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The Use of Biosensors for Biomonitoring Environmental Metal Pollution

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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]; *Lycopersicum 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 (H_2O_2) acts as a signaling molecule in response to HM and other stresses [27]. Under a pollution scenario by HM, H_2O_2 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 m^2 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 m^2, 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 Cr^6; 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].

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Conflict of interest

The authors declare that there is no conflict of interest.

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