Original article

Health risk assessment by toxic metals in little egrets (*Egretta garzetta*) and food chain contaminations

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ABSTRACT

This study analysed heavy metals from little egret (*Egretta garzetta*). Egret's Eggs, egg shells, food (fish and insects), blood, meat samples (thigh, liver, and chest), water, soil and sediments samples were collected from the two selected sites of the study area. Samples were analysed on flame atomic absorption spectrometer after acid digestion. Detected metals were found almost inline of concentrations when compared with the both sites. Among detected metals Mn was found higher in concentration ($\mu g/g$) i.e. 18.509 followed by Zn i.e. 9.383, Ni, Cu, Pb and Cd. Sediment exhibited higher levels ($\mu g/g$) of metals (25.061) followed by the meat (19.044) egrets food (18.825), excreta (16.26), blood serums (4.577), eggs (3.626) and water samples (2.432). The level of metals in sediments of the study are showed environmental concerns. Health risks were also investigated that were compared to guidelines of WHO and FAO threshold limits. It was found a marginal health risk to life through detected metals. This study revealed that little egret are good bio-indicator for the screening and investigation of contaminates presence in the environment.

1. Introduction

Egretta garzetta is a bird of size ranged from 55 to 65 cm in length with the measurement of 88 to 106 cm from tip to tip with weight ranged from 350 to 550 g. During breeding season two elongated nape plumes appeared on the breast and back of the bird while bill and eyes turned red or blue (Hancock et al., 2010).

In certain aquatic ecosystems egrets are considered excellent source of food being provided to the not only to the invertebrates but the higher organisms.

Heavy metals are present in environment due to natural and anthropogenic activities which are composed of metalloids those include nickel, arsenic, and zinc. These metalloids even in a smaller quantity can cause toxicity to living organisms. Heavy metals can generate from point sources and non-point sources of pollution including mining areas, foundries, smelters, and other industrial activities which ultimately contaminate the environment. Due to prolonged stay of such metals in environment they are placed to type of inorganic toxicants. Due to anthropogenic activities heavy metals incorporate directly from geological cycle and contribute as an environmental toxicity. Certain heavy metals such as Cambium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb) and Cobalt (Co) are absorbed in living organism from bottom line of ecosystem and cause toxicity (Dauwe et al., 2005). Such heavy metals become part of the proteins and lipid contents in the living organism and cause negative impacts during cellular metabolic activities (Koivula and Eeva, 2010). In the newly hatched baby birds Cd cause disease like cancer and in male baby bird destruction of testicular (Burger and Gochfeld, 2009).

Certain investigations on this bird showing that for the assessment of heavy metals different body part of the bird can be used such as feathers, liver, blood (Malik and zeb., 2009). Eggs (Burger an Gochfeld., 2000a, 2000b) and its shell (Ayes., 2007) can also be used for the investigation of different metals in this bird, partic-

ularly the female bird is considered a mean of excretion of these toxic heavy metals (Lam et al., 2005). The concentration of Cd in tissues of aquatic birds is reported higher when compared with other metals.

The massive traffic, industries and burning of fossil fuels are adding Pb and Cd in the environment locally and globally. These metals do not have any positive or known use for living biota particularly the fauna (Kenntner et al., 2003). In the trace metals shows Cd is considered the utmost toxic metal while Pb is lethal to birds (Battaglia et al., 2003). In birds' physique state is also influenced by Zinc (Kim et al., 2009). After soaking up these metals can flow around bird's physique and later it assembles in eggs and excrete (Burger, 2002).

The Pollution problem of heavy metals has increased in in recent decade in the South Asia (Hordoy et al.,1992 In states like Costa Rica and Florida certain studies have been conducted which showed ecotoxicology effects in the nesting birds. (Burger et al.,1993). Regular and long-term research is needed to unveil the problem of accumulation of heavy metals in the aquatic as well as terrestrial organisms. It is mandatory to understand that how these toxic contaminants bioaccumulate and bio-transform in different species of birds.

2. Methodology

2.1. Study area

This study was conducted in Mirpur which is a district of the state of Azad Jammu and Kashmir (AJK), Pakistan 33°8' 35'' North, 73° 44' 51'' East. It is adjoining area of northern Districts Jhelum and Rawalpindi of Punjab Province of Pakistan. Its elevation from sea level is 459 m and possess a mega water body namely Mangle Dam which holds about 100sq Km area.

2.2. Sampling

Samples were collected from two colonies of little egret from Jabbi area of District Mirpur AJK, two colonies were selected which along Jabbi stream and were just opposite to each other. Preliminary survey was conducted in March 2015 to finalize the sampling procedure, but sapling was done during the mints of April-June 2015. The collected samples were comprises of eggs, egg shells, liver, blood, chest, thigh, excreta, water, soil and food of egrets like (insects, fish)

2.3. Digestion

2.3.1. Eggs and egg shells

In the beginning with help of spatula eggs were broken down and white of eggs and Yellow of eggs were transferred to the respective label bottles, which were centrifuged at 2200 rpm for one minute. Then 1 ml of white of egg and yellow of eggs was transferred to a glass flask having volume 200 ml and Nitric Acid (HNO$_3$) 5 ml was added, and flask stirred for 2 min. After this Perchloric acid (HCLO$_4$) 1 ml was added to flask and again stirred for 2 min.

Egg samples were heated in order to dry the added acid the temperature of hot plat was gradually increased (50°c-250°c.) until the acids are completely dried out. A white colour material was obtained. The rest of the samples were also treated with the same method for digestion.

2.3.2. Sediments

Sediment samples 1 gm were taken in a tube, added Aquaregia (HCL, HNO$_3$) in 3:1 ratio 15 ml and left for overnight simply. After this samples were heated at 150°c and then added 5 ml HCLO$_4$ was

added to the soil samples which were heated again till dryness. For filtration Whatman filter paper was used, to raise the volume up to 25 ml the distilled water and remaining solution was added.

2.3.3. Water

Water sample 250 ml was filtered through Whattmann filter paper. Filter paper was digested in HCl and HNO$_3$ (1:5). The mixture was heated until transparent solution obtained. Solution was again filtered after cooling by using Whattmann filter paper.

2.3.4. Filtration

Distilled water was used to add in all samples when the digestion was completed; all samples filtered through filter papers and filtrates were collected in round bottom flask of capacity (50 ml) and kept at 4°c till further analysis.

2.4. Laboratory analysis

Flame Atomic Absorption Spectrophotometer (FAAS) was used for the deduction of heavy metals. The results were obtained subsequently while placing the standard dilutions in FAAS.

2.5. Statistical analysis

Basic descriptive statistics, Hierarchical agglomerative cluster analysis (HACA) are the statistical analysis of data. For the identification of sources of heavy metal contamination of egret species feeding habitats, Ward's linkage method and Pearson distance matrix was used in cluster analysis.

3. Results

All samples associated with little egret have shown that there are seven heavy metals namely Mn, Zn, Ni, Cu, Cd, Pb and Co metals were present with different concentrations. Three metals Pb, Co and Cd are toxic non-essential whenever present in any sample. The rest of metals like Mn, Zn, Ni and Cu are essential elements and useful in metabolic activity of the bird but these essential heavy metals become toxic or lethal for human and birds when found beyond the permissible limits.

Two sites were selected for sample collection from the study area. The samples used for the detection of metals were; egg content, egg shell, food samples (fish, insect), meat samples (liver, chest and thigh), blood sample, excreta sample, water samples, soil sediments. The concentrations of metals were different in samples from two sites and the concentration of heavy metals in site one is given in the Fig. 2.
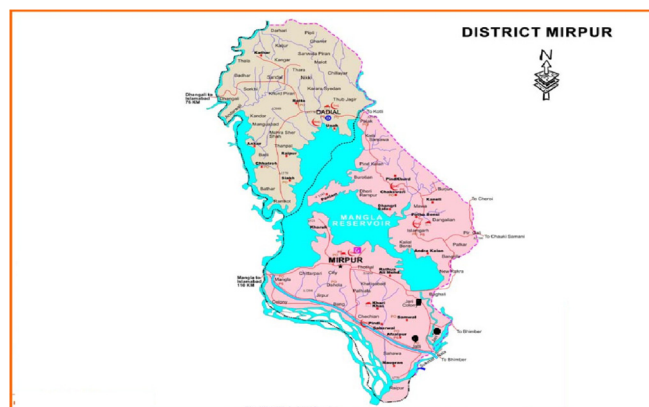

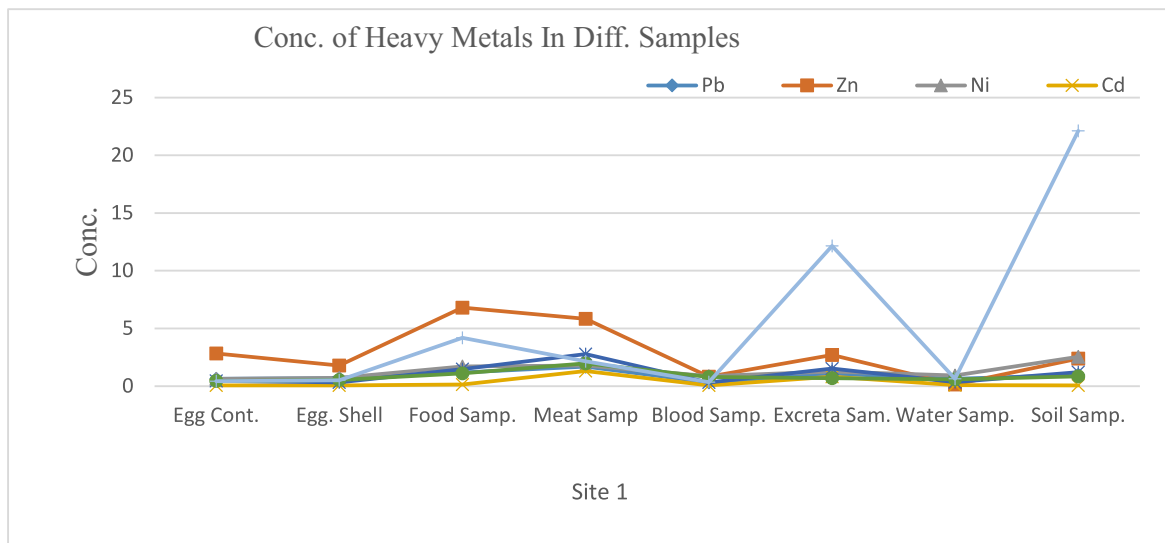**Fig. 1.** Map of the study Area (source: pakimage.com).

Fig. 2. Graphical presentation of heavy metals in different samples at site 1.

The mean concentration of heavy metals in egg content sample collected from little egret of site 1 was in order, Zn > Pb > Ni > Cu > Co > Mn > Cd with the mean concentration of 2.8418, 0.6407, 0.6325, 0.4641, 0. 4485, 0.4285 and 0.715 respectively. The concentration of heavy metals in egg shell samples of site 1 collected from little egret was little bit different from the concentration of heavy metals in egg content by following the order, Zn > Pb > Co > Mn > Cu > Ni > Cd with the mean concentration of 1.7825, 0.7042, 0.5447, 0.5051, 0.3198, 0.712 and 0.0748 respectively. The concentration of heavy metals in food samples (Fish and Insects) was different from the previous one with the following order, Zn > Mn > Ni > Cu > Pb > Co > Cd with the mean concentration of 6.8106, 4.1908, 1.7033, 1.4761, 1.1925, 1.1231 and 0.1518 respectively. Whereas the mean concentration of heavy metals in Meat samples (Liver, Chest and Thigh) was in the following order, Zn > Cu > Mn > Co > Ni > Pb > Cd with the mean concentration of 5.8229, 2.782, 2.1581, 1.980, 1.7988, 1.6982 and 1.3136 respectively. The order of heavy metals in blood samples was Ni > Zn > Co > Mn > Cu > PB > Cd with the mean concentration of 0.8875, 0.8247, 0.7865, 0.3469, 0.3185, 0.322 and 0.0768 respectively. The concentration of heavy metals in the excreta samples of site 1 was similar to some extent with the following order, Mn > Zn > Cu > Cd > Ni > Pb > Cd and Co with the mean concentration of 12.1389, 2.7158, 1.5172, 1.2583, 1.0839, 0.8195 and 0.6823 respectively. The mean concentration of heavy metals in the water samples was in the following order Ni > Pb > Mn > Co > Cu > Zn > Cd with the mean concentration of 0.9323, 0.6353, 0.6325, 0.5797, 0.3475, 0.1432 and 0.087 respectively whereas the mean concentration of heavy metals in soil sediments was in the following order Mn > Ni > Zn > Cu > Pb > Co > Cd with the mean concentration of 22.1174, 2.5486, 2.832, 1.2071, 0.9842, 0.8593 and 0.0784 respectively.

The concentrations of metals were different in samples from two sites and the concentration of heavy metals in site one is given in the Fig. 3. The mean concentration of heavy metals in egg content sample collected from little egret of site 2 was in order, Zn > Ni > Pb > Co > Mn > Cu > Cd with the mean concentration of 1.7045, 0.4487, 0.4452, 0.3545, 0.3052, 0.2751, and 0.0488 respectively. The concentration of heavy metals in egg shell samples of site 2 collected from little egret was little bit different from the concentration of heavy metals in egg content by following the order, Zn > Ni > Pb > Mn > Cu > Co > Cd with the mean concentration of 1.135, 0.5219, 0.4359, 0.3676, 0.3187, 0.357 and 0.0657

respectively. The concentration of heavy metals in food samples (Fish and Insects) at site 2 was different from the previous one with the following order, Zn > Mn > Ni > Pb > Cu > Co > Cd with the mean concentration of 7.2166, 6.7769, 2.0984, 1.8035, 1.7529, 1.0204 and 0.3637 respectively. Whereas the mean concentration of heavy metals in Meat samples (Liver, Chest and Thigh) at site 2 was in the following order, Zn > Ni > Co > Cu > Mn > Pb > Cd with the mean concentration of 5.2003, 2.9812, 2.7309, 2.6373, 2.5355. 2.3489 and 2.335respectively. The order of heavy metals in blood samples was Zn > Cd > Mn > Ni > Co > Pb > Cu with the mean concentration of 1.1308, 1.186, 1.0569, 0.8267, 0.6987, 0.4372, and 0.3433 respectively. The concentration of heavy metals in the excreta samples of site 2 was similar to some extent with the following order, Mn > Zn > Cu > Ni > Pb > Cd > Co with the mean concentration of 6.3071, 1.4035, 1.2439, 1.1571, 0.7956, 0.6096 and 0.805 respectively. The mean concentration of heavy metals in the water samples was in the following order Ni > Mn > Co > Pb > Cu > Cd > Zn with the mean concentration of 0.5974, 0.4408, 0.4242, 0.3748, 0.2269, 0.0692, and 0.476 respectively whereas the mean concentration of heavy metals in soil sediments was in the following order Mn > Ni > Zn > Cu > Pb > Co > Cd with the mean concentration of 10.6005, 3.8185, 1.6163 1.1961, 1.17, 0.9522, and 0.5998 respectively.

3.1. Statistical analysis in hierarchical dendrogram of metals

Basic descriptive statistics, Hierarchical agglomerative cluster analysis (HACA) for analysis of data. Ward's linkage method and Pearson distance matrix was used in cluster analysis (SI Figs. 1-8).

4. Discussion

Results showed that there was slight difference among the two colonies of little egrets the heavy metals like Co, Cu, Cd, Ni, Zn, and Pb. This non-significant variation can be associated to various foraging environment. The accumulation of heavy metals in birds depends upon pattern of diets being taken, body size, condition of body of bird etc. (Kojadinovic et al., 2007). Another reason for concentrations variations is egg laying time of the bird, metabolic differences, mechanisms of detoxification of an enzyme etc.

From both colonies of Jattali headworks Head the mean concentration was calculated 0.682 ± 0.956. This concentration was com-
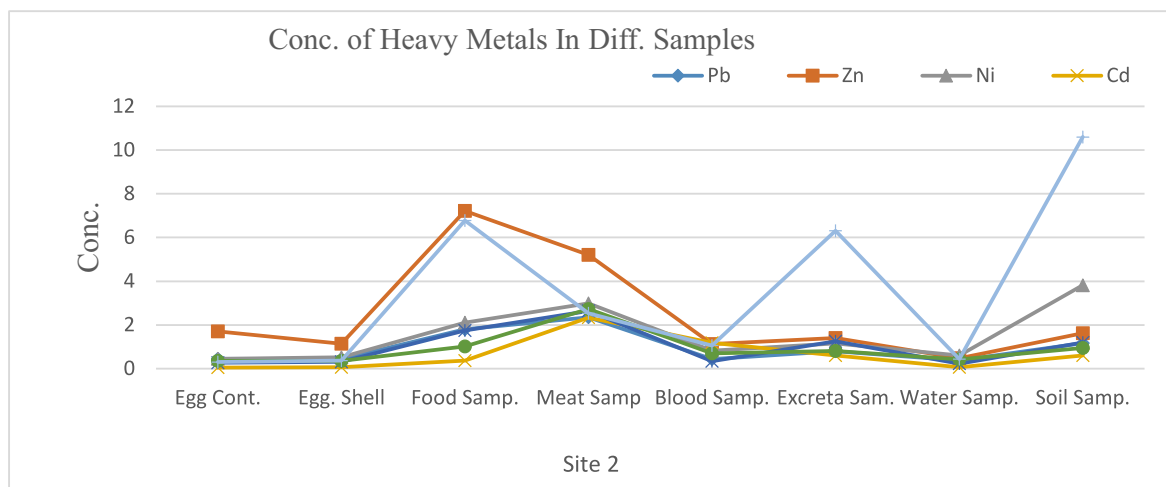
Fig. 3. Graphical presentation of heavy metals in different samples at site 1.

pared with common Eider Glaucous-winged Gull (0.30 μg/g) from Alaska (Burger and Goch Feld 2007). The concentration of Cd in present study (0.980 ± 0.544) was calculated below the uncertain threshold effect stage. The Cd concentration (1.163 ± 0.707 μg/g) in prey sample of little egret when compared with *Black tailed god-wit* (4.27, 3.07 μg/g) found lower (Roodbergen et al., 2008.) Similar Cd results were also compared with *Black crowned night* and *Grey heron* (174 and 172 μg/g) from Pyeongtaek, China (Kim and koo., 2008) and results of this study were also found lower.

In *present study the Pb concentration in the contents of eggs of little egrets was calculated (1.633 ± 0.315)* while Bostan et al., (2007) reported higher concentrations Pb (6.74 μg/g) and (73.50 μg/g) in Cattle egret respectively. A similar study was conducted from Alaska Burger and Goch Feld (2007) and they also reported higher concentration of Pb. The Pb concentration in liver of little egret Pb (0.606 ± 0.141) was recoded lower as compared to (<10 μg/g wet weight) Muhammad Shahbaz et al., (2013)

In current study the mean concentration of Pb in soil samples were calculated (2.135 ± 0.403) which was much lower (9.60 – 29.40 μg/g) calculated by Shahbaz et al., (2013).

Similarly, the Mean concentration of Ni was calculated (2.093 ± 0.811) in present study was higher than (0.50 – 0.76 μg/g dry weight) reported by Shahbaz et al., (2013). In prey samples The Ni concentration was calculated (2.251 ± 0.42) that was found higher (1.77 μg/g) when compared with a study conducted by (Rood Bergen et al., 2008) for Black tailed godwit. Boston et al., (2007) also conducted a study on cattel egert from Pakistan and found higher concentration of Ni (24.80 μg/g).

In case of Co the results of current study showed higher concentration of Co(1.639 ± 0.351) than a study conducted by Shahbaz et al., (2013) and calculated (3.79 μg/g) concentration of Co. The concentration of Mn in egg contents of little egret was calculated (1.022 ± 0.255), which was lower than a separate study (0.51–3.55 μg/g) conducted by Shahbaz et al., (2013).

Zn concentration in a balance state contributes for metabolism but its exertive or beyond the limit concentration become toxic for the birds. The mean concentration of Zn was calculated (4.928 ± 0.418) in recent study, this concentration is much lower when compared with the study conducted by Shahbaz et al., (2013) on Islam headwork and Trimu headwork's Heronries of little egrets (52.35 – 58.21 μg/g. In current study the concentration of Cu in the egg content was found higher (1.019 ± 0.334) when compared with a study conducted by Braune and Nobel (2009). They suggest that in most of the living organisms heavy metal concentration usually lesser (1 ppm-2.00 μg/g). The concentration of Zn in faeces in present

study was calculate (3.189 ± 0.917) higher than (202.57 ± 1.65 μg/g) faeces of vulture bird investigated by Bravo et al., (2005). The variation among different bird species is due to differences in bioavailability and diet (Adendorff. 1993).

During current investigation the concentration (0.648 ± 0.372) of Cd found lower than the concentration found in fecal samples of vulture bird from Venezuala state (13.93 ± 1.18 ppm) by (Bravo et al., 2005). Although Cd is considered the most hazardous heavy metal present in both environment and food with longer persistence (Lee et al., 1994). The heavy metal Mn have a vital role with birds excretion its exposure to avian embryos has teratogenic impacts (Hashmi et al., 2013). Bravo et al., (2005) calculated the highest concentration of Ni (15.19 ± 1.33 μg/g), while in current study the concentration of Ni was (1.205 ± 0.373), Higher concentration can damage the respiratory system, deoxyribonucleic acid (DNA) of the bird (Vanwyk et al., 2001).

With in the same study are two little egret species from two different sites showed non-significant difference among the assessed metals like Mn, Co, Cd, Cu, Pb, Ni and Zn. Samples like egg contents, egg shell, meat, Food, excreta, blood, water and sediments also have slight variations among the concentrations levels may be because of foraged in different habitats before their breeding.

Heavy metals are being incorporated in the food chain through pollutions caused by anthropogenic and some natural activities and most effected arears of world are from developing countries like Pakistan, India, Bangladesh and Nepal (Karn and Harada., 2001).

5. Conclusion and recommendations

The results of current study depicted that all samples from study area associated with little egrets were contaminated with heavy metals. There was no significant difference among the heavy metals concentrations of both sites (birds colonies) of study area. From the studied metals the maximum concentration Mn was calculated and the order of accumulation of heavy metals on the basis of concentration in the study area was sediments > meat samples > food > excreta > blood > egg content > eggshell > water samples. The higher concentration in of sediments study area is might be due to anthropogenic activities which are of environmental concern. The bird little egret is considered largely scattered species and elevated on food chain. This study shoes that such birds can be a wonderful tool for examining the environmental toxicants. It is also concluded that this bio can

be used as useful bioindicator for the assessment of organic and inorganic pollutants. Further comprehensive investigations and research should be performed to explain the destiny and ecotoxicological after-effects of heavy metals in avian species. Such investigation can portray the actual understandings of heavy metals and other pollutants, which could enable investigators to comprehend that how toxic contaminants move across the food chain. The temporal and continuous monitoring is also required for in the study area.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

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