CHAPTER 3

Metals and Metalloids in Terrestrial Systems: Bioaccumulation, Biomagnification and Subsequent Adverse Effects

Reinier M. Mann[1,*], Martina G. Vijver[2] and Willie J.G.M. Peijnenburg[2,3]

[1]Centre for Ecotoxicology, University of Technology Sydney, Australia; [2]Leiden University, Leiden, The Netherlands and [3]National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Abstract: Metals and metalloids are elemental substances that occur naturally in the Earth's crust, and are variously incorporated into biological systems as structural components or proteins. Imbalances in the environmental concentrations of several metals present a challenge to ecosystems because the species that form part of these ecosystems are often not equipped to regulate internal concentrations of these elements, or employ detoxification mechanisms that serve to biomagnify these elements in the food chain. This review examines the trophic movement of metals and metalloids within terrestrial ecosystems and the consequences of biomagnification and toxicity on populations. Several elemental contaminants are given special emphasis, including copper, zinc, arsenic, selenium, molybdenum, cadmium, mercury and lead. All these elements are of high historical importance and continue to be deposited within the biosphere.

INTRODUCTION

Elemental chemicals have a tendency to stick to clayish, peaty or organic-rich materials, and contamination of terrestrial systems generally occurs because soils are capable of acting as a sink for metals and other elemental chemicals [1]. The elemental contaminants of most concern are predominantly metals such as cadmium, lead, mercury or copper, among others. However, a few, like selenium and arsenic are metalloid elements. For the sake of simplicity in this discussion we have used only the term 'metal' to describe both types, even though examples may include metalloid elements like selenium.

Metals in soils originate from two separate sources: from geogenic processes related to the occurrence of metal-bearing geological formations, and from anthropogenic sources. Information on the sources, fate, transport and toxicity of metals and metalloids can be found in Chapter 1 and 2 of this book. Once bound within soils, metals are persistent, because elemental contaminants cannot degrade further (unlike complex organic pollutants), although they can undergo various reversible changes in speciation depending on the chemical environment [2].

Many trace elements are essential for life functions [3], and plants and animals possess various mechanisms for the accumulation of sufficient amounts of trace elements from their environment. These same mechanisms can also facilitate the uptake of non-essential metals [4, 5]. The uptake and retention of a metal (or any other chemical) by an organism is termed bioaccumulation. Bioaccumulation of essential as well as non-essential elements is dependent on both the chemical availability of the metals within the environment and the organism's capacity for uptake and subsequent excretion. A full overview on bioaccumulation is given in Hodson *et al.* [6], in which the different definitions of bioavailability currently in use are reviewed.

The severity of impact on ecosystems will reflect the concentrations of bioavailable metals in the soil. The concentrations of individual metals are dictated by the source of each contaminant. For example, mercury (Hg) contamination occurs predominantly as a consequence of atmospheric deposition, and is therefore rather diffuse. By contrast, a major source of cadmium (Cd) contamination has historically been through the application of rock-phosphate fertilizers, thereby selectively elevating Cd concentrations in agricultural soils. Very high levels of soil contamination usually only occur in the proximity to metal smeltering activities, and an examination of studies conducted in these environments are instructive about the relative movements of metals within local ecosystems.

One such study was conducted by Hunter *et al.* [7-9] in the vicinity of a copper refinery within Merseyside in north-west England. Copper (Cu) and Cd content of the soils within a 1 km radius of the refinery typically exceeded 500

***Address correspondence to Reinier M. Mann:** Centre for Ecotoxicology, Department of Environmental Sciences, University of Technology Sydney, NSW 2007, Australia; Present Address: Hydrobiology, Brisbane, Australia; Email: reinier.mann@hydrobiology.biz

Francisco Sánchez-Bayo, Paul J. van den Brink and Reinier M. Mann (Eds)

and 5 mg/kg, respectively [7]. In this highly impacted area, floral diversity was reduced to a few metal tolerant species compared to a reference site. However, invertebrate diversity, as represented through pitfall trapping, was not greatly affected, with the exception of a reduction in abundance of isopods (woodlice) and oligochaetes (earthworms) within a 1 km radius of the refinery. The site also supported small mammals; specifically field voles (*Microtus agrestis* L.), wood mice (*Apodemus sylvaticus* L.) and common shrews (*Sorex araneus* L.).

All organisms within this contaminated site accumulated metals to varying degrees, and following the ratio of Cu:Cd through the various trophic levels illustrates the variability in accumulation potential for different metals. The Cu:Cd ratio in the soil close to the refinery was 716:1. Vegetation at the refinery bioaccumulated both Cu and Cd, however the ratio was reduced to 37:1. The ratio of Cu:Cd in the herbivorous field voles was reduced further to 5:1. Among the various herbivorous, detritivorous and predatory invertebrate taxa the Cu:Cd ratio varied markedly, but within the diet of the carnivorous common shrew the ratio averaged 10:1 and was reduced further to 1:3 in the shrew itself [8, 9]. These changes in Cu:Cd ratios illustrate the element specific mobility of Cu and Cd within terrestrial food chains. In this example the change in ratio of Cu:Cd occurs because copper is an essential element that can be regulated by homeostatic mechanisms. In the study cited here [9], shrews within the vicinity of the refinery accumulated large body burdens of Cd, whereas their Cu burdens remained low. Unlike copper, cadmium is a non-essential metal and organisms have only limited capacity to eliminate it from their bodies and tend to pass it on to consumers/predators. It is notable that despite the large body burdens of Cd, shrews persisted in the contaminated environment, and this will be discussed further later in the chapter.

This chapter will examine the metal- and species-specific movements of metals in terrestrial ecosystems, and where examples exist, the consequences of bioaccumulation and biomagnification of metals for populations of terrestrial organisms. Illustrations of metal transfer through the aquatic food chain are included to provide a complete picture, thereby improving our ability to make general statements or to fill gaps of knowledge for terrestrial ecosystems.

What is Biomagnification?

The process whereby pollutants are transferred from food to an organism resulting in higher concentrations compared with the source is called biomagnification. There are two main groups of substances that biomagnify:

1. Novel (synthetic), lipophilic organic substances that are not easily degraded or metabolized because organisms lack previous exposure and therefore, have not evolved specific detoxification and excretion mechanisms. These substances are consequently known as 'persistent organic pollutants' or POPs.
2. Metals which, by definition, are not degradable because they are elements. Because metals are a natural part of the environment, organisms, particularly those subject to naturally high levels of metal exposure, have developed mechanisms to sequester and excrete them. Problems arise when organisms are exposed to higher concentrations of metals than usual, and which they cannot excrete or detoxify rapidly enough to prevent damage. Some of these metals are transferred in organic forms, like methylmercury, organoselenium and organotin, and like POPs will readily bioaccumulate.

These pollutants biomagnify along food chains because successive trophic levels consume relatively large quantities of biomass (food) to obtain the resources required for metabolic functioning. If that biomass is contaminated, the contaminant will be taken up in large quantities by the consumer. Lipophilic contaminants within consumed biomass are subsequently absorbed and stored in the bodies of the consumers rather than eliminated along with other waste products. If the consumer is eaten by another consumer organism, the fat tissue is digested and the contaminant is then stored in the tissues of the latter consumer. In this way, the contaminant builds up in the fatty tissues of the subsequent consumers and the concentration of the contaminant in their tissues becomes higher with each trophic level. Water-soluble pollutants usually do not biomagnify in this way because they would dissolve in the bodily fluids of the organism and be excreted. Thus the principle of biomagnification is based on the fact that the mass of the contaminant is largely conserved along the food chain, while the biomass decreases [10].

The extent to which biomagnification occurs between a consumer and its food/prey can be expressed as the biomagnification factor (BMF). The BMF can be used to predict ecological risk of chemicals [11, 12]. Determining the biomagnification for a food chain experimentally, by studying the transfer within a chain of prey and predators, is rather simple, although it may not be very practical as all possible chemical exposure routes to organisms (water, food and soil/sediment) must also be taken into account [13]. The total bioaccumulation of a metal in species of each trophic level within a specific food chain corresponds to the chemical concentration in the organism relative to the concentrations in its surrounding environment and in its diet, respectively. Hence, bioaccumulation (expressed as the bioaccumulation factor (BAF)) is the sum of two processes: bioconcentration, which is accumulation *via* the exposure medium (expressed as the bioconcentration factor (BCF)) and biomagnification which is uptake *via* food only (expressed as the biomagnification factor (BMF)). Although BAF is the sum of BCF and BMF, it should be noted that summing both factors in a numerical way requires much care because of the differences in units for BCF and BMF [14].

Are Metals Biomagnified?

Hendriks and Heikens [15] modelled metal kinetics in a food chain by means of empirical regressions based on mean values. In this modelling, it was concluded that despite taxonomic variability, metal concentrations diminish with increasing trophic levels. Also for marine ecosystems, Gray [16] concluded that biomagnification of metals is not a universal rule. This is in agreement with earlier conclusions of Laskowski [17], who proposed that, based on the mean concentrations that accumulate in successive trophic levels, biomagnification of Cd and Cu does not lead to high concentrations in carnivorous predators. In contrast to this view, van Straalen and Ernst [18] suggested that trophic movement of metal cannot be examined by generalizing about the body burdens of different trophic levels (*i.e.* using the statistical means of body burdens), but must be examined by following the path of each metal through each species, because different species will have different capacities to accumulate metals. The difference between these two views is illustrated in Fig. (**1**). Scheme A (Fig. **1A**) provides a simplistic hierarchical view of a trophic cascade in which all consumers can be allocated to discrete trophic levels. Alternatively, scheme B (Fig. **1B**) provides a model with more complicated interactions between different trophic levels, and when examined in this way, metal biomagnification may be manifest in some trophic pathways, but not in others.

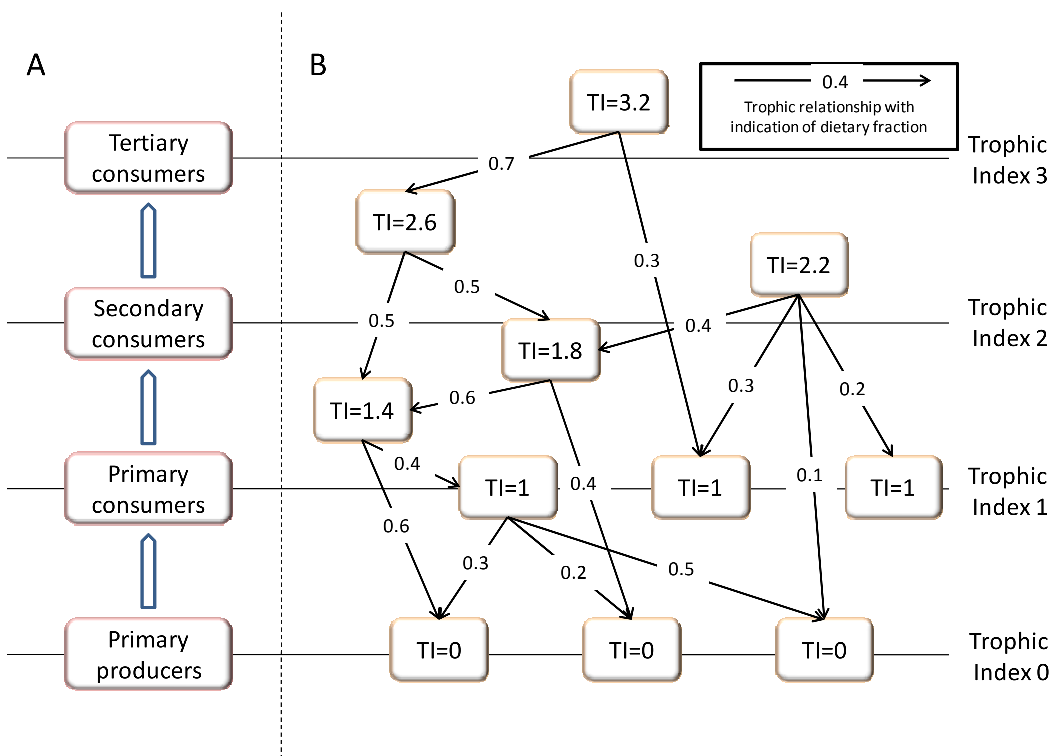

Figure 1: Schematic diagram of two biomagnification models. **A** provides a simplistic hierarchical scheme which places all consumers in subsequent trophic levels. **B** provides a more realistic "food web" representation where each organism has an associated trophic index (TI). TI is calculated as $TIi = 1 + \Sigma\ (TIj \times DFi,j)$, where TIi is the Trophic Index of the species i, TIj is the Trophic Index of the species j and DFi,j is the fraction of the species j in the total diet of species i. The TI for primary producers is set at 0. Redrawn and modified from Alonso [21].

Croteau *et al.* [19] concluded that predictive relationships between metal concentrations in predators and prey was only possible if their prey can be identified, and if the concentrations of metals in their prey is known. This information can subsequently be used in a bioaccumulation model. In the field situation, stable isotope ratios of carbon and nitrogen can be used to ascribe trophic positions of species within a food web. These ratios provide insights into time-integrated energy flows and food web structures [20]. The isotope ratios of carbon can be used to identify food sources, whereas the nitrogen isotopes can be used to infer the trophic position of an organism. After defining these relationships, pollutant concentrations can be compared to trophic levels inferred with the isotopic techniques, and a better understanding of habitat-specific food webs can be gained.

There is ongoing discussion as to whether biomagnification of metals occurs within aquatic and terrestrial systems, and more importantly, whether it can occur to the point that there is a detrimental effect on middle or top-order predators. Food web complexity (*i.e.* as represented in Fig. **1B**) makes predicting the trophic movement of metals rather difficult. Up to now, most information about biomagnification in field situations, especially quantitative relationships, is limited to comparative assessments between food chains, because the complexity of the process and the variability of data increase when moving to food-chain assessments.

TROPHIC MOVEMENT OF METALS AND FACTORS INVOLVED

Partitioning of metals is dictated by the environmental compartments present (especially solid and liquid phases) and the size of those compartments. Within the compartments, various environmental factors affect the bioavailability of metals for bioaccumulation [22-24] The environmental factors that influence the fate and partitioning of metals include pH, sorbing ligands in the exposure matrix, and the amounts of competing ions. Also, metal specificities affect bioaccumulation [25], as do species-specific characteristics such as the excretion capacity of organisms [10, 26] and the trophic level of the species.

Metal Specificity

Some metals are known to biomagnify. The best understood example is that of mercury (Hg), which biomagnifies to a great extent, but only when present in the lipophilic organic form, methyl mercury (MeHg). Methyl mercury is formed under anoxic conditions through microbial methylation [27], is readily bioaccumulated by algal species [28] and fungi [29] and subsequently biomagnified through trophic transfer [28, 30]. High body burdens of Hg are usually found in top-order marine predators [31] like the toothed whales. Among terrestrial fauna, particularly in birds, high body burdens in Hg usually occur as a consequence of consumption of aquatic invertebrates [32-34] or fish [34]. Similarly, semi-terrestrial mammalian predators in aquatic ecosystems (e.g. mink, otters, polar bears) also bioaccumulate Hg [35, 36], as do wholly terrestrial carnivores for which fish forms a large proportion of the diet [37].

The extent to which Hg biomagnification occurs in these food chains requires knowledge of the dietary preferences of the species involved. For example, polar bears are known to bioaccumulate Hg from their prey. However, the concentrations of Hg reported in polar bear liver, although high (between 1 and 200 μg/g dry wt) is rarely higher than in their prey food (seals) [38]. The reason for the apparently low level of trophic transfer of Hg between seals and polar bears likely lies in the polar bear's dietary preference for the skin and fat which have relatively little of the bioavailable MeHg, and the low bioavailability of inorganic Hg in seal liver [39].

Biomagnification of other metals is more difficult to demonstrate, although there are theoretical reasons to suspect that Se and Cd could biomagnify under some circumstances [40]. Gray [16] reviewed 35 papers on the subject of biomagnification of metals in aquatic systems and judged that there was little evidence, with the exception of MeHg, for the biomagnification of metals, despite the fact that 28% of papers (2 of 7) that examined biomagnification of organotin, did demonstrate biomagnification. Recently Se biomagnification has been demonstrated in a short aquatic food chain in the field [41]. In terrestrial systems, Se has not been demonstrated to biomagnify [42] although elevated tissue concentrations of selenium have been found among small resident mammals and birds nesting in the vicinity of a Se contaminated site [43, 44].

In aquatic systems, and contrary to prevailing views, Croteau *et al.* [40] demonstrated that Cd is progressively enriched among trophic levels in discrete epiphyte-based food webs composed of macrophyte-dwelling invertebrates (the first link being epiphytic algae) and fishes (the first link being gobies) [40]. In the same food web, Cu was not similarly enriched. Biomagnification of Cd has also been demonstrated in terrestrial food chains (see below).

Nickel (Ni) and thallium (Tl) also have the potential for transference along aquatic food chains. Dumas and Hare [45] demonstrated that the majority of both metals (58 to 83%) was assimilated by predatory alderflies (*Sialis velata*) feeding on aquatic invertebrates that had previously accumulated Ni and Tl from contaminated sediment, and indicates that these metals are easily transferred along the aquatic food chain and that food is an important source for biomagnification of these elements. Thallium in particular is known to bioaccumulate in plants grown in contaminated soils [46, 47]. However, very little information is available about the biomagnification potential of Tl in higher trophic fauna, although high levels of Tl were reported in greater white-toothed shrew (*Crocidura russula*) 19 months after the collapse of a tailing dam at the Los Frailes Mine in Aznalcóllar that resulted in extensive contamination with various metals in Doñana National Park, Spain [48].

Dietary Exposure to Metals

In aquatic systems, all organisms are subject to the diffusive mechanisms that allow metals to passively enter tissues. Bioconcentration occurs when organisms sequester those metals internally (*i.e.* when assimilation is higher than excretion), and thereby maintain an inward diffusion gradient. Bioconcentration is distinct from bioaccumulation. Bioaccumulation takes into account the internalization and retention of contaminants *via* all routes including ingestion and absorption across membranes such as gills, whereas bioconcentration is particular to aquatic organisms that accumulate contaminants across exposed membranes. All aquatic organisms, including fish, possess membranes that are exposed to the water column (e.g. gills) where diffusion of metals can occur. With this distinction in mind, Gray [16] observed that with passive uptake (e.g. fish) biomagnification does not occur as opposed to dietary uptake (e.g. birds) where biomagnification may be observed. In aquatic systems, up to the trophic level of fish, there is usually no need to assume that food is the major route for contaminant intake and therefore, that biomagnification is not so important. However, Gray [16] also observed that organisms that have aerial respiration (e.g. sea birds, reptiles and marine mammals) must take in contaminants *via* food rather than their body surface and are likely to show biomagnification. Therefore it can be concluded that aquatic systems react differently on the transfer of metals through the food chain than terrestrial food chains.

Transfer from Soil to Plant to Grazing Fauna

Many plants are able to bioaccumulate metals and some plant species are even able to hyperaccumulate various metals at levels exceeding their concentrations in ores [49]. It has been suggested that metal accumulation by plants may be a defence strategy to discourage consumption by herbivores [50, 51], although it may be more accurate to say that avoidance of plants with high metal burdens establishes an evolutionary selection pressure for hyperaccumulation among plants [51]. Similarly, some species of herbivores have evolved to utilise metals bioaccumulated in the ingested plant biomass as a defence against subsequent predation [52]. The implication here is that some animal consumers/predators are able to detect and selectively ingest or avoid metals in prey/food items.

However, in the absence of metal avoidance behaviours, trophic biomagnification of metals might be expected. In a study examining a floodplain area in The Netherlands with elevated metal concentrations [53], the most dominant plant species was the stinging nettle *Urtica dioica*. The stinging nettle contained only very low metal concentrations, far below the maximum values found in plants from non-polluted sites. Nevertheless, the main herbivore feeding on these plants, the snail *Cepaea nemoralis*, did contain metal concentrations that were much higher than background values [53]. Cadmium in particular was accumulated to very high levels, with consequent negative effects on reproduction [53, 54]. Similarly, substantial Cd accumulation was also reported among snails (*Helix aspersa*) in mesocosm studies [55, 56]. However, these studies indicated that up to 40% of the accumulated Cd, and even higher proportions of accumulated lead (Pb) and zinc (Zn) are bioconcentrated directly from the soil [56]. Exclusively dietary accumulation of Cd has been demonstrated in aphids. In an examination of the trophic movement of Cd and Zn between wheat grown on Cd-contaminated soils, and aphids (*Rhopalosiphum padi* and *Sitobion avenae*), aphids were demonstrated to bioaccumulate both Cd and Zn up to ten times the concentrations in wheat [57, 58].

Bioaccumulation of metals through grazing is not only dependent on metal levels in the plants consumed, but effectively depends on a delicate interplay between internal processes regulating concentrations of both essential and non-essential elements below the concentrations at which toxicosis occurs. A well known example is a two-stage process leading to copper toxicity in sheep and cattle. Copper toxicity among domestic ruminants generally occurs as a consequence of consumption of Cu-contaminated water, vegetation contaminated with Cu-based insecticides or fungicides or pasture that has been top-dressed with Cu salts or swine and poultry manure [59]. Initially, in the first stage, there is the steady accumulation of copper in the liver over time. Under normal circumstances, Cu is absorbed from the diet and transported in the bloodstream to the liver for storage. Excess Cu from the diet is stored in the liver and is released into the blood as needed for regular body functions. The circulating Cu level tends to remain constant regardless of the amount of excess Cu accumulating in the liver. When dietary intake of Cu is high, Cu can build up in the liver over a matter of weeks, months, or more than a year depending on a variety of factors without any clinical signs. However, with increased accumulation above the detoxification capacity of the animal, there is a sudden release of copper from the liver into the bloodstream. This rush of Cu into the sheep's bloodstream causes causing massive hemolysis, renal and liver failure and ultimately death in two to five days [59]. Copper toxicity among ruminants is usually recognised following veterinary examination of domestic stock, whereas copper toxicity among wildlife is seldom documented.

Despite the capacity of most animals to obtain and regulate Cu within narrow limits, Cu deficiency has also been observed among wild ruminants and domestic stock as a consequence of the presence in the diet of elements like molybdenum (Mo) and sulphur (S). Copper deficiency occurs because of the formation of insoluble Cu-Mo-S complexes that are excreted by the grazers [60]. Molybdenosis, or molybdenum induced Cu deficiency is likely the cause of death and disease among wild moose (*Alces alces*) in Sweden [61]. In such cases, total Cu levels in blood plasma are an unreliable guide to Cu status. Similarly, Cu levels in plants or in soil in themselves are also not reflective of actual bioaccumulative levels of essential elements for grazers as levels of other elements affecting the effective Cu dose also need to be taken into account.

Transfer to Higher Predators

Cadmium is the one metal that has been demonstrated to be biomagnified along terrestrial food chains. As indicated above, herbivorous invertebrates can biomagnify the Cd ingested from their food plants. High body burdens among herbivorous/detritivorous invertebrates occur because of high dietary-Cd assimilation efficiencies [up to 100%, 62, 63, 64] and low rates of elimination [e.g. 65]. With successive trophic levels in terrestrial food chains, the occurrence of biomagnification becomes less predictable. Some invertebrate predators have developed physiological mechanisms that allow them to avoid accumulating Cd from their prey. Using the example of aphids cited above, Merrington et al. [66] and Green et al. [67] demonstrated that two predators of aphid, lacewings (Mallada signata) and ladybird beetles (Coccinella septempunctata), did not biomagnify Cd contained in their aphid prey. In the case of the beetles, Cd was assimilated by the larvae, but was subsequently sequestered in pupal exuviae. Another example is the spider Dysdera crocata, which preys upon isopods. Isopods are known to accumulate large body burdens of metals, including Cd; however, when maintained exclusively on a diet of isopods with high body burdens of Cd, D. crocata did not assimilate Cd [68]. The absence of net assimilation in this spider occurs because of the breakdown of the digestive cells in the midgut diverticulae where metals temporarily accumulate, with subsequent release of metals into the lumen of the midgut prior to excretion [69]. In contrast, wolf spiders (Pirata piraticus), when provided with Cd contaminated fruit flies, assimilated nearly 70% of Cd from its prey without any elimination [70].

Among terrestrial vertebrates, Cd assimilation efficiencies are relatively low [<10%, 71, 72, 73], indicating that vertebrate digestive physiology presents an efficient barrier against Cd assimilation [74, 75]. However, overall bioaccumulation can still be expected to be high among some taxonomic groups, particularly homeothermic animals with high metabolic demands (and high food intakes) and long-lived animals. The species-specific differences in capacity to biomagnify Cd is best illustrated by an examination of the numerous studies that have reported accumulation of Cd among carnivorous shrews (*Sorex araneus*) and herbivorous voles (*Microtus agrestis* and *Myodes* [syn. *Clethrionomys*] *glareolus*). Shrews are particularly interesting because their high metabolic rates require them to consume >80% of their body weight each day. The field data presented in Fig. (**2**) comprise several studies and locations, mainly diffusively polluted floodplain soils and former mining areas. Studies were selected for inclusion if Cd concentrations in food items, *i.e.* earthworms and plants, were measured. As small mammals predominantly accumulate Cd *via* ingestion, absorption from water and inhalation could be disregarded as negligible. Binding of Cd to the metal-binding protein metallothionein, with subsequent storage in the liver and kidney is the main detoxification mechanism of small mammals and results in very low elimination rates. Any decrease in the concentration of Cd in the body could be attributed to elimination *via* growth dilution only.

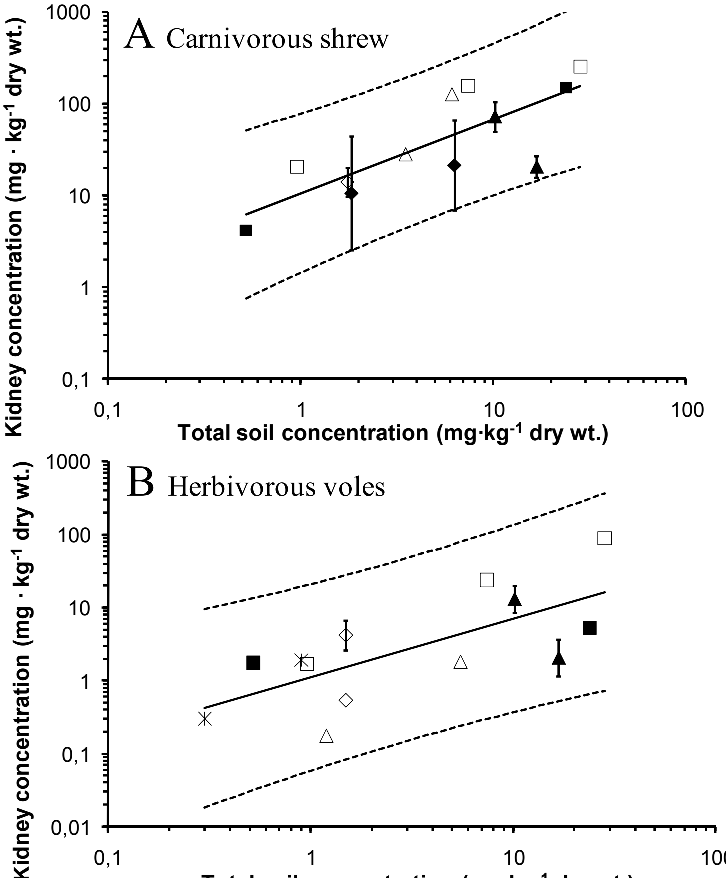

Figure 2: Cadmium accumulation in kidneys of carnivorous shrews (A) and herbivorous voles (B) (mg/kg dry weight) compared to total metal concentrations (mg/kg dry weight) in soils of various origins. ▲ = Biesbosch [76], ◆ = Rhine [77], ◇ = ADW [78, 79], △ = near closed smelter (Budel) and industrially polluted area (Arnhem) [80], □ = near Cd / Cu refinery, 1 km from refinery and reference location [9, 81], ■ = near mine and reference location in UK [82, 83], ✱ lead mine (Frongoch) and reference site [84]. Full line represents empirical regression. Upper and lower dashed lines represent the 97.5th and 2.5th percentile of the field data, respectively. The error bars represent the 95% confidence intervals and were plotted where possible. Figure adapted from Veltman *et al.* [85].

Linear regression analysis was performed to relate Cd concentrations in kidney, liver and whole body of small mammals to total soil concentrations. Results show a significant relationship between total soil concentrations and Cd concentrations in kidney and liver of carnivorous shrew and herbivorous voles (Fig. (**2**), only kidney data shown). Cadmium concentrations in above ground parts of plants were generally lower than concentrations in earthworms when exposed to similar soil-Cd concentrations. This was in agreement with the observation that Cd concentrations in voles were generally lower than in the shrew. Additionally, a large variation in Cd levels in plants was observed when related to total soil concentrations. As a consequence, regressions of Cd accumulation in herbivorous voles have a lower explained variance compared to carnivorous shrews based on total soil concentrations.

Transfer from Soil to Higher Fauna

Direct uptake of metals from soil by wildlife may be an important pathway for metal accumulation as well. Wildlife may ingest soil deliberately, or incidentally when they ingest soil-laden forage or organisms that contain soil in their intestine (e.g. earthworms) [e.g. 86]. Because the concentrations of metals in ingested soil may be higher compared to the metal concentrations in prey items, the soil can be an important pathway of exposure to predators as well.

Beyer *et al.* [87] provided an overview on the estimates of soil ingestion by wildlife based on experimental data, field captured animals and modelling. The authors found that between 3% and up to 30% of the ingested diet of wildlife consists of soils and/or sediments. Examples of carnivorous predators are raccoons (*Procyon lotor*) with 9% direct soil ingestion and red fox with 3%, whereas sandpipers consumed sediments at a rate of 7 to 30% of their diets. Highest rates among the herbivorous eaters studied were Canada geese (*Branta canadensis*) and the black-tailed prairie dog (*Cynomys ludovicianus*) (up to 8%).

Excretion of Metals by Predators

The capacity for excretion differs between predatory species. A high excretion capacity implies that biomagnification is lower. Reinfelder *et al.* [88] suggested that trophic transfer potential could be described from biodynamic parameters - weight-specific ingestion rate, assimilation efficiency (AE), and a rate constant of loss. Hendriks and Heikens [15] based their studies on these parameters as well. Higher concentrations at higher trophic levels can be explained by the fact that elimination rates decrease with increased body size [12, 89, 90]. The main reason for an absence of biomagnification among aquatic trophic chains is the observation that most marine organisms can fairly easy eliminate metals [16].

Predator Specificity (Gender & Life-Span)

Some authors indicate that females accumulate higher concentrations of metals than males of the same species. For example, some studies suggest that male and female moles (*Talpa europaea*) accumulate different heavy metal concentrations in their tissues. Kormanicki [91] observed that females have higher levels of Cd in the femur and stomach and higher levels of Zn in the gonads, spleen and skin. Pankakoski [92], in an examination of the same species in Finland indicated that Pb accumulation was also higher in females than males. The consequences of female biased accumulation for local population viability are not studied and remain unknown.

Gender variation of metal burdens was also observed by Deng *et al.* [93] in a study on metal levels in great tit (*Parus major*) and greenfinch (*Carduelis sinica*) in the western mountains of Beijing (China), but the differences did not follow a specific pattern. In general, trace metal concentrations in different body parts were similar between males and females in both species. However, in the liver and feathers, there were significant gender related differences, and the pattern was species specific. In the great tit, males possessed higher chromium (Cr) and Ni in the liver and Se in the feather than females, while females had higher levels of Cd in these body parts. In the greenfinch, males had higher concentrations of arsenic (As) and Zn than females in the feather, while females had higher concentrations of Zn and Se in the liver. Janssens *et al.* [94] found no general age- or gender-related differences in metal levels across a pollution gradient except for As and iron (Fe), where a significant interaction between site and gender was observed. Actually, the results of these authors suggest that feathers of great tits might be useful biomonitoring tools because they reflect the environmental contamination by heavy metals well.

Nam *et al.* [95] on the other hand observed no gender-related variation in two populations (Lake Biwa and Mie) of great cormorants (*Phalacrocorax carbo*) from Japan for most of the trace elements that they studied, except for higher hepatic strontium (Sr) concentrations in males from Lake Biwa. In both populations, some elements revealed tissue-specific accumulation. For example, most of the burden of Mo, silver (Ag) and Cd was in liver, Tl and Cd in kidney, Cu, rubidium (Rb) and caesium (Cs) in muscle, and vanadium (V), Sr and barium (Ba) in bone. Hepatic V, muscular Hg and Tl, and Cd in liver, kidney and muscle increased with growth. There was little gonad-specific accumulation of any metal. Thus this study countered the hypothesis that enhanced excretion of metal in the eggs laid by females reduces female metal burdens. Site specific variation in elemental concentrations in stomach contents also indicated that dietary sources tended to be the main factor in regional variations observed between the two colonies studied. Concentrations of toxic Hg and Cd in the liver of cormorants from the two colonies were lower than those from other areas, implying relatively low exposure to these metals. Concentrations of V, Co, Ag, Cd, Cs, Hg, Tl, Pb and Bi in liver remained more or less at the same level between 1993 and 2003, while hepatic Cr, Mn, Cu, Zn, Se, Rb, Sr and Ba showed apparent decrease.

The life span of organisms is also important when considering the bioaccumulation of metals because it affects the total period of time that an animal is exposed to contaminants. The older an organism is, the longer it has been exposed to any contaminant. Bioaccumulation will have a linear relationship with the exposure time of an organism, particularly when excretion is negligible. Overall, the life-span of an animal is likely to be a more important factor affecting metal burdens in specific predators than gender variation [96-98, but see 99].

Metal Sequestration in Prey

The transfer of metal from prey to predator is dependent on two highly variable factors. First, the capacity of predators to minimize net assimilation of metals contained within their prey is variable. As indicated above, the digestive physiology of vertebrates and some invertebrate predators provides an effective barrier against metal assimilation and accumulation. Second, the bioavailability of metal sequestered in the prey is also variable. Some plants (e.g. members of the family Brassicaceae) and animals (e.g. crustacean, oligochaete species) are able to accumulate large tissue burdens of metal. They are able to avoid the damaging effects of reactive forms of essential and nonessential metals and to selectively control utilization of essential metals, by sequestering them in non-toxic forms. This is effectively a kind of elimination. In the case of plants, metals appear to be sequestered predominantly as granular deposits in cell vacuoles [100]. Similarly, soil invertebrates are able to store metals such as Cd in special hepatopancreatic cells as inert granules [101, 102].

The form in which metals are stored in prey species has implications for bioavailability of metals to higher trophic levels (predators) [103-105]. In an attempt to discriminate between the various forms of sequestered metal, Wallace *et al.* [106, 107] defined various sub-cellular partitions based on a centrifugal protocol that partitioned animal tissue into five separate fractions: metal rich granules (MRG), cellular debris, organelles, heat stable proteins (HSP) and heat denatured proteins (HDP). Using this protocol in an examination of the relationship between sub-cellular Cd distribution in an oligochaete and its trophic transfer to a predatory shrimp [108], the authors proposed that only metal present in the soluble fraction (*i.e.* organelles and protein fractions) of prey is available to the predator. Similar conclusions were drawn in a study using bivalves as prey; the authors again concluded that the metal partitioning to organelles, HDP and HSP, comprise a sub-cellular compartment that is “trophically available” to predators [106]. This model seems to have established a degree of acceptance and provides a pragmatic approach to resolving sub-cellular metal distribution, and has been shown to yield useful insights with a variety of species: e.g. bivalves, perch and earthworms. However, refinements to the protocol will be required before it can be used to predict bioavailability of prey-bound metal because subsequent studies have demonstrated that the soluble fractions were not always 100% bioavailable and that the insoluble fractions were not always 100% non-bioavailable [105, 109].

CONSEQUENCES OF BIOMAGNIFICATION FOR POPULATIONS DECLINES/SHIFTS OR ECOSYSTEM EFFECTS

Direct Poisoning by Metals

Occasionally, human activities can lead to deposition of metal in such high concentrations that biomagnification is not required before symptoms of toxicity can be observed in wildlife, and result in the direct poisoning of higher vertebrates with metals. The most obvious example of this is through the use of lead shot for hunting and lead fishing sinkers. A recent study by Mateo *et al.* [110] reported up to 148 lead shot pellets per square meter in wetlands in southern Spain. Poisoning occurs when animals ingest spent shot. Waterfowl can ingest lead shot directly while feeding, and galliform birds can ingest shot as grit for their gizzards. Also, predators and scavengers ingest lead shot or lead shot fragments that are embedded in carcasses or that persists in the gizzards of birds [111, 112].

The first studies of Pb poisoning in waterfowl were conducted in the USA in as early as 1959 [113]. Since then, various scientific studies have examined Pb pollution in wetlands and the possible consequences of direct or indirect Pb poisoning on water birds. In a laboratory study, a single lead shot experimentally imbedded into the crop of ring-necked ducks (*Aythya collaris*) caused mortality or severe symptoms of debilitating toxicosis among 87% of birds [114]. Lead is a non-specific poison affecting numerous body systems, and sublethal exposure has variously been reported to result in (i) weakness of contaminated birds making it vulnerable to predation; (ii) alteration of energy requirements that will consequently be a handicap for birds on migration; (iii) reductions in clutch size; and (iv) result in eggshell thinning and embryo malformations [111, 115]. Most of these toxicological studies were conducted on species of ducks and swans.

Lead poisoning among waterfowl has resulted in several population declines. Population declines of mute swans (*Cygnus olor*) in the UK between the 1960s late 1980s were associated with ingestion of lead sinkers. Their numbers subsequently increased following the ban on sales of small lead sinkers in the late 1980s. Similarly, up to 50% of recorded mortalities among common loons (*Gavia immer*) in Canada were also associated with ingestion of lead fishing sinkers [116]. Additional investigations to see if Pb poisoning could have a similar effect on waders (common snipe *Gallinago gallinago* and jack snipe *Lymnocryptes minimus*) concluded that Pb poisoning also affects waders to a similar extent to that found in ducks, thus confirming previous studies on aquatic birds [117]. The use of lead sinkers for recreational fishing and lead shot for waterfowl hunting has been restricted in the USA and various other countries since the 1980s/1990s. However the incidence of Pb poisoning among raptors has not decreased in the USA and it seems likely that the continued use of lead shot for upland hunting has shifted the emphasis from water birds to predatory birds [116, 118].

Mercury poisoning among mammals is exemplified by Minamata disease. Minamata disease is manifested as severe neurological pathologies among victims exposed to Hg-contaminated food. In Minamata, Japan (the location from which the syndrome takes its name), poisoning resulted among human inhabitants following consumption of fish contaminated with biomagnified Hg (see below). However, similar neurological pathologies have been observed among people and birds following the use of organo-mercurial fungicides for the protection of grains and as a consequence of ingestion of contaminated seed [31].

Arsenic poisoning in animals and humans is caused by several different types of inorganic and organic arsenical compounds. Toxicity varies with factors such as oxidation state of the As, solubility, species of animal involved, and duration of exposure. Organic forms appear to have a lower toxicity to mammals than inorganic As. Research has shown that arsenites (trivalent forms) have a higher acute toxicity than arsenates (pentavalent forms) [119]. In mammals, As is known to promote cancer of the bladder, lung, and skin. Arsenic poisoning among the human population of Bangladesh occurs as a consequence of direct consumption of ground water, which has naturally high concentrations of As. However, because groundwater is also used for irrigation, contamination of rice (the staple crop in Bangladesh) is also a likely source of As [120]. Many plants are able to accumulate As, and some are able to hyperaccumulate this metalloid. For example, the Chinese brake fern (*Pteris vittata*) is able to hyperaccumulate As (more than 1000 mg As/kg of shoot dry weight) as As(V), reduce it to As(III), translocate it through the xylem with water and minerals as an As(III)-S compound, and then store it as As(III) in the fronds [121], thus making trivalent As available to herbivores. It is not known to what extent As poisoning occurs in animals that feed on plants that accumulate As, but because there is no threshold below which As intake is regarded as safe for humans, there is clearly potential for poisoning among other herbivorous species following the consumption of plants.

The occurrence of arsenic poisoning in Bangladesh is a similar scenario to that of *itai-itai* disease in post-WWII Japan. *Itai-itai* (meaning 'painful' in Japanese) disease was the name given to cases of cadmium poisoning among the human population living around the Jinzu River that had been contaminated with Cd originating from a zinc mine further upstream. *Itai-itai* disease is characterized by symptoms of osteoporosis and osteomalacia associated with renal dysfunction resulting from chronic Cd poisoning. Poisoning occurred because people either drank or cooked with the river water, or because they ate rice that had been irrigated with Jinzu River water [122]. Kobayashi *et al.* [122] performed multiple regression analyses, and found a strong correlation between consumption of Cd-contaminated rice and the occurrence of renal tubular dysfunction, indicating that Cd poisoning in the human population has occurred as a consequence of biomagnifications of Cd through rice.

Populations Under Threat from Biomagnification

Bioaccumulation of metals as a consequence of biomagnification or bioconcentration is frequently observed among lower trophic levels; however, elevated tissue burdens of metals do not necessarily translate into shifts in community or population structure. Again the studies by Hunter *et al.* [7, 8] are instructive. As indicated earlier, the vegetation structure was only altered in the highly metal-contaminated zone around the copper refinery, which was dominated by a few metal tolerant plant species. Although de-vegetation and dominance of contaminated soils by metal-tolerant plant species has been demonstrated in other sites with high levels of metal contamination [e.g. 123, 124, 125], moderate levels of metal contamination generally have little effect on plant communities.

Another notable difference between the highly contaminated zone near the refinery and less contaminated areas studied by Hunter *et al.* [8] was the lower abundances of isopods and oligochaetes in the most highly contaminated zone. Other studies have also shown earthworms to be sensitive to high levels of metal contamination in soils [126]. However, at sites where metal concentrations are moderate, soil community shifts are often subtle or masked by other environmental factors [127] and indicates that bioaccumulation of metals in lower trophic levels does not necessarily impact on invertebrate community structure [128].

However, impacts in vertebrate consumers/predators might be expected to be more pronounced because of the large volumes of prey taken. For example, godwits (*Limosa limosa*) are migratory waders that breed and feed at numerous contaminated sites in The Netherlands [129]. Although their residency at contaminated sites is temporary, godwits feeding on worms that were known to accumulate metals, in turn accumulated Pb, Hg and Cd from worms. *Limosa limosa* is listed in the so-called Dutch Red List (a list of endangered species that are protected by special legislation) and is therefore a conservation priority species. Numerous factors are implicated in the population declines of this species and it is not possible to isolate metal contamination as an important factor.

Arsenic poisoning has been implicated in declines of small mammals in alpine regions of the Snowy Mountains of Australia, although the causal links remain speculative. Bogong moths (*Agrotis infusa*) migrate annually from the agricultural plains to alpine regions to estivate and carry with them large body-burdens of As. Between the time when arsenic was first reported from two mountain estivation sites in the summer of 2000/2001 [130] and the following summer, the amount of arsenic found in moths increased by at least an order of magnitude [131]. Over this period, populations of the herbivorous broad-toothed rat (*Mastacomys fuscus*), the insectivorous dusky antechinus (*Antechinus swainsonii*) and the omnivorous mountain pygmy possum (*Burramys parvus*) declined, and As was detected in the faeces of *A. swainsonii* and another non-declining species, *Rattus fuscipes*. Because the declines in mammals were across several trophic groups (*i.e.* herbivores and insectivores), As poisoning is not likely to be the predominant causal factor, but may be a co-factor that needs to be considered in the declines of rare species like the mountain pygmy possum (*B. parvus*). The source of As in the moths was not known [130, 131], but it is presumed to come from the breeding grounds of the moths in the agricultural plains of southern Queensland and northern NSW [130]. As-based pesticides have been used in Australia since the early 1900s [132, 133] and continue to be used to a lesser extent in the form of the organoarsenic herbicides (e.g. monosodium methylarsonate; MSMA).

MSMA has also been applied widely in British Columbia, Canada to control outbreaks of Mountain Pine Beetle [134]. As a consequence, beetle larvae accumulated between 1.3 and 700 μg As/g (dry weight). Subsequent feeding by insectivorous woodpeckers and other forest passerines breeding within 1 km of MSMA stands contained elevated blood concentrations of total arsenic (geometric mean = 0.18 μg/g; range = 0.02 to 2.20 μg As/g) [135]. This range of whole blood concentrations is similar to the range of concentrations found among zebra finches provided orally with monomethylarsonic acid (MMA(V)) at doses between 8 and 72 μg/g/day over 14 days [136]. Oral doses of this magnitude were found to cause weight loss among adult finches after 14 days [136] and mortality among nestling zebra finches after 20 days [137], indicating that natural field populations of passerine birds may be affected in MSMA treated areas.

As indicated in the introduction, various small mammals are able to persist in highly contaminated sites. Of particular note is the persistence of common shrews (*Sorex araneus*) in sites with high levels of Cd contamination. Shrews are higher order predators in terrestrial environments, and because of their high metabolic demands, they consume large quantities of prey. In Cd-contaminated sites, shrews accumulate very large body burdens of Cd. In the study by Hunter *et al.* [81], shrews in the vicinity of a cadmium/copper refinery accumulated from their prey Cd residues in excess of 200 μg/g and 500 μg/g (dry weight) in the kidney and liver, respectively. Similar liver-Cd concentrations have also been reported by other authors [80, 138, 139]. It remains unclear if the health of shrews in these studies was compromised by the high body burdens of Cd. In an earlier report, Hunter *et al.* [140] described lesions in the kidney and liver of shrews from the same location and with similar Cd burdens. Later laboratory studies [73, 141] demonstrated reduced weights (but no mortality) among shrews exposed to Cd-contaminated diets for a period of 12 weeks. In those studies, test animals accumulated kidney and liver Cd burdens in excess of 1000 μg/g. It seems likely that shrews have evolved to tolerate high levels of metals [142].

Other species do not appear to be so tolerant. Damek-Poprawa and Sawicka-Kapusta [143] described histopathological changes in the liver and kidneys of herbivorous bank voles (*Clethrionomys glareolus*) with much lower tissue burdens of Cd in kidney (33 μg/g dry weight) and liver (16 μg/g dry weight). Cadmium is also known to compromise reproduction [144], and accumulation of heavy burdens of Cd may be limiting recoveries of European badgers (*Meles meles*). Van den Brink and Ma [145] found that the quantities of Cd and Zn in the kidneys of badgers in the different regions of The Netherlands were negatively correlated with the increase in the number of breeding dens (setts) in these regions and hence with the number of cubs born. The highest kidney Cd burdens (101 to 405 μg/g dry weight) were found among adult female badgers living close to rivers where earthworms (a major prey species) accumulated large burdens of Cd.

Although studies like those conducted by Hunter *et al.* [9, 81] and Mertens *et al.* [138] provide evidence that small mammal communities can persist even in highly contaminated environments without apparent detriment, other studies show that community structure is likely to be altered in the face of severe metal contamination. The Tar Creek Superfund Site in Oklahoma, USA, has a long history of metal contamination as a consequence of mining activities, and elevated tissue concentrations of Cd have been documented in several non-mammalian inhabitants [146, 147]. Although tissue-metal concentrations were not reported, Phelps and McBee [148] reported reduced species diversity among small mammal assemblages at Tar Creek compared to reference locations. At reference sites, several small mammals were recorded; however, in the contaminated sites, white-footed mice (*Peromyscus leucopus*) predominated, and in greater numbers than found in reference sites. White-footed mice have previously been demonstrated to dominate small mammal assemblages in disturbed environments [149, 150].

The capacity for white-footed mice to dominate in disturbed environments is note-worthy. In the case of the study by Levengood and Heske [150], the authors cited very high soil concentrations for Cd, Hg and Se (as well as other metals) in a wetland site that had received sediments dredged from Lake DePue, Illinois, in 1982. The wetland is periodically inundated as a management strategy for the conservation of waterfowl. White-footed mice, because of their arboreal habit, were able to utilise this habitat and persist with tissue burdens of Se that have been shown to be toxic to rats in laboratory studies. The white-footed mouse is an omnivore, and likely consumes invertebrates as well as plant seeds. Tissue metal (Cd and Pb) concentrations in grasshoppers and crickets were high (2.5 to 4.8 μg/g), and it seems likely that mice were exposed to relatively high levels of metal in their diet. However, apart from Se, kidney- and liver-metal concentrations remained comparatively low, indicating that the white-footed mouse was not under threat as a consequence of biomagnification of toxic metals. The mechanism by which white-footed mice are able to avoid bioaccumulation of metals is unknown, although tolerance to high levels of metals and other contaminants is likely related to the efficiency by which this species can detoxify reactive oxygen species as well as highly efficient DNA repair mechanisms [151].

Some metals only enter the higher terrestrial food chain after commencing their trajectories through aquatic food chains. Selenium and Hg are two such metals. Selenium is bioaccumulated in aquatic habitats and organoselenium compounds can be bioconcentrated over 200,000 times by zooplankton when water concentrations are in the 0.5 to 0.8 μg Se/L range. Inorganic selenium bioaccumulates more readily in phytoplankton than in zooplankton. Phytoplankton can concentrate inorganic selenium by a factor of 3000. Further biomagnifications occurs along the food chain, as predators consume selenium rich prey. A water concentration of 2 μg Se/L is considered highly hazardous to sensitive fish and aquatic birds. Compounding this trophic biomagnification, selenium poisoning can also be passed from parents to offspring through the egg, and selenium poisoning may persist for many generations [152]. As indicated above, selenium migrates into terrestrial ecosystems when birds or other taxa feed on aquatic organisms. The best studied example comes from California in the USA.

In the 1980s Kesterson National Wildlife Refuge, CA was contaminated with Se as a consequence of subsurface irrigation drainage. Selenium and other trace elements were leached from agricultural soils in the San Joaquin Valley, and the excess water was transported to Kesterson as a wildlife management strategy. Elevated body burdens of selenium were found in all animal taxa examined, including mammals, birds and reptiles [152]. The concentrations of Se in livers taken from waterfowl typically ranged between 20 and 100 μg/g and were significantly higher than in similar birds sampled from a reference site [153]. Selenium, although an essential element required for normal development, is toxic even when exposure is only slightly higher than essential requirements. Among waterfowl in Kesterson, particularly among eared grebes (*Podiceps nigricollis*), very low hatching rates were ascribed to selenium-induced embryotoxicosis [154]. More recent studies in Kesterson have continued to report elevated level of Se in passerine species such as starlings (*Sturnus vulgaris*) and small mammals, but without associated effects on hatching success or population declines [43, 44].

As indicated above, mercury enters the terrestrial food chain when predators eat aquatic species that have bioaccumulated the metal. The best known example of Hg poisoning in a terrestrial mammal is that of Minamata disease among human inhabitants of Japan following consumption of contaminated fish downstream of a Hg-contaminated river. Minamata disease is characterized by severe neurological disorders, and similar neurological symptoms of poisoning among mammalian predators such as predatory birds and mink have also been reported [31, 39]. Among species of mink (*Mustela* sp.), a lowest observable adverse effects level (LOEL) for Hg of 5 μg/g (wet weight) in the brain has been established in laboratory studies [36]. Brain Hg concentrations of this order of magnitude (0.5 to 5.0 μg/g wet weight) are common among wild mink [36], and some authors have speculated that mercury poisoning may be responsible for declines in mink (*Mustela vison*) in Georgia, North Carolina, and South Carolina [155], although the co-occurrence of chlorinated organic compounds in mink tissues was also implicated.

Biomagnification of metals in higher predators, such as predatory birds, large carnivorous mammals and reptiles [156-158], is more difficult to demonstrate. Predatory birds and large mammals are more sparsely distributed and far more mobile, having home ranges that go well beyond localised contamination. Contaminated prey, though likely forming part of their diets, will be diluted with prey from less contaminated sites. In the case of reptiles, a combination of low metabolic rate typical of poikilothermic animals and relatively low assimilation efficiencies [72, 159], is likely to reduce the risk of metal bioaccumulation. However, reptiles in general are under-represented in the ecotoxicology literature [160] and it would be premature to suggest that reptiles were not at risk from exposure to metals.

CONCLUSIONS

Direct metal poisoning of higher vertebrates is found sporadically, and examples thereof are mostly found for Pb. Magnification in the food chain *via* prey having elevated body burdens of metals is shown more often. The level of biomagnification that can be expected within the field situation is difficult to predict, and depends on five factors, namely: (i) metal specificity; (ii) exposure route of the predator; (iii). excretion possibilities of organisms; (iv) predator specificity such as gender and life span, and (v) metal sequestration in the prey organism.

In general, carnivorous populations show higher biomagnification compared to herbivorous organisms. Aquatic food chains are less at risk than terrestrial food chain when it comes to biomagnification of metals and metalloids. To assess biomagnification effects at community level, unraveling ecosystem complexity is necessary before species most exposed and at risk can be identified. Therefore, for the purpose of setting environmental quality objectives, it is important to include biomagnification as well.

The final objective of studies that examine biomagnification and toxicity of metals is to provide sufficient protection to all biological organisation levels. Observations in the laboratory and field have demonstrated that secondary poisoning of, for example, worm-eating birds and mammals may be more critical than direct exposure of soil organisms. In such cases, quality criteria that are protective of lower trophic levels, may not provide protection for top predators.

FUTURE PROSPECTS

Several metals are familiar as common contaminants in our environment by virtue of the fact that they have been used for many decades or centuries for industrial and agricultural purposes. Indeed, the various phases in human civilization are characterised by our use of specific metals. The Copper Age (c. 3200–2300 bc), the Bronze Age (2300–700 bc) and the Iron Age (700–1 bc), mark the discovery and adoption of these metals. The more recent industrial ages have seen the use of many other metals including those which now present a pollution hazard, such as Cd, Pb and Hg. Much of our knowledge about the environmental risks posed by metals comes from research on these metals. However, the industrial age is far from spent, and recent decades have seen increased reliance on several other metallic elements. In the immediate future the continued and increased reliance on coal combustion for electricity production will result in increased diffuse contamination of soils and oceans with various elements, including Hg, As, Cd, Cu, Pb, Se, Zn and various others because they are volatile species which are not retained by flue gas filtration systems [161]. Therefore, it can be expected that the environmental consequences of bioaccumulation and subsequent biomagnification of various metals, particularly Hg, Se and Cd are yet to be fully realised.

Other, less abundant metals are also increasing in our environment, due to use of a multitude of metals in innovative new applications like the broad area of nanotechnology and related fields (nanofood, nanocosmetics, nanopharmaceuticals, etc). Some of these new metal-based products will follow well understood metal uptake kinetics. However others may potentially find entry into biological systems *via* yet to be discovered modes of bio-uptake. Only time will tell if biomagnification and subsequent toxicity of these innovative combinations of metals and metalloids will occur.

ACKNOWLEDGMENT

Martina Vijver was supported by a NWO VENI-grant, project number 863.08.023.

REFERENCES

[1] Alloway BJ, Ed. Heavy metals in soils. 2nd Edition ed. London: Blackie Academic and Professional. Chapman and Hall; 1995.

[2] Campbell PGC, Chapman PM, Hale B. Risk assessment of metals in the environment. In: Hest RE, Harrison RM, Eds. Issues in Environmental Sciences, no 22: Chemicals in the Environment: Assessing and Managing the Risk: The Royal Society of Chemistry; 2006. pp. 102-31.

[3] Mertz W. The essential trace-elements. Science 1981; 213: 1332-1338.

[4] Ballatori N. Transport of toxic metals by molecular mimicry. Environ Health Perspect 2002; 110: 689-694.

[5] Zalups RK, Ahmad S. Molecular handling of cadmium in transporting epithelia. Toxicol Appl Pharmacol 2003; 186: 163-188.

[6] Hodson M, Vijver M, Peijnenburg W. Bioavailability. In: Swartjes F, Ed. Dealing with Contaminated Sites, from Theory Towards Practice; in press.

[7] Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem.1. Soil and vegetation contamination. J Appl Ecol 1987; 24: 573-586.

[8] Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 2. Invertebrates. J Appl Ecol 1987; 24: 587-599.

[9] Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 3. Small mammals. J Appl Ecol 1987; 24: 601-614.

[10] Janssen MPM, Bruins A, De Vries TH, van Straalen N. Comparison of cadmium kinetics in four soil arthropod species. Arch Environ Contam Toxicol 1991; 20: 305-312.

[11] Gobas FAPC, Morrison HA. Bioconcentration and biomagnification in the aquatic environment. In: Boethling RS, Mackay D, Eds. Handbook of Property Estimation Methods for Chemicals. Boca Raton, London: Lewis Publishers; 2000. pp. 189-231.

[12] Mackay D, Fraser A. Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ Pollut 2000; 110: 375-391.

[13] Veltman K. Bioaccumulation modeling of organic chemicals and metals based on chemical properties and species characteristics [PhD-thesis]. Nijmegen, The Netherlands: Radboud University; 2009.

[14] Peijnenburg WJGM, Jager T. Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. Ecotoxicol Environ Saf 2003; 56: 63-77.

[15] Hendriks AJ, Heikens A. The power of size. 2. Rate constants and equilibrium ratios for accumulation of inorganic substances related to species weight. Environ Toxicol Chem 2001; 20: 1421-1437.

[16] Gray JS. Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 2002; 45: 46-52.

[17] Laskowski R. Are the top carnivores endangered by heavy-metal biomagnification? Oikos 1991; 60: 387-390.

[18] van Straalen NM, Ernst WHO. Metal biomagnification may endanger species in critical pathways. Oikos 1991; 62: 255-256.

[19] Croteau MN, Hare L, Tessier A. Difficulties in relating Cd concentrations in the predatory insect *Chaoborus* to those of its prey in nature. Can J Fish Aquat Sci 2003; 60: 800-808.

[20] Peterson BJ, Fry B. Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 1987; 18: 293-320.

[21] Alonso E, Tapie N, Budzinski H, *et al.* A model for estimating the potential biomagnification of chemicals in a generic food web: preliminary development. Environ Sci Pollut Res 2008; 15: 31-40.

[22] Peijnenburg W, Baerselman R, de Groot AC, *et al.* Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the oligochaete *Eisenia andrei*. Ecotoxicol Environ Saf 1999; 44: 294-310.

[23] Spurgeon DJ, Hopkin SP. Effects of variations of the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*. Pedobiologia 1996; 40: 80-96.

[24] van Gestel CAM, Koolhaas JE. Water-extractability, free ion activity, and pH explain cadmium sorption and toxicity to *Folsomia candida* (Collembola) in seven soil-pH combinations. Environ Toxicol Chem 2004; 23: 1822-1833.

[25] Nahmani J, Hodson ME, Black S. Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. Environ Pollut 2007; 149: 44-58.

[26] Grodzinska K, Godzik B, Darowska E, Pawlowska B. Concentration of heavy-metals in trophic chains of niepolomice forest, S Poland. Ekol Pol 1987; 35: 327-344.

[27] Morel FMM, Kraepiel AML, Amyot M. The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst 1998; 29: 543-566.

[28] Mason RP, Reinfelder JR, Morel FMM. Bioaccumulation of mercury and methylmercury. Water Air Soil Pollut 1995; 80: 915-921.

[29] Fischer RG, Rapsomanikis S, Andreae MO, Baldi F. Bioaccumulation of methylmercury and transformation of inorganic mercury by macrofungi. Environ Sci Technol 1995; 29: 993-999.

[30] Pokorny B, Al Sayegh-Petkovsek S, Ribaric-Lasnik C, *et al.* Fungi ingestion as an important factor influencing heavy metal intake in roe deer: evidence from faeces. Sci Total Environ 2004; 324: 223-234.

[31] Tan SW, Meiller JC, Mahaffey KR. The endocrine effects of mercury in humans and wildlife. Crit Rev Toxicol 2009; 39: 228-269.

[32] Longcore JR, Haines TA, Halteman WA. Mercury in tree swallow food, eggs, bodies, and feathers at Acadia National Park, Maine, and an EPA Superfund site, Ayer, Massachusetts. Environ Monit Assess 2007; 126: 129-143.

[33] Evers DC, Savoy LJ, DeSorbo CR*, et al.* Adverse effects from environmental mercury loads on breeding common loons. Ecotoxicology 2008; 17: 69-81.

[34] Eagles-Smith CA, Ackerman JT, De La Cruz SEW, Takekawa JY. Mercury bioaccumulation and risk to three waterbird foraging guilds is influenced by foraging ecology and breeding stage. Environ Pollut 2009; 157: 1993-2002.

[35] Klenavic K, Champoux L, Mike O, Daoust PY, Evans RD, Evans HE. Mercury concentrations in wild mink (*Mustela vison*) and river otters (*Lontra canadensis*) collected from eastern and Atlantic Canada: relationship to age and parasitism. Environ Pollut 2008; 156: 359-366.

[36] Basu N, Scheuhammer AM, Bursian SJ, *et al.* Mink as a sentinel species in environmental health. Environ Res 2007; 103: 130-144.

[37] Kalisińska E, Lisowski P, Salicki W, Kucharska T, Kavetska K. Mercury in wild terrestrial carnivorous mammals from north-western Poland and unusual fish diet of red fox. Acta Theriol 2009; 54: 345-356.

[38] Braune BM, Norstrom RJ, Wong MP, Collins BT, Lee J. Geographical-distribution of metals in livers of polar bears from the Northwest Territories, Canada. Sci Total Environ 1991; 100: 283-299.

[39] Basu N, Scheuhammer AM, Sonne C, *et al.* Is dietary mercury of neurotoxicological concern to wild polar bears (*Ursus maritimus*)? Environ Toxicol Chem 2009; 28: 133-140.

[40] Croteau MN, Luoma SN, Stewart AR. Trophic transfer of metals along freshwater food chains: evidence of cadmium biomagnification in nature. Limnol Oceanogr 2005; 50: 1511-1519.

[41] Stewart AR, Luoma SN, Schleckat CE, Doblin MA, Hieb KA. Food web pathway determines how selenium affects aquatic ecosystems: a San Francisco bay case study. Environ Sci Technol 2004; 38: 4519-4526.

[42] Vickerman DB, Trumble JT. Biotransfer of selenium: effects on an insect predator, *Podisus maculiventris*. Ecotoxicology 2003; 12: 497-504.

[43] Santolo GM. Selenium accumulation in European Starlings nesting in a selenium-contaminated environment. Condor 2007; 109: 862-869.

[44] Santolo GM. Small mammals collected from a site with elevated selenium concentrations and three reference sites. Arch Environ Contam Toxicol 2009; 57: 741-754.

[45] Dumas J, Hare L. The internal distribution of nickel and thallium in two freshwater invertebrates and its relevance to trophic transfer. Environ Sci Technol 2008; 42: 5144-5149.

[46] Scheckel KG, Lombi E, Rock SA, McLaughlin MJ. *In vivo* synchrotron study of thallium speciation and compartmentation in *lberis intermedia*. Environ Sci Technol 2004; 38: 5095-5100.

[47] Babula P, Adam V, Opatrilova R, *et al.* Uncommon heavy metals, metalloids and their plant toxicity: a review. Environ Chem Lett 2008; 6: 189-213.

[48] Sánchez-Chardi A. Tissue, age, and sex distribution of thallium in shrews from Doñana, a protected area in SW Spain. Sci Total Environ 2007; 383: 237-240.

[49] Reeves RD, Baker AJM. Metal-accumulating plants. In: Raskin I, Ensley BD, Eds. Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. London: John Wiley & Sons, Inc; 2000. pp. 193-229.

[50] Quinn CF, Freeman JL, Galeas ML, Klamper EM, Pilon-Smits EAH. The role of selenium in protecting plants against prairie dog herbivory: implications for the evolution of selenium hyperaccumulation. Oecologia 2008; 155: 267-275.

[51] Freeman JL, Quinn CF, Lindblom SD, Klamper EM, Pilon-Smits EAH. Selenium protects the hyperaccumulator *Stanleya pinnata* against black-tailed prairie dog herbivory in native seleniferous habitats. Am J Bot 2009; 96: 1075-1085.

[52] Boyd RS. High-nickel insects and nickel hyperaccumulator plants: a review. Insect Sci 2009; 16: 19-31.

[53] Notten MJM, Oosthoek AJP, Rozema J, Aerts R. Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. Environ Pollut 2005; 138: 178-190.

[54] Notten MJM, Oosthoek AJP, Rozema J, Aerts R. Heavy metal pollution affects consumption and reproduction of the landsnail *Cepaea nemoralis* fed on naturally polluted *Urtica dioica* leaves. Ecotoxicology 2006; 15: 295-304.

[55] Gimbert F, Mench M, Coeurdassier M, Badot PM, de Vaufleury A. Kinetic and dynamic aspects of soil-plant-snail transfer of cadmium in the field. Environ Pollut 2008; 152: 736-745.

[56] Scheifler R, De Vaufleury A, Coeurdassier M, Crini N, Badot PM. Transfer of Cd, Cu, Ni, Pb, and Zn in a soil-plant-invertebrate food chain: a microcosm study. Environ Toxicol Chem 2006; 25: 815-822.

[57] Merrington G, Winder L, Green I. The uptake of cadmium and zinc by the bird-cherry oat aphid *Rhopalosiphum padi* (Homoptera: Aphididae) feeding on wheat grown on sewage sludge amended agricultural soil. Environ Pollut 1997; 96: 111-114.

[58] Merrington G, Winder L, Green I. The bioavailability of Cd and Zn from soils amended with sewage sludge to winter wheat and subsequently to the grain aphid *Sitobion avenae*. Sci Total Environ 1997; 205: 245-254.

[59] Roubies N, Giadinis ND, Polizopoulou Z, Argiroudis S. A retrospective study of chronic copper poisoning in 79 sheep flocks in Greece (1987-2007). J Vet Pharmacol Ther 2008; 31: 181-183.

[60] Frank A. A review of the "mysterious" wasting disease in Swedish moose (*Alces alces* L.) related to molybdenosis and disturbances in copper metabolism. Biol Trace Elem Res 2004; 102: 143-159.

[61] Frank A, Danielsson R, Jones B. The 'mysterious' disease in Swedish moose. Concentrations of trace elements in liver and kidneys and clinical chemistry. Comparison with experimental molybdenosis and copper deficiency in the goat. Sci Total Environ 2000; 249: 107-122.

[62] Zidar P, Drobne D, Štrus J, Blejec A. Intake and assimilation of zinc, copper, and cadmium in the terrestrial isopod *Porcellio scaber* Latr. (Crustacea, Isopoda). Bull Environ Contam Toxicol 2003; 70: 1028-1035.

[63] Calhôa CF, Soares AMVM, Mann RM. Cadmium assimilation in the terrestrial isopod, *Porcellio dilatatus* – is trophic transfer important? Sci Total Environ 2006; 371: 206-213.

[64] Laskowski R, Hopkin SP. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. Environ Pollut 1996; 91: 289-297.

[65] Witzel B. The influence of zinc on the uptake and loss of cadmium and lead in the woodlouse, *Porcellio scaber* (Isopoda, Oniscidea). Ecotoxicol Environ Saf 2000; 47: 43-53.

[66] Merrington G, Miller D, McLaughlin MJ, Keller MA. Trophic barriers to fertilizer Cd bioaccumulation through the food chain: a case study using a plant-insect predator pathway. Arch Environ Contam Toxicol 2001; 41: 151-156.

[67] Green ID, Merrington G, Tibbett M. Transfer of cadmium and zinc from sewage sludge amended soil through a plant-aphid system to newly emerged adult ladybirds (*Coccinella septempunctata*). Agric Ecosyst Environ 2003; 99: 171-178.

[68] Hopkin SP, Martin MH. Assimilation of zinc, cadmium, lead, copper, and iron by the spider *Dysdera crocata*, a predator of woodlice. Bull Environ Contam Toxicol 1985; 34: 183-187.

[69] Hopkin SP. Ecophysiology of Metals in Terrestrial Invertebrates. London: Elsevier Applied Science; 1989.

[70] Hendrickx F, Maelfait J-P, Langenbick F. Absence of cadmium excretion and high assimilation result in cadmium biomagnification in a wolf spider. Ecotoxicol Environ Saf 2003; 55: 287-292.

[71] Andersen O, Nielsen JB, Nordberg GF. Nutritional interactions in intestinal cadmium uptake - possibilities for risk reduction. Biometals 2004; 17: 543-547.

[72] Mann RM, Serra EA, Soares AMVM. Assimilation of cadmium in a European lacertid lizard: Is trophic transfer important? Environ Toxicol Chem 2006; 25: 3199-3203.

[73] Dodds-Smith ME, Johnson MS, Thompson DJ. Trace-metal accumulation by the shrew *Sorex araneus*. 1. Total-body burden, growth, and mortality. Ecotoxicol Environ Saf 1992; 24: 102-117.

[74] Mann RM, Sánchez-Hernández J-C, Serra EA, Soares AM. Bioaccumulation of Cd by a European lacertid lizard after chronic exposure to Cd-contaminated food. Chemosphere 2007; 68: 1525-1534.

[75] Franklin NM, Glover CN, Nicol JA, Wood CM. Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. Environ Toxicol Chem 2005; 24: 2954-2964.

[76] Hamers T, Van den Berg JHJ, van Gestel CAM, van Schooten FJ, Murk AJ. Risk assessment of metals and organic pollutants for herbivorous and carnivorous small mammal food chains in a polluted floodplain (Biesbosch, the Netherlands). Environ Pollut 2006; 144: 581-595.

[77] Hendriks AJ, Ma WC, Brouns JJ, Deruiterdijkman EM, Gast R. Modeling and monitoring organochlorine and heavy-metal accumulation in soils, earthworms, and shrews in Rhine-delta floodplains. Arch Environ Contam Toxicol. 1995; 29: 115-27.

[78] Wijnhoven S, Leuven R, van der Velde G*, et al.* Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species- and location-specific characteristics. Arch Environ Contam Toxicol 2007; 52: 603-613.
[79] Wijnhoven S, Van der Velde G, Leuven R, Eijsackers HJP, Smits AJM. Metal accumulation risks in regularly flooded and non-flooded parts of floodplains of the River Rhine: extractability and exposure through the food chain. Chem Ecol 2006; 22: 463-477.
[80] Ma WC, Denneman W, Faber J. Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch Environ Contam Toxicol 1991; 20: 266-270.
[81] Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 4. Tissue distribution and age accumulation in small mammals. J Appl Ecol 1989; 26: 89-99.
[82] Andrews SM, Johnson MS, Cooke JA. Distribution of trace-element pollutants in a contaminated grassland ecosystem established on metalliferous fluorspar tailings.1. Lead. Environ Pollut 1989; 58: 73-85.
[83] Shore RF. Predicting cadmium, lead and fluoride levels in small mammals from soil residues and by species-species extrapolation. Environ Pollut 1995; 88: 333-340.
[84] Milton A, Cooke JA, Johnson MS. Accumulation of lead, zinc, and cadmium in a wild population of *Clethrionomys glareolus* from an abandoned lead mine. Arch Environ Contam Toxicol 2003; 44: 405-411.
[85] Veltman K, Huijbregts MAJ, Hamers T, Wijnhoven S, Hendriks AJ. Cadmium accumulation in herbivorous and carnivorous small mammals: meta-analysis of field data and validation of the bioaccumulation model optimal modeling for ecotoxicological applications. Environ Toxicol Chem 2007; 26: 1488-1496.
[86] Rich CN, Talent LG. Soil ingestion may be an important route for the uptake of contaminants by some reptiles. Environ Toxicol Chem 2009; 28: 311-315.
[87] Beyer WN, Connor EE, Gerould S. Estimates of soil ingestion by wildlife. J Wildl Manage 1994; 58: 375-382.
[88] Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci Total Environ 1998; 219: 117-135.
[89] Connell DW. Environmental routes leading to the bioaccumulation of lipophilic chemicals. In: Connell DW, Ed. Bioaccumulation of Xenobiotic Compounds. Boca Raton, Florida: CRC Press; 1990. pp. 60-73.
[90] Leblanc GA. Trophic level differences in the bioconcentration of chemicals - implications in assessing environmental biomagnification. Environ Sci Technol 1995; 29: 154-160.
[91] Komarnicki GJK. Tissue, sex and age specific accumulation of heavy metals (Zn, Cu, Pb, Cd) by populations of the mole (*Talpa europaea* L.) in a central urban area. Chemosphere 2000; 41: 1593-1602.
[92] Pankakoski E, Hyvärinen H, Jalkanen M, Koivisto I. Accumulation of heavy-metals in the mole in Finland. Environ Pollut 1993; 80: 9-16.
[93] Deng HL, Zhang ZW, Chang CY, Wang Y. Trace metal concentration in great tit (*Parus major*) and greenfinch (*Carduelis sinica*) at the western mountains of Beijing, China. Environ Pollut 2007; 148: 620-626.
[94] Janssens E, Dauwe T, Bervoets L, Eens M. Heavy metals and selenium in feathers of great tits (*Parus major*) along a pollution gradient. Environ Toxicol Chem 2001; 20: 2815-2820.
[95] Nam DH, Anan Y, Ikemoto T*, et al.* Specific accumulation of 20 trace elements in great cormorants (*Phalacrocorax carbo*) from Japan. Environ Pollut 2005; 134: 503-514.
[96] Nygård T, Lie E, Røv N, Steinnes E. Metal dynamics in an Antarctic food chain. Mar Pollut Bull 2001; 42: 598-602.
[97] Rogival D, Scheirs J, Blust R. Transfer and accumulation of metals in a soil-diet-wood mouse food chain along a metal pollution gradient. Environ Pollut 2007; 145: 516-528.
[98] Sánchez-Chardi A, López-Fuster MJ, Nadal J. Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula*, from the Ebro Delta (NE Spain): sex- and age-dependent variation. Environ Pollut 2007; 145: 7-14.
[99] Fritsch C, Cosson RP, Coeurdassier M*, et al.* Responses of wild small mammals to a pollution gradient: host factors influence metal and metallothionein levels. Environ Pollut 2010; 158: 827-840.
[100] Wojcik M, Vangronsveld J, D'Haen J, Tukiendorf A. Cadmium tolerance in *Thlaspi caerulescens* - II. Localization of cadmium in *Thlaspi caerulescens*. Environ Exp Bot 2005; 53: 163-171.
[101] Köhler HR. Localization of metals in cells of saprophagous soil arthropods (Isopoda, Diplopoda, Collembola). Microsc Res Tech 2002; 56: 393-401.
[102] Morgan AJ, Turner MP, Morgan JE. Morphological plasticity in metal-sequestering earthworm chloragocytes: morphometric electron microscopy provides a biomarker of exposure in field populations. Environ Toxicol Chem 2002; 21: 610-618.
[103] Rainbow PS. Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 2002; 120: 497-507.

[104] Vijver MG, van Gestel CAM, Lanno RP, van Straalen NM, Peijnenburg WJGM. Internal metal sequestration and its ecotoxicological relevance: a review. Environ Sci Technol 2004; 38: 4705-4712.
[105] Monteiro M, Santos C, Soares AMVM, Mann RM. Does subcellular distribution in plants dictate the trophic bioavailability of cadmium to *Porcellio dilatatus* (Crustacea, Isopoda)? Environ Toxicol Chem 2008; 27: 2548-2556.
[106] Wallace WG, Lee B-G, Luoma SN. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar Ecol Prog Ser 2003; 249: 183-197.
[107] Wallace WG, Luoma SN. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). Mar Ecol Prog Ser 2003; 257: 125-137.
[108] Wallace WG, Lopez GR, Levinton JS. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar Ecol Prog Ser 1998; 172: 225-237.
[109] Zhang L, Wang WX. Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish. Limnol Oceanogr 2006; 51: 2008-2017.
[110] Mateo R, Green AJ, Lefranc H, Baos R, Figuerola J. Lead poisoning in wild birds from southern Spain: a comparative study of wetland areas and species affected, and trends over time. Ecotoxicol Environ Saf 2007; 66: 119-126.
[111] Fisher IJ, Pain DJ, Thomas VG. A review of lead poisoning from ammunition sources in terrestrial birds. Biol Conserv 2006; 131: 421-432.
[112] Gangoso L, Álvarez-Lloret P, Rodríguez-Navarro AAB, *et al.* Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources. Environ Pollut 2009; 157: 569-574.
[113] Bellrose FC. Lead poisoning as a mortality factor in waterfowl populations. Ill Nat Hist Surv Bull 1959; 27: 235-288.
[114] Mautino M, Bell JU. Experimental lead toxicity in the ring-necked duck. Environ Res 1986; 41: 538-545.
[115] Fox GA. Perturbations in terrestrial vertebrate populations: contaminants as a cause. In: Albers PH, H. HG, Oulendorf HM, Eds. Environmental Contaminants and Terrestrial Vertebrates: Effects on Populations, Communities, and Ecosystems. Pensacola, Florida: SETAC Press; 2000. pp. 19-59.
[116] Scheuhammer AM, Norris SL. The ecotoxicology of lead shot and lead fishing weights. Ecotoxicology 1996; 5: 279-295.
[117] Beck N, Granval P, Olivier G-N. Techniques d'analyse du régime alimentaire animal diurne de bécassines des marais (*Gallinago gallinago*) du nordouest de la France. Gibier Faune Sauvage 1995; 12: 1-20.
[118] Clark AJ, Scheuhammer AM. Lead poisoning in upland-foraging birds of prey in Canada. Ecotoxicology 2003; 12: 23-30.
[119] Kingston RL, Hall S, Sioris L. Clinical observations and medical outcome in 149 cases of arsenate ant killer ingestion. J Toxicol Clin Toxic 1993; 31: 581-591.
[120] Kile ML, Houseman EA, Breton CV*, et al.* Dietary arsenic exposure in Bangladesh. Environ Health Perspect 2007; 115: 889-893.
[121] Ma LQ, Komar KM, Tu C, *et al.* A fern that hyperaccumulates arsenic - a hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils. Nature 2001; 409: 579.
[122] Kobayashi E, Suwazono Y, Dochi M, Honda R, Kido T. influence of consumption of cadmium-polluted rice or Jinzu River water on occurrence of renal tubular dysfunction and/or *itai-itai* disease. Biol Trace Elem Res 2009; 127: 257-268.
[123] Khan AG, Chaudhry TM, Hayes WJ*, et al.* Physical, chemical and biological characterisation of a steelworks waste site at Port Kembla, NSW Australia. Water Air Soil Pollut 1998; 104: 389-402.
[124] Karjalainen AM, Kilpi-Koski J, Vaisanen AO, *et al.* Ecological risks of an old wood impregnation mill: application of the Triad approach. Integr Environ Assess Manage 2009; 5: 379-389.
[125] Galbraith H, Lejeune K, Lipton J. Metal and arsenic impacts to soils, vegetation communities and wildlife habitat in southwest Montana uplands contaminated by smelter emissions. 1. Field-evaluation. Environ Toxicol Chem 1995; 14: 1895-1903.
[126] Yeates GW, Orchard VA, Speir TW, Hunt JL, Hermans MCC. Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological-activity. Biol Fertil Soils 1994; 18: 200-208.
[127] Rutgers M. Field effects of pollutants at the community level - experimental challenges and significance of community shifts for ecosystem functioning. Sci Total Environ 2008; 406: 469-478.
[128] Haimi J, Matasniemi L. Soil decomposer animal community in heavy-metal contaminated coniferous forest with and without liming. Eur J Soil Biol 2002; 38: 131-136.
[129] Roodbergen M, Klok C, van der Hout A. Transfer of heavy metals in the food chain earthworm black-tailed godwit (*Limosa limosa*): comparison of a polluted and a reference site in The Netherlands. Sci Total Environ 2008; 406: 407-412.
[130] Green K, Broome L, Heinze D, Johnston S. Long distance transport of arsenic by migrating Bogong moths from agricultural lowlands to mountain ecosystems. Victorian Naturalist 2001; 118: 112-116.
[131] Green K. Migratory Bogong moths (*Agrotis infusa*) transport arsenic and concentrate it to lethal effect by estivating gregariously. Arct Antarct Alp Res 2008; 40: 74-80.

[132] Smith E, Smith J, Smith L, *et al.* Arsenic in Australian environment: an overview. J Environ Sci Health A 2003; 38: 223-239.
[133] EPA N. Assessment of orchard and market garden contaminated sites - discussion paper. Chatswood, Australia: NSW Environment Protection Authority; 1995.
[134] Morrissey CA, Dods PL, Elliott JE. Pesticide treatments affect mountain pine beetle abundance and woodpecker foraging behavior. Ecol Appl 2008; 18: 172-184.
[135] Morrissey CA, Albert CA, Dods PL, Cullen WR, Lai VWM, Elliott JE. Arsenic accumulation in bark beetles and forest birds occupying mountain pine beetle infested stands treated with monosodium methanearsonate. Environ Sci Technol 2007; 41: 1494-1500.
[136] Albert CA, Williams TD, Morrissey CA, Lai VWM, Cullen WR, Elliott JE. Dose-dependent uptake, elimination, and toxicity of monosodium methanearsonate in adult zebra finches (*Taeniopygia guttata*). Environ Toxicol Chem 2008; 27: 605-611.
[137] Albert C, Williams TD, Morrissey CA, Lai VWM, Cullen WR, Elliott JE. Tissue uptake, mortality, and sublethal effects of monomethylarsonic acid (MMA(V)) in nestling zebra finches (*Taeniopygia guttata*). J Toxicol Env Health Part A 2008; 71: 353-360.
[138] Mertens J, Luyssaert S, Verbeeren S, Vervaeke P, Lust N. Cd and Zn concentrations in small mammals and willow leaves on disposal facilities for dredged material. Environ Pollut 2001; 115: 17-22.
[139] Andrews SM, Johnson MS, Cooke JA. Cadmium in small mammals from grassland established on metalliferous mine waste. Environ Pollut A 1984; 33: 153-162.
[140] Hunter BA, Johnson MS, Thompson DJ. Cadmium induced lesions in tissues of *Sorex araneus* from metal refinery grasslands. In: Osborn D, Ed. Metals in Animals. Huntingdon, Cambs: Institute of Terrestrial Ecology; 1984. pp. 39-44.
[141] Dodds-Smith ME, Johnson MS, Thompson DJ. Trace-metal accumulation by the shrew *Sorex araneus*. 2. Tissue distribution in kidney and liver Ecotoxicol Environ Saf 1992; 24: 118-130.
[142] Marques CC, Sánchez-Chardi A, Gabriel SI, *et al.* How does the greater white-toothed shrew, *Crocidura russula*, responds to long-term heavy metal contamination? - A case study. Sci Total Environ 2007; 376: 128-133.
[143] Damek-Poprawa M, Sawicka-Kapusta K. Histopathological changes in the liver, kidneys, and testes of bank voles environmentally exposed to heavy metal emissions from the steelworks and zinc smelter in Poland. Environ Res 2004; 96: 72-78.
[144] Thompson J, Bannigan J. Cadmium: Toxic effects on the reproductive system and the embryo. Reprod Toxicol 2008; 25: 304-315.
[145] Van den Brink NW, Ma WC. Spatial and temporal trends in levels of trace metals and PCBs in the European badger *Meles meles* (L., 1758) in The Netherlands: implications for reproduction. Sci Total Environ 1998; 222: 107-118.
[146] Hays KA, McBee K. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 2007; 16: 353-361.
[147] Beyer WN, Dalgarn J, Dudding S*, et al.* Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Arch Environ Contam Toxicol 2005; 48: 108-117.
[148] Phelps KL, McBee K. Ecological characteristics of small mammal communities at a superfund site. Am Midl Nat 2009; 161: 57-68.
[149] Linzey AV, Grant DM. Characteristics of a white-footed mouse (*Peromyscus leucopus*) population inhabiting a polychlorinated-biphenyls contaminated site. Arch Environ Contam Toxicol 1994; 27: 521-526.
[150] Levengood JM, Heske EJ. Heavy metal exposure, reproductive activity, and demographic patterns in white-footed mice (*Peromyscus leucopus*) inhabiting a contaminated floodplain wetland. Sci Total Environ 2008; 389: 320-328.
[151] Ungvari Z, Krasnikov BF, Csiszar A*, et al.* Testing hypotheses of aging in long-lived mice of the genus *Peromyscus*: association between longevity and mitochondrial stress resistance, ROS detoxification pathways, and DNA repair efficiency. Age 2008; 30: 121-133.
[152] Lemly AD. Selenium Assessment in Aquatic Ecosystems. Alexander DE, Ed. New York: Springer; 2002.
[153] Ohlendorf HM, Hothem RL, Bunck CM, Marois KC. Bioaccumulation of selenium in birds at Kesterson Reservoir, California. Arch Environ Contam Toxicol 1990; 19: 495-507.
[154] Ohlendorf HM, Hothem RL, Welsh D. Nest success, cause-specific nest failure, and hatchability of aquatic birds at selenium-contaminated Kesterson Reservoir and a reference site. Condor 1989; 91: 787-796.
[155] Osowski SL, Brewer LW, Baker OE, Cobb GP. The decline of mink in Georgia, North Carolina, and South Carolina - the role of contaminants. Arch Environ Contam Toxicol 1995; 29: 418-423.
[156] Van den Brink NW, Groen NM, De Jonge J, Bosveld ATC. Ecotoxicological suitability of floodplain habitats in The Netherlands for the little owl (*Athene noctua vidalli*). Environ Pollut 2003; 122: 127-134.

[157] Dehn LA, Follmann EH, Thomas DL*, et al.* Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci Total Environ 2006; 362: 103-123.
[158] Albrecht J, Abalos M, Rice TM. Heavy metal levels in ribbon snakes (*Thamnophis sauritus*) and anuran larvae from the Mobile-Tensaw River Delta, Alabama, USA. Arch Environ Contam Toxicol 2007; 53: 647-654.
[159] Hopkins WA, Roe JH, Snodgrass JW, *et al.* Effects of chronic dietary exposure to trace elements on banded water snakes (*Nerodia fasciata*). Environ Toxicol Chem 2002; 21: 906-913.
[160] Hopkins WA. Use of tissue residues in reptile ecotoxicology: a call for integration and experimentalism. In: Gardner SC, Oberdörster E, Eds. Toxicology of Reptiles. Boca Raton, FL: Taylor & Francis; 2006. pp. 35-62.
[161] Pavageau MP, Pecheyran C, Krupp EM, Morin A, Donard OFX. Volatile metal species in coal combustion flue gas. Environ Sci Technol 2002; 36: 1561-1573