Cadmium (Cd) is regarded as one of major the presence of hazardous substances in the environment, with a broad variety toxicity to organs and an extended half-life of removal. Cd exposure for farmed ruminants can come from industrial processing and intensive agricultural operations, which can pollute feed and water. It was noted that most of the wastes produced by nearby factories in Al Fallujah city are either strewn around the highways or use as landfills while the sewage is used for irrigation, while sheep graze on the edges of runways and other sites that might have been contaminated with hazardous materials. EF (enrichment factor) According to the findings, the concentration of Cd in the soils of Al Fallujah city was exceptionally high, and according to sediment content criteria, the soils in Fallujah city were contaminated with high concentrations of Cd, revealing the influence of human and industrial activity on Cd accumulation in the soil. Cadmium levels in liver, kidney and muscle from 216 samples for 72 slaughtered sheep in slaughterhouse at Al Fallujah city, Iraq during the period of October to December 2020 were analyzed. The values of Cadmium detected in sheep liver, kidney and muscle samples were 0.138 ± 0.020, 0.432 ± 0.090, and 0.037 ± 0.003 mg/kg, respectively which refer to high concentration of Cd i

Abstract

Survey for Cadmium Concentration in Liver, Kidney and Muscle of Slaughtered Sheep in Butcher Shops of Fallujah City, Iraq

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Introduction

Cadmium (Cd) is the most dangerous heavy metal in the industrial and environmental elements due to the long half-life (may reach 30 years) and wide range of negative health effects, including carcinogenic, hepato-toxic, nephro-toxic, skeletal, and reproductive effects. It can build up in different tissues, especially the liver and kidneys, amplifying the negative health effects (1). According to the European Community (EC) 488/2014, the maximum level for Cd in sheep liver is 0.50 mg/kg and in kidney 1.0 mg/kg; while in sheep meat (excluding offal) is 0.050 mg/kg fresh weight (2).

The united states of environment protective agency (USEPA)’s Cd in drinking water has a legal maximum of 0.005 parts per million (ppm)8, and The World Health Organization (WHO) recommended safe limits of Cd in both wastewater and soils for agriculture is 0.003 ppm15,16. While target value of soil (0.8 mg/kg) specified to indicate desirable maximum levels of elements in unpolluted soils Source (1).

The urban soils of Fallujah city are exceptionally high enriched with Cd, according to the enrichment factor (EF), exceeded (WHO/FAO) guideline, and according to geoaccumulation index (Igeo), Cd in soil may come from a variety of sources anthropogenic and industrial, such as combustion of fossil fuels, phosphate fertilizer, and atmospheric deposition (3).

Phosphate fertilizers, fossil fuel burning, and other industrial operations clearly contribute considerably more to human exposure than cadmium production among anthropogenic sources of cadmium. Natural sources of cadmium in soils include underlying bedrock and transported parent material like glacial till and alluvium, and sources that are anthropogenic: which agricultural amendments, aerial deposition, and sewage sludge are all possible sources of cadmium in soils (4).

Cd exposure is frequently combined with industrial emissions from smelting and mining operations. Cadmium environmentally published via the smelting of other elements, the burning of fossil fuels, the incineration of waste, and the usage of phosphate and fertilizers derived from sewage (5). Heavy metals may be present in factory wastewater, which build over time in soil deposits along waste water routes as well as in creatures that live near them. Human exposure to polluted wastewater is common, especially in densely populated regions or when wastewater is utilized for agricultural purposes (6).

Main industrial purposes paints, electroplating, plastic stabilizers, and Ni-Cd batteries are all sources of Cd (7). Animal and man absorb cadmium by means of air, water, consuming directly of contaminated livers and kidneys, as well as, contaminated vegetables (8).

Several factors impair Cd bioavailability, retention, and toxicity, including nutritional status (decrease in Fe stores), multiple pregnancies, and health manner or diseases. (9). Cd has the capacity to traverse a variety of membranes and bind to ligands with a high affinity once inside the cell. Cd is a recognized carcinogenic in humans. (10).

Absorbed cadmium bound to metallothionein accumulates at the highest concentrations in sheep's liver and kidneys and other ruminant species, according to research. (11).

The mainly ways of Cd are absorbed through the respiratory and gastrointestinal tracts, as well as through the skin. a little. Usually, the most serious occupational threat for humans and animals is Cd absorption into the lungs, 10- 40% of Cd inhaled then retained (12), in the lungs, mucociliary and alveolar clearance is primarily responsible. The GI tract absorbs very little Cd. (13).

Within 48 hours after administration, ingested Cd is spread to the spleen, adrenals, liver and duodenum in rats. In the kidneys, accumulation is
slower, and high peaks are achieved on the sixth day. For small doses, cadmium concentrations in other organs remaining low, with the kidneys and liver accounting for around half of the all cadmium in the body. (14).

The kidney has the largest cadmium (Cd) concentrations, followed by the liver. Muscles constitute a minor proportion of the total body Cd, despite accounting for a vast proportion of body fat. Muscle meat is therefore well protected from swallowed Cd. (15).

The extended half-life of cadmium is considered to be caused by a metal-binding protein (Metallothionein) found in the kidney. When the body's amount of Cd rises, so does the level of metallothionein. Although metallothionein is found in numerous other organs, it is unclear why the kidneys are the primary location of Cd accumulation. The cadmium—metallothionein complex is really more toxic than Cd alone (16).

The most common endogenous excretion route for Cd is feces. Urinary Cd will increase 50 to 100-fold when there is a lot of Cd in the kidney, which induces proteinuria. (17). The degree of heavy metal toxicity varies based on exposure pathways, environmental conditions, animal diet, lactation level, and animal breed. (18).

Renal tubular dysfunction is caused by cadmium toxicity, as shown when a re-absorption decreased in the proximal tubules. Cadmium has a negative effect on the of reproductive organs, and induced follicular atresia in the ovary (19), it causes a degenerative alteration in the testes with lowering of spermatozoa motility (20).

Anemia, swollen joints, degeneration or retarded testicular development, liver and kidney injury, scaly skin, decreased growth, and high mortality are all common clinical signs of Cd toxicity in mammals (21).

In Iraq and middle east

Cadmium in liver showed significant differences in samarra than Tikrit and beiji: Tikrit, Samarra and Baiji the results was: 0.37, 0.50, 0.37ppm respectively. Also Cadmium in kidney showed significant differences presence in a low level of cadmium in Samarra 0.25ppm, then Tikrit and Baiji 0.37 and 0.40 ppm respectively (22).

In Mosul the results for cadmium showed that muscle, liver, and kidney samples from slaughtered cattle had mean values of 0.009, 0.0591, and 0.0979 mg/kg, respectively, and that only 1.33 percent and 2.67 percent of liver and kidney samples exceeded the EC's maximum acceptable limits, while all muscle samples had levels within the EC's cadmium limit. (23).

The greatest concentration of cadmium in the liver was found in sheep in Ramadi during the autumn and evening seasons (30.88 micro g / g liver), while the lowest concentration was found in beef in al-Baghdadi city during the summer season (9.66 g / g liver) (24).

In Egypt the cadmium residual level in muscles of sheep was 0.7±0.03 ppm, for liver 0.35±0.02 ppm and 0.21±0.1 ppm for kidney (25). While in Iran the mean fresh weight concentrations of cadmium in sheep muscle, liver, and kidney were around 0.0017 mg/kg, 0.0743 mg/kg, and 0.02290 mg/kg, respectively; the mean concentration of cadmium in tissue samples was typically lower than the European Commission's maximum permissible limit (26). The tissues of sheep in Saudi Arabia had significantly mean concentrations of Cd (μg/kg ww) 1.79 ± 0.66 in the liver; 1.18 ± 0.37 in the kidney and 0.44 ± 0.17 in the muscle (27).

Material and methods

Animals

A total of 216 livers, kidneys, and muscle samples were taken at random from 72 slaughtered
Awassi sheep (just males) ages 17.3±0.06 months in slaughterhouse at Al Fallujah city, Iraq during the period of October to December 2020 ((72 samples in October, 81 samples in November and 63 samples in December)) as shown in table-1. Only healthy animals were used in the study, and samples were taken from exact portion of the same organ, including the liver's lobus caudatus, the left kidney's cranial half, and the triceps muscle. During transport to the laboratory, plastic tubes were used to store the samples tissues were stored in and put in a cooler jar, where they were preserved at 20°C awaiting analysis.

Sample preparation and extraction

One gram (1g) of every sample was weighed and combined with 10 mL in a 3:2 ratio HNO3 (65%v/v): the combination was left to digest overnight in the cold before being cooked for 3 hours in a water bath at 70 ºC with stirring every 30 minutes to ensure full digestion. When the mixture be cold, the digest was putting into 20 mL standard flasks, washing with de-ionized water, until metal analyses, prepared sample solutions were stored in acid-leached polyethylene bottles at 25°C (28).

Measurement of cadmium concentrations

Metal's concentrations were read in a UNICAM series 969 Atomic Absorption Spectrophotometer (AAS) (UK), the amount of cadmium (Cd) was measured at 228.8 nm using hollow cathode lamps and an air-acetylene flame.

Statistical Analysis

Normality, mean values, standard errors (SE), Correlation coefficient and significance of correlation were calculated using (IBM SPSS Statistics for Windows, Version 20.0. IBM Corp, Armonk, NY). The results are represented as mean ±SE and P≤0.05 or P≤0.01 was considered significant.

Results and Discussion

In the present study results of examined kidney and liver samples showed exceeding maximum permissible limits (MPL) according to EC Regulation 1881/2006, as modified by EC Regulation 488/2014 ((the maximum amount of Cd in sheep liver is 0.50 mg/kg and 1.0 mg/kg of fresh kidney weight; whereas in sheep meat, excluding offal, the maximum level is 0.50 mg/kg.is 0.050 mg/kg fresh weight)), the Std. Error Mean values of Cadmium detected in sheep liver, kidney and muscle samples were 0.138±0.020 mg/kg, 0.432 ±0.090 mg/kg, and 0.037±0.003 mg/kg, respectively table-2-.

There are differences between mean concentrations of Cd in organs for each month, for liver mean was highest in October (0.162±0.021) and lowest in December (0.117±0.023), also the highest mean of kidney in October (0.473±0.084) and lowest in November (0.401±0.080), while for muscle mean was highest in in November (0.041±0.003) and lowest in December (0.033±0.003) as shown in figure -1-, table-1-.

Two hundred sixteen livers, kidneys, and muscle samples for 72 slaughtered sheep were detected for Cadmium concentration, found 142 (65.74%) of total samples were positive or with toxic concentrations ,74 (34.25%) was negative or below of toxic levels. On the other hand, 58 (80.55%) of liver samples were positive, 14 (19.44%) were negative, while 62 (86.11%) of kidney samples were positive, 10 (13.88%) were negative, and 22 (30.55%) of muscle samples were positive, 50 (69.44%) were negative as showed in table -3- which are means that the higher concentration of Cd was found in kidney and liver respectively.

The results of this study show, that liver Cd concentration was agreement with Kramarova et al., 2005(Slovakia) who found 0.16 mg/kg (29), Froslie et al., 1985(North Norway) 0.180 mg/kg
Liver Cd concentration in present study disagreed with Schulz-Schroeder, 1991 (Germany) who found 0.271 mg/kg (32), Abou-Arab, 2001 (Egypt) 0.261 mg/kg (33), Mariam et al., 2004 (Lahore) 0.33 mg/kg (34), Caggiano et al., 2005 (Italy) 0.33 mg/kg (35), Prankel et al., (U.K.) 0.44 mg/kg, Kramarova et al., 2005 (Slovakia) 0.258 mg/kg (29), also Bazargani-Gilani et al., 2016 (Iran) found 0.21 mg/kg (36).

While this study for liver Cd concentration was higher than Reykdal and Thorlacius, 2001 (Iceland) 0.045 mg/kg (37), Husain et al., 1996 (Kuwait) 0.044 mg/kg (38), Jorhem et al., 1999 (Sweden) 0.031 mg/kg (39), Langlands et al., 1988 (Australia) 0.03 mg/kg (40), Salisbury et al., 1991 (Canada) 0.060 mg/kg (41), Liu (2003) China 0.49 mg/kg (42), Kazemeini et al., 2010 (Iran) 0.074 mg/kg (43), and Akoto et al., 2014 (Ghana) 0.07 mg/kg (44).

This study for kidney Cd concentration was agreement with Froslie et al., 1985 (Norway) 0.547 mg/kg (30), Schulz-Schroeder, 1991 (Germany) 0.547 mg/kg too (32), Prankel et al. (U.K.) 0.36 mg/kg, Husain et al., (1996) Kuwait 0.301 mg/kg (38).

The study found kidney Cd concentration was lower than Langlands et al., 1988 (Australia) 0.96 mg/kg (40), Abou-Arab, 2001 (Egypt) 0.880 mg/kg (33), Liu 2003 (China) 1.83 mg/kg (42), Caggiano et al., 2005 (Italy) 6.71 mg/kg (35), Bazargani-Gilani et al., 2016 (Iran) 1.93 mg/kg (36).

Also in this study, kidney Cd concentration was higher than Nuutamo et al., 1980 (Finland) 0.140 mg/kg (45), Salisbury et al., 1991 (Canada) 0.170 mg/kg (44), Jorhem et al., 1999 (Sweden) 0.12 mg/kg (39), Reykdal and Thorlacius, 2001 (Iceland) 0.058 mg/kg (37), Kazemeini et al., 2010 (Iran) 0.229 mg/kg (43), Akoto et al., 2014 (Ghana) 0.18 mg/kg (44).

The study for muscle Cd concentration was agree with Langlands et al., 1988 (Australia) 0.03 mg/kg (37), and Abou-Arab, 2001 (Egypt) 0.020 mg/kg (30).

Muscle Cd concentration, of this study was higher than Vos et al., 1988 (Netherland) 0.003 mg/kg (46), Jorhem et al., 1999 (Sweden) 0.0019 mg/kg (39), Larsen, 2002 (Denmark) 0.0018 mg/kg (47), Gonzalez-Weller et al., 2006 (Spain) 0.0012 mg/kg (48), Kazemeini et al., 2010 (Iran) 0.001 mg/kg (43).

This study muscle Cd concentration was lower than Liu 2003 (China) 0.17 mg/kg (42), Caggiano et al., 2005 (Italy) 0.16 mg/kg (35).

It's difficult to make comparison between this study and other studies owing to the variations due to:

1- Analytical methodologies employed may be different.

2- Season and physiological conditions for animals.

3- Sex, age and animal species.

4- The nature of the feed and water sources.

5- Level and type of heavy metals contamination in order to prevent harmful of exposure to these heavy metals.

6- The nature and quality of the soil in the study area.

This study found that Al Fallujah city has a high residual concentration of Cd in sheep (kidney and liver) than that recommended by FAO and WHO, this may because this area have more industrial activities and many vehicles burn gasoline which may contaminated the forages and water by these elements.

Conclusion

Cd is a naturally occurring heavy metal that is
found in greater quantities in Cd-rich soils; nevertheless, industrial and agricultural operations account for more than 90% of Cd in the surface environment. Smelting, mining, alloy processing, and companies that utilize Cd as a dye in their production processes, as well as phosphate fertilizers, are all possible sources of Cd for farmed ruminants, this might indicate possible health risks among consumers, particularly youngsters, as a result of the increased meat intake in the research location. The results obtained from this study showed that mean concentrations of Cd in the liver and kidney of sheep were significant.

Table -1- The Mean of (liver, kidney and muscle) sheep Cadmium concentration samples for each three months of the study.

| Months   | Tissue | No. of samples | Std. Error Mean |
|----------|--------|----------------|----------------|
| October  | Liver  | 24             | 0.162±0.021    |
|          | Kidney | 24             | 0.473±0.084    |
|          | Muscle | 24             | 0.037±0.003    |
| November | Liver  | 27             | 0.135±0.015    |
|          | Kidney | 27             | 0.401±0.080    |
|          | Muscle | 27             | 0.041±0.003    |
| December | Liver  | 21             | 0.117±0.023    |
|          | Kidney | 21             | 0.421±0.105    |
|          | Muscle | 21             | 0.033±0.003    |

Table (2) Values of Cadmium detected in sheep liver, kidney and muscle samples

| Tissue | No. of tested samples | Positive Samples | Negative Samples |
|--------|-----------------------|------------------|------------------|
| Liver  | 72                    | 58               | 14               |
|        |                       | 80.55%           | 19.44%           |
| Kidney | 72                    | 62               | 10               |
|        |                       | 86.11%           | 13.88%           |
| Muscle | 72                    | 22               | 50               |
|        |                       | 30.55%           | 69.44%           |
| Total  | 216                   | 142              | 74               |
|        |                       | 65.74%           | 34.25%           |

Table (3) Cadmium percentages in liver, kidney and muscle sheep samples.

| Tissue | Std. Error Mean |
|--------|-----------------|
| Liver  | 0.138±0.020     |
| Kidney | 0.432±0.090     |
| Muscle | 0.037±0.003     |

Figure (1) the Mean of (liver, kidney and muscle) sheep samples for three months of the study.
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