

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



The Use of Biosensors for Biomonitoring Environmental Metal Pollution

*Efraín Tovar-Sánchez, Ramón Suarez-Rodríguez,
Augusto Ramírez-Trujillo, Leticia Valencia-Cuevas,
Isela Hernández-Plata and Patricia Mussali-Galante*

Abstract

The use of biosensors for biomonitoring environmental health has gained much attention in the last decades. The environment is continuously loaded with xenobiotics released by anthropogenic activities that pollute ecosystems, putting their integrity at risk. Therefore, there is an urgent need to study the negative effects of xenobiotics, specifically chemical agents. Biosensors or organisms that integrate exposure to pollutants in their environment and which respond in some measurable and predictable way are useful tools to study the extent of chemical pollution and its consequences across levels of biological organization. Among chemical pollutants, heavy metals are among the most toxic elements to nearly all living organisms. Wildlife is chronically exposed to complex metal mixtures in which effects on ecosystem health are difficult to assess. Therefore, different organisms may serve as biosensors to estimate detrimental effects of metal pollution. In this chapter, we will analyze bacteria, small mammals, some plant species, and lichens as biosensors for environmental metal pollution. Also, we will assess the importance of using different biomarkers on biosensors.

Keywords: heavy metals, environmental health, bacteria, small mammals, plants, lichens

1. Introduction

Human activity generates increasing amounts of new compounds that are released into the environment without prior knowledge of their potential toxicity or impact in living organisms. Heavy metals (HM) and metalloid such as arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), and aluminum (Al) are major environmental pollutants, particularly in industrial areas. Heavy metals are generated as a result of anthropogenic activities such as metal-working industries, cement factories, mining industry, smelting plants, refineries, and traffic and heating systems [1]. HM and their ions are ubiquitous and by definition are metals having atomic weights between 63.5 and 200.6 g mol⁻¹ and specific gravity greater than 5 g cm⁻³ [2]. Living organisms require small doses of some essential HM, including cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (V),

strontium (Sr), and zinc (Zn). However, in the case of essential metals and very toxic metals, excessive levels and, respectively, even small doses influence both the ecosystem and human health [3]. Nonessential HM which affect the surface water systems are Cd, chromium (Cr), Hg, Pb, As, and antimony (Sb).

Some metals have been classified as toxic, persistent, and accumulative elements. According to the Agency for Toxic Substances and Disease Registry [4], among the 10 most hazardous substances to human health, four are toxic metals: Pb, Hg, As, and Cd. Interestingly, Pb and Hg rank first among the most harmful metals to plants, followed by Cu, Cd/As, Co/Ni, and Mn [5]. Pb and Hg have been reported as mutagenic agents in plants [6]. Due to its chemical similarity to phosphorous, arsenic may interfere with several physiological and biochemical processes [6]. Cd does not appear to have any physiological function, with the exception of the marine diatom *Thalassiosira weissflogii* that possesses a carbonic anhydrase with Cd as its metal center [7]. Cu, Ni, Co, Zn, and Mn are all plant micronutrients. They participate in prosthetic groups and as cofactors of many proteins and are therefore essential for growth and development and, however, at high concentrations cause oxidative stress [8]. Al is another toxic element with significant implications for agriculture, because 30% of the world's land areas consist of acid soils [9].

Exposure to toxic metals can result in inhibition of seed germination, photosynthesis, and plant growth and consequently causes yield losses. These symptoms are normally related with overproduction or reactive oxygen species (ROS), changes in the permeability and structure of cell membranes, imbalance of mineral nutrients, incorporation of the metal into S-containing molecules, and cell cycle disruption [10]. Also, environmental metal exposure can affect all levels of biological organization. HM bioaccumulation in plants, lichens, small mammals, and bacteria might respond to this chemical stress on the molecular, cellular, or morphological scale and, at population, community and even ecosystem levels [11]. These different types of responses to toxic stress induced by HM (biomarkers) offer a powerful tool for documenting the extent of exposure and the effects of environmental metal contamination [12], revealing the potential use of plant, lichens, small mammals, and bacterial species as biosensors.

2. The use of plants as biosensors of heavy metal pollution

The use of plants as biosensors has a long history. For decades, they were used as a part of ecological risk assessment of agricultural and industrial chemicals, solid wastes, food additives, and chemically and radioactively polluted soil and water. However, the use of plants as environmental biomonitors has some drawbacks. Despite being higher eukaryotes, plants have completely different mechanisms of uptake, distribution, storage, compartmentalization, and metabolism of various pollutants [13].

2.1 Plants as biosensors

Vegetal species have the ability to absorb metals, particularly those essential for their development and growth through their root system from the soil, water, and overground vegetative organs from the atmosphere [14]. Also, these chemicals may be transported, transformed, stored, and accumulated in different cells and plant tissues. Genotoxicity or DNA damage is an early effect biomarker at the molecular level that has been used in several plant species exposed to HM. Metal binding to the cell nucleus causes damage including DNA base modifications, inter- and intramolecular cross-linkage of DNA and proteins, DNA strand breaks, rearrangements,

and de-purination [15]. The use of DNA strand breaks as biomarker of genotoxicity is common in plants, for example, *Bacopa monnieri* (Plantaginaceae) exposed to Cd [16]; *Nicotiana tabacum* and *Solanum tuberosum* (Asteraceae) exposed to Cd, Cu, Pb, and Zn [17]; *Pisum sativum* (Fabaceae) exposed to Cr (VI) [18]; *Lycopersicon esculentum* (Solanaceae) and *Cucumis sativus* (Cucurbitaceae) exposed to Cu [19]; and *Prosopis laevigata* (Fabaceae [20]), *Vachellia farnesiana* (Fabaceae [21]), *Pithecellobium dulce* [22], and *Wigandia urens* exposed to Pb, Cu, and Zn [23]. These alterations at the DNA level can trigger changes at the biochemical level that lead to diverse effects at the cellular, physiological, or morphological level, as early effects of exposure to HM in plants [18, 24].

Among the responses at the cellular level that occur in plants as a result of exposure to HM, we can mention oxidative damage, the production of chelating agents, and alterations in cell division [25]. In the case of oxidative damage, reactive oxygen species (ROS) production indirectly influences the production of antioxidant enzymes [26]. For example, the hydrogen peroxide (H₂O₂) acts as a signaling molecule in response to HM and other stresses [27]. Under a pollution scenario by HM, H₂O₂ levels increase in response to Cu and Cd treatment as it has been reported in *Arabidopsis thaliana* [28], in Hg exposure in tomato (*Lycopersicon esculentum* [29]), and in response to Mn toxicity in barley (*Hordeum vulgare* [29]). As a consequence of oxidative stress, the plants experience cellular damage and accumulate metal ions that disturb cellular ionic homeostasis [30]. To minimize these detrimental effects, plants have evolved detoxification mechanisms based on chelation and subcellular compartmentalization. Chelation of HM is a detoxification strategy and the best characterized classes of HM chelator in plants are phytochelatins (PCs) and metallothioneins (MTs) [25, 30, 31].

PCs are capable of chelating HM, thereby reducing the concentration of cytotoxic free metal ions [32]. In particular, the synthesis of this type of chelant proteins is quickly active with the presence of Cd, Cu, Zn, Ag, Au, Hg, and Pb [33]. For example, Cd is not essential for plant growth, but it is readily taken up by many plant species. Higher plants react to excess Cd by stimulating sulfate absorption [34] and production of PCs involved in Cd chelation and transport into vacuoles [35, 36]. Increased availability of Cd for root uptake may cause considerable alterations in mineral nutrition [37, 38], lipid biosynthesis [39], photosynthetic rate [40], and nitrogen metabolism [41] in plants. Consequently, this led to a severe growth inhibition [42, 43] and finally death [44]. PC induction has been reported in copper-tolerant plants of *Mimulus guttatus* (Phrymaceae [45]), *Brassica juncea* (Brassicaceae) following the intracellular accumulation of Cd [46], *Rauwolfia serpentina* (Apocynaceae), *Arabidopsis thaliana* (Brassicaceae), and *Silene vulgaris* (Caryophyllaceae) exposed to As [47] and *Lotus japonicus* (Fabaceae) exposed to Cd [48].

Metallothioneins (MTs) are proteins that play a key role in the binding and transport of various metals [49]. The structure of these highly conserved proteins is linked to their role in the homeostasis of essential metals such as Zn and Cu and detoxification of toxic elements such as Cd and Hg [50]. In wheat (*Triticum*) and in rice (*Oryza sativa*), MTs are induced by metal ions, such as Cu and Cd; in *A. thaliana* MT gene expression is activated in response to Cu and Cd [51]. MTs bind metal ions in *Cicer arietinum* (Fabaceae), *Quercus suber* (Fagaceae), and *Triticum aestivum* (Poaceae) exposed to Zn and Cd [52, 53].

Also, HM mixtures affect cell division [54]. In general, Pb, Cd, Fe, and Zn reduce the synthesis of the cell wall components, causing damage to the Golgi apparatus and other cell organelles. The inhibition of mitosis is also limited by links between HM and cell wall pectin, which becomes more rigid and limits both the expansion and size of the intracellular space [55]. For example, Lerda found that Pb

reduces the frequency of mitotic cells and increases the frequency of aberrant cells in onion (*Allium cepa*) [56]. Also, in corn plants (*Zea mays*), Eun and colleagues found that HM intervene in cellular division affecting the microtubules, which produces weakness in the cellular structure and the formation of binucleate cells in metaphase [57]. The aforementioned effects have been related mainly with the Pb and their synergies with other metals such as Al and Cu.

Likewise, HM interfere with ionic homeostasis and enzyme activity, resulting in physiological alterations which involve single organs (such as nutrient uptake by the roots) followed by more general processes such as germination, growth, photosynthesis, plant water balance, primary metabolism, and reproduction [30, 58]. Indeed, visible symptoms of heavy metal toxicity include chlorosis, leaf rolling and necrosis, senescence, wilting and stunted growth, low biomass production, limited numbers of seeds, and eventually death [58]. For example, reduction in morphological attributes of height, coverage, or biomass derived from exposure to HM has been reported in *Arundo donax* (Poaceae), exposed to As, Cd, and Pb [59]; *Zea mays* (Graminaceae) exposed to Cd, Fe, Ni, and Zn [60]; *Prosopis laevigata* (Fabaceae [61]), *Pithecellobium dulce* (Fabaceae [22]), *Vachellia farnesiana* (Fabaceae [21]), and *Wigandia urens* [23] exposed to Cu, Pb, and Zn.

Although the immediate effects of exposure to metals occur at the molecular and cellular levels, they can be extended to higher levels of biological organization: populations, communities, and ecosystems.

In the last decade, one of the emergent effects at the population level that has been evaluated in environmentally exposed populations to HM is shifts in their genetic pool, which were defined by Mussali-Galante and collaborators as permanent biomarkers [12]. Particularly, populations can undergo changes in their diversity and genetic structure [62] in two ways: (1) increased genetic variation as a result of mutations induced by genotoxic agents or (2) decreased genetic variability as a result of bottlenecks or selection [12]. In fact, these changes in the genetic reservoir of populations exposed to HM have been proposed as an indicator of ecosystem health [12]. In general, plant species in which alterations on its genetic diversity have been reported as a consequence of HM exposure include *Taraxacum officinale* (Asteraceae [63]), *Silene paradoxa* (Caryophyllaceae [64]), *Thlaspi caerulescens* (Brassicaceae [65]), *Pinus sylvestris* (Pinaceae [66]), *Thlaspi caerulescens* (Brassicaceae [67]), *Cistus ladanifer* (Cistaceae [68]), *Arabidopsis halleri* (Brassicaceae [69]), and *Prosopis laevigata* (Fabaceae [70]).

HM exposure may have consequences in higher levels of biological organization such as plant communities; however, few studies have examined these effects [71–73]. Shifts in diversity and species richness, changes in dominant species, changes in species composition, and biodiversity loss may be some of the emergent effects. For example, in a study carried out by Martínez-Becerril, it was documented that the vegetal community (trees, shrubs, and herbaceous) associated to mine tailings differed in its species composition compared to reference sites. Likewise, a significant reduction in species richness and diversity was documented [74]. Similarly, a reduction in plant species diversity in grassland contaminated by Cu and Cd [75] and a reduction in vegetal abundance with the increase of HM soil concentration in a metallophyte plant community [76] has been reported using plant communities as the point of interest.

Additionally, HM bioaccumulation in plants may also affect its interactions with other species. It has been suggested that metal accumulation by plants may be a defense strategy to discourage consumption by herbivores [77]. This defensive role of HM has been reported, for example, in the hyperaccumulator *Stanleya pinnata* (Brassicaceae) against black-tailed prairie dog herbivory in seleniferous habitats [78, 79]. Similarly, some species of herbivores have evolved to use metals

bioaccumulated in the ingested plant biomass as a defense against subsequent predation [80]. Even, metals can also affect plant-pollinator interactions as reported in *Impatiens capensis* (Balsaminaceae) in sites contaminated with Al and Ni [81].

At the ecosystem level, bioaccumulation within successive trophic levels (biomagnification) has been well documented for some metals. Under this scenario, the plants (primary producers) represent an important step in metal transfer since they constitute the base of the food chain. Therefore, certain metals can be transported from plants to higher levels of the food chain, representing a threat to biodiversity and to ecosystem integrity [82]. For example, HM transfer along the trophic chain has been reported for the Ni hyperaccumulator plant *Alyssum pintodalsilvae* (Brassicaceae) that transfers Ni to grasshoppers (herbivore) and spiders (carnivorous insect), the spiders having higher Ni concentrations [71]. Boyd and Wall found similar results suggesting that Ni could be passed from herbivorous to carnivorous insects [83]. Even, it has been documented that the transfer of HM reaches animals such as small mammals, reporting higher HM levels in carnivorous or omnivorous mammals in comparison to those that feed only by plants [84–86]. This process has also been reported in plants not considered hyperaccumulators. For example, Notten and collaborators report that *Urtica dioica* (Urticaceae) that is distributed in areas with elevated metal concentrations contained only very low metal concentrations [87]. However, the snail *Cepaea nemoralis*, which is the main herbivore feeding on these plants, did contain metal concentrations that were much higher than background values [87, 88]. These studies demonstrate that HM can be transferred among invertebrate species, mobilizing metals from one trophic level to another [84–86]. Finally, these studies evidenced the importance of vegetal species for the evaluation of HM impact on the trophic chain levels, as well as their incorporation and biomagnification patterns.

2.2 Lichens as biosensors

Lichens may be considered as one of the most commonly applied organisms as biosensors [89]. They are symbiotic organisms of fungi and algae and have been widely used in biomonitoring of air pollution [90]. Some of the most commonly used lichen species for toxic metals biomonitoring are *Parmelia sulcata*, *P. caperata*, *Hypogymnia physodes*, and *Xanthoria parietina* [89, 91]. Lacking a protective cuticle and roots, lichens absorb and retain nutrients and trace elements, including HM from dry and wet atmospheric deposition that exceed their physiological requirements. They tolerate these high concentrations by sequestering elements extracellularly as oxalate crystals or lichen acid complexes [92, 93].

A plethora of metal toxicity symptoms, including loss of cell membrane integrity, potassium leakage, disruption of ultrastructure, chlorophyll degradation, and oxidative stress, have been reported in lichens [94–96]. However, their expression depends on the metal and lichen species involved [97, 98]. For example, it is widely known that Cu causes cell membrane damage and adversely influences the photosynthetic apparatus of lichens [99] and also affects fungal and algal ultrastructure [100]. Also GSH (reduced glutathione), the precursor of PCs, is the principal low-molecular thiol and nonenzymatic antioxidant in lichens [101]. It plays a critical role in cellular defense against oxidative damage caused also by HM. For example, in a laboratory study, Pawlik-Skowronska and collaborators found that Cd, Pb, and Zn induced biosynthesis of PCs in the widespread epiphytic lichens *Xanthoria parietina*, *Physconia grisea*, and *Physcia adscendens* [102].

Toxic metal effects reported on lichens include discolored or pinkish necrotic patches and an absence of growth [103]. However, there are tolerant species, some normally associated with HM. For example, *Stereocaulon* species colonized polluted

road-site during the period of high Pb emissions [104], and *Vezdaea leprosa* occurs alongside motorway crash barriers in Germany and the UK [105]. So, characteristic lichen assemblages occur on metalliferous soils polluted by industrial emissions and on abandoned mine wastes [105–107] or lichen communities growing on trees [108]. Hence, bark and soil HM contents play a major influence in determining the composition of epiphytic lichen floras [108–110]. Several studies have evidenced the metal influences on epiphytic lichen abundance, cover, richness, and species diversity. For example, in some studies in coniferous forests, correlations between epiphytic lichen abundance and Mn supply were detected [98, 111]. Specifically, a decreasing cover value of the foliose lichen *Hypogymnia physodes* with increasing Mn concentrations in bark or stemflow were repeatedly found in stands of *Picea abies*. In another example, the lichen community associated to *Vachellia farnesiana*, *Prosopis laevigata*, and *Pithecellobium dulce* in exposed sites to HM (Pb, Cu, and Zn) showed higher richness and species diversity values as compared with a reference site [108]. In conclusion, lichens have proved to be very effective organisms as biosensors to detect HM in the environment.

2.3 Transgenic plants as biosensors

The alternative use of transgenic plants in horticulture, forestry, and construction seems to be more appealing for the public. In this respect, design and production of transgenic plants for environmental biomonitoring and cleaning up polluted areas can be action for more favorable public perception of genetically modified organisms [13].

Recently, substantial progress in generation and exploitation of transgenic plants as biomonitors has been made [112, 113]. One of the important advantages of transgenic biosensors is the ability to customize the assay in accordance with monitoring needs. This not only makes transgenic biosensors more sensitive to a particular pollutant but also allows for easy scoring.

Classically, a major approach to addressing these issues has been based on selective breeding or genetic engineering of plants in order to increase their baseline hardiness and/or ability to efficiently utilize resources [114]. Concurrent with these approaches have been efforts to develop and apply technologies toward monitoring and understanding the physiological responses of plants to stress [115–117]. This second, more recent approach is based on leveraging the finely tuned and highly sensitive mechanism plants which have developed to sense, to respond, and to adapt to changes in the environment [118].

Appreciation of this internal decision-making process in plants has led to the development of methods to monitor relevant natural physical phenomena, such as changes in the chlorophyll fluorescence spectra [115, 116]. Another route has been the direct engineering of plants to act as “vital reporters” both of their own health and of internal decision-making processes (so-called biosensors). By coupling knowledge of the genetic cascade stress responses with reporter proteins [e.g., beta-glucuronidase (GUS), luciferase (LUC), or fluorescent proteins (FP)], it is possible to visualize genetic events linked to/associated with stress responses [115, 119]. Indeed, prior research has demonstrated that both endogenous and synthetic promoters can be used as “biomarkers” for a variety of stress conditions, with the appropriate choice of promoter depending on a number of factors, including ease of interpretation, signal-to-noise ratio, and the timeliness of data acquisition [115, 120]. Thus, biomarkers have the potential of informing the researcher, in real time, of the magnitude of a wide variety of physiological events. In particular, the use of FPs has distinct advantages; namely, FP outputs are observable using widely available equipment (e.g., a fluorescent microscope) and require no exogenous

additives [118]. With the increasing availability of portable meters for measuring fluoresce [121], it is now feasible to transit this technology from the lab to the greenhouses and the fields.

3. The use of small mammals as biosensors of heavy metal pollution

3.1 Small mammals as biosensors

Small mammals (SM) are frequently used to monitor environmental contamination with HM such as Pb, Cd, Cr, Zn, Al, silver (Ag), As, Co, Cu, Fe, Mn, magnesium (Mg), nickel (Ni), Hg, selenium (Se), strontium (Sr), and Mo. These animals have been used mainly because they are found in the intermediate positions of trophic chains and they are small, have diverse diets, are relatively easy to capture, and have wide geographic distribution (which allows to compare between populations of contaminated and non-contaminated sites). The liver, kidneys, bone, muscle, brain, testicles, teeth, and blood are the main target organs for HM. Conducting studies with SM is important because they allow to make inferences about the bioavailability and bioaccumulation of HM, the biotransformation mechanisms of HM among different species, and the sources of exposure associated with the diet; they also allow to determine which species are susceptible to HM, which is an important step for the evaluation of the biomagnification of HM. Most of the monitoring studies of HM use SM belonging to two orders: Soricomorpha (shrews and moles) and Rodentia (squirrels, rats, mice, voles). The present chapter will focus on two species of the order Rodentia that belong to the families Muridae and Cricetidae. The life history characteristics of both species are described below.

Family Muridae: *Apodemus sylvaticus*; common names include long-tailed field mouse, small wood mouse, and wood mouse. Its conservation status is a minor concern [122]. It has 32 subspecies. Its geographical distribution includes Europe (with the exception of Finland and northern Russia) and some regions of North Africa. It is found at altitudes up to 3300 m.a.s.l. and has been recorded in a variety of seminatural habitats that include forests, moors, steppes, arid Mediterranean scrub, and sand dunes. It is also found in artificial habitats such as suburban and urban parks, gardens, vacant lots, pastures, crops, fields, and forest plantations. It is an omnivorous species that feeds at ground level; its diet includes plants/seeds (70–80%) and invertebrates (20%). It eats tree seeds, fleshy fruits, mushrooms, flowers, and aerial parts of plants. It also consumes fern leaves (*Culcita macrocarpa*) and oak acorns (*Quercus*) [123]. It has also been reported to eat worms, which could be an important source of HM for this species [124]. It is predated by snakes (*Hemorrhois hippocrepis*), eagle-owls (*Bubo bubo*), barn owls (*Tyto alba*), and foxes (*Vulpes vulpes*), among others. It is nocturnal and lives in galleries dug at shallow depths, in crevices, or tree holes. The home range of males is larger than that of females, and it becomes larger for males during the reproductive period. The home range of males can be up to 1.44 ha and 0.49 ha for females [123]. However, some studies estimate that the home range of the males of this species can be up to 2500 m² and that its activity range is 56.4 m [125]. The males are polygamous, and, during the breeding season, they travel long distances in search of reproductive partners. There are reports of attacks against intruders and subordinate males, which are thus expelled from the territory and which are displaced from the territories. Unlike males, females have exclusive territories. Their fertility rate is 1–7 l/year, each with an average of 5 pups. The maximum recorded life span in the wild is 12 months [123].

Family Cricetidae: taxonomic name, *Myodes glareolus*; its synonym is *Clethrionomys glareolus*. The common name is bank vole. Its conservation status is a

minor concern [126]. A total of 30 subspecies have been reported. It has a wide geographical distribution that includes the British islands, Europe, and Russia. To the north, it can be found beyond the Arctic Circle; to the south, they are found in northern Turkey and Kazakhstan. This species is not found in southern Iberia and the Mediterranean islands. It inhabits altitudes of 2400 m, including open forests, bushes, and hedges [126]. Bank voles are mainly herbivorous, consuming fleshy fruits, seeds, tender leaves, mushrooms, moss, flowers, and roots. They gnaw the bark of young trees and feed on the cambium, but they can also consume earthworms. It is predated by raptors such as the tawny owl (*Strix aluco*), the barn owl (*Tyto alba*), as well as small and medium carnivores [127]. Its home range is estimated to be up to 1000 m², and its activity ranges up to 35.7 m [125]. During breeding seasons, the males cover large areas that include the territories of several females. The females have exclusive territories. The mating system is polygynous. The females have 3 or 4 l/reproductive period, with an average of 4 or 5 pups/l. Gestation lasts between 18 and 22 days; the lactation period lasts approximately 18 days. The average life span is between 12 and 13 months, but under extreme conditions they can live for 3 months [127].

3.2 *Apodemus sylvaticus* as a biosensor

There are studies that show that *Apodemus sylvaticus* populations inhabiting contaminated areas bioaccumulate metalloids and HM. For example, Erry and collaborators quantified the concentration of As in *A. sylvaticus* and *C. glareolus* in five sites contaminated with As. These authors found that the organisms of both species accumulate similar concentrations of As in contaminated sites. The concentration of As in the liver and kidneys of the animals inhabiting the contaminated sites was higher than those of animals inhabiting the control site. The concentration of As in those organs was associated with the concentration of As in the stomach contents. Thus, the authors suggest that the animals were exposed to As through the diet and that the two species of mice bioaccumulate As in various organs [128]. Sánchez-Chardi and collaborators compared a population *A. sylvaticus* population inhabiting a control (non-contaminated) site with a population inhabiting a site contaminated by leachates containing potentially toxic elements. They found that the mice inhabiting the leachate site bioaccumulated Cd, Fe, Zn, Cu, Mn, Mo, and Cr, compared with the animals inhabiting the control site. The mice in the leachate site also showed low weight index and a high relative weight of the kidney, as well as high plasma values of glutamic pyruvic transaminase (GPT), an indicator of liver damage. They also showed greater genotoxicity than the animals of the control site. The authors suggest that the morphological and physiological changes observed in the population of *A. sylvaticus* inhabiting the leachate site indicate that this species is more sensitive than *Crocidura russula*, the other studied species inhabiting the site, and that the leachates affected the health of *A. sylvaticus* [129].

The comparison between *A. sylvaticus* and species of the order Soricomorpha, particularly shrews, showed that *A. sylvaticus* is more sensitive to renal toxicity caused by exposure to HM than *C. russula*. Nevertheless, shrews can bioaccumulate more HM. Sánchez-Chardi and collaborators compared populations of *A. sylvaticus* and *C. russula* inhabiting a non-contaminated site (control) with populations of the same species inhabiting a site contaminated by leachates containing potentially toxic elements. In both species inhabiting the contaminated the site, the histological analysis of the liver showed signs of necrosis and apoptosis, inflammation of preneoplastic nodules, and vacuolization. The kidneys were altered mainly in *A. sylvaticus* (necrosis and tubular inflammation), which suggested that this species is more sensitive to renal toxicity than *C. russula* [130]. However, some authors

mention that shrews can bioaccumulate more metals and metalloids than *A. sylvaticus*. Mertens and collaborators found that, in contaminated sites (a dredged material deposit), the shrew *Sorex araneus* bioaccumulates more Cd than *A. sylvaticus* and *C. glareolus*. This could be explained by the dietary habits of the studied species, since the diet of *Sorex araneus* consists of invertebrates, including insects and molluscs, while *A. sylvaticus* and *C. glareolus* are mainly herbivorous [131].

Studies by Wijnhoven and collaborators on floodplain species (*A. sylvaticus*, *C. glareolus*, *C. russula*, *M. agrestis*, *M. arvalis*, *M. minutus*, *S. araneus*) found that two species of shrew had higher concentrations of HM compared to the other species; the highest concentrations were found in the shrew *S. araneus*, which has insectivorous and carnivorous habits. Only Cu concentrations were higher in *C. glareolus* than in *A. sylvaticus* and *M. agrestis*. The differences in the concentrations of HM may be due to variations in exposure time (age of the individual), the heterogeneity of the concentrations of HM in soil, the movement of the animals to the other sites, and their feeding patterns. The accumulation of HM in the studied species could also be a risk factor for their predators, potentially altering the structure of their communities and the dynamics of the ecosystem [86].

Cooke and collaborators studied three mammalian species, *A. sylvaticus*, *M. agrestis*, and *S. araneus*, associated with a site contaminated with Pb, Cd, and F. The total accumulation levels of these three compounds in the studied species had the following order: *S. araneus* > *M. agrestis* > *A. sylvaticus*. The stomach contents of *S. araneus* showed that it had the highest intake of Pb, F, and Cd [132]. The differences in bioaccumulation are due to differences in daily intake, in the efficiency of digestion and assimilation, and to other physiological, biochemical, and behavioral factors. Similarly, Drouhot and collaborators found that *Crocidura russula* accumulated more As than *A. sylvaticus*, *Mus spretus*, and *Microtus arvalis*. They also mention that the differences in the accumulation of As between species and within the same species are due to variations in diet, foraging behavior, differences in metabolism, amount of ingested soil, and mobility of the organisms [133].

Some authors have used *A. sylvaticus* in distance gradient studies of contaminated areas. Scheirs and collaborators studied the concentration of metals (Cd, Co, Cr, Cu, Fe, Mn, Pb, and Zn) in soil and the genotoxicity found in *A. sylvaticus* along a distance gradient. The authors reported that the concentration of HM and the genetic damage found in *A. sylvaticus* was higher near the most contaminated areas [134]. Rogival and collaborators studied the accumulation of As, Cd, Cu, and Pb and Zn in *A. sylvaticus* mice inhabiting five sites along a distance gradient, in the soil of the sites, and in the mice's diet (acorns and two species of earthworms: *Dendrodrilus rubidus* and *Lumbricus rubellus*). They observed a gradient in the exposure to metals, beginning on the foundry (most contaminated site), in all the studied elements (soil, diet, and rodent), but not for the essential metals analyzed (Cu and Zn). The concentrations of As, Cd, and Pb in acorns were higher in the sites closest to the foundry. In earthworms, the concentrations of the five metals were higher near the foundry. The transfer of metals occurred mainly from the diet to the mice in the case of Pb and Cd [124]. Another study conducted by Tête and collaborators found that the concentrations of Pb in the liver and kidneys of *A. sylvaticus* followed a distance gradient from the contamination source (foundry). In contrast, the concentrations of Cd in the liver and kidneys of mice varied along the contamination gradient, forming a bell curve. Unlike the results of bioaccumulation, renal alterations (necrosis, lymphocyte infiltration) did not show an increase associated with a distance gradient. The results showed that *A. sylvaticus* is chronically exposed to Pb and Cd and that there is kidney damage present in the species [135].

3.3 *Myodes glareolus* as biosensor

Myodes glareolus, or *Clethrionomys glareolus*, has been used mainly in studies of bioaccumulation of metals and metalloids. Wijnhoven and collaborators analyzed several species of small mammals living in a contaminated floodplain. They found that, in almost 40% of the population of *C. glareolus*, the concentration of Cd exceeded the lowest level at which adverse effects are produced. The other two species, *Microtus agrestis* and *M. arvalis*, showed less ecotoxicological effects [136]. Topashka-Ancheva and collaborators evaluated other small mammals: *A. flavicollis*, *M. macedonicus*, *C. glareolus*, *P. subterraneus*, *M. arvalis*, *M. rossiaemeridionales*, and *C. nivalis*. They found that *C. glareolus* had a higher concentration of Cu and Cd in the body compared to the other species. The concentrations of Cu, Zn, Pb, and Cd in *C. glareolus* were significantly higher than in *A. flavicollis* in both the whole body and in the liver (except for Pb in the liver, which was higher in *A. flavicollis*). The authors suggest that the differences between species are due to the position of each species in the trophic chain, their diet, and lifestyle [137]. Damek-Poprawa and Sawicka-Kapusta compared the populations of a control site and two contaminated sites close to a steel and zinc foundry. No damage was found in *C. glareolus* inhabiting the control site, but there were histopathological changes in the kidneys and liver of the rodents inhabiting the contaminated sites. The concentration of Pb and Cd in liver, kidney, and femur tissues was higher in the rodents living in contaminated areas [138]. Erry and collaborators studied populations of *A. sylvaticus* and *C. glareolus* in a site contaminated with As and in a control site. Many species of rodents living in the contaminated site accumulated more As in the spleen, lung, muscle, and femur than those living in the control site. The concentrations of As in the liver, femur, and hair were higher in *A. sylvaticus* than in *C. glareolus* in both the contaminated and the control sites. The authors mention that these results could be due to the high water exchange and urinary excretion of *C. glareolus* compared to *A. sylvaticus*, which could make *C. glareolus* susceptible to renal toxicity [139].

As shown in the studies on *A. sylvaticus* and *C. glareolus*, the differences between both species are a function of diet, metabolism, mobility, and lifestyle; thus, the monitoring of environmental contamination with metals and metalloids should use small mammals belonging to different taxa in order to determine the real impact of HM on organisms and on trophic chains.

4. The use of bacteria as biosensors of heavy metal pollution

4.1 Bacteria as biosensors

Microorganisms are primary producers in many environmental ecosystems and play an essential role in the nutrient cycle, and they are very abundant and ubiquitous. The microbes proliferate rapidly, are easily detectable and easy to sample, and respond quickly to environmental changes, like temperature, pH, or the presence of contaminants including heavy metals. These characteristics make microorganisms good candidates as pollution biosensors [140]. In this sense, bioluminescent bacteria such as *Aliivibrio fischeri* and *Photobacterium phosphoreum* have been used to monitor water and soil contaminated with HM [141, 142]. This bioassay is carried out using the natural bioluminescence emitted by these bacteria and is based on the decrease of this fluorescence when the bacteria grow in samples of water or soil contaminated with different heavy metals such as Zn, Cu, Cd, Hg, and Cr, among others [141–144]. In the case of *A. fischeri*, the test has been developed commercially and is distributed under the name of Microtox[®]. However, this method is

sensitive to different pollutants such as antibiotics, pesticides, toxins, and organic compounds, which makes it a non-specific method for the detection of heavy metals [145]. Another bacterium proposed as a bioindicator is *Vogesella indigofera*; under normal conditions this bacterium develops a blue color due to the indigoidine production; when it grows in the presence of Cr⁶, the bacteria decreases the pigment production, and this decrease is dependent on the concentration of Cr⁶; at 150 µg/ml the bacteria are entirely white and rough [146]. *Serratia marcescens* is a Gram-negative bacterium that produces a red pigment known as prodigiosin; when the bacterium grows in sub-inhibitory concentrations of Cd, Cr, and Pb, the pigment production decreases drastically, so the authors propose it as a bioindicator of heavy metal contamination [147].

The presence of heavy metals in the environment exerts an intense selection pressure on the organisms that live there; an increase in its concentration and the high rate of horizontal gene transfer can select heavy metal-resistant microorganisms. Therefore, the resistance and detoxification genes have been used as biomarkers for the study of contaminated environments using molecular techniques such as quantitative PCR and real-time quantitative reverse transcription PCR. Within these genes are those involved in the resistance to As, *ACR3(1)* (arsenite efflux pump), *aioA* (arsenite oxidase), *arsB* (arsenical efflux pump), *arsC* (arsenate reductase), and *arsM* (arsenic methyltransferase) [148–151]; those that confer resistance to Cu, *copA* (Copper-exporting P-type ATPase), and *cusA* (copper export system) [152, 153]; for Cd, Zn, and Co resistance, *czcA* (Cd/Zn/Co efflux pump) [154]; for Hg, *hgcA* (mercury methylating protein) and *merA* (mercuric reductase) [155]; the *mr* [140] that encodes to metallothionein a cysteine-rich and heavy metal-binding protein [156]; and *sodA* [140] which codes for a superoxide dismutase, involved in the protection of toxicity against heavy metals [157]. Another technique to measure the presence and abundance of genes involved in resistance to heavy metals is through the use of genetic microarrays such as the GeoChip, commercially available [158]. With the use of this microarray, it was possible to correlate the presence of *arsC*, *copA*, *cueO* (multicopper oxidase), *merB* (alkylmercury lyase), *metC* (cystathionine beta-lyase), *tehB* (tellurite methyltransferase), and *terC* (tellurium resistance protein) genes in sediments and waters contaminated with Cd, Cr, Cu, Hg, and S [158, 159].

High concentrations of heavy metals affect microbial populations and therefore their processes. Thus, the evaluation of microbial processes represents good biomarkers of exposure in different environments. Within the parameters most used are the monitoring of enzymatic activities of the carbon and nitrogen cycle, soil respiration, microbial mass, and the ecosystem biodiversity [160, 161]. Microbial biodiversity is drastically affected by contamination with heavy metals. In general, it is observed that a higher concentration of heavy metals decreases bacterial species. However, with the massive sequencing of DNA, some bacterial groups that could serve as biosensors of contamination were identified, for example, the study carried out by Schneider and collaborators finds that the bacterial groups γ -*Proteobacteria*, *Verrucomicrobia*, and Chlamydiae showed a consistent response to Pb content across contrasting ecosystems. The phyla *Chlamydiae* and γ -*Proteobacteria* were more abundant, while *Verrucomicrobia* were less abundant at high contamination level. So, they conclude that such groups and ratios thereof can be considered as relevant bioindicators of Pb contamination [162]. In soils contaminated with Cu, it was observed that at increased concentrations, bacterial richness was negatively impacted and enhanced relative abundance of *Nitrospira* and *Acidobacteria* members and a lower representation of *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria*, suggesting a promising role as bioindicators of copper contamination in soils [163].

Acknowledgements

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

Conflict of interest

The authors declare that there is no conflict of interest.

Author details

Efraín Tovar-Sánchez¹, Ramón Suarez-Rodríguez², Augusto Ramírez-Trujillo², Leticia Valencia-Cuevas¹, Isela Hernández-Plata³ and Patricia Mussali-Galante^{3*}


1 Laboratorio de Marcadores Moleculares, Centro de Investigación en Biodiversidad y Conservación, Autonomous University of Morelos State, Cuernavaca, Morelos, Mexico

2 Laboratorio de Fisiología Molecular de Plantas, Centro de Investigación en Biotecnología, Autonomous University of Morelos State, Cuernavaca, Morelos, Mexico

3 Laboratorio de Investigaciones Ambientales, Centro de Investigación en Biotecnología, Autonomous University of Morelos State, Cuernavaca, Morelos, Mexico

*Address all correspondence to: patricia.mussali@uaemmx

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Sanità di Toppi L, Gabbrielli R. Response to cadmium in higher plants. *Environmental and Experimental Botany*. 1999;**41**:105-130. DOI: 10.1016/S0098-8472(98)00058-6
- [2] Srivastava NK, Majumder CB. Novel biofiltration methods for the treatment of heavy metals from industrial wastewater. *Journal of Hazardous Materials*. 2008;**151**:1-8. DOI: 10.1016/j.jhazmat.2007.09.101
- [3] Oehme I, Wolfbeis S. Optical sensors for determination of heavy metals ions. *Mikrochimica Acta*. 1997;**126**(3-4): 177-192. DOI: 10.1007/BF01242319
- [4] Agency for Toxic Substances and Disease Registry (ATSDR). CERCLA Priority List of Hazardous Substances. Atlanta, GA: Department of Health and Human Services; 2007. Available from: <http://www.atsdr.cdc.gov/cercla/07list.html>
- [5] Kopittke PM, Blamey FPC, Asher CJ, Menzies NW. Trace metal phytotoxicity in solution culture: A review. *Journal of Experimental Botany*. 2010;**61**:945-954. DOI: 10.1093/jxb/erp385
- [6] Patra M, Bhowmik N, Bandopadhyay B, Sharma A. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany*. 2004;**52**:199-223. DOI: 10.1016/j.envexpbot.2004.02.009
- [7] Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FMM. A cadmium enzyme from a marine diatom. *Nature*. 2005;**435**:42. DOI: 10.1038/435042a
- [8] Hansch R, Mendel RR. Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology*. 2009;**12**: 259-266. DOI: 10.1016/j.pbi.2009.05.006
- [9] Horst WJ, Wang Y, Eticha D. The role of the root apoplast in aluminum-induced inhibition of root elongation and in aluminum resistance of plants: A review. *Annals of Botany*. 2010;**106**: 185-197. DOI: 10.1093/aob/mcq053
- [10] Benavides MP, Gallego SM, Tomaro ML. Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*. 2005;**17**:21-34. DOI: 10.1590/S1677-04202005000100003
- [11] Johnson A, Singhal N, Hashmatt M. Metal-plant interactions: Toxicity and tolerance. In: Khan M, Zaidi A, Goel R, Musarrat J, editors. *Bio-management of Metal-Contaminated Soils, Environmental Pollution*. Vol. 20. Dordrecht: Springer; 2011. pp. 29-63. DOI: 10.1007/978-94-007-1914-9_2
- [12] Mussali-Galante P, Tovar-Sánchez E, Valverde M, Rojas E. Biomarkers of exposure for assessing environmental metal pollution: From molecules to ecosystems. *Revista Internacional de Contaminación Ambiental*. 2013;**29**: 117-140
- [13] Kobalchuk I, Kovalchuk O. Transgenic plants as sensors of environmental pollution genotoxicity. *Sensors*. 2008;**8**:1539-1558. DOI: 10.3390/s8031539
- [14] Stankovic S, Kalaba P, Stankovic AR. Biota as toxic metal indicators. *Environmental Chemistry Letters*. 2014;**12**:63-84. DOI: 10.1007/s10311-013-0430-6
- [15] Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: A review. *Environmental Chemical Letters*. 2010;**8**:199-216

- [16] Vajpayee P, Dhawan A, Shanker R. Evaluation of the alkaline comet assay conducted with the wetlands plant *Bacopa monnieri* L. as a model for ecogenotoxicity assessment. *Environmental and Molecular Mutagenesis*. 2006;**47**:483-489. DOI: 10.1002/em.20217
- [17] Gichner T, Patková Z, Száková J, Demnerová K. Toxicity and DNA damage in tobacco and potato plants growing on soil polluted with heavy metals. *Ecotoxicology and Environmental Safety*. 2006;**65**: 420-426. DOI: 10.1016/j.ecoenv.2005.08.006
- [18] Maldonado M, Rodríguez C, López A, Wrobel K, Wrobel K. Global DNA methylation in earthworms: A candidate biomarker of epigenetic risks related to the presence of metals/metalloids in terrestrial environments. *Environmental Pollution*. 2011;**159**: 2387-2392. DOI: 10.1016/j.envpol.2011.06.041
- [19] İşeri İÖD, Körpe DA, Yurtcu E, Sahin FI, Haberal M. Copper-induced oxidative damage, antioxidant response and genotoxicity in *Lycopersicon esculentum* mill. And *Cucumis sativus* L. *Plant Cell Reports*. 2011;**30**:1713-1721. DOI: 10.1007/s00299-011-1079-x
- [20] Murillo HAI. Detección de daño genotóxico en *Prosopis laevigata* de los jales de la Sierra de Huautla, Morelos, México provocado por metales pesados [thesis]. Mexico: Universidad Nacional Autónoma de México; 2015
- [21] Santoyo-Martínez M. Bioacumulación, daño genotóxico y cambios en la estructura y morfología foliar de *Vachelia farnesiana* en los jales de Huautla, Morelos [thesis]. Mexico: Universidad Autónoma del Estado de Morelos; 2016
- [22] Castañeda-Bautista JA. Estudio ecotoxicológico de los jales mineros de Huautla, Morelos: El caso de *Pithecellobium dulce* (Roxb.) Beth. (Fabaceae) [thesis]. Mexico: Universidad Autónoma del Estado de Morelos; 2016
- [23] Cobarrubias-Escamilla DL. Evaluación del daño genotóxico y cambios morfológicos de *Wigandia urens* expuesta a metales pesados en presas de jales abandonados [thesis]. Mexico: Universidad Autónoma del Estado de Morelos; 2017
- [24] Ferrer A. Intoxicación por metales. *Anales Sis San Navarra*. 2003;**26**:141-153
- [25] Manara A. Plant responses to heavy metal toxicity. In: Furini A, editor. *Plant and Heavy Metals*. Netherlands: Springer; 2012. p. 27-44. DOI: 10.1007/978-94-007-4441-7_2
- [26] Romero-Puertas MC, Corpas FJ, Rodríguez-Serrano M, Gómez M, del Río LA, Sandalio LM. Differential expression and regulation of antioxidative enzymes by cadmium in pea plants. *Journal of Plant Physiology*. 2007;**164**:1346-1357. DOI: 10.1016/j.jplph.2006.06.018
- [27] Dat JF, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F. Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences*. 2000;**57**:779-795. DOI: 10.1007/s000180050041
- [28] Maksymiec W, Krupa Z. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. *Environmental and Experimental Botany*. 2006;**57**:187-194. DOI: 10.1016/j.envexpbot.2005.05.006
- [29] Cho U-H, Park J-O. Mercury-induced oxidative stress in tomato seedlings. *Plant Science*. 2000;**156**:1-9. DOI: 10.1016/S0168-9452(00)00227-2

- [30] Yadav K. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany*. 2010;**76**: 167-179. DOI: 10.1016/j.sajb.2009.10.007
- [31] Cobbett C, Goldsbrough P. Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annual Review in Plant Biology*. 2002;**53**:159-182
- [32] Hirata K, Tsuji N, Miyamoto K. Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. *Journal of Bioscience and Bioengineering*. 2005;**100**:593-599. DOI: 10.1263/jbb.100.593
- [33] Cobbett CS. Phytochelatins and their roles in heavy metal detoxification. *Current Opinion in Plant Biology*. 2000; **3**:211-216. DOI: 10.1104/pp.123.3.825
- [34] Nocito FF, Pirovano L, Cocucci M, Sacchi GA. Cadmium-induced sulfate uptake in maize roots. *Plant Physiology*. 2002;**129**:1872-1879. DOI: 10.1104/pp.002659
- [35] Clemens S. Molecular mechanisms of metal tolerance and homeostasis. *Planta*. 2001;**212**:475-486. DOI: 10.1007/s004250000458
- [36] Inohue M. Phytochelatines. *Brazilian Journal of plant physiology*. 2005;**17**:65-78. DOI: 10.1007/s004250000458
- [37] Ben Ammar W, Nouairi I, Tray B, Zarrouk M, Jemal F, Ghorbal MH. Effets du cadmium sur l'accumulation ionique et les teneurs en lipides dans les feuilles de tomate (*Lycopersicon esculentum*). *Société de Biologie*. 2005;**199**:157-163. DOI: 10.1051/jbio:2005016
- [38] Dražić G, Mihailović N, Lojić M. Cadmium accumulation in *Medicago sativa* seedlings treated with salicylic acid. *Biologia Plantarum*. 2006;**50**: 239-244. DOI: 10.1007/s10535-006-0013-5
- [39] Nouairi I, Ben Ammar W, Ben Youssef N, Ben Miled Daoud D, Ghorbal MH, Zarrouk M. Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. *Plant Science*. 2005;**170**:511-519. DOI: 10.1016/j.plantsci.2005.10.003
- [40] Pietrini F, Iannelli MA, Pasqualini S, Massacci A. Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis*. *Plant Physiology*. 2003;**133**:829-837. DOI: 10.1104/pp.103.026518
- [41] Gouia H, Suzuki A, Brultfert J, Ghorbal MH. Effects of cadmium on the co-ordination of nitrogen and carbon metabolism in bean seedlings. *Journal of Plant Physiology*. 2003;**160**:367-376. DOI: 10.1078/0176-1617-00785
- [42] Agrawal V, Sharma K. Phytotoxic effects of Cu, Zn, Cd and Pb on in vitro regeneration and concomitant protein changes in *Holarrhena antidysenterica*. *Biologia Plantarum*. 2006;**50**:307-310. DOI: 10.1007/s10535-006-0027-z
- [43] Scebba F, Arduini I, Ercoli L, Sebastiani L. Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. *Biologia Plantarum*. 2006;**50**:688-692. DOI: 10.1007/s10535-006-0107-0
- [44] Mohanpuria P, Rana NK, Yadav SK. Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. *Environmental Toxicology*. 2007;**22**:368-374. DOI: 10.1002/tox.20273
- [45] Salt DE, Thurman DA, Tomsett AB, Sewell AK. Copper phytochelatins of *Mimulus guttatus*. *Proceedings of the*

Royal Society B. 1989;**236**:79-89. DOI: 10.1098/rspb.1989.0013

[46] Haag-Kerwer A, Schafer HJ, Heiss S, Walter C, Rausch T. Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. *Journal of Experimental Botany*. 1999;**50**:1827-1835. DOI: 10.1093/jxb/50.341.1827

[47] Schmöger ME, Oven M, Grill E. Detoxification of arsenic by phytochelatins in plants. *Plant Physiology*. 2000;**122**:793-801. DOI: 10.1104/pp.122.3.793

[48] Ramos J, Naya L, Gay M, Abian J, Becana M. Functional characterization of an unusual phytochelatin synthase, LjPCS3, of *Lotus japonicus*. *Plant Physiology*. 2008;**148**:536-545. DOI: 10.1104/pp.108.121715

[49] Costa PM, Caeiro S, Diniz MS, Lobo J, Martins M, Ferreira AM, et al. Biochemical endpoints on juvenile *Solea senegalensis* exposed to estuarine sediments: The effect of contaminant mixtures on metallothionein and CYP1A induction. *Ecotoxicology*. 2009;**18**: 988-1000. DOI: 10.1007/s10646-009-0373-7

[50] Hassinen VH, Tervahauta AI, Schat H, Kärenlampi SO. Plant metallothioneins—Metal chelators with ROS scavenging activity? *Plant Biology*. 2011;**13**:225-232. DOI: 10.1111/j.1438-8677.2010.00398.x

[51] Zhou J, Goldsbrough PB. Functional homologs of fungal metallothionein genes from *Arabidopsis*. *The Plant Cell*. 1994;**6**:875-884. DOI: 10.1105/tpc.6.6.875

[52] Domènech J, Mir G, Huguet G, Capdevila M, Molinas M, Atri-an S. Plant metallothionein domains: Functional insight into physiological metal binding and protein folding.

Biochimie. 2006;**88**:583-593. DOI: 10.1016/j.biochi.2005.11.002

[53] Freisinger E. Metallothioneins in plants. In: Sigel A, Sigel H, Sigel RKO, editors. *Metal Ions in Life Sciences*. Cambridge, UK. 2009;**5**:107-153. DOI: 10.1039/9781847558992-FP007

[54] Pál M, Holrváth E, Janda T, Páldi E, Szalai G. Physiological changes and defense mechanisms induced by cadmium stress in maize. *Journal of Plant Nutrition and Soil Science*. 2006; **169**:239-256. DOI: 10.1002/jpln.200520573

[55] Prasad M, Strzalka K. *Heavy Metal Stress in Plants*. Berlin: Springer; 1999. 138 p. DOI: 10.1007/978-3-662-07745-0_6

[56] Lerda D. The effect of lead on *Allium cepa* L. *Mutation Research Letters*. 1992;**281**:89-92. DOI: 10.1016/0165-7992(92)90041-F

[57] Eun S, Shik-Youn H, Lee Y. Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiologia Plantarum*. 2000;**110**:357-365. DOI: 10.1111/j.1399-3054.2000.1100310.x

[58] Furini A, editor. *Plants and Heavy Metals*, Springer Briefs in Biometals. The Netherlands: Springer; 2012. p. 83

[59] Guo Z, Miao X. Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L.) in soil contaminated with arsenic, cadmium and lead. *Journal of Central South University of Technology*. 2010;**17**: 770-777. DOI: 10.1007/s11771-010-0555-8

[60] Tovar-Sánchez E, Cervantes-Ramírez T, Castañeda-Bautista J, Gómez-Arroyo S, Ortiz-Hernández L, Sánchez-Salinas E, et al. Response of *Zea mays* to multimetal contaminated soils: A multibiomarker approach.

Ecotoxicology. 2018;**27**:1-17. DOI:
10.1007/s10646-018-1974-9

[61] Hernández-Lorenzo B. Análisis de la anatomía y morfología de *Prosopis laevigata*, por acumulación de metales pesados en la sierra de Huautla, Morelos [thesis]. Mexico: Universidad Autónoma del Estado de Morelos; 2014

[62] Bickham J, Sandhu S, Herbert P, Chikhi L, Athwal R. Effects of chemical contaminants on genetic diversity in natural populations: Implications for biomonitoring and ecotoxicology. Mutation Research. 2000;**463**:33-51. DOI: 10.1016/S1383-5742(00)00004-1

[63] Keane B, Collier M, Rogstad S. Pollution and genetic structure of North American populations of the common dandelion (*Taraxacum officinale*). Environmental Monitoring and Assessment. 2005;**105**:341-357. DOI: 10.1007/s10661-005-4333-2

[64] Mengoni A, Barabesi C, Gonnelli C, Galardi F, Gabbrielli R, Bazzicalupo M. Genetic diversity of heavy metal-tolerant populations in *Silene paradoxa* L. (Caryophyllaceae): A chloroplast microsatellite analysis. Molecular Ecology. 2001;**10**:1909-1916. DOI: 10.1046/j.0962-1083.2001.01336.x

[65] Basic N, Besnard G. Gene polymorphisms for elucidating the genetic structure of the heavy-metal hyperaccumulating trait in *Thlaspi caerulescens* and their cross-genera amplification in Brassicaceae. Journal of Plant Research. 2006;**119**:479-487. DOI: 10.1007/s10265-006-0011-x

[66] Prus-Glowacki CE, Wojnicka-Półtorak A, Kozacki L, Fagiewicz K. Effects of heavy metal pollution on genetic variation and cytological disturbances in *Pinus sylvestris* L. population. Journals of Applied Genetics. 2006;**47**:99-108. DOI: 10.1007/BF03194607

[67] Jiménez-Ambriz G, Petit C, Bourrié I, Olivieri I, Dubois S, Ronce O. Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and non-contaminated sites in southern France: Role of gene flow, selection and phenotypic plasticity. New Phytologist. 2007;**173**:199-215. DOI: 10.1111/j.1469-8137.2006.01923.x

[68] Quintela-Sabarís C, Glusppe V, Castro-Fernández D, Fraga M. Chloroplast microsatellites reveal that metalicolous populations of the Mediterranean shrub *Cistus ladanifer* L have multiple origins. Plant and Soil. 2010;**334**:161-174. DOI: 10.1007/s11104-010-0368-4

[69] Godé C, Decombeix I, Kostecka A, Wasowicz P, Pauwels M, Courseaux A, et al. Nuclear microsatellite loci for *Arabidopsis halleri* (Brassicaceae), a model species to study plant adaptation to heavy metals. American Journal of Botany. 2012;**99**:e49-e52. DOI: 10.3732/ajb.1100320

[70] Fuentes-Reza A. Bioacumulación y análisis de la estructura y diversidad genética de *Prosopis laevigata* por exposición a metales en Huautla, Morelos [thesis]. Mexico: Universidad Autónoma del Estado de Morelos; 2017

[71] Peterson LR, Trivett V, Baker AJM, Aguiar C, Pollard AJ. Spread of metals through an invertebrate food chain as influenced by a plant that hyperaccumulates nickel. Chemoecology. 2003;**13**:103-108. DOI: 10.1007/s00049-003-0234-4

[72] Boyd RS, Wall MA, Jaffré T. Nickel levels in arthropods associated with Ni hyperaccumulator plants from an ultramafic site in New Caledonia. Insect Science. 2006;**13**:271-277. DOI: 10.1111/j.1744-7917.2006.00094.x

[73] Bourioug M, Gimbert F, Alaoui-Sehmer L, Benbrahim M, Aleya L,

- Alaoui-Sossé B. Sewage sludge application in a plantation: Effects on trace metal transfer in soil-plant-snail continuum. *Science of the Total Environment*. 2015;**502**:309-314. DOI: 10.1016/j.scitotenv.2014.09.022
- [74] Martínez-Becerril C. Efecto de los metales pesados en jales mineros sobre la comunidad vegetal de la microcuenca de Huautla y el daño genotóxico en su herbívoro dominante *Peromyscus levipes* (Rodenta: Muridae) [thesis]. Mexico: Universidad Autónoma del Estado de Querétaro; 2009
- [75] Hunter B, Johnson M, Thompson D. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. *Journal of Applied Ecology*. 1989;**26**: 89-99. DOI: 10.2307/2403653
- [76] Becerril JM, Barrutia O, Plazaola JG, Hernández A, Olano JM, Garbisu C. Ecosistemas especies nativas de suelos contaminados por metales: Aspectos ecofisiológicos y su uso en fitorremediación. *Ecosistemas: Revista científica y técnica de ecología y medio ambiente*; 2007;**16**:50-55
- [77] Boyd RS. Plant defense using toxic inorganic ions: Conceptual models of the defensive enhancement and joint effects hypotheses. *Plant Science*. 2012;**195**:88-95. DOI: 10.1016/j.plantsci.2012.06.012
- [78] 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. DOI: 10.1007/s00442-007-0907-8
- [79] 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. *American Journal of Botany*. 2009;**96**:1075-1085. DOI: 10.3732/ajb.0800287
- [80] Boyd RS. High-nickel insects and nickel hyperaccumulator plants: A review. *Insect Science*. 2009;**16**:19-31. DOI: 10.1111/j.1744-7917.2009.00250.x
- [81] Meindl GA, Ashman TL. The effects of aluminum and nickel in nectar on the foraging behavior of bumblebees. *Environmental Pollution*. 2013;**177**:78-81. DOI: 10.1016/j.envpol.2013.02.017
- [82] Peralta-Videa JR, Lopez ML, Narayan M, Saupe GJ. The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. *The International Journal of Biochemistry & Cell Biology*. 2009;**41**: 1665-1677. DOI: 10.1016/j.biocel.2009.03.005
- [83] Boyd RS, Wall MA. Responses of generalist predators fed high-Ni *Melanotrichus boydi* (Heteroptera: Miridae): Elemental defense against the third trophic level. *The American Midland Naturalist*. 2001;**146**:186-198. DOI: 10.1674/0003
- [84] 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). *Environmental Pollution*. 2006;**144**:581-595. DOI: 10.1016/j.envpol.2006.01.020
- [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. *Environmental Toxicology and Chemistry*. 2007;**26**:1488-1496. DOI: 10.1897/06-518R.1
- [86] Wijnhoven S, Leuven RSE, Van der Velde G, Jungheim G, Koelemij EI, de

Vries FT, et al. Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: Importance of species- and location-specific characteristics. Archives of Environmental Contamination and Toxicology. 2007;**52**:603-613. DOI: 10.1007/s00244-006-0124-1

[87] Notten MJM, Oosthoek AJP, Rozema J, Aerts R. Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. Environmental Pollution. 2005;**138**:178-190. DOI: 10.1016/j.envpol.2005.01.011

[88] Notten MJM, Oosthoek AJP, Rozema J, Aerts R. The landsnail *Cepaea nemoralis* regulates internal Cd levels when fed on Cd-enriched stinging nettle (*Urtica dioica*) leaves at low, field-relevant concentrations. Environmental Pollution. 2006;**139**:296-305. DOI: 10.1016/j.envpol.2005.05.007

[89] Paoli L, Corsini A, Bigagli V, Vannini J, Bruscoli C, Loppi S. Long-term biological monitoring of environmental quality around a solid waste landfill assessed with lichens. Environmental Pollution. 2012;**161**:70-75. DOI: 10.1016/j.envpol.2011.09.028

[90] Purvis OW, Pawlik-Skowrońska B. Lichens and metals. In: Avery S, Stratford M, van West P, editors. Stress in Yeasts and Filamentous Fungi. Amsterdam: Elsevier; 2008. pp. 175-200. DOI: 10.1016/S0275-0287(08)80054-9

[91] Nimis PL, Lazzarin G, Lazzarin A, Skert N. Biomonitoring of trace elements with lichens in Veneto (NE Italy). Science of the Total Environment. 2000;**255**:97-111. DOI: 10.1016/S0048-9697(00)00454-X

[92] Giordani P, Modenesi P, Tretiach M. Determinant factors for the formation of the calcium oxalate minerals, weddellite and whewellite, on the

surface of foliose lichens. The Lichenologist. 2003;**35**:255-270. DOI: 10.1016/S0024-2829(03)00028-8

[93] Pawlik-Skowrońska B, Purvis OW, Pirszel J, Skowroński T. Cellular mechanisms of Cu tolerance in the epilithic lichen *Lecanora polytropa* growing at a copper mine. The Lichenologist. 2006;**38**:267-275. DOI: 10.1017/S0024282906005330

[94] Branquinho C. Lichens. In: Prasad MNV, editor. Metals in the Environment: Analysis by Biodiversity. New York: Marcel Dekker; 2001. pp. 117-115

[95] Paul A, Hauck M, Langenfeld-Heyser M. Ultrastructural changes in soredia of the epiphytic lichen *Hypogymnia physodes* cultivated with manganese. Environmental and Experimental Botany. 2004;**52**:139-147. DOI: 10.1016/j.envexpbot.2003.01.001

[96] Sanita di Toppi L, Marabottini R, Vattuone Z, Musetti R, Favali MA, Sorgona A, et al. Cell wall immobilisation and antioxidant status of *Xanthoria parietina* thalli exposed to cadmium. Functional Plant Biology. 2005;**32**:611-618. DOI: 10.1071/FP04237

[97] Branquinho C, Brown DH, Catarino F. The cellular location of Cu in lichens and its effects on membrane integrity and chlorophyll fluorescence. Environmental and Experimental Botany. 1997;**38**:165-179. DOI: 10.1016/S0098-8472(97)00015-4

[98] Hauck M, Paul A. Manganese as a site factor for epiphytic lichens. The Lichenologist. 2005;**37**:409-423. DOI: 10.1017/S0024282905014933

[99] Garty J, Tamir O, Levin T, Lehr H. The impact of UV-B and Sulphur- or copper-containing solutions in acidic conditions on chlorophyll fluorescence in selected *Ramalina* species. Environmental Pollution. 2007;**145**:266-273. DOI: 10.1016/j.envpol.2006.03.022

- [100] Tarhanen S. Ultrastructural responses of the lichen *Bryoria fuscescens* to simulated acid rain and heavy metal deposition. *Annals of Botany*. 1998;**82**: 735-746. DOI: 10.1006/anbo.1998.0734
- [101] Kranner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabentheiner E, et al. Antioxidant and photoprotection in a lichen as compared with its isolated symbiont partners. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;**102**:3141-3146. DOI: 10.1073/pnas.0407716102
- [102] Pawlik-Skowrońska B, di Toppi LS, Favali MA, Fossati F, Pirszel J, Skowronski T. Lichens respond to heavy metals by phytochelatin synthesis. *New Phytologist*. 2002;**156**:95-102. DOI: 10.1046/j.1469-8137.2002.00498.x
- [103] Seaward MRD. Some observations on heavy metal toxicity and tolerance in lichens. *The Lichenologist*. 1974;**6**:158-164. DOI: 10.1017/S0024282974000260
- [104] Ernst G. *Vezdaea leprosa*—Spezialist am strassenrand. *Herz*. 1995; **11**:175-188
- [105] Gilbert OL. *Lichens*. London: Harper Collins; 2000
- [106] Aptroot A, van den Boom PPG. *Pyrenocollema chlorococcum*, a new species with a chlorococcoid photobiont from zinc-contaminated soils and wood. *Cryptogamie Bryologie Lichenologie*. 1998;**19**:193-196
- [107] Cuny D, Denayer FO, de Foucault B, Schumacker R, Colein P, Van Haluwyn C. Patterns of metal soil contamination and changes in terrestrial cryptogamic communities. *Environmental Pollution*. 2004;**129**: 289-297. DOI: 10.1016/j.envpol.2003.10.009
- [108] Eligio-González S. Comunidades líquénicas asociadas a *Pithecellobium dulce*, *Acacia farnesiana* y *Prosopis laevigata* en jales mineros y sitios testigos en Huautla, Morelos [tesis]. Cuernavaca, Morelos: Universidad Autónoma del Estado de Morelos; 2016
- [109] Gauslaa Y, Holien H. Acidity of boreal *Picea abies*-canopy lichens and their substratum, modified by local soils and airborne acidic depositions. *Flora*. 1998;**193**:249-257. DOI: 10.1016/S0367-2530(17)30845-9
- [110] Díaz-Escandón D. Líquenes cortícolas como indicadores ambientales en los alrededores de mina de azufre El Vinagre (Cauca) [thesis]. Santiago de Cali, Colombia: Programa Académico de Biología; 2012
- [111] Hauck M. Epiphytic lichen diversity and forest dieback: The role of chemical site factors. *The Bryologist*. 2003;**106**:257-269. DOI: 10.1639/00072745(2003)106[0257:ELDAFD]2.0.CO;2
- [112] Kobalchuk I, Kovalchuk O, Arkhipov A, Hohn B. Transgenic plants are sensitive bioindicators of nuclear pollution caused by the Chernobyl accident. *Nature Biotechnology*. 1998; **16**:1054-1059. DOI: 10.1038/3505
- [113] Kovalchuk O, Titov V, Hohn B, Kobalchuk I. A sensitive transgenic plant system to detect toxic inorganic compounds in the environment. *Nature Biotechnology*. 2001;**19**:568-572. DOI: 10.1038/89327
- [114] Varshney RK, Bansal KC, Aggarwal PK, Datta SK, Craufurd PQ. Agricultural biotechnology for crop improvement in a variable climate: Hope or hype? *Trends in Plant Science*. 2011;**16**: 363-371. DOI: 10.1016/j.tplants.2011.03.004
- [115] Liew OW, Chong PCJ, Li B, Asundi AK. Signature optical cues: Emerging technologies for monitoring plant

- health. *Sensors*. 2008;**8**:3205-3239. DOI: 10.3390/s8053205
- [116] Chaerle L, Lenk S, Leinonen I, Jones HG, Van Der Straeten D, Buschmann C. Multi-sensor plantimaging: Towards the development of a stress-catalogue. *Biotechnology Journal*. 2009;**4**:1152-1167. DOI: 10.1002/biot.200800242
- [117] Wee CW, Dinney JR. Tools for high-spatial and temporal-resolution analysis of environmental responses in plants. *Biotechnology Letters*. 2010;**32**:1361-1371. DOI: 10.1007/s10529-010-0307-8
- [118] Hines G, Modavi C, Jiang K, Packard A, Poolla K, Feldman L. Tracking transience: A method for dynamic monitoring of biological events in *Arabidopsis thaliana* biosensors. *Plant*. 2015;**242**:1251-1261. DOI: 10.1007/s00425-015-2393-2
- [119] Meyer AJ, Brach T, Marty L, Kreye S, Rouhier N, Jacquot JP, et al. Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. *Plant Journal*. 2007;**52**(5):973-986. DOI: 10.1111/j.1365-313X.2007.03280.x
- [120] Antunes MS, Morey KJ, Smith JJ, Albrecht KD, Bowen TA, Zdunek JK, et al. Programmable ligand detection system in plants through a synthetic signal transduction pathway. *PLoS One*. 2001;**6**(1):e16292. DOI: 10.1371/journal.pone.0016292
- [121] Millwood RJ, Halfhill MD, Harkins D, Russotti R, Stewart CN Jr. Instrumentation and methodology for quantifying GFP fluorescence in intact plant organs. *BioTechniques*. 2003;**34**(3):638-643. DOI: 10.2144/03343pf02
- [122] Schlitter D, van der Straeten E, Amori G, Hutterer R, Kryštufek B, Yigit N, Mitsain G. *Apodemus sylvaticus*. The IUCN Red List of Threatened Species. 2016:e.T1904A115059104. DOI: 10.2305/IUCN.UK.2016-3.RLTS.T1904A22423831.en
- [123] Sánchez-González B, Navarro-Castilla A, Hernández MC, Barja I. Ratón de campo-*Apodemus sylvaticus*. In: Salvador A, Barja I, editors. *Enciclopedia Virtual de los Vertebrados Españoles*. Madrid, España: Museo Nacional de Ciencias Naturales; 2016. Available from: <http://www.vertebradosibericos.org/>
- [124] Rogival D, Scheirs J, Blust R. Transfer and accumulation of metals in a soil-diet-wood mouse food chain along a metal pollution gradient. *Environmental Pollution*. 2007;**145**:516-528. DOI: 10.1016/j.envpol.2006.04.019
- [125] Wijnhoven S, Van Der Velde G, Leuven RSEW, Smits AJM flooding ecology of voles, mice and shrews: The importance of geomorphological and vegetational heterogeneity in river floodplains. *Acta Theriologica*. 2005;**50**(4):453-472. DOI: 10.1007/BF03192639
- [126] Hutterer R, Kryštufek B, Yigit N, Mitsain G, Palomo LJ, Henttonen H, et al. *Myodes glareolus*. The IUCN red list of threatened species 2016:e.T4973A115070929. DOI: 10.2305/IUCN.UK.2016-3.RLTS.T4973A22372716.en
- [127] Luque-Larena JJ, Gosálbez Noguera J. *Myodes glareolus* (Schreber, 1780). In: Palomo LJ, Gisbert y J, Blanco JC, editors. *Atlas y libro rojo de los mamíferos terrestres de España*. Dirección General para la Biodiversidad -SECEM-SECEMU, Madrid, España. 2007. 398-400
- [128] Erry BV, Macnair MR, Meharg AA, Shore RF. Arsenic contamination in wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) on

- abandoned mine sites in Southwest Britain. *Environmental Pollution*. 2000; **110**(1):179-187. DOI: 10.1016/S0269-7491(99)00270-5
- [129] Sánchez-Chardi A, Peñarroja-Matutano C, Ribeiro CA, Nadal J. Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The wood mouse, *Apodemus sylvaticus*. *Chemosphere*. 2007; **70**(1):101-109. DOI: 10.1016/j.chemosphere.2007.06.047
- [130] Sánchez-Chardi A, Peñarroja-Matutano C, Borrás M, Nadal J. Bioaccumulation of metals and effects of a landfill in small mammals part III: Structural alterations. *Environmental Research*. 2009; **109**(8):960-967. DOI: 10.1016/j.envres.2009.08.004
- [131] 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. *Environmental Pollution*. 2001; **115**:17-22. DOI: 10.1016/S0269-7491(01)00096-3
- [132] Cooke JA, Andrews SM, Johnson MS. Lead, zinc, cadmium and fluoride in small mammals from contaminated grassland established on fluorspar tailings. *Water, Air, and Soil Pollution*. 1990; **5**:43-54. DOI: 10.1007/BF00211502
- [133] Drouhot S, Raoul F, Crini N, Tougard C, Prudent AS, Druart C, Rieffel D, Lambert JC, Tête N, Giraudoux P, Scheifler R. Responses of wild small mammals to arsenic pollution at a partially remediated mining site in southern France. *Science of the Total Environment*. 2014; **470-471**:1012-1022. DOI: 10.1016/j.scitotenv.2013.10.053.14
- [134] Scheirs J, De Coen A, Covaci A, Beernaert J, Kayawe VM, Caturla M, et al. Getoxicity in wood mice (*Apodemus sylvaticus*) along a pollution gradient: Exposure-, age-, and gender-related effects. *Environmental Toxicology and Chemistry*. 2006; **25**(8): 2154-2162. DOI: 10.1897/05-419R.1
- [135] Tête N, Durfort M, Rieffel D, Scheifler R, Sánchez-Chardi A. Histopathology related to cadmium and lead bioaccumulation in chronically exposed wood mice, *Apodemus sylvaticus*, around a former smelter. *Science Total Environment*. 2014; **481**: 167-177. DOI: 10.1016/j.scitotenv.2014.02.029
- [136] Wijnhoven S, Leuven RS, van der Velde G, Eijsackers HJ. Toxicological risks for small mammals in a diffusely and moderately polluted floodplain. *Science Total Environment*. 2008; **406**(3):401-406. DOI: 10.1016/j.scitotenv.2008.05.059
- [137] Topashka-Ancheva M, Metcheva R, Teodorova S. A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria I: Monitoring points and bioaccumulation features. *Ecotoxicology Environmental Safety*. 2003; **54**(2):176-187. DOI: 10.1016/S0147-6513(02)00052-0
- [138] 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. *Environmental Research*. 2004; **96**(1): 72-78. DOI: 10.1016/j.envres.2004.02.003
- [139] Erry BV, Macnair MR, Meharg AA, Shore RF. The distribution of arsenic in the body tissues of wood mice and bank voles. *Archives of Environmental Contamination and Toxicology*. 2005; **49**(4):569-576. DOI: 10.1007/s00244-004-0229-3
- [140] Bouskill NJ, Barnhart EP, Galloway TS, Handy RD, Ford TE. Quantification of changing *Pseudomonas aeruginosa* *sodA*, *htpX* and *mt* gene abundance in

- response to trace metal toxicity: A potential in situ biomarker of environmental health. *FEMS Microbiology Ecology*. 2007;**60**:276-286. DOI: 10.1111/j.1574-6941.2007.00296.x
- [141] An J, Jeong S, Moon HS, Jho EH, Nam K. Prediction of Cd and Pb toxicity to *Vibrio fischeri* using biotic ligand-based models in soil. *Journal of Hazardous Materials*. 2012;**203-204**: 69-76. DOI: 10.1016/j.jhazmat.2011.11.085
- [142] Qu R, Wang X, Liu Z, Yan Z, Wang Z. Development of a model to predict the effect of water chemistry on the acute toxicity of cadmium to *Photobacterium phosphoreum*. *Journal of Hazardous Materials*. 2013;**262**:288-296. DOI: 10.1016/j.jhazmat.2013.08.039
- [143] Wang X, Qu R, Wei Z, Yang X, Wang Z. Effect of water quality on mercury toxicity to *Photobacterium phosphoreum*: Model development and its application in natural waters. *Ecotoxicology and Environment Safety*. 2014;**104**:231-238. DOI: 10.1016/j.ecoenv.2014.03.029
- [144] Tsiridis V, Petala M, Samaras P, Hadjispyrou S, Sakellaropoulos G, Kungolos A. Interactive toxic effects of heavy metals and humic acids on *Vibrio fischeri*. *Ecotoxicology Environment Safety*. 2006;**63**:158-167. DOI: 10.1016/j.ecoenv.2005.04.005
- [145] Abbas M, Adil M, Ehtisham-Ul-Haque S, Munir B, Yameen M, Ghaffar A, et al. *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: A review. *Science of the Total Environment*. 2018;**626**: 1295-1309. DOI: 10.1016/j.scitotenv.2018.01.066
- [146] Gu JD, Cheung K. Phenotypic expression of *Vogesella indigofera* upon exposure to hexavalent chromium, Cr⁶⁺. *World Journal of Microbiology and Biotechnology*. 2001;**17**:475-480. DOI: 10.1023/A:1011917409139
- [147] Cristani M, Naccari C, Nostro A, Pizzimenti A, Trombetta D, Pizzimenti F. Possible use of *Serratia marcescens* in toxic metal biosorption (removal). *Environmental Science and Pollution Research*. 2012;**19**:161-168. DOI: 10.1007/s11356-011-0539-8
- [148] Poirel J, Joulian C, Leyval C, Billard P. Arsenite-induced changes in abundance and expression of arsenite transporter and arsenite oxidase genes of a soil microbial community. *Research Microbiology*. 2013;**164**:457-465. DOI: 10.1016/j.resmic.2013.01.012
- [149] Jiang Z, Li P, Jiang D, Wu G, Dong H, Wang Y, et al. Diversity and abundance of the arsenite oxidase gene *aiiA* in geothermal areas of Tengchong, Yunnan, China. *Extremophiles*. 2014;**18**:161-170. DOI: 10.1007/s00792-013-0608-7
- [150] Zhai W, Wong MT, Luo F, Hashmi MZ, Liu X, Edwards EA, et al. Arsenic methylation and its relationship to abundance and diversity of *arsM* genes in composting manure. *Scientific Reports*. 2017;**7**:42198. DOI: 10.1038/srep42198
- [151] Zhang SY, Zhao FJ, Sun GX, Su JQ, Yang XR, Li H, et al. Diversity and abundance of arsenic biotransformation genes in paddy soils from southern China. *Environmental Science and Technology*. 2015;**49**:4138-4146. DOI: 10.1021/acs.est.5b00028
- [152] Besaury L, Bodilis J, Delgas F, Andrade S, De la Iglesia R, Ouddane B, et al. Abundance and diversity of copper resistance genes *cusA* and *copA* in microbial communities in relation to the impact of copper on Chilean marine sediments. *Marine Pollution Bulletin*. 2013;**67**:16-25. DOI: 10.1016/j.marpolbul.2012.12.007
- [153] Li XF, Yin HB, Su JQ. An attempt to quantify Cu-resistant microorganisms in a paddy soil from Jiaying, China.

Pedosphere. 2012;**22**:201-205. DOI: 10.1016/S1002-0160(12)60006-X

[154] Roosa S, Wattiez R, Prygiel E, Lesven L, Billon G, Gillan DC. Bacterial metal resistance genes and metal bioavailability in contaminated sediments. *Environmental Pollution*. 2014;**189**:143-151. DOI: 10.1016/j.envpol.2014.02.031

[155] Liu YR, Yu RQ, Zheng YM, He JZ. Mint: Analysis of the microbial community structure by monitoring an Hg methylation gene (*hgcA*) in paddy soils along an Hg gradient. *Appl Environ Microbiol*. 2014;**80**:2874-2879. DOI: 10.1128/AEM.04225-13

[156] Geslin C, Llanos J, Prieur D, Jeanthon C. The manganese and iron superoxide dismutases protect *Escherichia coli* from heavy metal toxicity. *Research in Microbiology*. 2001;**152**:901-905. DOI: 10.1016/S0923-2508(01)01273-6

[157] Ruttkay-Nedecky B, Nejdil L, Gumulec J, Zitka O, Masarik M, Eckschlager T, et al. The role of metallothionein in oxidative stress. *International Journal of Molecular Science*. 2013;**14**:6044-6066. DOI: 10.3390/ijms14036044

[158] Saxena G, Marzinelli EM, Naing NN, He Z, Liang Y, Tom L, et al. Ecogenomics reveals metals and land-use pressures on microbial communities in the waterways of a megacity. *Environmental Science and Technology*. 2015;**49**:1462-1471. DOI: 10.1021/es504531s

[159] Jie S, Li M, Gan M, Zhu J, Yin H, Liu X. Microbial functional genes enriched in the Xiangjiang River sediments with heavy metal contamination. *BMC Microbiology*. 2016;**16**:179. DOI: 10.1186/s12866-016-0800-x

[160] Brookes PC. The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility Soils*. 1995;**19**:269-279. DOI: 10.1007/BF00336094

[161] Xia X, Lin S, Zhao J, Zhang W, Lin K, Lu Q, et al. Toxic responses of microorganisms to nickel exposure in farmland soil in the presence of earthworm (*Eisenia fetida*). *Chemosphere*. 2018;**192**:43-45. DOI: 10.1016/j.chemosphere.2017.10.146

[162] Schneider AR, Gommeaux M, Duclercq J, Fanin N, Conreux A, Alahmad A, et al. Response of bacterial communities to Pb smelter pollution in contrasting soils. *Science of the Total Environment*. 2017;**605-606**:436-444. DOI: 10.1016/j.scitotenv.2017.06.159

[163] Nunes I, Jacquiod S, Brejnrod A, Holm PE, Johansen A, Brandt KK, Priemé A, Sørensen SJ. Coping with copper: legacy effect of copper on potential activity of soil bacteria following a century of exposure. *FEMS Microbiology Ecology*. 2016;**92**:fiw175. DOI: 10.1093/femsec/fiw175