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Metallic trace elements in blood, wool, kidney and liver of sheep from a mine area of marrakech-Morocco

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ABSTRACT

This study evaluated the contribution of anthropogenic pollution to toxic metal residues(Cd, Cu, Pb and Zn) in sheep in Draa Lasfar mining area of Marrakech(Morocco). Samples of blood, wool, liver and kidney from animals aged 24-60 months were obtained from the mining area(50 sheep) and from a rural area(12 sheep). Samples were acid-digested, and levels of metals determined by atomic absorption spectrophotometry. Sheep from Drâa Lasfar mining area showed higher tissue levels of Cd, Cu, Pb and Zn than sheep from the rural area. The most effect of pollution on Cd, Pb and Zn were seen in the kidney (PFCd-k=3,7 ; PFPb-k=1,7 and PFZn-k=1,4), and on Cu in the liver(PFCu-l=2,2). As these trace elements are known to be highly toxic compounds to which chronic exposure results in severe diseases or even death, there is an urgent need to initiate an extensive epidemiological study of people consuming animal products originating from this area.

INTRODUCTION

Although metals are present naturally in the Earth's crust at various levels and many metals are essential for cells(e.g. copper(Cu), iron(Fe), manganese(Mn), nickel (Ni), zinc (Zn)), all metals are toxic at higher concen trations[14,49,77].

A variety of human activities, notably industrial and mining process have been responsible for the wider diffusion of heavy metals into the environment[47].

The accumulation of heavy metals and metalloids in agricultural soils is of increasing concern due to the food safety issues and potential health risks as well as its detrimental effects on soil ecosystems[19,14,49].

The threat of heavy metals to human and animal health is aggravated by their long-term persistence in the environment[21,77], they may be transferred and accumulated in the bodies of animals or human beings through food chain, which will probably include teratogenesis, anomalies in reproduction[45,46], cause DNA damage and carcinogenic effects by their mutagenic ability[15,39,49,77].

The main aim of the present study was to determine the presence of some trace elements in blood, kidney, liver and wool of ovine reared and slaughtered in two villages near a mine area(District of Draa Lasfar) located in north-west of the Mrabtine zone at approximately 10Km in the west of Marrakech city (figure 1).

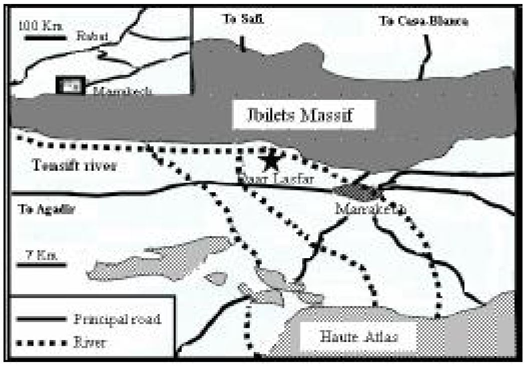

Figure 1 : Draa Lasfar mine localisation in marrakech region

The liver and the kidney were examined because they accumulate the highest concentrations of heavy metals[63,61,44]. The wool was examined because the current scientific approach is to use a biological material which is easy to collect and to preserve[34,35,36,30]. Its analysis can make it possible to detect and quantify the presence of toxic metals (such as mercury, lead, cadmium, arsenic.) in the animals' organism and thus reveal any exposure to these pollutants[30,66].

Toxic accumulation kinetics

Several investigations have studied the transfer of heavy metals from soil to animals either by direct contamination or via the vegetation[57,50].

Derache[16]defined the ratio Ci-Cs/Ci, known as the extraction-ratio or elimination-ratio that measures the rate of extraction(Ke) of a pollutant. He also defined a clearance of the toxic substance which represented the virtual volume of tissues completely removed from the substance per unit of time.

$$Cl = Ke \times V$$

The process of elimination of metallic trace elements is exponential[16].

$$dCt/dt = -Ke.Ci \quad dCt/Ci = -Ke.dt$$

$$\int dCt/Ci = -\int Ke.dt$$

$$LnCt/Ci = -Ke.t. \qquad (1)$$

$$Ct/Ci = \exp(-Ke.t)$$

$$Ct = Ci.\exp(-Ke.t)$$

In addition, expression of pollutant concentration accumulated in the organism is:

$$Ca = Ci - Ct$$

(1) becomes

$$Cs = Ci(1-\exp(-Ke.t))$$

MATERIAL AND METHODS

Sampling

50 sheep were the subject of this study. They belong to owners of Draa Lasfar area. The majority of the sheep was born and/or reared at least one year on the spot. They are distributed as following:

- 8 sheep of less than two years including 4 females and 4 males.
- 12 sheep between two and three years including 4 females and 8 males.
- 4 sheep between three and four years including 1 female and 3 males.
- 26 sheep of more than four years including 11 females and 15 males.

All the sheep receive the same feed ration composed of bad grasses(Couch grass mainly), they water from the same source: the Tensift river receiving the Draa Lasfar mine wastewater.

Control samples were collected from 12 sheep raised in a rural area. The rural area selected was at approximately 35Km in the southern part of the Marrakech region, to minimize the possible influence of atmospheric deposition from the industrial region(the prevailing wind direction in Marrakech is from the northwest).

Before sampling blood, approximately 10g of wool of each animal are taken on from the nape of the neck then placed in plastic bags and numbered.

Sampling blood was carried out by puncture in the jugular vein after careful disinfection using the cotton soaked with surgical alcohol. Approximately 3ml of blood were collected aseptically with needles of single use and stored in a heparinised tube. Tubes are numbered and placed in a refrigerator.

Liver and kidney (including both cortex and medulla) samples were collected immediately after slaughter. All samples were packed in numbered plastic bags and immediately transported to the laboratory and stored at -18°C until analysis.

Preparation of samples

1. Liver and kidney

Approximately 1g sub-samples were dried to a constant weight at 80^0C and then powdered. Accurately weighted aliquots of approximately 100mg of each powder organ were digested in 2ml of concentrated nitric acid at 250^{0}C for 4h. The volume was adjusted to 20ml double distilled water[64].

2. Blood

Approximately 2ml of blood sub-samples were dried to constant weight at 85^{0}C. Dried samples were cold digested in 1.5ml of concentrated nitric acid overnight[44].

3. Wool

The measurement of trace elements in wool is not without its own inherent problems. In fact, analysis must only take into account the internal (endogenous) fraction[59,30] which highlights the importance of removing the external (exogenous) contaminant fraction coming from the metal-rich dust deposited on the hair[59].

This part of work, the objective was to compare two washing procedures to select the most effective method in fully or almost fully removing the trace elements of external origin, that is, those settling on the wool, without affecting the hair matrix and removing the internal fraction which alone reflects trace metal accumulation in the organism.

Two washing procedures were compared. The first washing was carried out as it's described by Mehennaoui[51]; the wool is soaked in ebullient water overnight. One then carries out a rinsing with water demineralised and the need a brushing to remove any macroscopic particle.

A second washing procedure is based on the use of a rock product(Ghassoul) mixed with ebullient water until obtaining a consistent paste. The wool is soaked and kept in the pasty mixture at least 30min. the wool is then washed abundantly with bi-demineralised water.

Approximately 2g sub-samples were dried to a constant weight at 80^{0}C. A precisely weighed 50mg test specimen of washed/non-washed wool was carefully dried at constant temperature to constant weight. This specimen was introduced into a polystyrene tube, and 1ml nitric acid 8N was added[54,42].

The corked tube was preserved at ambient temperature for 24h. During this preliminary phase, most of the sample dissolved in the acid. To perfect dissolution, the corked tubes were placed for 1h in a boiler at 60^{0}C[42]. Corks were maintained in place by pressure(plate plus weight).

TABLE 1: Results of trace elements concentrations in non-washed and washed wool with the two different methods

		No-washed	Washed with water	Washed with rhassoul
Cd	M	0.11	0.10	0.10
	SD	0.05	0.05	0.07
	Max	0.25	0.28	0.34
	Min	0.06	0.04	0.04
	CV	0.42	0.55	0.71
Cu	M	6.48	5.99	4.41
	SD	6.15	4.86	2.67
	Max	33.36	27.64	14.77
	Min	1.78	2.08	1.84
	CV	0.95	0.81	0.60
Pb	M	45.65	14.32	6.78
	SD	39.03	15.70	5.84
	Max	181.45	68.63	21.18
	Min	11.34	1.85	0.22
	CV	0.85	1.10	0.86
Zn	M	140.14	112.81	92.35
	SD	53.37	20.43	20.83
	Max	383.48	151.30	140.21
	Min	87.61	75.59	48.11
	CV	0.38	0.18	0.23

M: mean; SD: Standard deviation; Max: maximum value; Min: Minimum value; CV: coefficient of variation

The recovered liquids were diluted in a suitable amount of bi-demineralised water for trace element analysis.

Analysis

Trace metal concentrations were determined by flame atomic absorption spectroscopy for Copper and zinc, and by graphite furnace atomic absorption spectroscopy for cadmium and lead. Metal concen trations were expressed as mean individual values ± standard deviation.

RESULTS

Washing procedure

TABLE 1 summaries results of trace elements variation concentrations in non-washed and washed wool with the two different methods. It shows that both of the two washing methods could decrease the trace elements concentration in the wool matrix but at different degrees. This decrease is undoubtedly due to the removal of contaminants bound onto the wool.

The results show also that washing with ebullient water had more difficulties to remove trace elements from the wool than Rhassoul. Thus, ebullient water removing 12.3% of Cd, 7.5% of Cu, 68.5% of Pb and 19.5% of Zn seems to be less efficient than Rhassoul removing 15.8% of Cd, 31.9% of Cu, 85.1% of Pb and 34.1% of Zn.

Rhassoul was the subject of many recent studies [12,13,55,32,33,17,48]. From the mineralogical point of view, Rhassoul contains in addition to the stevensite (trioctaedric smectite) other minerals such as quartz, gypsum, dolomite or sepiolite but in small proportions [69].

In order to maintain the electro-neutrality of the constitutive layers of the smectite, their negative charge resulting of isomorphic substitutions of Al3+by Mg2+/Fe2+ or from the deprotonation of the layers edges is compensated by the adsorption of an equivalent quantity of ions(Ca, Mg, K, Na, H…), they are compensation anions[5].

These counter anions are localised on external surfaces of the particles like between the unit layers. Because substitutions are localised mainly in the octahedral layer (external surface), the compensation anions are slightly related to the surface of the particles and can be exchanged by other cations present in the medium(Cd, Cu, Pb, Hg, Zn...).

Tissues accumulation

Descriptive statistics on blood, wool, liver and kidney concentrations of cadmium, copper, lead and zinc analysed in the 50 sheep(30 males and 20 females) from the mining area and in the 12 sheep(6males and 6 females) from a rural area, are shown in TABLE 2 and 3.

The obtained results show a difference in the capacity of accumulation of these trace elements by sheep, it varies according to the targeted organ, the trace element in question, the age of animal and its sex.

Results show that sheep from the mining area showed significantly higher trace elements levels than sheep from the rural area in all tissues analysed except zinc in wool.

Cd levels were very low in both groups of animals, and a large of samples of all tissues types did not contain detectable concentrations. This was particularly pronounced for wool.

In both groups of animals, Cd levels were significantly higher in the kidney than in the liver ($p<0.001$). These levels varied from one animal to another, they spread from 2,1mg/kg to 14,7mg/kg wet weight. Levels in both organs (liver and kidney) were significantly higher than in wool and blood.

TABLE 2 : Cd, Cu, Pb and Zn levels in liver, kidney, wool (mg/kg) and blood (mg/l) of female sheep raised in the industrial area and the rural area

	Mining area				Rural area				PF
	GM	SD	Max	Min	GM	SD	Max	Min	
Cd									
Liver	1.7	0.7	3.2	0.9	0.9	0.1	1.2	0.8	1.9
Kidney	5.5	3.3	14.7	2.1	1.6	0.8	3.2	0.9	3.4
Wool	0.11	0.08	0.40	0.10	0.09	0.02	0.12	0.07	1.2
Blood	3.9	2.2	8.4	0.4	2.9	1.4	4.4	1.3	1.3
Cu									
Liver	116.9	48.9	231.8	47.8	53.0	30.4	125.5	30.3	2.2
Kidney	24.2	23.0	125.5	17.5	20.3	0.3	20.6	18.5	1.2
Wool	4.3	2.1	10.3	1.5	4.2	0.7	5.0	3.2	1.0
Blood	311.1	98.6	510.0	143.3	253.3	43.5	296.7	206.7	1.2
Pb									
Liver	6.7	2.9	13.7	3.7	4.0	1.0	5.6	2.8	1.7
Kidney	5.7	2.3	12.7	2.6	4.6	1.2	4.7	3.7	1.4
Wool	10.8	10.2	41.6	1.1	6.9	1.0	7.9	5.9	1.6
Blood	38.7	3.5	50.8	33.4	34.0	13.0	47.7	18.0	1.1
Zn									
Liver	65.4	18.3	98.0	36.2	65.0	13.5	69.1	31.2	1.0
Kidney	84.2	35.6	231.1	38.1	62.9	13.0	77.0	38.1	1.3
Wool	11.4	10.3	31.6	0.4	11.7	8.4	26.4	6.2	1.0
Blood	418.4	95.9	632.7	268.0	354.1	81.9	428.3	257.3	1.2

TABLE 3: Cd, Cu, Pb and Zn levels in liver, kidney, wool (mg/kg) and blood (mg/l) of male sheep raised in the industrial area and the rural area

	Mining area				Rural area				PF
	GM	SD	Max	Min	GM	SD	Max	Min	
Cd									
Liver	1.6	1.0	5.4	0.8	1.0	0.1	1.2	0.9	1.6
Kidney	5.1	2.6	11.5	2.2	1.3	0.1	1.5	1.1	3.9
Wool	0.01	0.07	0.30	0.10	0.05	0.01	0.07	0.05	2.0
Blood	3.2	2.0	5.0	0.7	2.1	1.4	2.0	2.3	1.4
Cu									
Liver	122.8	60.6	270.9	29.5	55.0	41.3	137.5	32.8	2.2
Kidney	31.5	30.0	141.1	13.6	23.2	4.2	25.0	17.7	1.4
Wool	4.4	2.7	14.8	1.8	4.2	0.7	3.8	1.8	1.0
Blood	282.2	148.2	807.4	96.1	191.4	61.8	256.6	168.0	1.5
Pb									
Liver	7.0	3.0	13.4	3.1	5.9	1.4	8.4	4.8	1.2
Kidney	7.6	2.9	16.1	4.4	3.9	0.2	4.7	3.7	1.9
Wool	6.8	5.8	21.2	0.2	6.6	5.3	18.7	1.7	1.0
Blood	36.4	15.8	62.0	6.0	35.3	13.0	47.7	18.0	1.0
Zn									
Liver	57.7	18.9	103.5	29.9	55.8	17.5	69.1	31.2	1.0
Kidney	93.4	32.8	188.1	31.2	63.0	12.8	76.9	47.1	1.5
Wool	9.2	2.1	14.0	4.8	9.3	0.3	9.6	8.7	1.0
Blood	466.1	141.9	824.7	252.0	354.5	81.9	407.3	284.0	1.3

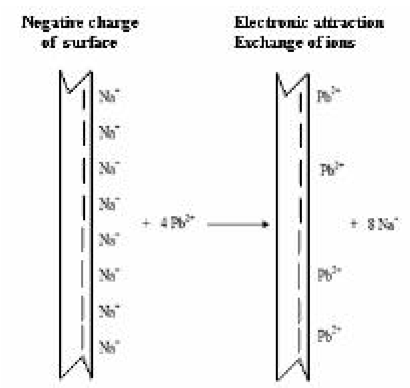

Figure 2 : Electronic attraction and exchange of the compensation cations by metallic pollutants

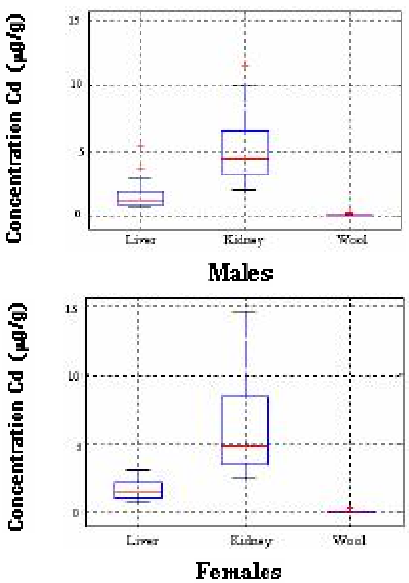

Figure 3: Cd levels in liver, kidney, wool (mg/kg) of male and female sheep raised in the industrial area

The maximum pollution factor(PF), calculated as ratios of Cd(in general trace element) levels in the mining zone to the same element levels in the rural zone(control), was noted for kidney(3.4 for females and 3.9 for males).

Results show also that copper levels were significantly higher($p<0.001$) in liver and blood than in kidney and wool. The most marked effect of pollution on copper was seen in the liver (PF=2.2 respectively for males and females), and to lesser extent in the kidney (PF =1.4 for males and PF=1.2 for females). No significant copper accumulation was noted in the wool of sheep(PF=1).

Lead levels were significantly higher in blood than in the other tissues, in witch an equal Pb repartition in liver and kidney was noted ($p<0.05$) for males but not for females. these levels (7,0±2, 8mg/kg in the liver and 6,9±2, 9mg/kg in the kidney) remain higher than those noted by Baxter et al.[2] (4,3µg/g in kidney), Vos et al.[72] (0,42µg/g in kidney and 0,96µg/g in liver) and those published by Hestrom and West[25] (0,1µg/g in the kidneys and 1,8µg/g in the livers). Nevertheless, several authors[18,1] noted contents more than in our study (12µg/g in the kidneys)(7,1µg/g in the kidneys). The most marked effect of pollution on lead was noted in kidney for males(PF=1.9) and in wool for females (PF=2). No significant lead accumulation (PF=1) was seen in the blood of sheep (males and females).

Zn levels were significantly higher in blood than in kidney($p<0.001$) and levels in both tissues were significantly higher than in liver and wool. Zn levels in no case differed significantly between sheep from mining and rural areas, with PFs being close to 1 for all tissues except kidney, in witch The most marked effect of pollution on zinc was noted(PF =1.5 for males and 1.3 for females). No significant zinc accumulation was seen in the liver and wool of sheep (males and females).

DISCUSSION

It is clear from the results of this study that environment contamination has a significantly effect on toxic metal levels in Drâa Lasfar sheep. The results show cadmium to concentrate primarily in the kidney, Cu in the liver, Pb in blood and Zn in the kidney. This unequal distribution amongst the organs is related to differences in the specific physiological functions of these elements and depends on their relative abundance in intracellular ligands able to bind metals, such as metalloproteins[29,28].

Results show that sheep have a high capacity to accumulate Cd in kidney(figure 3). The same observation was made by several authors[70,47] who reported in their work that kidney is the principal organ of cadmium accumulation.

Cd levels varied from one animal to another. This difference in renal cadmium concentration is undoubtedly explained by the effect of the age on its accumulation. The study of this interaction age-cadmium accumulation revealed the existence of a positive correlation between these two parameters. In the same optics, several authors confirmed this report[72,41,20,38,53,67].

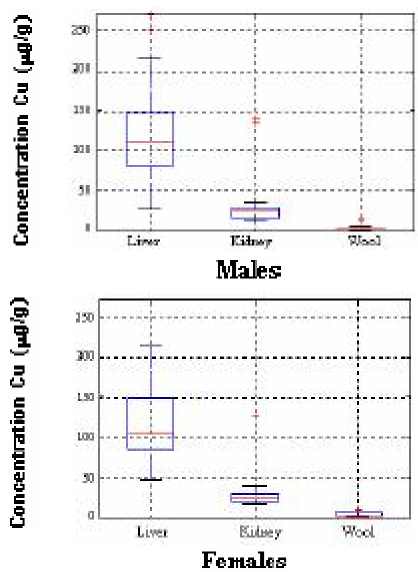

Figure 4: Cu levels in liver, kidney, wool(mg/kg) of male and female sheep raised in the industrial area

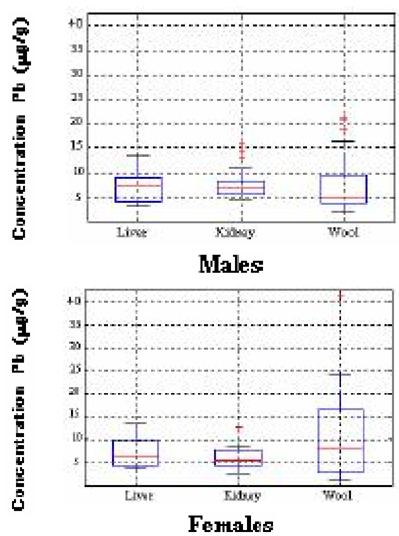

Figure 5 : Pb levels in liver, kidney, wool(mg/kg) of male and female sheep raised in the industrial area

The results show also that the renal cadmium concentrations were slightly higher in the female kidney than in the male to such a degree that all correlations between the sex effect and the cadmium accumulation were rejected($p<0,05$). Kottferova & Korenekova[40] announced in their work that this light increase in renal cadmium concentration can be explained by the longest biological half-life of cadmium in the females that in the males[8,44]. This difference in cadmium conservation aptitude between males and females could be due to a more effective metallothionein synthesis [74,44].

The cadmium levels in blood and wool were generally low(TABLE 1). These results are consistent with previous works[44]. In fact, the low sheep blood cadmium contents can be explained by studying the mechanisms implied in its transport in the organism[73] reported in their work that the intestinal absorption of cadmium is characterized by a rapid rate accumulation in the intestinal mucous membrane and a low rate of diffusive transfer in systemic circulation. Other works[73,46] postulated that in response to a cadmium exposure, there is an induction of metallothionein synthesis in the enterocytes. The synthesis of this protein at high concentrations can act as a barrier which would reduce the amount of Cd entering into systemic circulation and consequently traps it in the small intestine mucous membrane[73]. The retention of cadmium by the enterocytes would consequently reduce the cadmium quantity loaded to target organs (such liver and the kidney)[8,52].

Copper levels show a high capacity of sheep to accumulate it in the liver (figure 4). These results coincide perfectly with works of Terres Martos et al.[68].

Lopez et al.[43], Bohosiewicz et al.[7] and Falandysz [18], reported in their works that there is no indicative reference value of copper toxicity. In this connection[11,27], had noted some clinical signs when copper hepatic levels reached 150mg/kg wet weight. Gummow[23] made the same observations for about 152mg/kg. Perrin[58] also made the same observations but for weaker hepatic copper concentrations for about 126 mg/kg. In our study, 23% of the cases exceed 150mg/kg.

Several studies made on the copper accumulation in the kidney[11,23] recorded clinical signs for a copper renal level of 15mg/kg. Blood et al.(1992) reported in their work that this level is not a good indicator of copper impregnation in the organism since the hepatic contents in copper were normal. In our study, the renal contents of copper were higher than the limit fixed by Buck et al. [7] for 90% of the studied cases. Levels of copper in the blood of sheep of Drâa Lasfar mining area showed that there is not a significant difference($p<0,05$) between males and females, with higher concentrations in female blood. This result was justified by Terres Martos et al.[68] by their higher gut absorption efficiency for copper than males. In the same optic, Piscator[60] justifies this result by the influence of female oestrogens on copper

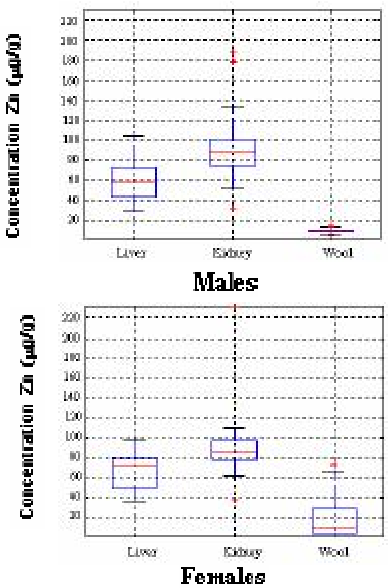

Figure 6 : Zn levels in liver, kidney, wool (mg/kg) of male and female sheep raised in the industrial area

metabolism.

Lopez et al.[43], Bremner & Beattie[10] and Bremner[10] reported in their works that copper is a poor inductor of metallothionein synthesis, but it can be fixed by a competition phenomenon with zinc for the metallothionein binding sites. In fact, Cu has a higher avidity to metallothionein than Zn; Cu can compete with and displace Zn from the metallothionein even after Zn has induced its synthesis[9,10].

Lead levels show that sheep have a low capacity to accumulate Pb in all analyzed tissues; they accumulated it at low levels (8,8µg/g in wool, 6,9µg/g in liver and 6,6µg/g in kidney) (figure 5).

Until these last years, it was traditionally considered that lead penetration through the intestinal wall is weak and ranging between 5 and 10%, correspondent to 90%(to 95%) of Ingested lead fecal elimination[16]. This report can justify the low lead level found in tissues sheep. In fact, the calcium, which has certain common properties with lead, can compete with Pb for certain intestinal proteins responsible of Pb absorption[3]. In the same optic, the phosphate ion decreases lead absorption in considerable proportions(from 63 to 10%), this action is pronounced for simultaneous calcium absorption[24]. This same antagonistic action was observed with iron and at less degree, with zinc.

Zinc levels show an unequal distribution of this essential metal in analysed tissues, they show also a high capacity of sheep to accumulate it in the Kidney(84,2µg/g for females and 93,4µg/g for males)(figure 6). Zinc organotropism in analyzed tissues depends especially on the organs physiological function[65]. In addition to the strong blood irrigation of kidney and its purifying properties, the presence of proteins rich in thiols-groups explains this strong accumulation capacity; capacity especially ensured by the presence of the metallothionein, whose synthesis is strongly induced by the exposure to zinc synthesis[75,76,62,56,6].

Generally, zinc is complexed to plasmatic organic ligands[22] such albumin and certain acid-amino. In this form, zinc is easily exchangeable and can bind to various tissue proteins (mainly metallothionein) in liver and kidney. A weak part of circulating zinc is trapped by α-2 macroglobulin and this complex can dissociate only in liver[26].

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