Heavy Metals in the Liver, Kidney, Brain, and Muscle: Health Risk Assessment for the 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 the liver, kidney, muscle, and brain of nine species of birds from the Chahnimeh Sistan from Iran to assess the 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 than in the other tissues of other birds. There were no significant gender differences in heavy metals in all tissue. The 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 they were for fish predators and omnivorous species. Short-distance migrant birds had significantly higher median levels of heavy metals in the liver and kidney than long-distance migrant birds. Ni levels in the liver and kidney tissues in 56% of birds were higher than the critical threshold levels for effects in birds. Our data indicate that environmental exposures to metals were higher in the wintering populations of birds in the Chahnimeh of Sistan from Iran than elsewhere. Concentrations of Zn, Pb, and Cd in a small percentage of birds were above toxicity levels. However, 56% of liver and kidney samples for nickel 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 may provide health risk to people consuming them.

Keywords Heavy metals · Birds · Sistan · Health risk assessment · Consumption

Introduction
Aquatic environments accumulate pollutants from runoff and atmospheric deposition. While aquatic habitats are dynamic, they have a limited capacity to accept man-made waste without adverse effects on biota. With further technology advancement and the increased 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 [1]. Besides heavy metals deriving from different industrial and agricultural sources, rocks and volcanoes are an additional source [2]. The increase of heavy metals in the biotic and abiotic environment is of great concern because of their adverse human health effects [3]. Small quantities of heavy metals such as lead, cadmium, and chromium and high concentrations of essential elements such as copper and zinc, in living tissues, have caused major concerns due to their serious health effects in birds [4].

Birds are well suited for biomonitoring because their biology is well-known, they have a relatively long lifespan (up to a dozen or more years), 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 [4, 5]. 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 [6]. Also, birds are used as an indicator of environmental pollution on local, regional, and global scales [5]. Local species (that feed locally) can be compared with those that migrate in (and therefore represent contamination over a larger geographical area) [7].

The process of bioaccumulation of heavy metals in birds is very complex and influenced by many factors, including climate, geographical conditions, physicochemical differences, and the mobility and bioavailability of metals [8]. Behavioral factors such as migration, foraging methods, grit collection, and position in the food chain influence exposure as well [9–11]. Metals are absorbed in the body, enter the blood circulation, and then exhibit different levels in tissues in relation to reaction to lipids, solubility, and transport in different specific cells [9]. 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 [9, 10, 12].

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 [13, 14]. The levels of Pb are examined in the bone or brain because of their accumulation over a lifetime and the effect they have on the nervous system [15, 16]. In recent years, human activities that increased the 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 [17].

Increased anthropogenic pollution has resulted in increased levels of organic matter, nutrients, and heavy metals in water, sediment [18–21], and fishes [22, 23] from Chahnimeh, Iran. Some of the pollutants coming from agricultural and industrial activities in Iran and Afghanistan have run off into the Helmand River, which supplies water to the Hamoun International Wetland and to human-used wells [22]. 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 Chahnimeh reservoirs of the Sistan region in eastern Iran. We examined metal differences as a function of migration, sex, species, and feeding habits using the liver, kidney, brain, and muscle samples. We also compared the levels to those published in the literature and examined the risk of metals for endangered species of waterfowl in the Chahnimeh of Sistan. These birds were given to us for studies after the Environmental Protection Agency removed them from fishermen who had hunted them illegally. Although sample sizes per species are low, this represents the first metal 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 (C) (*Phalacrocorax carbo*, *n* = 6), great crested grebe (G) (*Podiceps cristatus*, *n* = 10), black-winged stilt (B) (*Himantopus himantopus*, *n* = 10), moorhen (M) (*Gallinula chloropus*, *n* = 6), shoveler (S) (*Anas clypeata*, *n* = 8), marsh sandpiper (MS) (*Tringa stagnatilis*, *n* = 6), Eurasian spoonbill (E) (*Platalea leucorodia*, *n* = 2), and northern lapwing (N) (*Vanellus vanellus*, *n* = 2) were purchased from Chahnimeh of Sistan fishermen during February and March in 2019. Birds were weighed and stored in plastic bags that were cleaned with acetone and water. Samples were kept at −20 °C until dissection and analysis.

**Analytical Procedure**

Birds were thawed, and liver, kidney, brain, and pectoral muscle tissues were collected. Samples (1–3 g wet weight) were placed into 150 mL Erlenmeyer flasks; 10 mL 65% HNO₃ (Suprapure, Merck, Darmstadt, Germany) was added to the Erlenmeyer flasks and was slowly digested overnight after 5 mL HClO₄; 70% was added to each sample (Suprapure, Merck, Darmstadt, Germany) [24]. For digestion, we used a hot plate (sand bath) at the first step at 200 °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 analyzed. 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 of 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 averaged 88%, 90%, 95, 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 a sample, blank samples were injected three times for analysis, and the result was 3 times the standard deviation of the procedural blanks (0.08, 0.05, 0.07, and 0.1 µg/g dw in Cd, Ni, Pb, and Zn respectively). The precision and accuracy of the applied analytical method were 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, the analyses of three blank samples and of three reagent blanks were performed. 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 were 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, and Ni = 1.3 ± 0.1, and the certified values for the used material amounted to Zn = 88 ± 60, Pb = 0.23 ± 0.4, Cd = 21.58 ± 3, and Ni = 1.2 ± 0.13 (6 replications for 0.8 g sample with the recovery between 88 and 105%). 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 of dw.

Statistical Analysis

For data analysis, we used SPSS (version 20.0). The data were tested for normality using a Kolmogorov–Smirnov test. To determine normal distribution and homogeneity of variance of heavy metals levels in the tissue samples, we used the Kolmogorov–Smirnov test, and data were not normal. To normalize our data, we use log-transformation (log10), and, after normalizing all data, we used parametric statistics. To test differences in total heavy metal 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 indicated statistical significance.

Risk Assessment

To assess the health effects and compare them with standards, we converted g/dry weight to g/wet weight. The dry weight/wet weight ratio was assumed to be approximately 0.3 for all species [25, 26]. In this study, the target hazard quotient (THQ) was computed according to the guidelines of the US Environmental Protection Agency, and the level of absorption of heavy metals was considered 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) for 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:

\[ \text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{MS} \times C}{\text{RfDo} \times \text{BW} \times \text{AT}} \times 0.001 \]

When the target hazard quotient (THQ) is > 1, systemic effect may occur, and in fact, the THQ is the ratio between exposure and reference dose [27]. The reference dose (RfDo) (µg/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 was about 182.5, the exposure duration (ED) is 72 years, the meal size (MS) is about 182.5 g [28] and 20 g for kidney and liver [29]. C is the metal concentration (µg/g w.wt). The target hazard quotient (THQ) was computed according to the guidelines of the US Environmental Protection Agency, and the level of absorption (THQ) was computed according to the guidelines of the US

\[ \text{THQ} = \sum \text{THQ}_{\text{toxicant 1}} + \text{THQ}_{\text{toxicant 2}} + \cdots + \text{THQ}_{\text{toxicant n}} \]

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:

\[ \text{EDI(µg/g/daily)} = \frac{\text{MSSxC}}{\text{BW}} \]

\[ \text{EWI(µg/g/week)} = \frac{\text{MSSxC}}{\text{BW}} \]

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Results and Discussion

Total Heavy Metal Concentrations in the Liver, Kidney, and Brain and Muscles in Wild Birds from Iran

The levels of heavy metals in the brain, liver, kidney, and pectoral muscle are shown in Tables 1 and 2. The highest median toxic concentrations were of Ni, followed by Pb and Cd; the kidney and liver had the highest levels of Ni. The brain had the highest concentration of Pb (2.7 µg/g dw). For Zn (an essential element), the levels were the highest in the brain (34.50 µg/g dw), followed by the kidney (21.30 µg/g dw), liver (7.30 µg/g dw), and muscle (7 µg/g dw). Studies have shown that there is a homeostatic regulation of the intracelluar essential metals in birds [34–39].

Reports of Zn toxicity for wild birds in the liver are > 122 µg/g dw [36], > 440 µg/g dw [40], and 700–1830 µg/g dw [41]. Zn levels 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 [42], and our results show that none of the birds was above this level. Zn levels in bird livers of this study were much lower than those of birds in Kanibarazan wetland [43] and Gomishan and Anzali wetlands [44] but similar to birds in Miyankale and Gomishan wetlands in Iran [45]. Compared to the levels elsewhere in the world, Zn levels in livers of this study were lower than those of waterfowl in Chesapeake Bay, USA (103-107 µg/g dw) [35]; Donana National Park, Spain (52.5–138.9 µg/g dw) [46]; Eastern Poland (30.2–279.38 µg/g dw) [47]; Atlantic Canadian (84.7–173 µg/g dw) [48]; Chaun, Northeast Siberia, Russia (82–201 µg/g dw) [49]; four Spanish wetland (94–144 µg/g dw), Lake Biwa and Mizum coast, Japan (100–259 µg/g dw) [50]; and Eastern Austria (38.2 µg/g dw) [51].

We considered < 6 µg/g dw Pb in the liver and/or kidney to be indicative of “background” Pb exposure; individuals were considered to be “Pb exposed” when concentrations exceeded 6 µg/g dw in the liver or kidney and were “Pb poisoned” when kidney levels exceeded 20 µg/g dw or when liver levels exceeded 30 µg/g dw [52]. Birds such as shovelers (Anas clypeata), greylag geese (Anser anser), snow geese, brant geese (Branta bernicla), mallards, and black ducks from Northern California, the USA [53], Canada (gosling) [54], four wetland in Spain [55], and northern Idaho, USA [56], had levels of Pb in the livers higher than the threshold level of threat exposure to Pb in the livers. But birds in the Kanibarazan wetland [43], Miyankaleh, and Gomishan wetlands [45] from Iran; Eastern Poland [47]; Donana National Park, Spain [36]; Illinois River [57]; and Eastern Austria [51] were > 5 µg/g dw, indicating the possibility of Pb toxicity.

In birds from Iran in this study, the mean Pb concentrations were 0.57–4.7 µg/g dw in the liver and 0.60–8.73 µg/g dw in the kidney. The concentrations of Pb in the liver were lower than those observed from Kanibarazan wetland, Iran [43]; Gomishan and Miyankaleh, Iran [45]; Ebro Delta [55]; Lake Biwa and Mizum coast, Japan [50]; Hondo, Spain [42]; and four Spanish wetlands [55] and were much higher than those observed from Atlantic Canadian, Canada [48]; wetland in Northwestern Poland [58]; Eastern Poland [47]; an Illinois river, USA [57]; Eastern Austria [51]; and Donana National Park, Spain [46]. The concentration of Pb in kidney was higher from Kanibarazan wetland, Iran [43]; Gomishan and Miyankaleh wetlands, Iran [45]; Donana Park, Spain [36]; Lake Biwa and Mizum coast, Japan [50]; a wetland in Northwestern Poland [58]; and lower in birds from Chesapeake Bay, USA [35].

Concentrations of Cd > 3 µg/g dw and > 8 µg/g dw in the liver and kidney suggest toxic exposure [59], and levels greater than 40 µg/g dw and 100 µg/g dw in the liver and kidney, respectively, indicate toxicities [60]. 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 [59]. In birds from Iran, the mean cadmium concentrations were 0.43–3.94 µg/g dw in the liver and 0.47–7.47 µg/g dw in the kidney. The concentrations of Cd in liver were (1) similar to those found in birds from Ebro Delta, Spain [55]; Lake Biwa and Mizum coast, Japan [61]; and the Chesapeake Bay, USA [35]; (2) were much lower than those observed from Pacific northwest Canada [34], Chaun, Northeast Siberia, Russia [49]; and (3) were much higher than those observed from Zator and Milicz, Poland [62], Mississippi flyway [63], Eastern Poland [47], and an Illinois river [57]. The concentration Cd in kidney was similar to those found in birds from Donana National Park, Spain [36], and Illinois river, USA [57], and were lower than the Zator and Milicz, Poland [62]; Chaun Northeast Siberia, Russia [49]; and Pacific Northwest Canada [34] and were higher than Lake Biwa and Mizum coast, Japan [61]; a wetland in Northwestern Poland [58]; Kanibarazan wetland, Iran [43]; and Gomishan and Miyankaleh, Iran [45].

According to studies, Ni concentrations > 10 µg/g dw in the kidney and > 3 µg/g dw in the liver are toxic in wild birds [64]. In this study, 56% of Ni concentrations in the liver and 56% of Ni concentrations in the kidneys were higher than the toxicity level. In birds, Ni concentrations in the liver and kidney are seldom studied. Concentrations of Ni in livers of birds in this study were higher than those from Connecticut, USA [65]; Gdansk Bay, Poland [66]; San Francisco Bay, USA [67]; Jamaica Bay, USA [68]; Wrangel Island, Russia (Hui 1998); and Florida Lake from South Africa [69]. Concentrations of Ni in the kidney of birds in this study were higher than those from Southwest Atlantic coast, France [2]; Gdansk Bay of the Baltic Sea, Poland [70]; and a wetland in Northwestern Poland [58].
| Common name/species                      | Weight (g) | Length (cm) | Liver | Kidney | Brain | Muscle |
|-----------------------------------------|------------|-------------|-------|--------|-------|--------|
|                                        |            |             | Zn    | Pb     | Cd    | Ni     | Zn    | Pb   | Cd   | Ni   | Zn    | Pb   | Cd   | Ni   |
| Cormorant (C) (Phalacrocorax carbo)     | 177.0      | 77.6        | 5.83  | 0.50   | 0.43  | 2.87  | 6.23  | 0.60 | 0.47 | 2.97 | 8.70  | 0.73 | 0.67 | 0.10 |
|                                        | 180.2      | 78.0        | 5.60  | 0.50   | 0.40   | 2.80  | 6.30  | 0.60 | 0.50 | 3.00 | 7.50  | 0.60 | 0.60 | 0.10 |
|                                        | 170.0      | 75.0        | 5.50  | 0.50   | 0.40   | 2.70  | 5.80  | 0.50 | 0.40 | 2.70 | 6.90  | 0.60 | 0.50 | 0.10 |
|                                        | 180.2      | 80.0        | 6.40  | 0.50   | 0.50   | 3.10  | 6.60  | 0.70 | 0.50 | 3.20 | 11.70 | 1.00 | 0.90 | 0.10 |
| Great crested grebe (G) (Podiceps cristatus) | 860.6      | 50.0        | 7.62  | 0.66   | 0.60   | 3.66  | 21.54 | 1.78 | 1.54 | 10.54 | 27.88 | 2.12 | 2.08 | 0.24 |
|                                        | 870.0      | 50.0        | 7.10  | 0.60   | 0.60   | 3.60  | 21.30 | 1.00 | 1.20 | 10.40 | 27.20 | 1.70 | 2.10 | 0.20 |
|                                        | 799.0      | 47.0        | 5.10  | 0.30   | 0.40   | 2.50  | 6.90  | 0.70 | 0.50 | 3.30 | 19.70 | 1.10 | 1.50 | 0.20 |
| Shoveler (S) (Anas clypeata) n=8       | 528.0      | 45.5        | 6.63  | 0.57   | 0.50   | 3.20  | 11.73 | 1.02 | 0.90 | 0.57 | 30.28 | 2.75 | 2.25 | 0.25 |
|                                        | 527.5      | 45.0        | 6.60  | 0.60   | 0.50   | 3.20  | 11.75 | 1.05 | 0.90 | 0.57 | 25.55 | 2.15 | 1.90 | 0.20 |
|                                        | 500.0      | 42.0        | 6.00  | 0.50   | 0.50   | 3.00  | 8.30  | 0.60 | 0.60 | 3.90 | 22.60 | 2.10 | 1.70 | 0.20 |
| Black-winged stilt (B) (Himantopus himantopus) | 180.40     | 38.6        | 52.32 | 4.70   | 3.94   | 25.50 | 59.72 | 5.60 | 4.64 | 32.92 | 39.06 | 3.40 | 2.30 | 0.30 |
| Marsh sandpiper (MS) (Tringa stagnatilis) | 100.0      | 25.6        | 11.83 | 1.07   | 0.90   | 5.40  | 84.37 | 7.83 | 6.70 | 43.03 | 98.37 | 9.43 | 7.47 | 0.87 |
| Moorhen (M) (Gallinula chloropus)      | 253.3      | 28.3        | 15.60 | 1.23   | 1.20   | 7.57  | 98.97 | 8.73 | 7.47 | 47.37 | 62.53 | 3.50 | 4.73 | 0.53 |
| Northern lapwing (N) (Vanellus vanellus) | 149.5      | 26.5        | 2.00  | 2.00   | 2.00   | 1.55  | 58.00 | 5.15 | 4.25 | 28.35 | 60.60 | 5.25 | 4.35 | 0.50 |
| Eurasian Spoonbill (E) (Platlea leucorodia) | 1549.0     | 78.5        | 6.50  | 0.70   | 0.50   | 3.35  | 6.45  | 0.49 | 0.49 | 3.10 | 7.05  | 0.60 | 0.50 | 0.10 |
| Total number = 50                      | 403.2      | 45.0        | 17.40 | 1.61   | 1.39   | 8.44  | 43.95 | 3.93 | 3.34 | 22.09 | 41.32 | 3.44 | 3.11 | 0.36 |
|                                        | 240.0      | 42.0        | 7.30  | 0.70   | 0.60   | 3.60  | 21.30 | 2.00 | 1.20 | 10.90 | 34.50 | 2.70 | 2.60 | 0.30 |
|                                        | 1.7        | 25.0        | 2.10  | 0.30   | 0.20   | 1.60  | 5.80  | 0.50 | 0.40 | 2.70 | 6.90  | 0.60 | 0.50 | 0.10 |
|                                        | 1550.0     | 80.0        | 154.10| 13.90  | 11.60  | 75.40 | 152.30| 13.40| 11.50| 74.50 | 121.20| 11.60| 9.20 | 1.10 |

Table 1 The concentrations of trace metals (μg/g dw) in the brain and liver, kidney, and brain and muscle of waterfowl from the Chahnimeh of Sistan.
In birds, Pb concentrations in the brain >5 µg/g dw are indicative of poisoning [15], and concentrations >16 µg/g dw indicate an advanced state of exposure in birds [71]. In this study, none of the levels of Pb in the brains was higher than the toxic limit threshold.

**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 for 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). The accumulation of pollutants in the internal organs of their bodies is affected by the contaminant level of the food and water ingested. Although the liver and kidney are sites of detoxification, they reflect long-term bioaccumulation [5], while the muscle and brain are sites of accumulation but not of detoxification [72].

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 [70, 73]. Relatively low (up to 0.4 ppm wet wt) lead (Pb), but not cadmium (Cd), levels were recorded in the brain of pelagic seabirds [74, 75]. Redknobbed coots (Fulica cristata) from industrialized and polluted regions of South Africa had Pb levels in the brain that increased to 25 ppm dw — 2 and 4 times as much as in the kidneys and liver [69]. These studies on the accumulation of heavy metals in the brain of birds should be further compared to other studies of birds, both the same and other species. 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 very 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 was higher than other studies from other parts of the world, including Zator and Milicz, Poland [62]; a wetland in Northwestern Poland [58]; Gdansk Bay Baltic Sea, Poland [70]; Nilgiris, Tamil Nadu, India [76]; a lagoon of Marano, Italy [77]; Bjørøya and Jan Mayen Artic [78]; and Pomeranian Bay, Poland [79].

The highest Ni levels were found in the kidneys, the liver and muscles showed slightly lower levels, and the lowest levels were found in the brain (Figures 1 and 2). A significant difference was observed in Ni levels between the 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 diet and feeding habits [80]. 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 [11].

In our study, birds were divided into four groups, invertebrate predator, fish predator, fish and crab predator, and omnivore to examine the effects of type of food on metal levels, using published data [80, 81]. In the fourth group, we had only the Eurasian spoonbill ($n = 2$), 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 [82]. The reason for the increase in mercury in this bird compared to other birds was that its long legs allowed access to deeper 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 [83]. 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 that they fed on agricultural lands irrigated by farmers (and thus were exposed to contaminants in the water). We, and others [81], 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 from 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 [84]. In our study of heavy metals, birds that are invertebrate predators compared to birds that are predators...
Table 2 The concentrations of trace metals (µg/g dw) in the brain and liver, kidney, and brain and muscle of waterfowl from the Chahnimeh of Sistan and effect habitat birds

| Feeding habits              | Liver | Kidney | Brain | Muscle |
|-----------------------------|-------|--------|-------|--------|
|                             | Zn    | Pb     | Cd    | Ni     | Zn    | Pb     | Cd    | Ni     | Zn    | Pb     | Cd    | Ni     | Zn    | Pb     | Cd    | Ni     |
| Fish predator               |       |        |       |        |       |        |       |        |       |        |       |        |       |        |       |        |
| Number = 16                 | Mean  | 6.95   | 0.60  | 0.54  | 3.36  | 15.80 | 1.34  | 1.14  | 7.70  | 20.69 | 1.60  | 1.55  | 0.19  | 6.40  | 0.56  | 0.49  | 3.10  |
|                             | Median| 6.70   | 0.55  | 0.50  | 3.15  | 8.70  | 0.80  | 0.65  | 4.15  | 22.50 | 1.35  | 1.60  | 0.20  | 6.85  | 0.60  | 0.50  | 3.20  |
|                             | Minimum| 5.10 | 0.30  | 0.40  | 2.50  | 5.80  | 0.50  | 0.40  | 2.70  | 6.90  | .60   | .50   | 0.10  | 5.20  | 0.10  | 0.40  | 2.30  |
|                             | Maximum| 11.40| 1.10  | 0.90  | 5.40  | 46.80 | 4.30  | 3.60  | 23.10 | 37.90 | 3.60  | 2.90  | 0.30  | 7.30  | 0.80  | 0.60  | 3.70  |
| Omnivores                   | Mean  | 10.47 | 0.86  | 0.80  | 5.07  | 49.11 | 4.33  | 3.71  | 23.57 | 44.10 | 3.07  | 3.31  | 0.37  | 7.17  | 0.64  | 0.56  | 3.44  |
| Number = 14                 | Median| 7.30  | 0.60  | 0.50  | 3.40  | 15.10 | 1.40  | 1.20  | 7.50  | 35.10 | 2.20  | 2.60  | 0.30  | 7.00  | 0.70  | 0.50  | 3.50  |
|                             | Minimum| 6.00 | 0.50  | 0.50  | 3.00  | 8.30  | 0.60  | 0.60  | 3.90  | 22.60 | 1.40  | 1.70  | 0.20  | 5.30  | 0.40  | 0.40  | 2.60  |
|                             | Maximum| 20.80| 2.00  | 1.60  | 10.20 | 136.50| 11.50 | 10.40 | 66.60 | 104.70| 6.20  | 8.00  | 0.90  | 10.50 | 1.00  | 0.80  | 5.00  |
| Invertebrate predator       | Mean  | 33.24 | 3.20  | 2.71  | 16.14 | 69.11 | 6.31  | 5.31  | 35.82 | 61.29 | 5.67  | 4.63  | 0.54  | 7.90  | 0.60  | 0.54  | 3.41  |
| Number = 18                 | Median| 18.20*| 1.80* | 1.50* | 8.70* | 60.20*| 5.50* | 4.50* | 30.10*| 49.40*| 4.30* | 3.60  | 0.50  | 7.20  | 0.60  | 0.50  | 3.50  |
|                             | Minimum| 2.10 | 0.30  | 0.20  | 1.60  | 14.40 | 1.40  | 1.00  | 6.90  | 31.40 | 2.70  | 2.30  | 0.30  | 6.30  | 0.50  | 0.50  | 2.90  |
|                             | Maximum| 154.10| 13.90 | 11.60 | 75.40 | 152.30| 13.40 | 11.50 | 74.50 | 121.20| 11.60 | 9.20  | 1.10  | 7.70  | 0.80  | 0.60  | 3.70  |
| Fish and crab predator      | Mean  | 6.90  | 0.70  | 0.50  | 3.40  | 6.50  | 0.50  | 0.50  | 3.20  | 7.20  | 0.60  | 0.50  | 0.10  | 7.30  | 0.70  | 0.60  | 3.40  |
| Number = 2                  | Median| 6.90  | 0.70  | 0.50  | 3.40  | 6.50  | 0.50  | 0.50  | 3.20  | 7.20  | 0.60  | 0.50  | 0.10  | 7.30  | 0.70  | 0.60  | 3.40  |
|                             | Minimum| 6.90 | 0.70  | 0.50  | 3.40  | 6.50  | 0.50  | 0.50  | 3.20  | 7.20  | 0.60  | 0.50  | 0.10  | 7.30  | 0.70  | 0.60  | 3.40  |
|                             | Maximum| 6.90 | 0.70  | 0.50  | 3.40  | 6.50  | 0.50  | 0.50  | 3.20  | 7.20  | 0.60  | 0.50  | 0.10  | 7.30  | 0.70  | 0.60  | 3.40  |

*Significant difference between the concentrations of Zn, Pb, Cd, and Ni in the tissues of the liver and kidney of invertebrate predator with omnivores and fish predator (P < 0.05)
at higher trophic levels had higher concentrations of heavy metals in the liver [85–87].

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 metal levels between the internal organs of the kidney and liver for all four elements studied, but there was no statistically significant difference between the two groups of birds for brain and muscle tissue (Table 3). The birds in the southern wetlands

Fig. 1 Location map of the study area

Fig. 2 Median concentration µg/g dw in difference tissues of birds
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 [81, 88]. Heavy metal levels are high in this region of Iran, Caspian Sea, in fishes, macroalgal, sediment, and water [89–92]. High levels of heavy metals in the south Caspian Sea might explain the high level of these heavy metals in local migrants.

Lower median concentrations of heavy metals (Cd, Pb, Ni, and Zn) in the liver and kidney were detected in the long-distance migrant birds than in the local migrants ($P < 0.05$) (Figure 1). Low usage of heavy metals and pesticides in breeding regions birds (Siberia or Eastern Europe) [88] 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 ($P > 0.001$, $r > 0.603$), but none of the elements was positively correlated with the other elements among tissue. This shows that the pathways and sources of entry for the elements studied are similar, but the pathways for 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 [38, 48]. Positive correlations of Pb or Cd with other elements in tissues have been reported in birds from Korea [93, 94], Cory’s shearwater (*Calonectris diomedea*), and black-backed gulls (*Larus fuscus*) from England [95]; seabirds from Chaun, northeast Siberia, Russia [49]; and feral pigeons (*Columba livia*) from Korea [61].

### Health Risk Thresholds

One of the non-essential element in the body is Pb that can cause neurotoxicity, nephrotoxicity, and other health effects [96]. Both the Spanish legislation and Australian National Health and Medical Research Council (ANHMRC) proposed 2.0 $\mu$g/g ww as the maximum permitted level of Pb in food [97, 98]. The median level of Pb in muscle tissue in 6 species of birds (except for Eurasian spoonbill, great crested grebe, and moorhen) was lower than the Spanish legislation and ANHMRC guidelines. The median level of Pb in the liver and kidney of birds was higher than the levels allowed in the Spanish legislation and ANHMRC guidelines (except for cormorant); Eurasian spoonbill also had higher level in the kidney than these guideline (Fig. 3). The action level for human health is 1.7 $\mu$g/g ww Pb [99] (Fig. 3). In contrast to these maximum permitted levels for Pb, the Institute of Turkish Standards for Food (ITSF) and the European Commission (EC) introduced the permissible threshold level of 0.1 and

| Zn (µg/g dw) | Pb (µg/g dw) | Cd (µg/g dw) | Ni (µg/g dw) |
|-------------|-------------|-------------|-------------|
| Liver       | Kidney      | Brain       | Muscle      |
| Long-distance migrants | Local migration |
| (C, G, S, MS, N, E) | (B, M) |
| Mean        | Median      | Minimum     | Maximum     |
| 7.45        | 2.01        | 2.10        | 20.10       |
| 0.77        | 0.20        | 0.30        | 0.77        |
| 0.68        | 0.20        | 0.30        | 0.68        |
| 3.58        | 1.60        | 3.20        | 3.58        |
| 29.60       | 12.30       | 5.80        | 12.30       |
| 0.85        | 0.50        | 0.50        | 0.85        |
| 14.44       | 5.90        | 0.40        | 5.90        |
| 2.67        | 6.70        | 2.70        | 6.70        |
| 19.50       | 3.00        | 1.50        | 3.00        |
| 0.70        | 0.50        | 0.50        | 0.70        |
| 13.90       | 2.00        | 1.00        | 2.00        |
| 11.60       | 1.00        | 1.00        | 1.00        |
| 5.30        | 3.00        | 5.30        | 3.00        |
| 20.20       | 3.00        | 3.00        | 3.00        |
| 10.40       | 3.00        | 3.00        | 3.00        |
| 75.40       | 11.50       | 11.50       | 11.50       |
| 0.90        | 0.60        | 0.60        | 0.60        |
| 10.50       | 1.00        | 1.00        | 1.00        |
| 6.00        | 0.50        | 0.50        | 0.50        |
| 1.00        | 0.40        | 0.40        | 0.40        |
| 4.60        | 0.30        | 0.30        | 0.30        |
| 6.30        | 0.30        | 0.30        | 0.30        |
| 10.50       | 1.00        | 1.00        | 1.00        |
| 1.00        | 0.80        | 0.80        | 0.80        |
| 3.70        | 0.50        | 0.50        | 0.50        |

*Significant difference between the concentrations of Zn, Pb, Cd, and Ni in the tissues of the liver and kidney of long-distance migrants and local migration ($P < 0.05$).
The median level in flesh muscle, liver, and kidney of all birds in this study was 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 the ANHMRC, USFDA, and Western Australian authorities was 2, 3.7, and 5.5 µg/g ww, respectively. In our study, none of the birds exceeded this median level of 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 the ANHMRC, USFDA, and Western Australian authorities [97, 98]. Cadmium levels in the kidney were higher than the ANHMRC threshold in all birds except the cormorant and the Eurasian spoonbill. Also, the great crested grebe, with a Cd level of 4 µg/g ww, was higher than both the ANHMRC and USFDA guidance, and the rest of the birds were higher than all three guideline ANHMRC, USFDA and Western Australian authorities (Fig. 3). In this study, the levels of Cd in the muscle, liver, and kidney of all birds were greater than these thresholds.

The permissible limit of Ni in food by the US Food and Drug Administration is 10 µg/g ww [99]. According to this guideline, the levels of Ni in the muscle, liver, and kidney of all birds, except cormorant, were higher than the permissible limit. The permissible limit of the FAO Ni is 13 µg/g ww in food [102], and the levels of Ni in muscle of birds were lower than this limit, except for the liver in black-winged stilt, marsh sandpiper, moorhen, and northern lapwing (13 µg/g ww), and the levels in the kidney of all birds (except cormorant and Eurasian spoonbill) were higher than the FAO guideline (Fig. 3). The Food and Nutrition Board (FNB) [103] introduced the permissible limit of Ni as 4 µg/g ww. Accordingly, the levels of all muscle, liver, and kidney in all birds in the present study were higher than this limit, and the consumption of edible parts of all birds poses a threat for the health of people in this region.

In our study, the HQ for any of the metals in the muscle for most of birds was < 1, but the ∑HQ was > 1 in moorhen birds (Table 4). The HQ of Pb in liver was > 1, but in the other birds it wasn’t; the ∑HQ was not higher than 1 for any other birds (Table 4). In the edible parts, the level of HQ was high, and except cormorant, in other birds, the level ∑HQ was between 1.24 and 4, which was due to the high level of HQ in the kidneys and muscle of birds in this region (Fig. 4). The ∑HQ of each metal we examined was > 1, suggesting that people would experience health risks from consumption of birds from the Chahnimeh reservoirs (Fig. 4). On the other hand, values of the ∑HQ index for total exposure were > 1 for birds, indicating that the estimated exposure is a major health concern. Studies in the wetlands of northern Iran showed that the pochard is not suitable for consumption [107].

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 [29]. In this study, we...
|                | Zn  | Pb  | Cd  | Ni  |
|----------------|-----|-----|-----|-----|
| **PTWI**       |     |     |     |     |
| 7000           |     | 25  | 7   | 35  |
| **PTWI70**     | 490,000 | 1750 | 490 | 2450 |
| **PTDI**       | 70,000 | 250 | 70  | 350 |

|                | Kidney | Liver | Muscle | Total | Kidney | Liver | Muscle | Total | Kidney | Liver | Muscle | Total |
|----------------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
| Cormorant (C)  | EDI    |       |        |       |        |       |        |       |        |       |        |       |
|                | 5.9    | 5.5   | 24.2   | 35.6  | 0.5    | 0.5   | 1.3    | 2.3   | 0.5    | 0.4   | 1.9    | 2.7   |
|                | EWI    | 41.4  | 38.8   | 178.0 | 258.3  | 3.8   | 3.3    | 9.8   | 16.9   | 3.2   | 3.0    | 13.7  |
| Great crested grebe (G) | EDI | 20.5  | 7.3    | 32.0  | 59.7   | 1.7   | 0.6    | 3.2   | 5.5    | 1.5   | 0.6    | 2.5   |
|                | EWI    | 143.7 | 50.8   | 235.4 | 429.9  | 11.9  | 4.4    | 23.3  | 39.6   | 10.4  | 3.9    | 18.1  |
| Shoveler (S)   | EDI    | 11.2  | 6.3    | 28.7  | 46.2   | 1.0   | 0.6    | 2.4   | 4.0    | 0.9   | 0.5    | 2.2   |
|                | EWI    | 78.3  | 44.3   | 211.1 | 333.7  | 7.0   | 3.9    | 17.9  | 28.8   | 6.0   | 3.3    | 16.3  |
| Black-winged stilt (B) | EDI | 56.9  | 49.8   | 31.4  | 138.1  | 5.3   | 4.5    | 2.6   | 12.5   | 4.4   | 3.8    | 2.4   |
|                | EWI    | 398.2 | 348.8  | 231.4 | 978.4  | 37.3  | 31.4   | 19.5  | 88.2   | 30.9  | 26.3   | 17.5  |
| Marsh sandpiper (MS) | EDI | 84.1  | 11.3   | 32.7  | 128.1  | 7.5   | 2.8    | 13.3  | 6.4    | 0.9   | 2.5    | 9.8   |
|                | EWI    | 589.0 | 79.0   | 240.7 | 908.7  | 52.2  | 7.2    | 21.0  | 80.4   | 44.8  | 6.0    | 18.5  |
| Moorhen (M)    | EDI    | 94.2  | 14.8   | 37.5  | 146.6  | 8.3   | 1.2    | 3.4   | 12.9   | 7.1   | 1.1    | 2.9   |
|                | EWI    | 534.6 | 107.2  | 276.5 | 918.3  | 50.5  | 9.2    | 25    | 84.6   | 40.1  | 8.2    | 21.5  |
| Northern lapwing (N) | EDI | 55.5  | 2.0    | 33.4  | 90.9   | 5.1   | 1.9    | 2.7   | 9.6    | 4.3   | 1.5    | 2.6   |
|                | EWI    | 388.4 | 13.8   | 246.2 | 648.3  | 35.4  | 13.2   | 19.7  | 68.3   | 29.9  | 10.5   | 19.1  |
| Eurasian spoonbill (E) | EDI | 6.2   | 6.5    | 33.1  | 45.8   | 0.5   | 0.6    | 3.1   | 4.3    | 0.5   | 0.5    | 2.5   |
|                | EWI    | 43.3  | 45.7   | 243.6 | 332.6  | 3.4   | 4.5    | 23.1  | 31.0   | 3.4   | 3.5    | 18.8  |
examined the EWI and EDI and then compared it with the provisional permissible tolerable weekly intake (µg/kg body weight/week) (PTWI), PTWI 70 that is PTWI for 70 kg person (µg/week) and permissible tolerable daily intake (PTDI) for a 70 kg person (µg/day). The PTWI, PTWI 70, and PTDI depend on metal levels and the dietary use of different foods. The Food and Agriculture Organization and the World Health Organization in 2004 established the 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 [108]. Also, the PTWI according to the guidelines of FAO/WHO (2011) is 35 and 7000 µg/kg body weight/week for nickel and zinc, equaling 2450 and 490,000 µg/week for a 70 kg person, respectively [109].

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, and N 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 the level of 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, the EWI in kidney was higher of the PTWI, and also the level of Ni in the liver of birds B, MS, N, and M was higher of the PTWI (Table 4). The level of EWI of Ni in edible parts B, MS, N, and M was higher than the PTWI, and also the level of Ni in kidney B, MS, N, and M was higher than the PTDI (Table 4). The results of this study show that people in this area should not use “edible” parts of the birds examined, 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 [107, 110].

Conclusion

In this study, the 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 the levels in other tissues. Differences in metal 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 the effect level threshold; 56% of the liver and kidney 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 particularly avoid eating kidney and liver tissue.

**Author Contribution** All authors have contributed to conception and design, acquisition of data or analysis, interpretation of data, drafting the article, and critical reviewing.

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**Data Availability** The authors declared that the data and materials for this work are available.

**Declarations**

**Ethics Approval and Consent to Participate** All procedures performed in studies involving collection of birds were in accordance with the ethical standards of the institutional and/or national research committee of the University of Zabol with reference number IR.UOZ.REC.1399.005.

**Competing Interests** The authors declare no competing interests.

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