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DETERMINATION OF LEVELS OF LEAD AND CADMIUM IN VARIOUS MEAT TISSUES OF BEEF, GOAT AND LAMB

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Abstract
Lead (Pb) and cadmium (Cd) levels from different fresh meat tissues from beef, goat and lamb were determined in Lebanon for the first time. The inter- and internal distribution levels were estimated and compared. Their potential exposure risk from dietary intake was also studied. A total of two hundred and forty (n = 240) fresh meat tissue samples (n = 80 beef, n = 80 goats, n = 80 sheep) were collected over several months from different abattoirs. Each individual sample was then divided into subsamples for subsequent analysis and quality control purposes. Acid-assisted high-pressure microwave digestion in a closed vessel was used to digest all samples, while graphite furnace atomic absorption spectroscopy was used to quantify the toxic metals. The data showed that average lead levels in goats ranged from 2.66 to 69.84 µg/kg for the different tissues studied. In beef, it ranged from 0.50 to 41.34 µg/kg. While in lambs, levels ranged from 1.59 to 95.95 micrograms/kg. Meanwhile, cadmium levels ranged from 2.39 to 635.86 µg/kg in goat, from 0.55 to 173.72 µg/kg in beef, and from 1.37 to 688.95 µg/kg in lamb. Based on the distribution between levels of lead and cadmium in different tissues, it was found that beef tissues contain lower levels than lamb or goat meat. None of the samples exceeded the maximum permissible limit for lead, while for cadmium, beef tissue is safer for consumption. However, liver and kidney tissue in goat, beef and lamb should be minimized in all cases, especially for children.

Keywords: Lead and cadmium , meat tissues , environmental.

1- INTRODUCTION

Humans can be exposed to heavy metals through food contaminations, water, air, soil, local materials (Fort et al., 2014) , animal tissues, and plants (Dabak et al., 2015). The intake of such metals can cause harm to the body and other negative effects (Fort et al., 2014).Trace elements might become harmful when exposure to them exceeds certain levels (Tack, 2014). Non-essential elements can be toxic even at very low concentrations due to their accumulation in the body and non-degradability (Khan et al., 2010; Islam et al., 2015). While there are many pathways of exposure to heavy metals, the impact of such metals can be classified into carcinogenic impacts (Fu et al., 2014). General signs which may be related with heavy metal intoxication are gastrointestinal disorder, stomatitis, diarrhea, tremor, paralysis, hemoglobinuria (causing a rust-red color to stool), convulsion, and pneumonia (Duruibe et al. 2007). Lead is the main contaminant present in the nature and can be found in the hydrosphere, soils and rocks (Bosch et al., 2015). It is a dense, bluish-gray, odorless, has low melting point, and occurs in the crust of the earth (Nava-Ruiz et al., 2012). The electrical conductivity of lead is poor but processes good resistance to chemical corrosion. In the periodic table lead belongs to Group 4A with an atomic number of 82 and an atomic mass is 207.2g/mol. In nature Lead occurs in two forms; organic and inorganic. Lead (Pb(0)), lead (Pb(2+)), and lead (Pb(4+)) are the three inorganic forms. The divalent form is the most numerous and dominant where its salts can form highly soluble components in water and can invert to the elemental form (Heavy metals, n.d., www.lenntech.com/heavy-metals   Sources and Uses of Lead Lead is a widely dispersed toxic metal in nature and has been used for over 9,000 years (Dongre et al., 2012). It naturally occurs in hydrosphere, soils and rocks (Bosch., 2015). Lead is emitted to the environment by natural and anthropogenic sources. Most lead is released to the ecosystem by human activities such as mining, smelting, fly ash, commercial waste product, irrigation, inorganic fertilizers, phosphate fertilizers, geologic plant materials, vapor from heavy metals, combustion of gasoline, sewage sludge, battery plants, and industrial plants, paints, exercises of military, shooting ranges and hunting (Jenni et al., 2015, Nava-Ruiz et al., 2012). Lead is emitted to the ecosystem by natural sources such as forest fires, volcano eruptions, wind-blown dusts, and sea salt particles (Nagajyoti et al., 2010). Lead is also used in batteries, gasoline, pipes, paints, varnishes, welding (Khan et al., 2010) and cosmetics, dyes (Fang et al., 2014) and X-rays, ceramic . Lead can be found in the atmosphere, water and soil, due to many different sources.

Lead exposure is a main health issue (De Almeida et al., 2010). Exposure of lead is through dietary and non-dietary exposures, and the major human exposure to lead usually comes from the food chain (Winter-Sorkina et al. 2003). Main routes of exposure to this metal include contaminated food, water, air and soil (Tobwala et al., 2014). Food can be polluted during production, storage, transport, industrial processing (Valadez-Vega et al., 2011) (EFSA, 2010) for example, can explain most of the potential exposure routes through which people can obtain such toxic metals. Absorption through the skin is very weak, however, it is not considered to be serious route of exposure (Health Canada, 2011).

Lead is one of the most health hazards for humans because it causes damage to the gastrointestinal, anemia and nervous system (Valadez-Vega et al., 2011), renal dysfunction, cardiovascular diseases, auditory disorder, and visual inability (Afridi et al., 2010). IQ deficits and attention dysfunction (De Almeida et al., 2010), damage to liver, kidneys, hematopoietic system, reproductive system (Fang et al., 2014), DNA damage (Hu et al., 2014), breast cancer (Alatise et al., 2010), interventions in the enzymatic systems (Gonzalez-Weller et al., 2006) are all various possible health problems. Absorbed lead is transported firstly by the blood and soft tissues where the half-life is from 28-30 days, while the is considered as potential carcinogens for humans and classified as a class 2A carcinogens according to the International Agency for Research on Cancer (IARC classification)

For children, the impact on memory decrease and learning disabilities are fundamentally caused by the action and effect of lead on many brain functions (Nava-Ruiz et al., 2012). Lead is more harmful and accumulates in the brain where it passes the blood-brain barrier (BBB) and collects in the gray matter (Nava-Ruiz et al., 2012). Lead poisoning in young children leads to decrease in the cognitive deficits that mean reduced IQ scores even at low concentration of exposure (Nava-Ruiz et al., 2012). Studies have indicated a relation between lead blood concentrations and decrement in IQ score where scores have decreased from 1 to 5 points with a lead blood concentration of 10μg/dL (Ministry for the Environment, 2010)

Cadmium (Cd) occurs naturally in the environment. It is toxic (Gabriella Notarachille et al., 2014), and comparatively volatile, slivery-white and soft. In the periodic table, it belongs to group IIB with an atomic number of 48 and an average atomic weight of 112 g/mol. The element is present in the earth's crust, mostly linked to copper, zinc and lead ores, and can be found at very low concentrations ranging from 0.1 to 1ppm . The Environmental Protection Agency (EPA) considers cadmium as one of the toxic metals that occur among 13 toxic metal kinds on the priority pollutant list (Behbahani et al., 2013). Being 10 times more toxic than lead, it is considered to cause greater risks (Valadez-Vega et al., 2011). Cadmium can have two valence states; the elemental Cd(0), and the ion Cd(+2) , where the former is rare. The bivalent ion is usually found in the form of soluble salts such as $CdCl_2$ and $CdSO_4$, and almost insoluble salts like CdS and $CdCO_3$. Cadmium is classified as nonessential toxic metal Cadmium having the ability to bio-accumulate in the human body (Winter-Sorkina et al., 2003).

Cadmium is one of the most important toxic and main contaminants of the environment due to the rapid extension of industrialization (Wu et al., 2010), and agriculture field (Maciak et al., 2011). Like lead, Cd occurs in the environment due to natural sources and anthropogenic sources (Behbahani et al., 2013). In natural sources the volcano and rock weathering are the main causes of pollute in the environment, also anthropogenic activities have negative effect on the environment such as industrial and agricultural activities (Maciak et al., 2011). It generally occurs in the earth crusts linked with zinc ores (Cheng et al., 2014), and is mixed with other metals (copper, tin, silver and lead) to form metallic alloys (Water UK, n.d.). Cadmium is mainly released by mining, smelting, manufacturers of nickel-cadmium batteries, plastic stabilizers, refineries (Notarachille et al., 2014). Sources of cadmium include food, cigarette and alcoholic beverages (Mao et al., 2010), and can also be found in phosphorites, marine phosphates, and sedimentary rock (Cheng et al., 2014), phosphate fertilizers and sewage sludge (Phillips et al., 2011), irrigation water (Hu et al., 2014). Cd is used in nickel-cadmium batteries, in electroplating, coating for other metals, alloys, pigments, and semiconductors (Behbahani et al., 2013). Pollution environment with cadmium leads to food, water and air contamination that cause risk to the human health.

There are several pathways for human exposure to lead and cadmium as discussed earlier. Food chain is the major source of accumulation of many heavy metals. Specifically, meat is an important factor in the human diet because it represents the major source of proteins (Lukacova et al., 2015). This makes them a main source of lead and cadmium, thus leading to several diseases related to the toxicity of such metals. Accordingly, consuming meat becomes an important concern and thus studying its level of heavy metals becomes important to estimate the health risks derived from their consumption. Consequently, domestic animals that are generality sensitive and more extremely exposed to major doses of lead (Rudy, 2009) is of major interest in this study. The extent of lead and cadmium exposure depends on many factors such as animal's specie; age, and the way they are bred (Rudy, 2009). Individuals consuming their meat, thus, should be aware of the effects heavy metals on their health. For the measurement of such levels, higher sensitive techniques are required to determine the low concentrations of lead and cadmium in meat. Graphite

Furnace Atomic Absorption Spectroscopy (GFAAS) is one of the most important techniques that may be used to determine such levels (Damin, 2007).

2- Expermental and Methodology

Analytical Methods :A set of analytical techniques have been applied for the detection and quantification of heavy metals. Graphite furnace atomic absorption spectroscopy (GFAAS) is the most common method used. In addition, inductively coupled plasma-mass spectrometry (ICP-MS), flame atomic absorption spectroscopy (FAAS) (heavy metals), and inductively coupled plasma-optical emission spectrometry (ICP-OES) may also be used (Demirezen, 2006). These methods vary in their limits of detection, accuracy, and precision. In addition, they vary widely in cost, sample preparation, process of calibration and amounts of reagents and provisions needed. Such factors are taken into consideration in the choice of the adopted method where minute concentrations of analyses are now possible to be detected, with very low interferences.

Apparatus: Before digestion, samples were dried overnight in a programmable oven. Samples were digested using Thermo Ethos 1 (Milestone, Rainbow S.A.R.L.) microwave digestion oven. For detection study, a Thermo-Electron M series graphite furnace atomic absorption spectrometer (GFAAS) equipped with deuterium and Zeeman background correction (Zeeman Furnace GF95Z) together with an autosampler (FS95) was used (Thermo Electron Corporation, Milestone, distributed by Rainbow S.A.R.L.). The sample atomization is accomplished in specialized graphite tubes (Thermo Elemental Omega Platfrom Extended Lifetime Cuvettes). Coded hallow cathode lamps of lead and cadmium (Thermo-electron Corporation, Germany) were included in the GFAAS's carousel, where the lamp selection was done automatically based on the metal to be analyzed(Al-Chaarani, N, 2009). During all phases of heavy metal analysis, except for the atomization step, 99.999% pure argon gas was used as an internal inert gas having a flow of 300mL/min.

Reagents

Throughout the digestion and detection steps, Double Distilled de-ionized Water (ddH2O) obtained by water treatment using Milli-Q system (Millipore, Numelab) was used. Working standards for lead and cadmium were prepared using standard solutions supplied by Romil-Pure chemistry (Standard solutions 1000ppm element reference solution). Concentrated nitric acid 65% and hydrogen peroxide (30%) of high quality and purity were used for the sample digestion. Purified nitric acid was also used for the preparation of wash solution and diluents for the Graphite Furnace

Atomic Absorption Spectrometer (GFAAS). Ascorbic acid 99.99% pure and trace metal basis (purchased from Sigma-Aldrich) was used as pure chemical matrix modifier for lead detection, while magnesium nitrate 99.99% pure and trace metal basis (purchased from Sigma-Aldrich) was used as pure chemical matrix modifier for cadmium detection in the GFAAS. Certified Reference Material (CRM) (SRM1577C Bovine Liver NIST national institute of standards and technology) was used to validate all analytical procedures utilize

Optimization Conditions Cadmium and lead are volatile elements in nature with a wide range of volatility that is dependent on heat applied. Thus caution should be taken into consideration for choosing the optimum conditions for the heating program of the GFAAS and whether or not matrix modifiers should be used.

Samples : Fresh meat samples (100-200g each) of different tissues (liver, kidney, fat, bone marrow, heart, lung, muscle, and spleen) were purchased from various abattoirs in North Lebanon. The eight kinds of tissues were obtained from the same organism (lamb, beef, or goat) after slaughter and the procedure was repeated from other organisms and from several places. A total of 240 samples of lamb, beef, and goat were collected. For each organism, the eight various tissues were collected twice from each one organism, were labeled correctly and stored in acid pre-cleaned 50-mL conical tubes at -20˚C until treatment.

Sample Analysis by GFAAS: Prior to analyzing the samples in the GFAAS, the instrument was programmed according to the kind of analyses to be detected. The optimized parameters for lead and cadmium were entered into software of the instrument. Mother solutions of 10ppb for Pb and 1 ppb for Cd) for the calibration curve were prepared by using 1000ppm stock solutions and by using 10 fold dilutions each time, so as to diminish the analytical preparation errors as much as possible. The mother solutions were used by the GFAAS's auto-sampler in order to create automatically a specified five-point calibration curve specific for each metal. All dilutions were done using 0.5% nitric acid solution. A 1% nitric acid solution used to wash the auto-sampler injection tube between each dilution so as to prevent any cross-over contamination between the calibration points and the samples (Al-Chaarani, N, 2009).

3- Results

The levels of distribution of lead and cadmium within various meat tissues of beef, goat and lamb have been determined . Since such studies have not been carried out in our area, it becomes important to do this work and become aware of the risk from consuming such tissues.

The work focused on the levels of distribution of lead and cadmium in various meat tissues obtained from various organisms and their possible consumption risk. Such quantitative data may be looked at from various aspects in order to assess the levels of the metals in question and their possible health risk. First, the data clearly reveals that Pb and Cd were detected in all samples thus suggesting that the two toxic metals are found and spread in our environment. Considering the inter-level distribution of Pb and Cd in different tissues, generally, beef meat tissues have shown the lowest concentrations of both metals in comparison to goat and lamb . This suggests that the consumption of beef tissues may provide less exposure to either metal as opposed to goat and lamb. This may be due to many factors such as the size/weight of the animal, the distribution characteristics of the metal within the organism as well as the feeding habits and environment in which the animal was brought up. The way of 'feeding' may be considered as the most important factor in the area where cows are generally raised indoors while goats and lambs are raised on pasture. Similarly Oskarson et al (1992) have reported comparable observations where Pb levels in cows (muscle tissue) raised on pasture were higher than those raised indoors (Oskarsson et al., 1992). However, liver from lamb gave as close as 96% of the MAL of lead .Since the data reports mean concentrations of resamples for both metals, a closer look at the individual samples prior to taking the mean, reveals that 20% of goat liver, 40% of lamb liver, and 10% of lamb kidney samples have exceeded the MAL of Pb, whereas none of the samples in cow have done so. This once more, suggests that consumption of beef tissues may be less hazardous as opposed to consuming tissues from goat or lamb. On the other hand, and in comparison of the mean values of Cd obtained with the MAL of Cd (50 μg/kg) (Dailos, et al., 2006), cow heart tissues, as well as liver and kidney tissues of all three organisms studied have exceeded the MAL of Cd. Similarly, and by extracting samples information from the raw data for Cd prior to mean calculations, 80% of liver and kidney sample tissues in goat have exceeded the MAL of Cd, while 20% heart, 40% liver and kidney of cows, and 70% of liver, 90% of kidney of lamb, have all exceeded the Cd MAL. The results obtained in this study are very similar to those reported elsewhere (Ihedioha, et al., 2012).

According to many studies between 1995 and 2014 (Nkansh, et al., 2014; Stoyke, et al., 1995; Pompe-Gotal et al., 2002), the high accumulation of such metals in the liver and kidney was found to be directly related to their function as storage and excretory organ, respectively. In addition, going back to the fact that cows are raised indoors while goats and lambs are raised on pastures strongly suggests that the two latter species are more vulnerable to higher exposures to such toxic metals since there are no limits on what they feed on. Such unidentified sources of toxic metals could very well be attributed to

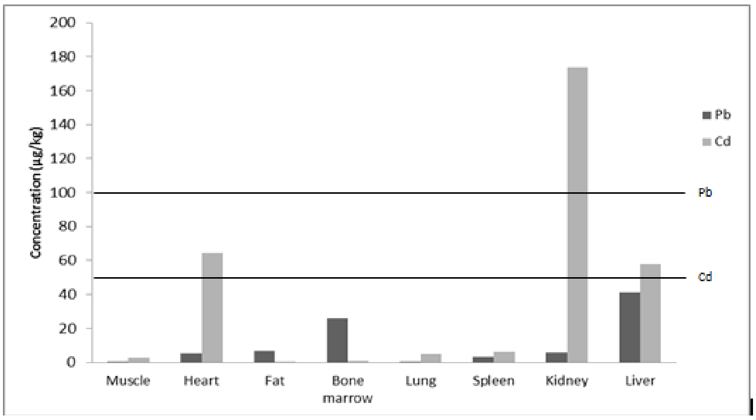

Figure 1.1: Lead and Cadmium Concentrations in Different Tissues of Beef.

of metals from the environment onto plants, municipal wastes, lead and nickel-cadmium batteries, electronic wastes, all of which are dumped and burnt in our area and are non-responsibly discarded. Considering the intra-level distribution of Pb and Cd in the different tissues studied, liver and kidney tissues of all organisms studied showed the highest amounts of bioaccumulation as opposed to all other tissues within each organism (Figures 1.1 ,1.2 and 1.3). This data strongly suggests that consumption of liver and kidney tissues from any of the three organisms may present a health risk if included regularly in the diet.

According to provisional tolerable weekly intake levels (PTWI) for Pb (Obeid, et al., 2014), and assuming a weekly consumption of 1 kg of the most contaminated meat tissue (lamb liver, 95.95 µg/kg,) by a 60 kg individual, 6.4% of the PTWI will be reached. For a 14-year old child however, 27.4% of the PTWI is reached suggesting that in the case of Pb, no major health concerns are present. In the case of cadmium however, and considering the most contaminated tissue (lamb kidney, 688.95 µg/kg,) 164% of Cd's PTWI is reached by a 60kg individual. The matter is much worse for a 14-year old child where 703% of the PTWI is reached. This suggests once again that beef meat tissues are safer for consumption but nevertheless, tissues from liver and kidneys of goat, beef and lamb should be minimized in all cases, especially for children

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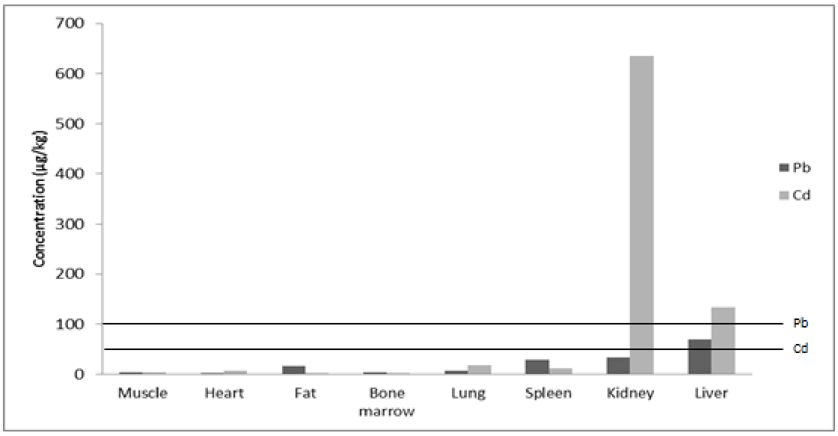

Figure 1.2: Lead and Cadmium Concentrations in Different Tissues of Goat.

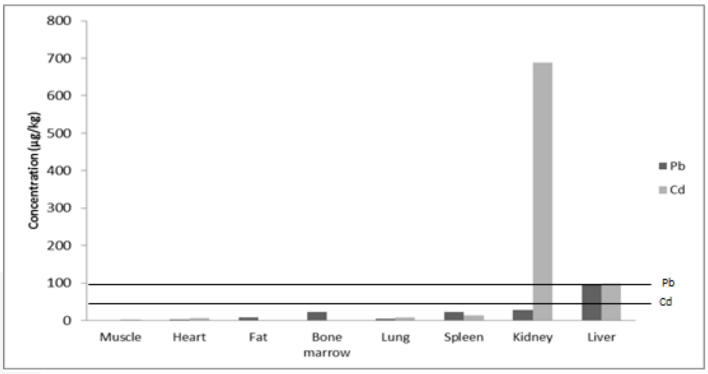

Figure 1.3: Lead and Cadmium Concentrations in Different Tissues of Lamb.

4- Conclusions and Recommendations

In this study the concentrations of lead and cadmium were analyzed and determined in various meat tissue samples from cow, goat and lamb in Lebanon. Since both metals have been detected in all of the samples, this will raise serious concerns on the extent of pollution in our environment. Based on the inter-level distribution of both Pb and Cd in the tissues of cow, goat and lamb, beef tissues were found to contain relatively lower levels of the metals in question, suggesting that beef in general may be considered safer for consumption as opposed to goat and lamb. Based on the intra-level assessment of both metals the liver and kidney tissues were found to contain high levels of both metals to a point where their MALs have been far exceeded. Regarding health risks from consuming such products, it is worth noting that consumption of goat and lamb should be minimized as opposed to beef. Of course, this is a generalization which an individual can use as a guideline and is not meant to be used since many more studies should be carried on the subject for definitive results.

The study also strongly suggests that consumption of liver and kidney tissues from any type of organism should be highly minimized since such organs were found to accumulate the highest amounts of Pb and Cd in all species studied. In light of this, researchers in the area as we as national health organizations should strongly engage in constant monitoring and evaluation of levels of toxic metals in the environment and in foodstuffs so as to provide some information on the extent of contamination and its sources and perhaps may be able to take measures in controlling such levels in the future. This study is the first of its kind in Lebanon, more research should be done while determining the presence of other heavy metals that pose greater health risks. This is a onetime study that does not lead to any definite results, however, it places the spotlight over an important issue that must be further investigated by governmental and world health organizations repeatedly, in order to give certain and conclusive and ongoing results. In addition to research, awareness programs and advisories caring for the public health and aiming to increase individual knowledge regarding the toxicity of such elements are essential to give the consumer full awareness and knowledge allowing them to balance between the benefits of meat and the risks derived from their consumption.

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