Chapter 21

Heavy Metal Pollution as a Biodiversity Threat

Efraín Tovar-Sánchez, Isela Hernández-Plata,
Miguel Santoyo Martínez,
Leticia Valencia-Cuevas and Patricia Mussali Galante

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http://dx.doi.org/10.5772/intechopen.74052

Abstract

Heavy metals exert their toxic effects through different mechanisms. Lately, increasing attention has been focused on understanding the long-term ecological effects of chronically exposed populations and communities and their consequences to the ecosystem. The long-term exposure to heavy metals in the environment represents a threat to wild populations, affecting communities and putting ecosystem integrity at risk. Therefore, this type of exposure represents a threat to biodiversity. In the field, metal exposure is generally characterized by low doses and chronic exposures. This type of exposure exerts alterations across levels of biological organization. Distribution and abundance of populations, the community structure and the ecosystem dynamics may be altered. This chapter will focus on how chronically metal exposures in the field affect negatively populations and communities becoming a threat to biodiversity. Also, attention is put on the tools that enable to characterize and analyze the detrimental effects of heavy metal exposure on wild populations. Hence, the use and development of biomarkers in ecotoxicology will be discussed.

Keywords: biological organization levels, biomarkers, ecosystem health, ecotoxicology, biodiversity

1. Introduction

Heavy metals can be emitted into the environment by natural sources and anthropogenic activities, being the anthropogenic activities the main causes of emission. Among these, mining operations represent the greatest threat to ecosystem integrity due to the persistence of heavy metals in the environment, which persist for hundreds of years after the cessation of mining operations [1]. In environmental exposures, these toxicants exert their effects trough different mechanisms, being chronic exposures at low doses of complex metal mixtures the responsible for the effects observed in wild animal populations and communities, with implications at the ecosystem level [2]. Therefore, this type of exposure represents a threat to biodiversity.

Exposed individuals integrate exposure to contaminants in their environment and respond in some measurable and predictable way, being these responses observed across different levels of biological organization [3]. Hence, to better understand the ecological consequences of metal exposure, the use of biological markers or biomarkers is necessary. Biomarkers are tools that enable the analysis of the extent of exposure and the effects of environmental chemical contamination [4]. These measures offer valuable predictors of ecologically relevant effects. However, in ecotoxicology, where exposed populations, communities and the consequences at the ecosystem level are the point of interest, the use of biomarkers is not an easy task, since the responses to toxic chemical stress become less specific and many variables interfere with physiological responses. In this context, Bickham and coworkers explain that although the damage from xenobiotic exposure is at the cellular or genetic levels, effects can be observed at higher levels of biological organization (emergent effects) [5]. It is important to take into consideration that the biomarker response must be tightly and regularly connected to responses at these higher levels, particularly if the biomarkers are to be used as effect indicators [6]. Also, biomarkers of exposure (external dose, internal dose; bioaccumulation levels), biomarkers of biological effective dose (DNA adducts) and biomarkers of effect (DNA breaks) must be used to analyze the relationship between the cellular and genetic effects with ecological responses.

At the population level, some considerations must be taken into account when analyzing the emergent effects. Some of these effects are: Shifts in sex proportions, age structure alterations, low reproductive success, inbreeding, genetic structure and diversity alterations, low fitness and population declines [7]. However, these effects are not specific to environmental metal exposures. Hence, differences in biomarker response among populations of a species may be taken carefully by analyzing geographical influences, habitat influence, population vulnerability (specific to the population in question) and exposure history [6]. In the last decade, one of the emergent effects that has been evaluated in environmentally exposed populations are shifts in their genetic pool, which were defined by Mussali-Galante and collaborators as permanent biomarkers [2].

At the community level: Shifts in diversity and species richness, changes in dominant species, changes in species composition and biodiversity loss may be some of the emergent effects. However, due to the complexity of species interactions, such effects cannot be accurately predicted from effects at the population level, as was recognized many years ago [8–10].

Studies assessing community level responses to environmental metal stress are mostly conducted in aquatic ecosystems using invertebrate and fish communities. Among the few studies conducted in terrestrial ecosystems, insect communities are the point of interest [11, 12]. In these type of studies bioaccumulation levels are analyzed in different invertebrate groups and the relationship between bioaccumulation levels and community effects (mainly species richness and composition) is examined.

At the ecosystem level, biomagnification (bioaccumulation within successive trophic levels) has been well documented for some metals. Trophic chain effects have been observed where individuals that feed lower on the food chain generally are exposed to lower metal concentrations. In these type of studies, the primary producers (plants) represent an important step in metal transfer since they constitute the foundation of the food chain. Hence, certain metals can be transported from plants to higher strata of the food chain, representing a threat to biodiversity and to ecosystem integrity [13].

2. Heavy metal effects on terrestrial wild animal populations and communities

Heavy metal (HM) exposure affect the health and survival of the individuals, resulting in negative impacts in the subsequent levels of biological organization, like populations and communities. The first step in ecotoxicological studies is to determine HM concentrations in soil and in the organism in question and then, to analyze the effects at the population level using emergent properties like male/female ratio, age class, reproductive success, inbreeding, genetic diversity and fitness. At the community level, species richness and diversity, dominant species, and species composition [7] are some of the characteristics that have been analyzed. In general, the Shannon-Wiener diversity index is one of the most used parameters in ecotoxicological studies. For a better understanding of the effects of HM on terrestrial ecosystems, the study of the functioning of detritivore soil communities [14–16] has been incorporated, where parameters such as biomass, soil organic matter content, microbial respiration, microbial biomass carbon, and the phosphatase activity have been analyzed.

2.1. Heavy metal accumulation in terrestrial invertebrates

Heavy metals may enter the trophic chain trough primary producers and invertebrates that live in soils [17]. Invertebrate are widely used in ecotoxicological studies due to their easy capture, wide distribution, high abundance, their key ecological roles such as soil decomposers, constitute the first step in trophic chains, low mobility, and are in close contact with soils [18–20]. For example, Gramigni et al. detected a relationship between HM (Zn, Ni, Mn, Cd and Pb) in soils and their bioaccumulation in ants *Crematogaster scutellaris* [21].

In invertebrate communities HM bioaccumulation has been observed in target organs. For example: Wilczek and Babczyńska found that spiders (*P. amentata*, *L. triangularis*, *M. segmentata*, *A. diadematus*, *A. marmoreus*) had higher bioaccumulation levels of Cu, Zn and Cd in their hepatopancreas than in the gonads [22]. Also, Gramigni et al. documented that ants (*Crematogaster scutellaris*) bioaccumulate Mn and Zn in their intestines, being Zn accumulated specifically in Malpighi tubules and low Zn concentrations were find in fat tissue [21]. In contrast, Ni, Pb, and Cd did not bioaccumulate specifically in target organs.

Some of the most invertebrate Phyla used in ecotoxicological studies are Arthropoda and Annelida [18]. Bioaccumulation patterns depend on the species or taxonomic group in question (Class, Order, Family, Genera). For example, Wilczek and Babczyńska studied different spider species (*Pardosa amentata*, *Linyphia triangularis*, *Metellina segmentata*, *Araneus diadematus*, *Araneus marmoreus*) [22]. They found that the spider *P. amentata* bioaccumulates more Zn in the hepatopancreas than other spider species that inhabit in the same polluted site. Also, HM bioaccumulation differences have been observed in different taxonomic classes. It was evidenced that bioaccumulation of Cr, Cu, Ni, Pb and Zn in the order Araneida (Class Arachnida) were higher than those detected in for the order Coleoptera (Class Insecta) [17]. Moreover, they found an effect of HM bioaccumulation depending on the analyzed order, being the order Araneida who presented a positive correlation between Zn bioaccumulation in spiders and Zn concentrations in soils. In contrast, in the order Coleoptera a positive correlation was register between Cd and Pb bioaccumulation and soil concentrations, these differences may be attributed to feeding preferences between both orders (predators and herbivores).

2.2. Heavy metal effects on populations and communities of terrestrial invertebrates

It has been documented that HM bioaccumulation in organisms may modify their body size. For example: Jones and Hopkin studied woodlice populations (*Porcellio scaber*) in polluted environments (Zn, Cd, Pb and Cu) [23]. The author reported that HM bioaccumulation had an effect on head size independently of the gender (male vs. female), being bigger in individuals from contaminated sites. They propose that environmental stressors generate costs in individuals because of the detoxification process, a fact that results in negative effects on their health and survival rates. Hence, HM exposure may have consequences in higher levels of biological organization such as populations and communities.

In ecotoxicology, gradient studies are necessary. They offer the visualization of gradual changes in HM soil concentrations in polluted sites and their relationship with population distribution and abundance and with some community structure parameters. Spurgeon and Hopkin found a negative correlation between the distance from the pollution source and HM concentrations (Pb, Cd, Zn and Cu) [24]. Also, a negative correlation was registered between HM concentration and the absolute abundance of six earthworm species (Phyllum Annelida: *L. rubellus*, L. cataneus, *L. terrestris*, *A. rosea*, *A. caliginosa*, *A. chlorotica*). Particularly, the species *L. rubellus*, *L. castaneus* and *L. terrestris* were present in most sites, whereas the species *A. rosea*, *A. caliginosa* and *A. chlorotica* were absent at the nearest sites to the pollution source. These differences were attributed to differences in calcium metabolism between earthworm species. Also, earthworm communities (*L. terrestres*, *L. rubellus*, *L. castaneus*, *A. chlorotica*, *A. rosea*, *A. caliginosa*, *A. longa*, *E. tetraedra*, *M. minúscula*, *O. cyaneum*) in a polluted site (Pb, Cd, Zn and Cu) were studied [25]. They found that the relative abundance and the Shannon-Weiner diversity index were lower on the nearest sites to the pollution source and higher in the farthest sites from the pollution source. However, the dominance index (Berger-Parker) was higher in sites near the pollution source because *L. rubellus*, *L. castaneus* and *L. terrestres* were the dominant species in those sites. The study by Jung et al. demonstrated that although the Shannon-Wiener diversity index values were similar between contaminated and less contaminated sites (Pb, Cd) for six different spider families, the abundance of the Linyphiidae family was correlated with metal concentrations in soils and enabled the discrimination between contaminated and less contaminated sites [12]. In contrast, others analyzed the community of the Phyla Annelida and Arthopoda, they found that absolute and relative abundances of all the organisms was lower in the most polluted sites [26]. Specifically, the earthworm species *A. caliginosa* had higher densities in non-polluted sites and was absent in polluted sites, on the contrary, the larvae of the coleopteran Hoplinae predominated in highly Zn polluted sites.

Also, there are studies that have shown that HM exposure does not affect some taxonomic groups. For example, Zaitsev and van Straalen studied the mite community (Phyllum Arthropoda) from contaminated soils (Pb, Zn, Cu, Fe, Cd) [19]. They evidenced that although a metal contamination gradient was found in the soils, this gradient was not detected in bioaccumulation of HM in mites, and no effects were found in the community structure. Likewise, Migliorini et al. did not find differences between the abundance of some arthropod groups (Collembola, Protura and Diplura), in contaminated sites by Pb and Sb, while other groups were absent (Symplyla) [20]. Hence, HM exposure affects differently the community structure of different invertebrate groups. Through the study of the functioning of detritivore soil communities [14–16] some parameters like biomass, soil organic matter content, microbial respiration, microbial biomass carbon, and the phosphatase activity are analyzed as biomarkers for HM effects at the community level. Hobbelen and colleagues studied millipedes, isopods, and earthworms in contaminated zones (Zn, Cu, Cd), where no correlation was found between community structure (richness and density) and soil metal content [14]. On the contrary, Zn concentration correlated positively with biomass of the earthworm *Lumbricus rubellus*. On the other hand, the soil organic matter content explained the variation in species density, showing that HM concentration in soils is not the only variable that influences the community structure.

The aforementioned studies evidenced that community structure and function of terrestrial invertebrates, facilitates the evaluation of HM impact on the first trophic chain levels, as well as their incorporation and biomagnification patterns. Therefore, studies assessing HM bioaccumulation in other trophic levels like terrestrial vertebrates complete the knowledge of the effects of HM in the ecosystem health.

2.3. Heavy metal bioaccumulation effects on health of terrestrial vertebrates

In wild vertebrates, information regarding HM bioaccumulation and their effects on target organs is vast. Some examples of wild vertebrate species used in ecotoxicological studies are: Brown bears (*Ursus arctos*), Gray wolfs (*Canis lupus*), Eurasian badgers (*Meles meles*) and Pine martens (*Martes martes*) [27], bank vole (*Clethrionomys glareolus*), yellow-necked mouse (*Apodemus flavicollis*) [28–30], wood mice (*Apodemus sylvaticus*) [31, 32], tuco-tuco (*Ctenomys torquatus*) [33], greater white-toothed (*Crosidura russula*) [34], *Peromyscus melanophrys*, pygmy mice (*Baiomys musculus*) [7, 35].

In general, studies on HM bioaccumulation on wild life, have detected an effect of the study species and the target organ on bioaccumulation patterns. For example, Bilandžić and collaborators analyzed HM bioaccumulation on wild carnivores, the authors report that the highest Cd concentration was present in kidney and liver of the Eurasian badger (*M. meles*) [27]. While Cu concentration in liver decreased among studied species showing the next pattern: Eurasian badger > Brown bear > Pine marten > Eurasian lynx > Gray wolf. The Eurasian badger registered the highest concentrations in muscles (As, Cu, Pb), liver (As, Cd, Cu, Pb) and kidneys (Cd, Pb) and the Pine marten accumulated the highest concentrations in kidneys (As, Cu, Hg). However, in the scientific literature, contrasting results about the effects of HM exposure on the health of the individuals are present. In this context, Levengood and Heske showed that white-footed mice that inhabit in a Cd and Zn polluted site, registered the highest Cd, Cu and Zn concentrations in liver, in comparison to unexposed individuals [36]. In spite of the bioaccumulation levels observed, they did not detect changes in the health of the individuals (reproductive and fitness parameters).

In contrast, other studies have detected histological changes in exposed individuals to HM. Damek-Poprawa and Sawicka-Kapusta found that yellow-necked mouse individuals that live in a polluted site, bioaccumulate more Pb and Cd that unexposed individuals [28]. In particular, individuals bioaccumulate Pb in their femur and Cd in kidneys. Also, histopathological studies showed that exposed individuals presented multiple organ alterations in liver, kidneys and testicles. Similar results were found for wood mice and the greater white-toothed living in landfill zones [31].

Studies assessing the effects of HM bioaccumulation on population and community parameters are scarce, a fact that may be attributed to sampling technique, which is influenced by the size and mobility of the individuals and trapping success (e.g. site perturbance, water availability, predator activity, migration index, etc.), among others. Therefore, in order to infer the population health status, some studies have considered the gender, age, (age class), reproductive condition, litter size (number of embryos, placental scars; embryos/scars per female) embryos weight, trap success, and condition index. In this regard, Santolo documented that male and female individuals of the deer mice exposed to Se from a contaminated site, had a lower condition Index than those from the reference site [37]. In addition, the ratio of males to females age class and reproductive condition were similar between individuals from both sites. Except from individuals from the polluted site that their reproductive condition was lower. This last result suggest that Se exposure affects negatively rodent populations, among other factors.

In addition, in terrestrial vertebrate communities, changes or alterations in community parameters may be due to the competitive selection of the most tolerant species. Moreover, some species may be opportunistic and HM residues may serve a protection mechanism against their predators [38].

Other parameters used as population level biomarkers are: residual index "RI" (linear regression between body weight and body length without tail) and kidney size proportion. RI is used as "energy reserve" measure. Individuals with positive RI values are considered as better fitted individuals, and the increase in kidney relative weight suggest the presence of a stressor [38].

2.4. Heavy metal effects on genetic diversity of exposed populations

Although HM exposure has immediate effects at the molecular and cellular levels, they may extend to higher levels of biological organization, like the genetic structure and diversity of the exposed populations [3]. Chronic exposures at low doses is one of the factor implicated in changes in the genetic pool of the populations, especially if chemical agents are capable of inducing DNA damage, such as HM. In general, there are four mechanisms by which HM exert their effects on the genetic diversity of exposed populations: (1) Some HM are genotoxic, mutagenic and alter DNA repair processes, increasing the mutational load of the individuals; (2) HM exposure favors the presence of tolerant genotypes and the elimination of intolerant ones, changing the genetic composition of the exposed population; (3) HM may cause bottlenecks and (4) alter migration patterns, increasing or decreasing genetic flow between populations [39–41].

Exposed population to HM pollution may have two types of response on genetic diversity levels: (a) increase in genetic diversity levels as a consequence of induced mutations by genotoxins or (b) decrease in genetic diversity levels as a result of bottlenecks [7]. In both cases, these responses are the consequence of the adaptation of the population to polluted environments [3, 41–44].

In general, studies where 11 mammal species were analyzed for the effects of HM exposure (being Cd, Zn, Cu and Pb the most common) on genetic diversity, the pattern found was that 45.4% of the analyzed species displayed a decrease in genetic diversity levels in comparison to non-exposed populations. Some examples are: *Peromyscus melanophrys*: As, Pb, Cd, Cu, Zn [7], *Cognettia sphagnetorum*: Cu [45]; *Talitrus saltator*: Cd, Hg, Cu, [46]; *Pachygrapsus marmoratus*: As, Pb, Cd, Co [47]; *Ficedula hypoleuca*: Cd, Zn, Pb, Cu, Ni, Al, As, Cr, Se [48]. In contrast, an increase in genetic diversity levels in 36.4% of the analyzed species was found, like *Lumbricus rubellus*: Cd, Zn, Cu, Pb [49]; *Cepaea nemoralis*: Cd, Cr, Cu, Ni, Pb, Zn [50]; *Parus major*: Cd, Zn, Cu, Pb, Ni, Al, As, Cr, Sn [48] and *Larus argentatus* (steel), [51]. Meanwhile, the remaining 18.2% of the studied species did not register changes in genetic diversity levels, such as: *Apodemos sylvaticus*: Cd, Zn, Cu, Pb, Ni, Al, Ag, As, Co, Mn, Fe [42] and *Succinea putris*: Cd, Cr, Cu, Ni, Pb, Zn [50].

Changes in genetic diversity levels as a consequence of metal pollution may serve as a biomarker of permanent effects. Mussali-Galante et al. defined permanent biomarkers as changes in genetic structure and diversity due to metal pollution that cannot be the same as they were before the exposure [2].

Finally, polluted environments may be considered as unique systems because their different origin, pollution type and degree are specific for each site. Additionally, edaphic characteristics, weather and vegetation type differ between sites. Hence, it is expected that there will be differences among exposed sites that may or may not alter populations and communities; therefore, the use of bioindicator o sentinel species becomes important. Species that represent biological diversity in terms of feeding preferences, life cycles, trophic chain position, etc. are the point of interest. This last initiative permits to identify susceptible species to environmental stressors such as HM. Basu et al. suggested that sentinel species should have: wide geographical distribution, high abundance, capacity to bioaccumulate HM, easy capture and sampling, low mobility and well known biology [52]. In terrestrial environments, small mammals are commonly used because of their similar physiological systems to humans.

3. Heavy metal effects on ecosystems

Ecosystems are open thermodynamic systems of matter and energy effluxes, which maintain stable from the balance of their biotic and abiotic components [53]. Ecosystem stability may be altered because of the incorporation of HM, derived from mining activities [54].

HM incorporation in the ecosystem depends mainly on their bioavailability and thereafter, through their incorporation into the trophic chain, reaching their highest concentrations in the last levels of the chain, a process known as "biomagnification" [55, 56]. Under this situation, ecosystems may be or may not be affected by HM, depending on the magnitude and exposure time, or if one of the functions that maintain the ecosystem integrity is compromised (e.g. nutrient cycles, energy efflux) due to biodiversity loss [57].

Many ecotoxicological studies that assess HM effects on terrestrial ecosystems have focused on the analysis of HM concentrations in soils, their bioavailability and bioaccumulation, but few have analyzed their biomagnification trough the trophic chain and their effects on ecosystem integrity [54].

3.1. Heavy metal incorporation into the ecosystems

The first step of HM incorporation into the ecosystems is because their bioavailability potential and soil mobility, where metallic cations adhere to negative charged particles like clay and organic matter, when metals separate from these soil particles, they enter the soluble soil fraction, being bioavailable and having the potential to bioaccumulate in different organisms [56]. Microorganisms, plants and invertebrate species have mechanisms to incorporate trace metals for their development and survival (e.g. Cu, Ni, Fe, Co, Mn and Mg), however, these can be toxic in higher concentrations. Also, these same mechanisms facilitate the entrance of non-trace metals (As, Cd, Hg and Pb) in the organisms, which are highly toxic at low concentrations [58].

Microorganism are vital elements of soils, they participate in nutrient and inorganic element recycling, like minerals and trace metals, for plants that constitute the first trophic level in terrestrial ecosystems [59]. However, HM pollution may affect microorganism communities, generating changes in their structure and biodiversity, which in turn, has consequences on the soil processes in which they participate [60, 61]. For example, development alterations and in biochemical processes of microorganism have been reported [61–63]. Such alterations affect organic matter decomposition process, reducing nutrient accumulation and availability for plants and compromising matter and energy fluxes at the base of the trophic chains [61]. On the other hand, soil invertebrates can bioaccumulate HM because of their feeding preferences, like crustaceans, snails, and earthworms that inhabit leaf litter and feed on organic matter with high HM concentrations [64, 65]. In fact, it has been proven that these invertebrate groups had the highest HM concentrations in comparison to beetles or butterflies, or even higher concentrations than some vertebrate groups [18, 64].

3.2. Heavy metal transfer along food chain

In particular, it has been suggested that HM hyperaccumulation by plants as a defense mechanism against herbivores, may transcend to higher trophic levels. For example, the plant *Streptanthus polygaloides* (Brassicaceae) is a Ni hyperaccumulator species, that is consumed by the herbivore *Melanotrichus boydi* (Hemiptera) who bioaccumulates Ni in its body as a defense mechanism against the predator species *Misumena vatia* (Araneae) [66].

HM transfer along the trophic chains varies depending on the type of metal, the trophic level in question and the number and type of species that integrate it. For example, [18] report that

Cd, Cu, Pb and Zn concentrations among invertebrate groups registered the next pattern: Isopoda > Lumbricidae > Coleoptera which is attributed to their different feeding patterns [57]. Another interesting example is that Cd is more mobile towards herbivores and their predators, while Zn is less efficient in its transfer to higher trophic levels [67, 68].

HM transfer along the trophic chain has been reported, for example for the Ni hyperaccumulator plant *Alyssum pintodalsilvae* (Brassicaceae), that transfers Ni to grasshoppers (herbivore) and spiders (carnivorous insect), having the spiders higher Ni concentrations [69]. Similar results were reported by Boyd and Wall [66]. These studies demonstrate that HM can be transferred among invertebrate species, mobilizing metals from one trophic level to another, reaching animals such as small mammals [70–72]. These last studies evaluated HM concentration in small mammals, reporting higher HM levels in carnivorous or omnivorous mammals in comparison to those that feed only by plants. So, HM transfer along the trophic chain not only depends on the magnitude of exposure, but on the species type, season, gender, age and metal type [73].

Additionally, HM bioaccumulation in plants may also affect interactions with their pollinators, since HM can transfer to nectar, a fact that alters pollinators feeding patterns, suggesting that metals and metalloids such as Se found in pollen and nectar affect negatively the pollinators, which results in changes in plant communities due to the nonappearance of pollinators on such plants [74–77].

Most of the studies about metal transfer along trophic chains in terrestrial ecosystems focus on at least three trophic chains levels. In contrast, a study by Hsu and collaborators in more than three trophic levels, they report high levels of Cd, Hg, Pb and Sn and their biomagnification in all analyzed levels, being the snails and the earthworms the groups who registered the highest metal concentrations [64].

All the aforementioned studies have evaluated HM transfer in small trophic chains in terrestrial ecosystems, the majority of them analyze three trophic levels. These studies are of great importance because the information generated helps to understand the general patterns of HM transfer along trophic chains, especially for the most common metals like Cd, Cu, Pb and Zn. Also, these studies highlight that HM transfer, assimilation and excretion in organisms along the trophic chain, can have extended effects, at the individual level (altering their health and physiology) at the population level (modifying population dynamics, abundance, distribution and their genetic pool) at the community level (altering species richness and diversity), affecting then, the ecosystem dynamics. Therefore, is very important to conduct studies where more than three trophic chain levels are analyzed and to integrate new biomarkers (e.g. stable isotope techniques; which enable to follow HM transfer along trophic chains by knowing the extent of the pollutant flux in the chain [78]).

4. Conclusions and perspectives

Chronic environmental metal exposures exert their negative effects on individuals health, having consequences at the population and community levels, putting ecosystem integrity at risk. However, the recognition and use of biomarkers in ecotoxicology has been a difficult task, due to the unspecific responses and multiple variables that affect physiological responses to toxic stress. Therefore, it becomes necessary that ecotoxicological studies include: HM concentrations in soils, bioaccumulation parameters in vertebrate and invertebrate species, the relationship between these biomarkers with morphological, anatomic and physiological alterations that may alter population parameters. In particular, the use of bioindicator or sentinel species is necessary in order to evidence the consequences of HM exposure in wild populations.

Terrestrial invertebrates have been used as an ideal system to evaluate community responses to environmental chemical stress, due their easy capture, wide distribution, great abundance, low mobility and close contact to HM from soils. Especially, earthworms and arthropods are the most studied organisms. On the contrary, the studies that evaluate HM effects on vertebrate community structure are scarce, probably due to their body size, mobility and sampling difficulties. However, when working with vertebrates, an excellent alternative has been the study of small mammal species that serve as good bioindicators and the results may be easily compared to humans. Also, a methodological strategy in many studies has been the use of pollution gradients in order to visualize slight changes in HM concentrations along the soil gradient and to relate these changes to some community structure parameters. At the moment, we can conclude that HM affect differently the community structure and the community functioning of the different animal groups studied so far.

At the community level, the search for new biomarkers continues. In this context, abundance changes in different guilds that conform the community may also be used as a biomarker since changes in abundance or guild disappearance in exposed communities may serve as an ecological response to chemical stress.

At the ecosystem level, ecotoxicological studies are very limited. Trophic chain alterations, biomagnification and modifications in nutrient and energy cycles have been reported. Studies generally asses HM transfer along three trophic levels, such studies have concluded that metal flux depends on the biology of the species, on the trophic position in the chain and on the metal type or metal mixture in question. Mainly, HM transfer from plants to invertebrate herbivores (insects) and from insects to other invertebrates (spiders) or predator vertebrates (small mammals) has been the point of interest. The information from these studies has gained attention, especially because human beings represent the last level of the trophic chain, such as in the case of agroecosystems. It is desirable to use as biomarkers in ecosystem studies, measures of stable carbon and nitrogen isotopes for evaluation of HM transfer along terrestrial trophic chains.

Finally, it necessary that future efforts integrate different biological and ecological responses across all levels of biological organization as a result of biomarker approaches. Moreover, study designs should be more rigorous, including multispecies and multibiomarkers that permit the evaluation of HM exposure in a more realistic way, which in turn will allow to predict, understand and resolve in a better way HM pollution problems worldwide.

Acknowledgements

We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the scholarships to IHP and to MSM.

Conflict of interest

The authors declare that there is no conflict of interest.

Author details

Efraín Tovar-Sánchez[1], Isela Hernández-Plata[2], Miguel Santoyo Martínez[1], Leticia Valencia-Cuevas[1] and Patricia Mussali Galante[2]*

*Address all correspondence to: patricia.mussali@uaem.mx

1 Laboratory of Molecular Markers, Center for Research on Biodiversity and Conservation, Autonomous University of Morelos State, Cuernavaca, Morelos, México

2 Laboratory of Environmental Research, Center for Biotechnology Research, Autonomous University of Morelos State, Cuernavaca, Morelos, México

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