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Methodologies for assessing exposure to metals: speciation, bioavailability of metals, and ecological host factors

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Abstract

Host factors play a role in the bioavailability of metals, making it critical to understand their nature and how to measure them, as well as how to measure bioavailability with respect to host factors. The host factors that are critical to consider during all phases of bioavailability studies are age, gender, size, genetic characteristics, behavior (food chain considerations), and interactions between all of them. Some of these vulnerabilities are unique to individuals, populations, species, or communities. There are many interactions between and among metals, the species of metals, and the physical environment (pH, salinity). Some factors enhance uptake and absorption, whereas others moderate it. Moreover, some metals have greater effects on invertebrate organisms, whereas other metals (or species thereof) affect vertebrates more strongly. Fish and wildlife are useful as sentinel species and bioindicators because they can help us understand the risk to the organisms themselves, to the ecosystem, and to humans.

1. Introduction

The importance of metals to ecosystems and their component parts was initially evaluated by measuring metal levels in air, water, and soil, and inferring potential effects on organisms. Subsequently, laboratory experiments were conducted to examine the effects on organisms of particular exposures. These experiments, as well as field observations of metal concentrations in media and in organisms, and of effects, have led to the realization that a wide range of other influences contribute to those effects. These include bioavailability and a range of host factors, including age, gender, size, and susceptibility.

Methods to measure and evaluate the effects of metals in the environment are complicated not only by bioavailability and susceptibility, but by measurement tools and detection limits, environmental conditions, and the interrelatedness of the metals themselves (Rattner and Heath, 1995). Factors within the hosts themselves influence both levels and effects, as well as the measurement tools and methodologies used to assess them. Furthermore, exposure can be measured by either levels or effects.

Two fact, speciation and bioavailability, are frequently interlinked because the speciation of the metal is often related to the bioavailability of different metals. In this article we consider speciation of individual metals and the effect that speciation has on bioavailability. Then we offer a more general discussion on bioavailability and the host factor(s) involved.

In aqueous systems, bioavailability is often correlated with the free-metal concentration, because the free ion is often the most bioavailable form of a dissolved metal. This is sometimes referred to as the free-ion activity model (Campbell and Tessier, 1996). Nevertheless, this is not a universal truth; for example, Simkiss (1983) found that chloro complexes of mercury were strongly lipophilic and more readily taken up than Hg^{2+}.

An important point when considering free-ion concentration of metals is that, until recently, most published reports reported exposures as much too high being because of background contamination. Techniques for cleaning samples (for example, washing of

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[1] Halfway through the writing of this article, my esteemed friend and colleague, Dr. David Peakall, passed away. For over 20 years I have learned from him, collaborated with him, and enjoyed his company and companionship. I will miss him dearly. This paper is a tribute to his long commitment to ecotoxicology and improved environmental health, and I feel the responsibility strongly.

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feathers) have reduced external contamination. Furthermore, with improved instrumentation, detection levels are now several orders of magnitude lower than in the past.

Bioavailability can be measured or calculated by a number of methods, including laboratory studies and field studies examining metal concentration in different organisms at different trophic levels and on different parts of the food web. Bioavailability has traditionally been defined to include the availability of metals to organisms as well as the availability of metals to tissues within organisms once inside the organisms. Although laboratory experiments, including biokinetic models, are useful in examining bioavailability, it is the behavior of metals in ecosystems that is salient for ecological risk assessment.

Table 1
Host factors affecting vulnerability to metal contamination for ecological receptors (after Burger and Gochfeld, 2001)

Exposure differences
Habitat related
Temporal patterns
Food chain effects
Individual differences
Age related
Gender related
Size and weight
Nutrition
Individual (genetic)
Family or species (taxa) vulnerabilities
Individuals vs populations

2. Evaluation methods for metal toxicity considering host factors

Effects of metals on organisms must be considered within a context of physical influences affecting transport and fate, as well as vulnerabilities that are unique to individuals, species, populations, and communities. Also, a distinction can be made between the host factors that affect external dose (how much enters the organisms itself) and internal dose (how much reaches the target organ). For example, food chain effects (Fig. 1) are often external, whereas other factors affect both external and internal dose.

Different individual organisms and species are affected in various ways, depending upon the breeding cycle, foraging methods, geographical ranges, and life history strategies (Table 1). Host factors that must be considered when developing methods to assess the effects of metals include exposure differences, food chain effects, age- and gender-related vulnerabilities, family or species vulnerabilities, as well as genetic and individual differences. Life history parameters (i.e., life span, age of parental dependency, migratory behavior) affect exposure and influence bioavailability. Understanding the relative role of each of these factors requires controlled laboratory experiments (Burger and Gochfeld, 2000, 2001).

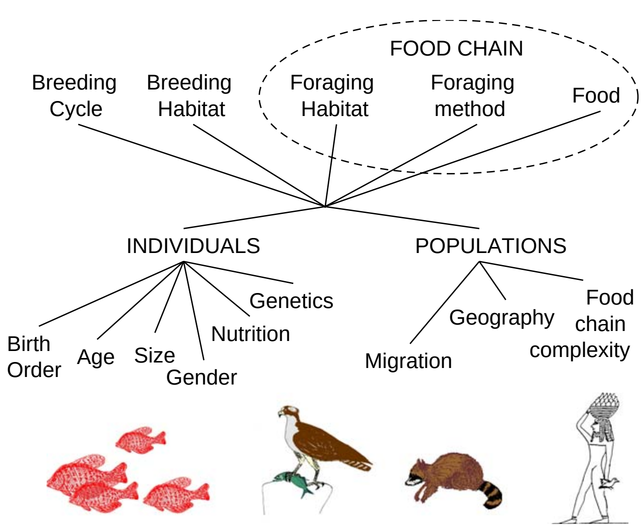

Fig. 1. Schematic of the host factors that affect bioavailability of metals, or other contaminants.

All other factors being equal, biota are exposed to different levels of contaminants because they occupy different habitats (e.g., different levels in the water column) and are active at different times. For example, some invertebrates and fish come to the surface at night (to avoid predation), making them vulnerable to predators that forage at night. Some birds, mammals, and sea turtles, for example, migrate from region to region, moving from one ecoregion to another, where bioavailability of metals varies dramatically.

Food chain effects, or bioaccumulation within successive trophic levels, has been well documented for some groups of organisms, as well as for some metals (see later). Organisms that feed lower on the food chain generally are exposed to lower levels of metals, although this varies because plants can bioaccumulate high levels of some metals; such plants are often used for bioremediation. Other host factors that must be considered are age- and gender-related vulnerabilities and differences in susceptibility. For example, young that are limited to a given space, as birds would be in a nest, are normally exposed to the food that parents can obtain within a reasonable foraging distance of their breeding colonies. On the contrary, animals that move from one region to another are exposed to metals with different bioavailabilities within that region. Such ecoregions can have profound effects on metal uptake, and on other anthropogenic exposures as well.

Many animals show gender-based size dimorphism, which leads to differences in the kinds of prey eaten or the size of prey eaten. This in turn can lead to different contaminant loads, because in many organisms, such as fish, metal levels increase with size and age (Lacerda et al., 1994; Bidone et al., 1997; Park and Curtis, 1997). From a human health perspective, this means that exposure can be reduced by eating smaller fish, as well as fish of different species (Burger et al., 1999)

Some animal species or families are more vulnerable because of not only their relative role on the food chain, but of their foraging methods or habitats, or their breeding habitats. Seals that leave the water to breed on beaches near industrial regions will be exposed to higher levels of contaminants that those hauling out on remote, more pristine beaches. Similarly, birds that nest on islands in urban estuaries and bays will be exposed to higher levels of contaminants than those nesting on remote, offshore islands. Some species differences may relate to internal toxicodynamics, leading to species differences even within closely related organisms (Lock and Janssen, 2001a). For example, seabirds seem to survive methylmercury exposure better than other birds, perhaps because they can demethylate mercury, allowing them to live with tissue levels that far exceed the values considered to produce lethal effects (Eisler, 1987; Burger and Gochfeld, 1997a; Thompson and Furness, 1989).

Finally, the relative distribution and size of populations of an animal species must be assessed when developing monitoring methods, because endangered or threatened populations are more at risk. The permissible methods are more restricted; invasive or lethal methods may be neither permitted nor desirable. Less invasive sampling techniques involving feathers (birds), hair (mammals), or tail clippings (reptiles), may be required. Animals that die for other reasons (from collisions with aircraft or cars, or from predators or floods) may prove useful for assessing internal metal levels.

3. Speciation and bioavailability of individual metals

3.1. Aluminum

In freshwater systems the speciation and solubility of aluminum are highly pH dependent. An outline of the system is shown in Fig. 2 (Spry and Wiener, 1991). Solubility is lowest between pH 6 and 7, with 90% of the aluminum existing as a colloidal solid. The solubility increases a 100-fold between pH 6 and pH 4.7.

The toxicity of Al to fish is primarily due to effects on osmoregulation by action on the gill surface (McDonald et al., 1989). Al is readily accumulated on and in the gill, but little is found in blood or internal organs. Thus the embryo is the life stage least sensitive to Al, whereas the

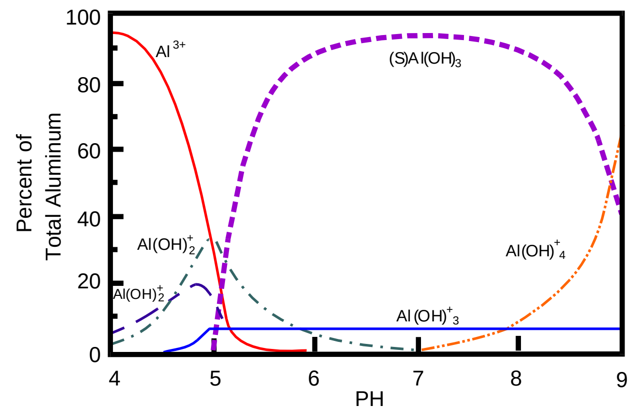

Fig. 2. Chemical speciation of aluminum for a 1 μmol/mL solution at 25°C and a total ionic strength of 100 mmol/mL. S indicates precipitated species (drawn after Spry and Wiener, 1991).

fry stage (small larval fish) is the most sensitive; then sensitivity decreases with age (Cleveland et al., 1986). Aluminum toxicity is interactive with that of the hydrogen ion and usually occurs at pH values ranging from 0.3 to 0.6 pH units above that at which the hydrogen ion causes some lethality. The toxicity of aqueous Al is reduced by Ca and dissolved oxygen (Spry and Wiener, 1991).

There are many reports of Al lethality to fish especially from Scandinavia (Henriksen et al., 1984; Skogheim et al., 1984). The relative contribution of low pH and elevated Al is difficult to determine and varies among geographic regions (Schindler, 1988).

3.2. Arsenic

The speciation of environmental arsenic has been extensively studied in the marine environment (Phillips, 1990; Neff, 1997) but little data seem to be available for other systems. The interest in marine ecosystems arises from human health concerns due to the high levels of arsenic found in some seafood. For example, Bohn (1975) found values ranging from 15 to 307 μg/g (dry wt.) in several species of fish from the west coast of Greenland, and plaice (*Pleuronectes platessa*) from the North Sea ranging from 12 to 216 μg/g (Falconer et al., 1983).

The dominant form of As in oxygenated marine and brackish water is as arsenate, As(V); the more toxic arsenite, As(III), usually composes only a small percentage of the total As content, which in the ocean is ~1.7 μg/L. Arsenate dominates in oxidized sediments, but reducing sediments reduce arsenate to arsenite, primarily in association with sulfide minerals. Marine algae accumulate arsenate, reduce it to arsenite, and then convert it to a wide range of organoarsenic compounds. Arsenobetaine $(CH_3)_3As\ CH_2CO_2$ is the dominant form, but many other compounds including, arsenocholine, several arseno-sugars, and arseno-lipids have been reported (Phillips, 1990).

The hazards of As in the marine environment were assessed by Neff (1997), who concluded that, although the concentration of As in seawater exceeds by two orders of magnitude the US EPA water-quality criterion for human health, it does not represent a serious hazard to marine animals and ecosystems. In heavily contaminated areas the concentrations approach, and sometimes exceed, those that could be toxic to sensitive species of phytoplankton. Marine animals have only a limited ability to bioconcentrate inorganic As from seawater but can bioaccumulate organoarsenic compounds, Thus nearly all of the As in the tissue of marine animals is in the form of arsenobetaine. Fortunately, this compound has a low toxicity to vertebrates and is readily excreted.

The effects of As in other organisms may be relatively low because of low bioconcentration, but this requires additional study. Arsenic levels in seabirds and marine mammals are generally low and rarely exceed 1 μg/g wet wt. (Norstrom et al., 1986; Ohlendorf et al., 1985).

3.3. Cadmium

Transfer of cadmium from terrestrial to aquatic systems is not very efficient; 94–96% remains in the soil. The cadmium that reaches the water, however, generally accumulates in vegetation more quickly than in biota (Huckabee and Blaylock, 1973). In water, 20% of the cadmium remains in suspended particles (Huckabee and Blaylock, 1973). Organocadmium compounds have not been reported in the environment, although a few unstable dialkyls can be synthesized in the laboratory. Thus, the formation of organocadmium compounds does not appear to be involved.

In freshwater, Cd speciation is controlled largely by pH, oxidation potential, and complexing ligands. For pH values 8, the dominant species is Cd^{2+}; at higher pH it is precipitated as the carbonate and thus is not bioavailable. Cadmium can form complexes with fulvic and humic acids; the extent to which this occurs depends on the concentration of the ligands and the ionic strength. As salinity increases, the dominant species becomes chloro-complexes, with $CdCl^{+}$ dominant in estuarine waters and $CdCl_2$ in oceanic water. It is generally accepted that only free Cd^{2+} can be accumulated by organisms, whereas chloro-complexes are not taken up. There is no evidence for biomagnification within marine or freshwater food webs (Jensen and Bro-Rasmussen, 1992).

In soil the most important influence controlling metal mobility is pH. In the pH range 4–7.7 the absorption capacity of soil was found to increase two to three times with each increase of 1 pH unit (Christensen, 1984). The uptake by plants varies greatly among species, virtually no Cd was taken up by peas and beans, whereas appreciable concentrations were found in cabbage and lettuce. There are also substantial differences in the Cd concentration in various parts of the plant. In general they decrease in the order roots > leaves > fruiting parts > seeds (Jensen and Bro-Rasmussen, 1992). The effects of the binding of Cd by metallothionein are considered later.

Cadmium is a teratogen, carcinogen, and possible mutagen (Eisler, 1985). Adverse effects in fish and wildlife are probable when Cd concentration exceeds 3 μg/kg (ppb) in freshwater, 4.5 μg/kg in saltwater, 1000 ppb in the diet, or 100 $\mu g/m^3$ in air (Eisler, 1985). Liver concentrations in vertebrate kidney or liver that exceed 10 μg/g (ppm, wet wt.) should be viewed as causing sublethal effects, and 200 μg/g (wet wt.) in the kidney is usually life-threatening (Eisler, 1985). Nonetheless, there are few reports of Cd-induced injury to terrestrial wildlife; these ecosystems are less affected. Cadmium levels of 40 μg/g in the kidney can be considered the threshold concentration for effects, although for seabirds, the threshold may be higher (Furness, 1996).

3.4. Lead

To confirm laboratory experiments that demonstrated the possibility of methylation of lead in sediments (Jarvie et al., 1975; Wong et al., 1975), the possibility of this occurring in the environment was examined. Harrison and Laxen (1978) concluded that tetraalkyl lead is formed in the environment. On the basis of their calculations of how much tetraalkyl lead would be expected in rural sites. They found that the level there was higher than elsewhere and attributed this to a natural source. This can hardly be called firm evidence, as the authors claimed. However, the conversion of inorganic lead to organic lead is a difficult process and probably does not normally occur in nature.

Studies in the highly contaminated Mersey Estuary in the United Kingdom (Ripley and Towner, 1984) found that although the effluent at Ellesmere Port contained di-, tri-, and tetraalkyl Pb compounds tetraalkyl form disappeared rapidly. The more stable di- and trialkyl Pb compounds were considered to be the cause of mortality of seabirds in the upper reaches of the estuary in the late 1970s. A route of bioavailability that is unique to Pb is the ingestion of lead shot by birds. Waterfowl are exposed to Pb through the ingestion of spent lead shot, presumably thinking that it is grit or food. Considerable mortality of waterfowl has been reported in the United States and Europe (summarized in Pain, 1992). Raptors that predate or scavenge on waterfowl are also at risk. Lead poisoning has been observed more frequently in bald eagles (*Haliaeetus leucocephalus*) than any other nonwaterfowl species. Approximately 200 cases of lead poisoning were diagnosed at the US National Wildlife Health Research Center (Locke and Friend, 1992). The widespread mortality of the national bird was one of the main reasons for the ban in 1991 on the use of lead shot in wildlife hunting in the United States. The progress that has been made toward the banning of lead shot has been reviewed by Scheuhammer and Norris (1996).

The two main sources of environmental Pb, lead shot and leaded petroleum, are decreasing. Using stable isotope ratios, Scheuhammer and Templeton (1998) were able to show that the Pb in juvenile herring gulls (*Larus argentatus*) came predominantly from leaded gasoline, whereas that in waterfowl and raptors it came from shotgun pellets (Pain, 1996). Smelters are a contemporary source of environmental Pb (ATSDR, 1999); this accounts for continued elevated Pb levels in some organisms. Moreover, smelting leads to atmospheric transport and deposition in remote areas. The potential for external contamination rather than internal assimilation exists, and in measuring Pb in hair or feathers it is critical to wash them carefully to remove external contamination (Walsh, 1990).

Lead is a neurotoxin that causes behavioral deficits in fish, birds, and mammals within days of exposure to sublethal concentrations, and these effects can persist after removal from the contaminant (Weber and Dingel, 1997; Burger and Gochfeld, 1997b, 2000). Lead also causes deficits or decreases in survival, growth rates, development, behavior, learning, and metabolism, as well as increased mucus formation in fish (Eisler, 1988). Calcium can reduce the uptake, and therefore the effects, of lead in fish (Varanasi and Gmur, 1978). Little information is available on the levels of Pb in muscle that are associated with impairments in the fish themselves, but levels of 50 μg/g in the diet are associated with reproductive effects in some carnivorous fish, and dietary levels as low as 0.1–0.5 μg/g are associated with learning deficits and abnormal social behavior in some mammals (Eisler, 1988).

3.5. Mercury

The methylation of mercury by microorganisms in sediments was demonstrated by Jernelöv (1969). Methylation was aided by high organic content and aerobic conditions Under anaerobic conditions conversion is slower, and if sulfur is present the highly insoluble mercuric sulfide is formed. Speciation is critical for mercury toxicity; methylmercury crosses the blood–brain barrier, whereas inorganic mercury does not; moreover, methylmercury is nearly completely absorbed across the intestine, whereas only a small percentage of inorganic mercury is absorbed (Scheuhammer, 1987).

Ph is critical in the uptake of Hg in freshwater systems. The uptake of Hg in the biota of two similar small lakes with differing pH, neither of which received any point source emissions, was examined by Scheuhammer and Graham (1999). They found that the concentration of Hg in fish was significantly higher in the acidic lake (pH 5.2–5.6) than in the circumneutral lake (pH 6.3–6.9) and in some individuals in the acidic lake the levels were high enough to be hazardous to breeding piscivorous birds such as the common loon (*Gavia immer*), whereas no individuals in the circumneutral environment approached this threshold (0.3 μg/g wet wt.). Meyer et al. (1998) found a strong negative linear relationship between the Hg level in the blood of loons and the pH of the lake on which they were born (Fig. 3, Meyer et al., 1998). They also found a relationship between chick exposure to Hg and productivity. As the authors point out, there is also the possibility that reduced fish abundance in lakes with low pH may be a factor in declining loon populations.

There are associations among metals (Thompson, 1996); some enhance absorption or uptake, whereas others inhibit it. The protective effect of Se on Hg toxicity has been known for about 30 years (Berlin, 1978; Ganther et al., 1972; Satoh et al., 1985). Studies in various organisms have found a tendency for the two to be positively correlated in tissues (Eisler, 1985; Caurant et al., 1994; Kuehl and Haebler, 1995; Wagemann et al., 1996). The concentration of Hg and Se were found to be highly correlated in the livers of marine mammals (ratio of 1:1) over a range of concentrations from 1 to 1000 μg/g (Koeman et al., 1975). A strong correlation was also found in the livers of seabirds, although here the Hg:Se ratio was 1:5 (Ohlendorf et al., 1986). More variable ratios have been found in fish (reviewed in Cuvin-Aralar and Furness, 1991). The high levels of Hg reported in marine mammals, with values as high as 510 μg/g wet wt. of Hg and 270 μg/g wet wt. Se (Wagemann and Muir, 1984), without showing any signs of toxicity suggest that the presence of the two elements together may provide a protective effect.

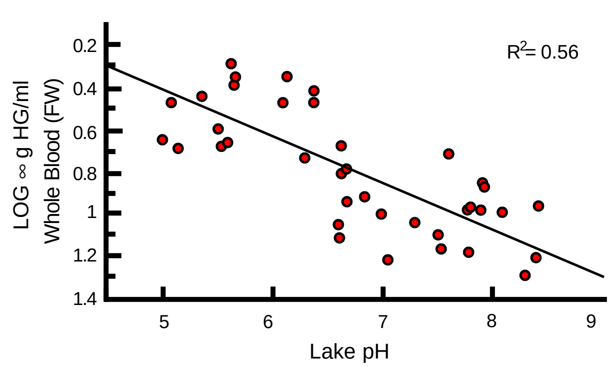

Fig. 3. Linear relationship of log (chick blood Hg) concentration (μg/mL) and lake Ph on freshwater lakes in Northern Wisconsin ($N = 45$, drawn after Meyer et al., 1998).

Five possible mechanisms of protection have been reviewed by Cuvin-Aralar and Furness (1991): (1) redistribution, (2) competition for binding sites between Hg, and Se, (3), formation of a Hg–Se complex, (4) conversion of toxic forms of Hg, and (5) prevention of oxidative damage. These mechanisms are not mutually exclusive. The two considered most important are formation of a Hg–Se complex and redistribution. The formation of a Hg–Se–protein complex would explain both the 1:1 ratio Hg:Se found in marine mammals and a decrease in toxicity, because the complex bis(methylmercuric) selenide linked to protein is much less toxic than methylmercury. Mercury uptake is not decreased by the presence of selenium, nor does selenium enhance mercury elimination. However, there is a redistribution to less sensitive tissues (e.g., kidney to muscle), giving some degree of protection.

The creation of reservoirs, often for hydroelectric projects such as the Churchill River diversion in Canada, has led to marked rises in rates of Hg production by microorganisms in sediments (Jackson, 1988). This phenomenon is primarily due to stimulation of microbial activity by submerged terrestrial organics concomitant with oxygen depletion. The levels of Hg in the muscle of northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) before the flooding of Southern Indian Lake in 1976 were mainly in the range of 0.2–0.3 μg/g which had risen to 0.5–1.0 μg/g by 1978–1982; however, higher values were found in some lakes (mean level of 2.90 μg/g in Notigi Lake and 2.56 μg/g in Rat Lake (Bodaly et al., 1984) with little sign of decrease within 5–8 years of impoundment.

The risk to avian species of wildlife of Hg exposure has been reviewed by Scheuhammer (1991); the lowest level in food known to cause reproductive effects is 1–2 fg/g (dry wt.) in the common loon (Barr, 1986), whereas other species examined have effects in the 2–6 fg/g range.

The natural occurrence of Hg in marine systems may have allowed some organisms to evolve mechanisms to deal with it. Other species may be particularly vulnerable because of increased susceptibility or increased uptake. Levels of Hg in fish and seabirds high on the food chain can be sufficiently high to cause effects both to themselves and to organisms that consume them, including humans (Law, 1996; Burger and Gochfeld, 1997b). Koeman et al. (1975) examined the concentration of methylmercury and total mercury in the marine ecosystem. They found that whereas in plankton most of the mercury was inorganic, in fish and seabirds methylmercury predominated.

3.6. Selenium

Although the greatest abundance of selenium is in igneous rocks, the bioavailability is low. More important as a source of selenium to wildlife is the selenium in sedimentary rocks such as shales and limestones. Selenate is the predominant inorganic form in the alkaline soils of semiarid regions, and selenite predominates in the soils of humid regions. Because selenate is more readily taken up by plants, high levels in plants are more likely to occur in arid areas. However, the major human-related cause of Se introduction and mobilization is the procurement, processing, and combustion of fossil fuels; it is highly concentrated in the mineral fraction remaining after coal is burned (Lemly, 1996).

Some plants, for example the vetches (*Astragalus* species), can accumulate Se from the soil to high concentrations in their tissues mainly as analogs of methionine. The Se concentration in such plants can be as high as 2000 fg/g (Stadtman, 1974). Thus plants are a major route of concentrating Se from soil and making it bioavailable to grazing animals. The addition of sulfate to the soil reduces Se uptake by plants, whereas phosphates increase plant concentration (Girling, 1984). Plants can convert inorganic Se into organic compounds. Nonaccumulator species of plants contain selenomethionine, whereas accumulator plants convert Se into soluble seleno-amino acids such as selenocystine (Girling, 1984). Selenomethionine is the predominant form ingested by animals, because accumulator plants are not normally eaten unless food is scarce. Besser et al. (1993) examined the uptake of selenate, selenite, and selenomethionine in a mesocosm consisting of algae, daphnids, and fish. They found that the bioconcentration factors (BCFs) of selenomethionine were much greater than either of the inorganic forms. The BCFs for selenomethionine were 16,000 for algae, 200,000 for daphnids, and 5000 for bluegills (*Lepomis macrochirus*).

The levels of Se in aquatic organisms in Kesterson Reservoir in California, which is contaminated with Se, were studied by Saika and Lowe (1987). There was considerable seasonal variation, but in one study, a water concentration of 0.04 μg/L was associated with 9.3 μg/g (dry wt.) in sediment, 133 μg/g in detritus, 214 μg/g in algae, 273 μg/g in rooted plants, 24–220 μg/g in aquatic insects (several species), and 223 μg/g in mosquitofish.

3.7. Tin

Although the organotins, especially tributyltin (TBT), have caused imposex in bivalves, leading to population declines (reviewed in Matthiessen and Gibbs, 1998), the source of the problem was direct leaching out of organotins from paints used on boats and not the formation of organotins in the environment. Being moderately lipophilic, TBT has the potential to bioaccumulate; it is rapidly degraded by fish, birds, and mammals, and thus adverse effects are seen only at lower trophic levels.

3.8. Zinc

Although cadmium pollution around zinc smelters (Cd occurs at 0.3% of Zn production) has been widely studied (see, for example, review by Stoeppler, 1991) Zn has been much less studied. Zinc is an essential element and has been considered nontoxic, but some workers recently have concluded that around smelters it is that is the metal causing the most damage to detrivorous soil invertebrates such as earthworms (*Eisenia fetida*; Hopkin and Spurgeon, 2001). Lock and Janssen (2001b) also concluded that the Zn:Cd ratio in soils is so high that the risk of Zn ecotoxicity to such terrestrial invertebrates as the earthworm (*Lumbricus* spp.), the potworm (*Enchyraeus albidus*) and the springtail (*Folsomia candida*) will be much greater in comparison with Cd ecotoxicity.

Vasquez et al. (1994) studied the compartmentalization of Zn in the Zn hyperaccumulator, *Thlaspi caerulescens*. In this plant species, in contrast to other plant species that were intolerant of high Zn concentrations, they observed the formation of Zn-rich crystals in the vacuoles of the epidermal and subepidermal cells of the leaves. The levels of sulfur were high; the S-containing compound was thought to be glucosinolates, but positive identification was not made.

Other influences such as pH also affect uptake. For example, Posthuma et al. (1998) showed that soil acidity was a major determinant of Zn partitioning and Zn uptake in two oligochaete worms (*Eisenia andrei* and *Enchytraeus crypticus*). Uptake of both Zn and Cd increased with increasing pH in a midge larva (*Chironomus riparius*, Bervoets and Blust, 2000).

4. Bioavailability of metals

Availability of metals from soil to organisms varies considerably among soil types and among species of organisms. For example, Lock et al. (2000) found that the toxicity of Zn and Cd to the earthworm (*Enchyraeus albidus*) varied by two orders of magnitude for a range of different soils. The principal influences affecting bioavailability were pH and cation-exchange capacity.

Plette et al. (1999) have developed equations to calculate the amount of metal bound reversibly to the exchange sites. Because the systems are chemically heterogeneous, two-component Langmuir–Freundlich equations were used. These workers examined copper binding to maize root cells and to fungal and yeast cells in a sandy soil, as well as cadmium binding with bacteria in a clay and a sandy soil. They found that although pH was the most important factor, calcium concentration (as a competitor for the same adsorption sites as the metal ions) was also important.

An additional problem arises with combination of metals. For example, van Gestel and Hensbergen (1997) found that the water solubility of Cd was substantially increased by the presence of Zn, whereas Cd did not affect the water solubility of Zn.

The effects of organic matter in water on the toxicity of metals to fish has been examined by Richards et al. (2001). These workers exposed rainbow trout (*Oncorhynchus mykiss*) to a combination of metals (Pb, Hg, Cd, Cu, Ag, and Co) and then added natural organic matter (NOM) collected from three sites in Ontario. The chemical composition of NOM varies considerably depending on the input source. Autochthonous NOM (that produced from phytoplankton within the water column) is composed mainly of open-chained carbon compounds enriched with carbohydrates and nitrogen compounds. Allochthonous NOM (that produced by washing in from the surrounding catchment) is rich in humic and fulvic acids and is highly colored. It was found that allochthonous NOM decreased the concentration of metals on the fish gills more than autochthonous NOM. Detailed characterization of NOM samples to determine the number of metal-binding sites per gram remains to be done, but preliminary work suggests than the ratio of specific fluorescence to specific absorbance can be used.

Wang (2001) compared the metal uptake rates and absorption efficiency of metals (Cd, Cr, Se, and Zn) in three species of marine bivalves. He found a significant negative correlation between the metal absorption efficiency and the clearance rate, implying that in an individual that was pumping a greater amount of water, there was coupled a lower absorption efficiency. It was concluded that metal availability from the aqueous phase was directly related to the physiological condition of the animal.

Two major types of cellular adaptation are known to occur after increased exposure to metals that can affect the bioavailability of the metal to the organisms (Cherian and Nordberg, 1983). One of these processes involves the binding of metals to nuclear proteins and also the formation of distinct inclusion bodies. The other mechanism is a cytoplasmic process involving a specific metal-binding protein, metallothionein.

A number of metals, notably Pb, Hg, Al, and Cu are accumulated intranuclearly. Intranuclear lead inclusions have been reported and show a dense compact central core surrounded by a fibrous zone (Moore et al., 1973). These inclusion bodies are believed to provide a major site for metal binding and prevent the lead from reaching target sites.

The metallothioneins are a family of low-molecular-weight, cysteine-rich metal-binding proteins that occur throughout the animal kingdom as well as in plants and eucaryotic microorganisms. That their synthesis can be induced by a wide variety of metal ions including cadmium, copper, mercury, cobalt, and zinc has led to their frequent use as a biomarker to show the presence of high levels of metals in the environment. Cherian and Nordberg (1987) have summarized the evidence for the involvement of metallothionein in limiting cell injury. Basically, the bioavailability of toxic metals is reduced by the induction of metallothionein and binding of metals to it. This has been shown to reduce toxicity to metals (e.g., certain enzyme systems, that are inhibited by Cd^{2+} are not inhibited by Cd-metallothionein). Hopkin (1989) found that 90% of the Cd and other heavy metals in woodlice from metal-contaminated sites was stored within the hepatopancreas. The metals are stored in sulfur-rich granules that are probably breakdown products of metallothionein. There is no evidence of remobilization of the metals; the granules can be excreted by voiding the contents of the cell into the lumen of the digestive system and subsequent elimination in the feces. Thus, the granules represent a storage detoxification system.

Some plants, termed metallophytes, naturally contain high concentrations of metals and are thus able to grow on metal-contaminated soils. Other plants have evolved distinct strains that are resistant to metals (Schat and Bookhum, 1992). Selection against metal tolerance on unpolluted soils has been demonstrated (Hickey and McNeilly, 1975), implying a cost for metal tolerance. Even when grown at optimum metal concentration, tolerant plants usually grow slower than nontolerant ones (Wilson, 1988). However, van Strallen and Hoffmann (2000) reviewed the data on metal tolerance and concluded that there is no strong evidence for costs of tolerance in the sense of increased energy allocation to detoxification.

5. Sentinel and indicator species

Sentinel species provide a useful tool for following heavy-metal exposure over time as well as serving as an early warning. A wide range of sentinel species have been used to assess the uptake of pollutants. The value of sentinel species depends partly on how metals accumulate in the organisms, and the amounts that cause effects. A useful organism must be sufficiently sensitive to the effects of a metal to provide early warning, yet not so sensitive that it reflects changes that are not biologically meaningful, both for the organism and for species that consume it. In addition, sentinels should be relatively easy to monitor over long periods of time and large spatial areas, and such monitoring should be economically feasible. Using sentinel species for regional or national biomonitoring schemes also requires public support; this can impose limits on the species selected (Peakall, 1992).

Residue analysis in sentinel species has been a useful indicator of environmental health (Keith, 1996). The many variables affecting metal toxicodynamics and elimination in animals, influencing their usefulness as sentinels. Factors affecting toxicodynamics include age, gender, seasonal and yearly variation, geographical variation, and trophic level (Burger, 1993; Watras et al., 1998; Snodgrass et al., 2000). Furthermore, the organisms in all ecosystems are not equally vulnerable to metals (Burger, 1997). There are many routes of elimination besides excretion, including sequestration in feathers, skin, scales, and hair, elimination from the salt gland, and in females, sequestration into eggs, young, and eggshells (Burger, 1993, 1994; Burger et al., 1994, 2000a, 2000b).

Bivalves have been extensively used as sentinels (Phillips and Rainbow, 1993; Rainbow and Blackmore, 2001; Wang, 2001). This group of organisms are relatively sedentary, filter large amounts of water, and are very widely distributed. Indeed the most global of biomonitoring programs is based on mussels (Goldberg, 1986). There are considerable differences among bivalves in feeding habits and life cycle characteristics. Other marine organisms that have been used include algae and amphipods, for which the need for reliable taxonomic identification is critical (Rainbow, 1995).

Caged fish have been used as sentinel species and have been valuable in the biomonitoring of estrogenic compounds (Harries et al., 1997). For example, eggs and fry are affected by chronic exposure to Cd (Prager, 1995), but there is a great deal of variation among species, with salmonids being more sensitive than other fish species (Wren et al., 1995). Later juvenile stages of fish are more sensitive than embryos, Cd is less toxic for fish living in hard water (compared to soft), and low water pH reduces Cd toxicity for algae and fish (Wren et al., 1995). Thus in using fish as sentinels, water chemistry, age of the fish, and species must be carefully considered. In addition, Ca-deficient diet in vertebrates enhances the overall toxicity of Cd, suggesting the importance of considering this influence.

Birds have been used extensively as sentinel species, or as bioindicators, because they are often numerous, large, and diurnal, and have public appeal. Moreover, top-level predators have often provided early warning of environmental insults, including heavy metals. Weseloh et al. (1994) used white Pekin ducks, a domestic form of the mallard (*Anas platyrhynchos*), to monitor organochlorine and metal contamination in the Great Lakes. Recently Levengood and Skowron (2001) compared the bioavailability of several metals to wild and sentinel mallards. They considered that examination of the gizzards of birds shot by hunters gave a more reliable assessment of bioavailability, because the wild species ate a wider range of food than the sentinel birds. They concluded that the use of sentinel species can result in inflated estimates of risk.

Mammals can be used as indicators of exposure and effect, particularly when they have an important or keystone role in ecosystems. Mammals are also useful bioindicators when consumed by both humans and other carnivores. As an example of this dual value, raccoons (*Procyon lotor*) were used to assess contamination at the Savannah Riversite in South Carolina because they consume fish, which can be contaminated with mercury, radiocesium, and other pollutants and are eaten by a variety of consumers, including humans (Burger et al., 2000c). Fish likewise serve this dual function.

6. Outstanding methodological issues

A number of methodological issues complicate the literature concerning the effects of metals on organisms, populations, and ecosystems. These include changes in technology over the years, use of wet vs dry wt., correlation of levels with effects (in both the laboratory and the field), and metal mixtures. Changes in technology mainly influence detection levels, which in turn affects the number of samples with detectable levels and our ability to compare tissue levels over long time periods.

Laboratory studies normally give the exposure level and effects, and field studies often report levels in tissues and effects. However, often exposure levels, levels in tissues, and effects (Burger and Gochfeld, 1997a) are all necessary to correlate the metal levels found in the field with known effects that have been documented in controlled laboratory experiments.

Perhaps the most challenging to our ability to measure and evaluate the effects of metals are metal mixtures, because the combinations that occur in nature are endless, and because biological processes influence their effects. Although it is possible to envision controlled experiments examining the effects of two metals in the laboratory, the combination of three or more becomes problematic, particularly given the possible variations in concentrations. Moreover, scientists that measure metals often do not examine the levels of other contaminants, such as organics, making it more difficult to evaluate effects in nature. The examination of mixtures is further complicated by differences in the organs affected.

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References

ATSDR, 1999. Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry. US Department of Health and Human Services, Public Health Service, Atlanta.

Barr, J.F., 1986. Population Dynamics of the Common Loon (*Gavia immer*) Associated with Mercury-Contaminated Waters in Northwestern Ontario. Canadian Wildlife Service, Occasional Paper No. 56, 25pp, Ottawa, Canada.

Berlin, M., 1978. Interactions between selenium and inorganic mercury. Environ. Health Perspect. 25, 67–69.

Bervoets, L., Blust, B.L., 2000. Effects of pH on cadmium and zinc uptake by the midge larvae *Chironomus riparius*. Aquat. Toxicol. 49, 145–157.

Besser, J.M., Canfield, T.J., La Pont, T.W., 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ. Toxicol. Chem. 12, 57–72.

Bidone, E.D., Castilhos, Z.C., Santos, T.J.S., Souza, T.M.C., Lacerda, L.D., 1997. Fish contamination and human exposure to mercury in Tartarugalzinho River, Northern Amazon, Brazil. A screening approach. Water Air Soil Pollut. 97, 9–15.

Bodaly, R.S., Hecky, R.E., Fudge, R.J.P., 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. Can. J. Fish. Aquat. Sci. 41, 682–691.

Bohn, A., 1975. Arsenic in marine organisms from West Greenland. Mar. Pollut. Bull. 6, 87–89.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev. Environ. Toxicol. 25, 203–311.

Burger, J., 1994. Heavy metals in avian eggshells: another excretion method. J. Toxicol. Environ. Health 41, 307–320.

Burger, J., 1997. Method for an approach to evaluating susceptibility of ecological systems to hazardous chemicals. Environ. Health Perspect. 105, 843–848.

Burger, J., Gochfeld, M., 1997a. Risk, mercury levels, and birds: relating adverse laboratory effects to field biomonitoring. Environ. Res. 75, 160–172.

Burger, J., Gochfeld, M., 1997b. Lead and neurobehavioral development in gulls: a model for understanding effects in the laboratory and the field. Neurotoxicology 18, 495–506.

Burger, J., Gochfeld, M., 2000. Effects of lead on larids: a review of laboratory and field studies. J. Toxicol Environ. Health B 3, 59–78.

Burger, J., Gochfeld, M., 2001. Effects of chemicals and pollution on seabirds. In: Schreiber, E.A., Burger, J. (Eds.), Biology of Marine Birds. CRC Press, Boca Raton, FL, pp. 485–525.

Burger, J., Marquez, M., Gochfeld, M., 1994. Heavy metals in hair of opossum from Palo Verde, Costa Rica. Arch. Environ. Contam. Toxicol. 27, 473–476.

Burger, J., Stephens, W., Boring, C.S., Kuklinski, M., Gibbons, J.W., Gochfeld, M., 1999. Ethnicity and risk: fishing and consumption in people fishing along the Savannah river. Risk Anal. 19, 427–438.

Burger, J., Trivedi, C.D., Gochfeld, M., 2000a. Metals in herring and great black-backed gulls from the New York bight: the role of salt gland in excretion. Environ. Monit. Assess. 64, 569–581.

Burger, J., Gochfeld, M., Rooney, A.A., Orlando, E.F., Woodward, A.R, Guillette Jr., L.J., 2000b. Metals and metalloids in tissues of American alligators in three Florida lakes. Arch. Environ. Contam. Toxicol. 38, 501–508.

Burger, J., Lord, C.S., McGrath, L., Gaines, K.F., Brisbin Jr., I.L., Gochfeld, M., Yurkow, E.J., 2000c. Metals and metallothionein in the liver of raccoons: utility for environmental assessment and monitoring. J. Toxicol. Environ. Health 60, 243–261.

Campbell, P.G.C., Tessier, A., 1996. Ecotoxicology of metals in the aquatic environment: geochemical aspects. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: A Hierarchical Treatment. CRC Press, Boca Raton, FL.

Caurant, F., Amiard, J.C., Amiard-Triquet, C., Sauriau, P.G., 1994. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hug, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Mar. Ecol. Prog. Ser. 103, 207–219.

Cherian, M., Nordberg, M., 1983. Cellular adaptation in metal toxicology and metallothionein. Toxicology 28, 10–15.

Christensen, T.H., 1984. Cadmium soil sorption at low concentrations. 1. Effect of time, cadmium load, pH, and calcium. Water Air Soil Pollut. 21, 105–114.

Cleveland, L., Little, E.E., Hamilton, S.J., Buckler, D.R., Hunt, J.B., 1986. Interactive toxicity of aluminum and acidity to early life stages in brook trout. Trans. Am. Fish. Soc. 115, 610–620.

Cuvin-Aralar, M.L.A., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotoxicol. Environ. Saf. 21, 348–364.

Eisler, R., 1985. Cadmium hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service Report No. 85 (1.2), Washington, DC.

Eisler, R., 1987. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service Report No. 85 (1.1), Washington, DC.

Eisler, R., 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service Report No. 85(1.14), Washington, DC.

Falconer, C.R., Shepherd, R.J., Pirie, J.M., Topping, G., 1983. Arsenic levels in fish and shellfish from the North Sea. Exp. Mar. Biol. Ecol. 71, 193–203.

Furness, R.W., 1996. Cadmium in birds. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 389–404.

Ganther, H.E., Goudie, C., Sunde, M.L., Kopecky, M.G., Wagner, R., Sang-Hwang, O.H., Hoekstra, W.G., 1972. Selenium relation to decreased toxicity of methylmercury added to diets containing Tuna. Science 72, 1122–1124.

Girling, C.A., 1984. Selenium in agriculture and the environment. Agric. Ecosyst. Environ. 11, 37–65.

Goldberg, E.D., 1986. The mussel watch concept. Environ. Monit. Assess. 7, 91–103.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J.P., Tylor, T., Zaman, N., 1997. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environ. Toxicol. Chem. 16, 534–542.

Henriksen, A., Skogheim, O.K., Rosseland, B.O., 1984. Episodic changes in pH and aluminum-speciation kill fish in a Norwegian salmon river. Vatten 40, 255–260.

Hickey, D.A., McNeilly, T., 1975. Competition between metal tolerant and normal plant populations; a field experiment on normal soil. Evolution 29, 458–464.

Hopkin, S.P., 1989. Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, Barking, UK.

Hopkin, S.P., Spurgeon, D.J., 2001. Forecasting the environmental effects of zinc, the metal of benign neglect in soil ecotoxicology. In: Rainbow, P.S., Hopkin, S.P., Crane, M. (Eds.), Forecasting the Environmental Fate and Effects of Chemicals. Wiley, Chichester, pp. 91–95.

Huckabee, J.W., Blaylock, B.G., 1973. Transfer of mercury and cadmium from terrestrial to aquatic ecosystems. In: Dhar, S.K. (Ed.), Metal Ions in Biological Systems. Plenum Press, New York, pp. 125–160.

Jackson, T.A., 1988. The mercury problem in recently formed reservoirs of northern Manitoba (Canada): effects of impoundment and other factors on the production of methyl mercury by microorganisms in sediments. Can. J. Fish. Aquat. Sci. 45, 97–121.

Jarvie, A.W.P., Markall, R.N., Potter, H.R., 1975. Chemical alkyation of lead. Nature 255, 217–218.

Jensen, A., Bro-Rasmussen, F., 1992. Environmental cadmium in Europe. Rev. Environ. Contam. Toxicol. 125, 101–181.

Jernelöv, A., 1969. Conversion of mercury compounds. In: Miller, M.W., Berg, G.G. (Eds.), Chemical Fallout: Current Research on Pesticides. Charles C. Thomas, Springfield, IL, pp. 68–74.

Keith, J.A., 1996. Residue analysis: how they were used to assess the hazards of contaminants to wildlife. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 1–48.

Koeman, J.H., van de Ven, W.S.M., de Goeij, J.J.M., Tjioe, P.S., van Haaften, J.L., 1975. Mercury and selenium in marine mammals and birds. Sci. Tot. Environ. 3, 279–287.

Kuehl, D.W., Haebler, R., 1995. Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphin (*Tursiope truncatus*) collected during an unusual mortality event in the Gulf of Mexico. Arch. Environ. Contam. Toxicol. 28, 494–499.

Lacerda, L.D., Bidone, E.D., Giumaraes, A.F., Pfeiffer, W.C., 1994. Mercury concentrations in fish from the Itacaiunas-Parauapebas River System. Carajas region. Amazon Ann. Acad. Bras. Sci. 66, 373–379.

Law, R.J., 1996. Metals in marine mammals. In: (Beyer, W.N., Heinz, G.H., Redmon-Norwood, A. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 357–376.

Lemly, A.D., 1996. Selenium in aquatic systems. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publisheres, Boca Raton, FL, pp. 427–458.

Levengood, J.M., Skowron, L.M., 2001. Use of a stimulated gizzard bioavailability of metals and other elements to waterfowl. Ecotoxicology 10, 299–304.

Lock, K., Janssen, C.R., 2001a. Zinc and cadmium body burdens in terrestrial oligochaetes: use and significance in environmental risk assessment. Environ. Toxicol. Chem. 20, 2067–2072.

Lock, K., Janssen, C.R., 2001b. Cadmium toxicity for terrestrial invertebrates: taking soil parameters affecting bioavailability into account. Ecotoxicology 10, 315–322.

Lock, K., Janssen, C.R., De Coen, W.M., 2000. Multivariate test designs to assess the influence of zinc and cadmium bioavailability in soils on the toxicity to *Enchytraeus albidus*. Environ. Toxicol. Chem. 19, 2666–2671.

Locke, L.N., Friend, M., 1992. Lead poisoning of avian species other than waterfowl. In: Pain, D.J. (Ed.), Lead Poisoning in Waterfowl. Spec. Publ. No. 16. International Waterfowl and Wetlands Research Bureau, Slimbridge, Gloucestershire, UK, pp. 19–22.

Matthiessen, P., Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyl-mediated endocrine disruption in mollusks. Environ. Toxicol. Chem. 17, 37–43.

McDonald, D.G., Reader, J.P., Dalziel, T.R.K., 1989. The combined effects of pH and trace metals on fish ionoregulation. In: Morris, R., Taylor, E., Brown, D., Brown, J. (Eds.), Acid Toxicity and Aquatic Animals. Cambridge University Press, Boston, pp. 221–242.

Meyer, M.W., Evers, D.C., Hartigan J, J., Rasmussen, P.S., 1998. Patterns of common loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, USA. Environ. Toxicol. Chem. 17, 184–190.

Moore, J.E., Goyer, R.A., Wilson, M.H., 1973. Lead induced inclusion bodies: solubility, aminoacid content and relationship to residual acidic nuclear proteins. Lab. Invest. 29, 488–494.

Neff, J.M., 1997. Ecotoxicology of arsenic in the marine environment. Environ. Toxicol. Chem. 16, 917–927.

Norstrom, R.J., Schweinsberg, R.E., Collins, B.T., 1986. Heavy metals and essential elements in livers of the polar bear (*Ursus maritimus*) in the Canadian Arctic. Sci. Total Environ. 48, 195–212.

Ohlendorf, H.M., Anderson, D.W., Boellstorff, D.E., Mulhern, B.M., 1985. Tissue distribution of trace elements and DDE in brown pelican. Bull. Environ. Contam. Toxicol. 35, 183–192.

Ohlendorf, H.M., Lowe, R.W., Kelly, P.R., Harvey, T.E., 1986. Selenium and heavy metals in San Francisco Bay diving ducks. J. Wildlife Manage. 50, 64–71.

Pain, D.J. (Ed.), 1992. Lead Poisoning in Waterfowl. Special Publ. No. 16. International Waterfowl and Wetlands Research Bureau, Slimbridge, Gloicestershire, UK. 105pp.

Pain, D.J., 1996. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 251–264.

Park, J.G., Curtis, L.R., 1997. Mercury distribution in sediments and bioaccumulation by fish in two Oregon reservoirs: point-source and nonpoint-source-impacted systems. Arch. Environ. Contam. Toxicol. 33, 423–429.

Peakall, D., 1992. Animal Biomarkers as Pollution Indicators. Chapman & Hall, London.

Phillips, D.J.H., 1990. Arsenic in aquatic organisms: a review, emphasizing chemical speciation. Aquat. Toxicol. 16, 151–186.

Phillips, D.J.H., Rainbow, P.S., 1993. Biomonitoring of Trace Aquatic Contaminants. Elsevier, London. 371pp.

Plette, A.C.C., Nederlof, M.M., Temminghoff, E.J.M., van Riemsdijk, W.H., 1999. Bioavailability of heavy metals in terrestrial and aquatic systems: a quantitative approach. Environ. Toxicol. Chem. 18, 1882–1890.

Posthuma, L., Notenbloom, J., De Grot, A.C., Peijnenburg, W.J.G.M., 1998. Soil acidity as major determinant of zinc partitioning and zinc uptake in two oligochaete worms (*Eisenia andrei* and *Enchytraeus crypticus*) exposed in contaminated field soils. In: Sheppard, S.C., Bembridge, J.D., Holmstrup, M., Posthuma, L. (Eds.), Progress in Earthworm Ecotoxicology. SETAC Press, Pensacola, FL, pp. 111–127.

Prager, J.C., 1995. . Environmental Contaminant Reference Databook, Vol. 1. Van Nostrand Reinhold, New York.

Rainbow, R.S., 1995. Biomonitoring of heavy metal availability in the marine environment. Mar. Pollut. Bull. 31, 183–192.

Rainbow, P.S., Blackmore, G., 2001. Barnacles as biomonitors of trace metals availabilities in Hong Kong coastal waters: changes in space and time. Mar. Environ. Res. 51, 441–463.

Rattner, B.A., Heath, A.C., 1995. Environmental factors affecting contaminant toxicity in aquatic and terrestrial vertebrates. In: Hoffman, D.J., Rattner, B.A., Burton Jr., G.A., Cairns Jr., J. (Eds.), Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, FL, pp. 519–537.

Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. Environ. Toxicol. Chem. 20, 1159–1166.

Ripley, J.P., Towner, J.V., 1984. The distribution of alkyl lead species in the Mersey Estuary. Mar. Pollut. Bull. 15, 153–158.

Saika, M.K., Lowe, T.P., 1987. Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch. Environ. Contamin. Toxicol. 16, 657–670.

Satoh, H., Yasuda, N., Shimai, S., 1985. Development of reflexes in neonatal mice prenatally exposed to methylmercury and selenite. Toxicol. Lett. 25, 199–203.

Schat, H., Bookum, W.M.T., 1992. Genetic control of copper tolerance in *Silene vulgaris*. Hereditary 68, 219–229.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: a review. Environ. Pollut. 46, 263–295.

Scheuhammer, A.M., 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds. Environ. Pollut. 71, 329–375.

Scheuhammer, A.M., Graham, J.E., 1999. The bioaccumulation of mercury in aquatic organisms from two similar lakes with differing pH. Ecotoxicology 8, 49–56.

Scheuhammer, A.M., Norris, S.L., 1996. The ecotoxicology of lead shot and lead fishing weights. Ecotoxicology 5, 279–295.

Scheuhammer, A.M., Templeton, D.M., 1998. Use of stable isotope ratios to distinguish sources of lead exposure in wild birds. Ecotoxicology 7, 37–42.

Schindler, D.W., 1988. Effects of acid rain on freshwater ecosystems. Science 239, 149–157.

Simkiss, K., 1983. Lipid solubility of heavy metals in saline solutions. J. Mar. Biol. Assoc. UK 63, 1–7.

Skogheim, O.K., Rossseland, B.O., Sevaldrud, I.H., 1984. Death of spawners of Atlantic salmon in River Ogna, SW Norway, caused by acidified aluminum-rich water. Rep. Inst. Freshwater Res. Drottningholm 61, 197–202.

Snodgrass, J.W., Jagoe, C.H., Bryan Jr., A.L., Burger, J., 2000. Effects of trophic status, and wetland morphology, hydroperiod and water chemistry on mercury concentrations in fish. Can. J. Fish Aquat. Sci. 57, 171–180.

Spry, D.J., Wiener, J.G., 1991. Metal bioavailability and toxicity to fish in low-alkaline lakes: a critical review. Environ. Pollut. 71, 243–304.

Stadtman, T.C., 1974. Selenium biochemistry. Science 183, 915–922.

Stoeppler, M., 1991. Cadmium. In: Merian, E. (Ed.), Metals and their Compounds in the Environment. Wiley VCH, Weinhiem, pp. 803–851.

Thompson, D.R., 1996. Mercury in birds and terrestrial mammals. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 341–356.

Thompson, D.R., Furness, R.W., 1989. The chemical form of mercury stores in South Atlantic seabirds. Environ. Pollut. 60, 305–317.

Varanasi, U., Gmur, D.J., 1978. Influence of water-borne and dietary calcium on uptake and retention of lead by coho salmon (*Oncorhynchus kisutch*). Toxicol. Appl. Pharmacol. 46, 65–75.

Vasquez, M.D., Poschenreider, C., Bercelo, J., Baker, A.J.M., 1994. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J and C Presl. Bot. Acta 107, 243–250.

van Gestel, C.A.M., Hensbergen, P.J., 1997. Interaction of Cd and Zn toxicity for *Flosmia candida* Willem (Collembola: Isotpmidae sp.) in relation to bioavailability in soil. Environ. Toxicol. Chem. 16, 1177–1186.

van Strallen, N.M., Hoffmann, A.A., 2000. Review of experimental evidence for physiological costs of tolerance to toxicants. In: Kammenga, J., Laskowski, R. (Eds.), Demography in Ecotoxicology. Wiley, Chichester, pp. 147–161.

Wagemann, R., Muir, D.C.G., 1984. Concentrations of heavy metals and organochlorines in marine mammals: overview and evaluation. Can Field Naturalist 86, 123–125.

Wagemann, R., Innes, S., Richard, P.R., 1996. Overview and regional and temporal differences of heavy metals of whales and ringed seals in the Canadian Arctic. Sci. Total Environ. 186, 41–66.

Walsh, P.M., 1990. The Use of seabirds as monitors of heavy metals in the marine environment. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, FL, pp. 183–204.

Wang, W.-X., 2001. Comparison of metal uptake rate and absorption efficiency in marine bivalves. Environ. Toxicol. Chem. 20, 1367–1373.

Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A., Wente, S.P., 1998. Bioaccumulation of mercury in pelagic freshwater food webs. Sci. Tot. Environ. 219, 183–208.

Weber, D.N., Dingel, W.M., 1997. Alterations in neurobehavioral responses in fishes exposed to lead and lead-chelating agents. Am. Zool. 37, 354–362.

Weseloh, D.V.C., Struger, J., Hebert, C., 1994. White pekin ducks (*Anas platyrhynchos*) as monitoring of organochlorine and metal contamination in the Great Lakes. J. Great Lakes Res. 20, 277–288.

Wilson, J.B., 1988. The cost of heavy metal tolerance: an example. Evolution 42, 408–413.

Wong, P.T.S., Chau, Y.K., Luxon, P.L., 1975. Methylation of lead in the environment. Nature 253, 263–264.

Wren, C.D., Harris, S., Harttrup, N., 1995. Ecotoxicology of mercury and cadmium. In: Hoffman, D.J., Rattner, B.A., Burton, G.A., Cairns, J. (Eds.), Handbook of Ecotoxicology. Lewis Publisheres, Boca Raton, FL, pp. 392–423.