Heavy Metals Contamination In Mammalian Wildlife of Talchaper Blackbuck Sanctuary Vs Dhavadoli Protected Area of Western Rajasthan, India

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Abstract:- Our aim to establish the metal (Pb, Cd, Cr, Cu and Zn) levels of Mammalian scat samples deriving from sanctuaries of western Rajasthan and comaprision between the concentrations of Talchaper Blackbuck Sanctuary, Churu and Dhavadoli Protected Area, Jodhpur. Restrictions on the sampling because of Wildlife Protection Act (1972) prevents taking of samples of living tissues to analyse body burdens of contaminants that the wildlife may be carrying, fecal samples were analysed. According to our results the concentrations of metals (µg/g (ppm) dry weight) in fecal samples were higher in the samples of Talchaper then the Dhavadoli. They were in the range of 9.86 to 24.24 (Pb), ND to 0.49 (Cd), 2.45 to 6.62 (Cr), 12.19 to 22.69 (Cu) and ND to 12.61 (Zn) in Talchaper whereas in Dhavadoli, 0.76 to 1.55 (Pb), 0.72 to 1.26 (Cd), 1.14 to 4.71 (Cr), 12.21 to 17.7 (Cu) and 4.53 to 9.61 (Zn). Vegetation, soil, salt and water of Talchaper Blackbuck Sanctuary, Rajasthan showed good concentration whereas Dhavadoli samples showed background concentrations of heavy metals (Pb, Cd, Cr, Cu & Zn).

Index term:- Scat, Heavy metals, Bioindicator, Wild mammals, Western Rajasthan.

1 INTODUCTION

Human health and the environmental contamination are the primary concern of human race. The insult of the environment started from the day when human species, which was till then only the gatherer of natural products, turned into agricultural race. The wildlife, over the years, however, adjusted to these changed circumstances. However a duel assault in the form of urbanization and industrialization has led the human civilization right to the doorsteps of the natural habitats. Large tracts of the natural vegetation were denuded forcing wildlife to withdraw and almost getting cornered into small pockets of wildlife reserves. As if this insult was not enough many of the factories spewing pollutants were located too close to the wildlife reserves. Metal roads were laid traversing through these green tracts. The traffic of automobiles started unmindful of the inconvenience to the wildlife and the pollution threatening the fauna. The wildlife harbored in these reserves are at the risk of getting exposed to automobile exhaust, industrial gases and suspended particulate matters. Anthropogenic activities near or within the wildlife habitats are threatening the wildlife with exposure to a variety of environmental contaminants.

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Several studies have reported concentrations of metals in wild mammals living in highly contaminated area near smelters [1], chlor-alkali plant [2], [3], verges of heavily-used highways [4] and mines or mine waste sites [5-6], [7]. A wide range of physiological and ecological effects of air pollutants in animals has been reported [8]. The effects ranged from physiological effects including death, to ecological effects such as behavioral changes. Environmental changes can be monitored biologically and non-biologically, directly in the field or using field samples in laboratory. Non-biological monitoring, since organisms generally integrate the effects of environmental contaminants over a period of time. However, using analytical chemistry in conjunction with appropriate biomarkers and bio-indicators can actually improve the environmental monitoring. Wildlife sanctuaries i.e. Talchaper blackbuck sanctuary, Churu and Dhavadoli Protected area, Jodhpur., are situated in north-western Rajasthan. Talchaper sanctuary is also famous for blackbucks hence it is also called as blackbuck sanctuary. It is situated 12 kms away from sujangarh (churu distict) and 215 kms from the capital city of Jaipur. It is a flat saline depression and is a unique ecosystem. The total area of sanctuary is 7.9 kms. The habitat is dry and arid and soil is full of minerals and salts. The main salty vegetation is Daabh grass., Desmostachya bipinnata, Lana., Haloxylon salicornicum etc. and fauna like desert fox., Vulpes vulpes, Jungle Cat., Felis chaus, Black Naped Hare., Lepus nigricollis, Nilgai., Boselaphus tragocamelus, Jackal., Canis aureus, Chinkara., Gazella gazelle etc. with Black Buck., Antilope cervicapra being the main herbivore can also be seen. The sanctuary also has many water holes calles as Talabs. There is also a salt extraction plant established on Chandwas talab, where salt is extracted in a traditional way. There is a motarable road that bisects the sanctuary in two halves where vehicles are playing. Dhavadoli is one of the main sanctuary of Rajasthan which also gives protection to blackbucks primarily. This sanctuary is situated in Doli

village which lies on Barmer-Jodhpur highway and is 55 kms away from Jodhpur city, total area is 165 hectares is also protected by Bishnois due to their religious faith. Wild animals observed in this area are desert fox., Vulpes vulpes, Jackal., Canis aureus, Chinkara., Gazella gazelle, Hare., Lepus nigricollis, Nilgai., Boselaphus tragocamelus, Black Buck., Antilope cervicapra, mongoose., Herpestes edwardsii and many resident birds. There is hardly any vehicular movement and urban settlement nereby. Various methods were employed to assess and draw a concentration profile of a variety of pollutants that might reach the wildlife habitats and wildlife itself. In fact the human race in its selfish design has used wildlife species as biological indicators to study the ambient concentration of the toxicants in his own ecosystem, both urban and industrial. However, mammals, which are much closer to human beings, are rarely used. In one such study rats, captured from either side of the highways indicated that the body concentration of the lead was directly proportional to the distance from the highway [9]. Bat was the first mammal used by analysis of its guano as bio-indicator for pesticidal pollution as well as mercury exposure [10], [11], [12] and analysis of feces for Cd intake in humans [12],[13]. Sileo et al. [14] recorded concentration of cadmium, lead, zinc, copper in the feces of deer killed near smelters to check the degree of metals pollution. A pilot study to monitor Pb contamination in wild herbivores from the protected areas of Rajasthan, India [15] suggests that exposure to heavy metals can be studied using herbivore dung as a bio-indicator. In the continuation of this, study was also done in mammalian fauna of Keoladeo National Park, Bharatpur [16] and Sariska Tiger Reserve, Alwar [17]. Scat samples of the mammals, vegetation, and soil samples clearly indicate the extent to which the mammalian fauna is exposed to metal contamination. Restrictions on the sampling because of Wildlife Protection Act (1972) prevents taking of samples of living tissues to analyze levels of contaminants that the wildlife may be carrying. However, the method of sacrificing or killing of animal may appear more scientific, but is certainly ethically unsound. Given the concern for loss of animal lives for scientific investigation, and the increasing biological poverty of the planet earth, there is an urgent need for developing biological indicator which will not involve killing of animals. To overcome this problem it was proposed to use feces / scat / fecal matter as bio-indicators or as a biomarkers to study exposure to heavy metals.

2. Materials and Methods

2.1 Sampling Procedure

In the field (Sanctuaries of western Rajasthan) scat sampling was totally opportunistic type. Fresh scat samples of wild mammals of reserves were collected with the help of forest staff from different sites. To ascertain the source of contamination water and vegetation(aquatic as well as xerophytic) samples of these parks were also collected. Another, suspected source of contamination was suspended particulate matter settling on the ground, hence soil samples were also taken from different sides of parks. Samples were brought to the laboratory and kept in freeze for metal analysis. Scat samples of the following mammalian species were collected; Blackbuck., Antilope cervicapra, Nilgai., Boselaphus tagocamelus, Chinkara., Gazella gazelle, Desert fox., Vulpes vulpues , desert hare., Lepus nigricollis dayanus, Jackal., Canis aureus, and vegetation samples were Daabh grass., Desmostachya bipinnata, Bean.,Vicia dasycarpa ,as well as aquatic vegetation i.e. Dunaliella saliva, Anabaena sp., from Talchaper Blackbuck Sanctuaty; Blackbuck., Antilope cervicapra, Nilgai., Boselaphus tagocamelus, Chinkara., Gazella gazelle, desert fox., Vulpes vulpes, Jackal., Canis aureus, Hare., Lepus nigricollis, as well as xerophtic vegetation i.e. Vilayati babul., Prosopis juliflora, kair**.,**Capparis deciduas, Indian Jujube.,Ziziphus mauritiana, Khipp.,Leptadenia pyrotechnica from Dhavadoli Protected Area, Jodhpur. Hence Talchaper sanctuary has salt extraction plant, salt samples were also collected. Scat and soil, salt samples were stored in the plastic zip lock bags and water samples in the sterilized plastic containers.

2.2 Sample Treatment

For analysis of sample 0.5 gm of dry scat / vegetation / soil were weighed and taken in the hard Borosil glass tube. Concentrated nitric acid and perchloric acid were added to each sample in 4:1 ratio. Sample was kept in water bath for 5 to 6 hours or until it was digested completely and became clear. When the sample was clear 3 to 4 drops of H2O2 (30%) were added to neutralize and to dissolve the fat. After cooling each sample was diluted upto 10 ml with deionized water and transferred to sterilized Borosil glass vial and stored at room temperature prior to analysis. Water samples were transferred into beakers, cleaned with double distilled water, and concentrated keeping on a hot plate in a flame hood adding 12 to 15 ml of analytical grade HNO3. The heating was continued till such time the sample became colorless and clean. However, samples were never allowed to dry completely. By and large, nitric acid alone was adequate for complete digestion of water samples. HClO4 was added only to those samples which had high organic matter which were always treated in advance (pre-treated) with nitric acid before adding perchloric acid. If necessary, more HNO3 was added and volume brought down to the lowest quantity (10 to 25 ml) before precipitation occurred. After completing thedigestion, beakers were allowed to cool. Samples were diluted upto 10 ml with double distilled water.

2.3 Analytical Determination

Using spectro-photometry method, heavy metal concentrations (Pb, Cu, Mn, Zn, Cd, Co) were measured in all biological samples of sanctuaries. Entire metal analysis was done by using GBC Advanta ver. 1.31 Atomic Absorption Spectrophotometer at 217 nm for lead, 228.9 nm for cadmium, 324.7 nm for copper, 213.9 nm for zinc and 357.9 nm for chromium. Results are presented in µg/g (ppm) dry weight and µg/ml (ppm) wet weight. In stastically airthmetic mean, standard deviation and standard error were calculated.

3 Results and Discussion

Wildlife sanctuaries studied in Western Rajasthan were Talchaper blackbuck sanctuary, Churu and Dhavadoli Protected area, Jodhpur. The scat/fecal matter sample analysis shows the presence of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu) and Zinc (Zn) in varying concentrations. In Talchaper, concentration of lead was observed in the range of 9.86 to 24.24 ppm d/w whereas it

was 0.76 to 1.55 ppm d/w in Dhavadoli. Cadmium was in range of ND to 0.49 ppm d/w in Talchaper, whereas it was 0.72 to 1.26 ppm d/w in Dhavadoli. Concentration of chromium was 2.45 to 6.62 ppm d/w in Talchaper, whereas it was 1.14 to 4.71 ppm d/w in Dhavadoli. Concentration of copper was 12.19 to 22.69 ppm d/w in Talchaper and 12.21 to 17.7 ppm d/w in Dhavadoli. Zinc was in range of ND to 12.61 ppm d/w in Talchaper whereas in Dhavadoli it was in range 4.53 to 9.61 ppm d/w in the fecal samples of wild mammals. Lead concentration was found in as follows in different mammals. In blackbuck, Antilope cervicapra, it was Talchaper (24.24±2.24 ppm d/w) > Dhavadoli (1.55±0.98 ppm d/w). In Chinkara., Gazella gazelle, the order was Talchaper (16.1±0.21 ppm d/w) > Dhavadoli (0.97±0.69 ppm d/w). In Talchaper, Concentration of lead in Desert hare, Lepus nigricollis dayanus it was Talchaper (9.86±0.35 ppm d/w) > Dhavadoli (1.02±0.09 ppm d/w). In Nilgai., Boselaphus tagocamelus, in Talchaper it was (12.89±1.43 ppm d/w) > Dhavadoli (1.21±0.76 ppm d/w), in jackal, Canis aureua it was 11.54±1.91 ppm d/w (Talchaper). In Dhavadoli concentration of lead in Desert fox., Vulpes vulpues it was 0.76±0.39 ppm d/w. (Table 1,2) Cadmium concentration was found in as follows in different mammals. In blackbuck, Antilope cervicapra, it was Dhavadoli (1.13±0.04 ppm d/w) > Talchaper (0.07±0.03 ppm d/w). In Chinkara., Gazella gazelle, the order was Dhavadoli (0.72±0.13 ppm d/w) > Talchaper (ND). In Talchaper, Concentration of lead in Desert hare, Lepus nigricollis dayanus it was Dhavadoli (0.74±0.47 ppm d/w) > Talchaper (0.02±0.01ppm d/w). In Nilgai., Boselaphus tagocamelus, Dhavadoli (1.26±0.07ppm d/w) > Talchaper it was (0.72±0.13 ppm d/w), in jackal, Canis aureua it was ND (Talchaper). In Dhavadoli concentration of lead in Desert fox., Vulpes vulpues it was 0.99±0.11 ppm d/w. (Table 1,2) Concentration of chromium was found as follows in different mammals. In blackbuck, Antilope cervicapra, it was Talchaper (6.16±0.18 ppm d/w) > Dhavadoli (2.21±0.07 ppm d/w). In Chinkara., Gazella gazelle, the order was Talchaper (2.45±0.13 ppm d/w) > Dhavadoli (1.14±0.72 ppm d/w). In Talchaper, Concentration of lead in Desert hare, Lepus nigricollis dayanus it was Talchaper (2.99±0.31 ppm d/w) > Dhavadoli (2.62±0.35 ppm d/w). In Nilgai., Boselaphus tagocamelus, in Talchaper it was (5.57±0.55ppm d/w) > Dhavadoli (4.71±0.77 ppm d/w), in jackal, Canis aureua it was 6.62±0.7 ppm d/w (Talchaper). In Dhavadoli concentration of lead in Desert fox., Vulpes vulpues it was 3.12±0.56 ppm d/w. (Table 1,2) Copper concentration was found as follows in different mammals. In blackbuck, Antilope cervicapra, it was Talchaper (22.69±2.71 ppm d/w) > Dhavadoli (14.16±0.58 ppm d/w). In Chinkara., Gazella gazelle, the order was Talchaper (21.3±0.97 ppm d/w) > Dhavadoli (12.21±2.01 ppm d/w). In Talchaper, Concentration of lead in Desert hare, Lepus nigricollis dayanus it was Talchaper (14.08±2.26 ppm d/w) > Dhavadoli (13.35±1.39 ppm d/w). In Nilgai., Boselaphus tagocamelus, in Talchaper it was (22.31±2.55 ppm d/w) > Dhavadoli (17.7±1.06 ppm d/w), in jackal, Canis aureua it was 12.19±2.91 ppm d/w (Talchaper). In Dhavadoli concentration of lead in Desert fox., Vulpes vulpues it was 16.6±1.45 ppm d/w. (Table 1,2) Concentration of zinc was found as follows in different mammals. In blackbuck, Antilope cervicapra, it was Talchaper (8.32±1.04 ppm d/w) > Dhavadoli (8.12±1.10 ppm d/w). In Chinkara., Gazella gazelle, the order was Dhavadoli (7.13±0.89 ppm d/w) > Talchaper (ND). In Talchaper, Concentration of lead in Desert hare, Lepus nigricollis dayanus it was Talchaper (12.61±1.6 ppm d/w) > Dhavadoli (4.53±1.21ppm d/w). In Nilgai., Boselaphus tagocamelus, in Talchaper it was (10.18±2.0 ppm d/w) > Dhavadoli (9.61±1.18 ppm d/w), in jackal, Canis aureua it was 3.18±1.02 ppm d/w (Talchaper). In Dhavadoli concentration of lead in Desert fox., Vulpes vulpues it was 6.34±0.06 ppm d/w. (Table 1,2) The analysis of soil and water indicates that highest concentration of lead was found in Talchaper sanctuary (soil., 15.6±3.05 ppm d/w, water., 12.66±1.46 ppm w/w)amongst all. While other metals i.e. cadmium, chromium, copper and zinc were present in background concentrations. (Table 1, 2) Analysis of metals in vegetations (Aquatic and Xerophytic) of these sanctuaries showed that the highest concentration of lead was also found in Talchaper vegetation which range was 9.78 to 22.1 ppm d/w (Table 1). Salt of Talchaper sanctuary was also analysed for metal concentrations and it also showed good concentration of lead (22.98±5.45 ppm d/w) and other metals (Table 1). Heavy metal concentrations were found in considerable amount in the biological samples collected from sanctuaries of western Rajasthan. Amongst them, samples collected from Talchaper sanctuary showed highest concentration of metals. There is a motarable road which is state highway that bisects this sanctuary in two halves, where two and four wheelers are plying regularly. These vehicles passing through the sanctuary are all time emitting exhaust laced with particulate matter containing metals. This smoke spewed by overloaded vehicles, has ultimately settled down on the vegetation, soil and water bodies. This is the reason that salt also showed high concentration of metal. As consequence apart from inhalation, wild mammals are exposed to metals through food and water. Whereas Dhavadoli Protected Area have very little vehicular movement and there is no urban settlement nearby. This is reason that most of the biological samples of these sanctuaries are showing background concentrations. Leonzio and Massi et.al. [18] had shown that metal concentration in feces normally equals that in food. Obviously the additional exposure was through plausible route of inhalation. The load of lead in fecal matter almost exceeded what is present in the food material. Earlier studies have quantified deposition of metals in the vicinity of the highway or traffic dense area, either by measurement by dry depositions fluxes at various distances from road, or by calculating soil and vegetation concentrations and assuming that the soil acts as long term store, hence effectively integrating the deposition [19], [20]. Lead concentrations as high as 6835, 1180 and 682 ppm dry weight have been reported in soil, vegetation and invertebrates, respectively [21], [20]. Metals belong to the group of foreign materials that are excreted into bile and their ratio of concentration in bile verses plasma is greater than 1.0 and may be as high as 10 to 1000. Since liver is in a very advantageous position for removing toxic materials from blood after their absorption, it can prevent their distribution to other parts of the body. Furthermore, because the liver is the main site of biotransformation of toxic agents the metabolites may be excreted into bile [22]. Lead is absorbed in gastrointestinal tract by two steps process. It is first absorbed from lumen and then excreted

into the intestinal fluid [23]. Upon oral ingestion about 5 to 10 % of lead is absorbed and usually less then 5% of what is absorbed is retained [24]. Thus about 99.5 % of total ingested lead is excreted through feces. Out of this 90% is coming out without being absorbed and 9.5% after being absorbed and metabolized leaving only 0.5% to be deposited in various body tissues. Our study has firmly established the value of fecal matter analysis as bioindicator of heavy metal contamination. At least our study holds out a promise where scat can be used, since it does not involve either disturbing or killing of an animal, as useful bioindicator.

4 Conclusions

Our results shows that fecal matter can use as good bio-indicator for gross metal exposure and it provide a less expensive or better means of assessing long-term trends in pollution or other forms of environmental change. This method is completely non-invasive one to conserve the wildlife.

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TABLE 1

METALS CONCENTRATIONS IN BIOLOGICAL SAMPLES OF TALCHAPER BLACKBUCK SANCTUARY, CHURU, RAJASTHAN

S.N	Species	N	Pb (ppm)		Cd(ppm)		Cr (ppm)		Cu (ppm)		Zn (ppm)	
			Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.
	Scat of Mammals											
1	*Gazella gazelle*	20	16.1±0.21	0.08	*ND	-	*2.45±0.13	0.03	21.3±0.97	0.23	*ND	-
2	*Antilope cervicapra*	15	#24.24±2.24	0.57	0.07±0.03	0.006	6.16±0.18	0.04	#22.69±2.71	0.60	8.32±1.04	0.23
3	*Boselaphus tragocamelus*	15	12.89±1.43	0.36	#0.49±0.06	0.015	5.57±0.55	0.142	22.31±2.55	0.65	10.18±2.0	0.51
4	*Lepus nigricollis dayanus*	12	*9.86±0.35	0.10	*0.02±0.01	0.002	2.99±0.31	0.08	14.08±2.26	0.82	#12.61±1.6	0.46
5	*Canis aureus*	8	11.54±1.91	0.55	*ND	-	#6.62±0.7	0.20	*12.19±2.91	0.98	3.18±1.02	0.29
	Vegetation											
6	Dunaliella saliva	10	17.3±1.71	0.54	2.12±0.66	0.20	#17.33±0.85	0.26	17.18±1.16	0.36	11.33±0.45	0.14
7	Anabaena sp.	8	#22.1±0.90	0.91	#2.98±0.84	0.89	12.76±1.1	0.79	21.22±0.88	0.43	#12.11±0.44	0.31
8	*Vicia dasycarpa*	12	*9.78±1.82	0.52	0.75±0.09	0.025	*2.29±0.03	0.008	*12.84±1.02	0.29	4.11±0.16	0.21
9	Fruit of *Vicia dasycarpa*	12	12.78±1.99	0.57	0.38±0.03	0.008	3.66±0.51	0.14	#22.07±2.63	0.75	3.2±0.51	0.14
10	*Desmostachya bipinnata*	10	10.08±2.42	0.76	0.31±0.15	0.04	4.99±0.35	0.11	18.19±0.07	0.02	*ND	-
11	Salt	9	#22.98±5.45	1.81	1.84±0.16	0.05	6.5±0.82	0.27	11.74±0.18	0.06	10.2±1.05	0.35
12	Water	9	12.66±1.46	0.48	0.73±0.03	0.01	1.77±0.23	0.07	12.19±1.21	0.07	5.09±1.61	0.53
13	Soil	11	15.6±3.05	0.91	0.48±0.03	0.009	7.34±0.1	0.03	12.24±1.66	0.5	7.13±1.07	0.32

*N=Number of samples, ND= Not detectable, * =Lowest Mean values, # = Highest Mean values, Metal concentration in µg/g (ppm) dry weight and µg/ml (ppm) wet weight.*

TABLE 2
METALS CONCENTRATIONS IN BIOLOGICAL SAMPLES OF DHAVADOLI PROTECTED AREA, JODHPUR

S.N.	Species	N	Pb (ppm)		Cd(ppm)		Cr (ppm)		Cu (ppm)		Zn (ppm)	
			Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.
	Scat of Wild Mammal											
1	*Antilope cervicapra*	14	#1.55±0.98	0.26	1.13±0.04	2.95	2.21±0.07	0.018	14.16±0.58	0.16	8.12±1.10	0.29
2	*Gazella gazelle*	21	0.97±0.69	0.15	*0.72±0.13	0.02	*1.14±0.72	0.15	*12.21±2.01	0.43	7.13±0.89	0.19
3	*Boselaphus tragocamelus*	25	1.21±0.76	0.15	#1.26±0.07	0.014	#4.71±0.77	0.154	#17.7±1.06	0.21	#9.61±1.18	0.23
4	*Lepus nigricollis*	20	1.02±0.09	0.21	0.74±0.47	0.09	2.62±0.35	0.51	13.35±1.39	0.11	*4.53±1.21	0.40
5	*Vulpes vulpues*	13	*0.76±0.39	0.37	0.99±0.11	0.78	3.12±0.56	0.41	16.6±1.45	0.31	6.34±0.06	0.51
	Vegetation											
4	*Prosopis juliflora*	10	1.05±0.38	0.12	1.01±0.06	0.018	3.32±0.24	0.075	#17.08±1.54	0.48	4.15±0.56	0.17
5	*Capparis decidua*	8	1.13±0.56	0.19	#1.08±0.02	0.007	#5.37±0.41	0.14	12.41±0.98	0.34	*3.19±1.06	0.37
6	*Ziziphus mauritiana*	12	*0.94±0.78	0.23	0.69±0.03	0.008	4.88±1.46	0.42	*7.52±0.14	0.04	4.01±0.76	0.21
7	*Leptadenia pyrotechnica*	8	#1.18±0.91	0.32	*0.41±0.03	0.01	*1.77±0.71	0.25	10.38±0.24	0.08	#10.38±1.28	0.45
8	Soil	10	1.47±0.73	0.23	0.34±0.02	0.006	15.06±1.94	0.61	8.41±0.32	0.29	3.15±0.73	0.23
9	water	12	0.61±0.11	0.09	0.15±0.06	0.08	0.92±1.12	0.06	11.13±0.98	0.53	9.15±0.63	0.43

*N=Number of samples, ND= Not detectable, * =Lowest Mean values, # = Highest Mean values, Metal concentration in µg/g (ppm) dry weight and µg/ml (ppm) wet weight.*