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BIOACCUMULATION OF HEAVY METALS IN WATER, ALGAE, SEDIMENTS, EGG SHELLS AND FEATHERS OF *ANAS PLATYRHYNCHOS* FROM RIVER SWAT AND BARANDU KP, PAKISTAN

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ABSTRACT

The concentration of selected heavy metals in water, algal flora and sediments and their bioaccumulation in eggshells and feathers of *Anas platyranchos* were measured at target sites along River Swat and River Barandu in Khyber Pakhtunkhwa, Pakistan. The order of HMs in water and sediment samples at River Swat was Cd > Zn > Ni > Cu > Cr > Pb and Zn > Cd > Ni > Cu > Cr > Pb, respectively. While in River Barandu, it was Cu > Cd > Zn > Ni > Cr ≈ Pb and Cu > Zn > Cd > Ni > Pb > Cr, respectively. The concentration of Cu, Zn, Ni, Cd and Pb was below the WHO permissible limits while the concentration of Cr exceeded the permissible limits in the water of River Swat, however, in River Barundu the selected HMs were under the safe limit. The target HMs in both rivers taken by *A. plathyrnachos* via water, sediments and food showed higher bio-concentration factor when compared to bio-sedimentation factor. Comparatively, River Swat was found to be more polluted than River Barundu for the selected HMs. The higher rate of bioaccumulation in the eggshells and feathers of *A. platyranchos* investigated in the present study can be considered a reliable biomarker to assess the biomagnification of pollutants in an aquatic food chain.

1. INTRODUCTION

Anthropogenic and natural activities are severely impacting the aquatic ecosystem. The release of HMs through natural phenomenon (erosion, sedimentation, decomposition) and various anthropogenic activities (agricultural, industrial, and mining) are the major causes of HMs accumulation in the biota (Nawab et al., 2016; Liu et al., 2020; Alipour et al., 2013). HMs are stable, non-biodegradable and can biomagnified through biota (Velásquez & Dussan., 2009). HMs are defined by different authors as "any naturally occurring elements having atomic number > 20, and density > 5 g/cm and become positively charged when takes part in any chemical reaction (Duruibe et al., 2007; Sharma & Agrawal, 2005; Järup, 2003). HMs are further categorized into essential and non-essential based on their role in biological reactions. Essential HMs includes Copper (Cu), Iron (Fe), Zinc (Zn) and Nickle (Ni), and non-essential HMs such as Mercury (Hg), Lead (Pb) Arsenic (As) and Cadmium (Cd), the latter having no known role in living organism (Gündoğdu et al., 2016).

The essentialities of these HMs depend upon the required level and cause toxicities when their concentrations exceed than the required level (Abbas et al., 2018). On the other hand, the non-essential HMs cause toxicity even in small amounts (Velásquez & Dussan, 2009). Both classes of HMs have the potential to bio-magnified from bio-concentration and bio-accumulation and eventually reach to aquatic organism (Avenant-Oldewage & Marx, 2000). Some HMs are metabolized or eliminated while others accumulate in target tissues or organs such as liver, muscles, feathers and maternally transferred to egg and developing embryo (Orłowski et al., 2016). Keeping in view the toxicities, ubiquities and persistence in the aquatic environment, these compounds have become the focus of ecological research (Samanta et al., 2005; Singh & Singh, 2006).

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HMs are usually monitored in the aquatic ecosystem by measuring their concentrations in water and sediments (Ekeanyanwu et al., 2010). HMs accumulated in sediments are considered as a future source of contamination (Fishar and Ali, 2005) and cause threatening situations to aquatic organisms (Qi et al., 2010). Health risks assessment by using living organisms to reflect the concentrations of HMs in particular system is commonly referred as bio-indicator (Liu et al., 2020). Organisms at different trophic level are widely used as representative species for measuring the concentrations of particular pollutants (Malik & Zeb, 2009). Avian species have been widely used as bio-indicators particularly for monitoring of HMs (Ansara-Ross et al., 2013). Among the bird species, mallards (*A. platrhynchos*) have been used as a bio-indicator because they are flightless, common worldwide, omnivores and their body style have modified for both aquatic and terrestrial habitats (Abbasi et al., 2015). Bird feathers and eggshells have been widely used as non-destructive tools to determine the concentration of HMs (Valladares et al., 2010). Feathers and eggshells have advantages over the internal organ because feathers can be plucked from both live and dead individuals (Burger & Gochfeld, 1995) and eggshells can be easily collected without any costs (Orłowski et al., 2014; Costa et al., 2012; Berglund et al., 2011; Ayas, 2007; Dauwe et al., 2002). In this context the current study aimed to compare the concentration of HMs in water, sediments, eggshells, and feathers of *A. platyranchos* from river Swat and river Barandu Khyber Pakhtunkhwa (KP), Pakistan.

2. MATERIALS AND METHODS

Study area

The sampling was done through five different spots from River Swat and River Barundu. The River Swat originates in high glaciers in Upper Swat flowing through southwards of the distract and meet the River Panjkora at Bosaq Bridge in distract Malakand (Ali et al., 2017) and eventually discharge into river Kabul near Charssada. Through its way the river Swat gains contaminations from domestic sewages, car washes and different factories and Markets. Barandu is the only river in district Buner which passes through several urban areas. River Barandu is considered as a primary source of water for common uses, fishing, irrigation, and industrial purposes for local population (figure 1).

Mallards (A. platyranchos)

The mallard (*A. platrhynchos*) has various features that attribute to the criteria as potential bio-indicator and give measures of exposure and toxic effects of environmental pollutions. Their beak have been evolved for capturing small fishes, small invertebrates such as mollusk, tadpoles, arthropods, crustaceans, worms, small aquatic plants, roots and varieties of seeds and also uptake sediments from aquatic environment (Alipour *et al.*, 2013). Their exposure to a wide variety of environmental contaminants and hence have the tendency to accumulate HMs in various organs, make them as potential bio-indicator to assess the health status of environment.

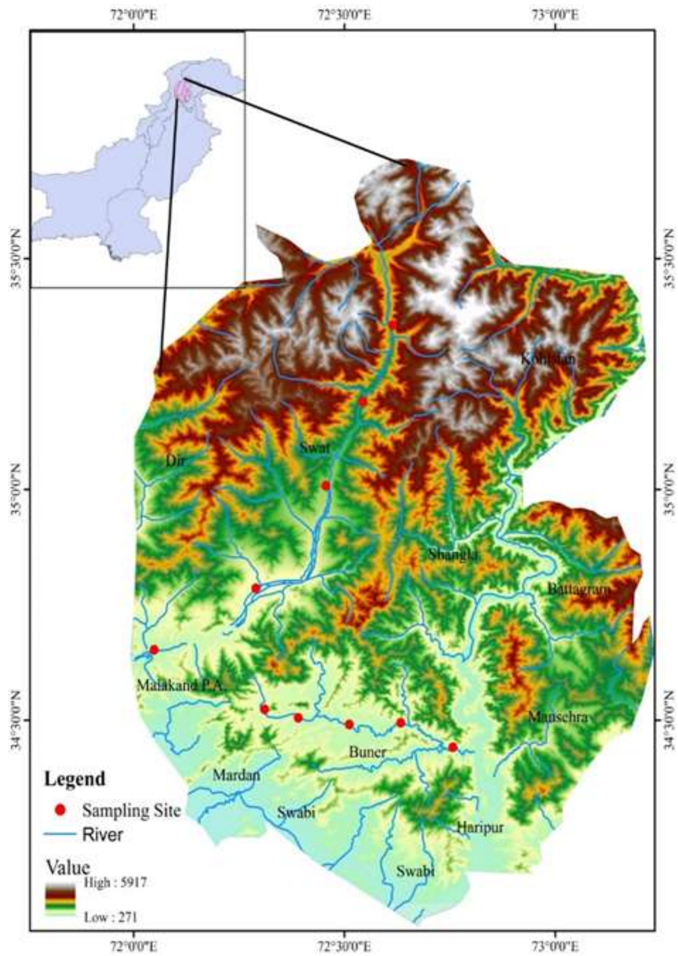

Figure 1. The study area sampling sites (red points) along the river Swat and river Barandu.

Samples Collection, Preparation and Analysis

Water, sediments, eggshells, and feathers sample were collected from the selected sites along river Swat and river Barandu as shown in figure 1. In total, thirty water, sediments, eggshells samples and 90 feathers including body feathers, wing feathers and tail feathers of mallards were collected. Water samples were collected from the surface of river in 500 mL polyethylene bottles. Sediments samples were collected through a 6″ galvanized steel sampler with 0.5" core diameter. Eggshells were collected from foraging and nestling sites of mallards and were washed thoroughly with double deionized water to remove any attached contaminants. Mallards were captured and a few feathers were plucked from body, wing, and tail by using a scissor without harming the birds. The captured birds were released immediately after samples collection.

Metal extraction from Sample

Water samples were filtered through Whatman filter paper (0.45 µm) to remove the suspended materials into 500 mL polyethylene bottles. The filtered water samples were stored at 0-5 ºC. The sediments were also filtered through Whatman filter paper (0.45 µm) for the removal of large particles, dried and passed through a 1 mm neat plastic sieve. The sieved samples were grinded to a powdered form with the help of a mortar and pestle. The feathers were cut into small pieces with the help of a sterilized stainless scissor and heated at 70ºC for 72 hours till dry. The eggshells were washed thoroughly rinsed with double distilled water and chopped into minute particles with a sterilized stainless scissor and heated at 70 ºC for 72 hours.

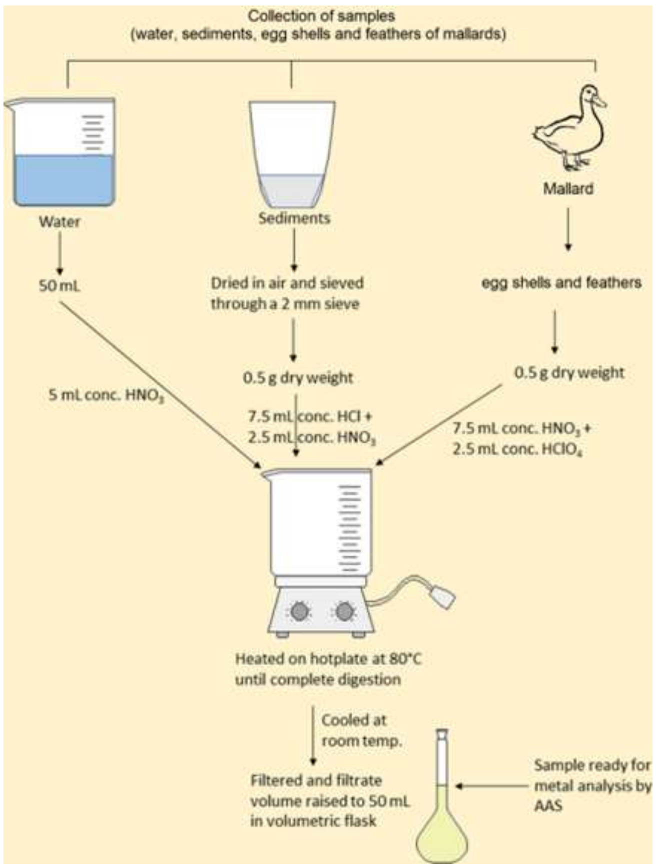

Figure 2. Experimental design and details of sample preparation. (Please cite the sources- the graphical abstract for sample preparation was made by the principal author of the same manuscript and included in MPhil thesis)

A 50 ml water was taken in a 100 ml beaker and 5 mL HNO_3 was added to it. After the addition of HNO_3, the beaker was placed on a hot plate at 80 °C until the sample volume was reduced to 50 ml. A 0.5 g of dry sediments and feathers was separately weighted on an analytical balance (Mettler Toledo, Melbourn, UK). The dried sediments were acidified by 7.5 ml HCL + 2.5 ml HNO_3 for digestion and kept on a hot plate at 80 ºC. The sample was heated until the brown fumes ceased, and a yellow solution was obtained. The solution was cooled at room temperature and filtered through Whatman filter paper (0.45 µm). The filtrate was diluted to 50 mL with deionized water. The collected feather samples (body feather, wing feather, and tail feather) and eggshells of 0.5 g were taken in a separate China dish and placed in an oven at 80 ºC for 72 hours. The feather and eggshells were digested with 7.5 ml HNO_3 and 2.5 ml $HClO_4$. After acidification and digestion, the mixture was heated on a hot plate until the samples were completely dissolved. Details of the experimental procedure are shown in figure 2. The prepared samples were transferred to 60 mL clean polyethylene bottles and the concentration of Cu, Zn, Ni, Cd, Cr and Pb was analyzed by a flame atomic absorption spectrophotometer (Perkinelmer).

Bio-concentration Factors (BCF) and Bio-sedimentation Factor (B.S.F)

The bio-concentration factors and bio-sedimentation factors in feathers and eggshells from aquatic ecosystem (water and sediments) was calculated according to the following equations (Evans & Engel, 1994).

$$BCF = \frac{\text{M feather or eggshells}}{dx\text{M water}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

$$BSF = \frac{\text{M feather or eggshells}}{\text{M Sediment}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where, M represent the metal concentration in feathers or eggshells, metal concentration in water or sediments.

Data analyses

Data was analyzed with SPSS software (version 21). Mean metal concentrations at different River sites were compared using one-way ANOVA (Turkey test) and *P* value of 0.05 was considered for statistical significance.

3. RESULTS AND DISCUSSION

HMs concentration in river's waters

The contamination of water with HMs mostly occurs when HMs find its way into water bodies via, chemical weathering of rocks and soil, agricultural runoffs, industrial waste discharge, mining, batteries, lead based paint, gasoline, and improper domestic waste discharge (Ali & Khan, 2021). Industrial effluent, agriculture runoff and domestic sewage containing different HMs are continuously released into fresh water which changes the pH, salinity. and temperature of water (Liu et al., 2020). The concentration of selected HMs at different spots in water of River Swat was in the order of (Khwazakhela > Madyan > Kabel > Batkhela > Chakdara). Showing an increasing pattern downstream which may be attributed due to increased anthropogenic activities downstream along the river's side which tally with the findings of (Ali et al., 2017; Ali & Khan, 2021; Liu et al., 2020). Zn was found to be higher in all spots followed by Cr and Ni while Pb was found below the detectable limit in some spots (Table 1). The overall order of average HMs concentration in water samples along river Swat was observed as Zn (0.032 mg/L) > Cu (0.005 mg/L) ≈ Ni (0.005 mg/L) > Cd (0.004 mg/L) ≈ Cr (0.004 mg/L) > Pb (0.00 mg/L). The concentration of selected heavy metals in the study area was below the WHO permissible limit for drinking water except Cr, which may probably be due to the overuse of biocides and mining in the region. It has been suggested that Cr is an essential nutrient for living organisms and plays a beneficial role by interaction with toxic elements. However, Cr can cause deleterious effects when its concentration exceeds the allowable limits (Orłowski et al., 2014). River Swat water is mainly contaminated through the anthropogenic activities, agriculture and industrial effluents and weathering of parent rocks containing HMs (Khan et al., 2013). Similarly, the concentration order for selected HMs in water at various spots of River Barundo was Elai > Pacha Kalay > Dagger > Torwarsak > Dewana Baba. Throughout the target sites at Barandu river, the concentration of Cu was found highest while Cr and Pb were observed below the detectable level. HMs concentration in water samples along river Barandu, was as Cu (0.080 mg/L) > Zn (0.040 mg/L) ≈ Cd (0.040 mg/L) > Ni (0.010 mg/L) > Cr (0.00 mg/L) ≈ Pb (0.00 mg/L) (Table 1). It has been emphasized that Barandu water are not suitable for domestic uses due to the high load of marble effluents from nearby industries (Mulk et al, 2016). Among all assessed HMs during this study, the highest concentration of Zn was found in river Swat and Cu in river Barandu. Though both rivers water was found below the WHO permissible limits for drinking water in the studied HMs except Cr in river Swat, it is essential to accurately assess the impact of environmental factors on the deposition of these HMs by surveying in a different season (Khan et al., 2013; Ali et al., 2021).

Concentration of HMs in sediments of river Swat and river Barandu

Average HMs concentration in sediment's samples along river Swat, the overall observed order of HMs was as Zn (26.21 mg/Kg) > Cd (5.24 mg/Kg) > Ni (2.81 mg/Kg) > Cu (0.55 mg/Kg) > Cr (0.49 mg/Kg) > Pb (0.00 mg/Kg). At Madyan the highest concentration was noted for Zn (17.96 mg/Kg dry weight) and lowest concentration for Pb (0.00 mg/Kg dry weight). At Khwazakhela the mean of Zn concentration remained highest (54.43 mg /kg dry weight) and Pb concentration lowest (0.00 mg /kg dry weight) in the sediments. At Kabal, the highest concentration was observed for Zn (18.23 mg/Kg dry weight) and lowest concentration for Pb (0.00 mg/Kg dry weight). At Chakdara the highest concentration was observed for Zn (22.57 mg/Kg dry weight) and lowest concentration for Pb (0.00 mg/Kg dry weight). At Batkhela, the highest concentration was observed for Zn (17.87 mg/Kg dry weight) and lowest concentration for Pb (0.00 mg/Kg dry weight). While average HMs concentration in sediments along river Barandu, the overall observed order of HMs was as Cu (20.35 mg/Kg) > Zn (10.28 mg/Kg) > Cd (4.79 mg/Kg) > Ni (1.74 mg/Kg) > Pb (0.32 mg/Kg) > Cr (0.00 mg/Kg). At Pacha Kaley the highest concentration was observed for Cu (22.83 mg/kg dry weight) and lowest for Cr (0.13 mg/kg dry weight). At Torwarsak the highest concentration was observed for Cu (22.60 mg/kg dry weight) and lowest for Cr (0.24 mg/kg dry weight). At Elai the highest concentration was observed for Cu (19.17 mg/kg dry weight) whereas the lowest concentration was for Cr (0.21 mg/kg dry weight). At Daggar the highest concentration was observed for Cu (22.47 mg/kg dry weight) and the lowest for Pb (0.20 mg/kg dry weight). At Dewana Baba the highest concentration was observed for Cu (14.70 mg/kg dry weight) and the lowest for Pb (0.42 mg/kg dry weight) (Table 2). In sediment, the concentration of heavy metal present was high as compared to water because sediment act as reservoir for pollutant which are being considered as a future source of contamination (Addo-Bediako et al., 2018). The sediment quality is mostly affected by HMs contamination potentially impacting the future of water resources (Fishar and Ali, 2005; Velásquez & Dussan, 2009).

Bioaccumulation of HMs in different feathers and eggshells of A. plathynchos in river Swat and River Barundo

Feathers are used as a non-destructive tools for HMs assessment when studying rare or endangered birds (Beyer et al., 1997). The level of contamination in feathers exhibits the circulating level of those contaminants in a system. It is important to mention that some HMs accumulation in feathers depends on the types of feathers (wing, tail and body feathers), and age of the birds (Tsipoura et al., 2008; Berglund et al., 2011). Therefore, different types of feathers were selected for analysis to precisely assess the bioaccumulation of HMs in the selected region.

The mean concentration of HMs in the body feathers of A. plathrynchos followed the order as Zn (28.60 mg/Kg) > Cu (9.90 mg/Kg) > Cd (3.47 mg/Kg) > Ni (0.67 mg/Kg) > Cr (0.002 mg/Kg) > Pb (0.00 mg/Kg) across river Swat. At Chakdara the highest concentration was observed for Zn (23.47 mg/Kg dry weight) and the lowest concentration for Cr and Pb (BDL) (Table 3). Similarly, mean concentration of HMs in the body feathers of *A. plathrynchos* followed the order as Zn (35.01 mg/Kg) > Cu (20.76 mg/Kg) > Cd (5.04 mg/Kg) > Ni (1.20 mg/Kg) > Pb (0.33 mg/Kg) > Cr (0.00 mg/Kg) across river Barandu. The mean concentration of Zn was highest (39.33 mg/Kg dry weight) while the Cr was found below the detection limit at Pacha

Kalay. The mean concentration of Zn was highest (38.23 mg/Kg dry weight) whereas Cr was below the detection limits at Torwarsak (Table 4). While the average mean concentration of HMs in the wing feathers of *A. plathrynchos* followed the order as Zn (23.40 mg/Kg) > Cu (18.64 mg/Kg) > Cd (3.32 mg/Kg) > Ni (0.87 mg/Kg) > Cr (0.01 mg/Kg) > Pb (0.00 mg/Kg) across river Swat (Table 3). And mean concentration of HMs in the wing feathers of *A. plathrynchos* followed the order as Zn (29.69 mg/Kg) > Cu (15.23 mg/Kg) > Cd (4.35 mg/Kg) > Ni (0.88 mg/Kg) > Cr (0.00 mg/Kg) ≈ Pb (0.00 mg/Kg) across river Barandu (Table 4). In the wing feathers at Pacha Kalay, the highest concentration was observed for Zn (30.70 mg/Kg dry weight) and lowest concentration for Cr and Pb (below the detection level). While in tail feathers the mean concentration of HMs in the tail feathers of *A. plathrynchos* followed the order as Zn (26.32 mg/Kg) > Cu (9.99 mg/Kg) > Cd (2.22 mg/Kg)> Ni (0.72 mg/Kg) > Cr (0.0226 mg/Kg) > Pb (0.00 mg/Kg) across river Swat (Table 3). While mean concentration of HMs in the tail feathers of *A. plathrynchos* followed the order as Zn (33.02 mg/Kg) > Cu (20.77 mg/Kg) > Cd (6.72 mg/Kg) > Ni (0.94 mg/Kg) > Pb (0.08 mg/Kg) > Cr (BDL) across river Barandu (Table 4). In Elai the highest concentration was observed for Zn (29.80 mg/Kg dry weight) and lowest concentration for Cr which was below the detection limits.

The mean concentration of selected HMs in the eggshell of *A. plathrynchos* followed the order as Cu (11.98 mg/Kg) > Cd (4.58 mg/Kg) > Zn (3.18 mg/Kg) > Ni (1.69 mg/Kg) > Cr (0.07 mg/Kg) > Pb (0.00 mg/Kg) across river Swat (Table 3). At Chakdara the highest concentration was observed for Cu (31.83 mg/Kg dry weight) and lowest concentration for Pb (BDL). While mean concentration of HMs in the eggshell of *A. plathrynchos* followed the order as Cu (14.03 mg/Kg) > Zn (5.19 mg/Kg) > Cd (3.31 mg/Kg) > Ni (1.75 mg/Kg) > Pb (0.35 mg/Kg) > Cr (BDL) across river Barandu (Table 4). It has been suggested that HMs in the eggs of birds is a way of excretion of these toxins (Metcheva et al., 2011). Variability among the HMs concentration depend upon the inter or intra species, feeding behavior and metabolic rate (Costa et al., 2012). Accumulation of HMs in specific tissues depends upon the uptake, storage and elimination (Kalay & Canli, 2000). Concentration of heavy metal in feather reflects the long term of exposure as compared to blood which reflect the most recent exposure (Tsipoura et al., 2008). In water the level of pollutants were less and in the top food chain it was increasing in different organ of organism due to bio magnification (Tsipoura et al., 2008).

Eggshell	Mean ±Sd.	7.33 ±0.67	4.50 ±0.17	1.84 ±0.06	1.83±0.31	BDL	0.38±0.03

Bio-concentration factor (BCF) and Bio-sedimentation factor (BSF)

In Madyan Cu followed the same pattern in descending order both for Bio Concentration factor and Bio-sedimentation factors as wing feather > tail feather > eggshell > body feather. For Zn the bio-concentration factor and bio-sedimentation factor followed the same pattern in descending order as Body feather > tail feather > wing feather > eggshell. For Ni the bio-concentration factor and bio-sedimentation factor followed the same pattern as eggshell > wing feather > tail feather > body feather. For Cd the bio-concentration factor and bio-sedimentation factor also followed the same pattern eggshell > wing feather > tail feather > body feather. For Cr the bio-concentration factor is NBCF, and bio-sedimentation factor exhibited the pattern as Body feather > eggshell > tail feather > wing feather and. For Pb the bio concentration factor and bio-sedimentation factors were abbreviated NBCF and NBSF because Pb concentration in water and sediment were below the detection limits. In Madyan over all bio-concentration factors were higher than bio-sedimentation factor (Table 5). While in Torwarsak the bio-concentration and bio-sedimentation factor for Cu followed the same pattern as wing feather > tail feather > eggshell > body feather. For Zn the BCF and BSF followed the same order as body feather > tail feather > wing feather > eggshell. For Ni the BCF and BSF followed the same order as eggshell > body feather > tail feather > wing feather. For Cd the BCF and BSF followed the same pattern as tail feather > wing feather > body feather > eggshell. For Pb the BCF and BSF followed the same order as body feather > eggshell > tail feather > wing feather. For Cr the BCF and BSF were abbreviated as NBCF and NBSF due to the absence of Cr in water and sediment (Table 6).

4. CONCLUSION

In both rivers water the detected value for all selected HMs were below the WHO permissible limits except Cr in river Swat which exceeded the WHO permissible limits for drinking water which may cause health risks due to the consumption of Cr contaminated water. River Barandu is entirely contaminated with directly discharging of marble industrial effluents and domestic sewage. *A. plathynchos* has the potential to be used as representative bio-indicator for monitoring the health status of both rivers.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Mean concentrations of selected HMs in water (mg/L) of river Swat and Barandu.

River Swat	Statistics	pH	Cu	Zn	Ni	Cd	Cr	Pb
Madyan	Min-Max	7.10-7.80	0.002-0.005	0.03-0.043	0.004-0.006	0.012-0.015	0.003-0.009	BDL
	Mean	7.45	0.003	0.035	0.005	0.013	0.005	
Khwazakhela	Min-Max	7.20-8.00	0.004-0.008	0.042-0.044	0.003-0.005	0.008-0.010	0.001-0.006	BDL
	Mean	7.6	0.006	0.04	0.004	0.009	0.003	
Kabal	Min-Max	6.90-7.90	0.005-0.008	0.031-0.035	0.005-0.006	0.006-0.009	0.001-0.003	BDL
	Mean	7.4	0.007	0.033	0.006	0.007	0.002	
Chakdara	Min-Max	7.5-7.80	0.004-0.006	0.017-0.021	0.005-0.007	0.004-0.008	0.001-0.003	BDL
	Mean	7.65	0.005	0.019	0.006	0.006	0.002	
Batkhela	Min-Max	6.70-8.10	0.003-0.007	0.023-0.033	0.005-0.009	0.003-0.006	0.002-0.160	BDL
	Mean	7.4	0.005	0.028	0.007	0.005	0.008	
Avg. Con.			**0.005**	**0.032**	**0.005**	**0.004**	**0.004**	**BDL**
River Barandu								
Pacha Kalay	Min-Max	7.0-8.0	0.100-0.120	0.071-0.080	0.008-0.008	0.045-0.055	BDL	BDL
	Mean	7.5	0.11	0.075	0.008	0.05		
Torwarsak	Min-Max	6.70–7.50	0.058-0.065	0.029-0.032	0.008-0.009	0.060-0.078	-	-
	Mean	7.2	0.061	0.03	0.008	0.068		
Elai	Min-Max	7.50-7.70	0.120-0.140	0.029-0.030	0.007-0.008	0.047-0.060	BDL	BDL
	Mean	7.7	0.13	0.029	0.008	0.054		
Daggar	Min-Max	7.10-7.80	0.060-0.066	0.031-0.033	0.007-0.009	0.022-0.033	BDL	BDL
	Mean	7.45	0.063	0.0317	0.008	0.028		
Dewana Baba	Min-Max	7.21-7.50	0.007-0.27	0.032-0.037	0.006-0.010	0.003-0.006	BDL	BDL
	Mean	7.3	0.017	0.034	0.008	0.004		
Avg. Con.			**0.08**	**0.04**	**0.01**	**0.04**	**-**	**-**

Table. 2. Mean concentrations of selected HMs in sediments (mg/kg) of river Swat and river Barandu.

River Swat		Cu	Zn	Ni	Cd	Cr	Pb
Madyan	Min-Max	0.48-0.78	17.9-18.10	1.48-1.59	4.1-5.20	0.5-54	BDL
	Mean	0.65	17.96	1.54	4.5	0.51	
Khwazakhela	Min-Max	0.480-0.770	48.7-59.00	4.3-4.50	3.5-4.20	1.15-1.40	BDL
	Mean	0.64	54.43	4.37	3.86		
Kabal	Min-Max	0.35-0.46	17.3-19.2	2.9-4.80	6.0-7.50	0.49-0.55	BDL
	Mean	0.4	18.23	3.73	6.83	0.52	
Chakdara	Min-Max	0.48-0.60	22.3-23	2.64-2.78	5.5-6.0	0.05-0.09	BDL
	Mean	0.56	22.57	2.7	5.8	0.073	
Batkhela	Min-Max	0.41-0.59	16.9-18.6	1.64-1.77	4.9-5.80	0.09-0.13	BDL

	Mean	0.48	17.87	1.7	5.22	0.11	
Avg. Con.		**0.55**	**26.21**	**2.81**	**5.24**	**0.49**	**-**
River Barandu							
Pacha Kalay	Min-Max	20.20-25.20	5.22-5.96	1.42-1.59	5.00-6.10	0.10-0.19	0.33-0.33
	Mean	22.83	5.69	1.49	5.63	0.13	0.33
Torwarsak	Min-Max	21.70-23.50	5.70-6.10	1.50-1.62	5.30-7.00	0.15-0.32	0.41-0.45
	Mean	22.6	5.87	1.54	6.27	0.24	0.42
Elai	Min-Max	17.50-20.60	12.30-13.00	1.60-1.94	3.40-5.50	0.14-0.320	0.14-0.36
	Mean	19.17	12.6	1.75	4.5	0.21	0.22
Daggar	Min-Max	20.70-24.80	20.60-24.50	1.90-3.05	5.40-6.60	0.04-0.70	0.03-0.40
	Mean	22.47	21.93	2.32	5.92	0.28	0.2
Dewan Baba	Min-Max	12.60-16.80	5.19-5.40	1.52-1.760	1.00-2.00	0.46-0.53	0.40-0.44
	Mean	14.7	5.32	1.61	1.63	0.5	0.42
Avg. Con.		**20.35**	**10.28**	**1.74**	**4.79**	**0.27**	**0.32**

Table 3. Bioaccumulation of selected HMs in different feathers of *A. plathynchos* from river Swat.

River Swat	**Statistics**	**Cu**	**Zn**	**Ni**	**Cd**	**Cr**	**Pb**
Madyan							
Body feather	Mean ±Std.	7 ± 0.50	36.07 ±0.45	0.34 ±0.05	1.6 ±0.56	0.09 ±0.01	BDL
Wing feather	Mean ±Sd.	17.70±1.57	25.70±2.22	0.74±0.01	4.63±0.81	0.04±0.02	BDL
Tail feather	Mean ±Sd.	13.50±1.411	26.90±1.30	0.52±0.03	2.53±0.45	0.05±0.01	BDL
Eggshells	Mean ±Sd.	9.70±0.80	3.27±0.55	1.717±0.206	5.13±1.27	0.06±0.036	BDL
Khwazakhela							
Body feather	Mean ±Sd.	9.1±0.85	31±0.70	0.68±0.1	1.93±0.55	BDL	BDL
Wing feather	Mean ±Sd.	6.50±0.50	23.63±1.40	0.71±0.08	1.77±0.64	BDL	BDL
Tail feather	Mean ±Sd.	6.74±1.00	26.50±0.61	0.67±0.08	2.07±0.311	0.063±0.11	BDL
Eggshells	Mean ±Sd.	3.80±0.76	3.33±0.21	1.41±0.15	3.16±0.57	0.13±0.04	BDL
Kabal							
Body feather	Mean ±Sd.	10.92±0.88	24.47±2.51	0.67±0.12	3.73±0.38	0.01±0.01	BDL
Wing feather	Mean ±Sd.	12.47±0.56	23.67±1.54	0.66±0.05	2.93±0.55	0.01±0.012	BDL
Tail feather	Mean ±Sd.	7.96±1.05	26.23±0.38	0.56±0.03	1.87±0.36	BDL	BDL
Eggshells	Mean ±Sd.	9.77±1.03	3.13±0.12	1.64±0.09	3.19±0.76	0.07±0.01	BDL
Chakdara							
Body feather	Mean ±Sd.	11.23±0.75	23.47±1.45	0.71±0.10	5.37±1.0	BDL	BDL
Wing feather	Mean ±Sd.	22.70±0.92	20.877±0.32	0.73±0.06	3.50±0.56	BDL	BDL
Tail feather	Mean ±Sd.	9.30±0.81854	25.83±1.07	0.87±0.11	1.85±0.22	BDL	BDL
Eggshells	Mean ±Sd.	31.83±1.53	3.00±0.10	1.79±0.11	6.67±0.93	0.04±0.01	BDL
Batkhela							
Body feather	Mean ±Sd.	11.22±2.21	28.07±1.42	0.93±0.07	4.73±1.02	BDL	BDL
Wing feather	Mean ±Sd.	33.83±0.83	23.13±1.25	1.50±0.30	3.75±0.95	BDL	BDL
Tail feather	Mean ±Sd.	12.43±1.23	26.13±0.31	0.98±0.10	2.77±0.25	BDL	BDL
Eggshells	Mean ±Sd.	4.80±0.98	3.17±0.23	1.90±0.10	4.77±0.32	0.06±0.04	BDL

Table 4. Bioaccumulation of selected HMs in different feathers of *A. plathynchos* from river Barandu.

River Barandu	Statistics	Cu	Zn	Ni	Cd	Cr	Pb
Pacha Kalay							
Body feather	Mean ±Sd	13.63±2.21	39.33±0.58	1.26±0.10	5.63±2.26	BDL	0.54±0.40
Wing feather	Mean ±Sd.	8.93±1.38	30.70±1.50	0.83±0.10	2.37±1.39	BDL	BDL
Tail feather	Mean ±Sd.	25.57±1.16	34.57±1.12	1.03±0.05	4.90±1.05	BDL	0.25±0.13
Eggshell	Mean ±Sd.	15.67±1.16	4.50±0.17	1.87±0.15	6.73±0.76	BDL	0.38±0.02
Torwarsak							
Body feather	Mean ±Sd.	13.43±1.15	38.23±2.48	1.32±0.14	5.43±1.21	BDL	0.43±0.11
Wing feather	Mean ±Sd.	23.37±2.40	34.70±3.28	0.78±0.047	7.23±1.24	BDL	BDL
Tail feather	Mean ±Sd.	19.00±1.00	36.30±0.56	0.90±0.06	9.37±0.57	BDL	0.00±0.06
Eggshell	Mean ±Sd.	18.50±2.34	4.93±0.209	1.96±0.10	4.00±0.50	BDL	0.40±0.04
Elai							
Body feather	Mean ±Sd.	25.33±3.19	32.00±0.90	1.02±0.12	3.27±0.64	BDL	0.15±0.12
Wing feather	Mean ±Sd.	8.13±0.81	27.13±1.70	0.94±0.10	1.87±0.15	BDL	BDL
Tail feather	Mean ±Sd.	17.50±0.87	29.80±2.82	0.89±0.04	6.27±1.10	BDL	0.13±0.12
Eggshell	Mean ±Sd.	7.50±0.56	6.77±0.61	1.34±0.11	2.27±0.64	BDL	0.21±0.06
Daggar							
Body feather	Mean ±Sd.	19.60±0.61	33.97±1.50	1.22±0.09	7.77±1.91	BDL	0.30±0.26
Wing feather	Mean ±Sd.	14.13±1.43	30.33±1.74	0.85±0.10	6.60±1.15	BDL	BDL
Tail feather	Mean ±Sd.	15.43±1.30	32.90±1.14	0.94±0.03	5.57±0.68	BDL	BDL
Eggshell	Mean ±Sd.	21.17±1.59	5.23±0.39	1.76±.13	1.73±1.10	BDL	0.37±0.05
Dewana Baba							
Body feather	Mean ±Sd.	31.83±3.25	31.53±3.63	1.16±0.20	3.10±1.49	BDL	0.22±0.06
Wing feather	Mean ±Sd.	21.57±2.91	25.57±1.77	0.99±0.15	3.70±1.11	BDL	BDL
Tail feather	Mean ±Sd.	26.33±1.07	31.53±1.21	0.96±0.10	7.50±0.62	BDL	0.03±0.06
Eggshell	Mean ±Sd.	7.33±0.67	4.50±0.17	1.84±0.06	1.83±0.31	BDL	0.38±0.03

Table 5. Bio-concentration and bio-sedimentation factors of selected HMs in eggshell and feather samples of *A. plathrynchos* from river Swat (water and sediments).

				Feather types (mg/Kg)				Bio-concentration Factor				Bio-sedimentation factor			
River Swat	Metals	Water (mg/L)	Sediments (mg/Kg)	Body feather	Wing feather	Tail feather	Eggshell (mg/Kg)	BCF b	BCF w	BCF t	BCF eg	BSF b	BSF w	BSF t	BSF eg
Madyan	Cu	0	0.65	7	17.7	13.5	9.7	NBCF	NBCF	NBCF	NBCF	10.76	27.23	20.76	14.92
	Zn	0.04	17.97	36.07	25.7	26.9	3.27	901.75	642.5	672.5	81.75	2.03	1.43	1.49	0.18
	Ni	0	1.54	0.34	0.74	0.52	1.72	NBCF	NBCF	NBCF	NBCF	0.22	0.48	0.33	1.11
	Cd	0.01	4.5	1.6	4.63	2.53	5.13	160	463	253	513	0.35	1.02	0.56	1.14
	Cr	0	0.51	0.09	0.04	0.05	0.06	NBCF	NBCF	NBCF	NBCF	0.18	0.07	0.09	0.12
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF	NBSF
Khwazakhela	Cu	0.01	0.64	9.1	6.5	6.73	3.8	910	650	673	380	14.14	10.1	10.46	5.9
	Zn	0.04	54.43	31	23.63	26.5	3.33	775	590.75	662.5	83.25	0.56	0.43	0.48	0.06
	Ni	0	4.37	0.67	0.77	0.67	1.41	NBCF	NBCF	NBCF	NBCF	0.15	0.16	0.15	0.32
	Cd	0.01	3.87	1.93	1.77	2.07	3.16	193	177	207	3116	0.49	0.45	0.53	0.81
	Cr	0	1.26	BDL	BDL	0.06	0.13	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	0.05	0.1
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF	NBSF
Kabal	Cu	0.01	0.4	10.92	12.47	7.96	9.77	1092	1247	796	977	27.25	31.11	19.86	24.37
	Zn	0.03	18.23	24.47	23.67	26.23	3.13	815	789	874.33	104.33	1.34	1.29	1.43	0.17
	Ni	0.01	3.73	0.67	0.66	0.6	1.64	67	66	60	164	0.17	0.17	0.15	0.43
	Cd	0.01	6.83	3.73	2.93	1.87	3.18	373	293	187	318	0.54	0.42	0.27	0.46
	Cr	0	0.52	0.01	0.01	BDL	0.07	NBCF	NBCF	NBCF	NBCF	0.01	0.01	NBSF	0.12
	Pb	BDL	BDL	BDL	BDL	BDL	0.33	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF	NBSF
Chakdara	Cu	0.01	0.56	11.23	22.7	9.3	31.83	1123	2270	930	3183	20.15	40.72	16.68	57.11
	Zn	0.02	22.57	23.47	20.87	25.83	3	1173	1043.5	1291.5	150	1.03	0.92	1.14	0.13
	Ni	0.01	2.7	0.71	0.73	0.87	1.79	71	73	87	179	0.26	0.27	0.32	0.66
	Cd	0.01	5.8	5.37	3.5	1.85	6.67	537	350	185	667	0.92	0.6	0.31	1.15
	Cr	0.01	0.07	BDL	BDL	BDL	0.04	NBCF	NBCF	NBCF	4	NBSF	NBSF	NBSF	0.59
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF	NBSF
Batkhela	Cu	0.01	0.48	11.22	33.83	12.43	4.8	1122	3383	1243	480	23.52	70.92	26.06	10.06
	Zn	0.03	17.87	28.077	23.13	26.13	3.17	935.9	771	871	105.66	1.57	1.29	1.53	0.17
	Ni	0.01	1.7	0.93	1.5	0.98	1.9	93	150	98	190	0.54	0.88	0.57	1.11
	Cd	0	5.22	4.73	3.75	2.77	4.77	NBCF	NBCF	NBCF	NBCF	0.9	0.71	0.5	0.91
	Cr	0.01	0.11	BDL	BDL	BDL	0.06	NBCF	NBCF	NBCF	7.11	NBSF	NBSF	NBSF	0.53
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF	NBSF

Table 6. Bio-concentration and bio-sedimentation factors of selected HMs in eggshell and feather samples of *A. plathrynchos* from river Barandu water (mg/L) and sediments (mg/Kg).

				Feather types (mg/Kg)				Bio-concentration Factor				Bio-sedimentation factor		
River Barandu	Metals	Water	Sediments	Body feather	Wing feather	Tail feather	Eggshell	BCF b	BCF w	BCF t	BCF eg	BSF b	BSF w	BSF t
Pacha Kalay	Cu	0.11	22.83	13.63	8.93	25.57	15.67	123.9	81.18	232.45	142.45	0.59	0.39	1.11
	Zn	0.08	5.69	39.33	30.7	34.57	4.5	491.62	383.75	432.12	56.25	6.9	5.39	6.07
	Ni	0.01	1.49	1.263	0.83	1.03	1.87	126.3	83	103	187	0.84	0.55	0.68
	Cd	0.05	5.63	5.63	2.37	4.9	6.73	112.6	47.4	98	134.6	0.99	0.42	0.86
	Cr	BDL	0.13	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF
	Pb	BDL	0.33	0.54	BDL	0.25	0.38	0.53	NBCF	0.25	0.38	1.62	NBSF	0.76
Torwarsak	Cu	0.06	22.6	13.43	23.37	19	18.5	223.83	389.5	316.66	308.3	0.59	1.03	0.84
	Zn	0.03	5.87	38.23	34.7	36.3	4.93	1274.3	1156.66	1210	164.33	6.51	5.91	6.18
	Ni	0.01	1.54	1.32	0.78	0.9	1.95	132	78	90	7531.38	0.85	0.5	0.58
	Cd	0.07	6.27	5.43	7.23	9.37	4	77.57	103.28	133.85	57.14	0.86	1.15	1.49
	Cr	BDL	0.24	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF
	Pb	BDL	0.42	0.43	BDL	0	0.4	0.43	NBCF	0.003	0.4	1.02	NBSF	0.008
Elai	Cu	0.13	19.17	25.33	8.13	17.5	7.5	194.84	62.53	134.61	57.69	1.32	0.42	0.91
	Zn	0.03	12.6	32	27.13	29.8	6.77	1066.66	904.33	993.33	225.66	2.53	2.15	2.36
	Ni	0.01	1.75	1.02	0.94	0.89	1.34	102	94	89	134	0.75	0.53	0.5
	Cd	0.05	4.5	3.27	1.87	6.27	2.27	65.4	37.4	125.4	45.4	0.72	0.41	1.39
	Cr	BDL	0.21	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF
	Pb	BDL	0.23	0.15	BDL	0.13	0.21	0.14	NBCF	0.13	0.21	0.64	NBSF	0.58
Daggar	Cu	0.06	22.47	19.6	14.13	15.43	21.17	326.66	235.5	257.1	352.83	0.87	0.62	0.68
	Zn	0.03	21.93	33.97	30.33	32.9	5.23	1132.33	1011	1096.66	174.33	1.54	1.38	1.5
	Ni	0.01	2.32	1.22	0.85	0.94	1.76	122	85	94	176	0.52	0.36	0.4
	Cd	0.03	5.92	7.7667	6.6	5.57	1.73	258.89	220	185.66	57.66	1.31	1.11	0.94
	Cr	BDL	0.28	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF
	Pb	BDL	0.2	0.3	BDL	BDL	0.37	0.3	NBCF	NBCF	1.83	1.5	NBSF	NBSF
Dewana Baba	Cu	0.02	14.7	31.83	21.57	26.33	7.33	1591.5	719	1316.5	366.5	2.16	1.46	1.79
	Zn	0.03	5.32	31.5333	25.57	31.53	4.5	1051	852.33	1051	150	5.93	4.8	5.93
	Ni	0.01	1.61	1.16333	0.99	0.96	1.84	116.3	99	96	184	0.72	0.61	0.59
	Cd	0	1.63	3.1	3.7	7.5	1.83	NBCF	NBCF	NBCF	NBCF	1.89	2.26	4.59
	Cr	BDL	0.51	BDL	BDL	BDL	BDL	NBCF	NBCF	NBCF	NBCF	NBSF	NBSF	NBSF
	Pb	BDL	0.42	0.22	BDL	0.03	0.37	0.42	NBCF	0.03	0.37	0.52	NBSF	0.03