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Research Article

Assessment of metal concentration in White-Breasted Waterhen (*Amaurornis phoenicurus*) from Phool Nagar and Baghiana Kalan, District Kasur, Pakistan

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Abstract

The White-breasted Waterhen (*Amaurornis phoenicurus*) is a member of the Rallidae family. The overhunting of local communities and anthropogenic contamination of aquatic ecosystems pose a growing threat to this water bird. This study was carried out at two sites to determine how metals affect White-breasted Waterhens. One site was a pond in the Phool Nagar village of Lamby Jageer, which is a natural site (site 1). The second site was a contaminated site that was obtained from Rohi Nala Baghiana Kalan (site 2). Birds from these sites were collected, slaughtered, and organs such as breast and thigh muscles, liver, heart, feathers, skin, and gizzard were separated. Waste samples were also collected from the intestine. Samples were prepared by chemical digestion and analyzed with atomic absorption spectroscopy to see the concentrations of sodium (Na), calcium (Ca), potassium (K), iron (Fe), zinc (Zn), lead (Pb), and cadmium (Cd), mercury (Hg), and chromium (Cr). Results indicated higher concentrations of essential metals such as Na, Ca, and Fe, with lower levels of Zn and K. Notably, non-essential heavy metals like Hg and Cr were not found at any site, whereas site 2, which was a contaminated site, only showed Cd and Pb in birds. The study concludes that the White-breasted Waterhen has potential importance for conservation as it is a source of halal meat and essential metals. However, the detection of non-essential metals highlights the adverse effects of environmental pollution on these birds.

Keywords: Contamination, Heavy metals, Pollution

Introduction

Birds bring immense value to ecosystems, captivating humans with their stunning plumage, distinctive vocalizations, and melodious songs. As sensitive indicators of environmental well-being, fluctuations in bird populations often signal broader habitat changes (O'Connell et al. 2000; Kessler et al. 2004; Niemi and McDonald 2004; Fogal et al. 2005; Alam et al. 2023). It is well known that complex ecosystems with diverse species are biologically more stable (Kushlan 1993). However, numerous waterbird species face threats, primarily or partially, due to excessive hunting pressures, highlighting the need for conservation efforts (Sadam et al. 2022).

The white-breasted waterhen (*Amaurornis phoenicurus*) is a member of the rail and crake family (Prompiram et al. 2023). It usually inhabits drains, marshes, and the banks of verdant rivers. These birds are known for their beauty and live around estuaries, freshwater marshes, and in marine habitats (Tropical Birds Tuttle Publishing 2014). Soil and water contamination is primarily caused by human activity (Yuce et al. 2006; Akhtar et al. 2021). According to Morais et al. (2012), there is a substantial risk to environmental health due to the significant growth of heavy metals such as Cd, Pb, Mn, Cu, and Cr in the environment, especially because of mining activities. Even though certain heavy metals are necessary in minute amounts, at larger concentrations they can become hazardous, remain in the environment, and worsen pollution. Reducing these metals' bioavailability, mobility, and toxicity is usually the main goal of pollution control techniques (Mohammed et al. 2011).

Due to the metals' accumulation in tissues and disruption of biological processes, exposure to lead, mercury, chromium, and cadmium in water birds can cause severe poisoning, poor reproductive performance, organ damage, and decreased survival rates (Lucia et al., 2010). When present in excess, even necessary elements like potassium (K), sodium (Na), calcium (Ca), iron (Fe), zinc (Zn), and calcium (Ca) can become poisonous (Jomova et al. 2022). Often, natural biogeochemical cycles are not enough to eliminate these man-made materials, especially when their concentrations are markedly increased in the environment (Zak et al. 2023). According to Parker et al. (2018), birds that inhabit areas high in heavy metals are subjected to these toxins either by eating metal-rich soil or grit that is essential for their gizzards to operate properly, or by absorbing their food. The amount of metal that birds accumulate can also change with age (Berglund et al. 2011).

According to Mishra et al. (2019), one of the main causes of ecological deterioration is rising metal levels. The white-breasted waterhen was chosen for this study because, although it has been noticeably declining recently, it was once widely distributed in the adjacent areas of the district of Kasur. The amounts of heavy and essential metals in the various organs of this species, as well as the consequences of environmental exposure to this bird, are not well documented, making this study unique.

Material and methods

The present study was designed to see the presence of essential and heavy metals in the organs of the White breasted waterhen (*Amaurornis phoenicurus*).

Selection of sites

The samples were collected from two different sites, and samples of White breasted waterhen (*Amaurornis phoenicurus*) were taken from two different sites one site was a pond in Phool Nagar village Lamby Jageer (Coordinates: 31°12′18″N 73°56′35″E/31.2050°N 73.9430°E/31.2050; 73.9430) that was less contaminated or natural site while the other was taken from Rohi Nala Baghiana Kalan (Coordinates: 30°56′56″N 73°44′36″E/30.94889°N 73.74333°E/30.94889; 73.74333) that was more contaminated site as compared to first one as this site received water from houses, factories and agricultural lands. Both sites are in the Kasur district (Fig. 1).

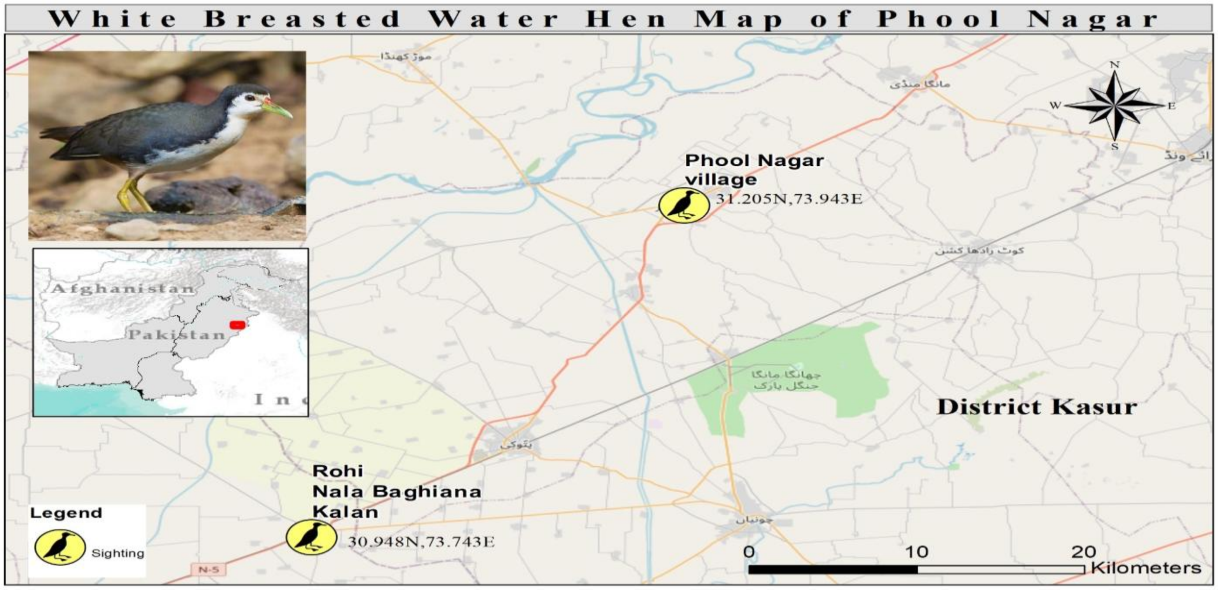

Figure 1. A map of the district of Kasur showing the location of the two study sites

Collection and Dissection of White Breasted Waterhens

White breasted water hens were captured by trapping nets and dissected in the laboratory using a dissection kit. All the organs, such as liver, skin, feathers, heart, gizzard, waste, thigh, and breast, were separated, cleaned carefully. and placed in properly labeled zipper bags. Samples were kept in the refrigerator at 4°C. The 5 g weights of each sample were measured in crucibles and were heated until the samples turned to ash. After that, all samples along with crucibles were put into an electric furnace carefully for about 24 hours at about 500°C to turn them into white ash.

Solution preparation of samples

All the charred samples were taken out of the furnace, and 5 ml of HCl (Hydrochloric acid) was added and diluted by adding 2 ml of water. All the samples were boiled and then cooled to room temperature, filtered, and water was added to make a 20 ml solution. All solutions were placed at room temperature to be used for further analysis (Yasmeen and Asif, 2022). For the Iron (Fe) test a 5 mL of the solution was taken and one mL of 2 Normal HCl was added to each test tube. Then, one ml of a 10 % solution of Potassium thiocyanate (KSCN) was added to every sample solution, including the blank. All these samples were taken into quartz cells and put into the spectrophotometer, and readings were taken one by one. A flame photometer was used for the determination of Na, K, and Ca.

Statistical Analysis

Statistical analysis was done by using SPSS version 22. ANOVA was applied to find the significant differences between the concentrations of metals in different samples and independent sample t test was applied to find the significant difference between two sites.

Results

White breasted waterhen was collected from site 1 that was natural Phool Nagar and site 2 was Rohi Nala at Baghiana Kalan. Different essential metals such as Na, Ca, K, Zn, and Fe were studied along with heavy metals Hg, Cr, Pb, and Cd in different samples of white breasted waterhen.

Concentration of Sodium (Na) in different samples of WBH from sites 1 and 2

Sodium (Na) levels were found to be lower in the waste samples at site 1 compared to site 2 of white-breasted waterhen, which was opposite to its trend of bioaccumulation in organs (Fig. 2).

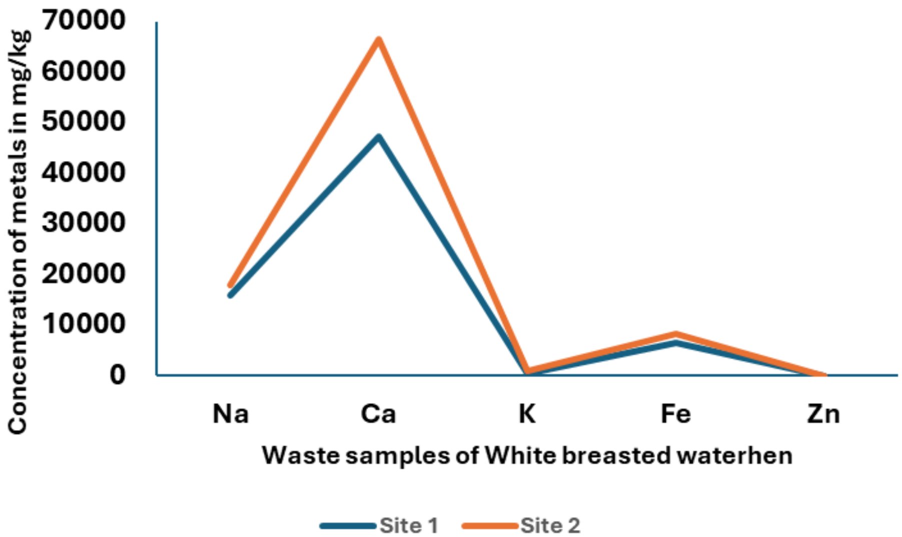

Figure 2. Concentration of metals in waste samples of White breasted waterhen

It was noticed for the organs that Na had highest levels in the heart of the white-breasted waterhen and lowest in the gizzard (279.64 ±0.02 mg/kg) (Fig. 3). Moreover, Sodium patterns were studied at two sites and found highest in all samples of site 1 except gizzard as compared to site 2 (Fig. 3).

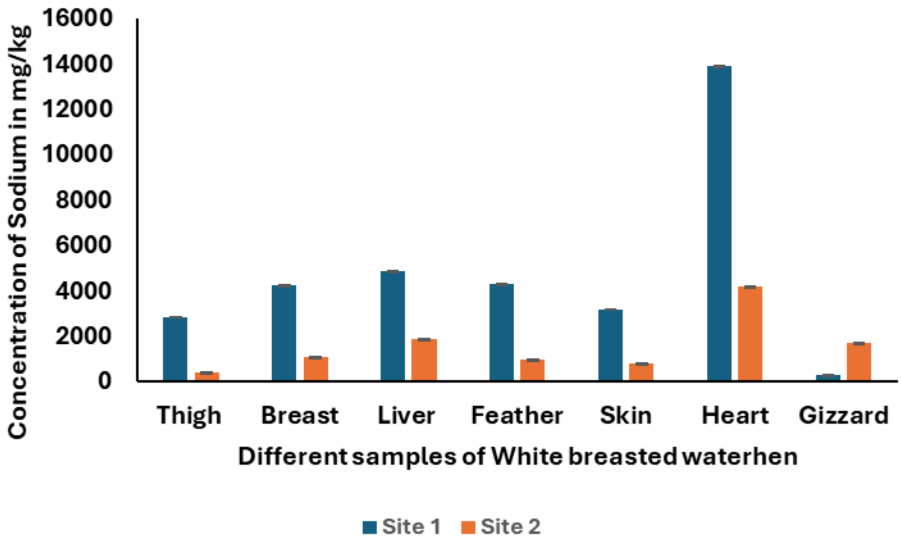

Figure 3. Concentration of Sodium in White breasted Waterhen

A general pattern for sodium concentrations in various samples was noted, indicating that the bird's heart had the highest level, followed by the liver, feathers, breast, skin, thigh, and gizzard. The white-breasted chicken at site 2 followed the same pattern as site 1. Notable variations were seen in Na between the two sites by an independent sample t-test at a p value <0.05.

Concentration of Calcium (Ca) in different samples of WBH from sites 1 and 2

The largest concentration of calcium was found in the waste of the white-breasted waterhen, with 7,318.61 ±0.00 mg/kg at site 1 and 9,196.42 ±0.05 mg/kg at site 2. Waste from both places had higher levels of calcium, most likely because of consuming sand or dirt high in calcium. (Fig. 2). The skin at site 2 (114.68 ±0.04 mg/kg) and the thigh at site 1 (284.74 ±0.02 mg/kg) had the lowest calcium levels (Fig. 4).

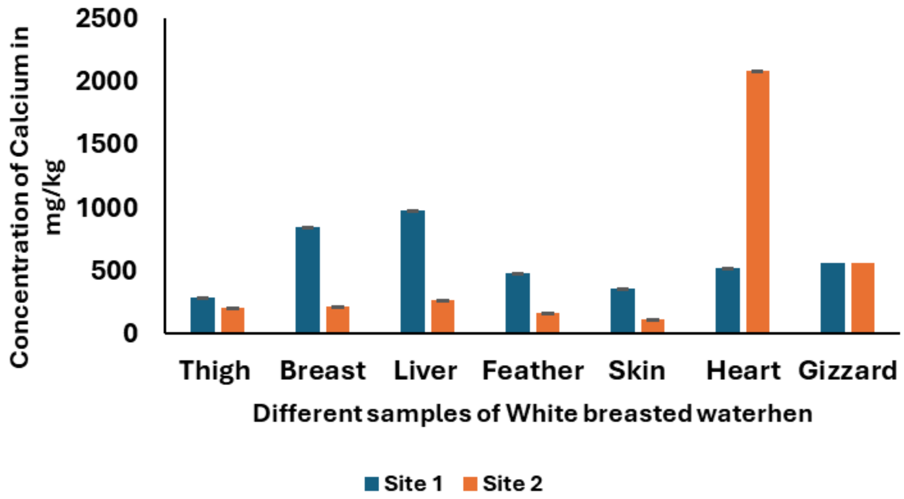

Fig. 4. Concentration of Calcium in White breasted Waterhen

The trends in calcium concentration were recorded as highest in Heart, followed by Gizzard > Liver > Breast > Thigh > Feather > Skin for Site 1 and Liver > Gizzard > Heart > Feather > Skin > Thigh for Site 2. By using a one-way ANOVA and an independent sample t-test, significant variations in Ca were observed between sites and among various organs with p values <0.05.

Concentration of Potassium (K) in different samples of WBH from sites 1 and 2

The element potassium, which is necessary for all living things, was found in every sample taken from the white-breasted waterhen. At Site 1 (natural): The waste had the greatest potassium levels (Fig. 2), followed by the skin, feathers, gizzard, heart, liver, breast, and thigh. The heart had the highest potassium amounts at the contaminated Site 2, while the levels in the waste, liver, gizzard, thigh, breast, feathers, and skin decreased in that order. The sites' patterns for potassium concentration varied, Twith site 1's heart displaying the greatest levels and site 2's heart at site 2 (Fig. 5). By using a one-way ANOVA and an independent sample t test, significant variations in K were observed between locations and among various organs with p values <0.05.

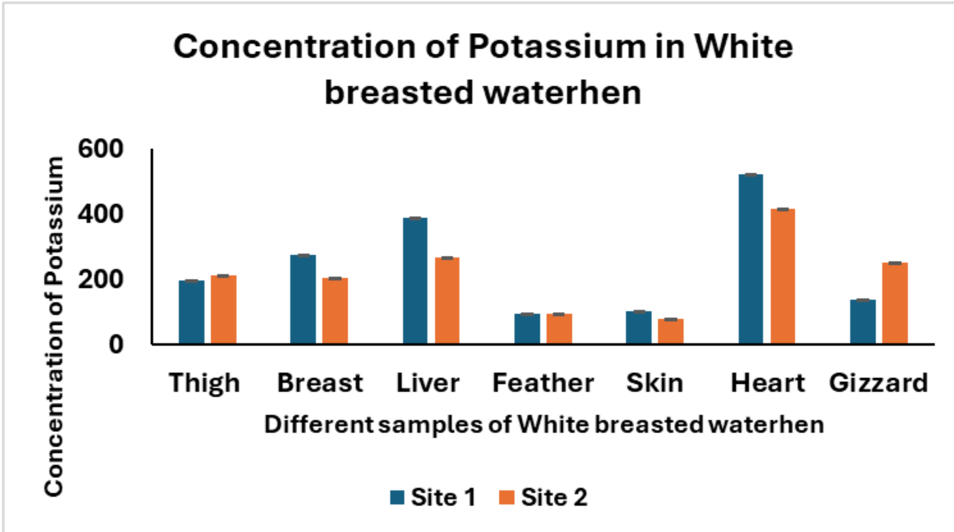

Figure 5. Concentration of Potassium in White breasted Waterhen

Concentration of Iron (Fe) in different samples of WBH from sites 1 and 2

All samples contained iron, which is necessary for hemoglobin and overall health in birds. Iron waste levels were higher at Site 1, indicating either high food intake or high metabolic processing (Fig. 2). The tissues with the highest concentration of iron were found to be in feathers, followed by skin, thigh, heart, liver, breast, and gizzard. Similar trends were seen at Site 2 (contaminated), where the iron concentration in trash was highest and in thighs was lowest. These findings suggest that accumulation patterns remained constant notwithstanding contamination. The concentration was found in the following order: gizzard followed by liver, feather, heart, skin, breast, and thigh (Fig. 6). By using a one-way ANOVA and an independent sample t test, significant differences in Fe were observed between sites and among various organs with p values less than 0.05.

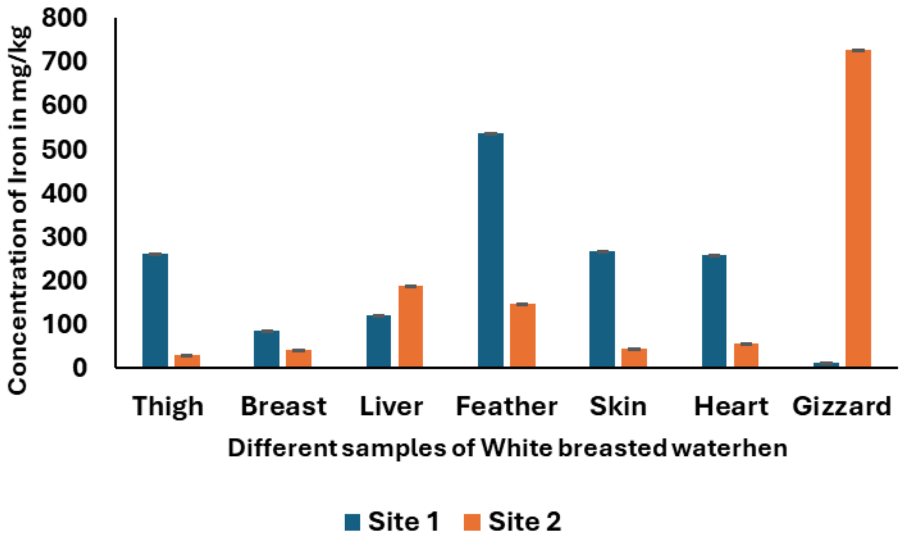

Figure 6. Concentration of Iron in White breasted Waterhen

Concentration of Zinc (Zn) in different samples of WBH from sites 1 and 2

Zinc (Zn) was detected in both sites, but the distribution patterns were different. For example, Site 1 (natural) revealed that the gizzard had the lowest concentration of Zn and the heart the greatest, with the least amount of Zn discovered in waste or feathers. This showed that the accumulation in vital organs is higher than in less important organs, with the following trend observed: Heart > Breast > Liver > Thigh > Skin > Gizzard. Site 2 (contaminated): The liver had the highest concentration of zinc, the skin the lowest, and the heart showed no zinc at all. This pattern points to different levels of accumulation that could be brought on by contaminated environments. Liver > Feather > Gizzard > Waste > Thigh > Breast > Skin were the trends observed in (Fig. 7). Significant differences were noticed in Zn among different organs at p value <0.05 by one-way ANOVA and between sites by independent sample t test.

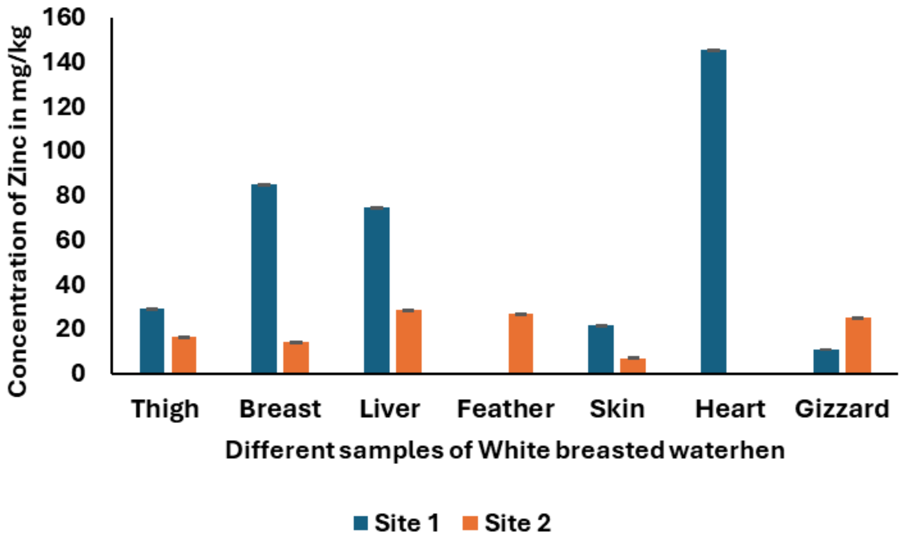

Figure 7. Concentration of Zinc in White breasted Waterhen

Concentration of Heavy Metals in different samples of WBH from site 1 and site 2

Cd and Pb were not detected in any sample of the white breasted waterhen at site 1, and Hg and Cr were not detected in the samples of birds at both sites. While Cd was present in all the samples except the thigh at site 2. Waste contains the utmost amount of Cd, while the gizzard contains the least. Cd follow the decreasing trend as follows: Waste (15.58 ±0.35 mg/kg) >Heart (13.29 ±0.57 mg/kg) >Liver (5.29 ±0.12 mg/kg) >Feather (4.52 ±0.71 mg/kg) >Breast (3.70 ±0.21 mg/kg) >Skin (2.52 ±0.28 mg/kg) >Gizzard (0.30 ±0.21 mg/kg) and Pb was detected in the samples of feathers of birds at site 2 (Table 1). Significant differences were noticed in Pb and Cd at p value <0.05 between sites by an independent sample t-test.

Table 1. Heavy metals in white breasted waterhen at site 2

Samples	**Cd**	**Pb**
Breast	3.70±0.21	ND
Liver	5.29±0.12	ND
Feather	4.52±0.71	1.39±0.02
Skin	2.52±0.28	ND
Heart	13.29±0.57	ND

Waste	15.58±0.35	ND
Gizzard	0.30±0.21	ND
Thigh	ND	ND

ND= Not Detected

Discussion

The white-breasted waterhen, an aquatic bird with economic and aesthetic value, is facing a decline in population across various sites in Kasur. Several factors contribute to this decrease, one of which is the increase in traffic, particularly towards site 2, due to the development of tourist attractions like Rana Resort. The heavy traffic not only disturbs the natural habitat but also generates noise pollution, which interferes with the birds' reproductive behavior. As a shy species, the white-breasted waterhen tends to hide in response to perceived threats, reducing its chances of successful breeding. This aligns with Lima (2009), who also noted that environmental changes can affect bird reproductive behavior. Another significant factor is water pollution. The birds typically nest near water bodies that have become increasingly contaminated by municipal and industrial waste, as well as agricultural runoff. Such pollution poses a serious threat to aquatic life and the birds that depend on these habitats, as highlighted by Dhanaraj (2024).

Additionally, hunting for birds’ halal meat has contributed to its decline. Sadam et al. (2022) reported that overhunting and illegal trading are major threats to this species. Similar findings have been reported by Wickramasinghe and Diwakara (2017), who stated that overhunting is responsible for the decline of 46% of bird species, and by Idowu and Morenikeji (2015), who also pointed to hunting and poaching as causes of decreasing bird populations. Dutta and Mohapatra (2017) further noted the role of local communities in the decline of bird species, suggesting that social and cultural factors also play a role in the dwindling numbers of the white-breasted waterhen.

The analysis of essential metals like sodium, calcium, potassium, iron, and zinc in the white-breasted waterhen revealed significant variations between the two study sites, indicating the impact of environmental conditions on metal accumulation in different organs. Sodium, vital for cellular function, showed the highest concentration in waste at the natural site, suggesting natural dietary intake, while at the contaminated site, it was highest in the heart, indicating possible disruption of physiological processes (Clancy et al. 2015). Calcium, crucial for bone structure, was most abundant in waste at both sites, likely due to the ingestion of calcium-rich soil or sediment and Bourassa et al. (2022) also reported the reason for high calcium intake via food. While a lower level of calcium is recorded in muscle tissues that might be due to its less storage in muscles rather than bones, eggshells, and teeth (Ciosek et al. 2021). Potassium, essential for nerve and muscle function (Pohl et al. 2013), followed a similar trend with higher levels in waste and heart, indicating its systemic importance. At site 1 the levels are high in waste separated from intestine while higher levels in heart tissues at site 2 showed its bioaccumulation due to pollution as findings of Borghesi et al. (2017). Iron, integral to hemoglobin, showed the highest accumulation in waste at both sites, reflecting dietary intake or metabolic processing, with a notable shift in the trend at the contaminated site, suggesting environmental stress. Zinc, important for immune function, had the highest concentration in vital organs like the heart at the natural site and the liver at the contaminated site, with variations possibly due to contamination affecting absorption and distribution are also evidenced from literature by Kosik-Bogacka and Łanocha-Arendarczyk (2019) and Leal et al. (2023) studies. These findings underscore the ecological significance of essential metals and highlight how environmental contamination can alter their distribution within aquatic birds, potentially affecting their health and survival (Sievers et al. 2018).

In a recent study, heavy metals were analyzed in birds from two sites: one with a clean pond environment and the other with contamination from household and agricultural waste. Cadmium (Cd) and Lead (Pb) were detected in samples from the contaminated site, indicating pollution. A similar study conducted in the European Union emphasized the importance of water birds as bio-indicators of environmental health (Stankovic and Stankovic 2013). Feathers from various birds at different sites were examined for heavy metals such as Cd, Hg, Cr, Co, As, Sb, and Ge. The study found that these metals have a higher affinity for feathers than for keratin, which is naturally present in feathers (Innangi et al. 2019). The presence of these metals in feathers serves as an indicator of the quality of water and feed available to the birds (Dmowski 1999; Einoder et al. 2018; Squadrone et al. 2019; Vizuete et al. 2019). Moreover, with very few exceptions, it was found that the organs of white-breasted waterhens at site 1 had higher levels of important metals such as Na, Ca, K, Fe, and Zn than those at site 2. The presence of heavy metals, which were exclusively found in the organs of the waterhens from that location, may be the cause of the lower levels of these critical elements in the samples from site 2. This could be because heavy metals can compete with essential metals for binding sites, obstruct metabolic processes, and form complexes that lower the essential metals' bioavailability, all of which can impede their uptake and utilization.

Conclusion

This study revealed significant differences in metal concentrations, reflecting environmental contamination. In white-breasted waterhens, levels of sodium, calcium, potassium, iron, and zinc varied between natural and contaminated sites, with higher concentrations in vital organs from the polluted site. The presence of toxic metals like Cd and Pb in birds from the contaminated area highlights the harmful effects of pollution on avian species. These findings underscore the importance of using water birds as bioindicators and the need for stricter environmental regulations to protect these species and their habitats.

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