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Determination of lead and cadmium in kidney, liver and the muscles of Camels and Sheep slaughtered in Libya.

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This study was designed to determine the levels of two toxic elements (lead and cadmium) in kidney, liver and the muscles of camels and sheep. The samples of slaughtered animals were collected from different western regions (Zawia, Sorman and Egilate) in Libya. The Atomic Absorption spectrophoto-meter was used to carry out the measurements of heavy metals of samples. A recognized highly significant effects ($P \leq 0.01$) of species; Parts of body, region and interaction among them on residual of lead and cadmium in kidney, liver and muscle were measured. The concentration of lead and cadmium were 107.42 and 53.0 µg/kg in camels, which higher than values measured for sheep (75.32 and 17 µg/kg). The concentration of lead and cadmium were 143.33 and 50 µg/kg; 93.66 and 45 µg/kg and 53.16 and 28 µg/kg in kidney, liver and muscle, respectively. Regarding the effect of region, the concentration of lead and cadmium were 118.62 and 48 µg/kg; 81.25 and 41µg/kg and 90.29 and 34 µg/kg for Zawia, Sorman and Egilate regions, respectively. The reference values of lead and cadmium concentration in kidney and liver reported by Egyptian Organization for Standardization (EOS) and FAO are 0.5 mg/kg (500µg/kg) and 0.1 mg/kg (100µg/kg), respectively. Thus, the mean concentrations of lead and cadmium in kidney, liver and muscle of slaughtered camels and sheep in this study were safe and lower than permissible limit recommended. Information about levels of these metals is very important to the consumers because they are awareness concern with healthy food especially meat.

INTRODUCTION

In Libya, like other countries, meat of goat, sheep, camel, beef, chicken and their liver and kidney are a major source of protein to the population and are widely consumed. Also, meat and meat products are important as human diets because they provide many trace elements required for human nutrition. Some of these are considered essential (Cr, Mn, Fe, Cu and Zn), but others are not (Cd, Hg, Pb etc.). The levels of these trace elements depend on factors such as environmental conditions, type of pasture, portability of water and genetic characteristics of livestock (Brito *et al*., 2006).

The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations. Instances of heavy metal contamination in meat products during processing have been reported (Santhi *et al*., 2008 and Brito *et al*., 2005). In other cases, contaminated animal feed and rearing of livestock in proximity to polluted environment were reported responsible for heavy metal contamination in meat (Miranda *et al*., 2005 and Sabir *et al*., 2003).

Heavy metals remain in ground water and soil yet they tend to accumulate and are very toxic at certain levels. Living organisms normally require some of these heavy metals up to certain limits and in case excess accumulation occurs it will lead to severe detrimental effects. Due to the grazing of cattle on contaminated soil, higher levels of metals have been found in beef and mutton (Sabir *et al*., 2003). Also, Gonzalez *et al*.(2006) recorded the levels of toxic metals (lead and cadmium) in meat product exceeding recommended limits. With advancing and increasing industrialization, many metals are entering into the environment. These metals stay permanently because they cannot be degraded. They enter into the food chains and from there they ultimately make their passage into the tissue. Lead, cadmium, mercury and arsenic are among the main toxic metals, which accumulate in food chains and have a cumulative effect (Cunningham and Saigo, 1997). Heavy metals often have direct physiologically toxic effects and are stored or incorporated in living tissues. A study made by Horky *et al*. (1998) observed that the distribution and localization of some heavy metals in tissues of some calf organs were detected, the most affected organs, which showed higher levels of trace metals, were livers, kidneys and small intestines. Contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006).Toxic symptoms of cadmium are kidney dysfunction, hypertension, hepatic injury and lung damage (John and Jeanne, 1994). Also, effects of toxic of lead are shown on haemopoietic, nervous, gastrointestinal and renal systems (Baykov *et al*., 1996).

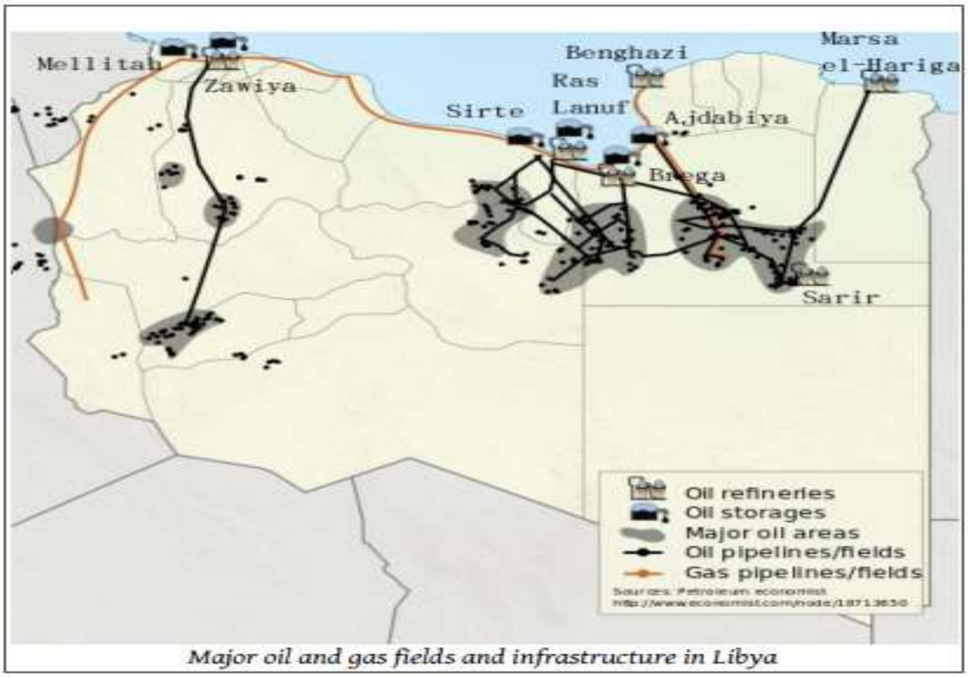
The objectives of this study was to determine residual concentrations of lead (Pb) and cadmium (Cd) metals in camels and sheep meat and to recognize role of different locations of western regions in Libya, parts of animals' body (liver, kidney and muscle) and interaction between them on Pb and Cd concentration. in camel and sheep meat.

MATERIALS AND METHODS

Sampling and sample preparation

Sampling and preparation of bovine liver, kidney and muscle samples

One hundred and eight samples consisting of muscle, liver and kidney parts from camel and sheep were collected from market in different areas (Zawia, Sorman and Egilate) of western Libya (Fig. 1).



Fig(1): Location of areas under study.

A slice of muscle sample of approximately 150 g was taken with a clean stainless steel knife from each carcass and placed in a plastic bag. All samples were placed in a cooler box at 4 C° and transported to the laboratory within the same day. On arrival at the laboratory, the samples were stored in a deep freezer at –22C° until analysis. Prior to analysis the samples were thawed, cut into small pieces using a stainless steel knife and about 50.0 g of each sample was transferred into a 250 ml conical flask and kept in a freeze-drier overnight. The samples were then freeze-dried for 24 h. Freeze-dried samples were homogenised using a blender (500 W, stainless steel bottom, 1.5 l glass jug, BBG52). The blender was cleaned well between samples, first with diluted detergent followed by tap water and then de-ionized water. The powdered samples were stored in air sealed cartel round bottles.

About 300 g of homogenised freeze-dried meat samples were transferred into each of XP-1500 Plus microwave digestion vessels. To each vessel, 1.00 ml of de-ionised water and 3.00 ml of 65% HNO3 were added. The vessels were then sealed and placed in the MARS 5 microwave digestion system.

Determination of lead and cadmium

Each homogenised sample (2g) was placed in a porcelain crucible. To avoid cross-contamination between the samples, single use plastic tools used to transfer the material. Each sample was then dried in oven at 60 to 80ºC for at least 12 hours. The crucibles with the samples introduced in muffle ovens and burned to ash at 450ºC. The temperature in the muffle oven increased at a rate of approximately 50ºC per hour and maintained at 450 ºC during 18 to 24 hours. The white ashes obtained with this procedure dissolved in 5% nitric acid to a volume of 50 ml.

The Atomic Absorption spectrophotometer (Thermo Electron Corporation, Perkin-Elmer, model 4100 ZL Zeeman, Germany) used to carry out the measurements. It equipped with a graphite furnace tube with an automatic sampler. All solutions were prepared in triplicate.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) to examine significance of species , area, parts of carcass and their interaction on heavy metals (Cd and Pb) concentrations in meat samples. Duncan's Multiple range test was used for comparing the differences among means using SAS program (SAS, 2006).

RESULTS AND DISCUSSION

Residual lead and cadmium in meat

1-Lead:

Means ± Standard error and level of significance for effects of species; region and parts of body on residual of lead in meat are presented in Table (1).

A Highly significant effect (P≤ 0.01) was observed for the previous factors on lead concentration in meat. The concentrate. of lead in camel meat (107.42 **μg/kg)** was higher than sheep meat (75.32 **μg/kg**).

Table (1): Means ± Standard error and level of significance for factors affecting lead in meat (μg/kg) .

Item	Lead
Species	**
Camel	107.42 ± 5.56 a
Sheep	75.32 ± 2.30 b
Region	**
Zawia	118.62 ± 9.11a
Sorman	81.25 ± 3.64 c
Egilate	90.29 ± 571 b
Parts of body	**
Liver	93.66±1.17 b
Muscle	53.16±0.15 c
Kidney	143.33 ±7.88a
Species x Region	**
Camel x Zawia	137.40± 12.51
Sorman	86.90 ± 4.70
Egilate	97.96 ± 8.05
Sheep x Zawia	81.07 ± 2.59
Sorman	69.95 ± 4.69
Egilate	74.95 ±4.17

** Highly significant at level 1% (p≤ 0.01)
means with different letters (a, b and c) are significantly different

In respect of effect of region, the highest value was 118.62 μg/kg in Zawia followed by 90.29 μg/kg in Egilate and the least was 81.25 μg/kg in Sorman.

With regard to the effect of muscle and official parts (kidney and liver) on lead concentration, the highest value was 143.33 μg/kg in kidney followed by value 93.66 μg/kg in muscle and the least value was 53.16 μg/kg in liver.

Concerning the interaction between species and region on lead concentration, the highest values were 137.40 and 81.07 in Zawia followed by 97.96 and 74.95 in Egialte and the least values were 86.90 and 69.95 μg/kg in Sorman area for both camel and sheep species, respectively.

Means ± Standard error and level of significance for factors affecting lead concentration. in different parts of both camel and sheep meat are shown in Table (2).

Table (2): Means ± Std. error and level of significance for factors affecting lead in different parts of animal body (μg/kg).

Item	Kidney	Muscle	Liver
Species	**	**	**
Camel	170.67 ± 8.75 a	54.23 ± 2.08 a	98.94 ± 0.77 a
Sheep	88.66 ± 0.89 b	52.63 ± 2.84 b	83.08 ± 0.69 b
Region	**	**	**
Zawia	191.06 ± 3.28 a	68.93 ± 0.99 a	95.87 ± 2.73 a
Sorman	105.92 ± 0.81 c	47.46 ± 0.20 b	90.36 ±1.15 b
Egilate	133.02 ± 0.63 b	43.11 ± 0.17c	94.73 ± 0.07a
Species x Region	**	**	**
Camel x Zawia	239.67 ± 3.28	69.75± 2.79	103.77± 0.46
Sorman	116.52 ± 0.81	49.84 ± 0.17	94.34 ±0.15
Egilate	155.83 ± 0.63	39.32 ± 0.24	98.73 ±115
Sheep x Zawia	93.84 ± 0.60	68.29 ± 2.79	86.75±0.60
Sorman	84.72 ± 0.07	42.71 ± 0.25	82.42± 0.06
Egilate	87.41 ± 0.06	50.71± 0.18	80.08± 0.07

** Highly significant at level 1% (p≤ 0.01)

a, b and c means with different letters are significantly different.

The effects of species, region and interaction between species and area were highly significant (P≤ 0.01).

Concerning the effect of species on the parts of body , the concentration. of cadmium in liver were 170.67 and 88.66 μg/kg, followed by kidney(98.94 and 83.08 μg/kg) and the least in muscle (54.23 and 52.63 μg/kg) for camel and sheep, respectively.

The region had a highly significant effect (p≤ 0.01) on lead concentration. in different parts of the body. The Kidney in Zawia had the highest value (191.06) followed by Egialte (133.02) and the least was Sorman (105.92 μg/kg). The muscle in Zawia had the highest value (68.93) followed by Sorman (47.46) and the least was Egialte region (43.11μg/kg). The liver had the same trend of kidney for lead concentration, the highest value was 95.87 in Zawia, then Egialte (94.73) and the least was Sorman (90.36 μg/kg).

Regarding effect of interaction between species and region on lead concentration, the kidney in Zawia region showed the highest estimates (239.67 and 93.84) followed by Egialte (155.83 and 87.41) and the least was Sorman (116.52 and 84.72) for camel and sheep meat, respectively.

The muscle in Zawia region also showed the highest estimates (69.75 and 68.29) but followed by Sorman (49.84 and 50.71) then Egialte (39.32 and 42.71) for camel and sheep meat, respectively.

The highest estimates for liver were in Zawia (103.77 and 86.75) followed by Egialte (98.73 and 82.42) and the least in Sorman (94.34 and 80.08 μg/kg) for camel and sheep meat, respectively.

2- Cadmium:

Means ± Standard error and level of significance for effects of species; region and parts on residual of cadmium in meat are presented in Table (3).

Table (3): Means ± Std. err. and level of significance for factors affecting cadmium in meat

Item	Cadmium (mg/kg)
Species	**
Camel	0.053 ± 0.001 a
Sheep	0.017± 0.001 b
Region	**
Zawia	0.048 ± 0.003 a
Sorman	0.041 ± 0.003 b
Egilate	0.034 ± 0.002 c
Parts	**
Liver	0.045 ± 0.003 b
Muscle	0.028 ± 0.002c
Kidney	0.050 ± 0.00 3a
Species x Region	**
Camel x Zawia	0.063 ±0.003
Sorman	0.053 ± 0.002
Egilate	0.043 ±0.001
Sheep x Zawia	0.019 ±0.001
Sorman	0.016 ±0.001
Egilate	0.015 ±0.001

** Highly significant at level 1% ($p \leq 0.01$)

a, b and c means with the different letters are significantly different

A highly significant effect ($P \leq 0.01$) for the previous factors was observed on cadmium concentration in mutton. The concentration. of cadmium in camel (0.053) was higher than sheep meat (0.017 μg/kg).Concerning effect of region, the highest estimate was in Zawia (0.048) followed by Sorman (0.041) and the least in Egilate region (0.034).

Regarding the effect of parts on cadmium concentration, the highest value was in kidney (0.050 mg/kg) followed by liver (0.045) and the least in muscle (0.028).

The effect of interaction between species and area on cadmium concentration. was highly significant ($P \leq 0.01$). The highest estimates were in Zawia (0.063 and 0.019) followed by Sorman (0.053 and 0.016) and the least in Egilate region (0.043 and 0.015 mg/kg) in both camel and sheep, respectively.

Table (4): Means ± Std. err. and level of significance for factors affecting cadmium in different parts of animal carcass (mg/kg).

Item	Kidney	Muscle	Liver
Species	**	**	**
Camel	0.065 ± 0.002 a	0.035± 0.0008 a	0.058 ± 0.002 a
Sheep	0.018 ± 0.0006 b	0.013± 0.0004 b	0.019 ± 0.004 b
Region	**	*	**
Zawia	0.060± 0.005 b	0.030± 0.003 b	0.054 ± 0.005 b
Sorman	0.047 ± 0.007 a	0.029 ±0.003 a	0.045 ± 0.006a
Egilate	0.042 ± 0.004c	0.024± 0.002c	0.036± 0.003c
Species x Region	**	**	**
Camel x Zawia	0.080 ± 0.0004	0.038 ± 0.0009	0.070± 0.001
Sorman	0.062± 0.0005	0.038 ± 0.0005	0.058± 0.001
Egilate	0.054± 0.0009	0.031± 0.0008	0.046± 0.0005
Sheep x Zawia	0.021± 0.0001	0.013± 0.0001	0.021 ± 0.0001
Sorman	0.016± 0.0005	0.015± 0.0004	0.019± 0.0001
Egilate	0.017± 0.0002	0.011 ± 0.0001	0.018±0.0001

** Highly significant at level 1% ($p \leq 0.01$)

a, b and c means with the different letters are significantly different.

Means ± Standard error and level of significance for factors affecting cadmium concentration. in different parts of both camel and sheep meat are shown in Table (4).

The effects of species, region and interaction between species and area were highly significant ($P \leq 0.01$). Concerning effect of species on the parts of body, the concentrations of cadmium were 0.065 and 0.018 in kidney, followed by those in liver (0.058 and 0.019) while the least were in muscle (0.035 and 0.013 mg/kg) for camel and sheep species, respectively.

The region had a highly significant effect ($p \leq 0.01$) on cadmium concentration in different parts of the body.

The Kidney in Zawia showed the highest estimate (191.06) followed by Egialte (133.02) and the least in Sorman (105.92 μg/kg). The muscle in Zawia showed the highest estimate (68.93) followed by Sorman (47.46) and the least in Egialte region (43.11μg/kg). The liver had the same trend as kidney for lead concentration, the highest in Zawia (95.87) followed by Egialte (94.73) then Sorman (90.36 μg/kg).

Regarding the interaction between specie and region on cadmium concentration, the kidney showed in Zawia region the highest estimates (0.080 and 0.021) followed by Sorman (0.060 and 0.017) then Egialte (0.054 and 0.016 mg/kg) for camel and sheep meat, respectively.

The highest estimates of muscle was in Zawia (0.038 and 0.015) followed by Sorman region (0.038 and 0.013) then the least in Egilalte region (0.031 and 0.011 mg/kg) for camel and sheep meat, respectively.

The highest values for liver were in Zawia (0.070 and 0.021) followed by Sorman (0.058 and 0.019) and the least were in Egialte region (0.046 and 0.018 mg/kg) for camel and sheep meat, respectively.

DISCUSSION

The results of present study indicated highly significant ($p \leq 0.01$) effects for species; region and parts of the body on residual levels of lead and cadmium (μg/kg) in camel and sheep meat.

The means of lead averaged 107.42 and 75.32 μg/kg in camel and sheep meat, respectively. These values are higher than values reported by Wojciechowska *et al*., (1990) (0.025-0.047 mg/kg); Licata *et al*. (2004) (0.05); and Simsek *et al*. (2000) (0.018-0.049) in cattle and the value of 0.05 mg/kg by Coni *et al*.(1996) in goat. On the other hand, present values are lower than that reported by Alais (2000) (0.95 in cattle) and value of 0.17 by Caggiano *et al*. (2005).

Regarding Cd concentration, the means averaged 0.053 and 0.017 mg/kg in camel and sheep meat, respectively. These values are higher than values reported by Wojciechowska *et al*., (1990) (0.025-0.047 mg/kg); Alais (2000) (0.02-.0.05 mg/kg) and Licata *et al. (*2004) (0.0228 mg/kg) in cattle. On the other hand, present values are lower than those of Swaileh *et al.* (2001) (0.34-0.57) and Caggiano *et al*. (2005) (0.15) in goat.

Increased concentrations of heavy metals in the body of domestic animals result in low fitness of animals and reproduction problems as well as immunity decline and occurrence of cancerous and teratogenic diseases (Bires *et al*., 1995). It is also known that heavy metals excretion such as cadmium (Cd), lead (Pb) and mercury (Hg) are significantly lower in offspring (Oskarsson *et al*., 1995). The assessment of dump site soils for the concentration of hazardous metals is imperative for healthy crop production.

In respect of status of muscle and official parts (kidney and liver) with heavy metals in this study. The results noticed that both kidney and liver had greater levels of lead compared with muscle. Also, cadmium level in liver was higher than muscle. These results may be due to that both kidney and liver are responsible for the detoxification and excretion of xenobiotics (Pitot and Dragan, 1995). Spices difference in Pb residues had been observed by (Aslam, 2010) who found higher values of this metal in kidney and liver of goats compared to cattle, being the highest in goat kidney. Bala *et al*. (2013) found significant difference in the concentration of lead and cadmium in liver and kidney of slaughtered cattle ($P < 0.05$).

Heavy metals in general may play a role in normal physiological processes but can accumulate to toxic levels, particularly in certain organs such liver and kidney. Thus, most countries of the world are giving great attention to the production of safe and healthy meat and other edible tissues for human use and many researchers studied the metal toxicity in the meat and other organs of animals. As these metals are toxic in nature and even at relatively, low concentrations can cause adverse effects. Contamination of heavy metals mostly occurs in meat products during processing (Santhi *et al.*, 2008). Bioaccumulation and toxicity of heavy metal residues in animal tissue as hazardous potential is well documented (Satoh *et al.*, 2002 and Alonso *et al.*, 2000).

In this regard, a study was performed on liver and kidneys of cattle to find the heavy metal accumulation in these organs. The results suggested that cadmium gradually and progressively accumulated in animal tissues, especially in kidneys (Jukna *et al.*, 2006). Some researches done were able to substantiate the general perception that certain heavy metals such as Cd and Pb are significantly more likely to be found in kidney and liver even with the lower detected levels than in meat (muscle tissue). Thus, meat of animals graze in areas polluted with heavy metals can be safe for human consumption when liver, kidney and bones are discarded, as toxic metals appear to bioaccumulate in these tissues. (Olsson *et al.*, 2002 and Caggiano *et al.*, 2005).

In the current study Zawia region found higher in residual concentrations of Pb and Cd in both camel and sheep meat compared to Sorman and Egilate regions. This may due to more industrial activities, mainly oil and gas , in this region, yet many vehicles burn gasoline containing lead and forages and water contaminated with these elements.

Zawia located 50 km west of Tripoli, it is Libya's second-biggest refinery. Zawia has an output yielding roughly 25 percent fuel oil, 40 percent middle distillates and 35 percent light distillates when running on good quality crude (APS, 2005).

In animals, which produce meat for human consumption, the relative levels of certain metals in different organs can be very important for several reasons. The first is that animals graze in different areas and may metals consumed, caused residues not of dangerous levels but they bioaccumulate over time. The method developed for analysis of animal organs for heavy metals has the advantage of having better detection levels than traditional methods and is more cost effective as several elements can be analyzed simultaneously. It thus becomes possible to differentiate physiologically 'safe' levels and be able to predict the speed at which metals can bioaccumulate in tissues more accurately.

Krajnc *et al.*, (1983) found that contamination in animals occurs through forage, feed and water while in human cadmium contamination can occur by the utilization of dairy products and meat. He also studied the relationship between cadmium concentration in organs of cattle and cadmium contents in soil and found that contamination in cattle organs is due to the feeding on forages growing on contaminated soils.

Also, Smith *et al (*1991) reported that heavy metal pollution in rural areas is due to disposal of industrial effluents and sewage sludge which causes problem for grazing animals because they depose heavy metals on pastures grasses or forages

Many people and animals are exposed to environmental lead in the form of industrial wastes, leaded gasoline, forages and water contaminated with this element and other anthropogenic sources. Air pollution is very common in big cities because vehicles burn gasoline containing lead. It is thought that lead is responsible for number of deaths (Oroian *et al.*, 2007).

It has been found that cadmium has not a single physiological function within the human body. Therefore, attention has diverted to its biohazardous potential. Once cadmium is absorbed, it accumulates in the body even throughout the life (Bernard, 2004). Even low concentration of cadmium can adversely affect the number of metabolic processes in animal body (Nordberg *et al.*, 2007). Cadmium intoxication can lead to kidney, bone and pulmonary damages (Godt *et al.*, 2006). Literature indicates that excessive intake of cadmium in cattle can lead to loss of appetite, anemia, poor growth, abortions and teratogenic effects. Excessive intake of cadmium alters the metabolism of zinc and copper in animals (Wright *et al.*, 1997).

It is very important to get knowledge of toxic metal accumulation in livestock and their adverse effects on animals and humans. Lead contamination is most commonly observed in forages and vegetables grown in area having lead melting furnace. Lead toxicity is frequently observed in farm animals, especially in those grazing on pasture in the vicinity of metallurgic complexes and also close to busy roads. Species susceptibility to lead has described in cattle, particularly the young one. The milk of dairy cows contains small amount of lead (Smirjdkova *et al.*, 2005).

In Spain, Marinda *et al.* (2005) conducted a study on cattle of industrial and rural areas of Asturias (northern Spain) to determine lead concentration. Their observations indicated that samples collected from cattle of industrial area have high concentration of lead especially in liver and kidneys than that of rural area.

In Egypt, Abou Doina (2008) performed a study in Cairo for detection of lead residues in different animal muscles , consumable organs and species. It has been noticed that kidney sampled from cattle near heavy traffic and urban areas contained the highest concentration of lead which was 0.198 and 0.490 mg/kg, respectively, while kidneys of buffaloes collected from industrial area contained the highest concentration of lead 0.790 mg kg-1. He concluded that concentrations of lead in liver and kidney was found to be the highest in industrial area than other areas. Besides, untreated waste effluents of industry which find their way to irrigation channels and hence pollute the fodder through soil. The other sources of Pb contamination are automobile exhaust gasses, which arise from antiknocking agents added to gasoline making the environment highly polluted with Pb (Marium *et al.*, 2004).

Although contamination of animal feed by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized.

CONCLUSIONS

-In current study, residual levels of Pb and Cd in the muscles and officials (liver and kidney) of camel and sheep were lower than that recommended by EOS and FAO .

-Genetic variation due to species has been depicted as residual levels of Pb and Cd in muscles and officials have been found higher in camel compared to sheep meat.

-The area of Zawia have higher residual concentrations of Pb and Cd in both camel and sheep meat compared to Sorman and Egilate areas. This may because this area have more industrial activities mainly oil and gas , have many vehicles burn gasoline containing lead and forages and water contaminated with this elements.

- It's difficult to make comparison between this study and other studies owing to the variations due to analytical methodologies employed, physiological conditions and age of the animals, the nature of the feed and level & type of heavy metals contamination.

-In order to prevent harmful of exposure to these heavy metals, awareness of the sources and uses, modes of entry in the body, toxic effects and safe limits must be established.

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