Journal of Wildlife and Ecology. (2019). 2(2):1-7

Research Paper **Open Access**

Fecal matter as a bio-indicator tool for heavy metal pollution

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Peer Reviewed

Citation: Sidra, S., R. Saleem, S. Ghafoor, H. Azeem, I. Imtiaz, Z. Parveen. 2019. Fecal matter as a bio-indicator tool for heavy metal pollution. Journal of Wildlife and Ecology. 3(2): 1-7.

Received: 21, 03, 2019
Accepted: 12, 05, 2019
Published: 01, 06, 2019

Competing interests: The authors have declared that no competing interests exist.

Funding: Authors have no source of funding for this work.

Availability of data: Authors have included all data in the manuscript that were collected during the field survey.

ABSTRACT

Environmental pollution can be determined by the help of bio-indicator species and various biological approaches. Heavy metals are one of the most persistent groups of toxins that threat the well being of the living systems. While the most common methods applied for determining the accumulation of heavy metals in living organisms involves killing the animal for tissue analysis, the current study concentrated on alternative methods for heavy metal analysis. Fecal matter was employed as a tool for detecting day to day exposure to air borne heavy metals in captive animals. Three species i.e. chinkara (*Gazelle bennetti*), Indian long eared hedgehog (*Hemiechinus collaris*), and grey partridge (*Perdix perdix*) were selected for this purpose. Fecal samples were collected from each animal kept in captivity at Captive Breeding and Research Centre, University of veterinary and animal Sciences. Water and soil samples were also collected from each enclosure. The samples were digested in acid and levels of Lead (Pb), Nickel (Ni) and Chromium (Cr) were detected under AAS. While the levels of heavy metals were observed in varying ranges, it was concluded that fecal matter can be used as reliable tool for detecting short term exposure to heavy metals in animals.

Key words: Atomic absorption Spectroscopy, Lead, Nickel, Chromium

INTRODUCTION

Environmental pollution can be determined by sources of biological approaches, with the support of bio-indicator animals that give knowledge on their environmental quality. Among the numerous pollutants present in the environments, heavy metals are known for their ability to bio accumulates within living tissues and bio magnify along a food chain. Also they possess toxic properties when present in large amounts (Govind and Madhuri, 2014).

Emission of these metals in the environment appear through a vast range of passageways and processes, in addition to air (e.g. during ignition, eradication and processing), to the soil and to the surface waters (drainage and discharge from shipping and storage). Atmospheric emissions have great burden to the human health, because of the epidemic potential and dispersion for exposure which usually occur. Lead emissions generally are related to transport and road and hence dispersed over space. Cadmium emissions are commonly related with non-ferrous metallurgy and fuel ignition. Humans are forcefully exposed to harmful physical, biological and chemical constituents of food, air, soil or water. The exposure does not result only from the existence of a toxic constituent in the environment but from contact as well (Bergulend *et al.,* 2001).

Rapid urbanization has led to a rise in dumping of pollutants, radio nuclides, heavy metals and different kinds of inorganic and organic components into the environment. The main source of metals pollution is industrial wastes. The metal poison is mixed in aquatic system via sewage, smelting process, drainage and leaching of wastes (Praveena *et al.,* 2013). The emission of these elements to environment is a severe problem. Particularly in urbanized and industrialized regions metals polluted sediments and soils are spread over a wide range (Han *et al.,* 2002; Wei and Yang, 2010). That is why levels are high nearest to the emission sources due to atmospheric deposition. These emitted metals are also subject to wide range atmospheric transport, ultimately reaching to remote regions.

Global pollution by lead from human measures can be dated back to Roman and Greek times (Hong *et al.,* 1994) because these elements do not decompose with time and worldwide their levels are increasing due to increasing combustion, fossil fuels, rapid population growth and rapid increase in industrial movements (Smodis and Bleise, 2000). Sources of mobile particulate matter in urban surrounding are motor vehicles and industries. Street dust is an efficient tool for estimating the degree of contamination and source of these metals (Kowalczyk *et al.,* 1982). Various analyses have described concentrations of metals in wild animals in extremely polluted areas near liquefier (Beyer *et al.,* 1985), other sources such as Chlor like alkali plant (Dustman *et al.,* 1972; Wren, 1985), profoundly used highway sides (Clark, 1979), quarries or quarry garbage sites (Roberts *et al.,* 1978; Andrew *et al.,* 1984). When these metals are consumed above an approved limit, they cause adverse effects to body. While these metals show particular symptoms of their toxicity, following common symptoms have been described linked with zinc, copper, lead, arsenic, aluminum and cadmium: diarrhoea, stomatitis, ataxia, paralysis, gastrointestinal disorders, pneumonia,

convulsion and hemoglobinuria to stool causing rust-red colour when fumes and elusive vapours are smelled (McCluggage, 1991). The exposure to metals can be mutagenic, neurotoxic, carcinogenic and toxic.

In urban environment the caged wild animals are exposed to air pollution. While human beings are exposed to a variety of pollutants, animals also have no escape. Particularly in the captive animals expose to heavy metals has been detected in many studies in those of Gupta and Bakre (2013). In order to assess the level of contamination in captive animals, a relatively new approach is useful i.e. sampling of fecal matter. Fecal matter has great advantage because it does not involving killing or disturbing the wild captive animals. However how much of the actual exposure is represented in the fecal matter of the animals needs to be investigated hence this study.

MATERIALS AND METHODS

Study site: University of Veterinary and Animal Sciences, Ravi campus covers an area of 4.4 km^2. The Ravi campus is divided into four blocks namely A, B, C, and D.A C and D block are academic departments while B block contain residential colony and livestock research center. The department of Wildlife and Ecology is located in C block. The department has kept a number of animals under captivity for research purpose. The enclosures in A-block are located near the highway (Multan Road, N-26). Moreover, there are some industries near the campus area. Consequently, vehicular exhaust and dust from the road as well as industrial emissions can accumulate on the surface of enclosures and increase the pollution levels.

Selection of animals: There are a total of 18 species housed in Ravi Campus. Among them, 3 are mammals while the rest are birds with one reptilian species as well. Among these, the following three species were selected on the basis of their distance from the road:

***Chinkara* (*Gazella bennetti*):** The enclosure of Chinkara is located adjacent to the national highway. The enclosure is large and spacious (100 x 200m) with grasses growing all over the surface. A total of seven Chinkara (*Gazella bennetti*) were housed in the selected enclosure. Among them, two were males; and two were females while three fawns were also present.

Indian long eared hedgehog (*Hemiechinus collaris*): Seven hedgehogs were captured from Kalabagh in 2016 and kept in an enclosure located in A Block at a distance of 0.5km near the national highway. The enclosure was 6 x 16.4 m in size containing four adults and three young hedgehogs.

***Gray Partridge* (*Perdix perdix*):** Gray partridges (*Perdix perdix*) were kept in a small caged enclosure located at a distance of 1km from the main road. There were three adult male and three female partridges in the selected enclosure.

Sampling of fecal matter: The animals were marked using food grade dyes for easy identification and to avoid confusion. The marked animals were kept under observation so that fecal matter could be collected from the specified animal. Hedgehogs are nocturnal animals and spend most of the day in burrows. Therefore for collection of fecal sample, the hedgehogs were temporarily shifted for 2 hours into separate card board boxes. As the marked animal passed out fecal matter, it was collected in polythene bags and labeled accordingly. Similarly fecal

matter from all the marked partridges was collected. The samples were air dried before preparing them for heavy metal sampling.

Sampling of soil: Soil samples were collected in order to detect the level of heavy metals in soil of Chinkara enclosure. Three samples from varying depths were collected so as to check the penetration of metals into soil from surface. Sample 1 (SS1) was collected from the top soil of the enclosure. Sample 2 (SS2) was collected from a depth of 30 cm (1 foot) and sample 3 (SS3) was collected from a depth of 60 cm (2 feet). Since the enclosures of gray partridge and Hedgehog were cemented, the dust depositing on the surface of the enclosures was brushed off and collected in a zipper bag to assess the concentration of heavy metals in settling dust. The samples were contained in separate polythene bags and labeled accordingly. The samples were air dried ground and sieved to remove any pebbles or grit before sending to the laboratory for analysis.

Collection of water sample: Groundwater was the source of drinking water in all enclosures. The sampling bottle was rinsed thoroughly with water provided to the animals. The sample was then collected in the bottle and tightly closed for further analysis.

Digestion of samples: The fecal samples were digested before heavy metal analysis. A stock solution (Nitric acid and Perchloric acid) was prepared in the concentration of 3:1 in which the samples were to be digested. Fecal matter was air dried before proceeding with digestion. Dry fecal matter was weighed and 1 gm. fecal matter was added to 10 ml of stock solution. The samples were then heated at a temperature of 150^{o}C for thirty minutes. The temperature was then increased up to 250^{o}C till end point was achieved in the form of clear solution or vine green colour. The digested sample was then diluted with distilled water up to 25 ml and filtered to remove any residues. The samples were then transferred to sterilized Borrosil glass vial and stored at room temperature prior to analysis. The soil samples were air dried and then sieved to remove any large particles, grit and stones etc. from the sample. The samples were processed by Soultanpour method for analysis. The fecal samples along with water and soil samples were sent to the Metals and Minerals detection lab (M2 lab), Department of Environmental sciences, UVAS for heavy metals detection via atomic absorption (Z-8230 Polarized Zeeman atomic Absorption Spectrophotometry).The metals selected for sampling were Lead, Chromium, and Nickel.

RESULTS AND DISCUSSION

The levels of heavy metals were found in varying levels. Lead was found to be absent in the water samples collected from all three sites while Ni concentration was 0.9 ppm and Cr level was 1.05 ppm. Likewise lead was present only in the surface soil sample but absent in samples collected from a depth of 30cm and 60cm (Table 1).

It is evident from the results thatlead is present only on the surface soil layer hence not naturally existing in the soil. The absence of lead in water samples also reflects this finding since the source of drinking water in Pattoki is ground water. It can therefore be speculated that vehicular emissions is the prime source of Lead in the area. On the other hand Nickel and Chromium are naturally found

in the earth crust and hence in the soil and water samples. However their levels were not too high to cause any acute affects on the animal health.

Table 1: Levels of selected heavy metals from soil samples collected from different enclosures (SS = soil sample).

Heavy metals	Chinkara			Partridges	Hedgehogs	Average ± St Dev
	SS1	SS2	SS3	SS4	SS5	
Pb (ppm)	0.15	0.00	0.00	0.45	1.65	0.45± 0.69
Cr (ppm)	1.8	1.65	1.8	1.65	0.00	1.38±0.78
Ni (ppm)	0.00	6.15	0.9	2.85	2.55	3.11± 2.20

The levels of heavy metals in fecal samples were found to be much higher than the levels in soil or water. Since even trace amounts of Pb, Cr and Ni can result in genotoxicity (Restrepo *et al.,* 2000; Xu *et al.,* 2008; Boyle and Duarte, 2008), the observed concentrations of these trace elements in fecal matter of selected animals is an indication of high exposure (Table 2). The WHO recommended limits of Pb, Cr and Ni in the human body are 0.1ppm, 0.05 ppm and 0.2 ppm respectively while their permissible levels in animal bodies are unknown. However one important thing to consider is the body size. While human beings are large sized individuals as compared to the animals in our study, it can be speculated that the reported levels in our study can prove to be more toxic owing to the smaller body size of the study animals.

Table 2: Mean concentrations of selected trace metals in fecal matter of animals.

Sr.	Value	Partridge	Hedgehogs	Chinkara
Pb (ppm)	*Average*	3.83	0.54	2.00
	Std. Dev	8.01	0.68	2.92
	Max	20.00	1.75	7.00
	Min	0.00	0.00	0.00
Cr (ppm)	*Average*	3.25	5.43	5.55
	Std. Dev	0.69	3.56	3.15
	Max	4.25	13.00	9.75
	Min	2.50	2.50	1.75
Ni (ppm)	*Average*	10.42	9.43	9.20
	Std. Dev	4.76	3.50	3.87
	Max	17.25	12.75	13.75
	Min	4.00	4.25	3.25

In general, ingestion of Pb through food and water is the major exposure pathway for Pb in humans. Individuals will absorb more Pb in their food if their diets are deficient in Ca, Fe, or Zn (Hubbart, 2012). In addition to atmospheric deposition, agricultural practice is a source of Pb in soils from mineral and organic fertilizers (WHO, 2007). According to Krystofova *et al*., (2009) unstable levels of Pb in the environment vary according to industrial production, urbanization, climate changes, and many other factors. While increasing

urbanization and industrialization has caused arise in pollution, animals can be used as a good tool to bio-monitor the levels of trace heavy metals in the environment. Thus quantitative and semi quantitative analysis of pollution concentrations can be done through them. The current study also concluded that the use of fecal matter can be used as a reliable tool to determine the ambient pollution levels and its exposure in animals.

Availability of data: We have included all data in the manuscript that were collected during the field survey.

Authors' contributions: Sidra has designed project, Saleem, Ghafoor, Azeem, Imtiaz and Parveen collected data and Sidra written this article; while Sidra critically analysis this article and approved as final.

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