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REVIEW

# Biota as toxic metal indicators

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**Abstract** Metal in the environment arises from both natural sources and human activities. Toxic metals in air, soil, and water have become a global problem. They are potential hazards to aquatic, animal, and human life because of their toxicity, bioaccumulative, and non-biodegradable nature. The major impacts of metal pollutants can be stated as ecosystem contamination and health problems of exposed human populations. Those problems have been a cause of increasing public concern throughout the world. Some trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions, and catalyze enzymatic reactions. But even metals that are biologically essential can be harmful to living organisms at high levels of exposure. An increasing concentration of heavy metals in the environment can modify mineral and enzyme functions of human beings. During the last two decades, the interest in using bioindicators as monitoring tools to assess environmental pollution with toxic metals has increased. Bioindicators are flora and fauna members, which are collected and analyzed to measure the levels of metal contaminants. Bioindicators therefore identify health hazards. Various living organisms, such as microbes, fungi, plants, animals, and humans, are used to monitor toxic metals from air, water, sediment, soil, and food chain. Here, we review recent bioindicators, toxicity assessment, and ecological effects.

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## Introduction

In natural systems, potentially toxic heavy metals can originate from rocks, ore minerals, volcanoes, and weathering releases of metals during soil formation transported to the surface and/or aquifer waters (Szyczewski et al. 2009). In the last few decades, the pressure from the activities of the urban population has been intense and anthropogenic emissions of potentially toxic trace metals have accelerated considerably. Anthropogenic impacts of toxic metals are related mostly to the mining, extraction, and refining stages and can be the cause of substantial air, water, and soil pollution (Norgate et al. 2007). Heavy metals once released into the environment—the air, water, and soil—do not disappear, but accumulate in soils, sediments, and biota.

Metals serving as micronutrients in living organisms usually occur in trace amounts that are precisely defined for each species. Both, their deficiency and high excess, badly affect living organisms. They, also directly or indirectly, throughout air, water, and food (plants, animals) get into human bodies. The excessive content of metals in the human body may in many ways affect the body (Stankovic et al. 2011a, b; Jovic et al. 2012). Therefore, the content of heavy metals needs to be known not only in water, air, soil, and sediment, but also in biological samples: plants, animals, and finally, in humans (Stankovic and Stankovic 2013).

Metals fall into one of two categories: essential and non-essential. Essential metals or micronutrients are required for the optimal functioning of biological and biochemical processes in organisms, while non-essential elements have no known biological functions and exert their toxicity by competing with essential metals for active enzyme or membrane protein sites (Torres et al. 2008). Metals that are biologically essential have the potential to be harmful to humans and other living organisms at high levels of exposure (Stankovic et al. 2011a; Stankovic and Jovic 2012). Together with essential nutrients, living organisms also take up heavy metals and can accumulate them.

Living organisms are commonly known as toxic metal bioindicators and their exposure to metals can be measured by either levels or effects. The widespread development and application of plants and animals as bioindicators has occurred primarily since the 1960s, using various animals like birds, mollusks, and mammals (Holt and Miller 2011). As shown in the review of Burger (2006), over 40 % of the bioindicator papers were about metal pollution, wherein fish, plants, invertebrates, and mammals were the dominantly used bioindicator species. For aquatic metal pollution, the commonly used bioindicators mainly contained organisms including plankton, insects, mollusks, fishes, plants, and birds (Zhou et al. 2008; Lam and Wang 2008; Jovic et al. 2011; Hargreaves et al. 2011; Joksimovic et al. 2011a, b; Markovic et al. 2012; Joksimovic and Stankovic 2012; Kitowski et al. 2012).

Higher plants, animals, algae, fungi, bacteria, and lichen have been used as bioindicators in air, soil, and water pollution surveys over the past few decades. Metal content in bioindicators depends not only on the metal concentrations in air, water, soil, sediment, and environmental conditions, but also on the biological factors of the organisms themselves. One of the largest problems associated with the exposure to toxic metals is their potential for bioaccumulation and biomagnification causing heavier exposure for some organisms than toxic metals present in the environment alone. This article is an abridged version of the chapter by Stankovic and Stankovic (2013) in the series Environmental Chemistry for a Sustainable World (http://www.springer.com/series/11480).

## Toxic metals

Metals normally occur in nature and some are essential to life, but can become toxic through accumulation in organisms. Only a few of the numerous metals present in the environment are essential and necessary in minute amounts to all living organisms, the so-called micronutrients. Micronutrients, such as Cu, Zn, Fe, Mn, Co, Mo, Cr, and Se, are required by humans in small quantities, a few milligram or microgram per day, and Ca, Mg, Na, P, and S, are also required, but in larger quantities, 100 mg or more per day, for the optimal functioning of biological and biochemical processes in humans (Stankovic and Stankovic 2013). Micronutrients are involved in the functional activities of living organisms.

Heavy metals occur naturally and from anthropogenic sources in the ecosystems with large variations in concentrations. Pb, Cd, Cr, Cu, Zn, Ni, As, and Hg are the most common heavy metal pollutants, and Hg, Pb, and Cd are of the greatest concern. They can be bioaccumulated through the food chain posing a toxic risk to species higher in the food chain and to humans (Stankovic and Stankovic 2013). The classification of elements from the periodic table, according to their toxicity and uptake, whether easily exposed to organisms, is presented in Table 1.

### Toxic metals in the environment

World mining activities are known to release significant amounts of toxic metals into the surrounding environment, Fig. 1. About 90 % of mine outputs of Cd, Cu, Zn, Ni, and Pb were consumed in the last century (Nriagu 1996). These elements are present at low levels in soil, rock, and water, but the process of world metal mining may release quantities harmful to the health of people and ecosystems, as well as the electric power industry, the primary metal industry, and world metal mining operations. Metals from

**Table 1** Classifications of elements according to toxicity and their uptake (Wood 1974)

| Not critical | Toxic, partially dissolved or easily exposed | Very toxic and easily exposed |
|---|---|---|
| Na, C, F, K, S, Sr, H, Cl | Ti, Ga, Hf, Rh, Nb, Ir | Be, As, Au, Cu, Pd, Pb |
| P, Li, Mg, Al, O, Br, Si | La, Zr, Os, Ta, Ru, Re | Co, Se, Hg, Zn, Ag, Sb |
| Fe, Rb, Ca, N | W | Ni, Te, Tl, Pt, Sn, Cd, Bi |

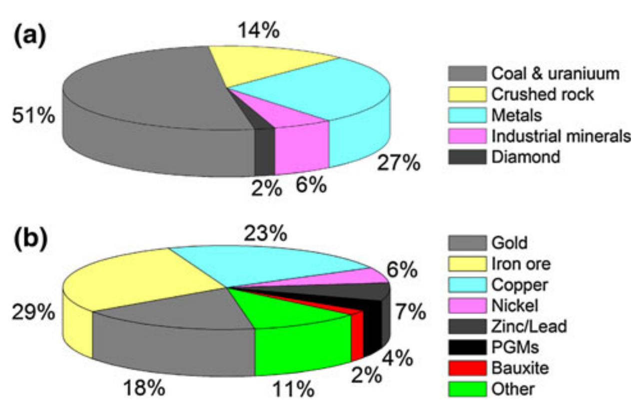


**Fig. 1** **a** Value of world mining activities, **b** world metals value at mine (Östensson 2006)

these sources are dispersed in the environment and they contaminate air, water, and soil. They, directly or indirectly, through air, water, plants, and animals, get into human bodies too.

The metals classified as heavy metals are: Cu, Co, Cr, Cd, Fe, Zn, Pb, Sn, Hg, Mn, Ni, Mo, V, and W (Szyczewski et al. 2009). Within the group of heavy metals, one can distinguish both the elements essential for living organisms (micronutrients) and the elements whose physiological role is unknown, and thus they are "inactive" toward plants, animals, and people. The metals serving as micronutrients in living organisms usually occur in trace amounts that are precisely defined for each species (Szyczewski et al. 2009). Both their deficiency and excess affect the psychophysical development of organisms as well as adults in a harmful way.

Metals are commonly considered as simulators or inhibiting factors of life processes, due to which they may appear toxic for living organisms. This depends on their concentration, ability to form complexes, and degree of oxidation. The strongest toxic properties are the characteristics for inorganic metals compounds, which are easily soluble and they can easily penetrate cell membranes and get into organisms. The free-metal ionic activity may be more important in producing metal toxicity than the total concentration of a metal (Szyczewski et al. 2009).

### *Mercury (Hg)*

The main natural sources of Hg are the emissions from the geothermal and volcanic activity (WHO 2007). The largest anthropogenic source of Hg on a global scale is the combustion of coal and other fossil fuels, including metal and cement production, forest fires, and waste disposal. Annually, anthropogenic pollution reaches approximately 2,000 t of this element, most of which comes from burning coal, milling of non-ferrous metals, and gold mining by amalgamation (Kalisinska et al. 2012). According to official emission data, the total Hg anthropogenic emissions in Europe was 413 t/year in 1990 and 195 t/year in 2003 (WHO 2007).

The total concentrations of Hg in natural waters are normally very low, below 1.0 ng/L; in drinking water, it is in the average of 25 ng/L and the WHO guideline value is 1.0 μg/L (WHO 2007). Dissolved total mercury values are: in an open ocean, 0.5–3.0 ng/L; coastal seawater, 2.0–15 ng/L; freshwater lakes and rivers, 1.0–3.0 ng/L. In the European top soils, Hg concentrations range from 10 to 160 μg/kg, reaching a median value of 40 μg/kg (WHO 2007). An inorganic-Hg form can be converted biologically to methylmercury (MeHg) in soil and water and enters the human body readily via the dietary route (Stankovic and Stankovic 2013).

### *Arsenic (As)*

The total annual anthropogenic As emissions were estimated as 28,000–54,000 tones/year, but this range does not include natural As emissions to the atmosphere, including volcanism, forest fires, and for example, significant amounts of As are released through hydrothermal activities in Yellowstone National Park (Reimann et al. 2009). Plants generally show low As concentrations, much lower than the supporting soils; the suggested value for the world reference plant of As is 0.1 mg/kg (Nagajyoti et al. 2010). The majority of plants have mechanisms for avoiding As uptake, but mosses and lichens consistently show higher As values than other terrestrial plants (Serbula et al. 2012).

The toxicity of As has been well known at least since Roman time (Stankovic and Stankovic 2013). The most carcinogenic of all substances named in current drinking water regulations is As. The drinking water action limit for As was quite recently lowered to 10 μg/L (from 50 μg/L). The As concentration in air is generally very low and ranges from 0.4 to 30 ng/m$^3$; in seawater from 1.0 to 8.0 μg/L, while in marine sediments it ranges from 1.0 to 60 mg/kg. The world-average value for As in soil is 5.0 mg/kg, but varies considerably across geographic regions (Reimann et al. 2009).

### *Lead (Pb)*

Pb is released into the atmosphere from natural and anthropogenic sources. Natural Pb emissions originate from volcanoes, forest fires, and biogenic sources. Major anthropogenic emission sources of Pb include the combustion of fossil fuels from traffic, non-ferrous metal production, and iron and steel production (WHO 2007). The levels of Pb in the environment vary between 4 and 20 mg/g of dust; uncontaminated waters contain Pb in concentrations ranging from 0.001 to 0.06 mg/L, while seawater contains up to 0.03 μg/L (Bardi 2010). In top soils, Pb concentrations are spatially heterogeneous and vary from below 10 mg/kg up to >70 mg/kg. The median value is estimated to be 22.6 mg/kg in the European soil.

In general, ingestion of Pb through food and water is the major exposure pathway for Pb in humans. Individuals will absorb more Pb in their food if their diets are deficient in Ca, Fe, or Zn (Hu 2002). The EU ambient air quality guideline for Pb is 0.5 μg/m$^3$ and in the immediate vicinity of specific industrial sources, the value was 1 μg/m$^3$ until 2010 (WHO 2007). The limit value for Pb in drinking water in the EU is currently 25 μg/L and will be reduced to 10 μg/L by 2013 (WHO 2007). Levels of Pb in soils range from 5.0 to 30 mg/kg. In addition to atmospheric deposition, agricultural practices are a source of Pb input to soils from mineral and organic fertilizers (WHO 2007). According to Krystofova et al. (2009), levels of Pb in the environment are not stable and vary according to industrial production, urbanization, climate changes, and many other factors.

*Cadmium (Cd)*

Cd is released to the biosphere from both natural and anthropogenic sources too. The total Cd emission to air from the natural sources is estimated at about 150–2,600 t (WHO 2007). The global Cd production increased with a factor of four from 1950 to 1990 and in the recent decade the production has slightly decreased. Global emission of Cd into air until the year 2000 was closed to 3,000 t/year and in Europe 257 t in 2003. Emissions of Cd in EU countries have decreased by 50 % and the dominant sources of Cd are atmospheric deposition and commercial phosphate fertilizers (OECD 1994).

On the basis of the Cd contents in surface soils from many parts of the world, the average value lies between 0.07 and 1.1 mg/kg. Values above 0.5 mg/kg usually reflect anthropogenic Cd inputs (WHO 2007). There are three main anthropogenic sources of terrestrial Cd: atmospheric deposition, agricultural application of phosphate fertilizers, and use of municipal sewage sludge as a fertilizer on agricultural soils. It has been reported that 90 % of the Cd in soil remains in the top 15 cm (WHO 2007). Cd levels of up to 5.0 mg/kg have been reported in sediments from river and lakes, and from 0.03 to 1.0 mg/kg in marine sediments. The average Cd content is about 5.0–20 ng/L in seawater, in European rivers roughly varies from 10 to 100 ng/L (OSPAR 2002) and in drinking waters Cd concentrations usually vary between 0.01 and 1.0 μg/L (WHO 2007).

*Chromium (Cr)*

Cr(VI) exists in soils naturally and is the sixth most abundant element in the Earth's crust. Cr is found in all phases of the environment, including air, water, and soil. Cr occurs naturally in Irish agricultural soils in concentrations between 5.0 and 250 mg/kg and in various soil types ranging from 1.04 to 3,015 mg/kg worldwide (Boyle and Kakouli-Duarte 2008). It is also present as a result of human practices, and mostly associated with industry.

Naturally occurring in soil, Cr ranges from 10 to 50 mg/kg depending on the parental material (Shanker et al. 2005). In freshwater, Cr concentrations generally range from 0.1 to 117 mg/L, whereas values for seawater range from 0.2 to 50 μg/L. In the atmosphere, Cr concentrations vary widely from the background concentration of 0.0012 μg/m$^3$ (Shanker et al. 2005). Cr has been extensively shown to induce general environmental toxicity, as well as more specific effects of an acute and chronic toxicity nature, such as neurotoxicity, dermatoxicity, genotoxicity, carcinogenicity, and immunotoxicity. It is believed that Cr inflicts more damage during Cr(VI) reduction to Cr(III), a process considered to be initiated in the cell by glutathione (Boyle and Kakouli-Duarte 2008).

## Air, water, and soil metal pollution

*Air*

Atmospheric pollution causes serious damage to human health and to all natural ecosystems. Among the many inorganic air pollutants originating from anthropogenic activities, heavy metals such as As, Cd, Cr, Hg, and Pb are of a major concern due to their toxicity. One of the most important sources of Cd, Cr, and Pb in the urban environment is road traffic (Melaku et al. 2008). Other anthropogenic air pollutant sources are constructions and agricultural activities, mining and mineral processing, wind-blown dust, and power plants. Coal-fired power plants are responsible for 99 % of Hg emissions. The Hg concentration in air is, in most areas, close to the mean global background value, which is 1.5–2.0 ng/m$^3$ (WHO 2007). In recent years, emissions of Hg into the air in Europe have been declining.

According to Harmens et al. (2008), the main sources of Pb emissions come from manufacturing industries (41 %) and road traffic (17 %). Pb levels in the ambient air in Europe have decreased in recent decades, that is, between 1990 and 2003 they fell by 50–70 %. In the air of rural areas, Pb concentrations are between 0.05 and 0.10 μg/m$^3$. Natural Pb content is estimated to be 6.0 ng/m$^3$ in the atmosphere and the air quality guideline recommended the Cd level of 5.0 ng/m$^3$ to prevent any further increases in Cd level in agricultural soils (WHO 2007).

*Water*

Heavy metals in aquatic systems can be naturally produced by the slow leaching from soil/rock to water, which are usually at low levels, causing no serious effects on human health. Nowadays, the industrial and agricultural development promotes the rapid increase in water metal pollution. Metals can accumulate in aquatic organisms and persist in water and sediments (Sevcikova et al. 2011).

Aquatic heavy metal pollutions usually represent high levels of Hg, Cr, Pb, Cd, Cu, Zn, and Ni in water systems (Zhou et al. 2008). Cu, Ni, Cr, and Zn are essential trace metals to living organisms, but become toxic at higher concentrations. Heavy metals including Hg, Cr, Cd, Ni, Cu, As, and Pb introduced into environmental water system may pose high toxicities on the aquatic organisms (Stankovic and Stankovic 2013). Concentrations of inorganic Hg in surface and ground waters are generally below concentrations known to cause adverse health effects, but MeHg in freshwater fish originates from the soil inorganic Hg and direct atmospheric deposition (Chen et al. 2008). Although the anthropogenic Hg emission in Europe decreased approximately by 50 % after 1990, the MeHg concentration remained the same in freshwater fish (WHO 2007).

### *Soil*

Soil is a fundamental natural resource for agriculture. Initially, heavy metals are naturally present in soils. The presence of heavy metals in the environment has accelerated due to human activities. The contamination of soil mainly occurs through air pollution, wastewater intake, and use of fertilizers in agriculture. The soil environment is a major sink of heavy metals. An extensive literature review of the urban soils trace metals contaminations worldwide is given by Wong et al. (2006). Purely theoretically, every 1,000 kg of "normal" soil contains 200 g Cr, 80 g Ni, 16 g Pb, 0.5 g Hg, and 0.2 g Cd (CAOBISCO 1996). In areas of agricultural and industrial activity, higher concentrations of heavy metals, in comparison to background levels, can be detected (Babula et al. 2008) and may disturb the soil ecosystem, plant productivity, and also pose threat to human health and ecosystems (Musarrat et al. 2011).

Trace metal assessments of urban soils frequently examined to detect metals that were traditionally significant for the environment and health, particularly Cu, Zn, Pb, and Cd (Wong et al. 2006). In the EU, the limit value for Cu in soils is 50–140 mg/kg and the mean levels vary between 13 and 24 mg/kg; the Zn soil level usually falls in the range of 10–300 mg/kg, and in the EU, the limit Zn value for soil is between 150 and 300 mg/kg dw. The limit values in the EU for Pb, Cd, and Hg are between 50 and 300, 1.0 and 3.0, and 1.0 and 1.5 mg/kg dw, respectively (Council Directive, 86/278) (Serbula et al. 2012).

The mean Pb concentration is estimated at 25 mg/kg on the world scale for surface soils. The average contents of Cd in soils are between 0.07 and 1.1 mg/kg, and for various soils, the mean concentrations of Hg do not exceed 0.4 mg/kg on the world scale for surface soils (Serbula et al. 2012). The world-average value for As in soils is 5 mg/kg (Reimann et al. 2009) and the EU limit for As in agricultural soil is 20 mg/kg (Bhattacharya et al. 2010). As(III) is more soluble and more mobile, and is much more toxic than As(V) in soils (Lai et al. 2010), but in well-aerated soils, arsenate [As(V)] is the predominant form. Currently, about half of the world's land and 70 % of total water are used for agriculture (Reijnders and Huijbregts 2009) and consequently soil pollution problems arise from agrochemicals being used.

## Biota

Biota growth and development are essential processes of life and propagation of the species. They are mainly depending on external resources present in water, soil, and air. The presence of toxic metals in the external environment leads to changes in the growth and development pattern of the biota. Metals are commonly considered as simulators or inhibitors of life processes, due to which they may appear toxic for living organisms (Szyczewski et al. 2009). Various metals play a key role to maintain and control various vital functions of living organisms. The essential metals have biochemical and physiological functions in living organisms. Two major functions of essential metals are: (a) participation in redox reaction and (b) direct participation as an integral part of several enzymes (Nagajyoti et al. 2010). The availability of metals in medium varies, and metals such as Cu, Zn, Fe, Mn, Mo, Cr, Ni, and Co are essential micronutrients whose uptake in excess results in toxic effects (Nagajyoti et al. 2010) and can damage living organisms, or in some cases, the organism can be injured or die (Szyczewski et al. 2009).

### Fauna

Metals uptake by fauna occurs through diffusion as well as ingestion of food. Metals can be stored in the skeletal structure and intracellular matrices of fauna organisms, and excreted in feces and eggs. Fauna organisms have additional defense mechanisms, such as heavy metal-binding proteins (Stankovic and Stankovic 2013). In comparison to plants, non-sessile animals can avoid a certain number of environmental or anthropogenic stressors by their mobility.

Mollusks have been successfully used as bioindicators in monitoring aquatic programs (Stankovic and Jovic 2013; Stankovic and Stankovic 2013). Terrestrial ecosystems were much less considered than the aquatic environment. It can be stated that terrestrial bivalves, gastropods, and especially the other mollusks classes have not yet received the attention they probably deserve, in terms of their ecological importance. A number of invertebrate species are known to be efficient accumulators of trace elements (Stankovic and Stankovic 2013).

Terrestrial vertebrates, including wild animals, absorb toxic metals via food, water, air, and through the skin and accumulate them mainly in the liver, kidney, and also in the brain (Kalisinska et al. 2012). Prey, predatory birds and mammals, are accumulators of significant amounts of organic Hg by feeding on beans and small warm-blooded vertebrate herbivores contaminated with Hg (Basu and Head 2010). Toxicity of methylmercury (MeHg) and Cd in mammals has been well documented (Basu and Head 2010).

Birds are primary and secondary consumers feeding on plants, invertebrates and vertebrates. Colonial waterbirds (herons, storks, ibises, pelicans, cormorants, gulls, terns, and marine birds) often have been used or proposed as indicators of Pb, Cd, As, Se, and Hg. Toxic metals such as Cr, Mn, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, and Hg have been studied in several terrestrial, aquatic, and seabirds, as birds are ordinarily at the top of the food web (Horai et al. 2007; Hargreaves et al. 2011).

### Flora

Uptake of elements into plants can happen via different routes: elements can be taken up via roots from soil and sediment and transported to the leaves; also, they may be taken up from the air or by precipitation directly via the leaves. Trace elements may also be taken up via both aforesaid ways, from water if they are aquatic. Many factors influence the plants' metal uptake and include the growing parameters (T, pH, soil aeration, Eh condition, light), the root system, the type of leaves, the type of plant size, fertilization, competition between the plant species, the availability of the elements in the soil/sediment/water or foliar deposits, soil moisture, and plant energy supply to roots and leaves (Nagajyoti et al. 2010).

Absorbed substances may be transported, converted, stored, and accumulated in the different cells and tissues of the plant. Accumulation and distribution of metals in the plant depend on the plant species, the metal level in the soil, water and air, the element species, pH, cation exchange capacity, bioavailability, vegetation period, and other factors (Filipovic-Trajkovic et al. 2012). Plants might react to environmental stress on the cellular biochemical, or morphological scale, and at species or population level (Johnson et al. 2011).

Plants need macronutrients (N, P, K, S, Ca, and Mg) for their development, and essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo for their metabolic needs (Nagajyoti et al. 2010). These metals in high concentrations in plant tissues might have phytotoxic effects, sometimes resulting in plant death (Szyczewski et al. 2009). Heavy metals such as As, Cd, Cr, Hg, and Pb are non-essential elements and toxic to plants. The levels of heavy metals in plants, both terrestrial and aquatic, vary widely because of the influence of environmental factors and the type of plant itself. The metal ranges observed in plants are presented in the review paper of Nagajyoti et al. (2010).

Babula et al. (2008) claim that essential elements are important constituents of pigments and enzymes, mainly Cu, Ni, Zn, Co, Fe, and Mo for algae and higher plants, but all metals/metalloids, especially Cd, Pb, Hg, and Cu are toxic in higher concentrations because of disrupting enzyme functions, replacing essential metals in pigments or producing reactive oxygen species (ROS). The similarity of certain heavy metals to essential heavy metals, for example, couples Cd–Zn, Se–S or As–P, predestinates their high toxicity due to the possibility to replace essential metals in enzymatic systems (Babula et al. 2008).

Plants contribute to the circulation of heavy metals in the food chain through active and passive absorption thereof, accumulation in tissues as well as subsequent being grazed by animals or consumed by humans. Plants have the ability to absorb all metals, especially those essential for their growth and development through the root from the soil, water, and through over-ground vegetative organs from the atmosphere. Levels of heavy metals in various species of plants growing in the same habitats may vary considerably (Wisłocka et al. 2006).

## Human-health implications

Humans can be exposed to metals through inhalation of dust or gaseous particles, or ingestion through food and drink. The 11 elements of highest concern within the European Community are: As, Cd, Co, Cr, Cu, Hg, Mn, Fe, Zn, Ni, and Pb. Some of these elements are actually necessary for humans in trace amounts (Co, Cu, Cr, Mn, Ni), while others are carcinogenic or toxic, affecting the central nervous system (Hg, Pb, As, Mn), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr) (Rai and Pal 2002; Chen et al. 2008; Lavery et al. 2009).

Exposure to heavy metals continues, although adverse health effects of heavy metals have been known for a long time (Rai and Pal 2002). Different levels of human exposure to heavy metals can lead to three different effects: acute effects in which symptoms appear immediately after exposure to heavy metals during a short exposure period; chronic effects are a result of low-level exposure over a long period of time, and finally, lethal effects can be defined as responses that occur when physical or chemical agents interfere with cellular and subcellular processes in the organism at the high level thus causing death (Kakkar and Jaffery 2005). Figure 2 depicts the deposition of metals in humans.

Metals are significant to humans because some of them are most important trace elements in various metabolic enzymes and constituents of cells: Zn, Cu, and Fe form important component of cell and they are the co-factors in several enzymes, while organically chelated $Cr^{3+}$ ion acts as a co-factor in insulin hormone response controlling carbohydrate metabolism in humans (Rai and Pal 2002). The daily need for Cr in humans should be around 50 µg. International Agency for Research on Cancer (IARC) has assessed that Cr(VI) compounds are carcinogenic to humans (Rai and Pal 2002). Other heavy metals such as

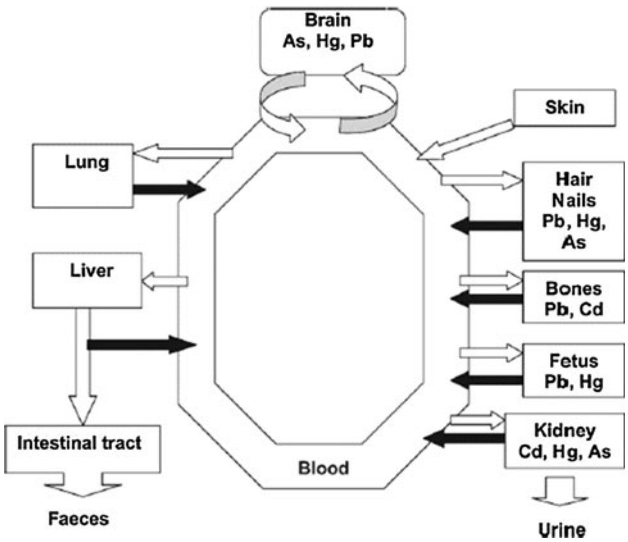


Fig. 2 Deposition of metals in humans (Kakkar and Jaffery 2005)

Hg, Cd, As, and Pb are toxic and have no known vital or beneficial effects on humans (Stankovic et al. 2011a) and their accumulation in the body over time can cause serious illness (Stankovic and Stankovic 2013).

The main source of Cd exposure in the general population is food. Chronic Cd poisoning induces disturbances in Ca metabolism accompanied by softening of bones, fractures, and skeletal deformations, and it became known as "itai-itai" disease (Stankovic et al. 2011a). Liver and kidney tissues are the two main sites of Cd storage and these organs accumulate considerable amounts of Cd, about 40–80 % of the body burden. Important health endpoints include kidney and bone damage and cancer (WHO 2007).

Hg is considered to be a highly toxic metal for living organisms. Human poisoning with MeHg is observed in various parts of the world (Stankovic and Stankovic 2013). Even at very low concentrations, Hg and its compounds present potential hazards due to enrichment in food chain. Human exposure to MeHg occurs mainly through the diet, more specifically, the consumption of fish. The importance of As as a health hazard, also known as a "slow killer," is now well recognized. As is the most common cause of acute heavy metal poisoning in humans, and does not leave the body once it has entered. There is no medicament available for chronic As toxicity. The poison also attacks internal organs, notably the lungs and kidneys, which can result in illnesses including cancer. Long-term ingestion of As contaminated drinking water produces gastrointestinal, skin, liver, and nerve tissue injuries and cancer (Stankovic and Stankovic 2013).

Even the Romans were aware that Pb could cause serious health problems (Rai and Pal 2002). Pb causes serious health hazards to humans, especially to young children, affecting the membrane permeability of kidney, liver, and brain cells, resulting in either reduced functioning or a complete breakdown of these tissues since Pb is a cumulative poison. The full impact of Pb poisoning on the health of children and adults is becoming clearer to most countries, and many governments have begun to take action in this respect (Stankovic and Stankovic 2013).

With regard to essential and non-essential elements, the Joint Food and Agricultural Organization and World Health Organization (FAO/WHO) have established the provisional tolerable weekly intake (PTWI) level, which is defined as an upper intake limit above which adverse health effects might be expected in humans (FAO/WHO 2004, 2007, 2010, Table 2).

Table 2 Tolerable intake levels for trace elements appointed by the FAO/WHO (http://www.inchem.org/ November 6, 2009)

| Element | Provisional tolerable weekly intake (PTWI) (mg/kg body weight) | Provisional maximum tolerable daily intake (PMTDI) (mg/kg body weight) |
|---|---|---|
| Arsenic | 0.015 | – |
| Cadmium | 0.007 | – |
| Copper | – | 0.50 |
| Iron | – | 0.80 |
| Lead | 0.025 | – |
| Mercury | 0.005 | – |
| Methylmercury | 0.0016 | – |
| Zinc | – | 1.00 |

## Regulations

Today, compelled by the growing environmental and health awareness of the public, assessments of an array of trace metals in soils, sediments, water, air, as well as foods are demanded by regulatory guidelines. Those routinely regulated trace metals include As, Ba, Cd, Co, Cr, Cu, Hg, Pb, Mo, Ni, V, and Zn (Stankovic and Stankovic 2013). The significance of heavy metals for the environment quality has been reported in several European Union Directives: Directive 2004/107/EC for As, Cd, Hg, Pb, and Ni monitoring in an air, Directive 2008/1/EC for integrated pollution prevention and control in Europe, Directive 2008/50/EC on ambient air quality.

The aim of a directive is to protect the environment by monitoring water, air, sediment, soil, biota, and to establish common quality rules for their chemical analysis. Within the EU countries, free access to environmental information is guaranteed according to the information directive 90/313/EEC (EEC 1990). The first EU directive in the field of nature protection was the Bird Directive in 1978 (79/409/EEC) (EEC 1979). Intensive Monitoring of Forest Ecosystems was established in 1994 within the framework of the UN-ECE ICP Forest, UN/ECE and EC (2000). Monitoring of heavy metals and other air toxicants is planned according to the directive of the CAFE (Clean Air for Europe), Directive 2004/107/EC (EC 2004).

The increasing bioindicator importance is also encouraged within the European Union's water framework directive (WFD) (EC 2000). The directive aims to achieve a good ecological and chemical status in all European water bodies, such as rivers, lakes, and coastal waters, and requires that the assessment of the ecological status of a system be accomplished primarily utilizing biological indicators. Among the wide range of bioindicators, five biological elements as bioindicators are listed within the WFD: phytoplankton, macroalgae, angiosperms, benthic invertebrates, and fish (Frontalini and Coccioni 2011). The Marine Strategy Framework Directive 2008/56/EC (MSFD) is formulated as follows: "concentrations of contaminants are at levels not giving rise to pollution effects." The recent adoption of human biomonitoring (HBM) as Action 3 in the Environment and Health Action Plan 2004–2010 of the European Commission COM (2004) has motivated the implementation and application of HBM in the European environment and health research.

## Bioindicators

A bioindicator is an organism or a part of an organism or a community of organisms, which contains information on the quantitative aspects of the quality of the environment; exposure of organisms can be measured by either levels or effects. The importance of metals to ecosystems can be evaluated by measuring metal levels in air, water, and soil, and potential effects on organisms. In practice, bioindicators can be any animal, plant, or microbial systems that can be used to formulate conclusions about the environmental conditions they are continuously exposed to (Stankovic and Stankovic 2013). According to Hodkinson and Jackson (2005), a bioindicator is a species or a group of species that reflects biotic and/or abiotic levels of contamination of an environment. Organisms used as metal pollution bioindicators must meet certain criteria: the body must constantly accumulate and tolerate large amounts of toxic metals, it must be tied to a single place to make it a true "representative" for the soil, air, and water environmental area; it must be available for collection, identification, and handling; it must have sufficient tissue for chemical analysis and a long life span to ensure sampling over a longer period of time.

### Animals as bioindicators

The animal species mostly used as bioindicators are zooplankton, invertebrates, and vertebrates. Animal species have been commonly used as indicators of aquatic ecosystems. Generally, metal accumulation by animals is favored by their limited ability to excrete these contaminants directly after their uptake because of metal inactivation by binding to MTs (Zhou et al. 2008).

#### *Zooplankton*

Plankton is composed of phytoplankton and zooplankton microscopic organisms that float freely within oceanic currents and in other bodies of water. Primary source of food in the aquatic food chain is phytoplankton that use chlorophyll to convert energy (from sunlight), inorganic chemicals (like nitrogen), and dissolved carbon dioxide gas into carbohydrates. Phyto- and other plankton are zooplankton's food. The zooplankton genus *Daphnia* is an important link in freshwater trophic chain, as it both consumes phytoplankton and represents a food source to invertebrate and vertebrate predators. The freshwater *Daphnia magna* is one of the oldest and most widely used zooplankton species as a test organism in aquatic toxicology (Ratte et al. 2003). *D. magna* is the most commonly tested freshwater species in acute and chronic tests on toxic metals, such as Cu, Cd, Zn, and Se in water (Lam and Wang 2008).

#### *Invertebrates*

Mollusks play important ecological roles in the different aquatic and terrestrial ecosystems of the world due to their ubiquitous distribution and enormous species number. Although mollusks are basically a marine group of animals, terrestrial and freshwater mollusks have also been successfully used to obtain information on the quality of terrestrial and freshwater ecosystems and to quantify contaminants in their environment. This is particularly the case with the two most diverse classes of mollusks, gastropods, and bivalves (Moloukhia and Sleem 2011).

Gastropods represent the only mollusks class in terrestrial ecosystems and consequently, snails are the only mollusks which can be used for bioindication and biomonitoring purposes in these environments. Particularly terrestrial snails can be utilized as accumulation indicators of metal pollution. Fritsch et al. (2011) compared the concentrations of Cd, Cu, Pb, and Zn in the grove snail and the glass snail, a herbivorous and a carnivorous species, respectively. Based on their results, toxic metal accumulation in snails and small mammals is governed by ecological (diet, habitat, and mobility) and physiological (assimilation and excretion of toxic metals) characteristics of animals (Fritsch et al. 2011).

Since 1976, bivalves have been used to assess the levels of contamination in marine ecosystems, and certain systematic groups, notably mussels and oysters, have been extensively studied worldwide. Within a single species, accumulation can be a function of age, size, sex and genotype, nutritional, and reproductive status. Factors that influence metal bioaccumulation are physical and chemical water properties: temperature, pH, dissolved oxygen, salinity, sediment grain size, and hydrological features of the system (Stankovic et al. 2011a; Stankovic and Jovic 2013).

Marine mussels, oysters, and clams are the most commonly used bivalve groups for toxic metals bioindicators (Jovic et al. 2011; Markovic et al. 2012; Stankovic and Stankovic 2013). Bellotto and Miekeley (2007) confirmed that the mussel *Perna perna* is an efficient toxic metal indicator in Asia–Pacific, Brazil and in tropical areas. Freshwater mollusks have also been used as toxic metal indicators and the most frequently used freshwater bivalve as a toxic metal indicator is the zebra mussel *Dreissena polymorpha*. The zebra mussel, a widespread invasive species in Europe and North America, is an important toxic metal bioindicator of freshwaters (Stankovic and Jovic 2013).

*Vertebrates*

The diet appears to be the most important factor that affects the levels of metals in the tissues of animals. Usually, the higher levels of metals in plants and animals from lower trophic levels mean the greater concentrations of metals in the tissues of higher level of animals, such as fish, birds, and mammals.

*Fish*

Fish are used as test organisms in aquatic toxicology because of their top position in the trophic chain and their role as food for humans. In fishes, trace metals, such as Mn, Co, Fe, V, Cu, Zn, and Se, are necessary in small amounts for metabolic processes, but Ni, Pb, Cr, Cd, Hg as non-essential elements perform no biological role for fish and become toxic above certain concentrations. Fish are well recognized bioindicators of environmental changes and are adequate for water monitoring programs (Hauser-Davis et al. 2012). At present, the main database of fish toxicity data exists for freshwater species. Comparative studies of toxicity data of freshwater fish and marine fish species show that marine species are more sensitive than freshwater species for the majority of substances tested (Chovanec et al. 2003). Gills, liver, kidney, and muscle fish are the organs mostly used for toxic metals investigations. The mobility of many fish species makes it difficult to identify not only the exact source of pollution, but also the time and duration of pollution exposure.

*Birds*

Birds can play an important role as heavy metal bioindicators. The general biology and ecology of birds are well known and birds are easy to identify, but birds are used as often as they could be as metal bioindicators. One disadvantage is that many species are migratory, making it difficult to determine where exposure occurred (Burger and Gochfeld 2004).

Birds have been successfully used to indicate temporal and spatial trends in toxic metal pollutions in terrestrial and aquatic ecosystems (Hargreaves et al. 2011; Zhang and Ma 2011; Kitowski et al. 2012). Birds have been proposed as useful biomonitoring species of pollutants from the year 1993. In many European areas, and also in other continents, birds have proven to be very useful bioindicators of Hg, especially sea- and waterbirds (Burger and Gochfeld 2004; Kitowski et al. 2012). Burger and Gochfeld (2004) analyzed methyl and inorganic Hg separately. MeHg makes up more than 90 % of the total mercury in liver, kidney, muscle, and feathers of birds. Feathers are good indicators of metal pollution (Zhang and Ma 2011). There is usually a significant correlation between concentrations of Pb in feathers and those in internal tissues, including blood (Burger and Gochfeld 2004). The quantities of a metal incorporated into the feather represent the body level at the time of feather growth. Waterbirds' eggs were used as an indicator to detect heavy metals concentrations or their temporal-spatial trends, as they are easier to obtain and can be saved for a longer period as compared to soft tissues (Zhang and Ma 2011).

Hargreaves et al.(2011) investigated As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, and Hg concentrations in the tissues, food, and abiotic environment of Arctic shorebirds and showed significant elements biodilution from soil to invertebrates to shorebirds. The highest levels of metals were recorded in the tissues of carnivore seabirds (Jakimska et al. 2011). The level of Hg concentration was found to be significantly lower in young birds than in adults. It can be either stored in internal tissues such as kidneys and liver or it can be excreted in feathers and eggs (Kitowski et al. 2012). Over the last decade, the international council for the exploration of the sea (ICES) has established a working group on "Seabird Ecology," dealing with the question of the usefulness of seabirds as indicators of the marine environment state.

*Mammals*

Mammals represent useful organisms for biomonitoring purposes and can be used when both temporal and spatial information is required. Among the numerous members of the class of mammals, free-ranging animals or "wildlife" fit best the requirements of a biomonitor. This is because they depend exclusively on the quality of food, water, and air in their habitat. They consume flora or fauna that reflect the local soil, water, and air contamination. Any contamination present will influence the animal and can have an effect on its health. The metal toxic levels in mammals depend on their diet composition and are often influenced by food-chain effects: whether they eat other animals or/and plants.

An omnivore is a kind of animal that eats other animals as well as plants (generally only the fruits and vegetables). Some omnivores will hunt and eat carnivores, herbivores, and other omnivores. A carnivore is an animal that gets food from killing and eating other animals. The most widespread predator in the order Carnivora is the red fox *Vulpes vulpes*. It lives in almost the entire northern hemisphere, from the Arctic Circle to North Africa, Central America, and the Asian steppes, with the exception of Iceland, the Arctic islands and parts of Siberia (Kalisinska et al. 2012). A herbivore is an animal that gets its energy from eating only plants, including grasses. Therefore, potentially toxic trace of Pb, Cd, Hg, As accumulates in mammals (Pokorny 2006; Rudy 2010 and Kalisinska et al. 2012).

Although many wildlife species could be used for toxic metals biomonitoring, the available literature concentrates on only a few species: wild boar, red deer, brown hare, and red fox (Pokorny 2006; Rudy 2010 and Kalisinska et al. 2012). Several taxes of small mammals can also be used for toxic metals biomonitoring of terrestrial (Sanchez-Chardi et al. 2007) and for aquatic ecosystems (Basu et al. 2007). The population of the European brown hare (*Lepus europaeus*) in Central Europe represents one of the best biomonitors for agriculturally used land (Rudy 2010). A very good bioindicator is the roe deer (*Capreolus capreolus*), the species abundant in nearly all parts of Europe in agricultural and forest areas, which is one of the most suitable species for bioindication of toxic metals pollution in terrestrial ecosystems (Pokorny 2006).

In the past decade, marine mammals, especially seals and dolphins, have been accepted as bioindicators due to their long life span and their position at the very top of the marine food chain (Agusa et al. 2011; Bellante et al. 2012; Kakuschke et al. 2012). Marine mammals are extremely susceptible to Hg contamination from both natural and anthropogenic inputs (Wintle et al. 2011; Kakuschke et al. 2012). Metal concentrations were measured in their liver, kidney, and muscle tissue, as well as in blood and plasma (Kakuschke et al. 2012).

### *Absorption and distribution of toxic metals among mammalian organs*

Usually liver and kidney samples are analyzed in order to monitor the Pb, As, Hg, and Cd exposure of an animal (Kakuschke et al. 2012; Bellante et al. 2012). The distribution pattern of Pb and As within these organs is not as uniform as it is found with Cd (e.g., Bellante et al. (2012)). Cd is a non-essential element for mammals. The retention rate of Cd increases in mammals when proteins, Cu, Zn, Fe, or vitamin D, are low in their diet. In addition to dietary uptake, another exposure pathway for Cd is the inhalation of contaminated air. Inhalation of particles containing Cd is the most important exposure pathway in areas with elevated atmospheric Cd levels (Tataruch and Kierdorf 2003).

After absorption in the lung and gut, Cd is transported via the bloodstream to body stores, particularly the liver. In blood, more than 95 % of Cd is bound to protein in the blood cells. In the liver, Cd is bound to MT (Zhou et al. 2008), and the formed complex is transported to the kidneys; approximately 50 % of the total Cd burden of the body is found in the liver and kidneys (Jakimska et al. 2011). In the kidneys, Cd has a very long retention time. Since the kidneys are the main target organ for Cd, they are the best tissue samples for the analysis of Cd in mammals (Tataruch and Kierdorf 2003; Bellante et al. 2012). In muscles, Cd concentrations are low.

The level of gastrointestinal absorption of Hg in animals depends on its chemical form. In its inorganic form, Hg is absorbed up to about 7 % from food, but on the other hand, the absorption of MeHg can be as high as 95 %. The organ distribution of Hg in mammals follows the sequence: kidneys > liver > spleen > brain, in descending order. Concentrations in blood and muscle are low (Tataruch and Kierdorf 2003).

The literature on Hg and Cd in mammals indicates that concentration levels are influenced by feeding patterns. Among terrestrial mammals, Hg concentrations increased from herbivores to omnivores and carnivores. For example, herbivores such as mule deer and various species of rabbits usually contained <1.0 mg Hg/kg fresh weight in liver and kidney, but carnivores such as red fox (*Vulpes vulpes*) contained 10 mg/kg ww (Kalisinska et al. 2012).

Animals as accumulative monitors of heavy metal pollution have some advantages over plants, such as area-related results and comparability to man (Pokorny 2006). Mammalian wildlife has physiological systems that are similar to those of humans in mediating the uptake, distribution, metabolism, and elimination of toxicants (Basu et al. 2007). Humans and many species of mammalian wildlife inhabit similar ecosystems and are exposed to common climates, food sources, and pollutants.

## Plants as bioindicators

Algae, fungi (micro and macro), and other plant communities play a fundamental role for nutrition and life on earth. The main sources of trace elements in them are their growth media. As non-mobile organisms, they are always exposed to the environmental conditions: air, soil, and water pollutants at their sites of growth. Various physiological and biochemical processes in them are affected by metals. Algae and plants growing in metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass production, and metal accumulation.

Accumulation and distribution of heavy metals in the plant depend on the plant species, the levels of the metals in the soil/sediment, water and air, the element species and bioavailability, pH, vegetation period, and multiple other factors (Nagajyoti et al. 2010). The quantity or level of heavy metal absorption in a plant not only depends on the concentration levels of the metals in the physical and chemical composition of the soil/sediment and water, but also varies in different parts of the plant. Plants also have the ability to accumulate heavy metals which have no known biological function (Filipovic-Trajkovic et al. 2012).

Elements are often classified as macronutrients or micronutrients, and as either essential or non-essential for the plant, whereas their concentrations are generally indicated as deficient, sufficient, or toxic. Plant nutrients yet identified and best known as essential are C, H, O, N, P, K, S, Ca, Mg (as macronutrients) and B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Si, Na, and Zn (as micronutrients). When these metals are present in bioavailable forms and at excessive levels, they have the potential to become toxic to plants (Nagajyoti et al. 2010).

Biomaterials such as micro- and macroalgae, fungi, lichens, mosses, tree bark, and leaves of higher plants have been used to detect the deposition, accumulation, and distribution of metal pollution in soil, water, and air. Accumulation and distribution of heavy metals in algae and plants depend on the species, the levels of the metals in the soil, water and air, the element species and bioavailability, pH, cation exchange capacity, vegetation period, and multiple other factors. Plants are able to minimize the adverse effects of excess heavy metals by regulating the distribution and translocation thereof within their organs or cells (Hossain et al. 2012).

### Lower plants

*Algae*, *Fungi*, *Lichen*, *and Moss* In terrestrial environments, bacteria, fungi, algae, and other lower plants play the dominant role in the biochemical cycling of heavy metals. In aquatic environments, algae play a key role in biogeochemical cycling of metals and their accumulation in sediments. For example, the uptake of toxic metals by phytoplankton is the first step in the bioaccumulation in aquatic food webs. Micro- and macroalgae also play an important role in the removal of toxic metals (Azizi et al. 2012; Rybak et al. 2012). Metals sequestered by microalgae are a major contributor to the metal load of the water column as well as to the metal content of sediments (Torres et al. 2008).

Some green algae or phytoplankton like *Scenedesmus subspicatus*, *Chlorella vulgaris*, or *Pseudokirchneriella subcapitata* are in use as standard bioindicators representing primary producers (Ratte et al. 2003). For example, *Klebsormidium*-dominated algal mats are good indicators of high Fe concentration in water, whereas the presence of *Fucus vesiculosus* suggests heavy metal pollution in marine environment. Algal and fungal biomasses are reported to show efficient metal removal from wastewater. The adsorption of heavy metals by algae is highly variable, depending on the metal, the taxon, and other conditions (Das et al. 2009).

Mosses and lichens, due to their bioaccumulative properties, are probably the most frequently used organisms as bioindicators of an aerial heavy metal contamination (Backor and Loppi 2009; Blagnytė and Paliulis 2010; Serbula et al. 2012). Mosses have been applied to measure heavy metal levels and trends within and around urban and industrial areas (Suchara et al. 2011). The use of fungi in the monitoring of heavy metal pollution is limited but some fungal groups are better bioaccumulators than others (Stankovic and Stankovic 2013).

Fresh- and seawater macrophytes are probably the main source of metals for many animals feeding on them, like invertebrates and fish, which are commonly consumed as human food. Therefore, the investigation of metal concentrations in the macroalgae species may provide useful information on the transfer of potentially toxic elements from abiotic compartments, water and sediment, to higher consumers including man. The use of marine macroalgae as bioindicators for trace metal pollution is currently very common (Joksimovic et al. 2011b; Joksimovic and Stankovic 2012). Freshwater and sea macroalgae are able to accumulate trace metals, reaching concentration values that are thousands of times higher than the corresponding concentration in water (Vardanyan et al. 2008; Akcali and Kucuksezgin 2011; Wolff et al. 2012).

### Higher plants

Higher plants have been used as bioindicators in areas with significant air pollution in the absence of mosses, but the use of plants in the bioindication of heavy metal levels is not routinely practiced yet. Different plant organs (leaves, flowers, bark, or roots) from naturally occurring wild plants and trees, and cultivated plants (vegetables and fruits) were evaluated as possible bioindicators of heavy metal pollution (Filipovic-Trajkovic et al. 2012). The most often used parts of higher plants are leaves/needles and a bark of different trees (Serbula et al. 2012).

Trace elements taken up by leaves can be translocated to other plant organs according to Filipovic-Trajkovic et al. (2012) and Serbula et al. (2012). Unlike Pb, Cd contamination cannot be removed from plants by washing/rinsing; it is already distributed throughout the organism. Generally, it is accepted that the normal Cd concentrations in plants are between 0.2 and 0.8 mg/kg and toxic concentrations of Cd are defined as ranging from 5.0 to 30 mg/kg, while Zn is not considered to be highly phytotoxic and the toxicity limit for Zn (300–400 mg/kg) depends on the plant species as well as on the growth stage. According to Kabata-Pendias and Pendias (1992), normal concentrations of Pb in plants are 0.1–10 mg/kg dw; element uptake and release depend on plant species, growth stage, and composition of the soil/sediment, especially Ca. Generally, toxic concentrations of Pb are defined as range of 30–300 mg/kg (Filipovic-Trajkovic et al. 2012).

Distribution of heavy metals in plants is unequal and the largest is in the tree bark. After the tree bark, heavy metals are mostly accumulated in the roots, then in the leaves, and as well as in the plant fruits. In over-ground organs, the highest amounts of Pb are found in the leaves and then in the fruits and vegetables (Filipovic-Trajkovic et al. 2012). Hg that presents in the soil has very low availability to plants as the roots function as a barrier. When organmercuric compounds were still in use as fungicides, it was shown that in wheat, barley, oats, and corn, Hg was transferred from the seed dressing into the new seedling. During growth, dilution of the mercury occurred and the concentration in the grain was reduced (Tataruch and Kierdorf 2003).

## Toxic metal bioindicators

The oldest and widely used species for toxic metal bioindicators are fish, bird, and mosses species, while species representing soil organisms are currently becoming more important in toxic metals bioindication. Representative species traditionally used for risk assessment of chemicals are various freshwater species representing bacteria, microalgae, invertebrates, and fishes and among the terrestrial species mainly used are the earthworm *Eisenia fetida* and some higher plants. The standardized bioindicators of the different trophic levels for the most commonly studied toxic metals in the environment compartments (air, soil, and water) are shown in Fig. 3. Up to now, toxic metals distributions were considered more homogeneous in aquatic systems than in terrestrial ones (Stankovic and Stankovic 2013).

Toxic metal biomonitoring in the air using mosses and lichens has been established for many years in Europe and North America. The terrestrial plants are promising indicators for the soil metal pollution. Biomonitoring based on

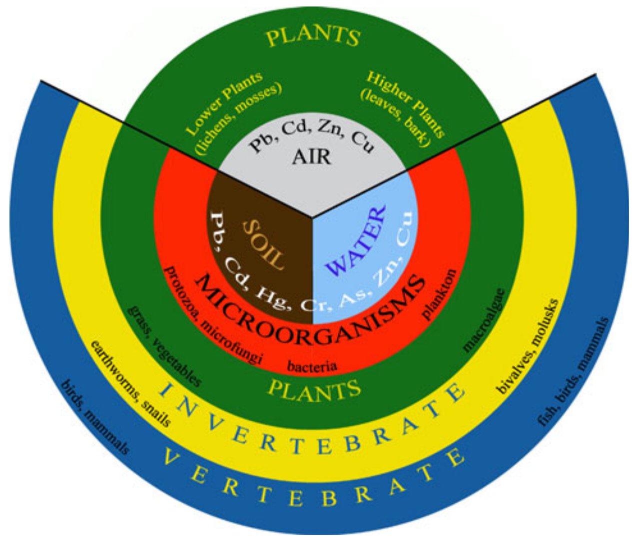


**Fig. 3** The most analyzed heavy metals in the biota of environmental compartments from reviewed literature (Stankovic and Stankovic 2013)

plants as heavy metal air pollution indicators has been widespread all over the world since the 1970s. Foliage and bark of trees are widely used in this regard (Dmuchowski and Bytnerowicz 2009).

### Bioindicators for air metal pollution

Numerous different bioindicators are used in monitoring air pollution: bacteria, fungi, mosses, lichens, grasses, agricultural crops, and plants (Stankovic and Stankovic 2013). Which plant species will be used as bioindicators also depends on how widely they are distributed throughout the region. The usage of air pollution bioindicators usually covers metal toxicity in traffics, smelters, mining industries, industrial pollution, coal-burning power plants, and agriculture (Harmens et al. 2008; Blagnytė and Paliulis 2010; Suchara et al. 2011; Paoli et al. 2012).

Typical examples of biological indicators of air pollution are lichens and mosses (Conti and Cecchetti 2001; Blagnytė and Paliulis 2010). Lichens and mosses are mostly used for atmospheric trace elements bioindication due to their capacity to accumulate and store heavy metals and other toxins. How lichens and mosses do not have roots, they are able to be direct bioindicators of the air pollution (Suchara et al. 2011). Lichens and mosses may be considered as the most commonly applied organisms as bioindicators. The most commonly used lichen and moss species for toxic metals biomonitoring were *Parmelia sulcata*, *Hypnum cupressiforme*, *Hylocomium splendens*, and *Pleurozium schreberi* (Paoli et al. 2012; Blagnytė and Paliulis 2010). Lichens are symbiotic organisms of fungi and algae, and have been widely used in biomonitoring of air pollution by trace elements and can be used to monitor air quality changes in urban areas over intervals of several years (Paoli et al. 2012). Naturally growing mosses have been widely used as effective bioindicators of metal air pollution. Moss appears to be an excellent bioindicator for the next monitoring elements and their uptake by mosses decreases in the order: Cu > Pb > Ni > Co > Cd > Zn, Mn (Harmens et al. 2008). Moss still appears to be one of the best air pollution bioindicators; however, based on literature search of Aboal et al. (2010), it could be concluded that mosses can be bioindicators for certain metals, such as Pb or Cd, probably because these elements are of almost exclusively atmospheric origin.

According to Harmens et al. (2008), many European countries have used mosses in national and multinational surveys of atmospheric-metal deposition. Different moss types are currently widely used as bioindicators since they obtain most of their nutrients directly from the air and by dry deposition. The procedures for the moss as a bioindicator, the methods of collection, processing and analyses of moss samples are outlined in the International Moss Monitoring Manual (Markert et al. 2011). But it is important to note that a unique species that can be a suitable indicator for biomonitoring of toxic metal pollution all over the world has not been found yet. For this reason, different species of mosses are useful as bioindicators in different parts of the world (Blagnytė and Paliulis 2010).

In the last decades of the twentieth century, a rapid increase in bioindication studies of pollutant loads in higher plants is observed (Stankovic and Stankovic 2013). Depending upon the type of the tree, the deployment of heavy metal content and manners of accumulation show a great variety: in some trees heavy metals are filtered out by the leaves from the air, while in others they are taken up by their crown or by their roots. Different plant organs (leaves, flowers, stems, or roots) from naturally occurring wild plants and trees, and cultivated plants (vegetables and fruits) were evaluated as possible bioindicators of heavy metal pollution too (Filipovic-Trajkovic et al. 2012). Filipovic-Trajkovic et al. (2012) found the highest amounts of heavy metals in the leaves, especially Pb, followed by the fruits and vegetables. They concluded that fruits and vegetables were metal avoiders. Tree barks of different tree species are used as metal bioindicators for longer term of air pollution (Baslar et al. 2009). Tree bark and higher plants leaves started to be used to detect the deposition, accumulation, and distribution of air metal pollution on large-scale air pollution (Kord et al. 2010; Serbula et al. 2012).

### Bioindicators for aquatic metal pollution

Specific programs for monitoring toxic metals in aquatic systems were undertaken as early as the 1960s using various animals like invertebrates, mollusks, and vertebrates, like mammals and birds (Stankovic and Stankovic 2013). Heavy metals play an important role as substances affecting aquatic organisms. Aquatic heavy metal pollution usually represents high levels of Hg, Cr, Pb, Cd, Cu, Zn, As, etc., which, introduced into environmental water system, may pose high toxicities on the aquatic organisms (Zhou et al. 2008). Many pollutants are associated with sediments in aquatic systems. Living benthic foraminiferal taxa were studied in surface sediment samples (Frontalini and Coccioni 2011). Foraminifera have been proven to be successful candidates as part of an integrated monitoring aquatic pollution program. The statistical analysis reveals a strong relationship between trace elements, in particular Hg, Mn, Ni, Pb, and Zn, and the occurrence of abnormalities in foraminiferal taxa (Frontalini and Coccioni 2011).

Microorganisms, such as protozoa, green algae, or bacteria, reflect the water quality only 1 week or 2 weeks prior to their sampling and analysis, whereas insect larvae, worms, snails, and other macroinvertebrate organisms reflect the condition from more than a month, and possibly several years prior to sampling. Microorganisms have proven capability to take up heavy metals from aqueous solutions, especially when the metal concentrations range from <1.0 to about 20 mg/L (Ahmad 2006). Microbes such as bacteria exist at the lowest trophic level, so bacteria have the ability to detect toxic compound before other organisms. Thus, bioindicators using bacteria have been commercialized, such as the *Lux-Fluoro*, the Polytox™, the Deltatox™, and the Microtox™ (Ahmad 2006). The environmental monitoring of toxic metals by bioindicators like bioluminescent bacteria (BLB) in marine environment is reported by Ahmad (2006).

Phytoplankton, as the important elementary producer in marine and inland waters, plays the key role to the whole ecosystem. Some phytoplanktons are bacteria, some are protists, and most are single-celled plants. Among them, the common kinds are cyanobacteria, green algae, and fungi (Das et al. 2009). The aquatic alga species and amounts can directly reflect the water quality (Zhou et al. 2008). As an example, the green alga *Chlorella ellipsoidea* was reported to exhibit growth inhibition due to Cu, Zn, Ni, and Cd exposure. Zooplankton species *D. magna* is the most commonly tested freshwater species in acute as well as in chronic tests (Zhou et al. 2008). *Daphniidae* toxicity test is the essential assay for worldwide water quality assessment.

The usefulness of river plankton for the toxicity of metals is measured as their inhibitory effect on the photosynthesis of natural algal assemblages, such as *Aulacoseira granulata*, *Actinocyclus normanii*, *Stephanodiscus neoastrea*, and *Cyclotella meneghiniana*, among many others. Several algal species accumulate considerable amounts of metals and can thus be used as indicators for Cd, Cu, or Pb. Most metals are slightly to highly toxic to algae, with As, Cu, Hg, and Zn having the greatest toxic effects. Both cyanobacteria and green algae exhibit concentrations of various metals proportionally to ambient concentrations (Azizi et al. 2012).

Most of what is stated for freshwater indicators equally applies to bioindicators in the marine environment, such as bacteria and phytoplankton, but macroalgae in coastal marine waters are far more important as bioindicators than macrophytic algae in freshwaters. Accumulation of heavy metals from the surrounding seawater makes them ideal bioindicators. Red algae (*Gracilaria* sp.), often dominant macroalgae of sea communities, are frequently deployed for Cd, Cu, and Zn coastal monitoring (Jakimska et al. 2011). Among the green macroalgae, the genera *Ulva, Enteromorpha*, and *Posidonia*, have attracted considerable attention as toxic metal bioindicators (Stankovic and Stankovic 2013). Aquatic macroalgae have been used for monitoring the contamination level of various heavy metals in aquatic environments, such as Zn, Pb, Cd, and Hg, and many others, as these plants have the ability to accumulate metallic ions (Vardanyan et al. 2008; Joksimovic et al. 2011b; Luy et al. 2012.

Fish are used as test organisms in aquatic toxicology because of their top position in the trophic chain and their role as food for humans. During their life cycle, fish feed on algae, rotifers, microcrustaceans, macroinvertebrates, higher plants, and other small fish. Fish are one of the most frequently used groups of bioindicators in water bodies. In the review by Sevcikova et al. (2011), the most important and most studied metals in fish are Fe, Cu, Cr, Hg, and Pb. Freshwater mussels are used as bioindicators of Pb, Cd, Hg, and Zn (Stankovic and Jovic 2013). It appears that freshwater gastropods and freshwater species, in general, may be less sensitive than their marine relatives, although there are insufficient freshwater data to ascertain whether there is a real difference in sensitivity.

Various aquatic organisms occurring in rivers, lakes, seas, and marines are potentially useful as metal pollution bioindicators for sediments and waters, including fish, shellfish, oyster, mussels, clams, aquatic plankton, and macroalgae (Joksimovic and Stankovic 2012; Stankovic and Stankovic 2013). To achieve adequate geographical and temporal toxicant bioindication, mussels (the blue mussel—*Mytilus edulis*, the Mediterranean mussel–*Mytilus galloprovincialis*) and fish (Atlantic cod—*Gadus morhua*, herring—*Clupea harengus* and flounder—*Platichthys flesus*) were selected as state indicators (EEA 2003). Other marine species, like marine mammals (seal, sea lion) and seabirds can also be used for the biomonitoring of metal pollution (Jakimska et al. 2011).

## Bioindicators for soil metal pollution

Various abiotic and biotic soil characteristics can be used as indicators for evaluating soil health. With increasing heavy metal concentrations, the activities of soil microbes, soil enzymes, and nitrogen fixation are inhibited, and growth of microfloral communities such as fungi, algae, and photosynthetic bacteria is reduced. Plants and soil inhabiting organisms such as soil microflora, and microfauna (protozoa), fungi, nematodes, earthworms, mites, and insects have been used as biotic indicators of soil toxicity (Park et al. 2011).

There are many ways in which bacteria, microfungi, and algae can take up toxic metal ions from a soil. The environmental monitoring of toxic pollutants by bioluminescent bacteria (BLB) in terrestrial environment is reported; the presence of toxicants in the soil sample reduced light emission of the bioluminescent microorganisms. General reductions in microfungal numbers have often been noted in soils polluted with Cu, Cd, Pb, As, and Zn. The soil protozoa *Tetrahymena pyriformis* are used for Cu and Zn determination in soil (Ahmad 2006). Mosses as well as higher fungi have developed accumulation mechanisms with regard to heavy metals from the soil (Kalac 2010).

The types of soil invertebrates used in monitoring pollutant effects include: nematodes, oligochaetes (earthworms), gastropods, springtails, isopods, arachnids (Stankovic and Stankovic 2013). The soil nematode community has been suggested to be a useful indicator of the status of soil pollution and soil ecological status because of their influence on soil food webs and plant–soil interactions (Sochova et al. 2006). The concentrations of heavy metals such as Cr, Cd, Pb, Zn, and Ni influence the soil nematode community structure (Park et al. 2011). In the United States, a guide was accepted and described the use of nematode *Caenorhabditis elegans* in soil toxicity tests (Boyle and Kakouli-Duarte 2008).

By far, the most common invertebrate soil bioindicators used to assess soil metal contaminations are members of the Family *Lubricidae* and *Eisenia* spp. earthworms (Annelida, Oligochaeta) (Hirano and Tamae 2010). Earthworms (*E. foetida*) are capable of accumulating Hg and Cd, Cu, Pb, and Zn; significant positive correlations have been found between metal concentrations in the earthworm and in the soil (Hirano and Tamae 2010; Olayinka et al. 2011), impacted by the substrate they consume and the length of exposure. Earthworms may be available alternative to traditionally applied organisms in aquatic ecosystems, such as fish, because they are simple and they can provide indications of metals bioavailability in a short time at relatively low cost. They have been extensively studied, and are approved for use in toxicity testing by the US EPA and the European Economic Community and the Organization for Economic Cooperation and Development.

Besides aquatic and marine gastropods, terrestrial gastropods (snails) are recognized as adequate bioindicators, because of their ability to accumulate Pb, Zn, Cu, and Cd (Madoz-Escande and Simon 2006). They also exhibit a very wide distribution of a limited number of species, for example, *Deroceras reticulatum* snails are found across much of North America, Europe, North Africa, and Atlantic islands (Hall et al. 2008). Snails are known to play an important role in the diet of many species, including snakes, toads, beetles, and birds.

In the case of vertebrates, in general, only a few vertebrate species spend most of their time throughout the year in close contact with the soil ecosystem. Examples would include some small mammals, such as ground squirrels, and some larger mammals, such as fox (Stankovic and Stankovic 2013). Red fox is a species who lives in a wide geographical range (Europe and North America), but occurring in a small home range for the entire year. It has a high position in the trophic pyramid and accumulates various toxic metals, including Hg (Kalisinska et al. 2012).

Exposure among terrestrial vertebrates occurs through ingestion of contaminated biotic or abiotic matter, contaminant absorption through skin, or via inhalation of volatile, aerosolized, or particle-bound contaminants. In most cases, exposure of wild mammals to contaminants is likely through oral consumption, either by ingesting contaminants incorporated into dietary food and water. Dietary exposure in mammals is a function of age, sex, and season, with mammalian dietary range from pure herbivorous to exclusively carnivorous diets, with virtually all gradients in between, which also impacts their contaminant exposure (Smith et al. 2007).

The use of physiological and biochemical plant parameters in the bioindication of heavy metal soil contamination is not routinely practiced yet. Some responses of higher plants to soil heavy metal contamination have a certain potential. The symptoms of reduced root growth, reduced seed sprouting, necrosis, and chlorosis appear in susceptible plants grown in soils contaminated with heavy metals (Park et al. 2011). Plants, besides macronutrients, require essential micronutrients for their development: Fe, Zn, Mn, Ni, Cu, and Mo. Most of these micronutrients accumulate in the plant tissues for their metabolic needs, but they never exceed 10 mg/kg. Yildiz et al. (2010) have reported the normal natural concentration intervals for toxic metals in terrestrial plants such as the following: Cd: 0.2–2.4 μg/g, Ni: 1–5 μg/g, Zn: 20–400 μg/g, Fe: 70–700 μg/g, Pb: 1–13 μg/g, Mn: 20–700 μg/g; they found that the level of accumulation in the high plant sample was soil-oriented. Higher levels of these metals and other heavy metals in plant tissue might have phytotoxic effects, sometimes resulting in death (Winkelmann 2005). But high levels of heavy metals in the soil do not always indicate similar high concentrations in plants (Brej 1998).

### Metal toxicity bioindicators for humans

For human contaminants, the approach firstly involves examining the contaminants in all the media, which form pathways for human exposure—food, drinking water, air, and soil, and then setting out data on contaminants in different human tissues: blood, urine, hair, nails, etc. The use of hair as an indicator is not new. Over 200 years ago, hair was analyzed to measure As levels in the body (Hubbart 2012). Hair can provide a more permanent record of trace elements associated with normal and abnormal metabolism, as well as trace elements assimilated from the environment (Hashem and Abed 2007). Furthermore, hair is easily collected, and may better reflect the total body pool of certain elements than either blood or urine as short-term indicators (Hubbart 2012). For example, hair is a long-term exposure bioindicator to MeHg: once Hg is incorporated into the hair, it remains unchanged (WHO 2007).

To detect the presence of As, Hg, or Pb, placenta, urine, finger/toenails, and human milk have been repeatedly used as indicators of humans' toxic metals exposure (Smolders et al. 2009). Heavy metals such as Hg, Pb, and Cd are the well-known toxicants to cross the placenta and to accumulate in fetal tissues (Gundacker and Hengstschläger 2012). Unlike urine, blood, placenta, and human milk, hair and fingernails can record the level and changes of elements in the body over a long period of time (Ayodele and Bayero 2010).

Humans can be affected directly by air and water metal pollutions, as well as indirectly through contaminated food supplies. Metals that are biologically essential have the potential to be harmful to humans and other living organisms at high levels of exposure (Freisinger 2010), because they irreversibly bind to active sites of enzymes; destroying normal metabolism by producing high-level toxicity and excessive content of heavy metals in human body may affect the body and psychophysical development (Szyczewski et al. 2009).

## Biomarkers of metal toxicity in living organisms

A conventional monitoring system of environmental metal pollution includes measuring the level of selected metals in the whole organism or in respective organs. However, measuring only the metal content in particular organs does not give information about its effect at the subcellular level. Therefore, the evaluation of biochemical biomarkers, metallothioneins (MTs), phytochelatins (PCs), and antioxidant enzymes (catalase—CAT, superoxide dismutase—SOD, glutathione S-transferases—GST and glutathione peroxidases—GPXs, lipid peroxidation—LP), may be useful in assessing metal exposure and the prediction of potential detrimental effects induced by environmental metal contaminants (Stankovic and Stankovic 2013).

Toxicity does not depend on total accumulated metal concentration. Accumulated metal concentrations should be interpreted in terms of different trace metal accumulation patterns dividing accumulated metals into two components: metabolically available metals and stored detoxified metals. The relationship between metal accumulation and toxicity is influenced by physiological activity of living organisms (Shariati and Shariati 2011). After penetrating the plasma membrane, the incoming metal is bound immediately by ligands and distributed between sites of storage, efflux, or toxic action.

A number of trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions, and catalyze enzymatic reactions (Nagajyoti et al. 2010). For example, Cu, Zn, and Fe are essential as constituents of the catalytic sites of several enzymes. Although some heavy metals are essential micronutrients for animals, plants, and many microorganisms, depending on the route and dose, all heavy metals demonstrate toxic effects on living organisms via metabolic interference and mutagenesis. The mechanisms by which metals exert their toxicity in living organisms are very diverse, especially their involvement in oxidative biochemical reactions through the formation of reactive oxygen species (ROS) (Torres et al. 2008; Nagajyoti et al. 2010). Heavy metals are involved in toxic redox mechanisms through the generation reactive oxygen species, associated with oxidative damage to important biomolecules, and molecular mechanisms of metal toxicity and carcinogenicity (Vlahogianni and Valavanidis 2007).

Metal ions can penetrate inside the cell interrupting cellular metabolism and in some cases can enter the nucleus. The entrance of the metal into the cell can mobilize several metabolic pathways and genetic processes to neutralize the source of toxicity (Azevedo and Rodriguez 2012). The entrance of certain metals into the nucleus can enhance the synthesis of RNA that codes from metallothioneins (MTs). MTs are low-molecular-weight peptides found mainly in the cytosol, lysosomes, and nucleus, high in the amino acid cysteine that contains a thiol group (-SH), which enables MTs to bind heavy metals (Nordberg and Nordberg 2009).

MT in the physiological system has several roles, especially in the metabolism and kinetics of metals: transport and detoxification of metal ions and protection from metal toxicity, free radical scavenging, storage of metal ions, metabolism of essential metal ions, immune response, genotoxicity and carcinogenicity, chemoresistance, and radiotherapy resistance (Shariati and Shariati 2011). Considering the heavy metal detoxification significance of MTs, these proteins seem to be more specifically involved in responses to heavy metals and can serve as the environment heavy metal pollution biomarkers (Valavanidis et al. 2006; Nordberg and Nordberg 2009; Shariati and Shariati 2011; Hauser-Davis et al. 2012). MTs and other selectively metal-binding proteins have found a comparable attention and application for aquatic studies, in aquatic mollusks and fish, especially snails and mussels, gastropods, insect, crustaceans, mussels, fishes, as well as in terrestrial surveys (Dallinger et al. 2000).

MTs are low-molecular-weight proteins with many sulfhydryl groups binding a variety of metals, showing a strong affinity toward certain essential and non-essential trace elements, such as Cu, Zn, Cd, and Hg. So far, MTs have been identified in a large number of species throughout the animal kingdom, and although a variety of biochemical data prove MTs to be structurally well defined, but their biological function is still under discussion (Freisinger 2010). A number of studies demonstrated that the synthesis of MTs can be induced by certain trace elements, but also by organic chemicals and other non-chemical stress factors, like infections, starvation, and injuries. Nevertheless, it has been shown that Zn, Cu, Cd, Hg, and other trace elements are the most potent inducers of MT synthesis (Nordberg and Nordberg 2009).

It has been concluded that the detoxification of metals is the primary biological function of these proteins. The involvement of MTs in Cd detoxification of terrestrial gastropods has been proven in detail for a number of species (Dallinger et al. 2000). MTs are found in all tissues of fish, particularly in the liver and kidney, and play an important role in the intracellular regulation of the essential metals Zn and Cu. MT concentrations in fish tissues increase by fish exposure to Cu, Zn, Cd, or Hg and the affinity of Cd and Hg to MTs is even higher so that they may displace essential metals (Zhou et al. 2008). Increased levels of stress proteins reflect the gradual impact on the fish metabolism ranging from adaptive to degenerative responses with severe consequences for fish survival.

MT can be used as an indicator in both environmental and biological monitoring reflecting human exposure to metals. Metal-binding proteins have special functions in the detoxification of toxic metals and also play a role in the metabolism of essential metals. MTs are involved in the regulation of the essential metals Cu and Zn and in the detoxification of Cd and Hg (Zhou et al. 2008; Freisinger 2010; Sevcikova et al. 2011). The synthesis of this protein probably represents the body's defense mechanism against the toxic Cd and Hg ion. MT in urine can be used as a sensitive biomarker for metal-induced nephrotoxicity and MT is an established biomarker in biomonitoring of human Cd exposure. MT mRNA in lymphocytes in humans has been suggested as an indicator of susceptible groups in relation to metal exposure (Nordberg and Nordberg 2009).

Both animal and plant cells are capable of generating—via multiple sources—a number of different reactive oxygen species (ROS), including the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical (·OH); all ROS are harmful to organisms at high concentrations

(Torres et al. 2008). The potential of oxygen-free radicals and other reactive oxygen species that able to damage tissues and cellular components in biological systems, called oxidative stress, have become a topic of significant interest for environmental toxicology studies (Valavanidis et al. 2006). Metal ions possess the ability to produce reactive radicals, resulting in DNA damage, LP, and depletion of protein sulfhydryls. When ROS levels exceed antioxidant defenses, the cells go into oxidative stress which causes membrane LP and changes in the activity of the antioxidant defense enzymes (Vlahogianni et al. 2007), like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), and no enzymatic antioxidants, such as glutathione, vitamin E, ascorbate, B-carotene, and urate.

Antioxidant defense enzymes play an important role in cellular antioxidant defense systems and protection from oxidative damage by ROS. Heavy metals are involved in toxic redox mechanisms through the generation of ROS, associated with oxidative damage to important biomolecules and molecular mechanisms of metal toxicity and carcinogenicity. Although there are considerable gaps in the knowledge of cellular damage, response mechanisms, repair processes, and disease etiology in biological systems, free radical reactions and the production of toxic ROS are known to be responsible for a variety of oxidative damages leading to adverse health effects and diseases (Valavanidis et al. 2006).

Many enzymes need cofactors to work properly, such as $Fe^{2+}$, $Mg^{2+}$, $Cu^{2+}$, and $Ca^{2+}$. The substitution of one heavy metal ion by another leads to the inhibition or/and loss of enzymatic activity. Antioxidant enzyme activities, oxidative damages such as lipid peroxidation—LP, and metal content in the marine species have manly been studied (Stankovic and Stankovic 2013). LP by ROS is considered to be a major mechanism by which heavy metals can cause tissue damage. The negative correlations found for LP with SOD and mainly GST activity highlighted the importance of these enzymes in preventing oxidative damage in mussels. CAT activity was also positively correlated with SOD and GST activities, which emphasizes that the three enzymes respond in a coordinated way to metal-induced oxidative stress (Semedo et al. 2012). Correlations between metal accumulation and biomarkers of oxidative stress, such as LP, CAT, and SOD in marine mollusks were found (Vlahogianni and Valavanidis 2007; Duarte et al. 2011; Giarratano et al. 2011). The study of Vlahogianni et al. (2007) showed that seasonal variations of the antioxidant defense enzymes and LP concentrations in mussels can be used as potential biomarkers of metal toxicity for long-term monitoring in marine coastal ecosystems.

Heavy metal toxicity is one of the major abiotic stresses also leading to hazardous effects in plants. A common consequence of heavy metal toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG). Both can cause lipids peroxidation, protein oxidation, inactivation of enzymes, DNA damage, and/or interact with other vital constituents of plant cells. Higher plants have evolved a sophisticated antioxidant defense system to scavenge ROS and MG (Hossain et al. 2012).

Heavy metal toxicity results in the accumulation of excessive ROS inside the plant cell. For example, Cu can directly generate ROS, whereas Cd is a redox-inactive heavy metal and can only generate ROS indirectly by enzyme inactivation. Potentially a very important mechanism of heavy metal detoxification and tolerance in plants under heavy metal stress is chelation of heavy metals in the cytosol or intracellular fluids. Plants make two types of peptide metal-binding ligands: phytochelatins (PCs) and metallothioneins (MTs) (Hossain et al. 2012).

MTs and PCs have been identified in a wide variety of plant species and in some microorganisms (Hegelund et al. 2012; Hossain et al. 2012). PCs and MTs are different classes of cysteine-rich heavy metal-binding protein molecules. MTs are cysteine-rich polypeptides encoded by a family of genes. In contrast, PCs are a family of enzymatically synthesized cysteine-rich peptides (Hossain et al. 2012). PCs are a family of Cys-rich polypeptides, although the most common PC forms have 2–4 peptides (Hossain et al. 2012).

PCs are structurally related to glutathione (GSH), and numerous physiological, biochemical, and genetic studies have confirmed that GSH is the substrate for PC biosynthesis. GSH is a tripeptide with thiol group (-SH) of cysteine. It can be synthesized in the human body from the amino acids. While all cells in the human body are capable of synthesizing glutathione, liver glutathione synthesis has been shown to be essential. In animal cells, GSH is catalyzed by glutathione S-transferase enzymes. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. GHS has a vital function in Fe metabolism (Kumar et al. 2011) and it is the major free radical scavenger in the brain (Gawryluk et al. 2011).

Recent plant molecular studies have shown that GSH by itself and its metabolizing enzymes act additively and coordinately for efficient protection against heavy metal damage in plants. PC synthase is primarily regulated by the activation of the enzyme in the presence of heavy metals (Hossain et al. 2012). The biosynthesis of PCs is induced by many heavy metals, including Cd, Hg, Ag, Cu, Ni, Au, Pb, As, and Zn; however, Cd is by far the strongest inducer.

Plants are not able to metabolize or eliminate Cd (Hossain et al. 2012). They adopt the strategy of making Cd-GSH and Cd-PCs complexes. Zhang and Ge (2008) found a close relationship between Cd level and GSH content as well as enzyme glutathione *S*-transferase (GST) activity in rice, suggesting that these two parameters of antioxidant defense system may be used as biomarkers of Cd-induced stress in plants. Glutathione peroxidases (GPXs) are key enzymes of the antioxidant network in plants. Cuypers et al. (2002) suggested that peroxidase activity can be used as a potential biomarker for heavy metal toxicity in plants. Similarly, a significant increase in GPX activity was also observed in red onion exposed to a variety of Hg, Pb, Cr, Cu, Zn, or Cd concentrations suggesting that the elevated activity of GPX was a result of heavy metal-induced free radical generation (Fatima and Ahmad 2005).

Research on plant MTs lags behind what is known about the vertebrate forms (Freisinger 2010). The large diversity in the metal-binding regions of plant MTs suggests that they have the ability to bind a greater range of metals than their animal counterparts and, consequently, a greater range of function (Cobbett and Goldsbrough 2002). In plants, MTs are extremely diverse (Hossain et al. 2012) and their role in the detoxification process has not been conclusively shown (Freisinger 2010). The high metal ion binding capacity of MTs suggests a role in metal ion storage, metabolism, and trafficking of essential $Cu^{+}$ and $Zn^{2+}$ ions, as well as the detoxification of non-essential metal ions such as $Cd^{2+}$ and $Hg^{2+}$ in living organisms. The precise MTs role in living organism remains elusive (Freisinger 2010).

PCs have been shown to play an important role in the detoxification of certain heavy metals in both plants and animals. PCs play a wider role in heavy metal detoxification in biology than previously expected, but it appears that some organisms probably do not express a PCs synthesis. There is, for example, no evidence for PC synthase in mouse and human genomes. Organisms with an aquatic or soil habitats are more likely to express PCs (Cobbett and Goldsbrough 2002).

## Conclusion

Without question, economic growth and social development are critical for improving environment, human health, and well-being. The toxic metals are widely distributed in the environment and their early identification is fundamental to prevention or control of the damages to humans and ecosystems. A wide range of legislation now exists in Europe and other countries to address the release of metals into the environment, including water, soil, and air, as well as toxic metal control of food.

In the last few decades, investigations have focused on searching for bioindicators such as microorganisms, plants, and animals that accumulate toxic metals, even man. Bioindication is the use of an organism, a part of an organism, or a community of organisms, in order to obtain information on the quality of its/their environment. Thus, the use of bioindicators should help to describe the natural environment and to detect and assess human impacts. Considering all the results presented in Stankovic and Stankovic (2013), it can be concluded that Zn, Cu, Pb, Cd, and Hg are the most intensively investigated elements in air, water, and soil bioindicators. Their concentrations depend on the investigated species and environmental compartment.

The largest number of bacteria, microorganisms, plant, and animal species are used as bioindicators of heavy metals in the aquatic ecosystems. The lowest number is used for the soil contamination. Mosses are particularly effective bioindicators of aerial heavy metal levels and trends within and around urban and industrial areas. Higher plants have appeal as indicators in air pollution monitoring in highly polluted areas where mosses are often absent. The use of plants in the bioindication of heavy metal contamination is not routinely practiced. Some responses of higher plants as bioindicators of soil contamination to heavy metals have the potential.

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