Trace metal concentration in wild avian species from Campania, Italy

Abstract: This study was conducted to determine heavy metals concentrations in tissues of 94 birds belonging to different species from coastal areas of Naples and Salerno (Southern Italy) in order to provide baseline data concerning trace element levels in wild birds living in or close to an area characterized by high anthropogenic impact. Additional aim of this study was to verify if diet influenced metal accumulation, so birds were classified as birds of prey, fish eating birds and insectivorous birds. Kidney was the primary organ for Cd accumulation in all groups considered, Pb was accumulated preferentially in bone, whereas Hg showed high values in liver of fish eating birds. Zn showed the highest mean concentrations, while Cu levels were one order of magnitude less. The variance analysis with respect to feeding habits disclosed only a lower accumulation of Hg in insectivores with respect to the other groups. For all metals, the concentrations measured in tissues should be considered indicative of chronic exposure to low, "background" environmental levels and/or to the presence of low bioavailable metals in the environment. In addition, metal levels were not of toxicological concern, being always well below the toxic thresholds defined for each metal.

Keywords: Birds • Heavy metals • Campania • Feeding habits

1. Introduction

Due to their industrial use and persistence in the environment some heavy metals can be responsible for environmental contamination and can be absorbed by animals through air, water and food and hence become available for bioaccumulation through food chain steps. Bio-magnification is prominent in the local environment especially in non-migrating predator avian species. These local, upper trophic level species play a very important role as bio-indicators of environmental contamination [1,2]. There are several data on metal concentrations in a wide variety of terrestrial and aquatic wild birds [3-12]; other studies have compared trace metal concentrations in birds with different feeding habits, belonging also to different trophic levels [13-16]. Coastal areas of Campania (Italy) show a great variety of habitats - woods, cultivated areas, wetlands and various naturalistic areas - where different resident avian species live; the same areas are also involved in migratory routes. Despite the presence of protected areas (e.g. Cratere degli Astroni Natural Reserve, 16 km from Naples), the degradation level of coastal areas continues to be high, for they are also the most inhabited, cultivated and industrialized areas. Despite these pollution sources, little information is currently available on metal accumulation and its effects on wildlife residing along the coasts of Campania. A previous study analysed Cd concentrations in Italian sparrows living in urban and rural areas of the Campania Region [17]; in the same area the concentration of total PCBs in gulls and kestrel were far higher than those estimated to elicit immunosuppressive effects [18]. Moreover, an epidemiological study detected an area in the provinces of Caserta and Naples where human cancer mortality and congenital malformation rates were significantly
increased [19]. Lastly, the Campania Region has recently been problematic for waste treatment and in 1994 it even declared a state of emergency.

In this study, we measured the concentrations of five heavy metals either toxic (Cd, Hg and Pb) or essential (Zn and Cu) in tissues of 94 wild birds (20 different species) from the Campania Region, Italy, to provide data concerning trace element levels in wild birds living in or close to this area characterized by high anthropogenic impact; an additional aim of our work was to compare groups of species with regards to different feeding habits (birds of prey, fish eating and insectivores).

2. Experimental Procedure

2.1. Sampling area characteristics
All animals used in this study were collected along the Campania coasts in Naples and Salerno provinces (40° 50’ 29.52” N, 14° 09’ 32.99” E) (Fig. 1). The area comprises a huge variety of habitats - cultivated areas, woods, wetlands and naturalistic areas - where more than 100 different avian species reside, and is characterized by severe environmental degradation.

2.2. Birds
Bird sampling was performed by “Cratere degli Astroni” Natural Reserve staff from 1998 to 2002. All birds (94) were found dead or died after collection from trauma. No bird was killed for this study and the sampling was conducted in accordance with national guidelines for the protection of wild species.

Species sharing the same feeding habits were grouped together for convenience as listed in Table 1: a) hawks, falcons and owls were included in the group of birds of prey (N=52), b) gulls, herons and grebes in fish eating bird group (N=31), c) other species feeding mainly on insects and terrestrial invertebrates were grouped as insectivorous birds (N=11).

2.3. Metal determination
Analytical determinations were conducted on liver, kidney and bone tissue collected during necropsy performed at Veterinary Faculty of Naples “Federico II” University, and stored at –20°C until analyses. Liver, kidney and bone were excised if their conservation status or dimensions were in good enough condition.

For each sample two 0.1-0.5 g aliquots were mineralized following standardized methods; samples for Cd, Pb, Zn and Cu analyses were supplemented with 2 mL of nitric acid and 0.5 mL of hydrogen peroxide, while samples for Hg analyses were supplemented with nitric acid only. All reagents were from Merck, Darmstadt (Germany); acids were of Suprapur grade.

Metal concentrations were determined by atomic absorption spectrometry using a Varian SpectrAA 600 instrument with a graphite furnace for Cd and Pb, an IL-11 Instrumentation Laboratories flame atomic spectrometer for Zn and Cu and a Varian SpectrAA 10 cold vapor generator for Hg. Two blanks were run during each set of analysis to check the purity of the chemicals and the accuracy of the method was checked with reference materials (CRM 278: lyophilized mussel, Community Bureau of Reference, BCR, Brussels). All values of reference materials were within certified limits. Detection limits were 0.5 ng g⁻¹ wet wt for Pb, 0.2 ng g⁻¹ for Hg, 2 ng g⁻¹ for Cd, 80 ng g⁻¹ for Zn and 120 ng g⁻¹ for Cu.

Metal concentrations in tissues were expressed as µg g⁻¹ on a wet weight basis.

2.4. Statistical analysis
Feeding habits (birds of prey, fish eating birds, insectivorous birds) were considered grouping factors, so all species were considered in statistical analysis even when only one bird or one tissue was available. Statistical analysis was performed using the STATISTICA 6.0© Program (StatSoft Italia S.r.l.) applying analysis of variance on metal tissue concentrations. If any data were below the limit of detection they were substituted by half of the limit of detection itself [20]. Birds presenting tissue concentrations making them outliers (observations numerically distant from the rest of the data, calculated as from Barnett and Lewis [21]) were not included in statistical analysis. Metal concentrations were reported as trophic level mean ± SE, median, minimum and maximum.

3. Results and Discussion

Metal concentrations in liver, kidneys and bone of birds are reported in Tables 2-4, where data are grouped on the basis of feeding habit (Table 1). Thus the fact that only one single bird could be present per species has little or no importance from a statistical point of view, as it enters in a bigger pool of data (birds of prey n=52, fish eating birds n=31, insectivorous birds n=11).

There was a major difference between the concentration and distribution of toxic and essential trace metals: Cd, Hg and Pb concentrations showed high individual variability and in many samples were below the LOD, whereas Cu and Zn were present at detectable concentrations in all samples examined.
3.1. Toxic trace metals

Cd concentrations (Fig. 2a and Tables 2-4) in birds of prey and fish eating birds were significantly higher in the kidney (p <0.05) (respectively 0.97±0.34 and 0.91±0.26 µg g⁻¹ w.w.) than in the liver (respectively 0.43±0.16 and 0.39±0.18 µg g⁻¹ w.w.), whereas the paucity of data in insectivores precluded a statistical analysis.

Table 1. Species subdivision according to feeding habits.

| Feeding habits          | Species                                   | N   |
|-------------------------|-------------------------------------------|-----|
| Birds of prey (Total 52)| Kestrel (Falco tinnunculus)               | 7   |
|                         | Common buzzard (Buteo buteo)              | 16  |
|                         | Sparrowhawk (Accipiter nisus)             | 3   |
|                         | Scops owl (Otus scops)                    | 5   |
|                         | Tawny owl (Strix aluco)                   | 8   |
|                         | Little owl (Atheno noctua)                | 10  |
|                         | Eurasian hobby (Falco subbuteo)          | 1   |
| Fish eating birds (Total 31)| Yellow-legged gull (Larus cachinnans)     | 22  |
|                         | Little bittern (Ixobrychus minutus)       | 4   |
|                         | Great crested grebe (Podiceps cristatus)  | 2   |
|                         | Black-headed gull (Larus ridibundus)      | 1   |
|                         | Black-crowned night-heron (Nycticorax nycticorax) | 1 |
|                         | Grey heron (Ardea cinerea)                | 1   |
| Insectivorous birds (Total 11)| Honey buzzard (Pernis apivorus)          | 2   |
|                         | Eurasian blackbird (Turdus merula)        | 3   |
|                         | Great spotted woodpecker (Dendrocopos major) | 1   |
|                         | Eurasian woodcock (Scolopax rusticola)    | 2   |
|                         | Eurasian hoopoe (Upupa epops)             | 2   |
|                         | Common cuckoo (Cuculus canorus)           | 1   |

Table 2. Metal concentrations in liver; values are expressed in µg g⁻¹ wet weight.

| Metal | Feeding habits       | N   | Mean±SE | Median | Min   | Max   |
|-------|----------------------|-----|---------|--------|-------|-------|
| Cd    | Birds of prey        | 45  | 0.43±0.16 | 0.055 | <LOD  | 6.23  |
|       | Fish eating birds    | 31  | 0.39±0.19 | 0.049 | <LOD  | 2.67  |
|       | Insectivorous birds  | 11  | 0.56±0.27 | 0.072 | <LOD  | 2.27  |
| Hg    | Birds of prey        | 45  | 1.24±0.55 | 0.32  | <LOD  | 25.6  |
|       | Fish eating birds    | 31  | 3.17±2.4  | 0.43  | <LOD  | 79.4  |
|       | Insectivorous birds  | 11  | 0.23±0.07 | 0.07  | <LOD  | 0.63  |
| Pb    | Birds of prey        | 27  | 0.06±0.01 | 0.013 | <LOD  | 0.90  |
|       | Fish eating birds    | 16  | 0.07±0.02 | 0.012 | <LOD  | 0.47  |
|       | Insectivorous birds  | 4   | 0.28±0.14 | 0.035 | <LOD  | 1.08  |
| Zn    | Birds of prey        | 47  | 91.9±7.73 | 79.7  | 23.5  | 224.6 |
|       | Fish eating birds    | 31  | 95.1±8.18 | 84.4  | 35.0  | 212.7 |
|       | Insectivorous birds  | 11  | 82.1±15.5 | 52.9  | 36.7  | 178.3 |
| Cu    | Birds of prey        | 46  | 8.64±1.00 | 7.05  | 1.66  | 42.9  |
|       | Fish eating birds    | 30  | 7.83±1.05 | 5.59  | 3.15  | 27.8  |
|       | Insectivorous birds  | 11  | 11.5±2.36 | 9.53  | 1.87  | 27.7  |
most soils, surface waters and plants. The main route of exposure to Cd in animals is via food. Once ingested, the metal is rapidly cleared from the blood and primarily accumulated in the liver bound to metallothioneins, small cysteine-rich proteins induced at the transcriptional level by Cd [22,23]. Cd-thionein is then transported to the kidney for long-term storage, as recently reported for woodcock [24]. In this scenario, due to the extremely long biological half life of the metal, Cd concentrations in the liver and especially in the kidney could be related to chronic environmental exposure.

A wide range of Cd concentrations have been reported in the kidney of birds: species exposed to low environmental doses show Cd levels below 1 μg g\(^{-1}\) w.w. [10,12,15,25], while species exposed to high doses can bioaccumulate the metal to values that may represent a serious health threat: e.g. white tailed ptarmigan, which feeds on willows with high Cd content, shows toxic renal Cd concentrations as high as 100 μg g\(^{-1}\) w.w. [26]. Intermediate values (12.1 μg g\(^{-1}\) w.w.) have been found in the kidney of Eurasian woodcock [23] and in Italian sparrows living in urban (10.8 μg g\(^{-1}\) w.w.) and rural (3.92 μg g\(^{-1}\) w.w.) areas of the Campania region [17]. The low levels of Cd in our samples, generally below 1 μg g\(^{-1}\) w.w., and the absence of significant differences related to feeding habits could be ascribed to low environmental contamination, or to a low bioavailable metal fraction in the environment. Higher Cd levels were observed in liver (1.2±0.9 μg g\(^{-1}\) w.w.) and kidney (3.3±1.5 μg g\(^{-1}\) w.w.) of little owls. Little owl is a non-migratory species that could be considered a good bioindicator of local pollution since it is at the top of the trophic chain, feeds close to cities where Cd contamination is potentially high, and thereby is continuously exposed to the metal. Our data are consistent with a partial environmental exposure and are higher than those previously reported for the same species from the Bologna-Parma area (Italy) and France, where mean liver values were 0.17 ±0.08 μg g\(^{-1}\) w.w. [10] and 0.38 μg g\(^{-1}\) w.w. [27]. Anyway, the values are lower than 3 mg kg\(^{-1}\) d.w. in liver and 8 mg kg\(^{-1}\) d.w. in kidneys, which might indicate an increased environmental exposure [28].

Among the other species we have considered in the present study, seabirds, especially seagulls, have been used as bioindicators of Cd environmental levels due to their worldwide distribution and their position in the food chain [3,13,29-31]. In addition, they show wide ecological adaptability and feed either in natural environment or in heavily anthropized areas. We have compared Cd concentrations in liver and kidney of 3 different species of seagulls from Italy (Table 5); geographic differences in Cd levels are evident: specimens living in the Venice Lagoon are clearly exposed to a higher Cd environmental contamination than gulls living in other Italian areas. Inter-specific differences were also found: Cd concentrations in liver and kidney followed this decreasing order regardless of the geographical

| Metal | Feeding habits | N  | Mean±SE | Median | Min   | Max   |
|-------|---------------|----|---------|--------|-------|-------|
| Cd    | Birds of prey | 45 | 0.97±0.34 | 0.135  | <LOD  | 13.87 |
|       | Fish eating birds | 29 | 0.91±0.27 | 0.41   | <LOD  | 2.67  |
|       | Insectivorous birds | 2  | -       | -      | <LOD  | 1.2   |
| Hg    | Birds of prey | 44 | 2.57±1.73 | 0.43   | <LOD  | 4.24  |
|       | Fish eating birds | 31 | 0.88±0.26 | 0.36   | <LOD  | 9.51  |
|       | Insectivorous birds | 2  | -       | -      | <LOD  | 0.05  |
| Pb    | Birds of prey | 22 | 0.02±0.01 | 0.007  | <LOD  | 0.22  |
|       | Fish eating birds | 14 | 0.03±0.01 | 0.019  | <LOD  | 0.09  |
|       | Insectivorous birds | 2  | -       | -      | <LOD  | 0.4   |
| Zn    | Birds of prey | 44 | 52.5±12.2 | 32.1   | 17.87 | 565   |
|       | Fish eating birds | 29 | 47.5±4.39 | 37.5   | 19.2  | 132   |
|       | Insectivorous birds | 2  | -       | -      | 24.9  | 91.8  |
| Cu    | Birds of prey | 45 | 5.03±0.38 | 4.36   | 1.68  | 15.7  |
|       | Fish eating birds | 29 | 5.69±0.35 | 5.35   | 3.83  | 12.8  |
|       | Insectivorous birds | 2  | -       | -      | 4.19  | 4.46  |
Area: black-headed gull > yellow-legged gull > herring gull, highlighting the importance of feeding habits (e.g., black-headed gulls feed on dumping sites) on metal body burden and species-specific biochemical adaptations.

Mercury (Hg) is one of the most toxic elements for birds. Spalding et al. [32] found that liver Hg concentrations > 6 mg kg⁻¹ correlated with malnutrition and mortality from chronic disease in Great white herons. This toxic element is of particular concern in seabirds, because Hg is methylated by bacteria in the aquatic environment to methyl mercury, which is then concentrated through the food chain [14, 30, 33]. Also in birds of prey, feeding habits seem to be quite important, for the metal can undergo huge biomagnification; in general, species higher up in the food chain accumulate higher levels of Hg [16, 34]. In fact, we found significantly higher concentrations of Hg in liver and kidney of fish-eating birds and birds of prey, similar to those reported by Alleva et al. [15] for liver of birds from central Italy. Presenting highest concentrations in kidney, birds of prey are assumed to be subject to a long-term constant exposure to Hg, with increasing risk of renal damage [35].

The lowest concentrations were found in insectivores in all the tissues examined. Observed data for insectivores are similar to those reported for various species from Texas by Mora et al. [36] and for spectacled eider from Alaska by Trust et al. [37]. For the insectivores a possible source of Hg may be methylmercury-based fungicides, which can accumulate in invertebrates, the prey of the birds considered [38].

Lead (Pb) is one of the most toxic heavy metals and plumbism could be one of the causes of death in wild animals, especially birds. The mean Pb concentrations we found are in agreement with data previously reported for birds living in North or Central Italy [10, 11] and far below the values suggested by other authors as indicative of environmental contamination in bones >5 µg g⁻¹ d.w. [28] and kidney > 10 µg g⁻¹ d.w. [39].

The main Pb source for birds is the oral ingestion of shotgun pellets [40]. Once solubilized in the acid

| Metal | Feeding habits | N  | Mean±SE  | Median | Min  | Max  |
|-------|----------------|----|----------|--------|------|------|
| Cd    | Birds of prey  | 52 | <LOD     | <LOD   | <LOD | <LOD |
|       | Fish eating birds | 31 | <LOD     | <LOD   | <LOD | <LOD |
|       | Insectivorous birds | 4  | <LOD     | <LOD   | <LOD | <LOD |
| Hg    | Birds of prey  | 52 | 0.05±0.01| <LOD   | <LOD | 0.74 |
|       | Fish eating birds | 31 | 0.11±0.03| 0.05   | <LOD | 0.85 |
|       | Insectivorous birds | 4  | 0.04±0.03| <LOD   | <LOD | 0.14 |
| Pb    | Birds of prey  | 27 | 0.37±0.22| 0.28   | <LOD | 5.08 |
|       | Fish eating birds | 23 | 0.36±0.2 | 0.113  | <LOD | 4.93 |
|       | Insectivorous birds | 3  | 0.12±0.02| <LOD   | 0.097| 0.15 |
| Zn    | Birds of prey  | 52 | 217±17.1 | 183    | 52.6 | 514  |
|       | Fish eating birds | 31 | 194±14.3 | 189    | 48.7 | 369  |
|       | Insectivorous birds | 4  | 198±56.5 | 217    | 66.7 | 293  |

**Table 4.** Metal concentrations in bone; values are expressed in µg g⁻¹ wet weight

| Metal | Feeding habits | N  | Mean±SE  | Median | Min  | Max  |
|-------|----------------|----|----------|--------|------|------|
| Cd    | Birds of prey  | 52 | <LOD     | <LOD   | <LOD | <LOD |
|       | Fish eating birds | 31 | <LOD     | <LOD   | <LOD | <LOD |
|       | Insectivorous birds | 4  | <LOD     | <LOD   | <LOD | <LOD |
| Hg    | Birds of prey  | 52 | 0.05±0.01| <LOD   | <LOD | 0.74 |
|       | Fish eating birds | 31 | 0.11±0.03| 0.05   | <LOD | 0.85 |
|       | Insectivorous birds | 4  | 0.04±0.03| <LOD   | <LOD | 0.14 |
| Pb    | Birds of prey  | 27 | 0.37±0.22| 0.28   | <LOD | 5.08 |
|       | Fish eating birds | 23 | 0.36±0.2 | 0.113  | <LOD | 4.93 |
|       | Insectivorous birds | 3  | 0.12±0.02| <LOD   | 0.097| 0.15 |
| Zn    | Birds of prey  | 52 | 217±17.1 | 183    | 52.6 | 514  |
|       | Fish eating birds | 31 | 194±14.3 | 189    | 48.7 | 369  |
|       | Insectivorous birds | 4  | 198±56.5 | 217    | 66.7 | 293  |

**Table 5.** Cd concentrations (µg g⁻¹ wet weight, mean±SD or SE for this work) in liver and kidney of 3 species of gulls from different areas of Italy.

Yellow-legged gull (Larus cachinnans)
- Campania Region (N=22) 0.52±0.28 1.21±0.39 This work

Herring gull (Larus argentatus)
- Elba Island (N=7) 0.20±0.09 0.94±0.34 [27]
- Po Delta (N=5) 0.88±0.46 2.06±1.19 [27]

Black-headed gull (Larus ridibundus)
- Po Delta (N=6) 0.90±0.27 3.22±0.74 [27]
- Venice Lagoon (N=5) 1.03±0.83 8.02±6.00 [3]
- Pesaro-Urbino Province (N=4) 0.48±0.53 - [15]
environment of the gizzard, lead is transported primarily to the red blood cells and then distributed by blood to mineralizing systems (bone) and soft tissues (liver). The higher concentrations of Pb in bones of birds of prey and fish eating birds, could be ascribed to previous exposure to Pb, mainly due to direct or indirect ingestion of lead shots \[40,41\]. Lower bone and higher kidney levels in insectivores can be explained by a different exposure to the metal, as confirmed by Hernandez et al. \[13\] in various avian species from Doñana Park, Spain. Low Pb concentrations in species feeding on invertebrates, thus not ingesting lead shots, can be considered indirect confirmation of results from studies on lead shot ingestion as the principal cause of Pb intoxication \[40-43\]. Nevertheless, some authors reported a certain risk for insectivore species feeding on worms and other terrestrial invertebrates, as they can accidentally ingest lead shots or contaminated soil \[6\].

3.2. Essential trace metals

Zn concentrations (Fig. 3a, Tables 2-4) follow a similar trend in all groups considered (bone > liver > kidney), with a maximum of 217±17 µg g\(^{-1}\) w.w. in bone and a minimum of 47.5±4.4 µg g\(^{-1}\) w.w. in kidney.

In the case of Cu, the metal concentrations in tissues were one order of magnitude less than those for Zn (Fig. 3b, Tables 2-4) and followed this decreasing order: liver>kidney>bone. In all groups considered, Cu concentrations in the liver (maximum value 11.5±2.4 µg g\(^{-1}\) w.w.) were significantly higher than in kidney (maximum value 5.69±0.35 µg g\(^{-1}\) w.w.) (p <0.05) and bone (maximum value 4.34± 0.38 µg g\(^{-1}\) w.w.) (p<0.05).

The extraordinary homogeneity in Zn concentrations in avian species living in different habitats is related to the role of Zn in biological systems. Zn has been shown to be essential to the structure and function of a large number of macromolecules and for over 300 enzymes \[44\]. It has both catalytic and structural roles in enzymes, while in zinc finger motifs it provides a scaffold organizing protein sub-domains for the interaction with either DNA or other proteins \[45\]. For this reason Zn is always maintained at constant intracellular levels through an efficient homeostatic control performed by sensor proteins, membrane transporters and metallothioneins participating in intracellular Zn transport, trafficking and storage \[46\]. Moreover, the induction of metallothioneins can counteract the effects of exposure to high environmental levels of Zn, shifting its metabolism to bone accumulation, typical of chronic exposure. This mechanism seems to be particularly efficient in fish eating birds because they present very low concentrations in kidney and high levels in bone.
our study tissue concentrations were never close to Zn toxic threshold for birds, fixed in 660 μg g⁻¹ w.w. [47]. We found values similar to those reported in birds by other authors [3, 23, 48-50] and referring to an environment not polluted by this metal.

The homogeneity of Cu concentrations in the avian species studied can be partially explained considering the essentiality of Cu for organisms. On the other hand, Cu redox activity can also lead to the production of oxygen free radicals, responsible for membrane lipid peroxidation, protein and DNA oxidation [51-53]. For this reason, organisms must tightly regulate Cu levels to provide enough metal for their cellular needs without developing the toxicity associated with the activity of free ions [54]. Higher Cu concentrations were found only in species (woodcock, blackbirds, hoopoe) feeding on earthworms, which extract their nutrients, including metals, by ingesting large amounts of soil and their body composition reflects the characteristics of the soil itself [24]. This finding is in agreement with data reported for Doñana Park, Spain [13].

4. Conclusions

To our knowledge this is the first work reporting data on trace metal concentrations in a consistent number of wild avian species from Campania Region. Determination of Cd, Hg, Pb, Zn and Cu in liver, kidney and bone failed to reveal toxic concentrations in tissues and values could be related to low environmental background contamination. Notwithstanding the presence of waste landfills in the vicinity of the sampling areas, none of the investigated species seem to be at risk of adverse effects. The behaviour and feeding habits of little owls and seagulls make these species useful bioindicators of metal contamination to be monitored in future to assess metal pollution.

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