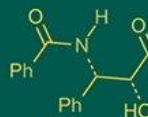


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Surveillance on heavy metal residues in muscle, liver and kidney of sheep slaughtered in Tamil Nadu

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Abstract

A study was undertaken to measure the concentration of heavy metal residues (cadmium, chromium, copper, iron, lead, nickel and zinc) in muscle, liver and kidneys of sheep collected from industrial and non-industrial areas of selected districts in Tamil Nadu viz., Coimbatore, Cuddalore and Salem at two distinct seasons (summer and rainy season). Fresh samples of sheep were randomly collected and subjected to acid digestion (wet ashing method) and subsequently analyzed for heavy metals using Inductively coupled plasma-optical emission spectrometry (ICP-OES). Data obtained from the study were statistically analyzed and interpreted. Results indicated that heavy metal concentrations varied from tissue to tissue within the same species. Samples collected from Coimbatore district has not revealed any excess metallic residues level than the permitted limit. Whereas tissues collected from Cuddalore and Salem district, had recorded slightly higher level of copper and iron in tissues of sheep (liver and kidney) than the maximum permissible limit set by FSSAI 2011 and significantly ($P < 0.05$) differed. From this study, it was concluded that, out of seven metallic residue concentration analyzed, four (cadmium, chromium, lead and zinc) were well below the maximum permissible level (MPL), prescribed by (FSSAI 2011) with the exception of copper, iron and nickel which had relatively higher concentration in all three tissues of sheep.

Keywords: Heavy metals, sheep tissues, cadmium, chromium, copper, lead and zinc

Introduction

India is endowed with rich livestock resources of 536.76 million, out of which sheep alone contributes 74 million and this population is progressively growing at the rate of 5.5% annually (AHS, 2024)^[1]. As per FAO (2025)^[11] report, India ranks 4th in the world in terms of total Meat production and the present meat production of India is estimated to be around 10.5 million tons, which is nearly 2.46% of the world's meat production. In India, sheep are most commonly raised under extensive system in which animals are allowed to graze on agriculture land, natural shrubs, bushes and pastures which makes them to perform more of exercise. Hence, the meat obtained from such animals is considered to be lean (low in fat and high in protein content) and it also contains several health promoting antioxidants like carnitine and aniserine. This attribute of mutton is one of the important reasons for fuelling demands in the domestic as well as global market. In Tamil Nadu, there are eight meat type sheep breeds viz., Madras red, Trichy black, Coimbatore, Nilgiris, Ramnad white, Kilakaraisal, Vembur and Nilagiri-sandyno, respectively (Thiruvankadan and Karunanithi, 2006)^[31].

Industrialization and urbanization are the two prime factors paved a way for environmental pollution. As percental pollution control board report, Tamil Nadu stands second place in the industrialization next to Maharashtra, but state owned pollution control board has regularly monitored and declared few places as industrially polluted area based on the level of pollutant prevailing in these places. Naturally, metals with a density of more than 5mg cm³ are considered heavy metals, and it generally includes elements such as arsenic, cadmium, chromium, copper, lead, nickel, molybdenum, vanadium and aluminum, other rare metals (Das and Das, 2018)^[8]. Humans are exposed to heavy metals mostly by ingestion and inhalation. Presence of heavy metal in high concentration was reported in water, soil and fodder from areas closer to industry (Singh *et al.*, 2019)^[30]. Among the different chemical pollutant, heavy metals is considered as one of the most important threats to human and animal's health as these metals cannot be degraded and stay permanently in the environment

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leading to bioaccumulation. Animals rose in such polluted areas or fed with feed and fodder grown from such areas, there is an ample opportunities to acquire and harbor higher level of these heavy metals in their produce viz., meat, milk and egg due to bioaccumulation and biomagnifications. Heavy metals gain entry into human body and results in accumulation in vital organs causing disorders such as necrosis of liver, damage of nephrons in kidney, hematological aberration and decline in growth rate (Mahimairaja and Shenbagavalli, 2007) [22]. It also gains greater significance owing to legal, commercial, bilateral relationship between the countries involved in export and import of meat and meat products. So the present study has been designed to evaluate the heavy metal residues in meat sold in Coimbatore, Cuddalore and Salem districts of Tamil Nadu.

Materials and Methods

The study was undertaken to estimate the heavy metal (cadmium, chromium, copper, iron, lead, nickel and zinc) residues in muscle, liver and kidneys of sheep collected from industrial and non-industrial areas of selected districts of Tamil Nadu, viz., Coimbatore, Cuddalore and Salem, as these areas are earmarked by the Tamil Nadu Pollution Control Board (TNPCB) as industrially polluted districts due to improper disposal of sewage or effluent into the environment. The procedures adapted for estimation of heavy metal residues have been discussed.

Collection of samples: Six hundred fresh samples viz., muscle, liver and kidneys of sheep, each weighing approximately 250 g (200 samples/each tissue) were randomly collected from various industrially polluted districts of Tamil Nadu viz., Coimbatore, Cuddalore, and Salem at two distinct seasons (summer and rainy). Out of these three districts, two distinct areas were ear-marked as industrial and nonindustrial area based on the number of industries and agricultural activities prevailing in that area. Special attention was focused while collecting samples from the identified regions, in order to get samples from the locally grown animals, under the guidance of Veterinary University Training and Research Centre's (VUTRC) and local veterinarians. All the samples were collected according to standard procedure prescribed by the (AOAC, 1990) [6], in order to minimize possible extraneous contamination and packed separately in clean polyethylene bags. The packed samples were then labeled (name of the sample, date and place of collection) and brought to the laboratory for further processing. Visible fat, blood clot and excessive connective tissues were removed manually, and stored in a deep freezer at -18 °C till digestion.

Processing of samples (Wet ashing of samples for heavy metal analysis): The frozen samples were thawed overnight in a refrigerator at (4±1 °C) and then digested by wet ashing method for the analysis of various heavy metals. Approximately 5 grams of samples were taken in 100 ml conical flask along with glass beads and 25 ml of triple glass distilled water added. About 10 ml each of concentrated nitric acid (Anal R grade) and 60% perchloric acid (Anal R grade) was added to the contents of the conical flask. The conical flask was heated for about 30 minutes at a temperature of 135 °C. Subsequently it was reduced to 70 °C and maintained, with intermittent shaking till the solution become clear. The solution was quantitatively transferred

into a 50 ml volumetric flask and made upto the mark with triple glass distilled water and mixed well and samples was used for heavy metal analysis.

Preparation of standard solution: The stock standard solutions and the working solutions for cadmium, chromium, copper, iron, lead, nickel and zinc were prepared as per standard methods in the manual ICP-OES (700 series). Calibration was done with multi-element calibration standards-(Calibration mix 3). Calibration of instrument was done on multiple wavelengths in aqueous medium linear range of 0.01ppm to 10ppm for cadmium, chromium, copper, iron, lead, nickel and zinc elements to measure the sample without dilution to reduce dilution error and have additional capacity of measuring simultaneous estimation of higher concentration of percentage and level elements along with ppm and ppb level elements.

Detection and quantification of heavy metals by ICP-OES: The main steps in analyzing samples by ICP-OES may be stated quite simply: The elements to be determined are selected. Solutions of the samples are prepared, using the conventional techniques of quantitative chemical analysis. A set of calibrating solutions is prepared. Each solution contains accurately known concentrations of the analyte elements, and the range of concentrations for each element in the set is chosen to include the expected concentration of that element in the sample solutions. The calibrating solutions and sample solutions are sprayed into the plasma and the intensities of appropriate emission lines are recorded. Calibration graphs ('analytical working curves') are prepared for each element from the emission intensities of the calibrating solutions. The concentrations of the elements in each sample solution are determined from the calibration graphs. The concentrations in the original sample are then calculated from the measured concentrations of the elements in the sample solution and the known dilution factor. Automated spectrometers, with computers and programmable sample changers, are generally used. This can perform graph calibration and calculation. Inductively coupled plasma-optical emission spectrometer (ICP-OES) consists of four major components viz., the sample introduction system, the excitation source, the spectrometer, the detection system.

Estimation of metals (Cd, Cr Cu, Fe, Pb, Ni and Zn): Calibration of instrument was done on multiple wavelengths in aqueous medium linear range of 0.01ppm to 10ppm for below mentioned elements to measure the sample without dilution to reduce dilution error and save time and have additional capability of measuring simultaneous estimation of higher concentration of percentage level elements along with ppm and ppb level elements.

Wavelength used for quantification of elements: Cadmium (Cr)-214.439, Chromium (Cr)-267.716, Copper (Cu)-324.754, Iron (Fe)-238.892, Lead (Pb)-220.353, Nickel (Ni)-216.555, Zinc (Zn)-213.857.

Statistical Analysis: Statistical differences between the different areas are (industrial and non-industrial area) of selected district of Tamil Nadu were determined by using the statistical package SPSS 14.0 version (SPCC Inc., Chicago, USA 2005)

Results and Discussions

The range of metallic residues viz., cadmium, chromium, copper, iron, lead, nickel and zinc in the tissues of sheep collected from the three districts (Coimbatore, Cuddalore and Salem) of Tamil Nadu are presented in Table 1, 2 and 3 respectively.

Cadmium: Highest concentration of cadmium was found in kidneys (0.91ppm) collected from industrial area during rainy season (In Cuddalore) whereas lowest concentration in muscle (0.01ppm) collected from non-industrial area during summer season (In Coimbatore). No significant difference was observed for cadmium concentration within the three tissues of sheep. In India, FSSAI, (2011) ^[13] has prescribed a standard of (1.5ppm) for cadmium in food. However, none of the samples in this study had cadmium content exceeding the permissible limit. This might be due to raising of sheep in uncontaminated grazing area and not accessibility to sewage sludge contaminated lands or fertilized land with super phosphate pointed out by (Goyer, 1991) ^[15]; and also animals are not accessed to cadmium contaminated areas viz., mines, smelting plants and metallurgical, galvanotechnical and dye works (Bartik and Piskac, 1981) ^[7]. Out of three tissues, the highest concentration of cadmium was observed in kidney tissues; may be due to the detoxification function of the organ where this metal get accumulated and kidney is considered as a target organ for cadmium toxicity. This suggests that cadmium pollution is not severe in the areas which were studied. The probable reason might also be the practice of slaughtering animals at young age, as these heavy metals are being bio accumulative in nature; the chances of deposition in the tissues are very rare. Kazemeini *et al.*, (2009) ^[19] who found the concentration of cadmium in muscle, liver and kidneys of 60 animals around 1 to 6 years old sheep carcass slaughtered in Flavarjan abattoir, Isfahan were 0.002, 0.07, 0.02 (mg/kg) respectively. These values were lower than the maximum acceptable concentration proposed by European Commission (EC, 2004) ^[10].

Chromium: Highest concentration of chromium was found in liver (2.11ppm) collected from industrial area during summer (In Cuddalore) whereas lowest concentration in muscle (0.01ppm) collected from industrial area during rainy (In Coimbatore). Significant difference was not observed within the three tissues of sheep for chromium content. The level of chromium in all the tissues of sheep were much below 30 ppm which was considered as minimum tissue level for chromate poisoning. The concentration reported in the present study were below the concentration obtained by Premanand *et al.* (2014) ^[28] who reported chromium content of 4.31ppm in muscle and 2.83 ppm in liver and 2.87 in kidney of sheep tissue collected from animals slaughtered at Puducherry Municipality slaughter house. Abraham *et al.* (2008) ^[3] reported that chromium concentration was below detectable level in muscle, liver and kidney of sheep where as for goat tissues the values (1.38-1.80 ppm) were little higher than those recorded in the present study. These variations in levels of chromium in sheep and goat tissues might be due to variation in chromium contents of the forages/grasses/plants on which the animals had grazed. Moreover, high concentration of chromium is found in tissues of animals which are grown in the vicinity of metal processing unit, tannery, refinery, asbestos product units which are the major

sources of atmospheric chromium emission (Towill *et al.* 1978) ^[33]. The level of chromium recorded in the tissues of sheep and goat collected from industrial area and non-industrial area of different districts of Tamil Nadu were below the level reported by Harrison and Staples (1955) ^[16] who has stated that the tissue levels of 30ppm in the liver and 4µg/ml in the whole blood is confirmed of chromate poisoning. In India, there is no specific standard limit for chromium content in fresh meat. The low level of chromium recorded in the tissues of sheep and goat collected from industrial and non-industrial area in different districts of Tamil Nadu, might be due to non accession of animals to graze in the potential anthropogenic source of chromium viz., electroplating factories, leather tanneries, wood preserving, metal processing, chromium plating, alloy preparation, petroleum refining and manufacturing of automobile parts and textile manufacturing facilities (Panov *et al.*, 2003 ^[27]; Huang *et al.*, 2004) ^[17]. Lower than this value was reported by Gawdaman *et al.*, (2010) ^[14] who found that the chromium content in tissues of cattle collected from open market in Puducherry region were 1.77 ppm in kidneys and 1.34ppm in liver.

Copper: Highest concentration of copper was found in liver (26.84ppm) collected from industrial area during summer (In Cuddalore) whereas lowest concentration in muscle (0.03ppm) collected from the non-industrial area during rainy (In Coimbatore). Highly significant difference ($p < 0.01$) was noticed within the tissues of sheep collected from non-industrial area whereas significant difference ($p < 0.05$) in industrial area for both the seasons. The maximum permissible limit prescribed by FSSAI (2011) ^[13] for copper in meat products is 20 ppm. However, the concentration of copper in all three tissues of sheep recorded in the present study was higher than the prescribed limit (irrespective of area and season). These values were lower than those reported by Abraham *et al.* (2008) ^[3] who recorded copper concentration to the tune of 5.17, 17.36 and 5.85 ppm in muscle, liver and kidney samples of sheep respectively from Chennai region. In comparison to goats, sheep accumulated higher concentration of copper in muscle and liver. But goat kidney had higher level of copper. These species differences might be due to their differences in grazing pattern, age, exposure to different copper levels through feeds and fodder. The reason for variation among tissues within a species might be due to the variation in their composition and biological activity. The lower level of copper in the tissues of sheep and goat collected from industrial and non-industrial areas of selected district of Tamil Nadu might be due to non-exposure of animals to the environmentally contaminated areas, excessive amounts of copper supplementation or feeds that have not been contaminated with copper compounds used for agricultural or industrial purposed (Underwood, 1977) ^[34]. Out of three tissues analyzed for copper, the higher concentration was observed in liver tissues followed by kidneys and muscle; is due to the metabolic function of the organ where this metal gets accumulated. According to Bartik and Piskac (1981) ^[7] certain plants or vegetable compounds influenced copper storage in liver of sheep. For example, long term feeding of clover caused toxic retention of copper in the liver and its action was combined with the effects of other factors. Grazing on heliotrope by sheep for a long time also caused accumulation of copper in the damaged liver. Higher than this value was reported by Abraham *et al.*, (1998) ^[4] who

found that the highest copper concentration of 117.36 and 88.95ppm in liver of sheep slaughtered at Madras Veterinary College as well as market samples respectively, without any pathological lesion on post-mortem. Mariam *et al.*, (2004) [23] reported that the copper content was 81.51

and 5.01ppm in lean meat; 93.24 and 318.82ppm in liver and in kidneys 5.42 and 6.40ppm in beef and mutton, respectively. Gawdaman *et al.*, (2010) [14] found a copper concentration in liver of 6.3ppm in cattle slaughtered at Puducherry region.

Table 1: Mean $\pm$ S.E. of heavy metal contents (ppm) in muscle, liver and kidneys of sheep collected from Coimbatore district

Elements (ppm)	Season	Area(s)	Muscle	Liver	Kidneys
Cadmium (Cd)	Summer	Industrial	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00 ^A	0.05 $\pm$ 0.00
		Non-industrial	0.01 $\pm$ 0.00	0.14 $\pm$ 0.06 ^B	0.08 $\pm$ 0.00
	Rainy	Industrial	0.22 $\pm$ 0.04 ^B	0.26 $\pm$ 0.05	0.31 $\pm$ 0.04 ^B
		Non-industrial	0.02 $\pm$ 0.00 ^A	0.14 $\pm$ 0.06	0.08 $\pm$ 0.00 ^A
Chromium (Cr)	Summer	Industrial	0.23 $\pm$ 0.01	0.26 $\pm$ 0.09 ^B	0.15 $\pm$ 0.07
		Non-industrial	0.04 $\pm$ 0.00	0.02 $\pm$ 0.00 ^A	0.01 $\pm$ 0.00
	Rainy	Industrial	0.01 $\pm$ 0.00	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00
		Non-industrial	0.03 $\pm$ 0.00	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00
Copper (Cu)	Summer	Industrial	1.97 $\pm$ 0.44 ^{aA}	8.00 $\pm$ 1.65 ^{bA}	2.79 $\pm$ 0.56 ^{aB}
		Non-industrial	4.95 $\pm$ 0.83 ^{bB}	17.04 $\pm$ 2.74 ^{cB}	1.28 $\pm$ 0.09 ^{aA}
	Rainy	Industrial	0.26 $\pm$ 0.04 ^{aB}	3.69 $\pm$ 1.58 ^c	1.60 $\pm$ 0.23 ^b
		Non-industrial	0.03 $\pm$ 0.00 ^{aA}	5.15 $\pm$ 0.85 ^b	1.71 $\pm$ 0.24 ^a
Iron (Fe)	Summer	Industrial	5.96 $\pm$ 0.83 ^{aA}	41.46 $\pm$ 7.07 ^c	30.29 $\pm$ 4.67 ^{bA}
		Non-industrial	20.42 $\pm$ 5.33 ^B	32.50 $\pm$ 5.85	32.22 $\pm$ 5.87 ^B
	Rainy	Industrial	9.05 $\pm$ 2.10 ^a	20.19 $\pm$ 2.13 ^{bA}	43.49 $\pm$ 3.03 ^c
		Non-industrial	11.03 $\pm$ 1.72 ^a	30.39 $\pm$ 2.73 ^{bB}	42.66 $\pm$ 4.59 ^c
Lead (Pb)	Summer	Industrial	0.08 $\pm$ 0.03 ^a	0.03 $\pm$ 0.00 ^a	0.23 $\pm$ 0.07 ^{bB}
		Non-industrial	0.02 $\pm$ 0.00	0.04 $\pm$ 0.00	0.03 $\pm$ 0.00 ^A
	Rainy	Industrial	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00	0.09 $\pm$ 0.02
		Non-industrial	0.02 $\pm$ 0.00	0.03 $\pm$ 0.00	0.04 $\pm$ 0.00
Nickel (Ni)	Summer	Industrial	0.33 $\pm$ 0.05 ^B	0.72 $\pm$ 0.21 ^B	0.51 $\pm$ 0.01 ^B
		Non-industrial	0.03 $\pm$ 0.01 ^A	0.07 $\pm$ 0.00 ^A	0.02 $\pm$ 0.00 ^A
	Rainy	Industrial	0.09 $\pm$ 0.03 ^A	0.18 $\pm$ 0.04	0.19 $\pm$ 0.04 ^A
		Non-industrial	0.17 $\pm$ 0.02 ^{aB}	0.28 $\pm$ 0.02 ^b	0.23 $\pm$ 0.03 ^{bB}
Zinc (Zn)	Summer	Industrial	9.44 $\pm$ 1.93	7.74 $\pm$ 1.69 ^B	3.42 $\pm$ 0.55 ^B
		Non-industrial	6.43 $\pm$ 1.50 ^c	4.09 $\pm$ 0.39 ^{aA}	2.17 $\pm$ 0.27 ^{aA}
	Rainy	Industrial	2.09 $\pm$ 0.25 ^a	6.25 $\pm$ 0.99 ^{bB}	5.42 $\pm$ 0.54 ^{bB}
		Non-industrial	2.29 $\pm$ 0.23	2.09 $\pm$ 0.28 ^A	2.17 $\pm$ 0.27 ^A

Means bearing different superscripts in a row (^{abc}) and in a column (^{AB}) differ significantly **-significance at ($p < 0.01$); *-significance at ($p < 0.05$)

Table 2: Mean $\pm$ S.E. of heavy metal contents (ppm) in muscle, liver and kidneys of sheep collected from Cudallore district

Elements (ppm)	Seasons	Area(s)	Muscle	Liver	Kidneys
Cadmium (Cd)	Summer	Industrial	0.24 $\pm$ 0.07 ^{bB}	0.25 $\pm$ 0.03 ^{bB}	0.14 $\pm$ 0.05 ^{aB}
		Non-industrial	0.03 $\pm$ 0.00 ^A	0.04 $\pm$ 0.00 ^A	0.06 $\pm$ 0.00 ^A
	Rainy	Industrial	0.02 $\pm$ 0.00	0.04 $\pm$ 0.00 ^B	0.91 $\pm$ 0.00
		Non-industrial	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00 ^A	0.02 $\pm$ 0.00
Chromium (Cr)	Summer	Industrial	1.67 $\pm$ 0.34 ^{aB}	2.11 $\pm$ 0.33 ^{bB}	1.71 $\pm$ 0.35 ^{aB}
		Non-industrial	0.05 $\pm$ 0.00 ^A	0.06 $\pm$ 0.00 ^A	0.08 $\pm$ 0.01 ^A
	Rainy	Industrial	2.90 $\pm$ 0.97	0.97 $\pm$ 0.26 ^B	0.92 $\pm$ 0.20 ^B
		Non-industrial	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00 ^A	0.03 $\pm$ 0.00 ^A
Copper (Cu)	Summer	Industrial	9.95 $\pm$ 2.23 ^{aB}	26.84 $\pm$ 5.54 ^{bB}	10.61 $\pm$ 2.86 ^{aB}
		Non-industrial	1.10 $\pm$ 0.03 ^{aA}	5.09 $\pm$ 1.18 ^{aA}	1.43 $\pm$ 0.43 ^{aA}
	Rainy	Industrial	3.75 $\pm$ 1.03 ^{bB}	11.45 $\pm$ 2.9 ^{cB}	1.66 $\pm$ 0.42 ^{aB}
		Non-industrial	0.16 $\pm$ 0.03 ^{aA}	3.23 $\pm$ 0.73 ^{cA}	0.61 $\pm$ 0.12 ^{bA}
Iron (Fe)	Summer	Industrial	36.27 $\pm$ 4.1 ^{aB}	77.26 $\pm$ 10.2 ^{cB}	46.49 $\pm$ 5.26 ^{bB}
		Non-industrial	1.33 $\pm$ 0.11 ^A	2.29 $\pm$ 0.32 ^A	4.16 $\pm$ 0.72 ^A
	Rainy	Industrial	17.27 $\pm$ 3.3 ^B	6.26 $\pm$ 1.22 ^B	27.09 $\pm$ 3.92 ^B
		Non-industrial	2.54 $\pm$ 0.22 ^A	2.41 $\pm$ 3.80 ^A	5.06 $\pm$ 0.62 ^A
Lead (Pb)	Summer	Industrial	0.09 $\pm$ 0.03 ^B	0.13 $\pm$ 0.03 ^B	0.09 $\pm$ 0.02 ^B
		Non-industrial	0.01 $\pm$ 0.00 ^A	0.04 $\pm$ 0.00 ^A	0.02 $\pm$ 0.00 ^A
	Rainy	Industrial	0.04 $\pm$ 0.01 ^B	0.03 $\pm$ 0.00 ^B	0.06 $\pm$ 0.02
		Non-industrial	0.03 $\pm$ 0.00 ^A	0.02 $\pm$ 0.00 ^A	0.04 $\pm$ 0.01
Nickel (Ni)	Summer	Industrial	0.19 $\pm$ 0.03	0.26 $\pm$ 0.02 ^B	0.18 $\pm$ 0.03 ^B
		Non-industrial	0.29 $\pm$ 0.15	0.03 $\pm$ 0.00 ^A	0.02 $\pm$ 0.00 ^A
	Rainy	Industrial	0.34 $\pm$ 0.06 ^B	0.28 $\pm$ 0.03 ^B	0.27 $\pm$ 0.02 ^A
		Non-industrial	0.01 $\pm$ 0.00 ^A	0.04 $\pm$ 0.01 ^A	1.07 $\pm$ 0.42 ^B
Zinc (Zn)	Summer	Industrial	32.43 $\pm$ 4.54 ^{cB}	19.43 $\pm$ 3.34 ^{aB}	28.50 $\pm$ 4.01 ^{bB}
		Non-industrial	2.71 $\pm$ 0.38 ^A	1.21 $\pm$ 0.17 ^A	1.48 $\pm$ 0.14 ^A
	Rainy	Industrial	22.19 $\pm$ 4.29 ^{bB}	16.59 $\pm$ 2.08 ^{aB}	16.02 $\pm$ 7.89 ^{aB}
		Non-industrial	4.79 $\pm$ 0.49 ^A	3.97 $\pm$ 0.43 ^A	3.19 $\pm$ 0.27 ^A

Means bearing different superscripts in a row (^{abc}) and in a column (^{AB}) differ significantly **-significance at ($p < 0.01$); *-significance at ($p < 0.05$)

Iron: Highest concentration of iron was found in liver (123ppm) collected from industrial area during rainy (In Salem) whereas lowest concentration in muscle (1.33ppm) collected from non-industrial area during summer (In Cuddalore). Highly significant difference ($p<0.01$) was noticed for both the areas during rainy season whereas only in industrial area during summer. Abou-Arab (2001) [2] reported iron content (mg/g wet weight) for sheep and goat collected from Kasuwan Shance Market in Maiduguri metropolis, Borno state, Nigeria was 3.76, 3.98 in liver, 3.07, 2.51 in kidneys, respectively. Higher than this value

was reported by Adei and Forsen-Adaboh (2008) [5] who found the iron content which ranged from 341.6-376.6, 327.8-374.2 (mg/kg) in liver of sheep and goat collected from Kumasi and Accra market, respectively. Medeiros *et al.*, (1989) [24] who had recorded the range of iron content in ovine muscle sample were 12.90 to 15.30mg/kg. Nwude *et al.*, (2010) [26] reported that the level of iron in muscle of cattle slaughtered during three different seasons ranged from not detectable at the onset of rainy season; 0.92 to 1.40 at the peak of rainy season and 0.70 to 1.50 mg/kg during the dry season.

Table 3: Mean $\pm$ S.E. of heavy metal contents (ppm) in muscle, liver and kidneys of sheep collected from Salem district

Elements (ppm)	Seasons	Area(s)	Muscle	Liver	Kidneys
Cadmium (Cd)	Summer	Industrial	0.01 $\pm$ 0.00	0.05 $\pm$ 0.01	0.04 $\pm$ 0.00
		Non-industrial	0.03 $\pm$ 0.01 ^a	0.07 $\pm$ 0.01 ^b	0.03 $\pm$ 0.00 ^a
	Rainy	Industrial	0.09 $\pm$ 0.01 ^a	0.11 $\pm$ 0.02 ^b	0.08 $\pm$ 0.01 ^a
		Non-industrial	0.10 $\pm$ 0.02	0.19 $\pm$ 0.06	0.12 $\pm$ 0.00
Chromium (Cr)	Summer	Industrial	0.33 $\pm$ 0.13	0.95 $\pm$ 0.41	0.60 $\pm$ 0.19
		Non-industrial	0.54 $\pm$ 0.08	1.06 $\pm$ 0.23	0.31 $\pm$ 0.09
	Rainy	Industrial	1.32 $\pm$ 0.29 ^B	1.37 $\pm$ 0.32 ^B	0.93 $\pm$ 0.22 ^B
		Non-industrial	0.04 $\pm$ 0.00 ^{bA}	0.01 $\pm$ 0.00 ^{aA}	0.03 $\pm$ 0.00 ^{aA}
Copper (Cu)	Summer	Industrial	1.97 $\pm$ 0.27 ^a	26.82 $\pm$ 5.45 ^{bB}	25.00 $\pm$ 3.97 ^b
		Non-industrial	2.29 $\pm$ 0.588	24.13 $\pm$ 4.74 ^{aA}	2.71 $\pm$ 0.66
	Rainy	Industrial	2.68 $\pm$ 0.45 ^B	12.55 $\pm$ 8.70 ^B	5.24 $\pm$ 0.46 ^b
		Non-industrial	0.24 $\pm$ 0.13 ^{aA}	2.89 $\pm$ 1.14 ^{bA}	0.27 $\pm$ 0.03 ^a
Iron (Fe)	Summer	Industrial	50.51 $\pm$ 3.93 ^{bB}	87.21 $\pm$ 21.33 ^c	31.61 $\pm$ 6.53 ^a
		Non-industrial	18.61 $\pm$ 2.88 ^{bA}	53.19 $\pm$ 6.15 ^c	38.71 $\pm$ 3.71 ^b
	Rainy	Industrial	36.27 $\pm$ 4.10 ^{bB}	123.81 $\pm$ 28.4 ^{bB}	30.90 $\pm$ 4.08 ^a
		Non-industrial	21.61 $\pm$ 1.90 ^{bA}	53.19 $\pm$ 6.17 ^{bA}	38.71 $\pm$ 3.71 ^a
Lead (Pb)	Summer	Industrial	0.14 $\pm$ 0.03 ^B	0.08 $\pm$ 0.01 ^B	0.16 $\pm$ 0.01
		Non-industrial	0.01 $\pm$ 0.00 ^A	0.04 $\pm$ 0.00 ^A	0.07 $\pm$ 0.02
	Rainy	Industrial	0.15 $\pm$ 0.03 ^B	0.17 $\pm$ 0.02	0.14 $\pm$ 0.01 ^B
		Non-industrial	0.02 $\pm$ 0.00 ^A	0.01 $\pm$ 0.00	0.08 $\pm$ 0.00 ^A
Nickel (Ni)	Summer	Industrial	0.21 $\pm$ 0.07	0.39 $\pm$ 0.08	0.24 $\pm$ 0.02
		Non-industrial	0.15 $\pm$ 0.03 ^a	0.38 $\pm$ 0.07 ^b	0.27 $\pm$ 0.04 ^a
	Rainy	Industrial	0.39 $\pm$ 0.11 ^B	0.29 $\pm$ 0.06	0.17 $\pm$ 0.03
		Non-industrial	0.04 $\pm$ 0.01 ^A	0.17 $\pm$ 0.05	0.19 $\pm$ 0.03
Zinc (Zn)	Summer	Industrial	24.12 $\pm$ 4.56 ^B	46.84 $\pm$ 7.39 ^B	44.16 $\pm$ 7.55 ^B
		Non-industrial	4.61 $\pm$ 1.17 ^A	13.34 $\pm$ 3.14 ^A	19.83 $\pm$ 3.15 ^A
	Rainy	Industrial	34.29 $\pm$ 3.80 ^B	37.47 $\pm$ 5.15 ^{Bb}	29.58 $\pm$ 3.22 ^{aB}
		Non-industrial	1.89 $\pm$ 0.27 ^{aA}	2.40 $\pm$ 0.47 ^{bA}	1.77 $\pm$ 0.24 ^{aA}

Means bearing different superscripts in a row (^{abc}) and in a column (^{AB}) differ significantly **-significance at ($p<0.01$); *-significance at ($p<0.05$)

Lead: Highest concentration of lead was found in kidneys (0.23 ppm) collected from industrial area during summer (In Coimbatore) whereas lowest concentration in muscle (0.01 ppm) collected from non-industrial area during summer (In Cuddalore). Significant difference ($p<0.05$) was noticed within the tissues of sheep collected from industrial area during summer. The concentrations of lead in these tissues of sheep were above the permissible limit of 2.5 ppm prescribed by FSSAI (2011) [13]. The concentration reported in the present study were below the concentration obtained by Premanand *et al.* (2014) [28] who reported a lead content of 4.83ppm in muscle and 4.58 ppm in liver and 4.41 in kidney of sheep tissue collected from animals slaughtered at Puducherry Municipality slaughter house. Abraham *et al.* (2008) [3] reported that lead contents were below detectable level in organs and muscle of sheep and goat reared and slaughtered at Madras Veterinary College, Chennai, India whereas in market slaughtered goats level of lead were 27.39 $\pm$ 0.62, 29.84 $\pm$ 1.16, 27.06 $\pm$ 0.54 ppm in kidney, liver and muscle, respectively. Higher concentration of lead in muscle and organs of sheep or goat were attributed to air

pollution due to vehicular emission, eating of wall papers by sheep and goats and water pollution. In India, FSSAI, (2011) [13] has fixed a specific standard for lead (2.5ppm) in the food. The level of lead recorded in this study was much lower than the permissible limit. The lower level of lead in these tissues of sheep might be due, the recent years, there has been a remarkable decline in lead levels in animal tissues throughout the developed countries; attributable to the phasing-out of leaded petrol. Moreover, these animals might not be exposed to the source of lead dioxide particulates or vapours of alkyl lead that had been evaporated from automobile fuel system would have deposited in the plants grown on the road sides or the adjoining area and there were chances for animals to consume these plants during grazing to get accumulated in the tissues, Goyer (1991) [15]. On contrary to this value Fonglan *et al.*, (1998) [12] who found that the mean value of lead in sheep tissues viz., muscle, liver and kidneys collected from industrial areas of Tamil Nadu were 4.20, 12.56 and 1.38mg/kg, respectively.

Nickel: Highest concentration of nickel was found in kidney (1.07ppm) collected from non-industrial area during rainy (In Cuddalore) whereas lowest concentration in muscle (0.01ppm) collected from non-industrial area during rainy (In Cuddalore). Significant difference ($p < 0.05$) was noticed within the tissues of sheep collected from non-industrial area during rainy season. The permissible limit of nickel in food was below 0.5mg/kg, according to WHO and USSR standard. Miranda *et al.*, (2009) [25] studied metal accumulation in cattle raised in serpentine soil area and observed that high percentage of the animals showed tissue concentration of nickel and copper. Korenekova *et al.*, (2002) [20] reported the highest nickel in the analyzed biological material of cattle slaughtered on agricultural farms in the fallout region of metallurgical plant was 0.231 mg/kg. Iwegbue *et al.*, (2008) [18] reported that the range of nickel concentrations (Ni) in chicken meat, chicken gizzard and turkey meat consumed in southern Nigeria were 0.01-1.37 (mg/kg). Higher than this value was reported by Tomovic *et al.*, (2010) [32] who found the content of nickel in the *M. Semimembranosus* of sixty-nine pigs from ten different genetic lines produced in Vojvodina. Nickel levels ranged from 12.93 to 80.18 μ g/100 g, with a general average of 32.41 μ g/100 g. The average level of nickel was found to be higher than the levels observed in pork in some developed countries.

Zinc:

Highest concentration of zinc was found in liver (46.84ppm) collected from industrial area during summer (In Salem) whereas lowest concentration in liver (1.21ppm) collected from non-industrial area during summer (In Cuddalore). Highly significant difference ($p < 0.01$) was noticed within the tissues of sheep collected from industrial area during rainy season whereas significant difference ($p < 0.05$) was noticed in non-industrial area during summer.

The concentration of zinc in muscle, liver and kidney of sheep were well below the permissible limits (50 ppm) prescribed by FSSAI (2011) [13]. Similar results were reported by Sarican *et al.* (1976) [29] who reported a zinc concentration in muscle of sheep (28.80 μ g/g). Abraham *et al.* (2008) [3] reported lower concentration of zinc range in kidney, liver and muscle of sheep (5.10-14.05 ppm). Similar result was reported by Langlands *et al.*, (1987) [21] reported that the survey conducted for zinc content in muscle, liver and kidneys of sheep raised in Australia were 38.2, 39.9 and 24.4mg/kg respectively. He also pointed out the concentration of copper in liver of 29.0 mg/kg collected from Victoria and 85.0 mg/kg which was collected from New South Wales. Lower than this value was noticed by Delowski (1973) [9] who investigated zinc concentration in tissue of cattle slaughtered at two different seasons. He found the zinc concentration of 0.24, 0.20mg/100ml in blood; 0.125, 0.125mg/100ml in plasma; 0.126, 0.09mg/100ml in serum, 3.42, 2.85mg/100g in liver, 2.32, 2.02mg/100g in spleen, 2.61, 2.25mg/100g in pancreas and 1.9, 1.9mg/100g in kidneys for summer and winter season, respectively. Finally, he concluded that concentration of zinc in the tissues of cattle was higher during summer than the winter season. Gawdaman *et al.*, (2010) [14] reported that the level of zinc in tissues of cattle and pig slaughtered in Puducherry region were 34.80, 38.65ppm for muscle, 42.41, 34.12ppm; for liver and 22.78, 39.46ppm; for kidneys, respectively. Higher than this value was reported

by Fonglan (1998) [12] who found the concentration of zinc (mg/kg) in kidneys of sheep collected from industrial and non-industrial areas of Tamil Nadu were 64.75 and 64.42, respectively.

Conclusion

Present study indicates that, the concentration of heavy metal in liver tissue, industrial area and summer season is higher than the other tissue, area and season. Out of seven heavy metals (Cadmium, Chromium, Copper, Iron, Lead, Nickel and Zinc) studied, mean concentration of four heavy metals (Cadmium, Chromium, Lead and Zinc) were below the Maximum permissible limit (MPL) prescribed by (FSSAI, 2011) except copper, iron and nickel which had relatively higher concentrations in all three tissues of sheep. However, the study points towards the fact that long term assessment and monitoring of harmful heavy metals in sheep, with much robust number of samples, is very much essential from the point of ensuring consumer safety. In addition, appropriate steps need to be taken to prevent heavy metals from entering into the food chain. This study will also help to fix standards for metals like chromium, iron and nickel for which no standards are available in FSSAI, (2011) [13].

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