Rhizosphere-induced heavy metal(loid) transformation in relation to bioavailability and remediation

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Abstract

Soil is the sink and source of heavy metals (both geogenic and anthropogenic) and plants are the ecosystem regulators, balancing the chemistry of life on earth. However, roots are the only connection between soil and plants, which are the real engineers of ecosystem dynamics responsible for environmental balance and stability. The plant-soil interface termed as 'rhizosphere' is a typical zone of soil where the physical, chemical and biological characteristics are different from bulk soil (outside the rhizosphere region). This is mainly controlled by physiological response from plants to the environmental changes through exudation of chemicals from root region and the cascade of chemical (changes in pH and redox potential, release of anions and nutrient transformation) and biological (microbial association) events that follow. The other adaptive mechanisms include root length and area as affected by temperature, moisture and nutrient content of the soil. In the recent years, advanced technologies have lead to significant findings at the micro-level in rhizosphere research, targeting the role of root-soil interface towards nutrient availability and agricultural productivity. However, with increasing human activities (including agriculture), undesirable quantites of heavy metals are being added to the environment thereby resulting in soil contamination. This review will discuss in detail on the processes involved in the (im)mobilisation of heavy metals in and around the root region as affected by chemical (pH and root exudates) and biological (microorganisms) components.

Keywords: Metal(loid)s, bioavailability, phytoremediation, rhizosphere, transformation, root exudates

1. Introduction

'Heavy metal(loid)s' generally includes elements (both metals and metalloids) with an atomic density greater than 6 g/cm³ [with the exception of arsenic (As), boron (B), and selenium (Se)]. This group includes both biologically essential [e.g., cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn),

and zinc (Zn)], and non-essential [e.g., cadmium (Cd), lead (Pb), and mercury (Hg)] elements. The essential elements (for plant, animal, or human nutrition) are required in low concentrations and hence are also known as 'trace elements' or 'micro nutrients'. The non-essential elements

are phytotoxic and/or zootoxic and are widely known as 'toxic elements'. Both groups are toxic to plants, animals and/or humans at extremely high concentrations. Build-up of these soil-borne elements in the human food chain depends on the amount and source of heavy metal(loid) input, soil properties, rate and magnitude of plant uptake and influenced by their bioavailability, and the extent of absorption by animals (Adriano, 2001).

With increasing demand for safe disposal of wastes generated from agricultural and industrial activities, soil is not only considered as a source of nutrients for plant growth, but also used as a sink for the removal of contaminants from these waste materials. As land treatment becomes an important waste management practice, soil is increasingly being seen as a major source of heavy metal(loid)s reaching the food chain, mainly through plant uptake and animal transfer. Such waste disposals have led to significant build up of a wide range of metal(loid)s in soils, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), selenium (Se), and zinc (Zn). Since metal(loid)s do not undergo chemical degradation, they persist for a long time in soil, posing threat to soil and environmental health (Adriano *et al.*, 2004).

There are a number of remediation strategies including physical (e.g. replacement of contaminated soil with clean soil), chemical (e.g. immobilisation using lime and phosphate compounds) and biological (bioremediation using microorganisms and/or plants) methods. The first two approaches are expensive and hence plant-aided remediation termed as 'phytoremediation' is increasingly being researched for restoration of heavy metal(loid) contaminated sites (Robinson *et al.*, 2006). In this approach, plants are generally used to extract the heavy metal(loid)s and the process is termed as phytoextraction. In some cases where the phytoextraction is not viable

due to bioavailability factors, *in situ* immobilization (e.g. phytostabilisation) is also considered as a phytoremediation strategy.

Any phytoremediation process starts at the soil-plant interface or the root zone region termed as 'rhizosphere' and hence the process of heavy metal(loid)s transformation mainly occurs in this region. With increasing demand for safe disposal of wastes generated from agricultural and industrial activities, rhizosphere soil is not only considered as a source of nutrients for plant growth, but also used as a sink for the removal of contaminants from these waste materials. The complexity of the rhizosphere region, the sources and dynamics of heavy metal(loid)s in soil and the role rhizosphere in heavy metal(loid) transformation will be presented in this review, with focus on bioavailability of heavy metal(loid)s and remediation of the contaminated soil.

2. Sources of heavy metals

Soil is the major sink for contaminant chemicals including excessive nutrients and undesirable quantities of heavy metals, which also makes it as a major source of these materials. Similarly, sediments in water are considered as the ultimate sink for these chemicals. Heavy metals reach the soil environment through both pedogenic (or geogenic) and anthropogenic processes. Arsenic (As) is the best example of a heavy metal(loid) released by natural processes. For example, in countries like India, China and Bangladesh, As release from ground water is a serious issues which were found to be transported from the sedimentary rocks of Himalayan origin over several centuries (Mahimairaja *et al.*, 2005; Yu *et al.*, 2007; Armienta *et al.*, 1997; Del Razo *et al.*, 1990). Majority of the naturally occurring sources are not readily available to plant uptake compared to the ones that are released from human activities (Lamb *et al.*, 2009; Naidu and Bolan, 2008; Naidu *et al.*, 1996). Most anthropogenic

activities, such as industrial processes, manufacturing and the disposal of domestic and industrial waste materials release heavy metals which are highly available for plant uptake (Table 1; Adriano 2001; McLaughlin *et al.* 1996). Atmospheric pollution from Pb-based petrol is a major issue in many developing countries where there is no constraint on the usage of leaded gasoline. While sewage sludge is the major source of metal inputs in Europe and North America. Phosphorus (P) fertilizers are considered to be the major source of heavy metal(loid) input, especially Cd, in Australia and New Zealand.

The main source of P for agricultural usage is phosphate rocks (PRs), which also contains considerable quantities of heavy metal(loid)s (Table 2). Addition of P compounds to soils not only helps to overcome the deficiency of some of the essential trace elements, such as Mo, but also introduces toxic metals such as Cd. In the case of Mo, P addition results in surface complexation reactions along with soil components including aluminium oxides, thereby reducing the adsorption of Mo (Goldberg, 2010). Wu *et al.* (2001) studied Mo adsorption in soil in the presence of equimolar phosphate concentration and found that phosphate decreased Mo adsorption in soil. Most agricultural soils fertilised with PRs for over several years are prone to Cd toxicity thereby affecting the quality of food crops and consequently affecting the overall food chain (Bao *et al.*, 2011). It is important to stress that PR deposits vary in their Cd content, leading to the variation in Cd contents of manufactured P fertilizers. With rich source of Cd from P fertilizers, large quantities of Cu are used in agriculture, horticulture and animal industries as formulations of Cu containing fungicides, such as copper oxichloride and 'Bordeaux' mixture, and as a growth promoter in piggery and poultry units (Adriano, 2001; Bao *et al.*, 2011). Copper containing fungicides are quite effective in controlling many fungal diseases and are also accepted in 'organic' farming (Komárek *et al.*, 2010). Even with increasing interest in 'organic' farming, the widespread use of Cu fungicides

is likely to continue in vineyards and citrus orchards. Accumulation of Cu in agricultural soils resulting from continuous use of Cu fungicides and sludge application has been reported in many countries. One of the major consequences of excessive accumulation of Cu in soils is its toxicity to plants and microbial communities.

Another prominent heavy metal is Cr from tannery and timber treatment effluents. Approximately, 6400 and 1600 tons of tannery and timber treatment effluents, respectively, are generated annually in New Zealand, and these effluents are considered to be the major sources of Cr contamination into aquatic and terrestrial environments. Chromium is used as Cr(III) in the tannery industry and as Cr(VI) in the timber treatment industry. Cr(VI) is highly toxic and carcinogenic even when present in very low concentrations in water. Large scale use of tannellised timber (treated with CCA) as fence post and in vineyards can also result in the release of Cu, Cr and As to soil environment.

3. Transformation of heavy metal(loid)s in soils

The general mechanisms involved in the transformation of metal(loid) ions in the soil lead to retention (mediated by sorption, precipitation, and complexation reactions) or loss (plant uptake, leaching, and volatilization) of heavy metal(loid)s (Figure 1). Although most metal(loid)s do not undergo volatilization related losses, some metal(loid)s such as As, Hg, and Se tend to form gaseous compounds (Mahimairaja *et al.*, 2005; Bolan *et al.*, 2013). The lower the metal(loid) solution concentration and the more sites available for sorption, the more likely that sorption/desorption processes will determine the soil solution concentration. However, the fate of metal(loid)s in the soil environment is dependent on both soil properties and environmental factors.

Table 1. Sources of heavy metals in soils and their expected ionic species in soil solution

Metal	Density (g/cm ³)	Ionic species in soil solution	Contaminant sources	Toxicity ^a
Arsenic (As)	5.73	As(III): As(OH) ₃ , AsO ₃ ³⁻ ; As(V): H ₂ As ₄ ⁺ , HAsO ₄ ²⁻	Timber treatment, paints, pesticides, geothermal	Toxic to plants, humans and animals
Cadmium (Cd)	8.64	Cd ²⁺ , CdOH ⁺ , CdCl ⁺ , CdHCO ₃ ⁺	Electroplating, batteries, fertilizers	Toxic to plants, humans and animals
Chromium (Cr)	7.81	Cr(III): Cr ³⁺ , CrO ₂ ⁺ , CrOH ²⁺ , Cr(OH) ₄ ⁺ ; Cr(VI): Cr ₂ O ₇ ²⁻	Timber treatment, leather tanning, pesticides, dyes	Cr(VI) toxic to plants, humans and animals ^b
Copper (Cu)	8.96	Cu ²⁺ (II), Cu ⁺ (III)	Fungicides, electrical, paints, pigments, Timber treatment, fertilizers, mine tailings	Toxic to plants, humans and animals
Lead (Pb)	11.35	Pb ²⁺ , PbOH ⁺ , PbCl ⁺ , PbHCO ₃ ⁺ , PbSO ₄ ⁰	Batteries, metal products, preservatives, petrol additives	Toxic to plants, human and animal
Manganese (Mn)	7.21	Mn ²⁺ , MnOH ⁺ , MnCl ⁺ , MnCO ₃ ⁰	Fertilizer	Toxic to plants
Mercury (Hg)	13.55	Hg ²⁺ , HgOH ⁺ , HgCl ₂ ⁰ , CH ₃ Hg ⁺ , Hg(OH) ₂ ⁰	Instruments, fumigants, geothermal	Toxic to humans and animals
Molybdenum (Mo)	10.2	MoO ₄ ²⁻ , HMoO ₄ ⁺ , H ₂ MoO ₄ ⁰	Fertilizer	Toxic to animals
Nickel (Ni)	8.90	Ni ²⁺ , NiSO ₄ ⁰ , NiHCO ₃ ⁺ , NiCO ₃ ⁰	Alloys, batteries, mine tailings	Toxic to plants and animals
Zinc (Zn)	7.13	Zn ²⁺ , ZnSO ₄ ⁰ , ZnCl ⁺ , ZnHCO ₃ ⁺ , ZnCO ₃ ⁰	Galvanizing, dyes, paints, timber treatment, fertilizers, mine tailings	Toxic to plants

^amost likely to observe at elevated concentrations in soils and water. ^bwhile Cr(VI) is very mobile and highly toxic, Cr(III) is essential in animal and human nutrition and generally immobile in the environment

Table 2. Metal concentration in phosphate compounds from various phosphate rock sources (Adriano 2001; McLaughlin *et al.* 1996; Syers *et al.* 1986).

Phosphate compound ^a	Concentration (mg/kg)								
	As	Cd	Co	Cu	Zn	Mn	Ni	Pb	Hg
GPR	4	38	3	15	393	7			
NFPR	7	3	5	4	57	212			
JPR	12	4	<1	8	235	5			
NCPR	23	48	2	9	400	7	9-51	<1 – 51	0.4 – 2.1
SPR	5	11	3	6	178	91			
MPR	3	8	6	4	90	151			
NIPR	3	100	6	8	1010	122			
APR	7	12	4	12	560	2			
MIPR	2	10	<1	6	220	2			
CRP		2	4	5	95	100			
IRP			109	32	187	975		962	
SSP			77	15	165	890		488	
TSP			47	49	418	75		238	
DAP			16	7.2	112	307		195	

^aPhosphate rocks: GPR - Gafsa phosphate rock, NFPR - North Florida phosphate rock, JPR – Jordan phosphate rock, NCPR – North Carolina phosphate rock, SPR –Sechura phosphate rock, MPR- Mexican phosphate rock, NIPR – Nauru Island phosphate rock, ARP – Arad phosphate rock, MIPR – Makatea Island phosphate rock, CRP, Chatham Rise phosphorite, IPR – Indian phosphate rock; Phosphate fertilizers: SSP – Single super phosphate, TSP – Triple superphosphate, DAP – Diammonium phosphate.

Figure 1. Possible reactions involved in physical, chemical and biological transformation of metal(loid)s in soil

3.1. Sorption/desorption process

Electrostatic attraction and/or specific bond formation are the two mechanisms behind the retention of charged solute species (ions) to the charged soil surface, which can be broadly grouped into specific and non-specific retention (Bolan *et al.*, 1999; Sparks, 2003; Li *et al.*, 2006). The former involves chemical bond formation between the ions in the solution and those in the soil surface, whereas in non-specific adsorption, the charge on the ions balances the charge on the soil particles through electrostatic attraction (Li *et al.*, 2006; Sposito, 1984; Zenteno *et al.*, 2013). A dynamic equilibrium occurs between metal(loid)s in solution and soil's solid phase, which is controlled both by soil properties and soil solution composition. While soil pH is the main factor influencing the sorption processes of the metal(loid)s, the nature of inorganic (HPO_4^{2-} , NO_3^- , Cl^- , and SO_4^{2-}) and organic (citrate, oxalate, fulvic, dissolved organic carbon) ligand ions present in soil affects the concentration of metal(loid)s in soil solution (Bolan *et al.*, 2003; Harter and Naidu, 1995). The inorganic anions can either form ion pair complexes with metal(loid)s thereby reducing their sorption (Hong

et al., 2010) or they can increase the negative charge on soil particles, thereby increasing the sorption of cationic metal(loid)s (Naidu *et al.*, 1994).

Most of the heavy metal(loid) ions in soil solution decreases with increase in pH because of increase in pH dependent surface charge on oxides of Fe, Al and Mn; chelation by organic matter, or precipitation as metal(loid) hydroxides (Mouta *et al.*, 2008; Stahl and James, 1991). Generally, an increase in soil pH increases the removal of both CEC and the total amount of heavy metal(loid)s from the soil (Shuman, 1986; Violante *et al.*, 2010). Complexation reactions between metal(loid)s and inorganic/organic ions could also contribute to metal(loid) retention, where the organic component of soil constituents possessing ligands forms chelates with metal(loid)s (Bolan *et al.*, 2011; Harter and Naidu, 1995). With increasing pH, the carboxyl, phenolic, alcoholic, and carbonyl functional groups in soil organic matter dissociate, thereby increasing the affinity of ligand ions for metal(loid) cations. The extent of metal(loid)-organic complex formation, however, varies with a number of factors including temperature and concentration. All these interactions are controlled by solution pH and ionic strength, the nature of the metal(loid) species, dominant cation, and inorganic and organic ligands present in the soil solution.

3.2. Precipitation/dissolution

Soils with high pH and high concentration of heavy metal(loid) ions have more chances of precipitation as a dominant process of metal(loid) immobilization, where the presence of anions such as SO_4^{2-} , CO_3^{2-} , OH^- , and HPO_4^{2-} facilitates the process (Naidu *et al.*, 1997; Hong *et al.*, 2007; Ok *et al.*, 2010). For example, McGowen *et al.* (2001) observed a decrease in the leaching of Cd, Pb, and Zn using activity-ratio diagrams indicating P (diammonium phosphate) induced decrease in solution metal(loid) concentrations, as facilitated by the formation of metal(loid)-P precipitates. Similarly, an increase in pH as a result of liming typically enhanced the retention of metals (Adriano, 2001; Bolan and Thiagarajan, 2001).

In the presence of iron oxyhydroxides, co-precipitation of metal(loid)s has also been reported at low pH and often such interactions lead to significant changes in the surface chemical properties of the substrate. For example, Lu *et al.* (2011) confirmed that co-precipitation of Pb^{2+} with ferric oxyhydroxides occurred at $\sim\text{pH } 4$ and is more efficient than adsorption in removing Pb^{2+} from aqueous solutions at similar sorbate/sorbent ratios and pH. Sorption of arsenate (As(V)) onto ferrihydrite; and Ni^{2+} and Cr^{3+} onto hydrous iron oxides showed that co-precipitation was more efficient process than sorption for metal(loid) removal from aqueous solutions. In another instance, Violante *et al.* (2007) noticed that As(V) was desorbed by P from a ferrihydrite on which As(V) was added than from a Fe-As(V) co-precipitate.

3.3. Oxidation/reduction

Metal(loid)s such as As, Cr, Hg, and Se, are most commonly subjected to microbial oxidation/reduction reactions, thereby affecting their speciation and mobility (Table 3). Redox reactions influence the speciation and mobility of metal(loid)s. For example, metals generally are less soluble in their higher oxidation state, whereas the

solubility and mobility of metalloids depend on both the oxidation state and the ionic form (Ross, 1994). In the case of sediments and soils, As(III) can be oxidized to As(V) by bacteria (Bachate *et al.*, 2012; Battaglia-Brunet *et al.*, 2002). The strong affinity of As(V) towards inorganic soil components, results in the immobilization of As after the oxidation process. Under well drained conditions, As is usually present as As(V) in soils, whereas under reduced conditions, As(III) dominates in soils, but elemental arsenic [As(0)] and arsine (H_3As) can also be present. The reduction and methylation reactions of As in sediments are generally mediated by bacterial degradation of organic matter coupled with reduction and use of sulfate as the terminal electron acceptor (Kim *et al.*, 2002).

Contrastingly, in the case of Cr, oxidation of Cr(III) to Cr(VI) can enhance Cr mobilization and bioavailability. It is primarily mediated abiotically through oxidizing agents such as Mn(IV), and to a lesser extent by Fe(III), whereas reduction of Cr(VI) to Cr(III) is mediated through both abiotic and biotic processes (Choppala *et al.*, 2013). Chromate (Cr(VI)) can be reduced to Cr(III) in environments where a ready source of electrons (Fe(II)) is available. Suitable conditions for microbial Cr(VI) reduction occur where organic matter is present to act as an electron donor, and Cr(VI) reduction is enhanced under acidic rather than alkaline conditions (Choppala *et al.*, 2013; Hsu *et al.*, 2009; Figure 2).

In most living systems, heavy metal(loid)s generally undergoes reduction rather than oxidation. For example, Se undergoes reduction by chemical reductants such as sulfide or hydroxylamine, or biochemically by glutathione reductase (Zhang *et al.*, 2004). In the case of Hg, microorganisms, particularly bacteria play a major role in reducing reactive Hg(II) to non-reactive Hg(0), which may be subjected to volatilization losses. Hg(II) is reduced to Hg(0) by mercuric reductase, and the dissimilatory metal(loid) reducing bacterium *Shewanella oneidensis* has been shown to reduce Hg(II) to Hg(0), which requires the presence of electron donors (Wiatrowski *et al.*, 2006).

Table 3. Oxidation-reductions reactions of metal(loid)s in soil.

Metal(loid)	Redox reactions	References
Cr(VI)	Addition of organic amendments (e.g. poultry manure, biosolids) enhanced the rate of Cr reduction in soil. The extent of Cr(VI) was influenced by the amount of dissolved organic carbon present in the soil and amendments.	Bolan <i>et al.</i> , 2003
Cr(VI)	Presence of DOC in soil as a result of soybean meal and rice bran addition, reduced resin extractable Cr(VI) in soil	Chiu <i>et al.</i> , 2009
Cr(VI)	Chromate leaching was reduced in soils in the presence of elevated organic matter because of reduction followed by retention on cation exchange sites or precipitation	Park <i>et al.</i> , 2004
Cr(VI)	Bacteria reduced most of Cr(VI) in anaerobic conditions and lactate was more effective as a bio-stimulant for reduction by acting as an electron source.	Banks <i>et al.</i> , 2006
As	The mixture of sewage sludge and poultry litter reduced As(V) to more mobile and toxic As(III).	Jackson and Miller, 1999
Se	Application of both insoluble (casein) an soluble (Casamino acids) to Se contaminated soil enhanced dimethyl selenide production and subsequent removal of Se from soil through volatilization	Zhang and Frankenberger Jr., 1999

Figure 2. Effect of carbon based amendments on maximum Cr reduction (mg/kg) in three different types of soils (Choppala *et al.*, 2013). Soil 1: Calcic red sandy loam; Soil 2: Calcic red clay; Soil 3: Tannery waste contaminated. Control: Unamended; BC: Black carbon; CMB: Chicken manure biochar
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3.4. Methylation/Demethylation

Another process of heavy metal(loid) transformation that occurs with the help of living system is methylation where toxic metal(loid)s are removed from soil by converting them to methyl derivatives that are subsequently removed by volatilization (Frankenberger and Losi, 1995). Methylated derivatives of As, Hg, and Se can originate from chemical and biological mechanisms and this frequently results in altered volatility, solubility, toxicity, and mobility. The major microbial methylating agents are methylcobalamin ($\text{CH}_3\text{CoB}_{12}$), involved in the methylation of Hg, and S-adenosylmethionine (SAM), involved in the methylation of As and Se. Although methylation of metal(loid)s occurs through both chemical (abiotic) and biological processes, biological methylation (biomethylation) is considered to be the dominant process in soils and aquatic environments. Biomethylation may result in metal(loid) detoxification, since methylated derivatives may be excreted readily from cells, and are often volatile and may be less toxic, e.g., organoarsenicals.

Microorganisms in soils and sediments act as biologically-active methylators, where organic matter serves as a methyl donor for both biomethylation and abiotic methylation in soils and sediments (Frankenberger and Arshad, 2001; Loseto *et al.*, 2004). For example, methylation of Hg is controlled by low molecular weight fractions of fulvic acid in soils (Battaglia-Brunet *et al.*, 2002). Similarly, Lambertsson and Nilsson (2006) suggested that organic matter and alternative electron acceptors influenced methylation of Hg in the sediments. Mercury is methylated through both biotic and abiotic pathways, although microbial methylation mediated mainly through

dissimilatory sulfate- and iron-reducing bacteria is generally regarded as the dominant environmental pathway (Rodriguez *et al.*, 2004).

In the case of As, biomethylation is effective in forming volatile compounds such as alkylarsines, which could easily be lost to the atmosphere (Lehr, 2003; Yin *et al.*, 2011). Methylated As species could result from direct excretion by algae or microbes or from degradation of the excreted arsenicals or more complex cellular organoarsenicals (Li *et al.*, 2009).

Selenium biomethylation is of interest because it represents a potential mechanism for removing Se from contaminated environments, and it is believed that methylated compounds, such as dimethyl selenide (DMSe) are less toxic than dissolved Se oxyanions. Fungi are more active in the methylation of Se in soils, although some Se-methylating bacterial isolates have also been identified (Oremland *et al.*, 2004). Dimethyl selenide can be demethylated in anoxic sediments as well as anaerobically by an obligate methylotroph similar to *Methanococcoides methylutens* in pure culture. An anaerobic demethylation reaction may result in the formation of toxic and reactive H_2Se from less toxic DMSe. Aerobic demethylation of DMSe is likely to yield Se(VI), thereby retaining Se in the system.

4. The rhizosphere

The rhizosphere, first described in 1904 by the German agronomist Lorentz Hiltner, has been the focus of intensive research for many years because of its importance in plant nutrition and pathogenesis. The importance of the rhizosphere has been well known for a long time as evidenced by the statements of Hiltner before an assembly of Deutsche Landwirtschafts-Gesellschaft. Hiltner

stated, “The nutrition of plants in general certainly depends upon the composition of the soil flora in the rhizosphere.” He further admitted, “If plants have the tendency to attract useful bacteria by their root excretions, it would not be surprising if they would also attract uninvited guests which, like the useful organisms, adapt to root excretions. Nowadays, the research on rhizosphere has been directed towards its influence on the transformation of pollutants in soils (Vysloulžilová *et al.*, 2006, Yang *et al.*, 2010). Anderson *et al.* (1993) stated, “Microbially mediated humification processes in the rhizosphere may have an important influence on the persistence and bioavailability of toxicants in surface soils”.

The rhizosphere is a zone at the root-soil interface, controlled by plant root and root released metabolites, where a plethora of mutually interacting physical, chemical and biological processes operates within this zone. The health of both the plant and the soil associated is dependent on this rhizosphere region and its biochemical reactions. Although attempts have been made to unravel some of these processes, the understanding of the intricacies of this unique ‘twilight zone’ is still in its infancy. It is for this reason that noted microbiologists, G.D. Bowen and A. Rovira, correctly described the rhizosphere zone as - ‘the hidden half of the hidden half’. Numerous research throughout the last hundred years helped in dividing rhizosphere into three zones based on their relative proximity to the root and their resultant influence (Figure 3). The endorhizosphere is the inner layer which includes cortex and endodermis, where microbes and cations (heavy metals) can occupy the space between cells. The rhizoplane or the middle layer consists of root epidermis and mucilage, found adjacent to the root. The ectorhizosphere

is the outermost layer leading into the bulk soil. Plant root systems are inherently complex and diverse and hence rhizosphere is not a region of definable size or shape. However, it depends on the physical, chemical and biological properties of the rhizosphere region, which change both radially and longitudinally along the root (Fageria and Stone, 2006). For example, Cd accumulation occurs in most plants only in the roots but restricted to other parts of the plant (Nocito *et al.*, 2011). Hence, physiological response by plants also play an important role in heavy metal resistance. In an experiment conducted by Sghayar *et al.* (2015), the role of thiol biosynthesis and metabolism were investigated for determining Cd tolerance in barley cultivars and they found strong effect on Cd accumulation.

The properties of rhizosphere vary according to the plant species, where the width has been shown to extend from 2–80 mm from the root surface. Concentration of root exudates and extent of microbial activity are useful indicators of demarcation of rhizosphere and bulk soil zone, generally termed as ‘edaphosphere’. Although the chemical and biological features help in identifying the rhizosphere, it is challenging to separate the zone physically from the surface of the root. There has been continuous efforts on determining the ideal ratio between rhizosphere and bulk soil known as R/E ratio, which was found to be between 2 and 20, which can be termed as rhizosphere effect (Badalucco and Kuikman, 2001). There have been abundant information on rhizosphere chemistry and biology, mainly targeting nutrients for agricultural production. However, research work on the role of rhizosphere and their mechanisms in remediating heavy metal(lloid)s from soil are scattered and relatively less.

Figure 3. A section of soil showing bulk soil and rhizosphere soil with three regions of rhizosphere – Ectorhizosphere, rhizoplane and endorhizosphere (with metal(loid)s and microorganisms).

5. The role of rhizosphere processes in phytoremediation

Phytoremediation or plant-assisted bioremediation refers to the use of plants to:

- stabilise or immobilise contaminants in soils or sediments which can be termed as *phytostabilisation* or *phytoimmobilisation*, respectively
- volatilise some metals and metalloids by the formation of volatile compounds by the action of rhizosphere microorganisms or after uptake in plant organs – *phyto-volatilisation/rhizo-volatilisation*
- extract metals / metalloids via uptake in harvestable plant parts, i.e. typically shoots, which can be termed as *phytomining*
- remove organic pollutants via microbial degradation in the plant rhizosphere or by metabolising them after uptake in plant organs – *phyto-degradation/rhizodegradation*

Wenzel (2009) pointed out that the action of plant rhizosphere is one of the key factors in establishing phytoremediation crops under (typically) harsh conditions. Selection of suitable plants not only enable uptake of specific heavy metals (phytoextraction) but also helps in controlling the bioavailability of the pollutant (phytoimmobilisation) and thereby degradation through ageing (phytodegradation). Since the processes are initiated at the rhizosphere region, terminologies such as rhizoremediation, rhizodegradation and rhizovolatilisation can also be used, alternatively. There are numerous reviews on the rhizosphere action in phytoremediation over the past two decades (Anderson *et al.*, 1993; Anderson and Coats, 1994; Dzantor, 2007; Fitz and Wenzel, 2002; Kuiper *et al.*, 2004; McGrath *et al.*, 2001; Meharg and Cairney, 2000; Newman and Reynolds, 2004; Siciliano and Germida, 1998;

Wenzel, 2009). There are four main factors that influences rhizosphere processes – (1) rhizosphere modification; (2) pH; (3) root exudates and (4) microbial activity. These factors can act both independently and also in a coordinated way.

a. Rhizosphere modification

Roots react to both the physical (e.g. presence or absence of water) and chemical changes (e.g. nutrients) in the soil environment. The response of roots not only affects the plants but also the soil in the rhizosphere region, thereby bringing in changes to the pH, chemical equilibrium of the rhizosphere soil and microbial activity in the region. Addition of nutrients and/or organic amendments to the soil can affect the chemistry (e.g., pH, organic acids, soil solution composition) and biology (e.g., microbial community) of soil (Park *et al.*, 2011). The changes in soil chemistry due to soil amendment and plant growth can therefore influence the transformation, mobility and bioavailability of metal(loid)s (Walker *et al.*, 2003; Clemente *et al.*, 2010). The effect of organic amendments on some of the rhizosphere properties in relation to metal(loid)s dynamics is presented in Table 4. In the case of nutrients such as phosphorus (P), plant response to As and P interactions in the root zone appears to be system dependent. In nutrient solution culture, increasing amounts of soluble P at fixed As concentration reduced As phytotoxicity (Rumberg *et al.*, 1960). This was attributed to the inhibition of As uptake by roots in the presence of excessive amounts of P. Whereas in soil systems, P fertilizer addition has been shown to enhance the uptake of As due to an increase in

solubility of As. This was because of competitive adsorption of phosphate to soil components such as Fe and Al and the resultant desorption of As (Creger and Peryea, 1994; Peryea, 1991). Hence, P retention in soil plays an important role in As adsorption.

Generally, plants respond to nutrient deficiency by changing the structure of roots, altering the chemical environment in the rhizosphere region or recruiting the help of microorganisms. While the presence/absence of nutrients and water influences the root morphology, the exudates from the roots alter the chemical equilibrium of the root region by acidifying or changing the redox conditions within the rhizosphere or directly chelating with the nutrient. Exudates can liberate nutrients via dissolution of insoluble mineral phases or desorption from clay minerals or organic matter where they are released into soil solution and can then be taken up by the plant. The most limiting nutrients to plant growth are nitrogen (N) and (P). Even though 78% of the Earth's atmosphere is composed of nitrogen (N_2 gas), it is in a form that is only utilizable by nitrogen-fixing organisms. As such, inorganic forms of N (NO_3^- , NH_4^+) that can be used by plants are added to soils. Plants respond differently depending on the form of nitrogen in the soil. Ammonium has a positive charge, and thus the plant expels one proton (H^+) for every NH_4^+ taken up resulting in a reduction in rhizosphere pH. When supplied with NO_3^- , the opposite can occur where the plant releases bicarbonate (HCO_3^-) which increases rhizosphere pH. These changes in pH can influence the availability of other plant essential micronutrients (e.g., Zn, Ca, Mg).

Table 4. Selected references on rhizosphere modifications in relation to heavy metal(loid)s remediation in soil as affected by organic amendments.

Organic amendments	Mode of action	Observations	References
Biosolid	pH	The application of biosolids decreased pH of pore water of rhizosphere soil of <i>Lolium perenne</i> .	Santibanez <i>et al.</i> , 2008
Sugarbeet	pH	Mixed amendment of sugarbeet, <i>Aspergillus niger</i> and rock phosphate significantly decreased pH in the rhizosphere of <i>Cistus albidus</i> .	Caravaca <i>et al.</i> , 2005
Humic acid	Organic acid	Application of humic acid increased bioavailability of various heavy metal(loid)s and enhanced their translocation from root to shoot.	Bandiera <i>et al.</i> , 2009.
Biosolids	Organic acid	Biosolids enhanced the organic acid production in the rhizosphere of the <i>Zea mays</i> .	Koo <i>et al.</i> , 2006
Crop residues	Microbial activity	Soils amended with crop residues increased microbial diversity in wheat rhizosphere	Nelson and Mele, 2006
Food waste compost	Microbial and enzyme activity	Application of food waste compost significantly increased bacterial and fungal populations, soil enzyme activities in lettuce rhizosphere	Lee <i>et al.</i> , 2004
Soluble organic carbon	Microbial activity	Structural manipulation of rhizobacterial communities can be mediated by artificial exudates in the form of soluble organic carbon	Baudoin <i>et al.</i> , 2003
Wheat straw	Microbial activity	Addition of organic amendments significantly enhanced the microbial population in wheat rhizosphere	Barakah <i>et al.</i> , 1995
Humic acid	Enzyme activity	Application of humic acids increased plasma membrane H ⁺ ATPase activity in the root of <i>Zea mays</i> seedlings	Canellas <i>et al.</i> , 2002.

b. Rhizosphere pH

Rhizosphere pH is generally acidic and it can be attributed to a combination of mechanisms, including: (i) cation–anion exchange balance; (ii) organic acid release (e.g., citric, malaeic, lactic, oxalic, propanic, butyric acids); (iii) root exudation and respiration and (iv) redox-coupled processes involving changes in the oxidation state of Fe, Mn and N and consuming or producing of H⁺ (Hinsinger *et al.*, 2003). Application of organic amendments such as biosolids and manures can also produce organic acids extracellularly due to organic matter breakdown and often decreases the pH of rhizosphere soils (Caravaca *et al.*, 2005; Santibanez *et al.*, 2010). However, the contribution of organic acid exudation to the pH of soil solutions depends on the soil pH, the assemblage of organic acids, and to some extent the ionic strength of the soil solution (Jones, 1998). One of the mechanisms behind rhizosphere acidification is the release of H⁺ by roots in their immediate vicinity (Ernst, 1996). Javed *et al.* (2013) studied the rhizosphere pH and exudates of *Eriophorum angustifolium* (cotton grass) and found

that although root exposure of 2–25 ng/g Pb, Cu, Zn, As and Cd induced higher exudation of organic acids, but they did not influence the changes in pH. This was attributed to other organic molecules such as polypeptides (Wheeler and Irving, 2010). Within the rhizosphere, soil acidification is mainly caused by an imbalance in the carbon and nitrogen cycles caused by uptake of cations-anions and respiration (Tang *et al.*, 2005). A major source of H⁺ fluxes in the rhizosphere is related to the differential uptake of cations and anions by plant roots (Hinsinger *et al.*, 2003; Zhang *et al.*, 2009; Ryan *et al.*, 1993). Uptake of solution NH₄⁺ results in a loss of positive charge in solution, which is counter-balanced by a corresponding release of H⁺ into the rhizosphere (Marschner, 1995). Apart from this, N transformation and nitrate leaching have been suggested to be major causes of soil acidification (Bolan *et al.*, 1991). The crop type also plays a major role in rhizosphere acidification. Nitrogen fixing plants tend to decrease soil pH more than non-N fixing plants, since the alkaline release does not occur significantly as NO₃⁻ is not required. Tang *et al.* (2000) reported that the lupin (*Lupinus angustifolius*) and

subterranean clover (*Trifolium subterraneum*) without receiving nitrogen fertilizer produced approximately 1.4 mmol H⁺ per kg soil over the experimental period, on average. The impact of plant on the rhizosphere also varies temporally, and at different points along the plant root (Marschner, 1995).

Although metal(loid) uptake by plants is known to decrease with decreasing pH in nutrient culture, metal(loid) uptake increases in the soil environment (Weng *et al.*, 2003; 2004). This can be attributed to competitive adsorption of metal(loid)s with soil components, thereby increasing the chances of metal(loid) desorption. For example, higher Cd uptake was obtained for lettuce (*Lactuca sativa*) and Swiss chard (*Beta vulgaris*) grown on acid soils (pH 4.8 to 5.7) than on calcareous soils (pH 7.4 to 7.8) (Mahler *et al.*, 1978). Khan and Jones (2009) showed that the addition of lime resulted in the largest reduction in metal extractability with *diethylene triamine pentaacetic acid* and Ca(NO₃)₂ and phytoavailability of Cu, Fe and Zn.

In addition to bioavailability of metal(loid)s to plants, acidification also affects the leaching and residence time of many metal(loid)s in soil. Tyler (1978) observed that the amount of metal(loid)s released from the mor soils of Sweden increased with decreasing pH. Approximately 85% of the total Cd was released from the soil at pH 2.8. The time needed for a 10% decrease in the total concentration of Cd in the mor horizon through leaching was estimated to be 1.7 years at a pH of 2.8, 4-5 years at a pH of 3.2, and 20 years at a pH of 4.2.

5.1..Root exudates

Apart from the morphological changes and pH dynamics, plant roots also respond chemically by releasing organic compounds such as carbohydrates, organic acids (carboxylic and amino acids) and

protons which are termed as root exudates and the process being 'Rhizodeposition'. The exudates are generally water-soluble and includes secretions, lysates, gases and mucilage (Grayston *et al.*, 1997). These rhizodeposits are mainly C based compounds (e.g. organic acids) and hence attract microorganisms fuelling additional microbial growth within the rhizosphere (Lynch and Whipps, 1990). Tao *et al.* (2004) evaluated the root induced changes in the transformation of Cu and concluded that the exudation of organic compounds can influence the behavior of trace and toxic metals.

Certain low molecular-weight compounds released from plant roots (amino acids, carboxylic acids, sugars, and simple and flavonoid-type phenolics) (Uren, 2007) may form stable complexes with metal cations in the soil solution matrix, and can directly modify metal(loid) availability in the rhizosphere (Jones, 1998). The role of carboxylates has been examined for their potential impact on the biogeochemistry of metals through aqueous complexation and ligand exchange processes. Amongst the range of carboxylates exuded in the rhizosphere, malate, citrate and oxalate are expected to have the most dramatic effect due to their ability for complexation of metal(loid)s (Hinsinger, 2001). In a study by Bolan *et al.* (2013) on native Australian vegetation (grass, shrub and trees), organic acid exudated from the roots have reduced Cr and As. In the case of Cr, reduction was negatively correlated to bioavailability whereas the reduction of As from V to As (III) increased its bioavailability which can also be attributed to the reduction induced pH increase.

Organic exudates are also released from the roots when the plant is under stress from mineral deficiency and toxicity (Jones, 1998; Marschner, 1995). Iron deficiency has been known to induce root exudation of range of organic compounds to improve Fe acquisition in soils (Jones, 1998; Rengel, 1997; 2002). Exudation of siderophores, another organic

exudate, is an additional strategy utilized by plants under Fe deficiency (Crowley and Rengel, 1999). The siderophores released by plants for the purpose of Fe chelation are termed as phytosiderophores. Phosphorus deficiency is also well known to result in organic anion exudation in a range of plant species (Jones, 1998; Marschner, 1995; Pearse *et al.*, 2006; 2007). The release of organic acids such as oxalate, malate and piscidate is hypothesized to increase P sequestration from mineral surfaces; however, organic exudates production does not necessarily result in greater P uptake (Pearse *et al.*, 2007). Root exudate production has also been shown in response to metal(loid) exposure in soil solution (Jones, 1998; Bertin *et al.*, 2003; Das and Maiti, 2007). Bao *et al.* (2011) demonstrated the effect of Cd concentration on exudation of organic acids by the roots of *Solanum nigrum* and showed that an increase in Cd concentration in soil, increased the overall concentration of organic acids (Figure 4). The exudation of organic acids by plants or associated microbes may complex metal(oids) and reduce uptake, although increased uptake is more commonly observed with artificial introduction of organic acids (Zhao *et al.*, 2001). Studies show that Al and some heavy metals can elicit organic acid exudation in roots. Nian *et al.* (2002) compared Al induced organic acid exudation in wheat plants with that of Cu's effect and found that the Cu also induced an efflux of organic acid exudation, thereby playing a role in alleviating Cu toxicity in plants.

The promotion of root growth by improvement of soil physical, chemical and biological conditions is also likely to enhance the further modification of metal(loid) behaviour in the rhizosphere (Farinati *et al.*, 2009). Koo *et al.* (2006) found the total organic acid concentration was highest when biosolid and plant growth was combined, rather the single treatment. The biosolid provided a baseline of a wide range of organic acids, although the organic acid

composition changed considerably with addition of plants. However, the combined effect was greater than the single roles, possibly due to additional root growth. The interaction of organic amendments with the rhizosphere depends on the nutrient content, heavy metal(loid) availability and the ability of plant species to tolerate heavy metal(loid) exposure, however, limited data exists to date.

Organic amendments (compost, compost water extract, the hydrophobic and hydrophilic fractions of the DOC extracted from the compost) increased available C compounds in the rhizosphere soils amended. Organic amendments enhanced organic C release by maize plant roots. Similar observations were made by Caravaca *et al.* (2002), who reported an increased rhizodeposition in *Olea europaea* sub sp. *sylvestris* and *Rhamnus lycioides* treated with composted residue. Caravaca *et al.* (2005) demonstrated that sugar beet-residue amendment increased the total carbohydrates and soluble C-fraction (water-soluble C and water-soluble carbohydrates) in the rhizosphere of *Cistus albidus* L. and *Quercus coccifera* L.

c. Microbial activity

Root exudates not only deposits the C compounds in the rhizosphere region and alter the chemical composition of the rhizosphere soil, but also serve as food for microorganisms (Brant *et al.*, 2006). Singh *et al.* (2007) hypothesised that plants select for beneficial microbial communities in their rhizosphere by releasing certain metabolites targeting specific microbes. While penetrating into the soil, root tips exert tremendous pressure ($> 7 \text{ kg/cm}^2$), thereby ensuring smooth growth. The epidermal cells also secrete a viscous, high molecular weight insoluble substance called mucilage, which not only lubricates the root tips but also assists the plants in nutrient acquisition and improves soil quality by increasing water infiltration

and aeration (Bengough and McKenzie, 1997). This mucilage also attracts beneficial microorganisms and serves as a bait for root pathogens, where oxidation/reduction of metal(loid)s takes place to sequester the metal(loid)s (Hawes *et al.*, 2000).

Numerous studies indicate higher solubility of heavy metal(loid)s such as As, Cu, Zn, and Pb in soils with plants than control soil without plants, which can be attributed to exudates forming soluble complexes with the metal(loid)s. For example, Banuelos and Lin (2007) conducted a field study on the effect of methionine on Se transformation and found higher rates of volatile Se in vegetated plots compared to the control plots. They attributed the transformation to additional microbial activity as effected by root exudates of the plants. Bolan *et al.* (2013) observed that increased microbial activity in the rhizosphere of native vegetation contributed to the reduction in the concentration of As and Cr. In this regard, it is well known that plants produce root exudates containing organic acid anions, sugars, vitamins, amino acids, inorganic ions, and some enzymes, which likely have substantial impacts on microbial population development and biological activity. Hence, the interaction between plant roots and soil microbes can enhance metal bioavailability in rhizosphere.

Apart from the plant roots, some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microorganisms can also excrete extracellular polymeric substances (EPS), such as polysaccharides, glucoprotein, siderophores, lipopolysaccharide, soluble peptide etc. These substances possess a substantial quantity of functional groups which can coordinate with metal(loid) ions. A number of microbes are involved in EPS production viz., *Bacillus megaterium*, *Acinetobacter*, *Pseudomonas aeruginosa*, SRB, soil fungi and cyanobacteria (Satpute *et al.*, 2010; Flemming and Wingender, 2001; Suh *et al.*, 1999; Domozych *et al.*, 2005; Domozych, 2007).

Similar to the endorhizosphere of plant roots, the cell wall of microbes also plays a major role in metal(loid) adsorption/reduction (Hall, 2002). Some studies have even proposed complexation, ion exchange, adsorption (by electrostatic interaction or van der Waals force), inorganic precipitation, oxidation and/or reduction as part of metal uptake mechanisms by organisms (Volesky, 1990; Pietrobelli *et al.*, 2009).

In metal(loid) contaminated soil, the poor performance of plant growth and root development are major limiting factors for phytoaccumulation of metal(loid)s. To overcome these problems, improvement of the microbial activity in rhizosphere in addition of organic amendments is necessary. Plant growth promotion mechanisms by indole acetic acid (IAA), siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing microbes in the presence of metal are not well known. Most of recent studies assume that demonstrating the presence of all or even some of these activities is sufficient to elaborate the mechanisms. These findings suggest that (i) IAA promotes plant growth *per se* (Patten and Glick, 2002), (ii) ACC deaminase prevents the stress by-product ethylene which inhibits plant growth (Penrose *et al.*, 2001) and (iii) siderophores help plants to acquire sufficient Fe in the presence of overwhelming amounts of other (potentially competing) metal(loid)s (Burd *et al.*, 2000). The Ni resistant ACC deaminase-containing plant growth-promoting bacterium could decrease the toxicity of Ni to canola plants (Burd *et al.*, 1998), there have been a large number of reports of facilitation of metal(loid) phytoextraction through the addition of plant growth-promoting bacteria and fungi. A number of scientific evidences proved that the inoculation of plant growth-promoting bacteria facilitated plant growth and increased the uptake of metal(loid) by the plant (Rajkumar *et al.*, 2006; Ma *et al.*, 2009; Sheng and Xia, 2006). Pii *et al.* (2015) highlighted that plant growth promoting bacteria can positively affect the

plants at both physiological and molecular levels (e.g. inducing rhizosphere acidification, genetic regulation of ion uptake).

In soil, heavy metal(loid)s can have long-term toxic effects within ecosystems (Oliveira and Pampulha, 2006) and have a negative influence on biologically mediated soil processes (Lee *et al.*, 2002). It is generally accepted that accumulation of metal(loids) reduces soil microbial biomass (Yang *et al.*, 2007) and various enzyme activities, leading to a decrease in the functional diversity in the soil ecosystem (Crowley, 2008) and changes in the microbial community structure. However, metal(loid) exposure may also lead to the development

of metal(loid) tolerant microbial populations (Ellis *et al.*, 2003). For example, many microbes have been reported to reduce Cr(VI) under aerobic and anaerobic condition. Bio-reduction of Cr(VI) can be directly achieved as a result of microbial metabolism (Nanchaiah *et al.*, 2010) or indirectly achieved by a bacterial metabolites such as H₂S (Okeke, 2008). Therefore, C based root exudates along with rhizodeposition induced microbial metabolism can enhance rhizosphere process thereby influencing metal(loid) transformation in the rhizosphere region. Phytoremediation strategies can be drawn based on the root exudate and the resultant microbial metabolites for restoring metal contaminated sites.

Figure 4. Exudation of some low molecular organic acids by roots of *Solanum nigrum* as affected by varying Cd concentration in soil (Bao *et al.*, 2011). Control: No Cd (0 mg/kg); Cd1: 1 mg/kg; Cd2: 5 mg/kg; Cd3: 10 mg/kg

6. Conclusion

Plant pathologists have been very active in research relating to rhizosphere influence on the infection of a number of root diseases. Soil scientists on the other hand seem to have neglected the importance

of rhizosphere in the transformation of nutrients and contaminants in soil. A greater understanding of the biological (microbial activity) and chemical (exudates) changes in the rhizosphere will enable us to identify the processes involved in the mobilisation of nutrients and heavy metals added to soils.

Further study is required to describe the ecological and physiological characteristics of the microbial communities associated with plant roots and to identify the zone of influence of various plant species in relation to remediation of heavy metals.

Plant roots are known to provide not only physical support to the plants, but also help at absorbing moisture and acquisition of nutrients. The region around root and soil is a dynamic interface of several biogeochemical processes as mediated by exudation of chemicals and resultant microbial activity. Understanding and utilising these interactions for heavy metal remediation on a contaminated land is a challenge for generations to come. With the advent of new and precise technologies (e.g. high resolution imaging) for rhizosphere observation and analyses, developing reliable technologies for heavy metal remediation at field level requires collaboration of soil scientists, microbiologists, plant biologists and mathematicians.

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