COMPARISON OF NON-INVASIVE MATRICES FOR METALS AND TRACE ELEMENTS EXPOSURE IN WHITE BREASTED KINGFISHER (*HALCYON SMYRNENSIS*)

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ABSTRACT: Toxic trace elements have the natural tendency to bioaccumulate in the tissues of living organism and a constant exposure to these metals can cause adverse health issues specially in organism present at the top of the food chain (i.e., birds and mammals) due to the ability of trace elements to bio magnify along the trophic levels. The aim of the current study is to determine the tendency of selected eight toxic trace elements i.e., Cd, Cr, Cu, Zn, Mn, Fe, Ni and Pb to bioaccumulate in blood, feathers and preen oil of White Breasted Kingfisher (*Halcyon smyrnensis*) in selected study site i.e., Manga Mandi, a peri urban area near Lahore, Pakistan, and to estimate their trophic transfer potential by using food samples of white breasted kingfisher. All the selected trace elements were detected in almost all the samples in heterogenous concentrations. Fe showed the highest concentration among all selected metal in all the samples of feathers, blood, preen oil and the food samples, which signifies the effect of large number of steel industries found in the vicinity of the sampling site. Cd showed the least concentration in each sample. In general, feathers found to be the most contaminated tissue followed by preen oil and blood. However, BAF for Zn is found to be highest i.e., 5.497µg/g while BAF of Fe is found to be lowest in all the tissues as 0.05µg/g, 0.052µg/g and 0.04µg/g in blood, feathers and preen oil respectively. For BCF, Cr, Cd, Cu and Pb showed no concentration while Pb and Zn showed highest concentration in feathers i.e., 17.369µg/g and 127.8µg/g respectively, and Mn and Ni were found highest in preen oil as 1.627µg/g and 6.933µg/g respectively. This signifies soil to be more contaminated than water. Almost all metal showed TTF>1 except Cd which was below detection limit mostly. Mn showed highest likelihood to be bioaccumulated along the trophic level while Cu showed the minimum. Highest TTF value was recorded for preen oil, followed by blood and feathers.

Key Words: Trophic Transfer Level, Bioaccumulation Factor, Bioconcentration Factor, Toxic Trace Elements, Heavy Metals, White Breasted Kingfisher (*Halcyon smyrnensis*)

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INTRODUCTION

Toxic trace elements started to gain significant importance after industrial revolution due to their toxic effect on human and environmental health. (Ali *et al*., 2019) Toxic trace elements, commonly known as Heavy Metals, can transfer from non-living environment to living environment, have tendency to accumulate there and magnify with time along the food chain (Szynkowska *et al*., 2018). They are well-known environmental pollutants due to their ability to persist in environment. Persistence is not the alone factor but bio-accumulation and bio-magnification behaviour of heavy metals make them worst among the environmental pollutants (Abbas *et al*., 2018). Naturally they enter the biotic environment through volcanic eruption along with the lava, as the core and mantle of earth are full of them, and through the weathering of metal-bearing rocks. Many human activities are also responsible for heavy metal pollution in environment (Mishra *et al*., 2019), some anthropogenic sources include industrial activities that uses various chemicals through different process- mostly heavy metals are used as catalysts in industries, agricultural sector that uses different chemicals to improve the crop production and mining activities etc (Mahanty *et al*., 2021). Extraction of mineral resources through mining and then its refining through various industrial processes, and then use of these minerals for different activities in industrial and agricultural sector mainly for the economic development has led to mobilization of the toxic elements in the environment and this has disturbed the natural bio-geochemical cycle of these metals. The increased contamination of toxic trace elements in environment, in different aquatic and terrestrial ecosystems, is becoming a great concern of environment, animal and human health (Bradney *et al*., 2019). Persistent nature of heavy metals tends to bio-accumulate them in different tissues of living organism, which are then gradually bio-magnified along the food chain consequently contaminating the whole food chain and food web, which ultimately affect the health of animals, environment and humans (Majed *et al*., 2016). Most environmentally relevant heavy metals and metalloids include Copper (Cu), Nickle (Ni), Chromium (Cr), Zinc (Zn), Cadmium (Cd), Lead (Pb). Mercury (Hg) and Arsenic (As)(Ali, Khan, & Ilahi, 2019). If humans are long term exposed to these metals, then they can cause serious neurological, and physical processes which are degenerative in nature which may result in emergence of various diseases such as Alzheimer's disease, Parkinson's disease and even cancer in case of chronic long-term exposure (Rehman *et al*., 2018).

Usually, environmental bioindicator species are used to assess the concentration of heavy metal pollution in environment. In recent years, birds have been widely used as the bioindicator specie (Egwumah *et al*., 2017). Birds are widespread terrestrial animals, travel long distances for food and reproduction and are commonly present at top or near the top of the food chain making them prone to bioaccumulate many biomagnified environmental pollutants. Assessment of the heavy metal concentration at different levels of the food chain indicates high concentration of heavy metals by the end of food chain due to bioaccumulation and biomagnification, and that’s why birds are considered as one of the most effected animals to the toxic effect of trace elements as most of them are present near or at top of the food chain. (Einoder *et al*., 2018) Heavy metals accumulate in the feathers and eggshells of birds. Beside feathers, blood and preen oil are also used for this purpose. Feathers, blood and preen oil of birds reflect the background abundance of toxic trace elements in the birds and that’s why they can be considered as the indicator for detecting the heavy metal pollution in the ecosystem. However, external deposition and disconnection from blood can be some limitations in using these organs for biomonitoring, therefore internal tissues like kidney, liver and blood etc. are used for this purpose. (Tasneem *et al*., 2020; Kanwal *et al*., 2020). But killing birds for obtaining organs has its own ethical considerations and is widely criticized all over the world. Accumulation of toxic elements in bird’s organs depends upon its physiology as well as different other factors like excretion potential, detoxification potential, extent and type of exposure, distance from the source and the chemical nature of the pollutant. (Abbasi *et al*., 2015 b). Different bird organs including feathers, eggshells, blood, preen oil, liver, kidney, pelvic and pectoral muscles have been used in recent years in various experiments to assess the heavy metals. (Sani *et al*., 2020; Castro *et al*., 2011; Frantz *et al*., 2012). However, each tissue and organ have its own protocol regarding its use for such studies that should be considered depending upon study’s scope and extent.

White Breasted Kingfisher (*Halcyon smyrnensis*), also known as White-Throated Kingfisher is a tree kingfisher found throughout the world but commonly noted in tropical region of Asia. It has a compact body with a large head. They are commonly found near agricultural lands, marshes, swamps, ponds, lakes, mangrove swamps, estuaries and in parklands. They are carnivores and primarily feed on fish, insects, arthropods, aquatic crustacean, reptiles and amphibians. (McCallen, 2007). Studies have showed that bioaccumulation of different toxic trace elements has, affected the white breasted kingfisher too. High concentration of Cu, Mn, Co, Ni, As and Pb has been noted in different aquatic and terrestrial bird species with Cu having the highest concentration. Along with natural processes rapid urbanization and anthropogenic sources are also the reason of bioaccumulation of heavy metals in the birds and with time this can cause serious health issues as they have tendency to bio magnify. (Biswas *et al*, 2020)

Major source of exposure of toxic elements in birds is “ingestion”, through food and water, which the bioaccumulate in the tissues. (Dolan *et al*., 2017; Vizuete *et al*., 2018). However, heavy metals have different tendencies to bioaccumulate in different tissues which can be determined using Trophic Transfer Factor (TTF). It is a ratio between heavy metal concentration in bird (predator) to concentration in its food (prey). (Jara-Marini *et al*., 2009). Though heavy metals have been widely detected in different bird tissues (Dahmardeh Behrooz and Burger, 2021a, Dahmardeh Behrooz and Burger, 2021b; Berglund, 2018; Abbasi *et al*., 2015a; b; Tasneem *et al*., 2020; Kanwal *et al*., 2020) but their bioaccumulation factor has been hardly highlighted. Moreover, work on comparison of bioaccumulation factor among different tissues of birds has not been done much. Therefore, the aim of the study is: 1) To investigate the source and extent of alarming increase in the bioaccumulation of the heavy metals in the food chain of White Breasted Kingfisher (*Halcyon smyrnensis*). 2) To determine the Bioaccumulation Factor (BAF) and Trophic Level (TTF) in the feathers, blood and preen oil of *Halcyon smyrnensis* by comparing the food samples and environment of the *Halcyon smyrnensis.*

MATERIAL METHODS

Study Site: The sampling was done in the flood plains of River Ravi between Manga Mandi (31.3016^{o}N, 74.0697^{o}E) and Jaranwala (31.3454^{o}N, 73.4298^{o}E) a peri urban area near the city of Lahore, Punjab, Pakistan, between January 2022 to March 2022.

Sample Collection: A total of five individuals (n=5) of White-Breasted Kingfisher (*Halcyon smyrnensis*) and Food (n=9) were collected using mist nets from suburbs of Manga Mandi. Minimal invasive procedure was used for the sampling. Due to different ethnic and scientific considerations a limited scale sampling was conducted.

All sampling was done in accordance with the studies of WWF-Pakistan with the help of specialized and trained persons, and all the ethical and international standards regarding sampling of birds were strictly followed. Ethical Approval was taken from the Bioethical Committee of University of the Punjab, Lahore. A special permission was also granted from the Local Wildlife Department.

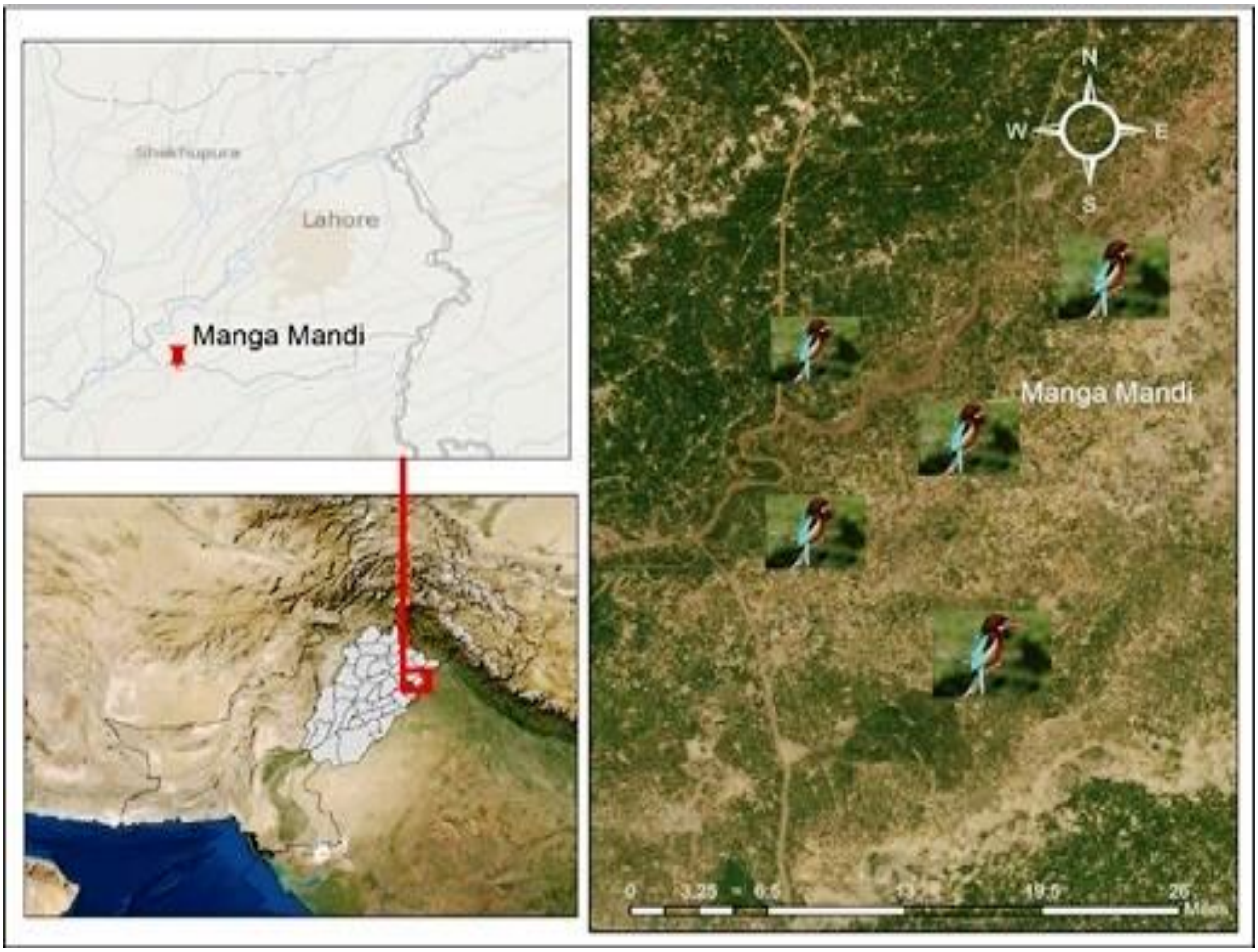

Figure 1 Location map of the sampling site (Manga Mandi) illustrating the sampling points of White Breasted Kingfisher collected from the premises of Lahore, Punjab, Pakistan

2-3 ml of blood was extracted from each of the live sampled bird (n = 4). No bird was harmed or killed in the procedure and the blood was collected with the help of a 26G needle fixed with 5ml syringe into a micro haematocrit tube by puncturing their brachial veins. (Zaman, *et al*., 2022).

After blood collection, 5-7 feathers (n = 5), weighing average 0.22 g, from each individual bird was collected and stored in polythene bags.

Immediately after the collection of feathers, preen oil was collected from each bird (n = 5), to an extent that no oil remained in the gland, using a sterile micro-capillary tube. Feathers around the preen glands were first plucked by using a cotton tipped applicator dipped in 0.9% NaCl solution so that preen oil doesn't get contaminated with feather bacteria. The samples were then weighed using a weighing sample and each sample weighed in between 0.6g-0.8g. (Alt, Mägi, Lodjak, & Mänd, 2020).

To determine the trophic transfer of the studied trace metals samples from the food chain of White Breasted Kingfisher were also taken. Food samples included three individuals of different species of fish (*Osteobrama cotio, Puntius ticto, Channa striata*), two live individuals of rodents (*Mus sp*.), two samples of frogs (*Bufo stomaticus*), one individual of wasp (*Vespidae*) and one sample of prawn (*Dendrobranchiata*).

For the bioconcentration study, sampling was from the habitat of White Breasted Kingfisher. Three samples of water and three samples of soil were taken.

Sterilized polythene bags were used to store each sample. After collection samples were immediately stored at -4^{o}C in a deep freezer before analysis.

Sample Preparation

Sample Storage: The glassware apparatus used in the experimental work and analysis was first thoroughly sterilized by dipping in 10% HNO_3 solution, followed by rinsing with deionized water and then properly oven dried. All the samples were frozen at -4^{o}C and then weighed using a weighing machine. Weight of each sample was between 0.6 g – 2g.

Acid Digestion: Acid Digestion of each sample was done on a hot plate before sending for the analysis. It is to be noted that different chemical concentration was taken for each different type of sample. For feathers, a solution of 13 ml HNO_3 and 14ml H_2O_2 in ratio of 13:14 was used. For blood, 0.5ml of each sample was digested at 110^{o}C in 5ml hydrochloric acid (95% HCl), Perchloric acid (70% $HClO_4$) and nitric acid (70% HNO_3) in ratio 1:8:8 respectively, for 2 hours. For Preen Oil, a solution of 1ml HCl, 8ml HNO_3, and 8ml $HClO_4$ was used. All the food samples were digested using solution of 5ml nitric acid (70% HNO_3) and 15ml of Hydrogen Peroxide (H_2O_2) in ratio 1:3. Prior to the digestion of fish, each sample was dissected and its alimentary canal was removed, sample was then weighed and oven dried at 120^{o}C for 120 minutes.

For soil, 0.5g of each sample was taken and oven dried for 60 minutes at 40^{o}C. Dried soil sample were then passed through a 0.2nm sieve. Then acid digestion was done using a solution of HNO_3, HCl and $HClO_4$ in 5:1:1. For water, 500 ml of sample was taken and double filtration was done using Whatman no 42 and the filtrate was collected in 250 ml plastic bottles.

Double Filtration: After acid digestion, 5ml distilled water was added in each sample and then placed for about two minutes to cool down. After cooling, each sample was filtered using Whatman Filter Paper no 42 and the filtrate obtained was raised to 50ml volume by adding distilled water. The solution was again filtered using Whatman no 42 in sterilized 250 ml plastic bottles.

Quality Assurance: Multiple samples (n=3) for each specimen were prepared. Procedural blanks (n=3) were also prepared for each type of sample.

Sample Analysis: For analysis, all the prepared samples, and the prepared procedural blanks of each sample, were sent to Pakistan Institute of Nuclear Science and Technology, Islamabad, Pakistan. Analysis of eight selected trace metals i.e., Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn was done using the technique ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy). When the concentration hits zero, the Limit of Quantification is computed as three times the standard deviation of a repeated study. LOQ obtained was 0.06 µg/L for Cu, 0.01 µg/L for Cr, 0.01 µg/L for Pb, 0.02 µg/L for Ni, 0.04 µg/L for Fe and 0.39 µg/L for Zn. The detection limits were obtained by analysing a spiked blank with seven duplicates and multiplying the standard deviation by 3.14, then doing this three times and taking an average. All of the concentrations were blank corrected and presented in arithmetic mean as (µg/ml) ± S.E.

RESULT AND DISCUSSION

General Metal Trends in Tissues of White Breasted Kingfisher: A detailed description of the observed concentrations (g/ml) of the eight chosen toxic trace elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in non-invasive tissues. i.e., Preen Oil, Feathers and Birds of White Breasted Kingfisher is given in the Table 1. Almost all the tissue samples showed some concentration for all of the metals. Fe showed highest concentration in all the samples. In the following study, the highest mean (min-max) concentration (µg/ml) for Fe at 3.416 (1.66-6.35), Pb at 0.148 (0.08-0.24), Zn at 1.704 (1.3-2.59), Cd at 0.012 (0.01-0.02), Cu at 0.1 (0.04-0.15) were recorded in the Feathers and for Mn at 0.586 (0.04-0.97), Ni at 0.104 (0.05-0.14), Cr at 0.134 (0-0.22) in Preen Oil of the White Breasted Kingfisher. The lowest mean (min-max) concentration (µg/ml) for Fe at 2.425 (1.58-3.65), Mn at 0.2675 (0.24-0.32), Ni at 0.045 (0.03-0.06) were detected in Blood, and for Zn at 0.092 (0.06-0.1), Cd at 0.0067 (0-0.01), Cu at 0.053 (0.05-0.06) and constant value of Pb at 0.04 were detected in Preen Oil of the White Breasted Kingfisher. Cr showed same value for lowest mean (min-max) concentration (µg/ml) in Feathers at 0.07 (0.04-0.13) and Blood at 0.07 (0.06-0.08) of Kingfisher. In general Fe, Mn, Ni and Zn showed 100% detection frequency in all the fourteen collected samples while Cd and Pb showed the lowest frequency of 64.29%. As for Preen Oil samples, 100% detection frequency was recorded for Fe, Mn, Ni and Zn, Cd and Pb showed 40%, while Cr and Cu showed 80% and 60% detection frequency, respectively. Blood samples showed 100% detection frequency for Cr too in addition to Fe, Mn, Ni and Zn while Cd, Cu and Pb showed 50% detection frequency. 100% detection frequency for all the samples of feathers was recorded for all the metals. Different concentration pattern was seen in each tissue i.e., preen oil, blood and feathers of the White Breasted Kingfisher. Trace metal concentration in preen oil was found to be highest for Fe followed by Mn, Cr, Ni, Zn, Cu, Pb and Cd respectively. For feathers, metals followed the trend highest for Fe, followed by Zn, Mn, Pb, Cu, Cr, Ni and Cd respectively. For the blood, metal trend is highest for Fe again followed by Mn, Zn, Cu, Cr, Ni equals to Pb and Cd at the end.

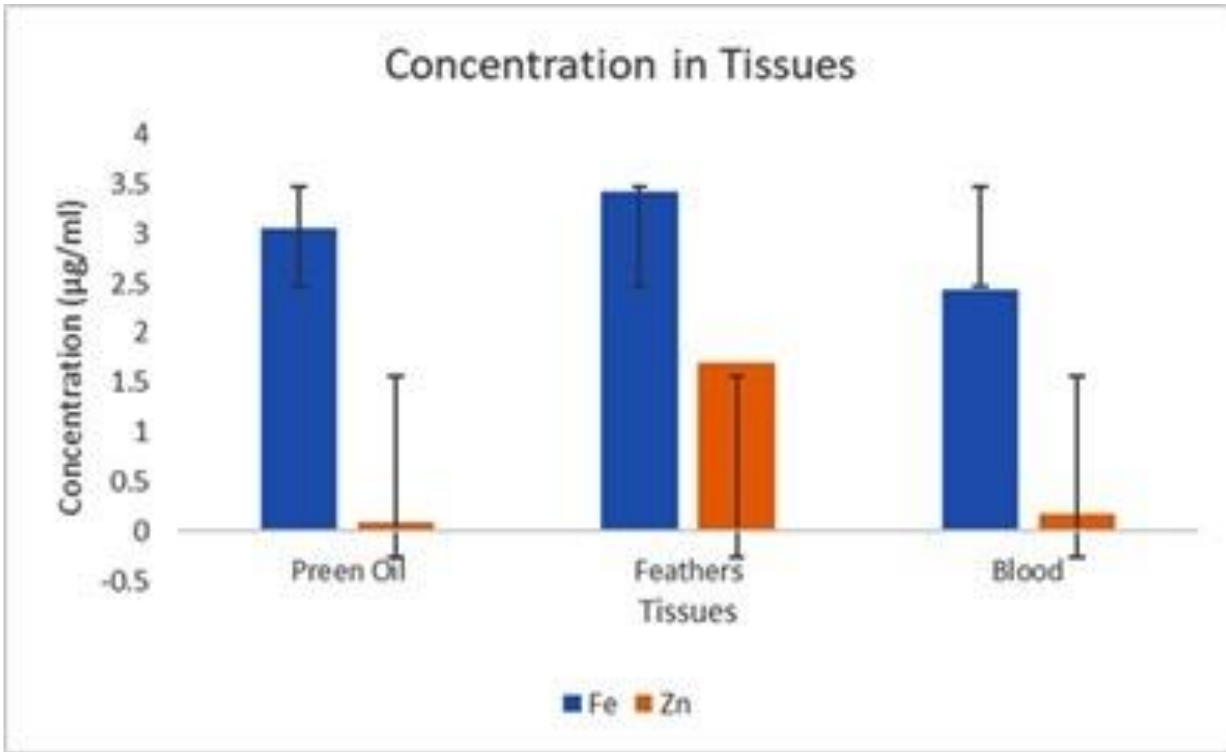

Figure 2 Mean Concentration of selected metals in the selected tissues of White Breasted Kingfisher

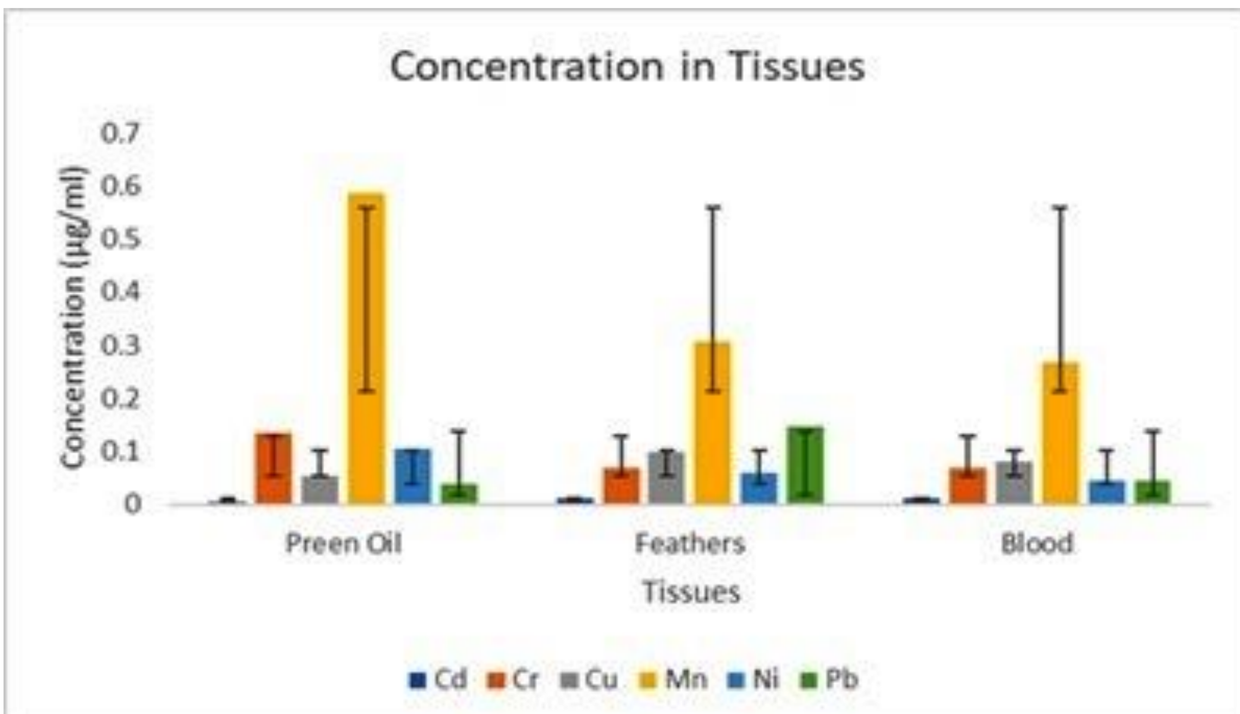

Figure 3. Mean Concentration of selected metals in the selected tissues of White Breasted Kingfisher

In general, Fe showed the highest concentration and Cd showed the lowest concentration. Fe concentration showed drastically high value as compared to other trace elements. May be this happened due to presence of large amount of steel industries in the vicinity of sampling site Manga Mandi, Lahore. A recent study conducted on distribution of toxic trace elements in black kite in different districts of Punjab, Pakistan also showed relatively high concentration of Fe (242.625mg/kg) in district Lahore among all the selected districts. Also, Fe was the most detected trace metal among all the metals in Lahore district depicting huge difference from other trace metals (Mahmood *et al*., 2022). Cd showed the least and negligible concentration in all the tissues, that can be considered a good sign as Cd is non-essential element and is considered as the most hazardous and toxic pollutant (Ali *et al*., 2019) as it can cause negative effect on bird's reproductive system by causing disruption in gametogenesis (Ali *et al*., 2019). Cd was also detected in very low concentration in different recent studies conducted in Lahore on House Crow (*Corvus splendens*) (Iqbal *et al*., 2021) Black Kite (Mahmood *et al*, 2022) and Cattle Egret (Zaman *et al*., 2022). In general, the concentration of toxic trace metals represented the trend as: Fe>Zn>Mn>Cr>Pb>Ni>Cu>Cd. Pb is non-essential trace metal and high concentration can cause physiological and neurological disorders in birds (Rodríguez-Álvarez *et al*., 2022). In our study, Pb demonstrated second lowest concentration after Cd in Preen Oil and Blood, but fourth highest concentration was observed in feathers. The reason for this can be high industrial and human activities in the area (Rutkowska *et al*., 2018) as feathers are exposed to Pb both endogenously and exogenously. Pb enters into the bloodstream but are gradually decreased and get deposited in the feathers and bones of birds (Rodríguez-Álvarez *et al*., 2022). A recent study conducted on feathers of birds from different districts of Azad Kashmir, Pakistan showed that high Pb accumulation was reported in areas with high traffic and industrial activities (Aziz *et al*., 2021). 34.75ppm is considered as the threshold level for Pb toxicity in avian feathers (Sidra *et al*., 2022) but fortunately concentration recorded in this study is much lower. Mn showed second highest concentration in Blood and Preen Oil, and third highest in Feathers. Mn is needed by birds for cell division, energy production and for different regulatory functions (Rodríguez-Álvarez *et al*., 2022) however high concentration of Mn in birds can have destructive effect on bird's immune system (Liu *et al*., 2013). A study conducted on house crow also showed high concentration of Mn (16.11mg/kg dw) in Lahore and northern Punjab region (Iqbal *et al*., 2021). Cr helps in blood sugar regulation (Rodríguez-Álvarez *et al*., 2022) but high concentration can cause issues in reproductive system in the avian species (Aziz *et al*., 2021). In our study, Cr showed third least concentration in feathers and blood but third highest concentration in preen oil although 100% DF for Cr was observed in feathers and blood samples, and 80% in preen oil samples. In general, it is the fourth most detected trace metals in tissues of white breasted kingfisher. High concentration of Cr in preen oil of White Breasted Kingfisher is strange as it's a general opinion that preen oil has its role in contaminating bird feathers during preening (Jaspers *et al*, 2019). A reason can be that preen oil is known to contain high number of lipophilic compounds and Cr is a fat-soluble element (Pacyna-Kuchta, 2022). A recent study on Cattle Egret in Manga Mandi, Lahore showed 0.09 µg/g Cr concentration in blood (Zaman *et al*., 2022) which is higher than recorded in this study in blood i.e., 0.07 µg/ml (1 µg/g = 1 ppm = 1 µg/ml). Zn is an essential metal for birds and is required for their proper metabolism and growth. In this study, feather showed the highest concentration of Zn and also showed significant difference from concentration in blood and preen oil. This was expected as Zn is required for the development and pigmentation of feathers and so tends to accumulate in feathers than other tissues (Rodríguez-Álvarez *et al*., 2022). Birds can tolerate high level of Zn as it is required for body's development. The threshold for Zn is 1200 µg/g (Solgi *et al*, 2020) which is much higher than the value recorded in this study. A recent study conducted on common mynas and bank mynas (n=30 and 20 respectively) in Lahore showed the Zn concentration in blood as 0.94 ppm and 0.97 ppm respectively which is higher than the value recorded in this study. In the same study, Zn recorded very high values in feathers i.e., 85.48 ppm and 92.26 ppm respectively (Sidra *et al*., 2022). Reason for this may be the high traffic and vehicular emission in the main city of Lahore. Ni is widely distributed trace metal and is helpful in hepatic functions (Rodríguez-Álvarez *et al*., 2022) but high concentration in birds can cause asthma and DNA disruption (Aziz *et al*., 2021). The normal concentration of Ni in birds is 500mg/kg (500ppm) (Iqbal *et al*., 2021) but the Ni recorded in this study is much lower than this value. Generally, Ni was the third least recorded metal and second least detected metal in blood and feathers. Preen oil showed comparatively high value but it was still very low to cause any lethal effect. Cu is used for the formation of many enzymes and helps in the proper functioning of the cell (Rodríguez-Álvarez *et al*., 2022). Our study showed the second lowest value for the Cu after Cd. In a recent study on Cattle Egret in Lahore Cu concentration was recorded 0.45 µg/g in blood (Zaman *et al*., 2022) which is higher than our recorded value.

One Way ANOVA Analysis run on SPSS showed significant concentration for all metals except Pb. Values higher than 0.05 are considered to have a significant threat over environment. Values recorded for metals Cd, Cr, Cu, Fe, Mn and Ni are 0.71, 0.187, 0.36, 0.72, 0.21 and 0.15 respectively. All of these values are higher than 0.05 which means that all of these trace metals are toxic for the health of birds and pose serious impacts over their tissues. While the value of Pb is 0.02 which means that it is less than 0.05 so its level of toxicity is much less so it won't pose effects on the health of kingfisher. Table 1 Illustrate the concentration of toxic trace elements in the targeted tissues of kingfisher.

Table 1: Illustration of targeted toxic trace elements concentration in different tissues (mean ± SD, min-max) i.e., preen oil, blood and feathers (µg/ml) of White Breasted Kingfisher (Halcyon smyrnensis) collected (n=5) from the premises of Lahore

Tissues	Cd Mean ± S.D Min-Max	Cr Mean ± S.D Min-Max	Cu Mean ± S.D Min-Max	Fe Mean ± S.D Min-Max	Mn Mean ± S.D Min-Max	Ni Mean ± S.D Min-Max	Pb Mean ± S.D Min-Max	Zn Mean ± S.D Min-Max
Preen Oil	0.0067 ± 0.005 0-0.01	0.134 ± 0.089 0-0.22	0.053 ± 0.005 0.05-0.06	3.058 ± 1.84 0.5-4.87	0.586 ± 0.398 0.04-0.97	0.104 ± 0.034 0.05-0.14	0.04 ± 0.02 0.04-0.04	0.092 ± 0.01 0.06-0.1
Feathers	0.012 ± 0.004 0.01-0.02	0.07 ± 0.035 0.04-0.13	0.1 ± 0.039 0.04-0.15	3.416 ± 2.28 1.66-6.35	0.306 ± 0.257 0.15-0.76	0.06 ± 0.025 0.03-0.1	0.148 ± 0.072 0.08-0.24	1.704 ± 0.56 1.3-2.59
Blood	0.01 ± 0 0.01-0.01	0.07 ± 0.008 0.06-0.08	0.08 ± 0.014 0.07-0.09	2.425 ± 0.96 1.58-3.65	0.268 ± 0.036 0.24-0.32	0.045 ± 0.013 0.03-0.06	0.045 ± 0.021 0.03-0.06	0.168 ± 0.041 0.13-0.22

General metal trend in Food Chain of Kingfisher: Anani and Olomukaro studied metals contamination in prawn and crab. Their study show that metal contamination is becoming the most worrisome problem around the world in freshwater ecosystems. This occurs due to persistence effect and accumulation of metals in aquatic system (Anani & Olomukaro, 2019). In our samples, the metals were detected in all the specimens from food chain except Cd. Cd didn't show any concentration in any of the specimen from food chain. Fe showed the highest concentration. Highest mean concentration of Fe was detected in Frog with a value of 3.606667 µg/ml, followed by rat (2.93µg/g), wasps (2.03 µg/g), fish (0.45µg/g) and lowest mean concentration was detected in prawns (0.31 µg/g). Cr wasn't detected in prawns and wasps, and very low concentration was detected in fish, rat and frog. Frog showed slightly high concentration of 0.025µg/g followed by same 0.02µg/g concentration in fish and rat. Cu wasn't detected in Fish and Rat, highest concentration was found in prawns (0.18 µg/g), followed by frog (0.053333 µg/ml) and lowest concentration in wasps (0.03µg/g). Highest concentration of Mn was detected in prawns with a value of 0.16 µg/ml, followed by concentration in frog 0.15 µg/ml and the lowest mean concentration was detected in rat (0.045µg/g). Ni showed highest mean concentration in frog (0.03µg/g) and lowest mean concentration in rat (0.015 µg/g). Pb was detected in all the specimens with frog showing highest mean concentration with a value of 0.16µg/g and the lowest mean concentration in fish having the value 0.033333 µg/ml. Zinc was detected in all specimens with highest concentration in frog (3.25µg/g), followed by mean concentration in rat (0.655µg/g) and the lowest mean concentration in wasps (0.3µg/g). General trend followed by the metals in food specimen of food chain in Kingfisher is: Fe>Zn>Mn>Cu>Pb>Ni>Cr

Table 2: Concentrations of toxic trace elements in different aquatic and terrestrial food/prey of Kingfisher (Halcyon smyrnensis) (µg/g wet weight) collected from the premises of Lahore.

Prey	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Fish (*Osteobrama cotio, Puntius ticto, Channa striata*)	ND	0.02	ND	0.45	0.115	0.02	0.033	0.585
Rat (Mus sp.)	ND	0.02	ND	2.93	0.045	0.015	0.085	0.655
Frog (*Bufo stomaticus*)	ND	0.025	0.053	3.61	0.15	0.03	0.16	3.25
Prawn (*Dendrobranchiata*)	ND	ND	0.18	0.31	0.16	0.02	0.05	0.48
Wasp (*Vespidae)*	ND	ND	0.03	2.03	0.09	0.02	0.06	0.3

Bio-accumulation, Bio-concentration, and Trophic Transfer factors: Bio-accumulation factor BAF, Bio-concentration factor BCF, and bio-magnification factor BMF also known as trophic transfer factor TTF, are used to explicate the accumulation of metals in birds and their prey and also in soil and water. Concentration of metals in birds to their environment is determined by BAF and BCF. In accordance with the study of Anani and Olomukaro, Heavy metal pollution in freshwater is increasing day by day specifically by food chain. BAF is calculated by the ratio of number of metals in tissues of birds to metal concentration in soil (Anani & Olomukaro, 2019). Calculating BAF help to know about the transfer capability of metals in the food chain while BMF calculation help to know about the relation between predator and prey and also to know about the trophic level transfer of metals throughout the food chain. Bio-accumulation in king fisher followed the trend as Zn>Cu>Cr>Cd>Ni>Mn>Fe. Pb was not detected in soil.

Formula:

***BAF** = Metals in tissues / metals in soil* (Aladesanmi *et al*., 2019)

The calculation results for BAF for selected metals has been shown in **Table 2**. These values are calculated in blood, feathers and preen oil. The accumulation of heavy metals is found to be highest in feathers followed by blood and then preen oil respectively. This is due to the fact that heavy metals get accumulated into the blood directly from food chain and then goes to feathers directly, thus this enhances the accumulation of heavy metals in the blood of kingfisher leading them to feathers. BAF of heavy metals is presented in **Table 2**. If the value of BAF appeared greater than 1 then it means that the accumulation of that selected metal is getting accumulated more inside the bird tissue rather than the environment from which it is taking in that specific metal. The BAF entries in the table show that no value for the Pb was detected in the samples. The BAF of Zn is found to be highest in 5.497µg/g while BAF of Fe is found to be lowest in all the tissues as 0.05µg/g, 0.052µg/g and 0.04µg/g in blood, feathers and preen oil respectively. Previous studies over the bio-accumulation factor in wildlife suggested that the BAF of Cu, Zn and Cd were found to be highest in feathers. The values of BAF ranges between 0.03 to 0.73µg/g for Cu, 0.22 to 0.57µg/g for Zn and 0 - 1.43µg/g in Cd. The BAF for Pb was also found negligible (Anbazhagan *et al*., 2021). Study done by Voigt on the body tissues of Neotropical fish *Geophagus brasiliensis* also showed that BAF for heavy metals including Cd (6.4µg/g), Cr (2.8µg/g), Fe (0.0µg/g), Mn (0.1µg/g), Ni (14.1µg/g), Cu (8.0µg/g), Pb (8.5µg/g) and Zn (5.9µg/g), were found to be highest in liver followed by gills and then in muscles. The trend was Liver> Gills>Muscles. Muscles having the lowest BAF is beneficial as it is considered as the most vital part of fish as it is consumed as a part of food by humans. If metal concentration increased in muscles of fish, then it may lead to cause serious health issues in humans as well (Voigt *et al*., 2014). A constant exposure to toxic metals at high concentration can diminish physiology of bird's body (Zaman *et al*., 2021, Lucia *et al*., 2014).

BCF is calculated by the ratio of concentration of metals in tissues to the concentration of metals in water. The trend followed by the metals for BCF is Zn>Fe>Ni>Mn. Cd, Cr, Cu and Pb were below detection level as they were not detected in samples.

Formula:

***BCF** = Metals in tissues / Metals in water* (Das *et al*, 2014)

Concentration of heavy metals in blood mainly represent exposure by diet while concentration in feather of such metal show exposure of metal in past to the body (Ulah *et al*., 2014). The BCF of selected metals are shown in **Table 2.** Concentrations of toxic trace elements are found highest in feathers followed by blood and then preen oil in the sample. This shows that accumulation of heavy metals was highest in feathers rather than blood and preen oil. The reason for this increased concentration in feather shows that high number of metals are getting deposited from the air as well as getting accumulation from the blood to the feathers by means of food chain. BCF of heavy metals in the tissues of kingfisher are presented in **Table 2.** In case of metals, the value of BCF should be less than 1. If the value increases greater than 1 then bio-accumulation of heavy metals will start taking place in the bird. The BDL entries show that no concentration of Cr, Cd, Cu and Pb were detected in the samples. While the concentration of Fe and Zn were found highest in Feathers as 17.369µg/g and 127.8µg/g, while Mn and Ni were found highest in preen oil as 1.627µg/g and 6.933µg/g respectively. The concentration values for Fe, Mn, Ni and Zn were higher than 1 so they are really unacceptable for the species as well as for the environment. Study of Falusi over bio-concentration factor in tropical crab in Nigeria represent concentration range of various metals in crab as Mn and Ni ranged between 0.09 - 0.83µg/g which is much lower than the concentration values obtained in our samples while values for Zn and Fe ranged from 4.89 - 5.5µg/g which are also much lower than our obtained concentration value (Falusi and Olanipekun, 2009). Another study done by Gyimah *et al*., for bioaccumulation factor and analysis of heavy metals in fish species showed that the concentration of Ni was found to be 24µg/g in the muscles of fish *Oreochromis niloticus*, which is much higher than the mean value of Ni found in our sample. For Cr and Cd, the values were found 7.0µg/g and 6.22µg/g respectively in fish muscle while BCF for Cr and Cd was not detected in our selected sample. Value for Ni in fish species was below the value provided by USFDA. For Cr and Cd, the detected values in fish muscles were found significant below 0.05µg/g (Gyimah *et al*., 2018). Biomagnification BMF is mainly referred by the term Trophic transfer factor TTF. Ali. H and Khan, E. refers that BMF is also called as biological magnification, an increase in the contaminant concentration in food chain along trophic levels. BMF of heavy metals are mainly used to demonstrate the connection of accumulation of trace metals in both the birds and their preys upon which they feed. This also helps to know the relation of prey with the predator and transfer of heavy metals in food chain at different trophic levels (Zaman, *et al*., 2022). This BMF was calculated by considering the ratio of prey and preen oil, blood and feathers of kingfisher.

Table 3: Bio-accumulation factor and bio-concentration factor calculated by the water and soil samples collected from the premises of Lahore.

Metals	Tissues	Bio-accumulation	Bio-concentration
Cd	Blood	0.933	ND
	Feathers	1.2	ND
	Preen oil	0.667	ND
Cr	Blood	1.02	ND
	Feathers	0.7	ND
	Preen oil	1.34	ND
Cu	Blood	1.352	ND
	Feathers	1.764	ND
	Preen oil	0.941	ND
Fe	Blood	0.050	16.459
	Feathers	0.052	17.369
	Preen oil	0.047	15.549
Mn	Blood	0.483	1.239
	Feathers	0.331	0.85
	Preen oil	0.634	1.628
Ni	Blood	0.647	5.467
	Feathers	0.473	4
	Preen oil	0.821	6.933
Pb	Blood	ND	ND
	Feathers	ND	ND
	Preen oil	ND	ND
Zn	Blood	2.898	67.35
	Feathers	5.497	127.8
	Preen oil	0.297	6.9

Bio-

Formula:

***TTF** = Metal in tissue / Metal in prey* (Zhang *et al*., 2021)

Concentration of toxic trace metals in all the prey species for king fisher is presented in **Table 3** as follows.

Contamination of fish and prawn with toxic metals shows the level of pollution of water with selected metals. Cd and Cu were not detected in fish while Cd and Cr were not found in prawn, this indicate that these metals were not present in water or their values were below detection level. Fe was detected in highest amount in frog 3.61µg/g, rat 2.93µg/g and wasp 2.03µg/g while Zn 3.25µg/g was found to be highest in frog.

Depending upon the values of TTF for predator-prey tissues, the results are shown in **Table 4**. Values of TTF which are greater than 1 show that the trophic transfer of heavy metal is likely while value less than 1 showed up to be unlikely for the heavy metal to be transferred to food chain at trophic levels (Tasneem *et al*., 2020). In general, all the metals detected have TTF>1 for all the tissues of king fisher except Cd as it was below detection level and also for Cd for the prey prawn and wasp. Mn was found to be in maximum range for TTF between 6 and 13 for the prey rat Mus sp. while minimum range TTF range between 0.02 and 0.5 for Zn was found in prey **B. stomaticus.** Generally, Mn showed greatest tendency of transfer at trophic level followed by Fe>Cr>Ni>Zn>Pb>Cu. Our study is in check with the results of potential for trophic transfer of toxic elements recorded previously with Zaman *et al*., 2020. In general, for all the metals, TTF was highest in preen oil then in blood followed by feathers for all metals. Mechanism for excretion and detoxification of metals mainly dominate the potential for trophic transfer (Zaman *et al*., 2020). For species, priority and propensity for diet may intercede in governing the trophic transfer potential of toxic metals (Soto-Jimenez *et al*., 2009). Kingfishers are mainly apex consumers so they are mainly placed at the top of a food chain as it feed over a wide variety of prey. It mainly consumes fish and other species like frogs, crustaceans, insects, reptiles and even small mammals. So, it depicts diverse potential of trophic transfer for toxic metals due to varying diet of it. Our study recommends a detail scrutiny with various preys throughout the year with different species of seasons mainly from terrestrial and aquatic food chains for better understanding of trophic transfer of toxic trace metals throughout the food chain.

Table 4: Trophic Transfer Factor (TTF) for all the blood, feather and preen oil for each of the prey species of Kingfisher (Halcyon smyrnensis):

Prey	Tissues	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Fish	Blood	BDL	5.1	BDL	7.193	3.878261	4.1	2.82	1.535
	Feathers	BDL	3.5	BDL	7.591	2.661	3	4.44	2.913
	Preen oil	BDL	6.7	BDL	6.796	5.096	5.2	1.2	0.157
Rat	Blood	BDL	5.1	BDL	1.105	9.911	5.467	1.106	1.371
	Feathers	BDL	3.5	BDL	1.166	6.8	4	1.741	2.602
	Preen oil	BDL	6.7	BDL	1.044	13.022	6.933	0.4706	0.140
Frog	Blood	BDL	4.08	1.438	0.898	2.973	2.733	0.588	0.276
	Feathers	BDL	2.8	1.875	0.947	2.04	2	0.925	0.524
	Preen oil	BDL	5.36	1	0.848	3.907	3.467	0.25	0.028
Prawns	Blood	BDL	BDL	0.426	10.442	2.788	4.1	1.88	1.871
	Feathers	BDL	BDL	0.556	11.019	1.913	3	2.96	3.55
	Preen oil	BDL	BDL	0.296	9.863	3.663	5.2	0.8	0.192
Wasps	Blood	BDL	BDL	2.556	1.595	4.956	4.1	1.567	2.993
	Feathers	BDL	BDL	3.333	1.683	3.4	3	2.467	5.68
	Preen oil	BDL	BDL	1.778	1.506	6.511	5.2	0.667	0.307

Limitations of the Study: The study has provided contrasting values for the bioaccumulation and trophic transfer level of selected toxic trace elements in the selected tissues of white breasted kingfisher. The study also gave a clue of potential of toxic trace elements to move from abiotic to biotic environment. However, the study has few limitations which can be considered for the purposeful explanation of the results and can be taken into consideration while going on for further studies. First of all, due to some legal and ethical considerations the sample size taken was very specified and small. Taking large number of samples can reduce any chance of statistical error. Similarly, some legal regulations limited our study to just three non-invasive or semi-invasive tissues. Using different internal organs of birds can help to get better and clear understanding of trend of bioaccumulation and trophic transfer of toxic elements in different body parts of the bird. Moreover, our study was limited to a specific area in a specific season. Expanding the scope of study to different time and space can help in better interpretation of the results. Using different species of birds can help to determine an accurate trend of bioaccumulation and biomagnification in the birds of that area. Finally, different ecological and biological factors like age, gender, reproductive and migratory status, habitat, distance from the source of pollution can also be considered for more meaningful results.

Conclusion: Our research showed a significant concentration of toxic trace metals in tissues of white breasted and through BAF, BCF and TTF it is evident that environment, habitat and food has significant role in heavy metals contamination of White Breasted Kingfisher. However, the potential to accumulate toxic trace metals is different for different tissues and for different metals. Feathers found to be most contaminated and preen oil to be least contaminated but Trophic transfer potential (TTF) was found to be highest for preen oil and lowest for feathers. High concentration in feathers can be an evident of external and internal deposition as feathers showed highest values for BAF and BCF. Fe was found significantly in high concentration in all tissues but it showed lowest value for BAF, highest for BCF and second highest for TTF signifying that it found its way through ingestion in white breasted kingfisher. Cd didn't show any value for TTF and BCF, but showed some minimum value for BAF signifying that it was accumulated through dermal contact. However, the concentration recorded in our study were more or less same as other studies conducted in the region. More studies should be done to understand the pathway and potential of accumulation of different trace metals in different tissues of white breasted kingfisher so significant measures can be taken to save its already declining population. This can be done by following the national and international standards, by making strict laws and imposing severe penalties in violation of these laws.

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