Sudan University of Sciences

And Technology

Collage of graduate studies

Screening of Some Heavy Metals in Food of Animal Origin in Rabak Locality at White Nile State, Sudan

فرز بعض المعادن الثقيلة في أغذية من أصل حيواني بمحلية ربك بولاية النيل الأبيض.

By:

Mogahed Awad Ahmed

B.V.M 2016 U of K

A thesis submitted for fulfillment of the requirements of College of Graduate Studies for the award of the Degree of Master of Veterinary Medicine, in Preventive Medicine

Supervisor: Prof. Mohamed Abdalslam Abdalla

Department of Preventive Medicine and Public Health, Sudan University of Science and Technology

March, 2022

Dedication

To my mother's soul,

to my father

my family,

my friends

with my best

wishes

Acknowledgements

I would like to acknowledge God Almighty as the ultimate source of all wisdom, diligence and guidance in this work that has been completed. Blessed be his name forever.

I would also like to have mercy on the soul of my former professor and supervisor, **Dr. Youssef Hussein Al-Mansoori**, and I ask God for his mercy and forgiveness.

My deepest gratitude and appreciation to **Prof. Mohamed Abdelsalam Abdalla**, the supervisor for his guidance, encouragement and technical advice, and for giving me the full confidence that resulted from my work. I wish him all the best and a happy life.

Thanks for the staff of the library of the Faculty of Veterinary Medicine, Sudan university of Science and Technology.

Finally, I would like to thank my colleague, Ahmed Alsmani Gamer Aldaowla for his help in statistical analysis.

ABSTRACT

This study was conducted in Rabak area, White Nile state for screening of some heavy metals (cadmium, copper, lead and zinc) in some tissues of fish, bovine and ovine during April 2020. Samples were collected from Rabak central fish market and Rabak slaughterhouse and processed in the laboratory of the National Center for Research in Khartoum. The results showed that the means concentration of Cd, was higher in fish muscle compared of bovine and ovine where as it was concentrated in ovine liver and bovine kidney. The lead is high in bovine tissue bovine liver where as high in bovine kidney. The copper is high ovine liver and ovine kidney. The high concentration of zinc in bovine muscle and bovine liver samples. The result showed that some samples were not confirm of some international standards European Commission standard (EC 1881, 2001) and FAO standards (FAO, 2003).

الخلاصة

أجريت هذه الدراسة بمنطقة ربك بولاية النيل الأبيض لفحص بعض المعادن الثقيلة (الكادميوم , النحاس ,الرصاص والزنك) في بعض أنسجة الأسماك والأبقار والضأن خلال شهر أبريل 2020. تم جمع العينات من سوق ربك المركزي للأسماك ومسلخ ربك ومعالجتها في معمل المركز القومي للبحوث في الخرطوم. وأظهرت النتائج أن متوسطات تركيز الكادميوم كانت أعلى في عضلات السمك مقارنة بالبقر والضأن حيث تركزت في كبد الضأن وكلية الأبقار. تحتوي أنسجة وكبد الأبقار على نسبة عالية من الرصاص وتحتوي كلى الأبقار على نسب عالية من الرصاص أيضاً . النحاس مرتفع في كبد الضأن وكلية الضأن. إرتفاع تركيز الزنك في عينات عضلات الأبقار وكبد الأبقار. أظهرت النتائج أن بعض العينات لم تكن مطابقة لبعض المعايير الدولية للمفوضية الأوروبية (EC 1881، 2001) ومعايير منظمة الأغذية والزراعة (الفاو ، 2003)

Table of contents

List of Tables

List of Acronyms

Cd	Cadmium
Cu	Copper
Pb	Lead
Zn	Zinc
Ec	European Commission
FAO	Food and Agriculture Organization
AAS	Atomic Absorption Spectrophotometer
SPSSS	Statistical Package for Social sciences
SD	Standard Deviation
Mg	Milligram
Kg	kilogram

List of Figures

Introduction

Metals are naturally occurring elements that are lustrous, good conductor of electricity, generally enter chemical reactions as positive ions or actions and are non-biodegradable. Metals are generally detoxified in organisms by hiding the active metal ions within a protein, deposition as insoluble form intracellular granules for long-term storage or excretion in the faces. Heavy metals are group of metals that are environmental pollutants and most of them have a density relative to water of greater than five. However, there are some metals for example Aluminum (Al) has a relative density of only 1.5 and considered extremely environmental pollutant. Heavy metals also include metalloids (semimetals), such as arsenic (As) (Walker *et al*., 2001). Although heavy metals are naturally occurring elements, most environmental pollution and plant and animal exposure result from anthropogenic activities (He *et al*., 2005; Shallari *et al*., 1998). Some of the heavy metals are essential for various biochemical and physiological functions and their deficiency leads to disease condition. These metals include cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) (WHO/FAO/IAEA, 1996). Other metals such as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) have no established biological functions and are considered as non-essential metals (Chang *et al*., 1996). Heavy metals affect cell organelles and some enzymes involved in metabolism, detoxification, and damage repair (Wang and Shi, 2001). The most toxic effects are induced by arsenic, cadmium, chromium, lead and mercury (Nordberg *et al*., 2015).

This study will be useful in determining the potential risk from the toxic effects of heavy metals and to make recommendations future implementations by the local health regulatory authorities. River water supports many lives. Farmers provide recreation and fishing to the communities and it may also be used for drinking purposes and irrigation. However, contamination of river water system by trace metals is of major concern and their determination has received great attention for monitoring environmental pollution since the events of Hg and Cd poisoning through fish. The pollution of aquatic environment with heavy metal has been worldwide problem during the recent years because they are indestructible and most of them have toxic effect to organism (Lafuente A, *et al*., 2004). Very few data are available on the presence of heavy metals in meat of animal origin in White Nile. Therefore, there is a serious need to establish a local database or conducting risk assessment studies in local animals and foodstuffs to evaluate the potential risk or threat to human from heavy metals. However, the consumer awareness world wide of these hazards has greatly affected the international animal products trade. Therefore, most countries have developed or started to develop their own monitoring system to meet the international residues standards in food of animal origin which was governed by the codex alimuntarus. In Sudan, very little work has been done in this field therefore this study aims to determine the presence of heavy metals in food produced in the White Nile State. Ignorance of the extent of heavy metal pollution causes heavy metals bioaccumulation in animals and human tissues resulting in disease conditions.

Objectives of the Study

1.General objective

The general objective of this study was to develop local and national database to assess potential risks or threats of heavy metals to human consuming meat from cattle, sheep and fish containing heavy metals.

2.Specific objectives

To evaluate the potential risks of heavy metals in the bovine, ovine tissues and fish in Rabak locality and determination of the levels of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn).

CHAPTER ONE

Literature Review

1. Meat

Meat is defined as those animal tissues, which are suitable for use as food. All processed or manufactured products, which might be prepared from those tissues, are included in the definition (Judge et al, 1990). Gunter and Peter (2007) defined meat as the muscle tissue of slaughter animals.

1.1. The nutritive value of meat

The nutritive value of meat is attributed to its protein, fat, carbohydrates, vitamins and minerals content (Mahassin, 2008). Red meat contains protein of high biological value and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fats. Although the nutritional composition of meat varies somewhat according to breed, feeding, season and meat cut. In general, lean meat has a low-fat content, moderate in cholesterol and rich in protein and many essential vitamins and minerals (Williams, 2007). Meat is essential for growth and maintenance of good health and mainly composed of proteins, fat, carbohydrate and some important essential elements. Need for mineral compounds depend on age, physiological state and feed intake as well as on living conditions (Ayesha *et al.*, 2014). Meat makes up an essential part of the food and is mainly composed of protein, fat and some important essential elements (Akan *et al*., 2010). It is also a good source of niacin, vitamins B6 and B12, phosphorous, zinc, and iron (Williams, 2007). Animal proteins have a high biological value (Ziegler, 1968) and the presence of essential amino acids makes it a complete protein (Bastin, 2007).

Apart from meat and meat products forming an important part of the human diet as well as an important source of a wide range of nutrients, they may also carry certain toxic substances (Fathy *et al*., 2011).

2. Fish

Fish as a food is consumed by many animal species, including humans. Three quarters of the Earth are covered by water so, fish has been an important part of the diet of humans in almost all countries in the world since the dawn of time. Fish is one of the cheapest sources of animal proteins and availability and affordability is better for fish in comparison to other animal protein sources. Fish serves as a healthy-food for the affluent world owing to the fish oils which are rich in polyunsaturated fatty acids (PUFAs), specifically omega-3 PUFAs and at the same time, it is a health-food for the people in the other extreme of the nutrition scale owing to its proteins, oils, vitamins and minerals and the benefits associated with the consumption of small indigenous fishes (Panda, 2015).

2.1. The nutritive value of fish

Fish is an important component of human diet. Fish contains proteins and other nitrogenous compounds lipids, minerals and vitamins and very low level of carbohydrates. Protein content of fish varies from 15 to 20% of the live body weight. Fish proteins contain the essential amino acids in the required proportion and thus, improve the overall protein quality of a mixed diet. The superior nutritional quality of fish lipids (oils) is well known. Fish lipids differ greatly from mammalian lipids in that they include up to 40% of long-chain fatty acids (C14 - C22) that are highly unsaturated and contain 5 or 6 double bonds. Fish is a good source of vitamin B complex and the species with good number of livers are good source of fat-soluble vitamins A and D. Fish is particularly a good source of minerals like calcium, phosphorus, iron, copper and trace elements like selenium and zinc. Besides, saltwater fish contains high levels of iodine also. In fact, fish is a good source of all nutrients except carbohydrates and vitamin C (Panba, 2015).

3. Heavy Metals:

Heavy metals can be very harmful to the human body even in low concentrations as there is no effective excretion mechanism (Ghosh *et al.,* 2012). Metals in general can be classified as toxic (cadmium, lead and mercury) and essential (cobalt, copper, zinc, iron). Toxic elements can be very harmful even at low concentration when ingested over a long time period due to their ability to accumulate in human and animal body (Ray, 1994). Of the 92 naturally occurring elements, approximately 30 metals and metalloids are potentially toxic to humans, Be, B, Li, Al, Ti, V, Cr, Mn, Cu, Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, Pt, Au, Hg, Pb, and Bi. Heavy metal is the generic term for metallic elements having an atomic weight higher than 40.04 (Ming-Ho, 2005). The sources of toxic metals in the environment are the fossil fuels, mining industries, waste disposals and municipal sewage. Farming and forestry also contribute to the metal content in the environment due to the uses of fertilizer, pesticide and herbicides (Jayasekara *et al*., 1992; Lukáčová and Golian, 2014). Metals, such as iron, copper, zinc and manganese, are essential metals since they play important role in biological systems, whereas mercury, lead and cadmium are toxic, even in trace amounts. The essential metals can also produce toxic effects at high concentrations. Only a few metals with proven hazardous nature are to be completely excluded in food for human consumption. Thus, only three metals, lead, cadmium and mercury, have been included in the regulations of the European Union for Hazardous Metals (EC, 2001). There has been a major environmental focus especially during the last decade. Sediments are important sinks for various pollutants like pesticides and heavy metals and also play a significant role in the remobilization of contaminants in aquatic systems under favorable conditions and in interactions between water and sediment. Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level (Rashed, 2001).

3.1. Source of heavy metals

Heavy metals enter the environment by natural and anthropogenic means. Such sources include: natural weathering of the earth's crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout and a number of others (Ming-Ho, 2005). Metals occur naturally in the earth's crust and their contents in the environment can vary between different regions resulting in spatial variations of background concentrations. The distribution of metals in the environment is governed by the properties of the metal and influences of environmental factors (Khlifi and Hamza, 2010). Heavy metals from manmade pollution sources are continuously released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystems is growing. Contamination with heavy metals is a serious threat because of their toxicity due to bioaccumulation and bio magnifications in the food chain (Demirezen and Uruc, 2006). Absorption of metals and metal compounds inhaled as particles are influenced by several processes that include deposition, mucociliary and alveolar clearance, solubilization and chemical binding (André *et al*., 2005). Instances of heavy metal contamination in meat products during processing have also been reported (Akan *et al*., 2010). Methods such as singeing off the hairs of the animals in flame fueled by various substances such as wood mixed with spent engine oil, plastics mixed with refuse or tires. These materials contain toxic substances such as heavy metals which can contaminate the meat and render them unfit for human consumption (Okiei *et al.* 2009).

3.2. Risk of heavy metals

After entering the body, the metals deposited in nasopharyngeal, tracheobronchial, or pulmonary compartments may be transported by mucociliary action to the gastrointestinal tract. Metals can also be phagocytized by macrophages (André *et al*., 2005). 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 (Santhi *et al*., 2008). These pollutants often have direct physiological toxic effects because they are stored or incorporated in tissues, sometimes permanently (Marian and Jonathan, 2014). Heavy metals such as mercury, plutonium, and lead are toxic metals that have no known vital or beneficial effect on organism and their accumulation over time in the bodies of animals and humans can cause serious ailments. Among all heavy metals, however, cadmium and lead are known as highly toxic (Reena *et al*., 2011). The toxicity of heavy metals depends on a number of factors. Specific symptomatology varies according to the metal in question, the total dose absorbed, and whether the exposure was acute or chronic. The age of the person can also influence toxicity. For example, young children are more susceptible to the effects of lead exposure because they absorb several times the percent ingested compared with adults (Soghonian and Sinert, 2008).

3.3. Classification of heavy metals

3.3.1. Essential heavy metals

Copper, chromium, zinc-nickel, cobalt and iron are essential metals required for all vital processes inside the body with optimum level. Otherwise, inadequate amount causes deficiency diseases and high-level cause's toxicity (Sivaperumal *et al.,* 2007).

3.3.2. Non-essential heavy metals

Non-essential heavy metals do not have biological roles and also called xenobiotic. When they are increased in concentrations, it will cause toxopathic effects in tissue; those involve aluminum, mercury, lead, Cadmium and others (Sfakianakis *et al.,* 2015).

4. Element of heavy metals

4.1. Lead (pb)

Lead as a toxicologically relevant element has been brought into the environment by man in extreme amounts despite its low geochemical mobility and has been distributed worldwide (Oehlenschläger, 2002). Lead still has a number of important uses in the present day; from sheets for roofing to screens for X rays and radioactive emissions. Like many other contaminations, lead is ubiquitous and can be found occurring as metallic lead, inorganic ions and salts (Harrison, 2001). Lead is a toxic metal that has no known vital or beneficial effect on organisms and its accumulation over time in the bodies of animals and humans can cause serious ailments. Lead may enter the atmosphere during mining, smelting, refining, manufacturing processes and by the use of lead containing products (Marian and Jonathan, 2014).

4.1.1. Source of lead contamination

The source of lead contamination of livestock is from the air, water they drink and food they eat. In general, lead accumulates in the plants and animals, while its concentration is magnified in the food chain (Halliwell *et al*., 2000). Food is one of the major sources of lead exposure; the others are air (mainly lead dust originating from petrol) and drinking water. Plant food may be contaminated with lead through its uptake from ambient air and soil; animals may then ingest the lead contaminated vegetation. In humans, lead ingestion may arise from eating lead contaminated vegetation or animal foods. Another source of ingestion is through the use of lead-containing vessels or lead-based pottery glazes (Ming-Ho, 2005).

4.1.2. Toxicity of lead

Lead is a metabolic poison and a neurotoxin that binds to essential enzymes and several other cellular components and inactivates them (Cunningham and Saigo, 1997). Toxic effects of lead are seen on haemopoietic, nervous, gastrointestinal and renal systems (Baykov *et al*., 1996). Lead has no essential function in humans, about 20% to 50% of inhaled, and 5% to 15% of ingested inorganic lead is absorbed. In contrast, about 80% of inhaled organic lead is absorbed and ingested organic Pb is absorbed readily. Once in the bloodstream, lead is primarily distributed among blood, soft tissue, and mineralizing tissue (Ming Ho, 2005). The bones and teeth of adults contain more than 95% of the total body burden of lead. Children are particularly sensitive to this metal because of their more rapid growth rate and metabolism, with critical effects in the developing nervous system (Castro-González and Méndez-Armenta, 2008). Lead is toxic heavy metal with widespread industrial use, but no known nutritional benefits. Chronic exposure at relatively low levels can result to damage to kidneys and liver and to immune, reproductive, cardiovascular, nervous and gastrointestinal systems (Okoye and Ugwu, 2010). Lead, for example, bio-accumulates in plants and animals. Its concentration is generally magnified in the food chain (Halliwel et al., 2000). Tuormaa (1995) reported that an excessive lead accumulation in children is known to cause hyperactivity, reduced intelligence and antisocial behavior. The main toxic effect of lead is nervous system dysfunction of the fetus and infants. In adults, it causes adverse blood effects, reproductive dysfunctions, damage to the gastrointestinal tract, nephropathies, damage to the central as well as the peripheral nervous system and interferences in the enzymatic systems (Rubio et al., 2005). Janefrances (2010) reported that, the lead intoxication leads as: encephalopathy in the central nervous system, disturbances in the kidney and liver functions progressing as far as necrosis, damage to the reproductive organs, premature births, spontaneous abortion, anemia and many metabolic deficiency symptoms.

4.2. Cadmium (Cd)

4.2.1. Source of cadmium

Cadmium is one of the metallic elements of most concern in the food and environment of man. The main exposure is encountered in industries dealing with pigment, metal plating, some plastics and batteries. Cadmium pollution (e.g., the emissions of a cadmium smelter or industry and the introduction of cadmium into sewage sludge, fertilizers and groundwater) can result insignificant human exposure to cadmium through the ingestion of contaminated foodstuffs, especially grains, cereals, and leafy vegetables (Smolder, 2001; Reesal *et al*., 1987). Airborne cadmium exposure is also a risk posed by the incineration of municipal waste containing plastics and nickel-cadmium batteries. Cigarette smoking constitutes an additional major source of cadmium exposure (Janference, 2010). However, for most people the primary source of cadmium exposure is food (WHO, 1992) since food materials tend to take up and retain cadmium. Cadmium in air, drinking water and food has the potential to affect the health of whole populations (WHO, 1994).

Tobacco smoke is one of the largest single sources of cadmium exposure in humans. Tobacco in all of its forms contains appreciable amounts of the metal. Because the absorption of cadmium from the lungs is much greater than from the gastrointestinal tract smoking contributes significantly to the total body burden (Figueroa, 2008; Ming-Ho, 2005). In general, for non-smokers and non-occupationally exposed workers, food products account for most of the human exposure burden to cadmium (ExtoxNet, 2003). In food, only inorganic cadmium salts are present. Organic cadmium compounds are very unstable. In contrast to lead and mercury ions, cadmium ions are readily absorbed by plants. They are equally distributed over the plant. Cadmium is taken up through the roots of plants to edible leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the cure of the kernel. Cadmium also accumulates in animal milk and fatty tissues (Figueroa, 2008). Therefore, people are exposed to cadmium when consuming plant and animal-based foods. Seafood, such as mollusks and crustaceans, can be also a source of cadmium (Castro and Méndez, 2008; WHO 2004b; WHO 2006). Food is one of the principle environmental sources of cadmium (Baykov *et al*., 1996). As cadmium moves through the food chain it becomes more and more concentrated as it reaches the carnivores where it increases in concentration by a factor of approximately, 50 to 60 times (Daniel and Edward, 1995).

4.2.2. Toxicity of heavy cadmium:

Cadmium is not known to have any beneficial effects, but can cause a broad spectrum of toxicological and biochemical dysfunctions (Funakoshi *et al*., 1995). Cadmium is a cumulative toxicant in the continental ecological cycling; it accumulates mostly in the liver and kidney (Zasadowski *et al*., 1999). Cadmium is naturally present in the environment: in air, soils, sediments, sand and even in unpolluted seawater. Cadmium is emitted to air from mines, metal smelters and industries using cadmium compounds for alloys, batteries, pigments and in plastics, although many countries have stringent controls in place on such emissions (Harrison, 2001). The renal toxicity of cadmium is reduced or abolished by increasing intakes of zinc, copper and selenium. The preventive effects of pretreatment with zinc and copper have been suggested to be the result of increased production of metallothione in the liver and renal cortex (Pizent *et al*., 2001). Cadmium accumulates in the human body affecting negatively several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system (Castro and Méndez, 2008). Other damages that have been observed include reproductive and development toxicity, hepatic, hematological and immunological effects (Apostoli and Catalani, 2011). Toxic effects of cadmium are kidney dysfunction, hypertension, hepatic injury and lung damage (John and Jeanne, 1994). Cadmium chloride at teratogenicity dose induced significant alterations in the detoxification enzymes in the liver and the kidney (Reddy and Yellamma, 1996). The health risks caused by lead and cadmium are well known and the levels in food as well as the migration of these metals from food containers are regulated (Tahvonen and Kumpulainen, 1994). Cadmium has a long eminence in the human body (between 10-40 years), especially in the kidneys (Rubio *et al*., 2006). In some cases, high concentrations of Cu and Zn in feeds for pigs and poultry lead to contamination of produced manure. If this is applied to agricultural land, as fertilizer, it might lead to pollution of land with these metals resulting in a pollution risk for other animals that are fed with the plants issued from that field (Poulsen, 1998). The biological half-life of Cadmium in the human kidney is long and has been estimated to be 10 to 30 years (Fox Spivey, 1987; Gabriel *et al.*, 2014). The risk associated with the exposure to heavy metals present in food product has aroused widespread concern in human health. Cadmium is primarily toxic to kidney, especially to proximal tubular cells. Bone demineralization is affected by cadmium toxicity directly by bone damage and indirectly as results of renal dysfunction (Marian and Jonatha, 2014).

4.3. Zinc (Zn)

4.3.1. Source of zinc

Zinc is available in most foodstuffs and beverages (ATSDR, 1992). Zinc is necessary for the function of a large number of metallic enzymes (ASTDR, 1992). It acts to diminish the toxicity of cadmium and cupper (Florence and Batley, 1980). Zinc is an essential element in animal and human diet. Too little zinc can cause problems; however, too much zinc is harmful to human health nausea and vomiting, epigastria pain, abdominal cramps and diarrhea (Plum *et al.*, 2010). Zinc is a common element in the human environment and is essential for many biological functions (Prasad *et al.*, 1963).

4.3.2. Toxicity of zinc

The acute zinc poisoning has been reported; its manifestations include nausea, vomiting, diarrhea, fever and lethargy and have been observed typically after ingestion of 4-8g of zinc (WHO, 1996). Toxicity of zinc seems to be low; however, various toxic reactions such as the metal fume fever in which the victim suffering from pulmonary distress, fever and gastroenteritis following ingestion of zinc salts have been reported in man (Murphy, 1970). Long-term feeding of very large amounts of zinc salts to rodents resulted in growth retardation, anemia, metabolic effects and ruminants appear more susceptible to zinc than rodents (Campbell and Mills, 1979). Underwood (1977) mentioned that zinc does not accumulated with continued exposure, but body content is modulated by homeostatic mechanisms that act principally on absorption and liver levels.

4.4. Copper (Cu)

Cupper acts as a cofactor in various redox enzymes, in mitochondrial respiration, iron absorption, and elastin synthesis. It is also required for the catalytic activities of many metalloenzymes like cytochrome C oxidase, superoxide dismutase, dopamine β-hydroxylase, lysol oxidase, and tyrosine (Tapiero *et al*., 2003, Llanos and Mercer, 2002).

4.4.1. Toxicity of cupper

The very high intakes from cupper can cause health problems such as liver and kidney damage, the ovine is more sensitive to cupper toxicity and determination of the Cu content in food is also an important subject with respect to human consumption (Lee and Stuebing, 1990). It is a lament toxic to prokaryotic and eukaryotic cells because it can bind to proteins and nucleic acids and cause the oxidation of lipids and proteins. Intoxication occurs by intakes voluntary or accidental contamination of drinks. The regulation of the intracellular activity of cupper as well as the mechanisms that maintain homeostasis of this element are considered crucial to maintain cell viability and prevent phenomena of toxicity (Florianczy, 2003).

5. Some international standards

5.1. European Commission (EC 1881, 2006)

The maximum levels of heavy metals in meat and meat product lead (0.1), Cadmium (0.05) and Mercury (0.50) mg/kg.

5.2. FAO Standard

The maximum levels of heavy metals in meat and meat product Pb (0.5) Cd (0.5) Hg (0.05) and as (0.5) mg/kg. (FAO, 2003)

5.3. Gulf standard (2015)

The maximum levels of heavy metals in meat and meat product lead (0.5), Cadmium (0.5) Mercury (0.1) and Arsenic (0.5) mg/kg. (Gulf standard, 2015)

5.4. China standard (2006)

The maximum levels of heavy metals in meat and meat product Pb (0.2), Cd (0.1,) Hg (0.05), as (0.05), Cr (1.0) and Ni (1.0) mg/kg. (China standard, 2006).

5.5. Australia New Zealand Food Standards (2015)

The maximum levels of heavy metals in meat and meat product lead (0.1), Cadmium (0.05), Mercury (0.5) and Arsenic (1.0) mg/kg. (Australia New Zealand Food Standards, 2015).

6. Pollution of the Aquatic Environment with Heavy Metals

Aquatic habitats, especially the freshwater ecosystems, are more subjected to pollution than other environments, because of water use in industrial processes as well as discharge of effluents from industry and urban development. Most aquatic ecosystems can cope with a certain degree of pollution, but severe pollution is reflected in a change in the fauna and flora of the community, which suffer such pollution. White Nile is the principal freshwater resource for Sudan, meeting nearly all demands for drinking water, irrigation, and industry. Contamination of the White Nile and its tributaries with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms (Rashed, 2004). Recently, chemical waste and agricultural drainage system help in spread of chemical pollution mainly heavy metal pollution such as Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd), Mercury (Hg), Nickel (Ni) and Chromium (Cr). The metal ions can react with constituents of the water or settle to the bottom and react with the sediments. Heavy metals have a greater chance of remaining in solution when complexes to chelating legends such as specific anions whose concentrations are determined by the pH of the surrounding environment. Metals precipitate as oxides/hydroxides at different pH regions and the amphoteric elements return to solution at higher PH. The hydroxide concentration (or pH) is therefore of great importance for the mobility of metals. Other factors also affect the fate of the metal ions like redox conditions and the presence of adsorbent sediments (Rashed, 2001).

7. Sources of aquatic pollution

7.1. Industrial Sources

Industrial processes such as mining and processing of metal ores, the finishing and plating of metals and the manufacture of metal objects. Metallic compounds which are widely used in other industries as pigments in paint and dye manufacture; in the manufacture of leather, rubber, textiles, paint, paper and chromium factories which are built close to water for shipping (Rashed, 2004).

7.2. Domestic Wastewater

Domestic wastewater contains substantial quantities of metals. The prevalence of heavy metals in domestic formulations, such as cosmetic or cleansing agents, is frequently overlooked (Rashed, 2004).

7.3. Agricultural Sources

Agricultural discharge contains residual of pesticides and fertilizers which contains metals (Rashed, 2004).

7.4. Atmospheric pollution

Acid rains containing trace metals as well as suspended particulate matter (SPM) input to the water body will cause the pollution of water with metals (Rashed, 2004).

8. Metals in Sediments

Heavy metals are a natural component of rocks and as the result of rocks weathering, they are transferred to soil and bottom sediments, where they are supplemented with metals originating from anthropogenic activity such as urbanization, industrialization, transportation and energy production (Xia *et al.*,2018). In the past, sediments and particulate matter have been considered as purely biotic material. This is obviously not the case and it is now well known that sediments contain large bacterial populations. Sediments are also complex mixtures of a number of solid phases that may include clays, silica, organic matter, carbonates and large bacterial populations. There are three possible mechanisms by which trace metals may be taken up by sediments and suspended matter: physicochemical adsorption from the water column, biological uptake by organic matter or organisms and physical accumulation of metal enriched particulate matter by sedimentation or entrainment.

Physicochemical adsorption direct from the water column happens in many different ways. Physical adsorption usually occurs when particulate matter directly adsorbs heavy metals straight from the water (Rashed, 2004).

CHAPTER TWO

Materials and Methods

1. Study area

The present study was carried out during April 2020 in the Rabak area. Rabak city, White Nile State is located on the Eastern bank of the White Nile, facing Kosti on the Western bank. It lies some 362 meters above sea level. Rabak is approximately 260 kilometers South of Khartoum and 340 kilometers West of the Ethiopian border. It is linked to the north of Sudan via the Khartoum–Rabak road; and it is linked by road Eastward to Sennar and Westward to Al-Ubayyid.

2. Collection of samples

A total of 210 samples of different types of fish, bovine and ovine were collected in plastic bags and transferred refrigerated in ice to a refrigerator (-20°c), then transferred to the laboratory for digestion and analysis in April 2020. These samples were classified into two groups

2.1. Fish samples

A total of 30 samples of fish muscle were collected from Rabak Central fish market.

2.2. Meat samples

A total of 180 bovine and ovine samples of meat were collected from Rabak slaughter-house as described in table 2.1.

3. Determination of total mineral concentration:

Total mineral concentration was determined for all samples by the dry ashing method described by The Perkin-Elmerin (1996). For meat samples, a dry ashing procedure was used. It requires minimum operator attention and there is no loss due to spattering, volatilization or retention on crucibles. After ashing, samples were dissolved in acid and diluted. The amounts of lead, cadmium, copper and zinc were determined by 210 / 211VGP Atomic Absorption Spectrophotometer. (AAS), USA.

4. Apparatus and equipment

-Sensitive scale.

-Plastic bag.

-What man filter 45.

-Stainless steel knifes.

-Glass vials 25 ml.

-Crucible dishes.

-Muffle furnace 550°C.

-Crucible vials.

-Heating block.

-Glass ware: funnels, volumetric flasks, pipettes, glass rod.

-Atomic Absorption Spectrophotometer. (AAS), USA.

5. Procedure of Meat Analysis

Briefly, 10 grams from the samples were weighed by a sensitive balance, chopped by a stainless-steel knife to prevent any possible contaminations, cut into small pieces to facilitate the burning process and then placed into an oven at 550 °C for 8 hours in a heat-resistant crucible**.** Then, samples were removed from the oven and left to cool until the next morning and 5 ml of HCl 20% was added to dissolve the samples completely. Filtration of samples was done into a 50 ml volumetric flask using filter paper whatman45. Volume was completed to 25 ml by distilled water. The liquid samples were introduced to Atomic Absorption Spectrophotometer (AAS) after preparation of the standard curve for each element.

6. Statistical analysis

The recorded data were subjected to One-way analysis of variance (ANOVA) and T test to assess the influence of different variables on the concentrations of heavy metals under study.

CHAPTER THREE

Results

The presence and distribution of the heavy metals: cadmium, copper, lead and zinc in fish muscle and ovine muscle, liver and kidney was investigated. Table 3.1. showed the presence of the heavy metals: Cd, Cu, Pb and Zn in all the samples examined (Mean ± STD). There were statistically significant differences ($P \geq 0.05$) between the concentrations of each of all the heavy metals under study in the fish, bovine and ovine tissues.

The distribution of the four heavy metals in muscle samples of fish, bovine and ovine showed statistically significant differences in Cd, Zn and Pb and there was no difference in Cu (Table 3.2). Comparing the levels of the four tested heavy metals in of bovine and ovine liver samples a significant difference in Cu, Pb and Zn but no significant difference in Cd.

The result showed that there were statistically significant differences in Cd and Cu in kidney samples of bovine and ovine but no difference in the Pb and Zn were detected.

Cadmium concentration of fish, bovine and ovine muscle, bovine and ovine liver and bovine and ovine kidneys are shown in figure 1., figure 2. And figure 3. Respectively.

Copper concentration of fish, bovine and ovine muscle, bovine and ovine liver and bovine and ovine kidneys are shown in figure 4., figure 5. And figure 6. Respectively.

Table 3.1. Mean and standard deviation of Cd, Cu, Pb and Zn concentrations in fish, bovine and ovine samples.

Species	Number of samples	Cd	Cu	Pb	Zn
Fish	30	0.075 ± 0.0405	0.685 ± 0.635	0.498 ± 0.226	3.305 ± 1.507
Bovine	90	0.054 ± 0.0405	0.685 ± 1.061	0.530 ± 0.749	4.414 ± 1.499
Ovine	90	0.055 ± 0.0226	6.118 ± 9.125	0.24 ± 0.151	3.99 ± 0.99
P-value		0.014	0.000	0.001	0.000

Table 3.2. Mean and standard deviation of Cd, Cu, Pb and Zn concentrations in muscles samples of fish, cattle and sheep.

Species	Cd	Cu	Pb	Zn
Fish	0.0757 ± 0.405	0.685 ± 0.635	0.4988 ± 0.227	3.305 ± 1.507
Bovine	0.3893 ± 0.209	0.715 ± 1.312	0.5939 ± 0.979	4.944 ± 1.565
Ovine	0.5056 ± 0.228	0.545 ± 0.364	0.2177 ± 0.114	3.912 ± 1.008
P-value	0.00	0.723	0.039	0.000

Table 3.3. Mean and standard deviation of Cd, Cu, Pb and Zn concentrations in bovine and ovine liver samples.

Species	Cd	Cu	Pb	Zn
Bovine	0.0389 ± 0.0209	0.730 ± 1.306	0.593 ± 0.979	4.944 ± 1.565
Ovine	0.0568 ± 0.0182	16.926 ± 8.60	1.493 ± 0.112	4.691 ± 0.7166
P-value	0.354	0.00	0.009	0.001

Table 3.4. Mean and standard deviation of Cd, Cu, Pb and Zn concentrations in bovine and ovine kidney samples.

Species	Cd	Cu	Pb	Zn
Bovine	0.0861 ± 0.057	0.609 ± 0.137	0.4033 ± 0.134	3.356 ± 0.506
Ovine	0.0595 ± 0.0259	0.882 ± 0.0259	0.354 ± 0.15	3.374 ± 0.764
P-value	0.008	0.000	0.653	0.757

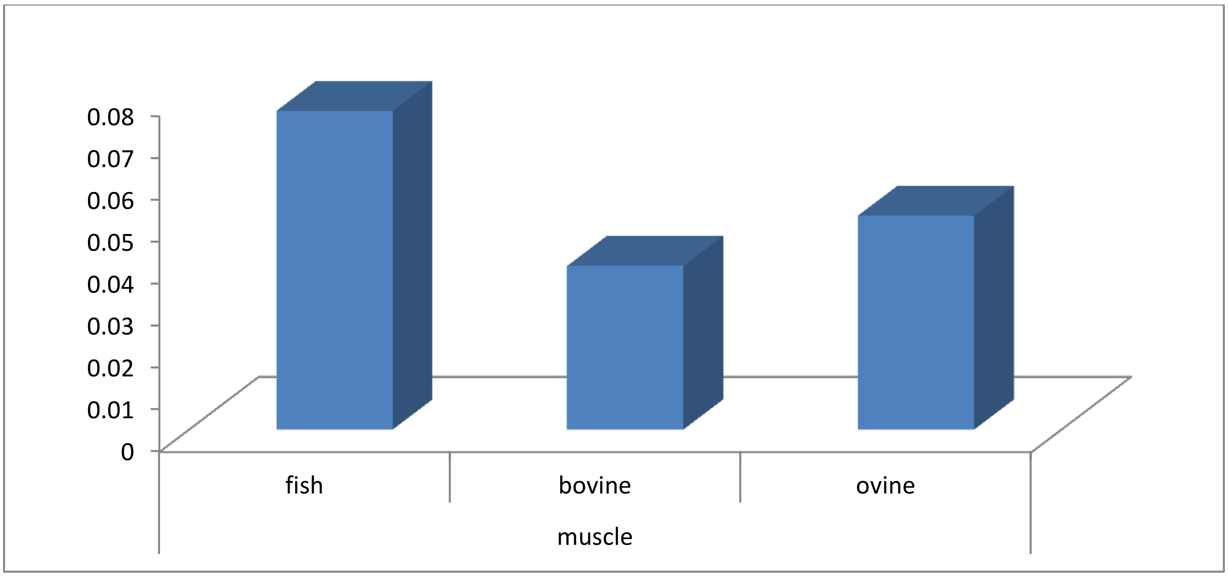

Fig. 3. 1. Mean concentration of cadmium in muscle samples of fish cattle and sheep.

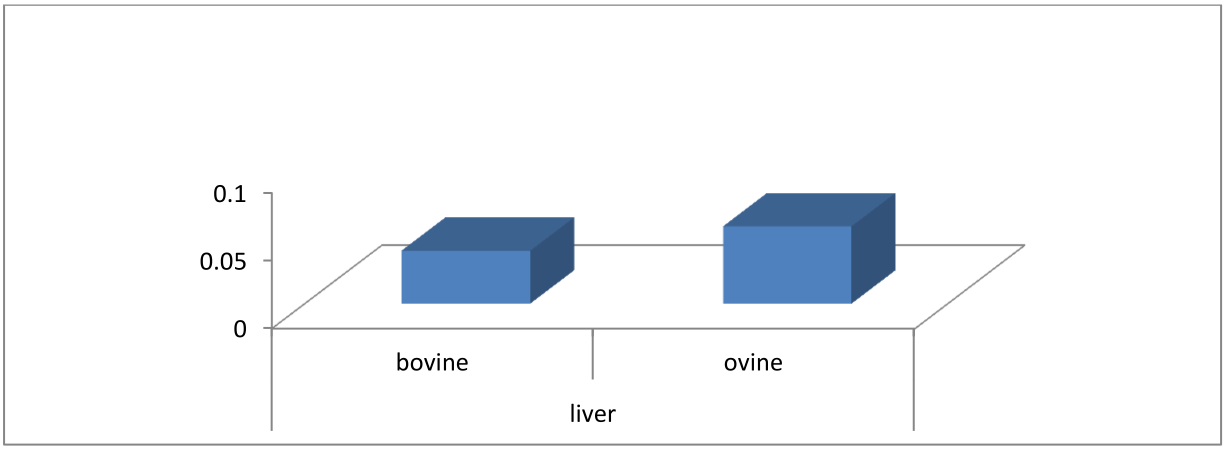

Fig. 3. 2. Mean concentration of cadmium in liver samples of cattle and sheep.

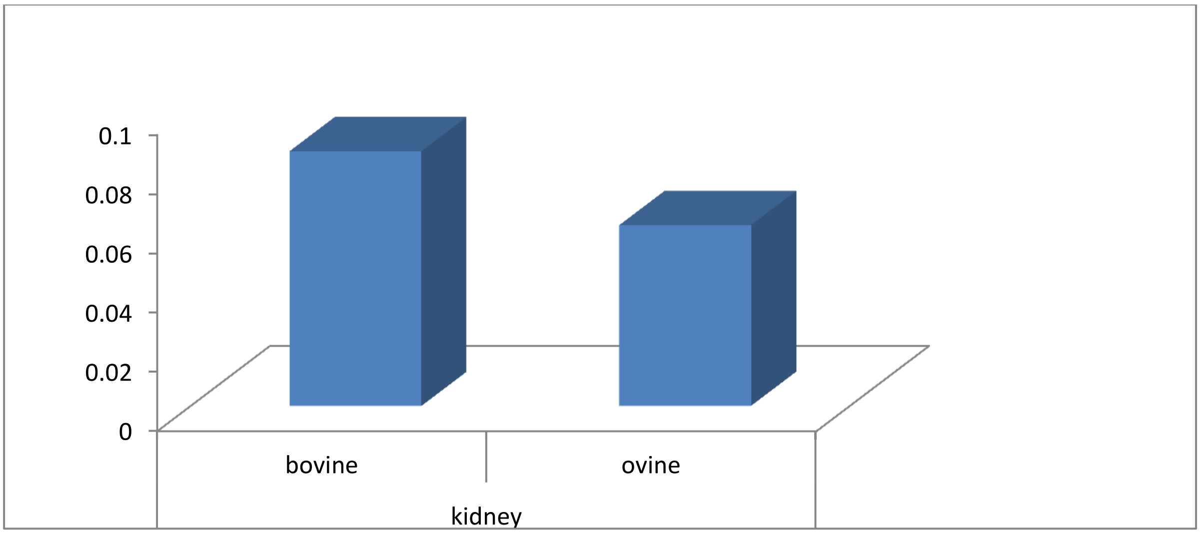

Fig. 3. 3. Mean concentration of cadmium in kidney samples of cattle and sheep.

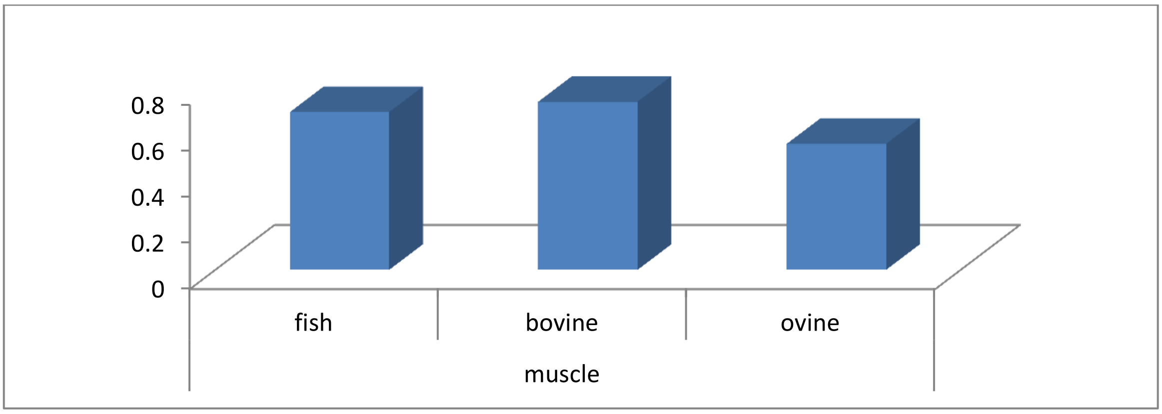

Fig. 3. 4. Mean concentration of copper in muscle samples of fish, cattle and sheep.

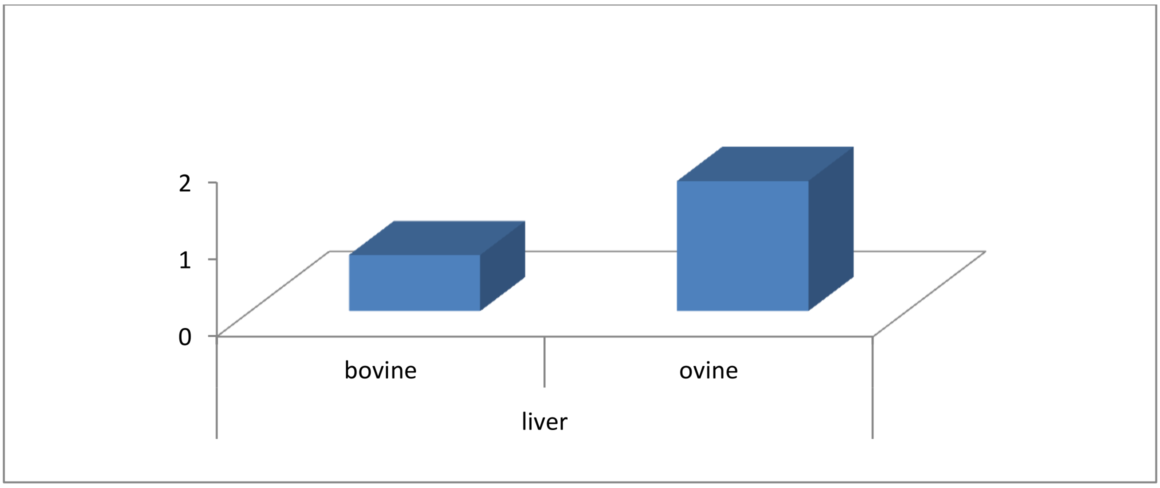

Fig. 3. 5. Mean concentration of copper in liver samples of cattle and sheep.

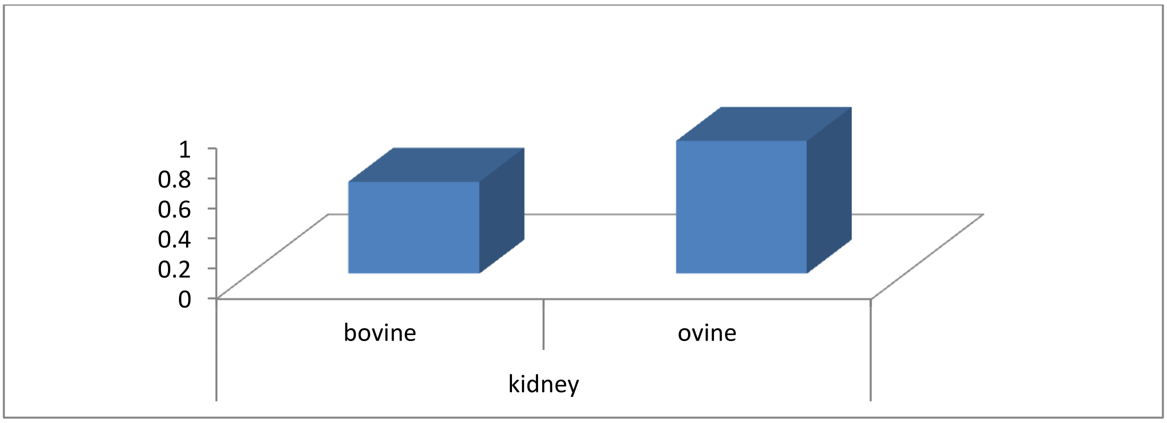

Fig. 3. 6. Mean concentration of copper in kidney samples of cattle and sheep.

Lead concentration of fish, bovine and ovine muscle, bovine and ovine liver and bovine and ovine kidneys are shown in figure 7., figure 8. And figure 9. Respectively.

Zinc concentration of fish, bovine and ovine muscle, bovine and ovine liver and bovine and ovine kidneys are shown in figure 10., figure 11. And figure 12. Respectively.

Figure (2) showed that the concentration of Cd is higher in fish muscles samples followed by the ovine muscle’s samples and bovine muscles samples. In liver samples the Cd is a little bit higher in ovine samples compared with bovine samples. The Cd was higher in the kidney samples of bovine than ovine.

In comparison between livers the lead is concentrated in a higher level in bovine samples than the ovine liver. In contrast, the lead is higher in the kidney of ovine than the kidney of bovine (figure 1).

Figure (2) showed that the concentration of Cd is higher in fish muscles samples followed by the ovine muscle’s samples and bovine muscles samples. In liver samples the Cd is a little bit higher in ovine samples compared with bovine samples. The Cd was higher in the kidney samples of bovine than ovine.

Figure (3) the copper concentration was relatively equal in the muscles of fish and bovine whereas it was lower in ovine muscles. The concentration of the copper was much high in liver samples of ovine compared to that of bovine samples. However, the concentration of copper is higher in kidney samples of ovine than bovine.

Figure (4) showed that the zinc concentration was high in bovine muscles samples followed by ovine muscles samples and low in fish muscles samples. In comparison of liver samples, the zinc is much higher in bovine liver than ovine liver. In contrast, in kidney samples the concentration of zinc in ovine is much higher than bovine.

Means and standard deviations of cadmium, lead, copper and zinc in fish cattle and sheep tissue samples are shown in table 3.5.

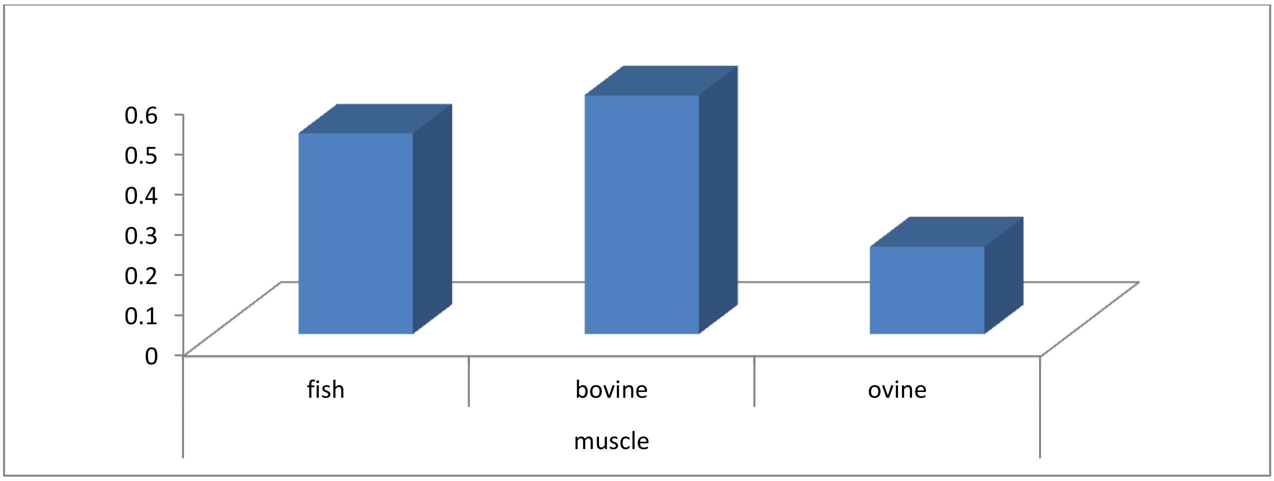

Fig. 3. 7. Mean concentration of lead in muscle samples of fish, cattle and sheep.

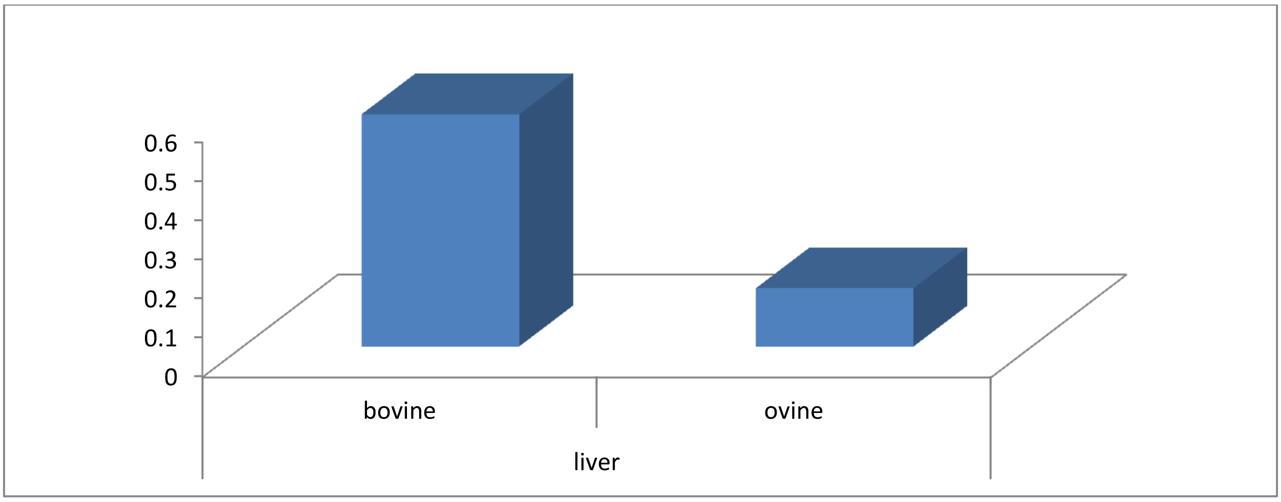

Fig. 3. 8. Mean concentration of lead in liver samples of cattle and sheep.

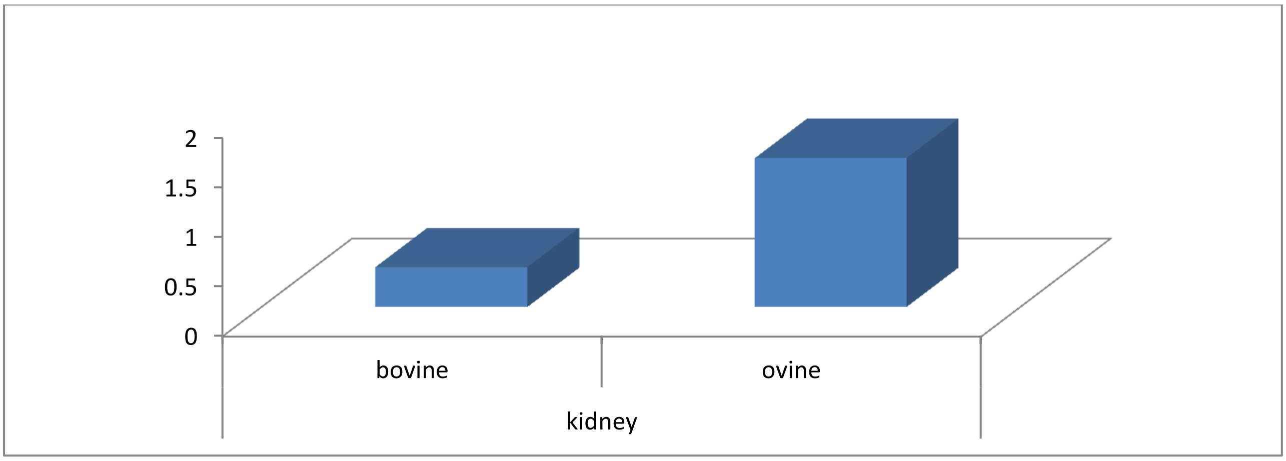

Fig. 3. 9. Mean concentration of lead in kidney samples of cattle and sheep.

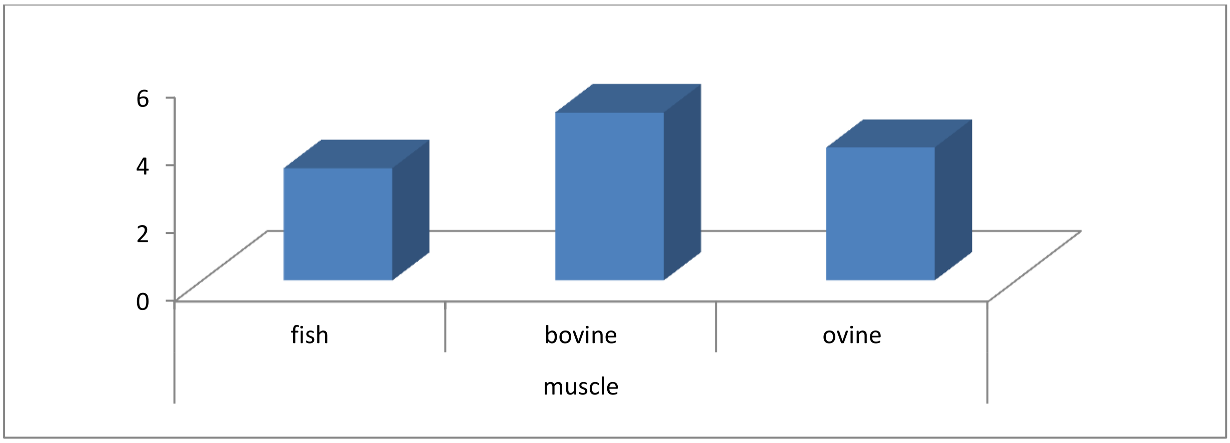

Fig. 3. 10. Mean concentration of zinc in muscle samples of fish, cattle and sheep.

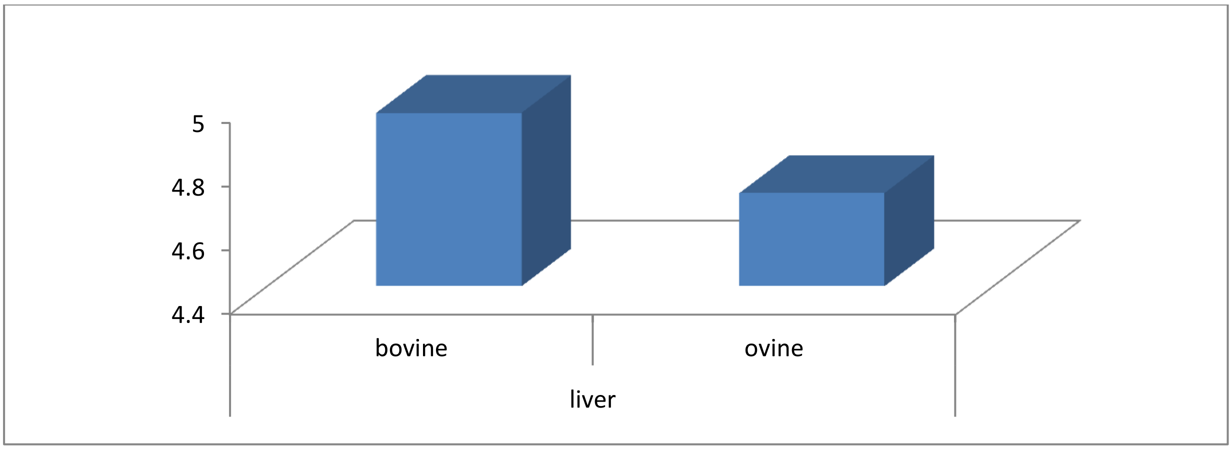

Fig. 3. 11. Mean concentration of zinc in liver samples of cattle and sheep.

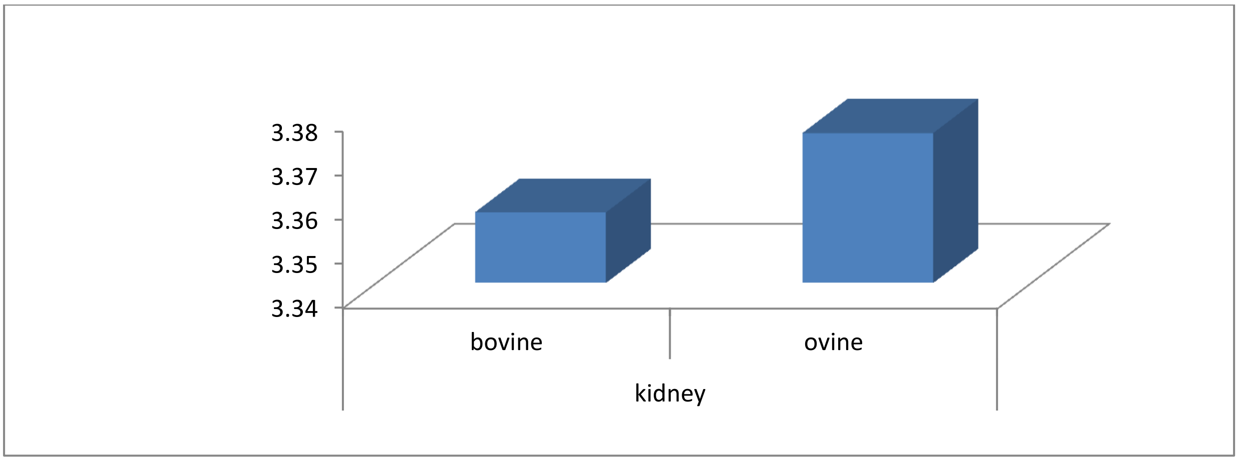

Fig. 3. 12. Mean concentration of zinc in kidney samples of cattle and sheep.

Table 5. Means and standard deviations of cadmium, lead, copper and zinc in fish cattle and sheep tissue samples.

Type	Metal	Means	S D	Minimum	Maximum
Fish	Cd	0.076	0.041	0.02	0.18
	Cu	0.685	0.636	0.15	3.73
	Pb	0.499	0.227	0.20	1.06
	Zn	3.305	1.507	1.72	9.66
Bovine muscle	Cd	0.039	0.021	0.00	0.10
	Cu	0.730	1.307	0.21	6.95
	Pb	0.594	0.979	0.10	4.43
	Zn	4.944	1.566	0.51	7.49
Bovine liver	Cd	0.039	0.0210	0.005	0.097
	Cu	0.730	1.307	0.208	6.953
	Pb	0.594	0.981	0.099	4.431
	Zn	4.944	1.566	0.507	7.490
Bovine kidneys	Cd	0.086	0.057	0.03	0.24
	Cu	0.611	0.137	0.40	0.89
	Pb	0.403	0.134	0.16	0.78
	Zn	3.356	0.507	2.18	4.17
Ovine muscle	Cd	0.051	0.023	0.019	0.099
	Cu	0.547	0.364	0.198	2.059
	Pb	0.218	0.115	0.032	0.499
	Zn	3.912	1.009	1.902	7.059
Ovine liver	Cd	0.057	0.0183	0.018	0.088
	Cu	1.693	8.600	2.114	33.849
	Pb	0.149	0.112	0.030	0.487
	Zn	4.692	0.711	2.723	6.567
Ovine kidneys	Cd	0.061	0.026	0.024	0.136
	Cu	0.882	0.383	0.208	1.922
	Pb	1.504	80.451	0.039	4.410
	Zn	3.374	0.765	0.492	5.348

Chapter Four

Discussion

This study aimed to detect and evaluate the concentration of heavy metals. In this study, heavy metals concentration namely, Cd, Cu, Pb and Zn in muscles of fish, bovine and ovine, livers and kidney of bovine and ovine were detected. The Cd concentration in the tissues of fish, bovine and ovine and also the liver and kidney of bovine and ovine is lower than the recommended value by the European Commission (0.05).Similar results was obtained by Niemi *et al* who justified that: Low cadmium level may be due to lower cadmium concentration in fertilizers Although, the bovine kidney (0.086) and ovine kidney (0.061) were a little bit higher. The copper is essential for good health but very high intakes can cause health problems such as liver and kidney damage (Agency for Toxic Substances and Disease Registry, 2004). The current study showed that the concentration of the copper was found very high in ovine liver and kidney, our results contradicted with Shafiq (2015). The copper is essential for good health but very high intakes can cause health problems such as liver and kidney damage (Agency for Toxic Substances and Disease Registry, 2004). The maximum copper concentration for meat and meat products has been proposed 0.90–30 mg/dl person. Former study conducted by Canli and Atli (2003), found the copper concentrations lower than our study.

Concerning the lead, our result detected higher concentration of lead and more than the permissible dose which reported by European Commission (0.4) except in ovine liver and ovine muscles, our results agreed with Amoidio cocchieri and foire (1987). Our finding in this study disagreed with Jorhem *et al* (1996), who stated that lead levels in meat tissues had been considerably reduced due to remarkable reduction in international lead emission from automobile.

The concentration of Lead was present in range of 0.15 - 1.5 mg/kg. This results was similar somewhat to 0.4 mg/kg (EC, 2001) and 0.5 mg/kg (FAO, 1983). However, the obtained result of lead in the samples exceeds the permissible All samples except Ovine muscle Ovine liver levels; 0.4 mg/kg (EC, 2001). Lead causes renal failure and liver damage in humans (Luckey and Venugopal, 1977).

Zinc concentration in this study was as following, (4.944), (4.944), (4.692), in bovine liver, bovine muscle and Ovine liver respectively, compared with ovine muscle, Ovine kidneys, bovine kidneys and fish which (3.912), (3.374), (3.356) and (3.305) mg/kg respectively. The concentrations of Zinc in this study was range between (3.31- 4.94) mg/kg. This results was more than the permissible limit (.05 mg/kg). Also, our results differs with Akan *et al* (2010), who was found zinc Concentrations ranged in beef, bovine, caprine and chicken from (0.10) to (2.04) mg/kg and agreed with Amani and Lamia (2012) which reported that zinc in beef and bovine (41.72) and (33.85) mg/kg, respectively. Also agree with Bended douche *et al* (2014) who found zinc concentrations in beef Mutton by (36.99) and (39.64) and mg / kg, respectively.

In the current study we find zinc more than permissible, while lead less than permissible, Numerous data shows that increased Zn supply may reduce Cd absorption and accumulation and prevent or reduce the adverse actions of Cd, whereas Zn deficiency can intensify Cd accumulation and toxicity. (Brzoska and Moniuszko-Jakoniuk 2001).

Conclusion and Recommendation

1. Conclusion

All fish, bovine and ovine samples were safe and free of heavy metals (cadmium, copper and zinc except lead) when compared to international standards. The essential elements of muscle of fish, bovine and ovine (muscle, liver and kidney) in this study were less than the standards limit. The study found that the concentrations of lead in each of the fish, bovine muscle, beef liver, bovine kidney and ovine kidneys were not comparable to international standards which may cause a potential risk to human.

2. Recommendation

- Further studies should be carried out in this field to produce muscle fish, bovine and ovine (muscle, liver and kidney) that match with international standards of food safety.
- Rationalizing the addition of compounds to raise the weight (such as arsenic compounds) in animals.
- Monitoring the nominations of the current factories and ensuring that their efficiency is not polluting the environment.

- Increasing control and conducting analyzes for heavy metals, not only in fish and meat present.
- In the markets but on all foods, by conducting a national survey of all foods, especially those foods that it is characterized by frequent consumption.
- Chemical hazard monitoring must be adopted by the government and other research institutions.

References

Agency for Toxic Substance and Disease Registry (ATSDR, 1992). Toxicological profile for zinc. Draft for public comments. U.S. Department of health and human services. Public Health Service. Agency for Toxic Substances and Disease Registry.

Akan, F.I. Abdulrahman, O.A. Sodipo and Y.A. Chiroma (2010). Distribution of Heavy Metals in the Liver, Kidney and Meat of Beef, Mutton, Caprine and Chicken from Kasuwan Shanu Market in Maiduguri Metropolis, Borno State, Nigeria. University of Maiduguri, Maiduguri, Nigeria. Research Journal of` Applied Sciences, Engineering and Technology **2 (8)**: 743-748.

Amani S. Alturiqi, Lamia A. Albedair (2012). Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. The Egyptian Journal of Aquatic Research Volume 38, Issue 1, 2012, 45–49.

André Luiz Oliveira da Silva, Paulo R.G. Barrocas, Silvana do Cuuto Jacub, Josino Custa Moreira (2005). Dietary intake and health effects of selected toxic elements. Brazilian Journal of Plant Physiology On-line version ISSN 1677-9452Braz. J. Plant Physiol. vol.17 no.1 LondrinaJan./Mar.2005 "http://dx.doi.org/10.1590/S1677042020050001000007"042020050001000007.

Apostoli, P. and Catalani, S. (2011). Metalions affecting reproduction and development. Metal Ions in Life Science. 8, 263-303.

Ayesha Mehmood, Raja Adil Sarfraz, Abdul Qudoos and Fareena Akbar (2014). Appraisal of Some Heavy Metals in Organ Meat from Non-industrialized Areas of Faisalabad, Pakistan. Global Veterinarian 12 (1): 98-103.

Bastin, S., (2007). Nutrition Value of Meat: Cu-op. Ext Service University of Kentucky-College of Agric. UK. Pp. 1-3.

Baykov, B. D., Stoyanov, M. P., Gugova, M. L. (1996). Cadmium and lead bioaccumulation in male chickens for high food concentrations.Toxicological and Environmental Chemistry, vol.54, no.1-4, p.155-159. http://dx.doi.org/10.1080/02772249609358308.

Baykov, B. D., Stoyanov, M. P., Gugova, M. L. (1996). Cadmium and lead bioaccumulation in male chickens for high food concentrations. Toxicological and Environmental Chemistry, vol.54, no.1-4, p.155-159."http://dx.doi.org/10.1080/02772249609358308"02772249609358308.

Bendeddouche.B, Zellagui R; Bendeddouche E. (2014). Levels of Selected Heavy Metals in Fresh Meat from Cattle, Ovine, Chicken and Camel Produced in Algeria. Annual Research and Review in Biology 4(8): 1260-1267, 2014 Science domain international www.sciencedomain.org.

Brzoska, M.M and Moniuszko Jakoniuk , J .(2001). Interactions between cadmium and zinc in the organism food chem. Toxicol.39(10):967-980.

Campbell, J.K. and Mills, C.F. (1979). The toxicity of zinc to pregnant Ovine . Environ. Res. 20: 1-13.

Canli, M., Atli, G., (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution 121, 129–136.

Castro-Gonzalez M.I,and Mendez-Armenta M (2008). Heavy metals: Implications associated to fish consumption, Environmental Toxicology and Pharmacology, 26, 2008,263-271.

Chang, LW.; Magos, L.; Suzuki, T., (1996). Toxicology of Metals. CRC Press, Boca, Raton. FL, USA.

Cherian M.G., Nordberg M., (1983). Cellular adaptation in metal toxicology and metallothionein, Toxicology, 28, 1-15.

China standard (2006). Specific Maximum Levels of Contaminations in Foods. Date: 8/14/2006 GAIN Report Number: CH6064 China, Peoples Republic of FAIRS 2006 Voluntary Report - public distribution.

Cunningham,W.P. and B.W. Saigo,(1997). Environmental Science Global Concern. 4thEdn. WMC Brown Publisher, New York, pp.: 389.

Daniel, B. And A.K.Edward, (1995). Environmental Science, Earth as a living planet, pp: 278–9. John Wiley and Sons, Inc. New York.

Demirezen, D. and K. Uruç, (2006). Comparative study of trace elements in certain fish, meat and meat products. Meat. Sic. 74: 255-260.

European Commission, (2001). Commission Regulation No. 466/2001 of 8 March 2001, Official Journal of European Communities 1.77/1.

European Commission (EC 1881, 2006). Setting maximum levels for certain contaminant test in foodstuffs, https://www.fsai.ie/uploadedFiles/Cunsol_Reg18812006.pdf72ExtoxNet"Extox Net.(2003) Cadmium contamination of food, Available from http://ace.orst.edu/info/extoxnet/faqs/foodcun/cadmium.htm2003"2003.

Extox Net, (2003). Cadmium contamination of food, Available from"http://ace.orst.edu/info/extoxnet/faqs/foodcun/cadmium.htm2003"2003.

Fathy A. Khalafalla, Fatma H. Ali, Fredi Schwagele, Mariam A. Abd-El-Wahab, (2011), Heavy metal residues in beef carcasses in Beni-Seuf abattoir, Egypt, Veterinaria Italiana; 47(3): 351-361.

Figueroa, E. (2008). Are more restrictive food cadmium standards justifiable health safety measures or opportunistic barriers to trade? An answer from economics and public health. Science of the Total Environment, 389, 1-9.

Florence, T.M., Batley, G.E. (1980). Chemical speciation in natural waters. CRC Critical Reviews in Analytical chemistry .pp 219-296.

Florianczy k. B (2003). Cupper in the organism-transport and storage in the cells. Ann Univ Mariae Curie Sklodowska 58: 85-88.

Food Agriculture Organization (FAO 2003). Heavy metals regulation "http://extwprlegs1.fao.org/docs/pdf/eri42405.pdf".pdf.

Food Standards Australia New Zealand, (FSANZ 2015) Code - Standard Contaminations and Natural Toxicants (2015). Standard 1.4.1 74 Contaminations and Natural Toxicants. Federal Register of Legislation. Australia government.

Fox Spivey M.R., (1987). Assessment of cadmium, lead and vanadium status of large animals as related to human food chain, Journal of Animal Science, 65, 1744-1752.

Funakoshi, T., Ohta, O.; Shimada, H. And Kojima, S. (1995). Effects of dithiocarbamaes and cadmium on the enzymatic activities in liver, kidney, and blood of mice. Toxical. Lett. 78: 183.

Gabriel Vasile Hoha, Elena Custăchescu, Ana Leahu, Benone Păsărin. (2014). Heavy metals contamination levels in processed meat marketed in Romania. Environmental Engineering and Management Journal September 2014, Vol. 13, No .9, 2411 - 2415 http://omicron.ch.tuiasi.ro/EEMJ.

Ghosh, A.K.; Bhatt, M.A.; Agrawal, H.P. (2012). Effect of longterm application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. Environmental Monitoring and Assessment 184: 1025-1036.

Gulf standardization Organization (2015). Standard of heavy metals in food. https://www.gso.org.sa/gso-website/gsowebsite/.../standards-services.

Gunter Heinz and Peter Hautzinger. (2007). Meat Processing Technology for Small- to medium scale Produce. Food and Agriculture Organization of the United Nations Regional Office for ASIA and the PACIFIC Bangkok, 2007 © FAO 2007 ISBN: 978- 974-7946-99-4.

Halliwel, D., Turoczy, N., Stagnitti, F. (2000). Lead concentrations in Eucalyptus sp. in a small coastal town. Bulletin of Environmental Contamination and Toxicology, vol.65, no. 5, p. 583-590. Retrieved from the web:"http://link.springer.cum/article/10.1007%2Fs0012800163"0012800163.

Harrison N (2001), Inorganic contaminations in food, In: Food Chemical Safety Contaminations, Watson, D.H. (Ed.), 2001,148 168. Hazard Substance Control, 38, 61–69.

He, Z.L.; Yang, X.E., and Stoffella, P.J. (2005). Trace elements in agro ecosystems and impacts on the environment. J. Trace Elem. Med. Biol., 19(2–3):125–140.

Janefrances Ngozi (2010). Assessment of level of some heavy metals in the muscle and internal organs of cows consumed in Enugu and Nsukka State, Nigeria.
"http://www.unn.edu.ng/publications%20/files/images/Ugwu_J_N_PG_PHD_05_40033.pdf".pdf.

Jayasekara, S.U., U. Samarajeewa and A.N. Jayakody, (1992). Trace metals in foods of animal origin in Srilanka. ASEAN Food J., 7: 105-107.

John, H.H. and I.R. Jeanne, (1994). Food additives, contaminations and naturaltoxins. In: Maurice E.S., A.O. James, S.L. Moshe and Febiger, (eds.) Modern nutrition in health and disease, 8thed. Part II.pp: 1597.

Jorhem , L., Sundstorm , B., Engman, J ., Astrand ., C., and Olssol., I (1996): Level of certain trace element in beef and pork imported to Sweden . Food addit contam ,13(7),737-745.

Judge, M. D.; Aberle, E. D.; Forrest. J.; Hendrick, H. and Merkel, R. A. (1990). Principles of meat science (2nded), Kendall Hunt, lowa USA.

Khlifi and Hamza-Chaffai, (2010). Head and neck cancer due to heavy metal exposure via tobaccu smoking and professional exposure. A Toxical Apply Pharmacal. 248(2):71-88.

Lafuente A, González-Carracedo A, Esquifino AI. Biometals (2004). 17: 451–6.

Lee YH, Stuebing RB (1990). Heavy metal contamination in the River Toad, Bufojuxtasper (Inger), near a copper mine in East Malaysia. Bulletin of Environmental Contamination and Toxiculogy.1990; 45:272-279.

Llanos RM, Mercer JF (2002). The molecular basis of cupper homeostasis cupper-related disorders. DNA Cell i 21: 259-270.22.

Luckey, T.D., Venugopal, B., (1977). Metal Toxicity in Mammals. Plenum Press, New York.

Lukáčová Anetta and Golian Jozef (2014). Lead in Meat and Meat Products Consumed by the Population in Slovakia. © 2014 The Journal of Animal and Veterinary Sciences Original Research Paper.

Mahassin Abd Elgany (2008). Effect of added camel meat on physicochemical properties of fresh beef sausage. M.Sc. degree in Meat Technology. Khartoum University.

Makanjuola, Olakunle Moses, (2016). Assessment of Heavy Metal in Raw Meat Sold in Some Notable Garages in Ogun State, South West, Nigeria International Journal of Research Studies in Biosciences (IJRSB)Volume 4, Issue 9, September2016, PP 10- 13ISSN 2349-0357 (Print) and ISSN 2349-0365 (Online)http://dx.doi.org/10.20431/2349-0365.0409003 www.arcjournals.org© ARC.

Marian Asantewah Nkansah, Jonathan Kweku Ansah (2014). Determination of Cd, Hg, As, Cr and Pb levels in meat from the Kumasi Central Abattoir. International Journal of Scientific and Research Publications, Volume 4, Issue 8, August 2014 1 ISSN 2250-3153.

Ming-Ho, Y. (2005). Environmental Toxicology: Biological and Health Effects of Pollutants, CRC Press LLC, 2nd Edition, Boca Raton, USAISBN 1-56670-670-2, 2nd.2005, Chap. 12.

Murphy, J.V. (1970). Intoxication following ingestion of elemental zinc. JAMA. 212:2119-2120.

Nordberg, G.F. Fowler, B.A. and Nordberg, M. (2015). Handbook on the Toxicology of Metals, 4th edn, Vol II. Academic Press, London, UK. pp. 582-610.

Niemi, A., Venalainen, E., Hirvi, T., Hirn, J. and Karppanen , E., (1991). The lead, cadmium and mercury concentration in muscle , liver and kidney from fishing pigs and cattle during 1987-1988. Z lebensm unters forsch ,192,427-429.

Oehlenschlager, J. (2002). Identifying heavy metals in fish in: Safety and Quality issues in fish processing, Bremner H.A, (Ed), Wood head Publishing Limited, Cambridge, 2002, 95-113.

Okiei W., Ogunlesi M., Alabi F., Osiughwu B. and Sojinrin, (2009). Determination of toxic metal concentrations in 82 flame–treated meat products, ponmo, African Journal of Biochemistry Research; 3(10): 332-339.

Okoye C. O. B., Ugwu J. N., (2010). Impact of environmental cadmium, lead, copper and zinc on quality of goat meat in Nigeria. Bulletin of the Chemical Society of Ethiopia, vol. 24, no. 1, p. 133-138."http://dx.doi.org/10.4314/bcse.v24i1.52975"1.52975.

Panba. B, Das. A, (2015). Conspectus of Inland Fisheries Management (ICAR) Publishers Central Council of Agricultural Research.

Perkin-Elmerin (1996). Analytical Method for Atomic Absorption Spectroscopy. Printed in the United State of America. Manual part No. 0303-0152 release D September 1996.

Pizent, A., Jurasivie, A. and Telisman, S. (2001). Blood pressure in relation to dietary cadmium intake, alcuhol cunsumption, blood lead and blood cadmium in female non- smokers. J. Trace Elem. Med. Biol., 15: 123-130.

Plum P., Rink L., Haase H., (2010). The essential toxin: impact of Zinc on human health, International Journal of Environmental Research and Public Health, 7, 1342–1365.

Poulsen H.D., (1998). Zinc and copper as feed additives, growth factors or unwanted environmental factors, Animal Feed Science and Technology, 7, 135-142.

Prasad A.S., Miale A., Farid Z., Schulert A., Sand stead H .H.(1963). Zinc metabolism in patients with the syndrome of iron deficiency anemia; hypogonadism and dwarfism J Lab clin Med. 1963, 61:537—549.

Rab Nawaz, Shafiq Ur Rehman, Shah Nawaz, Bushra Iftikhar (2015). analysis of heavy metals in red meat in district Peshawar Khyber pakhtunkhwa A. J. Med. Sci. (Peshawar, Print) July 2015, Vol. 23, No. 3: 166-171.

Rashed, M., N.(2004). Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int, 27: 27-33. DOI: 10.1016/S0160-4120 (01)00050-2.

Rashed, M., N. (2001). Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int, 27: 27-33. DOI: 10.1016/S0160-4120 (01)00050-2.

Ray S.; In J. W. Kiceniuk and S. Ray (Eds.) (1994). Analysis of contaminations in edible aquatic resources. VCH Publishers, Inc., pp.91–113.

Reddy, A.T.V. and K. Yellamma, (1996). Cadmium chloride induced alteration in the detoxification enzymes of rat liver and kidney. Pollut. Res., 15: 371–3.

Reena Singh, Neetu Gautam, Anurag Mishra, and Rajiv Gupta (2011). Heavy metals and living systems Indian J Pharmacol. 2011 May-Jun; 43(3):246–253.doi:10.4103/0253-7613.81505PMCID:PMC311333.

Reesal, M.R., Dufresne, R.M., Curbet, K. (1987). Adverse health effects from industrial and environmental cadmium. AOM Newsletters Vol 5.

Rubio C., Hardisson A., Reguera J.I., Revert C., Lafuente M.A., González-Iglesias T., (2006). Cadmium dietary intake in the Canary Islands, Spain, EnvironmentalResearch, 100, 123-129.

Rubio, C., T. González-Iglesias, C. Revert, J.I. Regueraand A.J. Gutiérrez (2005). Lead dietary intake in a Spanish Population (Canary Islands). J. Ag.

Santhi D, Balakrishnan V, Kalaikannan A, Radhakrishnan KT (2008). Presence of heavy metals in pork products in Chennai (India). Am. J.Food Technol. 3(3):192-199.

Sfakianakis, D.G., Renier, E., Kentour, M. And Tsatsakis, A.M. (2015). Effect of heavy metals on fish larvae deformities: A review. Environ. Res., 137: 246-255. DOI: 10.1016/j.envres.2014.12.014.

Shallari, S.; Schwartz, C.; Hasko, A., and Morel, J.L. (1998). Heavy metals in soils and plants of serpentine and industrial sites of Albania. Sci. Total. Environ. 19209:133– 142.

Sivaperumal, P., Sankar, T. V., Nair, V., (2007). Heavy metal concentration in fish, shellfish and fish products from internal markets of India vis-à-vis international standards Food Chem, 102:612-620. DOI: 10.1016 /j. foodchem.2006.05.041.

Smolders, E. (2001). Cadmium uptake by plants. Int. J. Occup. Med. Environ. Health.14: 177-83.

Soghoian, S., and Sinert, R. (2008). Toxicity, heavy metals."http://emedicine.medscape.cum/article/814960"814960-overview.

Tahvonen and Kumpulainen (1994). Lead and cadmium contents in pork, beef and chicken, and in pig and cow liver in Finland during 1991. Journal Food Additives and Contaminations, Food Additives and Contaminations, Volume 11, Issue 486

Tapiero H, Townsend DM, Tew KD (2003). Trace elements in human physiology and pathology. Cupper. Biomed Pharmacother 57: 386-398. Citation.

Tuormaa, T.E., (1995). The role of chromium, selenium and cupper in human and animal metabolism. J. Orthomolecular Med., 10: 149-164.

Underwood, E. J. (1977). Trace elements in human and animal nutrition. 4th Edn. Academic Press, New York, San Franciscu, London.

Walker, C.H.; Hopkin,S.P.; Sibly, R.M. and Peakall, D.B. (2001). Principles of Ecotoxicology, 2nd edn, Taylor & Francis, London, UK. P.P. 3-6

Wang S, Shi X. (2001). Molecular mechanisms of metal toxicity and carcinogenesis. Mol. Cell Biochem., 222:3–9.

WHO/FAO/IAEA (1996). Trace Elements in Human Nutrition and Health. WHO, Geneva, Switzerland.

Williams, P.G., (2007), Nutrient Cumposition of Red Meat, available:"http://ro.uow.edu.au/hbspapers/48"48.

World Health Organization (2003). Elemental mercury and inorganic mercury compounds: Human health aspects. Concise International Chemical Assessment Document 50, World Health Organization, Geneva.

World Health Organization WHO (1992). Cadmium. IPCS Environmental Health Criteria 134, Geneva.

World Health Organization WHO (1994). Cadmium. Environmental Health Criteria 134, Geneva.

World Health Organization WHO (1996). Trace elements in human nutrition and health. World Health Organization, Geneva.

World Health Organization WHO (2006). Evaluation of certain food contaminations. Sixty-fourth report of the Joint.

World Health Organization WHO. (2004). Evaluation of certain food additives contaminations.

Zasadowski, A.; Barski, D.; Markiewicz, K.;Zasadowski, Z.; Spodniewska, A, and Terlecka, A. (1999). Levels of 89 cadmium contamination of domestic animals (cattle) in the region of warmia and Masuria [Poland].Polish Journal of Environmental Studies, 8(6): 443-446.

Ziegler, P.T., (1968). The meat we eat, the interstate printers and publishers, inc, danville, illinois. pp.69-71 in , Dabuo C. E., (2011), metal and nutrient composition of processed cattle hide (welle) using four procedures, a thesis submitted to the School of Graduate Studies, Kwame Nkrumah University of Science and Technology (KNUST

Appendix

Table 6 Accepted level of heavy metal concentration for human consumption (mg/kg) according to some international's standards.

Heavy metals	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	References
European Commission	0.05	-	0.4	-	EC 1881, 2006
FDA RI	0.01	-	2	40	(Suyanto et al 2010)
FAO	0.1	-	0.5	-	(FAO, 1983)
LAEA-407	0.18	3.28	0.12	-	(Wyse et al., 2003)
EC, 2001	1.0	-	0.4	-	(EC, 2001)
Gulf (2015)	0.5	-	0.5	-	(Gulf 2015)
China (2006):	0.1	-	0.2	-	(China, 2006)