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The transfer of trace metals in the soil-plant-arthropod system

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Abstract

Essential and non-essential trace metals are capable of causing toxicity to organisms above a threshold concentration. Extensive research has assessed the behaviour of trace metals in biological and ecological systems, but has typically focused on single organisms within a trophic level and not on multi-trophic transfer through terrestrial food chains. This reinforces the notion of metal toxicity as a closed system, failing to consider one trophic level as a pollution source to another; therefore obscuring the full extent of ecosystem effects. Given the relatively few studies on trophic transfer of metals, this review has taken a compartment-based approach, where transfer of metals through trophic pathways is considered as a series of linked compartments (soil-plant-arthropod herbivore-arthropod predator). In particular, we consider the mechanisms by which trace metals are taken up by organisms, the forms and transformations that can occur within the organism and the consequences for trace metal availability to the next trophic level. The review focuses on four of the most prevalent metal cations in soil which are labile in terrestrial food chains: Cd, Cu, Zn and Ni. Current knowledge of the processes and mechanisms by which these metals are transformed and moved within and between trophic levels in the soil-plant-arthropod system are evaluated. We demonstrate that the key factors controlling the transfer of trace metals through the soil-plant-arthropod system are the form and location in which the metal occurs in the lower trophic level and the physiological mechanisms of each organism in regulating uptake, transformation, detoxification and transfer. The magnitude of transfer varies considerably depending on the trace metal concerned, as does its toxicity, and we conclude that biomagnification is not a general property of plant-arthropod and arthropod-arthropod systems. To deliver a more holistic assessment of ecosystem toxicity, integrated studies across ecosystem compartments are needed to identify critical pathways that can result in secondary toxicity across terrestrial food-chains.

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1.   Introduction

Trace metals in soils (metallic elements with typical concentrations of less than 1000 mg kg^{-1}) can be divided into two groups based on their biological function. The first group of elements, including Cu, Zn and Ni, are essential for the correct functioning of organisms (Marschner, 2012). The second group of trace elements have no known function in biological systems (Kabata-Pendias, 2010; Alloway, 2012a). Cadmium belongs to the second group when considering terrestrial ecosystems (Smolders and Mertens, 2012), although a biological role for Cd has been reported in marine diatoms (Lane and Morel, 2000; Xu et al., 2008). Both essential and non-essential trace metals are capable of causing toxicity above a certain threshold concentration. Extensive research has assessed the behaviour of trace metals in biological and ecological systems (e.g. Kabata-Pendias, 2010; Adriano, 2001; Peralta-Videa et al., 2009; Hooda, 2010; Alloway, 2012a; Jan et al., 2015). However, much of this research has typically focused on single organisms within a trophic level and not on multi-trophic transfer through terrestrial food chains, despite some notable field research and reviews on this topic (Fritsch et al., 2012; Nica et al., 2012; Orlowski et al., 2019; Pilon-Smits, 2019). This reinforces the notion of metal toxicity as a closed system, failing to consider one trophic level as a pollution source to another; therefore obscuring the full extent of its ecosystem effects. This holistic eco(system)toxicity concept needs addressing, and is the fundamental precept for this review, which we hope will underpin more integrated research efforts in the future.

In this review we address this knowledge gap, synthesising evidence on trophic transfers of metals and the underlying mechanisms in soil-plant-arthropod food-chains for four of the most prevalent and labile metal cations in terrestrial food chains (Cd, Cu, Zn and Ni). Only one previous review has considered metal transfers in terrestrial multi-trophic systems (Gall et al., 2015), but the authors took a much broader approach than we apply here, in terms of the number of metals and mammalian and human endpoints, and did not focus on the underpinning mechanisms. Given the

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relatively few studies that deal with the trophic transfer of metals and the large number of studies within each trophic level, this review has taken a compartment-based approach, where transfer of metals through trophic pathways is considered as a series of linked compartments (soil-plant-arthropod herbivore-arthropod predator). In each compartment we consider the input to the compartment, transport and transformations that occur in that compartment and its transfer to the next (higher trophic) compartment (Figure 1). The mechanisms by which trace metals are taken up by organisms, the forms and transformations that can occur within the organism and the effect that this may have on trace metal availability to the next trophic level are also explored.

[FIG. 1 NEAR HERE]

Metals are inevitably transferred from one lower (trophic) compartment to the next higher (tropic) compartment but the magnitude of transfer varies due to a complex interaction of chemical and physiological factors. The net effect of these factors can be expressed as biomagnification (bioaccumulation) factors or transfer coefficients. Transfer coefficients (TCs) are calculated by dividing the concentration in one compartment of the system by the concentration in the compartment below it (e.g. concentration in the arthropod divided by the concentration in the plant tissue) (Green et al, 2003; Green and Tibbett, 2008; Li et al., 2018). A transfer coefficient of less than one leads to the dilution of metal(s) in the higher compartment, and more than one such contiguous diluting transfer forms a benign pathway (Figure 2). A coefficient greater than one leads to the concentration of metals in the higher compartment, and more than one such contiguous concentrating transfer forms a critical pathway (van Straalen and Ernst, 1991) (Figure 2).

[FIG. 2 NEAR HERE]

The overall aim of the review is to identify the key factors controlling the transfer and toxicity of trace metals in excess in the soil-plant-arthropod system by addressing four research questions:

1. What determines the soil bioavailability of trace metals to plants?

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2. What are the physiological mechanisms that regulate uptake, transformation, accumulation and detoxification in plants and herbivorous and predatory Arthropods?

3. How do interactions between soil, plants and arthropods determine the magnitude of transfer between trophic levels?

4. How important is biomagnification in soil-plant-arthropod systems and what are the consequences where it occurs?

2. Soils

Potentially toxic trace elements are naturally present in soils, being residual to a lesser or greater extent from the parent material and other natural sources (e.g. volcano, wind dust, forest fires) (Oorts, 2012). Significant additions of some trace elements, including Cu, Ni, Cd and Zn have been made to many soils as a result of human activities, from common agricultural practices to direct industrial waste disposal (Alloway, 2013b). Since potentially toxic trace elements may be transferred through food webs, primarily as a result of plant uptake, there has been considerable attention devoted to the effect of trace element pollution in soils on the functioning of ecosystem components and the consequent risks to human health. The behaviour of trace elements in soils, however, is complex and, despite considerable recent advances, there is still no unifying theory able to predict trace element bioavailability from soil sources. Here we examine inputs and redistribution of the potentially toxic trace elements Cu, Ni, Cd and Zn in soils, and the chemical reactions controlling their distribution among solid- and aqueous-phase chemical species. We conclude with an analysis of current models which predict actual bioavailability, or chemical proxies of this elusive concept, as tools that can be applied in research focused on metal transfer across trophic levels.

2.1 Inputs of Cu, Ni, Cd and Zn to Soils

Trace elements occur naturally in the parent materials of soils, at concentrations which are broadly dependent on lithology. During the process of soil formation, trace elements become enriched or depleted in different soil horizons. This depends on the soil-forming environment and the predominant chemical reactions for each element (Adriano, 2001), for example, soil weathering and clay enrichment are associated with higher background levels of Zn (Mertens and Smolders, 2012). External inputs of trace elements include atmospheric deposition, such as from wind-blown dust, forest fires and volcanic ashes (Alloway, 2012b; Oorts, 2012), which is minimal in many natural environments (e.g., Gray et al., 2003) but may be locally important. Summaries of average concentrations of trace elements in different rock types have been presented by Kabata-Pendias (2010), Alloway (2012b) and Adriano (2001).

Anthropogenic activity has elevated concentrations of Cu, Ni, Cd and Zn in soils across the globe and most research into trace metal bioavailability has focused on such environments. Anthropogenic sources of trace metals have been reviewed exhaustively (e.g., Kabata-Pendias, 2010; Adriano, 2001; Naja and Volesky 2009; Alloway, 2012b). Industrial inputs include mining and ore processing, smelting and other metallurgical processes and a wide range of metal-utilizing industries. Agricultural activities contributing metals include use of fertilizers with trace element supplements or impurities, application of metal-contaminated organic wastes including sewage sludge or effluents from animal industries, and use of metal-containing pesticides, especially Cu and Zn in fungicides. Enhanced atmospheric deposition has also resulted from use and disposal of fossil fuels and their residues.

2.2 Mineralogy and chemistry of metals in soils

Trace elements are present in a range of chemical forms in soils. These may be inherited from unaltered parent material; alternatively, metal speciation in soil may reflect a combination of pedogenetic transformations and/or anthropogenic additions. It is unquestionable that the form of

trace elements is a major control on their availability or accessibility to organisms, and this has been reviewed thoroughly (Allen et al., 2001; Basta et al., 2005; McLaughlin et al., 2000; Nolan et al., 2003; Tessier and Campbell, 1987; Antoniadis et al., 2017). However, despite the importance of metal speciation in soils, methods for accurately determining speciation require further refinement. Total elemental analyses, which include all forms of metals in soils, do not generally correlate well with biological uptake (McLaughlin et al., 2000; Nolan et al., 2003; Kim et al., 2015). For example, the existence of metals in specific mineral phases in soils may be established using microprobe techniques such as EDS (having relatively high detection limits; Nielsen et al., 2015), synchrotron X-ray spectroscopic techniques (Kopittke et al., 2017), or TOF-SIMS (Time of flight secondary ion mass spectrometry; Arenas-Lago et al., 2016). Determination of soluble metals remains complicated by the difficulties in separating truly aqueous species from dispersed colloids. Techniques which target a conceptual fraction rather than a discrete species may, in fact, be more useful in predicting biological uptake than true speciation methods (McLaughlin et al., 2000; Zhang et al., 2001; Van der Ent et al., 2019).

General chemical properties of cadmium, copper, nickel and zinc

The chemical occurrence and reactions of Cd, Cu, Ni and Zn in natural environments have been reviewed thoroughly by several authors. The following is a brief summary of the significant chemical properties for these elements; (for more detail, we refer the reader to Adriano, 2001 and Alloway, 2012a). All four elements exist predominantly as divalent cations in natural systems (Cu^0 and Cu^+ may exist under some redox conditions, but are uncommon). The aqueous speciation of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} is mostly as stable complexes or ion pairs, as well as aquo- complexes and their hydrolysis products. The divalent cadmium ion is a soft Lewis acid, and shows a preference for reduced sulfur, some N-donor, or halide ligands. Divalent Cu^{2+}, Ni^{2+} and Zn^{2+} are intermediate Lewis acids and form ion pairs or complexes with a range of ligands, many with O– or N– containing functional groups, including sulfate, chloride and numerous organic ligands such as

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simple carboxylates and natural dissolved organic matter. All four elements form insoluble hydroxides, carbonates, phosphates and sulfides. A co-occurrence of Cd and Zn is often reported for both contaminated (Anju and Banerjee, 2011) and non-contaminated soils (De Oliveira et al., 2014), but a large meta-analysis did not find this to be the case for a wide range of soils globally (Hamon et al., 2004).

Metals in mineral phases

Many trace elements including Cu, Ni, Cd and Zn occur as discrete mineral phases containing the element of interest as the primary structural cation (such as hydroxides, carbonates, phosphates and sulfides), or as ions co-precipitated in variable proportions in the structure of more common minerals such as primary and secondary silicates, and oxyhydroxides of Fe, Al and Mn (Kabata-Pendias, 2010). While trace metals within primary or pedogenic mineral structures provide useful data on geochemical origins and soil-forming processes, these forms of elements are not usually considered to contribute to biological uptake due to their slow cycling within terrestrial ecosystems. Exceptions to this generalisation include trace metal ions coprecipitated with secondary iron or manganese oxyhydroxides (Wang and Jia, 2017), or present in sulfide minerals (Kabata-Pendias, 2010). Changes in soil redox potential can result in transient fluxes of dissolved metal ions from these minerals *via* processes such as reductive dissolution of oxides, or sulfide oxidation (Gambrell, 1994; Singh et al., 1996; Rinklebe et al., 2016). There is some evidence to show that, over a wide geographical range, several trace elements including Cu, Ni, Cd and Zn co-occur with iron minerals in soils (Hamon et al., 2004; De Oliveira et al., 2014). It should be noted, however, that despite the predominance of metals in mineral phases and their purported lack of bioavailability, correlations between total metal concentrations in soils and bioavailability as determined by plant uptake have been observed (e.g., McGrath et al., 2000). In some cases, metals in nanoparticulate phases in soils may contribute to enhanced bioavailability (e.g., Unrine et al., 2010; Watson et al., 2015), but in several cases the nanoparticles themselves can be assimilated by organisms and cause toxicity, such

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as observed in bacteria (Sirelkhatim et al., 2015), plants (Tripathi et al., 2017) and animals (Johnston et al., 2010). Studies on the bioavailability, transport and toxicity of nanoparticles in higher plants have been summarised in reviews from Miralles et al. (2012), Mustafa and Komatsu (2016), and Tripathi et al. (2017).

Chemisorption

Chemisorption (adsorption by formation of electron-sharing bonds) is likely to be very important in terms of bioavailability, based on either free ion activity concepts (exerting control on free ion activities) or on kinetically based models for bioavailability (since some desorption occurs over timescales which are likely to be relevant to replenishment of free ions). Chemisorption of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} at the water-particle interface occurs by surface complexation mechanisms. Such adsorption involves weakly acidic (*e.g.*, organic –COOH) or amphoteric (*e.g.*, terminal –OH on phyllosilicates or sesquioxides) functional groups forming coordinative bonds with metal cations. Typically, surface complexation of metal ions occurs at deprotonated sites or by displacement of H^+, so the reactions are favoured at high pH (Sposito, 1989). Chemisorbed trace metal cations in soils are most likely those targeted by common wet chemical extraction methods (*e.g.*, DTPA extraction, acetate buffer; Zhang et al., 2010). Chemisorption is commonly considered to show poor "reversibility" deduced from observations of desorption hysteresis (McLaren et al., 1981; Shaheen et al., 2013). The apparent lack of reversibility most likely reflects experimental factors; insufficient time for the forward reaction means that equilibrium is not achieved, and the difference in apparent equilibrium for the subsequent desorption phase is a consequence of the longer time elapsed. In some cases this may mean that rapid, strong adsorption is a metastable state, effectively a precursor for coprecipitation or physically/sterically constrained adsorption (eg., Rate et al., 1993 and McLaren et al., 1998).

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Ion Exchange

The occurrence of Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} as cations means that they can be retained by electrostatic sorption (*i.e.*, ion exchange) on negatively charged soil colloids such as phyllosilicates and organic matter. Since variable charge is more negative at high pH, the capacity of soils to hold cations electrostatically increases as pH increases (Antoniadis et al., 2017). The exchangeable fraction of metals usually represents a low proportion of total metal content in soils. Lower concentrations of exchangeable trace metals reflect the unfavourable thermodynamics of electrostatic sorption compared with other mechanisms, and competition at charged particle – water interfaces by high concentrations of di- or trivalent major cations (Ca^{2+}, Mg^{2+}, and Al^{3+}). Sorption reactions in soils can be considered to effectively form a continuum with respect to affinity for metal cations at concentrations at which high affinity chemisorption 'sites' are fully occupied by metal ions, therefore, lower-affinity sites then become more important for metal ion retention. As a result, in contaminated soils, compared with uncontaminated, there may be a higher proportion of weakly-bound metal ions, some of which may be held in ion-exchangeable form. Again, some wet chemical extraction methods (*e.g.*, 0.01M $CaCl_2$; Whitten and Ritchie (1991); Van der Ent et al., (2019) and review by McLaughlin et al. (2000)) appear to be designed to selectively determine trace metal cations in this fraction. The success of some of these methods, in determining fractions of metals which correlate well with plant uptake, may reflect the importance of weakly-sorbed exchangeable ions as a buffer for the soil solution pool. Although these correlations may occur for some elements, such as Cd, plant uptake of other metals (e.g. Cu, Ni and Zn) was shown to be poorly correlated to the "easily extracted" fraction in soils determined via the $CaCl_2$ method (Pauget et al., 2015).

Concentrations of metals in soil solution are usually very low compared with those in the solid phase, and reflect a combination of equilibrium with sparingly soluble and sorbed forms of metals, and formation of soluble inorganic and organic complexes (Wolt, 1994). A critical

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parameter is the activity or concentration of "free" metal ion, the value of which is central to the free-ion activity model of bioavailability discussed below.

Effect of residence time on metal availability

Whether trace metals such as Cu, Ni, Cd or Zn enter soil as ions or as components of amendments such as fertiliser or sewage sludge, short-term pedological processes act to change their speciation and bioavailability (*e.g*., Smolders et al. 2009; van Oort et al., 2018). Laboratory studies (e.g., Backes et al., 1995; Qiang et al., 2017) commonly demonstrate that, if metal ions react longer with soil components they desorb less and the desorbed fraction is released more slowly than for shorter reaction times. A contrasting concept is the "time bomb" hypothesis, where it is thought that metals applied in organic residues such as sewage sludge will increase in bioavailability in the medium- to long-term, following mineralisation of organic adsorbing phases and associated acidification (McBride, 2003; Bramryd, 2013). For example, the concentration of available Cd was shown to increase in soils under pig manure application only after 10 years (Xu et al., 2015). McGrath et al., (2000), however, found that Zn and Cd extractability from soil did not change significantly more than 20 years following sewage sludge application, results which do not support either increased or decreased bioavailability in the long-term. Some steps towards resolution of this issue have been made by Bergkvist and Jarvis (2004), who show by a modelling approach that long-term changes in metal bioavailability vary according to soil and sewage sludge properties and metal content, and that no universal outcomes exist.

2.3 Contemporary concepts for bioavailability of metals in soils

Bioavailability and bioaccessibility

It is significant that there is still some contention about how bioavailability should be defined for soil systems; many studies and reviews contain implicit operational definitions, or avoid making a definition altogether (e.g., Basta et al.*,* 2005; Nolan et al., 2003; Antoniadis et al., 2017). Semple

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et al. (2004) distinguish bioaccessibility and bioavailability, where a substance is *bioavailable* if a transfer from soil into an organism can occur, and *bioaccessible* substances are able to resupply the bioavailable pool, but are separated from organisms spatially or temporally, depending on their chemical or physical state. A common and often implicit assumption is of a continuum of "bioavailability" decreasing from free ions in solution to exchangeable ions, chemisorbed ions, and finally ions within mineral structures. In summary, bioavailability refers to a chemically active compound readily available to an organism, whereas bioaccessibility refers to a chemically inactive but potentially exploitable compound (Semple et al. 2007; Kim et al, 2015). Such discrimination is particularly interesting for bioremediation studies, in which the bioaccessible fraction can be a useful endpoint for assessment.

Methods for predicting bioavailability

It can be assumed, simplistically, that measurable fractions of metals in soils represent the bioavailable and/or bioaccessible component, without explicitly considering the biological uptake mechanism. This provides the rationale behind numerous chemical extraction techniques for measuring a fraction of metals in soils using electrolytes, dilute acids and/or buffers, or complexing agents, including many sequential extraction schemes (Lindsay and Norvell, 1978; Shuman, 1985; Tessier et al., 1979; Whitten and Ritchie, 1991). Further efforts, to improve the ability to predict bioavailability, have assumed that using an extractant which simulates the composition of earthworm gut fluids (e.g., relevant enzymes, anoxia) will more accurately reflect uptake and toxicity (Ma et al., 2009). Smith et al. (2010) showed that Cu, Pb and Zn extracted by a simulated earthworm gut extractant were correlated with some toxicity outcomes for plants, earthworms and collembolans in soils, out-performing total metal and other extractable metal concentrations. Using soil metal concentrations in combination with other soil chemical data has also been employed to predict metal bioavailability. A promising approach was presented by McBride et al. (1997), who proposed a semi-mechanistic model using total analyses of metals in soils in combination with

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measurements of soil parameters known to influence metal ion speciation (pH, organic carbon content) to successfully predict concentrations of metals in soil solution. More recent work by Smolders et al. (2009) showed that the best predictor of toxicity thresholds to plants or invertebrates (earthworms or collembolans), over a large range of soils, was the total concentration of metal in soil as a proportion of effective (unbuffered) cation exchange capacity (ECEC). This ratio needed to be corrected for leaching and/or aging of contaminated soils in the field, and the study also showed that neither soil pH nor free metal ion activity could reliably predict toxicity endpoints (Smolders et al., 2009). Nevertheless, Pauget et al. (2012), studying trace metals in snails, concluded that a good prediction of environmental bioavailability may come from focusing on total soil concentrations coupled with the influence of soil characteristics, such as pH and CEC. When considering both snails and plants, the accumulation of metals were influenced by very different parameters, mostly due to their physiological mechanisms (e.g. rhizospheric processes) and acquisition sources, i.e. superficial/deep soil layers for plants, and superficial soil and plant material for snails (Pauget et al., 2015). Thus, the authors concluded that bioindicators should complement chemical procedures to get better insights into contaminated areas.

A consensus has now emerged that bioavailability is a process that cannot be attributed to one single value, measured by a single method. There is a plethora of chemical techniques and biological systems devised for accessing trace metal bioavailability, such as invertebrate, plant and microbial tests - including single cell biosensors – which can measure different endpoints (toxicity, enzymatic activity, antioxidant compounds, fluorescence, bioluminescence etc.). Such methods have been thoroughly reviewed by Kumpiene et al. (2017).

Modelling trace metal bioavailability in soils

Metal availability in soils is the initial step from which a contaminant enters the food chain and moves up trophic levels. Therefore, models that can effectively predict trace metal

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biovailability in soil and plant uptake are the foundation for understanding transfer of trace metals in the soil-plant-arthropod system and for ecotoxicological risk assessments.

Free ion activity model(s)

The free-ion activity model of bioavailability assumes that metal uptake will be proportional to the activity of the free, uncomplexed metal ion in solution (Parker and Pedler, 1997). While some studies have shown a positive relationship between metal ion activity and plant uptake (Nolan et al., 2005; van Gestel and Koolhaas, 2004; Zhang et al., 2001; Shahid et al., 2011; Qiu and He, 2017), the free ion activity model does not account for resupply from soil solid phases which buffer metal ion activities, nor does it explicitly consider competition at the solution – organism interface or the influence of plant metabolism in the rhizosphere, and therefore would not necessarily be expected to apply in soils (Parker and Pedler, 1997; Parker et al., 2001). For instance, predicted free ion activities failed to explain metal concentrations in snails from soils contaminated by Cd, Pb and Zn, while a better prediction was obtained when total metal concentrations and soil variables were used as explanatory variables (Mourier et al. 2011). However, a recent model for 'free metal ion activity at the plant cell membrane' developed by Qiu and He (2017) accurately predicted Cu^{2+} and Zn^{2+} toxicity and uptake in *Hordeum vulgare* growing in soil, even when plants were exposed to a mixture of both metals; despite not accounting for internal transport mechanisms.

Metal-supply-based or kinetic models

Compelling evidence that metal ion uptake by plants is a function of both instantaneous dissolved concentration and the kinetics of resupply from soil solid phases comes from the work of Davison et al. 1999 and Zhang et al. (2001). The study by Zhang et al. (2001) showed that the "effective concentration" of Cu (C_E) measured by **diffusive gradients in thin films (DGT)**, was the best predictor of copper uptake by plants, followed by (in order) total dissolved Cu, free Cu^{2+} activity, and Cu extracted with EDTA solution. This confirms work showing that isotopically

exchangeable metal ions correlate well with metal uptake by plants (Hamon et al., 2002) and also older work showing plant uptake correlating with exchangeable (or neutral electrolyte-extractable) metal ions with relatively short extraction times (*e.g*., Whitten and Ritchie (1991); and discussed in the review by McLaughlin et al. (2000)). Several subsequent studies have confirmed the ability of DGT to predict phytoavailability under some soil conditions (e.g. (Mason et al., 2010; Perez and Anderson, 2009; Puschenreiter et al. 2013), but other studies do not show any relationship between metal ion fluxes measured by DGT and plant uptake. Oporto et al. (2009) show that at high soil Cd supply, where diffusion to the organism surface may not be limiting, DGT is not a good predictor of plant Cd uptake. In a study focusing on toxicity thresholds rather than uptake, Smolders et al. (2009) also showed that DGT could not predict Zn toxicity endpoints (EC_{50}) across a range of soils; the focus on toxicity presupposes that the range of soil Zn concentrations also reached high values. In contrast, Tandy et al. (2011) tested the prediction capacity of DGT for plant-available Cu and Zn in several soil types and at agronomical relevant concentrations, and found this method to be more accurate than the usual EDTA and DTPA methods. Two reviews (Degryse et al., 2009; Zhang and Davison, 2015) analysed a number of studies relating DGT metal analyses in soil to bioavailability. Both works concluded that DGT can validly predict plant uptake in situation where diffusion of metal ions to plant roots is limiting, but not where soil metal concentrations are high (in agreement with Oporto et al. (2009) and Smolders et al. (2009)). Labile metal complexes also contribute to DGT assays, and such complexes may or may not be taken up by plants (Degryse et al., 2009).

"Soil" versions of the Biotic Ligand Model

The biotic ligand model extends chemical equilibrium modelling to include the biological receptors on an organism as additional ligands for which complexation is competitive, as for other ligands, between metal ions and protons (Di Toro et al. 2001; Slaveykova and Wilkinson, 2005) (Figure 3; see later section on uptake by plant roots). A distinguishing feature of the biotic ligand approach is that metal ion toxicity is not affected by chemical gradients in the bulk solution (e.g.

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concentrations, pH), and its predictions depend on the amount of metal effectively bound to biological receptors, therefore toxic effects are *less* likely at lower pH, where other available metals (such as Ca^{2+} and Co^{2+}) will compete for the same biological carriers (Wang and Song, 2019). **The biotic ligand model (BLM)** has successfully predicted bioaccumulation and toxicity to fish and other aquatic organisms (Nolan et al., 2003; Blewett and Leonard, 2017). Weng et al. (2003), however, suggested that the magnitude of soil effects may overwhelm biotic ligand effects for Ni uptake; their work showed that the effect of pH follows that expected, if dissolved Ni^{2+} activity is controlled *via* adsorption or precipitation rather than competition for a biotic ligand. Conversely, a study by van Gestel et al. (2004), of Cd accumulation by collembolans, presented data to suggest that a BLM approach may be applicable for bioavailability in soil.

[**Fig. 3 near here**]

Thakali et al. (2006) showed that a terrestrial BLM predicts toxicity of soil-derived Cu^{2+} and Ni^{2+} to plants, such that root elongation could be predicted from the amount of Cu^{2+} or Ni^{2+} bound to a biotic ligand, while Lin et al. (2018) also predicted Cu toxicity effectively with BLM, in which H^{+}, Ca^{2+} and Mg^{2+} were shown to alleviate its toxicity. The BLM-based approach was also efficient in combined toxicity of Cu and Zn nutrient solution, also inversely correlated to Ca^{2+} and Mg^{2+} concentrations (Wang et al., 2017), while an adaptation of the BLM, for nonlinear metal relationships was successfully used to predict alleviation effects of Ca^{2+}, Mg^{2+} and K^{+} in Cd^{+2} toxicity in *Glycine max* roots (Chen et al., 2017). The predictive ability of these models, however, was restricted to the conditions of their study, which included a high range of total metal ion concentrations, to reflect the focus on toxicity and contaminated soils. Whether the terrestrial BLM approach would predict metal ion uptake in uncontaminated soils is not yet determined. The BLM approach implicitly assumes equilibrium, and in many cases metal ion bioavailability is likely to be controlled by rate-of-supply, with kinetics limited by diffusion or rate of release from a solid phase; this may be the reason for the success of DGT in predicting plant uptake in some studies (*e.g.*, Zhang et al., 2001; Zhao et al., 2006; Perez and Anderson, 2009; Ahumada et al., 2014).

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Interestingly, the BLM approach predicts phytotoxicity under the conditions (high soil metal contents) where DGT predictions have been shown to fail (Oporto et al., 2009).

Nevertheless, models such as the BLM need constant refining and calibration, an example is inclundig metal toxicokinetics (TK) into the equation, considering uptake/elimination rates and internal distribution (highly variable among different organisms), which may lead to a more predictive capability for metal bioaccumulation and toxicity (Ardestani et al., 2014).

2.4 Transport and mobility of trace metals in soils

Transport phenomena for trace elements in soils have been reviewed thoroughly by Carrillo-González et al. (2006). Generally, mobility of Cd, Cu, Ni and Zn is low relative to the rate of water movement in soils. This low mobility is a consequence of reactions which partition metal ions into solid phases: (co)precipitation; adsorption; and ion exchange. The thermodynamics of these reactions favour the existence of metals in solid phases, and the low observed mobilities also suggest that rates of metal ion release to the aqueous phase are slow. Factors which affect these equilibria (mainly metal ion identity and concentration, amount and type of adsorbing phases, pH, E_h) consequently affect metal transport, as do formation of soluble complexes or adsorption on mobile colloids. The intensity of sorption reactions in soils decreases in the approximate order Cu>Zn≈Ni>Cd (*e.g.*, Baker and Senft, 1995; Adriano, 2001; Young et al., 2012), with mobility increasing in the same order, but this ranking can vary with soil characteristics and is not replicated in all studies. Even for relatively weakly adsorbed elements like Cd, mobility is often observed to be low. The resulting small transport distances over observable time scales are shown by the retention of contaminant-derived metals in surface soil horizons, except in very acidic or sandy soils (Adriano, 2001). Over longer timescales, highly leached soils such as podzols show evidence of metal mobility in the form of near-surface depletion and enrichment in subsoil horizons (*e.g.*, Berggren, 1992). Mass transport of metal ions is likely to be dominated by preferential water flow, which is influenced by soil texture and structure (McLaren et al., 2004; Tack, 2010), with

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increasing recognition of colloid transport as a mechanism enhancing mass fluxes of metals (Sherene, 2010; Won et al., 2019). If the implications derived from use of the DGT technique (Zhang et al., 2001; Zhang and Davison, 2015) are valid, however, metal ion supply to plants seems more likely to occur by diffusion than mass transport. Diffusive transport depends on the concentration gradient, ionic diffusion coefficient and physical factors, such as water film thickness (Sparks, 1989; Carrillo-González et al., 2006). The plant root's ability to minimise diffusion distances is therefore critical, and this is discussed in later sections.

2.5 Rhizosphere soils: modification of trace metal chemistry by plants

Plants have various strategies for modifying the uptake of trace elements such as Cd, Cu, Ni or Zn from soil in the immediate vicinity of roots. Lowering of rhizosphere pH by H^+ release affects solubility and chemisorption equilibria, increasing soil solution concentrations of trace metal cations which are then susceptible to plant uptake (Marschner et al., 1986; Gomes et al., 2016). Production of metal-complexing ligands by plants also increases metal concentrations in solution, perturbing sorption and solubility equilibria as complexation reactions lower free metal ion activity (Merckx et al., 1986). Some metal-organic complexes have been shown, in solution culture, to be bioavailable for Cd (Cabrera et al., 1988) and Cu (Guo et al., 2008), as have been some complexes with simple inorganic ligands (Smolders and McLaughlin, 1996). Phytosiderophores (PS) released into the rhizosphere are also able to form PS-metal complexes and increase the bioavailability and uptake of Cu, Ni and Zn (Puschenreiter et al., 2017), however, the strength of binding and the capacity of roots to take up PS-metal complexes appears to vary among these metals (Kudo et al., 2007). Even though oxidation/reduction reactions in the rhizosphere are largely influenced by microbial activities (Seshadri et al., 2015; Ma et al., 2016), lowering of the redox potential of the rhizosphere is exploited by some plants as a mechanism for enhancing Fe and Mn uptake by reductive dissolution of Fe^{III} or Mn^{IV} oxyhydroxides (Marschner et al., 1986), but it is unclear whether this mechanism also affects uptake of associated trace elements such as Cd, Cu, Ni or Zn.

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Plants growing in anoxic soils can also add oxygen to their rhizosphere via aerenchyma, which can oxidise and dissolve sulfides and associated trace elements (Du Laing et al., 2009). Rhizosphere oxidation is also known to deposit ferric oxyhydroxides ("iron plaque") adjacent to roots (Begg et al., 1994), a process which co-accumulates trace elements such as Zn. As for rhizosphere reduction, however, the significance of rhizosphere oxidation for elements other than Fe and Mn is unclear (Du Laing et al., 2009), although new findings suggest that aerobic conditions tend to release Cd, Cu and Pb in soils (Rinklebe et al., 2016).

2.6 Soil bioavailability and multi-trophic metal transfer

The bioavailability of trace elements such as Cd, Cu, Ni and Zn in soil is a complex issue and, despite considerable progress being made, there are clearly ongoing issues with defining a bioavailability concept in soils and understanding the purely soil-related factors which affect the transfer of trace elements to organisms. The following discussion is informed by the basic soil chemical concepts described in the preceding sections, augmented by advances in understanding of the role of chemical and physical kinetics and in the ability of organisms themselves to modify trace element behaviour in soils by extracellular phenomena. A knowledge of soil processes alone cannot address within-organism transfers; the following section, therefore, focuses on the response of plants to the conditions created by soil processes, and the internal processes of the plants.

3. Terrestrial Plants

Terrestrial plants have developed a range of mechanisms to assimilate trace metals from soil environments with widely differing physical and chemical characteristics. In this section we discuss the plant physiological processes that govern the uptake, transport, transformation and storage of metals in plant tissues. These include the influence of roots and their associated mycorrhizal fungi on metal availability in the rhizosphere, and the factors regulating transport, complexation and sequestration of metals within the plant. All these processes affect the distribution and

concentrations of trace metals throughout the plant and consequently influence the degree of exposure of arthropods to trace metals.

3.1 Availability of trace metals to terrestrial plants

Prior to transport across the root plasmalemma a number of factors can affect trace metal availability to the plant, with the specific mechanisms employed varying among plant taxa. Plant roots typically increase the availability of metal ions to uptake proteins by acidifying the rhizosphere through the release of H^+ or carboxylic acids (e.g. Pang, et al., 2010) and consume or release O_2 altering the redox potential. In addition, the secretion of low-molecular weight compounds can mobilize metals directly by functioning as metal chelators, or indirectly by stimulating microbial activity in the rhizosphere (Marschner, 2012; Clemens, 2006; Parrotta et al., 2015).

A further factor affecting the availability and uptake of trace metals by terrestrial plants is the formation of symbiotic associations between the majority of plants and mycorrhizal fungi. Mycorrhizal fungi reside in the rhizosphere as spores, hyphae and propagules and colonise plant roots forming a link between the plant and the rhizosphere soil. The external mycelium of these commonly mutualistic associations plays a key role in nutrient uptake of most terrestrial plants by accessing nutrients beyond the diffusion zone of the root, and also accessing inorganic and organic nutrients not readily available to plants. Up to thirteen mycorrhizal types have been defined, the dominant ones being: arbuscular mycorrhizas (AM), ectomycorrhizas (ECM) and ericoid mycorrhizas (ERM) (Kariman et al 2018). Each mycorrhizal type differs in its structural characteristics and forms symbioses with particular groups of plants including grasses, forbs, crop plants, shrubs, orchids and trees. Their essential functions of bi-directional nutrient exchange of carbon from plant to fungus, and soil nutrients from fungus to plant are similar, with the exception of mycoheterotrophic plants (Bidartondo, 2005).

3.2 Uptake of trace metals by terrestrial plants

Mycorrhizal fungi

In addition to altering the availability of metals to plants in the rhizosphere, mycorrhizal fungi also enhance or regulate nutrient uptake and transport in plants. AM hyphae colonise many agriculturally important plants and take up nutrients such as P, N, Cu and Zn (Ryan and Tibbett, 2008; Cavagnaro, 2008; Watts-Williams et al., 2015), and are also important in natural and semi-natural ecosystems where they have a role in structuring plant communities (Standish et al., 2007; Bever et al., 2010). However, their contribution to nutrient uptake is particularly important for those nutrients that are diffusion limited due to their low mobility in soils, including P, Cu and Zn. For example, in *Trifolium repens*, 50-60 % of total Cu and Zn uptake was provided by AM hyphae (Li et al., 1991), and in *Solanum lycopersicum* 24% of Zn was delivered by the same pathway (Watts-Williams et al., 2015). Studies also show that the majority of Cu and Zn taken up by AM fungi is retained in the roots, although increased translocation to the shoots has also been recorded (Lee and George, 2005; Garg and Aggarwal, 2012; Zheng et al., 2015). For Cd and Ni evidence on the influence of AM colonisation on uptake is contradictory. In *Phaseolus vulgaris, Zea mays, Phleum pratense* and *Trifolium subterraneum* AM symbiosis was shown to increase uptake of Cd^{2+} with the majority of the Cd being retained in the roots (Arnold and Kapustka, 1993; Guo et al., 1996; Joner and Leyval, 1997). In contrast, in cucumber plants AM infection decreased concentrations of Cd in both the roots and shoots (Lee and George, 2005); similar results were also found in pigeonpea (Garg and Chandel, 2012), maize (Liu et al., 2014) and for both Zn and Cd in hyperaccumulator *Thlaspi praecox* (Vogel-Mikus et al., 2006). With respect to Ni uptake by AM fungi there have been very few studies performed with divergent results. For instance, AM infection reduced Ni concentrations in the roots and shoots of at least six plant species studied, typically by increasing biomass production (Guo et al., 1996; Lee and George, 2005; Orlowska et al., 2011; Amir et al., 2013; Shabani et al. 2016), whileAM fungi enhanced Ni uptake in soybean, lentil and sunflower plants in contaminated soils (Jamal et al., 2002; Ker and Charest, 2010).

ECM and ERM fungi mainly colonise trees and ericaceous shrubs. They play a key role in the mobilisation and uptake of organic and inorganic N and P providing access to nutrient sources not directly available to plants (Tibbett and Sanders, 2002; Cairney and Meharg, 2003), and are also known to mobilise trace metals by dissolving a variety of Cd, Cu and Zn-bearing minerals, including metal phosphates (Gadd et al., 2012). Plant species colonised by ECM and ERM fungi can also grow successfully on highly metal-contaminated sites (Colpaert et al., 2011; Saraswat and Rai, 2011; Wen et al., 2017). They exhibit decreased sensitivity to a wide range of essential and non-essential metals including Cd, Zn, Ni and Cu, however, metal tolerance or sensitivity varies widely between plant and mycorrhizal species (Bradley et al., 1982; Bücking and Heyser, 1994; Chen and Tibbett, 2007; Frey et al., 2000; Gadd et al., 2012; Hartley-Whitaker et al., 2000; Hrynkiewicz and Baum, 2013; Sell et al., 2005; Mrnka et al., 2012). The mechanistic basis of the role of ECM and ERM fungi in plant metal tolerance or decreased sensitivity is poorly understood. For ECM fungi a number of mechanisms have been postulated including extracellular binding, reduced uptake, metal efflux, intracellular chelation or compartmentation in the vacuoles by polyphosphate granules (Hartley, et al., 1997; Lanfranco, 2007; Colpaert et al., 2011; Luo et al., 2014). In the ECM fungus *Paxillus involutus* key mechanisms proposed for Cd tolerance are the complexation by metallothioneins (MT) or by phenolic compounds (Jacob et al., 2004), while two MT genes from *Laccaria bicolor* were shown to confer tolerance to both Cd and Cu (Reddy et al., 2014). However, as results on the effects of ECM colonisation on metal uptake and localisation are so varied it is likely that mechanisms used vary between plant species and their symbionts. For ERM fungi, the shoots of ERM plants generally contain less metal than non-mycorrhizal plants (Bradley et al., 1982). Indeed, *Calluna vulgaris* plants under ERM symbiosis were more tolerant to Cu toxicity, and presented lower shoot Cu accumulation than the non-mycorrhizal plants (Gibson and Mitchell, 2006). Therefore, avoidance or sequestration strategies are likely to contribute to the observed tolerance. According to Martino et al. (2000), in the ERM fungus *Oidiodendron maius*, mechanisms such as production of mucilage and extracellular pigments may be pivotal in Cd and

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Zn tolerance. Studies focusing on gene expression and characterization in *O. maius* are being carried out to explain the genetic mechanisms for Cd and Zn tolerance in this species (Daghino et al., 2016; Di Vietro et al., 2014; Khouja et al. 2013). These strategies would reduce the concentrations of metal entering the plant root/shoot, and consequently reduce exposure of arthropods to trace metals in contaminated soils.

Plant roots

Our understanding of the entry of cations into plant cells has been advanced through molecular investigations (Clemens, 2001; Kramer et al., 2007). A number of metal transporters have been identified in *Arabidopsis thaliana* and *Nicotiana spp.*, which are involved in the uptake, translocation and sequestration of metal ions, and many of these have also been studied in the metal hyperaccumulating plants, *Arabidopsis halleri* and *Noccaea* (*Thlaspi*) *caerulescens* (Bovet et al., 2006; Kramer et al., 2007; Broadley, 2007; O Lochlainn et al., 2011). At the plasma membrane, uptake of Zn^{2+} from the rhizosphere is mediated by metal-uptake transporters belonging to the ZIP family (Fox and Guerinot, 1998; Clemens, 2006; Campos et al., 2017; Zhang et al., 2017), with16 identified in *A. thaliana* (Colangelo and Guerinot, 2006). These proteins are ubiquitous in eukaryotes and transport Zn from the extracellular space into the cytoplasm (Gaither and Eide, 2001a) in plants and arthropods (see "uptake of trace metals by arthropods" section). ZIP transporters have a broad substrate range transporting Zn^{2+}, Cd^{2+}, Mn^{2+} and Fe^{2+} (Eide, 2006; Grotz et al., 1998; Korshunova et al., 1999; Pence et al., 2000; Wu et al., 2009; Milner et al., 2013; De Oliveira et al., 2020). As for the detoxification of Zn within the plant, members of the MTP (metal tolerance protein) family, from cation diffusion facilitator (CDF) family, are known to mediate Zn transport from the cytoplasm into the vacuole (Gustin et al. 2009; Migocka et al., 2015). Yellow-stripe 1 like (YSL) proteins are believed to mediate the uptake of metals, particularly Fe, which are complexed with phytosiderophores or nicotianamine (NA) (Waters et al., 2006; Socha and Guerinot, 2014). The most studied, YS1 from Maize, may also play a role in metal homeostasis as it

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can also transport Zn^{2+}, Cu^{2+} or Ni^{2+} (Haydon and Cobbett, 2007; Sinclair and Kramer, 2012), while two other members of this transporter family – *AtYSL4* and *AtYSL6* – are also involved in Ni^{2+} and Mn^{2+} homeostasis (Conte et al., 2013). Cd^{2+} is also transported by the Nramp (= Natural resistance associated macrophage proteins) transporter, AtNramp, which is a Fe^{2+} transporter and LCT1, also transports Ca^{2+} (Clemens et al., 1998; Thomine et al., 2000; Clemens, 2006; Migeon et al., 2010; Jha and Bohra, 2016). Nevertheless, as Cd is nonessential it is likely that it enters plant cells coincidentally, through transporters with low substrate-specificity; it is generally understood that Cd^{2+} uptake by plants happens by a carrier for Zn^{2+}, or even other divalent cations, such as Cu^{2+} or Fe^{2+}, or by Ca^{2+} and Mg^{2+} transporters/channels (Guerra et al. 2011, Clemens, 2006; Pottier et al., 2015). Analogous Nramp transporters have also been reported in arthropods (see section on uptake by arthropods). With the established importance of Cu^{2+} in processes such as electron transport, the COPT family of transporters involved in plant uptake of Cu were identified some time ago (Kampfenkel et al, 1995; Sancenon et al., 2004, Burkhead et al., 2009), with several other members identified recently (Andresen et al., 2018). The high affinity copper transporter, *Arabidopsis* COPT1, has a physiological role in root elongation and pollen development with the reporter gene highly expressed in embryos, trichomes, stomata, pollen and root tips (Sancenon et al., 2004). Unlike Zn or Fe, Cu is taken up mainly as Cu^{+} by COPT1, but plants may also take up Cu^{2+} by members of the ZIP family (Palmer and Guerinot, 2009). Proteins from the COPT family, which occur in a range of organisms, may be expressed during copper deficiency, exhibit tissue-specific patterns and be influenced by endogenous concentrations of Fe, Mn or Zn (Penarrubia et al., 2010; Yuan et al., 2011). For instance, a crosstalk between vacuolar Cu and Fe pools mobilisation in *A. thaliana* was shown to involve the Cu-transporter COPT5, Nramp3 and Nramp4. While COPT proteins were more expressed in *A. thaliana* exposed to Cd, suggesting that Cu uptake is an essential component for Cd resistance in this species (Gayomba et al., 2013).

As cation transporters generally appear to have broad substrate specificity, competition for uptake between ions of the same charge is highly likely. This is assuming that the number of

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binding sites is small in relation to the concentration of competing ions (Clarkson and Luttge, 1989) (see earlier discussion on biotic ligand model). Competitive uptake of Zn^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+} has been reported in a range of plants (Brar and Sekhon, 1976; Bowen, 1981, 1987; Cataldo et al., 1978, 1983). It therefore appears that multiple pathways exist for most metal ions.

3.3 Transport of trace metals in plants

From the cells on the surface of the root, ions are transported both symplastically and apoplastically through the root cortex to the endodermis. The endodermis then acts as a semi-permeable membrane across which ions can be regulated and actively transported into the pericycle (Punz and Sieghardt, 1993). There is evidence that the mechanisms sequestering trace metals in the roots and/or governing the loading of trace metals into the xylem fluid are important in regulating the translocation of trace metals from the root to the shoot (Hart et al., 1998a), such as by vacuolar sequestration in root cells (Zhang et al., 2020). However, the mechanism(s) by which cations are loaded into the xylem fluid are only partially understood (Kerkeb and Krämer, 2003), as a process mediated by membrane transport proteins which can be energy-dependent (Kadukova and Kadulicova, 2011; Mori et al., 2009). For Cd and Zn, these metals are taken up mainly by ZIP transporters and loaded into the xylem by heavy metal ATPases (Mendoza-Cozatl et al., 2011; Andresen et al., 2018). For instance, HMA4 is considered a key transporter involved in Cd and Zn hyperaccumulation, as well as in xylem loading (Ó Lochlainn et al., 2011; De Oliveira and Tibbett, 2018), with its gene highly expressed in the root pericycle of *A. halleri* (Hanikenne et al., 2008).

Transport of metal ions in the xylem vessels may be by simple bulk transport or by a chromatographic exchange process: the exact process appears to be dependent on the metal and the charge of the metal-ligand complex (Petit and van de Geijn, 1978). In both processes, trace metal translocation in the xylem is by mass flow in the transpiration stream (Luo et al., 2016). As a result, the quantity of trace metals translocated is proportional to the rate of transpiration (Grifferty and

Barrington, 2000). Since the flux of some cations is related to water flow, aquaporines and divalent-channel cations may mediate this process (Cabañero and Carvajal, 2007). The formation of chelate complexes may determine the transport of metals in plants, for example, NA is involved in Cu and Ni transport in the xylem (Kadukova and Kadulicova, 2011; Printz et al., 2016; Andresen et al., 2018). Citric and malic acids have also been linked to Cu transport in the xylem (Cao et al., 2020), however Cd was found to be in free ionic form in the xylem sap of *A. halleri* (Ueno et al., 2008). In addition to vertical translocation from root to shoot, there is also lateral translocation of trace metals through pits in the vessel walls. This results in the lateral translocation of approximately 12% hr^{-1} of the Cd^{2+} concentration within the xylem fluid (Petit and van de Geijn, 1978).

Translocation of trace metals within plants also takes place *via* phloem transport cells. Trace metals can enter the phloem sap by selective transport from the xylem vessels within the stem or by remobilisation from other plant tissues, especially at the nodes (Van Bel, 1990) and leaf vein network (Pate et al., 1975). In wheat plants the flag leaf and peduncle appear to be important sites for the transfer of trace metals between xylem and phloem (Herren and Feller, 1994, 1997; Zeller and Feller, 1999). Two distinct processes are involved in this transfer, the removal of metal from the xylem, and the loading of metal in phloem (Page and Feller, 2015). Transporters mediate uptake into the symplast, and distribution within the leaf may occur via the apoplast or the symplast pathways (Kadukova and Kadulicova, 2011). Control mechanisms for these processes are separate, for instance, at high concentrations Zn^{2+} is eliminated from the xylem in the stem of wheat, where it is retained and not loaded into the phloem (Herren and Feller, 1994). Factors affecting the loading and unloading of trace elements in phloem are thought to be pH, E_h, ionic strength and organic constituents within the phloem sap (Welch, 1995). For Cu, Fe and Zn, NA is likely to be important for the phloem loading and unloading (Curie et al., 2009). As in xylem fluid, trace metals in phloem sap are generally in the form of complexes rather than ions (Welch, 1995). Analyses in several plant species have shown that NA, glutathione and phytochelatins (PCs) are the main metal-ligand

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molecules found in the phloem sap, with NA forming complexes with Cu, Fe and Zn, and glutathione and PCs with Cd, Zn and Hg (Mendoza-Cozatl et al., 2011; Komal et al., 2015).

Movement of trace elements within phloem is by mass transport, driven by the loading of phloem within source tissues and the unloading of sucrose at sink tissues (Welch, 1995), and can be greatly influenced by metal-ligands in the plant (Mendoza-Cozatl et al., 2011). The mobility of metals within phloem varies with the metal and is also influenced by competition between metals and, at high concentrations, the toxic effects of metals on phloem transport (Herren and Feller, 1997; Zeller and Feller, 1999). In young wheat leaves, phloem mobility of Ni and Zn was higher than the mobility of Co, Cd and Mn (Riesen and Feller, 2005). Different ecotypes also have different phloem distribution patterns for the same metal, for instance hyperaccumulator *Sedum alfredii* is able to relocate 44% of Cd to other organs via phloem, while its non-hyperaccumulator ecotype only relocates 10% (Hu et al., 2019). Phloem transport cells are metabolically active and may regulate the concentrations of trace metals and the form in which they are present in phloem sap (Welch, 1995). Phloem translocation can result in the redistribution of Cd^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} from the stem, flag leaf and peduncle to the ears of winter wheat (Herren and Feller, 1997; Zeller and Feller, 1999; Riesen and Feller, 2005; Page and Feller, 2015) and a similar redistribution of Cd^{2+} may also occur in durum wheat (Cieslinski et al., 1996). This redistribution of trace metal ions to the developing grain may have an important influence on the exposure of sap feeding insects to trace metals.

Metal distribution and sinks within the plant

Overall, the translocation of trace metals typically results in a pattern of distribution within plants where metal concentration falls in the order root-shoot-reproductive organs (Lubben and Sauerbeck, 1991; Cieslinski et al, 1996; Merrington et al, 1997a; Kabata-Pendias, 2010; Gallego et al., 2012). Arthropod herbivory can affect this, with increased concentrations of Cd and Zn in the ears of wheat plants observed under aphid infestation (Green et al., 2005), possibly as a defence

mechanism against herbivory (Gall et al., 2015). The binding of metal ions through storage/detoxification mechanisms within the root and mycorrhizal fungi are probably responsible for the high concentrations of metals within this organ (Hart et al., 1998b; Bellion et al., 2006; Saraswat and Rai, 2011; Gonzales-Guerrero et al., 2016). However, there are large differences in the extent of root to shoot trace metal translocation among plant species (Jarvis et al., 1976). Cadmium is an exception to the normal pattern as it is found in higher concentrations in leaves and bark of some species such as poplars, flax and leafy vegetables such as spinach (Cieslinski et al, 1996; Marzilli et al., 2018), as well as plants considered to be hyperaccumulators (Kramer, 2010; Gallego et al., 2012).

Translocation to the reproductive organs occurs directly via the xylem pathway and also through redistribution via the phloem (Herren and Feller, 1994; Cieslinski et al, 1996; Herren and Feller, 1997; Zeller and Feller, 1998; Zeller and Feller, 1999; Mendoza-Cozatl et al., 2011; Printz et al., 2016). The xylem pathway translocates trace metals mainly to the vegetative parts of the reproductive organs, while the phloem pathway appears to be more important in translocation to the developing seed (Herren and Feller, 1994; Herren and Feller, 1997; Zeller and Feller, 1999; Mendoza-Cozatl et al., 2011; Deng et al., 2018). The relative importance of xylem and phloem pathways in translocation depends on (i) the extent of unloading of the metal from the xylem in the shoot, (ii) the relative mobility of the metal in the phloem and (iii) the control the plant can exert on the loading of the metal in the phloem. For instance, the majority of Zn^{2+} is unloaded from the xylem sap in the peduncle of wheat plants (Herren and Feller, 1994). Zn^{2+} reaches the developing ear by being loaded into the phloem in this site (Herren and Feller, 1994). Wheat can control the loading of Zn^{2+} into the phloem and thereby effectively regulate the Zn^{2+} concentration in the grain. In contrast, the extent of Cd^{2+} unloading in the peduncle is much less than Zn^{2+}, and therefore translocation to the ears of wheat occurs by both xylem and phloem pathways (Herren and Feller, 1997). Nonetheless, recent studies in rice showed that Cd deposition in grains was mediated by phloem transport, attributed to a transporter gene *OsLCT1* identified to be highly expressed during

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reproductive stages (Uraguchi and Fujiwara, 2012), while in wheat Cd was effectively remobilized from roots and stems, and accumulated in grains (Yan et al., 2019).

Several protein families are involved in the long-distance transport of trace metals in plants, with the majority identified in *A. thaliana*. The monovalent (Cu(I)) and divalent (Zn(II)/Cd(II)) cation transporting P_{IB}-type ATPases are crucial in Cu and Zn translocation from root-to-shoot, respectively (Hussain et al., 2004), while the ATPases HMA2 and HMA4 are a major mechanism for Cd translocation (Wong and Cobbett, 2009; Ó Lochlainn et al., 2011). Proteins of the IREG family are potentially involved in root-to-shoot transport of Ni and other transition metals (Kramer et al., 2007), as well as the NiCoT protein family (Czajka et al., 2019). Finally, it has been hypothesised that YSL transporters, which transport metal complexes with NA and other LMW compounds, may also be involved in long-distance transport of transition metals and remobilisation from senescent tissues to reproductive organs (Haydon and Cobbett, 2007; Kramer et al., 2007; Sinclair and Kramer, 2012; Printz et al., 2016).

3.4 Transformation of trace metals in plants

Trace metals taken up by plants are transformed within the plant for a number of reasons: to fulfil a range of metabolic plant functions; to detoxify excess concentrations of trace metals and render them metabolically inactive; and to transport the complex mix of ions found in xylem fluid, some of which can be reactive or easily immobilised by hydrolysis (Clemens, 2006). The complexing ligands are predominantly organic acids and anionic and cationic amino acids/peptides (Cataldo et al. 1988; Cobbett and Goldsbrough 2002; Andresen et al. 2018; Chen et al. 2018). The type of ligand bound to a metal ion is metal specific and each metal may be bound to more than one type of ligand. However, unbound, as well as bound, divalent metal ions also appear to be present within xylem fluid (Petit and van de Geijn, 1978; Cataldo et al. 1988; Mendoza-Cozatl et al., 2011; Lu et al. 2013).

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Zinc and nickel ions are complexed within the plant by organic acids and amino acids, but the actual ligands involved vary between plant species and plant tissues (Sinclair and Kramer, 2012). In grains and seeds, Zn^{2+} is localized in globoid crystals, protein bodies mainly consisting of phytate, which form a stable complex with Zn^{2+} (Welch, 1995). These complexes have also been identified in the roots, but not shoots, of a range of crop plants and have been implicated in restricting root-shoot transport of Zn (Vansteveninck et al., 1994). High citrate and malate concentrations in Zn tolerant plants have also been correlated with high internal Zn concentrations and Zn tolerance (Godbold et al., 1984; Harrington et al., 1996). Other compounds that are proposed to bind Zn in plants are organic acids, oxalate, phosphate, phytate and pectates (Sinclair and Kramer, 2012). Furthermore, in the hyperaccumulator *Thlaspi caerulescens* the majority of Zn^{2+} in the roots is complexed with histidine, but is transported to the shoot in the xylem as a hydrated cation, with a small proportion present as a Zn citrate complex. Once in the shoot, Zn is mainly complexed with citrate with small concentrations bound to histidine, oxalate and the cell wall (Salt et al., 1999). These complexes may be stored in the vacuoles of epidermal cells, and the cell walls of epidermal and mesophyll cells (Kupper et al., 1999; Frey et al., 2000). Generally, trichomes and epidermal cells accumulate the highest Zn concentrations, yet cell vacuoles probably make the greatest contribution in storing excess Zn in leaves (Sinclair and Kramer, 2012).

Nickel is mobile in the xylem and the phloem and is transported to the shoots in both hyperaccumulators and non-hyperaccumulators (Kochian, 1991; Riesen and Feller, 2005). In non-hyperaccumulators, Ni^{2+} is retained mainly in the roots, with a small proportion transported to the shoots. In hyperaccumulators Ni^{2+} is accumulated in the shoots: however, the mechanism by which this occurs differs between plant genus. In *Alyssum* spp. xylem and shoot Ni^{2+} concentrations are strongly correlated with the histidine concentration, with histidine involved in both Ni^{2+} transport into the xylem and storage in the leaf (Kramer, 2010). In the hyperaccumulator *Thlaspi goesingense* increased vacuolar storage and the resultant Ni^{2+} tolerance is responsible for the hyperaccumulator phenotype, not high rates of root-shoot transport (Kramer et al., 2000). However, histidine can

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sometimes suppress Ni transport into root vacuoles and might even enhance root-to-shoot Ni flux (Kramer, 2010). Nickel in the shoots of both hyperaccumulators and non-hyperaccumulators is located in the apoplast, cytoplasm and vacuole, with hyperaccumulators storing Ni^{2+} in the vacuoles of epidermal cells (Psaras et al., 2000; Van der Ent et al., 2020). Additionally, in hyperaccumulators from the *Noccaea* (*Thlaspi*) genus, a glutathione concentrations can be positively correlated with the capacity of plants to accumulate and tolerate high Ni concentrations (Freeman et al., 2004). In *Festuca arundinacea*, up-regulation of MT genes was also associated with Ni accumulation (Shabani et al. 2016). There are also variations in the ligands binding Ni^{2+} in different plant species, with citrate, malate, malonate, glutamine and histidine all identified as complexing Ni^{2+} in plant shoots (Kramer et al., 1996).

The two most studied metal binding ligands in plant cells are PCs and MTs (Cobbett and Goldsbrough 2002; Andresen et al. 2018). PCs are a class of non-protein structures with increasing repetitions of Gly-Cys terminated by Gly, they are enzymatically synthesised – by glutathione and phytochelatin synthase – and generally induced by metal and metalloid exposure (Rauser, 1990; Clemens, 2001; Garg and Kaur, 2013; Kaur and Garg, 2018). In contrast, MTs are small gene encoded cys-rich proteins which complex metals, particularly Cu, in animals, fungi and plants (Rauser, 1990; Hassinen et al. 2011).

PCs are important for the transport and detoxification of Cd^{2+} and to a lesser extent Cu^{2+} and Zn^{2+}. In the cytosol PCs chelate Cd^{2+} to form low molecular weight complexes which are translocated across the tonoplast via MTP and CDF proteins and sequestered in the vacuole of cells (Clemens, 2006; Garg and Kaur, 2013; Lin et al., 2016). Once in the vacuole these complexes combine with more Cd^{2+}, sulphur and other PC chains to form high molecular weight complexes (Salt and Rauser, 1995; Cobbett and Goldsbrough 2002; Kaur and Garg, 2018). There is also evidence from *A. thaliana* that PCs undergo long-distance root-to-shoot and shoot-to-root transport, with root-to-shoot transport resulting in reduced Cd^{2+} accumulation in roots (Gong et al., 2003; Chen et al., 2006).

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The mechanism of Cu^{2+} and Zn^{2+} complexation is less clear with conflicting evidence on the complexes involved. Cu^{2+} has a high affinity for peptide and sulfhydryl groups e.g. cysteine-rich proteins, as well as carboxylic and phenolic groups. Consequently, in roots and xylem sap more than 98% of Cu^{2+} is complexed (Marschner, 2012). PCs (cys-rich proteins) are involved in the homeostasis of Cu^{2+} and Zn^{2+} (Tennstedt et al., 2009). Their induction by Cu^{2+} and the formation of Cu-PC complexes has been proven using HPLC-ICP-AES in *Rubia tinctorum* root cultures (Maitani et al., 1996). There is no information available on the localization or storage of Cu-PC complexes; however, we postulate that they are located and transported in the same way as Cd-PC complexes, described above. In a study with several plant species growing in a contaminated mining area, Machado-Estrada et al. (2013) verified greater accumulation of Cu in stems of *Euphorbia prostrata*, whilst low accumulation of thiol-containing molecules, suggesting that PCs were not much involved in Cu accumulation. Unlike Cd, PCs would not be a primary factor in determining Cu tolerance (Lee and Kang, 2005; Hego et al., 2014) despite activating PC synthesis in algae (Mellado et al., 2012). In fact, PCs would be more pivotal in tolerance of non-essential metals, such as Cd and Hg, than in tolerance of toxic concentrations of essential metals (Hodson, 2012).

With respect to MTs, whilst MT-like gene sequences have been identified in plant tissues (Clemens, 2001), their function in plants is still unclear. There is some evidence they are involved in Cu tolerance and homeostasis, and are up-regulated under metal excess (Cobbett and Goldsborough, 2002). As yet there is still little molecular evidence of metal complexation by MTs within the plant (Clemens, 2006). However, several studies have shown that the heterologous expression of plant MT genes in yeast systems can increase tolerance and accumulation of metals such as Zn, Cd and Cu (Kohler et al., 2004; Guo et al., 2008; Yadav 2010; Sheoran et al., 2011; Bulgarelli et al., 2016; De Oliveira et al., 2020). In addition, MTs are involved in scavenging ROS (reactive oxygen species) in plant cells under stress through their Cys residues (Wong et al., 2004; Hassinen et al., 2011; Ruttkay-Nedecky et al., 2013).

3.5 Plants and multi-trophic metal transfer

Plants play a major role in regulating the transfer of metals from soil to arthropod, and should not be regarded as a neutral or passive carrier. Plants possess a variety of mechanisms for metal homeostasis and detoxification which result in metals being partitioned and complexed throughout the plant to varying degrees (see sections 3.3, 3.4). Essential metals such as Cu, Zn and Ni are sequestered mainly in the roots, with some restricted transport to shoots and reproductive organs. Cadmium, as a non-essential metal, has a higher propensity to accumulate in tissues other than the roots, with the exception of known excluder species, increasing the potential exposure of herbivorous arthropods, although this can vary between species and varieties. The complexation, transport, partitioning and sequestration of metals *in planta* can all affect the availability of metals and hence their subsequent transfer to herbivorous arthropods. These processes vary between plant species and also vary dependent on the internal metal load of the plant. There is also a potential feedback mechanism in-play as arthropod herbivory can affect the distribution of metals in plants (Green et al., 2005).

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4. Invertebrate herbivores

Invertebrate herbivory affects all terrestrial plant families, although the extent of herbivory will vary with the effectiveness of the plants defence mechanism (Kant et al., 2015), and can occur at a variety of stages in a plant's development and by a wide range of arthropod grazers. Indeed, the phylum Arthropoda consists of a diverse range of organisms, which is reflected in the diversity of trace metal concentrations found in the species of this phylum (Dar et al, 2019). This is the case among even closely related species feeding on similar diets and is derived from inter-specific differences in physiology (Glowacka et al., 1997; Janssen et al., 1991; Laskowski and Maryanski, 1993; Rabitsch, 1995; Sá et al., 2008), which results in some species exhibiting a poor ability to regulate internal trace metal concentrations (Dallinger, 1993; Janssen et al., 1991; Kramarz, 1999b). This section examines the physiology of arthropods that affects the transfer and accumulation of trace metals from their food.

4.1 Availability of trace metals to herbivorous arthropods

The most obvious factor governing the input of metals to herbivorous arthropods is the concentration and form of the trace metal in the plant tissues and cells consumed (section 2) which can affect the absorption of the metal by arthropods (Hare, 1992). For example, metals in an insoluble form, such as bound to cell wall components, may be digested and absorbed with difficulty, while metals in an ionic form and in dissolved complexes, such as in the vascular fluids, may be readily absorbed (Cataldo et al., 1987; Dar et al. 2017; Dar et al. 2019), although ingesting whole plant material appears to increase overall bioaccumulation (Butt et al. 2018). The transfer of metallic nanoparticles from plant to insects can also occur, as demonstrated by Kubo-Irie et al. (2016), in which TiO_2 nanoparticles were effectively taken up by the host plant (*Aristolochia debilis*), translocated to the leaves and eventually transferred to the larvae of the swallowtail butterfly (*Atrophaneura alcinous*).

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4.2 Uptake of trace metals by arthropods

By far the greatest input of trace metals into the soma of arthropods is through the ingestion of food (Janssen et al., 1993). Absorption of trace metals through the integument of terrestrial arthropods is thought to account for only a minor proportion of metal uptake (Janssen, et al., 1993; Vijver et al., 2005). Hence, the alimentary canal is the primary site of trace metal absorption. In arthropods, this organ consists of three regions, the foregut, midgut and hindgut. Both the foregut and hindgut are lined with cuticle, which leaves the midgut as the major site of nutrient and trace metal absorption (Chapman, 1998).

Trace metals are absorbed as free ions, which precludes uptake by simple diffusion over the plasma membrane. Uptake therefore proceeds via active transport proteins and facilitated diffusion through ion channels. The active transporters involved are closely analogous to those utilised by plant cells, but metal uptake in arthropods has received considerably less attention than in plants. Nevertheless, trace metal uptake by arthropod cells is complex with the uptake of a given metal potentially proceeding through more than one uptake system (Figure 4) and individual transport systems often exhibiting poor discrimination among elements, transporting several metals with similar physio-chemical properties. This later point is particularly important in the uptake of non-essential trace metals for which specific uptake mechanisms have not evolved.

[FIG. 4 NEAR HERE]

The primary uptake mechanism for the essential elements Cu and Zn have been clearly established. For Cu, the Cu^+ transporter Copper Transporter 1B (Ctr1B) is the most efficient importer of Cu into the enterocytes of larval *Drosophila melanogaster* (Balamurugan et al. 2007). Expression of Ctr1B is much lower in adult *D. melanogaster* and the expression of the related Cu transporter Ctrl1A suggests that this protein may be more important in Cu acquisition in adult flies (Zhou et al., 2003). Consequently, differing, although related, mechanisms can be active in the developmental stages compared to the adult stage, reflecting the nutrient demands of growth and development.

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Mammalian divalent metal transporter 1 (DMT 1, also designated Nramp2, SLC11A2, and DCT1) actively transports a number of divalent cations, including Fe^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+} (Bressler et al., 2004; Gunshin et al., 1997; Illing et al., 2007), but not Zn^{2+} or Pb^{2+}, into cells (Bannon et al., 2003; Sacher et al., 2001). Malvolio (Mvl), the insect orthologue of DMT 1 (Folwell et al., 2006; Martínez-Barnetche et al., 2007), is a functional Fe and Cu transporter (Martínez-Barnetche et al., 2007; Southon et al., 2008). Indeed, despite the demonstrated importance of Ctr transporters for Cu acquisition, functional Mvl also appears to be necessary for optimal Cu acquisition (Southon et al., 2008)

As described for plants, Zn^{2+} influx transporter proteins of the Zrt/IRT-like protein (ZIP) family function as the main mechanism for Zn uptake in Arthropods (Navarro and Schneuwly, 2017). The number of putative ZIP proteins sequences in the genome of *D. melanogaster* ranges between 8 and 10 (Gaither and Eide, 2001a; Taylor and Nicholson, 2003; Wang et al., 2009) and ZIP sequences are also reported from other Arthopods (Taylor and Nicholson, 2003). The importance of the ZIP transports for Zn uptake by *D. melanogaster* has been clearly established (Lye et al., 2012).

Cadmium has no known biological function in Arthropods and whilst Ni has a limited biological role in eukaryotes, Arthropods lack the genes for the enzyme utilising Ni as a cofactor and the gene coding for the eukaryotic Ni transporter (Zhang et al., 2009). Thus, in contrast to Cu and Zn, no specific uptake mechanisms exist for Cd or Ni in Arthropods and uptake must proceed through mechanisms evolved for the acquisition of essential elements.

The potential mechanisms for Cd uptake are principally the active transporters DMT 1 and ZIPs and Ca^{2+} channels. Investigations of Cd uptake via DMT 1 in whole mammals, mammal cells or using mammalian DMT 1 expressed in *Xenopus* oocytes, suggest that DMT 1 can play a significant role in Cd^{2+} uptake (Bressler et al., 2004; Okubo et al., 2003; Park et al., 2002; Ryu et al., 2004). Similarly, ZIP transporters from yeast, plants and mammals are able to to transport Cd^{2+}, but they have a lower affinity for Cd^{2+} than Zn^{2+} (Gitan et al., 1998; Clemens, 2006; Cohen et al.,

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2004; Dalton et al., 2005; Fujishiro et al., 2009; Girijashanker et al., 2008; Gitan et al., 1998). On this evidence and the conserved nature of transporters in eukaryotic cells, it is highly likely that Mvl and ZIPs transport Cd^{2+} into Arthropod cells, but this remains to be tested experimentally.

Research has also strongly implicated calcium channels in Cd uptake by invertebrates at the cellular (Braeckman et al., 1999) and organism level (Craig et al., 1999). However, He et al. (2009) suggested that based on reported affinities, transport by ZIP proteins could be more important to the transport of Cd into cells than either calcium channels or DMT 1 at environmentally relevant concentrations. Indeed, in MT-1 and –II knockout cells, ZIP 8 knockdown reduced Cd accumulation by 35%, but DMT 1 knockdown had a minimal effect (Fujishiro et al. 2009). Moreover, Cd resistance in two cell lines derived from the MT knockout cells was not related to changes in T-type Ca^{2+} channel expression (Fujishiro et al. 2009).

The studies discussed above strongly suggest that Cd can be taken up via multiple pathways. However, there is a tissue/cell specific expression of each type of transporter (Fujishiro et al., 2009; Girijashanker et al., 2008; Martínez-Barnetche et al., 2007; Wang et al., 2009), which results in various uptake pathways in different cells complicating attempts to elucidate critical Cd uptake pathways. Moreover, the relative contribution of these ones will almost certainly vary depending on the presence of competition from the transporters' intended substrate, the concentration of the intended substrate (which will affect expression of the transporter) and, in the case of secondary active transporters requiring H^+, the pH of the gut lumen (Gitan et al., 1998; Illing et al., 2007; Ryu et al., 2004; Wang et al., 2004).

Rapid accumulation of Ni is exhibited by some arthropods (Bednarska and Laskowski, 2008; Boyd, 2009; Bernardska et al., 2011; Green and Walmsley, 2013), which most likely occurs via iron and zinc transporters (Klein and Costa, 2015). DMT 1 actively transports Ni (Illing et al., 2007) and has been implicated in Ni uptake in rat jejunal segments (Muller-Fassbender et al., 2003). However, this raises an issue for some insects with a high midgut pH, such as the Lepidoptera and those that feed on hemicellulases (Chapman, 1998; Gullan and Cranston, 2005), as DMT 1 is a Me^{2+}/H^+

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symporter that requires an extra- to inter-cellular proton gradient for transport (Bannon et al., 2003; Elisma and Jumarie, 2001; Gunshin et al., 1997; Okubo et al., 2003); transport by DMT 1 appears to be negligible at pH < 7 (Elisma and Jumarie, 2001; Okubo et al., 2003). Typical midgut pH values for arthropods tend towards pH $6.0 - 6.5$, which should be sufficient for DMT 1/Mlv transport (Okubo et al., 2003; Martínez-Barnetche et al., 2007). Members of the ZIP family, which in the case of mammals have also been reported to transport Ni^{2+} (Dempski, 2012), are a second potential pathway for Ni uptake.

4.3 Transformations of trace metals in arthropods.

Once absorbed by cells, trace metals can be exported from the cell or be transformed in both the cytosol and within organelles. Transformations may be for the beneficial use of the organism or if the trace metal is in excess, to render the metal metabolically inactive to avoid toxicity (Figure 4). The diversity of the Arthropod phylum is reflected in the sites and nature of trace metal transformations (Sá et al., 2008). However, some generalities can be made. Beneficial transformations are principally the inclusion of trace metals into the active sites of enzymes, metallochaperones, respiratory pigments, transcription factors and into the exoskeleton (Chapman, 1998; Quicke et al., 1998; Schonfeld et al., 2002; Vijver et al., 2004). Transformations to avoid toxicity are principally biomineralisation *via* inclusion into mineral rich granules (MRGs, also called mineral spherites) or binding to specific metal sequestering proteins/peptides (Hopkin, 1989; Dallinger, 1993; Vijver et al., 2004; Pedersen et al., 2008).

Trace metals appear to be initially rendered metabolically inactive through binding to short chain peptides or proteins, predominantly glutathione and MTs (Martoja et al., 1983; Marioni and Watson, 1985; Singhal et al., 1987; Hopkin, 1989). The iron sequestering protein ferritin may also function in this role as it can bind Cd, Cu and Zn (Joshi and Zimmerman, 1988) and the genes coding for ferritin light and heavy chains in *D. melanogaster* are induced by Cd, Cu and Zn (Yepiskoposyan et al., 2006). In contrast, genes coding for metallochaperones show no increase in

transcription in response to elevated metal exposure, suggesting that they do not function in trace metal detoxification (Southon et al., 2004).

Glutathione and MTs are pivotal in the homeostasis and detoxification of trace metals in Arthropod cells under acute exposure. Glutathione is a tripeptide (γ-Glu-Cys-Gly), which, amongst a variety of roles, can bind metal(loid)s, including Cd, Cu, Ni, and Zn (Ballatori, 1994; Kręźel and Wojciech, 2004; Burford et al., 2005). Glutathione is present in relatively high (millimolar) concentrations within cells, which means that there is sufficient glutathione to rapidly bind and effectively detoxify metallic cations as they enter the cell (Singhal et al., 1987; Freedman et al., 1989; Ballatori, 1994; Conners and Ringwood, 2000; Zaroogian and Norwood, 2002). This is essential as there is a lag of several hours between increased metal concentrations and the rise in MT or MT-like protein (MTLP) levels in the cell (Everard and Swain, 1983; Ochi et al., 1988; Freedman et al., 1989). Binding of the metallic ions by glutathione appears to effectively protect cells from Cd (Singhal et al., 1987; Ochi et al., 1988) and Cu (Freedman et al., 1989) toxicity during this lag. Moreover, in both mammals and invertebrates, the formation of the MT-Cu complex appears to require glutatione, possibly because the formation of the complex requires glutatione to transport Cu to the MT molecule (Freedman et al., 1989; Ferruza et al., 2000; Zaroogian and Norwood, 2002).

The role of MTs in metal detoxification has been the subject of some debate, but *D. melanogaster* null mutants for all MT genes showed increased sensitivity to Cd and Cu stress (Egli et al., 2006; Yepiskoposyan et al., 2006), strongly suggesting that MTs provide a mechanism for the detoxification of trace metals. However, this mechanism does not function effectively for all trace metals. This is particularly evident for Zn in the Hexapoda, which in contrast to the situation in vertebrates, show a poor ability to synthesise MTs in response to Zn exposure and the MTs produced by them have a poor ability to bind Zn (Marioni and Watson, 1985; Marioni et al., 1986; Zhang et al., 2001; Sterenborg et al., 2003; Egli et al., 2006; Capdevila et al., 2010). Furthermore, MT null mutants of *D. melanogaster* show no increased sensitivity to Zn stress (Egli et al., 2006;

Yepiskoposyan et al., 2006). Little data are available for the other Athropod subphyla, but crustacean MT can bind both Zn and Cu (Valls et al., 2001; Capdevila et al., 2010), which suggests MTs may play a more prominent role in Zn homeostasis in at least this subphyllum. Overall, it has been suggested that MTs are more important for non-essential elements such as Cd, which lacks specific efflux pathways and needs to be detoxified by remaining tightly bound in the cytosol (Ardestani et al., 2014).

The relatively weak induction of and binding to MT/MTLPs by Ni suggests that MTs also do not play an important role in the regulation of Ni (Nielson et al., 1975; Barka et al., 2001; Amiard et al., 2006). Barka (2007) suggested that metalloproteins other than MTs bind Ni in the cytosol. However, the determination of Ni-MTLP levels is sensitive to the method used and that Ni binding to MTLPs may be underestimated (Geffard et al., 2010)

Typically 3-5 isoforms of MTs are reported for Arthropod species (Syring et al., 2000; Ahearn et al., 2004; Egli et al., 2006), although information is again lacking for many taxa. Isoforms differ in their ability to bind Cd and Cu such that one isoform will preferentially bind Cd and another Cu. Exposure to one of these metals results in the preferential induction of the gene for the MT isoform that preferentially binds that metal (Dallinger et al., 1997; Egli et al., 2006; Yepiskoposyan et al., 2006). Consequently, MTs provide a flexible system, allowing cells to respond to elevated concentration of one trace metal, whilst having a minimal affect on the homeostasis of essential metals.

Whilst glutatione/MTs function in the initial scavenging of trace metals, longer term storage appears to be the function of MRGs. Thus, the ultimate fate of metal bound to MT would be incorporation of the metal into intracellular MRG *via* a process of biomineralisation. This occurs in the lysosomal system of the cell (Hopkin, 1989; Barka, 2007). However, the processes involved in the transport of MT into the lysosome and incorporation of metal into MGRs is not fully understood. Moreover, excess Cu and Zn ions may also be 'scavenged' from the cytoplasm by importation directly into the lysosomes *via* transmembrane transporters such as Nramp2 (Martínez-

Barnetche et al., 2007; Sterling et al., 2007) and ZnT35C (Tejeda-Guzmán et al., 2018). Such a mechanism is analogous to vacuolar scavenging in plant cells. This mechanism would allow the lysosomal/MRG system to potentially contribute to the rapid removal of excessive trace metal ions from the cytosol prior to MT synthesis and could represent an important tolerance mechanism, as it does in plants.

Metal-rich granules are reported to be produced by the Hexapoda (Pawert et al., 1996; Pigino et al., 2005), Myriapoda (Vandenbulcke et al., 1998a; Köhler et al., 1995), Chelicerata (Ludwig and Alberti, 1988; Pigino et al., 2006) and Crustacea (Hopkin and Martin, 1982; Schill and Kohler, 2004). Metal-rich granules are produced in tissues where trace metals are accumulated (see below) and increase in number and/or metal concentration in relation to the exposure of the organism to trace metals (Martoja et al., 1983; Pawert et al., 1996; Vandenbulcke et al., 1998a; Vandenbulcke et al., 1998b; Schill and Kohler, 2004; Pigino et al., 2006). Metals incorporated in to MRGs are insoluble and therefore unavailable to take part in, or disrupt, the metabolic activity of the cell (Hopkin, 1989).

Hopkin (1989) described four types of MRG, termed A-D. Only types A and B sequester trace metals (Type C and D MRGs are described as sequestering Fe and Ca respectively). Type A granules are formed by concentric layers of calcium and magnesium phosphates in which Zn can be sequestered (Hopkin, 1989). Type B granules contain a high proportion of sulphur and chalcophilic elements such as Cd and Cu, but Zn may also be incorporated (Hopkin, 1989). Ni is reported to be incorporated into MRGs containing Ca, Mg, P, S and Cu and Zn (Barka, 2007), which suggests that it is found in both type A and B MRGs. Cu is more readily incorporated into the lysosomal/MRG system than Cd, which is more associated with MTLPs (Martoja, et al., 1983; Dallinger and Prosi, 1988; Barka, et al., 2001; Nunez-Nogueira et al., 2006). A relatively small proportion of Zn is found in MRGs, perhaps due to the instability of the Zn-thiolate complex in the low pH of lysosomes. Consequently, Zn could be liberated from MT before MRG formation with the result that Zn is exported back into cytosol (Amiard et al., 2006). The binding of Ni to MT is even less stable than

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for Zn (Nielson et al., 1985) and consequently only a small proportion of Ni is found in MRGs (Geffard et al., 2010).

Sites of trace metal sequestration

The taxonomic diversity of the Arthropoda is reflected in the diversity of the organs responsible for trace metal regulation and/or storage/detoxification. However, in all groups the efficiency of the MT/MRGs to sequester trace metals can efficiently prevent the majority of trace metals passing through the gut epithelia for distribution to the wider soma of the organism. As a result, the mid-gut and associated structures are the primary site of metal accumulation. In the sub-phyla Chelicerata and Crustacea, extensive accumulation of Cd, Cu and Zn occurs in well-developed mid-gut diverticulae, which form a structure called the hepatopancreas (Hopkin, 1989; 1990; Morgan et al., 1990; Köhler, 2002; Vijver et al., 2005). Very high concentrations of trace metals can be accumulated in this organ. For example, the hepatopancreas of the isopod *Porcellio scaber* can accumulate concentrations of over 1,250 mg kg^{-1} Cd, 19,000 mg kg^{-1} Cu and 21,000 mg kg^{-1} Zn (Hopkin, 1990).

The sub-phyla Myriapoda and Hexapoda lack developed mid-diverticula and the mid-gut itself is the principle site of Cd and Cu, and to a lesser extent Ni and Zn, storage/detoxification (Sohal et al., 1977; Aoki and Suzuki, 1984; Marioni and Watson, 1985; Marioni et al., 1986; Crommentuijn et al., 1994; Rabitsch, 1997; Köhler, 2002; Migula et al., 2007; Bednarska et al., 2019). For example, 92 % of the Cd in the body of the collembolan *Orchesella cincta* was found in the gut (Hensbergen et al., 2000).

The accumulation of Cd, Cu, Ni and Zn in the malpighian tubules (the organ responsible for osmo-regulation, ionic balance and excretion of nitrogenous waste in the Hexapoda, Myriapoda and Chelicerata) suggests that they function in the removal and storage/excretion of excess trace metals from the haemolymph (Martoja et al., 1983; Lindqvist et al., 1995; Rabitsch, 1997; Schofield et al., 1997; Bradley, 1998; Przybylowicz et al., 2003; Migula et al., 2007; Leonard et al., 2009; Yin et al.,

2017). The importance of this organ in trace metal storage/regulation can be judged by the fact that the highest concentrations after the mid-gut are found in the malpighian tubules (Rabitsch, 1997). Cd and Zn are stored in MRGs within the cells of malpighian tubules and this site may be important in the long-term accumulation of these elements (Sohal et al., 1976; Zierold and Wessing, 1990; Smit and Van Gestel, 1996; Tejeda-Guzmán et al., 2018). The form of Cu and Ni in the malpighian tubules is not currently understood, but storage in MRGs may be predominant.

Arthropods possess anthrocyte cells, which also remove substances from the haemolymph. Anthrocytes (also known as pericardial cells, garland cells or nephrocytes) are associated with the fat body and pericardium in the Hexapoda, Myriapoda and Chelicerata and gills of the Crustacea. These cells actively take up haemolymph by pinocytosis and principally function in the regulation of proteins in the haemolymph (Locke and Russell, 1998). However, large accumulations of Cd, Pb and Zn occur in MRGs within anthrocyte cells (Martoja et al., 1983; Vandenbulcke et al., 1998b; Nunez-Nogueira et al., 2006) and hence may have a significant role in removing trace metals from the haemolymph (Martoja, et al., 1983). Consequently anthrocytes may be central to the regulation of trace metals in the haemolymph of arthropod groups such as the Aphidae and Collembola that do not possess malpighian tubules.

Structural components of arthropods, such as the integument, can also be important sinks for trace metals. For example, Cd, Cu, Ni and Zn have been reported in arthropod exoskeletons (Hopkin and Martin 1983, Schmidt and Ibrahim 1994; Amyot et al. 1996; Schofield, et al. 2002; Green et al., 2003; Przybylowicz et al., 2003; Pourang et al., 2004; Boyd et al., 2007; Migula et al., 2007). The proportion of the total body burden of these metals found in the exoskeleton varies with metal and species (Orwlowski et al., 2020), but is typically in the range of 10% to 30% (Hopkin and Martin, 1983; Andrzejewska et al., 1990; Amyot, et al., 1996; Green, et al., 2003; Dar et al, 2015). Cu generally appears to be deposited in the exoskeleton to the greatest extent, followed by Zn > Cd > Ni (Amyot et al., 1996; Green et al., 2003). However, exceptions to this generalisation exist (Orlowski et al., 2020). For example, the centipede *Lithobius variegatus* had 45-60% of its Zn body

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burden in the exoskeleton, compared to 7-26% for Cu and 5 – 12 % for Cd (Hopkin and Martin, 1983). Transformation of trace metals into structural components is most striking in chewing herbivorous insects, the mandibles of which can contain up to 25% Zn (Schofield et al., 2002). The ovipositors of some hymenpoterous insects can also contain high Zn concentrations, up to 10 % by weight (Quicke et al., 1998). In both instances, the role of Zn in the exoskeleton is to increase hardness (Quicke et al., 1998; Schofield, et al., 2002). Moreover, in populations of the beetle *Lucanus cervus*, no correlation was found between abdomen and exoskeleton concentrations of Zn and Cu – indicating poor trafficking from gut to exoskeleton and more homeostatic control – while concentrations of Cd and Ni had a positive correlation between these two compartments (Orlowski et al., 2020).

Export of trace metals from cells

Metallic cations can leave cells through the Golgi secretory pathway or via active transport over the plasma membrane. As with plants, members of the Cation Diffusion Facilitator (CDF) family of proteins and P_{1B}-type ATPases are fundamental to trace metal efflux.

For Zn, the most important transporters are the Zinc Transport (ZnT) proteins, which are CDF members (solute carrier 30A family - SLC30A). For Cu, the P_{1B}-type ATPase ATP7 seems to be the only efflux transporter (Norgate et al., 2006). Both ZnT (ZnT35C and ZnT1) and ATP7 are expressed in *Drosphila* midgut cells (Southon et al., 2004; Norgate et al., 2006; Yepiskoposyan et al., 2006; Wang et al., 2009). They may be essential for the transfer of trace metals absorbed by the mid-gut epithelium into the wider soma and to prevent the build up of toxic concentrations of trace metals (Southon et al., 2004; Norgate et al., 2006; Yepiskoposyan et al., 2006; Bahadorani et al., 2010; Binks et al., 2010; Lye et al., 2012).

Both Cu and Zn are also exported from cells by the secretory pathway in golgi derived vesicles as part of the normal metabolism of metalloproteins (Eide, 2006; Lutsenko et al., 2007). Experimental evidence also suggests that Cd can leave cells when transported into golgi vesicles by

Ca^{2+}–ATPases instead of Ca^{2+} (Lauer et al., 2008). However, it is not clear if the secretary pathway has any role in preventing Cd toxicity.

No efflux transporters of Cd and Ni have been reported from arthropods. In mammals, there is evidence that the Fe^{2+} efflux transporter Ferroportin (FPN1, also designated Metal Transporter Protein 1, scl40a1, Ireg1) can also transport Cd^{2+} and Ni^{2+} (Ryu et al., 2004) and *A. thaliana* Ireg2 is also a Ni efflux transporter (Schaaf et al., 2006). Given the function of homologous transporters seems well conserved in eukaryotes, it is highly likely that this Fe pathway transports Cd and Ni. However, whilst FPN1 is strongly conserved from plants to animals and is present in arachnids, insects lack the gene for this protein (Tang and Zhou, 2013) and therefore it cannot explain Cd and Ni export in this class.

Another possible mechanism for Ni and Cd export is via CDF transporters. Cd can induce ZnT35C (Yepiskoposyan et al., 2006), suggesting that this Zn efflux transporter may transport Cd, while some members of the ZnT family can transport both Cd and Ni (Montanini et al., 2007). Direct Cd and Ni transport by arthropod ZnTs has not yet been shown and *D. melanogaster* with a truncated ZnT35C gene did not show increased sensitivity to Cd exposure (Yepiskoposyan et al., 2006), indicating that at best, efflux by this protein plays a minor role in preventing Cd toxicity. Consequently, the pathways for Cd and Ni export remain somewhat enigmatic.

Transport of trace metals within arthropods

The major mechanism for transporting substances absorbed through the gut around the body of Arthropods is *via* the open circulatory system in the haemolymph (Donker et al., 1990; Donker et al., 1996; Martin and Rainbow, 1998). Trace metals enter haemolymph over the basolateral surface of the mid-gut through the action of the transporters discussed in the preceding section. In the haemolymph, trace metals are predominately bound to proteins, with the free ion accounting account for as little as 0.01 % of the trace metal in haemolymph (Bryan 1966; Donker et al., 1990; Martin and Rainbow, 1998).

4.4 Regulation of trace metals by cells

Organisms and their constituent cells face a difficult balancing act to maintain a homeostatic supply of essential trace metals for the cell's metabolism, whilst preventing excess levels of essential and non-essential metals causing stress and damage. Consequently, the uptake, efflux and sequestration of trace metals can be regulated through a number of mechanisms. These include translation and post-translation control of the proteins involved.

To prevent the accumulation of a substance, individual cells can control the number of transport proteins localised to the plasma membrane and thereby control the flux of trace metals into the cell. ZIPs proteins involved with metal uptake are constantly trafficked between the plasma membrane and endosomes, with Zn deficiency decreasing endocytosis to increase the level of transporter in the plasma membrane (Bowers and Srai, 2018). Conversely, excess Zn rapidly changes the localisation of ZIPs from the plasma membrane to endosomes (Kim et al., 2004; Wang et al., 2004; Hara et al., 2017), reducing the flux of Zn into the cell. Degradation of the ZIP protein by lysosomes may follow endocyctosis (Hara et al., 2017; Bowers and Srai, 2018), potentially reducing the pool of ZIPs available for trafficking back to the plasma membrane and thereby maintaining reduced metal flux.

Copper dependent transcription level control, but not endocytosis, has been demonstrated for Ctr1B in *D. melanogaster* (Balamurugan et al., 2007). Simarly, Turski and Thiele (2007) found Ctr1A was largely localized to the plasma membrane, but they did not study the trafficking of the protein. Studies investigating the trafficking of mammalian Ctr1 demonstrated reversible trafficking of Ctr1 from the plasma membrane into endosomes following exposure of cells to Cu excess (Petris et al., 2003; Clifford et al., 2016), with recycling of Ctr1 back to the cell surface occurring rapidly (~15 min) following removal of excess Cu (Clifford et al., 2016). As with ZIPs, elevated Cu also resulted in the degradation of Ctr1 proteins in mammal cells (Petris et al., 2003).

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The ZnT Zn efflux transporters can also be trafficked to the plasma membrane in response to excess Zn and from the cells' surface under Zn deficiency to regulate Zn (Hara et al., 2017; Nishito and Kambe, 2019). Trafficking of the Cu efflux transporter ATP7 is less clear. The majority of ATP7 is reported to be localised to the plasma membrane (Norgate et al., 2006; Burke et al., 2008; Zhang et al., 2020), with some intracellular localisation, probably to supply Cu to the trans-golgi network. However, there is little evidence of trafficking between the two locations (Mercer et al., 2017). Consequently, transcription level control of ATP7 is likely the main mechanism of Cu efflux regulation.

Key to the transcription level regulation of free metal ions in the cell is the zinc finger transcription factor metal response element binding transcription factor 1 (or metal-responsive transcription factor 1; MTF-1). The MTF-1 protein has a strongly conserved zinc finger domain that is able to sense free Zn^{2+} in the cell through a direct and reversible interaction between the ion and zinc finger domain (Dalton et al. 1997). In insects, a second cysteine-rich domain senses Cu^+ through binding of the ion to this domain (Chen et al., 2008). MTF-1 can also sense Cd and Ni levels in the cell, possible via the release of Zn^{2+} into the cytoplasm following displacement of Zn from pools in the cell, particularly MTs, by these two metals (Zhang et al., 2003; Nemec et al., 2009). The interactions between Zn/Cu begin the activation MTF-1, which is translocated into the nucleus, subsequently binding to metal response elements in the promoter regions of specific genes, altering the transcription of these genes. Increased transcription is seen for the genes coding for MTs (Yepiskoposyan et a., 2006) and ferritin (Yepiskoposyan et a., 2006; Günther et al. 2012), increasing the cell's ability to sequester metals. Increased transcription also occurs for the genes for the efflux transporters ATP7, ZnT35C, ZnT63C (Yepiskoposyan et al., 2006; Günther et al. 2012) and FNP1 (Troadec et al., 2010), allowing the cell to export excess trace metals. MTF-1 can decrease expression of the ZIP10 and Ctr1B (Yepiskoposyan et al., 2006; Günther et al. 2012), potentially reducing uptake of Zn and Cu. However, there is no evidence that MTF-1 affects the expression of Ctr1A (Yepiskoposyan et al., 2006). Indeed, it seems for Cu that *D. melanogaster* is

not able to respond to potentially toxic Cu concentrations by decreasing uptake by the mid-gut and instead relies on the export of excess ions across the basolateral membrane into the haemolymph to prevent toxicity to the epithelial cells (Norgate et al., 2006; Southon et al., 2004).

Taken together, these mechanisms outlined above demonstrate that at the cellular level, Arthropods have the means to effectively deal with exposure to excess (and indeed, deficient) levels of trace metals, especially essential ones. However, for the organism, mechanisms that rely on the export of excess metal from the cell pass the problem on to other cells/tissues. Hence, the organism either has to excrete the excess metal from the body or have sufficient capacity to store the metal in a detoxified state. The latter strategy is risky in that when the storage/detoxification capacity of critical tissues/organs is exceeded, toxicity can quickly follow on further uptake of metals (Hopkin, 1990a; Crommentuijn et al., 1995; Santorufo et al., 2012). Hence, mechanisms for excreting of excess trace metals from the body have evolved.

4.5 Output of trace metals from arthropods

Trace metals not assimilated will be expulsed from the body in undigested food. For trace metals entering the cells of the mid-gut and associated structures, the principal mechanism of excretion is via cellular processes. These include the shedding of degenerated/sloughed cells, or the extrusion of MRGs into the lumen of the alimentary canal or hepatopancreas by holocrine/apocrine secretion (Sohal et al., 1977; Ludwig and Alberti, 1988; Dallinger, 1993; Vandenbulcke et al., 1998a; Przybylowicz et al., 2003; Pigino et al., 2006; Barka, 2007). These mechanisms can lead to the efficient excretion of trace metals from the body (Hopkin, 1990; Posthuma et al., 1992; Hensbergen et al., 2000; van der Fels-Klerx et al., 2016). However, some species retain MGRs and these tend to accumulate, and potentially biomagnify trace metals (Hopkin 1990; Dallinger, 1993; van der Fels-Klerx et al., 2016).

The exact nature and efficacy of excretion mechanisms is species specific (Janssen et al., 1991; Kramarz, 1999a and b). Even two closely related species with apparently identical digestive

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systems in terms of structure and physical function can utilise the same structures in different ways, resulting in very different excretion rates and therefore concentration with the body (Hopkin, 1990).

For assimilated trace metals passing through the mid-gut, there is experimental evidence that excretory/osmo-regulatory organs, such as the malpighian tubules of insects, can remove trace metals from the haemolymph and thence from the body (Donker et al., 1996; Martin et al., 2007). Evidence for this is most clear in the case of Zn. In larval *D. melanogaster*, ZIP71B/ZIP5 is expressed on the basolateral surface of the malpighian tubule cells, where it functions to influx Zn from the haemolymph (Yin et al., 2017). Several genes for the ZnT Zn efflux proteins are reportedly expressed in the malpighian tubules (Lye et al., 2012). Of these, d*ZnT35C* is expressed on the apical surface of malpighian tubule cells of larval *D. melanogaster* (Yin et al., 2017) and is thought to be fundamental to the removal of Zn from malpighian cells into the tubule lumen and regulation of Zn concentrations in the body (Yepiskoposyan et al., 2006; Yin et al., 2017). Cadmium is also secreted into the fluid in the tubule lumen at a rate that can significantly remove Cd from the haemolymph (Leonard et al., 2009). MRGs have also been reported in the lumen of the malpighian tubles (Spring and Felgenauer, 1996), suggesting that trace metals in this form can be excreted by this organ along with ionic forms.

The homologous structures of arachnids (malpighian tubules and coxal glands) and crustaceans (atennal glands) potentially have a similar role. The antennal glands of crustaceans can certainly remove Cd from the haemolymph (Rouleau et al., 2001), but a role in the excretion of trace metals from the body remains to be established.

Juvenile Arthropods and species which moult as adults can also lose trace metals through sequestration into the exoskeleton, which is then lost when shed during moulting or in the pupal exuviae (Andrzejewska et al., 1990; Lindqvist and Block, 1994; Hensbergen et al., 2000; Green et al., 2003; Przybylowicz et al., 2003; Raessler et al., 2005; Boyd, 2009; Dar et al., 2015). As with other aspects of trace metal physiology, the extent to which an element is eliminated by this mechanism can differ markedly between even closely related species (Raessler et al., 2005).

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4.6 Herbivorous arthropods and multi-trophic metal transfer

Herbivory is the crucial step responsible for introducing metal contaminants into the animal kingdom. The majority of trace metals taken up by the transport proteins of the mid-gut epithelium cells in arthropods are held there or in structures associated with the mid-gut, preventing toxicity by being either bound to polypeptides/proteins such as MTs or included into MRGs formed by lysosomes. A relatively small quantity of metal passes through the mid-gut into haemolymph, which distributes the metal around the soma. Thus, the processes that occur in the midgut epithelium are fundamental to the accumulation/excretion of trace metals. Trace metals retained in the midgut epithelia cells can be easily excreted by shedding of the cells containing accumulated metals or by the secretory activity of the epithelia cells. Trace metals passing through the midgut may be expelled via excretory organs, such as the malpighian tubules, or sequestered in other tissues from which excretion is difficult or impossible (for instance anthrocytes), leading to longer term retention and the potential for biomagnification into the next trophic level.

5. Predatory arthropods

5.1 Uptake, availability, transport, transformation and output of trace metals

The general sites and mechanisms of trace metal uptake, availability, transport, transformation and outputs in predatory arthropods do not appear to differ between herbivorous and predatory arthropods. Consequently, the major factor that separates the two trophic levels in terms of trace metal accumulation is diet. For instance, herbivorous arthropods tend to consume a small fraction of a plant, whilst predatory arthropods tend to consume most, if not all, of their prey. Moreover, the differing nature of the diets between the two trophic levels is likely to be reflected in their digestive enzymes (Chapman, 1998). The form of trace metals in the food of predatory arthropods will also differ. However, the assimilation efficiencies between trophic levels do not differ markedly

(Janssen et al., 1991), which may suggest that differences in availability are compensated for by the efficacy of the digestive enzymes.

The availability of metals in the food of predatory arthropods is understood to depend on the chemical form of the metal, principally whether the metals are bound to proteins/peptides (MTs, ferritin, glutathione, enzymes, respiratory pigments, etc.) or MRGs (Laskowski and Hopkin, 1996; Vijver et al., 2004). Protein bound metals, especially soluble proteins such as MTs, are thought to be readily available to predators (Laskowski & Hopkin, 1996; Vijver et al., 2004). In contrast, MRGs, by their very nature, are insoluble and as granule expulsion into the gut lumen appears to be an effective mechanism for excreting trace metals, metals do seem to be readily re-absorbed from MRGs. Hence, it seems that granules are poorly solubilised by digestive fluids (Hopkin 1989; Laskowski and Hopkin, 1996; Rainbow et al., 2006). However, the neogastropod mollusc *Nassarius festivus* can assimilate metals bound in MRGS (Rainbow et al. 2007). This could be related to the highly efficient digestive powers of this species, but the same authors found that a model of the invertebrate digestive system was also able to leach Cd and Zn from MRGs (Rainbow et al., 2007). Based on both experiments, metals bound to MT like proteins were more available than those bound to MRGs (Rainbow et al., 2007). Overall, the majority of trace metals in a cell is expected to be in chemical forms that can be absorbed by a predator (Vivjer et al., 2004).

Considering the above the feeding mechanism of predatory arthropods may be a key factor governing the uptake of trace metals (Hendrickx et al., 2003). This may be particularly important for Cd, as it is primarily accumulated within the soft proteinaceous tissues in arthropods (Hughes et al., 1980) and is particularly bound to MT in the midgut (Marioni et al., 1986; Hensbergen et al., 2000). Arthropods that feed by injecting digestive enzymes into their prey and sucking out the resultant partially digested tissues may therefore be feeding on tissues in which both the concentration and availability of Cd are high compared to the whole animal.

Experimental evidence suggests that predatory Arthropods that feed by piercing and sucking, such as spiders, lacewing larvae and pseudoscorpions, do accumulate higher concentrations of Cd

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(Green et al., 2006; Hendrickx et al., 2003; Janssen et al., 1991; Kramarz, 1999a; Scheifler et al., 2002; Babczyńska et al. 2011). However, Hopkin and Martin (1985) have shown that the spider *Dysdera crocata* did not assimilate Cd when fed highly contaminated woodlice. This species is a specialist predator of isopods, and may therefore have been adapted to the high concentrations of trace metals in its prey (Hopkin and Martin, 1985). Therefore, it cannot be generalised that all piercing-sucking predators assimilate high concentrations of Cd from their prey, especially when Cd accumulation patterns can diverge between male and female individuals within the same species, as verified in the spider *Agelena labyrinthica* (Babczyńska et al. 2011).

Knowledge of how feeding mechanisms affect Zn transfer between herbivore and predator arthropods is more limited. However, studies have shown similar Zn biomagnification in the piercing, sucking predator *Chrysoperla carnae* and two predatory beetles that consume the whole aphid, larval *Coccinella septempunctata* and *Bembidion lampros* (Green et al., 2006; Green et al., 1211 2003; Winder et al., 1999; Dar et al. 2017). Thus, the feeding mechanism possibly plays a more limited role in determining Zn accumulation in arthropods. This is perhaps due to the large extent to which Zn is bound to polypeptides/proteins, its even distribution in the soma and the ready leaching of Zn from MRGs (Lindqvist et al., 1995; Rainbow et al., 2007).

Copper can be incorporated into mineral granules (Hopkin, 1989) and to strongly induce synthesis of and to be bound to MTs (Dallinger, 1995). Hence, it can show biomagnification intermediate between Cd and Zn (Janssen and Hogervorst, 1993). However, some studies have indicated that Cu is effectively regulated via excretion and is not biomagnified (Crawford et al., 1995; Rabitsch, 1995; Babczyńska et al. 2011). Similarly, Ni does not appear to be effectively biomagnified in predators (Cheruiyot et al. 2013), although data on the forms of Ni in cells and transfer between trophic levels is currently insufficient to draw conclusions.

5.2 Predatory arthropods and multi-trophic metal transfer

The anatomy and physiology of predatory Arthropods does not differ dramatically from herbivorous Arthropods and thus it is not surprising that differences in metal accumulation do not commonly occur between the two trophic levels. However, several factors will affect the accumulation of metal within a predator. These are 1) the physiology of the predator (i.e. does it excrete metals or store/detoxify them); 2) the metal concentration the prey; 3) the form of the metal within the prey (i.e. protein/peptide bound or in MRGs); 4) feeding mechanism; 5) whether it is a specific predator adapted to metal concentrations/forms in its prey or a generalist with no adaptation; and 6) the level of contamination in the ecosystem. From this, generalist, liquid feeding predators that have prey species that poorly excrete metals and have a high proportion of metal bound to proteins would be the most vulnerable to metal pollution as their high exposure could overwhelm their ability to excrete and/or detoxify the metal, further increasing the risk of biomagnification to their own predators.

6. Metal accumulation across trophic levels

6.1 Accumulation strategies

Organisms tend to exhibit one of five strategies of metal accumulation on increasing exposure to trace metals: hyperregulator; accumulator-hyperregulator; accumulator-regulator; accumulator and hyperaccumulator (Figure 5). Here, we define exposure as both an increase in dose level or duration of an increased dose, with accumulation strategies generally holding true under both situations.

[**FIG. 5 NEAR HERE**]

Hyperregulators are able to maintain an almost constant concentration over a wide range of exposures. However, the physiological mechanism underlying regulation can become over burdened at high exposure and the concentrations can then increase, leading to toxicity. The shoots of many plant species exhibit this type of response, as do some arthropods (Gräff et al., 1997; Kramarz, 1999a).

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Accumulator-hyperregulators exhibit an initial increase in concentration with increasing exposure, but further exposure results in a decrease in concentration within the organism. This strategy can be observed as metal dose increases in arthropods (Crommentuijn et al., 1994), but is more commonly seen when invertebrates are exposed to an elevated concentration over time (Bednarska et al., 2011), with around 40% of invertebrate species exhibiting this type of response (Laskowski et al., 2010).

Accumulator–regulators undergo an initial rise in concentration, but the physiology of the organism reacts to prevent further accumulation. Both plants (Green et al., 2006) and arthropods (Janssen et al., 1991; Kramarz, 1999a and b) are reported to utilise this strategy. The fourth strategy of accumulator, is initially similar to that shown by accumulator-regulators, but the organisms do not show any sign of regulating trace metal concentrations. Instead, concentration increases linearly with exposure. Again, both plants (Baker, 1981) and arthropods exhibit this strategy (Janssen, et al., 1991; Kramarz, 1999a; Hendrickx et al., 2003).

The final strategy, hyperaccumulation, is characterised by organisms accumulating very high concentrations of trace metals even when exposed to low concentrations. This is seen in some metallophyte plants, but has not been reported for Arthropods. However, it has been suggested that herbivorous arthropods can use accumulated trace metals as an elemental defence against predators (Boyd and Wall, 2001). Indeed, increased Cu and Zn levels in *Spodoptera exigua* retarded the development and growth of predator *Podisus maculiventris* (Cheruiyot et al., 2013). Consequently, there is a possible evolutionary driver for some species of arthropods to develop hyperaccumulating physiology, and this warrants further investigation in areas of naturally occurring metalliferous soils.

For plants, accumulation is determined by the extent to which metals are transferred from roots to shoots. In the case of regulators, an effective root-shoot barrier exists that prevents metals reaching the pericycle of the root, which consequently restricts the metal pool available for loading into the xylem for transport to the shoot. Whilst this may protect photosynthetically active cells

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from the metabolic disruption caused by excessive metal concentrations, it also means that such plants cannot use the metal binding/detoxification capacity of the shoot. Thus, accumulator and hyperaccumulator plants can show increased tolerance to trace metals despite increasing the translocation of metals to the shoot. The physiological mechanisms involved are related to elevated loading of trace metal ions into the xylem due to increased cell to cell mobility and/or increased activity (V_{max}) of transporters effluxing ions from the cytoplasm into the xylem apoplast (Richau et al., 2009; Verbruggen et al., 2009).

In invertebrates, the dominant physiological processes determining the accumulation strategy may be those involved in the excretion of trace metals, as assimilation efficiencies show much less variation between taxa (Janssen et al., 1991). Excretion rate itself is probably determined by several factors, the importance of each differing among species. Species capable of regulating the concentration of trace metals in their bodies (hyperregulators, accumulator-hyperregulators, accumulator-regulators) most probably retain assimilated metal within the midgut, from where it can easily be excreted. The excretory organs would also be a potential route for excretion. However, it seems likely that this route would be secondary to processes in the midgut as trace metals entering the haemolymph have the potential to be taken up by sensitive organs, hence transfer through the midgut is undesirable.

Accumulator arthropods largely retain metals within their bodies. Indeed, excretion can even be almost entirely absent even when animals are returned to an uncontaminated diet (Janssen, et al., 1991). The physiological mechanisms underlying this behaviour are not clear, but may centre on the retention of metals in cells, tissues or organs lacking openings to the outside (such as anthrocytes) or the repeated re-absorption of metals excreted into the gut/excretory organ lumen. The evolution of a physiology that results in the retention of toxins seemscounter intuitive as invertebrates have a limited capacity to store trace metals in a detoxified state and when exceeded, soon suffer toxicity (Hopkin et al. 1990a; Crommentuijn et al., 1995). However, short lived species may be able to

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avoid the possible energetic and nutrient costs of excretions (i.e. replacing shed cells) by limiting excretion whilst having sufficient capacity to store/detoxify metals over their limited life time.

6.2 Multitrophic transfer and biomagnification

The differences in accumulation strategies shed light on the behaviour of trace metals within food chains, especially within the Arthropod components. Firstly, they help to explain the wide variation in trace metal concentrations reported in Arthropods, even under controlled laboratory conditions (Posthuma et al., 1992; Merrington et al., 2001; Sterenborg, et al., 2003; Sá et al., 2008; Green et al., 2010; Bednarska et al., 2019). The three accumulator strategies show a general increase in concentration with exposure (time). Thus, for a population all exposed to the same level of contaminant, older individuals in a population will tend to have higher concentrations in their bodies than younger individuals. Variability will be greatest in populations exhibiting an accumulator-hyperregulator type pattern, as few newly exposed individuals (juveniles and immigrants to the contaminated site) can exhibit concentrations in excess of 10 times greater than the steady state concentration of individuals exposed over longer periods (Bednarska et al., 2011).

The process of biomagnification within food chains (the increase in the concentration of a substance in an organism compared to its food) is acknowledged as a rare occurrence and is not a general characteristic of food chains (Janssen, et al., 1993). However, it has been suggested that critical pathways may be formed in which biomagnification of trace metals may endanger some components (van Straalen and Ernst, 1991). Due to a lack of research into the transfer of metals within food chains, the existence of such pathways remains mostly theoretical. However, a consideration of the accumulation patterns in Figure 5 supports the view that critical pathways can exist, but these are likely to be rare. Most obviously, food chains consisting of accumulators are going to result in biomagnification and this may well result in secondary toxicity to organisms in the upper trophic levels, especially generalist, liquid feeding predators. However, the inclusion of a hyperregulator or accumulator-hyperregulator in the chain would effectively block metal transfer to

higher trophic levels, preventing the formation of a critical pathway. In addition, in field conditions, the local habitat features (e.g. habitat edges) can influence multitrophic transfer of metals, especially for non-essential elements such as Cd (Orlowski et al., 2019). These factors may explain why critical pathways remain apparently rare and elusive.

Indeed this can be seen in Table 1, where we have compiled different experiments reporting Cu, Cd, Ni and/or Zn transfer across multiple trophic levels (in the field or in controlled environments), from soils to plants, to herbivore arthropods and predator arthropods. Transfer coefficients can vary greatly (Table 1), depending the metal, organism and trophic level, however it is evident that a critical pathway (TC > 1) is rare to occur for all compartments involved (although it happens for Zn in a few cases). It is also clear that studies which include all of the four compartments are lacking: predator arthropods and soils are less frequently reported (with herbivory being mainly the focus) from 27 studies, predators were absent in 12, while soil concentration values were not included in seven of them (Table 1).

[TABLE 1 NEAR HERE]

One important point that cannot be overlooked when considering the potential for secondary toxicity is that very few species are monophagous and most species are therefore exposed to pollutants through multiple pathways. In addition, arthopods are generally very good at assessing the quality of their food, including the presence of elevated trace metals and can avoid or reduce the consumption of contaminated food (Zidar et al., 2004; Bahadorani and Hilliker, 2009; Vesk and Reichman, 2009; Green et al., 2010). Thus, in a food web, a predator may reduce the consumption of accumulator species and focus consumption on less contaminated prey. This may still affect the population of a species due to increased intraspecific competition for a reduced food source, which in turn could result in reduced reproductive output or survival. Hence, absence of trace metal accumulation does not necessarily mean an absence of effect.

7. Conclusions

Trace metals may occur in soils through natural and anthropogenic inputs and are generally retained within soils for long periods, typically in the most biologically active surface horizons (Adriano, 2001). The major route by which trace metals can be transferred to organisms beyond the edaphic environment is through uptake by mycorrhizas and roots and their subsequent transfer to the above-ground biomass. The most labile trace metals in the soil to shoot pathway are Cd and Zn and this can result in the bioaccumulation of both metals in shoot tissues (Sauerbeck, 1991). Cu and Ni are moderately labile and tend not to biomagnify, with the exception of Nickel hyperaccumulators (~400 species from 4 families) found in metalliferous soils (Jaffre et al 2013). These metals have very low transfer coefficients in the soil-plant system, which results in much lower concentrations in shoot tissues than in the soil. It is clear, however, that the plant plays a major role in regulating the transfer of metals from soil to Arthropod, and should not be regarded as a neutral carrier.

Metal trafficking and binding to ligands within the plant can affect the availability of metals and hence their subsequent transfer to herbivorous Arthropods. At this trophic level biomagnification can occur in herbivorous arthropods (Merrington et al., 1997b; Devkota and Schmidt, 2001; Green et al., 2006, Green and Tibbett, 2008; Dar et al. 2017). As with the plant, it seems the physiology of the Arthropod is then crucial in determining biomagnification, and that may also occur in predatory Arthropods (Jansen et al., 1991; Hendrickx et al., 2003; Green et al., 2006; Orlowski et al., 2019). Although biomagnification is not a general property of plant-Arthropod and Arthropod-Arthropod systems, where a food chain consists of species that accumulate trace metals in sequence, a critical pathway can be formed, which could result in secondary toxicity. The effects of trace metal toxicity can be lethal or sub-lethal, such as loss of fecundity, which will affect entire communities and populations. Moreover, whilst populations can adapt to high levels of trace metal in their environment, this comes at the cost of a shorter life span, increased reproductive effort and decreased genetic diversity (Posthuma and van Straalen, 1993).

The key factors in controlling the transfer of trace metals through the soil-plant-Arthropod system are the type of metal, species and location in which the metal occurs in the lower trophic level as this will govern its fate and transfer to the higher trophic level (Green et al 2010; Laskowski and Hopkin, 1996). Regulation can occur in all compartments of the system (Figure 1; Table 1). This is controlled by the variation in the physiochemical environment in the soil and the physiology of plants and Arthropods. Without knowledge of all compartments it is difficult to predict the formation of a critical pathway.

Our knowledge of the physiology of essential trace metals has improved markedly in recent years, but similar efforts are needed to develop our understanding of the physiology of non-essential elements, such as Cd and Ni. Further research is also required to develop a full understanding of how predator guild may affect trace metal transfer and biomagification (especially under field or mesocosm conditions), as well as more dose-response investigations in arthropods, involving both physiological and gene expression analyses. It is also fundamental to learn the effects that interacting factors have on trace metal transfer and toxicity. These include: 1) how stress placed on plants by herbivory affects metal uptake and translocation in the plant; 2) how metal accumulation in herbivores affects predation through alteration of prey palatability/prey choice by the predator and 3) how stress caused by metal accumulation interacts with other ecological/environmental stressors, such as parasite infection, other pollutants (including interactions between different trace metals) and food scarcity, can affect fitness of exposed arthropods.

As this review has demonstrated, greater research effort is needed to deliver a more holistic assessment of ecosystem toxicity, in which integrated studies across ecosystem compartments will be crucial to identify critical pathways that can result in secondary toxicity through terrestrial food chains.

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Figure Captions

Figure 1. Conceptual compartmentalised model system for transfer of metals in a linear pathway from basal abiotic (soil) sources to higher biotic trophic levels in the soil-plant-arthropod system. Red circles represent metals and numbers 1 to 4 represent a trophic compartment.

Figure 2. Model compartment systems representing (a) benign (transfer coefficient < 1) and (b) critical (transfer coefficient > 1) pathways for dilution or concentration of metals in the soil-plant-arthropod system.

Figure 3. Conceptual diagram showing the interactions considered in the terrestrial biotic ligand model for metal ions (M^{2+}) (from Thakali *et al*., 2006).

Figure 4. Metal trafficking pathways in arthropod cells. MT – bound to metallothioneins, Glut – bound to glutathionone, A – incorporated into type A metal rich granule, B – incorporated into type B metal rich granule, ApoPro- transferred to apometalloproteins, MetChap – bound to metallochaperones, Pep – bound to low molecular weight polypeptides/proteins, organelle – sequestration within organelles other than lysosomes , SP – export via the secretory pathway, dashed lines – possible pathway Uptake proteins in bold are the primary proteins involved in metal uptake.

Figure 5. Models of organismal accumulation/regulation of metals by exposure. NB. Regulators may exclude and/or excrete metals, Hyper-regulator is partly synonymous with Excluder (after Baker 1981) but includes excretion.

Table 1. Total metal concentrations (M) and transfer coefficients (TC) of Cd, Cu, Ni and Zn within and between consecutive trophic levels: soil-plant-herbivorous arthropod-predatory arthropods.*

Compartment												
-- Soil --	------------ Plant ----- --------			------------- Herbivore ---------- ---			------------- Predator ---------- ---			Setting	Duration	Obs.	Ref.
[M]	Species	[M]	TC	Species	[M]	TC	Species	[M]	TC				
Zn: 30.7	*Triticum aestivum*	Zn: 18.8		*Sitobion avenae*	Zn: 112		*Benbidi on lar pros*	Zn: 112	Zn: 1	F/C	Plant: decimal stage 61-62 Predator: 9 d	sewage sludge amended soil	[1]
x	*Streptanthus polygatoides*	Ni: 2,900	x	*Melano tri chus boydi*	Ni: 780	Ni: 0.27	*Pholcus phalangioides*	Ni: 470	Ni: 0.6	C	Predator feed: 33 d	Ni in plant/herbivore is assumed from previous research	[2]
Cd: 0.12 Zn: 2.3	*Triticum aestivum*	Cd: 1.5 Zn: 57	Cd: 13 Zn: 25	*Rhopalosiphum padi*	Cd: 2.9 Zn: 324	Cd: 1.9 Zn: 5.7	*Mallada signata*	Cd: x Zn: 41	Cd: x Zn: 0.1	C	Plant: tillering +22 d Feed 1: 22 d Feed 2: 12 d		[3]

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Cu: 58.6 Ni: 43.1 Zn: 276	Forest litter (*Salix*, *Carpinus*)	Cu: 24.5 Ni: 43.1 Zn: 122	Cu: 0.4 Ni: 1.0 Zn: 0.4	*Orchesella cincta*	Cu: 6.2 Ni: x Zn: 79.3	Cu: 0.3 Ni: x Zn: 0.7	*Pardosa* sp.	Cu: 13 Ni: x Zn: 197	Cu: 2.1 Ni: x Zn: 2.5	F	x	invertebrates sampled in the same field site	[4]
x	*x*	x	x	Fruit flies	Cd: 54.5	x	*Pirata piraticus*	Cd: 142	Cd: 2.6	C	70 d	Fruit flies fed in medium with CdCl$_2$	[5]
Cd: 0.5 Zn: 91	*Hordeum vulgare*	Cd: 0.22 Zn: 143	Cd: 0.4 Zn: 1.6	*Sitobion avenae*	Cd: 0.36 Zn: 188	Cd: 1.6 Zn: 1.3	*Chrysoperla carnae*	Cd: 0.55 Zn: 249	Cd: 1.5 Zn: 1.3	C	Plant: tillering Feed 1: 10 d Feed 2: 11 d	sewage sludge amended soil	[6]
Cd: 0.16 Zn: 32.5	*Pisum sativum*	Cd: 0.15 Zn: 70	Cd: 0.9 Zn: 2.2	*Acyrthosiphon pisum*	Cd: 0.01 Zn: 135	Cd: 0.5 Zn: 1.9	x	x	x	F	Feeding: 72 d		[7]
x	*Berkheya coddii*	Cu: 10 Ni: 16,000 Zn: 71	x	*Anoplocnemis curvipes*	Cu: 65 Ni: 500 Zn: 180	Cu: 6.5 Ni: 0.03 Zn: 2.5	*x*	x	x	F	x		[8]
x	*Berkheya coddii*	Cu: 10 Ni: 16,000 Zn:	x	*Chrysolina clathrata*	Cu: 100 Ni: 250 Zn:	Cu: 10 Ni: 0.02 Zn:	*x*	x	x	F	x		[8]

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		71			150	2.1							

Table 1. Continued.

x	*Berkheya coddii*	Cu: 10 Ni: 16,000 Zn: 71	x	*Stenoscepa sp.*	Cu: 45 Ni: 3500 Zn: 220	Cu: 4.5 Ni: 0.22 Zn: 3.1	*x*	x	x	F	x		[8]
x	*Sebertia acuminata*	Ni: 6,900	x	*Bactrocera psidii*	Ni: 65 (420)	Ni: 0.01 (0.06)	x	x	x	F	x	adult (larvae) concentrations	[9]
x	*Berkheya coddii*	Cu: 15 Ni: 11,000 Zn: 78	x	*Stenoscepa sp.*	Cu: 51 Ni: 330 Zn: 430	Cu: 3.4 Ni: 0.3 Zn: 5.5	x	x	x	C	6 d		[10]
x	*Berkheya coddii*	Cu: 2.3 Ni: 12,500 Zn: 29	x	*Chrysolina pardalina* (larvae)	Cu: 47 Ni: 1,263 Zn: 1,080	Cu: 20.4 Ni: 0.1 Zn: 0.04	*Rhinocoris neavii*	Cu: 62 Ni: 516 Zn: 362	Cu: 1.3 Ni: 0.4 Zn:	F	x	Insects sampled in the same field site	[11]

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									0.3				
Cd: 18.5	*Triticum aestivum*	Cd: 10.5	Cd: 0.6	*Rhopalosiphum padi*	Cd: 13.5	Cd: 1.3	*Chrysoperla carnae*	Cd: 1.0	Cd: 0.1	C	Plant: 31 d Feed 1: 14 d Feed 2: 6 d	soil spiked with CdCl2	[12]
Cd: 8.4 Cu: 60 Zn: 793	*Rumex patientia × R. tianschanicus*	Cd: 0.31 Cu: 1.4 Zn: 8.4	Cd: 0.04 Cu: 0.02 Zn: 0.01	*Spodoptera litura*	Cd: 0.05 Cu: 6.9 Zn: 70	Cd: 0.2 Cu: 4.9 Zn: 8.3	x	x	x	F/C	Feeding: 6 d	paper includes metal transfer to chickens	[13]
Cd: 0.7 Zn: 100	*Triticum aestivum*	Cd: 1.2 Zn: 70	Cd: 1.7 Zn: 0.7	*Sitobion avenae*	Cd: 0.15 Zn: 130	Cd: 0.1 Zn: 1.9	*Coccinella septempunctata*	Cd: 0.12 Zn: 93	Cd: 0.8 Zn: 0.7	C	Plant: stage 37-51 Feed 1: 28 d Feed 2: 15 d	predator conc. estimated from daily mean intake × 15	[14]
Cd: 100	*Setaria viridis*	Cd: 15	Cd: 0.15	*Locusta migratoria*	Cd: 108	Cd: 7.2	x	x	x	F	x		[15]
Cd:	*Oryza*	Cd:	Cd	*Nilaparvat*	Cd:	Cd:	x	x	x	C	x		[1

0.3 7 Cu: 19. 3 Zn: 72. 3	*sativa*	2.7 Cu: 24 Zn: 104	: 7.3 Cu : 1.2 Zn : 1.4	*a lugens*	1.4 Cu: 25 Zn: 183	0.5 Cu: 1.1 Zn: 1.8							6]
Cd: 23 Cu: 76 Zn: 103 4	*Rumex dentatus*	Cd: 10 Cu: 30 Zn: 300	Cd : 0.4 Cu : 0.4 Zn : 0.3	*Acrida cinerea*	Cd: 6.0 Cu: 50 Zn: 330	Cd: 0.6 Cu: 1.7 Zn: 1.1		x	x x F x			average values from 5 sites	[1 7]
Cd: 1.7 Zn: 98	*Brassica juncea*	Cd: 5.5 Zn: 210	Cd : 3.2 Zn : 2.7	*Lipaphis erysimi*	Cd: 4.5 Zn: 250	Cd: 0.8 Zn: 1.2	*Coccinella septempunctata*	Cd : 2.5 Zn : 23 0	Cd: 0. 6 Zn: 0. 9	C	Plants: 61 d Feed 1: 21 d Feed 2: pupation	sewage sludge amended soil	[1 8]

Table 1. Continued.

Cd: 0.16 (67)	*Morus alba*	Cd: 0.0 3 (6.5)	Cd: 0.2 (0.1)	*Bombyx mori*	Cd: 0.0 2 (5.1)	Cd: 0.7 (0.8)			x x x C		Plant: 90 d Feeding: 5 d	control (soil + Cd(NO$_3$)$_2$)	[19]

Cd: 1.8 Cu: 15 Ni: 23 Zn: 165	Grasses (*L. perenne, P. pratense, P. pratensis*)	Cd: 2.5 Cu: 11 Ni: 12 Zn: 104	Cd: 1.4 Cu: 0.7 Ni: 0.5 Zn: 0.6	"Soil fauna"	Cd: 19 Cu: 38 Ni: 35 Zn: 740	Cd: 7.6 Cu: 3.5 Ni: 2.9 Zn: 7.1	x	x	x	F	x		soil fauna: several taxonomic groups	[20]
Cd: 0.8 Zn: 55	*Brassica juncea*	Cd: 1.7 Zn: 75	Cd: 2.1 Zn: 1.4	*Lipaphis erysimi*	Cd: 2.1 Zn: 150	Cd: 1.3 Zn: 2.0	*Coccinella septempuncta ta*	Cd: 1.5 Zn: 190	Cd: 0.7 Zn: 1.3	C		Plants: 61 d, Feed 1: 21 d, Feed 2: pupation	fly ash ameded soil	[21]
Cd: 2.6	*Solanum melongena*	Cd: 11	Cd: 4.2	*Dysmicoccus neobrevipes*	Cd: 5.?	Cd: 0.5	*Cryptolaemus montrouzieri*	Cd: 3.5	Cd: 0.6	C		Plant: flowering, Feed 1: 21 d Feed 2: pupation	soil spiked with CdCl$_2$	[22]
Cd: 2.6	*Solanum lycopersicum*	Cd: 11.5	Cd: 4.4	*Dysmicoccus neobrevipes*	Cd: 5.9	Cd: 0.5	*Cryptolaemus montrouzieri*	Cd: 3.8	Cd: 0.6	C		Plant: flowering Feed 1: 21 d Feed 2: pupation	soil spiked with CdCl$_2$	[22]
Cd: 1.6 Zn: 1.7	*Trifolium alexandrinum*	Cd: 5.9 Zn: 12.9	Cd: 3.7 Zn: 7.6	*Sitobion avenae*	Cd: 5.4 Zn: 14.7	Cd: 0.9 Zn: 1.1	*Coccinella septempuncta ta*	Cd: 6.0 Zn:	Cd: 1.1 Zn: 2.9	F	x		average values from 5 sites	[23]

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Cd: 1.6 Zn: 1.7	*Trifolium alexandrinum*	Cd: 5.9 Zn: 12.9	Cd: 3.7 Zn: 7.6	*Aiolopus thalassinus*	Cd: 35 Zn: 128	Cd: 5.9 Zn: 9.9	x	x	x	F	x	average values from 5 sites	[23]
Cd: 4.0 Cu: 532 Ni: 35.4 Zn: 1948	*Medicago sativa*	Cd: 0.25 Cu: 19.2 Ni: 3.1 Zn: 91.6	Cd: 0.06 Cu: 0.04 Ni: 0.08 Zn: 0.05	*Spodoptera exigua*	Cd: 0.06 Cu: 14.3 Ni: 0.6 Zn: 132	Cd: 0.2 Cu: 0.7 Ni: 0.2 Zn: 1.4	x	x	x	C	Feed: 8 days		[24]
Cd, Ni: 200	*Solanum melongena*	x	x	*Dysmicoccus neobrevipes*	Cd: 30.6 Ni: 31.5	x	*Cryptolaemus montrouzieri*	Cd: 4.4 Ni: 5.2	Cd: 0.14 Ni: 0.17	C	Plant: flowering Feed 1: 21 d Feed 2: pupation	soils spiked with metal solutions to 200 mg kg^{-1}	[25]
Cd, Ni, Zn: 200	*Solanum melongena*	x	x	*Dysmicoccus neobrevipes*	Cd: 30.6 Ni: 31.5 Zn: 33.	x	*Cryptolaemus montrouzieri*	x	x	C	Plant: flowering Feed 1: unclear Feed 2: pupation	soils spiked with metal solutions to 200 mg kg^{-1}	[26]

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								Cd:					
		Cd: 1.8			Cd: 2.7	Cd: 1.5		0.9	Cd: 0.6			uncontrolled	[27]
x	*Brassica napus*	Cu: 105	x	*Oulema melanopus*	Cu: 40	Cu: 0.4	*Coccinella septempuncta ta*	Cu : 180	Cu: 4.5	F	30 d	diet/other feeding sources	
		Zn: 6.0			Zn: 1.5	Zn: 0.3		Zn . 3..	Zn 2.6				

* - Some data extracted from figures; [M] metal concentration in the compartment (mg kg⁻¹); TC: transfer coefficient between compartment, values >1 indicate a critical pathway; Durations, timeframe of the experiment, assessment or animal feeding. Setting, F – field experiment/sampling; C – controlled environment (glasshouse, laboratory); x: absent, inapplicable or unclear information. In experiments with multiple concentrations assessed, preference was given to the highest in the initial compartments (soil or plant). Refs. [1] Winder et al., 1999; [2] Boyd and Wall, 2001; [3] Merrington et al., 2001; [4] van Straalen et al., 2001; [5] Hendrickx et al., 2003; [6] Green et al., 2006; [7] Green and Tibbett, 2008; [8] Boyd et al., 2006a; [9] Boyd et al., 2006b; [10] Boyd et al., 2007; [11] Migula et al., 2007; [12] Alonso et al., 2009; [13] Zhuang et al., 2009; [14] Green et al., 2010; [15] Zhang et al., 2012; [16] Wan et al., 2014 [17] Zhang et al., 2014; [18] Dar et al., 2015; [19] Zhou et al., 2015; [20] Chrzan, 2016; [21] Dar et al., 2017; [22] Wang et al., 2017; [23] Butt et al., 2018; [24] Garrouj et al., 2018; [25] Sang et al., 2018; [26] Du et al., 2019; [27] Orlowski et al., 2019.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Cd, Cu, Zn and Ni are prevalent and labile metal cations in food chains in terrestrial ecosystems.

- Current evidence on trophic transfer of metals and its underlying mechanisms are reviewed

- Compartment-based approach: metal transfer via trophic pathways are considered as a series of linked compartments

- Plants play a major role in regulating the transfer of metals from soil to Arthropods

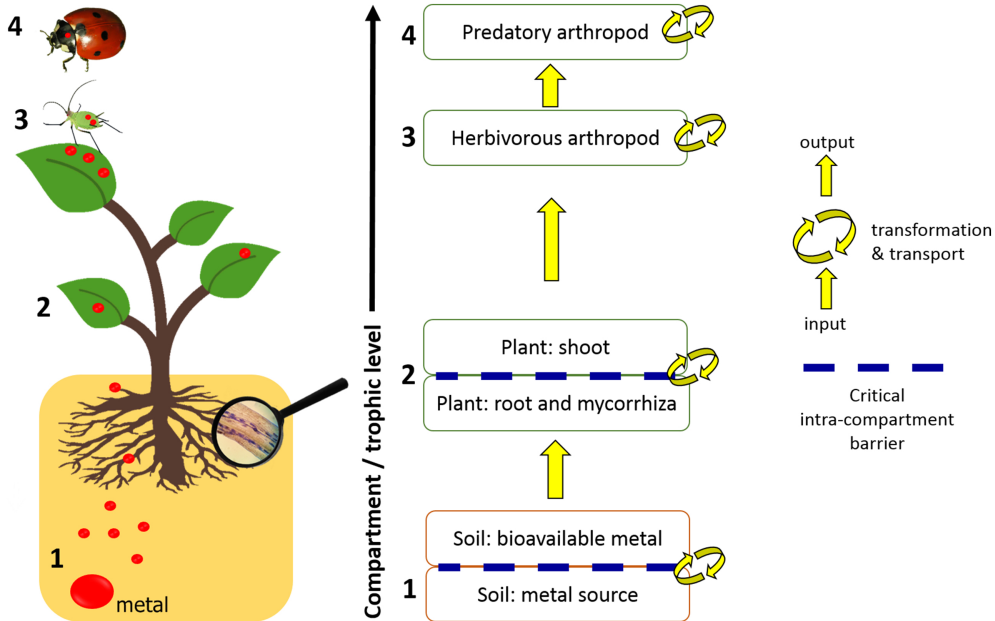- Biomagnification appears to be rare and dependent on accumulation/excretion strategies of arthropods

Figure 1

a) **Benign Pathway** — Trophic dilution / Metal concentration

- Predatory arthropod
- Herbivorous arthropod
- Plant
- Soil

b) **Critical Pathway** — Trophic concentration / Metal concentration
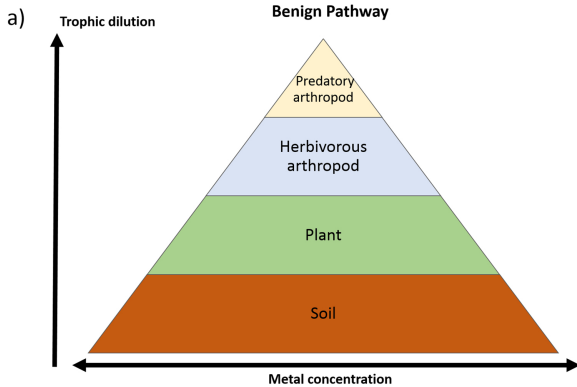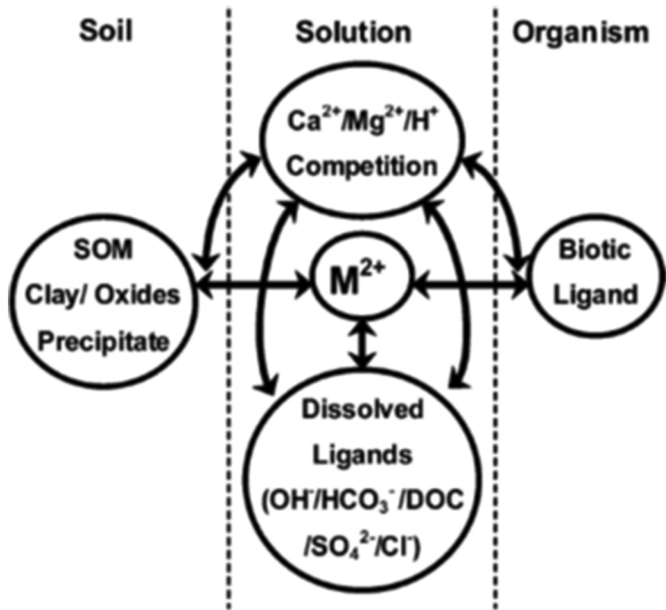
- Predatory arthropod
- Herbivorous arthropod
- Plant
- Soil
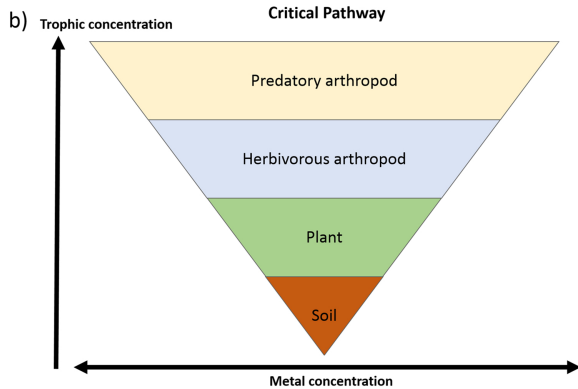
Figure 2

Figure 3

Figure 4

Figure 5