Heavy Metals in Liver, Kidney, Brain and Muscle: Health Risk Assessment for Consumption of Edible parts of Birds from the Chahnimeh Reservoirs Sistan (Iran)

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Abstract

The concentrations of four heavy metals zinc (Zn), lead (Pb), nickel (Ni) and cadmium (Cd) were determined in liver, kidney, muscle and brain of nine species of birds from the Chahmimeh Sistan from Iran, to assess metal levels and the potential risk to birds, and to the people who eat them. Significantly higher levels of all metals were found in the brain of waterfowl than in the tissues of other birds. There were no significant gender differences in heavy metals in all tissue. Levels of Pb, Cd, Ni and Zn in the liver and kidney varied as a function of feeding habitats; the median levels were significantly higher in invertebrate predators than fish predators and omnivorous species. Short distance migrating birds had significantly higher median levels of heavy metals in liver and kidney than long distance migrant birds. Ni levels in liver and kidney tissues in 56% of birds were higher than the critical thresholds levels for effects in birds. Our data indicate that environmental exposure to metals were higher in the wintering populations of birds in the Chahnimeh of Sistan from Iran. Concentration Zn, Pb and Cd in a small percentage of birds, and 56% nickel level in liver and 56% kidney concentrations in birds, were above toxicity levels. Determining the exposure frequency and daily intake of birds, the Hazard Quotient for edible tissues (kidney, Liver and muscle) of these birds showed that their consumption provides a health risk to people consuming them.

Introduction

Aquatic environments accumulate pollutants from runoff and atmospheric deposition. While these areas are dynamic, they have a limited capacity to accept man-made waste without adverse effects on biota. With further technology advancement and the development of industries, the volume of waste imported into water areas will likely increase. Heavy metals are pollutants of concern due to their toxicity, persistence, and accumulation in the tissues of living organisms. Generally, the main heavy metals of concern in the environment are from pesticides, chemical fertilizers, electroplating, preparation of paint, coal production, oil combustion, pigments, batteries, photovoltaic cells, greenhouse gas production processes, vehicles, synthetic plastic, extraction from foundry mines, leather product, urban waste incinerators and industrial waste (Kanwal et al. 2020). Besides heavy metals deriving from different industrial and agricultural sources, rocks and volcanoes are an additional source (Lucia et al. 2010). The increase of heavy metals in the biotic and abiotic environment is of great concern because of their adverse human health effects (Wu et al. 2016). Small quantities of heavy metals such as lead, cadmium and chromium, and high concentrations of essential elements such as copper and zinc, in living tissue tissues have caused major concerns due to their serious health effects in birds (Abbasi et al. 2015).

Birds are well suited for biomonitoring because their biology is well known, they have a relatively long lifespan, and they feed on different levels of the food chain, depending on the species. Birds are therefore one of the best indicators for evaluating heavy metals in the environment (Abbasi et al., 2015, Burger and Gochfeld, 2016). Birds are exposed to environmental pollutants from direct contact with contaminated water and food. Studies show that heavy metals accumulate in the organs of birds, especially waterfowl and other bird species that depend on rivers and other aquatic habitats to collect their food. High levels can be harmful and toxic to their reproduction and survival (Savinov et al. 2003). Also, birds are used as an indicator of environmental pollution on a local, regional and global scales (Burger and Gochfeld 2016). Levels in local species can be compared with those that migrate in (and therefore represent contamination over a larger geographical area)(Frederick et al. 1999).

The process of bioaccumulation of heavy metals in birds is very complex and under the influence of many factors, including climate, geographical conditions, physicochemical differences, and the mobility and bioavailability of metals (Aloupi et al. 2017). Behavioral factors such as migration, foraging methods, grit collection, and position in the food chain influence exposure as well (Beyer et al. 1998; Burger et al. 2003; Peakall and Burger 2003). Metals are absorbed in the body, enter the blood circulation, and then exhibit different levels in living things or tissues in relation to reaction to lipids, solubility and transport in different specific cells (Burger et al. 2003). Distribution and concentration of metals in various organs and tissues are influenced by various host characteristics. such as body nutritional status, weight, size, sex, homeostatic mechanisms of genetics, and interaction with nutrients or micronutrients (Honda et al. 1986; Burger et al. 2003; Peakall and Burger 2003).

Because of the key role the liver and kidney play in detoxification processes, heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni) and mercury (Hg), have been studied most extensively because of their toxicity (Kalisińska et al. 2010; Espin et al. 2016). Levels of Pb are examined in bone or brain because of their accumulation over a lifetime, and the effect they have on the nervous system (Pain 1996; Kalisinska 2000). In recent years, human activities that increase levels of heavy metals, such as intense agriculture, leakage of contaminated water to groundwater sources, drainage, and hunting, have posed a serious threat to wildlife (Angelidis and Albanis 1996; Hellenic Ornithological Society 2016).

Anthropogenic pollution has increased organic matter, nutrients, and heavy metals in water and sediment samples (de Luis et al. 2011; Rajaei et al. 2012; Sayadi et al. 2015; Bazrafshan et al. 2016) and fishes (Dahmardeh Behrooz et al. 2013; Ariyaee et al. 2015) from Chahnime, Iran. Some of the pollutants coming from agricultural and industrial activities in Iran and Afghanistan have runoff into the Helmand River, which supplies water to the Hamoun International Wetland and to human-used wells (Dahmardeh Behrooz et al. 2013). The amount of heavy metal contamination in birds in this area has not been studied.

The objective of this study is to assess heavy metal levels in birds wintering in the Chanimeh reservoirs of Sistan region in eastern Iran. We determined the levels of Cd, Pb, Ni, and Zn in brain, liver, kidney and muscle from nine species of birds in Chanimeh, in the Sistan region in Eastern Iran. We examined cadmium metal differences as a function of migration, sex, species, and feeding habits using liver, kidney, brain and muscle samples. We also compared levels to those published in the literature, and examined the risk of metals for endangered species of waterfowl in the Chanimeh of Sistan. Although sample sizes per species are low, this represents the first metals data of its kind from this region, and provides the first risk assessment for humans eating these birds.

Materials And Methods

Collection of Samples

Fifty individual birds of eight species, cormorant (*Phalacrocorax carbo*, n=6), great crested grebe (*Podiceps cristatus*, n=10), black-winged stilt (*Himantopus himantou*, n=10), moorhen (*Gallinula chloropus*, n=6), shoveler (*Anas clypeata*, n=8), marsh sandpiper (*Tringa stagnatilis*, n=6), eurasian spoonbill (*Platalea leucorodia*, n=2) and northern lapwing (*Vanellus vanellus* n=2) were purchased from Chanimeh of Sistan fishermen during February and March in 2019. Birds were weighed and stored in plastic bags that were previously cleaned with acetone and water. Samples were kept at -20^{o}C until dissection and analysis.

Analytical Procedure

Birds were thawed, and liver, kidney, brain and pectoral muscles tissues were collected. Samples (1-3 g wet weight) were placed into Erlenmeyer flasks with 150 Ml, ten mL 65% HNO_3 (Suprapure, Merck, Darmstadt, Germany) was added to the Erlenmeyer flasks, and was slowly digested overnight after five mL HClO; 70% was added to each sample (Suprapure, Merck, Darmstadt, Germany) (Mansouri et al. 2012). For digestion we used a hot plate (sand bath) at the first step at 200^{o}C, for about 6 h or until the solutions were clear after cooling. In the second step each sample was transferred to polyethylene bottles and deionized water was added until the sample equaled 25 mL. In each set of eight samples, one control sample was prepared and examined. Then the solution was filtered using a 0.45 µm nitrocellulose membrane filter. A Shimadzu AA 680 flame atomic absorption spectrophotometer was used for determining the concentrations heavy metals. The detection limits for Cd, Pb, Ni and Zn were 0.09, 0.04, 0.06 and 0.09 µg/g respectively. Also, the obtained recoveries for Cd, Pb, Ni and Zn gave average of 88% and 105% respectively.

Quality Control

Procedural blanks and certified reference material (CRMs, e.g. DOLT-2 (fish liver) and DORM-2) (fish muscles) were included in each sample batch. To determine the detection limit of heavy metals in the samples, blank samples were injected three times for analysis and the result with 3-times the standard deviation of the procedural blanks (0.08, 0.05, 0.07 µg/g dw and 0.1 in Cd, Ni, Pb and Zn respectively). The precision and accuracy of the applied analytical method was determined based on CRMs, e.g. DOLT-2 and DORM-2 heavy metal in sample. The results of our CRMs measurements were a good estimate of the real values. In each sample batch, procedural blanks and certified reference material DOLT-2 and DORM-2 were included. For each matrix, analyses of three blank samples and analysis of reagent blanks was performed. In order to estimate the accuracy and precision of the chemical analysis; sample blanks, standard blanks and three analytical duplicates with the concentration of 1.2 µg/g were injected and their mean and its 95% confidence interval was calculated. Quantification was based on multi-level calibration on the concentrations of 0.1, 0.5, 3, 15, 50 and 100 µg/g; and then the standard calibration curve was drawn with 99% accuracy. Two certified reference materials (DOLT-2 and DORM-2 from National Research

Council Canada, Institute for National) were included for QA/QC to check digestion efficiency and measurement accuracy. The certified values for the reference materials amounted to: Zn = 87 ± 2.5, Pb = 0.24 ± 0.3, Cd = 21.8 ± 5, Ni = 1.3 ± 0.12 and the certified values for the used material amounted to Zn = 88 ± 60, Pb = 0.23 ± 0.4, Cd = 21.58 ± 3, Ni = 1.2 ± 0.13 (6 replications for 0.8-g samples) (recovery 101% to 108%). The method's accuracy, understood as the degree of compatibility of results of multiple analyses of the same sample, reached up to 8% (relative standard deviation RSD). All concentrations are expressed in μg $g^{-1}$of dw.

Statistical analysis

For data analysis we used SPSS (Version 20.0). The data were tested for normality using a Kolmogorov-Smirnov test. For determine normal distribution and homogeneity of variance of heavy metals levels in the tissue samples we use Kolmogorov-Smirnov test and after normal data, we were employed the parametric statistics. To test differences in total heavy metals level of samples among groups we performed a one-way ANOVA, and then the Duncan's post hoc test for differences in level between areas was used. Spearman's rank correlation coefficients were used to test for correlation among various heavy metals from birds. A p-value < 0.05 was considered to indicate statistical significance.

Risk assessment

To assess the health effects and compare them with standards, we converted g/dry weight to g/wet weight (the ratio of dry weight to fresh weight is 0.3). In this study, the THQ was computed according to the guidlines of the United States Environmental Protection Agency and the level of absorption of heavy metals is equal to the absorption of ingestion (assuming that cooking does not affect the level of metals) (USEPA 1989). Furthermore, because of the lack of an oral reference dose (RfDo) of Pb, the value is specified as the permissible tolerable daily intake (PTDI) suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2013).

In this study, we calculated the THQ from the following equation:

$$THQ = \frac{EF \times ED \times MS \times C}{RfDo \times BW \times AT} \times 0.001$$

When THQ (Target Hazard Quotient) is > 1 systemic effect may occur and in fact the THQ is the ratio between exposure and reference dose (Copat 2014). The reference dose (RfDo) (μg/g/day) is an estimate with uncertainty of the daily exposure of human populations, including sensitive subgroups, without an appreciable risk of deleterious effects during a lifetime. The RfDo values used in this study were 0.001, 0.02, 0.004 and 0.3 for Cd, Ni, Pb and Zn respectively. The exposure frequency (EF) in this study is about 182.5, exposure duration (ED) is 72 years, meal size (MS) is about 95g (Sinkakarimi et al. 2013), and 20 g for kidney and liver (Mahmoud et al. 2015). C is the metal concentration (μg/g w.wt)(US EPA 2009; JECFA 2013). The body weight (BW) is 70 kg (US EPA 1989) and EF × ED = AT (average time).

$$THQ = \sum THQ_{toxicant\ 1} + THQ_{toxicant\ 2} + \ldots \ldots + THQ_{toxicant\ n}$$ (Chien et al. 2002).

Also, we calculated the estimated daily intake (EDI) and estimated weekly intake (EWI) based on daily and weekly consumption of birds (including liver and kidney muscle).

The estimated daily intake and estimated weekly intake were calculated as follows:

$$EDI\ (\mu g/g/daily) = \frac{MS \times C}{BW}$$

$$EDI\ (\mu g/g/week) = \frac{MS \times C}{BW}$$

Results And Discussion

Total Heavy Metal Concentrations in Wild Birds from Iran

Zinc: The levels of heavy metals in brain liver, kidney and pectoral muscle are shown in Table 1. The highest median toxic concentrations were of Ni, followed by Pb and Cd; in particular, kidney and liver had the highest levels of Ni. Brain had the highest concentration of Pb (2.7 µg/g dw). For Zn (an essential element), levels were the highest in brain (34.50 µg/g dw), followed by kidney (21.30 µg/g dw), liver (7.30 µg/g dw) and muscle (7 µg/g dw). Studies have shown that there is homeostatic regulation of the intracellular essential metals in birds (Di Giulio and Scanlon 1984; Cosson et al. 1988; Kim et al. 1998; Barjaktarovic et al. 2002; Gomez et al. 2004; Zaccaroni et al. 2011).

Reports of toxicity Zn for wild birds in liver are >122 ng/g dw (Gomez et al. 2004), > 440 n/g dw (Beyer et al. 2004). and 700–1830 ng/g dw (Sileo et al. 2003). Zn level in this study were lower than the Zn adverse threshold for wild birds. The normal level of Zn in the liver of mammals and birds usually does not exceed 525.0 µg/g dw (Taggart et al. 2009). and our results show that none of the birds were above the level.

Zn level in bird livers of this study were much lower than those of birds in Kanibarazan wetland (Alipour et al. 2016) and Gomishan and Anzali wetlands (Aazami and KianiMehr 2018), but similar to birds in Miyankale and Gomishan wetlands in Iran (Sinka-Karimi et al. 2015). Compared to levels elsewhere in the world, Zn level in livers of this study were lower than those of waterfowl in Chesapeake Bay, USA (103-107 µg/g dw) (Di Giulio and Scanlon 1984), Donana national park, Spain (52.5-138.9 µg/g dw) (Taggart et al. 2006), Eastern Poland (30.2-279.38 µg/g dw) (Komosa et al. 2012), Atlantic Canadian (84.7-173 µg/g dw) (Elliott et al. 1992), Chaun, north east Siberia, Russia (82-201 µg/g dw) (Kim et al. 1996), four Spanish wetland (94-144 µg/g dw), lake Biwa and Izum coast, Japan (100-259 µg/g dw) (Nam et al. 2005), and Eastern Austria (38.2 µg/g dw) (Plessl et al. 2017).

Lead: The threshold level for toxic exposure to Pb in liver and kidney of birds is > 6 µg/g dw weight (Clark and Scheuhammer 2003). In ours study one black-winged stilt (*Himantopus himantou*) was higher than the threat level exposure to lead in livers of birds (13.9 ng/g dry weight). Birds such as shovelers (*Anas clypeata*), greylag geese (*Anser anser*), snow geese, brant geese (*Branta bernicla*), mallards and black ducks from Northern California, USA (Hui et al. 1998), Canada (gosling) (Henny et al. 2000), four wetland in Spain (Mateo and Guitart 2003), and northern Idaho, USA (Blus et al. 1995) had levels of Pb in livers higher than the threshold level of threat exposure to Pb in livers. But birds in the Kanibarazan wetland (Alipour et al. 2016), Miyankaleh and Gomishan wetlands (Sinka-Karimi et al. 2015) from Iran, Eastern Poland (Komosa et al. 2012), Donana National Park, Spain (Gomez et al. 2004), Illinois River (Levengood 2003), Eastern Austria (Plessl et al. 2017) were >5 µg/g d.w, indicating the possibility of lead toxicity.

In birds, Pb concentrations in the brain >5 ng/g dw are indicative of poisoning (Kalisinska 2000), and concentrations >16 ng/g dw indicate an advanced state of exposure, birds (Havera et al. 1992). In this study, none of the levels of Pb in the brains were higher than the toxic limit threshold. In birds from Iran in this study, mean Pb concentrations were 0.57–4.7 µg/g dw. in liver and 0.60-8.73 µg/g dw in kidney. The concentration Pb in liver were lower than those observed from Kanibarazan wetland, Iran (Alipour et al. 2016), Gomishan and Miyankaleh, Iran (Sinka-Karimi et al. 2015), Ebro Delta (Mateo and Guitart 2003) , lake Biwa and Mie Izum coast, Japan (Nam et al. 2005), El Hondo, Spain (Taggart et al. 2009), four Spanish wetland (Mateo and Guitart 2003), and were m higher than those observed from Atlantic Canadian, Canada (Elliott et al. 1992), wetland in Northwestern Poland (Kalisińska et al. 2004), Eastern Poland (Komosa et al. 2012), an Illinois river, USA (Levengood 2003), Eastern Austria (Plessl et al. 2017), and Donana National Park, Spanish (Taggart et al. 2006). The concentration Pb in kidney were higher from Kanibarazan wetland, Iran (Alipour et al. 2016), Gomishan and Miyankaleh wetlands, Iran (Sinka-Karimi et al. 2015), Donana Park, Spain (Gomez et al. 2004), Lake Biwa and Mie Izum coast, Japan (Nam et al. 2005), a wetland in northwestern Poland (Kalisińska et al. 2004), and lower of Chesapeake Bay, USA (Di Giulio and Scanlon 1984).

Cadmium: Concentrations of Cd > 3 µg/g dw and > 8 µg/g dw in liver and kidney suggest toxic exposure (Scheuhammer 1987), and levels greater than 40 µg/g dw and 100 µg/g dw in the liver and kidney, respectively, indicate toxicities (Degernes 2008). In this study, except for one black-winged stilt, Cd concentrations of livers were far below the estimated toxic threshold; Cd concentration in one moorhen and one marsh sandpiper were far below the toxicity level (Scheuhammer 1987).

In our birds from Iran, mean cadmium concentrations were 0.43–3.94 µg/g dw in liver and 0.47-7.47 µg/g dw in kidney. The concentrations of Cd in liver were similar to those found in birds from Ebro Delta, Spain (Mateo and Guitart 2003), Lake Biwa and Mie,Izum coast, Japan (Nam and Lee 2006), and the Chesapeake Bay, USA (Di Giulio and Scanlon 1984), but were much lower than those observed from Pacific northwest Canada (Barjaktarovic et al. 2002), Chaun, Northeast Siberia, Russia (Kim et al. 1996), and were much higher than those observed from Zator and Milicz, Poland (Binkowski and Sawicka-Kapusta 2015), Mississippi flyway (Custer et al. 2003), Eastern Poland (Komosa et al. 2012), and an Illinois river (Levengood 2003).

The concentration Cd in kidney were similar to those found in birds from Donana National Park, Spain (Gomez et al. 2004), Illinois river, USA (Levengood 2003), and were lower than the Zator and Milicz, Poland (Binkowski and Sawicka-Kapusta 2015), Chaun Northeast Siberia, Russia (Kim et al. 1996), Pacific Northwest Canada (Barjaktarovic et al. 2002) and were higher than Lake Biwa and Mie Izum coast, Japan (Nam and Lee 2006), a wetland in Northwestern Poland (Kalisińska et al. 2004), Kanibarazan wetland, Iran (Alipour et al. 2016) and Gomishan and Miyankaleh, Iran (Sinka-Karimi et al. 2015).

Nickle: According to studies, Ni concentrations > 10 μg/g dw in kidney, and > 3 μg/g dw in the liver are toxic in wild birds (Outridge and Scheuhammer 1993). In this study, 56% of Ni concentrations in liver, and 56% of kidney concentrations in birds were higher than the toxicity level. In birds, Ni concentrations in liver and kidney are seldom studied. Concentrations of Ni in livers of birds in this study were higher than those from Connecticut, USA (Barclay et al. 1995) Gdansk Bay, Poland (Szefer and Falandysz 1986), San Francisco Bay, USA (Ohlendorf et al. 1986), Jamaica Bay, USA (Burger and Gochfeld 1985), Wrangel Island, Russia (Hui 1998), and Florida Lake from South Africa (Vaneeden and Schoonbee 1992). Concentrations of Ni in the kidney of birds in this study were higher than those from Southwest Atlantic coast, France (Lucia et al. 2010), Gdansk Bay of the Baltic Sea, Poland (Szefer and Falandysz 1987a), and a wetland in Northwestern Poland (Kalisińska et al. 2004).

Variation among Organs

In this study, the levels of heavy metals in muscle tissue were lower than in other tissues, and our results agree with other studies that reported that muscle tissue was not an active tissue for accumulating these heavy metals. Also, in this study, the brains of birds had the highest concentration of metals, except Ni ($P < 0.05$). The level of metal a body absorbs and accumulates depends on the level of exposure, the chemical form of an element, the interaction with other elements, and physiological factors of the bird species (Gochfeld and Burger 1987). Accumulation of pollutants in the internal organs of their bodies is greatly affected by the contaminant level of the food and water ingested. Although liver and kidney are sites of detoxification, they reflect long-term bioaccumulation (Burger and Gochfeld 2016), while muscle and brain only are sites of accumulation, but not of detoxification (Janaydeh et al. 2016).

If birds are exposed to high concentrations of Pb and Cd, these elements will be accumulating in high concentrations in the brains of these birds, such as in white-tailed eagle and scavenging gulls. Brain tissue levels are related to dietary contamination (Hulse et al. 1980; Szefer and Falandysz 1987b). Relatively low (up to 0.4 ppm wet wt) lead (Pb), but not cadmium (Cd), levels were recorded in the brain of pelagic seabirds (Morris et al. 1992; Rice 1992). Redknobbed coots (*Fulica cristata*) from industrialized and polluted regions of South Africa had Pb levels in brain that increased to 25 ppm dw - 2 and 4 times as much as in kidneys and liver (Vaneeden and Schoonbee 1992). These studies on the accumulation of heavy metals in the brain of birds should be further compared to other organs of the body. Different adaptations of birds to the environment, as well as the reaction and function of the brain against different contaminants, can be one of the factors affecting the absorption of contaminants in birds' brains. There are few studies of the levels of heavy metals in the brain tissue of birds. Compared to other studies, the level of heavy metals in brain tissues in this study were higher than other studies in other parts of the world, including Zator and Milicz, Poland (Binkowski and Sawicka-Kapusta 2015), a wetland in Northwestern Poland (Kalisińska et al. 2004), Gdansk Bay Baltic Sea, Poland (Szefer and Falandysz 1987b), Nilgiris, Tamil Nadu, India (Jayakumar and Muralidharan 2011), a lagoon of Marano, Italy (Leonzio et al. 1986), BjØrØya and Jan Mayen Artic (Malinga et al. 2010), and Pomeranian Bay, Poland (Kalisińska and Szuberla 1996).

The highest Ni levels were recorded in kidneys, liver and muscles showed slightly lower levels, and the lowest levels were found in the brain (Figure 2). A significant difference was observed in Ni levels between kidney and the liver, brain and muscles ($P > 0.05$).

Relationship between Metal Levels, Feeding Habits, and Migration Status

The most important factors that affect the concentration of metals among different species are the diet and feeding habits (Dahmardeh Behrooz et al. 2009a). Diet varies between different bird species depending on the foraging strategies and diet preferences. One of the key pathways for metals to enter the body of birds is through food, water, and by eating sediment, lead shot and grit (non-food items). The direct consumption of soil contaminated with metals is a major cause of increased contamination in their bodies, even if the contaminant levels in plants or their prey has not increased (Beyer et al. 1998).

In our study birds were divided into four groups: invertebrate predator, fish predator, fish and crab predator, and omnivor to examine the effects of type of food on metal levels, using published data (Mansoori 2008; Dahmardeh Behrooz et al. 2009a). In the fourth group, we had only the Eurasian spoonbill (n=1), so it was excluded from the statistical tests. Diet type had a significant effect on the levels of Zn, Pb, Cd and Ni in the kidney and liver, with invertebrate species having higher concentrations than fish predators and omnivores ($P < 0.05$). There were no statistically significant differences for brain and muscle levels for any of the metals examined.

In a study in Shadegan wetland from Iran on mercury pollution in three species of waders, black-winged stilt had higher levels of mercury in the feathers, liver, kidneys, and muscles than other birds in the study (Zamani-Ahmadmahmoodi et al. 2010). The reason for the increase in mercury in this bird compared to other birds was that its long legs allowed access to deeper parts of the water and stilts could hunt larger prey than invertebrates. Similarly, other authors found higher heavy metal levels in the larger species that had access to deeper sections of the water, and could hunt larger prey (Burger and Gochfeld 1992). In the present study, the reason for the increase in metals in the various organs of black-winged stilt, marsh sandpiper and northern lapwing was their feeding on agricultural lands irrigated by farmers. We, and others (D Mansoori, 2008), suggest that these species feed more on agricultural lands than do other species, remaining on the water for several days, rather than on the shores of the Chahnimeh from Sistan. Perhaps the use of chemical fertilizers and pesticides in agricultural lands has increased the exposure of birds to metals. This difference in metal concentration is most likely due to metal biogeochemical behavior, diet, and accidental ingestion of fine soil and sediment particle. However, it is impossible to separate soil selection/soil digestion and diet. Certainly these two exposure pathways are very effective in concentrating these metals because other metals are correlated with accidental ingestion of fine soil and sediment particle (Alloway 2012). In our study of heavy metals, birds that are herbivores compared to birds that are predators had higher concentrations of heavy metals in the liver (Okati 2013) (Parslow et al. 1982), (Brennan et al. 1992).

Birds of Chahnimeh reservoirs were divided into 2 groups of long-distance migrants, and local migrants that only go to the northern rivers and wetlands of Iran and do not leave Iran. It is noteworthy that there were differences in levels between the internal organs of the kidney and liver for all four elements studied, but no statistically significant difference was observed between the two groups of birds for brain and muscle tissue (Table 3). The birds in the southern wetlands from Iran migrate to northern wetlands in the provinces Gilan and Mazandarn in the southern Caspian Sea to avoid the hot summer months in south and southeast Iran (Mansoori 2008; Dahmardeh Behrooz et al. 2009b). Heavy metal levels are high in this region of Iran, Caspian Sea, in fishes, macroalga, sediment and water (Adel et al. 2016; Ebadi and Hisoriev 2017a, b; Malvandi 2017). These higher level of heavy metals in the south Caspian Sea might explain the high level of these heavy metals local migrants.

Lower median concentrations of heavy metals (Cd, Pb, Ni and Zn) in liver and kidney were detected in the long-distance migrant birds than the local migrants ($P > 0.05$) (Figure 1). Low usage of heavy metals and pesticides in breeding regions birds (Siberia or Eastern Europe) (Dahmardeh Behrooz et al. 2009b) that have migrated out of Iran might explain lower heavy metals in these birds.

Correlations among Heavy Metals

All four elements in this study were positively correlated with each other within organs ($P > 0.001$, $r > 0.777$), but none of the elements were positively correlated with the other element among tissue. This shows that the pathways and sources of entry for the elements studied are similar, but the pathways for the accumulation of these elements and the reactions of different organs of the body to these elements, are very different. A positive correlation between levels of Zn and Cd in the body of birds may protect them from the effects of increasing Cd in the body (Cosson et al. 1988; Elliott et al. 1992). Positive correlations of Pb or Cd with other element level in tissues have been reported in birds from Korea (Kim and Oh 2012), (Kim et al. 2009), Cory's shearwater (*Calonectris diomedea*) and black-backed gulls (*Larus fuscus*) from England (Stewart et al. 1995), seabirds from Chaun, northeast Siberia, Russia (Kim et al. 1996), and feral pigeons (*Columba livia*) from Korea (Nam and Lee 2006).

Health Risk Thresholds

One of non-essential element in the body is lead (Pb) that can cause neurotoxicity, nephrotoxicity, and other health effects (Garcia-Leston et al. 2010). Both Spanish legislation and Australian National Health and Medical Research Council (ANHMRC) proposed 2.0 μg/g ww as the maximum permitted level of Pb in food (Plaskett and Potter 1979; El-Sikaily et al. 2004). The median level of Pb in muscle tissue in 6 species birds (except for Eurasian spoonbill, great crested grebe and moorhen), was lower than the

Spanish legislation and ANHMRC guidelines. The median level of lead in the liver of birds was higher than the Spanish legislation and ANHMRC guidelines except for cormorant and in kidney of all birds except cormorant and Eurasian spoonbill were higher than this guideline (Figure 3). Also, 1 1.7 µgg w.w Pb is the action level for human health (USFDA 1993), and according to this guideline median level Pb in muscle of all birds (except cormorant), and liver and kidney in all birds, were higher than this guideline (Figure 3). In contrast to these maximum permitted levels for Pb, the Institute of Turkish Standards for Food (ITSF) and European Commission (EC) introduced the permissible threshold level of 0.1 and 0.5 µg/g ww respectively (EC 2001; Dirican et al. 2013). The median level in flesh muscle, liver, and kidney of all birds in this study were clearly higher than these guidelines, and according to these two guidelines, the health of the people of this region is endangered by consuming the muscle, and especially the liver of these birds.

The maximum permitted Cd level of ANHMRC, USFDA and Western Australian authorities are 2, 3.7 and 5.5 µg/g ww respectively. In our study none of the birds exceeded this median level Cd in muscle, but levels of Cd in liver of northern lapwing, moorhen, marsh sandpiper and black-winged stilt were higher than the threshold levels suggested by ANHMRC, USFDA and Western Australian authorities (Plaskett and Potter 1979; El-Sikaily et al. 2004). Cadmium levels in kidney were higher than the ANHMRC threshold in all birds except the cormorant and the Eurasian spoonbill. Also, the great crested grebe, with level 4 µg/g ww, was higher than both ANHMRC and USFDA guidance, and the rest of the birds were higher than all three guideline ANHMRC, USFDA and Western Australian authorities (Figure 3). In contrast to these maximum permitted levels, the Spanish legislation and EC threshold is 1 and 0.05 µg/g ww respectively (Plaskett and Potter 1979; El-Sikaily et al. 2004). In this study, levels of Cd in muscle, liver and kidney of all birds were greater than these thresholds.

The permissible limit of Ni food by the US Food and Drug Administration is 10 µg/g ww (USFDA, 1993). According to this guideline, the levels of Ni in muscle, liver and kidney of all birds, except cormorant, were higher than the permissible limit. The permissible limit of FAO Ni is 13 µg/g ww in food (FAO 1983), and level Ni in muscle birds were lower than this limit, except for liver in black-winged stilt, marsh sandpiper, moorhen and northern lapwing (13 µg/g ww), and levels in kidney of all birds (except cormorant and Eurasian spoonbill) were higher than the FAO guideline (Figure 3). The Food and Nutrition Board (FNB) (FNB, 2010) introduced the permissible limit of Ni as 4 µg/g ww. Accordingly, levels of all muscle, liver and kidney in all birds in the present study were higher than this limit, and consumption of edible parts of all birds pose a threat for heath people in this region.

The ANHMRC and WHO introduced an acceptable limit of 1000 µg/g ww for Zn in food. The level of Zn in muscle, liver and kidney in all birds of Zabol Chahnimeh reservoirs were below this toxic threshold (Plaskett and Potter 1979) (Cliton et al. 2008). (Figure 3).

Health Risk from Consuming Birds in Chanimeh Reservoir

In our study, the level HQ in any of the metals in the muscle of birds was not more than one, but the $\sum$HQ was higher than one in M. (Table 4). The level HQ of Pb in liver was higher than 1, but in the other birds it wasn't higher than 1, and even $\sum$HQ was not higher than 1 (Table 4). In kidney, the HQ of Pb in N, M, MS and B was higher than 1 and also, in these birds the $\sum$HQ was > 1. In the edible parts, the level of HQ was high and except C in the others birds between level $\sum$HQ was 1.24 to 4, and this due to the high level of HQ in the kidneys and muscle of birds in this region (Figure 4). The $\sum$HQ of each metal we examined was higher >1, suggesting that people would not experience health risks from consumption of birds from the Chahnimeh reservoirs (Figure 4). On the other hand, values of the $\sum$HQ index for total exposure were >1 for birds, indicating that the estimated exposure is a major health concern. Researchers' studies in the wetlands of northern Iran showed that the pochard is not suitable for consumption like our birds (Sinkakarimi et al. 2015a).

Estimated Human Daily and Weekly Toxic Elements Intake from Birds

Different metals in different concentrations have different effects on organisms, and some metals can show toxic effects even in low concentrations (Mahmoud et al. 2015). In this study, we examined the EWI and EDI and then compared it with Provisional permissible tolerable weekly intake (µg/kg body weight/week) (PTWI), PTWI 70 that is PTWI for 70 kg person (µg/week) and PTDI (Permissible tolerable daily intake) for a 70 kg person (µg/day). The PTWI, PTWI 70 and PTDI depends on metal levels, dietary use of different dietary foodstuffs. The Food and Agriculture Organization and World Health Organization in 2004 established Provisional permissible tolerable weekly intake for Pb and Cd of 7 and 25 µg/kg body weight/ week for people, equaling 490, 1750 µg/week for 70 kg (mean body weight of an Iranian person), respectively (JECFA 2004). Also, the PTWI according to guidelines of FAO/WHO (2011) are 35 and 7000 µg/kg body weight/ week for nickel and zinc, equaling 2450 and 490000 µg/week for a 70 kg person, respectively (JECFA 2011).

According to Table 4, none of the bird organs had levels of Zn, Pb, Cd and Ni that were higher than the level of PTWI70. In this study, the EWI of Pb in edible parts of birds B, MS, M, N and E was higher than PTWI, and this is due to the high level of EWI in the liver of these birds, while the level of lead in the muscle tissue of all birds was within the allowable range for PTDI, PTWI and PTWI 70.

For Cd, the level EWI in the edible parts was higher than PTWI in all birds, and the EDI level in edible parts birds G, B, MS, M and N was higher than the PTWT, which is due to the high level of EWI and EDI in the muscle and kidneys of these birds (Table 4).

In this study of Ni, the level EWI in muscle and edible parts was higher than PTWI in all birds, and level EWI was higher in B, MS, M and N of PTDI. The EDI in the birds B, N, M and MS was higher than the level of PTWI. Except C and E, in all birds that EWI in kidney them was higher of PTWI, also level Ni in liver birds B, Ms, N and M level EWI was higher of PTWI (Table 4). Level EDI of Ni in edible parts B, Ms, N and M was higher of PTWI and also level Ni in kidney M and Ms higher of PTWI, and finally, the data indicate that level EWI in edible parts B, MS, M and N was higher of PTDI (Table 4). The results of this study show that people in this area should not use edible parts, and the use of wild birds as daily and weekly food is a serious threat to the inhabitants of this area. This is contrary to the results obtained for birds in the wetlands of northern Iran, where EDI and EWI were within the permissible range and did not pose a threat to the people of the region (Sinkakarimi et al. 2015a, b).

Conclusion

In this study, levels of Cd, Pb, Ni and Zn were investigated in birds of Chahnimeh of Sistan from Iran. The level of all heavy metals (except nickel) in the brains of birds was higher than levels in other tissues. Differences in metals levels as a function of feeding habitat and migration were observed only in the kidney and liver tissues of birds. The levels of heavy metals in some birds were higher than an effects level threshold; 56% of the liver and kidneys samples of these birds were above the threat level. The results of this study show that birds in Chahnimeh of Sistan pose a risk to humans from heavy metal contamination. The data show that human consumption (using EDI, EWI and HQ) of the edible tissues of birds is not suitable: people of the region should avoid eating the edible tissues of wild birds and should avoid eating kidney and liver tissue.

Declarations

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Tables

Due to technical limitations the Tables are available as a download in the Supplementary Files.

Figures

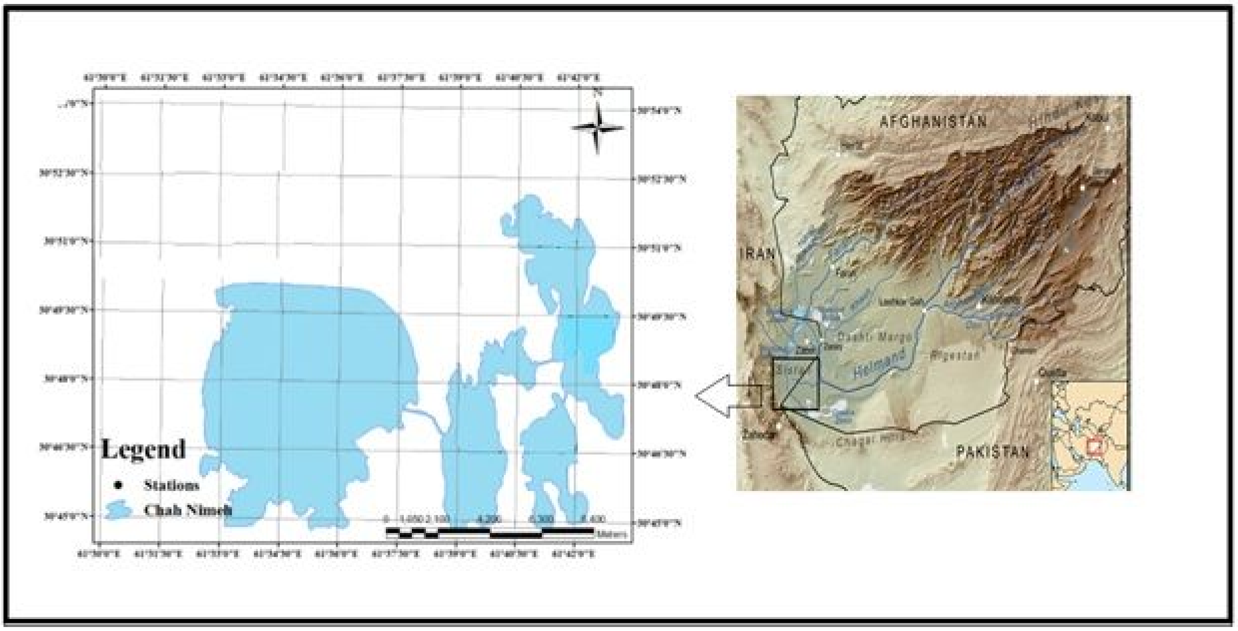

Figure 1

Location map of study area.

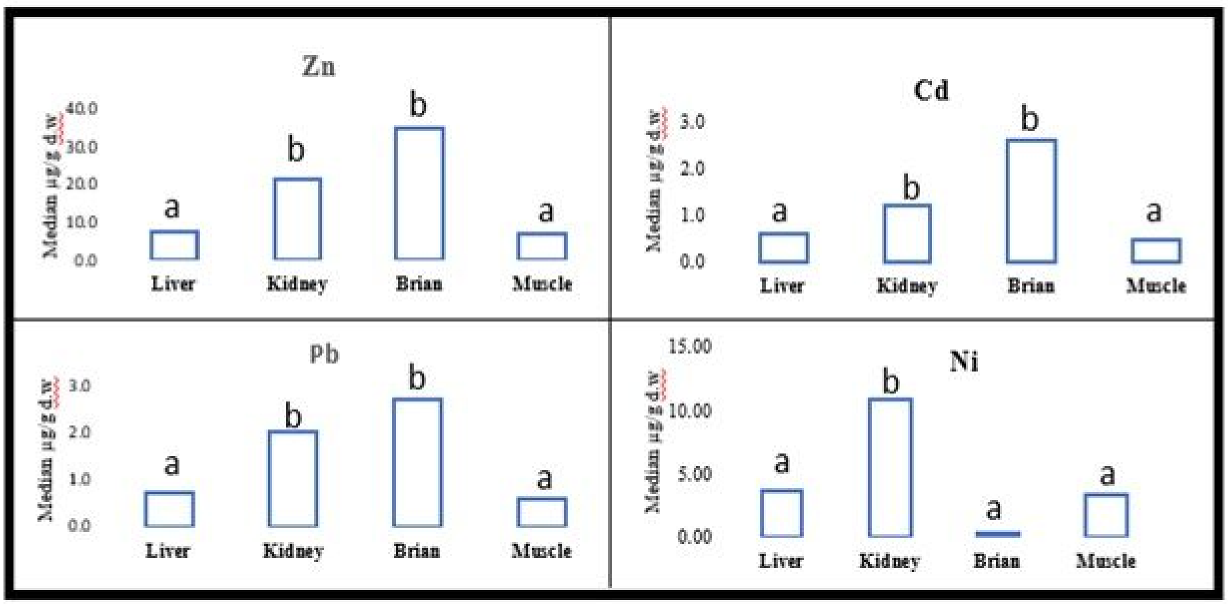

Figure 2

Median concentration µg/g d.w in difference tissues birds.

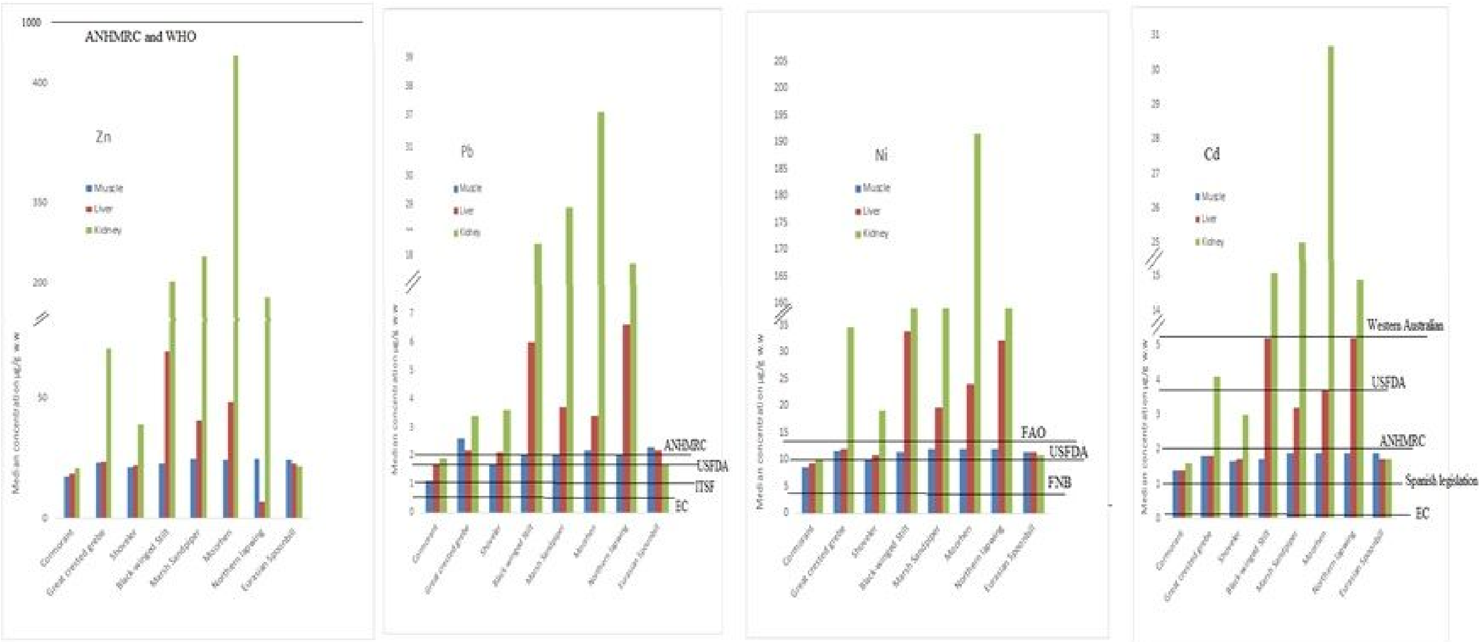

Figure 3

Median concentration heavy metal and compare with US Food and Drug Administration (USFDA), Australian National Health and Medical Research Council (ANHMRC), Turkish Standards for Food (ITSF), European Commission (EC), Food and Nutrition Board (FNB), Food and Agriculture Organization (FAO) and World Health Organization

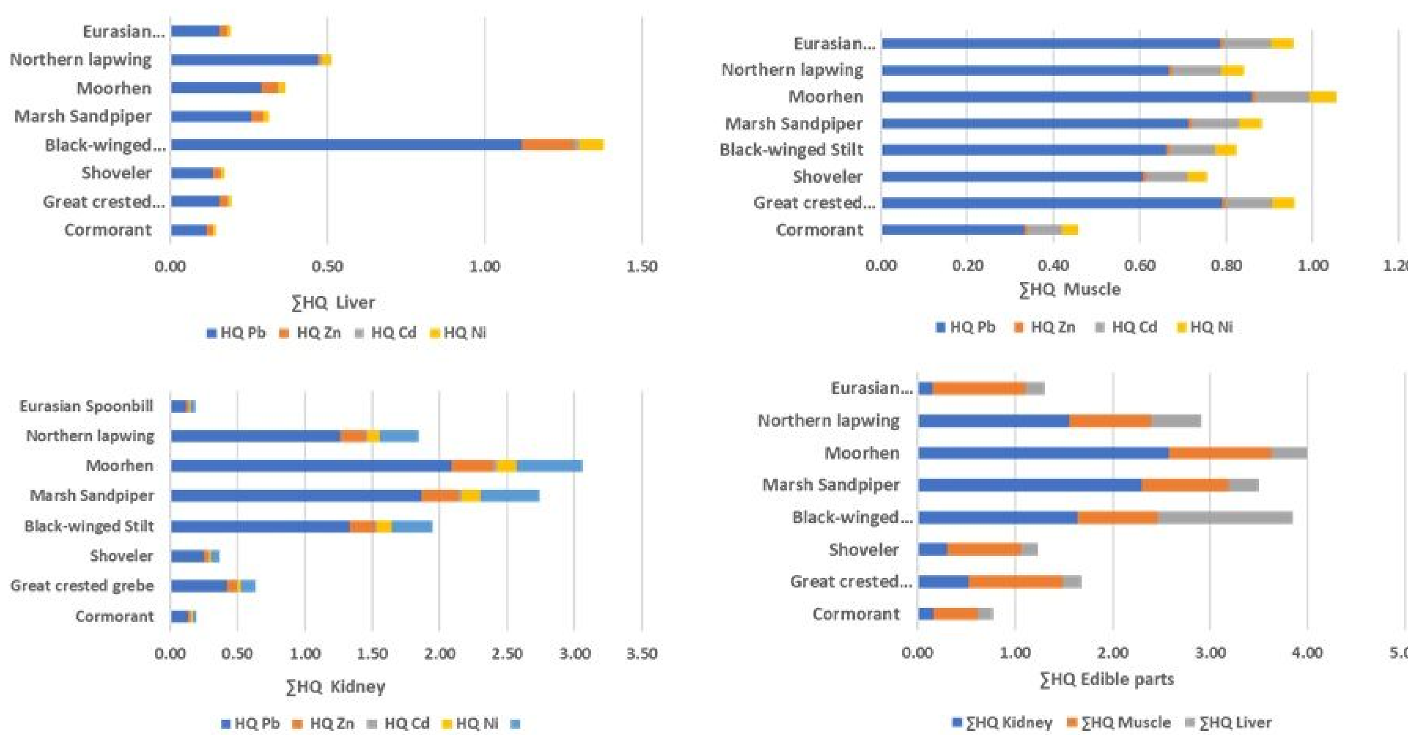

Figure 4

Estimated potential health risks for Zn, Pb, Cd and Ni via consumption liver, kidney, muscles and collected them (edible parts) Hazard quotients (HQ) and HQ HQ Pb + HQ CD + HG + HQ Ni + HQ Zn

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf