



Blood and offal tissue lead levels of Goats, sheep and chickens farmed near Battery Recycling smelters of Hyderabad Pakistan

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Abstract: Battery recycling is a common secondary lead smelting technique responsible for health hazards in humans and animals. In the present study live stock farms of goat, sheep and chickens in the vicinity of battery recycling complex in Hyderabad Sindh were surveyed for lead content in soil, pasture grasses, blood, and tissue samples derived from liver, kidneys, femur and chest muscles of animals. The lead content of soil and grass samples ranged between 70- 1310mg/kg and 20- 250 mg/kg respectively where as the blood lead levels of goats, sheep and chickens ranged between 3.32- 39.54µg/dl. The mean lead content of liver, kidney, and femur muscles of goat was found as 4.23± 1.37, 3.91 ± 0.86, and 1.25± 1.13 µg /dl respectively. The corresponding values in sheep were found as 6.02 ± 1.0, 6.58 ± 0.73, and 2.26 ± 0.46 µg/dl respectively. The elevated levels of lead were also found in chickens organs when compared with controls. All results being statistically significant indicated high impact of lead pollution from recycling complex on the health of animals.

Keywords: lead poisoning, lead toxicities, offal organs, soil lead, battery recycling

1.

INTRODUCTION

The coexistence of livestock farms and industrial establishments in the peri urban areas is a common feature of the big cities of Pakistan playing vital role in the socioeconomics of the urban people. Besides meeting nutritional requirements of the urban population, the farming of buffalos, cows, goats, sheep, and poultry also provide important raw materials for several industries. However this coexistence is not without problems in most of the cases as exposure of animals and birds to industrial waste may pose dangers to their lives. Hyderabad is second largest city of sindh having population of over two million. The city is surrounded by agricultural lands with potential for sustaining animal life in terms of availability of hay and crop residues as fodder and drinking water in abundance. This has led to extensive live stock farming which, as in the case of other cities of Pakistan, are also located in the proximity of several industries. The tons of toxic waste of these industries is not usually managed properly and disposed off on the open grounds without taking care of the dangers to animal and human life.

The recycling of exhausted lead acid batteries for reuse of metal in new devices is a common activity and reported as a major source of lead pollution in many parts of the developing world (Carvanos, *et al* 2013, Gottesfeld, *et al.*, 2011). The lead recycling is also a major industry of the Hyderabad with capacity to produce over one thousand metric tons of recycled lead per month. The industry uses the crude technology of open furnaces and the emitted lead containing fumes are

usually carried by winds and deposited on nearby hay and fodder used for grazing animals. The lead thus deposited has potential to produce acute or chronic poisoning in animals by regular exposure to varying quantities of the metal (De-Michelle 1984). Apart from dangers to life of animals, the metal accumulated in tissues, milk and eggs may also find way in to humans where it is implicated in several biological disorders including neuropathy, hematopoietic, renal insufficiency and the disease of the cardiovascular and reproductive systems (Tong, *et al.*, 2000) Hatch and funnel (1969) reported accumulation of lead in kidneys and liver of the cattle, Humpherys (1991) reviewed the effects of lead in horses, cattle, buffalo, goats sheep and swans. Krametter, Froetscher, *et al.*, (2007) reported toxic effects lead and mercury in cattle due to excessive accumulation of metals in liver and kidneys. Rodríguez-Estival, *et al* (2012) reported blood lead levels and aminolevulinatase dehydratase enzyme inhibition in cattle and sheep exposed to metal in a mining area. Similarly lead has also been reported in the eggs, liver, kidney, skeletal muscle, and ovarian tissues of the chickens (Trampel, *et al* 2003). The live stock herds and poultry farms, the main suppliers of meat, milk and eggs to Hyderabad city are also located in the vicinity of lead recycling smelters with proneness to accumulation of lead. To best of our knowledge no work has been reported in this regard therefore present study was carried out to determine the blood and tissue lead levels of goats, sheep and chickens, to analyze the contaminated soil and grasses of the grazing area for lead and to find out if there existed any correlation ship

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between the sources of exposure and lead levels of blood and various organ tissues of the animals.

2. MATERIALS AND METHODS

The battery recycling complex is spread over an area of three square kilometer with more than four hundred furnaces functioning in uncontrolled manner. The complex is surrounded by agricultural lands and pasture grounds where more than ten thousand heads of goats, sheep, cattle, and buffalo graze every day. A large number of poultry farms also exist in the area meeting nutritional requirements of the people of city and other areas. The soil of the area is of moderately calcareous and non saline type having abundance of hay and pasture grasses. The study was carried out on the groups comprising of 43 goats, 25 sheep, and 33 fully grown broiler chickens all selected using random sample design. The control animals for comparison purposes were derived from lead free areas and comprised of 21 goats 15 sheep and 25 chickens. 10 ml of blood sample was drawn using the jugular vein of each animal and wing vein of chickens in EDTA or heparin containing PTFE vials and subjected to hematological measurements for hemoglobin (Hb), Erythrocyte count (EC) Mean corpuscular volume (MCV) and packed cell volume (PCV) using hematology analyzer MSL VAO (China). A portion of blood (5ml) was also reserved for analysis of lead in blood by atomic absorption spectrometer. Prior to analysis of metal the blood samples were treated with Triton- 100, Ammonium hydrogen phosphate and Magnesium nitrate as described by Rodríguez-Estival (2012). For the purpose of analysis of lead in liver and kidney tissues the animals were clinically examined for symptoms of lead toxicity such as colic, ataxia, diarrhea, maniacal behavior and others. Based on the severity of symptoms 9 goats and 14 sheep were periodically slaughtered and the samples of respective tissues collected. 2 gram subsamples from each of the liver and kidney tissues were excised and processed for analysis of lead using method reported by Blanco-Panedo *et al.*, (2006).

The top layer of the soil of study area was used for determination of lead. The samples were collected in the radius of one kilometer around recycling smelters in order to know the effect of wind direction. The samples were dried ground, sieved and processed for acid digestion by per chloric, Hydrofluoric and Sulphuric acids as described in the literature (Allen *et al.*, 1989). The analysis of lead in vegetation of the area was carried out by excising the aerial parts of the plants at

the height of 10 centimeters from ground. Each of the samples was further chopped into small pieces, washed with deionized water, dried, homogenized and processed for acid digestion by per chloric, Nitric and Sulphuric acids as per recommended methods. (Allen *et al.*, 1989)

The statistical analysis of data was done by t test, one way Analysis of variance (ANOVA) and Pearsons correlation coefficients using statistical software Minitab 13.

3. RESULTS

General characteristics of the animals and control are given in (Table 1). Two sample T test was used to find the differences in age and weight of animals and controls. The quantitative levels of haematological parameters Haemoglobin, Erythrocyte count, Mean Corpuscular Volume and Packed Cell Volume of the animals and control groups shown in (Table 2).. One way analysis of variance (ANOVA) of each of the indices was used to detect the differences in exposed and control groups. The F values for Hb, EC, PCV and MCV in both goats and sheep found to be statistically significant at $p < 0.01$ indicating differences resulting from exposure. (Table 3). shows the descriptive statistics of blood lead for exposed and control groups of goats, sheep and chicken. The data was analysed by two sample t- test to detect the differences using significant level equal to 0.01.

Table 1. Some physical characteristics of exposed and control animals

Trait	Animal	
	Goat	sheep
<u>Total number of animals</u>		
Exposed	43	25
Control	21	15
<u>Number of male</u>		
Exposed	15	8
Control	8	6
<u>Number of females</u>		
Exposed	28	17
Control	13	9
<u>Age Months (Mean \pm SD)</u>		
Exposed	30 \pm 3.3	40 \pm 4.6
Control	31 \pm 2.7	43 \pm 3.6
<u>Weight kg (Mean \pm SD)</u>		
Exposed	55 \pm 2.4	62 \pm 2.5
Control	56 \pm 2.8	64 \pm 2.2
Breed	kamori	Kooka

Table 2. Mean \pm SD and one way ANOVA for some haematological indices of exposed and control animals

Animal	N	EC X (10^6 /L)	Hb (%)	MCV (fl)	PCV (%)
Goat Exposed	43	7.73 \pm 0.37	7.9 \pm 0.95	18.41 \pm 1.30	23.35 \pm 1.72
Control	21	12.21 \pm 0.99	10.67 \pm 1.28	23.48 \pm 2.06	25.39 \pm 2.0
<u>ANOVA</u> F P	-	689.58 < 0.01	88.41 < 0.01	143.09 < 0.01	16.64 < 0.01
Sheep Exposed	25	9.69 \pm 0.93	11.0 \pm 1.13	31.72 \pm 1.90	29.21 \pm 1.19
Control	15	13.16 \pm 0.55	13.85 \pm 1.04	33.66 \pm 1.46	35.93 \pm 1.35
<u>ANOVA</u> F P	-	168.92 < 0.01	51.46 < 0.01	11.45 < 0.01	268.24 <0.01

Table 3. Descriptive statistics of blood lead levels of groups of goat, sheep, chicken and respective control

Animal (control)	N (n)	Range (μ g/dl)	Mean (μ g/dl)	SD (μ g/dl)	Median (μ g/dl)	T	P
Goats	43	22.25-34.55	28.57	3.62	28.31	33.15	< 0.01
(control)	21	4.25-12.3	(8.31)	1.2	8.41		
Sheep	25	25.08-39.49	31.55	4.72	31.47	15.44	< 0.01
(control)	15	4.35-16.18	(12.15)	3.21	13.0		
Chicken	33	3.32- 5.96	4.85	0.858	4.88	-	-
(control)	25	(ND)	-	-			

The lead content (Mean \pm SD) of the soil and pasture grasses in relation to varying distances and directions from recycling complex are shown in (Table 4). The data was analysed using two way analysis of variance of mean lead levels at different distances and directions of recycling complex. To investigate whether wind direction had role in the soil and grass lead levels, the Pearsons correlation coefficients (r) were calculated for soil lead levels in different directions. The summary of the correlation data is given in (Table 5). The lead content of the tissues samples from liver, kidney and muscles of exposed and control animals along with analysis of data by t test is given in (Table 6).

4. DISCUSSION

The clinical examination of animals before commencement of study did not reveal the presence of any serious disease except 15 goats and 9 sheep who presented neurological symptoms of lead toxicity such as ataxia and lethargy in exposed groups. The animals of a given breed did not differ in respect of age among exposed and control groups but their mean weights differed significantly (Table 1). Generally the mean weights of exposed groups were lower than their controls. The exposed group of goats was found to have

blood lead in the range 22.25 to 34.55 μ g/dl with mean value of 28.57 μ g/dl. In the corresponding group of sheep these values were found to be still higher having range and mean levels as 25-39.49 and 31.55 μ g/dl respectively (Table 3). The results are in accordance with the findings by Palacios *et al* (2002) who reported blood lead levels in the range 20- 89 μ g/dl for horses living near the battery recycling plant and Rodríguez-Estival *et al* (2012) who found blood lead levels in the range 6-35 μ g/dl in cattle and sheep grazing in lead mining area. The data also shows the mean blood lead levels of sheep being higher than goats due to difference in grazing habits of the two groups of animals. Smith *et al* (2009) studied metal uptake by sheep grazing in the metal polluted plains. According to authors the accumulation of metals in sheep tends to high due to ingestion of high quantities of metal polluted soils. In the present study the blood lead levels above 25 μ g/dl were found to give rise to clinical signs of lead toxicity in goats and sheep which are slightly lower than the values reported by Rodríguez- Estival *et al* (2012). The haematological parameters of exposed animals showed significant effects of lead resulting in low values of Hb, EC, PCV and MCV in exposed groups. It has been reported

Table 4. Mean ± SD lead level of Soil and grass at various distances in different directions of recycling complex

Distance (meters)	Sample	Direction Lead Mean ±SD (mg/kg)							
		N (n)	NE (n)	E (n)	SE (n)	S (n)	SW (n)	W (n)	NW (n)
50	Soil	700±85.2 (5)	1100±150.2 (4)	510±46 (5)	520±55.1 (10)	400±61.1 (7)	1050±95 (5)	430±31.5 (6)	370±25 (6)
	Grass	50±12.3 (7)	230±76.7(6)	120±33 (6)	128±48.2 (8)	75±22.7 (7)	225±55 (9)	80±49.0 (7)	115±34 (5)
250	Soil	580±68.1 (7)	1310±110.6 (7)	240±47.3 (4)	300±32.2 (9)	300±47.5 (5)	1050±41.3 (4)	320±17.53 (7)	310±34.4 (5)
	Grass	40±17.5 (9)	240±58.2 (5)	90±14.6 (5)	67±23.5 (7)	65±13.6 (6)	65±13.1 (8)	55±15.4 (5)	90±15.5 (7)
500	Soil	310±55.2 (5)	900±73.4 (8)	200±33.2 (8)	260±57.5 (5)	183±34.5 (8)	1060±55.6 (7)	210±34.6 (4)	270±17.3 (6)
	Grass	35±19.4 (11)	170±25.7 (7)	55±9.6 (8)	55±17.3 (12)	34±8.7 (8)	123±13.5 (6)	51±7.5 (8)	60±9.7 (9)
750	Soil	224±76.1 (8)	1200±79.4 (6)	130±11.2 (7)	230±66.3 (11)	125±18.3 (7)	1110±72.4 (9)	175±16.8 (10)	230±26.8 (9)
	Grass	35±12.3 (7)	110±22.1 (5)	50±8.2 (4)	50±12.7 (8)	30±9.6 (7)	164± 18.9 (5)	35±6.6 (9)	47±5.7 (5)
1000	Soil	135±30.8 (10)	1280±90.7 (8)	70±10.1 (6)	170±44.5 (9)	60±14.2 (5)	1245±64.3 (7)	80±13.8 (5)	150±18.3 (6)
	Grass	22±8.6 (9)	210±24 (7)	40±7.5 (6)	36±9.5 (7)	22±6.8 (8)	190±23.8 (8)	30±5.5 (5)	38±7.9 (10)

Table 5. Correlations: N; NE; E; SE; S; SW; W; NW

	N	NE	E	SE	S	SW	W
NE	-0.041 0.948						
E	0.914 0.030	-0.275 0.654					
SE	0.902 0.036	-0.239 0.698	0.997 0.000				
S	0.994 0.001	-0.135 0.828	0.947 0.015	0.935 0.020			
SW	-0.755 0.140	0.431 0.469	-0.672 0.214	-0.648 0.237	-0.783 0.117		
W	0.985 0.002	-0.149 0.811	0.951 0.013	0.944 0.016	0.996 0.000	-0.804 0.101	
NW	0.723 0.168	-0.577 0.308	0.913 0.031	0.912 0.031	0.794 0.109	-0.715 0.175	0.822 0.088

Pearson correlation
P value

Table 6. Lead content of some offal organ and muscle tissue samples of exposed and control animals

Animal	Tissue lead (Mean ±SD mg/kg wet-weight)		
	Liver	Kidney	Muscle
<u>Goat</u> (n)			
Exposed (9)	4.23± 1.37	3.91 ± 0.86	1.25± 1.13
Control (5)	0.53 ± 0.26	0.72 ± 0.32	ND
<u>Sheep</u> (n)			
Exposed (14)	6.02 ± 1.0	6.58 ± 0.73	2.26 ± 0.46
Control (5)	0.12 ± 0.86	0.85 ± 0.43	0.22 ± 0.17
<u>Chicken</u> (n)			
Exposed (17)	0.60 ± 0.17	1.43 ± 0.23	0.031 ± 0.0116
Control (11)	ND	ND	ND

that lead inhibits the activity of delta aminolevulinatase enzyme which is a primary precursor of heme synthesis in animals and humans. The inhibition of enzyme results in low haemoglobin levels causing anaemia. It is also known that part of the ingested lead by humans and animals finds way into blood and where more than 80% of it remains bound to erythrocytes (Moore, 1988). This results in destruction of red cells as well as decrease in packed cell volume and related parameters. The haematological results in the present study are also indicative of these effects manifesting lower haemoglobin EC count and other indices of exposed animals than controls. Rodríguez-Estival *et al* also noted similar effects in cattle and sheep from lead mining areas.

The animals used for study in the present work had three years of history of grazing on pasture grasses around recycling complex. The elevated lead levels in animals were therefore studied in relation to presence of lead in pasture grasses and soils around recycling complex. The data given in table 4 shows very high levels of lead varying between 200- 1400 mg/kg and 30-200 mg/kg for soil and grass samples respectively. Similar results have been reported by low *et al* and Palacio *et al*. The latter workers found lead levels in the range 127-5657 mg/kg and 113-4741 mg/kg in soil and herbage samples respectively in the vicinity of lead recycling smelter. According to study the lead content decreased at far distances but they did not consider the role of winds which are main transporters of lead containing emissions from smelters in different directions. In the present work wind in the study area was variable speed and direction therefore soil lead content was correlated using different directions. The results of pearsons correlation coefficients thus obtained are shown in table 5. It may be seen that the r values are highly significant $p < 0.05$ for the pairs of E-N, SE-N, SE-E, S-N, S-E, S-SE, W-E, W-S, NW-E, NW-SE, and NW-W directions showing role of winds in deposition of metal.

In subcontinent the offal meat is frequently used in the preparation of special cuisines. However the offal organs liver and kidney of cattle goat sheep and birds like chicken have been reported to store toxic metals including lead cadmium mercury and others (Humphreys 1991, Liu 2003). It was therefore thought necessary to determine the lead levels of the kidney, liver and femur muscle samples of exposed animals and compare them with controls. The results are shown in table 6. The lead levels in kidney and liver samples of exposed animals were found to be higher than the normal background levels reported for respective animals (Humphreys 1991). These results differ from the findings reported by Abu- Arab (2001) who

determined lead content of offal organs of unexposed sheep and goats. The results further indicate that mean values of lead in liver and kidney were higher than those in femur muscle of the animals. In view of extensive use of chicken in human diet the analysis of lead in chicken meat was also carried out. The table 6 also shows the results of lead levels found in offal organs and chest muscle of chickens from a poultry farm near recycling complex. As compared to normal healthy unexposed chickens the lead levels of exposed chickens were found to be very high showing impact of lead pollution by recycling complex.

5. CONCLUSIONS

The blood lead levels in animals may vary among different species and individual animals also vary in their susceptibility to toxic effect of lead. The Battery recycling activities pose constant dangers to the health of live stock farms of goats, sheep and poultry birds in the peripheral areas of the Hyderabad city. High levels of lead in blood and tissues of exposed animals may through food chain find way into humans and cause several health problems. Offal organs have potential to store lead despite of its low concentration in blood therefore these organs can be hazardous for consumers and care should be exercised while using them as diet. The study also sets an example for monitoring the lead levels of animals brought on slaughter houses and their meat processed for sale in the markets.

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