely, in the rhizosphere of maize (*Zea mays* L.), they found lower pH (Fig. 1.7b) and subsequent higher available Cd concentrations relative to those occurring in the bulk soil.

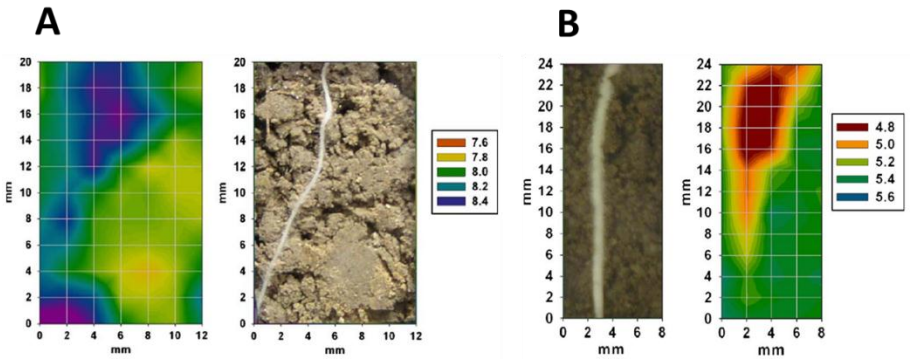


Fig. 1.7 2-D image of a typical rhizospheric pH pattern of (a) young alpine pennycress and (b) young maize roots obtained using planar pH optodes (Blossfeld et al., 2010).

The contrasting effects of plants may be attributed to differences in terms not only of plant species but also of soils investigated in those studies. Indeed, when considering pH changes in the rhizosphere, it should not be forgoten that the corresponding fluxes of protons can vary considerably according to the buffering capacity of the soil (Hinsinger et al., 2003). For a given efflux of protons, the pH will highly decrease or virtually not change if the soil is poorly or strongly, respectively, buffered (Fitz et al., 2003; Vysloužilová et al., 2006). However, in both cases, the protons will react with the soil solution and soil solid phase and might thereby affect the fate of heavy metals (Hinsinger and Courchesne, 2008).

2.3. Effects of root-induced changes in redox conditions

Root activity alters rhizosphere redox potential through respiratory oxygen (O) consumption and ion uptake or exudation (Fageria and Stone, 2006). Although reports in the literature of redox-driven changes in heavy metal speciation in the rhizosphere soil solution compared to the bulk soil are almost nonexistent, there is some evidence for changes in solid-phase speciation (Hinsinger and Courchesne, 2008). In anaerobic soils, rice as well as other wetland plants can release oxygen into the rhizosphere (Brix et al., 1996) and increase the redox potential (Flessa and Fischer, 1992; Begg et al., 1994). This induces the formation of Fe plaque and subsequent sorption and immobilization of metals such as Zn and Pb (Shuman and Wang, 1997; Yang et al., 2012) and metalloids such as As (Blute et al., 2004; Hu et al.,

2007). Recently, Lin et al. (2010) also found that an increase in redox potential in rice rhizosphere contributed to increase Cu solubility due to the oxidation of sulfur and organic compounds. It is also known that under reduced conditions, Fe and Mn, as well as associated ions (adsorbed or occluded), are solubilized. In well-aerated soils, reducing conditions however are mainly limited to the plant root surface (strategy I plants) where membrane-bound reductases promote the reduction of Fe^{3+} to Fe^{2+}, shifting the dissolution–precipitation equilibrium of Fe oxides toward dissolution (Wenzel et al., 2011).

2.4. Effects of root-induced changes in organic substances

Organic compounds are released in the soil from decomposing organic tissues and by all the organisms contributing to the rhizosphere: plant roots, mycorrhizas, free-living fungi, bacteria, algae, and microfauna (Hinsinger and Courchesne, 2008). Because of their high reactivity, they contribute to almost every biogeochemical reactions occurring in the rhizosphere, and thereby affect the speciation and mobility of heavy metals. In higher plants, a substantial proportion (20 – 60%) of C fixed during photosynthesis can be translocated below ground (Grayston et al., 1997; Kuzyakov and Domanski, 2000). Depending on root activity, 15 – 60% of this C fraction is used for root respiration and ultimately released as CO_2 (Lambers et al., 2002). Of the assimilates translocated below ground, up to 70% in perennial and up to 40% in annual plants enter the soil as organic rhizodeposition, corresponding to 800 – 4500 kg C ha^{-1} $year^{-1}$ (Lynch and Whipps, 1990; Swinnen et al., 1995; Kuzyakov and Domanski, 2000; Nguyen, 2003). Mass balance of the various pools of rhizodeposits is illustrated at Fig. 1.8 while the origin of these pools is depicted at Fig. 1.9. Rhizodeposition include lysates of sloughed-off cells and tissues resulting from root turnover, which account for up to 50 % of C translocated below ground (Grayston et al., 1997), and root exudates released from intact root cells. These latter can be subdivided into (1) diffusates, i.e. organic compounds continuously lost from plant roots by diffusion, (2) root exudates released as metabolic waste products, and (3) secretions with special functions in nutrient mobilization, detoxification, plant-microbial

signaling and defense reactions (Dakora and Phillips, 2002; Rengel, 2002; Walker et al., 2003; Neumann, 2007; Dennis et al., 2010). Root exudates may comprise 5 – 10% of the net fixed carbon in soil-grown plant (Jones et al., 2004). Very subtle changes within the rhizosphere can induce rapid shifts in the quality and quantity of root exudates (Dilkes et al., 2004), but the responsiveness of mucilages and sloughed-off root cells/tissues to such changes is unknown (Dennis et al., 2010). Moreover, by providing a major energy source for microbial growth, rhizodeposition stimulates the microbial activity in the rhizosphere (Paterson et al., 2007; Nannipieri et al., 2008; Denef et al., 2009). Microorganisms not only consume the organic compounds produced by roots, but also produce and release organic substances, notably low-molecular-mass organic acids (LMMOAs), and thus add to the total flux of organic C in the rhizosphere (Hinsinger and Courchesne, 2008). The outstanding importance of the rhizosphere for nutrients and heavy metals mobility is illustrated by the fact that the release of organic rhizodeposits, which can account for 30 – 40% of the total soil organic matter input, occurs into the rhizosphere soil, which comprises only 2 – 3% of the total soil volume (Grayston et al., 1997; Neumann, 2007).

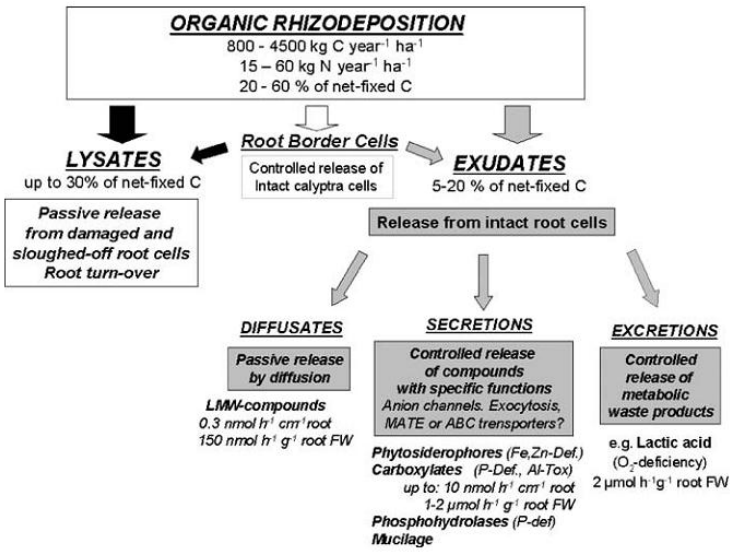


Fig. 1.8 Classification, quantities and release mechanism of organic rhizodeposition (Neumann, 2007).

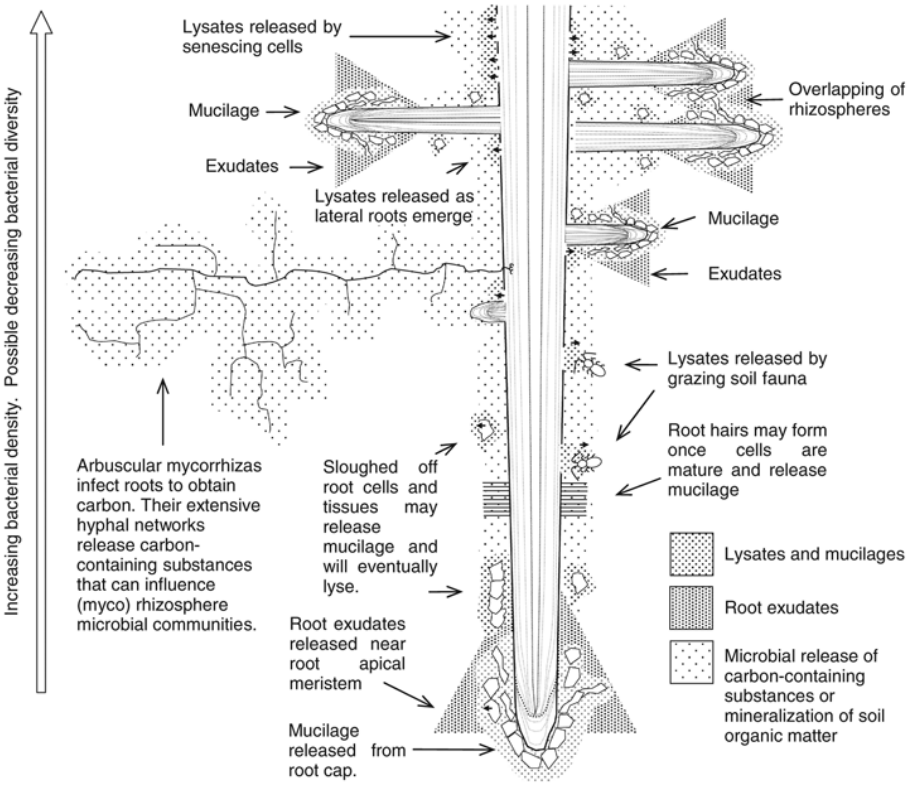


Fig. 1.9 Origins of various pools of rhizodeposits (Dennis et al., 2010).

The abundant organic ligands both actively and passively released by roots in the rhizosphere can form strong complexes or chelates with a range of metals (Hinsinger, 2001; Wenzel et al., 2011). For instance, Hamon et al. (1995) noted that while almost 100% of total soluble Zn and Cd were present as free metal ions in the solution of unplanted soil, 80 to 100% of Zn and 50 to 90% of Cd were complexed once radish had grown and produced complexing exudates. Similarly, root exudation was found to increase the proportion of complexed Co, Zn and Mn, respectively, from 6.4, 1.9 and 0.2 % under fallow to 61, 16 and 8% after 6 weeks of maize growth and to 31, 15 and 1% after 6 weeks of wheat growth (Merckx et al., 1986). Those complexing or chelating exudates may greatly influences the metal sorption-desorption onto and from soil components as well as their toxicity and phytoavailability (Violante et al., 2008). Li et al. (2011) showed that dissolved organic matter (DOM) derived from the rhizosphere of

hyperaccumulating ecotype of *Sedum alfredii* could significantly reduce metal sorption and increase its mobility through the formation of soluble DOM-metal complexes. Compared to the bulk soil, the rhizosphere of durum wheat was found enriched in DOM, which resulted in a decrease in free Cu^{2+} concentration in solution but, in the meantime, in a mobilization of Cu sorbed from the soil solid phase and an increase in the concentration of labile Cu in soil (Bravin et al., 2012). During organic anion exudation bursts, heavy metals as well as DOC from soil organic matter were strongly mobilized in the rhizosphere of *Lupinus albus* cluster roots (Dessureault-Rompré et al., 2008). Authors attributed the Al, Fe and Zn mobilization to complexation with exuded citrate while the Cu and Pb mobilization was due to their complexation with DOC derived from soil organic matter.

The release of carboxylic acids such as citrate, malate, oxalate, and malonate has been found to significantly increase in P- and Fe- (strategy I plants only) deficient plants, and several authors have reported a concomitant increase of other heavy metals such as Cu, Mn, and Zn (Dakora and Phillips, 2002). Phenolics and mucilage have also been suggested to play an important role in enhancing heavy metal bioavailability via complex formation in the rhizosphere (Morel et al., 1986; Quartacci et al., 2009). For instance, Degryse et al. (2008) found that root exudates of dicotyledonous plants (spinach and tomato) were able to mobilize Cu and Zn and that plants responded to Zn deficiency by exuding root exudates with higher metal affinity than those released at adequate Zn supply. Root exudates with higher metal affinity were suggested phenolics. One of the most remarkable groups of root exudates are the so-called phytosiderophores (Takagi et al., 1984; Marschner and Romheld, 1994). Phytosiderophores can be mugineic acids, whose the chemical structure as well as that of two derivatives are shown in Fig. 1.10. They are released by graminaceous plants (roots) by Fe acquisition mechanism (strategy II). They are secreted through efflux transporter (Nozoye et al., 2010) and then solubilize Fe in the soil, forming Fe-phytosiderophore complexes that are absorbed into root cells through a Fe-phytotransporter (Curie et al., 2001). Several studies have shown that such Fe-complexing compounds can efficiently dissolve Fe from poorly oxides, including goethite, which is a ubiquitous crystalline oxyhydroxide in soils (Kraemer, 2004; Reichard et al., 2005; Reichard et al.,

2007). However, although less documented than for Fe, phytosiderophores can also be released under Zn and Cu deficiency (Cakmak et al., 1996; von Wiren et al., 1996; Gries et al., 1998; Hopkins et al., 1998; Arnold et al., 2010). Indeed, because they can form strong complexes with metals (Dell'mour et al., 2010), phytosiderophores are capable of solubilizing not only Fe but also other metals such as Mn, Cu and Zn, thus mobilizing these metals from sparingly metal pools. For instance, Treeby et al. (1989) showed that Fe deficiency increased the release of phytosiderophores by cereals, which ultimately resulted in increased bioavailability of Cu, Mn and Zn. Similarly, Chaignon et al. (2002b) found that the growth of wheat on a Cu-contaminated soil under Fe deficiency resulted in a roughly threefold increase in the phytosiderophore release rate and in the bioavailability of Cu and Zn.

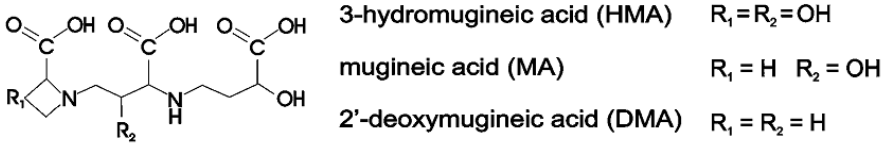


Fig. 1.10 Chemical structure of (1) mugineic acid and its derivatives (2) 2'-deoxymugineic acid and (3) 3-hydroxymugineic acid.

In contrast, it has also been reported that root exudation of organic ligands is one of the most important strategies used by plants to exclude metals by chelating them in the rhizosphere or in the apoplastic space and thus preventing their entry into the symplast (Zheng et al., 1998; Yang et al., 2000; Hill et al., 2002; Watanabe and Osaki, 2002). Secretion of high amounts of carboxylates, most commonly citrate and malate, has been observed in the rhizosphere of several plants exposed to phytotoxic Al concentrations (Kochian, 1995; Pellet et al., 1996; Ma et al., 2001; Ryan et al., 2001; Barceló and Poschenrieder, 2002; Ryan et al., 2009). Although much less documented in the literature, higher concentrations of organic exudates have also been found in the rhizosphere of plants exposed to other heavy metals than Al. Exposure to Ni led to greater exudation of Ni-chelating histidine and citrate in the rhizosphere of non-hyperaccumulator *Thlaspi arvense* than the hyperaccumulator *T. caerulescens* suggesting that exudation of both compounds help reduce Ni uptake and so play a role in a Ni-detoxification strategy (Salt et al., 1999). Qin et al. (2007) showed that

exposure to Cu stress induced root exudation of oxalate, malate and formate and that Zn exposure induced exudation of formate from poplar (*Populus tremula* L.). Cattani et al. (2006) observed that DOC concentrations in the rhizosphere of maize were threefold higher than in the bulk soil; this increase in DOC concentration was thought to contribute to the alleviation of Cu toxicity by promoting the formation of Cu-DOC complexes.

3. Phytostabilization

3.1. Concepts and current state

Soils contain many legacies from a less sustainable industrial past. Whereas water and air have much improved due to source-oriented measures, the soil does not (or very slowly) improve by itself after reducing or closing the sources. Soils and sediments are sinks for many contaminating substances and can only improve in a reasonable time if an active clean-up operation is performed. However, metal-contaminated soils are notoriously hard to remediate with conventional technologies (Marques et al., 2009) such as those described in Table 1.1. Although highly variable and dependent on the contaminants of concern, soil properties, site conditions, and so on, the usually enormous costs associated with the removal of metals from soils by means of traditional physicochemical methods explain why most companies tend to ignore the problem. Due to the fact that very often large areas are affected by heavy metal contamination, a removal is certainly difficult (Alkorta et al., 2004). Therefore, within the framework of the European Strategy for Soil Protection, the need to develop new, viable and *in situ* technologies for soil protection and remediation is a priority objective.

The last two decades have seen the emergence of eco-friendly, gentle remediation techniques using different plant species (Kidd et al., 2009). These phytotechnologies, collectively known as phytoremediation, are generally considered to be less invasive, less expensive, aesthetically pleasant and restorative of soil structure and functions compared to conventional, civil-engineering technologies (Cunningham and Berti, 1993; Salt et al., 1998). According to several authors (Barceló and Poschenrieder, 2003), conventional procedures raise the average cost per contaminated hectare of soil from 0.27 to 1.6 million $, while phytoremediation costs from about 10 to 1000 times less.

Table 1.1 Technologies for remediation of heavy metal contaminated soil (Marques et al., 2009).

Technology	Mechanism of action
Soil washing	The process separates coarse soil (sand and gravel) from fine soil (silt and clay), where contaminants tend to bind and sorb. This soil fraction must be further treated with other technologies.
Soil vapor extraction	Involves the installation of wells in the area of contamination. Vacuum is applied through the wells to evaporate the volatile constituents of the contaminated mass, which are subsequently withdrawn through an extraction well. Afterward, the extracted vapors are adequately treated.
Soil flushing	"Floods" contaminated soils with a solution that moves the contaminant to an area where they can be removed. Soil flushing is accomplished by passing an extraction fluid through soils using an injection or infiltration process. Recovered fluids with the absorbed contaminants may need further treatment.
Solidification	Encapsulates the waste materials in a monolithic solid of high structural integrity.
Vitrification	Uses a powerful source of energy to "melt" soil at extremely high temperatures (1600–2000 °C), immobilizing most inorganics into a chemically inert, stable glass product and destroying organic pollutants by pyrolysis.
Electrokinetics	Removes contaminants from soil by application of an electric field.
Thermal desorption	Contaminated soil is excavated, screened, and heated to temperatures such that the boiling point of the contaminants is reached, and they are released from the soil. The vaporized contaminants are often collected and treated by other means.
Encapsulation	Physical isolation and containment of the contaminated material. The impacted soils are isolated by low permeability caps or walls to limit the infiltration of precipitation.

Phytoremediation incorporates a range of phytotechnologies which differ according to the process by which plants remove, immobilize, or degrade contaminants (Pilon-Smits and Freeman, 2006). The number of these processes has recently increased with the development of new research domains, and consequently new terms have been introduced to describe several new techniques and findings in addition to the already existing studies (Fig. 1.11). Moreover, reviewing the common terms that have been used in the last two decades in relation to phytotechnologies, Conesa et al. (2012) showed the lack of normalization among researchers. For example,

rhizodegradation has been defined as the use of metabolic capabilities of plants and rhizosphere microorganisms to degrade organic pollutants (Wenzel, 2009). This process has also been referred to as phytostimulation, enhanced rhizosphere degradation, rhizosphere bioremediation, plant-assisted bioremediation, or plant-aided *in situ* biodegradation (Conesa et al., 2012).

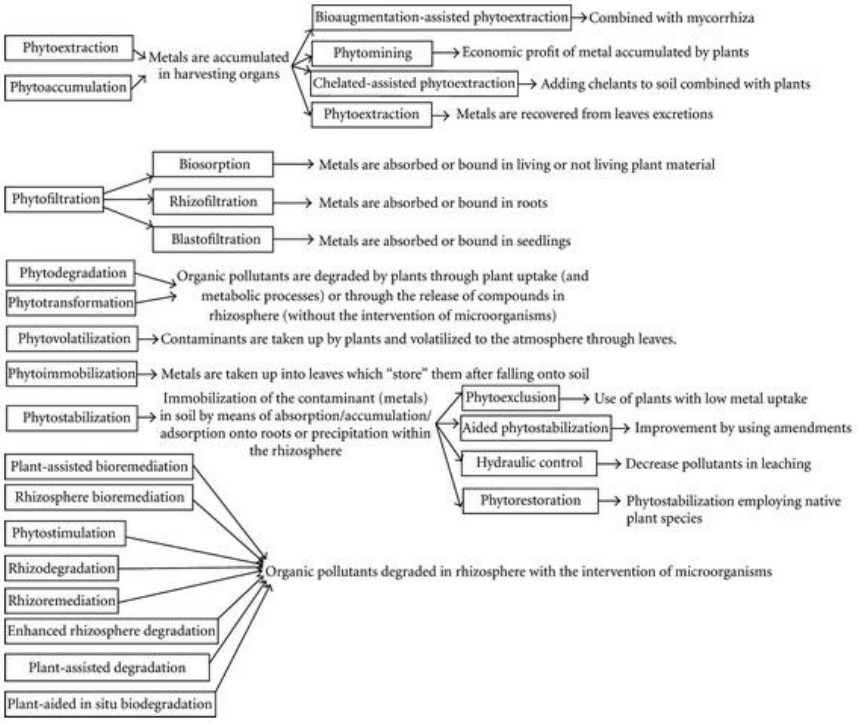


Fig. 1.11 Current classification of most frequently used phytotechnologies for soil remediation (Conesa et al., 2012).

The technique in which plants are used to remove contaminants through volatilization is called phytovolatilization (Moreno et al., 2005). Similarly, the process in which plants are used to remove heavy metals from soil and water and store them in harvestable tissue is called phytoextraction (Raskin, 1995; McGrath and Zhao, 2003). On the contrary to phytoextraction, phytostabilization is not a technology for real clean-up of contaminated soils, but a management strategy for stabilizing contaminants

which are potentially toxic (Vangronsveld et al., 2009). The purpose of phytostabilization (Fig. 1.12) is to establish a vegetation cover which will be effective in providing the necessary surface stability to prevent wind-blow of contaminated particulates, and in reducing water pollution by interception of a substantial proportion of incident precipitation (Vangronsveld et al., 1995; Tordoff et al., 2000). Plants may also help to contain contaminants within the vadose zone through accumulation by roots and precipitation within the rhizosphere (Bolan et al., 2011). Moreover, vegetative stabilization also improves the chemical and biological characteristics of the contaminated soil by increasing the organic matter content, nutrient levels, cation exchange capacity and biological activity (Arienzo et al., 2004). These chemical, biological and physical improvements accelerate the development of a viable nutrient cycle and self-sustaining vegetative cover and restore the affected area to some acceptable steady-state condition for secondary land use (Norland and Veith, 1995).

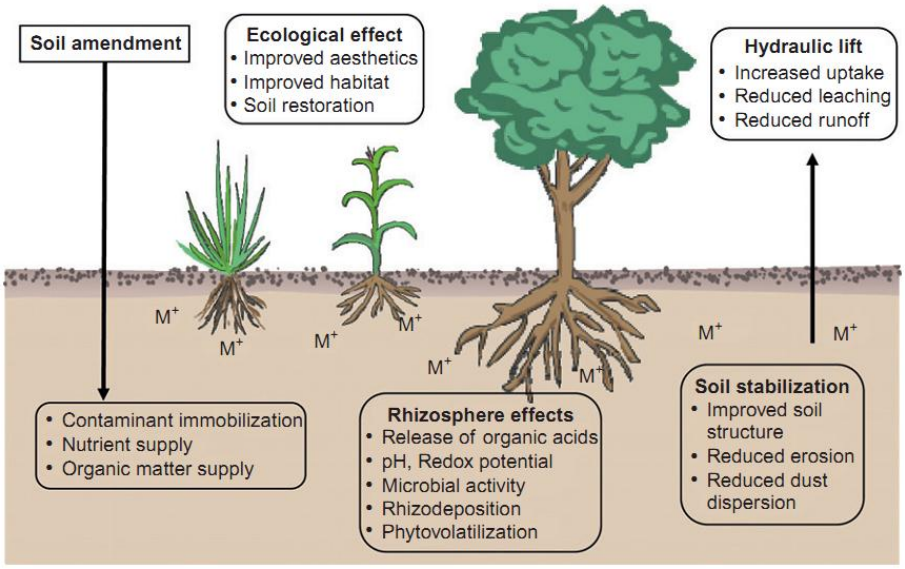


Fig. 1.12 Schematic diagram illustrating the potential action of phytostabilization on contaminants in soil (Bolan et al., 2011).

For heavy metals, the original concept of phytoremediation preferentially focused on phytoextraction, while phytostabilization received much less attention. Because phytoextraction aims at removing heavy metal from the

soil using plant, the research was mainly concentrated on the search for hyperaccumulator species or using biotechnology (study of metabolic mechanisms, genetic engineering) to increase metal uptake. This development resulted in many important findings in plant science that relate to plant-pollutant interactions and to the selection of plants species whith hyperaccumulation characteristics.

However, successful phytoextraction requires the cleansing of the soil to a level that complies with environmental regulations. Reviewing the current state of phytotechnologies to remediate soils, Conesa et al. (2012) stressed that field trials or commercial operations that demonstrate successful phytoextraction are conspicuously absent so far. Phytoextraction implementation is currently limited by the lack of commercially-available plant strains that can act in the absence of chemical modification of the contaminated soil. Rigorous evaluation of excluder, tolerant and metallophyte plant species is in progress, and there is increasing interest in exploiting biodiversity to phytoremediate a contaminated-soil. In soils that are contaminated with a number of metals, one or more elements may limit phytoextraction potential: e.g. high soil Cu content can reduce the growth of *Noccaea caerulescens*, and thus Zn and Cd phytoextraction (Mench et al., 2006). Enhancing metal desorption from soil to solution using synthetic chelates such as EDTA has been considered to promote phytoextraction (Huang et al., 1997; Lombi et al., 2001; Luo et al., 2005). However, in a critical review, Nowack et al. (2006) provide convincing evidence that at the level of chelatant addition that is required for significantly enhanced metal accumulation in the plant, only a small part of the mobilized metal complexes can be taken up. As a result, leaching of metals is unavoidable (Sun et al., 2001; Wenzel et al., 2003; Lambrechts et al., 2011b). The relatively high costs of the chelants and the risk of metal leaching they induce therefore restricts the use of this technique to sites that are not connected to groundwater (Nowack et al., 2006; Evangelou et al., 2007; Wenzel, 2009). The accumulation of high amounts of heavy metals in shoots brought additional issues of practicability for phytoextraction strategies, such as the further treatment of biomass and the entering of metals in the food chain. Calculations have also showed that soils with moderate or high heavy metal contents could not be remediated

by phytoextraction because it would take an unacceptable time to remove pollutants (Conesa et al., 2006; Robinson et al., 2009). As a result of these limitations, many of the pioneering companies which started in the phytoremediation business during the last 15 years by offering phytoextraction of radionuclides and heavy metals (e.g., Phytotech Inc.), stopped operating (Conesa et al., 2012).

Therefore, there has been a progressive shift away from phytoextraction towards phytostabilization, which seems having more scope of application (Dickinson et al., 2009). However, although more than two decades of research have focused on phytotechnologies, phytoextraction as well as phytostabilization are considered as a tool which is still qualified as "promising" rather than an effective remediation solution (Conesa et al., 2012). This highlights that the reliability of phytoremediation, even inside the scientific community, has not yet been achieved. As a result, nonscientific stakeholders in contaminated sites are still skeptical about its current applicability or future prospects. There is thus an urgent need to provide robust evidence not only of the efficacy of phytostabilization in immobilizing heavy metals but also of its ability to generate both economic and environmental benefits in order to make it commercially successful.

3.2. Improving phytostabilization using suitable plants, amendments and biochar

3.2.1. Choosing the plants

Selecting the plants suitable for the restoration is one of the key factors to carry out revegetation of heavy metal contaminated sites. Plant community tolerant to the metals plays a major role in restoration of heavy metal contaminated sites. Plants suitable for restoration should develop an extensive root system and a large amount of biomass while keeping the translocation of metals from roots to shoots as low as possible in soils with high metal concentrations. In addition, they must adapt to diverse site conditions, establish readily, and require little money and effort to maintain. Further, they must be able to survive and reproduce in contaminated soil (Bolan et al., 2011). Phytostabilization can be achieved by the spontaneous natural revegetation of the contaminated site. Indeed,

although metal contaminated soils usually present very unfavorable conditions for plant establishment, a few species of metal-tolerant plants can survive in these extreme environments and spontaneously colonize the site, thereby serving as an effective means of site stabilization (Smith and Bradshaw, 1979; Bradshaw, 1997; Whiting et al., 2004; Conesa et al., 2006; Orchard et al., 2009). Besides, some authors aim at determining the plant – soil relationships for such native pioneer species. The identification of these relationships allows to assess further revegetation options by selecting the most suitable plant species to revegetate contaminated soils and tailings (Conesa et al., 2006; Antosiewicz et al., 2008; Brunetti et al., 2009).

Plant tolerance to heavy metals is an essential condition for the phytostabilization of contaminated sites. When planting on a contaminated site, species or varieties are required that tolerate contaminants. All plants have basic heavy metal tolerance mechanisms but some species and varieties can survive in soil with inordinately high concentrations of heavy metals (Robinson et al., 2009). Mostly, such plants are only tolerant to the heavy metals that occur in the soil in which they grow, indicating that their tolerance mechanisms are element specific. According to Robinson et al. (2009), there are nine groups of tolerance mechanisms:

- Root avoidance
- External sequestration by bacteria, mycorrhizal fungi
- Exuding chelants that render the heavy metals unavailable for plant uptake
- Restriction of transport across plasmalemma into the cell
- Active efflux into the apoplast (pumping)
- Chelation of the heavy metals in the cytosol by phytochelatins, metallothieneins or organic acids
- Production of heat-shock proteins that repair cellular damage
- Storage of chelated heavy metals in the vacuole
- Abscission of organs, such as leaves, with a high heavy metal load.

Moreover, plant species are usually divided into (hyper)accumulators, indicators, and excluders according to their above-ground heavy metal concentration in relation to the heavy metal concentration in the soil (Fig. 1.13).

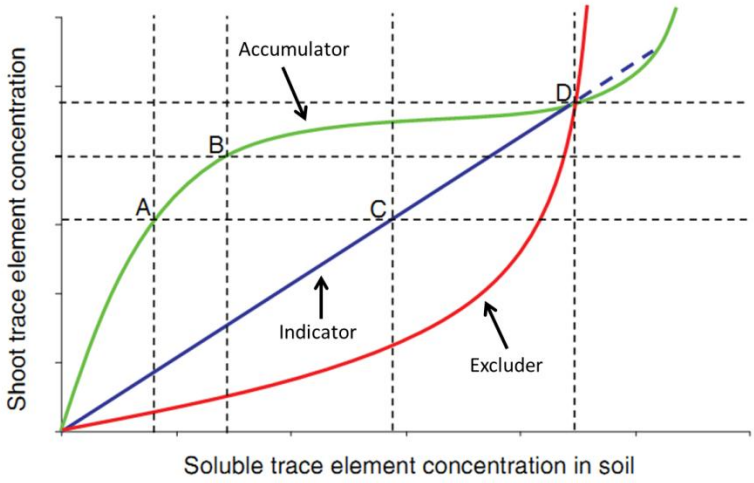


Fig. 1.13 Plant uptake of heavy metals as a function of soluble heavy metals in the soil (adapted from Baker, 1981 and Robinson et al. 2009). Points A and C are sufficient concentrations for accumulators and indicators. Point B is the optimal concentrations for accumulators. Concentrations above point D result in phytotoxicity.

Accumulator and hyperaccumulator plants (Fig. 1.13) actively accumulate heavy metals into the shoots when the shoots are below a sufficient concentration (Fig. 1.13, point A). Above this concentration, luxury uptake occurs until the shoot concentration reaches an optimal level (Fig. 1.13, point B). At higher concentration, roots may restrict heavy metal uptake until the threshold concentration is reached (Fig. 1.13, point D), where the mechanisms of homeostasis break down and phytotoxicity symptoms appear (Robinson et al., 2009). Hyperaccumulators have been defined as the plant species that are capable of accumulating metal(loid)s above the threshold concentrations of 10,000 mg kg^{-1} dry weight of shoots for Zn and Mn, 1000 mg kg^{-1} for Co, Cu, Ni, As and Se, and 100 mg kg^{-1} for Cd (Baker and Brooks, 1989). Nowadays, the bioconcentration factor (the shoot to root ratio of metal concentration, which measures the efficiency of the translocation) is used to define hyperaccumulators. A bioconcentration factor greater than 1 characterizes hyperaccumulator plants (McGrath and Zhao, 2003; Bolan et al., 2011). The potential of hyperaccumulators for phytostabilization still remains to be demonstrated. Generally, hyperaccumulators have a low biomass production, are exotic to most environments, and potentially facilitate the entry of metals into the food chain. Nevertheless, the hyperaccumulator *Alyssum bertolonii* has been

used successfully as a colonizing plant in mine spoil (Robinson et al., 2009). Here, the desirable property of the hyperaccumulator was its ability to tolerate nutrient imbalances and high Ni concentrations in the mine spoil, while adding organic matter to the spoil, thus allowing the spontaneous colonization of other species.

Indicator plants (Fig. 1.13) take up heavy metals in proportion to their soluble concentration in the soil. The shoot concentration indicates thus the soil concentration. Such an uptake implies that specific mechanisms for homeostasis are absent in the plant. Passive uptake occurs both above and below the sufficient tissue concentration (Fig. 1.13, point C; Robinson et al., 2009).

For nonessential metals, such as Cd, Pb and As, as well as essential metals that occur in high concentrations in soil, some plants exhibit active exclusion mechanisms (Fig. 1.13). In this strategy, the metals are maintained at a low tissue concentration, until the regulatory mechanisms are overloaded, or there is a disruption of the plasma membrane at the apoplast/symplast interface. When this occurs, heavy metals flood into the plant, resulting in reduced growth accompanied by chlorosis or necrosis (Robinson et al., 2009). Excluder plants are generally considered ideal candidates for the phytostabilization of soils with metal concentrations so high that they are phytotoxic for other species. Indeed, such plants can stabilize the soil surface, reducing erosion and leaching, while minimizing the risk that heavy metals enter the food chain via herbivores.

Besides metal tolerance and translocation, other criteria can be taken into account for the selection of plants for phytostabilization strategy. For instance, grasses (e.g. *Agrostis tenuis, Lolium perenne, Festuca rubra*) are considered as suitable candidates because of their good surface cover and their ability to form a dense rooting system (Smith and Bradshaw, 1979; Arienzo et al., 2004; Lambrechts et al., 2011). Leguminous (e.g. *Trifolium repens*, *Lupinus albus*), alone or in combination with grasses, have been suggested because of their ability to fix atmospheric nitrogen (Tordoff et al., 2000; Mains et al., 2006; Vazquez et al., 2006). Plants with economic interest such as energy crops (e.g. rapeseed, energy maize, miscanthus) have also been recommended in order to make phytostabilization

commercially attractive (Van Ginneken et al., 2007; Korzeniowska et al., 2011).

3.2.2. Classical amendments

In most instances, phytostabilization of a contaminated site is enhanced by the use of naturally occurring or artificial soil amendments (Fig. 1.12). These play three major roles when applied to contaminated soils: (i) improvement of the physical nature of the rooting medium, especially improving water and nutrient-holding capacity; (ii) supply of plant nutrients in a slow-release form, facilitating plant establishment; and (iii) *in situ* chemical immobilization of metals, reducing metal leachability and phytotoxicity (Alvarenga et al., 2008). A long lasting, if not permanent, effect is required. Amendments should be: inexpensive, readily available, easy to apply with low associated risks, devoid of disruptive or adverse effects, especially on the soil structure and fertility, compatible with plants used for revegetation, suitable for different contaminants, and in compliance with regulations (Berti and Cunningham, 2000). In addition to the listed considerations, some amendments may be useful to optimize plant growth by supplying nutrients and increasing soil water-holding capacity (e.g. phosphate fertilizers and organic materials).

Numerous organic and inorganic materials have been used to reduce solubility and bioavailability of heavy metals in contaminated soils. These amendments notably include liming material (Bolan et al., 2003; Geebelen et al., 2003; Alvarenga et al., 2008), phosphate (Basta and McGowen, 2004; Sneddon et al., 2006), zeolite (Oste et al., 2002a), alumino-silicates such as bentonite or fly ash (Geebelen et al., 2003; Ruttens et al., 2006a; González-Núñez et al., 2011) , Fe-based materials such as zero-valent iron, Fe oxides or red mud (Lombi et al., 2003; Hartley et al., 2004; Kumpiene et al., 2006; Komárek et al., 2013), and organic matter (Brown et al., 2003; Park et al., 2011b). Physical and chemical properties of these amendments have been discussed in several reviews (Knox et al., 2001; Bolan and Duraisamy, 2003; Adriano et al., 2004; Kumpiene et al., 2008; Kumpiene, 2010). These additives potentially reduce exposure by one or more of the following processes: sorption, redox reaction, precipitation, co-precipitation, ion exchange, complexation and excess of competing elements (see a few

examples in Table 1.2) (Berti and Cunningham, 2000; Mench et al., 2006). Moreover, beyond the metal immobilization in soils, an additional advantage to this technique is that some amendments are inexpensive and readily available in large quantities because they derive from bio-products or industrial by-products (Ciccu et al., 2003; Lombi et al., 2004; Guo et al., 2006; Kumpiene et al., 2008; Burgos et al., 2010). *In situ* metal immobilization using such materials may therefore be an enticing option to reclaim contaminated while effectively diverting materials from the waste stream and reusing them (Gadepalle et al., 2007). The cost-effectiveness of these amendments is a parameter of particular importance since the amount of amendments which is required for soil remediation is generally very high (1 - 10% w/w, i.e. 26 – 260 t ha^{-1}).

Table 1.2 Soil amendments in phytostabilization, suggested applicability to metal contaminants, and possible modes of contaminant inactivation (from Berti and Cunnigham, 2000).

Amendment type		Possible target contaminants	Suggested mode of inactivation
Phosphate materials	H3PO4, apatite, calcium orthophosphates, Na2HPO4, KH2PO4, other phosphate fertilizers, high-phosphate by-products (e.g. bone meal)	Pb	Formation of insoluble metal phosphate minerals, such as Pb pyromorphites
Hydrous Fe oxides	Iron rich or other by-products containing Fe oxides, isolated hydrous Fe oxides	As, Cd, Cu, Ni, Pb, Zn	Sorption of contaminants on oxide surface exchange sites, corprecipitation, or formation of contaminants – Fe compounds
Organic materials	Manure, composts, sludges, and other biosolids	As, Cd, Cu, Pb, Zn	Sorption of contaminants on exchange sites, or incorporation into the organic material
Inorganic clay minerals	Synthetic zeolites, natural aluminosilicates, or aluminosilicates by products from burning or coal refuse (e.g. fly ash)	As, Cd, Cu, Mn, Ni, Pb, Zn	Sorption of contaminants on mineral surface exchange sites, or incorporation into the mineral structure

3.2.3. Biochar: a promising amendment

3.2.3.1. Background

Biochar is a carbon-rich, fine-grained, porous substance, which is produced by thermal decomposition of organic material (e.g. wood, manure or leaves) under limited supply of oxygen (O_2), and at relatively low temperatures (<700°C) (Lehmann and Joseph, 2009a; Sohi et al., 2009). In order to distinguish biochar from charcoal, the definition adopted by the International Biochar Initiative (IBI) furthermore specifies the need for purposeful application of the material to soil for agricultural and environmental gains (Sohi et al., 2009). The use of biochar as a soil additive has recently stimulated a broad interest to simultaneously mitigate anthropogenic climate change while improving soil fertility and enhancing crop production (Lehmann et al., 2006). The idea of adding biochar to soil in order to increase its fertility is to be inspired by the ancient agricultural practices, by means of which dark earths in the Amazon Basin, *Terra Preta de Índio* (Fig. 1.14), were created (Manyà, 2012). These anthropogenic soils, which occupy at least 10% of Amazonia (Glaser and Birk, 2012), are very fertile areas interspersed with relatively infertile soils (Glaser et al., 2001). Although it is unclear if *Terra Preta* was produced intentionally or un-intentionally, literature data indicate that it results from the product of inorganic (e.g. ash, bones) and organic (e.g. biomass wastes, manure, excrements, urine and biochar) amendments to infertile Ferralsols incorporated by pre-Columbian inhabitants (Glaser and Birk, 2012). In these soils, aromaticity values estimated by extended X-ray absorption fine structure (EXAFS) and nonquantitative cross-polarization NMR suggest that char accounts for ∼25−50% of organic carbon (Solomon et al., 2007).

Fig. 1.14 Left: Typical Ferralsol profile. Right: typical adjacent *Terra Preta* profile. The topsoil horizons are dark grey or black colored and can reach a depth of more than 1 m. Potsherds, small bone and charcoal particles are characteristic for this horizon. Roots reach deeper down in higher density than in Ferralsols and signs of bioturbation and aggregates of biogenic origin can be found frequently (from Glaser et al., 2001, 2012).

Amazonian *Terra Preta* soils are not the only char-containing soils in the World. For instance, Mollisols are other fertile soils with high biochar content. These grassland-derived soils are typically classified as Phaeosems, Chernozems, and Kastanozems and can be extensively found in central North America, Ukraine, Germany, the Russian Federation, Argentina and Uruguay where they contribute to a significant part of the global grain production (Mao et al., 2012). The char in these soils is mainly attributed to grassland fires (Rodionov et al., 2010). Even in Wallonia, char-containing soils are ubiquitous (Fig. 1.15a). They result from the intensive charcoal production in forests by the "mound kiln" method (Fig. 1.15b) until the late 19th century for the needs of steel industries (Dufey and Hardy, accepted). Mound kiln sites can be identified in ploughed fields as dark circular or elliptical areas 25 to 40 m in diameter (Fig. 1.15c), and on forest floors as heightened domes with diameters of 6 to 15 m (Hardy et al., 2012). The characterization of these fascinating sites is currently carried out by Prof. J. Dufey, B. Hardy and colleagues at the Soil Science Laboratory of UCL (Louvain-la-Neuve).

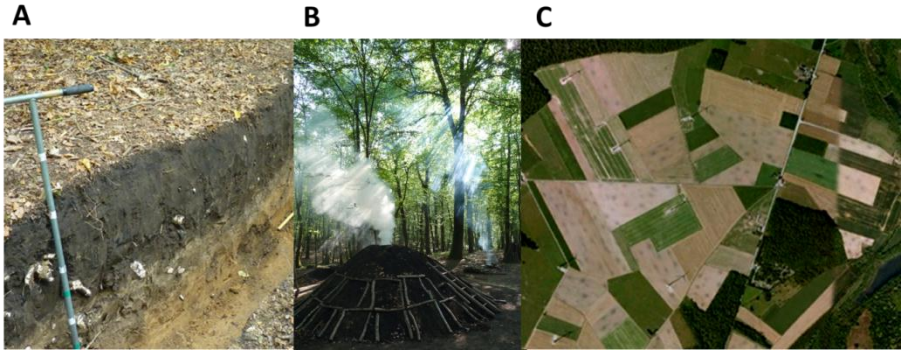


Fig. 1.15 Presence of char-containing soils in Wallonia. The picture shows (a) the layer of char accumulation due to repeated production of charcoal at this place by the (b) mound kiln method, and (c) the resulting dark area typically observed in ploughed fields (from Hardy et al., 2012).

One of the crucial properties of biochar that makes it attractive as a soil amendment is its highly porous structure, potentially responsible for improved water retention and increased soil surface area (Sohi et al., 2009). Furthermore, the addition of biochar to soil has been associated with increased nutrient use efficiency, either through nutrients contained in biochar or through physico-chemical processes that allow better utilization of soil-inherent or fertilizer-derived nutrients. As a result of the improvement of soil conditions for plant growth, biochar application to soils was generally found associated with an increase in crop yield, as reviewed by Manyà (2012) (Table 1.3). However, a few studies reported no increase in biomass production of wheat and radish for a high pH calcisol (Van Zwieten et al., 2010) and of rice for a gleysol (Haefele et al., 2011). According to Lehmann et al. (2003b), this can be explained by the fact that the available nutrients applied with biochar in this type of soils are not limiting, the CEC is already very high, and water stress does not occur.

Table 1.3 Summary of studies assessing the impact of biochar on crop yield (from Manyá, 2012 and included references).

Soil type	Biochar	Crop	Response
A weathered xanthic ferralsol from Central Amazonia (Brazil)	From secondary forestry wood. Addition rate: 67.2 and 135.2 Mg ha^{-1}.	Rice and cowpea	At a rate of 67.2 Mg ha^{-1} biomass increased by 20% (rice) and 50% (cowpea) compared to control (no biochar). At 135.2 Mg ha^{-1} biomass cowpea increased by 100%.
A weathered xanthic ferralsol from Central Amazonia (Brazil)	From secondary forestry wood. Addition rate: 11.0 Mg ha^{-1}.	Rice and sorghum	Stover and grain yields increased by 29% and 73%, respectively; compared to control treatment (only inorganic fertilizer).
A ferralsol (acidic soil) from New South Wales (Australia)	From mixtures of paper mill wastes and wood chips pyrolyzed at 550 °C. Addition rate: 10.0 Mg ha^{-1}.	Wheat, soybean and radish	Wheat: biochar improved yield by a factor of 1.3. When biochar was applied together to inorganic fertilizer, yields increased by a factor of 2.4 compared to using fertilizer alone. Soybean: biochar improved yield by a factor of 1.4 in the presence of fertilizer. Radish: dry biomass production was significantly increased by a factor of 1.5–2 both in the presence and absence of fertilizer. Biochar significantly increased both pH and CEC and reduced Al availability.
A carcisol (alkaline soil) from Victoria (Australia)	From mixtures of paper mill wastes and wood chips pyrolyzed at 550 °C. Addition rate: 10.0 Mg ha^{-1}.	Wheat, soybean, and radish	Wheat: yields were reduced by a factor of 2 both in the presence and absence of inorganic fertilizer. Soybean: biochar improved yield by a factor of 1.3 in the presence of fertilizer. Radish: biochar addition in the absence of fertilizer increased biomass production by a factor of 1.5. However, in the presence of fertilizer, yields were reduced by a factor of 2.
A clay-loam ferralsol from the oriental savanna of Colombia	Commercial wood charcoal. Addition rate: 20.0 Mg ha^{-1}.	Maize	Effect for 4 years (2003–2006). Maize grain yield did not significantly increase in the first year, but increases over the control were 28, 30 and 140% for 2004, 2005 and 2006, respectively.
A luvisol from Sydney (Australia)	From sewage sludge pyrolyzed at 550 °C. Addition rate: 10.0 Mg ha^{-1}.	Cherry tomato	Application of biochar improves the production of cherry tomatoes by 64% above the control soil conditions. The yield of production was found to be at its maximum when biochar was applied in combination with an inorganic fertilizer.

Table 1.3 (*Continued*)

Soil type	Biochar	Crop	Response
An anthraquic gleysol from Laguna (Philippines)	From rice husk partially burned in a combustion chamber Addition rate: 0.413 Mg ha^{-1}.	Rice	Effect for 4 years (2005–2008). Application of carbonized rice husks increased total organic carbon, total soil N, the C/N ratio, and available P and K. Biochar application decreased rice yields, especially in the first few seasons after application. For the entire period evaluated, rice yield decreased by 2.5% relative to control conditions.
A humic nitisol from Siniloan (Philippines)	From rice husk partially burned in a combustion chamber Addition rate: 0.413 Mg ha^{-1}.	Rice	Effect for 4 years (2005–2008). Application of carbonized rice husks increased total organic carbon, total soil N, the C/N ratio, and available P and K. For the entire period evaluated, rice yield increased by 8.9%.
A gleyic acrisol from Ubon (Thailand)	From rice husk partially burned in a combustion chamber Addition rate: 0.413 Mg ha^{-1}.	Rice	Effect for 4 years (2005–2008). Application of carbonized rice husks increased total organic carbon, total soil N, the C/N ratio, and available P and K. For the entire period evaluated, rice yield increased by 8.7%.
A typical ultisol from southern China	From rice straw pyrolyzed at low heating rate and at peak temperatures below 450 °C. Addition rate: 240 Mg ha^{-1}.	Maize	The effected of biochar amendment application on the 40-day maize dry matter production was marked: in the absence of NPK fertilizer, biomass production increased by 64%. In the presence of fertilizer, biomass production increased by a factor of 3 compared to using fertilizer alone.
A silt loam soil (with a subacid pH of 5.2) from the region of Tuscany (Italy)	A commercial charcoal obtained from coppiced woodlands (beech, hazel, oak, and birch). Addition rate: 30–60Mg ha^{-1}.	Durum wheat	Effect for 2 years (2009–2010). Biochar addition significantly increased grain production with respect to the control. On average, the grain yield increase ranged from 28% to 39%. No significant differences were observed between biochar rate treatments of 30 and 60 Mg ha^{-1}.

Besides this, a key property of biochar is its apparent biological and chemical stability. It is believed that biochar persists in the environment over millennia due to its biological and chemical recalcitrance caused by the polyaromatic backbone (Schmidt et al., 1999; Nguyen et al., 2008). The

existence of *Terra Preta* even today proves that biochar is stable over millennia in extreme environments such as the humid tropics (Glaser and Birk, 2012). This property can allow biochar to act as a carbon sink, as well as provide benefits to soil that are long-lived (Sohi et al., 2009). Indeed, the conversion of biomass to long-term stable soil carbon species can result in a long-term carbon sink, as the biomass removes atmospheric CO_2 through photosynthesis (McHenry, 2009). Moreover, three complementary goals can be achieved by using biochar applications for environmental management: soil improvement (from both productivity and pollution point of view; see below), waste valorization (if waste biomass is used for this purpose), and energy production (if energy is captured during the biochar production process) (Lehmann and Joseph, 2009a; Manyà, 2012). Thus, through the sequestration of plant-capured carbon by the biochar incorporation into the soil and the production of energy products during pyrolysis (bio-oil and syngas), the whole process becomes "carbon-negative", as illustrated by the example in Fig. 1.16, where a net C withdrawal from the atmosphere of 20% is suggested (Lehmann, 2007b).

Moreover, there may be additional and potentially important effects of biochar addition on the emission of other greenhouse gases from soil and on indirect emissions (Sohi et al., 2010). Key indirect emissions savings would include those associated with the manufacture of fertilizer not required to produce equivalent crop yield where positive effects on crop nutrient use efficiency have been achieved (Sohi et al., 2010). Moreover, preliminary researches suggest that nitrous oxide (N_2O) and methane (CH_4) emissions from soil may be significantly reduced by biochar application, possibly due to the increased soil aeration (Yanai et al., 2007; van Zwieten et al., 2009).

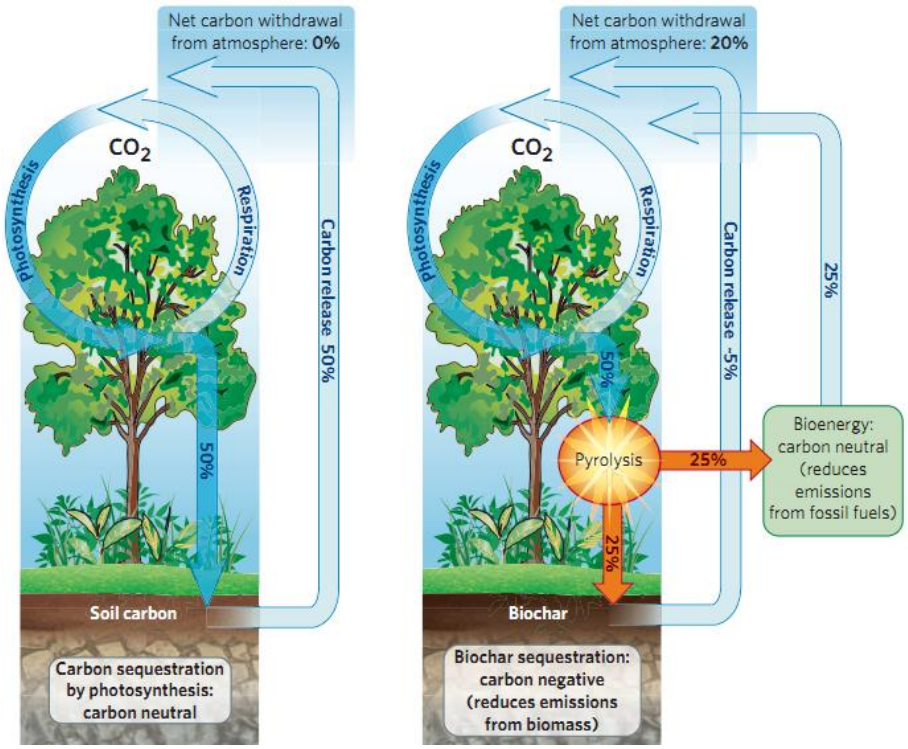


Fig. 1.16 Principle behind C sequestration with biochar soil application and bioenergy production compared to a normal system (Lehmann, 2007b).

Despite widespread interest in producing biochar for soil amelioration and potential climate change mitigation, substantial uncertainties nevertheless remain about the environmental impact and the sustainability of biochar production and application. According to Sparkes and Stoutjesdijk (2011), the main uncertainties and potential limitations of biochar application to soil include:

- the lack of research about the appropriate level of biochar application for different soil types;
- the effects of biochar on agrochemicals, notably because biochar can bind organic chemicals, such as herbicides, thereby reducing the amount of the chemical avalaible to kill target species (Jones et al., 2011);
- the availability of biomass because, although the global potential for biochar and bioenergy production is large, there is only a finite area of land available without compromising food production;

- the effect on soil albedo. Indeed, due to the ability of biochar to darken the color of soil, especially in soils with poor organic matter content, biochar application to soil increases solar energy absorption and decrease soil albedo. The subsequent increase in soil temperature could potentially accelerate cycling of nutrients and extend growth season in temperate climates (Sohi et al., 2010);
- the amount of time that biochar remains in soils (soil residence time). Although some authors calculate a biochar half life in soil of several hundred to thousands year, very few field trials have assessed soil residence over a range of conditions and biochar types;
- the effects on soil organic matter. On the basis of results showing an increase in soil organic matter decomposition after biochar application to soil, some researchers have suggested that increased rates of loss of soil organic matter would be more than compensated by increases in production of biomass both above and below ground. However, this has not yet been sufficiently demonstrated.

3.2.3.2. Biochar feedstock and production

Almost any form of organic material can be introduced into a pyrolyzer as the high temperature of the pyrolysis process neutralizes any organic toxins and pathogens in the feedstock (Laird et al., 2009). Feedstock currently used at a commercial-scale or in research facilities include wood chip and wood pellets, tree bark, crop residues (including straw, nut shells and rice hulls), switch grass, organic wastes such as distillers grain, bagasse from the sugarcane industry and olive waste, chicken litter, dairy manure, sewage sludge and paper sludge (Sohi et al., 2009).

Biochar can be produced at scales ranging from large industrial facilities down to the individual farm (Lehmann and Joseph, 2009b), and even at the domestic level (Whitman and Lehmann, 2009; Torres-Rojas et al., 2011), making it applicable to a variety of socioeconomic situations. Various pyrolysis technologies are commercially available that yield different proportions of biochar and bioenergy products, such as bio-oil and syngas

(Woolf et al., 2010). The gaseous bioenergy products are typically used to generate electricity; the bio-oil may be used directly for low-grade heating applications and, potentially, as a diesel substitute after suitable treatment (Elliott, 2007).

The balance between char and gas, oil and tar products in pyrolysis depends to a considerable extent upon the rate of heating (Table 1.4) (IEA, 2007). ''Slow'' pyrolysis (heating for seconds or minutes) may be deployed as a continuous process, where purged (oxygen-free) feedstock is transported into and through an externally heated kiln (gas flow removing volatiles, char emerging at the other end); ''fast'' pyrolysis depends on very rapid (ms to s) transfer of heat, typically to fine biomass particles blown through hot gases in confined oxygen-free chamber (variants on such equipment have also been used in slow ''downdraft'' systems) (Sohi et al., 2010).

Table 1.4 Fate of initial feedstock mass between products of pyrolysis processes (IEA, 2007).

Process	Liquid (bio-oil)	Solid (biochar)	Gas (syngas)
Fast pyrolysis: Moderate temperature (~500°C), short hot vapor residence time (<2s)	75%	12%	13%
Moderate pyrolysis: Low – moderate temperature, moderate hot vapor residence time (~10 – 20s)	50%	25%	25%
Slow pyrolysis: Low-moderate temperature, long vapor residence time (~5-30min)	30%	35%	35%
Gasification: High temperature (>800 °C), moderate vapor residence time (~10 – 20s)	5%	10%	85%

Pyrolysis engineers have traditionally sought to minimize char production as it has been viewed as a problematic low-value waste fraction. Consequently, there has been much focus of research effort on fast pyrolysis which maximizes condensable oil and also gasification. These produce predominantly oil or gas, yielding only small proportions of char. The development of integrated systems that produce energy and char at high efficiency by slow pyrolysis is still, therefore, mainly on the research scale, with technology commercially deployed only at a handful of locations (Sohi et al., 2010). However, reviewing the recent literature focused on

increasing charcoal yields, Manyà (2012) identified several variables and factors that play a critical role during the slow pyrolysis process; those include peak temperature (i.e. the highest temperature reached during the process), pressure, vapor residence time and moisture content. In general, low peak temperature, high pressure (1.0 – 3.0 MPa), high vapor residence time and high moisture contents (in the range of 42 – 62%) increase the charcoal yield. The biochar yield is also influenced by the biomass feedstock composition (holocellulose, lignin, extractives, and inorganic matter). Feedstock with high lignin content can produce higher biochar yields (Antal and Grønli, 2003; Demirbas, 2006). Moreover, inorganic matter, especially alkali and alkali earth metals, catalyzes biomass decomposition and char-forming reactions (Yaman, 2004). For instance, pretreating the biomass feedstock with hot water (at 80°C for 2 h) to reduce the mineral matter content was found to decrease charcoal yields (Teng and Wei, 1998).

3.2.3.3. Physico-chemical properties of biochar

Operating parameters during the pyrolysis process influence also the resultant physical properties of biochar. The pyrolysis peak temperature is expected to be the most important factors because the fundamental physical changes (i.e. the release of volatiles, the formation of intermediate melts and the volatilization of the intermediate melts) are all temperature dependent. The temperature ranges, however, under which these stages occur vary with feedstock. Heating rates and pressures are expected to have the second greatest influence since they affect the physical mass transfer of volatiles evolving at the given temperature from the reacting particles (Downie et al., 2009).

It is commonly accepted that each biochar particle comprises of two main structural fractions: stacked crystalline grapheme sheets and randomly ordered amorphous aromatic structures (Fig. 1.17) (Verheijen et al., 2010).

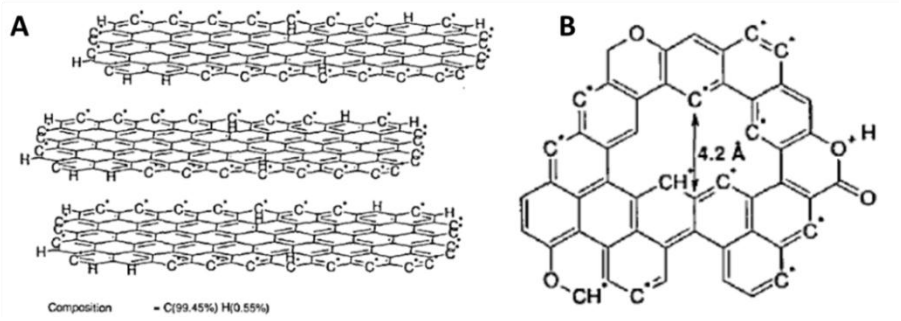


Fig. 1.17 Putative structure of charcoal (from Verheijen et al., 2010) showing (a) a model of a microcristalline graphitic structure is shown and (b) an aromatic structure containing oxygen and carbon free radicals.

Hydrogen, O, N, P and S are found predominantly incorporated within the aromatic rings as heteroatoms (Bourke et al., 2007). Biochar composition is highly heterogeneous, containing both stable and labile components (Sohi et al., 2009). On top of fixed C, volatile matter, mineral matter (ash) and moisture present in varying proportions (Table 1.5) are generally regarded as the major biochar constituents (Antal and Grønli, 2003). According to Chan and Xu (2009), total N, P and K in biochar were found to range between 1.8 to 56.4, 2.7 to 480 and 1.0 to 58.0 g kg^{-1}, respectively (Table 1.6). The variability can be attributed to different feedstocks and different conditions under which the various biochars were manufactured. Interestingly, total ranges of N, P and K in biochar are wider than those reported in the literature for typical organic fertilizers (Verheijen et al., 2010). Most minerals within the ash fraction of biochar are thought to occur as discrete associations independent of the carbon matrix, with the exception of K and Ca (Amonette and Joseph, 2009).

Table 1.5 Relative proportion range of the four main components of biochar (weight percentage) as commonly found for a variety of source materials and pyrolysis conditions (Verheijen et al., 2010).

Component	Proportion (%)
Fixed carbon	50 – 90
Volatile matters (e.g. tars)	0 – 40
Moisture	1 – 15
Ash (mineral matter)	0.5 - 5

Table 1.6 pH, C, N, P, K and carbonate contents of various biochars (from Chan and Xu, 2009 and included references).

Biochar feedstocks	pH	C ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	CO_3 (%)	Production conditions
Wood	-[a]	708	10.9	68	0.9	-	By local farmers
Green wastes	6.2	680	1.7	0.2	1.0	<0.5	450°C
Poultry litter	9.9	380	20	25.2	11,600	15	450°C
Sewage sludge	-	470	64	56	-	-	450°C
Unknown	9.6	905	56.4	2.7	51	-	Unknown
Broiler litter	-	258	7.5	48	30	-	700°C and steam activated
Broiler cake	-	172	6.0	73	58	-	700°C and steam activated
Bark of *Acacia mangium*	7.4	398	19.4	-	-	-	260°C – 360°C
Rice straw	-	490	13.2	-	-	-	500°C
Sugar cane bagasse	-	710	17.7	-	-	-	500°C
Coconut shell	-	690	9.4	-	-	-	500°C
Oil mallee tree after oil extraction	8.4	340	12	1.2	7.0	-	« Moki » method
Soybean cake	-	590	78.2	-	-	-	550°C
Eucalyptus deglupta	7.0	824	5.73	0.6	-	-	350°C
Mean	8.1	543	22.3	23.7	24.3	-	

[a]data not available

The complex and heterogeneous chemical composition of biochars is extended to its surface chemistry (Verheijen et al., 2010), which in turn explains the way biochar interacts with a wide range of organic and inorganic compounds in the soil (Atkinson et al., 2010; Joseph et al., 2010). Breaking and rearrangement of the chemical bounds in the biomass during processing results in the formation of numerous functional groups such as hydroxyl -OH, amino-NH_2, keton -OR, ester -(C=O)OR, nitro -NO_2, aldehyde -(C=O)H, carboxyl -(C=O)OH, occurring predominantly on the outer surface

of the grapheme sheets and surfaces of pores (Amonette and Joseph, 2009; Verheijen et al., 2010). Some of these act as electron donors, while others as electron acceptors, resulting on coexisting areas with properties ranging from acidic to basic and from hydrophilic to hydrophobic (Amonette and Joseph, 2009; Joseph et al., 2010), even though freshly produced biochars are usually hydrophobic in nature (Fig. 1.18) because of the non-polar surface characteristics (Lehmann et al., 2009; Bruun, 2011).

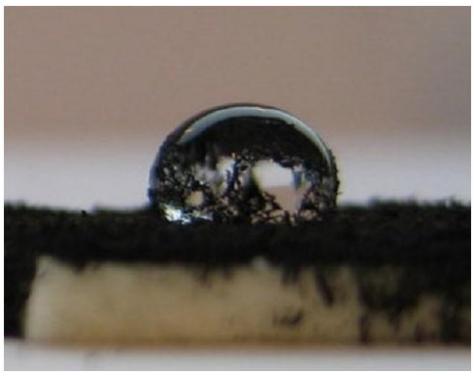


Fig. 1.18 Drop of water added to a dense layer of biochar (525 °C). The picture was taken 15 minutes after the addition, illustrating the great hydrophobicity of fresh FP-biochar, where water remained in a spherical form. Note the acute outer angle between the horizontal surface and the sides of the drop, which illustrates the hydrophobic characteristics of biochar (Bruun, 2011).

Pore sizes for biochar are usually reported according to standard IUPAC value ranges (Downie et al., 2009): macropores are > 50 nm internal diameter (ID), mesopores are 2-50 nm ID, and micropores are < 2 nm ID. The elementary porosity and structure of the biomass feedstock is retained in the biochar product formed (Downie et al., 2009). The vascular structure of the original plant material, for example, is likely to contribute for the occurrence of macropores in biochar (Fig. 1.19). In contrast, micropores are mainly formed during processing of the parent material. While macropores have been identified as a feeder to smaller pores (Zabaniotou et al., 2008; Downie et al., 2009), micropores effectively account for the characteristically large surface area in charcoals (Brown, 2009).

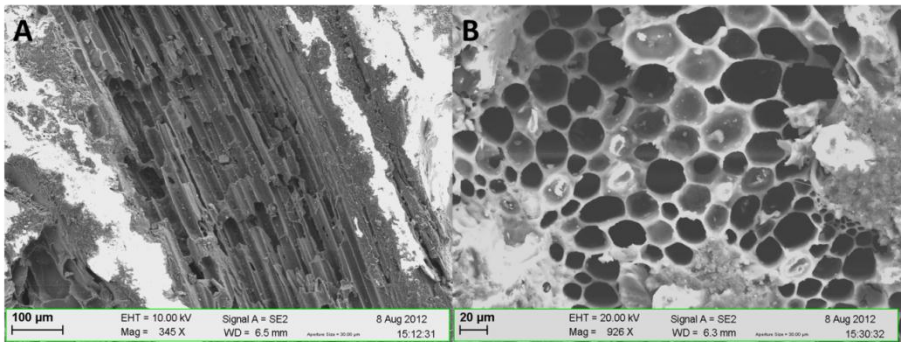


Fig. 1.19 Scanning electron microscope (SEM) image showing macroporosity of (a) longitudinal and (b) cross section of a miscantus-derived biochar . Operational conditions of production were an end temperature of 600°C and a residence time in the reactor of 30 minutes (personal source).

Cation exchange capacity (CEC) variation in biochars ranges from negligible to around 40 $cmol_c\ kg^{-1}$ (Verheijen et al., 2010) and has been reported to change following incorporation into soils (Lehmann, 2007a). This may occur by the continuous oxidation of biochar surfaces, which increase the number of the oxygenated functional groups, and by the adsorption of organic acids by the biochar (Liang et al., 2006; Cheng et al., 2008). Considering the very large heterogeneity of its properties, biochar pH values are relatively homogeneous, that is to say they are largely neutral to basic (Verheijen et al., 2010). Chan and Xu (2009) reviewed biochar pH values from a wide variety of feedstocks and found a mean of pH 8.1 in a total range of pH 6.2 – 9.6 (Table 1.6). Moreover, because some biochars can have fairly high concentrations of carbonates (Chan and Xu, 2009), it has been suggested that biochar can be valuable as a liming material for overcoming soil acidity (Van Zwieten et al., 2010). However, since pH and carbonate content of biochar can vary depending on the feedstock and the production conditions (Table 1.6), all biochars are not equally effective for liming the soil.

3.2.3.4. Biochar for phytostabilization

Besides the benefit of carbon sequestration, Lehmann and Rondon (2006) proposed the biochar application as a sustainable way to improve highly degraded (weathered) lands. Indeed, biochar may act as a soil conditioner that enhances plant growth by supplying and, more importantly, retaining nutrients and by providing other services such as improving soil physical

and biological properties (Glaser et al., 2002; Lehmann et al., 2003b). Such considerations gave rise to the idea of using biochar to permanently sequester C in contaminated soils while promoting their revegetation. In addition, since the porous structure of biochar, its high internal surface area and its ability to adsorb soluble organic matter, gases and nutrients are likely to provide a highly suitable habitat for microbes to colonize, grow and reproduce (particularly for bacteria, actinomycetes and arbuscular mycorrhizal fungi) (Thies and Rillig, 2009), biochar application to contaminated soils might assist the preservation and support of soil biodiversity and biotope for the microbiota (Beesley et al., 2011), which is usually limited in such toxic soils (Baath, 1989).

Moreover, biochar has not only a high potential for increasing fertility parameters but also many favorable properties for the immobilization of heavy metals such as microporous structure, active functional groups, and high pH and CEC (Jiang et al., 2012). However, although the capability of carbonaceous materials, such as biochar, to remove heavy metals from aqueous solutions and wastewaters has been explored (Mohan et al., 2007; Inyang et al., 2012; Ippolito et al., 2012; Kołodyńska et al., 2012; Xu et al., 2012b), application to real world contaminated soil systems has received surprisingly little systematic investigation to date (Beesley and Marmiroli, 2011). As reviewed by Beesley et al. (2011), the few studies which have been carried out showed generally a reduction of metal mobility but the opposite can also be observed. This is notably because all investigated biochars do not necessarily display similar properties. For instance, given the wide range of biochar pH (Table 1.6), the immobilization of heavy metals due to a liming effect subsequent to biochar application can considerably vary depending on the type biochar which is applied. Moreover, the mechanisms of mobilization/immobilization were dependent on the investigated soils. For instance, in a sandy soil with low intrinsic Cu retention, Uchimiya et al. (2011) suggested that CEC was the primary mechanism by which biochar improved Cu retention while, in a clay-rich, alkaline soil with higher intrinsic Cu retention capacity, sorption to mineral (ash) components of the biochar assisted retention (precipitation). Regarding Pb, Cao et al. (2011) suggested that its immobilization in a contaminated soil amended with dairy-manure biochar was due to reaction

of P originally contained in biochar with soil Pb to form insoluble hydroxypyromorphite $Pb_5(PO_4)_3(OH)$. In mine tailings amended with 0 – 10% orchard prune-derived biochar, Fellet et al. (2011) found that biochar application increased pH and CEC and reduced bioavailable (DTPA-extractable) concentrations of Cd, Pb and Zn. The application of a hardwood-derived biochar to sediment-derived canal bank soil induced significant reductions in concentrations of Cd and Zn in pore water collected during 60 days field exposure (Beesley et al., 2010). However, the same study revealed that Cu mobility increased after the biochar application because biochar increased DOC in soil pore water. Similarly, Hartley et al. (2009) found an increase of As mobility in the presence of biochar due to the higher release of DOC. This is in disagreement with the results of Gomez-Eyles et al. (2011) which showed a decrease in water soluble carbon and a concurrent decrease in Cu mobility after amendment of a hardwood-derived biochar, suggesting that the linkages are far from straightforward and will depend on local soil conditions (Beesley et al., 2011). In a shooting range soil, Uchimiya et al. (2012) found that oxidized biochars rich in carboxyl functional groups exhibited significantly greater Pb, Cu, and Zn stabilization ability compared to unoxidized biochars. As a result, slow *in situ* oxidation of biochar, which results in the formation of carboxylic, phenolic, and other oxygen-containing surface functional groups (Cheng et al., 2006), may result in greater ability to stabilize heavy metals in soils. However, the authors stress that the effects of biochar aging should be fully addressed by considering additional processes such as the coating of the biochar surface by mineral, organic, clay, and silt components of soil, root penetration (Fig. 1.20; Joseph et al., 2010), and transport (Zhang et al., 2010).

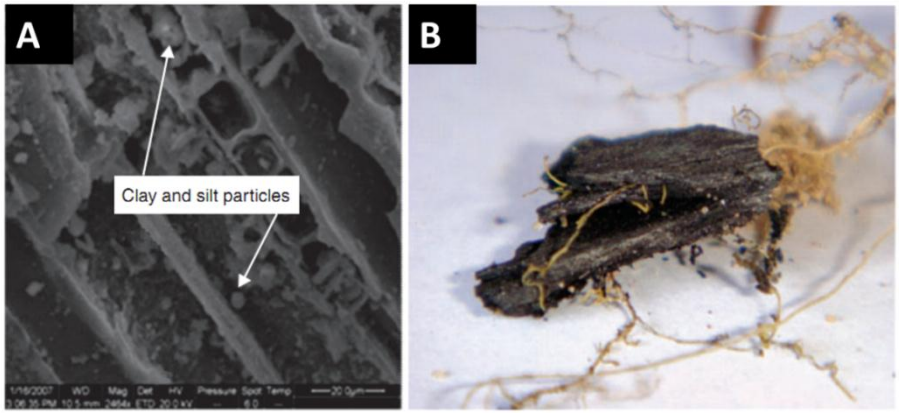


Fig. 1.20 Illustration of biochar aging in soils by (a) a SEM image showing clay and silt particles entering the macropores of the greenwaste biochar and (b) an optical image showing root hairs penetrating a biochar particle that has been in soil for 20 years (from Joseph et al., 2010).

Only a very limited number of studies have investigated the effect of biochar on the plant growth on metal contaminated soils so far. Hartley et al. (2009) suggested the feasibility of growing *Miscanthus* on an industrially As-polluted soil because low-soil plant transfer was found in the presence of biochar. According to the authors, one explanation may be increased availability of P limiting As uptake; the chemical similarity and behavior of phosphate (PO_4^{3-}) and As means they may compete with each other for uptake sites. The results nevertheless indicated a need to combine biochar with other amendment to obtain the highest biomass yield because the biochar alone could not enhance yield. In a soil originated from a former copper mine site, Karami et al. (2011) observed that harvestable amounts of Cu from ryegrass shoot were lower in the presence of biochar than in the presence of green waste compost. Indeed, although these authors found that Cu concentrations in shoots were slightly higher in the presence of biochar than compost, the biomass production was much more important in the compost-amended soil. In a pot experiment, Namgay et al. (2010) showed that biochar application decreased the concentration of As, Cd, and Cu in maize shoots whereas the effects were inconsistent on Pb and Zn concentrations in the shoots. Park et al. (2011a) investigated the metal immobilizing impact of chicken manure- and green waste-derived biochars, and their effectiveness in promoting Indian mustard growth. The authors

concluded that biochars could be used to enhance phytostabilization of metal contaminated soils as they significantly decreased Cd, Cu and Pb accumulation by plants while increasing the biomass production. The authors suggested that the metal immobilization by biochar might be due to both specific and non-specific adsorption but they did not test it. Chicken manure-derived biochar was the most effective in both the immobilization of metals and increasing plant growth, indicating that the effectiveness can vary depending on the type of biochar present.

Given all these considerations, the possibility of using biochar for phytostabilization purposes is not still fully understood. In particular, studies showed contradictory results regarding the predominant parameter influencing metal mobility when biochar is applied to the soil: pH, CEC, DOC and PO_4^{3-} release might play a significant role. Moreover, the effect of biochar on the metal mobility with aging is not elucidated.

4. Challenges, objectives and thesis outline

4.1. Challenges

In 2001, Sparks (2001) pointed that improving of the understanding of rhizosphere chemistry would be one of the most important future frontiers in soil chemistry. Although advances have been made in the past decade to unravel some of the multiple processes and interactions of heavy metals in the rhizosphere of terrestrial plants, Wenzel et al. (2011) recently stressed the importance to carry out further research to better address the inherent complexities. Similarly, in a recent review, Bolan et al. (2011) stressed that, to ensure a successful phytostabilization, much more investigations should be devoted to the effect of plant roots and associated microorganisms onto the chemistry and bioavailability of heavy metals in contaminated soils. Besides the rhizosphere effects, the litter accumulation due to the revegetation of the soil might also affect the success of phytostabilization. Indeed, in non-contaminated soils, Titeux and Delvaux (2009) for example showed that metal leaching could be highly controlled by the release of organic anions released from the leaf litter.

Phytostabilization has been seen above as a promising technology for soil remediation. However, across all studies, only limited attention has been paid to the leaching of heavy metals in relation with the application of metal immobilizing amendments (Ruttens et al., 2006a). Moreover, while some amendments, in particular organic amendments, have been used successfully in revegetation in metal contaminated areas by supplying sufficient nutrients, increasing soil water-holding capacity, and isolating toxic materials (Brown et al., 2003; Park et al., 2011b), results from column experiments (without plants) have shown that the incorporation of the same amendments can dramatically increase the heavy metal leaching (Schwab et al., 2007).

Moreover, it is important to keep in mind that, given the large extent of soil contamination in the World, metal contaminated soils provide a significant but hitherto neglected component of the global soil resource. According to Dickinson et al. (2009), the practical applications of phytostabilization are too often overlooked while there is huge scope for cross-cutting other environmental agenda, with synergies that involve the recovery and

provision of services from contaminated soils. Therefore, the authors argue that additional focuses on biomass energy and carbon sequestration are required for the justification and advancement of phytostabilization. To this end, sequestering biochar while cultivating bioenergy crops could be an attractive solution. Indeed, on the basis of the results of studies evaluating the effect of biochar on heavy metal immobilization as well as plant growth on metal contaminated soils, it appears that biochar application has a promising potential for phytostabilization. However, given the paucity of data and the lack of representativity, it is essential to conduct further investigations prior to implement it in the field. On the one hand, biochar incorporation into the soil is irreversible, so a misunderstanding of its effects could lead to dramatic consequences (e.g., a higher metal mobility), especially for multi-metal contaminated soils. On the other hand, it is essential to evaluate whether phytostabilization with biochar is more effective than with other cost-effective amendments. According to Beesley et al. (2011), since the costs associated with the use of biochar are not likely to be dissimilar from those of other soil amendments, in particular local wastes and by-products, the low density of biochar means that, on a weight basis, greater transportation costs could be a deciding factor in the application of biochars to some sites with large areas requiring remediation.

4.2. Objectives and thesis outline

With the above-mentioned gaps to bridge in mind, this thesis aims at gaining better insights about the impact of plants, amendments and biochar, alone or in combination, on the mobility of heavy metals in soils and the consequent implications for phytostabilization (Fig. 1.21). In order to determine the impact of rhizosphere activity on the release of heavy metals into the environment and the subsequent implications for phytostabilization, we assess in **Chapter 2** the effect of root activity on the release of Zn from a simplified system containing only one metal-bearing phase (the mineral smithsonite). Obviously, the occurrence of a unique metal phase in the environment is unlikely. Therefore, **Chapter 3** evaluates the effect of root activity on the release of Cd, Zn and Pb from two highly

metal contaminated soils. To this end, leaching column experiments as well as the analysis of Zn isotope distribution in the different pools of the system are carried out.

Besides the root activity, organic matter accumulation due to revegetation might also affect the release of heavy metals from the soil. **Chapter 4** thus investigates the impact of organic matter build-up in a long-term spontaneously revegetated slag heap on the leachability of Cd, Zn and Pb and the consequent implications for the use of plants for phytostabilization purposes.

Then, **Chapter 5** examines the feasibility to reduce simultaneously the leaching and the phytoavailability of Cd, Zn and Pb using six different cost-effective amendments, either inorganic or organic, when plants are growing for phytostabilization purposes.

With the idea in mind of using biochar for phytostabilization purposes, **Chapter 6** consists of a preliminary investigation of the effects of biochar application on the mobility of heavy metals in a contaminated soil. It is designed to explore the bioavailability and $CaCl_2$ extractability over time of Cd, Zn and Pb. It also assesses the impact of biochar application on the release of heavy metals upon acidifying conditions. Afterwards, **Chapter 7** examines the effects of rhizospheric processes of two plants, *Agrostis tenuis* and *Lupinus albus*, on the metal mobility in a soil amended with biochar.

Finally, in order to provide the recovery and provision of services from metal contaminated soils, **Chapter 8** evaluates the possibility to grow a bioenergy crop (rapeseed) on a metal contaminated soil while sequestering C through biochar incorporation. The effect of biochar application on both the Cd, Zn and Pb uptake and the biomass production is compared with the effect produced by the application of the best amendment found in Chapter 5.

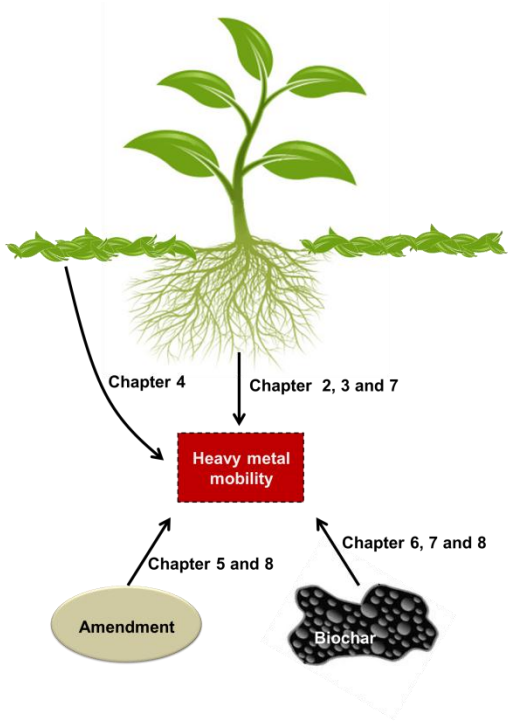


Fig. 1.21 Schematic thesis ouline.

For the elaboration of this thesis, we deliberately chose an approach in which each chapter could generate practical applications. With this scientific strategy, experimental conditions differ from a chapter to another one as they are selected in order to meet the specific objective of the chapter.

For example, the selection of the plant species used in each chapter is motivated by the chapter's need. Fast growth and direct indication of heavy metal bioavailability was the reason for choosing for *Lolium multiflorum* (Chapter 2 and 6), potential use for phytostabilization for *Agrostis tenuis* and *Lupinus albus* (Chapter 3, 4, 5 and 7) and potential use for energy biomass production for *Brassica napus* (Chapter 8). Similarly, the choice of the type of substrate is also guided by the chapter's objective, i.e. a single metal-bearing mineral for Chapter 2, a soil for which metal-bearing particles can be easily size-separated for Chapter 3, a soil being *in situ* covered by spontaneous vegetation for Chapter 4, and a soil which has been diffusely

contaminated by metal-rich atmospheric fallouts and which is suitable for pot experiments for Chapters 4, 5, 6 and 7.

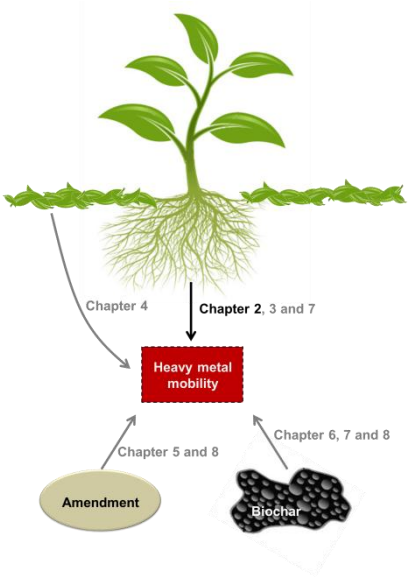
All chapters are presented in the format of a journal article so that they can be read independently from the others. Each chapter is nevertheless preceded by a short introduction that places it within the thesis. To date, Chapter 2 and 5 are published and Chapter 4 is in press. Chapters 6 and 8 have been submitted for publication. It is planned to submit Chapter 3 for the next two months. Because chapter 7 uses several results of Chapter 6, it will be submitted as soon as Chapter 6 is accepted.

Chapter 2

Zinc mineral weathering as affected by plants roots*

Where are we?

In order to determine the impact of rhizosphere activity on the release of heavy metals into the environment and the subsequent implications for phytostabilization, Chapter 2 consists of a preliminary investigation evaluating the potential effect of roots on the weathering of smithsonite ($ZnCO_3$), a common Zn mineral in soils.



1. Introduction

Since the Industrial Revolution, anthropic activities have generated large Zn inputs to soils. This is due, for instance, to atmospheric emission fallouts (Nriagu, 1996) or to the deposition of pyrometalurgical wastes which often have a high residual Zn content (Nriagu and Pacyna, 1988). Such contaminated soils are of increasingly major concern for environmental quality (Dudka and Adriano, 1997; He et al., 2005) and human health (Cui et

* Adapted from Houben, D. and Sonnet Ph. (2012) Zinc mineral weathering as affected by plant roots. *Applied Geochemistry*, 27, 1587 – 1592.

al., 2005) and their reclamation is thus necessary. Increasingly, the spontaneous or assisted establishment of vegetation on contaminated soils is regarded as a commonsense mitigation measure since it helps reduce the dispersion of contaminants by wind or water erosion, runoff and water percolation through the substrate (Vangronsveld et al., 1995). Yet, surprisingly, only a few studies have attempted to assess the potential effect of plant activity on the weathering of Zn-bearing minerals and, by extension, on the release of Zn into the environment (Banks et al., 1994; Zhu et al., 1999; Zhao et al., 2007).

The physico-chemical conditions in the rhizosphere, which is defined as the volume of soil influenced by root activity (Hinsinger, 1998), may differ drastically in many respects from those of the bulk soil (Marschner and Römheld, 1996), thereby having a considerable effect on the success of phytoremediation (Wenzel, 2009). For instance, the uptake of water and nutrients by roots may affect the ionic composition and the pH of the soil solution in the rhizosphere (Nye, 1981; Haynes, 1990; Hinsinger et al., 2003). Plant roots can also modify the rhizospheric solution composition through the exudation of organic compounds that stimulate the microbial activity (Nannipieri et al., 2008). Moreover, the release of organic root exudates in the rhizosphere increases the concentration of dissolved organic C (DOC) which has a strong affinity to polyvalent cations and, thus, affects the biogeochemistry of nutrients and pollutants in soils (Dessureault-Rompré et al., 2008). Thus, changes in soil induced by plants modify the weathering conditions and, as a result, an increase in mineral dissolution can be observed in the vicinity of roots (Courchesne and Gobran, 1997; Hinsinger et al., 2001, 1992).

The potential role played by plants in the weathering of Zn-bearing minerals needs to be investigated not only because of possible dissemination of the metal but also because Zn plays a dualistic role in the soil-plant system. Acting notably as a building block in all six enzyme classes, Zn is a vital micronutrient for most living organisms (Berg and Shi, 1996), including higher plants (Broadley et al., 2007). On the other hand, in excessive concentrations, Zn is toxic.

This study was conducted to gain a better insight on the weathering of a Zn-bearing mineral in soil when subjected to root activity. A continuous flow

experiment was set up in the laboratory to compare the amount of Zn released by smithsonite ($ZnCO_3$) in both the presence and the absence of *Lolium multiflorum* (Italian ryegrass). Smithsonite is a secondary Zn mineral, commonly found in the oxidized zones of ore deposits or as a replacement of calcareous rocks, derived by the alteration of primary Zn minerals, especially sphalerite (Gaines et al., 1997). Moreover, smithsonite is also frequently found in metallurgy wastes and in soils contaminated by atmospheric fallout (Van Damme et al., 2010). Italian ryegrass was chosen for its fast growth and because the *Lolium* group species in particular and grasses in general are often considered to be pioneer and suitable plants for covering contaminated substrates (Smith and Bradshaw, 1979; Arienzo et al., 2004). Moreover, it has also been shown that *L. multiflorum* has the ability to induce readily the weathering of minerals (Hinsinger et al., 1992).

2. Materials and methods

2.1. Smithsonite

The smithsonite used in the study came from the Moresnet (Belgium) Zn ore deposit. A complete description of this deposit can be found in Coppola et al. (2008). Smithsonite crystal aggregates were crushed using a cast iron mortar and pestle and the particles were dry sieved to isolate the fraction comprised between 500 and 1000 µm. Binocular observation and hand-picking were used to remove impurities from the smithsonite concentrate. The 500 - 1000 µm fraction was then rinsed repeatedly with deionized water in order to remove dust, dried (105°C; 72h) and then stored in a high-density polyethylene (HDPE) bottle at ambient temperature. Mineralogical purity of the concentrate was determined by X-ray diffraction (Bruker AXS D8 Advance, SOL-X detector) which showed that smithsonite was largely dominant, though minor impurities of calcite, dolomite and siderite were detectable. The elemental composition (HCl digestion) revealed that the Zn content of the concentrate was 51.5 wt.%, close to that of stoechiometric smithsonite (52.1 wt.%), while minor elements Ca (854 mg kg^{-1}), Fe (4450 mg kg^{-1}), Mg (2070 mg kg^{-1}) and Mn (651 mg kg^{-1}) were in the usual range for this mineral (Gaines et al., 1997). Prior to use, 1±0.001 g of smithsonite

particles were mixed with 9±0.001 g of quartz (500-1000 µm) to obtain a homogeneous thin Zn-rich substrate. In order to be able to insert the substrate underneath the root mat, the mixture was then inserted in a flat round bag (58 mm diameter) made by heat sealing two pieces of polyamide net (20 µm mesh size).

2.2. Nutrient solution

In the experiment two different nutrient solutions were used: a complete nutrient solution and a Zn-deficient nutrient solution. The chemical composition of the complete nutrient solution expressed in m*M* was (Rufyikiri et al., 2000; Rufyikiri et al., 2004): 1 $Ca(NO_3)_2.4H_2O$, 0.5 KCl, 0.25 K_2SO_4, 0.05 $MgCl_2$, 0.05 $MgSO_4$, 0.05 NaH_2PO_4, 0.08 FeNaEDTA, 0.08 H_3BO_3, and in µ*M*: 0.8 $ZnSO_4.7H_2O$, 8 $MnCl_2.4H_2O$, 0.8 $CuSO_4.5H_2O$, 0.8 $(NH_4)_6Mo_7O_{24}.4H_2O$. The composition of the Zn-deficient nutrient solution was identical to that of the complete nutrient solution except that no $ZnSO_4.7H_2O$ was added. In each nutrient solution, N was supplied in NO_3 form in order to avoid stimulating acidification of the rhizosphere (Haynes, 1990), which could mask the effects of other weathering agents. During the entire experiment, nutrient solution reservoirs were bubbled with ambient air in order to prevent anoxic conditions developping.

2.3. Experimental set-up

The design of the experimental device (see Appendix A for details) was based on Hinsinger et al. (2001). This device allowed those authors to collect elements released by a basalt powder under root activity while ensuring conducive growth conditions for plants. The experimental set-up is illustrated in Fig. 2.1.

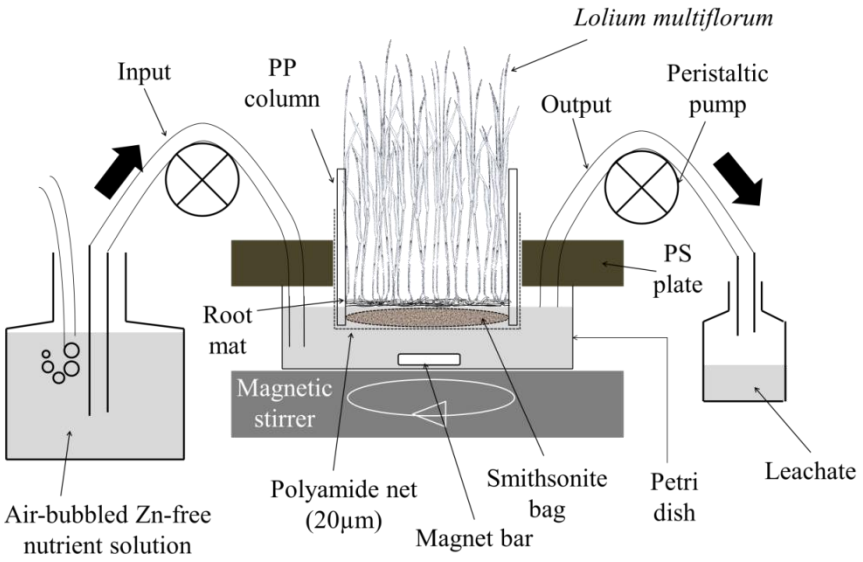


Fig. 2.1. Experimental device used for studying the effect of root activity on smithsonite weathering (based on Hinsinger et al., 2001).

A plant compartment was made up of a transparent polypropylene (PP) cylinder (60 mm in diameter and 60 mm in height), closed at the bottom by a polyamide net (20 µm mesh size). The cylinder was first inserted into a 60 mm diameter circular opening carved in a 100 × 100 mm polystyrene (PS) square plate so that the bottom of the cylinder was aligned with the base of the PS plate. The role of the PS plate was to keep the plant compartment afloat when put into a tank filled with deionized water so that the polyamide net was kept constantly wet. In each PP cylinder, 0.8±0.01 g of sterilized seeds (10 min in 2 *M* H_2O_2) of *Lolium multiflorum* Lam. (Italian ryegrass) was sown and germinated in the dark. After 3 days of germination, the deionized water was replaced with the complete nutrient solution and the seedlings were transferred to a controlled greenhouse under a photoperiod of 14 h (photon flux density of 120-180 µmol m^{-2} s^{-1}), constant temperature (20°C) and relative humidity (95%). This pre-growth period lasted 11 days to ensure a dense root mat on the polyamide net surface (Fig. 2.2).

Fig. 2.2. View of the dense root mat of *Lolium multiflorum* Lam. (Italian ryegrass) at the end of the pre-growth period before being put in contact with smithsonite.

After the pre-growth period, the root mat was briefly removed from the plant compartment in order to insert a bag containing the mineral mixture (quartz + smithsonite) between the polyamide net and the root mat. Each plant compartment was then put on top of an open polycarbonate Petri dish (90 mm diameter). The PP cylinder was then slid downwards through the PS plate so that a free space of 3 mm was left between the polyamide net at the bottom of the PP cylinder and the bottom of the Petri dish. A magnetic stir bar (1.5 mm thick and 15 mm long) was introduced into this free space and the whole system was placed on a magnetic stirrer. Each Petri dish was connected by opaque inlet tubing to its own nutrient solution reservoir and by opaque outlet tubing to its own HDPE collector bottle. The inlet tubing was attached so that the Zn-deficient nutrient solution was introduced near the middle of the Petri dish while the outlet tubing was attached at 8 mm from the bottom of the Petri dish in order to act as an overflow collector. An identical flow rate was obtained in both the inlet and the outlet tubing by using two parallel channels of the same peristaltic pump. A continuous open flow from the fresh nutrient solution reservoir (designated hereafter as the Zn-deficient nutrient solution) to the HDPE collector (designated hereafter as the leachate) was thus established with a constant rate of 130 cm^3 day^{-1}.

The Zn-deficient nutrient solution was allowed to flow into the device immediately after having brought the roots into contact with the smithsonite and quartz bag. However, leachates collected during the first four days were discarded in order to ensure that steady-state dissolution of smithsonite had been reached. After this 4-day equilibration period, the

plants of two devices were removed from the experiment and the roots and shoots were kept for analysis. During the 10 remaining days of the experiment, plant treatments were performed in three replicates while control treatments were carried out in duplicate. Control treatments consisted of carrying out the complete experimental procedure on a smithsonite and quartz bag without any plant compartment and covered with a film of plastic paraffin (Parafilm "M").

2.4. Leachate analysis

Leachates were analyzed 156, 192, 265 and 336 h after placing the smithsonite and quartz bag in contact with the plant roots. At each date of leachate collection, the volume of leached solutions was determined by weighing the HDPE collector bottles. A small aliquot was then sampled for pH determination and the remaining solution was filtered at 0.45 µm (Pall, Supor 450 membrane). The Zn concentration was measured by inductively coupled plasma – atomic emission spectrometry (ICP-AES; Thermo Jarrell Ash, IRIS Advantage). The concentration of dissolved organic C (DOC) was measured using a Dohrmann DC-180 C analyzer.

2.5. Plant analysis

Plants were harvested at the end of the 4-day equilibrium period or after 14 days of contact with the smithsonite and quartz bag. At each harvest, shoots and roots were separated and roots were rinsed with deionized water. Shoots and roots were then dried (60°C; 72 h), weighed and crushed. The Zn concentration in shoots and roots was determined by ICP-AES after mineralization by HNO_3 and *aqua regia* digestion. The amount of Zn taken up by plants from the beginning to the end of the leachate collection was calculated as the difference between the amount of Zn in vegetation at the end of the experiment minus the amount of Zn in vegetation at the end of the 4-day equilibrium period. It should be noted that, at each root harvest, no mycorrhizal fungi was detected by visual observation.

3. Results and discussion

3.1. Plant roots influence on pH and dissolved organic carbon (DOC)

Throughout the experiment (Table 2.1), the pH value in both systems was within the usual range reported for smithsonite-containing mine wastes, soils and sediments (i.e. 6.5-8.5) (Van Damme et al., 2010; Nannoni et al., 2011; Iavazzo et al., 2012). Because smithsonite is a carbonate mineral, the average pH in leachates in the presence or absence of plants was higher than the initial pH of the Zn-deficient nutrient solution (Fig. 2.3a). Moreover, compared to the control, the average pH in leachates in the presence of plants increased by 0.4 units. Similar observations were obtained by Blossfeld et al. (2010) for perennial ryegrass. The main process responsible for the pH variation in the rhizosphere is the release of H^+ or OH^- by roots to compensate charge imbalance due to unequal uptakes of cations and anions by plant roots (Hinsinger et al., 2003). Because N is strongly taken up by plants, its consumption greatly influences the cation-anion balances (Haynes, 1990). Since, in the study, NO_3^- was by far the dominant *N* source, the roots most likely released OH^- in the rhizospheric solution to counterbalance the excess negative charge taken up by the roots (Gahoonia et al., 1992), a mechanism which increased the leachate pH. Finally, higher CO_2 concentration due to root respiration could have also contributed to increase the pH of leachates from planted systems (Hamon et al., 1995). Indeed, CO_2 degassing until reaching equilibrium with the ambient pCO_2 had been attained increases the pH of the solution.

Table 2.1 pH, dissolved organic carbon (DOC) and Zn concentrations in the leachates over time in the absence (control) or presence of *Lolium multiflorum*. Values are mean ± standard deviation of two (control) or three (*L. multiflorum*) replicates.

Time (hours)	pH		DOC (mg l^{-1})		Zn (µg l^{-1})	
	Control	*Lolium multiflorum*	Control	*Lolium multiflorum*	Control	*Lolium multiflorum*
156	7.28±0.08	7.53±0.11	10.42±1.96	19.33±3.56	306±8	433±112
182	7.49±0.07	7.79±0.05	11.00±0.25	13.77±2.03	156±26	271±40
265	7.34±0.02	7.76±0.03	8.52±1.47	11.44±2.04	106±13	274±53
336	7.37±0.03	7.89±0.03	8.43±0.64	13.72±1.90	119±12	350±91

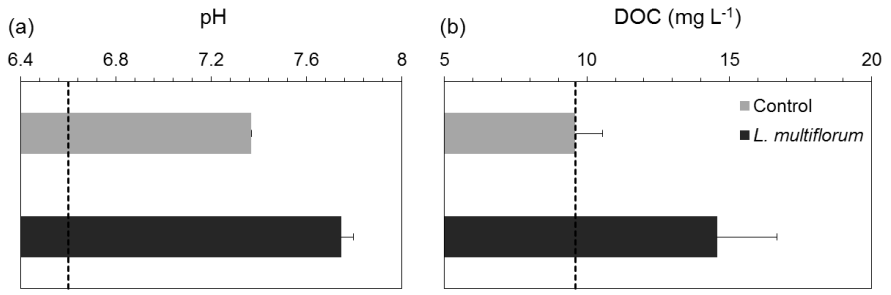


Fig. 2.3. Average pH (a) and dissolved organic C (DOC; b) concentrations in leachates in the absence (control) or presence of *Lolium multiflorum*. The dotted line represents the initial pH or DOC concentration value in the input Zn-deficient nutrient solution. Error bars denote standard deviation of two (control) or three (*L. multiflorum*) replicates.

The average DOC concentration in leachates from control treatments was not different from that in the Zn-depleted nutrient solution (Fig. 2.3b), indicating that no significant biotic activity occurred in the control system. The presence of DOC in control leachates was thus only due to the presence of EDTA from the nutrient solution. Conversely, the presence of plants significantly increased the DOC concentration (Table 2.1; Fig. 2.3b) as reported in previous findings (Hamon et al., 1995; Lorenz et al., 1997; Kim et al., 2010). Generally, the authors attributed this DOC increase to root exudation or to the stimulation of the soil organic matter solubilization/degradation caused by the rhizospheric activity (Khalid et al., 2007; Zhao et al., 2007). Since the rhizodeposits and the organic compounds released by root-associated microorganisms were the sole source of DOC in the study, it can be assumed that the additional DOC concentration measured in leachates only derived from rhizosphere activity. Production of DOC by microorganisms most probably occurred as a result of the stimulation of microbial activities by the presence of rhizodeposits such as root exudates (Dennis et al., 2010). Thus, in accordance with Phillips et al. (2011), DOC concentration of organic compounds produced by the rhizosphere activity (DOC_{rhizo}) at each sampling date was calculated as the concentration of DOC in the leachate from planted systems minus the average DOC concentration in the leachate from plant-free controls.

3.2. Kinetics of Zn release into leachates

On each sampling date, the Zn concentrations in leachates were higher in the presence of plants (Table 2.1). The cumulative release of Zn by the smithsonite and quartz bags in the leachates (µg g^{-1} smithsonite) in both the presence and the absence of plants was calculated and plotted against time (Fig. 2.4). During the span of the experiment, the cumulative release of Zn followed a straight line for both settings. Such a linear release (zero-order kinetic) is usually observed when a mineral is submitted to pure dissolution, provided the concentration of the released element is kept low (Raulund-Rasmussen et al., 1998). To quantify the effect of *L. multiflorum* on the Zn release in leachates, the rate of Zn release (*k*; µg g^{-1} s^{-1}) was calculated in leachates. This rate was obtained by regressing the total amount of Zn against time. Results clearly highlighted that, from the first to the last leachate sampling, plants enhanced the weathering of smithsonite as the rate of Zn release in leachates in the presence of plants ($k = 2.9 \times 10^{-4}$ µg g^{-1} s^{-1}) was twice as high than in the absence of plants ($k = 1.5 \times 10^{-4}$ µg g^{-1} s^{-1}). As a result, although the Zn amount released in the first leachate from the planted system (124 ± 12.0 µg of Zn) was similar to that in the control (99 ± 10.6 µg of Zn), the total amount of Zn leached at the end of the experiment (314 ± 30.0 µg of Zn) was 1.6 times higher in the presence of plants than that in their absence (198 ± 0.2 µg of Zn).

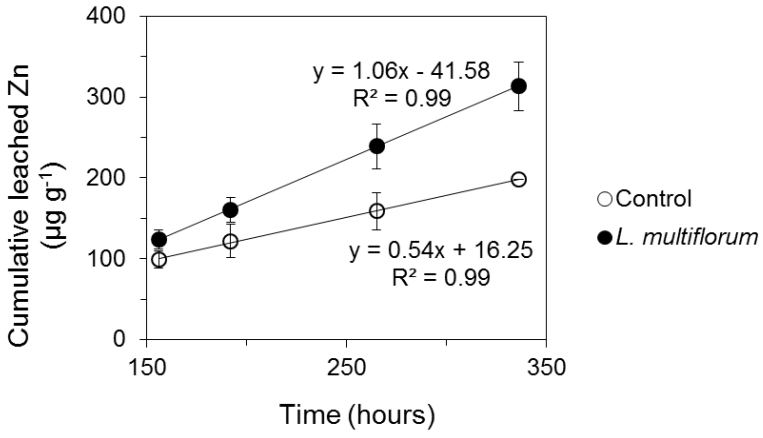


Fig. 2.4. Cumulative Zn released in leachates by smithsonite plotted against time in the absence (control) or presence of *Lolium multiflorum*. The slope coefficient of linear regressions (straight lines) indicates the rate of Zn release in leachates.

3.3. Mass balance of released Zn

In order to quantify the *L. multiflorum* impact on the total Zn release by smithsonite and, thus, on the weathering of the mineral, a mass balance has been calculated by summing the amounts of Zn transferred from smithsonite to the leachates and to the plants (Fig. 2.5). The results unambiguously showed that the presence of plants considerably accelerated the weathering of smithsonite as the total Zn amount released in the presence of *L. multiflorum* (445 ± 6.5 µg of Zn) was 2.25 times higher than that released in the control experiment (198 ± 0.2 µg of Zn). Overall, half of this Zn release increase went into the leachates (116 µg of Zn), while the other half went into the plants (118 µg of Zn), which indicates that the plants, and especially the shoots, acted as a significant Zn sink.

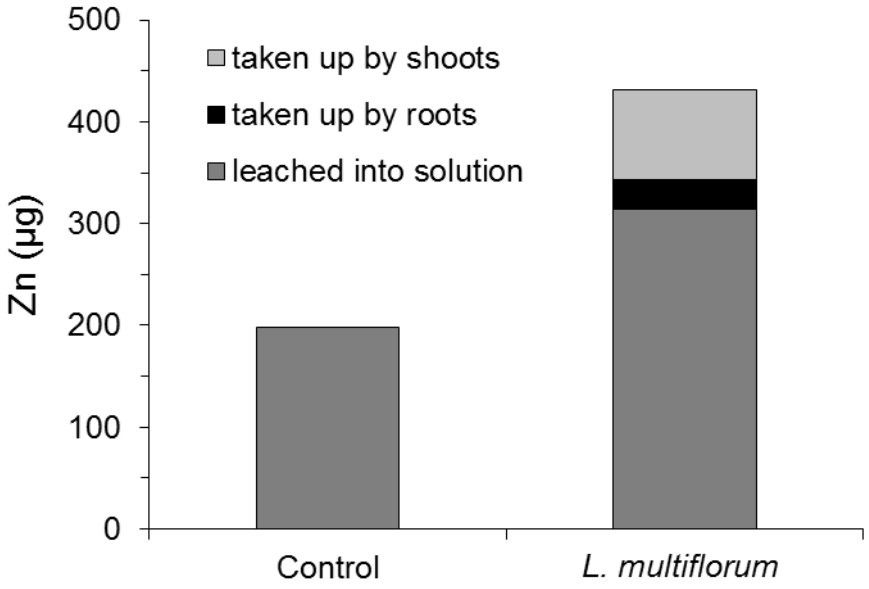


Fig. 2.5. Mass balance of total Zn released into leachates and taken up by roots and shoots in the absence (control) or presence of *Lolium multiflorum* at the end of the experiment.

3.4. Possible mechanisms for plant-induced weathering of smithsonite

Hinsinger et al. (1992) proposed that the primary cause for plant-induced mineral weathering is the uptake activity of the plant roots. For instance,

using trioctahedral mica (phlogopite) as the sole source for both K and Mg, they demonstrated that rapid weathering of phlogopite took place in the vicinity of the roots of *L. multiflorum*. Using experimental devices similar to those used in this study, Hinsinger et al. (2001) also came to the conclusion that the uptake activity of plant roots was probably responsible in part for the enhanced dissolution of basalt when in the presence of plants. By taking up, at fairly rates, major nutrients as well as nonessential elements, plants roots constantly depleted the solution. The removal of dissolution products shifted the dissolution equilibrium according to Le Chatelier's principle, thereby resulting in an increased dissolution of the rock. Accordingly, in the present experiment, Zn produced by the dissolution of smithsonite has probably been continuously absorbed by the plants. This sink effect of roots caused a Zn depletion in the solution, which could have induced further smithsonite dissolution to replenish the solution. A similar plant sink-induced dissolution effect has been observed by Bakhsh et al. (1990) in soil systems. These authors showed that in response to a decrease in soluble Zn concentration, brought about by ryegrass root uptake, the available source of Zn for plant uptake was maintained through the shift of Zn from insoluble to soluble pools. However, since smithsonite released spontaneously Zn in solution, even in the absence of plants, and the total Zn amount exported by plants was negligible relative to the total Zn amount in the smithsonite bag (i.e. less than 0.1%), the plant sink effect likely contributed for a minor part to the enhancement of the weathering of smithosnite.

Among the various factors governing biological weathering processes, pH is usually regarded as the most important (Kelly et al., 1998). For carbonate minerals, it has been shown that plants can increase their dissolution, and thus the associated release of bound-cations, by excreting H^+ (Bertrand et al., 1999). In the experiment, the pH increased in the leachates in the presence of plants. This increase would have normally hindered the dissolution of carbonate mineral (Lindsay, 1979). As the opposite was observed, it can be inferred that a change in rhizospheric pH was not the controlling factor which explains the increased Zn release in the presence of plants. A similar rhizospheric pH increase was observed by Kim et al. (2010) who postulated that higher Zn solubility in the rhizosphere was caused by

the marked increase in DOC, exuded from roots, compared to the bulk soil. Likewise, Birkefeld et al. (2006) suggested that dissolution of metal-containing minerals and slag particles in forest soils was accelerated as a result of an increase in DOC concentration. Therefore, in the study, the higher Zn release in the presence of plants was attributed to the increase in DOC concentration due to root exudation. This is supported by the strong correlation (r = 0.95, p < 0.001) observed between the Zn concentration in the leachates and the DOC_{rhizo} concentration (Fig. 2.6). Note that when all data are considered, Zn and DOC concentrations in leachates are also well correlated in the presence of plants (r = 0.89; p < 0.001) but not in their absence (r = 0.46; p > 0.05; Fig. H.1, Appendix H). Organic acids, especially carboxylic acids, exuded by roots are known to play a key role in mineral weathering (Jones, 1998) and can contribute, for instance, to phosphate solubilization (Hoffland et al., 1989; Kirk et al., 1999). Some root exudates or secretions, such as aliphatic and phenolic acids or phytosiderophores, can form chelates or strong complexes with metals (Mitsios and Danalatos, 2005). Typically, phytosiderophores are secreted by grass roots in response to a Fe-deficiency (Marschner and Romheld, 1994), but also to Zn-deficiency (von Wiren et al., 1996). They can thus directly enhance the dissolution of any solid phases which contain such metals. Although the nutrient solution in the experiment was Zn-deficient, it is unlikely that this initial deficiency induced any significant phytosiderophore production. The control experiments, in the absence of plants, showed that smithsonite spontaneously released Zn into leachates. Furthermore, shoots harvested at the completion of the experiment exhibited a Zn concentration of 82.0 ± 7.5 mg kg^{-1} dry weight (n = 3), well above the deficiency threshold for plants (<10-20 mg kg^{-1} dry weight; Kabata-Pendias, 2011). It is thus unlikely that phytosiderophores accounted for a significant fraction of the DOC measured in the root exudates. Other organic exudates probably contributed to the DOC enrichment measured in the leachates. These may have been released by such processes as the inherent leakiness of root cell plasma membranes or the concentration gradient between the roots and the solution (Farrar et al., 2003). In root exudates from ryegrass, Xu et al. (2007) detected high concentrations of low-molecular-weight organic acids (oxalic, tartaric, malic and succinic acids). Because such compounds have a

strong affinity for metals and form relatively stable chelates (Dakora and Phillips, 2002), their likely release by the plants in the experiment could thus have been involved in the enhancement of the weathering of smithsonite. The root exudates formed organo-Zn complexes which, in turn, modified the equilibrium. Two possible mechanisms have been proposed by Drever and Stillings (1997) and Turpault et al. (2009). By adhering to the mineral surface, these compounds enhance the extraction of Zn from the mineral particles by electron transfer. By chelating Zn^{2+} ions in solution, they help maintain a steep chemical activity gradient in the vicinity of the mineral surface that controls the mineral dissolution rate and they prevent any secondary mineral formation by keeping the solution undersaturated. Finally, in spite of surface sterilization of seeds, it is unlikely that microorganisms were totally absent and thus the contribution of their activity to smithsonite weathering should not be completely ruled out. For instance, rhizodeposits such as root exudates may provide the substrate required for the production of mineral weathering metabolites by the root-associated microorganisms (Grayston et al., 1997; Calvaruso et al., 2006) which might, in turn, increase the smithsonite weathering.

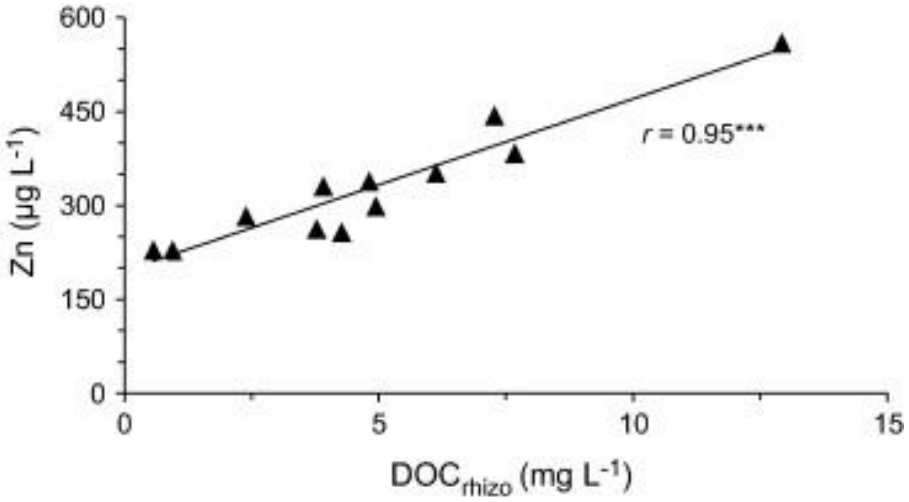


Fig. 2.6. Correlation between Zn concentration and dissolved organic C concentration of organic compounds produced by the rhizosphere activity (DOC_{rhizo}) in leachates. Marked correlation coefficient (*r**) is the Pearson's correlation coefficient significant at the 0.001 probability level.**

4. Conclusions and implications

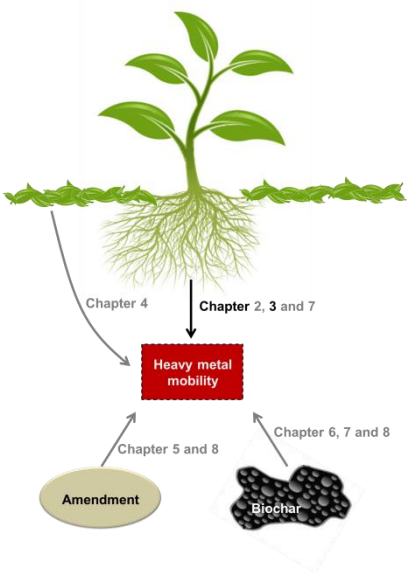
This study aimed at assessing the potential effect of roots on the weathering of smithsonite, a common Zn mineral. Although the presence of plants increased the pH of the solution surrounding smithsonite particles, it was shown that, compared to the plant-free controls, *L. multiflorum* root activity doubled the rate of Zn release from smithsonite to leachates and that the Zn concentration in leachates was controlled by DOC originating in organic compounds produced by the rhizosphere activity. Moreover, the total Zn amount released by smithsonite (defined as the sum of the Zn amounts lost by leaching and taken up by the plants) has been shown to be more than twice as high in the presence of plants compared to the controls. Although root-induced mobilization of Zn has been previously shown (Degryse et al., 2008), to the authors' knowledge, this study is the first to quantitatively assess the short-term effects of root activity on weathering of a Zn mineral and the subsequent Zn distribution into leachates, roots and shoots. As this was a laboratory study, *in situ* long-term studies could lead to different conclusions due to, for example, the presence of a complex, diverse soil microbial community and other solid soil constituents. Moreover, it must be stressed that the results of this study are highly dependent on the supplied form of N. If NO_3^- was replaced with NH_4^+, acidification of leachates would most likely have occured, thereby modifying the mineral dissolution. However, the results showing a significant increase in Zn release in the presence of plants suggests that the ability of plants to alter metal phases in soils should be taken into account when re-vegetation strategies are considered for the reclamation of metal polluted soils. Indeed, it is crucial to accurately assess all potential weathering mechanisms acting upon contaminated substrates because they initiate the process of dispersing metals to surrounding environments.

Chapter 3

Effects of plants on heavy metal release and Zn isotope fractionation in the leachate-soil-plant system

Where are we?

Chapter 2 demonstrates that root activity can increase the release of Zn into the environment by accelerating the weathering of metal phases. However, the study was specific to one mineral and thus did not consider other accompanying solid phases. Obviously, the occurrence of a unique metal phase in the environment is unlikely. Therefore, Chapter 3 aims at evaluating the effect of root activity on the release of Cd, Zn and Pb from two highly metal contaminated soils.



1. Introduction

Zinc is a metal which plays a dualistic role in the environment. For most living organisms, Zn is a vital micronutrient, notably because it acts as a building block in all six enzyme classes (Berg and Shi, 1996). In contrast, an excess of Zn causes deleterious effects on organisms. For instance, high levels of Zn in soil inhibit many plant metabolic functions, result in retarded

growth and cause senescence (Nagajyoti et al., 2010). While Zn is the most common crop micronutrient deficiency (Broadley et al., 2007), numerous soils have been contaminated by Zn worldwide since the Industrial Revolution. Large Zn inputs to soil are due, for instance, to atmospheric emission fallouts (Nriagu, 1996) or to the deposition of pyrometallurgical wastes, such as slag, which often have a high residual Zn content (Nriagu and Pacyna, 1988).

There is increasing evidence that plant-induced chemical changes can drastically alter the release of metals in soils (Kim et al., 2010; Bravin et al., 2012), even though studies have shown divergent results. For instance, in column experiments, in which either effluent were collected at the bottom of the column (Banks et al., 1994; Zhu et al., 1999; Keller et al., 2002) or soil solution was sampled using suction cups at various depths (Zhao et al., 2007; Huynh et al., 2008), plants were found to induce both mobilizing and immobilizing effects. A comprehensive understanding of the plant interactions with the medium and the subsequent plant-induced mechanisms that affect the release of Zn is thus required to fuel the process of assessing the biogeochemistry of Zn and the ecological risks arisen by its presence in soils. This requirement is amplified by the increasing use of phytotechnologies, such as phytostabilization, for the reclamation of contaminated soils. The purpose of phytotechnologies is notably to establish a vegetation cover which will be effective in providing the necessary surface stability to prevent wind-blow of contaminated particulates, and, more importantly, in reducing water percolation by interception of a substantial proportion of incident precipitation (Vangronsveld et al., 1995; Tordoff et al., 2000).

Stable Zn isotopes offer the opportunity for gaining new insights into the biotic and abiotic processes which control Zn release from the Earth's critical zone (Brantley et al., 2007). Recently, a number of studies have investigated Zn isotopes as potential tracers for metal fluxes in the anthroposphere (Sivry et al., 2008; Mattielli et al., 2009). Black et al. (2011) suggested that the Zn leaching from industrial sites could lead to the observed heavy signature in river systems, the magnitude of which being dependent on the source material and on the aqueous species that form. According to Borrok et al. (2008), the relative abundances of Zn isotopes in

natural waters may be used to probe biogeochemical reactions and to fingerprint and track sources of Zn. However, the understanding of Zn isotope ratios in the environment depends on their quantification in the various compartments and materials on earth and on their fractionation during the biogeochemical processes that drive the speciation of Zn (Jouvin et al., 2009). Since biogeochemical processes are important mechanisms that affect the isotope fractionation during Zn cycling in the environment (Bigalke et al., 2010), they need to be carefully assessed prior to use Zn isotopes for the identification of Zn sources (Weiss et al., 2007; Juillot et al., 2011). In particular, plant uptake has been recognized as a key process affecting the Zn isotope composition during its biogeochemical cycle, but the understanding of the controlling mechanisms and constraints are still limited at best (Jouvin et al., 2012). During plant uptake of Zn, an enrichment of the heavier isotopes in the roots compared to the nutrient solution followed by a depletion in the shoots compared to the roots have been reported (Weiss et al., 2005; Moynier et al., 2009; Caldelas et al., 2011). Arnold et al. (2010) observed heavy Zn isotope enrichment in efficient rice genotypes relative to the plant-available Zn. This observation was explained by the preferential complexation of heavy Zn isotopes by phytosiderophores released by roots and the subsequent uptake of this complex.

Moreover, besides the recycling of elements which are taken up by plants and ultimately return to soil through the litterfall, plant-induced changes in the rhizosphere could also directly affect the Zn isotope signature in the soil solution or leachates.

This study was conducted to gain a better insight about the effects of plants on the Zn release from the soil. Zinc isotopes were used to trace sources and processes controlling Zn mobility within the leachate-soil-plant system. Since Zn industries produced also large quantities of dusts and wastes rich in Cd and Pb, so that elevated levels of Zn in contaminated soils are often accompanied with high levels of Cd and Pb, we also investigated the effects of plants on the release of these metals from the soil. *Agrostis tenuis* Sibth. was chosen as model plant because it is a metal-tolerant plant which can successfully cover in a temperate climate any Pb/Zn waste with a moderate physical structure, be it calcareous or acidic (Tordoff et al., 2000).

2. Materials and methods

2.1. Site description

Two soils, with different sources of metal contamination, were collected in Belgium. Site 1 is located at Sclaigneaux near the city of Andenne in the alluvial plain of the Meuse River (hereafter referred to as Sclaigneaux). Although this 80 ha site is now a natural reserve accessible to the public, from the 1850's to the 1970's it was intensively subjected to heavy metal contamination due to atmospheric fallouts and slag deposits originating from the adjacent lead and zinc smelting plant (Société Dumont). A detailed description of the site and associated slag can be found in De Nul (2010). The study soil was collected in a bare area of a slag heap which is partly spontaneously revegetated by grasses and birches. Site 2 consists of an industrial dump site located at Angleur near the city of Liège (hereafter referred to as Angleur). The total site area measures around 1.9 m², and major parts of the tailings are neighbored by the Ourthe River and the Canal of the Ourthe. The dump contains waste material originating from a zinc smelting plant, which was operated between 1837 and 1905 by the Société des Mines et Fonderies de Zinc de la Vieille-Montagne. A detailed description of the site is available in Ganne et al. (2006). Although several parts of the site are still bare, it has been spontaneously colonized by a (pseudo-)metallophytre flora including *Armeria maritima* ssp. *halleri* and *Agrostis tenuis* Sibth. (Houben et al., in press; Chapter 4). The study soil was collected in a bare area. A picture of both sites is given in Appendix B.

2.2. Collection and preliminary characterization of soil samples

Composite bulk (10 kg) surface soil samples (0-10 cm) were collected from each site according to a random design. After homogenization and two-week air-drying in the laboratory, a subsample was crushed and dry sieved to a particle size comprised between 100 and 500 µm. The relative percentage (by weight) of this fraction within the fine Earth (0 – 2 mm) was

about 45% for both soils (Table 3.1). All the experiments of this study were conducted on this soil fraction.

Table 3.1 Grain size distribution (%, w/w) within the fine Earth (0 – 2 mm) for both soils.

	0 – 100 µm	100 – 500 µm	500 – 2000 µm
Sclaigneaux	12	45	43
Angleur	10	44	46

Table 3.2 presents selected properties of this 100 – 500 µm fraction, including pH (pH-H_2O), electrical conductivity, cation exchange capacity and exchangeable cations determined according to the procedures outlined by Page et al. (1982) and total metal content determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Thermo Jarrell Ash, IRIS Advantage) after calcination at 450 °C followed by acid digestion (HNO_3, $HCLO_4$, and HF), as described in Lambrechts et al. (2011b). Table 3.2 reveals that both sites are highly contaminated and that Zn for the soil of Angleur and both Zn and Pb for the soil of Sclaigneaux are the major cations on the exchange complex.

Table 3.2 pH, electrical conductivity (EC), cation exchange capacity (CEC), exchangeable cation (Exch-) and total metal contents of soils.

	Sclaigneaux	Angleur
pH	6.73	7.51
EC (µS cm^{-1})	53	26
CEC ($cmol_c$ kg^{-1})	5.32	7.84
Exch-Ca ($cmol_c$ kg^{-1})	0.84	1.56
Exch-K ($cmol_c$ kg^{-1})	0.03	0.04
Exch-Mg ($cmol_c$ kg^{-1})	0.17	0.07
Exch-Na ($cmol_c$ kg^{-1})	0.01	0.01
Exch-Zn ($cmol_c$ kg^{-1})	1.75	2.54
Exch-Pb ($cmol_c$ kg^{-1})	1.13	0.06
Total Zn (mg kg^{-1})	56,600	9470
Total Cd (mg kg^{-1})	147	56
Total Pb (mg kg^{-1})	18,500	1110

The mineralogy of the soils was determined by performing X-Ray diffraction spectra (Bruker AXS D8 Advance spectrometer, Cu Kα radiation). The heavy metal distribution in soils was analyzed using the Community Bureau of Reference (BCR) three-step sequential extraction scheme (Rauret et al., 1999). This method makes it possible to partition the metals into the operationally defined exchangeable, acid-soluble and specifically adsorbed

fraction (F1), iron and manganese oxyhydroxide-bound fraction (F2), and organic- and sulfide-bound fraction (F3). An additional fourth step consists in extracting the residual fraction bound to the mineral matrix (F4). The procedure is summarized in Table 3.3. After each separation by centrifugation, the supernatant was filtered (0.45 µm), and the concentrations of Cd, Zn, and Pb in the solution were measured by ICP-AES. The quality of the analytical data obtained with BCR scheme was controlled by applying the same method on certified reference materials (BCR-701; Certified Reference Material, European Commission, Brussels) and results were in good agreement overall with the certified values and were within the literature range reviewed by Sutherland (2010). Each soil analysis was performed in three replicates. All element concentrations are presented on a dry matter basis (i.e., dried at 105°C).

Table 3.3 Modified BCR (Community Bureau of Reference) sequential extraction scheme

Step[a]	Operational definition	Target phase	Chemical reagents and conditions
1	Exchangeable and weak acid soluble (F1)	Soil solution, carbonates, exchangeable metals	To a 1-g aliquot: add 40 ml of 0.11 M HOAc, shake for 16 h at 22±5 °C; separate extract from the solid residue by centrifugation at 3000 *g* for 20 min
2	Reducible (F2)	Iron and manganese oxyhydroxides	To Step 1 residue: add 40 ml of 0.5 M $NH_2OH.HCl$ from a 1 l solution containing 25 ml of 2 M HNO3 (pH 1.5), shake for 16 h at 22±5 °C; centrifuge at 3000 *g* for 20 min
3	Oxidizable (F3)	Organic matter and sulfides	To Step 2 residue: add 10 ml of H_2O_2 (pH 2-3), 1 h at room temperature; heat to 85±2 °C for 1 h; add a further 10 ml of H_2O_2 and heat to 85±2 °C for 1 h; add 50 ml of 1 M NH_4OAc (pH 2) and shake for 16 h at 22±5 °C; centrifuge at 3000 *g* for 20 min
4	Residual (F4)	Remaining, non-silicate bound metals	Digest Step 3 residue on a hotplate using $HF/HNO_3/HClO_4$ mixture in a Teflon crucible

[a]Steps 1-3 adapted from Rauret et al. (1999). Step 4 was added to determine the concentration of metals in the residue from Step 3.

2.3. Leaching column design and growth conditions

The design of the experimental device (for details, see appendix B) was based on Gommers et al. (2005). The columns consisted of PVC tubes with a 300 mm length and a 75 mm diameter. A multiholes drilled bottom end cap covered by a polyamide mesh (mesh size 20 µm) was fitted at the lower end of the columns. Each column was connected to a funnel channeling the leachates into a high-density polyethylene (HDPE) collector bottle. All apparatus were acid washed (pH 3, HCl) and abundantly rinsed with deionized water. Each column was filled in with a mixture consisting of 100 g of soil particles (100 – 500 µm) and 1900 g of washed quartz (500 – 1000 µm). The height of this mixture into the column was about 25 cm.

Prior to the sowing, the columns were arranged according to a randomized design in a controlled climate room (temperature of 20°C, relative humidity of 80%, 16-hour photoperiod and mean light intensity varying from 120 to 180 µmol m^{-2} s^{-1}). The columns were also slowly saturated with the nutrient solution whose the chemical composition expressed in mM was (Rufyikiri et al., 2000; Rufyikiri et al., 2004): 1 $Ca(NO_3)_2.4H_2O$, 0.5 KCl, 0.25 K_2SO_4, 0.05 $MgCl_2$, 0.05 $MgSO_4$, 0.05 NaH_2PO_4, and 0.08 H_3BO_3. Then, 0.5 ± 0.01 g of sterilized seeds (10 min in 2 M H_2O_2) of *Agrostis tenuis* Sibth. was sown in each column and the surface of the soil was covered by a thin layer (2 mm) of polyethylene beads in order to limit the surface from drying-out, prevent soil destructuration by drop impact and ensure watering flow homogeneity. Reference experiments were also conducted following the same procedure but without plants. One week after sowing, each column was connected by opaque inlet tubing to its own nutrient solution reservoir and started to be irrigated. The nutrient solution was carried from the reservoir to the column using a multichannel peristaltic pump which ensured that each column received the same volume of solution. However, according to the growth rate of the plants and related transpiration, the irrigating flow was modified during the course of the experiment so that the percolated volume from each column was at least 80 - 100 ml $week^{-1}$. Leachates were collected weekly for 7 weeks. At the completion of the experiment, shoots were cut using ceramic scissors. The material filling the tube was then extruded and the core was cut into 5-cm thick slices. The relatively large but contrasted sizes of grains between quartz and soil made it possible to

separate the roots from the adhering solid particles while enabling the recovery of the soil by sieving the soil-quartz mixture. Thus, after carefully removing and setting aside the roots, the material of each slice was air-dried and soil was separated from quartz by sieving at < 500 µm. Similar to Cappuyns et al. (2006), a single extractions with $CaCl_2$ (0.01 M) was performed to estimate the "actual" mobilization (i.e. porewater composition) of Zn, Cd and Pb in each slice (Houba et al., 2000). The pH (pH-$CaCl_2$) was also measured in the 0.01 M $CaCl_2$ extract.
In total, we set up 12 columns: 2 soils (Sclaigneaux and Angleur) × 2 vegetal cover conditions (*Agrostis tenuis* and plant-free soil) × 3 replicates.

2.4. Leachate analysis

At each date of leachate collection, the volume of solution was determined by weighing the HDPE collector bottles. A small aliquot was then set aside for pH determination and the remaining solution was filtered at 0.45 µm (Pall, Supor 450 membrane). Concentrations of Cd, Pb, Zn, Ca, Fe, K, Mg, Mn and Na were measured by ICP-AES. The concentration of inorganic anions (F^-, Cl^-, NO_3^-, $H_2PO_4^-$ and SO_4^{2-}) and NH_4^+ were determined by ion chromatography (HPLC; Dionex). The concentrations in dissolved organic carbon (DOC) were measured using a Dohrmann DC-180 carbon analyzer.
The aqueous speciation of Zn was obtained by calculation using the Visual MINTEQ 3.0 software and based on the Stockholm Humic Model (SHM). The complete description of this model for aqueous speciation is available in Gustafsson (2001). Computations were performed assuming that the solutions were in equilibrium with atmospheric CO_2 and using temperature, pH, DOC, anions and total dissolved cations as input data. To be consistent with the model optimization performed by Sjostedt et al. (2010), the ratio of active dissolved organic matter (DOM) to DOC was set at 1.65 and 100% of the active DOM was considered to be fulvic acids.

2.5. Plant analysis

At harvest, shoots and roots were repeatedly washed with deionized water to remove any adhering soil particles, dried (70°C; 96 h), weighed and ground. The Zn, Cd and Pb concentrations in shoots and roots was determined by ICP-AES after mineralization by HNO_3 and *aqua regia* digestion.

2.6. Chemical Zn purification and isotope analyses

Chemical treatments were carried out under a class 100 laminar flow hood in a class 1000 cleaned room (Université Libre de Bruxelles, Belgium). All the acids used for the isotope analysis sensus stricto were suboiled pro-analysi acids and the resistivity of mQ-water was 18 MΩ.

Zinc isotope analyses were performed on each root and shoot harvested at the completion of the column experiment and on all leachates collected after three and six weeks. The Zn isotope signature of both soils was analyzed in two replicates. Crushed soil and plant samples were calcinated for 24 hours at 450°C prior to be dissolved in concentrated HNO_3 and HF in Savilex® beaker on a hot plate until dryness. Leachates were evaporated. Then, dry residues of soil, plant and leachates were redissolved in 6 M HCl. Zn was purified using chromatographic micro-columns loaded with 0.2 ml AG1-X8 resin filled by successive addition of acids ($HCl/HNO_3/HBr$) according the methodology described by Couder (2011).

Zn isotopes were measured on a Nu plasma MC-ICP-MS in wet plasma mode (Université Libre de Bruxelles, Belgium). Mass discrimination effects were corrected by using simultaneously external normalization (Cu-doping method) and standard-sample bracketing with in-house Merck Zn and Cu standard solutions (calibrated against the JMC 3-0749L Zn and NIST SRM 976 Cu reference standards). During the period of data collection, repeated analysis of our in-house Zn and Cu standards gave a mean value of 0.00±0.03 ‰ (2SD).

The Zn isotope compositions in this study were expressed as
$\delta^{66}Zn = [(^{66}Zn/^{64}Zn)_{sample}/(^{66}Zn/^{64}Zn)_{standard} - 1] \times 1000$ (in ‰).

To express the isotope fractionation between two components A and B, we used $\Delta^{66}Zn_{A-B}$ that equals the difference $\delta^{66}Zn_A - \delta^{66}Zn_B$.

3. Results and Discussion

3.1. Geochemical forms of heavy metals in the soils

Table 3.4 depicts the concentrations as well as the proportion of metals in each operationally defined fraction of the BCR sequential extraction procedure. For Zn, the fractions followed the order: F1 > F2 > F4 > F3 in both soils (Table 3.4). The dominance of Zn in the first fraction of the BCR procedure (45 – 64 %; F1), indicates that Zn was readily adsorbed on the surface of several soil constituents such as clay minerals, organic matter and hydrous oxides and thus potentially highly mobile, which is in agreement with observations made by other authors for contaminated soils and tailings (Davidson et al., 2006; Rodríguez et al., 2009; Anju and Banerjee, 2010). For both soils, the concentration of Zn in the F1 fraction, which corresponds to 76 $cmol_c\ kg^{-1}$ and 23 $cmol_c\ kg^{-1}$ for the soils from Sclaigneaux and Angleur respectively, was largely higher than the concentration of Zn on the exchange complex (Table 3.2), suggesting that a significant part of Zn was specifically adsorbed. Given the soil pH (Table 3.2), the presence of Zn-bearing carbonates probably did not occur in the soil of Sclaigneaux while it could slightly arise in the soil of Angleur. In both soils, although Zn was mainly associated with the F1 fraction, a significant proportion of Zn was found in the reducible fraction (23 – 40 %; F2), i.e. the Zn pool associated to Fe-Mn oxides. This can be explained by the fact that the incoming pollutants initially existed as unstable chemical forms and their dissolution leads to the formation of precipitates, especially as easily to moderately reducible oxides and hydroxides (Song et al., 1999; Lee, 2006). Under superficial environmental conditions, remnant sulfide ore minerals that was incorporated in slag during the smelting process are quickly oxidized and associated metals are released and separated from sulphur at an early stage of mineral weathering (He et al., 2005; Rodríguez et al., 2009). Thus, in our case study, the very low presence of Zn in the F3

fraction (1 – 3%) is likely due to the weathering of metals sulfides (Verner et al., 1996; Anju and Banerjee, 2010) and the subsequent Zn redistribution among other soil components (Houben et al., in press).

Table 3.4 Concentration (mg kg^{-1}; mean ± standard deviation of three replicates) and fractionation (%) of metals into the four fractions (BCR procedure, see Table 3.3) in soils.

	Zn		Cd		Pb	
	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%
Sclaigneaux						
F1	24,880 ± 340	45	61 ± 0	47	6330 ± 80	33
F2	22,030 ± 700	40	44 ± 2	33	9930 ± 620	52
F3	583 ± 9	1	6 ± 0	5	803 ± 7	4
F4	7190 ± 370	13	10 ± 2	15	2030 ± 240	11
Angleur						
F1	7560 ± 310	64	31 ± 0	48	43 ± 4	3
F2	2720 ± 200	23	28 ± 0	43	1280 ± 130	75
F3	303 ± 3	3	2 ± 0	3	173 ± 9	10
F4	1146 ± 18	10	4 ± 0	6	203 ± 23	12

The Cd fractions followed the same order as Zn for both soils (F1 > F2 > F4 > F3, Table 3.4). This partition pattern indicates a high potential mobility and a similar environmental behavior for both metals. The same Cd distribution was found by Anju and Banerjee (2010) in old tailings and the authors also attributed the least Cd abundance in the F3 fraction to the sulfides weathering.

Unlike Zn and Cd, the highest percentages of Pb in both soils were found in the F2 fraction (52 – 75%; Table 3.4). The accumulation of anthropogenic Pb in the F2 fraction is consistent with other findings showing that Fe and Mn hydrous oxides are important scavengers of Pb and that they play a key role in controlling its mobility in the environment (Davidson et al., 2006; Rodríguez et al., 2009). In contrast to the soil from Sclaigneaux, the abundance of Pb in the F1 fraction of the soil from Angleur was very low (3%), which indicates a limited mobility and a low bioavailability of Pb in this soil. The difference of Pb distribution in the F1 fraction between both soils is likely due to differences in terms of both ores and processes which were used by both industries. Indeed, the smelting plant at Sclaigneaux produced Zn and Pb while the smelting plant at Angleur was only producing Zn. Finally, the oxidizable fraction (F3) did not account for a high part of the total Pb in both soils (4 – 10%). These low percentages of Pb in the F3

fraction were similar to those reported by other studies for old tailings and surrounding soils (Li and Thornton, 2001; Rodríguez et al., 2009). They suggest that, as for Cd and Zn, some Pb sulfide minerals remained in slag and that they underwent dissolution by weathering.

3.2. Biomass production and heavy metal uptake by plants

The biomass of both roots and shoots was significantly lower ($p < 0.001$) for plants grown on the Sclaigneaux soil. This is most likely due to a higher phytotoxicity in this soil since all the other experimental conditions were similar for both soils. *Agrostis tenuis* is usually considered a pseudo-metallophyte, i.e. a plant which grows on both metal-contaminated and non-contaminated soils (Baker, 1987). Its long known metal tolerance (Bradshaw, 1952) has promoted its use for the stabilization of metal contaminated soils (Smith and Bradshaw, 1979; Tordoff et al., 2000). In our experiments, irrespective of the soil, Zn, Cd and Pb were much more concentrated in roots than in shoots (Table 3.5), suggesting metal immobilization in roots. The same trends were observed by Dahmani-Muller et al. (2000) for Zn, Cd and Pb and by Denaeyer-De Smet and Duvigneaud (1974) for Pb and Zn in *A. tenuis* growing in polluted soils. Except for Zn in shoots, both roots and shoots of plants grown on the Sclaigneaux soil exhibited the highest concentrations in Zn, Cd and Pb (Table 3.5). This is probably related to the much higher metal concentration in the F1 fraction in this soil (Table 3.4). *Agrostis tenuis* is usually considered a Zn excluder, i.e. a plant which shows constant Zn concentration in shoots in response to high Zn concentration (Baker, 1981), and this likely explains why the Zn concentrations in shoots was similar for the soils. Zinc concentrations in shoots compare to values found by Denaeyer-De Smet and Duvigneaud (1974) and Couder (2011) for *A. tenuis* growing in soils polluted by atmospheric fallouts (480 mg kg^{-1} and 449 mg kg^{-1}, respectively).

Table 3.5 Concentration (mean ± standard deviation of three replicates) of Zn, Cd and Pb in roots and shoots of *Agrostis tenuis*

	Sclaigneaux		Angleur	
	Roots	Shoots	Roots	Shoots
Biomass (g)	0.15 ± 0.04	0.46 ± 0.12	0.33 ± 0.02	1.11 ± 0.09
Zn (mg kg^{-1})	7000 ± 1890	455 ± 44	1560 ± 350	537 ± 44
Cd (mg kg^{-1})	40.38 ± 9.55	2.47 ± 0.81	15.82 ± 3.85	0.93 ± 0.42
Pb (mg kg^{-1})	2860 ± 790	28.39 ± 2.11	158.2 ± 24.9	1.96 ± 0.19

3.3. Plant root influence on pH, dissolved organic carbon (DOC) concentration and volume of leachate

The mineral nutrition of plants is well known to influence the pH in the rhizosphere as roots release H^+ or OH^- to compensate charge imbalance due to unequal uptakes of cations and anions (Hinsinger et al., 2003). The plant nitrogen nutrition is an important factor influencing the cation-anion balances because nitrogen is strongly taken up by plants and can occur either as anion (NO_3^-) or cation (NH_4^+) (Haynes, 1990). In this study, nitrogen was exclusively supplied as nitrate in order to ensure that no H^+ would be exuded by the plant for its nitrogen nutrition. However, it was observed that the presence of plants induced the acidification of the leachates in both soils (Fig. 3.1). With the exception of the top layer of the columns (0-5cm), the pH (pH-$CaCl_2$) along the soil profile was likewise lower in the presence of plants (Fig. 3.2), indicating that more protons were present on the exchange complex in the planted soils than in the plant-free soils. A similar pH decrease in the rhizosphere of nitrate-fed tobacco was observed by Loosemore et al. (2004) who attributed it to an excess cation uptake. According to several authors (Bhandal and Kaur, 1992; Calba and Jaillard, 1997; You et al., 2009; Mokhele et al., 2012) this excess cation uptake could be explained by the decrease or the inhibition of anion uptake, and particularly nitrate uptake, induced by metal toxicity. Moreover, the stimulation of microbial activity, and especially nitrification, in the presence of plants could have also contributed to decrease the soil pH (Iizumi et al., 1980; Penton et al., 2013).

It is generally observed that the DOC concentration is higher in the presence of plants, and this is attributed to the exudation and the secretion of organic compounds by the rhizosphere activity (Khalid et al., 2007; Bravin

et al., 2012; Houben and Sonnet, 2012; Chapter 2). However, in this study, leachates from planted soils were generally not significantly enriched in DOC compared to leachates from plant-free soils (Fig. 3.1). It is well known that the microbial biomass thrives in the vicinity of an active root because rhizodeposits, especially organic exudates, provide the rich carbon source required for growth (Merckx et al., 1986; Dennis et al., 2010). The growth of microbial population may in turn rapidly consume the DOC produced by roots (Hamon et al., 1995) and this could therefore provide an explanation for the absence of DOC enrichment in leachates from planted soils. For both soils, a peak of DOC concentration was observed 2 weeks after the beginning of the leachate collect, regardless of the presence or the absence of plants. This is consistent with other studies which reported a DOC flush in solution of soils subjected to drying and then rewetting (Powlson and Jenkinson, 1976; Van Veen et al., 1985; Merckx et al., 2001). According to Merckx et al. (2001), the origin of the DOC flush upon rewetting is unclear but could come from the release of soluble C from organisms which were killed by the drying process. The authors also suggested that drying may release organic matter trapped in small pores, and that this material is not readsorbed upon rewetting because of the hysteresis effects accompanying the drying-rewetting event.

According to Zhu et al. (1999), the traditional view of the impact of vegetation on metal mobility is that it ends up reducing leaching because of water uptake through evapotranspiration. In this study, as expected, the presence of plants decreased the volume of leachates over the experiment (excepting at the beginning of the experiment, Fig. 3.1). The reduction was strongest for the Angleur soil, and this can be linked to the higher biomass production by *A. tenuis* on this soil (Table 3.5).

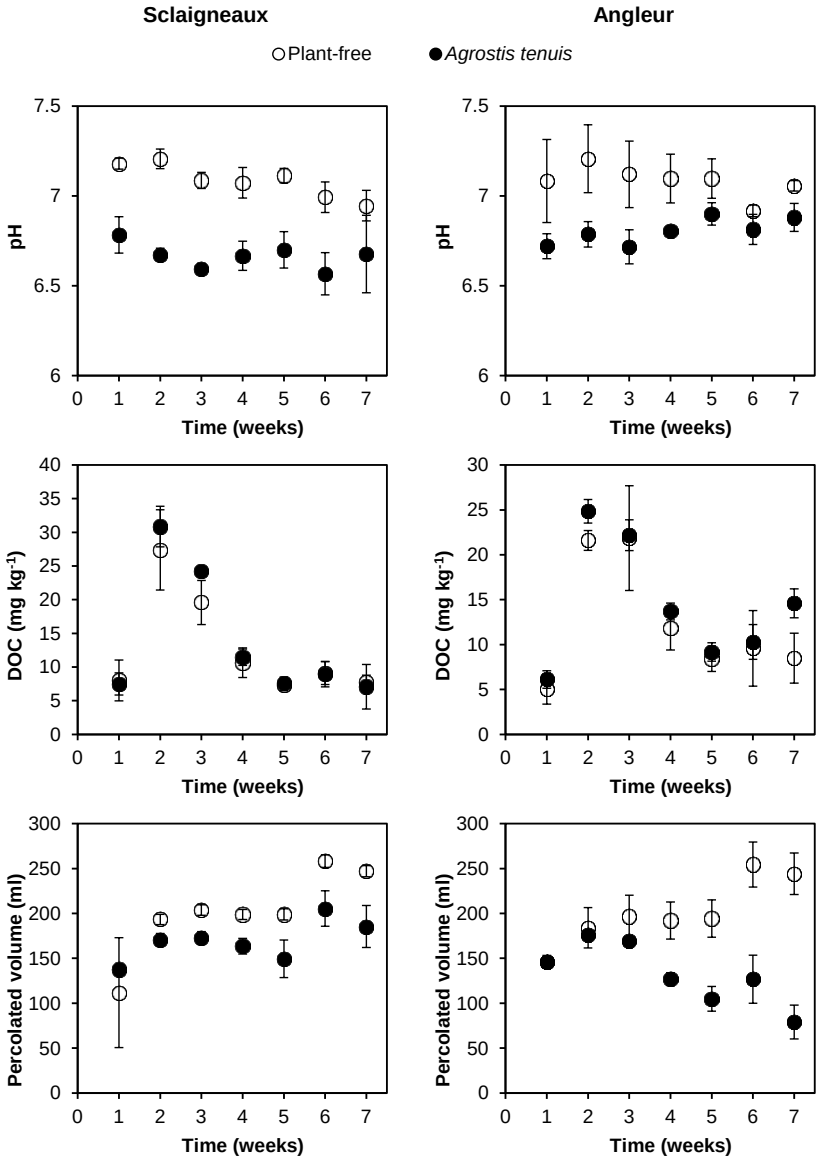


Fig. 3.1 Time evolution of pH, dissolved organic carbon (DOC) concentrations and percolated volumes of leachates from plant-free and *Agrostis tenuis*-planted soils (Sclaigneaux, left; Angleur, right).

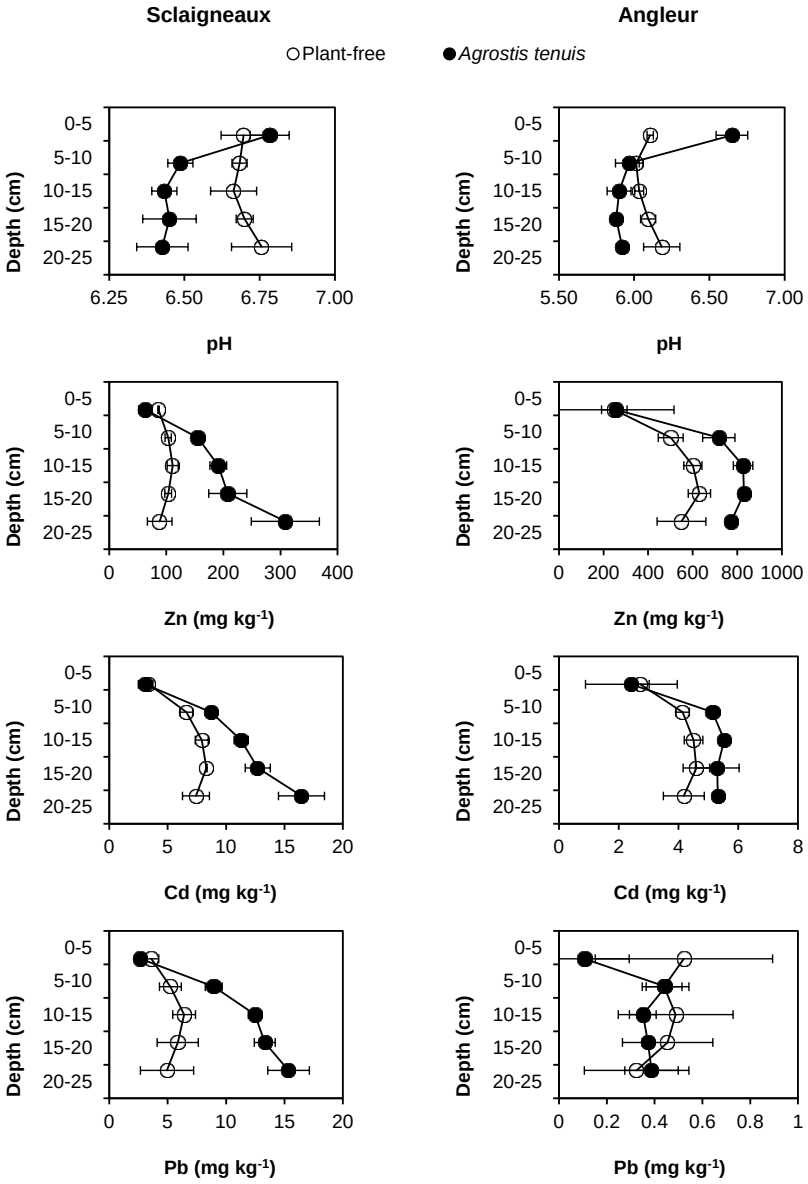


Fig. 3.2 Depth profiles of pH (pH-$CaCl_2$) and 0.01 M $CaCl_2$-extractable concentrations of Zn, Cd and Pb in soils in the absence (plant-free) or presence of *Agrostis tenuis* (Sclaigneaux, left; Angleur, right). The horizontal distance between corresponding symbols is a measure of the effect of the plant presence.

3.4. Heavy metal release as affected by plants

Although the volume of leachate was reduced in the presence of plant, the total amounts of Zn leached over the experiment were significantly higher, indicating a strong root-induced Zn mobilization (Fig. 3.3). The root-induced mobilization of Zn was strongest for the Sclaigneaux soil. In comparison with the plant-free soil, the total amount of leached Zn in the presence of plants increased by 4 times in this soil, while it only increased by 1.3 times in the Angleur soil. According to the time evolution of Zn concentration in leachates (Fig. 3.4), the root-induced Zn mobilization took place all over the experiment for the Sclaigneaux soil while it decreased at the end of the experiment for the Angleur soil. Moreover, with the exception of the top of the colums (0-5cm), the $CaCl_2$-extractable Zn concentrations along the soil profile were significantly higher in the presence of plants (Fig. 3.2), suggesting that root activity shifted Zn from a less soluble to a more soluble form. Major plant-induced processes responsible for metal mobilization include rhizosphere acidification, which causes dissolution of metal-bearing phases, and exudation of organic ligands, which causes complexation of metals in the soil solution (Hinsinger, 2001). In this study, the second process did likely not contribute for a significant part to the increased Zn mobilization because the DOC concentration (Fig. 3.1) and the Zn complexation with dissolved organic matter (Fig. 3.5) did not increase in the leachates from planted soils. By contrast, the close negative relationships between $CaCl_2$-extractable concentrations and pH along the soil profile (Fig. 3.6) suggest that the plant-induced acidification played a key role in the Zn mobilization. This is further supported by the lower pH and the concomitant higher Zn concentrations exhibited by the leachates from planted soils (Fig. 3.1, 3.4).

Similarly to Zn, the growth of *A. tenuis* induced a release of Cd and Pb. This release is attested by the fact that the total amounts of released metals (*i.e.*, the sum of the amount of metal transferred from the soil to the leachates and to the plants) were systematically higher in the presence of plants than in the absence of plants (Fig. 3.4). In the Angleur soil, however, the additional release of both Cd and Pb was, for the most part, taken up by the plants, which resulted in the total amount of leached metal being identical in presence as in absence of plants (Fig. 3.3). This high uptake of

the Cd and Pb mobilized by the root-induced processes, possibly occurred because the concentration of metals released were much smaller than those in the Sclaigneaux soil.

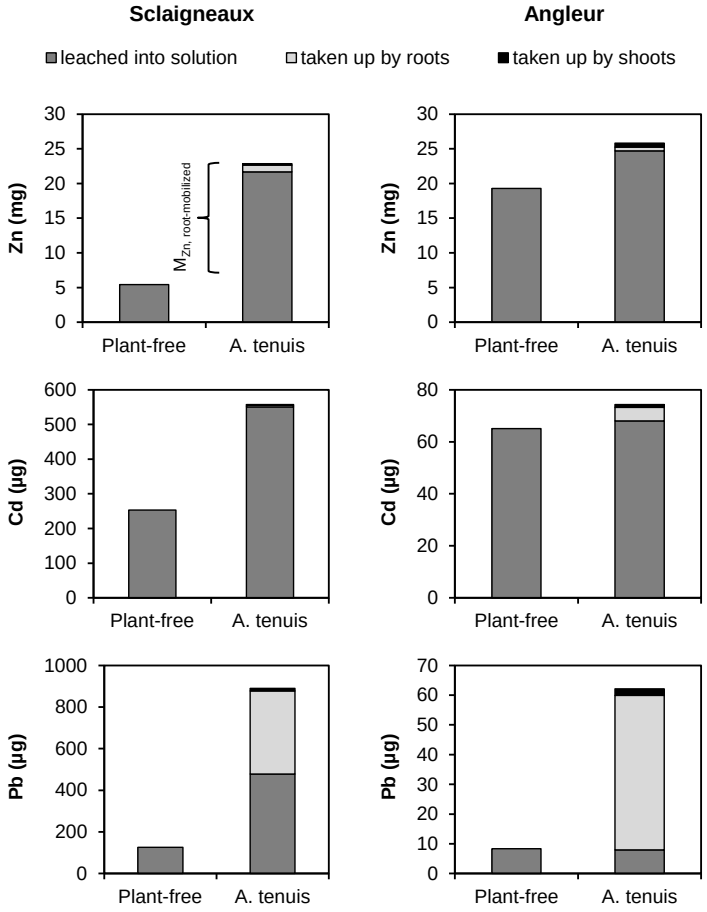


Fig. 3.3 Mass balance of total Zn, Cd and Pb released into leachates and taken up by roots and shoots in the absence (plant-free) or presence of *Agrostis tenuis* at the end of the experiment (Sclaigneaux, left; Angleur, right). The $M_{Zn,\ root\text{-}mobilized}$ represents the additional mass of Zn which is released due to root-induced Zn mobilization. Note the different y-axis between both soils for Cd and Pb.

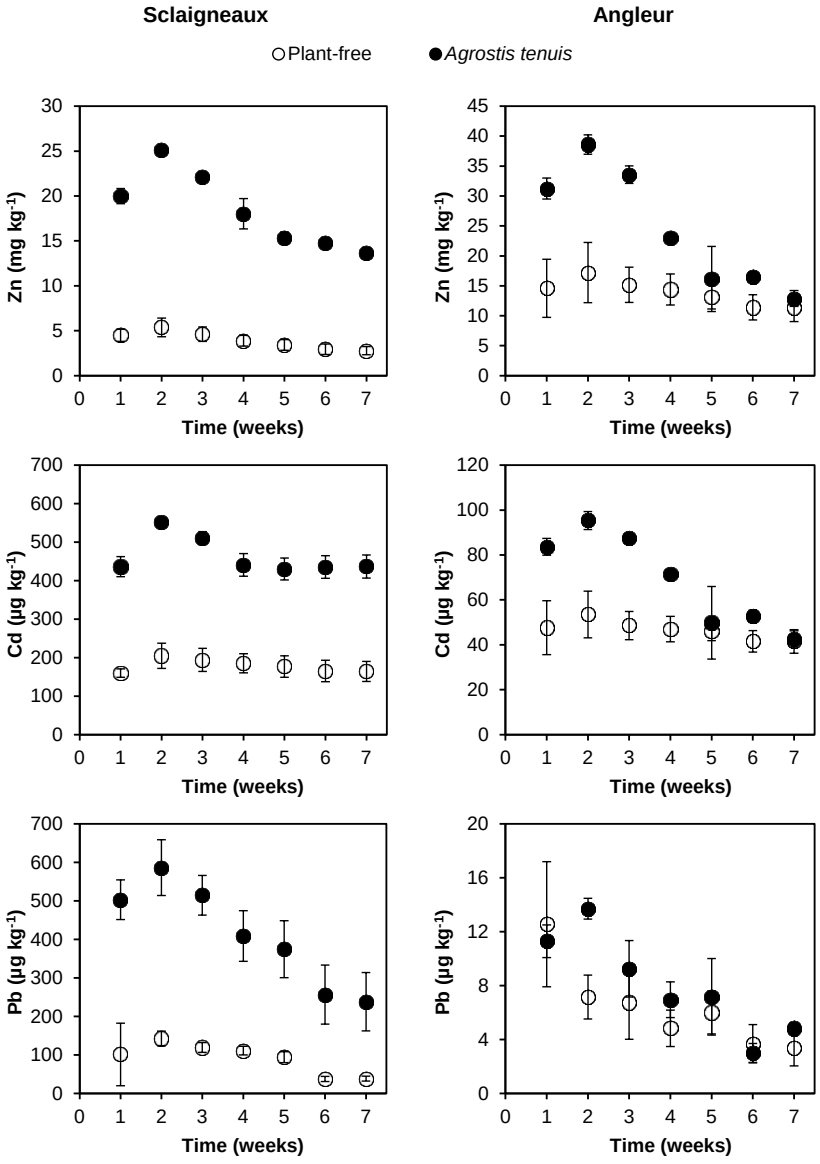


Fig. 3.4 Time evolution of Zn, Cd and Pb concentrations in leachates of plant-free and *Agrostis tenuis*-planted soils (Sclaigneaux, left; Angleur, right). The vertical distance between corresponding symbols is a measure of the effect of the plant presence.

Time evolution of concentrations (Fig. 3.4) and $CaCl_2$ extractable concentrations along the soil profile (Fig. 3.2) of Cd in both soils and of Pb in the Sclaigneaux soils were similar to that of Zn. Mechanisms for metal

mobilization in the presence of plants can thus be presumed to be similar for all three metals, i.e. mainly due to plant-induced acidification. This is further supported by the strong negative correlation between $CaCl_2$-extractable concentrations and pH along the soil profile (Fig. 3.6). However, the release of Pb in the Angleur soil was somewhat different since $CaCl_2$-extractable Pb concentrations along the soil profile did not increase in the presence of plants (Fig. 3.2) and did only poorly correlate with pH (Fig. 3.6). A likely explanation is that the susceptibility of Pb to be mobilized under slightly acidifying conditions is limited. This is consistent with Degryse et al. (2009) who showed that the solid-liquid partitioning of Pb was less pH-sensitive than that of both Cd and Zn. In our experiments, a limited mobilization is attested by the extremely low abundance of Pb measured in the first fraction of the sequential extraction procedure (F1; Table 3.4). As a consequence, the solubility of Pb in the Angleur soil was probably negligibly affected by the root-induced acidification. The increased Pb release in this soil in the presence of plants must thus be considered as due to the uptake activity of the *A. tenuis* roots which gradually absorbed the Pb as it was being released by the soil. In other words, according to Le Chatelier's principle (Law of Mass Action), the sink effect of roots caused a Pb depletion in the solution, which in turn induced further Pb release to replenish the solution (Hinsinger, 2001).

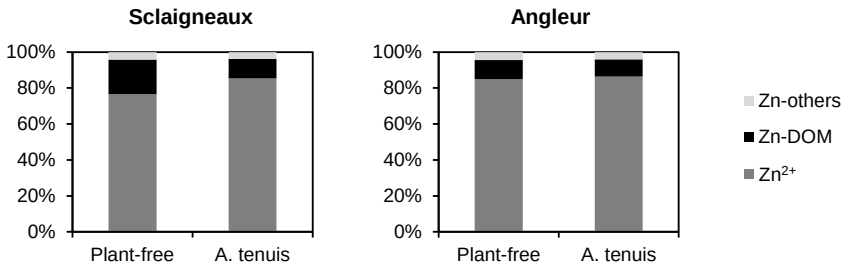


Fig. 3.5 Average speciation of zinc in the leachates of plant-free and *Agrostis tenuis*-planted soils (Sclaigneaux, left; Angleur, right) as computed by Visual MINTEQ 3.0. Zn^{2+}: metal as free ion; Zn-DOM: metal bound to dissolved organic matter; Zn-others: metal bound to other ligands.

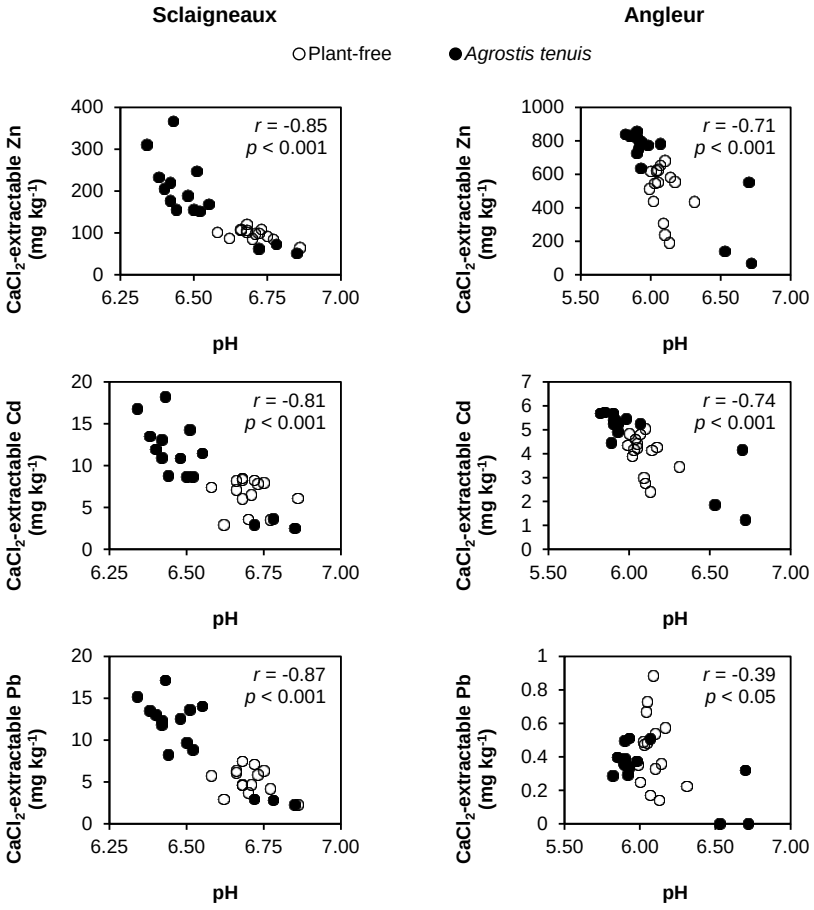


Fig. 3.6 Relationships between pH (pH-$CaCl_2$) and 0.01 M $CaCl_2$-extractable concentrations of Zn, Cd and Pb in soil profiles in the absence (plant-free) or presence of *Agrostis tenuis* (Sclaigneaux, left; Angleur, right).

3.5. Zinc isotope fractionation in the leachate-soil-plant system

3.5.1. Soils

Several studies showed that slag produced by metallurgical activities are characterized by an enrichment in heavy Zn isotopes (δ^{66}Zn values ranging from +0.2 ‰ to +1.5‰) relative to the Zn isotope composition of the initial ores (Dolgopolova et al., 2006; Sivry et al., 2008; Sonke et al., 2008; Bigalke

et al., 2010). The $\delta^{66}Zn$ value of the Angleur soil ($\delta^{66}Zn$ = 0.32 ± 0.01 ‰) is within this range, while the $\delta^{66}Zn$ of the Sclaigneaux soil ($\delta^{66}Zn$ = 0.09 ± 0.00 ‰) is slightly lower (Fig. 3.7). This reflects that slag was not the only source of Zn in the Sclaigneaux soil. Intense deposition of aerial dusts emitted by the stacks of the adjacent smelting plant occurred, as testified by the elevated Zn, Cd and Pb contents that are measured in the surrounding natural soil (see chapter 6). Zn isotope composition of dusts and aerial fallouts related to pyrometallurgical activities are known to be enriched in light Zn isotopes compared to slags, with negative $\delta^{66}Zn$ values down to -0.67 ‰ (Mattielli et al., 2009). X-ray diffraction (Table 3.6) revealed that the Sclaigneaux soil, unlike the Angleur soil, contains hemimorphite ($Zn_4Si_2O_7[OH]_2.H_2O$) and willemite (Zn_2SiO_4) which are Zn phases commonly found in slag (Nachtegaal et al., 2005). With smithsonite ($ZnCO_3$), hemimorphite and willemite are the major components of calamine, the primary Zn ore used at Sclaigneaux until the 1880s' (De Nul, 2010). Their presence at the soil surface might thus indicate that the bulks of the slag deposition stopped more than one century ago. It is likely thus that the investigated area has also been affected by subsequent atmospheric fallouts from the adjacent base metal facilities which remained in operation until the 1970s' (De Nul, 2010).

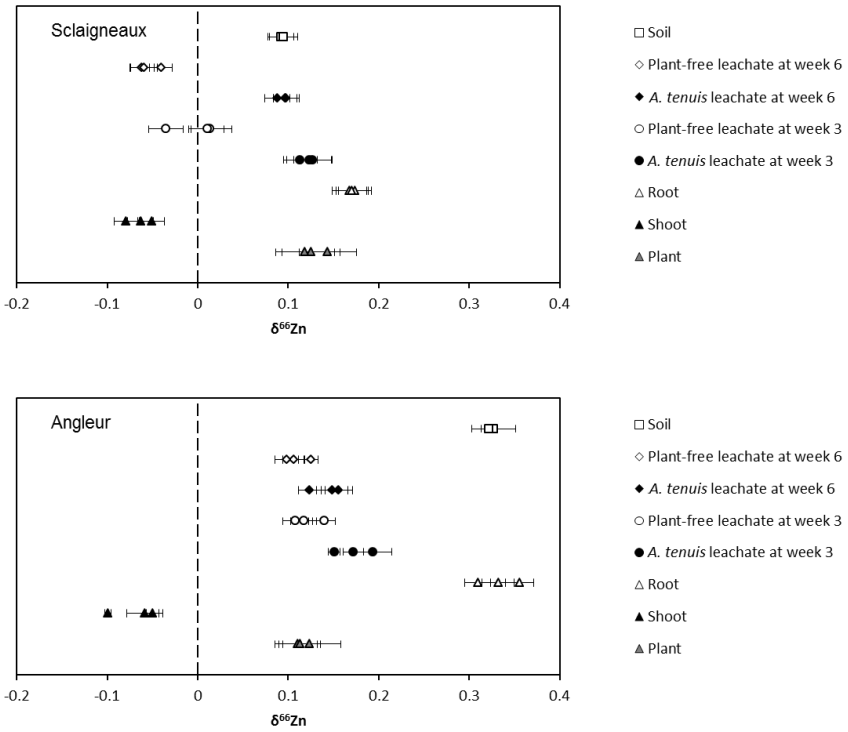


Fig. 3.7 Zn isotope compositions of soils, leachates from plant-free and from planted with *Agrostis tenuis* soils, root, shoot and whole plant. Results are presented as individual sample ± 2SEM. Isotope composition estimates for the whole plant $\delta^{66}Zn_{plant}$ (grey triangles) are calculated using the Zn masses and isotopic compositions of roots and shoots.

3.5.2. Plants

Similarly to Aucour et al. (2011), the Zn isotope composition of the whole plant $\delta^{66}Zn_{plant}$ was estimated according to the following:

$$\delta^{66}Zn_{plant}\,(M_{Zn,\,root} + M_{Zn,\,shoot}) = \delta^{66}Zn_{root}M_{Zn,\,root} + \delta^{66}Zn_{shoot}M_{Zn,\,shoot}$$

where $M_{Zn,\,root}$ and $M_{Zn,\,shoot}$ are the mass of Zn in root and in shoot respectively.

Compared to leachates from planted soils, the isotope composition of the whole plants showed only small variation ($\Delta^{66}Zn_{plant\,-\,A.\,tenuis\ leachate}$ = 0.02 ± 0.01‰ and -0.04 ± 0.02‰ for Sclaigneaux and Angleur, respectively; Fig.

3.7). This suggests that Zn uptake by roots of *A. tenuis* probably involved a transport system with no isotope selection.

However, although the Zn root uptake was almost not isotopically discriminatory, an isotope fractionation was observed within the plants (Fig. 3.7), suggesting that the translocation process was isotopically selective. The roots were enriched in heavy isotope relative to the whole plant ($\Delta^{66}Zn_{root\ -\ shoot}$ = 0.24 ± 0.01‰ and 0.40 ± 0.05‰ for Sclaigneaux and Angleur, respectively) which is in line with previous studies conducted elsewhere (Weiss et al., 2005; Viers et al., 2007; Moynier et al., 2009; Aucour et al., 2011; Jouvin et al., 2012; Tang et al., 2012). Similar to Tang et al. (2012), we estimated the Zn isotope fractionation in *A. tenuis* using a Rayleigh-type mass balance, where the isotope composition of the root Zn pool depends on the $\delta^{66}Zn$ of Zn taken up by plant ($\delta^{66}Zn_{plant}$) and the Zn proportion left in root after translocation (F_{root} = Zn mass in the root/Zn mass in the whole plant):

$$\Delta^{66}Zn_{root-plant} = \delta^{66}Zn_{root} - \delta^{66}Zn_{plant} = \Delta^{66}Zn_{translocation} \ln F_{root}$$

where $\Delta^{66}Zn_{translocation}$ represents the isotope discrimination during translocation process in the shoots. The data produced a very good fit between $\Delta^{66}Zn_{root-plant}$ and $\ln F_{root}$ (Fig. 3.8), thereby confirming that a large proportion of Zn enrichment in roots was due to the translocation process, including the radial Zn transfer to the xylem. The enrichment in heavy Zn in the roots can be in part explained by the Zn adsorption at the root cell walls as this process favors heavier Zn isotopes (Weiss et al., 2005) and has been reported as a major feature of the mechanism of tolerance to Zn in *A. tenuis* (Peterson, 1969; Turner and Marshall, 1972; Wainwright and Woolhouse, 1977). Besides Zn binding to cell walls, tolerance mechanisms may imply notably efflux pumping at the plasma membrane, chelation of metals in the cytosol by peptides such as phytochelatins and Zn vacuolar compartmentalization by tonoplast-located transporters (Hall, 2002; Broadley et al., 2007). All these processes are also expected to favor the heavy isotope (Caldelas et al., 2011). As a result of the preferential heavy Zn isotope sequestration in roots, the xylem sap was enriched in light isotope, which explains the lighter isotope composition of shoots.

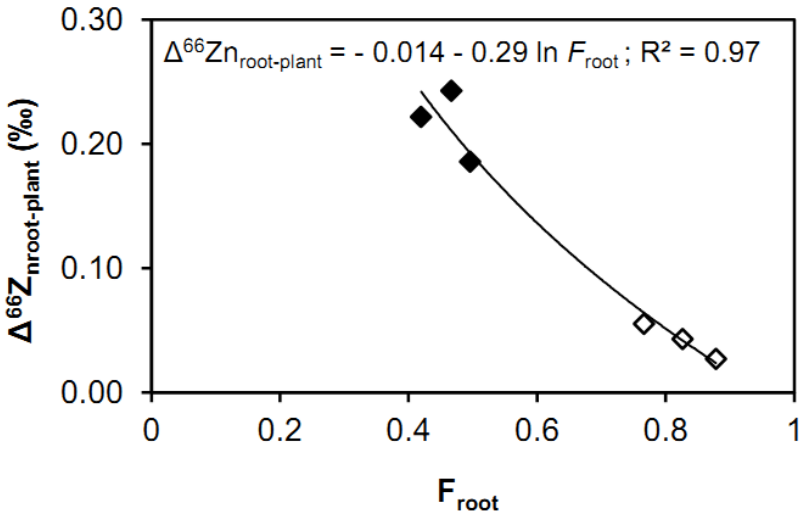


Fig. 9 Raleygh mass balance to model the Zn isotope fractionation between root and whole plant ($\Delta^{66}Zn_{root\text{-}plant}$), as a function of F_{root} during translocation. F_{root} is given as mass-ratio between root and whole plant. Open diamands: Sclaigneaux plants; dark diamonds: Angleur plants.

Interestingly, shoots of *A. tenuis* collected *in situ* by Couder (2011) at a soil Zn-contaminated by atmospheric fallouts showed both similar Zn concentrations (450 mg kg^{-1}) and similar isotope composition ($\delta^{66}Zn_{shoots}$ = -0.05 ± 0.02‰) as *A. tenuis* shoots harvested in the present study (i.e., 455 ± 44 mg kg^{-1} and $\delta^{66}Zn_{shoots}$ = -0.06 ± 0.01‰ for shoots from Sclaigneaux soil plants 537 ± 44 mg kg^{-1} and $\delta^{66}Zn_{shoots}$ = -0.07 ± 0.03‰ for shoots from Angleur soil; Table 3.5, Fig. 3.7). These seemingly constant values between the study of Couder (2011) and the present study tend to indicate that the Zn translocation at the root-shoot interface is strongly regulated by *A. tenuis*. Further researches on more samples should nevertheless be carried out to confirm this hypothesis.

Table 3.6 Mineralogical composition of Sclaigneaux and Angleur bulk soil determined using X-ray diffraction spectra.

Sclaigneaux	Angleur
Quartz	Quartz
Cristobalite	Anhydrite
Anhydrite	Mulite
Barite	
Mulite	
Hemimorphite	
Lead	
Willemite	

3.5.3. Leachates

For both soils, leachates from plant-free soils were enriched in light Zn isotope relative to the bulk soil (average $\Delta^{66}Zn_{\text{plant-free leachate – soil}}$ = -0.12 ± 0.03‰ and -0.21 ± 0.01‰ for Sclaigneaux and Angleur soils, respectively; Fig. 3.7), which confirms that spontaneous Zn release from the solid phase to the soil solution favors light isotopes (Arnold et al., 2010). The presence of plants significantly (p < 0.05) modified the Zn isotope composition of leachates as, for both soils, an enrichment in heavy Zn was observed in leachates from planted soils compared to leachates from plant-free soils (average $\Delta^{66}Zn_{\textit{A. tenuis}\text{ leachate – plant-free leachate}}$ = 0.14 ± 0.02 ‰ and 0.04 ± 0.01 ‰ for Sclaigneaux and for Angleur respectively; Fig. 3.7). Such an enrichment in heavy Zn in nutrient solution has been previously reported in hydroponic experiments, because the plant uptake depleted the solution in light Zn isotope (Aucour et al., 2011; Caldelas et al., 2011). However, in contrast to previous studies, the enrichment in heavy Zn that we observed in leachates from planted soils cannot be ascribed to the plant uptake since the amounts of Zn taken up by plants were very low compared to the total amount of leached Zn (4.5 – 5.5 %; Fig. 3.3). More importantly, the whole plants were isotopically similar to leachates from plant-free soils (Angleur) or even heavier (Sclaigneaux) (Fig. 3.7).

The Zn isotope composition of a solution can be seen as the result of the mass balance between the isotopically lighter free ions and the isotopically heavier organic complexes (Jouvin et al., 2009). Consequently, the isotopically heavier composition of leachates in the presence of plants suggests a higher proportion of complexed Zn in solution, possibly due to the release of phytosiderophores (Arnold et al., 2010). However, as

previously mentioned, small variations of DOC concentration were observed between plant-free and planted soils (Fig. 3.1). Moreover, calculated speciation (Visual MINTEQ) did not indicate any increase in organo-Zn complexes in the presence of plants and predicted free Zn^{2+} to be the dominant species in both cases (Fig. 3.5). Therefore, the heavier Zn isotope composition of leachates from planted soils can only be explained by the release of Zn from a pool that is heavier than the one which spontaneously supplied Zn to leachates in the absence of plants. This evidences the ability of plants to induce the mobilization of Zn from a less soluble pool. We hypothesize that this pool could be ascribed to the Zn-bound to Fe-Mn oxides. The reasoning is that root-induced acidification might cause a release of Zn from Fe-Mn oxides (Kirk and Bajita, 1995) and that Zn desorption upon acidification enriched the solution in heavy Zn isotopes (Balistrieri et al., 2008; Juillot et al., 2008). For instance, Balistrieri et al. (2008) showed that the fraction of Zn sorbed after 24 h at pH 7.5 on 2-Lines ferrihydrite was 86% of the initial Zn amount in solution (initial Zn concentration = 0.1 mM; solid/liquid ratio = 1:100) and that the Zn isotope signature of the solution was $\delta^{66}Zn$ = -0.22‰. At pH 7, the fraction of sorbed Zn was 60% and the $\delta^{66}Zn$ value of the solution was -0.11‰ while at pH 6.5, the fraction of sorbed Zn was 35% and the $\delta^{66}Zn$ value of the solution was +0.03‰. The authors showed a similar trend for goethite, the fraction of sorbed Zn being 96% and 53% and the $\delta^{66}Zn$ of the solution being 0.01‰ and 0.13‰ when the pH was maintained at 7.5 and 6.5, respectively.

3.5.4. Isotope composition of the root-mobilized Zn

The Zn isotope composition of the total Zn released by the soil in the presence of *A. tenuis* ($\delta^{66}Zn_{A.\ tenuis\ \text{total}}$) can be obtained from our measurements using the following equation:

$$\delta^{66}Zn_{A.\ tenuis\ \text{total}}\ (M_{Zn,\ \text{plant}} + M_{Zn,\ A.\ tenuis\ \text{leachate}})$$
$$= \delta^{66}Zn_{\text{plant}} M_{Zn,\ \text{plant}} + \delta^{66}Zn_{A.\ tenuis\ \text{leachate}} M_{Zn,\ A.\ tenuis\ \text{leachate}}$$

where $M_{Zn,\ \text{plant}}$ and $M_{Zn,\ A.\ tenuis\ \text{leachate}}$ are respectively the total mass of Zn taken up by plants and released into leachates in the course of the

experiment by planted soils. We can also write a second equation involving the Zn isotope composition of the total Zn released by the soil in the presence of *A. tenuis*:

$$\delta^{66}Zn_{A.\ tenuis\ \text{total}} (M_{Zn,\ \text{plant-free leachate}} + M_{Zn,\ \text{root-mobilized}})$$
$$= \delta^{66}Zn_{\text{plant-free leachate}} M_{Zn,\ \text{plant-free leachate}} + \delta^{66}Zn_{\text{root-mobilized}} M_{Zn,\ \text{root-mobilized}}$$

where $M_{Zn,\ \text{plant-free leachate}}$ is the total mass of Zn released into plant-free leachate in the course of the experiment and $M_{\text{root-mobilized}}$ is the additional Zn mass released in the presence of plants, i.e. $M_{Zn,\ \text{root-mobilized}} = M_{Zn,\ \text{Plant}} + M_{Zn,\ A.\ tenuis\ \text{leachate}} - M_{Zn,\ \text{plant-free leachate}}$ (Fig. 3.3). This equation allows us to obtain the average isotope composition signature of the root-mobilized Zn ($\delta^{66}Zn_{\text{root-mobilized}}$) from our measurements.

Calculation results show that $\delta^{66}Zn_{\text{root-mobilized}}$ was 0.15‰ and 0.28‰ for the Sclaigneaux and Angleur soils, respectively. Compared to the isotope signature of the Zn spontaneously released by the soil, i.e. without the action of the plants ($\delta^{66}Zn_{\text{plant-free leachate}}$), the isotope signature of the root-mobilized Zn was +0.18 ± 0.03‰ and +0.16 ± 0.01 (± SD, n = 6) heavier for the Sclaigneaux and the Angleur soil, respectively. This isotope fractionation due to root-induced Zn mobilization was thus apparently identical for both soils, which tends to suggest that similar mobilizing mechanisms acted in both soils.

3.5.5. Comparison with *in situ* observations

This study highlights that the presence of *A. tenuis* and the subsequent root-induced acidification of the soil can lead to a considerable enrichment in heavy Zn isotope in leachates relative to those from plant-free soils. As a consequence, on the long run, a soil covered by *A. tenuis* would progressively become isotopically lighter than the plant-free soil. Interestingly, this could explain, at least in part, the observation made *in situ* by Couder (2011) at the Angleur site. The author showed that the isotope composition of the soil of an area spontaneously long-term revegetated by *A. tenuis* ($\delta^{66}Zn$ = 0.29 ± 0.01‰ and 0.23 ± 0.01‰ for soils at 0-7 and 7-30 cm depth, respectively) was slightly but significantly lighter than that of the contiguous left bare soil ($\delta^{66}Zn$ = 0.37 ± 0.02‰ and 0.34 ±

0.04‰ for soils at 0-7 and 7-30 cm depth, respectively). In addition, Couder (2011) also found that the Zn isotope fractionation between the soil and the leachates *in situ* collected using zero-tension lysimeters placed at a depth of 7 cm (i.e. $\Delta^{66}Zn_{leachate\ -\ soil}$) was lower for the area covered by *A. tenuis* ($\Delta^{66}Zn_{A.\ tenuis\ leachate\ -\ soil}$ = -0.25 ‰) than for the plant-free area ($\Delta^{66}Zn_{plant\text{-}free\ leachate\ -\ soil}$ = -0.33 ‰), which is consistent with our laboratory findings (i.e. $\Delta^{66}Zn_{A.\ tenuis\ leachate\ -\ soil}$ = 0.01 ± 0.02‰ and $\Delta^{66}Zn_{plant\text{-}free\ leachate\ -\ soil}$ = -0.12 ± 0.03‰ for Sclaigneaux, and $\Delta^{66}Zn_{A.\ tenuis\ leachate\ -\ soil}$ = -0.17 ± 0.02‰ and $\Delta^{66}Zn_{plant\text{-}free\ leachate\ -\ soil}$ = -0.21 ± 0.01‰ for Angleur; Fig. 3.7). However, it must be kept in mind that this simplified reasoning does not take into account the isotope fractioning processes that can be induced by the buildup and degradation of litter. Future investigations should consequently be carried out to elucidate the role played by accumulated litter in the Zn release and isotope composition of leachates.

4. Conclusion and implications

This study shows that the presence of *Agrostis tenuis* Sibth. on contaminated soils can considerably increase the release of heavy metals, notably due to root-induced acidification, despite the reduction of the leachate volumes. This should be taken into account when phytostabilization strategies are considered for the reclamation of metal polluted soils. *Agrostis tenuis* sequesters heavy metals in roots and this induces Zn isotope fractionation within the plants, the heavier Zn isotope being preferentially sequestered in the roots. Our data suggest that while the Zn uptake by the plant has only a negligible effect on the Zn isotope composition of leachates, the root-induced mobilization effect considerably alters it because it promotes the release of heavy Zn isotopes from the soil to the solution. This heavy Zn isotope enrichment in leachates in the presence of plants also evidences that roots induce the release of Zn from another pool than in the absence of plants. Consequently, our results suggest that for the variation of Zn isotope signature in waters, the root-mobilization of Zn in the soils could be a more important factor than the Zn uptake by plants. This has implications notably for the use of Zn isotope as a

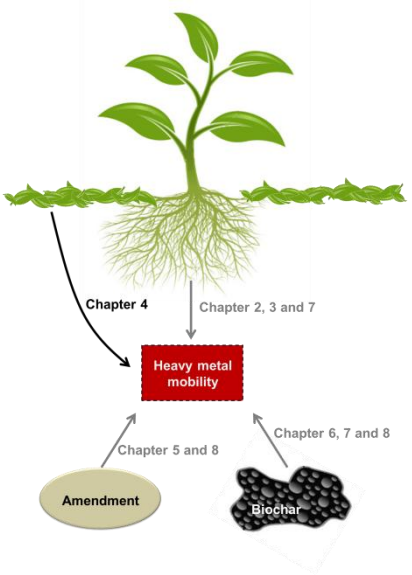
tracer of both biogeochemical reactions, including root activity, and source of pollution.

Chapter 4

Leachability of cadmium, lead and zinc in a long-term spontaneously revegetated slag heap: Implications for phytostabilization*

Where are we?

Chapter 2 and 3 evidence that metal mobilization from the soil may become intensified by the root activity, which, in turn, may negatively affect phytostabilization strategies. However, the success of phytostabilization should also consider the modifications of soil physico-chemical properties due to the long-term presence of plants, in particular because vegetation cover contributes to the soil organic matter. Therefore, Chapter 4 raised the question as to whether the impact of the long-term presence of plants and the subsequent changes in soil properties affect the heavy metal mobility.



* Adapted from Houben D., Couder E. and Sonnet Ph. (*in press*). Leachability of cadmium, lead and zinc in a long-term spontaneously revegetated slag heap: Implications for phytostabilization. *Journal of Soils and Sediments*, doi: 10.1007/s11368-012-0546-5.

1. Introduction

Throughout the nineteenth and the first half of the twentieth century, several zinc (Zn) smelting plants, now closed, operated in Europe. Between 1913 and 1932, the production of zinc in Europe varied from 404,800 to 693,300 tons/year (Musset, 1935). The pyrometallurgical process included retort furnaces, produced slag and scoria, which have been left behind as slag heaps. Zinc smelter slags from that period are known to present a high residual concentration of toxic elements such as As, Cd, Cu, Pb and Zn (Ettler et al., 2009). If left unattended, they constitute a source of toxic metal, which can have deleterious effects on the environment (Dudka and Adriano, 1997) and contaminate groundwater (Navarro et al., 2008). In addition, these slag heaps are frequently situated in densely populated areas, where they directly put human health at risk due to the contamination of vegetable garden and incidental ingestion of contaminated soils and dusts (Bosso and Enzweiler, 2008; El Hamiani et al., 2010).

Heavy metal toxicity, nutrient deficiency or poor physical structure of the substrate often inhibits vegetation establishment and colonization on mine and smelter sites (Ye et al., 2002). Nevertheless, a plant cover composed of metal tolerant species can spontaneously form (Bradshaw, 1997; Conesa et al., 2006). In some cases, this spontaneous revegetation could itself serve for phytostabilization (Barrutia et al., 2011) by creating a vegetative cap for the long-term stabilization and containment of waste. Conventional techniques for remediating metal contaminated soil, including landfilling, excavation and off-site treatment are cost-prohibitive and applicable only to relatively small areas. Phytostabilization has been suggested as a promising alternative because it offers the benefits of being implemented *in situ* at low cost as well as being less environmentally disruptive. The plant canopy limits eolian dispersion while the roots prevent water erosion and leaching (Mendez and Maier, 2008). As reviewed in detail by Bolan et al. (2011), the successful outcome of phytostabilization as a technique to remediate and revegetate contaminated sites depends on multiple factors including not only specific type of soils, plants and contaminants but also the more general surrounding environmental conditions.

The presence of plants may markedly modify the chemical conditions in the soil and, thus, also the fate of the metals (Marschner, 1995). The pH can be modified due to an unequal uptake of cations and anions by the roots (Haynes, 1990). The exudation of organic compounds by the roots can have an effect on mineral dissolution (Ochs, 1996; Houben and Sonnet, 2012; Chapter 2) and metal ion complexation (Banks et al., 1994; Jones, 1998; Nigam et al., 2001). The establishment of a vegetation cover can rapidly enrich the soil in organic matter (Coughtrey et al., 1979; Boucher et al., 2005; Sourkova et al., 2005). This enrichment can induce changes in the chemical and physical properties of the soil such as water infiltration, moisture holding capacity, sorption capacities, nutrient availability, etc. (Facelli and Pickett, 1991). Organic matter also provides a source of energy for soil biota, which controls the decomposition and mineralization of plant residues, thereby releasing both organic and inorganic compounds (Sourkova et al., 2005). The release of such compounds can, in turn, modify the release of metals from the soil (McBride et al., 1997; Titeux and Delvaux, 2009). When assessing the long-term success of any revegetation strategy, the influence of the plant species on the soil organic matter must thus be considered (Ottenhof et al., 2007).

Only a few studies have investigated the effects of revegetation on the heavy metal mobility in contaminated soil (Banks et al., 1994; Hamon et al., 1995; Zhu et al., 1999; Schwartz et al., 2001; Zhao et al., 2007; Nowack et al., 2010). Moreover, most of them were carried out for a relatively short period (i.e., from a few months to a few years) and overlooked the possible impact of organic matter accumulation. Additional information is thus required for assessing how the revegetation affects the long-term behavior of heavy metals, especially in terms of mobility and speciation (Mendez and Maier, 2008).

In this chapter, two plots having been long-term spontaneously revegetated by either *Armeria maritima* or *Agrostis tenuis* as well as a bare plot have been chosen from a slag heap in order to compare the differences of heavy metal behavior in each soil. Our objectives were to determine the speciation and the release of Cd, Pb and Zn in two long-term revegetated soils in comparison with a bare soil located in the same slag heap. Assessing these parameters is of the utmost importance to determine if spontaneous

revegetation is suitable for phytostabilization. For these purposes, sequential extractions [Community Bureau of Reference (BCR) scheme] and a leaching column experiment were carried out.

2. Materials and methods

2.1. Site description and soil sampling

Investigations were carried out at an industrial dump site located at Angleur (5 km from the city of Liège, eastern Belgium). The total site area measures around 19,000 m² and major parts of the tailings are neighbored by the Ourthe River and the canal of the Ourthe (Fig. 4.1). The dump contains waste material originating from a zinc smelting plant which was operated between 1837 and 1905 by the Société des Mines et Fonderies de Zinc de la Vieille-Montagne. Until 1885, the zinc ore consisted mainly of calamine, a mixture of smithsonite ($ZnCO_3$) and hemimorphite ($Zn_2SiO_4.H_2O$). Between 1885 and the end of the industrial activity, the ore primarily consisted of zinc sulfides. At the surface, a volume of 53,000 m³ of slag covers an area of about 8,000 m². The waste layer has an average thickness of 6-8 m. A more detailed description of the site is available in Ganne et al. (2006). When the plant closed, the site was spontaneously colonized by a special vegetation typical of zinc-contaminated soil. In his inventory of metallophyte species growing in the study site, Lambinon (1959) reported the presence of *Viola calaminaria*, *Noccaea caerulescens* subsp. *calaminare*, *Armeria maritima* ssp. *halleri* and *Silene vulgaris* ssp. *humilis*. Because of the presence of this typical flora, the site has been classified as a protected nature reserve.

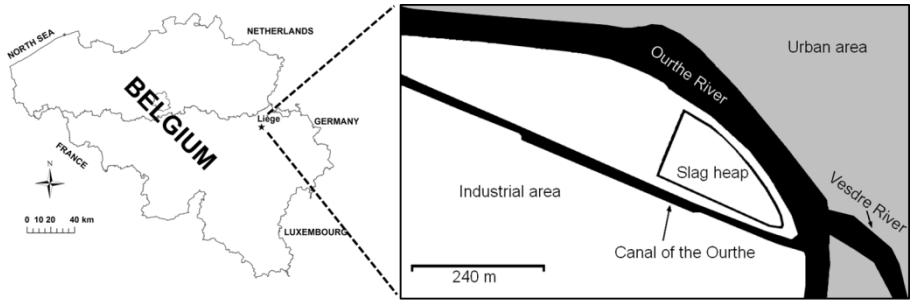


Fig. 4.1 Location of the study site

The site is exceptional in that it currently presents various plots covered by monospecific stands of different metal-tolerant plant species. Mapping the soil according to the vegetative cover made it possible to select three small contiguous plots. Two of the plots represent a homogenous monospecific stand and one plot is bare: "ARM" covered by the metallophyte *Armeria maritima* ssp. *halleri* (50°36'44.9'' N; 5°36'39.6'' E), "AGR", covered by the pseudometallophyte *Agrostis tenuis* Sibth. (50°36'44.2'' N; 5°36'39.6'' E), and "BAR" with no vegetation (50°36'44.2'' N; 5°36'39.8'' E) (Fig. 4.2). According to Couder (2011), the three soils contain a similar mixture of slag minerals, as identified by X-ray diffraction (quartz, magnetite, hematite, albite, Zn-spinel, willemite, illite and vermiculite) in addition to nondiffracting matter (vitreous slag, coal particles and amorphous soil minerals).

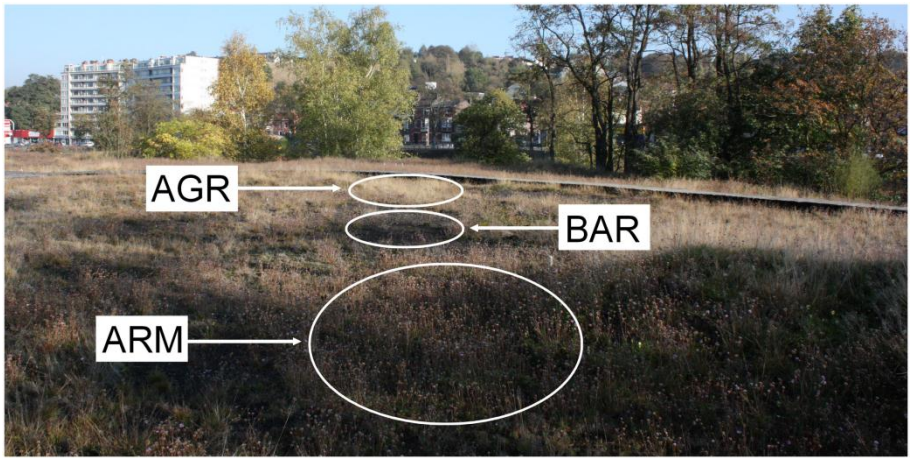


Fig. 4.2 Locations of the sampling plots in the study area. ARM and AGR are plots revegetated by *Armeria maritima* and *Agrostis tenuis*, respectively. BAR is a bare plot.

In each plot, soil was collected as a composite sample from the surface (0-7 cm) of three 0.2 × 0.2 m squares randomly selected from a 1 × 1 m². In the two revegetated plots, the 7-cm thick surface horizon was densely packed and consisted in a mixture of mineral and poorly humified organic matter. The upper horizons overlay an undifferentiated very dark gray (Munsell soil color chart) mineral-dominated horizon having a thickness of at least 30 cm and presenting traces of both brick and coal particles (Couder, 2011). *Armeria maritima* roots were exclusively confined to the upper horizon while a few *Agrostis tenuis* roots reached a depth of 10-12 cm. All soils were sieved through 2 mm after separating and removing the roots, and then air-dried during 3 days at room temperature, homogenized and maintained at 4°C prior to use. Roots and particles bigger than 2 mm were discarded.

2.2. Soil characterization

Organic C, following the Walkley and Black procedure, Kjeldahl N and pH (5 g of soil/25 ml of H_2O) were determined using the procedures outlined by Page et al. (1982). Determination of $CaCO_3$ was performed according to the Marcour method consisting in exposing soil samples to 0.5 N H_2SO_4 and estimating $CaCO_3$ concentrations by 0.5 N NaOH titration (Page et al., 1982). The grain size fractionation was achieved by quantitative recovery of sand (50 µm < diam. < 2 mm), silt (2 µm < diam. < 50 µm) and clay (diam. < 2 µm) after sonication and dispersion with Na-resin (IRP-120 resin) (Rouiller et al., 1972). The cation exchange capacity (CEC) (Chapman, 1965) and the Mehlich 3 extractable phosphorus (Mehlich, 1984) were determined. The metal composition of soils was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Jarrell Ash) after calcination at 450°C followed by acid digestion (HNO_3, $HClO_4$ and HF), as described in (Lambrechts et al., 2011b). Quality control was carried out using internal control samples, blank samples and triplicates analyses. All element concentrations are presented on a dry matter basis (i.e., dried at 105°C).

2.3. Heavy metal fractionation analysis

Heavy metal fractionation in soils was analyzed using the BCR three-step sequential extraction scheme (Rauret et al., 1999). This method makes it possible to fractionate the metals into the operationally defined exchangeable, acid-soluble and specifically adsorbed fraction (F1), iron and manganese oxyhydroxide-bound fraction (F2), and organic- and sulfide-bound fractions (F3). In addition, a fourth step consists in extracting the residual fraction bound to the mineral matrix (F4). The procedure is summarized in Table 3.3. After each separation by centrifugation, the supernatant was filtered (0.45 µm) and the Cd, Zn and Pb concentrations in the solution were measured by ICP-AES (Jarrell Ash).

Three independent replicates were performed for each soil sample and blanks were measured for each set of analyses. All analytical concentrations were expressed on a dry mass basis. The quality of the analytical data obtained using the BCR sequential extraction method was controlled by applying the same method on certified reference materials BCR-701.

Results of the metal fractionation of the reference material were in good agreement overall with the certified values (Table 4.1) and were within the range reported in the literature and recently reviewed by Sutherland (2010).

Table 4.1 Comparison of the results of the modified BCR sequential extraction of sample BCR-701 (mean ± standard deviation of 3 replicates) with certified values.

		Cd	Zn	Pb
Step 1	Obtained value	6.94 ± 0.05	205 ± 2	2.79 ± 0.03
	Certified value	7.34 ± 0.35	205 ± 6	3.18 ± 0.21
Step 2	Obtained value	3.98 ± 0.03	119 ± 1	127 ± 3
	Certified value	3.77 ± 0.06	114 ± 5	126 ± 3
Step 3	Obtained value	0.28 ± 0.00	46.5 ± 1	9.2 ± 1
	Certified value	0.27 ± 0.06	45.7 ± 4	9.3 ± 2

2.4. Leaching column design

The columns were made from 75-mm diameter PVC cylinder (see Appendix C for details). Each column was fitted with a multiholes drilled bottom end cap and connected to a funnel channeling the leachates into a high-density

polyethylene collector bottle. A Whatman no. 41 filter was placed within each end cap to prevent any loss of material at the bottom of the column. In the column, the soil was underlain by a layer of quartz wool to ensure free drainage. A 1-cm layer of quartz wool and a fritted glass disk were placed at the top of the soil column to ensure the homogeneity of the water supply and to prevent any disturbance by water droplets. All apparatus were acid washed (pH 3, HCl) and rinsed with deionized water before use.

We reconstituted 15 columns (three soils, five replicates). The weight of the soil in each column was calculated to represent a 1.4-cm layer, which corresponds to one fifth of the actual horizon height investigated *in situ*. The columns were arranged in a randomized design in a controlled climate room and maintained in the dark at constant temperature (10°C) and humidity (80%). Irrigation was performed manually by pipette. Two times a week, 7.6 mm of deionized water was evenly distributed to the top of each column. The temperature of the experiment and the quantity of water supply were selected on the basis of annual means of temperature and rainfall recorded in the investigated area. For the three soils, the field capacity was reached after about 25 days. After this rewetting period, the first leachates appeared and columns were freely drained for 30 days in order to leach dissolved salts formed during the previous soil drying. After this 55-day equilibrium period, leachates were sampled every 17 days. In total, five leachate samplings were carried out.

2.5. Physical and chemical analyses of the leachates

After sampling, the volume, pH and electrical conductivity (EC) of the leachates were directly determined and the solution was analyzed after filtration at 0.45 µm (Pall, Supor 450 membrane). Concentrations of cations were measured by ICP-AES (Jarrell Ash). Concentrations of dissolved organic carbon (DOC) were measured using a Dohrmann DC-180 carbon analyzer.

2.6. Statistical analysis

Average results for the different soils were compared using one-way analysis of variance (ANOVA) followed by Fisher's test ($p < 0.05$) for multiple comparisons. Prior to ANOVA, homogeneity of variances was tested using Levene's test. Pearson's correlation coefficients (*r*) were calculated to determine the relationships between the heavy metal concentrations in leachates and several other leachate parameters (pH, EC and DOC concentration). Three levels of significance were considered: $p < 0.05$, $p < 0.01$ and $p < 0.001$. All statistical analyses were carried out using XLSTAT (Addinsoft, ver. 2010.5.08).

3. Results and discussion

3.1. Soil characteristics, total metal contents, and relationships with vegetation

Selected properties of soils are presented in Table 4.2. Revegetated soils were more acidic (pH = 6.72 and 6.55 for ARM and AGR, respectively) than the bare soil (pH = 7.34). The ability of plant roots to alter soil pH is a well-known phenomenon and may be attributed to several mechanisms such as cation-anion uptake balance, organic anion release, exudation and respiration, and redox-coupled processes (Hinsinger et al., 2003). Moreover, the microbial activity and the decomposition of organic matter accumulated in revegetated soils may also contribute to soil acidification (van Breemen et al., 1983; Jing et al., 2007).

Table 4.2 Substrate characteristics, available phosphorus ($P_{Mehlich\ 3}$) and total metal contents. Results are means of three replicates.

	pH	$CaCO_3$	OC[b]	N	C/N	Grain size distribution			CEC	$P_{Mehlich\ 3}$	Total Cd	Total Zn	Total Pb
						Sand	Silt	Clay					
		(%)	(%)	(%)	(-)	(%)	(%)	(%)	($cmol_c\ kg^{-1}$)	($mg\ kg^{-1}$)	($mg\ kg^{-1}$)	($mg\ kg^{-1}$)	($mg\ kg^{-1}$)
ARM	6.72	ND[a]	14.9	1.14	13.0	61.5	25.6	12.9	44.7	50.36	51	52,900	6,000
AGR	6.55	ND	8.3	0.78	10.6	46.0	40.3	13.7	29.6	1.11	139	65,600	21,000
BAR	7.34	0.3	3.7	0.41	9.0	63.3	28.6	8.1	11.4	1.83	140	54,800	23,300

[a]ND: not detected
[b]OC: organic carbon

In the two revegetated soils, low C/N ratios (see Table 4.2) indicate that fresh litter was well degraded. However, the high organic C contents in AGR and, especially in ARM, indicate that mineralization of humified organic matter was considerably reduced. This is in accordance with the observations made elsewhere showing that high heavy metal pollution affects the organic matter decomposition mainly in the late stages of decay (Baath, 1989; Berg et al., 1991; Balabane et al., 1999). According to Ottenhof et al. (2007), the early stages of soil formation in mine waste materials consists of organic matter enrichment from pioneer species. With time, a well-developed Ah horizon should form. However, such an Ah horizon could not be observed in the field.

Overall, the investigated soils had very high Cd, Zn and Pb contents. The range of contents (51 – 140 mg kg^{-1} for Cd, 52,900 – 65,600 mg kg^{-1} for Zn and 6,000 – 23,300 mg kg^{-1} for Pb) is congruous to values previously found by Ganne et al. (2006) in various samples originating from the same site. Generally, the three soils presented comparable metal contents, although a lower Pb and Cd content was observed in ARM, reflecting the small-scale variation, which is characteristic of such polluted sites (Nowack et al., 2010).

The high content of metals in soils explains the presence of both *Armeria maritima* and *Agrostis tenuis*. Being an absolute metallophyte that hyperaccumulates Pb and Zn, *Armeria maritima* grows only on metal contaminated and naturally metal-rich soils (Baker, 1987) and disappears when the metal concentration in the soils decreases (Schwartz et al., 2001). As a pseudo-metallophyte, *Agrostis tenuis* grows on both metal contaminated and noncontaminated soils (Baker, 1987). The *Agrostis tenuis* metal tolerance has been long known (Bradshaw, 1952) and is mainly due to an exclusion mechanism, which immobilizes metals in roots (Dahmani-Muller et al., 2000). According to Tordoff et al. (2000), metal tolerant *Agrostis tenuis* cultivar can successfully cover in a temperate climate any Pb/Zn waste with a moderate physical structure (Table 4.2), be it calcareous or acidic. Moreover, hyperaccumulators and metal tolerant grasses respond positively to fertilization (Zhao et al., 1998; Tordoff et al., 2000; Schwartz et al., 2001). The improvement of soil fertility parameters such as CEC, N and organic C contents in both ARM and AGR compared to BAR and the

increased available P (Mehlich 3 extractable P) content in ARM (Table 4.2) probably contributed to self-sustaining the plant cover (Norland and Veith, 1995). The plants transferred significant quantities of metals to their shoots each year (Schwartz et al., 2001), which then decayed and formed a thick litter.

3.2. Effects of revegetation on metal fractionation

Pools of metal measured by sequential extractions are usually described as operationally defined, i.e. they are defined in terms of the specific protocol used to determine them. Despite this incertitude about the exact nature of the metal pools, sequential extraction still provides helpful information when comparing various treatments applied to the same initial soil (Hammer and Keller, 2002). Here, the modified BCR sequential extractions scheme (Rauret et al., 1999) was used to elucidate variations in the metal chemistry of revegetated and bare soils. The concentrations and the distribution of heavy metals among the four fractions are depicted in Table 4.3 and Fig. 4.3, respectively. The recovery, which is the ratio of the sum of the metal concentration in individual fractions to the total metal concentration in soils, was from 92.2% to 122.5%, which compares to the results achieved by other studies (Anju and Banerjee, 2010).

Table 4.3 Concentration of metals (mean ± standard deviation of three replicates) of the four fractions (BCR procedure) in the substrates revegetated by either *Armeria maritima* (ARM) or *Agrostis tenuis* (AGR) and in the bare substrate (BAR).

	Cd (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})
ARM			
F1	23.9 ± 0.4	39,502 ± 1560	585 ± 3
F2	12.1 ± 0.0	11,039 ± 244	460 ± 13
F3	5.56 ± 0.29	5,676 ± 206	4203 ± 44
F4	20.8 ± 2.9	7,607 ± 277	2,087 ± 69
AGR			
F1	73.5 ± 0.2	30,325 ± 147	2,369 ± 45
F2	27.9 ± 0.1	11,973 ± 281	1,942 ± 3
F3	9.95 ± 0.28	10,724 ± 244	13,383 ± 142
F4	30.0 ± 0.3	12,194 ± 513	4,875 ± 101
BAR			
F1	67.6 ± 0.2	21,583 ± 302	5,303 ± 110
F2	19.4 ± 0.5	8,883 ± 45	2,514 ± 54
F3	11.5 ± 0.5	12,484 ± 229	12,503 ± 131
F4	31.0 ± 1.0	11,870 ± 336	4,577 ± 79

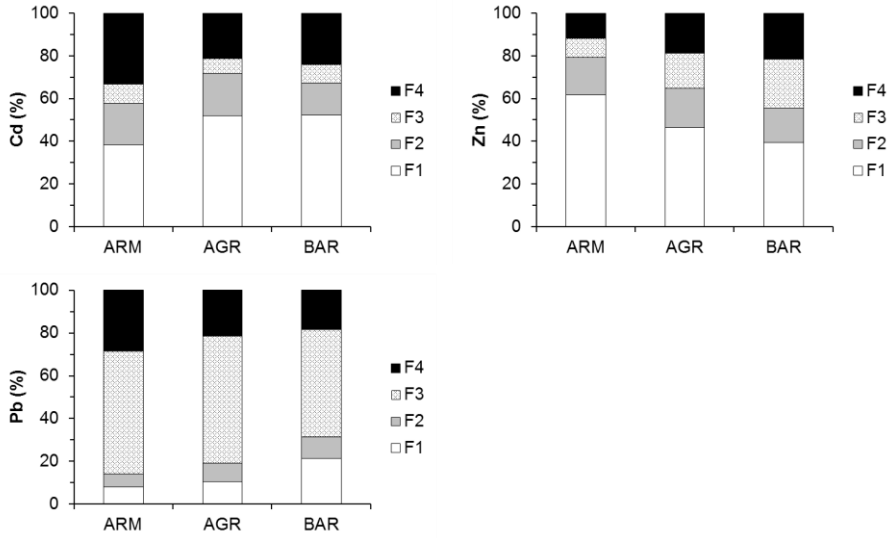


Fig. 4.3 Solid phase fractionation of Cd, Zn and Pb (average of three replicates) into the four fractions (see Table 3.3) in the substrates revegetated by either *Armeria maritima* (ARM) or *Agrostis tenuis* (AGR) and in the bare substrate (BAR).

The highest percentages of Cd and Zn in the three soils were found in the fraction dissolved in acetic acid (F1), which includes water-soluble, exchangeable and carbonate bound metals (Anju and Banerjee, 2010). This indicates that Cd and Zn are potentially highly mobile and that a pH decrease in soils could dramatically increase their release. This is in agreement with the results obtained by Ganne et al. (2006) on samples from the same site. Using pH_{stat} leaching test, they showed that Cd and Zn were the most mobile elements and that their release was highly increased by acidifying conditions. In our study, the high Cd and Zn contents in the F1 fraction of the bare soil could be interpreted as an indication of the presence of carbonates. The low alkalinity (Table 4.2) in the soil could have been conducive to the formation of $CdCO_3$ and $ZnCO_3$ (Lindsay, 1979). However X-ray diffraction did not detect these minerals, possibly because of their low concentration. In the two revegetated soils, the more acidic conditions rule out the hypothetical presence of Cd and Zn carbonate precipitates. In these soils, Cd and Zn could thus have occurred mainly on

the exchange sites at the surface of the soil constituents, such as clay minerals, hydrous oxides and organic matter. The litter build-up also probably contributed to the higher proportion of Zn in F1 in revegetated soils, since metals present in the leaves of metallophytes are mainly present in a soluble state (Perronnet et al., 2000; Boucher et al., 2005). Moreover, in Zn-contaminated soils covered by Zn-tolerant plants, Sarret et al. (2004) showed that, after the plant decay, Zn was predominantly bound to soil organic matter under a labile form. A shift of Zn from F3 and F4 to F1 in the presence of plants can be observed in Fig. 4.3. This is in agreement with Dahmani-Muller et al. (2002) who reported that the presence of metallophytes promoted the weathering of metallic minerals such as Zn-sulfides and that the subsequent amount of released Zn was partly redistributed onto the exchange sites of soil constituents. A contribution of microbial activity to the increase in metal solubility in the presence of plants should not be ruled out (Alford et al., 2010), although it is difficult to quantify (Becerra-Castro et al., 2012). Compared to the bulk soil, the rhizosphere of (pseudo-)metallophytes contains a large microbial population with a higher metabolic activity (Becerra-Castro et al., 2012). These microorganisms have the ability to increase the dissolution of metal from the nonlabile phase which, in turn, increases the metal concentration in the most mobile soil fraction (Whiting et al., 2001a). For instance, improvement of metal solubility due to increased production of acid and metal-solubilizing exudates were described in the presence of rhizosphere bacteria from metallophytes (Whiting et al., 2001a; Lodewyckx et al., 2002; Abou-Shanab et al., 2003).

Unlike Cd and Zn, most of the Pb was rather strongly bound since its distribution among F3 + F4 accounted for 69%, 81% and 86% of total Pb in, respectively, BAR, AGR and ARM. In the three studied soils, the oxidizable fraction (F3) was by far the most abundant Pb containing fraction. As underlined by Anju and Banerjee (2010), a particular drawback of the commonly used sequential extraction schemes is that F3 contains metals associated with both organic matter and sulfides. Although this study did not distinguish metals associated with organic matter from that associated with sulfides, this would be of great interest, especially when assessing the potential environmental impact of metallurgical wastes deposits. This

impact can be assessed on the basis of the metal fractionation in soils using, for instance, a risk assessment code (RAC), as suggested by Jain (2004). Since Pb sulfides weather faster in the presence of plants (Marseille et al., 2000), the Pb increase in F3 for the two revegetated soils can be attributed to the accumulation of organic matter from plant residues which is well-known to display a strong affinity for Pb (Davies, 1990). Unlike ARM and AGR, the second highest Pb fraction was F1 for BAR (21%). This difference is probably due to the higher pH which could promote the precipitation of Pb in this soil, possibly in the form of carbonate.

3.3. Effects of revegetation on leachate properties

3.3.1. pH, DOC and EC in leachates

The time-dependent patterns of pH, EC and DOC concentrations in leachates are shown in Fig. 4.4. Except for the last leachate sample, solutions from ARM exhibited the highest pH. Solutions from AGR were more alkaline than solutions from BAR only for the two first leachate samplings. Such slight pH increase in the presence of plants is characteristic of mineralization of low C/N plant residues (Boucher et al., 2005). Although a layer of sand was placed at the bottom of the column to ensure free drainage, the pH increase in revegetated soils could also be in part due to the occurrence of anaerobic spots, as suggested by the increase of Fe concentrations over time in leachates of ARM and, to a lesser extent, AGR (Fig. H.2, Appendix H). At the end of the experiment, leachates from all soils exhibited similar pH values (7.5).

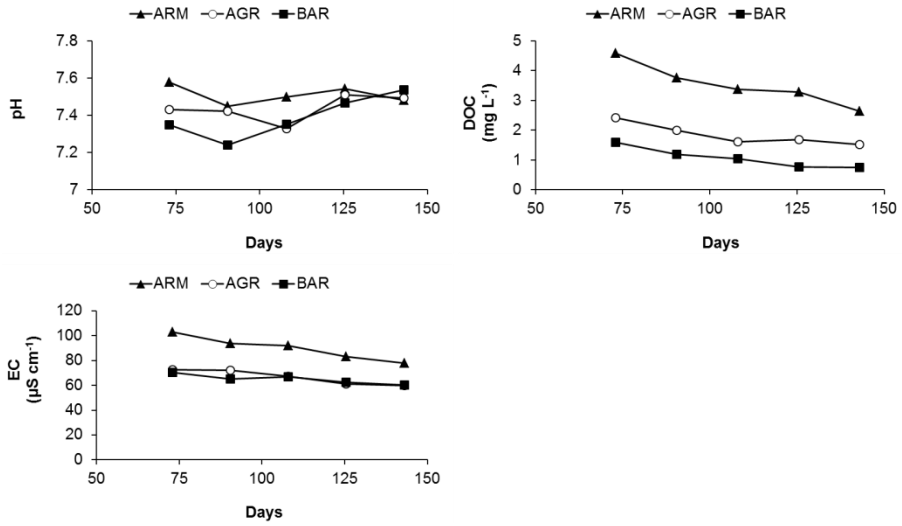


Fig. 4.4 Evolution of pH, DOC concentration, and electrical conductivity (EC) in leachates. Each point represents the average of five replicates.

Throughout the entire experiment, DOC concentrations were significantly ($p < 0.05$) higher in leachates from the two revegetated soils than from the bare soil (see Fig. 4.4). Moreover, leachates from ARM were systematically significantly ($p < 0.05$) more concentrated in DOC than leachates from AGR. The order relationship within the DOC concentrations in leachates is in accordance with the relative contents of organic C in the soils, that is ARM > AGR > BAR. Moreover, the differences in terms of DOC concentrations are also in accordance with the differences in C/N ratio between soils (Table 4.2), as previously reported (Godde et al., 1996).

Throughout the experiment, the electrical conductivity (EC) in leachates from AGR and BAR was very similar, while it was systematically significantly ($p<0.05$) higher in leachates from ARM, indicating a greater release of soluble electrolytes. This difference was likely caused by the degradation of litter which acted as a source of inorganic ions in solution (Nambu and Yonebayashi, 1999; Hongve et al., 2000) and also liberated organic solutes that could promote mineral weathering and thus the release of associated ions (Jones, 1998; Raulund-Rasmussen et al., 1998; Titeux and Delvaux, 2009).

3.3.2. Heavy metal leachability

The time-evolutions of heavy metal concentrations in leachates are depicted in Fig. H.2 (Appendix H). However, because the total metal content was not necessarily identical for all soils, it was not possible to determine reliably whether differences of metal concentrations in leachates between soils were due to differences in total metal content or due to differences in mobilizing/immobilizing properties. To overcome this problem, we preferred to compare the metal behavior in soils using the fraction of metals which was leached throughout the experiment. The leachability of heavy metals, defined as the percentage of metal exported to leachates with respect to the initial metal amount in the column, is presented as a function of time in Fig. 4.5.

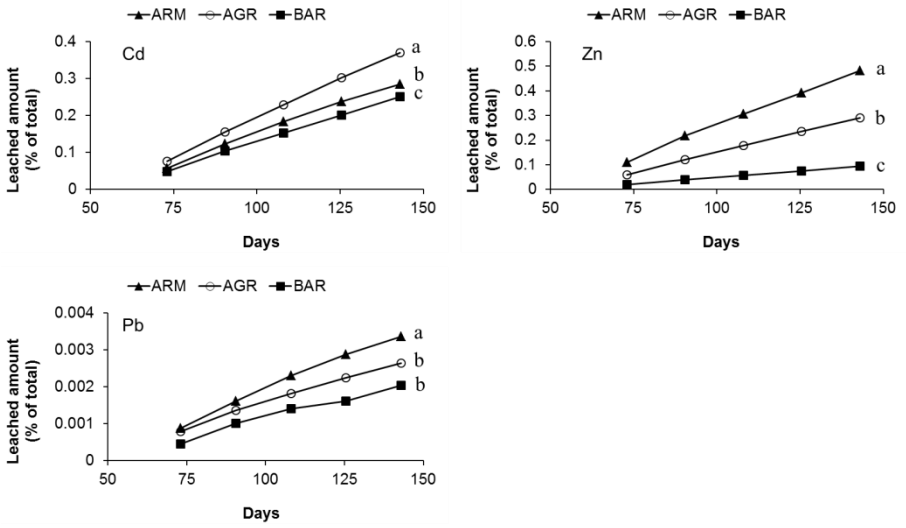


Fig. 4.5 Cumulated amount of Cd, Zn, and Pb leached as a function of time in the substrates revegetated by either *Armeria maritima* (ARM) or *Agrostis tenuis* (AGR) and in the bare substrate (BAR). Each point represents the average of five replicates. Total cumulated amounts (upper right end-point of each curve) marked by different letters are significantly different at the 5 % level according to the Fisher's multiple comparison test.

Lead leachability was very low compared to that of Cd and Zn. This observation, which was already reported for the same site (Ganne et al., 2006), can be related to the sequential extraction results showing that Pb was by far less represented in the mobile fraction (F1) than Cd and Zn (see

Fig. 4.3). At the end of the experiment, the leachability was in the 0.002-0.003% range for Pb, while it reached 0.25-0.37% and 0.09-0.48% for Cd and Zn, respectively.

Overall, the lowest leachability of metals occurred in the bare soil, even though Pb leachability for BAR and AGR was not significantly different ($p = 0.082$). Compared to the bare soil, the long-term presence of *Agrostis tenuis* induced a strong increase in Cd leachability. At the end of the experiment, the percentage of leached Cd from AGR reached 0.37% while it accounted only for 0.25% from BAR. Although its effect was statistically significant ($p < 0.05$) compared to the bare soil, the long-term cover of the slag heap by *Armeria maritima* only moderately increased the Cd leachability with a final percentage of released Cd equal to 0.28%.

According to the strong positive correlation coefficients between metal concentrations in leachates, the three metals displayed similar behavior in AGR and BAR. On the contrary, while Zn and Pb concentrations in leachates from ARM were significantly correlated ($r = 0.58^{**}$), their correlation with Cd was not statistically significant (Table 4.4). These data suggest similar behavior for Zn and Pb in ARM that is distinctly different from that of Cd.

3.4. Relationships between pH, EC, DOC concentration and heavy metal release

Although weak in some cases, a significant correlation was found between metal concentrations and DOC concentrations in leachates from both revegetated soils while only Pb concentrations were significantly correlated with DOC concentrations in the bare soil (Tables 4.4, 4.5 and 4.6). These results suggest the major role of DOC in metal mobilization when the soil is enriched in organic matter, as previously reported (Tyler, 1981; Kuiters and Mulder, 1993; Titeux and Delvaux, 2009). As a result, the higher metal leachability in revegetated soils can be explained by their greater DOC release. Similar increase in vertical mobility of metals with increasing dissolved organic compound concentrations were observed at numerous smelting sites and have been partly attributed to the complexation of metallic ions by DOC (Burckhard et al., 1995; Sterckeman et al., 2000; Ettler

et al., 2005). Degradation of plant residues, which concomitantly released both organic compounds and metals (Boucher et al., 2005), might provide an additional explanation for the close relationships between DOC and Cd, Zn and Pb concentrations.

Table 4.4 Pearson's correlation coefficients *r* between heavy metal and dissolved organic carbon (DOC) concentrations, pH and electrical conductivity (EC) in leachates from ARM (n = 5 columns × 5 sampling events).

	Cd	Zn	DOC	pH	EC
Cd	1	0.38	0.45*	-0.42*	0.57**
Zn	0.38	1	0.78***	-0.07	0.82***
Pb	0.37	0.58**	0.59**	-0.05	0.68***

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 4.5 Pearson's correlation coefficients *r* between heavy metal and dissolved organic carbon (DOC) concentrations, pH and electrical conductivity (EC) in leachates from AGR (n = 5 columns × 5 sampling events).

	Cd	Zn	DOC	pH	EC
Cd	1	0.76***	0.42*	-0.28	0.67***
Zn	0.76***	1	0.57**	-0.39	0.74***
Pb	0.66***	0.73***	0.76***	-0.12	0.52**

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 4.6 Pearson's correlation coefficients *r* between heavy metal and dissolved organic carbon (DOC) concentrations, pH and electrical conductivity (EC) in leachates from BAR (n = 5 columns × 5 sampling events).

	Cd	Zn	DOC	pH	EC
Cd	1	0.81***	0.26	-0.14	0.38*
Zn	0.81***	1	0.36	-0.10	0.29*
Pb	0.58**	0.53**	0.46*	-0.29	0.27*

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

The highly significant positive correlation between metal concentrations and EC in leachates from the two revegetated soils (Tables 4.4 and 4.5) suggested that metal ions may likewise have been mobilized through ion exchange (Al-Wabel et al., 2002). The higher metal leachability from ARM could, therefore, also be partly attributed to the enhancement of electrolytes in solution, in addition to the increase in the DOC concentration. According to Butler (2009), an interaction between both parameters could occur as the result of the ionic strength controlling the initial release of metal through a cation-exchange mechanism and organic compounds subsequently complexing the dissolved ions.

As previously mentioned, the behavior of Cd in ARM was somewhat different from that of both Zn and Pb. Interestingly, Cd concentrations, unlike Zn and Pb concentrations, were significantly negatively correlated with pH in leachates from ARM ($r = -0.42$, $p<0.05$; Table 4.4). It can be thus deduced that Cd release from ARM was not only controlled by DOC and EC but also by the pH of the solution. It is well documented that pH increase plays a major role in the metal immobilization through several processes including the increase of metal sorption onto negatives sites (Naidu et al., 1994; Bradl, 2004) and metal precipitation (Lindsay, 1979). As a result, besides the lower proportion of Cd in F1 (see Fig. 4.3), the lack of markedly higher Cd leachability in ARM (see Fig. 4.5) was likely due to the slightly higher pH in leachates (see Fig. 4.4), which counteracted the mobilizing effects of DOC and EC.

3.5. Implications for phytostabilization

The revegetation of slag heaps by pioneering (pseudo-)metallophytes is usually considered a commonsense mitigation measure for phytostabilizing the site. Results of this study nevertheless demonstrated that the long-term presence of such plants may increase the leachability of Cd, Zn and Pb in soils. Besides rhizosphere processes including root and microbiological activities, the long-term litter build-up in revegetated substrates may promote the metal leachability by releasing not only metals it contains itself but also weathering agents such as soluble organic compounds. In “assisted phytostabilization”, plant-induced metal mobilizing effects are suppressed by adding metal-immobilizing amendment in the soil (Bolan et al., 2011). However, as incorporating such amendments in soils can lead not only to the reduction of metal leaching but also to the alleviation of metal phytoavailability (Houben et al., 2012; Chapter 5) , it is likely that absolute metallophytes would disappear using this technique. Overall, although native (pseudo-)metallophyte communities occurring on heavy metal contaminated soils represent a major biological source for environmental phytotechnologies (Barrutia et al., 2011), it is concluded that the use of hyperaccumulator plants for phytostabilization should not be

recommended because of several reasons. First, hyperaccumulator plants have a low biomass and are thus less efficient in reducing the horizontal metal dispersion than larger plants. In addition, they also present high metal concentrations, which may jeopardize the food chain. Next, their rhizosphere activity as well as their litter accumulation and degradation increase the metal solubility and leachability in soil. Finally, their use is incompatible with the use of metal-immobilizing amendments. Therefore, the use of pioneer pseudo-metallophytes for phytostabilization should be preferred since such plants do not accumulate metal in their shoots, produce higher biomass and grow on both metal contaminated and noncontaminated soils and thus should also be able to grow on metal contaminated soils treated with metal-immobilizing amendments. Phytostabilization efficiency may also be further improved notably by enhancing the interactions between plants and plant growth-promoting bacteria, supplying fertilizers and selecting plants with high evapotranspiration rates, thereby reducing metal leaching (Bolan et al., 2011).

4. Conclusions

In the investigated soils, results of both sequential extractions and leaching column experiments showed that Zn and Cd had a great potential mobility while that of Pb was rather limited. However, the long-term spontaneous colonization of the slag heap by plants led to soil modifications that affected the leachability of these three metals. The correlations between metal and DOC concentrations in leachates suggested that long-term incorporation of organic matter in soil by plants greatly affected the fate of metals inside the soil. Moreover, responses of metal behavior to revegetation depended on the established plant species. The leachability of Cd and both Zn and Pb was the highest in the soils revegetated by *Agrostis tenuis* and *Armeria maritima*, respectively. Therefore, since revegetation of waste dumps, such as mine tailings and slag heaps, is usually regarded as a suitable option for site phytostabilization, the potential effect of plant activity and organic matter build-up on heavy metal release should be

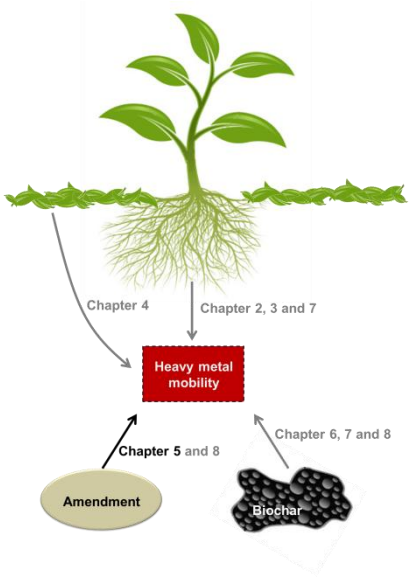
carefully considered prior to implementing this technique. In addition, further *in situ* investigations, especially at deeper horizons, are recommended in order to evaluate whether the enhancement of metal release due to the presence of plants does not outweigh the positive impact of plant establishment on the reduction of metal leaching and metal-rich particle dispersion. Overall, it is concluded that phytostabilization strategy should give preference to pioneer pseudo-metallophytes over hyperaccumulator plants.

Chapter 5

Heavy metal immobilization by cost-effective amendments in a contaminated soil: Effects on metal leaching and phytoavailability*

Where are we?

The results of the first three study chapters indicate that the use of plants for phytostabilizing metal contaminated sites may lead to a higher metal mobility within the soil due to both root activity and organic matter accumulation. In order to optimize the beneficial effects provided by the revegetation of contaminated soils, it is thus essential to reduce as much as possible the mobility of heavy metals. In this context, Chapter 5 examines the feasibility to reduce simultaneously the leaching and the phytoavailability of Cd, Zn and Pb using six different cost-effective amendments when plants are growing for phytostabilization purposes.



* Adapted from Houben D., Pircar J. and Sonnet Ph. (2012) Heavy metal immobilization by cost-effective amendments in a contaminated soil: Effects on metal leaching and phytoavailability. *Journal of Geochemical Exploration*, 123, 87 - 94.

1. Introduction

Since the onset of the Industrial Revolution, metal refining plants using pyrometallurgical processes have generated large emissions of heavy metals such as Cd, Zn and Pb. As the main target of such contaminants, a large number of soils are, nowadays, intensively contaminated by metals in widespread areas (Nriagu and Pacyna, 1988). As a result, degradation of the quality of the environment (Dudka and Adriano, 1997), human health (Cui et al., 2005; Pruvot et al., 2006) and surface and ground water (Rosner, 1998) is observed in the vicinity of such polluted soils. Although the restoration of these hazardous soils is essential, the use of most traditional remediation practices, including excavation and landfilling, is unfeasible on a large scale because these techniques are environmentally disruptive and cost-prohibitive. These concerns have prompted the emergence of cost-effective and less disruptive alternatives for soil remediation. Among these technologies, *in situ* immobilization of metals has received a growing amount of interest and is turning out to be a promising solution for soil remediation (Guo et al., 2006; Ruttens et al., 2006a). This technique aims at alleviating the risk of groundwater contamination, plant uptake and exposure of other living organisms by inactivating metals using metal immobilizing amendments (Boisson et al., 1999b). The main intent of the incorporation of amendments into contaminated soils is not to alter the total metal concentration but to impair the mobility and toxicity of metals by accelerating key immobilizing processes such as (ad)sorption, precipitation, complexation and redox reactions (Adriano et al., 2004). An additional advantage to this technique is that some amendments are inexpensive and readily available in large quantities because they derive from bio-products or industrial by-products (Guo et al., 2006; Kumpiene et al., 2008). *In situ* metal immobilization using such materials may therefore be an enticing option to reclaim contaminated soils while effectively diverting materials from the waste stream and reusing them (Gadepalle et al., 2007). In this context, significant efforts have been made to assess different potentially effective additives to remediate metal-contaminated soils (Vangronsveld et al., 1995; Berti and Cunningham, 1997; Basta and McGowen, 2004; Lombi et al., 2004). However, few studies have

simultaneously compared the effectiveness of a large number of amendments with contrasting properties to immobilize metals. Such comparisons are almost only available in specific literature reviews (Knox et al., 2001; Kumpiene et al., 2008). These comparisons should therefore be interpreted with caution since the results of each separate study are dependent on several parameters such as the soil properties or the method used to assess the amendment's ability to immobilize metals.

Moreover, the immobilization of metals by amendments is frequently combined with the revegetation of the contaminated soil, the so-called phytostabilization technique. The rationale is that the establishment of a suitable plant cover on the soil is helpful in preventing the dispersion of contaminants through erosion, runoff and percolation while increasing biodiversity as well as being aesthetically pleasant (Vangronsveld et al., 1995; Tordoff et al., 2000; Alkorta et al., 2004; Mench et al., 2006). Among suitable plants for phytostabilization, white lupin (*Lupinus albus* L.) appears to be an excellent candidate (Castaldi et al., 2005; Vazquez et al., 2006). Indeed, its nitrogen-fixation capacity, adaptability to poor acidic soils, tolerance to lime excess, high salinity, elevated heavy metal content in soils and several other biotic and abiotic stresses (Huyghe, 1997; Kerley, 2000; Ximenez-Embun et al., 2002; Pastor et al., 2003), allow white lupin to thrive in poor and contaminated soils.

Although the revegetation of contaminated sites offers many environmental benefits, excessive metal uptake by plants may nevertheless jeopardize the food chain (Love and Babu, 2006). Thus, implementing a phytostabilization strategy on contaminated soils requires a serious evaluation of the effects of amendments on the metal phytoavailability (Madejon et al., 2006). Moreover, despite the fact that various metals in soils show contrasting leachability and phytoavailability (Zhu et al., 1999; Marseille et al., 2000), evaluation of the simultaneous effects of amendments on these two transfer pathways has surprisingly been the subject of only limited attention (Ruttens et al., 2006a; Ruttens et al., 2006b; Lambrechts et al., 2011b). To address this problem, our study aimed at investigating the influence of cost-effective amendments on both the leaching and the phytoavailability of Cd, Zn and Pb. Since it is a potentially promising candidate for phytostabilization for the above-mentioned

reasons, white lupin was chosen as the study plant. As the speciation of heavy metals in solution can profoundly affect their biological and geochemical cycling (Krishnamurti and Naidu, 2008), the distribution of metals among their various physicochemical forms in leachates was also assessed. For this purpose, we simulated the metal speciation in leachates by means of a computer-based chemical equilibrium model (Visual MINTEQ 3.0). Cost-effective amendments were selected in order to cover a wide range of additives with different properties: $CaCO_3$, zerovalent iron (Fe^0) in the form of iron grit, fly ash, dehydrated cow manure, bentonite and bone meal.

2. Materials and method

2.1. Contaminated soil

The study site was located at Prayon (Liège province, eastern part of Belgium). From the 1930s to the 1970s, this area was intensively subjected to Cd-, Zn-, and Pb-bearing atmospheric fallouts originating from emissions from the local zinc industries. Soil samples were collected from the top 14 cm of the slightly acidic (pH = 5.8) loam contaminated soil. After sampling around 200 kg, the soil was dried at ambient temperature and sieved through a 2-mm plastic sieve. Sieved soil was then stored at 4°C prior to use. The soil CEC was 13.25 $cmol_c\ kg^{-1}$ and the soil organic carbon and total nitrogen contents were 3.91% and 0.31%, respectively. Its elemental composition is listed in Table 5.1.

2.2. Amendments

Six amendments were tested: (i) calcium carbonate ($CaCO_3$), (ii) iron grit (zero-valent iron; Fe^0), (iii) fly ash, (iv) dehydrated cow manure, (v) bentonite and (vi) bone meal. Calcium carbonate was of *pro analysi* grade (Merck). Iron grit was a by-product from machining tools in a machine shop. Fly ash was a by-product from a thermoelectric power plant. Bone meal, bentonite and manure were obtained from a commercial supplier and are

identical to those used for garden fertilization. The chemical compositions of iron grit, bone meal, fly ash, bentonite and manure were measured in our laboratory by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Jarrell Ash) after calcination at 450°C followed by either (i) Li-metaborate/Li-tetraborate fusion for major elements (Chao and Sanzolone, 1992) or (ii) acid digestion (HNO_3, $HCLO_4$ and HF) for trace metals. The pH of the amendments was also determined (5 g of soil/25 ml of H_2O). Data are presented in Table 5.1 and show that none of the amendments contained excessive metal concentrations compared to the soil metal content.

Table 5.1. pH and elemental composition of soil and some amendments. All concentrations are expressed in mg kg^{-1}.

	Soil	Iron grit	Fly ash	Manure	Bentonite	Bone meal
pH	5.8	6.96	8.65	8.36	9.22	6.86
Al	70200	ND[a]	124000	3420	80700	230
Ca	2070	1360	24900	18200	34700	150000
Fe	42100	995000	49800	2620	36200	405
K	29000	ND	28500	19900	3630	10700
Mg	7030	ND	8920	575	19700	3900
Mn	640	4690	765	225	320	15
Na	4450	ND	4280	4290	5780	8250
P	435	ND	1360	8535	360	71800
Si	285000	ND	226000	83500	227000	2500
Cd	33	ND	0.57	0.41	ND	ND
Zn	2090	ND	212	177	40	106
Pb	702	ND	97.60	5.88	3.26	0.02

[a]ND: not detected

2.3. Experimental design

A leaching pot experiment, based on the design of Marseille et al. (2000), was carried out to investigate the effect of amendments on metal leaching and uptake by plants (see Appendix D for details). A Whatman no. 41 filter followed by a quartz wool plug was inserted at the perforated bottom of each plastic pot in order to prevent coarse material from draining out of the pot and ensure free-drainage conditions. The base of each pot was connected to a funnel so that the leaching solution was channeled into a high-density polyethylene (HDPE) collector bottle. Each pot was filled with a mixture consisting of 500 g of contaminated soil, 250 g of washed sand to

prevent soil compaction, and a constant 25 g mass of amendment. Each mixture was individually prepared by thoroughly mixing the soil, sand and amendment in plastic flask containers by rotation. A control treatment was also prepared following the same procedure but without adding amendment. All treatments were performed in 5 replicates. Before sowing, the pots were placed in a controlled dark room and the mixtures were equilibrated during 10 weeks at 20°C and at 70 % of the water holding capacity (WHC).

After the equilibration period, the pots were transferred to a controlled phytotron (temperature of 20°C, relative humidity of 80%, 16-hour photoperiod and mean light intensity varying from 130 to 150 µmol m^{-2} s^{-1}) and were arranged according to a randomized design. Ten seeds of white lupin (*L. albus* L.) were sown in each pot and the surface of the mixture was then covered by a thin layer (2-3 mm) of polyethylene balls in order to limit the surface from drying-out, prevent soil destructuration by drop impact and ensure watering flow homogeneity. Pots were irrigated four times a week with deionized water and leachates were collected at 2, 6, 10 and 14 weeks after sowing. On irrigation days, each pot received the same irrigating amount of deionized water. However, according to the growth rate of the plants and related transpiration, the irrigating flow was modified during the course of the experiment so that the percolated volume from each pot was at least 25 ml $week^{-1}$. After 6 weeks, the less developed seedlings were pulled out so that each pot contained four plants. Shoots from the remaining plants were harvested 14 weeks after sowing.

2.4. Leachate analysis

At each date of leachate collection, the volume of solution was determined by weighing the HDPE collector bottles. A small aliquot was then set aside for pH determination and the remaining solution was filtered at 0.45 µm (Pall, Supor 450 membrane). Concentrations of Cd, Cu, Pb, Zn, Ca, Fe, K, Mg, Mn and Na were measured by ICP-AES spectrometry (Jarrell Ash). The concentrations of inorganic anions (F^-, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) and NH_4^+ were determined by ion chromatography (HPLC-Dionex). The

concentrations in dissolved organic carbon (DOC) were measured using a Dohrmann DC-180 carbon analyzer.

The aqueous speciation of Cd, Zn and Pb was obtained by calculation using the Visual MINTEQ 3.0 software and based on the Stockholm Humic Model (SHM; Gustafsson, 2001). The SHM uses a discrete-site approach to predict the metal-humic complexation. In this model, cations can form both monodentate and bidentate complexes. The model takes into account the electrostatic effects and the bulk of humic substances is considered to form gels. The complete description of the SHM for aqueous speciation is available in Gustafsson (2001). Additional details can be found in Gustafsson and van Schaik (2003) and Gustafsson and Kleja (2005). Computations were performed assuming that the solutions were in equilibrium with atmospheric CO_2 and using temperature, pH, DOC, anions and total dissolved cations as input data. To be consistent with the model optimization performed by Sjostedt et al. (2010), the ratio of active dissolved organic matter (DOM) to DOC was set at 1.65 and 100 % of the active DOM was considered to be fulvic acids.

2.5. Plant analysis

At the end of the experiment, the aerial parts of the plants were harvested, dried (60°C; 72 h), weighed and crushed. The P-, Cd-, Zn- and Pb-contents of the plants were then analyzed by ICP-AES after a tri-acid ($HClO_4$, HNO_3 and HF) dissolution.

2.6. Statistical analysis

Statistical analyses to compare the average results of the different treatments were performed using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p < 0.05$) for multiple comparisons. Prior to ANOVA, homogeneity of variances was tested using Levene's test and logarithmic transformation was applied to dependent variables when necessary. Paired comparisons between the treatments and the control were carried out using Student's *t*-test. Pearson's correlation coefficients (*r*)

were calculated to determine the relationships between the heavy metal concentrations in leachates and their pH and DOC contents. Three levels of significance were considered: $p < 0.05$, $p < 0.01$ and $p < 0.001$. All statistical analyses were carried out using XLSTAT version 2010.5.08. Results provided in the rest of the paper are presented as: mean value (± standard deviation, number of replicates).

3. Results

3.1. Leaching analysis

Flow-weighted mean metal concentration and speciation in leachates for all treatments are presented in Fig. 5.1. Flow-weighted mean metal concentration was calculated as the total mass of metal leached divided by the total amount of water percolated through each column over the duration of the experiment. The evolution of pH and the concentrations of DOC, Cd, Zn and Pb in leachates as a function of time are presented in Fig. 5.2a, b, c, d and e, respectively. Since the range of measured concentration values covers several orders of magnitude, the results are plotted on a logarithmic scale.

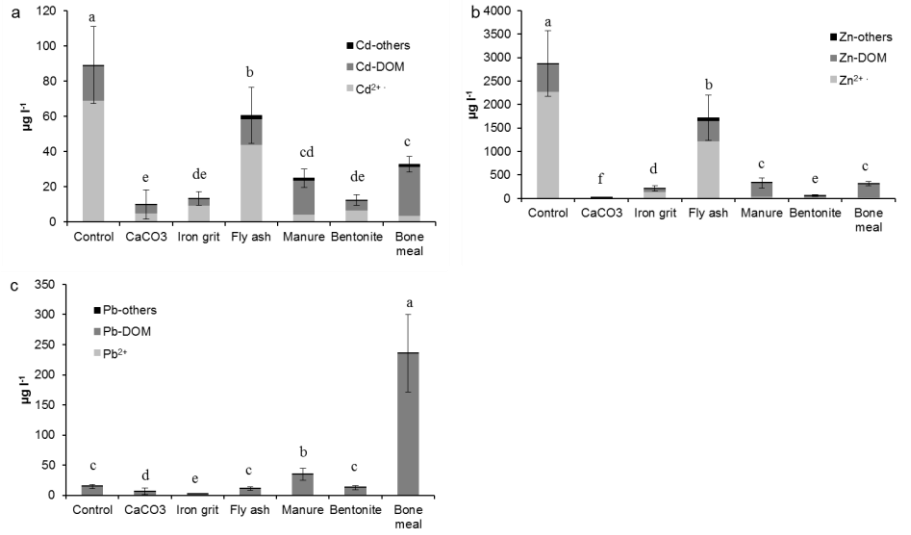


Fig. 5.1. Flow-weighted mean concentration (height of columns) and speciation of Cd (a), Zn (b) and Pb (c) in leachates. For each treatment, modeling according to Visual MINTEQ presents the speciation of metals among their ionic free, bound to dissolved organic matter (-DOM) and bound to other ligands (-others) species. Standard deviation (n = 5) is presented for the total dissolved metal concentration. Columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

3.1.1. pH and DOC release

Compared to the control, all treatments induced a significant ($p < 0.05$) increase in pH in leachates from the first sampling (Fig. 5.2a). After 14 weeks, this pH increase was still statistically significant ($p < 0.05$) for bone meal, bentonite, $CaCO_3$ and fly ash treatments. While the addition of iron grit and manure to the soil led to temporary increases in the leachate pH (Fig. 5.2a), no statistically significant differences with control pH leachates were found at the end of the experiment.

At the first leachate sampling, all treatments except fly ash significantly ($p < 0.05$) affected the release of DOC compared to the control (Fig. 5.2b). Bone meal followed by manure produced the greatest DOC release, whose concentration in leachates was multiplied by 69 and 16, respectively, compared to the control (see dark color of leachates in Appendix D). DOC concentrations in the first leachates originating from the $CaCO_3$ and bentonite treatments increased 5 and 2.5 times, respectively, whereas, in

the presence of iron grit, they decreased 2.5 times compared to the control. Over the course of the experiment, DOC concentration in leachates from the soil amended with bone meal strongly decreased so that, after 14 weeks, it reached a level that was not statistically significantly different from that of the DOC concentration in control leachates. Conversely, compared to the untreated soil, manure, $CaCO_3$ and bentonite treatments induced a significant DOC concentration increase ($p < 0.05$) in leachates at each collection period whereas an opposite trend was observed for the iron grit treatment. Finally, throughout the experiment, the addition of fly ash to the soil did not significantly affect the DOC concentration in leachates.

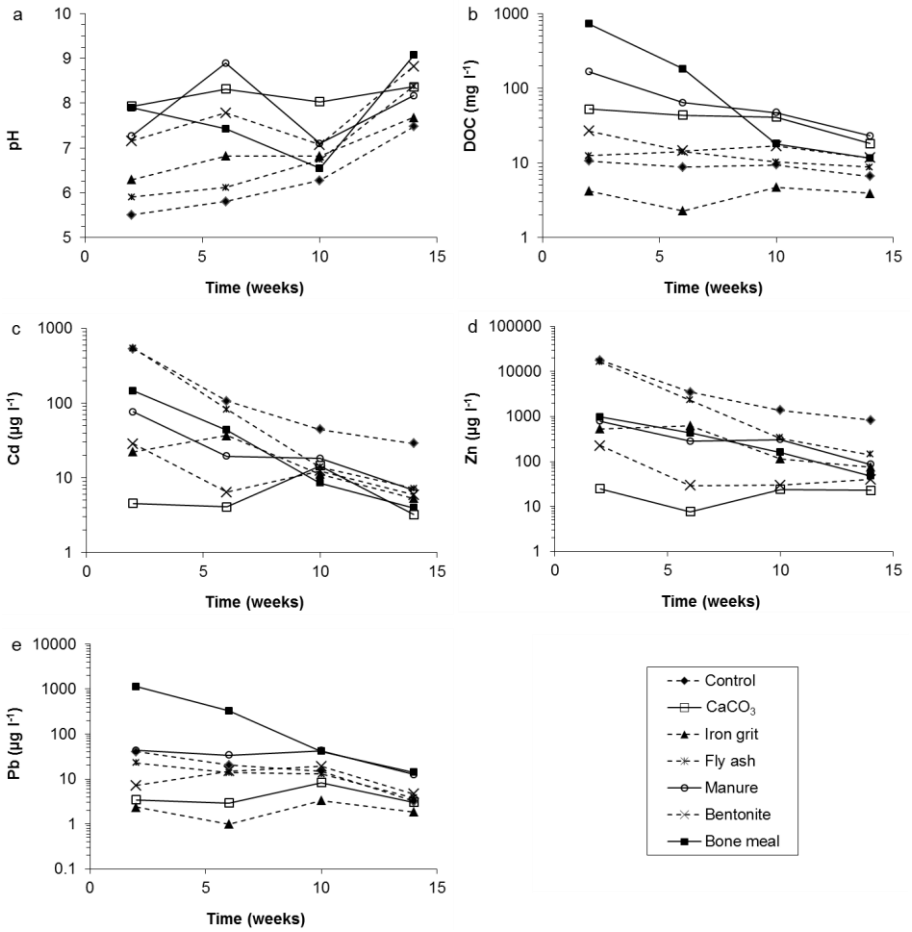


Fig. 5.2. Evolution of pH (a), dissolved organic carbon (DOC; b), Cd (c), Zn (d) and Pb (e) concentrations in leachates as a function of time. Each point represents the average of five replicates.

3.1.2. Metal leaching

Table 5.2 indicates that a very high positive Pearson's correlation coefficient ($r = 0.98$, $p < 0.001$) was found between Cd and Zn concentrations in leachates, whereas no correlation was detected between Pb and Cd or Zn concentrations.

Table 5.2 Pearson's correlation coefficients *r* between heavy metal and dissolved organic carbon (DOC) concentration and pH in leachates (*n* = 140).

	Cd	Zn	DOC	pH
Cd	1	0.98***	0.09	-0.49***
Zn	0.98***	1	-0.07	-0.51***
Pb	0.14	-0.02	0.97***	0.09

*** Significant at $p < 0.001$

The flow-weighted mean concentrations of Cd and Zn leached over the duration of the experiment from the untreated soil (control) were 98 (±22, n = 5) µg l^{-1} and 2875 (±702, n = 5) µg l^{-1}, respectively. Compared to the control, all treatments exhibited significantly ($p < 0.05$) lower flow-weighted mean concentrations of Cd and Zn in leachates. For Cd, flow-weighted mean concentration was decreased by 32.9%, 63.1%, 72.9%, 85.4%, 86.5% and 88.3% in the fly ash, bone meal, manure, iron grit, bentonite and $CaCO_3$ treatments, respectively. Flow-weighted mean Zn concentration was lowered by 41.2%, 89.0%, 89.1%, 92.9%, 97.9%, 98.5% in the fly ash, manure, bone meal, iron grit, bentonite and $CaCO_3$ treatments, respectively.

Regarding metal speciation, the Visual MINTEQ software showed that Cd and Zn mainly occurred as free ion (Cd^{2+} and Zn^{2+}, respectively) in the leachates from the control treatment (Fig. 5.1a, b). For Cd and Zn, all treatments significantly ($p < 0.05$) reduced the flow-weighted mean concentration of the ionic free form while the concentration of organo-metallic complexes only significantly ($p < 0.05$) decreased in the presence of $CaCO_3$, iron grit and bentonite (Fig. 5.1a, b).

After 2 weeks, leachates from all treatments except fly ash exhibited significantly ($p < 0.05$) lower Cd and Zn concentrations than that found in the control. At the end of the experiment, a significant reduction ($p < 0.05$) in Cd and Zn concentrations was observed for all treatments (Fig. 5.2c, d).

Moreover, a strong negative correlation was found between Cd and Zn concentrations and pH in leachates ($r = -0.49$, $p < 0.001$ and $r = -0.51$, $p < 0.001$; Table 5.2) over the course of the experiment.

The flow-weighted mean concentration of Pb from the untreated soil (control) for the overall the experiment was 15 (±3, n = 5) μg l^{-1} (Fig. 5.1c). In the presence of iron grit, $CaCO_3$, fly ash and bentonite, flow-weighted mean Pb concentrations were lowered by 83%, 57%, 25% and 14% respectively, though the effects of fly ash and bentonite were not statistically significant (Fig. 5.1c). Conversely, the addition of manure and, especially, bone meal greatly increased Pb leaching since the flow-weighted mean Pb concentration was 2.3 and 16 times higher, respectively, than in the control. At the first sampling, only bentonite and manure treatments did not induce a significant effect on Pb concentrations in leachates compared to the control (Fig. 5.2e). Inversely, Pb concentrations were significantly ($p < 0.05$) increased in the presence of bone meal and lowered by the fly ash, bentonite, $CaCO_3$ and iron grit treatments. After 14 weeks, Pb concentrations were still significantly ($p = 0.01$) lower in leachates from the soil treated with iron grit whereas all other treatments did not induce significant differences compared to the untreated soil (Fig. 5.2e).

Finally, in all leachates, organo-Pb complexes were dominant (Fig. 5.1c) and a very strong correlation ($r = 0.97$, $p < 0.001$; Table 5.2) was found between Pb and DOC concentrations in all leachates.

3.2. Plant analysis

3.2.1. Metal phytoavailability

At the end of the experiment, average concentrations of Cd, Zn and Pb in shoots of white lupin grown in the untreated soil (control), were, respectively, 11.6 (±2.8, n = 5) mg kg^{-1}DW, 1033.6 (±57.6, n = 5) mg kg^{-1}DW and 12.4 (±2.9, n = 5) mg kg^{-1}DW (Fig. 5.3a, b, c). Compared with the control, concentrations of Cd and Pb in shoots of plants grown in soil treated with iron grit were doubled while a slight decrease was observed for Zn. The addition of fly ash slightly reduced the concentration of both Zn and Pb in shoots while the bentonite treatment only decreased the Zn

concentration (Fig. 5.3a, b, c). Bone meal, $CaCO_3$ and manure were the most effective treatments. They reduced the phytoavailability of all of the considered metals. Cd, Zn and Pb concentration in shoots was lowered by 86.5%, 88.9% and 74.2%, respectively for bone meal, 82.2%, 87.5% and 54.5% respectively for $CaCO_3$ and 60.1%, 64.3% and 52.0% respectively for manure (Fig. 5.3a, b, c).

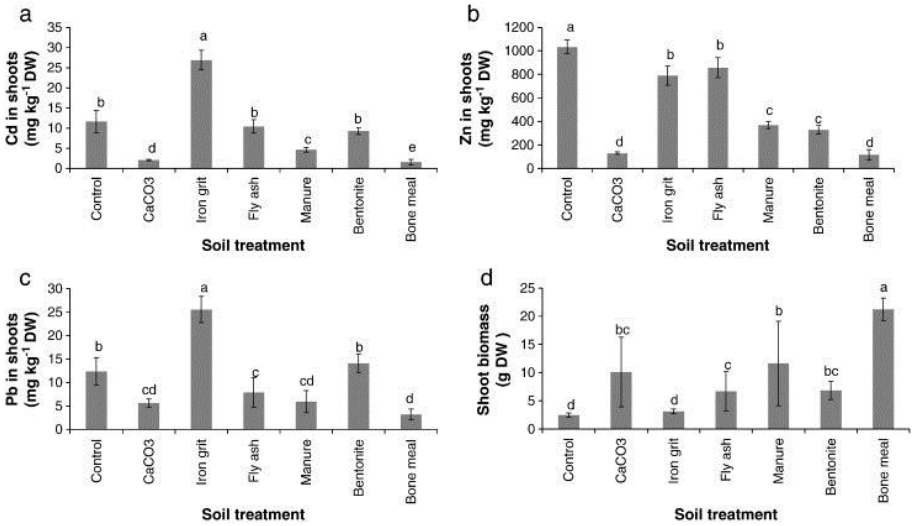


Fig. 5.3 Average concentration of Cd (a), Zn (b), and Pb (c) and biomass (d) of shoots of white lupin. Values are average (n = 5) ± standard deviation. Columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

3.2.2. Biomass production

At harvest, all treatments, except iron grit, showed a significant ($p < 0.05$) greater aerial biomass production than the untreated soil (Fig. 5.3d). Biomass of shoots was increased by 8.7, 4.8, 4.1, 2.8 and 2.7 times for bone meal, manure, $CaCO_3$, bentonite and fly ash, respectively, in comparison with the control.

4. Discussion

4.1. Heavy metal leaching

The evolution of metal concentrations as a function of time indicates that the positive or the negative effects of amendments on metal leaching were already strong at the very beginning of the experiment. Overall, the decrease of metal concentration in leachate induced by amendments was less pronounced at the end of the experiment. One probable explanation is that the soil pool containing the most soluble metal species of the control soil was progressively depleted due to the continuous leaching during the experiment.

In agreement with Schwab et al. (2007) and Sneddon et al. (2006), our correlations (Table 5.2) show that Cd and Zn display similar leaching behavior. However, treatments were systematically more efficient for reducing Zn as opposed to Cd concentrations. Conversely, the absence of any correlation between leachate concentrations of Pb and Cd or Zn (Table 5.2) indicates that the response of Pb to the incorporation of amendments into the soil was distinctly different from those of both Cd and Zn and, thus, probably governed by other parameters. A first explanation for this distinct metal behavior can be drawn from the Visual MINTEQ speciation results which showed that, in accordance with Holm et al. (1995), McBride et al. (1997) and Degryse et al. (2009), Cd and Zn speciation in solutions from the untreated soil was dominated by free ion (Cd^{2+} and Zn^{2+}) while Pb was mainly complexed with dissolved organic matter (Fig. 5.1).

The highly significant negative correlation coefficient between pH and both Cd and Zn, suggests that the increase of pH induced by the incorporation of amendments into the contaminated soil was one of the key parameters governing the release of Cd and Zn in our experiments. It is well known that in soils, a pH increase induces metal immobilization through several processes including the increase of metal sorption onto negatives sites (Cavallaro and McBride, 1980; Naidu et al., 1994; Bradl, 2004), or the precipitation of metals in the form of oxides, hydroxides, carbonates and phosphates (Lindsay, 1979). In soils treated with alkaline materials, precipitation and increased sorption were identified as the main mechanisms involved in the retention of metals (Lombi et al., 2003; Lee et

al., 2009). In our experiments, the addition of $CaCO_3$ into the soil increased the leachate pH up to 8 and this may explain why it lowered the flow-weighted mean Cd and Zn concentrations by 88.3% and 98.5%, respectively. However, although correlation coefficients between pH and both Cd and Zn concentrations in leachates were statistically highly significant (Table 5.2), they were not sufficiently high to ascertain whether the post-amendment decrease in metal leaching was solely due to an increase in pH. For instance, after 2 weeks, although $CaCO_3$ and bone meal treatments presented similar pH (Fig. 5.2a), bone meal was by far less efficient than $CaCO_3$ to decrease Cd and Zn concentrations in leachates (Fig. 5.1a, b). The same conclusion can be drawn by comparing bentonite and manure, the latter being less efficient to reduce Cd and Zn concentrations. Being organic additives, bone meal and manure greatly enriched the leaching solution in DOC (Fig. 5.2b). This enrichment is known to enhance the solubility of metals (Naidu and Harter, 1998) and has most probably been reinforced by the leachate alkalinity rise, as DOC chelating properties are known to be improved by an increase in pH (Oste et al., 2002b). Therefore, by maintaining a high concentration of organo-metallic complexes in solution (Fig. 5.1c), the strong DOC release by the bone meal and manure treatments likely partly counterbalanced or prevented the pH-induced immobilization effect of these amendments. The pH of leachate from iron grit treatment was only slightly higher than that of the control at the beginning of the experiment and this difference tended to diminish with time. This indicates that factors other than pH were responsible for the great efficiency of iron grit to reduce Cd and Zn leaching. As a zero-valent iron amendment, iron grit has initially no binding capacity for ions (Hanauer et al., 2011). Yet, applied to the soil, iron grit may rapidly oxidize, leading to the formation of amorphous iron oxides. According to Lambrechts et al. (2011b) who reported a similar pH trend in leachates of a soil treated with steel shot, oxidation of iron grit rapidly occurred in our experiment. Thus, the strong decrease in Cd and Zn concentrations is likely due to the formation of iron oxide on which metals may quickly sorb (an equilibrium time ranging from 24 to 72 h has been reported by Smith, 1996) or coprecipitate (Martinez and McBride, 1998; Boisson et al., 1999a; Lee et al., 2009). Finally, in addition to its liming-effect, (Fig. 5.2a), bentonite is able to

limit metal leaching through ion exchange and chemisorption (Kumpiene, 2010) and these two effects also probably contributed to the strong metal immobilization observed with this treatment.

The strong positive correlation between DOC and Pb concentrations (Table 5.2) suggests that the Pb response to the different treatments was strongly influenced by their effects on DOC concentrations. As reported by Titeux and Delvaux (2009), organic anions play a major role in Pb mobilization. Fulvic acids, which are important components of DOC (Christensen et al., 1998), can form stronger complexes with Pb than with other metals such as Cd or Zn. As a result, Pb mobility is favored in the presence of elevated DOC concentration (Impellitteri et al., 2002; Schwab et al., 2007). Compared to the control, Pb leaching was not affected by fly ash and bentonite treatments (Fig. 5.1c), likely because these amendments did not cause major changes in DOC concentrations (Fig. 5.2b). Inversely, in bone meal and, to a lesser extent, in manure treatments, the large DOC enrichment (Fig. 5.2b) increased the complexation of Pb by organic ligand (Fig. 5.1c) which led to a dramatic increase of Pb concentration in the leachates (Fig. 5.2e), as previously reported (Sneddon et al., 2006; Schwab et al., 2007). The immobilizing effect of $CaCO_3$ on Pb probably occurred not only because the pH-induced metal immobilization effect was higher than the DOC-induced mobilization effect but also because the massive input of calcium reduced the DOC-induced mobilization effect by forming stable Ca-DOC complexes (Romkens et al., 1996). Finally, iron grit was by far the most efficient amendment to decrease the Pb leaching. Besides their ability to sorb Pb (Geebelen et al., 2003), we observed that newly formed iron oxides were very effective at removing DOC from solution (Fig. 5.2b), which confirms previous works (McBride and Martinez, 2000). The DOC removal provides an additional explanation for the strong immobilization we observed for oxidized iron grit.

4.2. Heavy metal phytoavailability

Only $CaCO_3$ addition resulted in a strong reduction of metal plant uptake in addition to metal leaching for the three considered metals, confirming the

high importance of pH in the control of these two pathways (Basta et al., 2005). Additionally, massive Ca inputs might also contribute to the reduction of metal concentration in aboveground plant parts by inhibiting the translocation of metal from root to shoot (Bolan et al., 2003). Bentonite treatment only significantly decreased the phytoavailability of Zn. It is likely that Cd and Pb ions were not sufficiently strongly retained by bentonite due to their larger ionic radii which limit their introduction into interlayers and their complexation onto surface sites (Abollino et al., 2003). Unlike Zn and Pb, Cd phytoavailability was not reduced in the presence of fly ash. This reflects the low adsorption of Cd on fly ash as reported by Ahmaruzzaman (2010) and Wang and Wu (2006). Surprisingly, while iron grit induced strong metal leaching attenuation, this amendment doubled the phytoavailability of Cd and Pb. Similar observations were obtained by Lambrechts et al. (2011b). Authors postulated that the effect of iron grit was depressed at the vicinity of roots due to local rhizospheric acidification. Soil acidification by roots is a well-known phenomenon for *L. albus* (Neumann et al., 1999). It might be stimulated, among others, by a depletion of nutrients, such as phosphate, which are irreversibly sorbed on newly formed iron oxides (Hanauer et al., 2011). This is supported by the lower P content in shoots of plants grown on the soil treated with iron grit than on the control soil (respectively 439 $\pm$ 26 mg kg^{-1} DW and 710 $\pm$ 165 mg kg^{-1} DW; data not shown). Moreover, in a leaching column experiment, we previously showed that the oxidation of iron grit (Fe^0) incorporated in the present soil was accompanied by the reduction of nitrate into ammonium (Houben, 2011; Houben and Sonnet, unpublished). Therefore, the plant uptake of N under the form of ammonium instead of nitrate, which results in a net release of H^+ (Haynes, 1990), could have also contributed to increase the heavy metal phytoavailability. On the other hand, shoots of white lupin grown in soil treated with bone meal exhibited the lowest Cd, Zn and Pb concentrations (Fig. 5.3a, b, c). This result was rather unexpected considering the results of the leaching experiment. This might be explained by the fact that, in contrast to its effect on the total soluble metal concentrations in leachates, bone meal treatment strongly reduced the concentration of free ion metal species (Fig. 5.1), thereby lowering the metal availability for plant uptake

(Oste et al., 2002a). A similar explanation can be invoked for the strong decrease in metal phytoavailability that was induced by manure.

Obviously, white lupin biomass increased with decreasing metal concentration in shoots (Fig. 5.3). However, plants grown in soil treated by $CaCO_3$ had similar metal concentrations but lower biomass than plants harvested on manure and bone meal treatments. It is thus likely that the metal phytoavailability was not the sole parameter controlling production yield. The elevated biomass production in the presence of bone meal and, to a lesser extent, of manure most likely reflected an improvement in soil fertility. For instance, an increase in organic C content and a supply of nutrients such as P (Table 5.1) would promote plant growth (Cao et al., 2009). In accordance with Schwab et al. (2007), our results indicate that carbon-rich amendments may facilitate the revegetation of contaminated soils but their potential harmful effect on groundwater contamination due to metal leaching should be carefully considered.

5. Summary and conclusion

Our study aimed at simultaneously investigating the effects of six contrasted cost-effective amendments on both leaching and phytoavailability of Cd, Zn and Pb. Table 5.3, which summarizes our results, shows that the two metal transfer pathways we considered are differently affected by the introduction of amendments into the soil. Furthermore, Cd, Zn and Pb do not respond similarly to the same amendments. This highlights the difficulty in finding amendments that are equally effective for every target contaminant in multi-element contaminated soils. Overall, the addition of $CaCO_3$ turned out to be the best compromise to reduce both leaching and phytoavailability of the three considered metals. However, the effects of amendments on phytoavailability are plant-specific and they would be most likely different if another plant was used. Additional experiments on a wider range of plant species are thus required in order to generalize the results. In addition, the time span of our experiments was relatively short. Further investigations on the middle- and long-run are of utmost importance to examine the positive but potentially ephemeral

effect of some amendments, in particular those acting primarily as liming agents. Moreover, the reversibility of the fixation mechanisms should be tested in order to ensure the sustainability of the treatment. For instance, Lombi et al. (2003) showed that the mechanism of metal fixation in soil amended with lime were reversible while they were not when the soil was amended with materials rich in Fe- and Al-oxides (i.e. red mud). On the other hand, adverse effects of some treatments, such as the high leaching of Pb due to the fast release of DOC by bone meal, could diminish with time and lead to the formation of more stable compounds. In addition, the combination of several amendments should be tested to gain insight into their possible synergetic effects. For instance, the lack of effectiveness of iron grit to reduce metal phytoavailability, due to the depletion of the nutrient availability it induces, could be offset by the use of additional appropriate organic amendment.

Table 5.3 Overview of significantly ($p < 0.05$) positive (+, immobilization), negative (-, mobilization) and not significant (=) effects of treatments on flow-weighted mean concentration (leaching) and phytoavailability of heavy metals.

	Leaching			Phytoavailability		
	Cd	Zn	Pb	Cd	Zn	Pb
$CaCO_3$	+	+	+	+	+	+
Iron grit	+	+	+	-	+	-
Fly ash	+	+	=	=	+	+
Manure	+	+	-	+	+	+
Bentonite	+	+	=	=	+	=
Bone meal	+	+	-	+	+	+

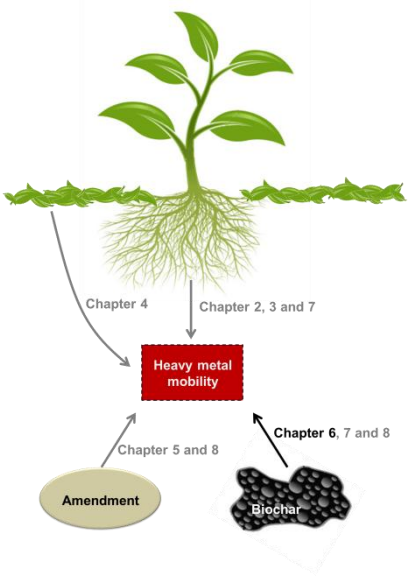
Our study stresses the necessity to evaluate the potential effect of amendments on simultaneous leaching and phytoavailability for each target metal prior to implementing *in situ* immobilization and revegetation. Nonetheless, we firmly believe that the use of suitable amendments in contaminated soils might be helpful to prevent risks for ecosystems due to vegetation or underlying groundwater contamination.

Chapter 6

Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar*

Where are we?

Chapter 5 shows that the application of adequate amendments to contaminated soils can considerably decrease the mobility of heavy metals. Considering biochar a potential amendment for phytostabilization purposes, Chapter 6 consists of a preliminary investigation devoted to gain insight into the effect of the application of three different rates of biochar (1%, 5% and 10%; w/w) onto a heavy metal contaminated soil on the fate of Cd, Zn and Pb.



* Adapated from Houben D., Evrard L. and Sonnet Ph. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Submitted to *Chemosphere*.

1. Introduction

Since the onset of the Industrial Revolution, anthropic activities have resulted in substantial increases in atmospheric metal concentration and the worldwide contamination of soils (Nriagu and Pacyna, 1988). The presence of excessive metal contents in soils may have serious consequences for surrounding ecosystems, groundwater, agricultural productivity and human health (Adriano, 2001). The restoration of hazardous soils is thus essential. Conventional remediation techniques (e.g. excavation, landfilling, soil washing) are nowadays recognized as inappropriate because they generate considerable disturbance in the environment and they are economically unfeasible on a large scale. An alternative technique consists in introducing *in situ* amendments into the contaminated soils in order to reduce the metal mobility while simultaneously creating conditions that promote plant growth (chapter 5). This technique has received a growing interest and is turning out to be a promising green and cost-effective alternative for soil remediation (Adriano et al., 2004; Kumpiene, 2010; Bolan et al., 2011; Houben et al., 2012).

Biochar, the product of biomass pyrolysis under minimal oxygen supply (Lehmann and Joseph, 2009a), has recently sparkled research interest due to the discovery that biochar-type substances explain the high amounts of organic carbon (Glaser et al., 2001) and the sustained fertility (Lehmann et al., 2003a; Marris, 2006) of the so-called *Terra Preta*, a dark fertile anthropogenic soil found in the Amazon Basin. The high C content of biochar and its chemical stability in soil have encouraged experimentation on its potential use for long-term C sequestration (Lehmann, 2007). It has also been shown that application of biochar in soils rapidly increases the soil fertility and plant growth by supplying and retaining nutrients while improving soil physical and biological properties (Glaser et al., 2002; Novak et al., 2009; Laird et al., 2010; Uzoma et al., 2011). Moreover, a few recent studies have shown promising results regarding the retention of heavy metals in soils in the presence of biochar (Beesley et al., 2010; Uchimiya et al., 2010; Fellet et al., 2011; Karami et al., 2011; Park et al., 2011a). Immobilizing mechanisms have been mainly attributed to the increase in both soil pH and CEC and the adsorption of metal-complexing DOC (Beesley

and Marmiroli, 2011; Uchimiya etr al., 2011; Karami et al., 2011). Therefore, biochar has been considered a potential amendment for promoting the establishment of a plant cover and phytostabilization strategies on contaminated soils (Beesley et al., 2011).

However, because the incorporation of biochar into the soil is irreversible, a full and comprehensive characterization of the effect of biochar on pollutants is of utmost importance prior to implement this technique in the field. Currently, generalizations about these effects are difficult to make because of the small number of existing studies and the lack of agreement over the role of biochar on the metal immobilization that was reported (Park et al., 2011a). Moreover, application to real soil systems has received little systematic investigation to date (Beesley and Marmiroli, 2011). In particular, the evolution of the metal bioavailability with aging has not still been the subject of deep investigation. Moreover, as pH is one of the most important parameter affecting the mobility of heavy metal in soils, examining the acid neutralizing capacity (ANC) of biochar-amended soils and obtaining the leaching curve of metals at decreasing pH is essential to determine whether these soils represent potential "chemical time bombs" (Stigliani et al., 1991). Investigating the response of metals in amended soils upon acidification and gaining insight about the reversibility of the interactions in the solid matrix can be achieved by means of pH-dependent leaching test (Carter et al., 2009; Rigol et al., 2009; González-Núñez et al., 2012). This test allows not only to assess the solubility changes in response to a pH change but also to determine the potential buffering capacity of the soil and its sensitivity to pH changes due to external stresses such as a re-acidification (Cappuyns and Swennen, 2008b).

In the present study, we investigated the effects of biochar application on both the bioavailability over time and the release upon acidification of heavy metals (Cd, Zn and Pb). To this end, a 56-days incubation experiment, a pot experiment with two harvests of ryegrass (*Lolium multiflorum* Lam.) and a pH-dependent leaching test were performed on a heavy metal-contaminated soil amended following three different rates of biochar (1%, 5% and 10%; w/w).

2. Materials and methods

2.1. Contaminated soil

The study site is located at Sclaigneaux (Namur Province, Belgium; Appendix E). From the 1850s to the 1970s the site was intensively subjected to Cd-, Zn-, and Pb-bearing atmospheric fallouts originating from the adjacent zinc and lead smelting plant. A total mass of 250 kg of surface soil (0-14 cm) was obtained by composite sampling of a 20 × 20 m² area that was colonized by metal-tolerant plant species (*Rumex acetosa* L., *Festuca nigrescens* Lam. and *Agrostis capillaris* L.). The soil was then air-dried for two weeks, crushed and sieved to a particle size of < 2 mm in diameter. Particle size analysis using the pipette method revealed that the soil was a sandy loam (USDA classification) with 64% sand, 24% silt, and 12% clay. Elemental composition of the studied soil was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Jarrell Ash) after calcination at 450 °C followed by either (i) Li-metaborate/Li-tetraborate fusion for major elements (Chao and Sanzolone, 1992) or (ii) acid digestion (HNO_3, $HClO_4$ and HF) for trace metals. Carbon and N concentrations were measured by gas chromatography after dry combustion (1800°C) with a Thermo Quest CHN autoanalyzer. The pH and the elemental composition are listed in Table 6.1.

Table 6.1 pH (pH-H_2O) and elemental composition of the soil and the biochar.

		Soil	Biochar
pH		6.57	10.24
Organic C	g kg^{-1}	190	535
N	g kg^{-1}	4.2	3.1
Ca	mg kg^{-1}	2380	10 470
K	mg kg^{-1}	4270	12 230
Mg	mg kg^{-1}	980	3950
Na	mg kg^{-1}	1450	1370
P	mg kg^{-1}	530	2910
Cd	mg kg^{-1}	24.0	0.1
Zn	mg kg^{-1}	2980	116
Pb	mg kg^{-1}	3110	n.d.[a]

[a]n.d. : not detected

2.2. Biochar

The biochar was industrially produced in a commercial pyrolysis reactor by Pyreg GmbH (Dörth, Germany). The biomass feedstock consisted of miscanthus (*Miscanthus* × *giganteus*) straw. Operating conditions of production were a residence time in the reactor of 30 minutes and an end temperature of pyrolysis of 600°C. In our experiments, the biochar was used as supplied, without prior washing to remove soluble salts. Elemental composition of biochar was determined as for the soil. Heavy metal content was very low and could be considered as negligible compared to the content in soil (Table 6.1).

2.3. Substrates preparation

The contaminated soil was mixed with 1%, 5% and 10% (w/w) of biochar, proportions that are identical to those of Fellet et al. (2011). Biochar treatments were compared to the untreated soil (control). Amended soils were thoroughly homogenized in large plastic containers and individually prepared prior to use.

2.4. Incubation experiment

2.4.1. Experimental set-up

An incubation experiment was conducted in order to assess the effects of treatments on a selection of physico-chemical properties. Each 1000-cm^3 plastic pot was filled with 200 g of mixture, covered with a perforated cap to limit water evaporation while ensuring gas exchange and incubated at 20±1°C for 56 days in the dark. In the pot, the mixture was underlain by a 2-cm layer of polymer granules to avoid potential water stagnation and development of anoxic conditions. All over the experiment, the moisture content of each mixture was kept at 75% water holding capacity (WHC) by adding water and weighing the pots on a weekly basis. Each treatment was performed in four replicates.

2.4.2. Chemical and physical analysis

After 1 hour, and 7, 14, 28 and 56 days, 2.5 g of each soil was sampled and subjected to a $CaCl_2$ (0.01 M) extraction based on the scheme proposed by Houba et al. (2000). The samples were transferred to a polypropylene centrifugation tube in which 25 ml of 0.01 M $CaCl_2$ was added and then shaken for 2 hours at 20°C. After shaking, the pH (pH-$CaCl_2$) was measured in the suspension and the extract was separated from the solid residue by centrifugation at 3000 *g* for 15 minutes. Heavy metal concentration in the extract was determined ICP-AES. At each sampling date, an additional small aliquot of soil was sampled in each pot in order to determine the soil dry weight and express the results on a dry matter basis (i.e. dried at 105°C).

2.4.3. Kinetic studies

The decrease or the increase of $CaCl_2$-extractable heavy metal concentration was fitted by the linear form of the kinetic Elovich equations ($q = a + b\ln t$), where q is the heavy metal decrease or increase, a and b are constants and t is the time (in hours) (Chien and Clayton, 1980; Sparks, 1989; Jalali and Khanlari, 2008). As recommended by Chien and Clayton (1980), the goodness of fit of the kinetic equation was assessed according to its coefficient of determination (R^2) and the standard error of estimate (SE) calculated from:

$$SE = \left[\sum \left(q_t - q'_t\right)^2 / (n-2)\right]^{1/2}$$

where q_t and q'_t are the measured and predicted amounts of released heavy metals at time t, respectively, and n is the number of measurements.

2.5. Pot experiment

A pot experiment was conducted to investigate how the addition of biochar affected the heavy metal bioavailability (Appendix E). Plastic plant pots (11.5-cm diameter, 10.5-cm height) were filled with 450 g of soil and biochar mixture to which 150 g of washed sand has been added to prevent soil compaction. Before sowing, the pots were placed in a controlled dark room and the mixtures were equilibrated during 4 weeks at 75% WHC. Each treatment was performed in four replicates.

After the equilibration period, the pots were transferred to a greenhouse glass and were arranged according to a randomized design. In each pot, 1.5 g of sterilized (10 min in H_2O_2 6%) seeds of Italian ryegrass (*Lolium multiflorum* Lam.) were sown. The surface of the mixture was then covered by a thin layer (1 - 2 mm) of quartz particles in order to limit the surface from drying-out, to prevent soil destructuration by drop impact and to ensure watering flow homogeneity. The trials were conducted under controlled greenhouse conditions (temperature 18-25°C, 16-hour photoperiod) with daily sprinkler watering. After 4 and 8 weeks, shoots were harvested by cutting 1 cm above the soil with ceramic scissors, dried (60°C; 72 h), weighed and crushed. The Cd-, Zn- and Pb-contents of the aerial parts were then analyzed by ICP-AES after a tri-acid ($HClO_4$, HNO_3 and HF) dissolution, as described in Lambrechts et al. (2011a).

2.6. pH-dependent leaching test

In order to gain insight about the effect of a re-acidification event on the metal release, the soil and amended soils were subjected to a pH-dependent extraction procedure, or "leaching tests", adapted from the CEN/TS 14429 test (CEN/TS, 2006). For each of the treatments, air-dried soil samples (2 g) were collected at the end of the incubation experiment. The experiment was carried out at fixed liquid-to-solid ratio (10 L kg^{-1}) and contact time (48 h) with leachants containing 15 different preselected amounts of acid (HCl) and deionized water. After having separated the liquid phase by centrifugation and filtration (0.45 µm), the final pH of the extracts was measured and the Cd, Zn and Pb concentrations were determined by ICP-AES (Jarrell Ash). Results are expressed on a dry matter basis (i.e. dried at 105°C).

2.7. Statistical analysis

Statistical analyses to compare the average results of the different treatments were performed using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p<0.05$) for multiple comparisons. Prior to ANOVA, homogeneity of variances was tested using Levene's test and

logarithmic transformation was applied to dependent variable when necessary. Paired comparisons between the treated and the control soils were carried out using Student's t-test. The relationships between parameters were determined using Pearson's correlation coefficients (*r*). All statistical analyses were carried out using XLSTAT (Addinsoft, ver. 2010.5.08).

3. Results and Discussion

3.1. Soil pH

Compared to the control, the soil pH (pH-$CaCl_2$) was significantly ($p<0.05$) increased by the addition of biochar throughout the incubation experiment (Fig. 6.1a), except for day 28 in biochar-1% treatment for which the increase was only significant at $p<0.064$. This pH increase is consistent with the alkali nature of the biochar (Table 6.1).

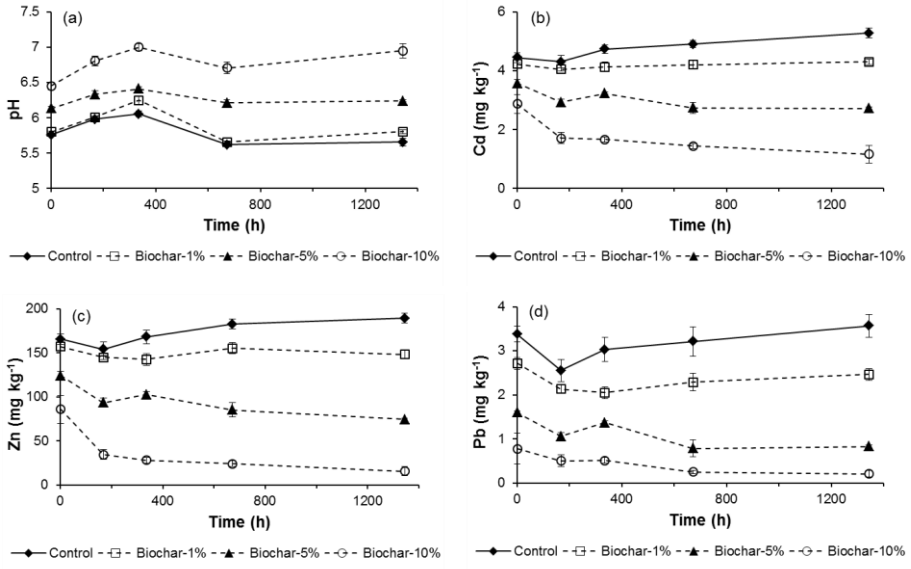


Fig. 6.1 Evolution of pH-$CaCl_2$ (a) and 0.01 M $CaCl_2$-extractable concentrations of Cd (b), Zn (c) and Pb (d) as a function of time. Each point represents the average of four replicates.

3.2. Heavy metal immobilization

The effect of biochar on heavy metal immobilization was assessed by monitoring $CaCl_2$-extractable metal concentrations released by soil and soil-biochar mixtures during the incubation experiment. The 0.01M $CaCl_2$ extraction provides information about the soil solution and exchangeable metal pools and can be regarded as an indicator of metal solubility, bioavailability and mobility in soils (Houba et al., 2000; Pueyo et al., 2004; Kalis et al., 2007; Lambrechts et al., 2011a). This procedure has also been used to study the influence of aging on the bioavailability of metals in soils (Lock and Janssen, 2003; Lu et al., 2009; Burgos et al., 2010; Schreck et al., 2011).

All over the experiment, $CaCl_2$-extractable metal concentrations were significantly lower ($p<0.05$) in the presence of biochar compared to the control (Fig. 6.1b, c, d). The application of biochar in increasing rates resulted in a correlative reduction of release of metals. This reduction can be attributed in part to the significant increase in soil pH due to the biochar liming effect (Fig. 6.1a). The existence of this effect is attested by the strong correlation between the $CaCl_2$-extractable metal concentrations and the soil pH (pH-$CaCl_2$) observed on the entire dataset of measurements without distinguishing between treatments (Fig. 6.2). The rise in soil pH induces metal immobilization because it favors metal precipitation, decreases metal solubility and promotes metal adsorption by increasing the net negative charge of variably charged soil constituents (Lindsay, 1979; McBride et al., 1997; Bradl, 2004). It is also likely that the higher pH brought about by the biochar application promoted metal adsorption on the biochar particles themselves since the density of cation exchange sites increases on the biochar surface with pH (Harvey et al., 2011). This is in agreement with Beesley and Marmiroli (2011) who demonstrated by scanning electron microanalysis that biochar rapidly reduces the concentration of both Cd and Zn in eluate from contaminated soil columns due to sorption of metals at the biochar surface. Similarly, Lehmann et al. (2003b) showed that the leaching of fertilizer (e.g. NH_4^+, Ca and Mg) was rapidly reduced in a soil amended with biochar due to electrostatic adsorption to an exchange complex created by the biochar addition.

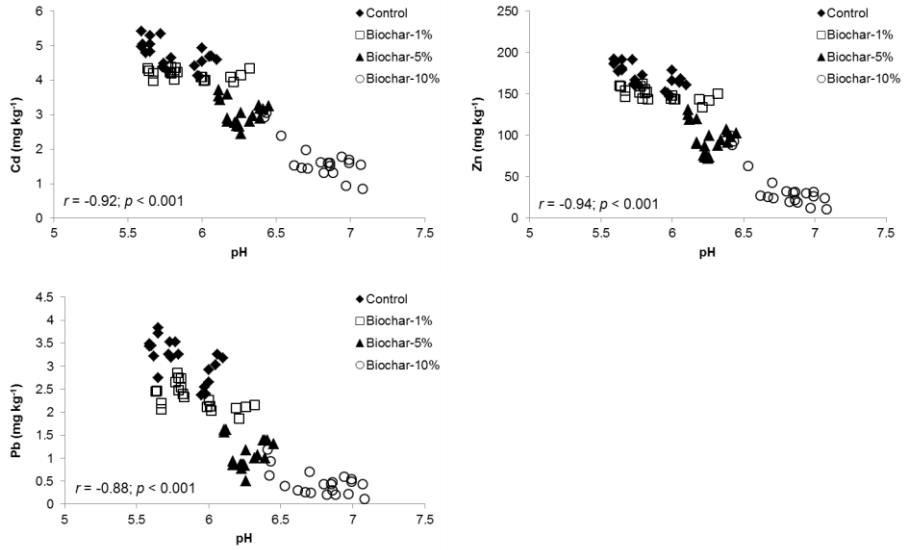


Fig. 6.2 Relationship between pH and metal concentrations in 0.01 M $CaCl_2$ extracts. Marked correlation coefficient (*r*) is the Pearson's correlation coefficient calculated for the whole dataset of measurements.

Compared to the control, the $CaCl_2$-extractability after 1 hour of incubation was reduced by 5%, 20% and 35% for Cd, 5%, 25% and 48% for Zn, and 19%, 52% and 77% for Pb in the presence of 1%, 5% and 10% of biochar, respectively. The pattern of this rapid metal immobilization was thus Pb>Zn>Cd. By analogy with humus-amended soils (Chaturvedi et al., 2006), the preferential fast adsorption exhibited by Pb over both Zn and Cd in the biochar-amended soils might be attributed to the greater affinity of Pb for functional groups (carboxylic and phenolic) situated on the surface of oxidized biochar particles. Being hard Lewis bases, the metal affinity for these functional groups is greater for Pb^{2+}, which is a borderline hard Lewis acid compared, than for Zn^{2+} and Cd^{2+}, which are soft Lewis acids.

In the presence of 5% and 10% of biochar, the initial fast metal sorption was followed by a secondary slow retention, as attested by the continuous decrease in $CaCl_2$-extractable metals with time (Fig. 6.1b, c, d). Compared to the situation after 1 hour of incubation, $CaCl_2$-extractable concentrations of Cd, Zn and Pb at the end of the experiment were respectively 1.3, 1.7 and 1.9 times lower for biochar-5% treatment and 2.5, 5.4 and 3.8 times lower for biochar-10% treatment. This attenuation is characteristic of aging which refers to processes by which the mobility and bioavailability of metals

decline with time (Bruemmer et al., 1988; Ma et al., 2006). Increases in sorption with time are usually attributed to the mechanisms with lower reaction rates such as diffusion into micropores of both inorganic and organic soil constituents and surface nucleation – precipitation (Bruemmer et al., 1988; Ainsworth et al., 1994; Sparks, 2003; Ma et al., 2006). Inyang et al. (2012) reported that the fast initial increase in Pb removal from aqueous solution by biochar was followed by a slowdown as sorption approached equilibrium. These authors suggested that the mechanism for the removal of Pb was diffusion-controlled, the diffusion rate being governed by the biochar pore size. Therefore, the slow secondary increase in metal retention observed in biochar-5% and treatment-10% biochar could be assigned to reactions of diffusion, precipitation and adsorption of metals in the micropores of biochar as they underwent a slow oxidation (Nguyen et al., 2008). Since aging reactions tend to become irreversible, the metal bioavailability decreases thus with aging time (McLaughlin, 2001). In the untreated soil, $CaCl_2$-extractable metal concentrations slightly increased with time indicating that, without biochar, aging did not reduce the release of metal (which was even slightly enhanced). No clear trend was observed for the biochar-1% treatment. A possible explanation is that biochar micropores were made unavailable for adsorbing metal because the organic substances present in the soil (e.g. humic acids) readily attached to the biochar surface, rendering inner pores inaccessible for metal diffusion and further adsorption (Kwon and Pignatello, 2005; Pignatello et al., 2006).

Table 6.2 Parameters, coefficient of determination and standard error of the estimate of the Elovich equation (*q* = *a* + *bln*t*, where q is the metal increase or decrease [mg kg^{-1}], *t* is the time [hour] and *a* and *b* are constants) used to describe the time-evolution of the 0.01M $CaCl_2$ extractability of Cd, Zn and Pb in soils.**

	Cd				Zn				Pb			
	a	*b*	R^2	SE	*a*	*b*	R^2	SE	*a*	*b*	R^2	SE
Control	4.3	0.1	0.42	0.3	159	2.4	0.25	13.9	3.2	-0.0	0.01	0.4
Biochar-1%	4.2	0.0	0.00	0.1	155	-1.2	0.27	6.3	2.6	-0.1	0.41	0.2
Biochar-5%	3.6	-0.1	0.79	0.3	125	-6.0	0.85	8.5	1.6	-0.1	0.70	0.2
Biochar-10%	2.9	-0.2	0.99	0.1	85	-9.7	0.99	1.7	0.8	-0.1	0.84	0.1

Characterizing the aging effect on heavy metal extractability was attempted by describing the evolution of heavy metal release with time by the Elovich equation. The Elovich equation has been successfully used to describe the kinetics of sorption and desorption of various inorganic substances, including heavy metals, on soils (Sparks, 1989; Taylor et al., 1995; Singh and Pandeya, 1998; Jalali and Khanlari, 2008) as well as other matrix such as bone char (Cheung et al., 2000). It usually well fits observations where reaction rate is very rapid at first and then declines slowly as the apparent equilibrium is approached (Chien and Clayton, 1980), as observed for metal behavior in biochar-5% and biochar-10% treatments (Fig.6.1). The Elovich equation slope (*b*) depicts the loss or the gain in metal extractability with time and can be used as an index of the rate of transformation from exchangeable fraction to more stable fraction. Based on Jalali and Khanlari (2008), we defined the constant *b* in Elovich equation as the transformation rate and we used it to compare the treatments or the heavy metals. The more negative the *b* value, the higher the transformation rate. The interpretation of the *b* value is nevertheless correct provided the model output predicts observations, which is only verified for biochar-5% and biochar-10% treatments, as revealed by R^2 and SE values (Table 6.2). In the soils amended with 5% and 10% of biochar, the *b* value for Zn (-6.0 and -9.7, respectively) was largely more negative than for Cd (-0.1 and -0.2, respectively) and for Pb (-0.1 and -0.1, respectively), indicating that, over the incubation period, Zn was much more easily transformed from exchangeable fraction to more stable fraction than both Cd and Pb. Generally, the diffusion rates of metals into micropores are determined by their ionic radii (Bruemmer et al., 1988), with the larger the ionic radius, the slower the reaction (Fischer et al., 2007). Assuming that metal diffusion occurred in biochar-5% and -10% amended soils, the much higher rate of transformation of Zn could be thus explained by its small ionic radius (0.74 Å) which favored its diffusion, compared to that of Cd (0.97 Å) and Pb (1.2 Å). Moreover, *b* values of Cd and Zn were the highest in the presence of 10% of biochar, suggesting that the transformation rate of exchangeable Zn and Cd fraction increased with the rate of biochar amendment. This could be due to the higher pH increase induced by this treatment. Indeed, pH has

been reported as the most important parameter determining the effect of aging on metal partitioning between soil and $CaCl_2$ extract, with the effect of aging becoming more important with increasing pH (Lock and Janssen, 2003; Ma et al., 2006). On the contrary, the rate of Pb transformation does not seem to be affected by the percentage of added biochar, the *b* values being close to each other regardless the presence of 5% or 10% of biochar.

3.3. Heavy metal bioavailability

Among accumulating bioindicator plants used for trace elements biomonitoring, ryegrass (*Lolium. multiflorum*) is frequently selected because of its high capacity for the accumulation of toxic substances and its tolerance against heavy metals (Klumpp et al., 2009). It does not present mechanisms of metal exclusion or hyperaccumulation during metal uptake and is therefore recommended as a valuable tool for bioavailability assessment (Lambrechts et al., 2011a). Therefore, in this study, metal concentrations in ryegrass shoots were considered an indicator of the metal bioavailability in soils. While biochar-1% treatment did generally not decrease the metal concentration in shoot in comparison with the control, the application of 5% and 10% of biochar significantly lowered the metal bioavailability (Fig. 6.3a, b, c). Compared to the control, Cd, Zn and Pb concentrations measured in shoots from biochar-5% treatment were respectively reduced by 40%, 53% and 30% at the first harvest and by 53%, 57% and 37% at the second harvest. For biochar-10%, they were reduced by 67%, 73% and 50% at the first harvest and by 75%, 74% and 59% at the second harvest. Thus, although the metal concentrations in shoots were higher at the second than at the first harvest (Fig. 6.3a, b, c), the reduction compared to the control was the highest at the second harvest. Even though a “dilution effect” due to the higher biomass production (Fig. 6.3d) should not be completely ruled out, this reflected a decrease in metal bioavailability with time, as previously suggested by results of $CaCl_2$ extraction.

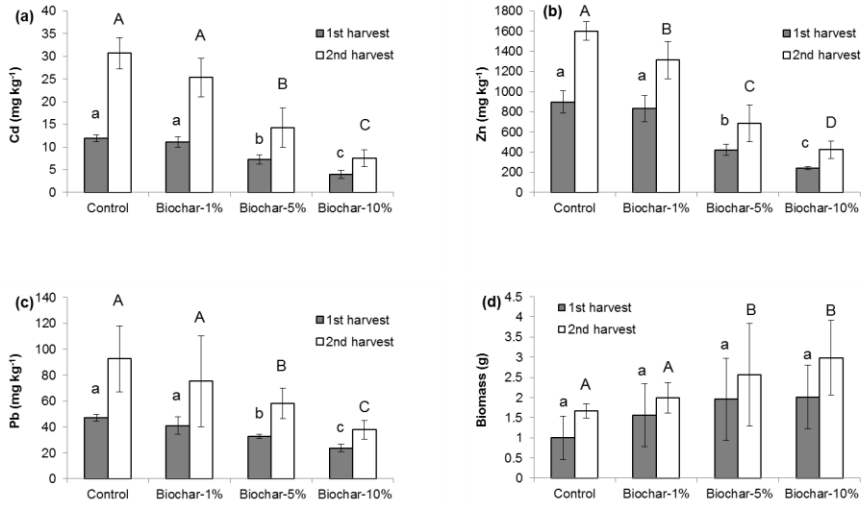


Fig. 6.3 Concentration of Cd (a), Zn (b), and Pb (c) and biomass (d) of shoots of ryegrass (*Lolium multiflorum*) at the first and the second harvests (4 and 8 weeks after sowing, respectively). Values are average (n = 4) ± standard deviation. Columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

3.4. Potential remobilization due to acidification

Although $CaCl_2$ extraction results showed that the incorporation biochar in soil was effective in immobilizing heavy metals, its performance at medium- and long-term has still to be ascertained. In order to assess the stability of the immobilization products, we adopted the method proposed by several authors (Carter et al., 2009; Lee et al., 2009; González-Núñez et al., 2012) which consists in determining the ability of heavy metals to remain insoluble upon acidification.

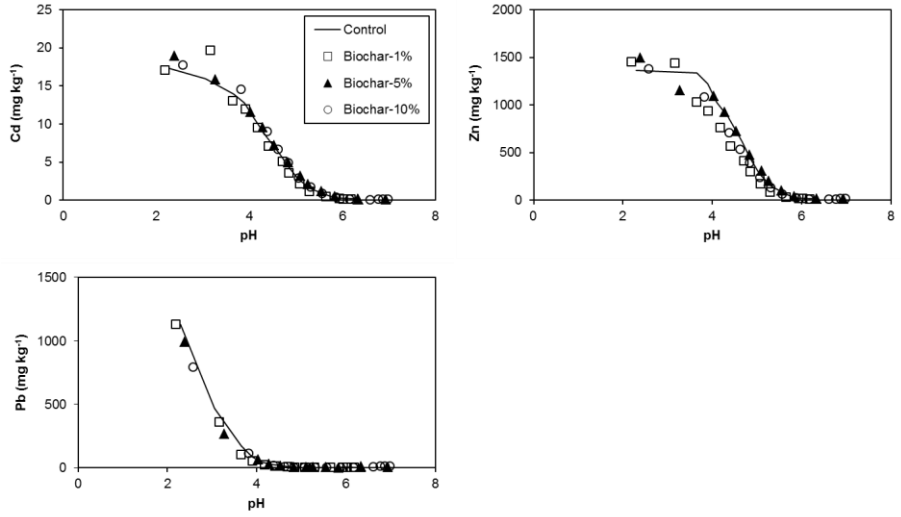


Fig. 6.4 Leaching curves of Cd, Zn, and Pb as a function of pH.

Fig. 6.4 shows that the response of Cd, Zn and Pb to change of pH was the same in biochar-amended soils as in the control. This indicates that the application of biochar did not lead to the formation new one or more metal-bearing phases that are resistant to pH change. It can be concluded that the reaction between the heavy metals and biochar were thus pH-dependent and reversible at acidic pH. However, the occurrence of a soil acidification depends on the ANC (acid neutralizing capacity) of soils which measures the overall buffering capacity of soils against acidification (Cappuyns and Swennen, 2008a). The amount of acid added to a soil-water suspension to keep the pH at a predefined constant value gives an assessment of the ANC of the soil. The soil ANC at pH 4 (ANC_{pH4}; a parameter often reported in the literature) was improved in the presence of 5% and 10% of biochar (Fig. 6.5). According to Xu et al. (2012a), protonation of oxygen-containing functional groups of biochar is the main mechanism for the increase of the ANC after biochar application to soils. According to Saarsalmi et al. (2001), alkaline ash present in biochar may also contribute to the increase of the ANC of soils. From a practical point of view, the knowledge of the ANC is helpful because it enables to assess the time required to reach a given soil pH and thus to determine to what extent a treatment can resist an acidification event. Using data reported by Cappuyns and Swennen (2008b) who considered that the total acid

deposition by rain for the year 2005 amounted in Flanders to 3810 equivalent of acids per hectare per year and assuming a quasi-constant emissions of SO_x, NO_x and NH_x compounds in the coming years, the time to reach pH 4 was calculated by applying the following equation:

$$Time\ (years) = \frac{ANC_{pH\,4}\ (mmol\ kg^{-1}) \times mass\ of\ soil\ (kg\ ha^{-1})}{3810 \times 1000\ (mmol\ ha^{-1}\ year^{-1})}$$

We calculated the mass of soil by considering a soil layer of 20 cm and a bulk density of 1300 kg m^{-3}. Computations showed that the time needed to reach pH 4 would be 56, 57, 69 and 93 years for the control and the biochar-1%, -5% and -10% treatments, respectively. The following results must however be regarded as a worst-case scenario since the considered experimental conditions do not occur in nature (Cappuyns and Swennen, 2008b). In all treatments, the acidification to pH 4 released significant quantities of Cd and Zn (Fig. 6.4). For Pb, the release dramatically increased below this pH (Fig. 6.4). Because, irrespective of the treatment, such an acidification could occur in less than one century as a result of acid deposition, all these soils are obvious examples of chemical time bombs. However, by improving the soil ANC and thus the time required to reach this hazardous soil pH, incorporation of 10%- and, in a lesser extent, 5%-biochar could delay the triggering of the bomb.

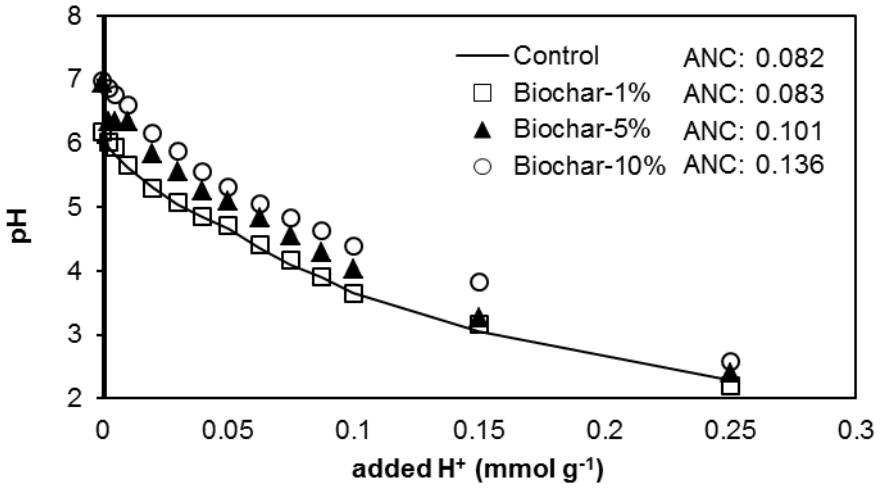


Fig. 6.5 pH titration curves and ANC_{pH4} values (mmol g^{-1}) of soils.

4. Conclusion

This study showed that the application of biochar on a heavy metal contaminated soil can help reduce the extractability as well as the bioavailability of Cd, Zn and Pb, notably because it raises the soil pH. Moreover, provided the soil is not subjected to subsequent acidification, the bioavailability of metals decreases gradually with time when the soil is amended by 5% or 10% of biochar. In the presence of 10% biochar, 0.01M $CaCl_2$-extractable concentrations of Cd, Zn and Pb after 56 days of incubation are respectively 2.5, 5.4 and 3.8 times lower than those measured after 1 hour of incubation. These aging reactions cannot be explained by pH changes over time and could be due in part to diffusion into biochar micropores. Although the metal release at a given pH is similar regardless to the presence or the absence of biochar, the incorporation of biochar into soil may help delaying the time when a hazardous pH is reached because it improves the ANC of the soil.

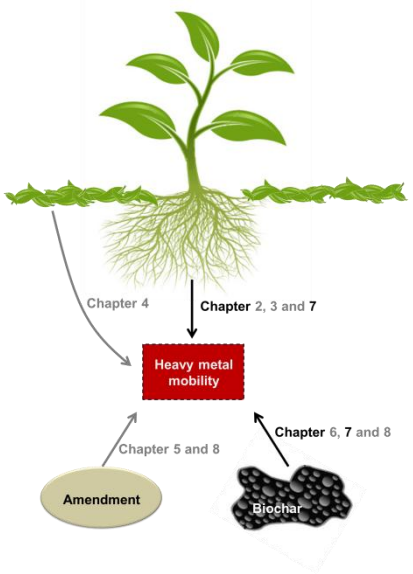
It is concluded by the authors that the application of industrially-produced biochar to soil for *in situ* immobilization of metals (and taking advantage of this for sequestering carbon) could be feasible provided soil pH is monitored over time.

Chapter 7

Effects of biochar and root-induced changes in the rhizosphere of *Agrostis tenuis* and *Lupinus albus* on heavy metal soil fractionation and plant uptake

Where are we?

As demonstrated by Chapter 2 and 3, the root activity can greatly affect the success of phytostabilization by inducing metal mobilization. Consequently, the examination of biochar application for phytostabilization purposes must include the understanding of the heavy metal mobility within the rhizosphere. Therefore Chapter 7 examines the effects of rhizospheric processes of two plants, *Agrostis tenuis* and *Lupinus albus*, on the metal mobility in a soil amended with biochar.



1. Introduction

Since the Industrial Revolution, anthropogenic activities have left a toxic footprint from mineral exploration and mining to ore beneficiation and metal refining (Mendez and Maier, 2008). Worldwide, it was estimated that 22 million hectares of land are contaminated with heavy metals (GACGC, 1994). Because of the lack of heavy metal degradation in the environment, there is a statutory requirement to remediate polluted sites to prevent exposure to humans and ecosystems. Increasingly, phytostabilization is suggested as an *in situ* cost-effective and environmentally friendly approach for the remediation of heavy metal-contaminated soils (Mench et al., 2006; Karami et al., 2011). This technique consists in installing a vegetation cover on contaminated areas for containing the contaminant within the vadoze zone (Bolan et al., 2011). The plant canopy limits eolian dispersion while the roots prevent water erosion and leaching and provide a rhizosphere wherein metals can precipitate and stabilize (Vangronsveld et al., 1995; Mendez and Maier, 2008).

Phytostabilization is usually aided by the *in situ* application of soil amendments that are effective in both the immobilization of metals and the supply of material conditions that promote plant growth and stimulate ecological restoration (Adriano et al., 2004; Vangronsveld et al., 2009; Park et al., 2011b; Houben et al., 2012). Of the numerous amendments proposed for phytostabilization strategies, biochar is gaining a growing attention (Beesley et al., 2011). Besides its attractive interest for replenishing C stocks and improving C sequestration on the long term in soils (Lehmann, 2007b; Woolf et al., 2010), biochar can act as a soil conditioner because its application may rapidly increase the soil fertility and plant growth by supplying and retaining nutrients while improving soil physical and biological properties (Glaser et al., 2002; Laird et al., 2010; Uzoma et al., 2011). Moreover, Chapter 6 as well as recent studies have shown that biochar application to contaminated soils may immobilize heavy metals and reduce their bioavailability (Uchimiya et al., 2010; Karami et al., 2011; Park et al., 2011a).

Although researches studying the effects of amendments on metal fate have mostly focused on changes in the bulk soil so far, it is increasingly

recognized that the enhancement of the phytostabilization processes requires a solid understanding of the complex interactions in the rhizosphere (Kidd et al., 2009; Wenzel, 2009; Hashimoto et al., 2011). Indeed, the physico-chemical conditions in this microenvironment may differ drastically in many respects from those in the bulk soil (Marschner and Römheld, 1996), thereby having a considerable effect on the solid speciation and, ultimately, the bioavailability of heavy metals (Hinsinger, 2001; Chaignon et al., 2002a; Hinsinger and Courchesne, 2008; Bravin et al., 2012; Houben and Sonnet, 2012). In particular, root-induced pH changes are recognized as a critical mechanism influencing metal solubility in the rhizosphere (Loosemore et al., 2004; Hinsinger and Courchesne, 2008). Since the biochar incorporation into contaminated soils is irreversible, it is thus essential to elucidate its effects on the rhizosphere response prior to implement it at field scale for phytostabilization purposes.

Therefore, the objective of this study was to gain a better insight about how the biochar application to contaminated soil affects the rhizosphere response of two plants commonly used in phytostabilization schemes (*Agrostis tenuis* and *Lupinus albus*) and, in turn, the speciation and the bioavailability of heavy metals (Cd, Pb and Zn). For this purpose, a "worst-case scenario" approach was adopted, which consisted in growing plants on a thin disk of soil without using a nutrient solution. This experiment enables us to explore what is the limit response of the rhizosphere when a contaminated soil is amended with biochar.

2. Materials and methods

2.1. Soil and biochar

The study soil was collected at Sclaigneaux (Namur province, Belgium). From the 1850's to the 1970's, it was intensively subjected to Cd-, Zn-, and Pb-bearing atmospheric fallouts originating from adjacent Zn and Pb smelters. After drying at ambient temperature and sieving (2 mm), the soil was amended with biochar on a 5% w/w basis (thereafter called biochar-5%). Biochar derived from miscanthus (*Miscanthus* × *giganteus*) straw and

was obtained from Pyreg GmbH (Dörth, Germany) using an industrial pyrolysis reactor (30 minutes of residence time and 600°C of end temperature of pyrolysis). Untreated (thereafter called control) soil was also part of the experimental design. Four 200-g pots were prepared per each treatment and were incubated at field capacity in darkness over a period of 56 days (for details, see chapter 6). The soils were then removed from the pots, dried at ambient temperature and stored in plastic bottles prior to the commencement of the current study. The main physical and chemical characteristics of the soils are presented in Table 7.1.

Table 7.1 Characteristics and total metal content of the soil (control) and the soil amended with 5% biochar (biochar-5%)

		Control	Biochar-5%
pH-$CaCl_2$		5.66	6.24
CEC	$cmol_c\ kg^{-1}$	7.38	7.92
Exchangeable-Ca	$cmol_c\ kg^{-1}$	3.65	3.99
Exchangeable-K	$cmol_c\ kg^{-1}$	0.12	1.06
Exchangeable-Mg	$cmol_c\ kg^{-1}$	0.80	0.94
Exchangeable-Na	$cmol_c\ kg^{-1}$	0.01	0.07
Mehlich 3-P	$mg\ kg^{-1}$	20.8	27.5
Organic C	$mg\ kg^{-1}$	190	207
NO_3-N	$mg\ kg^{-1}$	40.6	10.5
NH_4-N	$mg\ kg^{-1}$	3.5	10.5
Total Cd	$mg\ kg^{-1}$	24	25
Total Zn	$mg\ kg^{-1}$	3080	2940
Total Pb	$mg\ kg^{-1}$	2690	2590

2.2. Biotest experimental device

The design of the biotest used in this study was based on that of Lambrechts et al. (2011a) and is illustrated in Fig. 7.1 and Appendix F. The principle of this device is similar to that used by other authors (Chaignon et al., 2002a; Chaignon and Hinsinger, 2003; Loosemore et al., 2004; Bravin et al., 2010; Bravin et al., 2012) and consists in separating plant roots from soil with a 20-µm polyamide mesh to facilitate the collection of roots and rhizosphere. Briefly, 6 or 0.8 g of sterilized seeds (10 min in 2 M H_2O_2) of *L. albus* or *A. tenuis* respectively per pot were germinated in demineralized water for three days in darknrdd. Then, the demineralized water was replaced with nutrient solution and the seedlings were allowed to grow for 7 days under a photoperiod of 16 h (photon flux density of 120-180 µmol m^-

^{2} s^{-1}), constant temperature (20°C) and relative humidity (95%). The chemical composition of the nutrient solution expressed in mM was: 1 $Ca(NO_3)_2.4H_2O$, 0.5 KCl, 0.25 K_2SO_4, 0.05 $MgCl_2$, 0.05 $MgSO_4$, 0.05 NaH_2PO_4, and 0.08 H_3BO_3. This pre-growth period allowed the formation of a dense root mat.

At the completion of this pre-growth stage, plant compartments were transferred onto a thin layer of pre-incubated soil mixture (about 2-mm thick, 7.5 g dry soil). The whole system was placed on a polystyrene plate floating on 1-dm³ demineralized water. A polyamide cloth below the soil layer dipped into the demineralized water reservoir in order to maintain constant soil moisture by capillarity phenomenon.

After 21 days of contact, the soil layer under plants was considered as rhizosphere (Chaignon et al., 2002a). Similar to Bravin et al. (2012), unplanted control treatments, in which the soil had been incubated in similar devices without plants (thereafter called bulk soil), were carried out. In total, 24 devices were carried out: 2 treatments (control or biochar-5%) × 3 crop conditions (*A. tenuis*, *L. albus*, and uncropped/bulk soil) × 4 replicates.

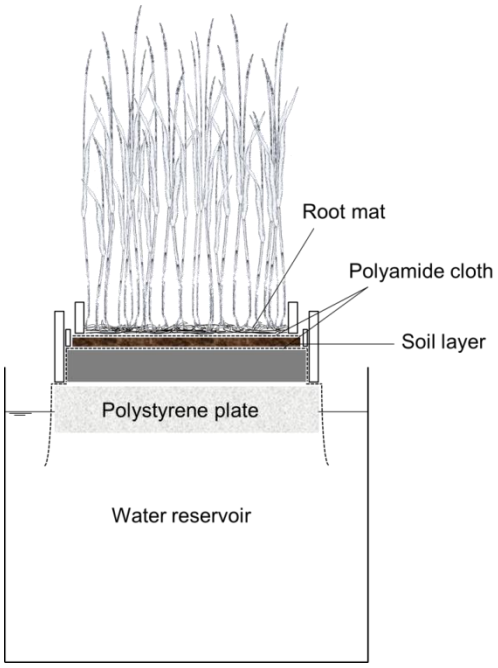


Fig. 7.1 Biotest experiment design (see Lambrechts et al., 2011 and Appendix F for more details)

2.3. Plant analyses

At harvest, shoots and roots were separated and roots were gently rinsed with deionized water. Shoots and roots were then dried at 60°C, weighed and crushed. The heavy metal (Cd, Zn and Pb) concentration in shoots and roots was determined by ICP-AES after mineralization by HNO_3 and *aqua regia* digestion. The plant's ability to translocate metals from roots to shoots was estimated using the translocation factor and calculated as the metal concentration in shoot divided by the metal concentration in root. The water uptake by plants throughout the biotest experiment was calculated as the difference between the total volume of water introduced during the experiment minus the water volume remaining in the reservoir at the plant harvest.

2.4. Soil analyses

At the completion of the biotest experiment, cropped soils (rhizosphere) and uncropped soils (bulk soil) were collected and dried at ambient temperature. Soil pH was measured in H_2O (soil to water ratio of 1:5) after shaking for 1h. The fractionation of Cd, Zn and Pb in soils was determined according to the sequential extraction scheme proposed by Tessier et al. (1979) which consists of five steps to extract heavy metals bound to the following operationally defined fractions: exchangeable (EXCH), bound to carbonates (CARB), bound to Fe and Mn oxides (OXI), bound to organic matter (ORG) and residual (RES). All metal concentrations are expressed on a dry matter basis (i.e., dried at 105°C).

2.5. Statistical analyses

Statistical analyses to compare the average results of sequential extractions and pH were performed using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p<0.05$). Paired comparisons between the control and the biochar-5% treatment were carried out using Student's t-test. Prior to statistical analyses, homogeneity of variances was tested using Levene's

test. Pearson's correlation coefficients (*r*) were calculated to determine the relationships between the heavy metal concentrations in sequential extraction fractions and the soil pH. Three levels of significance were considered: $p<0.05$, $p<0.01$ and $p<0.001$. All statistical analyses were carried out using XLSTAT (Addinsoft, ver. 2010.5.08).

3. Results and Discussion

3.1. Bulk soil and rhizosphere pH

In the bulk soil, the application of biochar showed a significant liming effect as the pH raised from 5.58 to 6.70 (Fig. 7.2). However, such a liming effect was not observed in the rhizosphere of both plants as their rhizopheric pH in the presence and in the absence of biochar were similar and significantly lower than bulk soil pH (Fig. 7.2).

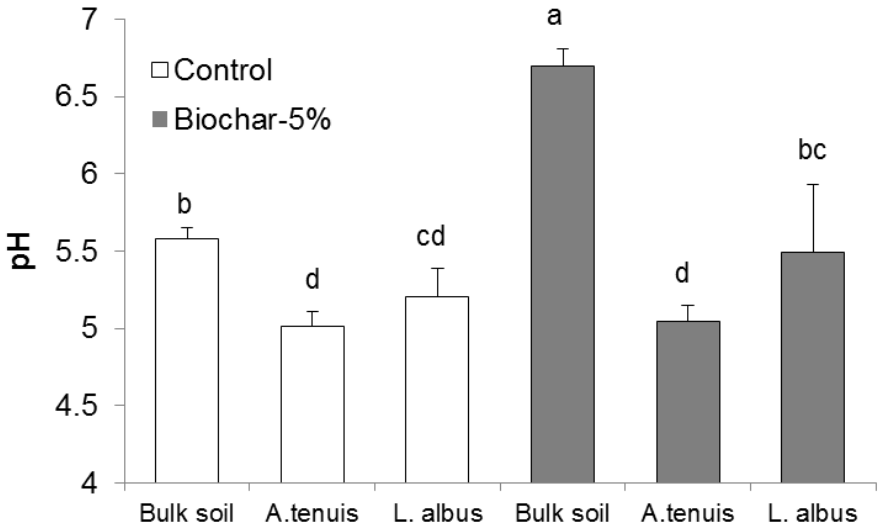


Fig. 7.2 pH in the bulk soil and in the rhizosphere of *Agrostis tenuis* and *Lupinus albus* in the control and biochar-5% treatment. Values are average (n = 4) ± standard deviations. Columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

Using the data of the titration curves found for these soils (Chapter 6), we calculated that the quantity of H^+ necessary to decrease bulk soil pH to

those measured in the rhizosphere was 0.026 and 0.017 mmol g^{-1}soil for *A. tenuis* and *L. albus* respectively in control and 0.052 and 0.038 mmol g^{-1}soil for *A. tenuis* and *L. albus* respectively in biochar-5% treatment. This data indicates that the net H^+ production in the rhizosphere was twofold more intense in the presence of biochar, whatever the plant. The main process explaining pH differences between the rhizosphere and the bulk soil is the release of H^+ or OH^- by roots to compensate charge imbalance due to unequal uptakes of cations and anions (Nye, 1981; Hinsinger et al., 2003). The high supply of exchangeable cations (Table 7.1) by the biochar can have promoted their absorption by plants and, in turn, increased rhizosphere acidification. For instance, Loss et al. (1993) showed that in K-enriched media, such as biochar-5% (Table 7.1), the K uptake by *Lupinus angustifolius* L. was exacerbated thereby leading to the secretion of large amounts of H^+ to compensate the charge imbalance in solution. It is also well-known that the rhizosphere pH is greatly influenced by the N uptake by roots and that a higher uptake of NH_4-N relative to NO_3-N increases the release of H^+ from roots (Haynes, 1990; Wang and Zabowski, 1998; Hinsinger et al., 2003). Consequently, the higher NH_4-N content while lower NO_3-N content in biochar-5% contributed likely to stimulate rhizosphere acidification. Taghizadeh-Toosi et al. (2011) proposed that one possible mechanism for the reduced NO_3-N content is the stronger adsorption of NH_4^+ by the biochar which reduced the soil NH_4-N pool available to nitrifiers. In addition, enhanced release of H^+ under Fe deficiency has been described as strategy I of Fe acquisition and has been found in all dicots species (Marschner, 1995). For *L. albus*, liming effect subsequent to biochar application may have resulted in decreased availability of Fe, and thus in enhanced H^+ efflux. N_2-fixation by *L. albus* can also contribute to rhizosphere acidification (Sas et al., 2002) but nodules were not found on *L. albus* roots, neither in the absence nor in the presence of biochar. This suggests that this process did not contribute to increase the H^+ production in the *L. albus* rhizosphere. Proteoid roots of *L. albus* can also acidify the rhizosphere by releasing citrate, malate and protons, especially under P deficiency (Dinkelaker et al., 1989; Neumann et al., 1999; Sas et al., 2001). However, it is unlikely that this process was exacerbated in the rhizosphere of biochar-amended soil and thus responsible of the higher acid production

since biochar application highly increased the available P (Mehlich 3-P) content (Table 7.1).

3.2. Effects of biochar on heavy metal fractionation in bulk soil

The fractionation of metals in soils was assessed using sequential extraction procedure according to the Tessier's scheme (Tessier et al., 1979). Although they provide operationally-defined results and may suffer from several drawbacks such as lack of selectivity and element redistribution during extraction, sequential extraction procedures were used extensively by researchers to elucidate the fractionation of heavy metal in soils, including the rhizosphere (Bacon and Davidson, 2008). According to Hammer and Keller (2002), their use is particularly justified when the aim is to compare differences in the same soil engendered by different treatment, such as the effect of amendment or plant growth on metal distribution

Results of chemical fractionation of metals in the bulk soil show that the biochar application depressed the amount of Cd and Zn in the EXCH pool while it increased that in the CARB pool (Fig. 7.3). The other Cd and Zn fractions were not significantly affected. The shift of Cd and Zn from the EXCH to CARB fraction induced by biochar application was most likely the result of the raise in soil pH which promoted the precipitation of metals (Lindsay, 1979). Our results coincide with those of Xian and In Shokohifard (1989) which showed that when the soil pH was changed, Cd and Zn were chiefly shifted between exchangeable and carbonate forms, while the other fractions were almost unchanged. Similarly, Jiang et al. (2012) suggested that the metal precipitation as a result of the pH increase brought about by biochar application was one of the mechanisms involved in the metal immobilization in a biochar-amended Ultisol. As for Cd and Zn, the biochar application decreased significantly the EXCH pool of Pb in the bulk soil (Fig. 7.3). However, the shift of Pb from the EXCH pool to the other pools was not obvious. Even though the CARB and RES pools were slightly increased, the differences were not statistically significant ($p>0.05$) compared to the control (Fig. 7.3).

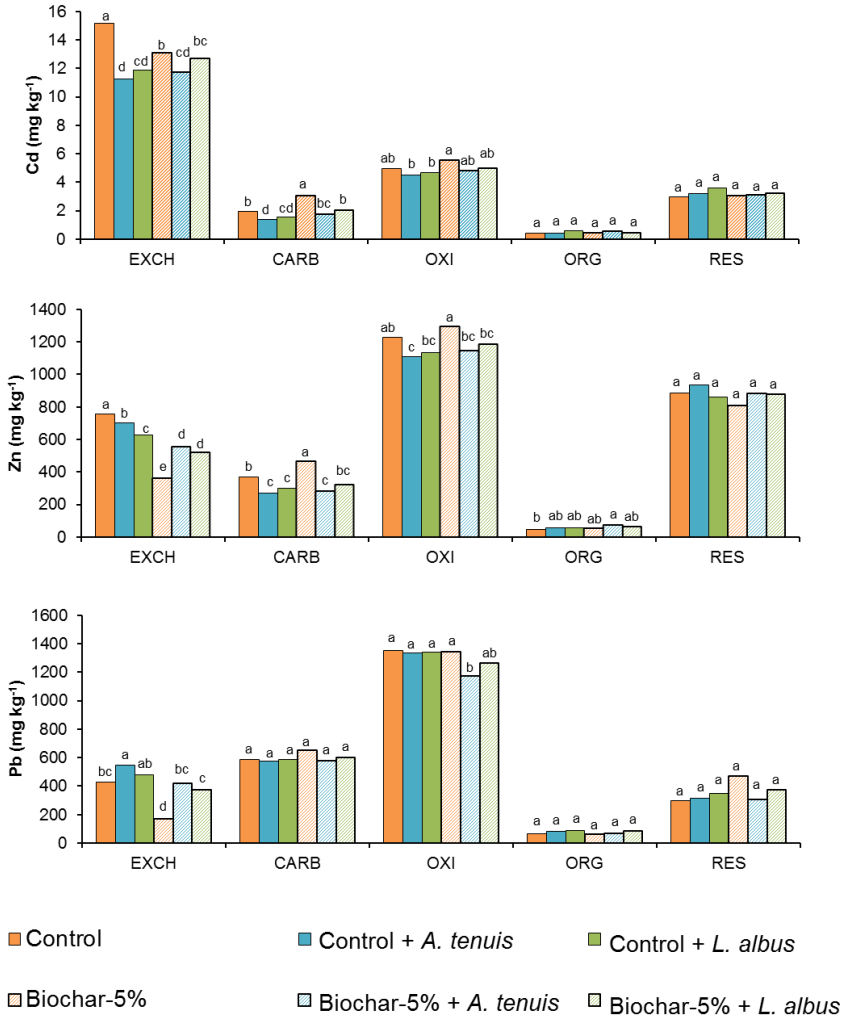


Fig. 7.3. Fractionation of Cd, Zn and Pb in the bulk soil and in the rhizosphere of *Agrostis tenuis* and *Lupinus albus* in the control and biochar-5% treatments (EXCH: exchangeable; CARB: bound to carbonate; OXI: bound to Fe and Mn oxides; ORG: bound to organic matter; RES: residual). For each fraction, columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

3.3. Effects of root-induced changes on heavy metal fractionation

In both the control and the biochar-5% soils, the growth of both *A. tenuis* and *L. albus* decreased the Cd amount in the CARB fraction (Fig. 7.3). This decrease can be attributed to the rhizosphere-induced acidification (Fig. 7.2) which is well-known to favor the release of metals associated with carbonates (Marschner and Römheld, 1996). The strong positive correlation between pH and Cd concentration in the CARB pool (Fig. 7.4) confirms the predominant role that the pH played in the control of the carbonate-bound Cd solubility. A similar trend was noticed for Zn, the CARB pool in the rhizosphere of both control and biochar-5% soils being significantly lower than that in the bulk soil (Fig. 7.3), likely as a result of the lower pH (Fig. 7.2). Irrespective of the treatments, the pool of Zn bound to Fe- and Mn-oxides (OXI) was also significantly depleted in the presence of plants. Since the metals associated with oxides can be released under acidic conditions (Xian and In Shokohifard, 1989), this Zn depletion can be explained by the root-induced acidification (Marschner and Römheld, 1996). This is mirrored by the strong positive correlation found between pH and Zn bound to Fe- and Mn-oxides pool (Fig. 7.4). Unlike Cd, the EXCH pool of Zn in the presence of biochar was significantly higher in the rhizosphere of both *A. tenuis* and *L. albus* than in the bulk soil. This suggests that, contrarily to Cd, a fraction of Zn solubilized from the CARB pool due to root-induced acidification was not taken up by plants but redistributed onto the exchange sites of soil and biochar particles. In agreement with Loosemore et al. (2004), the concentration of Cd and Zn in the EXCH pool is thus the final result from the concurrent dynamics of pH change and metal uptake induced by roots.

In both treatments, the rhizosphere activity of *A. tenuis* and *L. albus* increased the Pb amount in the EXCH pool compared to the bulk soil (Fig. 7.3). The reason of this Pb enrichment in the EXCH pool was probably also related to the root-induced acidification as suggested by the strong negative correlation between the amount of Pb in this pool and the soil pH (Fig. 7.4). The strong positive correlation between the amount of Pb in the CARB pool and the soil pH (Fig. 7.4) also suggests that root-induced acidification promoted the dissolution of carbonate-bound Pb pool and this

may have contributed to the increase of Pb in EXCH pool. However, this interpretation should be regarded with caution as the Pb amounts in the CARB pool were not statistically significantly lower in the rhizosphere (Fig. 7.3). Our results are in line with those Hashimoto et al. (2011) which demonstrated that Pb immobilization in a hydroxyapatite-amended was negatively affected by rhizosphere processes because of the root-induced acidification which increased Pb solubility.

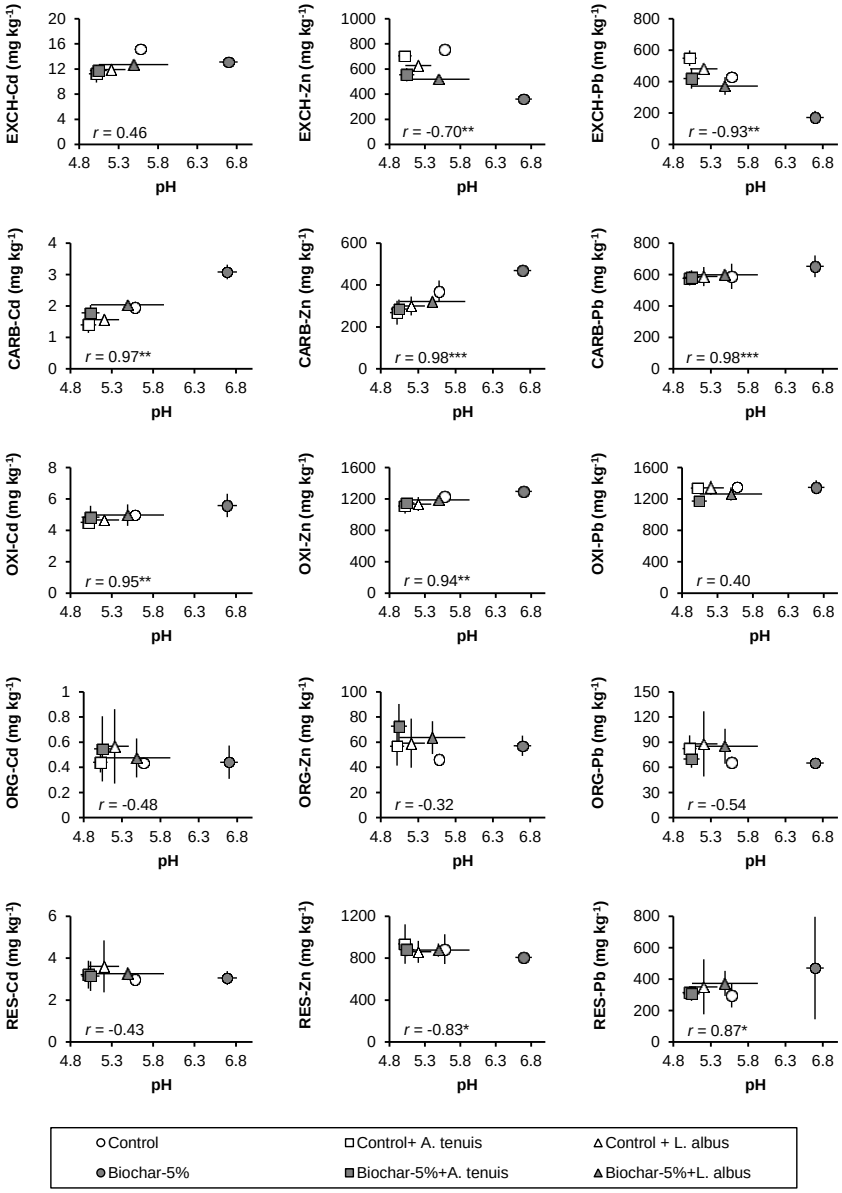


Fig. 7.4. Relationship between soil pH and metal fractions in soil (EXCH: exchangeable; CARB: bound to carbonate; OXI: bound to Fe and Mn oxides; ORG: bound to organic matter; RES: residual). Values are average (n = 4) ± standard deviations. Marked *r* correlation coefficient is the Pearson's correlation coefficient. *p<0.05; **p<0.01; ***p<0.001.

3.4. Effects of biochar and root-induced changes on heavy metal uptake and biomass production by plants

Except for Cd concentration in *A. tenuis* shoots and *L. albus* roots, the application of biochar did not significantly reduce the concentration of heavy metals in both roots and shoots of plants (Fig. 7.5), even though a general decrease trend was observed. The inefficacy of biochar application to reduce metal uptake by plants can be related to the effects of root-induced changes on metal distribution in the rhizosphere. Results of sequential extractions revealed that the main mechanism of Cd, Zn and to a lesser extent Pb immobilization was metal precipitation due to the pH increase. However, rhizosphere activity suppressed the liming effect of biochar and lowered the rhizosphere pH down to a level which was similar to that measured in the rhizosphere of plants grown in the absence of biochar. This root-induced acidification dissolved or hindered the formation of metal precipitates in the rhizosphere and thus counteracted the biochar-induced immobilization effect observed in the bulk soil. As a result, metal bioavailability at the soil-root interface was similar regardless of the presence or the absence of biochar. These results confirm the predominant role of pH in the control of metal bioavailability in biochar amended soils and coincides with our previous findings (Chapter 6) which showed that the release of Cd, Zn and Pb under identical acidic conditions was similar regardless of the presence or the absence of biochar into the soil.

The lack of significant reduction of metal concentration in plant parts after biochar application appears to contrast with results obtained by other studies (Park et al., 2011a) as well as by Chapters 6 and 8. However, by restricting physically the roots in small volumes of soil, the biotest used in this study “concentrates” the effects of the roots on the soil (Marschner, 1995). As a consequence, high root density intensified the effects of root-induced mobilization mechanisms (Whiting et al., 2001b), leading to an overestimation of rhizosphere effects (Neumann et al., 2009). Moreover, since the thin layer of soil was the sole source of nutrients for plants, this restricted reservoir most likely induced the intensification of rhizosphere processes. This is consistent with Chaignon and Hinsinger (2003) who, using similar biotest devices, showed that Cu bioavailability was systematically higher when the plants grew in the presence of deionized water instead of

nutrient solution. Results of the present study should thus be regarded as the results of the worst-case scenario. They allow defining the limitations for the use of biochar for phytostabilization strategies. In studies conducted using larger soil volume (e.g. pot or field experiment), we might expect a slower root-induced metal mobilization due to, for instance, a higher acid buffering capacity as well as a higher nutrient supply.

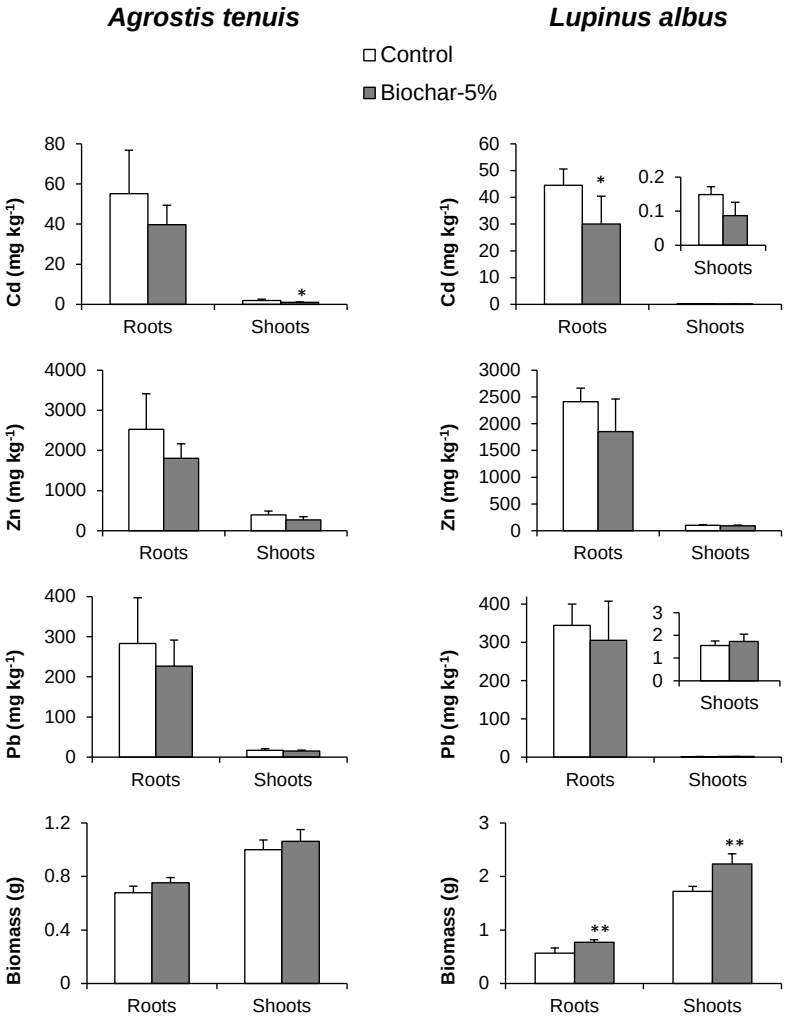


Fig. 7.5. Concentration of Cd, Zn and Pb and biomass production of roots and shoots of *Agrostis tenuis* (left) and *Lupinus albus* (right) in the control and biochar-5% treatments. Values are average (n = 4) ± standard deviations. * and **: Significant at the 0.05 and 0.01 probability level, respectively.

Irrespective of the treatment, translocation factors exhibited similar values (Table 7.2). This indicates that biochar application did not affect the root to shoot translocation of Cd, Zn and Pb in both *A. tenuis* and *L. albus*. Both plants immobilized Cd, Zn and Pb in roots, as indicated by their translocation factor < 1. This behavior is related to an exclusion strategy (Baker, 1981) and confirms that both species can suit for phytostabilization since such a restricted transport to aerial parts allow to avoid contaminant transfer into the food chain and metal accumulation on the soil surface, as metal-rich leaf litter is deposited (Kidd et al., 2009; Robinson et al., 2009; Houben et al., in press; Chapter 4). *Agrostis tenuis* was nevertheless less efficient for immobilizing metals in roots as this plant systematically exhibited the highest translocation factors (Table 7.2). In the roots of *L. albus*, Cd and Pb were more strongly immobilized than Zn as their translocation factor was approximately one order of magnitude lower than that of Zn. In agreement with this result, Page et al. (2006) reported a stronger retention of Cd over Zn in the roots. These authors suggested that Cd, which is a nonessential element by contrast to Zn, is recognized as a toxic compound at the root level and, as it is not needed in the shoot, is sequestered by *L. albus* in the roots to avoid damage to the shoots. In a lesser extent, *A. tenuis* also tended to immobilize more strongly Cd and Pb than Zn in roots. These results are consistent with those of Dahmani-Muller et al. (2000) which explained the higher translocation of Zn towards *A. tenuis* shoots by a greater diffusive transport of Zn.

Table 7.2 Translocation factors (= Metal concentration in shoots/Metal concentration in roots) for plants in the control and in the biochar-5% treatment.

	Agrostis tenuis		*Lupinus albus*	
	Control	Biochar-5%	Control	Biochar-5%
Cd	0.035	0.025	0.003	0.003
Zn	0.156	0.150	0.043	0.050
Pb	0.060	0.067	0.004	0.006

Contrarily to *A. tenuis*, the biomass of both roots and shoots of *L. albus* was significantly increased in the presence of biochar (Fig. 7.5). This biomass increase can be in part explained by the enhancement of nutrients contents in soil brought about by biochar application (Table 7.1). In a

phytostabilization scheme, higher biomass production is attractive because it reduces the metal dispersion by creating a larger physical barrier against soil erosion. Moreover, the associated higher water uptake (Table 7.3) is beneficial since under field conditions it would limit the metal percolation throughout the soil profile.

Table 7.3 Water uptake (ml) by *Agrostis tenuis* and *Lupinus albus* in the control and in the biochar-5% treatment. Values are average (n = 4) ± standard deviations.

	Control	Biochar-5%
Agrostis tenuis	488±73	491±55
Lupinus albus	462±77	565±8*

*Significantly different at the 0.05 probability level.

4. Conclusion

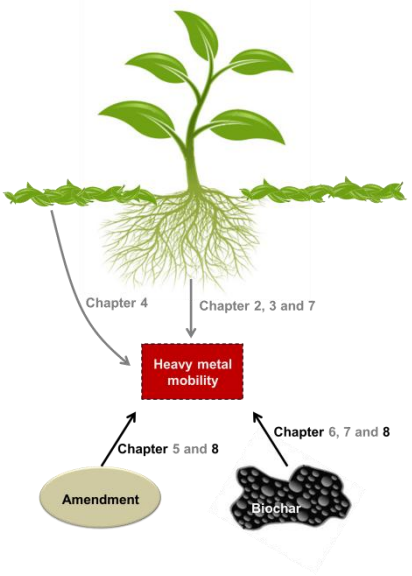
The biochar application to the bulk soil significantly reduced the exchangeable pool of Cd, Zn and Pb due to liming effect which subsequently promoted the metal shift into carbonate-bound pool. However, concentration of metals in roots and shoots of both *Agrostis tenuis* and *Lupinus albus* were generally not reduced in the presence of biochar. This was likely due to the high root-induced acidification in the rhizosphere which counteracted the liming effect of biochar and, in turn, increased the metal bioavailability. Although the study should be considered a worst-case scenario because experimental conditions induced the intensification of rhizosphere processes to the fullest extent, the results highlight that potential changes in rhizosphere pH need to be accounted prior to implement phytostabilization with biochar (or other amendments) in the field. Preventing soil acidification in the rhizosphere to avoid metal re-mobilization could also be achieved, notably by increasing the acid buffering capacity (e.g. by liming), supplying N as NO_3 and selecting less acidifying plants. Among the two tested plants, preference should be given to *Lupinus albus* over *Agrostis tenuis* as the first species displayed lower root-induced acidification. Additional results of this study also suggest that *Lupinus albus* could be more suitable than *Agrostis tenuis* for phytostabilization as it showed a lower root-to-shoot metal translocation,

lower metal concentrations in both roots and shoots, and higher biomass production as well as higher water uptake after biochar application.

Chapter 8

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.)*

Where are we?



With the aim to explore potential synergies between phytostabilization and climate change mitigation, Chapter 8 investigates the effects of biochar application on the Cd, Zn and Pb uptake and growth of a bioenergy crop (rapeseed, *Brassica napus*) on a metal contaminated soil. The effects of biochar addition on metal bioavailability and biomass production are compared to those induced by the application of $CaCO_3$ because (i) $CaCO_3$ was the best metal immobilizing amendment in the Chapter 5 and (ii) Chapter 6 and 7 show that the liming effect

* Adapted from Houben D., Evrard L. and Sonnet Ph. 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.). Submitted to *Biomass & Bioenergy*.

subsequent to biochar application is the main mechanism responsible of metal immobilization in biochar-amended soils.

1. Introduction

In recent years, biomass has been recognized as a major renewable and carbon-neutral energy source to replace declining fossil fuel resources (Muradov and Veziroğlu, 2008). In many countries, energy and climate policies include mandatory targets for the production of bioenergy, such as biofuel. For instance, the binding target for the use of biofuel in transport is 10% by 2020 for all Member States in the European Union (European Commission, 2009). However, the use of biofuel as an alternative to fossil fuel is currently restricted by the ability of the natural environment to provide both energy and food crops for an ever larger and energy-demanding population (Connor and Minguez, 2006). The need to feed 9 billion people by 2050 while conserving natural resources rules out the use of valuable arable land for energy production. Solutions should therefore be proposed for using disused or neglected plots containing degraded, polluted or reclaimed soils (Schröder et al., 2008).

Since the onset of the Industrial Revolution, anthropogenic activities such as metal mining, smelting and refining, have contaminated soils with heavy metals in many places throughout the world (Nriagu and Pacyna, 1988). Remediation of these hazardous soils by conventional practices, including excavation and landfilling, is unfeasible on large scale because these techniques are cost-prohibitive and environmentally disruptive. In contrast, phytoremediation, the use of vegetation for *in situ* restoration of contaminated soils, is generally considered a cost-effective and environmentally friendly approach (Arthur et al., 2005). However, it is increasingly recognized that the success of phytoremediation depends on its capacity to produce valuable biomass (Conesa et al., 2012). Since metal contaminated soils represent a significant but hitherto neglected component of the global soil resource (Dickinson et al., 2009), developing biofuel crops on these soils could provide some relief in the "fuel versus

food" dilemma (Licht and Isebrands, 2005; Van Ginneken et al., 2007). However, cultivating metal-contaminated soils is complicated because, in most instances, several growth-limiting factors for the plants, such as a high phytoxicity of the pollutants and poor fertility conditions, are acting simultaneously (Tordoff et al., 2000). Hence, soil amendments are usually necessary to render the substrate suitable for the plant establishment (Brown et al., 2003; Houben et al., 2012).

Biochar is the result of biomass pyrolysis under minimal oxygen supply (Lehmann and Joseph, 2009a). Amending the soil with biochar has increasingly attracted widespread attention for two reasons. First, because of its chemical stability, biochar is ideally suited for sequestrating C in soil (Lehmann, 2007b; Woolf et al., 2010). Second, biochar is often regarded as a soil conditioner because its application rapidly increases the soil fertility and plant growth by supplying and retaining nutrients while simultaneously improving the physical and biological properties of the soil (Glaser et al., 2002; Laird et al., 2010; Uzoma et al., 2011). In addition, several results show that the addition of biochar in soil might help reduce the phytoavailability of heavy metals (Karami et al., 2011; Park et al., 2011a). For these reasons, the application of biochar has recently been suggested as a sustainable means to promote the revegetation and the restoration of degraded lands (Beesley et al., 2011; Fellet et al., 2011).

The application of biochar to metal-contaminated soils could thus serve three purposes: to immobilize heavy metals, to improve the soil conditions thereby allowing energy biomass and biofuel production, and to sequester C by burying part of the produced biomass.

The objective of this study was to assess to what extent the application of different concentrations of biochar to metal-contaminated soils affected the bioavailability of Cd, Zn and Pb and the biomass production of rapeseed (*Brassica napus* L.). We selected rapeseed as the study plant because this species belonging to the Brassicacea family has received much attention due to its fast growth, elevated fully-harvestable biomass production and high energy potential while being tolerant to high metal concentrations (Vamerali et al., 2010; Brunetti et al., 2011; Romih et al., 2011; Witters et al., 2012b). Because the increase of soil pH subsequent to biochar application has been reported to be an important mechanism involved in

the metal immobilization (Beesley and Marmiroli, 2011), we compared the biochar treatments with a classical alkalinizing treatment (liming using $CaCO_3$). $CaCO_3$ also turned out to be the best metal immobilizing amendment in chapter 4.

2. Materials and methods

2.1. Study site

The study site is located at Sclaigneaux (Namur province, Belgium). Although this 55 ha site is now a natural reserve accessible to the public, from the 1850's to the 1970's it was subjected to intense Cd-, Zn-, and Pb-bearing atmospheric fallout originating from adjacent zinc and lead smelters. A total mass of 250 kg of surface soil (0-14 cm) was obtained by composite sampling of a 20 × 20 m^2 area that was colonized by metal-tolerant plant species (*Rumex acetosa* L., *Festuca nigrescens* Lam. and *Agrostis capillaris* L.). The soil was then air-dried for two weeks, crushed and sieved to a particle size of < 2 mm in diameter. Particle size analysis using the pipette method revealed that the soil was a sandy loam (USDA classification) with 64% sand, 24% silt, and 12% clay. The pH and elemental composition of the studied soil are listed in Table 8.1.

Table 8.1 pH and elemental composition of the soil and the biochar.

		Soil	Biochar
pH		6.57	10.24
Organic C	g kg^{-1}	190	535
N	g kg^{-1}	4.2	3.1
Ca	mg kg^{-1}	2380	10,500
K	mg kg^{-1}	4270	12,200
Mg	mg kg^{-1}	980	3950
Na	mg kg^{-1}	1450	1370
P	mg kg^{-1}	530	2910
Cd	mg kg^{-1}	24.0	0.1
Zn	mg kg^{-1}	2980	116
Pb	mg kg^{-1}	3110	n.d.[a]

[a]n.d. : not detected

2.2. Soil amendments

Commercial grade biochar was obtained from Pyreg GmbH (Dörth, Germany) who uses an industrial pyrolysis reactor and miscanthus (*Miscanthus* × *giganteus*) straw as feedstock. Operating conditions include a residence time in the reactor of 30 minutes and an end temperature of pyrolysis of 600°C. In our experiments, the biochar was used as supplied, without prior washing to remove soluble salts. Elemental composition of the biochar (Table 8.1) indicates that its heavy metal content was very low compared to that of the soil. Preliminary characterizations indicated that biochar CEC was 29.47 $cmol_c\ kg^{-1}$ and exchangeable Ca, K, Mg and Na concentrations were 8.45 $cmol_c\ kg^{-1}$, 31.22 $cmol_c\ kg^{-1}$, 3.20 $cmol_c\ kg^{-1}$ and 2.17 $cmol_c\ kg^{-1}$ respectively. The lime used for the comparison analyses consisted of calcium carbonate ($CaCO_3$) and was of *pro analysi* grade (Merck).

2.3. Substrate preparation

Untreated soil (control), soil treated by liming (lime) and soil treated by three concentrations of biochar amendment were used for this experiment. Biochar treatments were prepared by mixing the dry soil with 1% (biochar-1%), 5% (biochar-5%) and 10% (biochar-10%) of biochar (w/w). Similar to Paulose et al. (2007), liming treatment was prepared by mixing the soil with 5% of $CaCO_3$. Amended soils were thoroughly homogenized in large plastic containers and individually prepared immediately prior to use.

2.4. Greenhouse pot experiment

Plastic plant pots (16-cm diameter, 22-cm height) were filled with a mixture of 1800 g of soil, 600 g of washed sand (to prevent soil compaction) and 12 g of fertilizer (Osmocote® slow-release fertilizer, N:P:K 14:14:14). Before sowing, the pots were placed in a climate-controlled dark room and the mixtures were equilibrated during four weeks at field capacity. Each treatment had five replicates. After the equilibration period, the pots were transferred to a glass greenhouse and were arranged according to a randomized design. In each pot, 20 sterilized (10 min in H_2O_2 6%) seeds of

rapessed (*Brassica napus* L.) were sown (Appendix G) and the surface of the pot was covered by a thin layer (1 - 2 mm) of quartz grains; these prevented the surface from drying-out, prevented soil destructuration by drop impact and ensured watering flow homogeneity. The trials were conducted under controlled greenhouse conditions (temperature 18-25°C, 16-hour photoperiod) with daily watering. Four weeks after sowing, excess germinated seedlings were removed (first harvest) so that only 3 uniform plants per pot were allowed to grow for the following eight weeks. At the end of the experiment (i.e., 12 weeks after sowing), the shoots of the surviving plants were harvested (second harvest). The total duration of the rapeseed cultivation was similar to that used in the study of Brunetti et al. (2011).

When harvesting, the rapeseed shoots were cut at 1 cm above the soil using ceramic scissors. Plants were weighed for fresh biomass determination, then dried (60°C; 72 h) and re-weighed for dry biomass determination. Shoot water content (SWC) was calculated by subtraction. The nutrient (Ca, K, Mg and P) and heavy metal (Cd, Zn, and Pb) contents of the plant were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Jarrell Ash) after grinding and digesting the dried biomass in a tri-acid mixture ($HClO_4$, HNO_3 and HF) (Lambrechts et al., 2011a). The bioconcentration factor (BCF) was calculated as the ratio between the heavy metal concentration in the plant shoot and the total heavy metal concentration in the soil (McGrath and Zhao, 2003).

2.5. Substrate characterization

No substrate characterization was carried out on the soil at the end of the pot experiments as several parameters, such as available nutrient and metal contents, had been modified by both the plant growth and the Osmocote addition. Instead, 200 g of each treatment were placed in 1000-ml plastic pots and left at field capacity and room temperature to react in the dark. The trials were conducted in four replicates for 56 days. The duration of this equilibration period was specifically chosen to correspond to the same time period separating the introduction of the substrates in the plant pots and the first rapeseed harvest. After 56 days, the substrates

were air-dried and the cation exchange capacity (CEC) and the exchangeable (plant-available) nutrients (Thomas, 1982) were determined. The available P concentration was measured using Mehlich 3 extraction (Mehlich, 1984). Soil 0.01 M $CaCl_2$-extractable concentrations of Cd, Zn and Pb were determined according to Houba et al. (2000). The pH (pH-$CaCl_2$) was measured in the 0.01 M $CaCl_2$ extract.

2.6. Statistical analyses

Statistical analyses to compare the average results of the different treatments were performed using a one-way analysis of variance (ANOVA) followed by Fisher's test ($p<0.05$) for multiple comparisons. Prior to ANOVA, homogeneity of variances was tested using Levene's test and logarithmic transformation was applied to dependent variables when necessary. All statistical analyses were carried out using XLSTAT (Addinsoft, ver. 2010.5.08).

3. Results and Discussion

3.1. Soil characteristics

By the end of the incubation period, the addition of 5% and 10% of biochar slightly but significantly increased the soil CEC (Table 8.2). This increase of soil CEC in the presence of biochar is in agreement with earlier findings (Liang et al., 2006; Van Zwieten et al., 2010; Uzoma et al., 2011). According to Cheng et al. (2008), the soil CEC enhancement after biochar application should become much more important with time due to the continuous oxidation of the biochar surfaces and the adsorption of organic acids by the biochar.

Significant increases in exchangeable cations and Mehlich 3 extractable P were observed and correlated positively with the amount of biochar added (Table 8.2). Potassium was the nutrient which increased the most; its exchangeable content was multiplied by 2.8, 8.8 and 15.8 after the application of 1%, 5% and 10% of biochar respectively compared to the control. Similar to Laird et al. (2010), we attributed the increase in available

nutrient content with increasing levels of biochar to the presence of these nutrients in the biochar itself and especially in the ash it inevitably contains. According to Glaser et al. (2002), ash in biochar rapidly releases free bases such as K, Ca, and Mg ions into the soil solution thereby increasing the pH value of the soil and providing readily available nutrients for plant growth. Interestingly, the exchangeable K content in biochar was 12,210 mg kg^{-1} (31.22 cmolc kg^{-1}; section 2.2) which corresponds exactly to its total K content (12,230 mg kg^{-1}; Table 8.1). This suggests that virtually all the K in the biochar was potentially available for plant growth after its application to the soil.

The soil pH was clearly modified by the amendments. Table 8.2 shows that the pH (pH-$CaCl_2$) of the soil significantly increased with the concentration of biochar amendment, starting from 5.66 in the control to 6.95 in the biochar-10% treatment. A similar trend was observed by Fellet et al. (2011) using the same concentrations of biochar application. The increase in soil pH after the application of biochar may be attributed to the alkaline nature of biochar (Table 8.1). As previously suggested (Chan and Xu, 2009), biochar can thus act as a liming treatment.

Table 8.2 pH-$CaCl_2$, cation exchange capacity (CEC) and plant-available nutrient contents in the five substrates (exchangeable Ca, K, Mg and Na and Mehlich 3 extractable P). The means (n = 4) with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

		Control	Biochar-1%	Biochar-5%	Biochar-10%	Lime
pH-$CaCl_2$		5.66a	5.80b	6.24c	6.95d	7.83e
CEC	$cmol_c\ kg^{-1}$	7.38a	7.27a	7.92b	8.22b	7.02a
Ca	$cmol_c\ kg^{-1}$	3.65a	3.77ab	3.99b	4.36c	31.43d
K	$cmol_c\ kg^{-1}$	0.12a	0.34b	1.06c	1.89d	0.10a
Mg	$cmol_c\ kg^{-1}$	0.80a	0.84ab	0.94c	1.08d	0.90bc
Na	$cmol_c\ kg^{-1}$	0.01a	0.03a	0.07b	0.10c	0.01a
P	$mg\ kg^{-1}$	20.8b	22.1b	27.5c	45.2d	16.9a

3.2. Extractable (0.01 M $CaCl_2$) heavy metal concentration

Although current legislative frameworks for soil pollution are mainly based on total metal content, it is largely recognized that environmental risks inherent to the presence of heavy metals in soils are mainly dependent on their bioavailable concentrations (Pueyo et al., 2004). These can be

assessed by 0.01 M $CaCl_2$ extraction, which is widely reported as being a proxy for bioavailability of metals in soils to plants (Kabata-Pendias, 2004; Pueyo et al., 2004; Lambrechts et al., 2011a).

Table 8.3 0.01 M $CaCl_2$ Cd, Zn and Pb extractable concentrations in the five substrates. The means (n = 4) with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

		Control	Biochar-1%	Biochar-5%	Biochar-10%	Lime
Cd	mg kg^{-1}	5.27a	4.29b	2.71c	1.15d	0.21e
Zn	mg kg^{-1}	189a	148b	74.3c	15.8d	1.23e
Pb	mg kg^{-1}	3.57a	2.46b	0.83c	0.21d	0.10e

The $CaCl_2$-extractibility of Cd, Zn and Pb significantly decreased after the incorporation of biochar (Table 8.3). Reduction of the metal extractability increased with the concentration of biochar application. Compared to the control, incorporation of 1% biochar reduced $CaCl_2$-extractable Cd, Zn and Pb concentrations by 19%, 22% and 31%, respectively. In the presence of 5% biochar, the reduction reached 49%, 61% and 77%, while in the presence of 10% biochar, it reached 78%, 92% and 94% for Cd, Zn and Pb, respectively. Since pH is the most important parameter controlling the $CaCl_2$-extractability of metals in soils (Rieuwerts et al., 2006), the decrease in extractable metal concentrations can be attributed in a part to a significant increase in soil pH due to the addition of biochar (Table 8.2; Fig. 8.1). Similarly, other studies have previously suggested that raising the pH could be one mechanism by which metal mobility was reduced by biochar (Uchimiya et al., 2010; Beesley and Marmiroli, 2011). The very high reduction of metal $CaCl_2$-extractability after liming (96%, 99% and 97% for Cd, Zn and Pb, respectively) confirms that metal immobilization in the study soil was highly sensitive to pH elevation. According to the literature, the main responsible mechanisms for metal immobilization at elevated pH are the heavy metal precipitation in the form of oxides, hydroxides, carbonates and phosphates (Lindsay, 1979) and the reduction of heavy metal solubility (Cavallaro and McBride, 1980; McBride et al., 1997). In our experiment, the increase in soil pH probably further enhanced the adsorption of metals because it increased the negative charge not only on the soil components (Naidu et al., 1994; Bolan et al., 2003) but also on the biochar particles because biochar predominantly possesses pH-dependent charges (Chan

and Xu, 2009). It is likely that the slight CEC increase after biochar application (Table 8.2) also contributed to the metal immobilization because the $CaCl_2$-extractability of metals is known to be negatively correlated with CEC (Wang et al., 2004). Such a CEC effect should intensify with time because, as described above, the CEC in soils amended with biochar increases with time, thereby offering new sites for metal sorption.

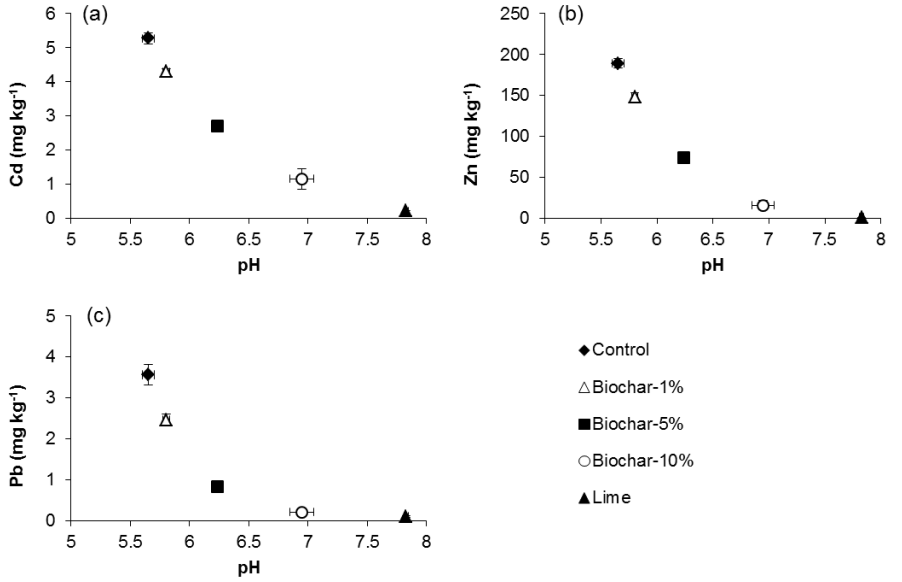


Fig. 8.1 Relationships between pH (pH-$CaCl_2$) and 0.01 M extractable concentrations of Cd (a), Zn (b), and Pb (c). Values are averages (n = 4) ± standard deviations.

3.3. Plant growth and metal uptake

Three weeks after sowing, visible signs of metal toxicity in the above ground parts of rapeseed (leaf chlorosis, dessication and growth retardation) were already marked for plants that grew on the untreated and the 1%-biochar amended soils (Fig. 8.2). Such symptoms are usual for rapeseed plants submitted to metal stress and their metabolic origins have been addressed by several studies (Baryla et al., 2001; Ben Ghnaya et al., 2009; Ashraf et al., 2011). The very low SWC as well as the limited biomass production measured in these plants at the first harvest (Table 8.4) are also symptoms commonly observed in metal-stressed plants (Shah et al., 2010). Moreover,

plants remaining in the pots after the first harvest were not able to grow any longer on the control and 1% biochar-amended soil. The mortality of rapeseed seedlings due to metal toxicity is consistent with other studies (Bernhard et al., 2005; Padmavathiamma and Li, 2009).

Fig. 8.2 Representative image showing the differences in rapeseed (*Brassica napus* L.) growth between soil and treatments three weeks after sowing, from left to right: untreated soil (control), biochar-1%, biochar-5% biochar-10% and lime treatments. Only one of the five replicates is shown for each treatment.

Table 8.4 Biomass, shoot water content (SWC) and Ca, K, Mg and P concentrations in rapeseed (*Brassica napus* L.) shoots at the first and the second harvests (4 and 12 weeks after sowing, respectively). The means (n = 5) with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test.

	Treatment	Biomass g DW plant^{-1}	SWC %	Ca mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	P mg kg^{-1}
1st harvest	Control	0.01a	32.0a	26,120b	33,630a	7521b	2545a
	Biochar-1%	0.02a	35.6a	29,520c	49,610b	9031c	2899a
	Biochar-5%	0.02a	84.1b	28,670bc	81,780d	9103c	3529b
	Biochar-10%	0.05b	90.3b	19,830a	89,260e	7067b	3754b
	Lime	0.05b	89.3b	33,420d	66,790c	4576a	3821b
2nd harvest	Biochar-5%	0.42a	91.6a	27,970a	54,030b	6943b	3224a
	Biochar-10%	4.08c	88.1a	25,140a	46,510b	4801a	3737ab
	Lime	1.31b	90.0a	26,910a	43,130a	4329a	4331b

The relationships between Cd, Zn and Pb concentrations in shoots from the first harvest and their respective $CaCl_2$-extractable contents in soil are depicted in Fig. 8.3. According to Fig. 1.13, the curve for Pb suggests that rapeseed behaved as an accumulator with respect to Pb (Fig. 8.3c) (Baker, 1981). Conversely, the curves for both Cd and Zn indicate that rapeseed tolerated Cd and Zn through an exclusion mechanism (Fig. 8.3a, b). According to Baker (1981), the *Brassica* species behaves as metal excluders, i.e., they tend to maintain constant metal shoot concentrations unless the soil metal concentration is above a critical value. Above this threshold, the tolerance mechanisms break down and unrestricted metal transport takes place which results in metal accumulation possibly up to lethal levels. Hernández-Allica et al. (2008) found that concentrations of 92 mg Cd kg^{-1},

10,916 mg Zn kg^{-1} and 328 mg Pb kg^{-1} in rapeseed shoots caused the inhibition of the shoot growth by about 100%. In our study, the shoot concentrations of Cd and Zn in rapeseed grown on both the control and the biochar-1% treatment were very close to their respective threshold values reported by Hernández-Allica et al. (2008) while Pb was much less concentrated (Fig. 8.4). Therefore, data suggest that in this study both Cd and Zn are toxic elements that were predominantly responsible for the death of the plants. However, these threshold values were assessed using mono-metallic and hydroponic cultures. Here, the simultaneous presence of elevated Cd, Zn and Pb concentrations in the soil most likely reinforced the soil toxicity since these metals may have strong synergetic effects on the growth of rapeseed (Angelova et al., 2008).

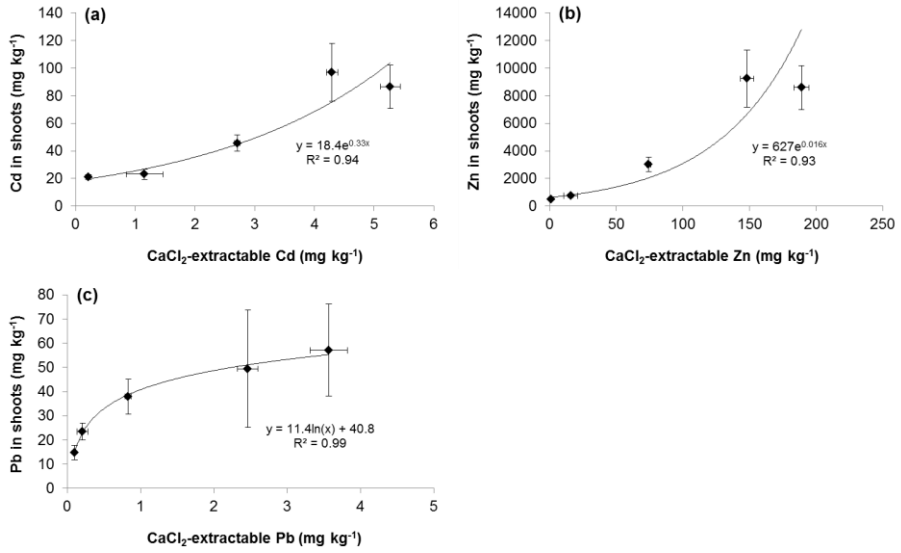


Fig. 8.3 Uptake response of rapeseed (*Brassica napus* L.) to 0.01 M $CaCl_2$ extractable Cd (a), Zn (b), and Pb (c) content in soil. Values are averages (n = 5 for metal in shoots, n = 4 for $CaCl_2$-extractable metal) ± standard deviations.

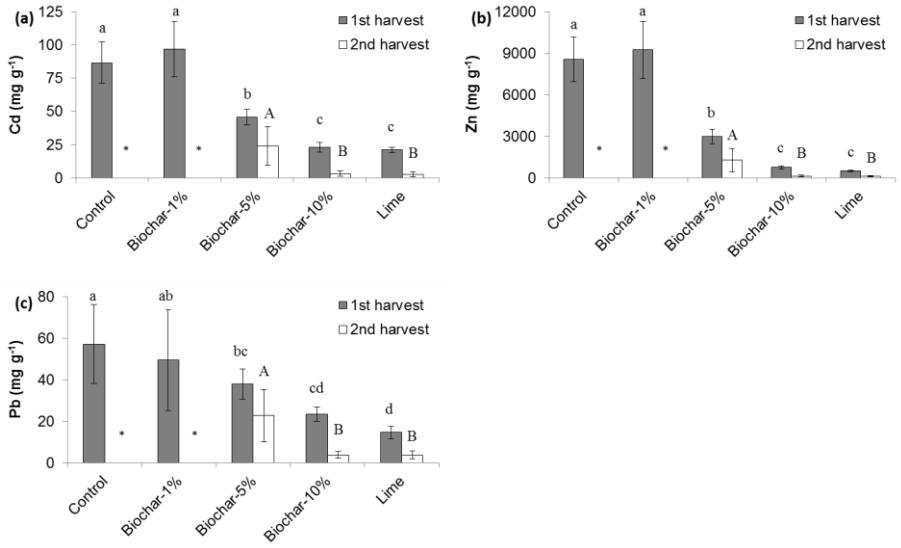


Fig. 8.4 Cd (a), Zn (b) and Pb (c) concentrations in rapeseed (*Brassica napus* L.) shoots at the first and the second harvests (4 and 12 weeks after sowing, respectively). Values are average (n = 5) ± standard deviation. Columns with the same letter do not differ significantly at the 5% level according to the Fisher's multiple comparison test. *No data are available for plants on the control and the biochar-1% treated soils at the second harvest because all plants had died.

The reduced metal bioavailability after liming and biochar-5% and -10% applications (Table 8.3) resulted in a significant decrease in metal concentration in shoots from the first harvest. As depicted in Fig. 8.4, the application of 5% and 10% of biochar lowered the metal shoot concentrations at the first harvest by 47% and 75%, respectively for Cd, 65% and 91% for Zn, and 33% and 59% for Pb. Compared to the control, the addition of lime had a similar impact to that of the biochar-10% treatment (Fig. 8.4). By alleviating the metal phytoavailability, the biochar-5% and -10% and the lime application enabled the plants to survive and grow without presenting any toxicity symptoms during the entire cultivation period (except a slight yellowing in shoots from the biochar-5% treatment). Moreover, the increase in biomass throughout the experiment (Table 8.4) was globally accompanied with a decrease in heavy metal concentrations in the shoots (Fig. 8.4).

At the end of the experiment, although both the liming and biochar-10% treatments were equally effective in reducing Cd, Zn and Pb concentrations in the shoots (Fig. 8.4), our results showed a significant increase in the

rapeseed biomass production when biochar was applied (Table 8.4). The biomass of plants harvested on the biochar-10% treatment was 9.7 and 3.1 times higher than that of plants grown on the biochar-5% and lime treatments, respectively. Although all the soil treatments received N:P:K fertilizer (Osmocote), the nutrients supplied by biochar and the improved soil conditions such as pH and CEC (Table 8.2) may have contributed to the high biomass production induced by this treatment. It can thus be inferred that higher biomass production in the presence of 10% biochar was not only due to the alleviation of metal phytotoxicity but also to the enhancement of soil fertility. This is consistent with other studies (Hossain et al., 2010; Uzoma et al., 2011) that reported higher plant productivity when biochar was applied and attributed this enhancement to the increase in soil available nutrients. The marked increase in K concentration in the shoots from the first harvest with increasing levels of biochar (Table 8.4) reflects the great improvement in soil K availability after biochar application (Table 8.2). Such a relationship was nevertheless less evident for the other nutrients. At the second harvest, the absence of any significant increase in nutrient concentrations in shoots in the presence of 10% biochar was probably due to a dilution effect caused by the much higher rapeseed biomass (Table 8.4).

3.4. Bioconcentration factor (BCF) and impact on phytoremediation strategies

For rapeseed grown on metal contaminated soils, Fornes et al. (2009), Brunetti et al. (2011) and Romih et al. (2011) reported BCF values ranging from 0.05 to 1.01 for Cd, 0.05 to 1.17 for Zn and 0.002 to 0.05 for Pb. Compared to these values, rapeseed grown on the control and 1% biochar-amended soils exhibited largely higher BCFs for Cd and Zn (Table 8.5), indicating that the uptake of Cd and Zn by the plants was unrestricted. On the contrary, in the biochar-5%, biochar-10% and lime treatments, metal uptake was restricted, even during the first harvest. The BCFs for Cd, Zn and Pb were in the range of, or lower than, the above-reported values, except for the BCF measured for Cd in plants grown on the biochar-5% treated soil (Table 8.5). According to McGrath and Zhao (2003), phytoextraction is not

feasible when plants have a BCF value lower than 1, regardless of how large the achievable biomass is. Our study shows that combining biochar soil incorporation with rapeseed cultivation for phytoextraction purposes would not be realistic since plants with a BCF value higher than 1 did not survive while the surviving plants exhibited BCF values lower than 1 (Table 8.5). Phytoextracting heavy metals to reach acceptable metal levels in the soil would consequently require an excessively long amount of time. However, the large decrease in heavy metal bioavailability and shoot concentration we observed as well as the increase in valuable biomass production make the combination of biochar incorporation into the soil and rapeseed cultivation both environmentally and economically suitable as a phytostabilization strategy (Conesa et al., 2012). Compared to the liming treatment, the application of biochar-10% should also be more efficient in reducing metal leaching since the much increased biomass production would most likely improve evapotranspiration, thereby reducing water percolation throughout the soil profile.

Table 8.5 Bioconcentration factor (BCF = shoot metal concentration/soil total metal concentration) of Cd, Zn and Pb in rapeseed (*Brassica napus* L.) at the first and the second harvests (4 and 12 weeks after sowing, respectively).

	Treatment	Cd	Zn	Pb
1st harvest	Control	3.61	2.88	0.018
	Biochar-1%	4.04	3.10	0.016
	Biochar-5%	1.90	1.01	0.012
	Biochar-10%	0.96	0.25	0.008
	Lime	0.88	0.17	0.005
2nd harvest	Biochar-5%	1.00	0.43	0.007
	Biochar-10%	0.14	0.05	0.001
	Lime	0.11	0.04	0.001

3.5. Practical implications

Recently, Witters et al. (2012a,b) provided evidence that phytoremediation, when used to produce bioenergy crops including rapeseed, could positively abate atmospheric CO_2 while being economically efficient. Energy can be obtained from such crops using low-temperature pyrolysis (Keller et al.,

2005; Laird et al., 2009). This technique generates biochar which can be incorporated into the soil to reduce greenhouse gas emissions (Gaunt and Lehmann, 2008; Lee et al., 2010). Investigating the potential use of ten by-products from different bioenergy chains as soil amendments, Cayuela et al. (2010) concluded that biochar was the ideal by-product for mitigating climate change because it contains highly stable C and does not promote N_2O formation and emission. Pyrolysing biomass harvested on metal-contaminated soils for production of both bioenergy (e.g. biofuels) and biochar followed by biochar incorporation into the soils could be therefore a suitable option for contributing to climate change mitigation. This would be feasible provided that feedstock biomass does not contain excessive metal levels because pyrolysis of metal-rich plants can lead to the production of metal contaminated by-products (Sas-Nowosielska et al., 2004). Studies on pyrolysis have found that rapeseed was one of the most interesting sources of biomass for this purpose (Onay and Mete Koçkar, 2004; Sánchez et al., 2009). In our study, rapeseed grown on biochar-10% treatment presented Zn and Pb concentrations of 147 mg kg^{-1} and 3.80 mg kg^{-1} respectively, which is within or below the "normal" range of metal concentration commonly found in shoots of various plant species (25 – 150 mg kg^{-1} and 5-10 mg kg^{-1} respectively; Kabata-Pendias and Mukherjee, 2007) while Cd concentration (3.29 mg kg^{-1}) was above the "normal" range of Cd concentration (0.01 – 0.2 mg kg^{-1}) but still lower than the excessive or toxic range of concentrations (5 – 30 mg kg^{-1} ; Kabata-Pendias and Mukherjee, 2007). These data suggest that the pyrolysis of rapeseed grown in the presence of 10% biochar will not yield new biochar containing excessive metal levels. As outlined by Kwapinski et al. (2010), clear guidelines for biochar production from various feedstock are nevertheless essential to ensure that biochar compositions meet acceptable standards.

An additional advantage of biochar incorporation is the improvement of soil fertility parameters such as nutrient contents, pH and CEC (Table 8.2) and water retention (Fellet et al., 2011) thereby lessening the need for fertilizer and irrigating water. Moreover, the agronomic benefits of biochar last longer than those offered by any other form of organic matter (e.g. manure or compost) commonly applied to land due to its greater efficiency in

retaining nutrients and keeping them available as well as its favorably long persistence in soil (Sánchez et al., 2009).

4. Conclusion

In this study, we demonstrated that the incorporation of biochar into metal-contaminated soils decreased the availability of Cd, Zn and Pb and improved the production of rapeseed (*Brassica napus* L.). This has implications for the use of contaminated soils for growing bioenergy crops combined with simultaneously sequestering C in the soil. Compared to liming, the application of 10% biochar presented similar efficacy in decreasing the heavy metal concentration in rapeseed shoots but tripled biomass production. Since the biomass that was harvested in the presence of 10% biochar did not present excessive metal concentrations, it can be pyrolyzed to provide both bioenergy (e.g. biofuels) and new non-polluting biochar. Our study shows that contaminated land that is unsuitable for growing food crops can be used in a scheme that combines soil reclamation through phytoremediation, biochar utilization and bioenergy production. Further studies including life cycle assessment and evaluation of external costs are needed to quantify the societal, economic and environmental pros and cons associated with this combination strategy.

Chapter 9

General discussion

1. Motivations

Being at the interface between the atmosphere and the Earth's crust, as well as the substrate for natural and agricultural ecosystems, the soil is open to inputs of heavy metals from many sources (Alloway, 1990). The relatively sudden occurrence of pollutants into the recipient ecosystems since the Industrial Revolution has clearly overwhelmed their self-cleaning capacity and, as a consequence, resulted in the accumulation of pollutants (Nriagu and Pacyna, 1988; Nriagu, 1996). Because the magnitude of the problem calls for immediate action, soil pollution has recently been attracting considerable public attention and many remediation technologies have been developed to treat contaminated soils (Garbisu and Alkorta, 2003).

Cleaning-up heavy metal contaminated soils with conventional remediation techniques is a costly enterprise. For instance, the European Environment Agency has estimated the overall cost for the remediation of contaminated sites in Europe to be between €59 and €109 billion (Commission of the European Communities, 2002). In addition to be cost-prohibitive, conventional techniques are environmentally disruptive and often inappropriate for the treatment of large contaminated areas. There is thus an urgent need for alternative, cheap and efficient methods to reclaim these soils. To this end, various "gentle" remediation technologies have been developed to remediate contaminated sites (Onwubuya et al., 2009) so as to maintain the environment as a sustainable source for future generation. Among them, phytostabilization has received a growing interest during the two last decades. The aim of phytostabilization is to promote the establishment of a vegetation cover which will be effective in providing the

necessary surface stability to prevent wind-blown erosion of contaminated particles, and in reducing water pollution by intercepting a substantial proportion of incident precipitation (Vangronsveld et al., 1995; Tordoff et al., 2000). Phytostabilization is usually enhanced by the use of amendments which favor plant growth and/or immobilize heavy metals within the soil (Adriano et al., 2004; Mench et al., 2006; Bolan et al., 2011). Among soil amendments, biochar is gaining a growing interest as, beyond its potential immobilizing effect on heavy metals, its incorporation in soils could improve soil conditions to sustain plant growth while sequestering carbon on the long run.

Because they initiate the process of dispersing metals to surrounding environments, it is, however, essential to accurately assess all potential metal mobilizing mechanisms acting upon contaminated substrates. Plants, amendments and biochar, alone or in combination, have the potential to modify physico-chemical properties within the soil which, in turn, may positively or negatively affect the mobility of heavy metals. Therefore, our scientific strategy was motivated by the aim of gaining better insights about how plants, amendments and biochar may affect the mobility of heavy metals and, thereby, the success of phytostabilization.

2. Heavy metal mobility as affected by plants

As a preliminary investigation (**Chapter 2**), we evaluated the potential effect of roots on the weathering of smithsonite ($ZnCO_3$), a common Zn mineral. In a continuous flow experiment we observed that, although the root activity of the fast growing *Lolium multiflorum* plant increased the pH of the solution surrounding smithsonite particles, the total amount of Zn released from the carbonate mineral was increased by a factor of 2.25 in the presence of plants. This increase was due not only to plant uptake but also to the enhancement of Zn release into leachates. The rate of Zn release from smithsonite to leachates in planted systems was twice as high compared to that in the plant-free systems. The strong correlation between concentrations of Zn and dissolved organic C produced by the rhizosphere activity in leachates suggested that organic exudates and secretions were closely involved in smithsonite weathering. This laboratory study evidences

the ability of plants to alter metal phases in soils and the need to carefully take into account the potential higher metal release into the soil solution when phytostabilization strategy is considered for the reclamation of contaminated soils. However, the study was specific to one mineral and thus did not consider other accompanying solid phases. This simplified situation obviously does not occur in the nature. Therefore, **Chapter 3** was conducted in order to assess the potential effect of plant growth on the metal release from two heavily metal contaminated soils. Because the use of Zn isotope is increasingly suggested to trace environmental Zn sources as well as to gain better insights on Zn biogeochemical cycle, this chapter also investigated the effect of plants on Zn isotope fractionation into the leachate-soil-plant system. In this column experiment, *Agrostis tenuis*, a metal-tolerant grass species which has been previously successfully used by other authors to cover metal contaminated soils, was grown for 7 weeks on a quartz-soil mixture and leachates were collected and analyzed weekly. Consistently with Chapter 2, the presence of plants significantly increased the release of Zn as well as that of Cd and Pb from the solid phase but the magnitude depended on the soil and the metal solid speciation. However, in disagreement with Chapter 2, plant-induced acidification appeared as the main mechanism responsible of the higher metal leaching, while dissolved organic C exuded in the rhizosphere did not play a significant role. This could be due to the fact that organic compounds produced by the rhizosphere activity were rapidly degraded due to the presence of a diverse and more active soil microbial community compared to that in Chapter 2. Zinc isotope analysis revealed that the Zn plant uptake was not isotopically discriminatory with respect to the leachate. However, the Zn tolerance strategy of *A. tenuis*, which promotes the sequestration of Zn in roots, induced Zn isotope fractionation within the plants, the heavier Zn isotope being preferentially retained in the roots. More importantly, the root-induced mobilization of Zn favored the leaching of Zn which was isotopiclally heavier than that spontaneously released by the soil in the absence of plants. This evidences that *A. tenuis* root activity mobilizes Zn from another pool than that which spontaneously releases Zn upon contact with the percolating solution. Interestingly, the isotope results of this study might provide an explanation for the isotope variations observed *in situ* at a

slag heap by Couder (2011) between lysimeter solutions collected under a bare area and those collected under an area covered by *A. tenuis*.

The findings of the two first study chapters demonstrate that heavy metal mobilization from the soil may become intensified by the root activity, which, in turn, may negatively affect phytostabilization strategies. However, according to Ottenhof et al. (2007), the success of phytostabilization should also consider the modifications of soil physico-chemical properties due to the long-term presence of plants, in particular because vegetation cover contributes to the soil organic matter. Therefore, **Chapter 4** raised the question as to whether the impact of the long-term presence of plants and the subsequent changes in soil properties affect the heavy metal mobility. Comparing the surface soils of a slag heap left bare to the same soil being spontaneously long-term revegetated by either *Armeria maritima* or *Agrostis tenuis*, we showed that the soil pH in the presence of *A. maritima* and *A. tenuis* was lower by 0.6 and 0.8 units respectively, than that of the bare soil (pH 7.34). As expected, the long-term presence of plants caused an enrichment in organic matter in soils as the soil organic carbon content in the presence of *A. maritima* and *A. tenuis* was 4.0 and 2.2 times higher, respectively, than that of the bare soil (organic C content = 3.7 %). Using a leaching column experiment, it was found that the leachability (i.e., the percentage of metal exported to leachates with respect to the initial metal amount in the column) of Cd, Zn and Pb was systematically higher in the revegetated soil. This higher metal leachability can notably be explained by the release of metals from decomposing plant residues. As suggested by the strong positive correlations between the concentrations of metals and those of organic anions in leachates, the decomposition of accumulated organic matter also contributed to increase the metal leachability by releasing organic anions which complex metal ions.

3. Improving phytostabilization using amendments

The results of the three first study chapters clearly indicate that the use of plants for phytostabilizing heavy metal contaminated sites may lead to a higher metal mobility within the soil due to both root activity and organic matter accumulation. In order to optimize the beneficial effects provided by

the revegetation of contaminated soils, it is thus essential to reduce as much as possible the mobility of heavy metals. This can be achieved by the incorporation of amendments into the soil, whose purpose is of course not to alter the total metal concentration but to impair the mobility and toxicity of metals by accelerating key immobilizing process such as adsorption, precipitation, complexation and redox reactions. An additional advantage to this technique is that it may use amendments which derive from bio-products (e.g. biosolids, manure, bone meal) or from industrial by-products, thereby consisting of an enticing option to divert such materials from the waste stream and reuse them. However, in phytostabilization studies, only limited attention has been paid to the leaching of heavy metals in relation to the application of metal immobilizing amendments (Ruttens et al., 2006a). Therefore, **Chapter 5** investigated the effect of cost-effective potentially immobilizing amendments ($CaCO_3$, iron grit, fly ash, manure, bentonite and bone meal) on both the leaching and the phytoavailability of Cd, Zn and Pb in a contaminated soil. To this end, a pot experiment using *Lupinus albus*, a promising plant candidate for phytostabilization, was conducted. The leachates percolating through the pots were regularly collected and analyzed. The results of this chapter indicated that the Cd and Zn leaching was reduced by all amendments, mainly due to alkalinity increase. The Pb leaching was strongly affected by the dissolved organic carbon (DOC) release. Therefore, bone meal and manure treatments, which highly increased DOC concentrations in leachates, increased the flow-weighted mean Pb concentrations by 2.3 and 16 times, respectively. Surprisingly, while the application of iron grit induced strong Cd and Pb leaching reductions, this amendment doubled the Cd and Pb concentrations in shoots of *L. albus*. Conversely, the addition of bone meal reduced the Pb concentrations in shoots by 74%, probably because Pb was predominantly present in solution as organo-Pb complexes (as predicted using Visual MINTEQ) that are unavailable to plants. The addition of bone meal and, to a lesser extent manure, also resulted in the highest production of biomass because of the improved soil fertility. This study provides additional evidence that the use of suitable amendments in contaminated soils might be helpful to prevent risks for ecosystems due to vegetation on underlying groundwater contamination. However, the dissimilar responses of Cd, Zn

and Pb to the same amendments highlight the difficulty in finding amendments that are equally effective for every target contaminant in multi-metal contaminated soils. This work also shows that the leaching and the uptake of metals may be differently affected by the incorporation of amendments into the soil. This stresses the necessity to evaluate the potential effect of amendments both on leaching and phytoavailability for each target metal prior to implement phytostabilization in the field. Overall, the addition of $CaCO_3$ turns out to be the best compromise because this treatment was the only one to reduce both leaching and phytoavailability of the three considered metals. However, it must be kept in mind that the immobilizing effect induced by the application of $CaCO_3$ is ephemeral and, therefore, the application of $CaCO_3$ should be renewed over time. Further investigations aiming at identifying amendments which are both cost-effective and with irreversible effects should thus be carried out.

4. Biochar: a promising amendment for phytostabilization?

4.1. Exploration

With the idea of using biochar for phytostabilization purposes, **Chapter 6** consisted of a preliminary investigation devoted to gain insight into the effect of the application of three different rates of biochar (1%, 5% and 10%; w/w) onto a heavy metal contaminated soil. Bioavailability and 0.01 M $CaCl_2$ extractability over time was investigated as well as the release of Cd, Zn and Pb upon acidification. The results of this chapter showed that the 0.01 M $CaCl_2$-extractability of metals significantly decreased with increasing rate of biochar application and that this effect could be mostly attributed to the raise in soil pH. After 1 hour of incubation, the application of 10% biochar reduced the Cd, Zn and Pb extractability by 35%, 48% and 77% respectively. In the presence of 5% and 10% of biochar, the metal extractability continued to decrease over the next 56 days, likely due to aging processes including the diffusion of metal into the micropores of biochar particles. Ryegrass (*Lolium multiflorum* Lam.) was allowed to grow in a pot experiment and harvested at 28 and 56 days after sowing. At each

harvest, the metal concentration in shoot decreased with increasing rate of biochar application but the reduction was more marked at the second harvest, reflecting the decrease of metal bioavailability over time. Examining the metal release when the soil is subjected to acidification, we found that, at a defined pH value, the metal concentration in solution was not affected by the presence of biochar. However, acid neutralizing capacity (ANC) increased with increasing levels of biochar application. This indicates that the time required to reach a hazardous pH can be predicted to be longer after biochar application. From this study, it was concluded that the application of biochar for *in situ* metal immobilization could be feasible provided soil pH was monitored over time.

As demonstrated by Chapter 2 and 3, the root activity can greatly affect the success of phytostabilization by inducing metal mobilization. Consequently, the examination of biochar application for phytostabilization purposes must include the understanding of the heavy metal mobility within the rhizosphere. In **Chapter 7**, we observed that, in bulk soil, the application of 5% biochar significantly reduces the exchangeable pool of Cd, Zn and Pb mainly due to the liming effect which subsequently promotes the metal shift into the carbonate-bound pool. In the rhizosphere of both *Agrostis tenuis* and *Lupinus albus*, a net release of H^+ was observed. This release was higher in the presence than in the absence of biochar, possibly because biochar application reduced the content of nitrate while it increased that of ammonium in the soil. Because of this root-induced acidification, the positive liming effect of biochar on metal immobilization noticed in the bulk soil was counteracted in the rhizosphere of both plants. As a result, the concentrations of metals in roots and shoots of both plants were virtually not reduced by the application of biochar. In accordance with the Chapter 6, Chapter 7 indicates that the immobilization of heavy metals in soils brought about by biochar application is pH-dependent. Therefore, in order to prevent the re-mobilization of metals due to root-induced acidification, the release of H^+ in the rhizosphere should be minimized. This could be achieved, for instance, by increasing the nitrate content in the soil. In the same line of thoughts, the reduction of metal concentrations in shoots after biochar application observed in the chapter 6 could be in part due to the

fact that root-induced acidification was limited because of the presence of fertilizer (Osmocote®) in which nitrate is the predominant form of nitrogen.

4.2. Synergetic effects

With the aim to explore potential synergies between phytostabilization and climate change mitigation, the last chapter (**Chapter 8**) investigated the effects of biochar application at different concentrations (1%, 5% and 10%) on the Cd, Zn and Pb uptake and growth of rapeseed (*Brassica napus*) on a metal contaminated soil. The effects of biochar addition were compared to those induced by the application of $CaCO_3$ because (i) $CaCO_3$ was found to be the best metal immobilizing amendment in the chapter 5 and (ii) chapters 6 and 7 suggested that the liming effect subsequent to biochar application is the main mechanism responsible of metal immobilization in biochar-amended soils. Each treatment was also amended with fertilizer (Osmocote®) in which nitrate is the predominant form of nitrogen. Twelve weeks after sowing, all plants cultivated on the untreated soil and on the soil amended with 1% biochar had died because of the soil toxicity. On the contrary, the plants grew normally on the soils that had the other treatments. Compared to $CaCO_3$, treatment by 10% biochar proved equally efficient in reducing metal concentrations in shoots but the rapeseed biomass production tripled as a result of the soil fertility improvement, i.e. an increase in CEC and available nutrient content. Thus, in addition to C sequestration, Chapter 8 proves that biochar incorporation into metal contaminated soils is full of promise as it could make it possible to cultivate bioenergy crops without encroaching on agricultural lands. Because shoots of rapeseed did not present excess metal concentrations, it was also suggested that, to further contribute to the reduction of CO_2 emission, the harvested biomass can in turn be used as feedstock for pyrolysis to produce bioenergy and new biochar. Moreover, besides the beneficial effect due to the C sequestration, the threefold increase in biomass production in the presence of 10% biochar compared with the $CaCO_3$ treatment might justify the use of biochar over cheaper amendments for phytostabilization. The practical implications of this study are arguments for the justification and the advancement of phytostabilization (Dickinson et al., 2009).

5. Economic implications for phytostabilization

Raising the question as to whether phytotechnologies are still a promising tool for the remediation of metal polluted soils, Conesa et al. (2012) recently argued that more effective and commercially feasible techniques were urgently required for the justification of their implementation in the field. These authors proposed to make phytotechnologies more commercially attractive by:

> (i) clearly distinguishing processes from techniques, to improve communication and cooperation by the commercial sector;
> (ii) exploiting new economic opportunities such as the production of bioenergy, biochar, and biofortified crops;
> (iii) applying economic studies and economic evaluations as well as developing new implementation protocols.

On the basis of these recommendations, we aimed here at estimating whether the phytostabilization of soils by incorporating biochar and growing rapeseed can be seen as an economically appealing solution for the remediation of metal contaminated soils (Fig. 9.1).

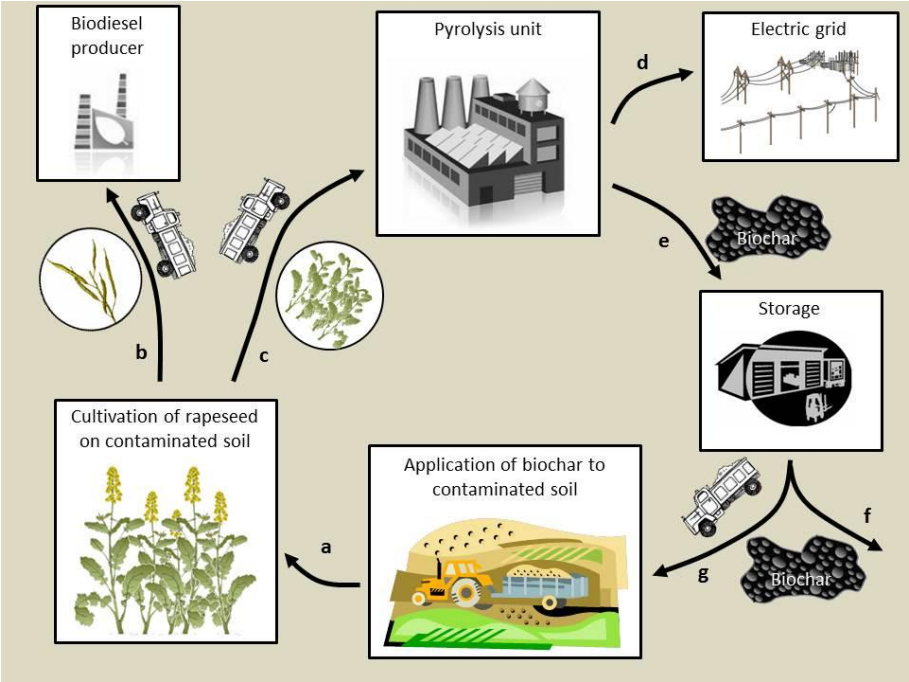


Fig. 9.1 Schematic representation of the phytostabilization of contaminated soils by applying biochar and growing rapeseed. The soil amended with biochar can serve for the production of rapeseed (a). At harvest, seeds are sold and transported to a biodiesel producer (b) and straws are sold and transported to pyrolysis unit (c). Pyrolysis unit produces electricity (d) and new biochar which is stored (e). New biochar can be exported (f) or applied on the contaminated soil (g).

5.1. Economic valuation of biochar application

The approach in evaluating costs related to the biochar application to soils was based on the figures given in the study of Shackley et al. (2011) which estimates the feasibility and costs of biochar deployment in the UK (considering an exchange rate £1 = €1.242). The authors considered three potential sizes of pyrolysis units (PUs): small-scale (~2000 oven dry ton per annum [odtpa]), medium-scale (~16,000 odtpa) and large-scale (~185,000 odpta). The small scale unit could take in feedstocks from a number of farms, small woodmills and from city green waste sources. The medium-scale unit is an industrial facility that produces electric power (and potentially heat) for a single large consumer or for supply to the national grid (with heat being supplied to a district heating system). The large-scale unit is a large bioenergy power plant (but still very small compared with a 1

GW coal power plant). Since the large-scale scenario contains many uncertainties and is unlikely to be developed on the short run, only the small- and the medium scale scenarios are considered for the estimation of the costs associated to the application of biochar for soil remediation.

The biochar production was calculated by Shackley et al. (2011) over a 10-year period with 2 years start-up conditions; hence reduced output compared to years 3 – 10: 61% of final efficiency is achieved in year 1, 67% in year 2 and 100% in year 3. The resulting biochar production yield over this period is 36% which represents 727 and 5396 tons of biochar per year for small-scale and medium-scale, respectively.

The costs and revenues associated with the biochar deployment are briefly detailed below and presented in Table 9.1; the reader is invited to consult the paper of Shackley et al. (2011) for more details. The **feedstock costs** used here were those related to rapeseed straws. These feedstock costs include also the effect of biochar production on nutrient balance in soils where straws are employed as a feedstock instead of being left on the field. It is assumed that (i) 100% of straw from a field would be removed, (ii) most (90%) of the phosphorus and potassium would be retained during pyrolysis while 45% of nitrogen is retained, and (iii) the biochar produced from the straw of a given field is returned to that same field. The nutrient content in rapeseed is assumed to be 7 kg N, 2.2 kg P_2O_5 and 11.5 kg K_2O per ton, with a value of €1.40/kgN, €1.80/kg P_2O_5 and €1.24/kg K_2O (Copeland and Turley, 2008). The **capital costs** include all design, equipment, construction, civil and commissioning costs and assume that the project lifetime is over 20 years and that the discount rate is 8%. The detailed assumptions for **transportation costs** are presented in Mortimer et al. (2009). For the small-scale unit, the **storage costs** was calculated on the basis of the cost of existing farm storage which is €0.44 per ton per week (cost of grain storage) and assuming storage for an average 20-week period. For medium-scale unit, it was assumed that biochar deployment will be contracted-out and storage will occur in specially constructed units at a cost of €161 m² floor space of dedicated facilities (200 × 100 × 5m, load factor 0.5, with a capital recovery of 0.149 over 10 years), which gives an annualized cost of €19 per ton of biochar. The **natural gas costs** are the costs of the gas used to initiate the pyrolysis. For the small-scale unit, the **labor costs** were calculated on

the basis of 1 person "full-time equivalent" using standard wage rates while the other operational **plant costs** were based upon farm-based pyrolyser. Both the labor costs and the plant costs for the medium-scale unit were from the data provided by a demonstration pilot-plant (1 manager, 1 administrator and 2 workers). The costs related to the **application of biochar to field** were considered similar for the small- and the medium-scale units. They are based on the assumption that a fertilizer spreader conveys 6 tons of biochar per journey and that it takes 1 hour for loading, wetting, transport to field, application and return journey. These costs integrate the cost for the use of a tractor and a spreader as well as the labor costs. Finally, the estimation of the total costs of biochar delivered to the field must include the potential revenues provided by the generation of electricity during pyrolysis. Shackley et al. (2011) assumed that electricity is generated during pyrolysis at about 25% efficiency of conversion. The corresponding electricity generation is about 0.74 MWh per ton of biochar. Assuming that the value of 1 MWh is €62.1 (market price), the income provided by the **sale of electricity** accounts for €46 per ton of biochar. In addition, policy incentives for the renewable energy can offer additional revenue. In Belgium, producers of green electricity receive a **green certificate** for every MWh (net) produced and sell it to electricity suppliers. The guaranteed minimum price is €65/certificate in Wallonia (http://www.cwape.be, 24/11/2012). These green certificates play a great role in the final cost of biochar (Table 9.1). Obviously, their values are dependent on the national/regional renewable energy policy. Considering all these costs and revenues, the total cost of rapeseed-derived biochar delivered to the field would account for 326 – 404€ per ton (Table 9.1).

Table 9.1 Costs of biochar produced from small-scale, medium scale and large scale pyrolysis units, €/t biochar applied to field (based on Witters, 2011).

	Small-scale unit	Medium-scale unit
Capital cost	108	125
Feedstock	170	183
Transport	12	27
Storage	9	19
Natural gas	14	14
Labor	51	60
Plant costs	61	75
Application to field	6	6
Sales of electricity	-46	-46
Green certificates	-48	-48
Net cost	337	415

5.2. Economic valuation of rapeseed cultivation

In order to assess the potential economic benefit which can be obtained by the cultivation of rapeseed on contaminated soils, we used the figures found in Witters (2011). In her PhD thesis, which focuses on the economic feasibility of phytoremediation in Flanders, Witters (2011) estimated the Adapted Gross Income (AGI) provided by such a strategy. The AGI is calculated as:

Adpated Gross Income (AGI)
= revenue (marketable products (main and rest) + animals + other + subsidies) – cost (third party labor + equipment + herbicides and pesticides + fertilizer + seed and planting material + animal related + crop related + fuel).

Table 9.2 gives an overview of agricultural costs and revenues for rapeseed when the seeds are sold to a biodiesel producer (instead of being pressed by the farmers themselves for using the oil for personal car or tractor use). On the basis of the average yield observed for 20 farmers in the Campine region, Witters (2011) considered a theoretical dry matter yield of 3 ton ha^{-1} for the seeds and 2.2 ton ha^{-1} for the green parts (straws). Planting costs include ploughing and harrowing. The author used an average market price of €208 ton^{-1} for seeds and of €50 ton^{-1} for straw. Remediating crops are eligible for single payments from the European Union (according to the

Common Agricultural Policy, CAP). For a Campine farmer, this single payment accounts for €450 per hectare. Finally, Witters (2011) considered that the distance from the closest biodiesel producer and the Campine region was about 120 km, leading to a transport cost of € 70 per ha. According to this scheme, the average gross income accounts for €548 ha^{-1} y^{-1}.

Since biochar application to contaminated soils may have beneficial effects on the rapeseed biomass production, we also calculated the AGI for a scenario in which biochar has been added to the soil prior to the sowing. According to Hammond et al. (2011), we assumed that biochar addition would result in a 10% increase in net primary productivity (the final dry matter yield would be thus 3.3 ton ha^{-1} for the seeds and 2.42 ton ha^{-1} for the straws) and a 10% decrease in fertilizer requirement. In this scenario, the final AGI would account for €639 ha^{-1} y^{-1}.

Table 9.2 Variable costs and revenues (€ ha^{-1} y^{-1}) for rapeseed cultivation (Witters, 2011).

	Without biochar	With biochar
Variable costs		
Planting		
Plant material	60	60
Planting	65	65
Maintenance		
Fertilizer	180	162
Herbicides	80	80
Fert. and herb. application	50	50
Stalk control	50	50
Harvest		
Seeds	150	150
Transport	70	77
Revenues		
Seeds	692	761
Straw	111	122
Single payment	450	450
Adapted Gross Income	548	639

5.3. Economic valuation of the combination of biochar application and rapeseed cultivation for phytostabilization purposes

Chapter 6 indicated that biochar applied at a concentration of 10% (w/w) can effectively reduce the mobility and the bioavailability of heavy metals in contaminated soils while sequestering carbon. According to this treatment and considering a soil layer of 20 cm and a bulk density of 1300 kg m^{-3}, the amount of biochar required to remediate one hectare would correspond to 260 tons. On the basis of Table 9.1, a rough estimation of the cost of the remediation of one hectare of a contaminated soil by applying 10% biochar would range between €87,620 and €107,900. This corresponds to €33.7 – €41.5 per ton of contaminated soil, which is less than conventional remediation techniques whose the cost usually ranges between €50 and €560 ton^{-1} soil (McGrath et al., 2001; Mulligan et al., 2001). Moreover, Chapter 8 demonstrated that the application of biochar to metal contaminated soils could sustain the cultivation of rapeseed without encroaching on agricultural lands. According to the Table 9.2, this strategy could create an annual income of €639 ha^{-1}, representing a commercially attractive argument for the implementation of phytostabilization when the soil remediation is mandatory. It should be noted that rapeseed can however only be grown once every three years. In the two intermediate years, Witters et al. (2012b) suggest to cultivate energy maize, a plant which has also the potential to generate appealing income. Note however that the economic value of the costs for phytoremediation using these estimations is highly dependent on subsidies (e.g. green certificate, single payment; Table 9.1 and 9.2). By maintaining or strengthening incentives, government could therefore considerably guide the remediation of soils using such technologies.

An additional argument which could convince policymakers to encourage the remediation of contaminated soils by the incorporation of biochar in combination with the cultivation of bioenergy crops is the abatement of CO_2 which can be reached by this strategy. Using life cycle assessment, Hammond et al. (2011) estimated that small- and medium-scale pyrolysis biochar units could abate 0.7 and 0.8 ton CO_2 per oven dry ton of feedstock. Considering that the biochar yield is 36%, the remediation of one hectare of

a contaminated soil by applying 10% of biochar (i.e. 260 tons) would result in an abatement of 506 – 578 ton CO_2. If the amended soil is used to produce rapeseed, an additional abatement can be obtained. According to Witters et al. (2012b), a net CO_2 avoidance of 3.2 ton CO_2 per hectare per year can be achieved when the seeds are used for biodiesel production. This CO_2 abatement is further increased when the rapeseed straws are used for biochar production and then applied to soils. Assuming a straw biomass of 2.42 ton ha^{-1} y^{-1}, this would result in an additional CO_2 abatement of 1.7 – 1.9 ton CO_2 per hectare per year.

This CO_2 abatement can be included in the economic analysis. In order to put a price on this environmental benefit, Witters et al. (2012a) suggested to use the marginal abatement cost of carbon (MAC; Appendix I) that would be required to reduce emissions to reach the global goal of stabilizing carbon concentration in the atmosphere at a level that avoids unacceptably dangerous climate change. Similar to Witters et al. (2012a), our analysis was based on the value given by the ExternE (External Costs of Energy) project of the European Commission which uses a MAC of €20 ton^{-1} CO_2. According to this figure, the external benefit of CO_2 abatement when applying 10 % of biochar to one hectare of a contaminated soil would range between €10,120 and €11,560. In addition, the CO_2 abatement provided by the cultivation of rapeseed could provide an annual external benefit of €98 - €102 per hectare. These numbers could contribute to making explicit the competitive advantage of phytostabilization compared to conventional techniques of soil remediation.

Moreover, some world areas, including Flanders and Wallonia, take the best available technology not exceeding excessive cost (BATNEEC) as a criterion for selecting remediation option. The BATNEEC tool uses a multicriteria analysis taking into account environmental, technical and financial aspects. The outcome of this BATNEEC analysis is a global score, based on information on the estimation of cost, the expected results, the expected environmental impact and the restriction for land use for a selection of soil remediation alternatives (Cappuyns, 2012). In Flanders, in view of the growing importance of a “green and sustainable” approach of soil remediation, OVAM (the Public Waste Agency of Flanders) has recommended the introduction of the CO_2-footprint, using a CO_2-calculator

developed by Tauw (a well-established European consulting firm), in the multi-criteria analysis for the BATNEEC evaluation of soil remediation (Bruneel et al., 2012). The CO_2 calculator developed by Tauw expresses the environmental impact of the different aspects of the soil remediation option (soil excavation, soil vapour extraction, chemical oxidation, etc and monitoring and aftercare) in tons of CO_2 (Cappuyns, 2012). Besides its lower cost, the CO_2 abatement achieved by phytostabilization using biochar soil incorporation and bioenergy crop cultivation is an additional parameter which could thus make this technique advantageous compared to conventional techniques when a BATNEEC approach is used for selecting the remediation option.

Conclusion and perspectives

1. General conclusion

From this thesis, several conclusions can be drawn about how plants, amendments and biochar affect the mobility of heavy metals in contaminated soils (Fig. 10.1). The presence of plants systematically increased the release of Cd, Zn and Pb from the soil to the soil solution relative to the plant-free situation. Higher mobilization of metals is due to the rhizosphere activity which acidifies the soil (Chapter 3 and 7) and releases soluble complexing organic compounds (Chapter 2). According to Zn isotope analysis, the leachates collected in the presence of plants are enriched in heavy Zn isotopes relative to those collected below plant free-soils (Chapter 3). This indicates that the Zn mobilized by roots originates from another pool than that of the Zn spontaneously released by the soil in the solution. Moreover, higher release of metals in the presence of plants is also caused by the degradation of the long-term accumulated plant residues. In fact, this process not only releases metals from decomposing plant residues but also induces the mobilization of metals from the solid phase due to the release of weathering agents, especially complexing organic anions (Chapter 4). Consequently, in order to optimize the benefits provided by the establishment of a plant cover for phytostabilization purposes, it is essential to apply metal immobilizing amendments to the soil. However, the effect of the amendment on the metal mobility must be fully understood prior to implement this strategy in the field. Indeed, while the application of some amendments such $CaCO_3$ successfully reduces both the leaching and the phytoavailability of heavy metals, some others may contrastingly affect these two pathways (Chapter 5). For instance, the application of bone meal or manure effectively reduces the metal uptake by *Lupinus albus* while it strongly increases the leaching of Pb because the amendments release considerable amounts of dissolved organic carbon. Among soil amendments that can be used for phytostabilization purposes,

biochar turns out to be a promising candidate. Its application to contaminated soils decreases the Cd, Zn and Pb mobility and bioavailability to an extent which is proportional to its rate of application (Chapter 6). Moreover, compared to classical $CaCO_3$ amendment, the application of 10% biochar triples the production of biomass by rapeseed while being equally efficient to reduce metal concentration in shoots (Chapter 8). This result is particularly meaningful because it indicates that, besides offering the potentiality of sequestering carbon, the application of biochar onto contaminated soils can ameliorate the phytostabilization of the site while providing economic and environmental incomes via the production of a valuable biomass usable for bioenergy purposes (Chapter 9). However, further studies are crucial because the mechanisms responsible for the metal immobilization after biochar application have been found to be predominantly pH-dependent, even though aging reactions may also occur (chapter 6). As a result, plant-induced acidification in the rhizosphere can counteract the metal immobilizing effect brought in by the application of biochar (chapter 7).

Raising the question whether the establishment of a plant cover on metal contaminated soils is necessarily a mitigation measure, our findings went somewhat against the grain. The requirement of using a wide range of techniques, including innovative tools (e.g. stable Zn isotopes), materials (e.g. biochar), and experimental designs (e.g. continuous flow experiment), exemplifies the difficulty in finding a satisfactory answer. From our results, it appears nevertheless that the optimization of the phytostabilization of contaminated sites requires the application of metal immobilizing soil amendments, among which biochar turns out to be full of promise.

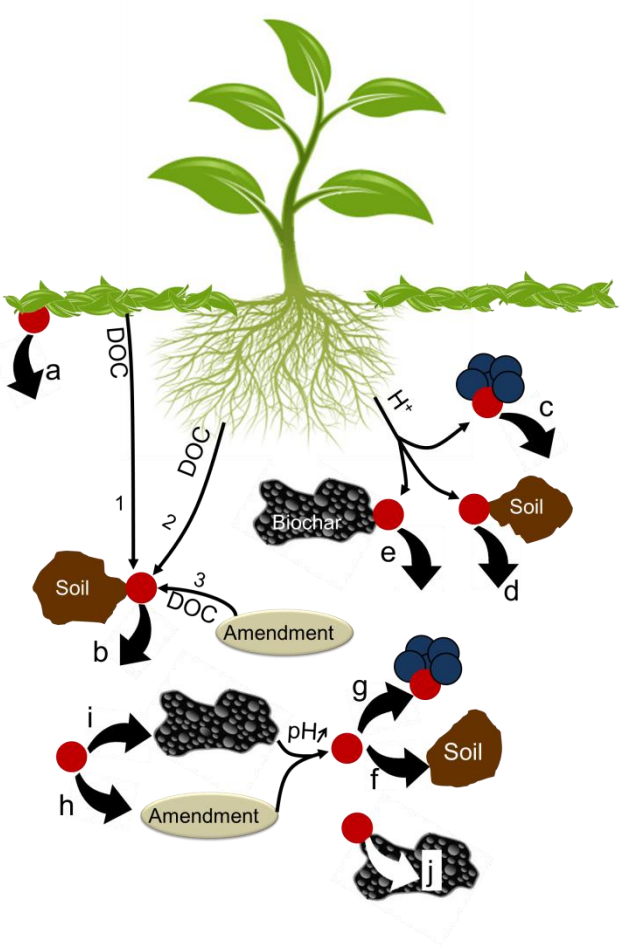


Fig. 10.1 Schematic illustration of the various mobilizing and immobilizing effects investigated in this thesis. Heavy metals can be mobilized due (a) to their release from decomposing plant residues (Chapter 4), (b) to the complexation with dissolved organic compounds originating from (1) the organic matter degradation (Chapter 4), (2) the root exudation (Chapter 2), and (3) the dissolution of organic amendments (e.g. manure, bone meal; Chapter 5), to the root-induced acidification (Chapter 3 and 7) which (c) dissolves metal precipitates and desorbs metal from the surface of (d) soil constituents and (e) biochar particles. Metal immobilization can be induced by the increase in soil pH subsequent to the application of amendments (Chapter 5) or biochar (Chapter 6, 7 and 8) which results in increasing (g) metal precipitation and (f) metal sorption onto the surface of soil constituents. The metals are also immobilized due to their sorption onto the surface of (h) amendments (Chapter 5) and (i) biochar particles themselves (Chapter 6, 7 and 8). Finally, the immobilization of heavy metals can increase with time due to aging reaction which involved (j) the metal diffusion into biochar micropores (Chapter 6).

2. Perspectives

Although the results of this work show that the presence of plants can markedly influence the mobility of heavy metals, many questions remain still open. For instance, the nature and the origin of DOC in the rhizosphere should be more fully identified. Further studies focusing on microbial activity and mycorrhizae should help understand their specific role in the success of phytostabilization. Investigations about the influence of different nutrient supplies could also provide relevant constrains about the best strategy to adopt in order to minimize the metal mobilizing effects induced by roots. Moreover, the results about the Zn isotopes, which show that plant growth can affect the Zn isotope signature of leachates, could create new perspectives for the use of Zn isotopes as tracers of the biogeochemical reactions. Analyzing the Zn isotope variation in leachates or soil solutions during the entire development of the plant could indicate at what time the plant induces the strongest Zn mobilization. Relating the Zn isotope signature of leachates with that of different soil constituents (e.g. by analyzing the isotope signature of extracts of sequential extractions) could also provide helpful information about the Zn pool which is preferentially mobilized by the rhizosphere activity.

In a more general context, the results of this work indicates that, more than the metal uptake, the release of weathering agents by plants, in particular by roots and decomposing plant residues, might play a significant role in the biogeochemical cycle of heavy metals. A deeper exploration of the role of plant in the weathering of metal phases should help understand past and predict future fluxes of metal within the Earth's critical zone. Likewise, further investigations on the effects of rhizosphere-induced weathering of metal solid phase on the isotope fractionation of metal in the liquid phase should help determine the importance of biological processes on the isotope signature of surface waters. Beyond the implications on phytostabilization, this kind of studies would be relevant at the global scale. Indeed, it is believed that, since the Devonian Period (400 to 360 million years ago), the spread of rooted vascular plants has considerably

accelerated the weathering of minerals, thereby affecting many process at the Earth's surface (Berner, 1997; Moulton et al., 2000).

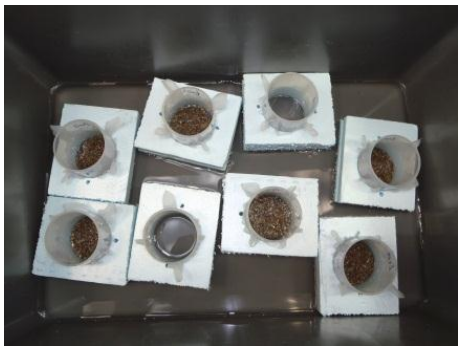
Since our results are derived from experiments carried out on a relatively short span of time, the fundamental question of their long-term persistence still remains unresolved. For example, the weathering effect of plants most likely depends on their stage of development. The immobilizing effects of some amendments may be possibly reversible with time due to, for instance, natural acidification of soil. Further researches studying the metal release upon acidification should therefore provide useful information regarding the reversibility of the immobilizing processes. On the other hand, aging reactions could increase the irreversibility of the metal immobilization. The 56-day incubation experiment (chapter 6) showed that the bioavailability of metals decreases with time in biochar-amended soils, possibly due to the diffusion and subsequent sorption of metals into biochar micropores. However, such aging process should be fully addressed because, in the same time, biochar pores may also be blocked by the substances it is adsorbing, especially organic matter, rendering inner pores inaccessible for diffusion and further adsorption. A preliminary experiment to elucidate the effect of aging processes on the metal immobilization in the presence of biochar would be to determine the metal release from amended soils subjected to wetting-drying cycles (Degryse et al., 2004), a method which artificially accelerates the aging reactions. Moreover, although our results indicate that biochar application decreases metal mobility in soils, it is unclear to what extent the metal retention is due to the creation of an additional metal binding capacity. This question could be resolved using isotope dilution technique performed at different constant pH (Lombi et al., 2003; Smolders et al., 2012). In order to identify the parameters influencing the metal binding capacity of biochar, such analyses could be extended to various biochars, differing in feedstock origin, pyrolysis conditions (e.g. temperature, residence of time) and age (e.g. freshly produced biochar *vs* char collected *in situ* in *Terra Preta*, Mollisols or disused mound kiln areas). Another promising perspective would be to identify areas containing several plots which were historically subjected to charcoal incorporation (e.g. due to charcoal production using the mound

kiln method) prior to be contaminated by metal-rich atmospheric fallouts. The comparison of the heavy metal distribution and speciation along the vertical profile in the char-containing soils with those in the adjacent non-amended soils should provide important information for the estimation of the long-term effect of biochar incorporation on the heavy metal fate. Other analyses including plants, micro-, meso- and macro-fauna of soils as well as ecotoxicity could be also coupled with this study in order to gain insights on the long-term impact of this strategy on the functioning of surrounding ecosystems.

A major challenge will be to achieve the adequate upscaling of observations, processes, and interactions to environmentally relevant scales for the management of contaminated sites. Our studies were performed only on the fine soil fraction (<2 mm) or on a specific grain size fraction, without considering the coarse farction. However, both natural and technic coarse fractions can also significantly contribute to the metal release in anthropogenic soils (El Khalil et al., 2008). Further examination on this soil fraction is therefore essential to properly evaluate the effects of plants and amendments on the mobility of heavy metals in disturbed soils and to develop models which could be applied at field scale. Investigating *in situ* mobilization of heavy metals using solution sampler (e.g. rhizon or lysimeter) placed at different depths under bare, revegetated or/and amended contaminated soils should help confirm, or not, our laboratory findings. A major point will be to identify which rate of amendment or biochar application represents the best compromise between environmental benefits and costs. In this context, promising perspectives of this work would be (i) to carry out life cycling assessment which could be used for putting forth preliminary estimates of net CO_2 equivalent abatement from biochar production and use as well as fom the use of industrial by-products as soil amendments, and (ii) to derive a provisional marginal abatement cost curve illustrating the cost-effectiveness potential of mitigation ability offered by the application of both biochar and amendments to contaminated soil.

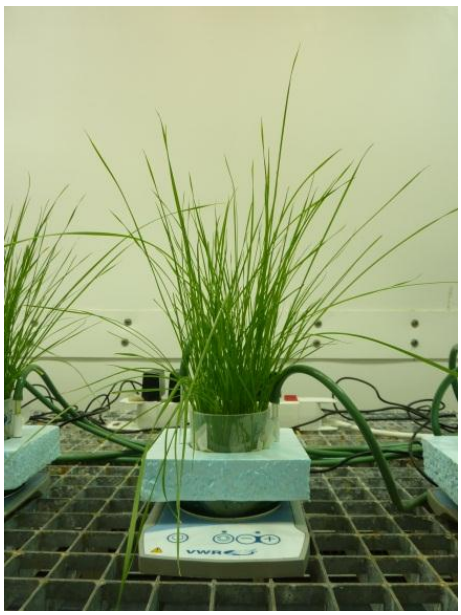
Appendix

Appendix A - Illustrations of the experimental set-up used in Chapter 2



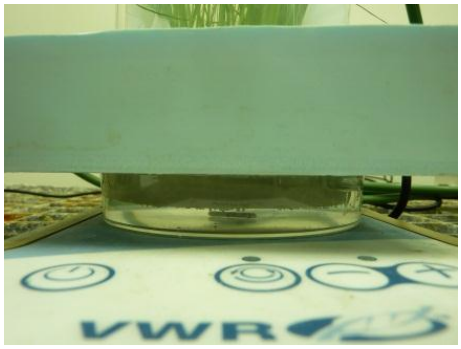
Germination

Quartz-smithsonite bag



Lolium multiflorum growth

Inlet and oulet tubing connected to the peristaltic pump



Magnetic stirrer and stir bar

Plant harvest

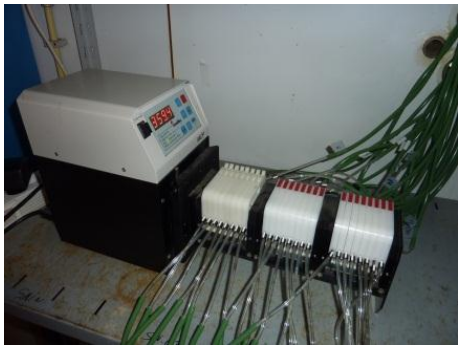
Appendix B - Illustrations of study sites and experimental set-up used in Chapter 3

Sclaigneaux site

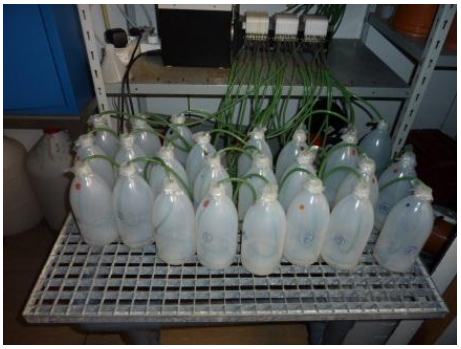
Angleur site

Agrostis tenuis growth

Polyethylene beads at the surface of the column



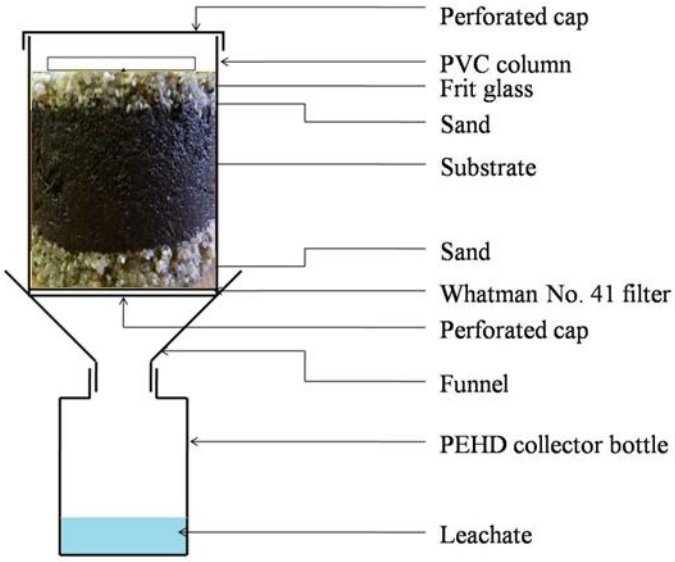
Inlet tubing connected to the peristaltic pump



Reservoirs of nutrient solution

Extrudation of the material filling the column

Appendix C - Illustrations of the column experiment used in Chapter 4



Leaching column design

Columns in phytotron

Appendix D - Illustrations of the leaching pot experiment used in Chapter 5

Lupinus albus germination

Leaching pot experiment in phytotron

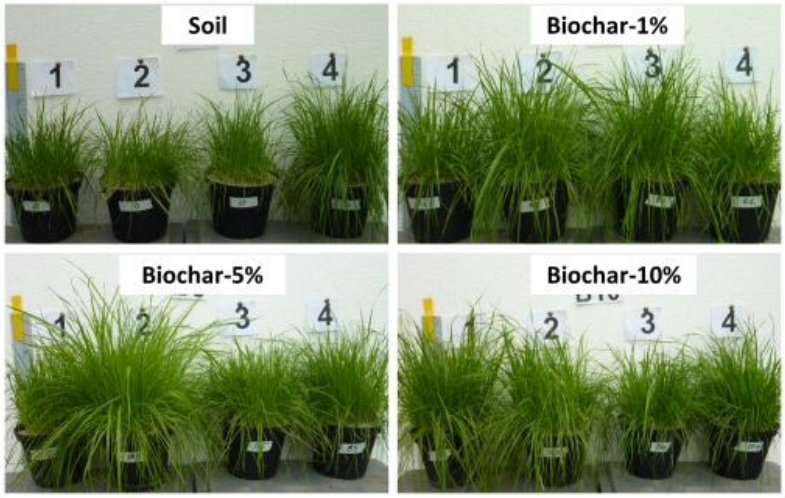
Examples of leachates collected at 2 weeks after sowing. From left to right: control, $CaCO_3$, bone meal and manure treatments; note the very dark color of leachates from bone meal and manure treatments due to the high concentration in DOC.

Appendix E – Study site and illustrations of the pot experiment used in Chapter 6

Study site

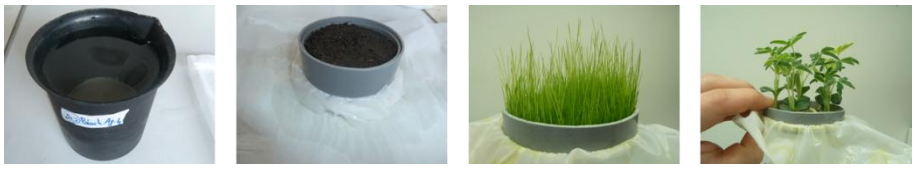
Soil sampling. Note the dark surface horizon due to the poor degradation of organic matter.

Lolium multiflorum growth in greenhouse glass



L. multiflorum at the firt harvest

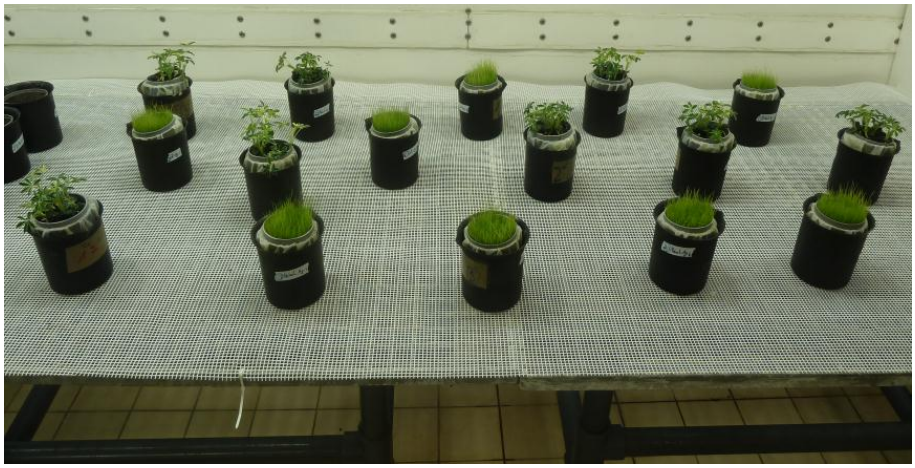
Appendix F – Illustrations of the biotest used in Chapter 7



From left to right : nutrient solution reservoir, soil compartment, *Agrostis tenuis* compartment, *Lupinus albus* compartment.

Root mat of *A. tenuis* (left) and *L. albus* (right) at the end of the pre-growth period.

Control (left) and biochar-5% (right) unplanted treatments (bulk soils).



Biotest in phytotron

A. tenuis harvest

Separation of roots and shoots of *L. albus*

Appendix G – Illustrations of the greenhouse pot experiment used in Chapter 8

Sowing of rapeseed

Rapeseed growth

Appendix H – Supplementary results

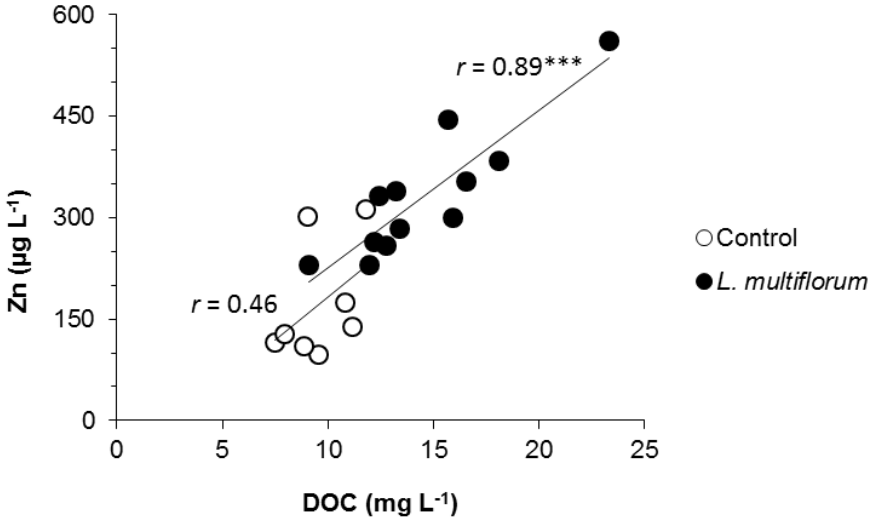


Fig. H.1 Correlation between Zn concentration and dissolved organic C concentration in leachates in the absence (control) or presence of *Lolium multiflorum* (Chapter 2). Marked correlation coefficient (*r*) is the Pearson's correlation coefficient. * significant at the 0.001 probability level.**

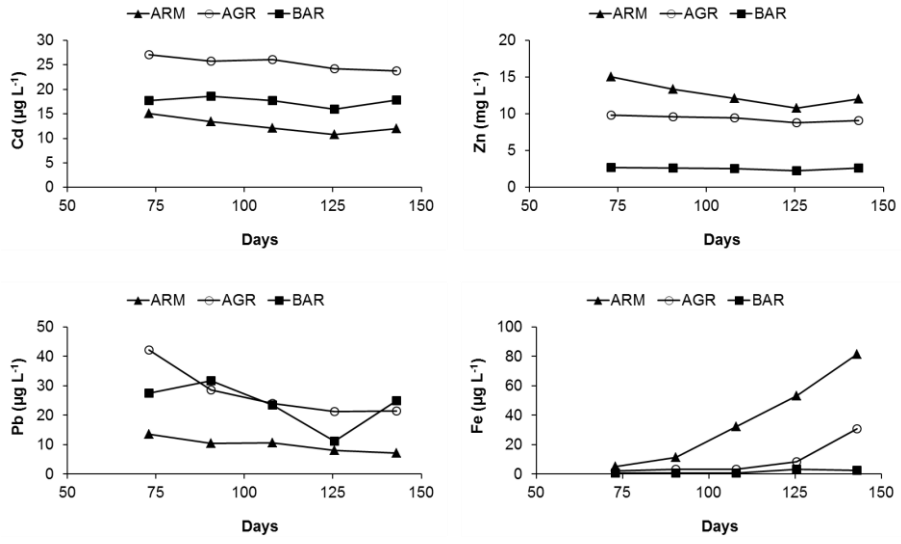
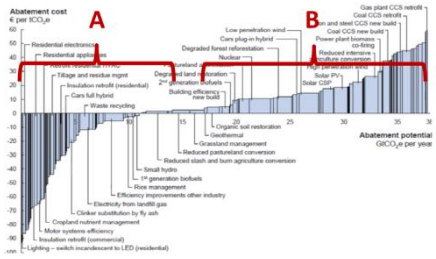
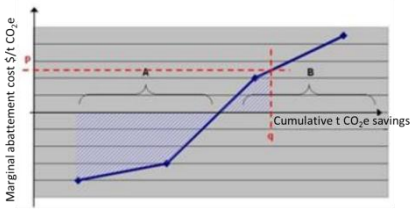


Fig. H.2 Evolution of Cd, Zn, Pb and Fe concentrations (Chapter 4). Each point represents the average of five replicates.

Appendix I – Marginal abatement cost curve

A marginal abatement cost curve (MACC) represents the relationship between the cost-effectiveness of different abatement options and the total amount of greenhouse gases abated. It reflects the additional costs of reducing the last unit of carbon and is upward-sloping: i.e. marginal costs rise with the increase of the abatement effort. MACCS can be derived in different ways, either as a histogram or as a curve (Bockel et al., 2012):

The histogram	The curve
	
The histogram assesses the cost and reduction potential of each single abatement measure. Each bar represents a single mitigation option. The width of the bar represents the amount of abatement potential available from the action (in $MtCO_2e$). The height of the bar represents the average unit cost of the action (cost per ton of CO_2e saved). The area (height * width) of the bar represents the total cost of the action, i.e. how much it would cost altogether in order to deliver all the CO_2 savings from the action. The total width of the MACC shows the total CO_2 savings available from all actions, and the sum of the areas of the total amount of bars represents the total cost of abatement for all actions. This type of MACC representation is easy to understand; the marginal cost and the mitigation potential can be unambiguously assigned to one option.	The curve indicates the cost, usually in \$/t CO_2e, associated with the last unit (the marginal cost) of emission abatement (in general in million tons of CO_2). The curve enables us to analyze the cost of the last abated unit of CO_2 for a defined abatement level while the integral of the abatement cost curve (the area under the curve) gives us the total abatement costs. For example here, the point (q,p) represents the marginal cost, p, of abating an additional unit of carbon emissions at quantity q. The integral of the area under the curve (hatched area) represents the total abatement costs of carbon emission reduction q.
In both cases, moving along the curve from left to right worsen the cost-effectiveness of low carbon options since each ton of CO_2e mitigated becomes more expensive. Different mitigation options will occupy different positions on the curve. Some options may be able to reduce emissions and save money (A), other options may reduce more emissions, but incur a positive cost (B).	

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List of Publications

A. International peer-reviewed journals

Houben, D., Meunier, C., Pereira, B., & Sonnet, Ph. 2011. Predicting the degree of phosphorus saturation using the ammonium acetate-EDTA soil test. *Soil use and management*, **27**, 283-293.

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Houben, D., Pircar, J. & Sonnet, Ph. 2012. Heavy metal immobilization by cost-effective amendments in a contaminated soil: Effects on metal leaching and phytoavailability. *Journal of Geochemical Exploration*,**123**, 87-94.

Houben, D., Couder, E. & Sonnet, Ph. *In press*. Leachability of cadmium, lead and zinc in a long-term spontaneously revegetated slag heap: Implications for phytostabilization. *Journal of Soils and Sediments*, doi: 10.1007/s11368-012-0546-5.

Houben, D., Evrard, L. & Sonnet, Ph. 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.). Submitted to *Biomass and Bioenergy*.

Houben, D., Evrard, L. & Sonnet, Ph. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Submitted to *Chemosphere*.

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B. Conference proceedings

Houben, D. & Sonnet, Ph. Zinc mineral weathering as affected by plant roots. *Eurosoil – Soil Science for the benefit of mankind and environment*, 2012, Bari, Italy.

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