Accumulation of Some Heavy Metals in Selected Tissues of Cape Hare, *Lepus Capensis* From Pakistan

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Abstract: Accumulation of selected heavy metals (Cd, Cr, Cu, Fe & Pb) was assessed in cape hare (*Lepus capensis*) from northern areas of Pakistan. Five different tissues (heart, lungs, kidneys, liver and muscles) of twelve males and nine females were studied for metal accumulation. A significant variation of heavy metals concentration was observed among different tissues of cape hare. The mean concentration of Cd was highest in heart (1.688 µg/g) and lowest in muscles (1.27 µg/g) and Cr in heart (86.6 µg/g) was highest and in the lungs (5.2 µg/g) was the lowest. Cu in liver (3.96 µg/g) was highest and lowest in lungs (2.793 µg/g) and Fe had highest value in lungs (154.9 µg/g) as compared to muscle (59.57 µg/g). While Pb was highest in lungs (40.31µg/g), minimum in kidneys (19.68 µg/g) and In relation to sex, higher mean concentration of heavy metals was observed in female as compared to male except Cr. It was found in all the tissues of males but not detected in the lungs, liver and muscles of female. The mean concentration of selected heavy metal was above the consumable limits determined by European Commission Regulation as: Cd-0.05 µg/g, Cr-1.0µg/g, Cu-5.0µg/g, Fe-10.0µg/g and Pb-0.1µg/g. This study concludes that consumable tissues of cape hare of northern areas of Pakistan specifically from Shangla, Mansehra, Haripur, Mirpur and Kotli have accumulated high concentration of heavy metals, may pose health risk when consumed, thus not suitable for human consumption.

Key words: Bioaccumulation, non essential heavy metals, trace metals, cape hare, wildlife, metal toxicity

INTRODUCTION

Heavy metals pollution is a serious problem at global level whether it is from natural or anthropogenic sources (Langner *et al.*, 2011 and Markert *et al.*, 2011). Rapid industrialization, mining, automobiles, agriculture and use of advance technology are the major sources of heavy metal pollution. Heavy metals from fertilizers are continuously entering in trace amounts (Al-Khashman *et al.*, 2006) and accumulated in the soil, from where they accumulate in the plants, then enter into the human food chain and cause a risk to human health (Pertsemli & Voutsa, 2007 and Ibrahim *et al.*, 2009). Once they contaminate food chain, will ultimately transferred to human body directly or indirectly (Kalpan *et al.*, 2010). Even at very small concentration they are toxic and cause risk to organisms as well as human (Oishi *et al.*, 2000). Along the food chain they are biomagnified, thus are more concerned at higher trophic levels (Rajaganapathy, 2006 and Aycicek *et al.*, 2008) and induce health impacts in human (Aschner, 2002).

Food chain, in any environment can work its magic across different species and help stack these heavy metals up, ultimately into humans (Singh *et al.*, 2007; Ghani, 2010). Any wild animal accumulate these heavy metals via food ingestion, dermal contact and respiratory pathway (Kolesarova *et al.*, 2008).
Cadmium is a toxic metal and pollute environment through various natural and anthropogenic sources (Bernard, 2008). Cadmium tends to biomagnifies in soft tissues of animal such as lungs, brain, liver, kidneys, placenta and bones. Cd being non essential heavy metal, is known to cause number of ailments in human such as pneumonitis, pulmonary edema, osteomalacia, osteoporosis, kidney dysfunction, kidney lesions, lung damages, structural and functional damages in reproductive tissues (Soylak *et al.*, 2002) thus decreasing fertility in male and female organisms (Telisman *et al.*, 2000), notorious as feminizing agent in anurans (Sharma, 2008), itai-itai and myocardial diseases as well as carcinogenic, mutagenic and teratogenic effects in mammals (Tellez-Rojo *et al.*, 2006; Govind & Madhuri, 2014).

The most toxic species of chromium are trivalent Cr^{+3} and hexavalent Cr^{+6} (Jaishankar *et al.*, 2014), and Cr^{+3} is an essential trace metal for mammals to maintain protein, lipid and glucose metabolism but Cr^{+6} is carcinogenic (Tarley *et al.*, 2001). Cr enters into the environment specifically from metallurgical, chemical and refractory industries (Tchounwou *et al.*, 2012). It impairs liver, kidneys, spleen and blood cells (Govind & Madhuri, 2014). Its high toxicity is based upon its strong oxidative effect which leads to the enzyme

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malfunction, respiratory diseases, oxidative stress, DNA damage, carcinogenic effects and organ failure (Boran *et al.*, 2014).

While copper enters into the environment through natural and several anthropogenic sources. Copper toxicity has induced copperiedus which leads number of serious ailments in humans (Vonk *et al.*, 2008; Brewer, 2010). The specific iron sources are industrial as well as natural. High dietary iron intake can cause iron overload leading to liver and heart diseases (Valko *et al.*, 2005), neurodegenerative disorders, induced diabetes, hormonal abnormalities (Fitzgerald, 2009), significant tissue damage (Brissot *et al.*, 2012) and immune system anomalies (Kohgo *et al.*, 2008).

Distinguished sources of lead (Pb) pollution are mainly industrial (Mañay *et al.*, 2008) and accumulated in brain, blood, bones, kidneys and thyroid gland. High concentrations of Pb in the body have caused abnormalities of kidneys, gastrointestinal tract, joint, reproductive and nervous system and hemoglobin synthesis (Raikwar *et al.*, 2008; Govind & Madhuri, 2014).

Cape hare, *Lepus capenses* is a small game animal that occupies mainly hilly habitats and thick forests. *L. capenses* is distributed throughout foot hills of Himalayan region ranging from Pakistan to India and feeds on grasses, forbs, crops and vegetables depending upon the availability of food type. It is a threatened species due to overhunting, habitat destruction, cutting of forest, forest fire and environmental pollution. Hunting of cape hare is a famous activity in the region and meat obtained is eaten in the social circles. This has created the interest to determine the heavy metal accumulation in wild meat that may cause deleterious effects in humans on consumption (Iqbal *et al.*, 2009; Yi *et al.*, 2011; Shahid *et al.*, 2013). The determination heavy metal accumulation in food stuff is useful in health risk assessment (Celechovska *et al.*, 2008; Idowu *et al.*, 2014). Objective of the present study was to assess the accumulation of heavy metals (Cd, Cr, Cu, Fe and Pb) in selected tissue of cape hare as well as accumulation in relation to their sex.

MATERIALS AND METHODS

Cape hare, *L. capensis* is found in the Northern areas of Pakistan including province Khyber-Pakhtunkhwa (KP), state Azad Jammu and Kashmir and foothills of Himalaya. During this study a total of 21 adult of *Lepus capensis* (12 males and 9 females) were collected including 6 individuals from District Mansehra, 6 from District Shangla and 5 from District Haripur (KP), 2 from Kotli and 2 from Mirpur (Azad Jammu and Kashmir). Adult animal collection was done under permission from Wildlife Department, Government of KP and Azad Jammu and Kashmir. They were transported to environmental biology laboratory at Department of Zoology, University of the Punjab, Lahore.

After morphological examination animals were dissected to obtain selected tissues. Tissues samples were chopped into small pieces with clean plastic knife, sealed in separate zip lock Teflon grade polythene bags (with all necessary information like specimen code, place, age, sex and place of animal collection) and kept at 40° C until acid digestion. The samples were pulverized in liquid nitrogen with glass mortar and a pestle (pre-cleaned with 10% HNO_3) and allowed to air-dry overnight in a closed chamber at room temperature to a constant weight (one gram). The dried samples were then acid-digested by adding 5 ml of concentrated HNO_3 (metal grade) according to Csuros and Csuros (2002). Heavy metal analysis of samples was carried out by using Atomic Absorption Spectrometer (Varian FS 240AA) by following standard conditions (Malik *et al.*, 2010). Standard curves were established by measuring different dilutions of Cd, Cr, Cu, Fe & Pb standards, the lowest dilution being 0.5µg/l for each metal. The accuracy and integrity of the sample analyses (in triplicate) were monitored by regularly running check standards and deionized water blanks.

According to European Commission Regulation (EC) No 1881/2006, the maximum level for Cd and Pb in meat of bovine animals is 0.050 µg/g and 0.1 µg/g respectively. While the permissible level for chromium (Cr) is 1.0 µg/g, Cu is 5.0 µg/g and Fe is 10.0 µg/g.

The data was subjected to SPSS. One way analysis of variance (ANOVA) test was applied to find out the correlation and regression relationships among different variables followed by the Tukey's test. An overall α value of 0.05 was used to assess significant differences. Data are presented as mean ± SD. Bartlett's test was used to assess the homogeneity of the variables.

RESULTS

A significant variation was found in accumulation of Cd, Cr, Cu, Fe and Pb among selected tissues (heart, lungs, kidney, liver and muscle) of all specimens of cape hare, *L. capensis*. The highest concentration of Cd metal was recorded in the heart (1.688 µg/g) while the lowest was in the muscles (1.27 µg/g) tissues. Cd accumulation in heart was significantly higher ($P<0.05$) than in muscles and non-significantly higher than other tissues. Accumulation of Cr in heart (86.6

µg/g) was highest and the lowest amount was detected in the lungs (5.20 µg/g). Similarly the highest concentration of other metals such as Cu, Fe and Pb were 3.95 µg/g in liver, 154.9 µg/g and 40.31 µg/g in lungs respectively while the lowest concentration was 2.79 µg/g in lungs, 59.57 µg/g in muscles and 19.68 µg/g in kidneys respectively (table 1).

Table 1: Concentration (µg/g dry weight) of Cd, Cr, Cu, Fe and Pb in the selected organs/tissues of cape hare, *Lepus capensis* collected from northern areas of Pakistan.

		Mean	Maximum	Minimum	Median	S.D
Heart	Cd	1.69	7.50	0.10	0.85	1.99
	Cr	86.6	121.8	63.3	74.6	31.0
	Cu	3.72	6.30	1.70	3.60	1.20
	Fe	98.6	328.6	3.0	90.3	106.0
	Pb	28.93	128.0	4.90	22.65	27.68
Lungs	Cd	1.46	3.30	0.20	0.90	1.18
	Cr	5.20	5.70	4.70	5.20	0.707
	Cu	2.79	6.60	1.00	2.60	1.60
	Fe	154.9	339.1	36.5	148.1	88.9
	Pb	40.31	126.5	16.10	26.60	32.90
Kidney	Cd	1.53	3.50	0.20	1.50	1.18
	Cr	80.70	80.70	80.70	80.70	*
	Cu	3.33	5.20	1.00	3.80	1.05
	Fe	83.5	159.7	3.6	90.5	65.6
	Pb	19.68	28.20	6.30	20.40	6.91
Liver	Cd	1.50	3.20	0.20	1.10	1.185
	Cr	54.7	88.4	35.9	39.7	29.36
	Cu	3.95	6.10	2.00	4.00	1.05
	Fe	133.1	212.5	96.80	124.3	39.2
	Pb	28.67	116.4	1.00	20.90	25.38
Muscle	Cd	1.27	3.40	0.10	0.60	1.18
	Cr	36.9	46.4	27.4	36.9	13.44
	Cu	3.45	7.60	1.50	2.80	1.71
	Fe	59.57	86.40	34.70	61.70	17.64
	Pb	25.61	139.0	0.30	22.60	27.80

In relation to sex, concentrations of heavy metals (Cd, Cr, Cu, Fe and Pb) accumulation in selected tissues (heart, lungs, kidneys, liver and muscle) are given in tables 2-5. In comparison of sex, significantly higher ($P<0.05$) median concentration of Cd was found in female heart (2.311 ìg/g) and kidneys (2.086 ìg/g) than in male heart (0.800 ìg/g) and kidneys (0.986 ìg/g). The accumulation of Cd in other tissues was also non-significantly higher in females than males (table 2).

Table 2: Concentration (µg/g dry weight) of cadmium (Cd) in selected tissues of cape hare, *L. capensis* in relation to their sex.

Cadmium (Cd)	Heart		Lungs		Kidney		Liver		Muscle	
Parameters	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Mean	0.800	2.311	1.140	1.571	0.986	2.086	1.367	1.425	1.100	1.370
Maximum	3.100	7.500	2.800	3.300	2.700	3.500	3.200	3.100	3.000	3.400
Minimum	0.100	0.100	0.300	0.200	0.200	0.900	0.400	0.200	0.100	0.200
Median	0.450	2.500	0.900	0.800	0.700	2.000	1.100	0.800	0.550	2.125
S.D	0.997	2.378	0.991	1.307	0.880	0.786	1.044	1.245	1.074	1.256

Chromium was detected in only two individuals; one male and one female. In female only heart (121.80 ìg/g) and liver (37.80 ìg/g) have showed accumulation of chromium while in male all tissues accumulated Cr with highest value (88.400 ìg/g) in liver and the lowest (5.200 ìg/g) in the lungs. Male had higher concentration of Cr in liver and female had higher concentration of Cr in heart.

The accumulation of Cu in tissues of male cape hare*, L. capensis* was in the order of heart>liver>lungs>kidneys >3muscles, while in females, liver>muscles>kidneys> heart>lungs. Higher mean concentration was accumulated in male heart (4.256 ìg/g), liver (3.858 ìg/g) and lungs (3.133 ìg/g) whereas in female liver (3.814 ìg/g) and muscles (3.640 ìg/g) have accumulated higher Cu concentration (table 3).

Table 3: Concentration (µg/g dry weight) of copper (Cu) in selected tissues of cape hare, *L. capensis* in relation to their sex.

Cadmium (Cd)	Heart		Lungs		Kidney		Liver		Muscle	
Parameters	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Mean	4.256	3.490	3.133	2.371	2.756	3.540	3.858	3.814	2.660	3.640
Maximum	5.100	6.300	6.600	4.100	5.200	4.100	6.100	5.500	5.000	7.600
Minimum	3.300	1.700	1.000	1.100	1.000	2.700	2.000	2.500	1.500	2.000
Median	4.100	3.350	2.700	2.200	2.300	3.600	3.850	3.900	2.350	3.300
S.D	0.642	1.493	1.983	1.081	1.285	0.568	1.143	0.941	1.150	1.599

Copper concentration in both male and female tissues was lower than iron and lead but higher than cadmium and chromium accumulation.

Iron (Fe) accumulation was also detected in selected tissues of cape hare. The order of accumulation in males was lungs>liver> heart>kidneys>muscles, while in females it was liver>lungs>kidneys>heart> muscles. Overall, mean iron concentration was higher in males among all tissues under study as compared to females, except kidney (table 4). Iron accumulation in the tissues of both males and females was observed the highest as compared to other metals (Cd, Cr, Cu and Pb).

Table 4: Concentration (µg/g dry weight) of iron (Fe) in selected tissues of cape hare, *L. capensis* in relation to their sex.

Iron (Fe)	Fe in Heart		Fe in Lungs		Fe in Kidney		Fe in Liver		Fe in Muscle	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean	163.3	106.9	209.2	127.0	88.2	117.6	164.0	155.0	59.79	58.48
Maximum	328.6	150.5	339.1	251.7	155.4	162.7	252.1	283.2	92.40	99.30
Minimum	71.6	63.7	89.8	36.5	60.0	65.6	103.4	96.8	35.20	8.00
Median	132.6	102.0	205.6	112.3	78.4	116.6	142.6	149.8	53.85	58.65
S.D	88.4	29.2	87.6	67.5	31.0	35.6	54.6	63.3	19.82	24.58

The order of accumulation of Pb in males was lungs>liver>heart>kidneys>muscles while in females it was lungs>heart>liver>muscles> kidneys. The overall lead accumulation in female tissues was higher in heart (30.60 ìg/g), lungs (45.90 ìg/g) and muscles (21.36 ìg/g), while in males it was higher in kidneys (20.20 ìg/g) and liver (31.7 ìg/g). Lead concentration in both sexes was lower than iron, but higher than cadmium, chromium and copper accumulation (table 5).

Table 5: Concentration (µg/g dry weight) of lead (Pb) in selected tissues of cape hare, *L. capensis* in relation to their sex.

Lead (Pb)	Heart		Lungs		Kidney		Liver		Muscle	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean	25.92	30.6	33.8	45.5	20.20	19.20	31.7	26.09	18.74	21.63
Maximum	63.30	128.0	83.5	126.5	28.20	25.80	116.4	57.20	26.20	28.90
Minimum	4.90	8.6	18.3	16.1	6.30	8.60	5.5	12.70	0.30	10.80
Median	26.50	20.5	26.0	29.3	18.30	21.10	20.8	21.00	24.70	22.60
S.D	15.91	35.0	24.6	39.8	7.75	6.63	32.6	16.03	9.31	6.42

Correlation analysis between heavy metals (Cd, Pb, Fe and Cu,) concentration and their accumulation in selected tissues (kidneys, lungs, muscles, heart and liver) revealed significant ($P<0.05$) positive and negative relationships. The correlation of chromium with other heavy metals was not possible because chromium was found in only 07 tissue samples of 2 individuals thus the data available for Cr was not enough to correlate chromium with other metals in 105 samples.

Correlation analysis showed significant ($P< 0.05$) relationships between metal accumulations in selected tissues. Cd concentration accumulated in kidney had a positive relationship with Cd accumulation in liver and negative relationship with Cu in lungs. Cd accumulation in liver showed positive correlation with Cd in heart. Cd accumulation in muscles showed positive correlation with Cd in liver and negative with Cd in lungs and Cu in liver. Cd accumulation in liver showed negative relationship with Cu in lungs and liver. Similarly Pb concentration in muscles had a significant positive correlation with Cu accumulation in heart and Pb accumulation in liver also had a significant positive relationship with Fe in lungs. Fe accumulation in kidney also showed positive correlation with Fe in lungs and negative in muscles. Fe in liver showed significant positive correlation with Cu accumulation in lungs and liver while Cu accumulation showed only positive correlation with Cu in liver.

DISCUSSION

The bioaccumulation of heavy metals (Cd, Cr, Cu Fe and Pb) was high in the selected tissues (heart, lungs, kidneys, liver and muscles) of cape hare (*Lepus capensis*). The high level of heavy metals accumulation in the tissues of cape hare provides the evidence of intensive environmental contamination (Kolesarova *et al.*, 2008). Increasing pollution in terrestrial and aquatic habitat can maximize the chances of bioaccumulation of heavy metals in them. Cape hare is placed at second trophic level along the food chain and vulnerable for heavy metal pollution because of its natural habits of efficient uptake of veggie nutrients. The highest concentration of heavy metals is expected to accumulate in the soft tissues of the animal i.e. liver, kidney, lungs, heart and muscle (Lias, 2013; Shahid *et al.*, 2013). High level of Cd, Fe and Pb accumulation was also observed in the internal tissues of small wild mammals in their natural environment in Slovakia (Martiniakova *et al.*, 2012).

The concentration of Cd accumulation was higher than the maximum level permissible by European Commission (EC) (Regulation, 2006) in all the tissues under study of *Lepus capensis*. The order of accumulation of Cd in different tissues was in heart>kidneys>liver >lungs>muscle (table 1). In the present study, the concentration of Cd in kidneys was lower as compared to Pb concentration which is in accordance with the findings of Massanyi *et al.* (2003), Josthna *et al.* (2012) and Shahid *et al.* (2013).

Chromium was detected in only 2 individuals of *L. capensis*; one male and one female that were collected from Shangla (KP) and Mansehra (KP). In total, 105 samples were analyzed for chromium and 98 samples were below detection limit. Chromium was present in high concentration in kidneys as compared to lungs, muscles and liver which is supported by the finding of Palaniappan & Karthikeyan, (2009) who assessed chromium accumulation in various tissues of a freshwater fish, *Cirrhinus mrigala* and wild hare (Shahid *et al*., 2013).

The concentration of Cu accumulation was significantly ($P<0.05$) high in different tissues of *L. capensis* with the order of accumulation as liver>heart>muscles>kidneys>lungs. The findings of the present study are in corroboration with other studies where Cu is generally associated with liver as being the most important organ of metabolism and tends to high accumulation of heavy metals (Gybina & Prohaska, 2006; Vonk *et al.*, 2011).

The accumulation of Fe was highest among metals analyzed in selected tissues of cape hare, *Lepus capensis*. The order of accumulation was observed a lungs>liver>heart>kidneys>mus- cles. It is because cape hare is an herbivore and consumes large amount of vegetarian diet that constitutes intake of high amount of Fe present in the leaves, rhizomes and stems of dry and green grasses (Crockett, 1984). Cape hare meat and viscera are consumed by human and if they are loaded with high concentration of Fe, it may cause Fe toxicity when combined with Fe as medicine or supplement (Altamura & Muckenthaler, 2009). The higher concentration of Fe in mammals have the ability to cause iron overload and hemochromatosis (Oruc *et al.*, 2009).

The Pb accumulation was higher than permissible level set by European Commission (EC) regulation with the order of accumulation as lungs>heart>liver>muscles> kidneys. High concentration of Pb in small wild mammal tissues such as liver and kidney is reported by Stansley & Roscoe (1996).

It was evident from the data that mean concentration of heavy metal was higher in female as compared to males in selected tissues of cape hare. Similarly the order of metal accumulation among both sexes was observed as Fe>Pb>Cu>Cd>Cr. Heavy metals accumulation in the sex of mole (*Talpa europaea*) showed higher trend of accumulation of heavy metals in females as compared to males in the study (Komarnicki, 2000). High median concentration in females as compared to male was also observed in another study on *Lepus nigricollis* (Shahid *et al.*, 2013). The high concentration of heavy metals in females as compared to males could be the reason that females are heavier and larger in size with more active life style than males. Moreover the feeding rate of females is higher than males because they give birth to 1-3 leverets per litter with as many as 4-6 litters per year. When females consumes more food to support their lifestyle and baby leverets, that may result into high uptake of different heavy metals and eventually accumulate in their tissues. Moreover the young hares consume the fecal pellets produced by their mothers, which they tend to do, it also increases the chances of bioaccumulation of heavy metals in their tissues from the beginning of their life (Montuire, 2001) and results in high concentration at adulthood.

Cadmium accumulation order in males was liver>lungs>muscles>kidneys>heart while in females it was heart>kidneys>lungs>liver>muscles. The tendency of cadmium accumulation in females was higher than the males (table 2). Cadmium concentration in both males and females was lower than iron, lead and copper but higher than chromium accumulation. The reingestion of fecal material by the wild hare to extract maximum nourishment from food and their efficient absorbance from the food having toxic metal content may also have caused the bioaccumulation of heavy metals (Watson, 2010).

According to another study, acanthocephalans parasitizing different mammals have the ability to bio-concentrate heavy metals in the tissue of their host (Sures *et al.*, 2002). Thus the presence of ectoparasites on the body of cape hare probably has influenced high accumulation of heavy metal in the tissues.

CONCLUSION

The high concentration of heavy metals in the tissues of *Lepus capensis* depicted it as ecological biomarker indicating the degree of metal contamination in their habitats. It demands serious attention of respective authorities towards the environmental pollution (Depledge & Galloway, 2005) particularly metal pollution. The consumption of contaminated meat of *L. capensis* is declared as toxic in view of the present study. The meat of wild hare, *L. nigricollis* has been already been declared not fit for human consumption (Shahid *et al.*, 2013).

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