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Review

# Do earthworms impact metal mobility and availability in soil? – A review

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*We review the impact of earthworms on metal mobility and availability and suggest areas for further investigation.*

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ABSTRACT

The importance of earthworms to ecosystem functioning has led to many studies on the impacts of metals on earthworms. Far less attention has been paid to the impact that earthworms have on soil metals both in terms of metal mobility and availability. In this review we consider which earthworms have been used in such studies, which soil components have been investigated, which types of soil have been used and what measures of mobility and availability applied. We proceed to review proposed reasons for effects: changes in microbial populations, pH, dissolved organic carbon and metal speciation. The balance of evidence suggests that earthworms increase metal mobility and availability but more studies are required to determine the precise mechanism for this.

## 1. Introduction and scope of review

Earthworms are an essential part of the soil fauna in most global soils, represent a significant proportion of the soil biomass and are regarded as a useful indicator of soil health and quality (Edwards, 2004). It is their important role in the decomposition of organic matter and subsequent cycling of nutrients that has led to their use as an indicator organism for the biological impact of soil pollutants and this in turn has lead to a large body of work on earthworm ecotoxicology (Spurgeon et al., 2003). Earthworms are also often the subject of inoculation programmes during the restoration of degraded lands (Butt, 1999) and inoculation of earthworms to metal-contaminated soils has been suggested (Dickinson, 2000) largely due to the role earthworms are known to play in soil formation at such sites (Frouz et al., 2007).

Earthworms can survive and reproduce in anthropogenically metal-contaminated soil (Spurgeon et al., 1994) and can also accumulate high concentrations of metals within their bodies (Hobbelen et al., 2006). There are many studies that have reported the impact of metals in soil on the inhabiting earthworms, as reviewed by Nahmani et al. (2007), but relatively few that have determined the influence of the earthworms on the chemistry of the metals in the soil.

The purpose of this review is to bring these studies together, in order to highlight what impact earthworms might have on metal mobility and availability in soils, why these impacts might occur and to propose future research needs to obtain definitive solutions to some of the apparent contradictions in the literature. A conceptual model of how earthworms may impact on metal chemistry in soils is shown in Fig. 1. The potential mechanisms identified in Fig. 1 by which earthworms may impact metal chemistry are addressed following a review of the different species of earthworm, different soil components and different types and forms of metals that have been used in studies and chemical extractions and bioassays that have been used to determine changes in metal chemistry in those studies. The studies reviewed in this paper are summarised in tabular form in the Supplementary data.

This review considers impacts of earthworms on metal mobility and bioavailability. Much has been written regarding bioavailability and its companion term bioaccessibility (e.g. Harmsen, 2007; ISO, 2005; Semple et al., 2004) and it is not the aim of this review to provide an extensive critique of these terms. In this review we use the term bioavailability to indicate either that metal which is taken up by an organism into its tissues during a bioassay (i.e. the term relates to a specific organism and a specific set of experimental conditions including time) or those metals that are extractable by a specified chemical procedure. The metals considered bioavailable by this latter procedure may come from a variety of pools in the soil including pore water, cation exchange sites, specific adsorption sites and metal-bearing minerals, it is the nature of chemical extractions that the precise pool from which metals are extracted cannot be identified. If the two broad methods for assessing bioavailability are compared, chemical extractions have the potential to remove metals from a soil that might not be classed as bioavailable by a bioassay due to the fixed duration of bioassays and

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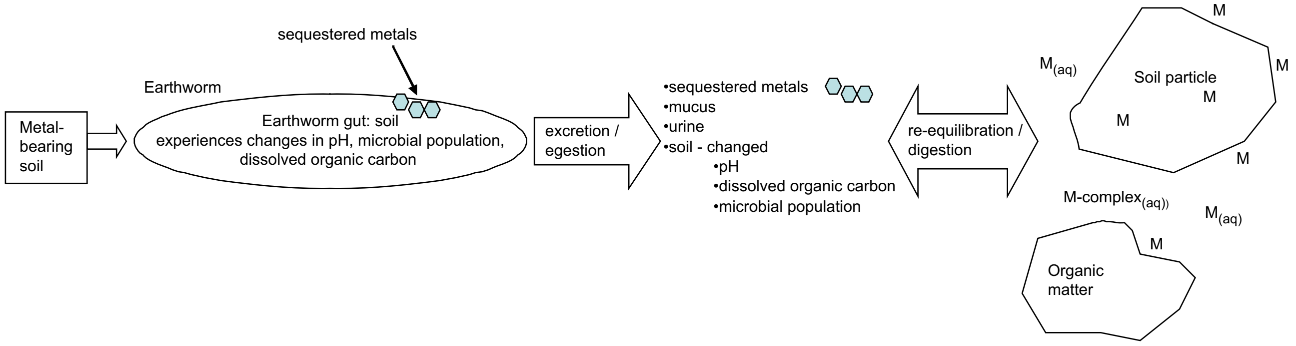

**Fig. 1.** Conceptual model of possible impacts of earthworms on soil metal chemistry. Ingested soil travels through the gut and is egested. The egested soil may have a different pH, bacterial population and dissolved organic carbon content, all of which may modify soil chemistry. Modified bacterial populations may impact on organic matter sorbed metals. pH and dissolved organic matter changes due to egestion of soil and/or excretion of mucus and urine may impact on sorbed metals. Some metals may be sequestered in earthworm tissues and subsequently excreted in a form different from the ingested metal.

the possible limited exploration of the soil by the organism used in the bioassay.

## 2. Species selection

Examples of epigeic, anecic and endogeic earthworms have all been used in studies investigating the impact of earthworms on metal availability and mobility. The epigeic standard toxicity test earthworm *Eisenia fetida* has been widely used to determine the effect of earthworm activity on metal extractability by chemicals (Udovic et al., 2007; Wen et al., 2006) availability to plants (Wen et al., 2004; Liu et al., 2005), humans (via a chemical proxy, Udovic and Lestan, 2007) and, the earthworms themselves (Currie et al., 2005) despite generally not being present in contaminated soils. Typically these studies record an increase in metal availability/mobility in the presence of earthworms. Other epigeic earthworms that have been studied within the context of changes in metal availability include *Eisenia veneta* (Sizmur and Hodson, 2008), *Dendrobaena rubida* (Ireland, 1975) and *Lumbricus rubellus* (Udovic and Lestan, 2007).

Anecic earthworms produce permanent vertical burrows lined with faeces and a mucus and bacteria-rich layer which lead to an increase in the volumes of leachate that pass through soil by the creation of channels of preferential flow (Subler et al., 1997; Farenhorst et al., 2000). However, as the water (or solution) spends less time in contact with the soil surfaces this does not necessarily lead to an increase in the leaching of elements from the burrowed soil. Whilst not common in highly contaminated soils, studies have been performed to investigate the impact of anecic species such as *Lumbricus terrestris* on the availability and mobility of metals in metal amended soils (e.g. Zorn et al., 2005a), mine contaminated soils (Ma et al., 2000) and sewage sludge-amended soils (Kizilkaya, 2004; Rada et al., 1996) and in particular, the enrichment and accumulation of metals in the burrow wall, known as the drilosphere (Tomlin et al., 1993). The effects of anecic earthworms of the *Pheretima* genus on the properties of metals in soil have also been extensively studied in Asia with particular attention given to environments polluted by mining activities (Ma et al., 2000, 2002) and the use of earthworms to promote phytoextraction (Cheng and Wong, 2002; Yu et al., 2005; Wang et al., 2006). These studies name the earthworms used as *Pheretima* sp. with Ma et al. (2000, 2002) and Cheng and Wong (2002) using *Pheretima guillelmi* alongside the unidentified *Pheretima* sp. As with the epigeic species, typically anecic species of earthworm appear to increase metal availability and mobility.

Endogeic species inhabit the mineral soil usually creating horizontal non-permanent burrows. This group of earthworms is probably best suited for determining the effect of earthworm activity on changes in the metal distribution in soils, as this is where they 'naturally' spend most of their time. However, this functional group is the least used in such studies, probably because they are not commercially available and are difficult to maintain in the laboratory. Species of the *Aporrectodea* genus have demonstrated the ability to increase metal availability to other invertebrates (Coeurdassier et al., 2007; Fritsch et al., 2008) and have been found to both increase (Stephens et al., 1994; Lukkari et al., 2006) and decrease metal concentrations readily extractable by plants and chemicals in amended soils (Zorn et al., 2005b; Lukkari et al., 2006). *Octolasion tyrtaeum* has been shown to increase extractable metals from contaminated and remediated soil (Udovic et al., 2007). In conclusion, all three ecological groups seem to increase the availability and mobility of metals in soils.

The life history and ancestral heritage of earthworms may also play a part in their impact on metal mobility and availability as genetic or physiological resistance has been known to allow populations to persist in metal-polluted environments (e.g. Arnold et al., 2008; Spurgeon and Hopkin, 2000). A population of the endogeic earthworm *Aporrectodea caliginosa tuberculata* from a contaminated site reduced metal availability, determined by a sequential extraction, more than the same species collected from an uncontaminated site (Lukkari et al., 2006). It has also been shown that the epigeic earthworm *L. rubellus* originating from metal-contaminated soil develops a form of resistance to As (Langdon et al., 1999) and that the speciation of the As inside the earthworm may play a part in the resistance mechanism (Langdon et al., 2002). This suggests that the speciation of the metals excreted by the earthworm may also differ in resistant earthworms. The exposure history of earthworms is therefore an important consideration when reviewing the mechanisms by which earthworms alter metal availability in contaminated soils.

## 3. Different soil components

The majority of studies have compared earthworm-worked to earthworm not-worked soil (e.g. Stephens et al., 1994; Devliegher and Verstraete, 1996; Rada et al., 1996; Ma et al., 2000, 2002, 2003, 2006; Cheng and Wong, 2002; Wen et al., 2004, 2006; Liu et al., 2005; Cheng et al., 2005; Yu et al., 2005; Zorn et al., 2005a,b; Lukkari et al., 2006; Wang et al., 2006; Coeurdassier et al., 2007; Dandan et al., 2007; Fritsch et al., 2008) but studies have also contrasted bulk soil to cast material (Ireland, 1975; Kizilkaya, 2004; Asawalam and Johnson, 2007; Udovic and Lestan, 2007; Udovic et al., 2007) and the drilosphere (Tomlin et al., 1993; Naftel et al., 2002). Results are similar in all cases with earthworm activity generally causing an increase in metal mobility and availability. Lukkari et al. (2006) found that metals were more chemically extractable in earthworm casts but that extractability decreased in the bulk soil compared to earthworm-free control soil. Protz et al. (1993) noted increases in metal concentration on burrow walls compared to bulk soil from sewage sludge-applied plots. Tomlin et al. (1993) report that burrows in a sewage sludge-applied plot are lined with faeces that contain higher concentrations of trace metals than the surrounding soil and that the soluble forms of these metals could be leached away from the burrow wall, accelerating the movement of metals from surface-applied sewage sludge to an underlying aquifer. Naftel et al. (2002) report a difference in the X-ray Absorption Near Edge Spectroscopy (XANES) spectra (Brown et al., 2005) of the Mn K-edge of the burrow walls compared to bulk (earthworm-inhabited) soils. This is attributed to a change in Mn species but not a change in oxidation state. Information on the metal concentrations in the linings of earthworm burrows may be valuable in assessing the leachability of metals to ground water for example. However, when assessing potential uptake by plants or other soil invertebrates, then measurements taken from casts or bulk soil are perhaps more appropriate.

## 4. Amended versus field-contaminated soils

Experiments on earthworms and metal-bearing soils are performed on either naturally metal-rich soil, contaminated soil or soil artificially amended with metals. Generally speaking, metals that are added as an artificial amendment are likely to be more bioavailable than concentrations that have built up over time, for example downwind from a metal smelting complex (Spurgeon and Hopkin, 1995). Studies that use chemically amended soils have the advantage of being able to control the exposure concentration and have an application in generating LC or $EC_{50}$ values or determining the effect of individual metals in isolation from other contaminants (Spurgeon et al., 1994), but they do not represent field conditions. Thus, for example, results that suggest that earthworms enhance phytoextraction (Cheng and Wong, 2002; Yu et al., 2005; Wang et al., 2006; Dandan et al., 2007) should be interpreted with care. Several authors report an increase in metal availability, assessed via chemical extractions, in chemically amended soils due to earthworm activity (Cheng et al., 2005; Currie et al., 2005; Yu et al., 2005; Zorn et al., 2005a; Wang et al., 2006; Dandan et al., 2007), while only Zorn et al. (2005b) shows evidence to suggest that *A. caliginosa* can decrease the availability of Zn in a 10-year aged amended soil. There are a few more examples of earthworm activity decreasing metal availability in contaminated soils (Ireland, 1975; Ma et al., 2000; Liu et al., 2005) but a larger evidence base suggests an increase in metal availability due to earthworm activity in both contaminated (Rada et al., 1996; Ma et al., 2002, 2003; Coeurdassier et al., 2007; Udovic and Lestan, 2007; Udovic et al., 2007) and uncontaminated (Stephens et al., 1994; Devliegher and Verstraete, 1996; Wen et al., 2004, 2006) soils.

Several studies have used soils amended with sewage sludge, with obvious implications for sewage disposal. Long-term plots of sewage sludge-amended soils indicate that the availability and speciation of the metals do not change very much after applications cease (McGrath and Cegarra, 1992; McBride et al., 2000). However, the breakdown of organic matter in sewage sludge by bacteria associated with earthworms is suggested as a mechanism for the increase in soluble metal concentrations in earthworm-worked soil (Rada et al., 1996).

Metals can be broadly divided into essential metals that are required for metabolic processes carried out by organisms, such as Zn or Cu, and non-essential metals that are not required and are not normally present in high concentrations in non-contaminated soils, such as Cd and Pb. There is evidence to suggest that earthworms respond differently to these two types of metal and while essential metals are regulated and excreted, non-essential metals are probably detoxified through binding with organic ligands or sequestration within inorganic matrices (Spurgeon and Hopkin, 1999). The difference between these two mechanisms may cause differences between the effects of earthworm activity on individual metals in the soil environment. Ireland (1975) described contrasting effects of *D. rubida* on the available Pb and Zn in earthworm casts compared to bulk soils. The earthworms changed the availability of Pb and Zn in different ways with Pb being made more available while Zn was made less available. Sizmur and Hodson (2008) report that water extractable Pb increased and Zn decreased after *E. veneta* inoculation to soil from metalliferous mining sites and Devliegher and Verstraete (1996) report a decrease in ammonium acetate–EDTA extractable Ni contrasting with an increase in a range of essential and non-essential metals after *L. terrestris* inoculation. Despite these examples, the vast majority of studies show the effect of earthworms on metal availability not to be metal-specific whether the effects be increases (e.g. Stephens et al., 1994; Rada et al., 1996; Ma et al., 2002, 2003; Kizilkaya, 2004; Wen et al., 2004, 2006; Asawalam and Johnson, 2007; Coeurdassier et al., 2007) or decreases (e.g. Liu et al., 2005; Lukkari et al., 2006; Ma et al., 2006) in metal availability. It can therefore be concluded that the individual metals are not as important as the earthworms themselves or soil properties when determining the impact of earthworms on metal availability.

## 5. Chemical methods to assess availability

A large suite of chemical methods are available for determining the bioavailability of metals in the soil environment (Peijnenburg et al., 2007). Many of these have been correlated with the uptake of metals into target organisms in bioassays but what is clear is that no single chemical extraction can be used as a measure of ‘bioavailable metals.’ It is for this reason that chemical methods for extracting metals are operationally defined.

The use of water as a metal extracting agent is perhaps the simplest method and is likely to yield the lowest metal concentrations. This fraction represents the most available portion of the total metal concentration in soil and it can be stated with a degree of certainty that this fraction is bioavailable. In uncontaminated soils, the water extractable fractions of Zn, Cu, Cr, Cd, Co, Ni and Pb (Wen et al., 2004), as well as Y, La, Ce, Pr and Nd (Wen et al., 2006), increased due to the activity of *E. fetida*. Likewise, in sewage sludge-amended soil, the water extractable Cd and Cu concentrations increased with increasing incubation time of *L. terrestris* (Rada et al., 1996). These studies show an increase in the availability of a selection of essential and non-essential metals due to the activity of ecologically contrasting earthworms in contrasting soil environments. In contrast, Sizmur and Hodson (2008) found a decrease in the water extractable concentration of Cu, Pb and Zn in *E. veneta* worked soil that had been amended with solid Cu sulphate, Pb nitrate and Zn sulphate more than 10 years prior to the experiment. They also observed a decrease in the concentration of water extractable As, Cu and Pb in earthworm-worked soil originating adjacent to metalliferous mining sites compared to earthworm-free soil. However, water extractable Zn in the mining site soil was found to be increased by the activity of *E. veneta*. Due to the relative simplicity of the measurement and the strong evidence that results may provide, it is perhaps surprising that water extractable metals are not used more often to show the effect of earthworm activity on metal mobility and availability.

A more complicated picture was presented when the faeces of *D. rubida* were compared to the bulk soil collected alongside a stream that flowed through a lead/zinc mine spoil. There was more water extractable Pb but less Zn in earthworm faeces than the bulk soil (Ireland, 1975). However, when acetic acid extractions (suggested to represent the plant available fraction) were considered, there was more extractable Zn but less Pb in earthworm faeces than the bulk soil (Ireland, 1975). These contrasting results illustrate the problem with using chemical extractions as indicators of bioavailability.

Another example of a weak extraction used for the determination of metal availability is ammonium acetate ($NH_4OAc$). This was applied to inoculated and earthworm-free Pb/Zn tailings amended with topsoil; the burrowing effects of the earthworm *P. guillelmi* decreased the concentration of extractable Pb and Zn (Ma et al., 2006). This was accompanied by a decrease in the plant tissue metal concentrations of the woody legume, *Leucaena leucocephala* grown in the soil containing earthworms. However, the total metal extracted by *L. leucocephala* increased as a result of increased plant growth. Houba et al. (1996) recommend a 0.01 M $CaCl_2$ extraction for use as an estimate of the bioavailability of metals in soils. This extraction has shown increasing extractability of Zn in soil worked by *L. rubellus* and *L. terrestris* (Zorn et al., 2005a) and decreasing extractability of Zn in soil worked by the endogeic *A. caliginosa* (Zorn et al., 2005b) in a 10-year-old Zn amended soil. The activity of earthworms also caused an increase in $CaCl_2$ extractable Cd and an increase in the root Cd concentrations of ryegrass.

The use of chelating agents in both the remediation of metal-contaminated soil through soil washing and in the extraction of the available fraction of the total metal concentration of a soil is well established. The use of strong complexing agents such as EDTA or DTPA is likely to extract a greater portion of the metal present than the weak acid or salt solutions discussed above (Peijnenburg et al., 2007). Evidence suggests, however, that chelating agents may not be a good predictor of plant uptake of metals as they are less affected by the pH and cation exchange capacity of the soil, although the chelator-extractable metal concentrations correlate well with the total metal content (Brun et al., 1998). When the activity of *Pheretima* sp. on the concentration of DTPA extractable Zn in different soils amended with a range of Zn concentrations was investigated, the different soils and the different concentrations of Zn yielded different effects showing no clear relationship (Cheng and Wong, 2002). However, a significant correlation between DTPA extractable Zn and Zn accumulation in ryegrass and Indian mustard was found in addition to an increase in DTPA extractable Zn due to the activity of anecic earthworms of the *Pheretima* genus (Wang et al., 2006). Further to this, when the earthworm *Metaphire guillelmi* was incubated in Cu contaminated soils, DTPA extractable Cu increased significantly compared to earthworm-free soil and this was mirrored by a significantly greater Cu content in ryegrass (Dandan et al., 2007). In another study, DTPA extractable Pb and Zn decreased in UK Pb/Zn mine spoil after inoculation with *L. terrestris* while in Chinese Pb/Zn mine spoil, DTPA extractable Pb increased due to the activity of *P. guillelmi* but decreased due to *Pheretima* sp. inoculation (Ma et al., 2000). The balance of evidence seems to indicate an increase in DTPA extractable Pb and Zn due to species of *Pheretima* in Chinese Pb/Zn mine tailings (Ma et al., 2002) with a strong correlation between DTPA extractable Pb and Zn and uptake into the roots of trees (*L. leucocephala*) and earthworm tissues (Ma et al., 2003).

It is often of interest to know which pools of metal have changed with changes in metal availability. Sequential extractions, in which extractants of increasing strength are applied to the soil, are an inexpensive method to determine this though it has to be remembered that the metal pools are operationally defined. A six step sequential extraction indicated few differences between earthworm casts and bulk soil in the fractionation of Pb and Zn in soils processed by *E. fetida*, *L. rubellus* and the endogeic earthworm *O. tyrtaeum* (Udovic and Lestan, 2007; Udovic et al., 2007). Other studies, however, have identified evidence for earthworms changing the availability of metals in soils through changes in speciation. Lukkari et al. (2006) applied a five-step sequential extraction to casts and bulk soil for both contaminated and uncontaminated soil inhabited by *A. caliginosa tuberculata*. The cast material contained more Cu and Zn bound to soluble fractions and less to iron and manganese oxides and soil organic matter fractions compared to bulk soil. However, bulk earthworm processed soil contained more Cu bound to the organic matter fraction and less to the soil carbonates and the iron and manganese oxides fractions compared to the earthworm-free bulk soil. Similarly the bulk earthworm processed soil contained more Zn in the organic matter and residual fractions and less in the iron and manganese oxide fraction than the earthworm-free bulk soil. These results indicate that earthworm activity decreased metal availability in the bulk soil. Earthworms collected from contaminated soil did this more efficiently than naïve earthworms. In contrast to this, *E. fetida* in uncontaminated soil was shown to increase the water extractable, exchangeable and carbonate bound metals by mobilising the organic matter and sulphide bound fractions (Wen et al., 2004). *L. terrestris* was shown to increase the exchangeable and acid soluble fractions of Cu and Cd while decreasing the oxidisable fraction (El-Gharmali, 2002) or increase the water extractable Cu and Zn while decreasing the exchangeable and organically bound metals (Kizilkaya, 2004) in sewage-amended soils.

## 6. Plant bioassays to assess availability

Although chemical extractions are easier and cheaper to carry out and almost certainly the method of preference when determining availability of contaminants on a large number of samples, for research purposes an observed effect on the availability of metals will undoubtedly hold more weight when validated with a bioassay. Plants are perhaps the simplest organism to consider as, for non-volatile elements, there is just one uptake route to consider, the uptake of metals dissolved in the water extracted by the roots. When investigating the effect of earthworms on the availability and mobility of metals both plant metal concentration and plant biomass need to be considered since changes in plant biomass due to earthworm activity will impact on mass of metal extracted from the soil even if plant metal concentration is unaffected. It is therefore strongly recommended to report both the concentration and biomass of whatever plant tissue (roots, shoots, leaves, etc.) is being discussed and the total uptake of metals derived by multiplying the concentration by the biomass.

The endogeic earthworms, *Aporrectodea rosea* and *Aporrectodea trapezoides* were used to determine the effect of earthworm activity on the foliar concentration of a range of elements in wheat grown in uncontaminated soil (Stephens et al., 1994). *A. rosea* significantly increased the concentrations of Ca, K, N, Na, Cu and Mn and *A. trapezoides* significantly increased the concentrations of Mn, Al, Ca, Fe, N, K and Na but it is noteworthy that these are all essential nutrients and were present at levels not known to be toxic to plants or earthworms. In a similar study, using uncontaminated soil, *E. fetida* increased the biomass of wheat with increases in the content of Zn, Cu, Cr, Cd, Co, Ni and Pb (Wen et al., 2004) and the rare earth elements Y, La, Ce, Pr and Nd (Wen et al., 2006) in both shoots and roots.

Earthworms of the genus *Pheretima* have been extensively used to investigate the effects of earthworms on the uptake of metals by various plants in Asia usually in the context of mine spoil re-vegetation or phytoextraction potential. In a soil amended with Zn, the biomass of the shoots and roots of both ryegrass and Indian mustard was increased due to *Pheretima* sp. activity with both the concentration of Zn in shoots increased and the uptake into shoots and roots significantly enhanced by the earthworm inoculation (Wang et al., 2006). *Pheretima* sp. was also introduced to soil amended with Cd, which resulted in an increase in the dry weight of the shoots but not the roots of ryegrass. The earthworms however increased the Cd concentration in the roots but not in the shoots. When these data are combined, the earthworms were found to increase the uptake of Cd in both the roots and shoots (Cheng et al., 2005; Yu et al., 2005). Soil amended with Cu to which casts of *Pheretima* sp. from uncontaminated soil were added resulted in an increase in the shoot and root biomass of ryegrass and an increase in Cu concentration and accumulation in the shoots. The Cu concentration in the roots however was decreased indicating the possibility that the casts promote Cu transfer from roots to shoots (Shufen et al., 2006). The earthworm *P. guillelmi* was introduced into Pb/Zn mine tailings followed by the transplanting of trees (*L. leucocephala*). The introduction of the earthworms was shown to improve the growth of the trees and enhance the uptake of Pb and Zn. As the earthworms also increased the root:shoot mass ratio, the enhanced uptake was suggested to be a direct result of the larger roots (Ma et al., 2003, 2006).

In contrast to the studies mentioned above, when sewage sludge amended with Cd and Cu was planted with cabbage and *E. fetida* introduced to some treatments, the cabbage in the earthworm inoculated soil was found to have lower concentrations of Cd and increased biomass (Liu et al., 2005). When willow trees were planted in pots filled with concentrated sewage sludge, the earthworm, *E. fetida* was found to reduce soil moisture and change the structure of the sludge whilst the biomass of the roots and shoots of the trees increased. However, there was no change in the concentration of metals in the trees (Kocik et al., 2007).

When ryegrass was planted in sewage sludge-amended soils, the presence of *L. terrestris* increased the concentrations of Cu and Cd in the plants (Rada et al., 1996). In a separate study involving *L. terrestris* and ryegrass in soils of differing Cd, Cu, Fe, Pb and Zn content, earthworms were shown to generally increase the concentration of trace elements in both the shoots and the roots apart from Fe and Pb in the roots (Abdul Rida, 1996). *M. guillelmi* was shown to increase the Cu concentration in the shoots and roots of ryegrass in Cu amended soil with the added observation of the earthworm being able to increase the transfer of Cu from the roots to the shoots (Dandan et al., 2007).

## 7. Animal bioassays to assess availability

The bioavailability of metals to animals is more complicated than the availability to plants, as multiple routes of exposure may exist. Metals can enter the body through ingestion, inhalation and dermal contact. A contaminant entering through the ingestion route may not be available but just accessible, as the metal may not be absorbed by the body but merely excreted in the faeces. In a study involving soil polluted by aerially deposited metallic trace elements, *A. tuberculata* was found to increase the accumulation of Cd, Cu and Zn to the snail *Helix aspersa* (Coeurdassier et al., 2007). The earthworms alone did not increase the water extractable metals so the suggested mechanism for this effect was that the snail faecal material had a higher dissolved organic carbon concentration than the soil. Mixing of the faecal material into the soil by the earthworms increased organo-metal complexation, increasing the available concentration of metals. *A. tuberculata* was also shown to increase the transfer of $^{137}$Cs from soil to snails (*H. aspersa*) in a container experiment. The reason suggested for this are changes in the structure and composition of casts compared to bulk soil due to digestive processes (Fritsch et al., 2008). When *E. fetida* was used in toxicity tests involving single and multiple-occupancy test containers, the decrease in the weights of the earthworms was greater in the multiple-occupancy tests and these earthworms also had higher Pb tissue concentrations in sterile soil amended with Pb (Currie et al., 2005). It was suggested that more mucus was produced by earthworms in the multiple-occupancy tests as a result of earthworm interactions and that this increased metal availability via the formation of organo-metal complexes.

Ruby's Physiologically Based Extraction Test (PBET) is a chemical protocol designed to predict the bioavailability of metals in soil to humans through the ingestion exposure route, and has been validated with rodent bioassays for Pb and As (Ruby et al., 1996). This chemical extraction is considered here with the studies involving the uptake into a test animal as this is as close as one can get to testing the bioavailability of toxic metals to humans for ethical reasons. In a soil remediated by leaching with EDTA and a non-remediated soil, the bioavailability (measured by PBET) of Pb in casts of *E. fetida* was significantly higher than that of Pb in *L. rubellus* casts and higher than that of the bulk remediated and non-remediated soil (Udovic and Lestan, 2007). When PBET was used in a similar experiment but using *E. fetida* and the endogeic *O. tyrtaeum*, it was found that Pb concentration in the stomach phase was significantly lower in earthworm casts in the remediated and non-remediated soils than the bulk soils themselves, however, the small intestine phases showed the opposite pattern (Udovic et al., 2007). These studies suggest that the earthworms used can change the chemistry of Pb in both remediated and non-remediated soils making Pb more available to humans via the ingestion route.

## 8. Mechanisms suggested for the changes in availability and mobility of metals due to the presence of earthworms

Relating changes in available metals to any one soil parameter is difficult because even controlled changes to a single soil property in the laboratory may have consequent effects on other soil properties that in turn affect metal mobility. This is particularly the case when determining the parameters that impact the bioavailability of metals to earthworms (Beyer et al., 1987). When using weak extractions to determine the availability of metals to earthworms the results obtained are variable and studies conducted in the field (Vijver et al., 2007) and in the laboratory using field soils (Hobbelen et al., 2006) have shown that no single parameter is universally better correlated with uptake into earthworms; sometimes pseudototal (aqua regia extractable) metal concentration gives a better correlation and sometimes pore water concentrations. This is reflected in the debate in the literature as to whether dermal or intestinal uptake is the dominant uptake mechanism for metals into earthworms (e.g. Vijver et al., 2003; Marinussen et al., 1997; Wallwork, 1983). One might expect pore water concentrations to correlate better with uptake if dermal uptake is dominant and pseudototal concentrations to correlate better with uptake if intestinal uptake is dominant. Either way, it can be hypothesised that the properties within the gut of the earthworm will significantly impact metal availability to the earthworm. Whether the availability increases or decreases will depend on the conditions in the bulk soil relative to the conditions in the earthworm's gut. It is however not clear which of the properties that the earthworms may alter are most important.

Alternatively, it has been suggested that a reason for the decrease in metal availability of worm-worked soil is due to accumulation (and therefore sequestration) of metal within the tissue of the earthworm itself (Liu et al., 2005). Conversely, reasons for increases in observed metal availability are suggested to be due to the decomposition of earthworm tissues with high metal burdens making these metals more available than they were to the earthworm that accumulated them (Ireland, 1975; Ma et al., 2002). Digesting the surviving earthworms to determine the metal body burden would show if earthworm 'sequestration' is a significant mechanism of detoxification of metals in polluted soils. In the environment, the mass ratio of earthworms to soil is small enough for any 'sequestration' effect to be too small to affect metal availability in a significant way. Further to this, evidence of increases in metal availability in earthworm mesocosms where mortality did not occur (Ma et al., 2000) suggests other mechanisms are occurring.

Having documented studies that investigate changes in availability of metals due to earthworm activity we now review suggested mechanisms for the changes.

### 8.1. *Stimulation of the soil microbial population*

Increases in the biomass of bacteria, actinomycetes and fungi have been found in the earthworm casts of soil where increases in the availability of metals to plants have been observed (Wen et al., 2004). A suggested mechanism for an increase in the availability of metals is the stimulation of bacterial populations which enzymatically degrade organic matter, releasing the organically bound metals into solution (Rada et al., 1996).

Earthworms are thought to affect soil microflora by comminution, burrowing, casting, grazing and dispersal. The ability of a microbial species to survive these processes depends on its ability to adapt to the conditions a particular earthworm may induce (Brown, 1995). Although it is thought that earthworms do feed preferentially on fungal rich soil or substrate, there is also evidence to suggest that they do not gain nutrition selectively as Pokarzhevskii et al. (1997) show that earthworms are ecosystemivorous feeding on entire soil microbial ecosystems. It can therefore probably not be said that earthworms affect metal availability by grazing a particular functional group of soil microorganisms that play an important role in the cycling of metals. Earthworms excrete mucus and urine into the soil environment which are thought to increase microbial activity although this effect is not proportional to the size of the earthworm indicating the possibility that earthworms release other stimulating substances in addition to the mucus and urine (Binet et al., 1998). Other explanations for increases in microbial activity associated with earthworm activity are improved aeration, higher organic matter contents and greater water availability associated with bioturbation (Tiunov and Scheu, 1999). In a review of the effect of the invasion of earthworms to ecosystems previously devoid of earthworms, McLean et al. (2006) conclude that where the microbial community has had time to adapt to earthworm activities, earthworms induce a shift to a smaller, more active microbial community though in the short term microbial activity increases in fresh casts may decline within a few hours. Additionally an overall decrease in the fungal:bacterial ratio occurs, possibly due to physical disturbance of fungal hyphae.

Much of the focus on the changes in the microbial biomass and activity as a result of earthworms has focused on the soil environment within the gut. An increase in numbers of bacteria, actinomycetes and micromycetes was observed in the intestine of *L. rubellus* during the passage of food (Kristufek et al., 1992). This may persist on soil excretion as the active microbial biomass was shown to be increased in the faeces of *A. caliginosa* compared to bulk soil (Kristufek et al., 1992). However, microbial biomass-C was not found to be different in *L. rubellus* faeces compared to earthworm-worked soil by Daniel and Anderson (1992) and Scheu (1992) reports that the percentages of active microorganisms were similar between faeces and soil. Sheehan et al. (2008) show that increases in the population densities of anecic, epigeic and endogeic earthworms all decrease the microbial biomass and activity reportedly due to an increase in consumption of the microorganisms by the earthworms or by competition for food.

Evidence exists that the digestion of organic matter by endogeic earthworms takes place through a mutualistic relationship with ingested microorganisms (Trigo and Lavelle, 1993) implying that although no bacteria are native to the earthworm gut *per se*, the conditions are such that certain types of organisms will survive to fulfil a beneficial role. This observation is strengthened with the report of an anoxic microzone within the earthworm gut that soil microorganisms experience which stimulates a portion of the ingested microorganisms including fermentative bacteria (Drake and Horn, 2006). It is therefore a possibility that earthworms, due to conditions within the gut, change the soil microbiological community or the active community, which will perform functions at different rates when compared to bulk soil.

The earthworm *L. terrestris* was used in a study whereby the effects of earthworm activity were spilt into Gut Associated Processes (GAP) and Nutrient Enrichment Processes (NEP). NEP were simulated through mechanical mixing to represent the effect of earthworm bioturbation. It was found that the NEP rather than the GAP are the cause of the observed increases in microbial populations in the literature. GAP were found to reduce the microbial population and activity both directly, through feeding on microorganisms, and indirectly, through reduction of the substrate (Devliegher and Verstraete, 1995). Increases in available metals in this experiment were reportedly due to both the GAP and NEP but mostly due to the GAP (Devliegher and Verstraete, 1996). Changes in the microbial population and activity did not relate to the changes in metal availability in this study. This suggests that in this case the changes in the microbial population are perhaps not very important in the effect of earthworm activity on metal availability.

### 8.2. *Alteration of soil pH*

It is well known that the pH of a soil is a primary factor in determining the proportion of the total concentration of metals that are dissolved in the soil solution (Sauve et al., 2000) and some studies have suggested that this is a mechanism whereby earthworms can increase the metal availability in soil by decreasing the soil pH (El-Gharmali, 2002; Kizilkaya, 2004; Yu et al., 2005). However, many more studies show that the effect of earthworm activity is to increase rather than decrease the soil pH, reportedly due to cutaneous mucus secretion (Schrader, 1994), while still observing increases in the availability of the metals (Ma et al., 2002, 2003; Wen et al., 2006; Udovic and Lestan, 2007; Udovic et al., 2007). This indicates that the alteration of pH is at least not the only mechanism for the increasing effect earthworms appear to have on metal availability. For example, changes in the availability of cations or anions in addition to pH changes will also impact on metal mobility, speciation and availability through competitive sorption and complexation reactions.

### 8.3. *Alteration of soil dissolved organic carbon (DOC)*

It is well known that changes in DOC in soil solution impact on the availability of metals (e.g. Antoniadis and Alloway, 2002; Steenbergen et al., 2005; Arnold et al., 2007) either through complexation with metals in solution or by bringing metals into solution through exchange on the soil surface. There is evidence to suggest that earthworms play a humifying role in the soil as humic acids were detected in earthworm-worked soil that were not present in the non-humified starting material (Businelli et al., 1984). Humic acids in particular are known to increase the availability of metals to plants through the formation of organo-metal complexes (Halim et al., 2003; Evangelou et al., 2004). Some authors suggest that increased uptake of metals by plants due to earthworm activity may be as a direct result of the release of metal-chelating organic materials released by earthworms forming organo-metal complexes (Currie et al., 2005; Wang et al., 2006; Wen et al., 2006; Udovic et al., 2007).

A significant correlation has been found between the effect of *E. fetida* increasing DOC and the concentration of water extractable metals (Zn, Cu, Cr, Cd, Co, Ni and Pb) (Wen et al., 2004). A similar positive relationship between the concentration of water extractable rare earth elements, Y, La, Ce, Pr and Nd, and DOC has been reported in uncontaminated soils (Wen et al., 2006). In addition, an increase in DOC was the explanation given for the effect of earthworms increasing the metal availability to snails as it is thought that the earthworms mixed the DOC-rich faecal material produced by the snails into the bulk soil, influencing the partitioning of metals (Coeurdassier et al., 2007). An increase in DOC has also been noted in a study which reported that the presence of the earthworm *M. guillelmi* increased the availability of Cu to plants (Dandan et al., 2007).

### 8.4. Metal speciation and sequestration within earthworm tissue

The main area of metal accumulation in earthworms is the chloragogenous tissue surrounding the posterior alimentary canal (Morgan and Morris, 1982). Two pathways for the intracellular binding of metals exist in the chloragogenous tissue. Pathway A binds metals in insoluble, O-donating, phosphate-rich granules, referred to as chloragosomes (Morgan and Morris, 1982; Morgan and Morgan, 1989; Cotter-Howells et al., 2005). Pathway B binds metals to low molecular weight, S-donating ligands which are believed to exhibit the characteristics of mammalian metallothionein within a vesicular organelle referred to as the cadmosome (Morgan and Morris, 1982; Sturzenbaum et al., 1998; Cotter-Howells et al., 2005; Demuynck et al., 2006, 2007).

Pathway A is thought to sequester Pb and Zn in an insoluble form by displacing Ca, thereby detoxifying by accumulative immobilisation and reducing the availability of these metals when in toxic concentrations (Morgan and Morgan, 1989). Evidence for this has been reported in both *D. rubida* and *L. rubellus* (Morgan and Morris, 1982; Morgan and Morgan, 1989).

Pathway B is thought to have different functions depending on the concentration of the metal in question in the soil. Two isoforms of earthworm metallothionein have been identified in earthworms, with wMT-1 used as a carrier for essential metals such as Zn and Cu at non-toxic levels and wMT-2 used to immobilize non-essential metals like Cd and essential metals such as Zn when they are present in concentrations above a toxic threshold (Morgan and Morgan, 1989; Sturzenbaum et al., 2001).

It is not clear how quickly metals rendered unavailable by either of the above pathways would become available again following excretion into the soil environment.

There is also evidence that As induces metallothionein expression in earthworms collected from metal-contaminated sites (Langdon et al., 2005) and evidence that resistance to arsenic toxicity is due to an inherited genetic adaption (Langdon et al., 2003). This highlights the importance of using earthworms collected from metal-contaminated soil that have an inherited genetic resistance to the metals present when assessing the effect of earthworms on metal mobility and availability in metal-contaminated soils.

## 9. Conclusions and recommendations

When studying the effects of earthworm activity on metal mobility and availability, care must be taken to ensure that the experimental design lends itself to the observation of environmentally relevant effects. The use of soils that have been contaminated in the environment rather than amended in the laboratory is recommended in order to provide an environmentally relevant test medium, metals in laboratory amended soils often behave differently to those in field-contaminated soils due to differing residence times of the metals. The use of earthworms that have a prior exposure history is recommended if the researcher is attempting to re-create field conditions since earthworms will adapt to their environment over time and hence their impact on metal chemistry may change with time. Naïve earthworms are recommended to test the effects of an inoculation programme. Differences in results due to different species and ecological groups should be identified and how these species interact to collectively affect metal speciation and availability in the soil environment would also be a useful observation.

When investigating the effects of earthworms on metal availability, a test organism should be used with the effect of exposure determined by a toxicological response or the measurement of the accumulated metal. This can then be combined with chemical techniques to strengthen conclusions and aid explanations of the mechanisms involved. When studying the uptake of metals by plants, care should be taken in expressing results. Both the concentration of metals within the plants and the total amount of metals taken up by the plants should be given.

There seems to be an overuse of chemical extractions to represent ‘available’ or ‘mobile’ fractions of the metals in the soil but the measurement of the metal content of extracted pore water is underrepresented. Observable changes in the water extractable metal fraction of an earthworm-worked soil will provide stronger evidence for the impact of earthworms on metal mobility and availability than the use of more involved chemical extractions.

The balance of evidence seems to suggest that earthworms increase the availability and mobility of essential and non-essential metals in both contaminated and uncontaminated soils but the mechanisms for this are not clear. Evidence suggests that the earthworms can stimulate microbial activity and alter pH or DOC. There is also evidence that some earthworms sequester metals within their chloragogenous tissues in two distinct structures but it is not clear if these persist in the soil environment. Any of these four (or five) mechanisms may be reasons for the reported impacts of earthworms on metal availability and mobility and warrant further investigation.

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2009.02.029

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