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Comparative assessment of invasive and non-invasive approaches for estimating metallic pollutants in the environment

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ABSTRACT

Metallic pollutants make an important category of environmental contaminants owing to their capability for bioaccumulation and biomagnification in the living systems. The methods employed for determining their concentrations in the living bodies may be invasive or non-invasive and their outcomes have often been questioned regarding their representativeness of the true exposure to the contaminants. The current study was hence designed to compare the outcomes both invasive and non-invasive methods used for exposure assessment of metallic pollutants in living beings. For this purpose, two common avian species bank myna (*Acridotheres ginginianus*), and common myna (*Acridotheres tristis*) were selected due to their high abundance in Lahore, Punjab, Pakistan. Trace metals' concentrations were detected in their liver, blood and feathers; former two being invasive methods while the latter approach to be non-invasive one. However a poor correlation was found among metallic pollutants in all three organs with lowest concentrations reported from the blood samples. The association between the trace metal concentrations in all organs was also non-significant. It can hence be concluded that no single organ can be a true representative of environmental exposure to metallic pollutants and combined approaches need to be investigated for determining extent of contamination in living bodies.

1. Introduction

Environmental pollution is increasing day by day in developing countries as a result of exponential increase of human population and technology developments (Xu et al., 2013). Environmental pollution, by heavy metals cause serious health impacts both for humans and wildlife (Klaassen, 2013; Ullah et al., 2014). Heavy metals are the environmental pollutants that are non-biodegradable and may accumulate in the higher levels of food chains, with respect to diet and habitat preferences of every single species (Boncompagni et al., 2003). Trace elements are the elements required in low concentration by the living beings to continue life but are essential (e.g. minerals and vitamins). Trace metals such as arsenic (As), copper (Cu), zinc (Zn), selenium (Se), chromium (Cr), manganese (Mn) and many more can enter the food chain, and have the aptitude to biomagnify (Bostan et al., 2007). Heavy metal exposure has been documented to threaten avian reproductive success (Anbazhagan et al., 2021).

To gauge the effect heavy metal contamination on environment, various bio-monitoring approaches have been used including the use of particular organisms that may reflect environmental contamination (Burger et al., 2008). Birds have been considered as potentially strong indicators of environmental pollution indicators since 1960s (Erwin and Custer, 2000), as some species being at the top of food chain, signaled any changes in lower trophic levels by showing responses (Boncompagni et al., 2003). Most bird species living in close proximity of human beings are more prone to envi-

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ronmental pollution and subsequent toxic effects (Malik and Zeb, 2009). Metal contamination in the eggs, dropping and feathers of birds have been world widely reported over the years and was documented to affect bird abundance (Muralidharan et al., 2004; Hashmi et al., 2013). Feathers mount up trace metals throughout their growth phase and harbor various metal absorption levels in different locations of the body (Bortolotti and Barlow, 1985; Dauwe et al., 2003; Varagiya et al., 2022). Moreover, feathers are considered to be a better indicator of environmental pollution due to higher accumulation of heavy metal as compared to other tissues (Zamani-Ahmadmahmoodi et al., 2010). While at present use of feathers is still considered an easy and effective means of documenting environmental pollution levels, it is not considered a true representative of elemental exposure. To control metal pollution, it is critical to select bird species that are sensitive to environmental changes and have predictable response. Common myna (*Acridotheres tristis*), House crow (*Corvus splendens*) and House Sparrow (*Passer domesticus*) are the birds that usually live in close proximity of human settlements and are known to accumulate varying levels of trace metals in their organs including feathers (Aziz et al., 2021). However it is yet to be established if the metal uptake by feathers can provide with a significant insight into metal accumulation within the body. The current study was hence deigned to investigate three of the most commonly used organs in toxicology assessments for heavy metal pollution i.e. feathers, blood and liver. Two abundant, least concern avian species associated with urban habitats i.e. bank myna (*Acridotheres ginginianus*), and common myna (*Acridotheres tristis*) were used in this study to biomonitor metal contamination in different organs and to assess if using the non-invasive approach (feathers) could be a more feasible option to detect heavy metal contamination in living beings.

Pakistan is one of developing countries facing serious environmental pollution in various regions, where urban, industrial and agricultural activities majorly contributes to environmental pollution (Malik and Zeb, 2009). Moderate heavy metal concentrations in the feathers of cattle egret have been documented in agricultural areas (Ullah et al., 2014), information from industrial and urbanized areas is scarce. Yet the industrial areas are more exposed to heavy metal pollution due to various urban and industrial effluents from tanneries steel manufacturing factories, and automobiles. Therefore, this study aimed to quantify metal concentration (zinc, lead and nickel) in liver, blood, and feathers of bank myna and common myna from district Lahore, to assess feasibility of invasive and non-invasive methods to monitor heavy metal pollution in urban areas. Zinc, lead and nickel were selected due to close association with industrial and vehicular emissions.

2. Materials and methods

2.1. Study area

Lahore is the 2nd biggest city of Pakistan which is provincial capital of Punjab. It lies between 31°15′ and 31°45′ north and 74°01′ and 74°39′ east with a total area of 1772 km (Demographia, 2017). It is also one of the most polluted urban areas of Pakistan because it has wide range of industries and a large motor pool which results in harmful environment for living beings (Ali et al., 2015). The environment of the city is hence laden with multiple pollutants including particulate matter, gaseous pollutants and trace metals.

2.2. Sample collection

For this study, two common avian species bank myna *Acridotheres ginginianus* (n = 20), and common myna *Acridotheres tristis* (n = 30) were captured from different areas of the Lahore city using mist nets (Gosler, 2004) and were transferred into cages and transported to the Department of Wildlife and Ecology, Ravi Campus University of Veterinary and Animal Sciences, Pattoki for further analysis. Liver, blood and feathers were taken from each bird for further processing. Approval from Ethical committee of the University was obtained prior to sampling.

Feathers (5–6 each) obtained from samples were first wiped with fresh water, washed with distilled water and finally cleaned with acetone three times to confirm removal of all external pollutants (Saeki et al., 2000). Samples were then oven-dried at 80 °C for 12 h, cut into smaller pieces and digested in Nitric acid (HNO_3) and Perchloric acid ($HClO_4$) in 3:1 ratio following Saeki et al. (2000) and Nighat et al. (2013). The digested samples were diluted with 15 ml ddH_2O and trace metal concentrations (lead, nickel and zinc) measured using Atomic Absorption Spectrophotometer (Janssens et al. 2001). Whole blood samples (2 ml each) were obtained through cardiac puncture and transferred into serum separating vaccutainers, thereby yielding approximately 1 ml of serum. The birds were euthanized and organs were excised out via dissection. Liver was selected for this study since it metabolizes trace metals essential for the body. The same protocol was employed for digestion of both samples as given above (Saeki et al., 2000).

The obtained results were then compared through one-way ANOVA for finding any significant association in metal concentrations in the selected organs and also through Pearson's correlation using SPSS ver 20.0 (IBM Inc.).

3. Results

A total of 150 samples including liver, blood and feathers were collected from two avian species including *Acridotheres tristis and Acridotheres ginginianus* from Lahore and analyzed for metal concentrations. Concentrations of trace metals were found to vary from organ to organ in both species (Tables 1a and 1b). Highest levels of Zn 85.48 ± 18.48 ppm and 92.26 ± 5.68 ppm were found in feathers of *Acridotheres tristis and Acridotheres ginginianus* respectively as compared to other organs as obvious in the Figs. 1 and 2. However, lowest levels of Zn, Pb and Ni found in blood of both species.

One-way ANOVA was conducted to find any significant difference in trace metal level in all three organs of the captured species. A statistically significant difference was found in trace metal concentrations in all three organs under investigation with p = 0.00 for all groups. To find out the exact difference among groups, Post Hoc multiple comparisons using the Tukey HSD test were run and it was observed that concentration of zinc differed significantly in all three organs in both species. On the other hand, the mean levels of lead in liver and feathers of both species were non-significantly different. In case of nickel, a non-significant difference was

Table 1a
Summary of trace metal levels observed in *Acridotheres tristis.*

Avian species *Acridotheres tristis* (n = 30)	Trace metals (ppm)	Organs	Mean + standard deviation
	Zn	Liver	25.70 + 17.30
		Blood	0.94 + 0.62
		Feathers	85.48 + 18.48
	Pb	Liver	2.82 + 3.09
		Blood	0.61 + 0.97
		Feathers	3.29 + 2.48
	Ni	Liver	3.13 + 5.17
		Blood	0.05 + 0.05
		Feathers	2.73 + 0.69

Table 1b
Summary of trace metal levels observed in *Acridotheres ginginianus*.

Avian species *Acridotheres ginginianus* (n = 20)	Trace metals (ppm)	Organs	Mean ± standard deviation
	Zn	Liver	7.10 ± 7.07
		Blood	0.97 ± 0.91
		Feathers	92.26 ± 5.68
	Pb	Liver	5.99 ± 8.63
		Blood	0.57 ± 0.65
		Feathers	2.54 ± 2.38
	Ni	Liver	2.10 ± 0.96
		Blood	0.05 ± 0.03
		Feathers	3.20 ± 0.61

observed for common myna, while the bank myna showed significant differences in all three organs.

Pearson's correlation showed a negative, weak correlation was observed for almost each variable (Table 2). Overall it can be concluded that the levels of selected trace metals in all three organs under investigation (Liver, Blood, and Feathers) did not show any significant association with each other.

4. Discussion

Trace metal contamination and their potential to bioaccumulate in living tissues of animals is a severe health crisis around the world due to the toxicity, abundant resources, non-biodegradable properties, and accumulative behavior of these metallic contaminants. While their concentrations may not be very high, they do become pronounced in higher trophic levels due to biomagnification and hence disrupting the ecological balance. It is therefore necessary to regularly monitor the levels of trace metals in the environment to sustain integrity of the ecosystem. Since avian species occupy large ecological niches, are at a higher trophic level and feed on variety of diets, their use as bioindicator species is more widespread.

It has been noted that levels of trace metals not only vary from organ to organ but also differ with age (Barbieri et al., 2010). Moreover, while avian species may bioaccumulate pollutants in their feathers during growth phase, the role of exogenous sources after gaining maturity cannot be neglected (Veerle et al., 2004). In the current study the trace metals selected for monitoring included lead, zinc and nickel which are associated with industrial and

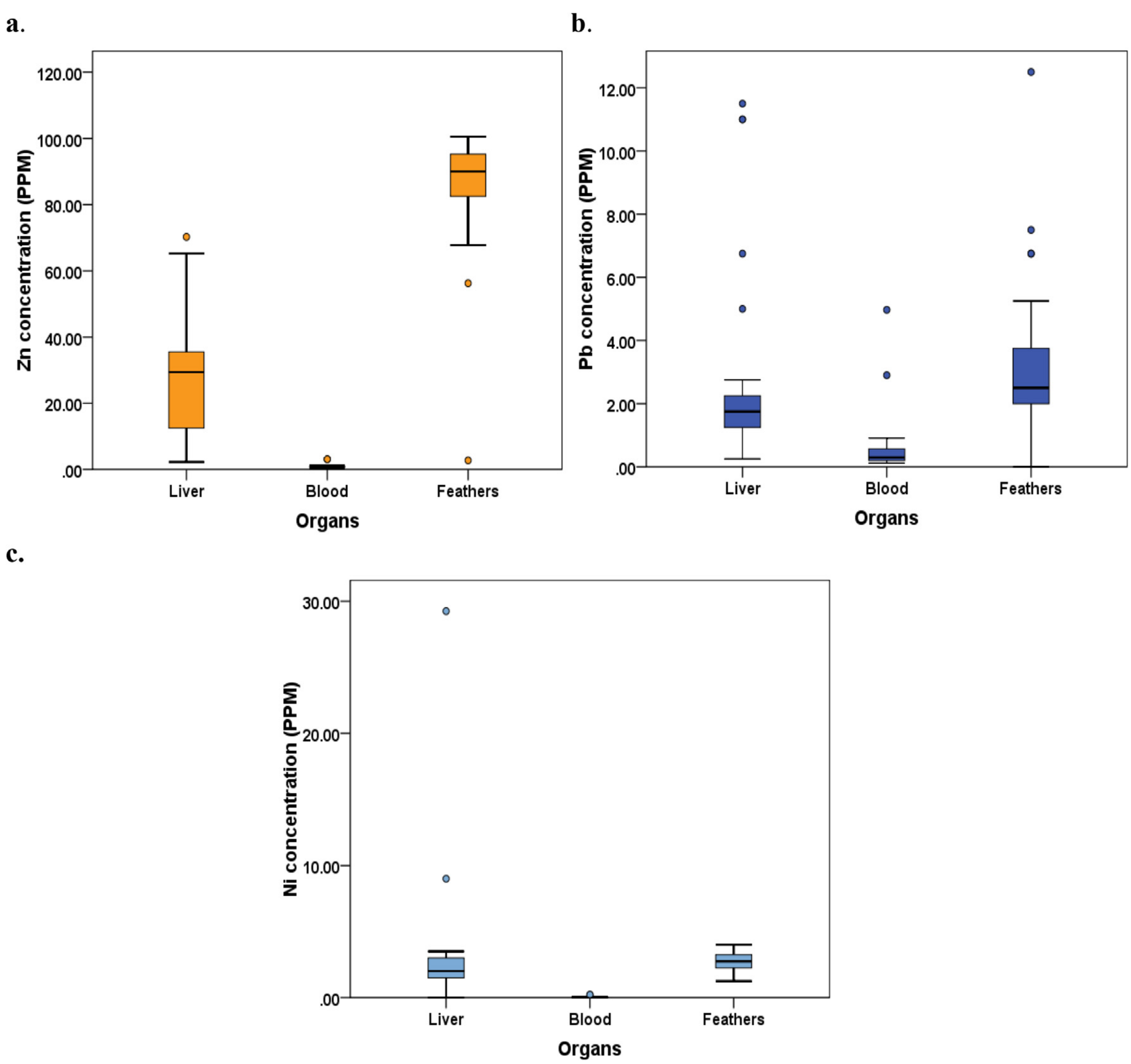

Fig. 1. Levels of trace metals observed in different organs of common myna *Acridotheres tristis* (a) Zn, (b) Pb, (c) Ni.

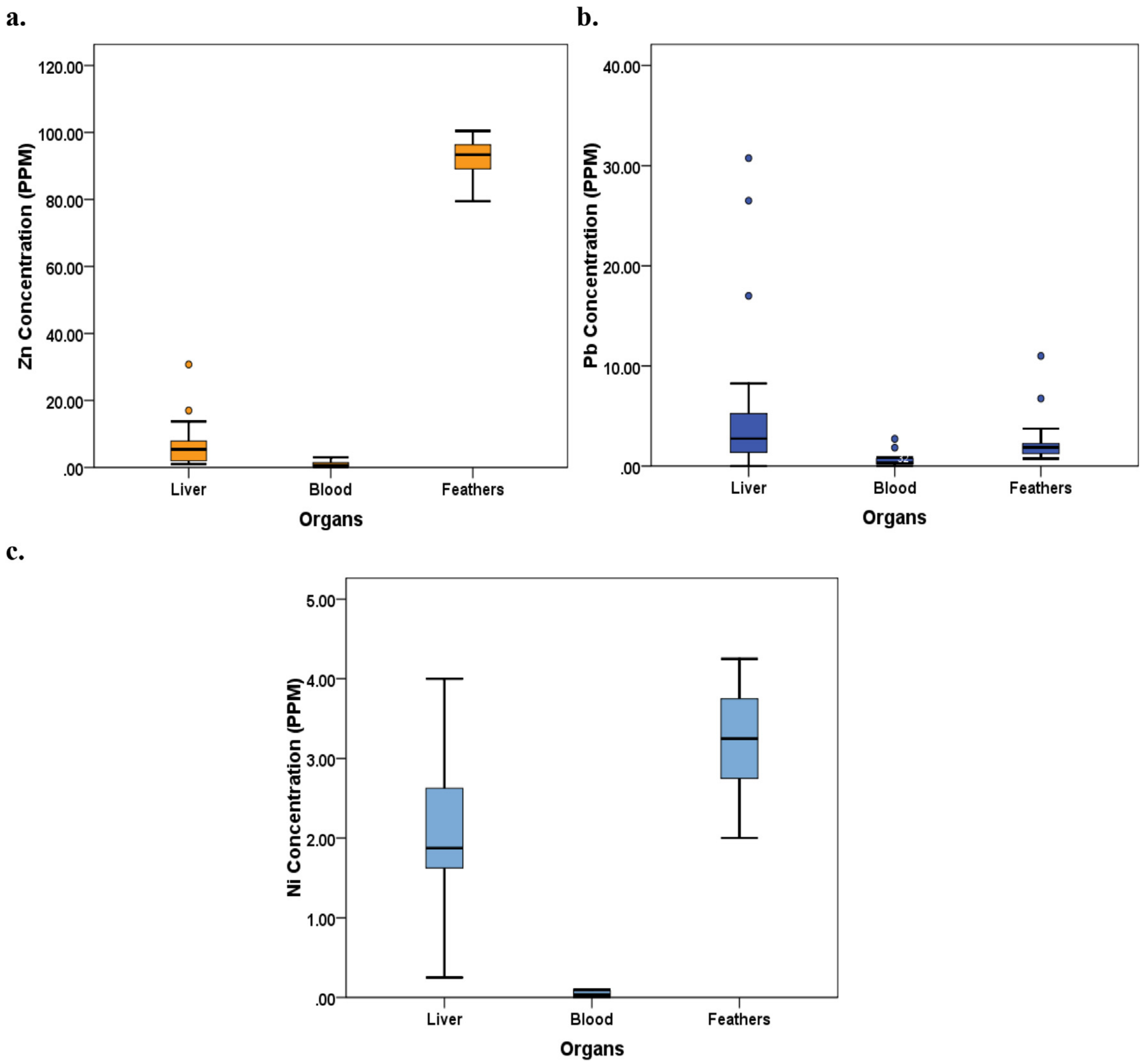

Fig. 2. Levels of trace metals observed in different organs of bank myna *Acridotheres ginginianus* (a) Zn, (b) Pb, (c) Ni.

Table 2
Pearson's correlation for the selected trace metals in all three organs

	Pearson's correlation R^2	
	Common myna	Bank myna
Zinc		
Liver vs Blood	−0.185	−0.048
Blood vs Feathers	−0.53	0.571
Feathers vs Liver	0.123	0.276
Lead		
Liver vs Blood	−0.132	−0.088
Blood vs Feathers	−0.289	−0.236
Feathers vs Liver	−0.035	−0.206
Nickel		
Liver vs Blood	−0.043	−0.041
Blood vs Feathers	−0.323	0.049
Feathers vs Liver	−0.244	−0.113

vehicular emissions. The level of zinc in the feathers of all species was observed to be elevated as compared to other metals ranging between 2.75 and 100.5 ppm in common mynas and 79.5–100.5 ppm in bank mynas. While Zn is an essential element for the body and a component of many enzymes, it enters the body from external means as well (Malik et al., 2010; Abdullah et al., 2015). Similar levels of zinc have been reported in different avian species from Pakistan (Movalli, 2000; Malik and Zeb, 2009; Nighat et al., 2013), Belgium (Dauwe et al., 2000) and China (Zhang et al., 2006; Deng et al., 2007) as well. These levels are however reported to be more endogenous in origin rather than coming from exogenous sources as in the case of lead and nickel (Veerle et al., 2004).

The levels of lead were observed to 0–12.5 ppm in common mynas and 0.75–11.0 ppm in bank mynas which are similar to those reported by Movalli (2000), Malik and Zeb (2009), and Nighat et al. (2013). Lead is a highly toxic trace metal and Pb concentrations of 34.75 ppm in avian feathers has been reported to affect the reproductive potential of avian species and cause stunted development (Burger and Gochfeld, 2000). Since its sources are primarily anthropogenic, lead is extensively stated as symbol of metal contamination by Metcheva et al. (2006), resulting from combustion processes, petroleum incineration, automobiles, leather manufacturing and oil leakage (Markowski et al., 2013). Lahore is a highly populous urban center with a large motor pool as discussed earlier. A study reported harmful levels of lead coming from pharmaceutical manufacturing units, urban manure, as well as waste material from tanneries (Qadir and Malik, 2011). The levels of nickel accumulating in feathers were found to be 1.25–4 ppm in common mynas and 2.00–4.25 ppm in bank mynas. These accumulated values of Ni were similar to those reported by (Malik and Zeb, 2009; Ullah et al., 2014) from Pakistan. Sources of nickel are also primarily exogenous (Janssens et al., 2004) coming from industrial actions, Nickel-Chromium platting, electrical appliances developing, battery and tanning manufacturing. A recent study by Aziz et al. (2021) has reported the levels of Cr, Pb and Ni in avian feathers from North-Eastern parts of Pakistan. However the levels of Pb and Ni reported by them in feathers of common myna (0.951 + 0.67 ppm and 0.543 + 0.39 ppm respectively) are lower than those reported in our study hereby highlighting the impact of highly urbanized environment upon the animals.

While the result obtained in this study are comparable to those reported by many others as discussed above, it is important to regularly monitor trace metal and other environmental pollutants on a regular basis. Avian feathers in this regard prove to be an easily accessible, non-destructive approach but as the results conclude, the levels of trace metals within each of three types of samples differ significantly. In fact a single organ cannot be a good representative of trace metal levels and as observed by Mukhtar et al. (2020) as well multiple organs need to be assessed for obtaining a complete picture of the exposure to environmental pollutants. As reviewed by Varagiya et al. (2022), feathers are considered a more feasible approach for heavy metal detection owing to multiple reasons including their being a non-destructive approach and easy to collect. However it has been noted that they may not be a suitable indicator for contamination of internal organs and can be instead utilized as early predictors for heavy metal contamination in the environment (Varagiya et al., 2022). It is also essential to devise mitigation measures for removal of such contaminants from the environment so as to sustain its integrity.

5. Conclusion

The current study offered the bio-monitoring of trace metals in three different organs of mynas in Lahore, Pakistan. The outcome of lead, nickel and zinc in excess amount is a sign of accumulation of metals in bird's feathers. However, the overall contamination levels were quite less in blood while liver also showed accumulation of trace metals, particularly lead in excess. However, the outcomes conclude that no single organ can be a true representative of environmental exposure to metallic pollutants and combined approaches need to be investigated for determining extent of contamination in living bodies.

Declaration of Competing Interest

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

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