

Incidence and Public Health Risk Assessment of Toxic Metal Residues (cadmium and lead) in Liver and Kidney of Ovine and Bovine from Algeria

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Abstract

This study intended to establish if Cd and Pb in the livers and kidneys of bovine and ovine slaughtered in the study area exceeded to concentrations maximum limit proposed by the European Commission (EC) 2006. After digestion of samples, Cadmium and lead concentrations were determined by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS). The results of our study have shown that lead and cadmium concentrations depend on age and species. The average fresh weight levels of liver and kidneys of bovine animals were about (0.319 mg/kg and 0.337mg/kg) for Cd and (0,502mg/kg and 0.497mg kg) for Pb, while in ovine, mean concentrations in the liver and kidneys were (0,241mg/kg and 0.232 mg/kg) for Cd and (0.259 mg/kg and 0.265 mg/kg) for Pb. Concentrations above the maximum limit proposed by the EC 2006 for Pb and Cd have been detected in some liver and kidney samples from bovine older than 4 years and in ovine over 1 year old .Statistical analysis revealed a very significant ($P<0.01$) difference in Cd concentrations in the liver and kidneys between age groups and species.

Keywords: Lead, cadmium, bovine, ovine, liver, kidney.

Introduction

Lead (Pb) and cadmium (Cd) are cumulative toxic metals thus, their widespread use and cumulative effect could lead to a severe environmental contamination and many public health problems (Saratug *et al.*, 2003 and Custer *et al.*, 2004). They are transmitted from mobile and stationary sources into the air and may reach to human food

(Sharkawy and Amal, 2003). Industrial and urban activities generate the accumulation of metallic waste in soils, which can be transferred to plants and therefore to animals, hence the importance of knowledge of soil-plant transfer- terrestrial organisms.

Ruminants bioaccumulate these toxic elements and are therefore the most exposed to Pb

and Cd, due to the grazing structure. Heavy metals have the ability to lodge in the reticulum of ruminants and provide a reservoir, which is continually absorbed into the bodies of these animals (Radosztitis *et al.*, 2007).

Ovine and bovine are the main sources of red meats, which have the potential to induce toxic human exposure. Offal's, especially liver and kidneys are the targeted organs for the bioaccumulation of metallic elements, and thus constitute a significant source of exogenous food intake. Differences in contamination levels of internal organs (kidney and liver) depend on the species and age of animals (Hecht, 1983; Kreiowska-Kuias, 1994; Studzinski *et al.*, 1992).

In Algeria, offal consumption is very important given its low cost and is part of some traditional diets. The level of heavy metal impregnation in the meat producing animal population is poorly investigated.

The aim of our study was to determine the levels of Pb and Cd in kidney and liver samples taken from bovine and ovine slaughtered in the country, as well as the potential risks of consumption of these organs.

Materials and methods

Ethical approval

Ethical approval is not applicable to this study as liver and kidney samples used for the analysis of heavy metals were collected from slaughtered bovine and ovine in Algeria.

Reagents

The chemicals and reagents used were of analytical high quality and they were mostly from Sigma (St. Louis, MO) and Merck (Mannheim, Germany). The HNO₃ was of suprapure quality (E. Merck, Darmstadt), standard solutions of lead and cadmium were purchased from Merck (Darmstadt, Germany) and double distilled deionized water (Milli-Q Millipore 18.2 M Ω -cm resistivity) was used for all dilutions.

Collection of Samples

A total of one hundred and eighty fresh liver and kidney samples were collected from bovine (n=80) and ovine (n=100) from the slaughterhouses in East and North of Algeria, from October 2016 to February 2017. The samples were taken only from healthy animals of three age groups: less than 12 months, between 1 to 4 years and over 4 years.

Mineralization of samples

Samples mineralization was done according to European Standard EN 13805 (2002). Briefly, 100g of each organ for analysis were stored in plastic falcon tubes and placed in a cooler box. Samples in the box were stored at -20°C during transfer to the laboratory up until sample preparation and analyses. At the laboratory the samples were freed of fat, major blood vessels and tendons, and homogenised.

All plastic and glassware were cleaned by soaking in diluted HNO₃ (10% v/v) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock Heavy-metal levels in examined tissues were measured according to the method described by Finerty *et al.* (1990). In summary, 1 g of each sample was mixed with 10 mL 3:2 HNO₃ (65%v/v): HClO₄ (70%v/v). The mixture was allowed to digest overnight in the cold and later heated for 3 h in a water bath at 70°C with swirling at 30 min intervals to ensure complete digestion. After cooling, the digest was transferred into 20 mL standard asks, rinsing with de-ionized water and made up to the mark. Prepared sample solutions were kept in acid-leached polyethylene bottles at room temperature until metal analyses.

The samples were firstly dried and then thermally degraded at 750°C.

Calibration and Assay of Heavy Metals

Before each series of measurements, commercialized calibration solutions were prepared (6 for Pb and Cd and 5 for Hg); then, calibration curves were established. For Pb and Cd, a standard (PerkinElmer Pure reference N9300281) was used at a concentration of 100 mg/L for Hg; a standard (AccuStandard reference AA34N-5) was used at a concentration of 1000 mg/L. Heavy metals concentrations were assessed using Atomic Absorption Spectroscopy (A.A.S) (UNICAM 929). For each heavy metal, there was a specific hollow cathode lamp and the machine was set at a particular wavelength for the metal to be analysed. The content of heavy metals in the assessed tissues was expressed in mg/kg of fresh mass. The dosage of Cd was carried out with a wavelength of 228, 8 nm and temperature programming: at 100°C, 800°C and 900°C. In contrast, the dosage of Pb was carried out with a wavelength of 217 nm and programmed at the same temperature. The calibration of the device was performed by the embodiment of 10 blank measurements.

Quality control of the analysis

Analytical performances were verified by processing Certified Reference Materials: For Pb and Cd, we used the cereals and derivatives BIPEA with internal reference number (15350395) and a known concentration of these metals (Cd=0.406 mg/kg w.w), and (Pb=0.851 mg/kg w.w). The results were in good agreement with the certified values located in these intervals: 0.3046-0.5075 for Cd, and 0.6128-0.10892 for Pb.

Statistical analysis was performed with Statview® software (Version 4.55, California - USA). The heavy metals concentrations were described by their means as well as their minimum and maximum values (the extent). The results were performed as mean \pm standard error (M \pm SE). The one way analysis of variance (ANOVA) was performed to compare the bioaccumulation of Cd and Pb in the liver and kidneys of bovine and ovine species. Z-test was used to determine differences between groups. The difference was considered significant at $P < 0.05$.

Results and discussions

The objective of this study was to determine Pb and Cd residues in cattle and sheep offal (liver

and kidney), the results were expressed in mg / kg.

Over of 180 samples (all samples combined), 48 samples (26.66%) contained high levels of Pb and Cd residues, with values exceeding the European certified reference value of (0.5 mg / kg) for lead in the liver and kidneys and 0.5 mg / kg and 1 mg / kg for Cd in the liver and kidneys respectively

Offal of cattle was more affected than that of sheep. In fact, out of 180 collected samples, 48 presented concentrations exceeding the EC limits, only 13 out of 100 samples were contaminated in sheep. Livers are the most contaminated compared to kidneys with a rate of 16,66 % (30/180) against 10% (18/180) for the kidney (Table 1).

Cadmium

The mean concentrations of Cd levels in the liver and kidney of bovine obtained in our study were (0.319 \pm 0.040mg/kg and 0.337 \pm 0.077mg / kg) respectively. The highest concentrations were observed in kidney with a maximum level of 1.865mg / kg followed by liver (0.82mg/kg).

The mean concentration of Cd in liver was higher among the age group >4years with 144 concentration of (0.474mg/kg \pm 0.055) in comparison

Table 1: The Lead and Cadmium concentration of liver and kidney of slaughtered bovine and ovine in Algeria (mgkg⁻¹ fresh weight).

| | Lead | | | | | | Cadmium | | | | | |
|---------------------------------------|-------------------------|-------------------------|------------------|------------------------|-------------------------|---------------|------------------------|-------------------------|-----------|-------------------------|-------------------------|-----------|
| | Liver | | | Kidney | | | Liver | | | Kidney | | |
| | Bovine | Ovine | P | Bovine | Ovine | P | Bovine | Ovine | P | Bovine | Ovine | P |
| No of samples | 40 | 50 | | 40 | 50 | | 40 | 50 | | 40 | 50 | |
| EC limits (mg.kg ⁻¹) | 0.5 | 0.5 | | 0.5 | 0.5 | | 0.5 | 0.5 | | 1 | 1 | |
| Mean \pm ES (µg.kg ⁻¹) | 0.502 \pm 0.051 | 0.259 \pm 0.021 | <0.001 | 0.497 \pm 0.07 | 0.265 \pm 0.030 | 0.0033 | 0.319 \pm 0.04 | 0.241 \pm 0.022 | NS | 0.337 \pm 0.077 | 0.232 \pm 0.030 | NS |
| Minimum | 0.078 | 0.001 | | 0.009 | 0.005 | | 0.024 | 0.030 | | 0.019 | 0.015 | |
| Maximum | 1.45 | 0.580 | | 1.475 | 0.880 | | 0.82 | 0.605 | | 1.856 | 1.019 | |
| No of samples exceeding the EC limits | 12 | 4 | | 9 | 4 | | 10 | 4 | | 4 | 1 | |

to the age category ≤ 4 years, where the concentration of Cd was $0.143\text{mg/kg} \pm 0.018$.

In the kidney, the mean concentrations of Cadmium were also higher among the age group > 4 years with the concentration ($0.589\text{mg/kg} \pm 0.1$) in comparison to the age category ≤ 4 years, where the concentration of Cd was (0.085 ± 0.01) (Table 1 and Table 2).

For ovine, mean Cd concentrations were $0.241 \pm 0.022\text{mg/kg}$ in liver samples and $0.232 \pm 0.033\text{mg/kg}$ in kidney samples. Nevertheless, only 4 (8%) liver samples and 1 (2%) kidney samples with a maximum level of 0.605mg/kg and 1.019mg/kg respectively, containing Cd concentrations greater than the limits proposed by EC were found.

The mean Cadmium concentrations for ovine, young and aged were $0.161 \pm 0.018\text{mg/kg}$ and $0.321 \pm 0.034\text{mg/kg}$ respectively in the liver samples, and $0.145 \pm 0.021\text{mg/kg}$ and $0.319 \pm 0.058\text{mg/kg}$ respectively in the kidney samples (Tab. 1 and Tab. 3).

Lead

The obtained results (Table 1) revealed mean lead concentrations in the liver of bovine and ovine,

were ($0.502 \pm 0.051\text{mg/kg}$ and $0.259 \pm 0.021\text{mg/kg}$), respectively. while in the kidney, mean Pb values in the kidney of bovine and ovine, were respectively, ($0.497\text{mg/kg} \pm 0.077$ and $0.265 \pm 0.03\text{mg/kg}$). the samples examined exceeded the limits imposed by the European Regulations in livers and kidneys.

The mean Pb concentrations were $0.27 \pm 0.024\text{mg/kg}$ and $0.734 \pm 0.067\text{mg/kg}$ in the liver samples, and $0.254\text{mg/kg} \pm 0.01$ and $0.739\text{mg/kg} \pm 0.132$, in the kidney samples for young and aged cattle, respectively. Whereas in sheep, concentrations in liver samples were $0.197 \pm 0.024\text{mg/kg}$ and $0.32 \pm 0.03\text{mg/kg}$, and in the kidney, we obtained the mean rates of $0.206 \pm 0.024\text{mg/kg}$ and $0.324 \pm 0.053\text{mg/kg}$, for young and aged animals, respectively (Tab. 2 and Tab. 3).

This study revealed that Pb and Cd have been detected in all tissues analysed. In general, the liver contained more Pb and Cd concentrations than the kidney.

From these results, very significant differences were observed between the accumulation of

Table 2: The Lead and Cadmium concentration of liver and kidney of slaughtered bovine in Algeria in relation to the age (mgkg^{-1} fresh weight) (Mean \pm SE).

| | Lead | | <i>P</i> | Cadmium | | <i>P</i> |
|---------------|---------------------------|--------------------------|------------|---------------------------|--------------------------|------------|
| | ≤ 4 years (Males) | > 4 years (Females) | | ≤ 4 years (Males) | > 4 years (Females) | |
| Liver | 0.270 ± 0.024^a | 0.734 ± 0.067^a | < 0.0001 | 0.143 ± 0.018^a | 0.474 ± 0.055^a | < 0.0001 |
| Kidney | 0.254 ± 0.031^a | 0.739 ± 0.132^a | 0.001 | 0.085 ± 0.01^b | 0.589 ± 0.132^a | 0.0005 |

(^{a,b}) Change in latter in the same column indicate significant difference between concentration of Lead and cadmium.

Table 3: The Lead and Cadmium concentration of liver and kidney of slaughtered ovine in Algeria in relation to the age (mgkg^{-1} fresh weight) (Mean \pm SE).

| | Lead | | <i>P</i> | Cadmium | | <i>P</i> |
|---------------|--------------------------|-------------------------|----------|--------------------------|-------------------------|----------|
| | ≤ 1 year (Males) | > 1 year (Females) | | ≤ 1 year (Males) | > 1 year (Females) | |
| Liver | 0.197 ± 0.024^a | 0.32 ± 0.03^a | 0.002 | 0.161 ± 0.018^a | 0.321 ± 0.034^a | 0.0001 |
| Kidney | 0.206 ± 0.024^a | 0.324 ± 0.053^a | 0.0468 | 0.145 ± 0.021^a | 0.319 ± 0.058^a | 0.0068 |

(^{a,b}) Change in latter in the same column indicate significant difference between concentration of Lead and cadmium.

heavy metal residues (Cd, Pb) in organs (liver and kidney) and animals' species (bovine and ovine)

From these results, clear interspecies differences in the accumulation of heavy-metal residues between cattle and sheep tissues were observed. Cattle, especially, had higher levels of both Cd and Pb than sheep did. The mean Cd concentrations recorded in our study were similar to those found by Al-Naemi (2011) in Iraq. Our results are in contrast with the ones found by Khalafallah *et al.* (2015); Bala *et al.* (2012); Akan *et al.* (2010), but they were lower than those obtained by Swaileh *et al.* (2009) and Mariam *et al.* (2004), in which the limits are higher.

The presence of Cd in animal tissues, especially livers and kidneys, was strongly related to Cd levels in animal feed (Kreuzer *et al.*, 1988). This was related to the differences in exposure, and other factors such as: age, species and food composition. Therefore, its presence in the samples constitutes a risk for public health.

Pb was considered one of the main environmental pollutants and one of the causes of accidental poisoning for domestic animals in particular. Lead absorbed accumulates in the bones, liver and kidneys. Lead levels in liver and kidneys have been generally related to lead levels in animal feed (Kreuzer *et al.*, 1988; Sharma *et al.*, 1982; Vreman *et al.*, 1986).

Cattle were more contaminated by Pb with a percentage of 22% and 30% in the kidney and liver respectively than sheep with a percentage of 8% each in the liver and kidney.

Higher Pb concentrations were found in kidneys and livers of both species (bovine and ovine). These concentrations were significantly higher than the limits set by the European Commission (EC) (0.5 mg / kg).

The lead levels found in this study were similar to most of the literature data as Al-Naemi, (2011) in Iraq, AbouDounia (2008) in Egypt, Kazemeini *et al.*, (2010). Our result is in contrast with the ones found by of Nriagu *et al.* (2009) in Jamaica; Khalafallah *et al.* (2015); Bala *et al.* (2013) in Niger, in which the limits are higher. The results showed a very significant ($P < 0.05$) difference between Cd and Pb levels in the studied organs due to different age categories. A positive correlation was found between age and the content of the studied metals. The highest rate of Cd has been found in kidney and liver in cattle samples, ranging over 4 years

old. As for sheep, the highest rate was found in the age group > 1 .

The highest Pb concentrations found in cows in the liver and kidneys would have been related to the age group > 4 years at intake. Concerning sheep, the highest rates were observed in the age group between 1 and 2 years. Our results have confirmed those of other authors (Salisbury *et al.* 1991; Lopez Alonso *et al.* 2000, 2002), who also demonstrated that there was a positive correlation between age, Cd and Pb concentrations in organs, which explains that the nature of these later were highly accumulative.

In our study, the highest levels of Cd and Pb were recorded in breeding dairy cows between 5 and 9 years older. These high levels can be explained by the high doses of mineral supplements received by these cows during gestation. Thus, minerals have been known for their interactions with Cd, such as calcium, zinc, copper, iron, and selenium (Andersen *et al.*, 1997). Studies have shown that exposure to Cd and Pb during pregnancy and lactation can have deleterious effects in offspring, resulting in developmental and behavioural deficits and adverse reproductive effects as Cd induces a decrease in relative testes weight and plasma testosterone, indicating interference with spermiogenesis and steroidogenesis (Pillai *et al.* 2012; Bonda *et al.* 2004; Luo *et al.* 2015).

Cattle and other animals constitute bio indicators of environmental contamination of heavy metals (Miranda *et al.*, 2009). The results of our study reflect excessive levels of exposure to heavy metals. The consumption of foods produced in contaminated areas and the ingestion or inhalation of contaminated particles are two principal exposure pathways of these potentially toxic metals in man. A close correlation has been reported between the heavy metals concentrations in bovine tissues and that in soil, food and drinking water (Sedki *et al.* 2003; Qiu *et al.*, 2008).

It was therefore possible that the animals in our study (cattle and sheep) were from a polluted area and dietary habits lead to the bioaccumulation of heavy metals in the liver and kidneys. In general, heavy metals such as Pb and Cd have slow removal rates, so that harmful levels could accumulate in tissues after prolonged exposure to low levels in the environment.

The consumption of offal's, particularly the liver, was well known in Algeria related to its high

iron content and also a major source of vitamins, proteins and lipids. This can result in indirect ingestion of Pb and Cd which constitute a serious problem for public health.

Conclusion

The results of this study revealed the presence of heavy metals (lead and cadmium) at different concentrations in all samples (liver and kidneys) analysed. Indeed, liver samples of cattle and sheep contained higher concentrations of Cd and Pb than the kidney.

As well as, lead and cadmium levels of liver and kidney samples in cattle were higher than liver and kidney samples in sheep. This was related to the differences of age of animals studied because age has a significant influence on the accumulation of toxic metals. Moreover, the effect of sex and age on heavy metal exposure has been well confirmed. On the other hand, the concentrations of these metals were high in these organs and the consumption of large quantities represents a potential health risk. Therefore, accurate care and sanitary inspection of livestock in these areas were recommended.

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