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College of Natural and Computational Sciences

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Master Thesis

AGRICULTURAL BENEFITS AND TOXICITY OF ANIMAL BONE ASHES: CHEMICAL COMPOSITION ANALYSIS

A Thesis Submitted to the Office of Graduate Program of Debre Berhan University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

BY

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DEBRE BERHAN UNIVERSITY
POST GRADUATE STUDIES

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

APPROVAL SHEET I

This is to certify that the thesis entitled **"Agricultural Benefits and Toxicity Level of Animal Bone Ashes: Chemical composition analysis"** submitted in partial fulfillment of requirements Degree of Master of Science in Chemistry**,** College of Natural and Computational Science**,** Debre Berhan University, and it is a faithful record of original research work carried out by **Demise Turuna Teshoma** under my guidance and supervision.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the thesis requirements.

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APPROVAL SHEET II

We, the undersigned members of the boarded of the examiners of the final open defense by **Demise Turuna Teshoma** have read and evaluated his thesis entitled "***Agricultural Benefits and Toxicity Level of Animal Bone Ashes: Chemical composition analysis***" and examined the candidates. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Masters of Science in Chemistry.

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STATEMENT OF THE AUTHOR

I declare   that all sources of materials used for this thesis have been duly acknowledged. This thesis had been submitted in partial fulfillment of the requirements for MSc degree at Debre Berhan University College of Natural and Computational Science and it is made available under internet Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or other.

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Table of Contents

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF APPENDIX FIGURES

Appendix Figure **Page**

ABSTRACT

The problem facing today, mostly in developing country is the deposits of wastes materials especially from meat industry. An emerging issue in world is the growing environmental treat caused by animal wastes. People slaughter animals for their economic product or other purposes and throw away the byproduct without knowing the problem come after it. To reduce this pollution the way of disposing wastes and recycling it in to other raw material is studied. The bone sample were collected and digested for the determination of their mineral constituents by Atomic Absorption and Ultra Violet Visible instruments. On the other hand, the waste material produced from animal wastes can be recycled and changed to raw material for soil amendments for plant growth. To be environmentally eco-friendly the sanction way of west disposal is advisable to use. In this study the content of bone ash sample indicates the presence of essential elements such as Calcium (4013±0.3), Potassium (137±0.007 and Phosphorus (1062.3±30.78) while there is in low amount some toxic metals or heavy metals such as Lead (37±0.0084), Cadmium (32.6±0.00034) and Zinc (179±0.0034).

Key words: Ash bones, AAS, heavy metals, Nutrient

CHAPTER 1

INTRODUCTION

1.1. Back ground

All living things die due to different factors such as disease, accidents, as well as natural disasters. The dead bodies of animals broken down in to their constituents by bacteria or fungi and the bone, which account (10-20)% of the live weight of animals remain to decompose over long period of time left above the ground or buried in shallow. In addition, global slaughter industry produces 130 billion kilogram of animal bone to produces meat as a side stream of their core business because of its big economic benefits and not getting good utilization of bone, which cause a huge waste of available source, it is not good for creating economic profits and protecting the environment

Due to bacteriological hazard and difficulty with storage, utilization process of wastes has become troublesome. This means the slaughter house as well as meat processing produce an enormous amount of animal meat as their income and produce a large quantity of animal bone as offal. However, animal bones are treated as tailpiece with no use value. This growing amount of bone residue, among other waste material, has considered one of the significant environmental challenges that the food industry facing today. An emerging issue in today's world is the growing environmental treat caused by animal wastes.

The stamping-out approach *(look figure 1 below*), which is traditionally the most common and successful method of disease eradication, requires technology for animal carcass disposal as an integral component. Some general principles for choosing a disposal option are enunciated as factors for consideration, however primary consideration must be given to disease control and eradication.

A summary of currently available technologies for animal carcass disposal is presented as a hierarchy based on their reliability for pathogen inactivation. The technologies listed include: rendering, incineration, pyre-burning, composting, mass burial or open-pit burial, licensed commercial landfill, mounding, fermentation, and examples of technologies under development *(Norman G. Willis 2003)*. As well a special consideration for the disposal of prion disease infected carcasses is discussed, where rendering, incineration, and alkaline hydrolysis are the preferred technologies.

However, there is a growing trend in society to reject the excessive waste of valuable animal products, the negative environmental and animal welfare outcomes, and the devastating economic impacts on agricultural industries as well as on national economies. This is creating pressure for alternatives to mass animal slaughter and carcass disposal.

Animal bones left to decay naturally above the ground or buried in shallow pots a hazard to ground water and surface water can jeopardize the health of the domestic livestock, wild life and pets. These wastes are disposed in ways that have been reported to cause the pollution of surface and underground waters and air quality (Odoemelan and Ajunwa, 2008); affect the health of residents living within the vicinity of the abattoirs, destroy affected water bodies thus affect fish yield, (Aina and Adedipe, 1991); discharge of blood and animal faeces into streams has been reported by Nwachukwu *et al*., (2011) to cause oxygen-depletion while humans may also be affected through outbreak of water borne diseases and other respiratory and chest diseases (Mohammed and Musa, 2012). Bone wastes are also ideal breeding grounds for disease causing organisms (pathogens). Our health is major factor when circulating the importance of proper disposal of dead animals. We don't want the animal remains to spread any disease to us through direct contact nor leach out disease through the ground. Remediation of waste contaminated by heavy metals is necessary in order to reduce the associated risks, make the land resource available for agricultural production, enhance food security, and scale down land tenure problems.

The rise in environmental awareness has led to the close examination of animal carcasses disposal methods to make sure the right method is used and that it is an efficient as possible. Disposing animal bones properly is important for many reasons including wealth, health, wellbeing of the surrounding animals, in addition when an animal is properly disposed of citizens. Getting rid of bones outside of our rubbish bin can be tricky. The dried bone of animals can result in massive volume of animal carcasses. Instead there are option of disposing this waste and increasing number of them. Since we appreciate the natural world, it is important to consider the wellbeing of living animals when we are choosing method of disposal. We wouldn't want to infect innocent animals. Toxic elements (heavy metals) are common to the environment and are responsible for both intentional poisoning and unintentional exposures that can lead to the adverse health effects and potentially death. Dangerous exposures can be prevented by recognizing and minimizing common sources of toxic elements in our diet, water, workplace and homes. Laboratory testing is an important tool for detecting and managing toxic element exposures; several analytical methods are available. However, the clinical value of an element testing is dependent up on collecting an appropriate specimen at an appropriate time, with consideration of many pre analytical variables that can compromise testing (Deborah E. Keil, et al., 2011)

Figure 1 : Bones disposed at the Trans-Amadi abattoir in Port-Harcourt (Akpa et al., 2014).

Some sanctioned way of disposing or getting rid of animal bones does cost money, it is best interest of our wallet to use those rather than damping remaining illegally. Obtaining comprehensive understanding of available carcass disposal technologies is important in order to be able to make an environmentally and economically sound decision when choosing a carcass disposal method.

Treated animal waste has found many applications among which the most important are; fertilizers, animal feeds, biodiesel/biogas, dielectric product, natural pigment, cosmetics, glues, ceramics and energy product. Thermal treatment is recognized as safe elimination process (Patrick Sharrock et al., 2019). During high temperature combustion, all of organic matter in materials, including proteins, is degraded to carbon dioxide, water, nitrous and Sulphur dioxides, etc.(Moeller 2015). Utilization of those wastes after thermal decomposition and calcinations make it applicable for different purpose. Bone and meat combustion residues mostly arise from bone combustion, they contain high amount of phosphate and calcium, the two major constituent of bone ashes (Deydier et al., 2005). The component of bone ash varies with animal used as a source of bone and the area from which sample collected. Researchers also identified that the largest quantities of dried bones are elements of compound calcium phosphate as weight percentage of calcium 38.97%, phosphorus 18.66% and oxygen 40% (Sumaiya Al Ghuazaili et al., 2019). The classification of bone material in to suitable category depends on defining its origin by its product and the only method of their utilization in burning or calcining process at higher temperature (Kinga krupa-zuczer et al., 2012). The availability of plant nutrients in the soil is fundamental to improve growth and crop yields.

1.2 Statement of problem

Growing amount of bone residue waste material has considered as one of the significant challenges such as brain, spinal cord, tonsils, etc. and sick animal corpses that food industry is facing today(Eric Deydier et al., 2005) and also they ere toxic to living things and tend to accumulate in plants and animals causing chronic adverse effects on human health (Vijaya, BG, Kiran and Negandrappa 2010). Because of need to address disposal of materials infected with pathogens, new regulation have come into effect for the transport and disposal of dead farm animals or carcasses. Instead of utilizing valuable bones material commercially, those have been usually considered and treated as slaughter house waste and are disposed in landfills and rendering plants. Insufficient management bone creates a variety of problems that endanger public health and environment in general. Speed of decision-making is critical at the time of such a crisis. So the following problems stimulate the researcher to this study:

- Which way animal offal processing is safe and economically useful?
- How to evaluate the alkalinity and fertility of bone ashes?
- What are the amount of elements (potassium, calcium, phosphorus, lead, cadmium and Zinc) in the bone ash and their properties?
- Why animal bone ash is toxic and detection of their level of their toxicity?

1.3 Objective of the study

1.3.1 General objective

- To investigate the agricultural benefit and toxicity level of animal bone ashes.

1.3.2 Specific objective

- To carry out characterization of elements of bone ashes.
- To testing of alkalinity of sample bone ashes.
- To evaluate the elements of agricultural chemistry (phosphorus, potassium and calcium)
- To review some toxic elements of bone ashes(lead, cadmium and Zinc)

1.4 Significance of the study

The purpose of this study is to share the observation and concerns with the rest of benefits and to briefly describe the methodology used in this paper for a suitable substitute. Knowledge of the basic chemistry, environment and associated health effects of the heavy metals accumulated in animal bones necessary in understanding of their speciation, bioavailability and remediation options. This paper reviews the observation and experiments that lead to conclusion and discusses to identify a satisfactory substitute. It provides the result of agricultural uses and toxicity of bone ashes elemental composition. The reader of this study could understand the agricultural benefits of essential elements of bone ashes and differentiate level of hazardous elements of bone ash.

1.5 Scope of the study

The area of this paper restricted to agricultural uses and toxicity level determination of animal (cow) bone ashes. It examines the transformation of bone ashes in to a slow release fertilization and possible ways to minimize toxicity of the toxic elements (Lead, Zink and Cadmium) on environment. The sample of bone was gained from nearest slaughter house of the researcher's Lalo kile by limited instruments like Atomic Absorption and UV-Vis spectroscopy.. The area of this research is limited due to the time and budget constraints

1.6 Limitation of the study

The problem faced the researcher during the studies are:

- The distance from the laboratory for experimental study
- Lack of transportation
- Failure of the instruments reading and problem with an electric power service.
- Refusal of permission from working institution.

CHAPTER 2

2 REVIEW LITRATURE

Bone ash is a white material produced by the calcination of bones. Bone is produced from animal, first processed to remove any adhering meat which is generally sold as pet food. The typical analysis of claimed bone is 67-85% calcium phosphate, 3-10% calcium carbonate, 2-3 magnesium phosphate and small amount of Cano and CaF_2. However it can vary significantly depending on the source of bones and process used.

It consists about 55.82% calcium oxide, 42.39% phosphorus pent oxide, and 1.79% water (refer the below table 1). But the exact composition of these compounds varies depending up on the type of bone ash being used. The formula the formula for the bone ash is Ca (OH) $(PO_4)_3$. To sreduce this trouble, the methods of dealing with certain waste disposal depending on its categories have been worked out. The classification of this material into suitable categories depends on defining it origin by this product and the only method of utilization is burning or calcining process at higher temperature. Bone meal is by products of rendering industry made from animal processing offal. Offal consists of those animal parts that are not suited for human consumption. Solid wastes of industrial wastes consist of undigested bones (Apka.Jackson et al 2014). Thermal treatment is recognized as safe illumination process (Patrick Sharrock et al., 2019).

Utilization of this wastes after thermal decomposition and calcination make it is applicable for different purposes. Bone combustion residues mostly arise from bone combustion; they contain high amount of phosphate and calcium, two major constituent of bone (Deydier et al., 2005).

Researchers also identified that the largest quantity of dried bones are elements of the compound calcium phosphate and as weight percentage of calcium 38.97 %, phosphorus 18, 66% and oxygen 40%. Those main components of bone can recycled properly and degrade because of the biodegradable property. Insufficient management of the bone creates a variety of problems that endanger public health and environment in general. To avoid this danger, these wastes are deposited in ways that have been reported to cause pollution of surface and under wastes and air quality. (Odoemelan and Ajunwa 2008)

Table 1: The Content of chemical Archeological human bone (in ppm) (*R. Allmäe, J. Limbo-Simovart, L. Heapost, E. Verš 2012)*

	Ca	Zn	Pb
Mean	27.4	108.8	1.1
Minimum	22.7	88.5	0.3
Maximum	32.2	194.4	2.4
STD	2.7	29.9	0.6

Bone ash is primarily composed of mostly calcium phosphate. Calcium phosphates have many applications and one of their applications is agriculturally as a fertilizers.

Fertilizers

Fertilizers are the substance that is added to the soil to increase the productivity of the soil. It uses soils to stabilize. Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities, which, after continued fertilizer, application may significantly increase their content in the soil (L.H. P. Jones, 1981). Metals, such as Cd and Pb, have no known physiological activity (Raymond A.Wuana and Felix E. Okieimen., 2011). Application of certain phosphatic fertilizers inadvertently adds Cd and other potentially toxic elements to the soil.

Phosphorus

It is the main component of limiting plant growth. The essential element of bone ash that plays a vital role in soil fertility is phosphorus. Phosphorus is essential for proper development of plants in addition with nitrogen and potassium. Phosphorus is essential for proper development of plants in addition with nitrogen and potassium. Plants need phosphorus in relatively large quantity and its role cannot play by any other elements (*Sultenfuss and Doyle 1999; Mohammad 2012).* The supply of phosphorus determine the development of roots, the condition of stem, the formation of flowers and fruits, the rate of plant nutrition, the efficiency and quality of crops, nitrogen fixation in legumes and resistance to both biotic and a biotic environmental factors *(Mohammad 2012*). The shortage of fertilizer phosphorus of assimilable forms of this component in the environment of plant reduces the yield quantity and biological quality *(Grzebisz et al., 2003*). Production of mineral phosphorus fertilizers is based almost entirely on phosphate. Example, animal bones (Seid, et al., 2012) phosphorus biofertilizers from bone ash and ash diluted equaled commercial fertilizers in terms of growing crop. It does not change the p^H of soil and it is contributed to effective waste management *(Magdalena Jastrbsk et al., 2016)* The compounds in the bone ash can also present in oxide form as shown in below table (look table 2 below)

Table 2: Oxide composition of bone ash using XRF (ZainalZakaria Hamdzun Haron, 2013)

Oxide	OA	GA	SA	UKA	JA
P_2O_5	44.7	43.9	43.3	57.1	55.9
CaO	51.7	53.6	55.4	41.7	41.1
SiO_2	1.08	0.19	0.24	0.55	1.38
Al_2O_3	0.44	0.15	0.15	0.33	1.30
Fe_2O_3	0.19	0.06	0,03	0.09	0.10
TiO_2	0.01	0.004	0.001	0.01	0.01
Na_2O	0.61	0.97	0.13	0.03	0.04
K_2O	0.08	0.03	0.03	0.03	0.04
MgO	1.15	1.07	0.63	0.20	0.21

The ash played a fascinating role in increasing soil shear strength (Gbanganathew et el., 2016). Bone ash in agriculture is mainly due to the presence of phosphorus. To grow and complete the life style, plants must acquire not only macro nutrient (N, P, K, S, Ca and Mg), but also essential micro elements. Some soils are deficient in the heavy metals that are essential for plant growth.

(M.M lasat 2000) Crops may be supplied with N, P, K, S, Ca and Mg as an addition to the soil or as a foliar spray.

Bone meal was first used as a fertilizer in the mid-nineteenth century. In places such as Abilene, Texas, buffalo bones were collected and used for fertilizer. Before 1900, the buffalo bone industry was supplemented by cattle bones. Today, the bones of cows as well as pigs and sheep, are most commonly used for bone meal. Bone meal is ground in to two types: a coarse grind and a fine grind. Fine grinds release nutrients in to soil faster. As bone meal is rich in calcium and phosphorus, but deficient in nitrogen, gardeners may add nitrogen-rich manure to balance the application.

Toxic elements (Heavy metals) of bone ashes

The heavy metals are typically identified as Zinc (Zn), Copper (Cu), Nickel (Ni), Cadmium (Cd), Lead (Pb), Mercury (Hg), Chromium (Cr), Molybdenum (Mo), Selenium (Se), Arsenic (As) and Fluoride (F).(refer the table 3 below)

Table 3: Mineral composition of the chicken's bone ash (K. Chojnaka and I. Michalak 2009)

Types	Element	bone ash conc.(in mg/kg) of dry mass
Micro elements	Mn	4.29
	Zn	215
	Cu	2.87
	Co	1.09
	Fe	298
	Mo	1.22
	Cr	6.48
	B	4.16
Alkali metals and Alkaline earth metals	K	13372
	Ca	225372
	Mg	5965
	Na	8861
	Ba	36.7
Toxic Elements	Cd	0.232
	Ni	3.73
	Pb	1.92
Other Elements	Ti	1.52
	Al	14.8
	Ag	0.305
	V	56.8

Cadmium (Cd)

Cadmium is located at the end of the second row of transition elements with atomic number 48, atomic weight 112.4, density 8.65 g cm^{-3}, melting point $320.9^{0}C$, and boiling point $765^{0}C$. Cadmium typically inorganic forms, is abundant in the earth's crust. Acute problems include lung and gastro-tract problems. Cadmium has a long residency in the body and has been associated with chronic kidney problems, bone damage, respiratory function and reproductive function (*Advice Sheet 18)*. Together with Hg and Pb, Cd is one of the big three heavy metal poisons and is not known for any essential biological function. In its compounds, Cd occurs as the divalent Cd (II) ion. Cadmium is directly below Zn in the periodic table and has a chemical similarity to that of Zn, an essential micronutrient for plants and animals. This may account in part for Cd's toxicity; because Zn being an essential trace element, its substitution by Cd may cause the Malfunctioning of metabolic processes *(P.G.C.Campbell 2006*).Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products.

Lead (Pb)

The content of lead in the bones refers to the influence of the surrounding environment. Lead gets into the organism either through the digestive tract or lungs and most of the lead getting into the organism is stored in the bones (Bronner F. 2008). When the animal gets older, the content of Pb in bones accumulates if the organism is exposed to the source of pollution. Biologically Pb behaves in the organism similar to Sr and Ba replacing calcium in the mineral part of the bone (Burton J. H. 2008). The appearance of lead in the organism is explained by the use of domestic vessels, which had the glazing containing lead, or the drinking water, which came from the pipelines containing lead (Smrčka V. 2005). In interpreting the chemical composition of the archaeological bones there are also certain possibilities for making mistakes. It is well known that the chemical composition of the bones, which were in the soil for a long time, may have changed, the joint effect of the temporal factor and the environmental.

Lead is the most common of the heavy metals and occurs naturally in soils. It is known to cause problems with the blood, including anemia. It is also associated with gastrointestinal and cardiovascular problems (*Advice Sheet 18)*. 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 (Marian Asantewah Nkansah, 2014).

Zinc (Zn)

Commonly associated with plant toxicity. In humans can cause anemia and organ damage. Bone ash itself is environmentally non-toxic at the beginning. However, recent evidence indicates that bone ash can be reduced up on contact with Aluminum alloy to produce metal phosphate. Metal phosphates can intern react with water or water vapor to liberate phosphine, (PH_3) a highly dangerous and toxic gas (Bierman et al 1995). Potential health effects of this chemical is

Inhalation: inhalation of this material may irritate respiratory tract.

Skin contact: prolonged contact with this material may cause skin irritation.

Eye contact: contact with this material may cause severe eye irritation with possible damage to the cornea.

Ingestion: ingestion of large doses may cause irritation gastric intestinal tract, causing symptoms such as abdominal pain, nausea, vomiting and diarrhea.

Toxic elements in animal bone ash constrains on the direct application of ash from the incineration of sludge as a fertilizers are associated with toxic element (Bierman et al 1995).

Many heavy metals are environmentally stable and non-biodegradable, toxic to living things and tend to accumulate in plants and animals causing chronic adverse effects on human health (Vijaya, BG, Kiran and Negandrappa 2010). Heavy metals can be divided according to their need different organisms. Those can be classified as essential and non-essential. Risks of heavy metal contamination of soil- plant system through application of Copper, Iron, manganese, Molybdenum and Zink are essential elements for plants, animals, and humans (Alysonrbs and Fabio 2014). Exposure to toxic elements (“heavy metals”) poses unique issues for human health. Metals differ from other pollutants in that they are neither created nor destroyed and occur naturally in the environment

Researcher also stated that arsenic cadmium, lead and mercury are not essential to any organisms. That means they are not essential for plants. They can cause toxicity and hurts up on exposition. The most common heavy metals found at contaminated sites in order of abundance are Lead, Chromium, Arsenic, Cadmium, Mercury (Patrick Sharrock et al., 2019). The fate and transport of a heavy metal in in soil depends significantly on the chemical form and speciation of the metal. Once in the soil, heavy metals are absorbed by initial fast reaction (minutes and hours), followed by slow adsorption reaction (days and years) and are therefore, re-distributed in to in to different chemical forms with varying bio availability, mobility and toxicity (J.Shiowantana et al 2001; J.Buekers 2007). This distribution is believed to be controlled to be controlled by reaction of heavy metals in a soil such as:

I) Mineral precipitation and dissolution,

II) Ion exchange, adsorption and desorption

III) Aqueous solution

IV) Biological mobilization and immobilization

V) Plant uptake(D.B.Levy1992)

Bone ash can be used alone as an organic fertilizers or it can be treated with sulfuric acid to form a single superphosphate fertilizer which is more water soluble.

$$Ca_3 (PO_4)_2 + 2H_2 SO_4 + 5H_2O \rightarrow 2CaSO_4.2H_2O + Ca (H_2PO_4)_2.H_2O$$

Bone ash is bone of animal such as cow, sheep, goat, fish, buffalo, camel and etc. burnt at a temperature around 1100^0C. For this purpose animal bone and tooth where utilized (Olutaiwoa.O.et al., 2018). Animal bones are part of the composite that form the body of animals. Carbonization is the process of conversion of an organic substance in to carbon or carbon containing substances or residue through pyrolysis or destructive distillation. (Abubakar et al., 2012).

The researcher must be careful in taking bone samples because different bones and also their parts may differ in the concentration of chemical elements (Smrčka V.2005). It is also known that the compact substance of bones is modeled more slowly and it is more inert to the effects of the other environment because of its compact structure (Ezzo J. A. 1994 and Grupe G. 1988) and this is why in taking samples the diaphysis of long bones should be preferred instead of taking ribs and flat bones because in them the sponge matter, being sensitive to pollution, is less protected. The chemical elements which are most often detected in the archaeological bones with the purpose of reconstructing the diet of the people in the past and the environmental effects are Ca, P, Ba, As, Mg, Sr, Al, Zn, Mn, Cu, V, Cd, Cr and Pb and also their interrelations are studied.

CHAPTER 3

MATERIALS AND METHODS

The apparatus used to prepare bone ash from animal bone and characterization, activation and decolorization performance are: P^H-meter, mortar and pestle, beakers, water bath, burette, and characterizing instruments are UV-visible and inductively coupled plasma. The reagents used are nitric acid, per chloric acid, hydrogen peroxide, Ammonium molybdates solution, potassium antimony tartarate, ascorbic acid, sulphuric acid and distilled water. The reason for using those chemicals and apparatus and instruments are due to the availability of the devices reliability and in case of reagents or solvents are because of their dissolving power.

3.1 Experimental sites

The bone sample for study purpose obtained from slaughter house of three different place of Lalokile Woreda in Oromiya region, 519km away from Ethiopian capital city Addis Ababa

3.2 Bone sample collection

Dry bones were collected from the slaughter house by poly ethylene Three different samples were taken from three different slaughter houses. Different sample bone from healthy cows in fresh state were taken The taken samples were crushed in to some smaller parts by hummer, assigned by the code as A, B and C separately and brought to laboratory for digestion.

3.2.1 Chemicals and reagents

Nitric acid, per chloric acid and hydrogen peroxide were reagent for sample digestion. Ammonium molybdates solution, potassium antimony tartarate, ascorbic acid and sulphuric acid also used for determination of phosphorus. Distilled water is the solvent used in experiment.

3.2.2 Materials, apparatus and instruments

Many analytical apparatus have been used to detect and quantify essential elements and toxic elements accumulated in sample of bone ash. Mass of the bone ash sample was Weigh by measuring balances. The sample heated by heating device. The milling process was done by mortar and pestle. Boiling processed by boiled by condenser, filtered by filter paper, the solution contained bone ash sample poured in to the beaker and burette. The alkalinity is measured by P^H-meter. Concentration is measured (determined) by UV-Vis and Atomic absorption spectroscopy (AAS).

3.3 Experimental methods and procedures

3.3.2. Sample preparation

One gram of the powder of bone sample was taken and treated with concentrated nitric acid and per chloric acid as well as hydrogen peroxide by the ratio of 4:3:0.5, respectively. Digestion was continued until the appearance of the solution become colorless. The colorless sample were poured into glass container of hundred milliliter and taken for determination.

3.3.3 The procedure of metal wet digestion

First phosphate standard was prepared. By using standard calibration curve was the unknown concentration of the analyte is calculated. Phosphate-containing sample digested for phosphate analysis by treating with reagent mixtures like ammonium molybdates solution, potassium antimony tartarate, 1.75% ascorbic acid and 5N sulphuric acid. When the treated sample gives a blue color the intensity of that solution is measured by UV-Vis in the same laboratory where sample digestion done. The rest digested sample prepared poured into the glass and obtained to the powerful instrument for reading heavy metal contents.

CHAPTTER 4

RESULT AND DISCUSSION

4.1 Determination of elements in bone ash

The elemental contents of the bones of animal (cow) collected and digested for analyses by Atomic absorption spectroscopy analysis and UV-Vis instruments reveals the following results indicated below tables.

Table 4: pH of the sample of the bone ash

Sample	Trial	pH of the bone ash
A_1	1	7.9
A_2	2	7.8
A_3	3	7.6
B_1	1	7.5
B_2	2	7.6
B_3	3	7.9
C_1	1	7.4
C_2	2	7.3
C_3	3	7.39

Three sample of bone ashes sample taken to laboratory for P^H study (A, B, C) three for each sample (triplets) to reduce error is digested and read by P^H-meter. The P^H =7.6 of the bone ash sample indicates it has slightly alkalinity property (Foth and Ellis 1997). The sample taken to laboratory for chemical composition analysis reveals the presences of investigated elements as summarized in below table (Table 5).

Table 5: Chemical composition of sample of bone ashes

Sample	Trial	Concentration in mg/kg					
		Phosphorus	Calcium	Potassium	Lead	Cadmium	Zinc
A_1	1	785.71	4045	94.60	49.46	34.16	1.879
A2	2	750	4049	94.45	49.48	34.13	187.2
A_3	3	732.14	4044	94.45	49.3	34.12	187.4
B_1	1	1285.14	3985	197.25	33.53	34.6	154.4
B_2	2	1257.14	3987	197.40	33.71	34.61	154.8
B_3	3	1342.86	3989	197.40	33.78	34.57	154.2
C_1	1	1135.71	4004	119.20	27.09	29.06	196.5
C2	2	1114.29	4007	119.30	27.13	29.18	195.8
C3	3	1157.14	4006	119.25	27.08	29.11	196

Three sample of bone ashes sample taken to laboratory for Lead content study (A, B, C) three for each sample(triplets) to reduce error is digested and read by Atomic Absorption Spectroscopy and UV-Vis for phosphorus. The concentration of the bone ash sample of each sample indicates its Mean (average) the sample (in mg/kg) is: for Phosphorus (1062.78), for Calcium (4013), for Potassium (137.3), for Cadmium (367), for Lead (326) and for Zinc(179). Generally, the result of the this study shows that the bone sample mineral elements after calculating total average of the three triplet sample and standard deviation summarized as below table

Table 6: Mineral contents of bone ash and their standard deviation.

Numbers	Parameters	Conc. Mean (mg/kg)	Mean± Stan. Deviat.(mg/kg)
1	pH	7.589	7.589 ± 0.14
2	P	1062.3	1062.3 ± 30.78
3	Ca	4013	4013 ± 0.03
4	K	137	137 ± 0.0074
5	Zn	179	179 ± 0.0034
6	Pb	36.7	36.7 ± 0.0008
7	Cd	32.6	32.6 ± 0.00034

The result of the this study shows that the bone sample mineral elements after calculating total average of the three triplet (A, B, C) sample and standard deviation

Chemical composition of the bone ash sample identified the elemental content of the bone ash sample as in comparison of obtained result with the standards:

Cadmium Concentration (in mg/kg)

It shows result for cadmium (32) is highest with all Moeller 2015(0.3), Edward Someus et al 2018 (0.3-1.34), and K. Kjnacka et al., 2009 (0.2)

Lead Concentration (in mg/kg)

For Lead (37) it is also the highest when compared with all of the standard : Moeller 2015 (8-17), Edward Someus et al., 2018 (1) and K. Kjnacka et al., 2009 (1.92).

Zinc concentration (in mg/kg)

For Zink (179), it is lower in case of K. Kjnacka et al., 2009 (215) and higher to Edward Someus et al., 2018 (75), also Moeller 2015 (113), and (*R. Allmäe et al., 2012)* with its value (108.8)

Phosphorus Concentration (in mg/kg)

For Phosphorus(1062.3) it is lower in comparison with Edward Someus et al., 2018 (104,000)

Calcium concentration (in mg/kg)

Calcium(4013) is lower in the case of both standards K. Kjnacka et al., 2009 (225,327), Edward Someus et al., 2018 (312,000) and higher when compared with(*R. Allmäe et al., 2012)* With its value(27.4).

Potassium concentration (in mg/kg)

Potassium is (137) is higher with Edward Someus et al., 2018 (1.91) and lower in case of K. Kjnacka et al., 2009 (13372).

CHAPTER 5

5.1 CONCLUSION

The study of the above result reveals that the bone ash sample with pH 7.6 which is bone ash sample range falls in slightly alkalinity range and it contains elements Ca, P, K, Pb, Cd, and Zn.. The essential elements for agricultural uses such as Potassium, Phosphorus, and calcium in the bone ash samples. This indicates the fertility of bone ash. Concentration of potentially Toxic elements: The relationship heavy metal loads to concentration of plant nutrient is in comparison to the most other phosphorus fertilizers such as phosphate rocks is very favorable. Mean heavy metal concentration, such as Cadmium, for instance, is relatively the same with the standard. So, the thermal destruction of animal carcasses yields calcium phosphate mineral residues. Such ashes should not be considered as waste but as valuable resource to replace or substitute for natural phosphate rock

5.2 Recommendation

Nowadays, brain, spinal cord, tonsils, etc. and sick animal corpses are considered as high risk wastes and must be incinerated. From this study it is understood that the safe way of animal bone processing is thermal treatment method. It reveals the treated bone ash sample majorly constituent of useful elements. Therefore for using the bone ash for agricultural activity as fertilizers is recommended. Using calcined or heat threated bones on land application increases plant growth and development.

This work underlines the possibility to use this natural material as a low cost pure phosphate source with small heavy metal content (Pb, Zn or Cd), contrary to most natural phosphate ores The awareness of using the bone ash sample recycling method should be practiced in order to reduce pollution that results from inappropriate and dangerous disposal method.

For further investigation the researchers recommended that the study of different animals content of nutrient level comparatively and preferring of bone sample in detail. Further progress in these areas should contribute value added outlets and help alleviate environmental concerns in a sustained development perspective.

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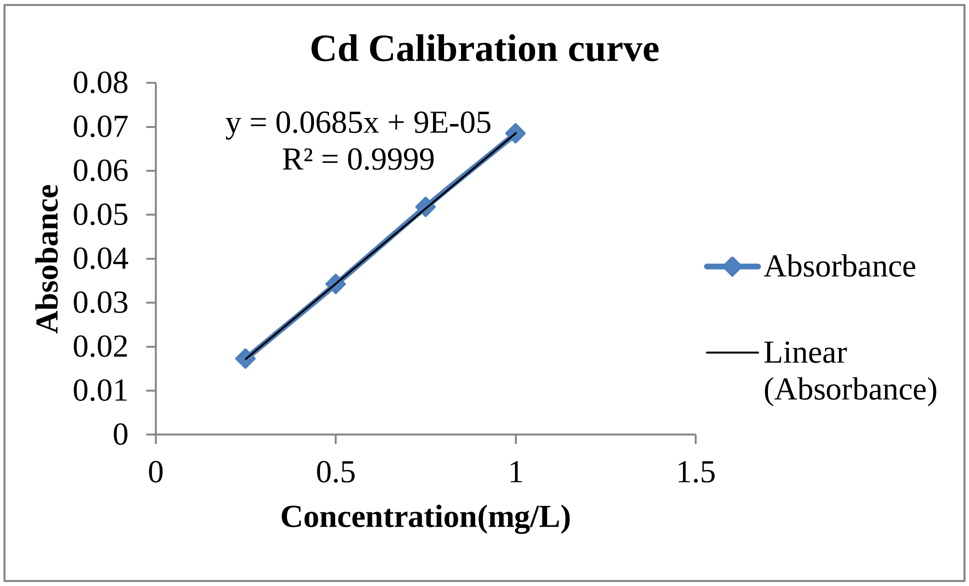
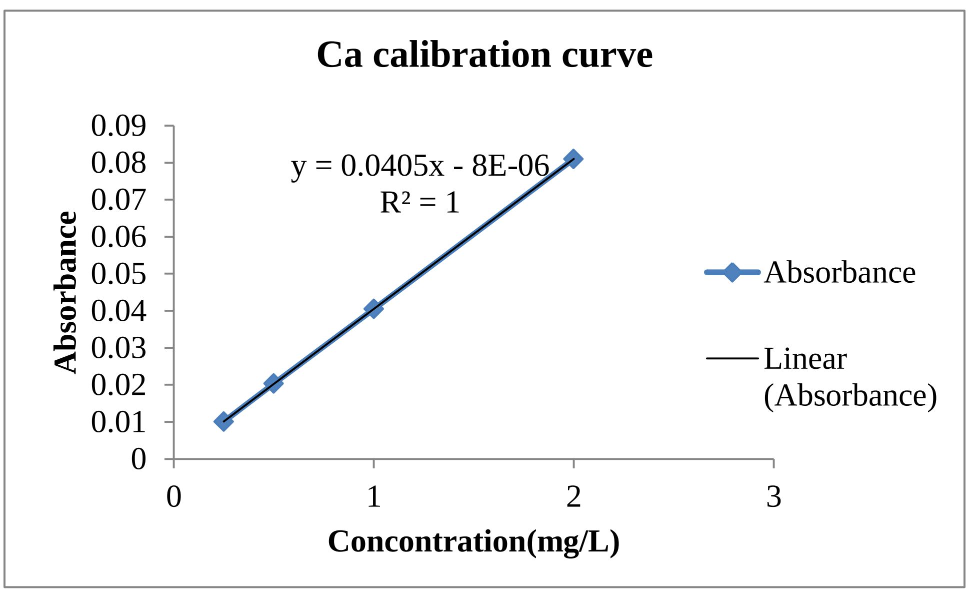
LIST OF GRAPH IN THE APPENDIX

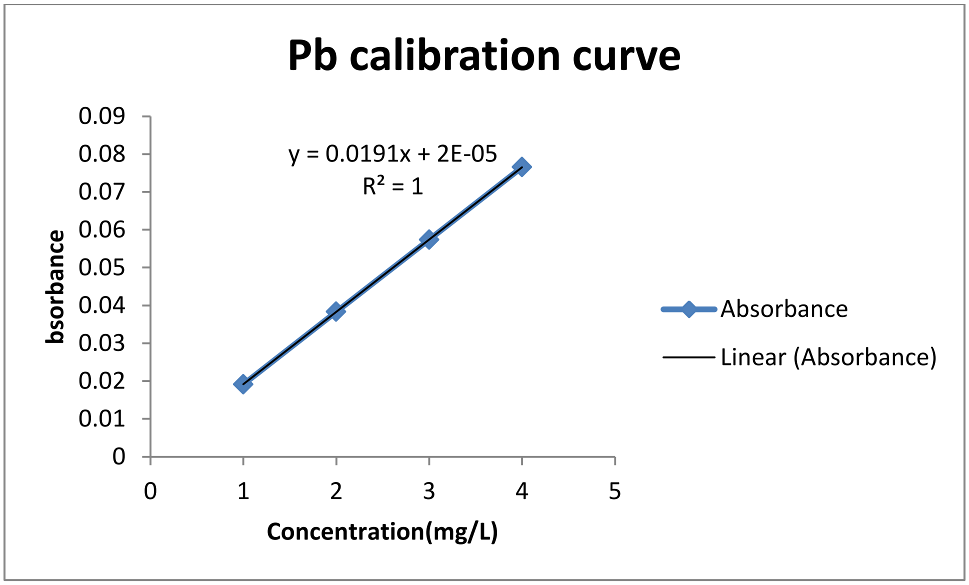
Appendix Figure 1 : Calibration Curve for Cadmium



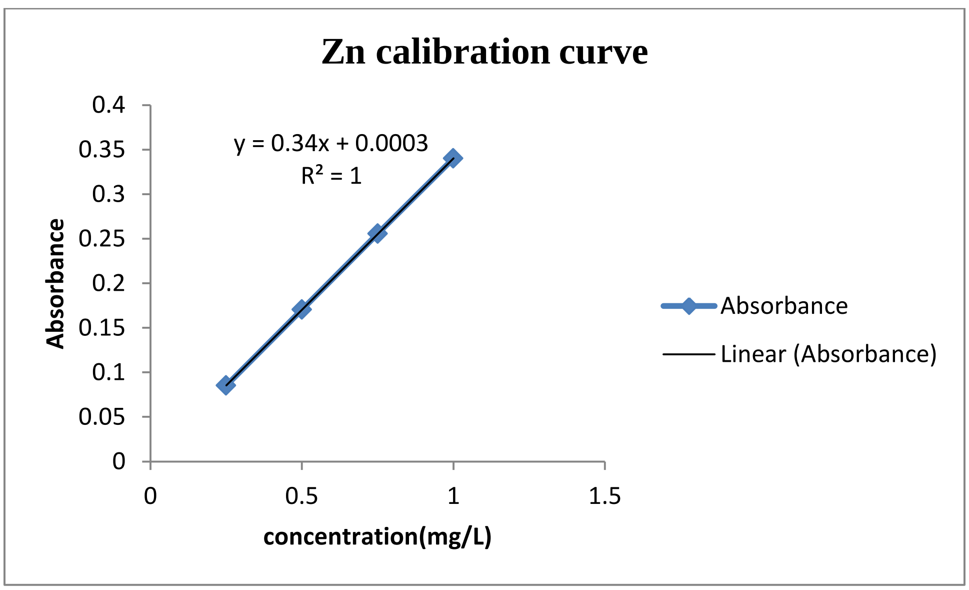
Appendix Figure 2: Calibration Curve for Calcium



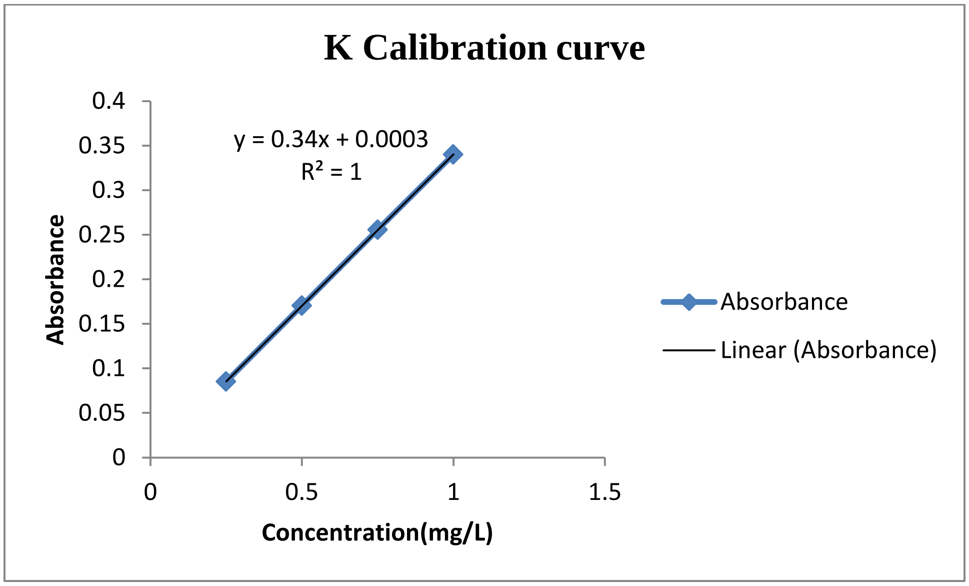
Appendix Figure 3 : Calibration Curve for Lead



Appendix Figure 4: Calibration Curve for Zinc



Appendix Figure 5 : Calibration Curve for Potassium



Appendix Figure 6: Calibration Curve for phosphorus