Chapter 2
Endothermic Animals as Biomonitors of Terrestrial Environments

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Abstract Since the late 1980s, wildlife toxicology has grown considerably as an important field of laboratory and field research. It focuses on the effects of various chemicals on the reproduction, health, and well-being of wildlife, including essential and nonessential elements. Deficiency of essential elements (e.g., copper, manganese, nickel, zinc, selenium) can lead to adverse effects in endothermic vertebrates, while their excess may result in significant intoxication or even death. However, the greatest concern is the contamination with highly toxic nonessential elements such as mercury, lead, cadmium, and arsenic.

Human activity results in the introduction of large amounts of essential and nonessential trace elements into biogeochemical cycles. Particularly exposed to excessive levels of trace elements are top avian and mammalian predators at the end point of biological pathways along which contaminants may accumulate in increasing concentrations. Determinations of trace elements in samples from selected species serving as biomonitors can be used to indirectly assess the condition of terrestrial ecosystems, including herbivorous, omnivorous, and predatory wildlife. Biomonitors are usually native species common in the area (involving hunted animals) but also invasive species (in Europe American mink and raccoon from North America; in the USA and Canada wild boar and common starling from Europe). Biomonitoring using terrestrial birds and mammals can be local, regional, or continental and is well developed in many countries of the Northern Hemisphere, especially in North America and Europe.

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1 Introduction

Since the 1950s, the dynamic growth of the world's population has been accompanied by growing levels of environmental pollution, resulting in a multifaceted destruction of natural biological systems, as observed both locally and globally. This has been accompanied by an increase in interest in environmental phenomena and developments in ecology, including ecotoxicology. Research has shown that measurements of the levels of various substances in air, water, and soil from chemical monitoring of the environment are still not sufficient to properly assess the health hazards to humans, animals, plants, and entire ecosystems. Currently, the use of living or deceased organisms in biotesting, bioindication, and biomonitoring is an established method of determining inorganic and organic contaminants and pollutions (Burger 2006a; Bealey et al. 2008; Holt and Miller 2011; Markert 2013).

The terms "bioindicator," "biomonitor," "bioaccumulator," and "biomarker" have all been used in varying ways to describe different approaches and techniques for studying biological responses to pollution of the air and other environmental components. In ecological and environmental sciences, the terms "biomonitor" and "bioindicator" have been and still are used interchangeably, and the terminology in this area can be ambiguous. However, since the early 1990s, we may observe a certain distinction in the use of these terms (Burger 2006a, b; Wilkomirski 2013; Sidding et al. 2016). Biomonitoring can include both a qualitative (bioindicator) and quantitative (biomonitor) approach in pollution control. For example, chemical analysis of biomonitors (an organism or its part or a community of organisms) contains information on the quantitative aspects of quality of the environment. A biomonitor is also a bioindicator, except that it quantifies the impact or eventual outcome on an organism or ecosystem and their health (O'Brien et al. 1993; Markert et al. 2003; Burger 2006a; Bealey et al. 2008). Large-scale biomonitoring uses plant and animal bioaccumulators, or organisms that accumulate various chemicals (including contaminants) in the tissues. Bioaccumulation is result of the biological sequestering of many substances often at a higher concentrations than that at which they occur in the surrounding environment or/and in food of animals.

2 Trace Elements

Some elements present in inorganic/organic forms in organisms are essential elements and others nonessential. In biochemistry, an essential trace element (or micronutrient) is a dietary mineral that is needed in very minute quantities (expressed in micrograms or milligrams) for the proper growth, development, and physiology of the organism. In humans the requirement per day is below 100 mg, with a deficiency leading to disorders that may even prove fatal.

In endothermic vertebrates, the biochemical functions of essential trace elements include enzyme activity, transport of oxygen (iron and copper), organization and

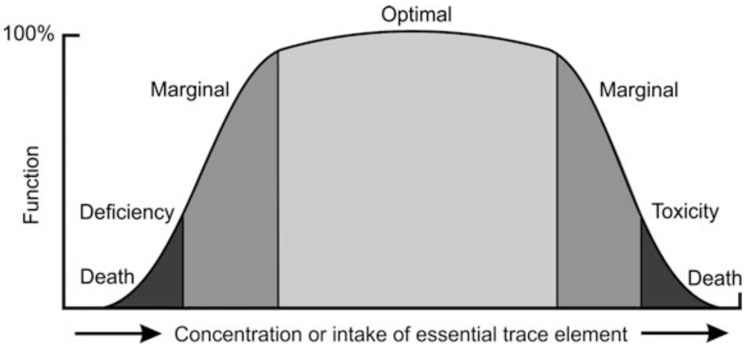

Fig. 2.1 Dependence of biologic function on the tissue concentration of essential trace elements (according to Aras and Ataman 2006)

structure of macromolecules, vitamin activity (cobalt and vitamin B_{12}), or hormonal activity, e.g., iodine and thyroid hormones (Taylor 1996). All essential elements may even be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period (Fig. 2.1). This aspect has been well recognized in humans, domesticated and laboratory animals, yet very poorly in wildlife (WHO 1973; Wada 2004; NRC 2005; Aras and Atman 2006; Lopez-Alonso 2012; Yatoo et al. 2013; Prashanth et al. 2015; Bhattacharya et al. 2016).

According to the National Research Council (NRC 2005), heavy metals (accepting as a criterion a density of at least 5.0 g cm^{-3}) such as cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) and two nonmetal elements (iodine (I) and selenium (Se)) are considered essential trace elements for higher animals. NRC (2005) classified nickel (Ni) as a possible essential element, while in the case of fluorine (F), which is nonmetallic, opinions are divided. Some researchers believe that F is an essential element for animals and humans while others consider the available evidence for indispensability to be insufficient. Elements essential to domestic, laboratory, or wild mammals and birds may not be essential to humans and vice versa.

In the document by the WHO (2002), the following trace elements are described as essential for human health: Cu, Zn, Fe, Cr, Mo, Se, Co, and I (the list includes Cr but its status as an essential element is controversial, where CrIII is beneficial for animals and humans but CrVI is a human carcinogen). The next smaller group is composed of Si (silicon), Mn, Ni, B (boron), and V (vanadium) with those elements classified as probably essential elements for humans. There are some differences between the NRC (2005) and WHO (2002) reports concerning the essentiality of elements for animals and humans, and the discussion on essentiality of some of those elements is still open (Aras and Ataman 2006; Bhattacharya et al. 2016; Maret 2016).

The term “trace element” is also used in analytical chemistry and geochemistry. In analytical chemistry it is an element whose average concentration is less than 100 mg kg^{-1} (<100 ppm) but in geochemistry it is less than 1000 mg kg^{-1}

(<1000 ppm) or 0.1% of a rock's composition. Elements from mineral deposits are activated as a result of natural processes, but their contribution to the biogeochemical cycles is very much driven by human economic activity, especially over the last 100–150 years (Klee and Graedel 2004). The natural distribution and concentration of elements in the Earth's crust are very diverse as a result of the geological structure, but they are subject to strong anthropogenic modification having a significant impact on the mineral composition of plants, animals, and people and consequently on their condition, health, and reproduction (Adriano 2001; Yaroshevsky 2006; Steinnes 2009; Kabata-Pendias 2011). Among the elements, which can occur in living creatures, special attention is paid to all the essential and some of the nonessential trace elements, the latter having no biological function. For a long time, the greatest concerns have been triggered by heavy metals which are highly toxic to endothermic vertebrates, such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As), the latter being metalloids (Nriagu and Pacyna 1988; Adriano 2001; Anke et al. 2001; Jarup 2003; Hubner et al. 2010; Tchounwou et al. 2012). Additionally, for several decades, there has been an increasing interest on other metallic xenobiotics such as silver (Ag) and aluminum (Al) due to their high neurotoxicity and increasing distribution in the environment, including man-made Ag and Al nanoparticles (Ray et al. 2010; Stensberg et al. 2011; Jaishankar et al. 2014; Karmakar et al. 2014). Table 2.1 summarizes the concentrations and densities of selected trace elements found in the Earth's crust, which in varying amounts accumulate in wild-living endothermic vertebrates (Selinus and Finkelman 2011). Some of them are classified as essential elements and some as nonessential elements, all of which are discussed in more detail in the respective chapters of this book. In the case of wildlife, the ranges of mean concentrations of elements are shown for the liver, because this organ plays a key role in trace element regulation, bioaccumulation, and detoxification (Vikøren et al. 2005; Horai et al. 2006; Neuschwander-Tetri 2007). In addition, the liver is one of the most extensively used biological materials in ecotoxicological studies for quantifying trends in medium- to long-term contaminant exposure, with most data in this field existing from hepatic tissue (Burger et al. 2000; Gamberg et al. 2005; Braune and Malone 2006; Taggart et al. 2006; Vikøren et al. 2011; Gall et al. 2015; Espin et al. 2016; Kitowski et al. 2017).

In the group of essential elements, mean concentrations in the liver may in some cases reach one (Cr, Cu, Mo, Zn) or three (Se) orders of magnitude higher than their level in the Earth's crust. Liver concentrations of nonessential and highly toxic elements such as Cd, Hg, Ag may also be three orders of magnitude higher than in the crust. In the case of As and Sn, average hepatic concentrations may exceed those in the crust by two or one order of magnitude, respectively.

Eukaryotic organisms, including vertebrates, have evolutionarily developed mechanisms that enable them to maintain a proper level of various essential trace elements and homeostasis (Zhang and Gladyshev 2010). Terrestrial vertebrates via physiological and anatomical means have regulated and/or stored essential elements, including heavy metals up to certain exposure levels such that metals may not be present in their bodies in a concentration, form, or place that can result in a toxic effect. In such regulation, the gastrointestinal tract and the liver play crucial roles in

Table 2.1 Density and concentration of selected elements in the upper continental crust (according to Yaroshevsky 2006), their biochemical status (NRC 2005), and range of mean hepatic levels in wild endothermic animals

Element	Density (g cm^{-3})	Crust concentration	Biochemical status and range of mean hepatic concentration (ppm dw)			No. of chapters in this book
			Essential	Probably essential	Non-essential	
Ag, silver	10.49	0.07 ppm			<DL–44.0	18
Al, aluminum	2.70	8.05%			0.20–25.35	12
As, arsenic	5.73	1.70 ppm			0.05–122.6	13
Cd, cadmium	8.65	0.13 ppm			0.05–163.2	14
Cr, chromium	7.14	83.0 ppm	0.05–150.0			3
Cu, copper	8.92	47.0 ppm	10–790			4
F, fluorine	1.70[a]	660 ppm			<2.6–270[b]	15
Fe, iron	7.87	4.65%	<100–4920			6
Hg, mercury	13.53	0.08 ppm			<DL–35	17
I, iodine	4.94	0.40 ppm	0.17–0.35[c]			5
Mn, manganese	7.47	1000 ppm	2.20–38.3			7
Mo, molybdenum	10.28	1.10 ppm	0.9–13.0			8
Ni, nickel	8.91	58.0 ppm		0.01–13.0		9
Pb, lead	11.34	16.0 ppm			0.30–48.0	16
Se, selenium	4.82	0.05 ppm	<0.20–23.0			10
Sn, tin	7.31	2.50 ppm			<0.01–15.0	19
Zn, zinc	7.14	83.0 ppm	21.0–297.4			11

ppm parts per million or mg kg^{-1}, *dw* dry weight, *DL* detection limit

[a] Density of F in g L^{-1}

[b] In literature no data on wild animals was found, just fluoride concentrations in the livers of control laboratory rat and fatal human cases because 99% of fluorine is retained in bones and teeth (Inkielewicz and Krechniak 2003; NRC 2005; Martinez et al. 2007)

[c] Data available for farm animals only

the uptake and transport of cations (e.g., Cu, Fe, Zn). The anionic group such as Mo and Se is more water-soluble and is less reactive with nitrogen, sulfur, phosphorus, and oxygen, as well as hydroxide groups, than are cations. They are absorbed very efficiently through the intestine. In general, total body burden is regulated by renal excretion (WHO 1996; Rutherford and Bird 2004; US EPA 2007; Lopez-Alonso 2012; Sakulsak 2012; Kim and Oh 2013). Toxic elements strongly affect some essential element metabolisms because they compete for binders for these elements in the biological system. Concentrations of various essential and nonessential elements in birds and mammals depend on many factors and processes, including their forms, oxidation state, and the amount in habitats; biotransformation, bioavailability, diet, and position in the food chains of endothermic vertebrates; absorption (in which the intestinal route is the most important); and the duration of exposure (Chapman 1996; Adriano 2001; Martelli et al. 2006; Diaz-Bone and van de Wiele 2010; García-Barrera et al. 2012; Bhargava and Bhargava 2013). Heavy metals (both essential and nonessential) and metalloids (such as Se, As) in wildlife are the most often analyzed pollutants (Burger 2006b; Jaishankar et al. 2014; Stankovic et al. 2014; Gall et al. 2015; Espin et al. 2016), wherein pollution is defined as contamination that does or can result in adverse biological effects to resident communities. All pollutants are contaminants (substances which are present at places where they should not be or at concentrations above background), but not all contaminants are pollutants (Chapman 2007). Unlike plants and lichens, domestic and wild animals do not usually show qualitative morphological and/or physiological changes as a consequence of chronic bioabsorption of trace elements and the undesirable effects caused by them, which would allow these animals to be considered as bioindicators. There are only a few examples in this field from areas where a natural excess of these elements is noticed. These include loss of hair and malformations of hooves as a result of excessive selenium in food sources and dental fluorosis as a result of a high uptake of fluoride dissolved in groundwater (James and Shupe 1984; Al-Dissi et al. 2011; Choubisa et al. 2011). Wildlife is very often used as a biomonitor where they chronically bioaccumulate trace elements and other substances, but the reaction of the animals to them are generally invisible. These substances qualitatively and quantitatively can be assayed in laboratories using highly specialized and sensitive equipment, from samples of the appropriate biota (Markert et al. 2003).

3 Terrestrial Endothermic Vertebrates as Biomonitors

Since the 1970s there has been a steady and dynamic growth in research and implementation of biomonitoring programs that use organisms from various taxonomic groups as biomonitors of environmental pollution. Of the terrestrial endothermic vertebrates, mainly wild animals but sometimes also breeding birds and mammals (including furbearers) are chosen (Wren 1984; O’Brien et al. 1993; López Alonso et al. 2002; Golden and Rattner 2003; Tataruch and Kierdorf 2003; Ji et al.

2006; Wolfe et al. 2007; Rabinowitz et al. 2009; Reis et al. 2010; Rajaganapathy et al. 2011; Kalisinska et al. 2012a). The consequence of this has been an increase in the number of reports in this field concerning wildlife. Of particular interest are persistent organic pollutants (including organochlorine pesticides and polychlorinated biphenyls (PCBs)), but much attention is also devoted to trace elements (Golden and Rattner 2003; Markert et al. 2003, 2008; Stolen et al. 2005; Burger 2006b; Hollamby et al. 2006; Holt and Miller 2011). Warm-blooded biomonitors can be used for information on:

- Essential and nonessential element concentrations and relations between them in selected species (especially in rare and threatened birds and mammals, common species, including game animals, which are used by humans as food and potentially valuable source of minerals but may also contained toxic contaminants)
- Concentrations and bioavailability of essential elements in an area of interest important for the detection of their deficiency or excess and which may be referenced in proper steps in the management and health protection both in animals and humans (e.g., level of selenium is significant in protection against mercury toxicity)
- Bioindicators can be used as information about various temporal and spatial changes occurring in a specific area (including those from anthropogenic and natural sources such as atmospheric deposition, floods), especially in the case of xenobiotic metals and metalloids
- Differences in trace element concentrations among animals from the same area (or from control and contaminated sites) and the various trophic levels
- Ecotoxicological situation of selected species which are widely distributed in various provinces, states, countries, and even continents

Depending on the purpose of the research or the biomonitoring program being implemented, one or several of the above points may be taken into account, but there may also be others not mentioned above (Talmage and Walton 1991; Stolen et al. 2005; Burger 2006a; Smith et al. 2007; Zhang and Ma 2011; Garcia-Fernandez 2014; Espin et al. 2016; Herzke et al. 2017). Species that are targeted as candidates of bioindicators of trace elements should have desirable characteristics including:

- Are sensitive and indicative of change
- Broad distribution with accompanying data
- Easily measured and readily observable
- Well-known ecology and life history
- Suitable for lab studies
- Significant to humans
- Economical/cost-effective
- Well-developed and usable with existing data
- Common enough not to impact populations

Lists of characteristics may differ from one another to a certain point, and a potential or suitable terrestrial candidate may fulfill only some of the desired features (O’Brien et al. 1993; Hollamby et al. 2006; Espin et al. 2016; Herzke et al. 2017). It seems that the list of avian species is much longer than that of mammalian species. This is due not only to the greater number of bird species found in the world, which is almost twice as much as mammals (9993 and 5416 species, respectively), but also from a much larger and more active group of people professionally and amateurly researching and observing birds (ornithologists, volunteers, and bird-watchers) compared to the analogous "mammalian" group (Jones and Safi 2011; Jetz et al. 2012). The highest biodiversity of birds and mammals is recorded in tropical regions. Mammalian and avian fauna in Europe in comparison to other parts of the Northern Hemisphere is poorly diversified, with the avifauna of eastern Asia about 50% richer than Europe and North America, and Western North America the richest region with 14% and 44% more species than eastern Asia and Europe, respectively (Monkkonen and Viro 1997). However, wildlife researchers and observers mainly operate in Europe and North America, which are dominated by animal species of temperate and boreal biomes, with a much better knowledge of their biology. For example, in Europe about 270 mammalian species and 400 avian species are noticed, and in continental North America (USA and Canada) over 710 and 540 species, respectively (Leveque and Mounolou 2003; Arita et al. 2005; Thuiller et al. 2014; Sauer et al. 2017). For biomonitoring goals, much more numerous and more diverse avian samples and information on them than mammalian have been gathered. It concerns terrestrial wildlife too (Frank 1986; Ma et al. 1991; Furness and Greenwood 1993; Golden and Rattner 2003; Rattner et al. 2005; Burger 2006b; Smith et al. 2007; Schmeller et al. 2012; Carneiro et al. 2016; Sauer et al. 2017).

3.1 Mammals as Biomonitors

In Europe, North America, and Asia (mainly in Korea and Japan), samples in which trace elements are determined usually come from several or a dozen selected species of wildlife found on those continents. Among inland mammals there are mainly representatives of the following animal groups: even-toed ungulates (ordo Artiodactyla), lagomorphs (ordo Lagomorpha including hares and rabbits), carnivores (ordo Carnivora), bats (ordo Chiroptera), and Micromammalia, which comprises both rodents (ordo Rodentia) and insectivores (ordo Insectivora). Many researchers prefer micromammals because of their frequent occurrence in the environment, small individual areas, relatively easy acquisition for research, and the possibility of comparison and/or verification of laboratory rodent species results. In addition, their small size makes it possible to assess trace element content in the whole body and an assessment of their transmission to predatory animals (Wren 1986; Ma et al. 1991; Talmage and Walton 1991; Shore and Douben 1994; Kramarova et al. 2005; Sánchez-Chardi et al. 2007; Wijnhoven et al. 2007; Mendez-Rodriguez and Alvarez-Castaneda 2014; Gall et al. 2015). In addition, micromammals are an important part of the diet of avian and mammalian predators and participate in the transmission of trace elements between the links of terrestrial food chains (Gall et al. 2015; Knopper et al. 2006; Herzke et al. 2017). However, the transformation of trace elements in these small mammals is poorly correlated with that occurring in humans and medium-sized long-lived mammals because micromammals have a much higher metabolic rate, usually a short life (1–2 years), and the samples taken, e.g., kidneys or brain, have very low mass, which may cause some analytical problems, including the risk of contamination of the research material (Speakman 2005; Wijnhoven et al. 2007).

Trace elements in the environment generally occur in low concentrations (including highly toxic metals), but their impact on long-lived organisms, including many animals and humans, lasts many years. In the indirect evaluation of their chronic impact on mammals, medium-sized carnivores have been used successfully such as canids (family Canidae: red fox (*Vulpes vulpes*), Arctic fox (*V. lagopus*), golden jackal (*Canis aureus*), and raccoon dog (*Nyctereutes procyonoides*)), mustelids (family Mustelidae: river otter (*Lontra canadensis*), Eurasian otter (*Lutra lutra*), American mink (*Neovison vison*) (previously *Mustela vison*), voloine (*Gulo gulo*), European badger (*Meles meles*), and martens among others), and raccoon (*Procyon lotor*) belonging to family Procyonidae (Wren 1984, Van den Brink and Ma 1998; Lord et al. 2002; Hoekstra et al. 2003; Millan et al. 2008; Heltai and Markov 2012; Kalisinska et al. 2016; Markov et al. 2016; Herzke et al. 2017). They are positioned on the top of the food pyramid, and their feed consists of field and forest rodents, hares, birds, seeds, fruits, or fish in various amounts in semiaquatic species (otters, American mink, raccoon). Many medium-sized carnivores are widely distributed in forest, agricultural, and urban landscapes of the Northern Hemisphere, with some species introduced into areas beyond their natural occurrence (Gehrt et al. 2011; Lesmeister et al. 2015; Poessel et al. 2017). For example, native North American raccoon and American mink are common as alien species in many European countries, while the raccoon dog present in Eastern and Central Europe originated from Asia (Genovesi et al. 2009). Fish-eating wildlife is particularly exposed to mercury biomethylated in water and sediments, and methylmercury product undergoing biomagnification in food chains. For this reason Hg achieves its highest concentrations in fish and piscivorous birds and mammals from the ends of food chains. In inland ecosystems fish-eating carnivores are preferred in studies on mercury contamination. Many reports concerning Hg (and sometimes other heavy metal levels) in American minks, river otters, and raccoons inhabiting North America have been published over the years (e.g., Wobeser and Swift 1976; Wren et al. 1980; Wren 1986; Lord et al. 2002; Wolfe et al. 2007; Sleeman et al. 2010; Basu 2012), but increasing numbers of European studies using American minks and raccoons have also been observed (Norheim et al. 1984; Kalisinska et al. 2012a, 2016, 2017; Brzezinski et al. 2014; Lanocha et al. 2014; Ljungvall et al. 2017).

In contrast to mesocarnivores, publications on trace element concentrations in large predatory Northern Hemisphere mammals, such as cats, are rare (e.g., Eurasian lynx (*Lynx lynx*), North American cougar (*Puma concolor*), bears, and wolves) due to their usually small population sizes, dispersion, and very large anthropogenic limitations of natural ranges, making it difficult to obtain biological samples from them and perform spatiotemporal comparisons (Gamberg and Braune 1999; Shore et al. 2001; Newman et al. 2004; Millan et al. 2008; Celechovska et al. 2006; Noel et al. 2014; Lazarus et al. 2017).

In ecotoxicology, herbivorous game mammals (especially deer; red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*) among others), moose/elk (*Alces alces*), reindeer/caribou (*Rangifer tarandus*), and hares play an important role. Determination of trace elements in these species allows not only identification of the adverse effects connected with excess or deficiency of micronutrients in the animals themselves and on their populations, identification of the contamination of the food chains by nonessential elements, and estimation of human health risks (Adriano 2001; O'Hara et al. 2003; Tataruch and Kierdorf 2003; Vikøren et al. 2005; Mysłek and Kalisińska 2006; Pedersen and Lierhagen 2006; Kursa et al. 2010; Al-Dissi et al. 2011; Ertl et al. 2016). Venison (mainly muscle and to a lesser extent the liver and other offal) is consumed as an important source of protein and micronutrients, but when it contains elevated amounts of toxic elements (e.g., Pb incorporated in tissues), this may contribute to consumer intoxication (Strmiskova and Strmiska 1992; Borch-Iohnsen et al. 1996; Frank et al. 2000; Wolfe et al. 2010; Roug et al. 2015; Skibniewski et al. 2015; Ertl et al. 2016) and a threat to wild mammalian and avian raptors and scavengers, including threatened species (Rogers et al. 2012; Haig et al. 2014; Behmke et al. 2015; Arnemo et al. 2016; Herring et al. 2016).

Omnivorous animals occupy an intermediate trophic position between herbivorous and carnivorous mammals. In Eurasia, one of the most widespread hunted species in this group is wild boar *Sus scrofa*, the progenitor of the domestic pig, and is very often used in European ecotoxicological studies (Santiago et al. 1998; Kursa et al. 2010; Rudy 2010; Amici et al. 2012; Danieli et al. 2012; Dlugaszek and Kopczynski 2013; Gasparik et al. 2017). In North America, the wild boar (feral hog) is classified as an invasive rapidly spreading species and is now abundant in the south and southwest of the USA (Snow et al. 2017; McClure et al. 2018). Although it is a hunted animal in the USA and its meat is often consumed by people, its tissues are rarely tested for the presence of trace elements. Therefore, this type of data is very seldom used for indirect assessment of environmental pollution and consumer health exposure in North America (Oldenkamp 2016; Oldenkamp et al. 2017; Smith et al. 2018). The trophic chain position of the raccoon in North America and Europe is similar to that of the wild boar. In an effort to protect native fauna, the populations of these (and other) species are being deliberately reduced outside their natural ranges, so their tissues can be easily obtained for ecotoxicological studies and intercontinental comparisons. Selenium concentration comparisons in this aspect in omnivorous wild-living mammals seem particularly interesting. Selenium is an element with a very uneven distribution in the earth's crust. Much of Central and Northern Europe's soils are Se-deficient, while North American soils are generally rich in this microelement; in some areas its levels are even excessive. A comparison of Se concentrations in wild boar muscles from Europe (Czech Republic) and the USA (Georgia) indicates that Se levels in the European population are an order of magnitude smaller than in the USA, at 0.10 mg kg^{-1} vs 1.0 mg kg^{-1} dw (Kursa et al. 2010; Oldenkamp 2016). Considering that Se counteracts the absorption of Hg from the diet, areas with an elevated amount of Hg and food poor in Se (e.g., fish) would exhibit increased Hg intoxication of animals compared to individuals of the same species from areas of comparable Hg concentration but more abundant in Se. In relation to raccoon and American mink from Poland, such a suggestion was put forward by Kalisinska et al. (2017) after comparisons of data on Se and Hg in the muscles of these species in Europe (NW Poland) and North America.

3.2 Birds as Biomonitors

Avifauna, especially inland birds, is the longest (over 100 years) and the most intensively methodically observed group of animals in Europe and North America. In contrast, in Asia large-scale observations were initiated as late as the 1970s–1980s (Bibby 2003; Li and Mundkur 2006; Keck 2015). Various bird monitoring programs in Europe and North America, from local to pancontinental, have been introduced for at least 50 years, and some of them include pollution testing (Lambert et al. 2009; Schmeller et al. 2012; Gomez-Ramirez et al. 2014; Ahrestani et al. 2017; Sauer et al. 2017). There are many examples in the history of ecotoxicology where birds have been used as sentinels of environmental and human health. Canaries used to be taken to mines to indicate dangerous concentrations of methane. A dramatic reduction in the populations of birds of prey showed the dangers associated with the widespread use of pesticides in agriculture, including DDT (dichlorodiphenyltrichloroethane), organochlorine substances, and alkyl mercury compounds. The use of the latter, highly neurotoxic and undergoing biomagnification in the trophic chains, resulted in the considerable exposure of piscivorous wildlife and humans to mercury (Scheuhammer 2008; Rabinowitz et al. 2009; Rattner 2009; Basu 2012; Holt et al. 2012; Espin et al. 2016).

Yet another and very spectacular example is the impact of lead contained in hunting ammunition on the health and fitness of individual birds and its effects at the population level. Waterfowl such as ducks and geese (also some landfowls) are a unique group in this respect, because they swallow small pebbles as gastroliths, which are retained in the gizzard and used to grind food. However, the birds do not distinguish pebbles from spent lead shot pellets. Incidental mortality from waterfowl hunting reached population-level effects when over two million ducks and geese (~2% of all waterfowl) in North America were poisoned annually by ingestion of spent lead shot deposited on the grounds and in sediments (Bellrose 1951). Waterfowl, in addition to shot pellets, also swallow leaded fishing gear used in recreational fishing, which eventually also results in the intoxication of animals and people. In addition, waterfowls and other game animals may retain hunting ammunition in their bodies, which can then be swallowed by predators and scavengers. Thanks to numerous field observations of professionals, bird watchers, volunteers, and ecotoxicological research, the use of DDT and pesticides containing mercury was eventually banned in many developed countries (Smith et al. 2007; Espin et al. 2016; Movalli et al. 2017). In the USA, the use of lead pellets in waterfowl hunting was discontinued, as in a few European Union countries. The scientific arguments and the strong voice of the public resulted in a change of policy in the USA and Canada which used the prevalence of lead poisoning among birds as the basis of policy and law introduced to reduce lead use at the continental level, including leaded petrol (Thomas and Guitart 2010; Golden et al. 2016). However, the problem of metallic lead poisoning of rare, endangered birds and the so-called flagship species remains one of the most important in wildlife toxicology, because lead pellets scattered in the environment are still swallowed by waterfowl and landfowl, and lead bullets used in large-game hunts contaminate viscera (offal) left by hunters in the field (Pain et al. 2009; Haig et al. 2014; Espin et al. 2016; Herring et al. 2016). Tranel and Kimmel (2009), based on data from Minnesota (USA), estimated that among terrestrial vertebrates such as reptiles, mammals, and birds, lead ammunition had the greatest effect on birds (about 95%), mostly water birds (38%), raptors, and scavengers (24%). In this respect, the situation may be similar in other parts of the world where hunters use lead ammunition (Pain et al. 2009; Saito 2009; Nadjafzadeh et al. 2013; Golden et al. 2016). Another source of intoxication of birds and humans with lead are remnants of paint containing this metal and leaded gasoline (Nriagu 1990; Cai and Calisi 2016). Therefore, birds are also used in the biomonitoring of cities, e.g., urban pigeons (Ohi et al. 1981; Dauwe et al. 2005; Deng et al. 2007; Roux and Marra 2007; Behmke et al. 2015; Cai and Calisi 2016; Pollack et al. 2017).

In addition to a large number of studies on lead in birds, there is also a considerable body of research on mercury, especially in North America (Rattner et al. 2000, 2005). In inland ecosystems, exposure to mercury is the highest among piscivorous species, and in North America key research in this field includes common loon (*Gavia immer*), bald eagle (*Haliaeetus leucocephalus*), osprey (*Pandion haliaetus*), mergansers, and grebes (DesGranges et al. 1998; Scheuhammer et al. 1998; Stout and Trust 2002; Mierzykowski et al. 2011, 2013; Rutkiewicz et al. 2011; Shore et al. 2011; Depew et al. 2012; Schoch et al. 2014). There are also many studies on other aquatic birds, especially game waterfowls (Gerstenberger 2004; Rothschild and Duffy 2005; Braune and Malone 2006). For a long time, it was thought that mercury is only marginally accumulated in terrestrial songbirds. This view changed after the publication of Cristol et al. (2008) which showed that in areas historically anthropogenically contaminated with mercury it is transferred from the river (the South River, Virginia, USA) and riverside areas to arthropods (spiders and insects) and then songbirds feeding on them. This discovery inspired broader studies on songbirds as sentinels of mercury in terrestrial habitats (Jackson et al. 2015). The flagship species in European studies on mercury contamination is the white-tailed eagle (*Haliaeetus albicilla*) (Norheim and Frøslie 1978; Falandysz et al. 2001; Kenntner et al. 2001; Kalisinska et al. 2016; Kitowski et al. 2017), but observational studies show this species is not at risk of mercury intoxication, as opposed to lead. However, elevated amounts of this metal were found in some of the common merganser (*Mergus merganser*) wintering on the southern coast of the Baltic Sea (Kalisinska et al. 2010).

In ecotoxicological studies, elements are rarely determined in many types of samples from wild birds to characterize their distribution in the body. Such exceptions include two papers describing the distribution of Hg and trace elements in piscivorous great cormorants (*Phalacrocorax carbo*) (Nam et al. 2005; Misztal-Szkudlińska et al. 2018) and one report concerning Hg in young osprey (DesGranges et al. 1998). The muscles of an adult cormorant had the largest amount of Cu (>65%), a significant part of Hg and Cr (about 35%), as well as about 30% of Se and Co accumulated in the body (Nam et al. 2005). In osprey nestlings, about 85% of absorbed Hg gets to feathers during their growth, and from the remaining a dozen or so percent, half of them accumulate in muscles (DesGranges et al. 1998). The quoted works show that the highest amount of Hg in soft tissues is found in the muscles of birds, but its distribution is strongly influenced by the intense transfer of Hg to feathers during their growth.

The usefulness of various bird tissues to monitor the abundance of the environment with elements essential to life and its contamination with toxic metals is constantly discussed. Although the samples most frequently selected in biomonitoring include liver and kidneys, it is important to study their concentration in the muscles and target tissues because of the transfer of various elements up the terrestrial food chains. Interpretation of the obtained concentrations of elements in avian samples requires their reference to threshold values, as in the book by Beyer and Meador (2011) for Cd, Hg, Pb, and Se. However, most trace elements have not been researched in such a thorough fashion for very large and species-diverse clusters of birds. Due to this lack of data, certain reference may come from values calculated for unanalyzed tissues based on the known concentration in the examined tissues through the use of appropriate equations (when the concentrations between these tissues correlate with each other) (Mochizuki et al. 2011; Ackerman et al. 2016; Evers 2018).

3.3 *Tissues of Terrestrial Vertebrates Used in Biomonitoring*

In wildlife toxicology, various types of biological samples may be collected from live animals captured then released (mainly feathers, hair/fur, blood; less frequently fragments of claws or oil from the uropygial gland) or from dead birds and mammals (most of all internal tissues such as liver, kidney, muscle, bone, and brain, but also external tissues). Studies on environmental contaminants, including toxic trace elements, often use avian eggs, with one egg usually taken from individual broods, assaying contaminants in the eggshell, whole egg content, or white and yolk separately (Leonzio and Massi 1989; Burger and Gochfeld 2003; Hashmi et al. 2015; Ackerman et al. 2016; Orlowski et al. 2016; Movalli et al. 2017; Pollack et al. 2017). In addition, researchers often use feathers (e.g., in nests or nearby), hair, mammalian scat, cervid antlers, and avian pellets. Those biological materials are taken mainly from endangered, threatened, or sensitive species; such noninvasive sampling methods are recommended as valuable tools to monitor wildlife and minimally affect free-ranging animals. So-called “postlethal” animal samples are obtained from those already killed by hunters, trappers, museum collectors, or vehicles or found in the field (Kierdorf and Kierdorf 2003; Pokorny 2006; Pauli et al. 2010; Movalli et al. 2017; Trapp and Flaherty 2017).

Different trace elements are deposited in different wildlife tissues at different rates and amounts. The liver, kidney, muscle, and bone from internal tissues are major locations where the largest part of the absorbed essential trace elements are deposited, but concentrations in these tissues are not necessarily representative of the entire body burden, and it can be difficult to detect trace element deficiencies within critical organs (Taylor 1996; Demesko et al. 2018). Essential and nonessential trace elements in different tissues and organs may be subject to temporary or long-term accumulation in various body parts, biotransformation (including methylation and demethylation), and removal mainly with feces and urine, and to a small extent also with saliva, sweat, tears and respiration (Nollet et al. 2008; Lopez-Alonso 2012; Jan et al. 2015; Prashanth et al. 2015). Additional methods of metal and metalloid excretion in birds are eggs and feathers and in mammals the fur (Burger et al. 1993; Burger 1994; Leonzio et al. 2009; Rendón-Lugo et al. 2017). The organ or tissue in which trace metal/metalloid toxicity occurs may differ from the organ or tissue(s) where the element bioaccumulates, which may be connected with its kinetics. Target organs (where the toxic effects are produced) may differ between species of endothermic vertebrates, mainly owing to differences in absorption, distribution, and excretion (US EPA 2007). Table 2.2 presents the main target organs/tissues of nonessential elements and internal body parts where the elements achieve typically highest levels in terrestrial endothermic animals.

Among the internal tissues of wildlife, a number of essential and nonessential elements are predominantly measured in the liver and kidney; however, fluoride and lead are mainly investigated in the bones (Mateo et al. 2003; Demesko et al. 2018). For the past two to three decades, nondestructive samples (hair, feathers, and blood) have been preferred, which are often taken from birds and bats (Russo and Jones 2015; Pauli et al. 2010; Wada et al. 2010; Langner et al. 2012; Lodenius and Solonen 2013; Stankovic et al. 2014; Gall et al. 2015; Flache et al. 2015; Ackerman et al. 2016). Sampling of live animals does not reduce the population, which is important in the case of their small numbers, especially with regard to protected species, and such action is usually socially acceptable. It is estimated that plumage and mammalian pelts contain the largest part of methylmercury (MeHg) accumulated in the body. Therefore these tissues are frequently used in the detection of mercury exposure in wildlife, but many other heavy metals are also investigated in these keratin structures. Feathers (similar to hair) are metabolically inert after their formation, so for those avian species with well-known molt schedules, the analyses of specific individual feathers provide unique chemical information of a very discrete time. For many bird species, the molt schedules are poorly recognized, and metal concentrations in feathers are highly variable within an individual bird. Therefore, proper interpretation of chemical results is very difficult or impossible. For these reasons some researchers state that feathers and hair have a low priority as preferred tissues for sampling in ecotoxicological studies (Furness and Greenwood 1993; Leonzio et al. 2009; Ackerman et al. 2016; Rendón-Lugo et al. 2017).

Table 2.2 Main target organ or tissue as well as internal body parts of terrestrial endothermic animals where nonessential trace elements are accumulated following chronic oral chronic

Element	Target organ or tissue	Organ or tissue with typically highest concentration	References
Ag, silver	Probably brain and liver	Bones	Connors et al. (1972), Horai et al. (2006), and Kuo et al. (2000)
Al, aluminum	Brain and bone	Brain, liver	Llacuna et al. (1995), Krewski et al. (2007), Al-Ganzoury and El-Shaer (2008), and Lucia et al. (2010)
As, arsenic	Liver	Liver	Liu and Waalkes (2008), Sanchez-Virosta et al. (2015), and Mandal (2017)
Cd, cadmium	Kidneys	Kidneys, liver	Martelli et al. (2006) and Wayland and Scheuhammer (2011)
F, fluorine	Skeleton and kidneys	Bones	Bird et al. (1992), Tsunoda et al. (2005), and Kurdi (2016)
Hg, mercury	Kidneys for inorganic Hg; brain for organic Hg	Kidney, liver	Evers et al. (2005), Clarkson and Magos (2006), Bridges and Zalups (2010), and Sleeman et al. (2010)
Pb, lead	Nervous system, mainly brain	Bones	Silbergeld et al. (1993), Nemsadze et al. (2009), Franson and Pain (2011), Flora et al. (2012), and Kalisinska et al. (2016)
Sn, tin	Probably bones and liver	Bones, liver	Kannan and Falandysz (1997), Harding et al. (1998), Nath (2000), ATSDR (2005), and Mizukawa et al. (2009)

Generally, metal levels in blood samples reflect short-term exposure (immediate dietary intake), the liver and kidney reflect longer terms, while the bones reflect the longest because their mineral remodeling occurs very slowly (Stankovic et al. 2014; Gall et al. 2015; Espin et al. 2016). Cadmium is bioaccumulated in bird and mammal kidneys almost over the entire lifetime, and a strong correlation between nephric Cd level and animal age is observed (Wayland and Scheuhammer 2011; Rendón-Lugo et al. 2017). Many trace elements achieve their highest concentrations in the liver and kidneys, (Table 2.2), but together these organs constitute no more than 4%–6% of the animal's body weight. The muscles (40%–50% of body weight) are most significant in the transfer of trace elements between animals from different trophic levels, depending on the type of consumer (Kalisinska et al. 2017). This is especially important in the case of Hg. The level of intestinal absorption of Hg in terrestrial vertebrates depends on its chemical form, and in animal muscle about 90% Hg is present as MeHg, which is almost completely absorbed from the digestive tract. Hg in the liver and kidney is mostly inorganic Hg with low intestinal absorption (<10%). In the kidneys and liver, the percentage of MeHg in total mercury (THg) can be small (especially when THg reaches high concentrations), which is why these organs play a small role in the transfer of Hg between animals. Unfortunately, few papers provide information about the absolute and relative weight of tissues and organs as well as the percentage composition of the consumer's diet, so it is difficult to estimate the amount of transfer of trace elements between different trophic levels. Among terrestrial birds and mammals, Hg concentration increases from herbivores to omnivores and carnivores, but in the case of other trace elements, this type of regularity is not always clearly determined (Tete et al. 2013; Stankovic et al. 2014; Kalisinska et al. 2017).

The most numerous group of ecotoxicological studies concerns a small group of trace elements (<10). They are dominated by toxic elements (Cd, Hg, Pb, As), usually analyzed in 1–3 types of biological samples. Publications in which several or dozens of elements were determined in samples obtained from terrestrial birds and mammals are much less numerous, but this has been made possible due to technical progress in chemical analysis (e.g., Harding et al. 1998; Falandysz et al. 2001; Horai et al. 2006; Deng et al. 2007; Dailey et al. 2008; Zimmerman et al. 2008; Ertl et al. 2016; Lazarus et al. 2017). In literature, data concerning trace elements in soft and hard tissues tend to be presented as mean wet/fresh or dry weight. In scientific studies, the diversity of samples and the multiplicity of the elements determined are subject to various comparisons and discussions. Then it is necessary to present concentrations of elements not only in the same units (mainly expressed as mg kg^{-1}, which is analogous to μg g^{-1} or ppm) but also selecting dry or wet weight. Conversion of wet weight to dry weight (or vice versa) requires knowledge of the percentage of water in the samples, but such information is seldom presented in the reports. Furthermore, samples are dried at temperatures ranging from 50 °C to 105 °C (not always to constant weight), depending on the methodology and the analytical requirements. Therefore, various comparisons use the average percentage of water in vertebrate tissues (Ackerman et al. 2016; Zukal et al. 2015). For the purposes of this book, the average water content in the four most commonly analyzed tissues of birds and mammals was calculated using data from seven and ten species, respectively (data for birds were taken from Honda et al. 1985; Cosson et al. 1988: Kalisinska et al. 2010, 2014; Binkowski et al. 2013; for mammals from Weiner 1973; Reinoso et al. 1997; Blus and Henny 1990; Gamberg et al. 2005; Rudy 2010; Sleeman et al. 2010; Kalisinska et al. 2012a, b; Lanocha et al. 2014). Table 2.3 shows the average percentage of water in the tissues of birds and mammals and also proposed coefficients for wet to dry mass conversion.

When collecting samples from wild mammals and birds, it is advisable to obtain and record important information about them, including species, sex, age, location (latitude and longitude), and season/year. This kind of data is needed for intra- and

Table 2.3 Mean moisture content in tissues of endothermic vertebrates and proposed conversion factors (CF) for normalization of wet weight assay results from tissue samples to dry weight

Parameter	Liver	Kidney	Muscle	Brain
Mammals				
Moisture in tissues (%)	70.9	75.5	74.6	77.0
CF	3.0	2.5	2.5	2.0
Birds				
Moisture in tissues (%)	70.2	74.3	71.4	79.9
CF	3.0	2.5	3.0	2.0

interspecies analyses of differences in the concentration of trace elements and may reveal time-spatial changes on a regional, continental, and even global scale (Tataruch and Kierdorf 2003; Hollamby et al. 2006; Burger 2007; Traas and van Leeuwen 2007; Zukal et al. 2015; Gochfeld 2017).

Depending on the assumed objective of research in wildlife toxicology, samples used in analysis may come from one or more species representing the same or different trophic categories (e.g., herbivores, omnivores, predators). Particularly important are studies analyzing the concentration of selected highly toxic elements (Hg, Pb, Cd) due to the range of research and the very large number of samples (sometimes exceeding 1000). For example, a study on Hg levels in North American birds analyzed blood samples of 102 songbird species from terrestrial habitats (Jackson et al. 2015). The review by Ackerman et al. (2016) compiled literature data on Hg in approximately 27,000 samples (eggs, blood, liver, muscle, and feathers) from 225 species of birds from various systematic groups found in western North America. They concluded that avian Hg concentrations were greatest in ocean and salt marsh habitats and lowest in terrestrial habitats. Their analysis identified multiple hotspots contaminated by the metal in the western part of North America. Finally, Jackson et al. (2016) studied Hg in the blood of 20 avian piscivorous species (including a few target species: bald eagle, osprey, common loon) and those species turned out to be much more exposed to Hg than non-piscivorous species including songbirds.

Biomonitoring of heavy metals in Europe uses bird species on a smaller scale. The leading role is played by diurnal and nocturnal avian raptors, mostly tested for lead and to a lesser extent mercury, the two most preferred metals in such studies (Gomez-Ramirez et al. 2014; Espin et al. 2016). Unlike birds, it is difficult to find extensive studies on toxic metals in North American and European mammals that would allow intra- and intercontinental comparisons (Tranel and Kimmel 2009; Yates et al. 2014). Such publications can only be found for mercury in otters. Mercury in white-tailed eagle and Eurasian otter has been of great interest in Europe for years and sporadically in Asia. In relation to these two species, their North American counterparts are the bald eagle and river otter, which have also been extensively studied. Below we present an example of intercontinental comparisons concerning hepatic mercury concentrations in these species (Fig. 2.2). Median hepatic Hg concentrations in both otter species were similar, but Hg levels in the bald eagle were higher than in the white-tailed eagle (Mann-Whitney *U* test, $p < 0.05$).

Also other piscivorous wildlife species are used in Hg biomonitoring, including both native and alien species occurring in Europe and North America, with well-known biology and reactions to Hg (Table 2.4). However, the volume of European research is much smaller than in North America (e.g., because of lower Hg contamination), and it is difficult to perform comprehensive intercontinental comparisons. For example, there are many American and Canadian papers on Hg in species such as American mink or raccoon (native mammals from North America introduced in Europe), but in Europe the research has been scarce so far. Birds such as common loon or common merganser are native to both continents, but the volume of research

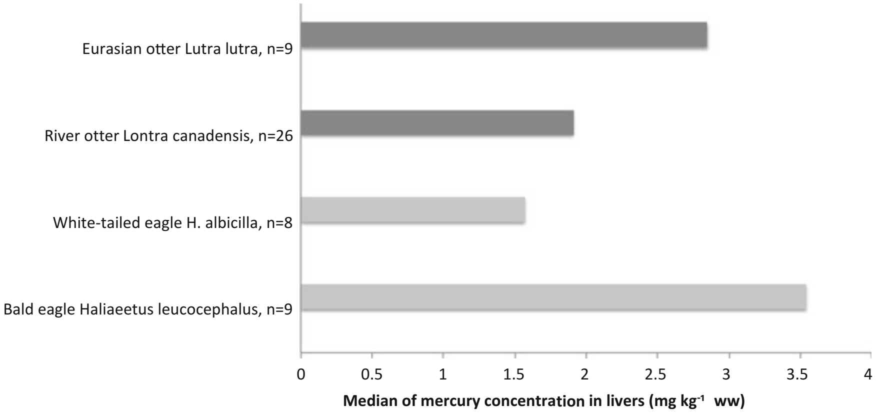

Fig. 2.2 Hepatic mercury concentrations in counterpart piscivorous species of Europe (Eurasian otter and white-tailed eagle) and North America (river otter and bald eagle). Data sources of Eurasian otter, Madsen et al. (1999), Kruuk et al. (1997), Gutleb et al. (1998), Lemarchand et al. (2010), Walker et al. (2010, 2011), Lodenius et al. (2014); river otter: Wren et al. (1980), Sheffy and Amant (1982), Kucera (1983), Halbrook et al. (1994), Evans et al. (2000), Facemire et al. (1995), Mierle et al. (2000), Fortin et al. (2001), Yates et al. (2005), Grove and Henny (2008), Klenavic et al. (2008), Strom (2008), Sellers (2010), Stansley et al. (2010), Mayack (2012), Keeyask Hydropower Limited Partnership (2012), Dornbos et al. (2013); white-tailed eagle, Norheim and Frøslie (1978), Holt et al. (1979), Falandysz et al. (2001), Kenntner et al. (2001), Kalisinska et al. (2014), Krone et al. (2004, 2006), Kitowski et al. (2017); bald eagle, Evans (1993), Wood et al. (1996), Stout and Trust (2002), Weech et al. (2003), Evers et al. (2005), Mierzykowski et al. (2011, 2013), Rutkiewicz et al. (2011)

Table 2.4 Candidates of mercury bioindicator species from terrestrial mammals and birds in North America and Europe

North America	Distribution category and remarks	Europe	Distribution category and remarks
Mammals		Mammals	
American mink *Neovison vison*	Native	American mink *Neovison vison*	Alien, common in Europe
Raccoon *Procyon lotor*	Native	Raccoon *Procyon lotor*	Alien common in Europe
River otter *Lontra canadensis*	Native	Eurasian otter *Lutra lutra*	Native, counterpart species to river otter
Birds		Birds	
Common loon *Gavia immer*	Native	Common loon *Gavia immer*	Native, mainly in Scandinavia
Common merganser *Mergus merganser*	Native	Common merganser *Mergus merganser*	Native
Bald eagle *Haliaeetus leucocephalus*	Native	White-tailed eagle *Haliaeetus albicilla*	Native, counterpart species to bald eagle

in North America is also much greater than in Europe (especially with regard to common loon). With time, when the number of European studies on Hg in their bodies will become sufficiently large (especially American mink and raccoon in Europe), it will be possible to deepen intercontinental comparative studies.

Biomonitoring potential is one of the few acceptable effects of introducing alien game animals. It is associated with good knowledge of the biology of most of these species (e.g., American mink, raccoon, wild boar, red fox), social approval for acquiring material for research from specimens during culling of their populations. Nevertheless, in various European countries and some parts of North America (rarely in Asia), biomonitoring programs for various contaminants in terrestrial ecosystems, including trace elements, are created mainly on the basis of selected native species of birds and mammals. An interesting European example is the Norwegian program "Environmental pollutants in the terrestrial and urban environment," now having been conducted for several years and based mainly on the research on the following animals: earthworms, brown rat (*Rattus norvegicus*), red fox, fieldfare (*Turdus pilaris*), Eurasian sparrowhawk (*Accipiter nisus*), and tawny owl (*Strix aluco*) (Herzke et al. 2017).

4 Conclusions

The collection and analysis of a sufficiently large number of diverse data on trace elements determined in many species of wildlife allow, among other things, to select candidate species as biomonitors accumulating specific elements in their tissues (e.g., piscivorous species for mercury biomonitoring) and identify existing threats from toxic substances for endangered species, localization of hotspots, and levels of human exposure to trace elements. In order to carry out comparisons in this respect on a large scale, i.e., covering the large terrestrial areas of the Northern Hemisphere, it would be necessary to focus on widespread and numerous species representing different trophic levels.

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