Chapter 15
Role of Earthworms on Phytoremediation of Heavy Metal-Polluted Soils

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15.1 Introduction

Heavy metal contamination of soil is a major concern in all parts of the world, in particular in emerging countries where there is an increasing need for soil for food. Heavy metals such as cadmium, copper, lead, mercury, and zinc remain in the soil where they accumulate as a result of activities such as mining and the application of urban sewage sludge for agriculture. The accumulation of heavy metals in the environment can affect the health of humans and animals. At microscale, heavy metals also have an adverse effect on bacterial populations which in turn affects the global functioning of ecosystems. Microorganisms play a key role in biogeochemical processes. Changes in the microbial communities may reduce their ability to maintain soil fertility in the long term. This has led to the recent development of techniques for cleaning up polluted soils and sites. One such technique is phytoremediation, which exploits the ability of certain plants to accumulate large amounts of heavy metals (Chaney et al. 1997; Salt et al. 1998; Padmavathiamma and Li 2007). Phytoremediation has many advantages: (1) it is the only method available for in situ extraction of heavy metals from soils; (2) it is economically viable as, at least theoretically, the energy required for the process is free (from the

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sun), and the harvestable parts of the plants that accumulate heavy metals can be used for energy, biological catalysts, insulators, etc.; and (3) it has a low environmental impact consistent with environmental protection policies and allows the soils to be exploited for crops after or at the same time as the soil is being remediated (Losfeld et al. 2012). However, phytoremediation has limitations: (1) the slow growth and low biomass require a considerable investment in time and/or money, and (2) the heavy metals accumulate slowly in the plants as the pools of heavy metals available to the plants at a given time are small. Chelating agents such as EDTA, DTPA, and citric acids have been tested successfully (Luo et al. 2005; Luo et al. 2006a, b) but may have undesirable effects such as toxicity for plants (Evangelou et al. 2007), and (3) plant growth is reduced by the phytotoxicity of the heavy metals (Shah and Nongkynrih 2007; Salt et al. 1998; Singh et al. 2003).

To improve the performance of phytoextraction, hyperaccumulating plants with high biomass (e.g., *Brassica juncea* or Indian mustard) are used. Recent research has concentrated on the role of the rhizosphere with a view to associating microbial bioaugmentation of soils with phytoextraction (Lebeau et al. 2008; Sessitsch et al. 2013; Wenzel 2009; Khan 2005), but few studies have considered the drilosphere compartment, the part of the soil influenced by earthworm secretions and castings (Aghababaei et al. 2014a; Du et al. 2014; Jusselme et al. 2012, 2013). However, earthworms as ecological engineers play an important role in their environment (Derouard et al. 1997; Bohlen et al. 2002; Dechaine et al. 2005; Tapia-Coral et al. 2006). The positive effects of earthworms on plant production (Table 15.1) have been extensively documented (Blouin et al. 2007, 2013; Wang et al. 2006) as well as their effects on heavy metal solubility and availability (Sizmur et al. 2011b, c, d). The interactions between heavy metals and earthworms depend on the earthworm species, the metal, and the physical and chemical properties of the soil (Weltje 1998; Morgan and Morgan 1999; Sizmur and Hodson 2009). Earthworms have an effect on metal speciation in soils, changing the bioaccessibility and bioavailability of the metals for other organisms, such as plants (Sizmur et al. 2011a). This chapter summarizes the current understanding of the interactions between earthworms, plants, and microorganisms in heavy metal-contaminated soil. It covers basic research as well as practical phytoremediation.

15.2 Earthworms as Ecosystem Engineers

The term "ecosystem engineers" was used by (Lawton 1994) to designate organisms that directly or indirectly influence the availability of resources to other species by causing physical state changes in biotic and abiotic materials. Earthworms in tropical soils are recognized as key ecosystem engineers as they modify, maintain, and create habitats (Jones et al. 1994; Lavelle 1996).

Earthworms (annelids, oligochaetes) are the dominant biomass of soil macrofauna in most terrestrial ecosystems. About 7,000 species have been

Table 15.1 Earthworm effects on plant biomass in heavy metal-polluted soils

Soil conditions	Plants	Earthworms		Plant biomass Shoot (g)	Root (g)	Reference
Pb pollution at 1,000 mg kg^{-1}	*Lantana camara*	*Pontoscolex corethrurus*	Without	21.52	19.11	Jusselme et al. (2012, 2013)
			With	31.77	24.21	
Pb/Zn mine tailings at 1,202 mg Pb kg^{-1}	*Leucaena leucocephala*	*Pheretima guillelmi*	Without	30.0		Ma et al. (2006)
			With	34.0		
Cu pollution 400 mg kg^{-1}	Ryegrass (*Lolium multiflorum*)	*Metaphire guillelmi*	Without	1.23	0.33	Dandan et al. (2007)
			With	2.67	0.45	
Zn pollution at 400 mg kg^{-1}	Ryegrass	*Pheretima* sp.	Without	1.79	0.67	Wang et al. (2006)
			With	2.66	1.11	
	Indian mustard	*Pheretima* sp.	Without	1.04	0.12	
			With	1.41	0.27	
Cd pollution at 20 mg kg^{-1}	Ryegrass (*L. multiflorum*)	*Pheretima* sp.	Without	1.78	0.53	Yu et al. (2005)
			With	1.96	0.46	

identified, divided into 15 families, most of which live in the tropics (Lavelle 1997). They play an important role in environmental functioning through various physical and biological mechanisms that preserve the structure of the soil and improve its fertility (Stork and Eggleton 1992; Lavelle 1997). By modifying the physical and chemical properties of the soil, they also change the habitats of microbial communities (Lavelle 1997).

15.2.1 Main Geographic Origins and Taxonomy

Earthworms are found in all tropical and temperate soils with a high level of diversity. Quaternary glaciers caused earthworms to become locally extinct so that they are found in greater abundance in the tropics. Earthworms fall into three broad ecological categories—epigeic, anecic, and endogeic—depending on morphology and behavior (Bouche 1977).

1. *Epigeic earthworms* (Bouche 1977) are small (10–30 mm) and generally live in litter and decomposed organic matter. They are also found in the feces of large herbivores or in damp woods during decomposition. They live on the soil surface and are, therefore, particularly susceptible to predation, climate variability, and anthropogenic activities such as surface plowing and the application of pesticides. Epigeic earthworms play an important role in recycling organic matter.
2. *Anecic earthworms* (Bouche 1977) are medium to giant worms (10–110 cm) living in vertical or subvertical burrows with varying degrees of branching that open onto the soil surface. By ingesting soil and burying organic matter, they mix the organic matter and mineral fraction from the different soil horizons. These species are found throughout the entire depth of the soil profile and have strong muscles enabling them to adapt to a relatively high soil compaction and withstand human pressures in cultivated soils.
3. *Endogeic earthworms* (Bouche 1977) vary in size (1–20 cm). They account for 20–50 % of the biomass of fertile land and live in the soil, burrowing in any orientation. They feed on the organic matter in the soil and in poorer soils may need to be very mobile to find all the food they need. Lavelle (1981) defined three subcategories of endogeic earthworms—polyhumics, mesohumics, and oligohumics—based on the richness of the soil organic matter they eat, oligohumic earthworms being those which ingest organic matter in the poorest soils.

15.2.2 Impact of Earthworms on Soil

The impact of earthworms on soil depends on their ecological category, endogeic and anecic having the greatest effect (Brown et al. 2000). The main physical activities of earthworms include (1) the creation of galleries in which they move and (2) excretion in the galleries (feces) or on the soil surface (casts). The drilosphere is the area where soil functioning is influenced by earthworm activities. It includes all dependent physical structures of earthworms such as the contents of the digestive tract, casts, and galleries as well as associated communities of invertebrates and microorganisms. The structure and relative importance of the drilosphere are determined by the climate, soil parameters, and quality of organic inputs (Lavelle 1997).

15.2.2.1 Galleries

The gallery network (size, orientation, etc.) depends on the ecological category of the earthworm. The number of galleries in the soil depends on the abundance of earthworms but can be up to several hundreds per m^2. In sites with large earthworm communities, the volume of the galleries contributes significantly to the pore size of the soil, providing passageways for air and water in the soil (Bouché and Al-Addan 1997). These galleries improve the porosity and aeration. Experiments have shown that, in microcosms, the galleries of *L. terrestris* earthworms significantly increased the water flow (Joschko et al. 1989). Field studies clearly support these findings by showing the transfer of water through the *L. terrestris* galleries (Edwards et al. 1992) and the strong correlation between the infiltration rate and the length, area, and volume of the galleries of the anecic earthworm *Scherotheca gigas mifuga* (Bouche 1977).

Moreover, galleries make it possible to transfer compounds from different soil horizons, both passively by percolation and infiltration and by the active role of anecic burrowing species. Earthworms line their galleries with mucus- and nutrient-rich droppings as they pass through the soil (Binet and Curmi 1992). This makes the walls of galleries richer in organic carbon and nitrogen than the surrounding soil. These relationships stimulate the development of a high density of bacteria throughout the gallery walls, increasing respiratory activity and enzymatic digestion. Tiunov and Scheu (1999) showed that microbial biomass was higher by a factor of 2.3–4.7 in the walls of *L. terrestris* galleries than in the surrounding soil. This microbial growth increased the soil respiration by a factor of 3.7–9.1 in forest ecosystems.

15.2.2.2 Casts

Earthworms ingest soil and excrete waste onto the soil surface or in the galleries. They produce casts on the surface amounting to between 200 and 250 t ha^{-1} $year^{-1}$ in temperate soil and 40–50 t ha^{-1} $year^{-1}$ in grassland, representing a soil thickness of 3–4 mm. Binet and Le Bayon (1998) evaluated the production of casts from 2.5 to 3.2 kg m^{-2} $year^{-1}$ (dry weight) in a temperate maize crop.

However, the production and abundance of earthworm casts depend on environmental conditions (climate, soil type), the earthworm species, and the vegetal cover.

For example, the disappearance of earthworm casts accounts for about 70 % and 20 % in rainy season and dry season, respectively. The casts are gradually incorporated into the soil matrix during the dry season (Binet and Le Bayon 1998).

The feeding behavior of earthworms leads to considerable variability in the composition of their casts. The physical structure of the casts provides microenvironmental conditions that differ from the initial soils. Some earthworms feed selectively on the parts of the soil that are rich in organic matter (Zhang and Schrader 1993; Doube et al. 1994). Doube et al. (1997) showed that *L. terrestris* and *A. caliginosa* preferentially consume a mixture of fine inorganic particles and organic material rather than organic material on its own. Fungi are also an important food source for many species of earthworms (Edwards and Fletcher 1988). Earthworms may also feed on protozoa, bacteria, and algae. During transit through the digestive tract of earthworms, these microorganism populations are modified by the physical and chemical conditions in the intestine. The surviving microorganisms (in particular fungal spores, protozoa, and resistant bacteria) are present in the inoculums that subsequently colonize the casts and are responsible for the microbial processes (Brown 1995). Parle et al. (1963a) showed that *L. terrestris* casts had higher concentrations of bacteria and actinomycetes after the soil had passed through the earthworm gut where conditions were favorable for their development. Fungal hyphae developed on the surface of casts (Parle et al. 1963b), a phenomenon that was also observed for the geophagous earthworm *Pontoscolex corethrurus* (Barois et al. 1987). Many studies have demonstrated that earthworms can stimulate soil microbial activity, although the density of bacterial and fungal populations may be reduced after transiting the gut of endogeic earthworms (Krišrtuek et al. 1992). Enzyme activities can provide information on the functional diversity of the microbial community. Tiwari et al. (1989) showed that phosphatase, dehydrogenase, and urease activities were more intense in casts. High phosphatase activity has also been found in fresh casts of the endogeic earthworm *A. caliginosa* (Aira et al. 2010). These enzyme activities can affect the bioavailability of mineral elements such as phosphorus (Satchell and Martin 1984).

15.3 Earthworms and Heavy Metals

Earthworms are more sensitive to heavy metals than other invertebrates living in soils (Bengtsson et al. 1992), and their ability to accumulate heavy metals is often greater than for other animal species (Beyer et al. 1982). However, the effects of heavy metals depend on the earthworm species, stage of development, lifestyle (where they live and what they eat), and their ability to adapt to contaminants. These effects also depend on the nature and chemical forms of the metal and the physical and chemical properties of the soil.

An increase in the heavy metal content in soil above acceptable levels reduces the density of earthworms (Pizl and Josens 1995) and also reduces weight gain (Spurgeon and Hopkin 1996), sexual development, and cocoon production

(Spurgeon and Hopkin 1999). Spurgeon and Hopkin (1999) reported a significant reduction in the survival rate of four species of earthworm with zinc levels ranging from 2,000 to 3,600 mg Zn kg^{-1} of soil. They also reported significant weight loss with zinc levels ranging from 1,200 to 2,000 mg Zn kg^{-1}. Lukkari and Haimi (2005) suggested that one of the potential mechanisms of adaptation to pollution is avoidance as earthworms placed in contaminated soil may be able to differentiate organic matter according to its level of contamination.

The ability of earthworms to accumulate heavy metals was recognized in the literature as early as the late nineteenth century (Hopkin 1989). Heavy metals accumulate in the digestive tissues of earthworms after ingestion or by dermal exposure as earthworms have no protective cuticle and are in continuous contact with the polluted soil. Many studies have determined the factors controlling bioconcentration: (1) the earthworm species and its ecological category, (2) the heavy metal species, (3) the physical and chemical properties of the soil, (4) the season, and (5) the distance from the source of contamination. Studying heavy metal accumulation in earthworm tissue might be a good bioindicator of heavy metal availability in soils (Lanno and Mccarty 1997; Conder and Lanno 2000; Paoletti 1999; Oste et al. 2001).

15.4 Earthworms and Phytoremediation

15.4.1 Evidence of the Effect of Earthworms on Phytoremediation

The effects of earthworms on phytoremediation performance were described for the first time by (Ma et al. 2003) who found that the presence of the anecic earthworm *Pheretima guillelmi* increased the amount of Pb (mostly in roots) extracted by the leguminous plant *Leucaena leucocephala*. These results are in line with those of (Wang et al. 2006) who reported an increase of Zn phytoextraction by ryegrass and Indian mustard when the soil was inoculated with the earthworm *Pheretima* sp. The additional accumulation of heavy metals by plants as the result of earthworms was confirmed by (Dandan et al. 2007) with Cu uptake by ryegrass in the presence of *Metaphire guillelmi*. In recent years (Ruiz et al. 2009), used soil microcosms to show that the epigeic earthworm, *Eisenia fetida*, significantly increased the growth of maize (*Zea mays*) and barley (*Hordeum vulgare*) and resulted in the accumulation of heavy metals (Cu, Cd, Pb, and Zn). The plant growth led to a threefold increase in Zn extraction. The presence of the anecic earthworm *L. terrestris* also significantly increased the phytoextraction of Pb and Zn by maize and barley, although to a lesser extent (Ruiz et al. 2011). More recently, (Jusselme et al. 2012) studied the interaction between *Lantana camara* which is a hyperaccumulating plant for lead and cadmium and the endogenous tropical earthworm *P. corethrurus* (Oligochaeta, Glossoscolecidae) commonly found in both polluted and unpolluted areas. In this study, which used Pb-spiked soil in microcosms (500 and 1,000 mg kg^{-1}), most of the earthworms introduced into the microcosms remained alive (>90 %) after 1 month, and all the soil was burrowed by earthworms. With *P. corethrurus*, an increase of shoot and root biomass was recorded as well as an increase of lead uptake by plants (Jusselme et al. 2012). Eventually, Du et al. (2014) showed that the influence of the earthworm *Eisenia fetida* on the accumulation of Cd in leaves or stems of corn resulted in a Cd concentration in the soil of more than 1,000 mg Cd kg^{-1}. Conversely, *Pheretima* sp. only improved the phytoextraction of Zn and Pb by ryegrass *Lolium multiflorum* in moderately contaminated soils after the third harvest.

All these studies clearly demonstrated that all types of earthworms have a clear effect on the phytoremediation by various plants of soils contaminated by heavy metals. Some of the mechanisms by which earthworms influence phytoremediation are described below.

15.4.2 Mechanisms by Which Earthworms Influence Phytoremediation

15.4.2.1 Interactions Between Earthworms and Plants

Most studies showed that earthworms affected the growth of hyperaccumulating plants, in particular the roots (Table 15.2). The overall health of the plant is often given as the main factor for the increase in heavy metal phytoextraction performance. For example, Wang et al. (2006) showed that soil bioaugmentation by earthworms increased the biomass of ryegrass and Indian mustard which resulted in greater uptake and accumulation of zinc. The positive effect of earthworms on growth and heavy metal accumulation by plants may be direct and/or indirect through a positive effect on soil microorganisms such as arbuscular mycorrhizal fungi (AMF) (Eisenhauer et al. 2009; Ortiz-Ceballos 2007; Ma et al. 2006; Gaur and Adholeya 2004) and almost all plant growth-promoting bacteria (PGPB) (Sinha 2010; Wu et al. 2012) that are themselves known to improve phytoextraction performance (Lebeau et al. 2008; Sessitsch et al. 2013). In return, the earthworms use root exudates as a nutrient source to survive in polluted conditions. Earthworms increase the dispersion rate of viable mycorrhizal propagules and actinomycetes such as *Frankia* and PGPB, some of which are nitrogen fixing (Wu et al. 2006). The effect of microbial stimulation on the amount of nitrogen fixed by the plants could be an important part of the positive effect of earthworms. The microorganisms increase the primary biomass by stimulating the plant growth in various ways. Firstly, PGPB increase the plant biomass and root surface as well as reduce the toxicity of heavy metals to the plant. The amount of ethylene produced by the plant during induced heavy metal stress can be reduced by the degradation of ACC (aminocyclopropane carboxylic acid), a precursor of ethylene, by ACC-deaminase produced by PGPB (Ma et al. 2009a, b, 2011; Braud et al. 2009;

Table 15.2 Earthworm effects on heavy metal phytoextraction

	Barley		Maize		*Lantana camara*		Indian mustard		Ryegrass		Tomato	
Heavy metals	Without E	With E	Without E	With E	Without E	With E	Without E	With E	Without E	With E	Without E	With mucus E
Cd												
Shoot	n.d	2.8 ± 0.4	n.d	n.d							161.6 ± 14.1	265 ± 13.1
Root	n.d	14.4 ± 3.8	n.d	n.d							117.4 ± 82.8	2474.5 ± 119.8
Cu												
Shoot	10.8 ± 2.1[a]	9.5 ± 1.4	7.65 ± 0.57	10.2 ± 2.0					52.98[b]	69.52		
Root	14.8 ± 1.3	18.5 ± 2.2	8.26 ± 0.58	17.6 ± 3.6					854.97	1312.5		
Shoot	18.4 ± 4.2	15.2 ± 3.0	6.89 ± 2.15	9.5 ± 4.3								
Root	16.8 ± 2.0	19.0 ± 8.0	7.16 ± 1.94	17.7 ± 4.7								
Pb												
Shoot	26.5 ± 5.3	32.0 ± 6.8	26.6 ± 3.8[c]	20.8 ± 6.8	225.97 ± 74.9[d]	522.77 ± 135.1			5.76	13.36		
Root	109.1 ± 26.8	165.0 ± 58.7	101.3 ± 14.25	263.1 ± 66.1	1645.9 ± 590.9[d]	3284.08 ± 328.1			185.9	228.4		
Shoot	17.0 ± 3.6	21.9 ± 4.5	13.8 ± 5.0	13.2 ± 3.7								
Root	27.9 ± 4.7	51.7 ± 6.5	15.3 ± 5.4	30.6 ± 16.8								
Zn												
Shoot	123.6 ± 25.8	267. 0 ± 24.9	246.9 ± 30.9[c]	351.2 ± 36.0					210.5	285.5		
Root	454.6 ± 80.2	1305.4 ± 268.0	271.5 ± 44.5	567.7 ± 139.1					742.5	909		
Shoot	76.9 ± 3.8	86.2 ± 7.3	77.1 ± 3.3	86.6 ± 11.8			503.8[e]	774.5	621.11[e]	996.04		
Root	144 ± 17	198 ± 8.7	60.3 ± 4.2	137 ± 14			101.9[e]	240.3	467.75	928.17		

[a] Ruiz et al. (2009)
[b] Wang et al. (2007)
[c] Ma et al. (2003)
[d] Jusselme et al. (2012)
[e] Wang et al. (2006)

Denton 2007; Dimkpa et al. 2009; Grandlic et al. 2009). Secondly, absorption of iron by the plant can be facilitated by bacterial siderophores (Crowley et al. 1988, 1992; Bar-Ness et al. 1992; Glick 2003). Thirdly, some authors (Tomati et al. 1988; Castellanos Suarez et al. 2014) have tested the hypothesis that bacterial phytohormones, in particular indoleacetic acid (IAA), that stimulate root growth and are activated in earthworm casts are responsible for the increase in the root biomass and the available heavy metal for plants. The exploration of the casts by roots facilitates the assimilation of large amounts of resources with a reduced investment in carbon. The resulting carbon gain reduces catabolism, increases chlorophyll synthesis, and improves the rate of CO_2 fixation, consequently accelerating plant growth. This excess energy can also enable the plant to respond to environmental stress such as heavy metal toxicity.

15.4.2.2 Interactions Between Earthworms and Heavy Metals

Many studies have addressed the impact of earthworms themselves and their activities on the dynamics of heavy metals in the soil in terms of solubility, extractability, and bioavailability (Abdul Rida 1996; Devliegher and Verstraete 1996; Wen et al. 2004; Udovic and Lestan 2007). The presence of the earthworm *Lumbricus terrestris* increased Cu availability by 6 % (Devliegher and Verstraete 1996). Some earthworms such as *L. terrestris*, *L. rubellus*, and *Aporrectodea caliginosa* can survive in heavy metal-polluted soils (Langdon et al. 1999; Morgan and Morgan 1999; Kızılkaya 2008). They influence the mobility and availability of metal through their burrowing and casting activity (Sizmur and Hodson 2009; Sizmur et al. 2011a, b, c, d). However, the effect of these organisms on the bioavailability of heavy metals for plants remains very modest in heavy metal-contaminated sites (Abdul Rida 1996).

It was shown that earthworms can influence the heavy metal availability in soils by mixing deep soils, humus, and biological material in the earthworm gut (Hobbelen et al. 2006; Cheng and Wong 2002). Ma et al. (2002) demonstrated that the concentration of the available Pb was increased by up to 48.2 % by earthworm inoculation, and (Cheng and Wong 2002) suggested that earthworm burrowing and feeding activities increased Zn availability.

At present, most results concerning the effects of earthworms on the availability of heavy metal have been obtained from artificially contaminated soils and/or microcosm experiments. (Smolders et al. 2009) stated that soils artificially contaminated with fully soluble metal sources do not represent conditions prevailing in naturally metal-rich contaminated soils, and (Spurgeon and Hopkin 1995) reported that heavy metals in artificially contaminated soils are likely to be more bioavailable than in "naturally" polluted soils. Therefore, further studies are required to determine the extent to which differences between the results under controlled conditions and field results can be explained by the differences between artificially contaminated and "naturally" polluted soils.

While the application of earthworms to soils to increase heavy metal availability has in some instances increased metal extraction from soils, it is also important to note that there are some drawbacks that may have practical implications. Although heavy metal migration through the soil is very slow (e.g., 0.01 cm $year^{-1}$ for Pb considered to be relatively immobile) (Kylander et al. 2008), if the available heavy metals exceed the capacity of metal absorption by plants, it is possible that, in some soils, the increase of metal availability by the earthworm activity may lead to faster diffusion.

15.4.2.3 Earthworms and Soil Enzyme Activities

The capacity of plants to absorb heavy metals depends on the plants' health. Plant health in turn depends on soil quality. Moreover, a phytoextraction process must aim not only to remove the heavy metal from the soil but, more importantly, to restore soil quality (Doran and Safley 1997). Therefore, soil quality indicators are needed to assess the overall performance of a phytoextraction process. Of the various biological indicators, soil enzyme activity has been suggested as a good indicator of soil quality (Alkorta et al. 2003) as this plays an important role in mineralization processes that convert organic compounds into inorganic compounds. The role of enzymes in soils is expressed as the quantity of nutrients released such as nitrate, phosphorus (P), and potassium (K) that are important for plant growth.

In heavy metal-contaminated soils, most enzymes are inactive as they are inhibited by the protein-binding capacity of metals (Alkorta et al. 2003). Enzymes such as xylanase, cellulase, alkaline phosphatase, and fluorescein diacetate (FDA) activities are affected by the amount of heavy metal in the soils although (Jusselme et al. 2013) concluded that lead pollution ranging between 500 and 1,000 mg kg^{-1} of soils does not inhibit soil enzyme activities. These discrepancies can be explained by the nature and degree of inhibition of heavy metals as the effects depend largely on soil type, heavy metal levels, and soil physical and chemical properties.

Earthworms play a major role in promoting soil health, in particular soil enzyme activities. A comparison of soil enzyme activities in the presence and absence of earthworms by (Jusselme et al. 2013) in a Pb-phytoextraction experiment with Pb ranging between 500 and 1,000 mg kg^{-1} of soil clearly showed that the presence of earthworms significantly increased most enzyme activities. This was particularly true in the root-adhering soil of *Lantana camara*, the hyperaccumulating plant used in this study. However, these authors also showed that the increased activities of *N*-acetyl-D-glucosaminidase and urease involved in the nitrogen cycle in the presence of earthworms resulted in lower nitrogen availability for plants. This may be explained by the complex interactions between plants, nutrient availability, and earthworms: (1) initially, the earthworm activities increase N availability, improving plant growth and health and so stimulating the metal phytoextraction process, but the uptake of available nitrogen by the plants increases too fast and the available N becomes too scarce by the end of phytoextraction process, and (2) the earthworm activities improve plant growth and heavy metal uptake by mechanisms not based on nitrogen. Blouin et al. (2006) showed that earthworms (*Millsonia anomala*) do not increase rice growth by improving nitrogen mineralization. Phosphorus (P) is also an important nutrient for plant growth and reproduction. Plants use phosphorus for root development, flower initiation, and seed and fruit development (Fuhrman et al. 2005). James (1991) showed that P availability in earthworm casts could contribute about 50 % of the plants' requirements. Satchell and Martin (1984) suggested that higher levels of P availability in earthworm casts were based mainly on increased phosphatase activity, involved in the hydrolysis of organic P compounds in the casts, although it has not been established whether the increase in the activity is due to earthworm-derived enzymes or to increased microbial activity. The increase in P availability as a result of an increase in enzyme activities by earthworms improves plant growth as well as Pb uptake (Jusselme et al. 2012). Unlike nitrogen and phosphorus, potassium does not form any vital organic compounds in the plant. However, the presence of K is vital for plant growth because K is known to be an enzyme activator that boosts the metabolism. Jusselme et al. (2013) demonstrated that earthworms increased FDA activity, which could lead to increased K availability for plant uptake.

Since enzyme responses depend on the type of enzyme, enzyme activity could be used as a biological indicator to assess heavy metal-contaminated soil functioning. This result can be explained by the interaction of plant/microorganisms/earthworms as shown in Sect. 15.4.2.3.

15.4.2.4 Interactions Between Earthworms and Soil Microorganisms

Trace metals are known to be toxic to soil microorganisms (Del Val et al. 1999; Giller et al. 1998). They reduce the microbial activity (Lorenz et al. 2006; Oliveira and Pampulha 2006), diversity (Hassan et al. 2011; Hu et al. 2007), and abundance (Liu et al. 2012; Pasqualetti et al. 2012). However soil functioning is a result of tight interactions between microorganisms, plants, and soil macrofauna. Soil microorganisms are in part influenced by soil macrofauna (Aira et al. 2002, 2007, 2010). It has been shown that earthworms have a significant effect on the composition, distribution, and activity of soil fungi, in particular by (1) ingesting fungal spores and even ingesting certain fungi, (2) creating microsites favorable to fungal development, (3) dispersing fungal species, and (4) transforming and redistributing soil organic matter (Brown 1995; Lavelle 2002; Scheu et al. 2002). Despite a large body of literature on the impact of earthworms and microorganisms on heavy metals in soils, only a few studies have addressed the question of earthworm-assisted heavy metal phytoextraction (Wang et al. 2006; Yu et al. 2005) although without considering the role of microorganisms (Aghababaei et al. 2014b).

The question arises whether the beneficial effect of earthworms on phytoextraction performance results from the stimulation of soil microorganisms. Although earthworms may be able to increase metal bioavailability in heavy metal-contaminated soil, the mechanism remains unclear. Sizmur and Hodson (2009) suggested four principal mechanisms by which earthworm activities may change heavy metal mobility and bioavailability: (1) modification of soil pH, (2) modification of soil dissolved organic carbon (DOC), (3) heavy metal speciation and sequestration within the earthworm tissue, and (4) stimulation of the soil microbial population.

Although heavy metals are toxic to soil microorganisms, Jusselme et al. (2012) showed that the total microbial activity and fungal richness index based on DGGE patterns increased with Pb pollution in the root-adhering soil of *L. camara*. This did not agree with previous results for other heavy metals (As, Cd, Hg) in long-term contaminated sites (Lorenz et al. 2006; Oliveira and Pampulha 2006). There may be various explanations for this discrepancy: (1) the duration of the exposition in this study was short (1 month vs. several years), and (2) the growth and activity of *L. camara* roots may have stimulated microbial activity as a result of a higher amount of rhizodeposits and counterbalanced the toxicity of Pb.

Microbial activity increases in the presence of earthworms as shown in many studies (Aira et al. 2008, 2010; Dempsey et al. 2013; Gómez-Brandón et al. 2012; Tao et al. 2009; Tiwari and Mishra 1993). As earthworms digest decaying substrates, they increase the pool size of nutrients available for microorganisms, promoting microbial growth. Microorganisms are largely dormant in the soil waiting for favorable conditions which are provided by earthworm burrowing and casting (Lavelle 2002). Earthworms significantly increase total fungal abundance and all fungal diversity indices, as has already been shown (Krišrtuek et al. 1992). Del Val et al. (1999) and Jusselme et al. (2013) showed that, in heavy metal-spiked soils bioaugmented with earthworms, the structure of the fungal community is modified: the appearance of new bands indicated that minor populations in uncontaminated soils became dominant in heavy metal-spiked soils as the result of their tolerance. The positive effect of earthworms on the abundance of cultivable fungi counteracted the negative effect of heavy metals in the polluted soils in spite of the higher bioavailable concentration of heavy metals in soils. The effects of earthworms on the activity, abundance, and structure of the fungal community may (1) increase fungal growth by means of decaying substrates (Brown 1995) and (2) select fungal populations more adapted to heavy metal contamination (Hui et al. 2012). Smith and Reed also showed that the activity of mycorrhizal fungi was increased by earthworms resulting in an increase in the exchange surface between plants and soil, increasing plant uptake and biomass. The effect of earthworms alone on Cd availability is greater than that of AMF in Cd-polluted soils, and interactions between these organisms have a much greater effect on soil microorganisms than on Cd availability. Thus, the presence of both earthworms and AMF could mitigate the effects of Cd on soil microbial life (Aghababaei et al. 2014b).

These results suggest a combined positive effect of earthworms and soil microorganisms on (1) the availability of heavy metals in soil and (2) the availability of nutrients leading to a higher plant biomass and increasing heavy metal absorption and accumulation by plants. Earthworms, as soil ecosystem engineers, are known to change the microbial composition and to stimulate its activity (Brown 1995; Binet and Le Bayon 1998), while soil microorganisms, as decomposers, improve nutrient mineralization and availability for plants (Berg and Laskowski 2005).

15.5 Conclusion: Mutualistic Interactions Between Plants, Microorganisms, and Earthworms

The bioaugmentation of earthworms in soils spiked by heavy metals modifies microbial functioning, counterbalances the effect of heavy metals on the fungal community (abundance, diversity, and structure) and promotes the phytoextraction of heavy metals by plants. Positive interactions between plants, microorganisms, and earthworms form a virtuous circle: improving any one of the interactions improves all the others (Fig. 15.1). Taking just the activation of microorganisms (PGPB and AMF), there is a benefit for both the plants and earthworms.

The activation by earthworms of microorganisms producing compounds which target heavy metals reduces the toxic effect of these metals and increases the heavy metal availability for the plants. The result is an indirect positive effect on plants exposed to heavy metal pollution (Fig. 15.2). Apart from the direct toxicity of heavy

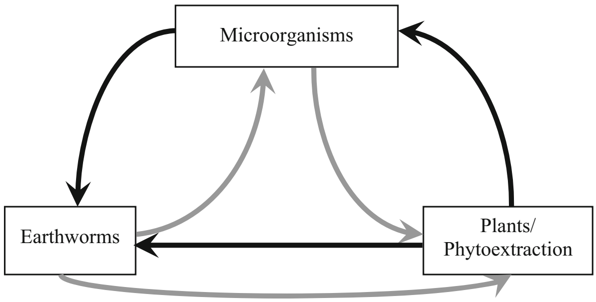

Fig. 15.1 Virtuous circle of interactions between plants, microorganisms, and earthworms

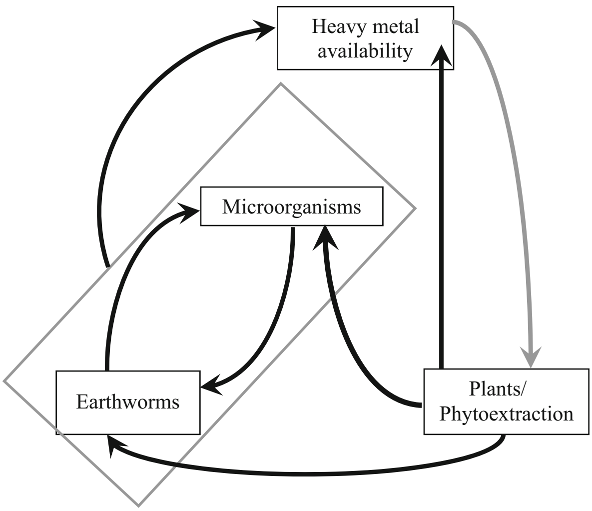

Fig. 15.2 Interactions between plants, microorganisms, and earthworms and Pb availability

metals to plants, improving any one of the interactions is beneficial for plants, microorganisms, and earthworms.

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