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Review Article

# Heavy Metals as Pollutants and Consequences on the Animal Kingdom

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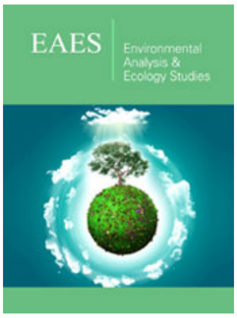

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## Abstract

Heavy metals are the term generally used for metals that have a density greater than 5kg/dm$^3$ and are generally toxic, their residues causing environmental pollution. Metals are considered important toxic pollutants that enter biogeochemical cycles and accumulate in natural and artificial ecosystems. Metals are continuously released into the biosphere by volcanic eruptions, natural weathering of rocks, but also by numerous anthropogenic activities, such as mining, fossil fuel combustion, industrial and urban wastewater, and agricultural practices. On a global scale, there is now evidence that anthropogenic activities have polluted the environment with heavy metals from the poles to the tropics and from mountains to the depths of the oceans.

**Keywords:** Heavy metals; Toxic pollutants; Metal bioavailability; Magnitude of the biomarker response

## Introduction

### The role of heavy metals as pollutants

The distribution of HMs in water, sediments and the atmosphere results from their presence in the Earth's crust. In their natural concentrations, HMs play an essential role in many biochemical processes in the body, but any concentration exceeding the background can become toxic. As a result of anthropogenic activities, current levels are higher than under natural conditions, representing a threat to organisms, since many HMs are harmful even in moderate concentrations [1].

The toxic potential of HMs depends on their bioavailability and physicochemical properties. These properties depend on the atomic structure of the HMs, as shown in the periodic table of elements. Metals are divided into the following categories: alkaline, alkaline earth, transitional, metalloid. Examples of metals that are of greater relevance to the environment in terms of toxic effects are the following: Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni), Tin (Sn), Vanadium (V), Zinc (Zn). Arsenic is also considered a dangerous metal, although from a chemical point of view it is actually a semi-metal (metalloid). Heavy metals are divided into three hazard classes depending on their toxicity to humans and the environment: Class I (extremely hazardous): Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb); and Class II (highly hazardous): Boron (B), Cobalt (Co), Nickel (Ni), Molybdenum (Mo), Copper (Cu), Antimony (Sb), Chromium (Cr), Selenium (Se), Zinc (Zn).

General sources of pollution of the marine environment are represented by: coastal cities and industries, wastewater and industrial residues, household waste and rainwater;

naval transport, dumping of waste into the sea; wrecks, lost or intentionally dumped ammunition, offshore drilling platforms, atmospheric deposition. Terrestrial sources that generate Heavy Metals (HMs) are mainly represented by wastewater treatment plants, manufacturing industries, mining, agriculture. HMs are transported either in dissolved forms in water or as an integral part of sediments. Once in the aquatic environment, they can follow several paths: dissolved in the water column, stored in sediments, volatilized into the atmosphere, taken up by organisms [2]. The HMs are also generated as a result of natural processes of rock erosion. This process is intensified as a result of mining extractive activities that thus expose various ores containing HMs. Leaks from tailings dumps and tailings ponds introduce substantial quantities of HMs into water resources. It is considered that, in the absence of appropriate measures, mining activities pose a great long-term risk in terms of the release of HMs into the environment. Any activity involving the extraction or processing of HMs is a source of fine metal particles dispersed in the atmosphere. Rusting and other forms of corrosion lead to the spread of HMs in the environment, during the use or storage of various metal equipment. The combustion of fossil fuels or various categories of waste also produces the release of HMs into the atmosphere. The greatest deposition of metal particles obviously occurs in the vicinity of mines, smelters, or other categories of metal processing activities, which represent the major sources of emission. But most particles are so small that they can be transported over enormous distances by the wind. In particular, mercury, which is present in gaseous form in the atmosphere, can be dispersed on a large scale, very far from the sources of origin. Road transport is also responsible for significant lead emissions, following the use of fuels containing lead compounds as an additive [3]. The HMs released into the atmosphere are deposited at ground level, where they remain for a long time. Under certain conditions, such as a decrease in pH, HMs in the soil, especially mercury and cadmium, are solubilized and reach water resources [4]. Understanding the mechanisms of interaction between HMs and marine organisms involves the following aspects: bioavailability and mode of uptake of HMs; intervention of possible protective mechanisms; susceptibility of organisms to the various effects produced by exposure.

## Bioavailability of heavy metals for marine organisms

Metal bioavailability is defined as the fraction of the total metal concentration that has the potential to accumulate in the organism. Factors that control metal bioavailability include: the biological characteristics of the organism (metal assimilation efficiency, feeding strategies, size/age, reproductive stage); metal geochemistry (water-sediment partitioning, metal speciation); and environmental physicochemical factors that influence the above factors (temperature, salinity, pH, ionic strength, dissolved organic carbon concentration, total suspended solids), [5]. Speciation of HMs in the marine environment is of fundamental importance because the bioavailability and toxicity of HMs depend on their chemical form in water. Speciation is in turn dependent on the specific physicochemical factors of the marine environment. HMs are present in the marine environment in different chemical forms (dissolved, colloidal or particulate), as a result of the balance between metal ions and inorganic and organic complexes [6]. The bioavailability of HMs is one of the determining factors of their accumulation in marine organisms. The uptake of HMs occurs directly from seawater through the permeable surfaces of the body, in the case of dissolved forms, and through food, in the case of particulate forms. The uptake of HMs from seawater is influenced by the speciation of the metal, the presence of organic or inorganic complexes, pH, temperature, salinity, redox conditions [7]. Intestinal uptake depends on similar factors, to which are added the feeding rate, intestinal transit time and digestion efficiency [8]. Numerous studies have shown that the hydrated free ion form is the majority bioavailable form for copper, cadmium and zinc [9], although exceptions have been reported [10].

Thus, the importance of other chemical forms of dissolved HMs, such as complexes formed with low molecular weight organic ligands, should not be neglected. It has been observed that the presence of organic ligands increases the bioavailability of cadmium in mussels and fish, due to the facilitation of the diffusion of the hydrophobic compound in membrane lipids. Organic compounds of some HMs can be much more bioavailable than ionic forms, the best example being organomercuric compounds that are lipid soluble and penetrate rapidly into the body, having an increased toxicity compared to mercury chloride [11]. Adsorption on suspensions affects the total concentration of HMs present in the water column. The mode of association of HMs in the particulate phase is also critical for the process of uptake by organisms through food ingestion. Sediments accumulate insoluble metal compounds, which can under certain conditions be released into the interstitial water, thus adding to the soluble or suspended HMs in the water column. HMs concentrations in sediments and suspensions are much higher than in seawater, so a small fraction of them can represent an important source for uptake, especially for filter-feeding and buried organisms. It is expected that fine-grained oxidized particles will represent the most important source of available HMs [12]. Numerous studies have shown that the bioavailability of HMs for sediment-feeding bivalve molluscs depends on the type of sediment particles. If the particles are coated with bacterial extracellular polymers or fulvic acids, the bioavailability of cadmium, zinc and silver is significantly increased. Binding to iron oxyhydroxides reduces the bioavailability of HMs present in sediment [13]. The nature of the different forms of HMs in the marine environment remains a variable that is not fully understood. Dissolved or particulate forms of HMs have different uptake and accumulation pathways and require in-depth studies. The specific uptake pathways of free ionic forms and those complexed with organic ligands need to be identified and characterized. It is not known whether there are specific mechanisms for different valence states or for different types of inorganic ionic complexes. The transfer of HMs along aquatic food chains is of interest to environmental health research for several reasons. First, the accumulation of HMs in marine organisms can ultimately result in the trophic transfer of HMs to humans, leading to a potential risk to public health from the consumption of contaminated seafood. The most famous and tragic example was the outbreak of Minamata disease in Japan, following the consumption of seafood containing high concentrations of methylmercury [14]. From an ecosystem health perspective, HMs can be toxic to marine organisms, disrupting ecosystem functioning through a wide range of harmful effects. Living organisms play an important role in the biogeochemical cycles of HMs in the marine environment. Factors that influence metal accumulation are the relative amounts of HMs present in the environment, as well as their chemical form. However, there is considerable variation in metal concentration between species, tissues, and even between individuals collected from the same location. This is because the uptake and elimination of HMs are determined by biological parameters, including the permeability of external surfaces, feeding strategies, amounts and types of internal ligands, the efficiency of excretory systems, nutritional status, growth, season and reproductive stage. Living organisms show a certain selectivity in the accumulation of HMs, and a distinction must be made between essential and non-essential metals. Essential metals such as copper, zinc, manganese, iron or cobalt are vital components of many enzymes and respiratory pigments. Consequently, marine organisms must provide their tissues with HMs in sufficient quantities for metabolic and respiratory needs. Deficiency of these HMs, but also accumulation above certain levels, produce harmful effects [15]. Non-essential metals (lead, arsenic, mercury, cadmium) are very toxic, even at very low levels, especially if they accumulate at metabolically active sites. The body is forced to limit the accumulation of non-essential metals or convert them into non-toxic forms. Toxic HMs interfere with the normal metabolic functions of essential elements. By binding to protein macromolecules, a disruption of normal biological function occurs. Metal-catalyzed formation of oxygen free radicals is implicated in the production of many pathological changes, including mutagenesis, carcinogenesis, and aging [16]. Thus, although HMs are essential components of life, they become harmful when present in excess. Increasing bioavailable levels in the marine environment are a problem for human health and marine ecosystems.

## Effects of heavy metal pollution on marine ecosystems

In recent decades, increased inputs of contaminants and habitat destruction have produced drastic changes in aquatic ecosystems. In this direction, scientific interest has increased in the following areas: the accumulation and toxic effects of contaminants on aquatic organisms; the uptake and accumulation of contaminants in marine resources intended for human consumption. The effects of pollutants can be detected at several levels of biological organization, from the level of the entire ecosystem to the subcellular and molecular levels. The most relevant ecotoxicological assessments, from an ecological point of view, are those that describe changes in the structure and function of ecosystems. These measurements are often difficult, time-consuming and do not allow the correlation of the degree of modification of the ecosystem with a particular level of contamination. At the cellular and molecular levels, pathological changes and biochemical markers that occur following exposure to pollutants have been identified [17]. Correlations have been established between specific pollutants present in certain concentrations and pathological or biochemical responses. However, correlating effects at the individual level with alterations at the community or population level is quite difficult. There are concerns about the relevance of applying physiological and biochemical methods for assessing the effects of pollution at the population level. Inter-individual variability in response to pollutants is of major importance, as it is the key to understanding the selection mechanisms that accompany ecological changes induced by pollution. It is considered that pollutants that do not exert a selection pressure do not cause significant biological effects at the ecosystem level, because they do not produce community restructuring. There is a wide range of methods available for assessing the effects of pollutants in the marine environment, of particular importance being the integrated assessment, with the use of several methods, each targeting a different level of biological organization. Interactions between pollutants and organisms involve several aspects [18]. The first stage in aquatic toxicology studies consists of assessing the type of pollutant, its bioavailability and the routes of uptake by the organism. The distribution of pollutants in the marine environment includes: accumulation in the benthic substrate, distribution in the water column and uptake by organisms. The fraction present in the water column (bound to colloids, particles or dissolved) and in food represents the bioavailable fraction. There are two major uptake routes: the respiratory system (gills) and the digestive system. Food as a source of uptake is especially important in benthic ecosystems, where pollutants associated with sediments are significant for aquatic ecosystems. The uptake of contaminants from sources leads to their concentration in tissues. The rate of bioconcentration depends on many factors, such as temperature, physiological state (sex, season) and the potential for biomagnification along the trophic level. The first stage of contaminant impact - organism is represented by the interaction with endogenous molecules. These interactions are classified into three main groups. The contaminant can be sequestered and then neutralized, and/or can have specific interactions with endogenous molecules (inhibition of some enzymes) and/or can be metabolized by the enzymes of the biotransformation system. All these interactions can lead to: long-term storage (neutralized fraction); direct or indirect toxic effects (after biotransformation); excretion of contaminants or their metabolites.

The toxic effects of pollutants have repercussions at the cellular, tissue or organismal level, thus modifying the integrity of the population and ultimately the entire ecosystem [19]. The response time to the impact of contaminants varies from hours for the molecular and cellular level, to several years at the population and community level. Specificity, in the sense of identifying the pollutant that causes an effect, can only be achieved at the molecular level. A physiological response, such as the effect on growth, represents a non-specific response to a series of environmental stimuli, providing a measure of the overall impact. Even if in this case the ecological relevance exists, it is necessary to elucidate the molecular mechanisms. The term biomarker defines the biological parameters that change in an organism exposed to environmental contaminants. The concept of biomarkers does not only mean biochemical measurements, but also includes: cellular pathology, physiological processes and even the behaviour of an organism exposed to pollutants [20]. This allows for sequential use of biomarkers, starting with non-specific (physiological) biomarkers and ending with specific biochemical and cellular biomarkers (e.g., mixed-function oxidase activity, metallothionein's, intracellular granules, tissue damage) [21]. The magnitude of the biomarker response, together with the determination of tissue concentrations of pollutants, contributes to the overall assessment of the impact of pollution. The behaviour of HMs in marine organisms is described by the mechanisms of uptake, storage, excretion and regulation. General models of metal uptake and accumulation will be discussed in an attempt to understand and explain the variability of tissue levels of HMs.

## Mechanisms of metals uptake by the organism

The motivation for numerous studies on HMs comes from the need to understand the impact of pollution on marine communities. There has been a tendency to investigate mainly the uptake of HMs in solution in seawater, although the nutritional route also has great importance in the uptake of HMs [22]. HMs present in soluble form in seawater penetrate the cells of marine organisms either by passive diffusion processes (invertebrates) or by means of a "carrier" molecule (fish) [23]. The absorption of HMs in solution in seawater occurs both through the general surface of the body and through specialized areas such as the gills or intestinal walls. In most large crustaceans and molluscs, the barrier through which HMs are taken up is represented by the respiratory surfaces, the rest of the body being protected by the calcareous or chitinous exoskeleton. Once metal ions have crossed the barrier and entered the body, they are rapidly bound by intracellular ligands. The presence of ligands with high affinity for HMs, such as glutathione and metallothionein's, in the cell ensures their continuous removal from the inner layer of the cell membrane, thus reducing the concentration of cations in the cell and maintaining a permanent gradient with respect to the external environment [24]. The uptake of HMs bound to suspended matter is mainly correlated with the activity of the digestive tract and represents an important source for organisms [25]. In marine invertebrates (molluscs, crustaceans), metal-bearing suspensions are taken up by endocytosis, an active transport mechanism. At the level of lysosomes, the biological material is degraded, in this way the metal becomes available for the cell or can remain bound to the resulting compounds [26].

## Mechanisms of metals accumulation and elimination

Once in the body, the metal will be accumulated and/or excreted. Accumulation can occur as a result of physiological mechanisms in the case of essential metals, which are directed to tissues to perform metabolic functions. At the tissue level, there may be needs for metal storage, in order to ensure a stock of HMs that meets metabolic requirements [27]. Excess metal ions, which exceed metabolic and storage needs, are toxic and must be removed from the vicinity of biologically important molecules. The metal can be eliminated from the body or bio transformed, before being stored in specific tissues, into inert non-toxic forms. Marine organisms have developed different strategies for taking up, storing or eliminating HMs. Either the penetration of metal ions is restricted, accompanied by mechanisms that ensure a low tissue requirement for essential HMs, or the penetration of all metal ions is allowed, accompanied by mechanisms for storing or eliminating excess HMs. HMs in the body can be initially stored in the tissues where they were taken up (gills, intestine, skin). They subsequently reach the tissues where detoxification, long-term storage or elimination takes place (digestive gland, kidney) [28]. Three main mechanisms for the elimination of HMs have been described in marine organisms [29]. One way, represented by loss at the body surface or gills, characterizes easily mobilized HMs, which are adsorbed on the external mucus or complexed to intracellular or extracellular ligands with low affinity. Another mechanism consists of elimination at the intestinal level. In invertebrates, HMs are eliminated with the waste products of intracellular digestion, which are excreted from the digestive gland into the intestine. Urinary excretion is another route of metal elimination.

## Research and regulation of tissue concentrations

Different tissue concentrations of HMs are explained by the specific properties of each tissue for uptake, retention and excretion. By combining these processes, some marine organisms are potentially able to regulate their concentrations of certain HMs in the body. There are many concerns regarding the regulatory capacity of marine organisms, in the context of their use as biological indicators of metal pollution. For an organism to be used as a pollution indicator, there must be a simple correlation between the level of HMs in the environment and in its tissues. Organisms capable of regulating their metal concentrations do not meet this criterion [30].

In some tissues, the levels of some HMs are maintained within narrow limits by regulatory mechanisms that do not involve the accumulation of excess metal. In other tissues, metal concentrations may be more variable. This may reflect either a less stringent regulation of uptake and excretion, or that the tissue has a storage capacity, allowing the accumulation of HMs in non-toxic, metabolically inert forms. HMs in these stores may be gradually eliminated from the body or may continue to accumulate throughout life. In some organisms, the entire body metal load may be found in a single tissue. Fluctuations in metal concentrations in certain tissues may be masked when the total content in the whole organism is analysed. Concluding that an organism has regulatory capacities solely on the basis of the fact that the total level shows small variations with increasing exposure concentration may be incorrect, if one neglects to observe a significant increase in metal levels in a target tissue or vital organ. Marine organisms take up HMs from food or water, transport them, store them, and excrete them, in order to maintain a continuous flux that controls the concentration of free cations in cells and fluids. In the cells of different tissues, HMs can reach high concentrations, due to the ability of some cells to accumulate excess HMs in a non-toxic form, by binding to soluble compounds or compartmentalization in membrane vesicles and granules [31].

The different biochemical processes involved in metal homeostasis do not show the same degree of activity in all cells of an organism. In the different organs of the same organism, HMs can accumulate differentially. The determining factor for metal concentrations in the organism is represented by their bioavailability from water and food. The nature of the metal (essential or non-essential, chemical properties) and the physiological state of the organism influence the uptake, distribution, accumulation in tissues and excretion [32]. The significance of HMs levels is discussed in relation to the health status of organisms and their use in the biomonitoring of metal pollution.

## Conclusion

Excess HMs have inhibitory effects on the development of marine organisms (phytoplankton, crustaceans, fish). They can affect the growth of molluscs, oxygen consumption, byssus formation, and the reproductive process. Histological changes occur in fish and crustaceans exposed to high concentrations of HMs, such as: changes in the appearance of the gills, necrosis, or fatty degeneration of the liver. Elucidating the effects at the cellular level allows us to understand the ways in which HMs can alter the metabolism and physiology of marine organisms. The cell membrane is the first target structure when HMs penetrate the cell. It has been demonstrated that HMs can bind to membrane proteins and phospholipids, altering their structure and functions. HMs can stimulate lipid peroxidation processes, a complex sequence of biochemical reactions, defined as "oxidative damage to polyunsaturated lipids." The whole process results in the production of compounds that are extremely toxic to the cell, due to their high reactivity towards other cellular components (soluble and membrane proteins, DNA). The biological role of essential HMs is correlated with their high affinity for the active groups of enzymes and structural proteins. The toxic effects of non-essential HMs can be produced by their tendency to substitute essential HMs and compete for biological ligands. Toxic HMs with high affinity for the active groups of proteins can affect the structure and function of these molecules and ultimately the physiology of the cell. The lethal effects of some HMs on crustaceans have been attributed to the inhibition of enzymes involved in cellular respiration. The histological changes observed in fish and crustaceans following chronic exposure to HMs are secondary effects produced by the disruption of nutrition following the inhibition of enzymes of catabolic pathways. The effects on growth and development have been attributed to the inhibition of enzymes involved in protein synthesis and cell division. It is considered that, depending on the metal, the degree of bioaccumulation and the vulnerability of the enzymatic systems, a multitude of effects are possible.

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