KARADENIZ TECHNICAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF FOREST INDUSTRY ENGINEERING

REMOVAL OF CHROMIUM, COPPER AND ARSENIC FROM CCA-TREATED WOOD SAWDUST USING CALIFORNIA RED WORM (*Eisenia fetida)*

MASTERS THESIS

Abdul-Rafiq MOHAMMED

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Abdul-Rafiq MOHAMMED

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DECLARATION

I Abdul-Rafiq MOHAMMED by this means declare that this thesis, entitled "REMOVAL OF CHROMIUM, COPPER AND ARSENIC FROM CCA-TREATED SAWDUST USING CALIFORNIA RED WORM (*Eisenia fetida)*" submitted to the graduate school of natural and applied sciences at Karadeniz Technical University to the best of my knowledge is the results of my own findings and that this work has never been presented here or elsewhere for the award of any form of degree except where references other researchers used have been duly acknowledged in the text. 21.12.2018

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SUMMARY

REMOVAL OF CHROMIUM, COPPER AND ARSENIC FROM CCA-TREATED SAWDUST USING CALIFORNIA RED WORM *(Eisenia fetida)*

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In testing for the efficacy of earthworms capability to remove heavy metals from wood protected with chromated copper arsenate (CCA) chemical wood preservative, California red worm *Eisenia fetida* was exposed to substrate made from a mixture of cow dung and wood sawdust from yellow pine (*Pinus sylvesteris*) CCA-treated utility pole. The period of exposure of the experiment spanned for twelve weeks. The study was done to ascertain the effects of substrate on the reproduction and total growth development of earthworms *Eisenia fetida*. Also, the new remediation methodology of using earthworm species to remove heavy metals from CCA-treated wood was tested to determine the amount of heavy metals extracted by *Eisenia fetida*. The results from ICP-MS analysis at the end of the exposure showed that the percentage removal of CCA heavy metals by earthworms from substrates with respect to the treatment levels and particle sizes of substrate was 52%, 50% and 49% for As, Cr and Cu respectively at treatment level T_2 for As and T_1 for both Cr and Cu. Arsenic was accumulated from substrate with unsoaked sawdust of 40 mesh while chromium and copper were accumulated from substrate with soaked sawdust of 60 and 40 mesh respectively. The metal bioaccumulation factor (BAF) which showed the inherent amount of heavy metals in tissues of earthworms was evaluated at the end of the experiment. It was revealed that arsenic (As) had the highest bioaccumulated heavy metal in tissues of *Eisenia fetida* followed by copper (Cu) and chromium (Cr). The population build-up and collective weight gained by earthworms was declined by treatment levels with high amount of CCA-treated sawdust (T_3 and T_4) as the period of exposure prolonged although substantial growth rate was attained at substrate level $T_{2.}$

Keywords: Bioaccumulation factor, California red worm, CCA, Cow dung, Yellow pine, ICP-MS analysis, Soaked sawdust, Unsoaked sawdust, 40 mesh, 60 mesh.

Yüksek Lisans Tezi
ÖZET
CCA İLE EMPRENYELİ ODUN ÖRNEKLERİNDEN BAKIR, KROM VE ARSENİĞİN
UZAKLAŞTIRILMASINDA KIRMIZI KALİFORNİA SOLUCANI *(Eisenia fetida)* KULLANILMASI

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Bu çalışmada, atıl halde CCA ile emprenyeli sarıçam (*Pinus sylvesteris*) tel direkleri Kırmızı Kalifornia solucanı (*Eisenia fetida*) kullanılarak bakır, krom ve arseniğin odundan uzaklaştırılmasına çalışılmıştır. CCA ile emprenyeli odun atıklarının ve inek gübresinden oluşan kompostun münferit veya oransal karışımlarının Kırmızı Kaliforniya solucanın çoğalmasına, gelişmesine ve toplam biokütle değişimine etkisi ve atıl hale gelen CCA ile emprenyeli tel direklerinde bakır, krom ve arseniğin şimdiye kadar hiç uygulanmayan yeni bir remidasyon yöntemiyle (Kırmızı Kalifornia solucanı kullanılarak) uzaklaştırılma miktarı tespit edilmiştir. Besin ortamı ve Kırmızı Kaliforniya solucanı ICP-MS analizlerine tabi tutulmuştur. Kırmızı Kalifornia solucanı 12 haftalık bir süre boyunca CCA ile emprenye edilmiş sarıçam odun tozu ile inek gübresinden oluşan bir karışımdan elde edilen besin ortamına maruz bırakıldı. CCA ile emprenyeli odun atıklarının ve inek gübresinden oluşan kompostun CCA oranı arttıkça solucan sayısı ve toplam biyokütlesi zamanla azaldığı tespit edilmiştir. Bununla birlikte, kompostların CCA miktarıda azalma olduğu belirtilmiştir. Çalışma sonunda değerlendirilen metal bioakümülasyon faktörü solucan dokularında en yüksek birikmiş ağır metal arsenik olmuştur ve ardından sırasıyla krom ve bakırı olduğu tespit edilmiştir.

Anahtar Kelimeler: Biyo-remidasyon, CCA, ICP-MS analizi, Kırmızı Kalifornia solucanı, Remidasyon, Sarıçam, İnek gübresi, Suda bekletilmiş talaşı Suda bekletilmemiş, 40 meş, 60 meş.

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LIST OF ABBREVIATIONS

ACA	: Ammoniacal Copper Arsenate
ACC	: Acid Copper Chromate
ACS	: American Cancer Society
ACQ	: Alkaline Copper Quaternary
ACZA	: Ammoniacal Copper Zinc Arsenate
ANOVA	: Analysis of Variance
As	: Arsenic
As_2O_5	: Arsenic pentoxide
ATSDR	: Agency for Toxic Substances and Diseases Registry
AWPA	: American Wood Preservers Association
AWPI	: American Wood Preservers Institute
BAFs	: Bioaccumulation Factors
BCFs	: Bioconcentration Factors
CCA	: Chromated Copper Arsenate
Cd	: Cadmium
CD	: Cow Dung
CFs	: Concentration Factors
Cr	: Chromium
CRD	: Completed Randomized Design
CrO_3	: Chromium trioxide
Cu	: Copper
CuO	: Copper (II) oxide or Cupric oxide
CZC	: Chromated Zinc Chloride
°C	: Degrees Celsius
EBs	: Experimental Boxes
H+	: Hydrogen
Hg	: Mercury
HNO_3	: Nitric Acid
H_2O_2	: Hydrogen peroxide
Ib/ft^3	: Pounds per cubic foot
ICP-MS	: Inducted Coupled Plasma-Mass Spectrophotometer

ISO	: International Standard Organization
ISSG	: Invasive Species Specialist Group
Kg/m^3	: Kilogram per cubic meter
Mn	: Manganese
Na_2HASO_4	: Sodium arsenic
NH_3	: Ammonium
OECD	: Organization for Economic Cooperation and Develo
Pb	: Lead
SS	: Soaked Sawdust
TS	: Treated Sawdust
TEDAŞ	: Turkish Electricity Distribution Company (Türkiye Elektrik Dağıtım Anonım Şirketi)
UCS	: Use Category System
UFs	: Uptake Factors
USA	: United State of American
US	: Unsoaked Sawdust
US	: Untreated Sawdust
USCPSC	: United States Consumer Product Safety Commissioı
USEPA	: United State Environmental Protection Agency
WPSC	: Wood Preservative Science Council
Zn	: Zinc

1. INTRODUCTION

The absolute reliance on steel and concrete in structural applications in recent times has been overwhelming. The use of wood for this purpose however cannot be overlooked. Wood is still a vital and versatile raw material element in our infrastructural applications. As a matter of fact, it is the only renewable natural resource accessible for this function. Notwithstanding all the unique qualities of wood one of its fundamental hindrances is its vulnerability to lose its quality in an inimical situation (Morrell, 2006). Water is the greatest enemy of wood in putting its durability to test. In dealing with this deficiency of wood there have been many ways available to preserve wood for its durability to stand a test of time. Some of the long-established known techniques of preserving wood entailed the utilization of naturally resistant hardwood species, supplementary preserved wood of non-durable species and devises to expel water from wood. In water prone conditions wood must either be naturally tenacious or preserved to be able to prevail. The insufficient availability of naturally durable woods in the wood industry has influenced the rise in the use of non-durable species that are preserved with chemicals. These chemicals used in the preservation of wood are of different varieties and greater number of them is melted in water and applied which subsequently incorporate heavy metals such as zinc, arsenic, lead, copper, cadmium and chromium into the wood.

Until recent times, the most frequently used chemical for wood preservation was a combination of arsenic, copper and chromium also known as chromated copper arsenate (CCA). In spite of the fact that CCA utilization has globally waned it continues to be one of the compelling chemicals used in the treated wood industry to preserve wood. It is also the type of wood preservative that all other water-based wood preservatives are referenced (Morrell, 2006). As the treated wood industry develops, new products become apparent, technology advances and environmental concerns intensifies (Freeman *et al.* 2003). Some of the environmental concerns has led to a widespread discussion on the usage of chromated copper arsenate (CCA) owing to the qualms associated with these heavy metals (chromium, copper and arsenic) released from CCA treated wood amidst in-service and out service periods via rainfall, leaching, or direct human contact (Hata *et al.* 2006).

Chromated copper arsenate (CCA), was discovered in India in the 1930s and was subsequently made known to other parts of the globe. Since the inception of CCA, it has become the frequently used preservative for open space structures and is very popular in the treated wood industry all over the world (Hata *et al.* 2006). CCA treated wood products for years had been protective against damages of insects and decay organisms (Leduc *et al.* 2008). The application of CCA treated wood materials increase day-in and day-out and it been projected to reaching higher amount with time (Huang and Cooper, 2000).

The environmental concerns of CCA chemical is an evergreen topic in the wood preservation industry that needs thorough investigation for resolution. This is because CCA chemical wood preservative contains heavy metals that are harmful to the environment and its inhabitants. Nonetheless the interest in ecotoxicological research in recent times has also been reawakened and many of these studies have suggested ways of recovering the environment from the lethal threats pose by both in-service and disposed out-of-service chemically treated wood products. Conclusions drawn from these studies had also involved different forms of remediation (Pare *et al.* 1998; Keener *et al.* 2001; Gupta *et al.* 2005; Suthar, 2008; Pattnaik and Reddy, 2010; Pattnaik and Reddy, 2011).

The volumes generated and controlling of abdicated CCA treated wood products varies from one country to the other. The wood industry in Japan for instance has willingly halted the production of CCA treated wood because of economic motives instead of environmental concerns. Whereas environmental and public cognizance in Australia has prompted the impending limitation of CCA uses for certain purposes but diffident to assist limitations on CCA treated woods sale and use (Hata *et al.* 2006). In contrast, there was a deliberate removal of CCA treated wood for residential use in the United States of America due to concerns of direct human contact (Lebow, 2010). This influenced the focus on the development of non-arsenic preservatives of wood in the United States of America (Freeman *et al.* 2003).

Though the available statistical data on the quantity of redundant CCA treated wood products around the world is not enough for decision making on the menace. According to the statistical data from Trabzon Electricity Distribution Company in Turkey, TEDAŞ (2009), there is still in use about 8 million pieces (3,000,000m^3) of treated utility poles. It was only in 2010 the value of 117,000 pieces (38.924m^3) was realized. Furthermore, information provided by the State Railways, according to Taşçıoğlu and Tufan, (2011) suggested that 5000m^3 of treated traverse were provided annually for the maintenance and repair of railway shears in Turkey. The quantum of wood materials treated with preservatives realized after their service life in the United States of America was 1,000,000m^3 in 1990 and

15,000,000m^3 in 2010 and this was also projected by Cooper (1993) to increase by 3,000,000m^3 by the year 2020.

With respect to European countries such as France and Germany, 2.1-2.4 million tons of treated wood materials have become obsolete by completing their life span (Helsen *et al.* 1998). In France, 26 million utility poles are said to be treated and used. The number of utility poles that have finished serving their purpose and have become obsolete is 500,000 tons per year. It was estimated that the piling up of the utility poles in their deserted state will continue for 50 more years (Helsen *et al.* 1998; Hingston *et al.* 2001).

The contamination caused by utility poles is normally brought to bear through the maintenance works done to prolong their durability which incorporate wood chemical preservatives into the environment. These maintenance works are necessary for utility poles because the initial chemical preservatives used on them lessen as time passes. Currently many utility poles around had been reapplied with preservatives or are yet to be applied again (Callahan, 2015). The service life of CCA treated utility poles can span for over 40-50 years although a study conducted by Gezer (2003) suggested that treated utility poles that spans for a maximum of 10-15 years was available in Black Sea region of Turkey. CCA treated wood products can be used for about 20-30 years in an open-air condition and will still have a significant amount of chemical preservatives when it out-of-service. Nevertheless, the problem of disposal and management of various organic wastes of which obsolete CCA treated wood products is not exceptional present a challenge to many countries in the world (Edwards and Arancon, 2004).

Discarding of CCA treated wood waste has been projected to rise globally as years pass by. The current traditional methods of disposing CCA treated wood products involve incineration which is practice more in Japan and Korea although studies have revealed how a gram of ashes remain from incineration of CCA treated wood material is lethal enough to kill 150 people (Hay *et al.* 2000). Landfill disposal, which is also common in Australia, and then recycle and reuse. However, recycle and reuse seem to be impeded by the complications in separating and sorting preserved and unpreserved wood waste materials (Hata *et al.* 2006) which unfortunately makes sorting unacceptable in practical and economical terms.

The information above is clear enough a reason on how serious the method of disposing obsolete treated wood materials contribute to exposing heavy metals to the environment and its inhabitants. The reprocessing of wood waste is vital for the efficient exploitation of the natural resource. This benign emerging innovation that has already begun in other developed countries is new to the developing world. There is a critical necessity to come about with innovation for reusing out-of-service CCA treated wood for other useful purposes owing to concerns raised about environmental pollution by disposed CCA treated wood products. In recent time, researchers have used different forms of strong acids which could remove and dissolve CCA compounds that had been fixed in CCA treated wood (Gezer *et al.* 2006). However, the present study is intended to diverge by using a bioremediation means involving a California red worm (*Eisenia fetida*). The study also involved a more of comparing a normal composting and vermicomposting activities.

The aim of this thesis work was to explore the possibilities of removing arsenic, copper and chromium from CCA treated utility pole sawdust using *Eisenia fetida* earthworm species which is recognized as tolerant to toxic chemicals and heavy metals.

1.1. Justifications of the Study

Recent studies and investigations related to CCA wood preservative have focused on chemical remediation to get rid of Cu, As and Cr heavy metals out of CCA contaminated media of different forms (Kartal and Kose, 2003; Clauses, 2004; Kazi and Cooper, 2006; Kakitani *et al.* 2006; Gezer *et al.* 2006). Chemical remediation is the most common method used in ecotoxicology studies. However, its implementation has not been safe and cost effective. In other words, chemical remediation method has not been a panacea for the very important problem at stake. In scope of this, it is quite unique to state that, this is one of the rare studies where California red worm (*Eisenia fetida*) was used to explore the likelihoods of extracting chromium, arsenic and copper from CCA preserved wood.

California red worm, *Eisenia fetida* has been the international number one earthworm species for chemical toxicity tests (OECD, 2016). Although *Eisenia fetida* is not an inbred deep soil-inhabit organism (Booth and O’Halloran, 2001) it is considered as a representative of other earthworm species due to its sensitivity to heavy metals and other chemicals. Little information is available on how *Eisenia fetida* responds to heterogenous metal contaminants like CCA even though this group of earthworms has been employed in standard ecotoxicity chemical trials (OECD, 2016). Many studies have also concentered on earthworms’ toxicity and single metal accrual (Weltje, 1998). However, specific growth and reproductive outcomes were achieved in a study where earthworms exposed to leachate from wood preservative made of multiple metal (Cu, Cr, As-CCA) as compared to single metal leachate (Cu-ACQ) (Leduc *et al.* 2008).

The reactions of *Eisenia fetida* to heavy metals in studies are available in large quantities of data. *Eisenia fetida* has also been the choice and a point of reference in the international toxicity test (ISO 993; 1998; OECD 2004). It is strong and can easily be grown in copious numbers in the laboratory as it develops within eight weeks, profusely reproduces and has lesser generation period as compared to other species and it is receptive to an extensive array of toxicants (Nahmani *et al*. 2007). However, owing to the fact that *Eisenia fetida* resides in organic rich niches such manure piles and dung (Bouche, 1972) it has been a source of criticism in accumulation and toxicity studies for not being a deep soil dwelling organism.

Eisenia fetida has also shown the capacity to tolerate heavy metals which are non-lethal to its body through results of many studies (Leduc *et al*. 2008). They tend to accommodate more nutrients for a lengthier period without any adverse influence on the environment. While consuming soil and organic substance they absorb heavy metals with the help of their fragile skin and intestine thereupon accumulating these metals in their body tissues (Hand *et al.* 1988; Singh and Sharma, 2002). Moreover, Cortet *et al.* (1999), reported that copper, zinc, lead and cadmium are accumulated and bioconcentrated in earthworms within certain environmental conditions. However, Spurgeon and Hopkin (2000) stated in their work that zinc in many cases was the only grave toxic metal to these organisms.

In line with the above, the study under consideration investigates with the aim of assessing possibilities of *Eisenia fetida* extracting and accumulating heavy metals from a substrate mixture of cow dung and sawdust from CCA-treated wood. Also, CCA-treated sawdust has not been popular in ecotoxicology research as a medium for earthworm exposure. On the other hand, earthworm production for the purposes of fertilizer and manure for amending soil fertility has gained much attention and has become important in recent years. Earthworms are known to produce manure that is highly rich in nutrients for agricultural purposes and for that matter they are easily accessible from dealers in fertilizers (Nahmani *et al.,* 2007). The transformation of biowaste by means of earthworms' species into manure for agricultural use is also fast becoming popular around the globe (Rajkhowa *et al.* 2015). For these reasons, the use of earthworms for heavy metals extraction was predicted to be an antidote to ecotoxicology as economically viable, socially acceptable and ecologically sound technology (Sharma *et al.* 2005).

This study was aimed to investigate and help curb contaminations caused by obsolete CCA treated wood products in the environment.

1.2. Problem Statement and Objectives

The major chemical wood preservatives used in the wood industry are mainly; pentachlorophenol, creosote and arsenicals or chromium, copper, and arsenic. Although CCA preservatives used to treat wood product such as playground equipment and other residential applications have been banned the utilization of CCA for other wood products is still available. These wood products contain dangerous leachable chemicals such as dioxins and arsenic. According to the American Cancer Society these dangerous chemicals are carcinogenic and are detrimental to human health and other organisms (A.C.S., 2016). Chemical wood preservatives have been classified among hazardous representatives of cancer (U.S.E.P.A., 2007). They are said to be agents of nervous system toxicants, reproductive defects and birth complications. There is also higher cancer risk of millions of times for those who are involved in pentachlorophenol administration works to poles and this is higher than the acceptable limit for people per the reports of U.S.E.PA., (1999).

Among all the chemical preservatives used in the wood industry, the most common and extensively used is the chromated-copper-arsenate, popularly known as CCA. This chemical preservative has since replaced the traditional creosote after its introduction in the wood industry. It is made up of three metals in the form of oxides and pressurized into wood by a process called ‘’Wolmanising’’ (Weis *et al.* 1995). Many studies have confirmed the toxicity level of CCA treated wood to many organisms in different forms (Weis and Weis, 2004; Smith, 2009; Reddy and Pattnaik, 2009). In most of these studies, it was realized that the release of metals from CCA treated wood products was via leaching. The risk regarding CCA treated wood became apparent from the fact that Arsenic, chromium and copper were drained and consequently carried into the environment via soils (Leduc *et al.* 2008). Although increased drying time of CCA treated wood was known to have lessen the leaching of heavy metals from chemically treated woods (Hingston *et al*. 2002) some quantities of these toxic metals were found to have leached out into the ecosystem from properly preserved wood especially when the wood was new (Weis and Weis, 2004).

Arsenic elements can be detected in different medium such as soils, freshwater sediments and sea, plants and marine organisms (Burguera and Burguera, 1997). Hingston *et al.*, (2000); Townsend *et al*. (2004) reported that the quantities of metals that can be leached from CCA treated wood depends on factors like pH, duration of contact between wood and leaching solution, type of wood, ionic strength of leaching solution and preservation method.

From the perspective of environmental hazard and ecological toxicity the deadly aspect of bioavailable heavy metal portions is usually in a minute quantity of the entire burden (Amir *et al.* 2004; Fuentes *et al.* 2006).

The following included the objectives of this research work;

- To investigate the effects of CCA individual components of CCA treated wood waste (sawdust) and the cow dung on the reproduction, development, and the total growth changes of the California red worm (*Eisenia fetida).*
- To investigate the amount removed in percentage of chromium, arsenic and copper and from the principal substrate (sawdust and cow dung) using California red worm (*Eisenia fetida).*

2. LITERATURE REVIEW

2.1. General Concept about Earthworms

Earthworms are tube-shaped, segmented natural invertebrates of agroecosystem belonging to the class *Oligachaeta*, order *Megadrilacea* and in the phylum Annelida (Haokip and Singh, 2012). They are significant soil creatures making up a sizable proportion of the entire biomass of invertebrates prevailing in soils of temperate and tropical regions. Earthworms are the majority soil invertebrate organisms in tropical, temperate and subtropical regions (Nainawat and Nagendra, 2001). They are the foremost multicellular organisms without backbone to have thrived in terrestrial locations (Kale and Karmegam, 2010). Earthworms are hermaphrodites however self-fertilization for reproduction does not take place within an organism. They are significant burrowing organisms that increase quality of soils in the ecosystem. For this purpose and the significant work done by earthworms in soils they are referred to as ecosystem engineers (Lavelle, 1997). Soil quality, crop yield and plant growth are all results of the important activities of earthworm species owing to the relative portly stature and their behavioral feeding pattern. Earthworms are also popularly known as “night crawler”, “anglerworm” (for fishing baits), “dew-worm”, and “rainworm”. Other are also known as megadriles and microdriles because of their literally bigger and smaller body size respectively. Earthworms are located in their numbers and active at locations with moistness as compared to distressed environments (WFF, 2012).

The burrowing activities of earthworms improve soil physical structure and increase the general qualities of soils. It increases plants moisture availability and uptake. Key processes like dynamics of chemical processes, soil nutrients unleashing, dynamics of organic matter and microbial activities are also influenced by earthworms casting, channeling activities, feeding behavior and physical characteristics like permeation and conglomeration. (Sharpley and Syers, 1976; Lavelle *et al*. 1983; Bostrom, 1987; Bouche *et al*. 1987; Scheu, 1987; Scheu, 1990; Lavelle and Martin, 1992; Pashanasi *et al.* 1992; Edward and Bohlen, 1996). The above processes all aid to provide plant growth catalysts and also facilitate the conversion of soil nutrients into absorbable state for plants use. Barois and Rajkhowaelle, (1986); Lavelle *et al*. (1995) have all reported on the existence of an interdependent relationship between earthworms species and microorganisms which aid in the conservation of microbial diversity and soil productivity.

Earthworms are the main organisms in charge of blending and distribution of soil particles. They contribute to soil quality via means of by taking halfway decayed litter from the top soil, digesting it, and moving it to other locations in the soil. These traits of earthworms make them suitable creatures for monitoring the effects of soils pollution (Fischer and Koszorus, 1992). Their presence in soil is an evidence of the caliber of the soil fertility. The impact of earthworms on soil activities varies between species and environmental types.

Earthworms' activities such as casting and burrowing can impact on the health and functionality of soil. Undecomposed organic materials are often seen on the topsoil of soils without earthworms which affects the soil structure. The availability of earthworms in their numbers in soils is a sign of the presence of other soil organisms that contribute to improve soil fertility (Pavithra, 2012).

Earthworms are significant source of food for different organisms of all types. The popular one among them is the use of earthworms as bait for fishing. They are also a source of medium through which contaminants are passed on to more advanced trophic levels. Earthworms are extensively used as pointers of soil contamination and health owing to the significant role they play in breaking down organic materials in soils which contribute to soil development and maintenance of soil structure (Fischer and Koszorus, 1992; Edwards and Bohlen, 1996). The earthworm family *Lumbricidae,* are known for the enhancement of macro porosity and aggregation of soil (Lee, 1985; Vetter *et al*. 2004). The casts and burrows produced by this group of earthworms contain a lot of macronutrients which improves the growth of plants roots in the soil (Edwards and Bater, 1992). They also improve the fertility of soils and coexist with other soil fauna organisms (Fragoso and Lavelle, 1992).

According to Fragoso and Lavelle, (1992) earthworm species in the tropics are predisposed to certain factors in their locations. These factors included soil condition (pH, nutrients content), seasonal changes and temperature. Their population is also affected by the annual rainfall observed in a typical rainforest zone. Annual rainfall of 3000mm is ideal for their survival but annual rainfall amount of 4000mm and 2000mm is respectively too wet and dry for earthworms' existence (Fragoso and Lavelle, 1992). Lavelle, (1988) also reported that earthworms are hardly seen in arid zones populated with termites and receive annual rainfall amount of 900mm or lower with temperatures above 35ºC and a longer dry season. Nevertheless, they withstand dry seasons by moving vertically lower and deeper into the soil causing periodical perpendicular change in earthworms' distribution in the soil (Fragoso and Lavelle, 1992) but lateral circulation of earthworms in the soil according Rossi, (2003);

Decaëns and Rossi, (2001) is haphazard and organized at different spaces. Earthworms have also been used in many waste managements and ecotoxicity studies where they were used as bioaccumulation factor of a medium (Langdon *et al*. 2003; Leduc *et al.* 2007; Nahmani *et al.* 2007; Mench and Bes, 2009; Pattnaik and Reddy, 2011). In all these cases, the ecological characteristics of earthworms were considered when using them for remediating contaminated soils or any contaminated media. For instance, the earthworm species *Eisenia fetida* is widely known to be considered as the approved and recognized earthworm species for researches of ecotoxicology test (Langdon *et al.* 2005; Peijnenburg and Vijver, 2009). They are also bred as baits for fishing and for vermicomposting purposes. They dwell only in organic-rich materials environs like composts and dung heaps and barely found in soils (Edwards and Arancon, 2004). These ecological variations of earthworms have substantial outcomes on the accumulation, sensitivity and exposure of heavy metals to earthworms.

For example, some features of earthworms like their place of abode and preferred mode of feeding can impact on the extent of contact with contaminated soil particles which may result in variations of earthworms’ exposure to heavy metals. Earthworms have special means of adjusting themselves to heavy metal exposure which aid them to dynamically control heavy metal bioconcentration in their body tissue (Hopkin, 1989). A report by Spurgeon and Hopkin (1999) confirmed a steady amassment of Cu and Zn in bodies of *Eisenia fetida* which signified substantial removal of these heavy metals and showing their distinct capability to accommodate heavy metals. Earthworms also have the capacity to hold themselves against lethal impacts of heavy metals by storing excess heavy metals, removal and detoxification of heavy metals (Vijver *et al*. 2004). The type of heavy metal and earthworm species influences the attachment of heavy metals to proteins like metallothionine as reported in an eco-physiological research (Spurgeon and Hopkin, 1999; Vijver *et al.* 2004). However, Spurgeon *et al.* (2000) and Nahmani *et al*. (2007) reported that the accrual of heavy metal levels is different with regard to earthworm species but reactivity of *Eisenia fetida* to heavy metal was found lesser than other species.

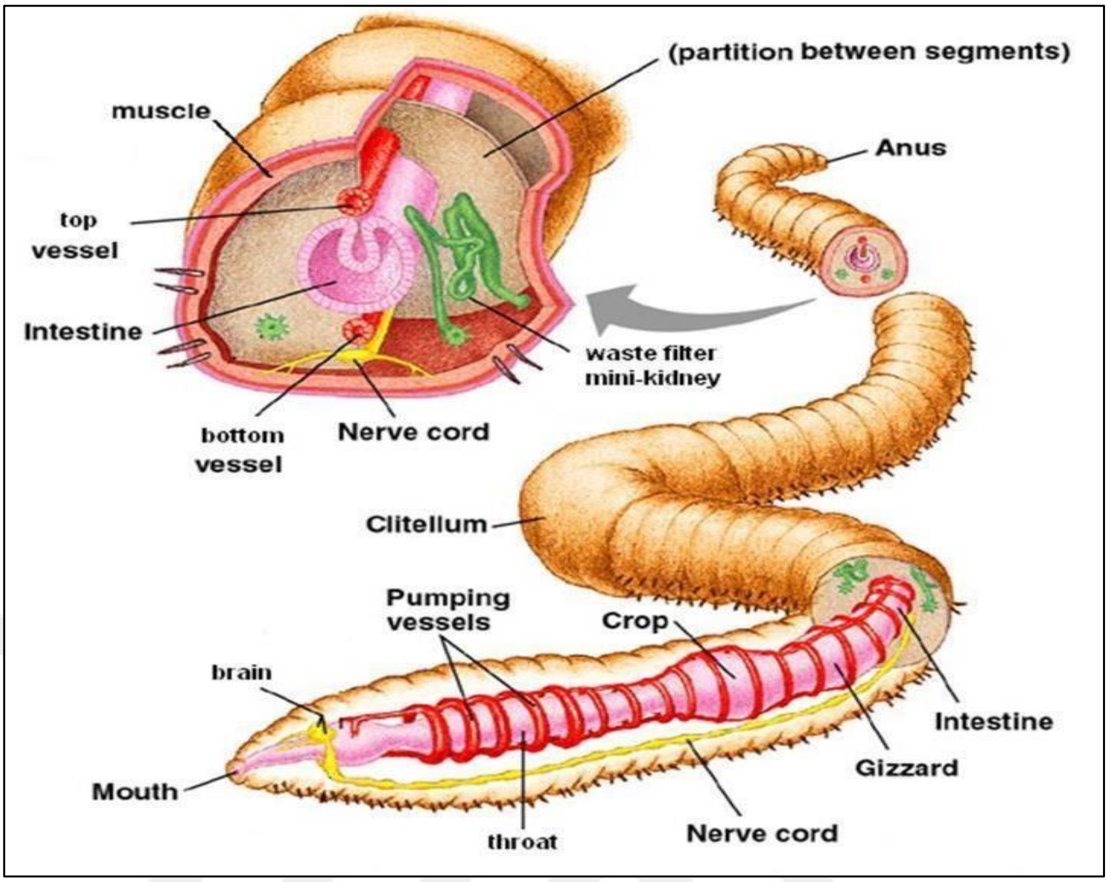

Figure 1. A labelled diagram of an earthworm

2.1.1. Categories of Earthworm

There are three types of recognized earthworms with regards to their environmental purposes, location of habitat and bodily characteristics such as body size, color and shape. In respect of the above three ecotypes of earthworms have been classified by researchers as follows;

A. Anecic earthworms

B. Epigeic earthworms

C. Endogeic earthworms (Bouché, 1977).

Each of these earthworm categories constitutes earthworm ranges with distinctive features. The epigeic earthworms like other fragile soil organisms are principally responsible for soil litter modification. On the other hand, the endogeics and anecics earthworm species impact on ecosystem layer processes and activities and also contribute significantly to soil health condition and their impact on soils is great and may influence properties and processes at the ecosystem level (Lavelle, 1997). Also, all the three ecotypes of earthworms identify above are used in one way or the other for burrowing purposes. Whiles endogeics are suitable for horizontally deep burrowing, anecics used for general burrowing purposes and epigeics which are popularly known as ground dwellers are used for the purpose of tillage with little burrowing (Hickman and Reid 2008).

Epigeic earthworms are typically located in the topmost part of the soil while the endogeic earthworm species are found in the first 10 to 20 cm close to the surface of soils. On the other hand, the anecics reside in the lowest alcove of the soil and are very scanty in numbers in tropics due to their place of habitat (Barois *et al*. 1999). The figure 2 illustrates categories of earthworms in their ecological habitats and location in the soil.

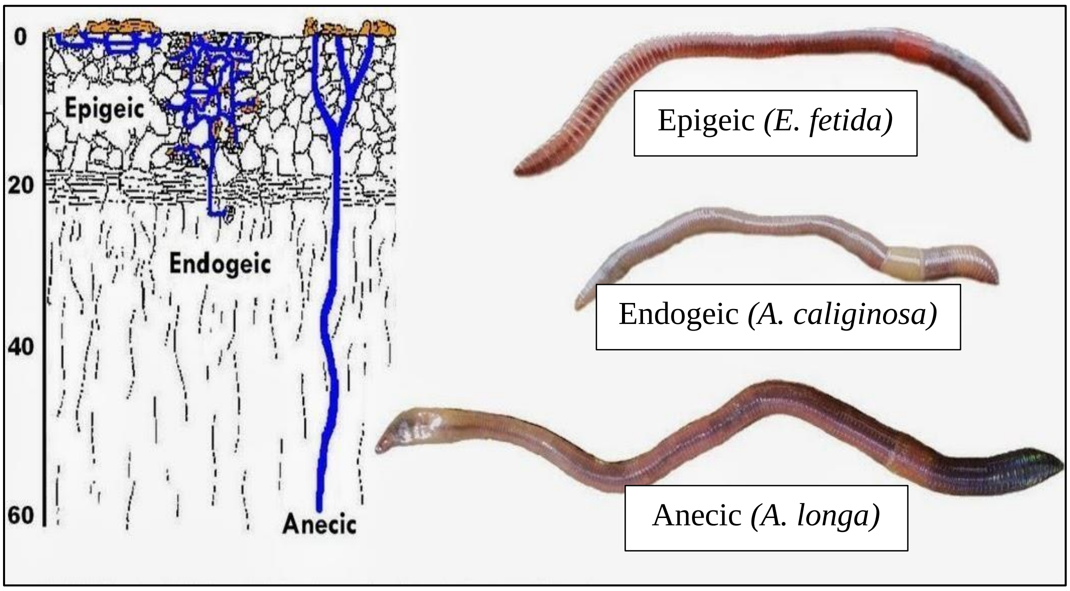

Figure 2. Earthworm ecotypes in their respective location in the soil. The figure was adapted from Qiu et al. (2013), and slightly modified

2.1.1.1. The Anecic Earthworm

The anecic earthworms are mostly dominant in biomass of soils of many temperate regions (Lavelle, 1983). They are primarily vertical burrowing species and they carry organic matter from the upper part of the soil to their fixed abode deep down the soil (Butt, 1993; Domínguez, 2004). These transferred organic matter and surface litter are fed on by anecics at the basal part of the soil. Anecics are large in body form and burrows to the upper layer of the soil solely for feeding purposes (Paoletti, 1999). Their presence in an area can be noticed by a feature known as ‘middens’ which is mostly found at the openings of burrows. This mound ring-shaped trait is mostly a combination of leaves, soil and other organic matters.

The activities of anecics earthworm species in soils may extremely alter soil essentials up to a depth of 1m or more. Earthworm species of anecic group may include; *Lumbricus terrestris and Aporrectodea longa.*

2.1.1.2. The Epigeic Earthworms

The epigeic earthworm species are normally smallish in body form and dwell in, comminute, feed on materials found on the surface of soil. These materials are mostly made of organic humus and litter devoid of soil particles (Lavelle, 1988). They are also found attached to plant root because they ingest leaf litter on the surface of soil (Domínguez, 2004). The feeding manner and mechanism used by epigeic earthworm species to refine litter renders rich amount of nutrients to the soil. They have a short period of guts transfer which make them rely on other microorganisms to assist them in the disintegration of litter in their guts. Some of the earthworm species belonging to this group of earthworms ecotype include; *Lumbricus rubellus* and *Eisenia fetida*.

2.1.1.3. The Endogeic Earthworm

Endogeic earthworm species are commonly found in the tropics and the sole class of earthworm prevalence in biomass of agroecosystems (Lavelle, 1983). They nourish themselves with soil minerals of the soil subsurface and dwell therein as their abode. They are geophagous species of earthworms that are hardly found in the layer of the topsoil (Bouché, 1977). Endogeics feed on varied qualities of soil minerals and produce greater and quality nutritious ground casts. These casts are mostly of two types; large and compacted cast and small but loose cast which contain some quantities of NH_3 and other substantial nutrients (Barois et al. 1999) as compared to undigested soil organic minerals. *Murchieona muldali, Aporrectodea caliginosa, Pontoscolex corethrurus, Aporrectodea rosea, Allolobophora chlorotica, Lampito mauritii* and *Aporrectodea icterica* are all members of endogeic earthworm species.

2.1.2. Distribution of Earthworms

All the earthworm ecotypes and categories discussed above are prevalent all over Europe and can also be found in other part of the world such as North America (Hendrix and Bohlen, 2002). There are about 6,000 species of earthworms discovered and about 120 of those species are widely distributed around the globe. Most of these earthworm species are of global and foreign origins. Different types of earthworm species have been discovered in different countries from different origins. The highest recognized earthworm species is reported to be in Australia. 650 indigenous species of earthworms with 75 other exotic species can be found in Australia. Also, a total of 182 earthworm species of 12 families have been reported in the North of Mexico, USA, and Canada sixty taxa out of them have been exotically introduced.

2.1.3. The California Red Worm (*Eisenia fetida)*

There are two recognized families of *Eisenia fetida* available and these have been divided into species. These two species include; *Eisenia foetida* and *Eisenia foetida andrei*. They are morphologically similar, but the former has an intersecting and crosswise stripping on their segment whereas the later has a variegated reddish color (OECD, 2015). The California red worm is an earthworm species that belongs to the epigeic earthworm ecotype and lives on the upper layer of the soil or in 10inches of the topsoil under the litter layer (Ranch, 2008). It has a dark red color skin with external structures of 35130mm in length which is greater than 70mm, 3-5mm in diameter, 80-120 segments and weighs about 1.4g. California red worm just like any other species of worm does not tolerate solar light because exposure to solar light results in their death within few minutes. *Eisenia fetida* is one of the earthworm species that has demonstrated a lot of prospects for the purposes of vermicomposting. The environmental requirements and biology of *Eisenia fetida* have been broadly studied and reported in different forms (Kaplan *et al.* 1980; Hartenstein *et al.* 1981; Reinecke and Venter, 1987; Edwards, 1988).

It is an earthworm species that multiplies massively (Hartenstein *et al.* 1979) and develops very fast (Neuhauser *et al.* 1980). It has also shown an inherent sign and promising mechanism for waste management (Hartenstein, 1981). It lives approximately four and a half years and is able to multiply and reproduce about 1,300 new earthworms annually under favorable conditions (vermiculture manual). Any medium that harbors *Eisenia fetida* earthworm species must have an adequate climatic condition. Severe heat or cold impedes the activities of *Eisenia fetida* species. However, the water content of the soil harboring them is very significant for the burrowing purpose of the earthworm to commute easily through the soil (Kooijman and Cammeraat 2010; Owojori and Reinecke 2010). With optimum temperatures at a particular place, it is possible to raise this type of earthworm species at any location. They are strained under 30^0C and can easily die under temperatures above 40^0C. They are also not responsive when the temperature is below 7^0C though still generating humus in smaller amount. They reach their maximum reproduction ability when they live between temperatures 14^0C and 27^0C. Their reproductive activeness for a significant longer period than it had been considered possible is a wide spread phenomenon (Venter and Reinecke, 1988).

Normally matured California red worm produces humus of quantity of their weight and consume an equivalent quantity of food every day. This implies that their activeness is dependent on the amount of food available to them (Klok, 2007). They are able to live through well-defined soil conditions. The multiplication of earthworm population was reported to have been as a result of the application of manure and waste water sludge at a polluted area (Tejada 2009; Eijsackers 2010; Tejada and Masciandaro, 2011). California red worm also avoids extreme soil conditions. Their activities and survival are impeded by conditions such as high content of salt, organic contamination, high concentration of heavy metals and severe pH which also changes the formation of their habitat (Lapied *et al.* 2009; Eijsackers, 2010; Irmler, 2010; Kooijman and Cammeraat, 2010; Owojori and Reinecke, 2010). They die in extreme alkaline or acidic conditions. For this reason, their food and environmental pH is between 6.2 and 7.8 but a pH of 7 is ideal.

Eisenia fetida simultaneously advances, eats, and makes tunnels in soils. It is during this process it leaves the excreta behind and turns the soil in its terrain much more fertile than the fertile soil from any artificial fertilizer. The excretes of California red worm are many times richer in nitrogen, potassium, phosphorus and calcium than artificial fertilizer (URL-1).

Figure 3. *Eisenia fetida* earthworms at different stages of their life cycle
(Photo taken by Mohammed, 2018)
A. Hatchling cocoon of *Eisenia fetida*
B. Juvenile of *Eisenia fetida*
C. Clitellum-matured *Eisenia fetida*
D. Bunch of *Eisenia fetida*

2.1.4. The Life Circle of the California Red Worm (*Eisenia fetida*)

California red worms are hermaphrodites with both male and female reproductive organs in a single organism (Ranch, 2008). A matured *Eisenia fetida* has a thick, light-colored pigment and saddle-like ring found in the skin of the worm known as clitellum. For the purposes of copulation earthworms place themselves in a horizontal position on the ground with their head pointed in corresponding directions. In this position they secrete mucus that sticks their bodies together. Earthworms then interchange sperms which are placed onto their skin. These sperms are later moved into an opening slightly apart from the clitellum where they are stored for a short while. During this process earthworm also expels their own eggs separately into openings near the openings of the sperms on their skin.

After this process they move away from each other and produce extra gummy mucus all over their clitellum which desiccates into a solidified band to strengthen their bodies.

Afterwards the earthworms withdraw from the solid band of mucus by sagging themselves out of it. The sperms and eggs pores are picked up from the gluey base as the solid mucus band moves over the opening keeping them. After earthworms have successfully withdrawn from the mucus band the end closes and it becomes a minute rigid cocoon with egg and sperm in it. The sperms obtained during the exchange period of sperms are utilized to produce more cocoons till it finishes. The fertilization of earthworms' eggs and sperms happens within the cocoon for juvenile worms to be reproduced. *Eisenia fetida* has an incubation period ranging from 32 to 73 days. It takes about 32 to 109 weeks for freshly hatched juveniles of worm mature and reproduces cocoons. They are able to produce cocoons in substantial numbers annually. However, this will depend on environmental conditions such as; moisture, temperature, and availability of food. When earthworms are fed well with three times of their weight of food, at least 75% of moisture availability and available temperatures between 20 and 25, their biomass double up within 3 to 4 months (Ranch, 2008).

According to Klok (2007), earthworms need enough food to be active. However, Neuhauser *et al*. (1980), demonstrated the available relationship between earthworms rate of weight gain and particle size of the food they consume; a smaller particle size of food leads to a higher growth of earthworms. Also, promising signs of population build-up and reproduction was observed in earthworms after several weeks of experimentation especially in the abundance of earthworm cocoons (Morgan, 2011). Eisenia fetida was reported to have increased in biomass and released some essential elements in a vermicompost study where different types of growth media were tested (Yüksek, 2016).

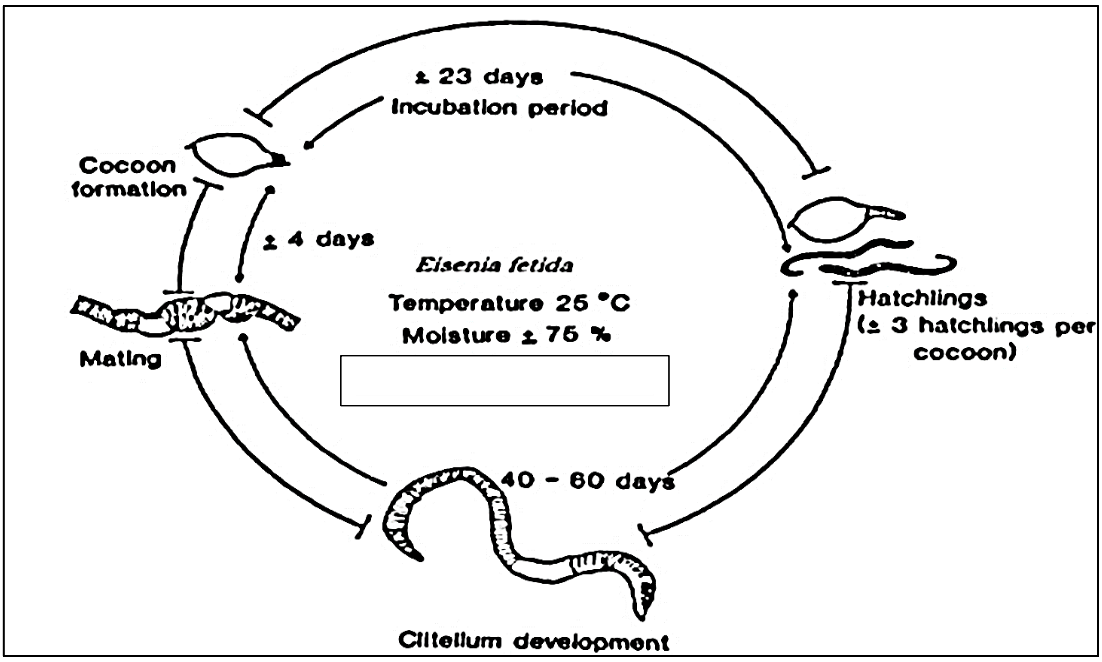

Figure 4. The life cycle California red worm (*Eisenia fetida*)
(Venter and Reinecke, 1988).

Figure 5. Eisenia fetida laying side by side in exchange of sperms for reproduction
(Photo taken by Mohammed, 2018).

2.1.5. Metal Accumulation by California Red worm (*Eisenia fetida*)

There is no doubt about the substantial evidence available on the capability of the three ecophysiological categories of earthworms accumulating several essential and nonessential metals from plant growth media. The epigeic ecotype of vermicomposting earthworm species like *Eisenia fetida* and *Dendrobaena veneta* are the most popular species that have been used for accumulation purposes. They are able to accumulate heavy metals from different contaminated soils. Due to anthropogenic activities many studies have reported the ability of this ecotype of earthworms to accumulate heavy metals from soils of metallic polluted and unpolluted soils used as control (Morgan *et al*. 1993; Peijnenburg, 2002; Peijnenburg and Vijver, 2009). The fragile and highly permeable body walls of earthworms coupled with their bio-metal accumulation characteristics reflect their detritivores lifestyle. However, earthworm bioaccumulate heavy metals and store them in a form of non-lethal but die of the metals when it reaches a critical concentration (Morgan and Morgan, 1988).

Mountouris *et al*. (2002) defined bioaccumulation as the mechanism by which organisms concentrate chemicals from their immediate habitat or environment into their body tissues. There are oodles of factors that regulate the accumulation of heavy metals into tissues of earthworm. These factors are known to be the characteristics of its habitat. Some of these factors include; oxides of manganese, aluminum, iron (Shea, 1988; Bendell-Young and Harvey, 1991; Bryan and Langston, 1992; Bendell-Young et al, 1994; Janssen *et al.* 1997), acid volatile sulphide (Chapman *et al.* 1998) and organic carbon content (Mahony *et al.* 1996). According to Luoma and Rainbow (2005), bioaccumulation by earthworms is mostly driven by physiological and physicochemical parameters. The environmental conditions, characteristics of metalloid and heavy metals and the intended species all put together regulate bioaccumulation dynamically. Vermeulen *et al*. (2009), have demonstrated in their study the influence of environmental factors on accumulation of Pb, Cu and As in earthworms tissues. However, pH level and organic matter content has also been reported to be a significant facilitator of concentration of the heavy metals Cd and Pb in tissues of organisms (Peijnenburg, 2002).

The targeted metal of concern in the usage of CCA wood preservative is widely known to be arsenic. But its availability to earthworm species also varies with respect to earthworm's location in the soil. According to Langdon *et al*. (2003), arsenic is more available to earthworm species that are not found deep in the soil due to the strong bond between arsenic and both organic and mineral matter in the soil. Bioaccumulation factor (BAF; also cited as uptake factor, UF, Concentration factors, CF, and bioconcentration factor, BCF) is the ratio of the total concentration of heavy metal in tissue of an organism to the total concentration of heavy metal in its immediate environment of habitat (substrate or medium used for a study). As used in many studies (Mountouris *et al.*, 2002; Leduc *et al.*, 2008; Pattnaik and Reddy, 2011; Singh *et al*., 2017) to ascertain the inherent concentration of heavy metals in an organism, the following formula was used to extrapolate the proportion of these metals allocation in organism and its environment;

$$\mathbf{BAF = C_{organism} / C_{habitat}}$$

Where;

$\mathbf{C_{organism}}$ means the total metals concentration in the tissues of the organism (ppm; mg/kg).

$\mathbf{C_{habitat}}$ means the total metals concentrations in the organism's habitat (ppm; mg/kg).

Pattnaik and Reddy, (2011), also interpreted the results calculated from the above formula as the comparison of the obtained answer to a unit (1); stating that when heavy metals concentration in the organism (earthworm tissues) is higher than that of its habitat (substrate), the bioaccumulation factor (BAF) will be more than one and less than one when the metals concentration in the organism's habitat is higher than that of the organism.

Heavy metals are also known to display different bioaccumulation behaviors in earthworms (Sample *et al.* 1998). Unfortunately, the bioaccumulation factor of several metals in organisms of water prone area (McGeer *et al.* 2003; DeForest *et al.* 2007) and earthworms (Neuhauser *et al.* 1995) have been reported to show strong inclinations in the direction of inverse relationships with extrinsic metal accumulation. Nieboer and Richardson, (1980), also stated that the bioaccumulation capacity of a given metal is not described by its bonding attractiveness or metabolic significance.

As reported by Morgan (2011), in relation to the utilization of bioaccumulation factor for risk evaluation, it is important for the calculated value to imply the integral characteristics of the chemical of concern. And it should not also be variegated with the variations in environmental conditions. It is also important for differences between bioconcentration and accumulation be known prior to analysis and interpretation of data from toxicity study. Bioconcentration or bioavailability in toxicity testing is not a broad feature in ecotoxicity studies as it can be species-inclined or organism-inclined (Giller *et al.* 1998). The accumulation of metals by an organism does not necessarily suggest bioconcentration

(Morgan, 2011) due to the analytical approaches employed, the variations in life style between species and the mechanism as well as duration of exposure to contaminants. In relation to this, bioaccumulation information on water prone organisms is used with vigilance in risk evaluation on the basis of specific cases (McGeer *et al.* 2002).

2.2. Wood Utility Poles

Utility poles have been and continue to be one of the most common features of any scenery. Utility poles have been made from wood since time immemorial. However, recent innovations and development have led to the introduction of utility poles made from steel, concrete and other form of materials. Wood utility poles have many functions in the architecture of a surrounding. They have primarily aided in channeling electrical cables from electricity companies to various households and institutions. In recent times, innovations have led to the covering of electrical cables beneath the ground nonetheless there are still available a substantial amount of utility poles in the system conveying wires of different forms for varied purposes. The American wood preservers association (AWPA) have reported of a significant amount of utility poles serving different purpose in the United States. These utility poles were said to be managed and used by companies of railway, electricity and telecommunication (A.W.P.A, 2005).

Utility poles are generally preserved with different forms of wood preservatives to ensure their durability against any form of attack. Different tree species have been used for utility poles in various countries. The suitability of a tree species to be used for utility pole is relative to the location of a country and the availability of these trees. The most common tree species used for this purpose in recent times is the pine species. The pine species and other tree species have been used for utility poles with treatment from chemical wood preservative. The most popular chemical wood preservative used is chromated copper arsenate (CCA) which is made of a mixture of copper, arsenic and chromium however creosote and pentachlorophenol (Penta) are the other two most popular preservatives used in treating utility poles (Callahan, 2015).

2.3. Wood Preservation

Wood preservation involves the impregnation of wood with chemicals that protect wood from biological deterioration and delay combustion due to fire. According to Lebow (2010), Wood preservatives seek to meet two broad criteria via;

- The provision of the desired wood protection in the intended end use.
- The achievement of the desired protection without presenting unreasonable risks to people or the environment because wood preservatives are considered a type of pesticides.

There are two types of processes used in the preservation of wood. These are pressure and non-pressure processes. The pressure process is the most common process in which chemical is impregnated into wood by a carrier fluid in closed vessels under pressures considerably above atmospheric pressure. Preservative chemicals used during wood preservation process are separated into two general categories (A.W.P.I., 1994) of the following;

1. Oil-borne preservatives
2. Waterborne preservatives

Waterborne and oil-borne preservatives have a purpose to prolong the service life of wood products by protecting them against insects and fungal attack. Wood exposed to outdoor atmosphere and those in direct contact with soil and water are more prone to decay and therefore need to be treated.

2.3.1. Oil-borne Preservatives

Oil-borne wood preservatives are some of the oldest preservatives, and their use continues in many applications. Wood does not swell from treatment with preservative oils, but it may shrink if it decreases in moisture content during the process of treatment. Volatile oils or solvents with oil-borne preservatives leave wood cleaner after treatment than heavy oils but may not provide much protection. Wood treated with some preservative oils can be glued satisfactorily, although special processing or cleaning may be needed to remove surplus oils from surfaces before spreading the adhesive. Oil-borne preservatives use oil to carry the treatment chemical into wood. These preservatives do not react with wood but are insoluble in water and have low volatility (Hunt and Garrett, 1967; Kollmann and Cote, 1968). The migration of whole oil can be a problem when the treating process does not release adequate internal pressure. But the primary loss of chemical occurs as water gets into contact with the wood surface there by producing slow depletion. Oil-borne preservatives include copper naphthenate, zinc naphthenate, pentachlorophenol and creosote. The most common of these are pentachlorophenol and creosote. Pentachlorophenol is a crystalline aromatic compound while creosote is a heavy black-brown liquid produced by condensing vapors from heated carbon-rich sources, such as coal or wood. The resultant preservative is sometimes mixed with tar oils and petroleum oils. Pentachlorophenol, creosote, and solutions with heavy, less volatile petroleum oils often help to protect wood from weathering but may adversely influence its cleanliness and fire performance. They have an odor and impart dark color to the wood and result in an oily surface which is difficult to paint. Oil-borne preservatives reduce surface checking and offer water repellency. The most common uses of creosote treated wood include railroad and bridge ties. Pentachlorophenol is used to treat utility poles and cross-arms (Milton, 1995). Wood treated with either of the two chemicals is flammable and contact with the skin may cause irritation. Also, wood treated with pentachlorophenol and creosote is not advisable to be used for indoor purposes.

2.3.2. Waterborne Preservatives

Waterborne preservatives are often used when cleanliness and paint ability of the treated wood are needed. Formulations intended for outdoor uses have shown high resistance to leaching and very good performance in service (Lebow, 2010). Waterborne preservatives are included in specifications for items such as lumber, timber, posts, building foundations, poles, and piling. Waterborne preservatives use water as the carrier fluid during the treatment process. The water in the process is made to evaporate from the wood shortly after treatment leaving behind the treatment chemicals. The water aspect of the treatment process may cause some drying and shrinkage at installation unless the wood is kiln-dried after treatment. Waterborne preservatives may also include both metal and organic based systems. Some of the metal systems react with wood, making them resistant to leaching. Organic systems are typically made water soluble by adding co-solvents. Once these systems dry, the chemical having low water solubility thereby resists leaching. Waterborne systems are often preferred because of the clean surface, paint ability, lack of odor, low fire hazard and low cost of the solvent. The biggest market for waterborne systems is residential applications.

The efficacy of waterborne metal preservatives is driven by fixation, where a series of reactions between preservative components and wood component results in the stabilization and in-solubilization of toxic elements in wood (Cooper *et al.* 1993). Salts formed by elements like copper, chromium, zinc, and arsenic contained in preservative solutions react to form insoluble preservative compounds (Hunt and Garrett, 1967). Copper based preservatives are the most commonly used waterborne preservatives due to their low cost and efficacy against insects and fungal attack. Copper is broadly toxic to fungi, causing membrane disruption and inhibiting many important enzymatic reactions. Low levels of copper are less effective against insect attack, although high copper levels are effective against most insects. The reaction sites for copper in wood are the carboxylic groups found in hemicelluloses (Thomason and Pasek, 1997), although Xiel *et al*. (1995), proposed that copper also interfaced with hydroxyl groups from phenolic and carboxylic groups in lignin. An ion exchange theory was also postulated by Dahlgren (1972) where weak acid groups in wood formed interfaces with copper cation by the exchange of H^+.

Metal oxides are the most common waterborne chemicals used in the wood industry. These chemicals include ammoniacal copper zinc arsenate (ACZA), chromated zinc chloride (CZC), acid copper chromate (ACC), ammoniacal copper arsenate (ACA) and chromated copper arsenate (CCA). The most widely used waterborne preservative among all these is the chromated copper arsenate, CCA which according to the A.W.P.I., (1994), represents over 90% of the U.S. waterborne preservative market. CCA is composed of oxides or salts of chromium, copper, and arsenic. The arsenic guards wood against insects while the copper in the wood acts as a fungicide. The chromium serves as a facilitator and injects the arsenic and copper into the wood.

2.3.3. Selection of Wood Preservatives

In choosing wood preservatives for wood treatment, the specific purpose of the wood product determines the type of wood preservative to be applied. Wood products are mostly used above ground level or below ground where it is in unswerving contact with water or soil. In each of these situations wood is prone to severe or low deterioration threats. For this reason, selection of appropriate wood preservatives with the above in mind is encouraged to guard wood against wide range of organisms and leaching resistance.

The American Wood Protection Association (AWPA) has developed standards known as use category system (UCS) to regulate and direct appropriate selection of wood preservatives based on the purpose and use of wood products. The standards simplify the mechanism of choosing right wood preservatives and their retentions for a particular purpose.

The system categorized treated wood products based on the sternness of the deterioration threat. That is based on the purpose of wood products from the lowest severity of deterioration risk to the highest severity as use category one, UC1, UC2, UC3, UC4 and UC5. However, some wood preservatives are appropriate for variety of purposes irrespective of the environmental conditions but might not be proper for purposes of constant human usage. Other factors considered for the standardization also included; surface dryness, adhesive bonding, possible odor, ease of finished purpose and cost. The American Wood Protection Association (AWPA) have also prepared use category system standards for CCA-treated products end use (Table 1)

Table 1. Summary of use category system (Lebow, 2010).

Use Category (UC)	Purpose Settings	Usage Environment	Common Agents of Destruction	General Application
UC 1	Above ground indoor construction (Dry)	Constantly guarded from sources of moisture and weather	Insects only	Indoor application and furnishings
UC 2	Above ground indoor construction (Damp)	Guarded from weather and other sources of moisture	Decay fungi and insects	Indoor application
UC 3A	Outdoor construction above ground (Coated and rapid water runoff)	Prone to all weather cycles	Decay fungi and insects	Coated millwork, siding and trim
UC 3B	Fresh water and close to ground	Prone to all weather cycles	Decay fungi and insects	Fence, deck, guardrail posts and utility poles
UC 4A	Fresh water and close to ground	Prone to all weather cycles	Decay fungi and insects	Fence, deck, guardrail posts and utility poles (low decay areas)

Continuation of Table 1

UC 4B	Fresh water and close to ground Critical components or difficult replacement	Prone to all weather cycles high decay potential	Decay fungi and insects	Permanent wood foundations, building poles, horticultural posts and utility poles (high decay areas)
UC 4C	Fresh water and close to ground Critical structural components	Prone to all weather cycles harsh environments with extreme decay potential	Decay fungi and insects	Land and freshwater piling, foundation piling and utility poles (severe decay areas)
UC 5A	Salt or brackish water	Constantly prone to marine water	Salt-water organisms	Piling, bulkheads, bracing
UC 5B	Salt or brackish water	Constantly prone to marine water	Salt-water organisms Such as Creosote-tolerant, *L. tripunctata*	Piling, bulkheads, bracing
UC 5C	Salt or brackish water	Continuous marine exposure (salt water)	Salt-water organisms	Piling, bulkheads, bracing

2.3.4. Application Method of Wood Preservatives

Before modern-day means of wood preservative application to guard wood for its durability there was a way out through a trial and error means of rubbing and brushing wood preservatives in the form of solution or oil on surfaces wood. It is through this means most effective preservatives and application processes were slowly developed. The demand increases of wood products including railroad ties and utility poles during industrial revolution era also helped fuel the invention of current application processes of wood preservatives. The goal of modern-day wood preservation processes, as compared to the early years is to warrant a down reaching, even infiltration with judicious cost without causing any threat to the environment. The methods of administering wood preservatives into wood have been divided into two main processes which include;

I. Pressure processes

II. Non-pressure processes

The main differences between the above methods of wood preservative application is the amount of force exerted during the process of application. Also, one is done in an enclosed medium while the other is done by following a laydown procedure and varies wide in the equipment used for the process.

Pressure process of wood preservative application to wood involves the process of plunging wood into an apparatus containing a preservative in high-pressure. More pressure is applied to force the preservative into the wood. This is done by placing wood on a tram and passing it through a long steel cylinder, which is then shut and filled with preservative as shown in Figure 6. The preservative is forced into the wood up till the preferred amount is osmosed by the wood with relatively deep penetration. Based on retention and the level of penetration of preservative, there are three main pressure processes used for applying preservative to wood;

- Empty Cell pressure process
- Full Cell pressure process
- Modified Full Cell pressure process

All the above mechanisms of pressure process of application of preservative to wood vary in detail but have the same general principle.

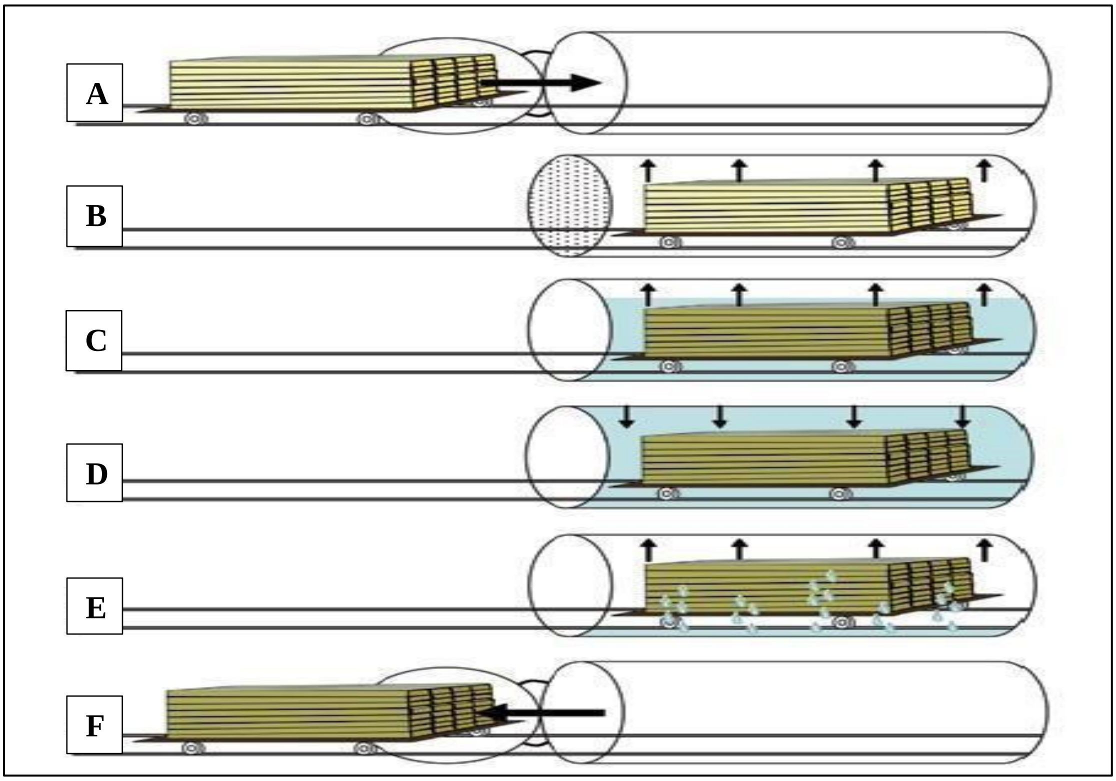

Figure 6. General procedures in pressure treating process (Lebow, 2010).

A. Untreated wood installed in a steel cylinder

B. Vacuum is applied to get rid of air out of the wood

C. The wood is plunged in a solution of a wood preservative with continuous vacuum

D. Pressure is applied to force the wood preservative into the wood

E. Wood preservative is pumped out and a final vacuum is drawn to remove excess wood preservative

F. Residual wood preservative is blown up and the wood is carried off the steel cylinder

Non-pressure process of wood treatment involves different processes in treating wood against damage. Unlike the pressure processes of wood treatment these processes vary extensively in retaining and infiltration levels of the wood preservative and subsequently the extent to which they can shield preserved wood. According to Lebow (2010), Non-pressure methods of wood treatment may include the following;

- ❖ Diffusion processes with waterborne preservatives
- ❖ Vacuum treatment and a variety of miscellaneous processes
- ❖ Surface application of preservatives by brief dipping.
- ❖ Soaking in preservative oils or steeping in solutions of waterborne preservatives

2.3.5. Wood Preservative Effectiveness

The effectiveness of a wood preservative is not only shown in the durability of a preserved wood but can be judged by virtue of the mode of treatment together with the magnitude at which the wood preservative can be retained and infiltrated into the treated wood. Furthermore, durability cannot be guaranteed of an effective wood preservative with below standard retention levels and low infiltration. However, wood treatment is induced by certain characteristics of wood such as moisture content of wood, permeability of heartwood, type of wood and quantity of heartwood and sapwood (Lebow, 2010).

The quantity of wood preservative retained in a wood after wood have been treated is known as its process retention level. Retention is calculated as kilograms of preservative per cubic meter (kg/m^3) or pounds per cubic foot (Ib/ft^3) of lumber. The retention level of a wood after preservation or the amount of preservative chemical used to treat a wood depends on the specific purpose of the wood product. Low retention value of 0.25 lb/ft^3 can be appropriate for above ground applications of wood such as timbers, plywood and lumber whereas heavy duty applications like columns, pilings and structural poles might require higher retention values between 0.8 and 2.5 lb/ft^3.

Furthermore, retention level of wood is influence by various characteristic of wood. Factors like wood species and permeability and quantity of sapwood is crucial for appropriate retention level of a wood. Wood species with high amount of sapwood is highly preferred in the wood treatment industry due to its permeability trait. Below is a table of various CCA treated wood applications and their retention requirement values as described by A.W.P.A. (1996).

Table 2. CCA-treated wood applications and their retention values

Application of Wood	Wood Retention Value (Kg/m^3)
Above ground usage: lumber, timbers, and plywood	4.00
Ground/Freshwater usage: lumber, timbers, and plywood	6.41
Salt-water splash/heavy duty usage: timbers, plywood and structural poles	9.61
Heavy duty/Freshwater usage: pilings and columns	12.81
Salt-water immersion: pilings and columns	40.05

2.4. Chromated Copper Arsenate

Chromated copper arsenate (CCA) is a globally prevalent wood preservative discovered in 1930 somewhere in India. CCA has been the first choice of chemical wood preservative in the wood treatment industry since its inception. It has also been the most popular wood preservative among outdoor products for their durability. It continues to be the main variety of chemical wood preservative utilized in various countries for different purposes (Hata *et al.* 2006). CCA is a kind of wood preserving chemical which consist of salts or oxides of arsenic, copper and chromium. The chromium component is used to link copper and chromium elements to cellular components of the wood while copper and arsenic aid as protective lethal component that deters organisms that might attack wood (Chirenje *et al.* 2003).

CCA has been extensively utilized in North America for many years to safeguard wood products from rotting and destruction caused by wood feeding organisms (Leduc, 2008). CCA chemical wood preservative has protected varieties of treated wood products used as exterior commercial and residential products. Notable among them may include; landscape timbers playground structures, railway tracks, utility poles and picnic structures (Stillwell *et al.* 2003). These products for many years have been protective against fungi, insects and other wood destroying organisms from damage. CCA treated wood products can be used for 20-30 years in an open-air condition. However, they will still contain a significant amount of chemical preservatives after they become obsolete of their purpose. Until 2004 when the use of CCA chemical wood preservative was declined by law, CCA treated wood treated also known as green treated wood in the United States monopolized the treated wood industry (Lebow, 2010).

Due to the concerns for the intensifying rate of CCA aftereffects in the environment, there was restraint on the use of CCA chemical wood preservative in many countries. The U.S EPA for instance has restricted the utilization of creosote, CCA and Penta only to the production of pilings, utility poles and the likes (ATSDR, 2001) to decrease direct public link with treated wood structures. Thus, it is prohibited to produce CCA treated lumber meant for residential structures. However, existing structures are not affected by the restriction. In replacing alternative preservatives for non-industrial application, the American Wood Protection Association came out with a list of allowable CCA uses which is based on specific commodity and the imperative ones (A.W.P.A., 2001). The most of these allowable uses are based on the standards for poles, piles and wood used in highway construction.

The primary advantages in the use of CCA-treated wood are that it produces no odor or vapor and its surface can be easily painted. At low retention values it does not change the general appearance of the wood, maintaining the aesthetic quality of natural wood. The wood is suitable for indoor purposes and is used for interior parts of a wood structure in contact with the floor. Drawbacks of the wood are a strong green color at high retention values. It should not be used in applications where it is in contact with food or drinking water. CCA is used to treat primarily lumber, timbers, posts, and plywood. Its use in treating other products, such as poles and pilings, has seen relative increases as well.

2.4.1. Types of Chromated Copper Arsenate (CCA)

CCA can be grouped into three types (A, B and C) base on the relative proportion of metals in the mixture of chemicals as showed in Table 3. Although several formulations of CCA have been used in the past, CCA type C has been the primary formulation and is currently the only formulation listed in the AWPA standards. CCA-C was found to have the optimum combination of efficacy and resistance to leaching though the earlier formulations (CCA-A and CCA-B) have also provided long term protection for treated stakes. CCA-C has an active composition of 47.5% chromium trioxide, 34.0% arsenic pentoxide and 18.5% copper oxide (Lebow, 2010). The amount of CCA chemical used to treat wood depends upon the intended application of the treated wood product. In the U.S for instance, wood use for above ground applications is treated using a minimum of 4kg of chemical per cubic meter (m^3) of wood product. Utility poles are treated at 6.4kg/m^3 and wood used for pilings within marine environments is treated at 40kg/m^3. The CCA chemical typically imparts a green color to the wood. At low retention levels the color is a very faint green whereas at high retention levels the color is a strong olive green (Solo Gabriele *et al.* 2003).

Table 3. CCA types and their chemical compositions (A.W.P.A., 1996).

Chemical composition of CCA	CCA-Type A	CCA-Type B	CCA-Type C
Chromium as CrO_3	65.5%	35.3%	47.5%
Copper as CuO	18.1%	19.6%	18.5%
Arsenic as As_2O_5	16.4%	45.1%	34.0%

2.4.2. The Science behind Chromated Copper Arsenate-Treated Wood

The chemistry behind CCA and its use in wood preservation is designed to keep the preservative in the wood so the product can perform as intended. Overtime, however, very small amounts of the preservative chemicals can be removed by contact with the surface of the wood. The chemical of concern in relation to chromated copper arsenate is arsenic. It is important to be noted that arsenic is a naturally occurring element which is exposed to human and other creatures every day in food, water, air and soil around us. There is an extensive amount of scientific literature related to arsenic in relation to CCA-treated wood, its toxicity, epidemiological studies and exposure assessments (W.P.S.C., 2009).

Arsenic compounds have been used by humans for several thousand years (O'Neill, 1995). Historically, arsenic was used for medicinal purposes in the treatment of ailments such as trypanosomiasis, amoebic dysentery, and syphilis (Ascue and Nriagu, 1994; Eisler, 1994). Arsenic compounds are currently used as pesticides, wood preservatives and in glass and electronics manufacture (Ascue and Nriagu, 1994). Sodium arsenate (Na_2HASO_4) is used to debark trees, in cattle and sheep dips and in the control of aquatic weeds (Ascue and Nriagu, 1994). The element is found in a large variety of samples such as sea and freshwater, sediments, soils, marine organisms, and plants (Burguera and Burguera, 1997).

However, concerns are mounting due to industrial use processes that release arsenic into the environment and, potentially, into human food chain (Langdon *et al.* 2003). Transition metals and arsenic among others are included in the group of elements known as "trace elements" which, together, constitute less than 1% of the Earth crust (Alloway and Aryers, 1994). At some concentration of that element its toxicity, the route of uptake and its bioavailability (Alloway and Ayers, 1994) soils exposed to industrial effluents, areas next to smelters and mine spoils have the greatest accumulation of arsenic and plants and soil have been shown to accumulate arsenic (Porter and Peterson, 1975; Mehang *et al.* 1994).

2.4.3. Uses of Chromated Copper Arsenate-Treated Wood

CCA-treated wood is used in marine facilities (piling and structures), utility poles and cross arms, piling for terrestrial and freshwater uses, commercial and agricultural construction (primary foundation) and highway structures such as bridge components, guardrails, and posts. CCA has a well-proven history of giving consistent long life to preserved wood products, both through over 50 years of laboratory and field testing as well as successful long-term use of products in challenging environments. Compared to non-wood products, benefits of treated wood products include; lower density, ease of field modification, structural flexibility and durability, aesthetics appeal, and that wood is a renewable resource.

CCA preservative adds benefits to wood including proven efficacy, long term product life and low cost. In addition, the treated product is clean, dry, non-slippery and paintable, low in odor and has a pleasing appearance. Over seventy years of safe use and the body of sound scientific and medical evidence proved that CCA pressure treated wood is safe when used as recommended. The service life of wood preserved with CCA is typically 20-30years which is 4-6 times longer than untreated wood. The extended service life of CCA-treated wood helps to reduce the harvesting of trees and saves this precious natural resource (W.P.S.C., 2009).

2.4.4. Chromated Copper Arsenate-Treated Wood and Arsenic

Throughout the world, arsenic is creating potentially serious environmental problems for human and other living organisms. Most reported arsenic problems in water supply systems have been found in groundwater, usually the drinking water source in rural areas, which have been caused by human activities such as mining, petroleum refining, sewage sludge, agricultural chemicals, ceramic manufacturing and coal fly ash (Viraraghavan *et al.* 1999). Natural causes include mineral weathering and dissolution caused by the changes of geochemical environments to reductive conditions (Namasivayam and Senthilkumar, 1998; Chris *et al.* 2000).

In all the above situations, very diminutive amounts of arsenic can be dislodged from the surface of treated wood through contact. Arsenic in those minute amounts is in a complex form and not readily available in terms of human exposure. All studies evaluating potential exposure to arsenic from contact with CCA treated wood confirmed that the levels are far below and is not distinguishable from background levels of arsenic content in normal diet and drinking water (A.T.S.D.R., 2007). With the viewpoint of dermal absorption, recent research published in the scientific literature confirmed that the arsenic in wood residues is poorly absorbed and does not result in detectable levels (Westel *et al.* 2004). The potential exposures to children using playing ground structures made from CCA treated wood and those who played on play sets made from other materials was studied and the conclusion was equally the same (Kwon *et al.* 2004).

In perspective approach, several recent publications such as Tsuji *et al.* (2007), and Georgopoulos *et al.* (2007) confirm that the very small potential exposure from contact with CCA treated wood is far below and indistinguishable from the background levels of arsenic content in diet and drinking water. The bioavailability of arsenic is difficult to measure in terms of its speciation, due to its ability to exist in both anionic and cationic states (Langdon *et al.* 2003). But speciation of arsenic in earthworm tissues appears to be in inorganic forms and may differ between species. It is possible that earthworm species can sequester arsenic in their tissues in less toxic forms than arsenate especially when the accumulation is over a prolonged period time. However, exposure time and method of introduction of the toxin may influence accumulation rates. Microbial activity can also produce highly toxic arsenine gas and methyl derivatives, under strong reducing conditions. Methylation has a significant role to play in the cycling and mobilization of arsenic (Langdon *et al.* 2003).

2.4.5. Disposal of Wood Preserved with CCA

There are several wood products available as part of many landscape designs and many of them have been preserved with chromated copper arsenate (CCA) wood preservative to sustain the longevity of their purpose. CCA-treated wood products are in wide variety of application of which some may include; post, plywood, poles and timbers. The service life cycle of wood is prolonged by CCA wood preservative and this can occur for 20-30 years (W.P.S.C., 2008). After the final stage of the service life of CCA wood product it replaced and discarded. In the United States for instance, there is an annual production of 6 million CCA-treated utility poles of which only 3% of them are discarded due to end of service life (Callahan, 2015). There are 3 main methods that have been recognized for disposing CCA-treated utility poles after their service life cycle. These methods may include; recycling for other purposes, disposing at the landfill site and incineration. The method of disposing obsolete CCA-treated wood at the landfill site is the widespread method practice. However, practicing any of the three methods comes with a repercussion of lethal chemicals discharge into the environment.

Rapid global urbanization together with human population growth poses difficulties in regulation and disposal of variety of organic waste. National and international statutory and policy changes introduced during the last decade reinforce the technical and public perception challenges. For instance, the European Union Landfill Directive (council directive 1999/31/EC) requires all member states to divert biodegradable municipal waste away from direct landfill disposal and into alternative forms of treatment. Sludge for example, coming from urban sources contains not only significant quantities of nutrients but also heavy metals and metalloids. Both essentials and nonessential metals in excess can interfere with soil microbial activities and plant growth, and they may also pose direct or indirect threats to wildlife, farm animals and human health through the consumption of tainted crops. For these reasons and despite the many potential and realized benefits of disposing of biosolids on land, tight regulatory controls on application rates to land have been formally introduced or recommended in several countries to limit the exposures of the biota to metal contaminant (Morgan, 2011).

Disposal of CCA-treated wood is under the authority of state and local solid waste management authorities. Many states and local governments have specific regulations, guidelines and recommendations for the management and disposal of discarded CCA-treated wood. CCA-treated wood used for residential purposes can be disposed with regular municipal trash (municipal solid waste not yard waste) in many areas; it should not be burned or used as compost or mulch. However, because state or local laws may be stricter than federal requirements, it is recommended that one contacts the waste management agency for their area when it comes times to replace any treated wood structure (W.P.S.C., 2009).

The quantities produced, and management of discarded CCA-treated wood is currently different in each country. The Japanese industry for instance has stopped producing CCA-treated wood voluntarily because of economic reasons rather than environmental or public concerns. However, in Australia, environmental and public awareness is influencing the imminent restriction of CCA for some uses but reluctant to support restrictions on CCA-treated timbers use and sale (Hata *et al.* 2006). Disposal of CCA-treated wood waste is predicted to increase in all countries as the years go by. The current traditional methods of disposing CCA-treated wood products involve incineration which is practiced in Japan and Korea, Landfill disposal, in Australia, and recycle and reuse. But recycling and reuse appear to be hampered by the difficulty in separation and sorting of treated and untreated wood waste streams (Hata *et al.* 2006). Unfortunately, sorting has been found to be economically and practically unacceptable.

3. MATERIALS AND METHODS

3.1. Experimental Setting

The research work was conducted at the Department of Forest Industry Engineering in Karadeniz Technical University, Trabzon-Turkey. All experimental activities took place in the laboratory of Wood Protection and Mycology. The preparation towards the experiment started in October 2017 with the actual experiment beginning from December 2017 to April 2018.

3.2. Materials Used in the Experiment

The major materials used in the experiment included the following;

- Tap water
- Cow dung (CD)
- Nylon mesh
- Laboratory Test Sieve Shaker
- Thomas-Wiley Laboratory Mill
- Wood sawdust (wet and dry for both treated and untreated sawdust)
- California red worms (*Eisenia fetida*)
- White transparent plastic boxes (30×19×14.5cm) afterwards referred to as experimental boxes (EBs) in the write-up.

Small sized multiple perforations were made on the lids of the experimental boxes for air circulation and proper respiration purposes of the earthworms. A bigger size was also drilled at the center bottom of the experimental boxes. This hole was covered with a nylon mesh with the help of a plastic glue to prevent earthworms from falling out and escaping from the EBs during the experimental period. The purpose of this hole was to collect the liquid leachate that will be draining out from the substrate in the EBs.

The treated sawdust was made from redundant out-of-service CCA-treated yellow pine (*Pinus sylvesteris*) utility pole which was obtained from the yards of Aksa Electricity (Çoruh Electricity Retail Sales Company Limited) in Arsin-Trabzon. The untreated sawdust on the other hand was made from waste pieces of wood of yellow pine obtained from the workshop of the department of Forest Industry Engineering. The earthworms, *Eisenia fetida* and the cow dung was obtained from LAZUTIM Organic Trading Company Limited in Rize, Turkey. The cow dung was used in the study as an inoculant to impel the process of vermicomposting (Karthikeyan *et al.* 2007; Pramanik *et al.*, 2007; Gupta and Garg, 2009). The experimental boxes used for the experiment were obtained from the market and had dimensions of 30×19×14.5cm.

The treated and untreated sawdust together with the cow dung mixed with water served as the main substrate for the experiment and at the same time as a captivating food source to the earthworms (Sughar and Singh, 2009). The main substrate and earthworms used were kept in the experimental boxes. The water was used as the binding agent for mixing the cow dung and the sawdust.

3.3. Experimental Design and Set up

The experimental design employed in the study was complete randomized design (CRD). The design involved five treatment/substrate levels with three replications to ensure a better estimation of experimental error (Gomez and Gomez, 1984). The experimental treatment/substrate levels were as follows;

- T_0 = 100g of untreated sawdust
- T_1 = 75g + 25g of untreated and CCA-treated sawdust
- T_2 = 50g + 50g of untreated and CCA-treated sawdust
- T_3 = 25g + 75g of untreated and CCA-treated sawdust
- T_4 = 100g of CCA-treated sawdust

Each treatment/substrate was mixed with equal amount of cow dung (900g) into experimental boxes with thirty specimens of earthworms introduced in them. The layout of the experiment for the substrates with the earthworms is shown below in Table (4);

Table 4. Table 4. Experimental design and layout for substrate with earthworms

TREATMENT	REPLICATION FOR UNSOAKED SAWDUST		REPLICATION FOR SOAKED SAWDUST	
	40 MESH	60 MESH	40 MESH	60 MESH
T_0	1, 2, 3	16, 17, 18	31, 32, 33	46, 47, 48
T_1	4, 5, 6	19, 20, 21	34, 35, 36	49, 50, 51
T_2	7, 8, 9	22, 23, 24	37, 38, 39	52, 53, 54
T_3	10, 11, 12	25, 26, 27	40, 41, 42	55, 56, 57
T_4	13, 14, 15	28, 29, 30	43, 44, 45	58, 59, 60

The rows in Table (4) represented the triplications of the treatment/substrate levels for the substrates while the columns represented the various treatment levels of the substrate.

3.4. The Experimental Procedure

The procedure used in this study involved three stages of different activities. These stages included the following;

- Preparation of the Sawdust
- Preparation of the Substrate
- Introduction of Earthworm specimens into the Substrate

3.4.1. Preparation of the Sawdust

This stage of the study started somewhere in mid-October 2017. A redundant out of service yellow pine (*Pinus sylvestris*) electric utility pole was obtained from the storage head office of Aksa Electricity (Çoruh Electricity Retail Sales Company Limited) located in Arsin, a suburb of Trabzon in the north-eastern part of Black Sea region in Turkey. The utility pole was known to have been preserved with type-C of CCA wood preservative (CCA-C) which is the most common formulation of CCA preservative in the United States of America. The preservative used constituted 18.5% of CuO, 34% of As_2O_5 and 47.5% of CrO_3 (A.W.P.A., 2001). The utility pole was also known to have been treated with a retention of $10kg/m^3$ in full-cell process of wood treatment.

The long utility pole obtained was cross-cut with a chainsaw machine into pieces of one meter long for easy transportation from the yard of Aksa Electricity to the experimental site on the university campus. Pieces of untreated waste wood were also obtained from the workshop of forest industry engineering department. The wood species used here was also from yellow pine tree species (*Pinus sylvestris*).

The outer layer (all layers before the heartwood) of the CCA-treated utility pole and untreated pieces of wood obtained were made into wood shavings at the workshop. After which the shavings were grinded with the aid of Thomas-Wiley's Laboratory Mill (model 4) into sawdust. Laboratory Test Sieve Shaker was then used to sieve the sawdust over 40 mesh (425 microns) and 60 mesh (250 microns) into two distinct particle sizes. The remains of the sawdust on top of both meshes were collected after sieving and used for the experiment.

Figure 7. Out-of-service CCA-treated utility poles used in the experiment (Photo taken by Mohammed, 2018)

Figure 8. Untreated (A) and CCA-treated (B) yellow pine (Pinus sylvestris) wood (Photo taken by Mohammed, 2018)

Figure 9. Thomas-Wiley's Laboratory Mill (A) and Laboratory Test Sieve Shaker (B) (Photo taken by Mohammed, 2018)

3.4.2 Preparation of the Substrate

The substrate used in the experiment served as the principal treatment and the source of food to the earthworms. The substrate was prepared in experimental boxes with dimensions 30×19×14.5cm. The substrate was made up of two main components (sawdust and cow dung) with three different combinations as follows;

- Cow Dung (CD) + Untreated Sawdust (US)
- Cow Dung (CD) + Untreated Sawdust (US) + CCA-Treated Sawdust (TS)
- Cow Dung (CD) + CCA-Treated Sawdust (TS)

Also, five different ratios of these combinations were prepared with three replications as the various substrate levels (Table 6).

Table 5. Substrate combination of CD, US and TS in grams

	SUBSTRATE COMBINATIONS	
COW DUNG(CD)	SAWDUST (40 MESH & 60 MESH)	
	UNTREATED SAWDUST (US)	CCA-TREATED SAWDUST (TS)
900g	100g	-
900g	75g	25g
900g	50g	50g
900g	25g	75g
900g	-	100g

The first combination represented the control of the experiment and the other combinations represented the various levels of the substrates. In all a total of 60 EBs of substrate were prepared. With each experimental box containing a total weight of 1000g of substrate. Also, substrates prepared were of soaked and unsoaked sawdust. Sawdust were soaked with respect to the various treatment levels before the substrates were prepared. This was done by measuring, mixing and soaking sawdust with water for two weeks in cylindrical glass containers.

The following procedures were followed in the preparation of substrates into each experimental box;

- 900g of cow dung was measured into an experimental box.
- The soaked sawdust was then added to the CD in the experimental box. The mixture was thoroughly stirred with the hand to achieve a homogeneous mixture.
- Water was then added to the mixture and once again stirred thoroughly for homogeneity and to achieve a moisture content conducive for the survival of the earthworms.
- The experimental boxes containing the substrate were place on a wooden structure with a plastic bottle underneath it to collect the draining leachate from the substrate.

The above procedure was repeated for substrates prepared with unsoaked sawdust. However, in all these cases, substrates were prepared separately for sawdust with particle sizes of 40 and 60 mesh. In all a total of 60 experimental boxes of substrate were prepared thus 30 boxes each for sawdust with particle sizes of 40 and 60 meshes. And within each of the particle sizes 15 boxes of the 30 boxes of substrate was made of soaked sawdust and the other 15 boxes made of unsoaked sawdust.

Figure 10. Soaked (B) and unsoaked (A) sawdust used in substrate preparation (Photo taken by Mohammed, 2018)

3.4.3. Introduction of Earthworm specimens into the Substrate

The California red worm (*Eisenia fetida*) was the earthworm species used for the experiment. Earthworms were kept in a plastic bucket after they were obtained from a supplier (LAZUTIM Organic Trading Company Limited). The bucket contained naturally fermented cow dung. This was kept overnight under 20^0C room temperature with a constant source of light at the laboratory. The earthworms were introduced into the substrate the next day after the substrate had been prepared. Thirty (30) specimens of preclitellated (sexually immature) earthworms of the same age and in good health conditions were randomly selected from the plastic bucket for introduction into the substrates.

These earthworms were thoroughly rinsed under running tap water and weighed on electronic balance for their initial collective weights before they were introduced into the EBs. The earthworms were added to the substrate by separating the substrate in the EBs into two parts and then earthworms were gently placed at the bottom of the EBs and covered with the substrate afterwards. An initial total of 1800 earthworms were used in the experiment. The total initial collective weights of these earthworms were 399.83g with an average of 6.66g.

Figure 11. Experimental boxes containing substrates and earthworms (Eisenia fetida)

(Photo taken by Mohammed, 2018)

3.5. Maintenance Activities duration the Exposure

The experimental substrate served as a source of feed to the earthworms during the period of exposure. However, there was no point in time during the experiment where any constituent of the substrate was added to the substrate in the EBs when it was seen exhausted by the earthworms. Meanwhile the temperature of the substrates was stabilized between 18°C and 20^0C for 90 days. The moisture content of the maximum water holding capacity of all the substrates were kept at an equivalent percentage of 60% for the entire duration of the experiment. Water in a plastic bottle was sprinkled on substrates in the EBs when substrates were observed dried. During the exposure, experimental boxes were opened early in the morning and closed in the evening for proper circulation of air. This was done on a daily base. There was also a constant source of light in the laboratory for both day and night throughout the experimental period.

3.6. Data Collection

Data from the experiment were scheduled and taken after six and twelve weeks of the experiment. After six weeks of experimentation, the first batch of data were taken on the following parameters;

- ❖ The number of earthworms that survived (including newly hatched ones) was taken after substrate exposure to earthworms. This was done by emptying substrate from the EBs into a broad plastic bowl, survived earthworms were picked by hand sorting, rinsed and counted.
- ❖ The collective weights of earthworms that survived were also taken after exposure to substrate. This was also determined by collectively weighing the earthworms in a petri dish on a scale balance.
- ❖ Data on mortality of earthworms were also taken as it is the predominant target used (included in OECD guideline) to assess and determine heavy metal toxicity in earthworm tissues (Gestel and Dis, 1988; Peijnenburg and Vijver, 2009). Data on mortality was extrapolated from the total number of surviving earthworms and newly hatched ones. Nonetheless, a continuous record keeping of observable dead earthworms in various EBs was also employed. For the

purpose of ICP-MS analysis of heavy metals, samples of substrate from various EBs were taken and stored.

The above process for data taking was repeated exactly for the same data after twelve weeks of exposure. However, earthworm samples were also taken after twelve weeks of exposure for ICP-MS analysis of heavy metal bioaccumulation.

3.7. Sample Preparation for ICP-MS Analysis

Three main sample data were taken for the purpose of ICP-MS heavy metals analysis of arsenic, chromium and copper. These samples included;

- *Eisenia fetida* earthworm samples
- Sawdust samples (treated and untreated)
- Substrate samples (mixture of cow dung and both CCA-treated and untreated sawdust)

The above solid samples taken were transformed into a liquid state by two different processes for easy ICP-MS heavy metals analysis. These processes included the following;

- Drying process of samples
- Acid digestion process of solid samples

3.7.1. Drying Process of Samples

During this process earthworms, substrate and sawdust samples were all oven dried separately to get rid of moisture content. For earthworm samples, a few numbers of them were taken from each experimental box into different petri dishes following a study procedure of Arnold and Hodson (2007), they were thoroughly rinsed to get rid of the substrate and other materials and they were left to empty the contents in their guts. This took place between 1 and 3 hours. Afterwards a subsequent rinsing of the earthworms was done and earthworms were oven dried with a heat temperature of 108^{0}C for 24 hours. The total dried weight of earthworms was recorded and the dried earthworms were stored for the next process. The substrates taken and stored after both six and twelve weeks were also oven dried at the same temperature and time as the earthworms. The purpose was to achieve a total dry solid sample of substrate. After which the total dry weight of each level of substrates was taken and stored. Samples of both CCA-treated and untreated sawdust were also taken, and oven dried at 108^0C for 24 hours for their total dryness. Thereafter the total dry weight was figured out and stored.

Figure 12. Oven dry of *Eisenia fetida* samples (Photo taken by Mohammed, 2018)

3.7.2. Acid Digestion Process of Solid Samples

Acid digestion process involves dissolving solid samples to primarily change metals into a soluble form for analysis on Plasma Emission Spectrophotometer or Atomic Absorption Spectrophotometer (Edgell, 1988). This is done by the addition of acids to samples and heated until complete decomposition of the solid samples is achieved. Acid digestion is said to be a very good method where decomposition of solid samples for release of analyte or extracting trace elements and cation are needed for analysis.

In this study the standard operating procedures of the United States Environmental Protection Agency (U.S.E.P.A, 1996) was adopted for acid digestion of the solid samples obtained from the experiment;

- Reagents used in the process included nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). These chemicals were in quantities of 65% and 35% respectively.
- A gram of oven dried substrate (mixture of cow dung and both CCA-treated and untreated sawdust) sample was measured into a beaker.

- ❖ The above was repeated for dried sawdust samples but for earthworm samples 0.25g was weighed because of the quantity of earthworm samples available after the exposure.
- ❖ 15ml of 65% of nitric acid (HNO_3) was added to substrate sample in the beaker.
- ❖ The above was also repeated for sawdust samples but for earthworm samples, 3.75ml of 65% of nitric acid (HNO_3) was used.
- ❖ The sample solution in the beaker was heated on a hot plate until the entire content in the beaker was soluble. The substrate and sawdust samples took an hour and 30 minutes to be soluble but earthworm samples became soluble very fast due to their fragile nature.
- ❖ The burning sample solution on the hot plate was refluxed with some drops of 35% of hydrogen peroxide (H_2O_2) with the help of a disposable transfer pipette.
- ❖ The reflexed sample solution was made to cool for 30 minutes.
- ❖ The cooled sample solution was then filtered through 0.45µm millex syringe filter into test tubes for analysis.

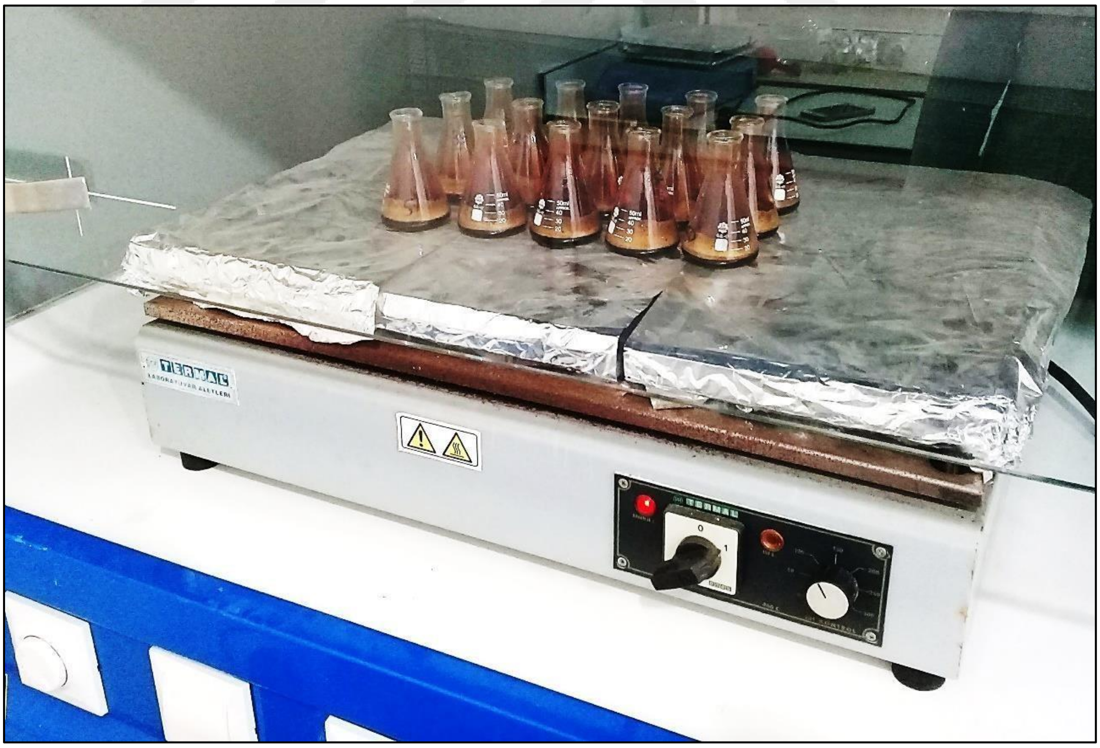

Figure 13. Heating of sample solution on hot plate (Photo by Mohammed, 2018)

Figure 14. Filtered sample solutions ready for ICP-MS analysis of heavy metal (Photo taken by Mohammed, 2018)

3.8. Calculations and Statistical Analysis

Heavy metal accrual by a species in its body tissue relative to the amount of these metals in its environment is referred to as bioaccumulation factor (BAF). In this study the BAF of CCA was evaluated at the end of the study as the concentration of the heavy metals in the tissues of earthworms *Eisenia fetida* in relation to the concentration in substrates after 12 weeks of exposure. The following formulae was used for the BAF of CCA as used by Mountouris et al, (2002) and Pattnaik and Reddy, (2011).

$$\mathbf{BAF_{Heavy\ Metal} = C_{earthworms} / C_{substrate}}$$

Where;

$BAF_{Heavy\ Metal}$ is the bioaccumulation for heavy metals

$C_{earthworm}$ is heavy metal concentration in earthworms (ppm)

$C_{substrate}$ is heavy metal concentration in substrate (ppm)

All three replications of the measurements taken were presented as a single mean value. The data collected were examined using SPSS computer software program. Analysis of variance (ANOVA) was run for all data taken and a post hoc test for homogeneity was done using Duncan test at significant level of $p \leq 0.05$. This was done to determine the significant effect of treatment levels of substrate, particle sizes of both soaked and dry sawdust used for the substrate and the time of exposure with the interest to establish their influence on earthworms' population growth and weight development with respect to heavy metal (CCA) removal from the principal substrate by California red earthworm *Eisenia fetida.*

4. RESULTS AND DISCUSSIONS

4.1. Effects of Treatment Levels on the Total Number of Earthworms

Analysis of results on growth parameters showed a progressive rate of development within the initial weeks of the experiment. The increase in earthworm's population in the form of quantity of worms and cocoons showed a substantial sequence although some insignificant number of earthworms was observed dead. Progressive increase in quantity of earthworms was observed at substrate levels T_0 (900g of cow dung + 100g of untreated sawdust) and T_1 (900g of cow dung + 75g of untreated sawdust + 25g of CCA-treated sawdust) until the 12th week of the experiment. But substrate levels T_2 (900g of cow dung + 50g of untreated sawdust + 50g of CCA-treated sawdust), T_3 (900g of cow dung + 25g of untreated sawdust + 75g of CCA-treated sawdust) and T_4 (900g of cow dung + 100g of CCA-treated sawdust) saw a continuous decline in earthworm's population after the 6th week through to the 12th week of the experiment. The substrate level T_4 which contained 100g of CCA-treated wood sawdust witnessed the highest rate of earthworms' mortality. However, there were few numbers that endured throughout the experimental period. These surviving earthworms were used in bioaccumulation analysis of As, Cu and Cr. The above findings contrasted with a study where earthworms *Eisenia fetida* were completely found dead after being fed and exposed to a substrate mixture of thermal power plant fly ash and cow dung for 30 days in a vermistabilization experiment. This according to the study was as a result of the cementing properties and perniciousness of the thermal power plant fly ash (Adarsh Pal *et al.,* 2016) which is not so as compared to CCA-treated wood sawdust used in the present study (figure 15).

Furthermore, earthworms found at substrate levels T_0 (900g of cow dung + 100g of untreated sawdust) and T_1 (900g of cow dung + 75g of untreated sawdust + 25g of CCA-treated sawdust) showed signs of biomass increase and fully developed clitellum after two weeks of exposure. The latter was an indication of sexual maturity of earthworms. This was coupled with visible pair of earthworms stacked together in substrates which was also a sign of mating between earthworms to exchange sperms for reproduction. The production of cocoons by adult *Eisenia fetida* earthworms were observed in significant numbers in the second week of the experiment but hatchling of these cocoons was delayed. As similar incident was reported in a study where *Eisenia fetida* achieved higher growth rate as compared to delayed in cocoon development and hatchling in a soil contaminated with leachate from CCA-treated wood (Leduc *et al*., 2008). This according to the study was because *Eisenia fetida* allotted more energy to growth than reproduction.

On the other hand, earthworms found in substrate levels with high amount of CCA-treated sawdust (T_3 and T_4) also displayed all the above signs after four weeks of exposure but their biomass development was slow, and their cocoon production was observed to be scanty although the number of cocoons produced was not one of the parameters considered in this study. Several studies have proved how the type of feeding material, nature and accessibility of food to earthworm species are critical factors in their development. These factors like other important factors such as moisture content and temperature also influence earthworm's total growth development (Evans and Guild, 1948; Neuhauser *et al.*, 1979; Reinecke and Viljeon, 1990; Elvira *et al.*, 1998). Earthworms have also demonstrated a sizable population growth over time with respect to the kind of food available to them (Degefe and Tamire, 2017). The observed slow pace in attaining sexual maturity and the scantiness in earthworms cocoon production in the present study at substrate levels T_3 (900g of cow dung + 75g of untreated sawdust + 25g of treated sawdust) and T_4 (900g of cow dung + 100g of CCA-treated sawdust) could be due to the high amount of CCA-treated sawdust in those substrate levels. And this can be related to what was alluded by Venter and Reinecke (1988) that favorable nutritive status is a prerequisite for growth development as well as sexual maturity in earthworms.

The comfortable build-up of earthworm population at substrate level T_0 (900g of cow dung + 100g of untreated sawdust) as compared to the rest of the substrate levels could be directly associated to the fact that *Eisenia fetida* found substrate level T_0 to be as appealing as their natural habitat because it did not contain any amount of CCA-treated sawdust. A comparable finding was reported of *Eisenia fetida* growing well in a nutritive medium of only cow dung over 600 days without any record of mortality (Venter and Reinecke1988) even though substrate used in this study was a mixture of sawdust and cow dung.

Moreover, the time-dependent change in total earthworms' population growth revealed a direct relationship between population growth and the time of exposure. As the time of exposure increased, the population growth of earthworms also increased in substrate levels with small or no amount of CCA-treated sawdust (T_0 and T_1). However, time-dependent change in total population growth of earthworms showed that as the time (weeks) of exposure of earthworms to substrate prolongs, the number of earthworms decreased in substrate levels with high amount of CCA-treated sawdust. This indicated that substrate with high amount of CCA-treated sawdust was not conducive for growth and development of

California red worm (*Eisenia fetida)* especially when it was over a long period of time (12 weeks). Nevertheless, substantial growth rate was observed in substrate with 25g of CCA-treated sawdust but not as the maximum population accrued at the same level in a study of vermistabilization of thermal plant fly ash and cow dung (Adarsh Pal *et al.*, 2016). The above is also valid in comparison with similar study where bioaccumulation of Cu As, and Cr in tissues of *Eisenia fetida* from leachate of chromated copper arsenate wood preservative resulted in the declining of growth and reproduction (Leduc *et al*., 2008). However, Sivasankari (2016), also reported a continuous substantial rise in earthworms population in a complete cow dung inoculant with respect to time.

The substrate level T_0 (900g of cow dung + 100g of untreated sawdust) had earthworm population build-up from the first week till the last week of the experiment without any disruption. This increase was followed by substrate level T_1 (900g of cow dung + 75g of untreated sawdust + 25g of CCA-treated sawdust) but fluctuations in increasing and decreasing until the final stage of the experiment was observed for substrate levels T_2 (900g of cow dung + 50g of untreated sawdust + 50g of CCA-treated sawdust), T_3 (900g of cow dung + 25g of untreated sawdust + 75g of CCA-treated sawdust) and T_4 (900g of cow dung + 100g of CCA-treated sawdust). The maximum mean number of earthworms was observed at substrate levels as 100 worms for T_0 (900g of cow dung + 100g of untreated sawdust), 58 worms for T_1 (900g of cow dung + 75g of untreated sawdust + 25g of CCA-treated sawdust), 44 worms for T_2 (900g of cow dung + 50g of untreated sawdust + 50g of CCA-treated sawdust), 30 worms for T_3 (900g of cow dung + 25g of untreated sawdust + 75g of CCA-treated sawdust) and 36 worms for T_4 (900g of cow dung + 100g of CCA-treated sawdust) as shown below in Figure 15.

From the above findings, chromated copper arsenate (CCA) can also be regarded and considered as a growth retarding chemical because it poorly supported the population growth of earthworms *Eisenia fetida* especially at substrate levels T_3 (900g of cow dung +25g of untreated + 75g of CCA-treated sawdust) and T_4 (900g of cow dung + 100g of CCA-treated sawdust). Chromated copper arsenic at certain quantity can also be considered as a growth media that impedes reproduction and slows down growth in earthworms as reported by Jager *et al.*, (2006).

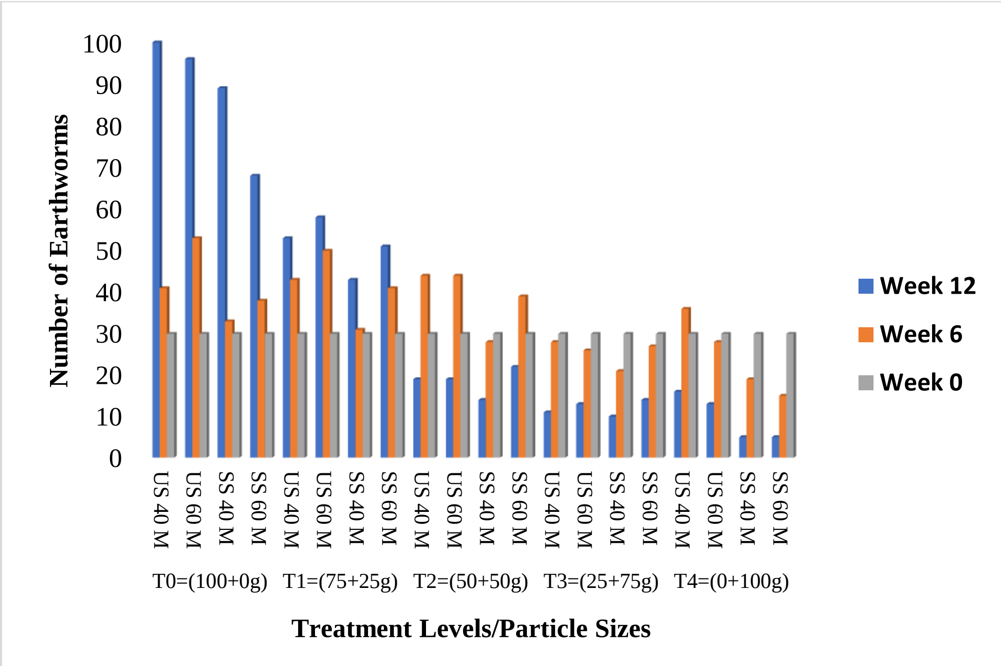

Figure 15. Total number of earthworms at different treatment levels

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh)

The statistical analysis of variance on the total number of earthworms with respect to the time of exposure and treatment levels of substrates showed highly significant difference within the various independent variables (p=0.000). The comparison test of effect between time of exposure and treatment levels on the total number of earthworms also showed highly significant difference between the independent variables (p=0.000) as shown in Table (6) . The post hoc test of homogeneity with Duncan test displayed 2 different groups for the time of exposure and 4 distinct groups for the treatment levels (Table 7).

Table 6. Analysis of variance on the total number of earthworms with respect to the time of exposure and treatment levels of substrates

Source of variance	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Sig.
Corrected Model	58158.11	14	4154.15	64.83	***
Intercept	199666.81	1	199666.81	3116.23	***
Time of Exposure	1215.81	2	607.91	9.49	***
Treatment Levels	26351.22	4	6587.81	102.82	***
Time of Exposure &Treatment Levels	30591.08	8	3823.89	59.68	***
Error	10572.08	165	64.07		
Total	268397.00	180			
Corrected Total	68730.19	179			

***means highly significant (p=0.000), **means significant ($0.004 \leq p \geq 0.018$), NS = not significant

Table 7. Results on Duncan test of homogeneity of the total number of earthworms with respect to time of exposure and treatment levels of substrates

Time of Exposure	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Initial Week	60	30.00	A
Week Twelve	60	33.57	B
Week Six	60	36.35	B
Treatment Levels			
T_4=(0+100g)	36	21.42	A
T_3=(25+75g)	36	22.56	A
T_2=(50+50g)	36	28.56	B
T_1=(75+25g)	36	40.78	C
T_0=(100+0g)	36	53.22	D

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

4.2. Effects of Treatment Levels on the Collective Weight of earthworms

The food sources of earthworms do not only influence the size of their population but also their reproduction rate and total growth development (Dominguez, 2004). Earthworms total biomass development with regards to body weight and length showed a relative consistent pattern of increase over the time of exposure. The total collective weights of earthworms at various substrate levels were almost the same at the initial stage of the experiment. The preliminary few weeks of the experiment witnessed a rapid increase where considerable variations in collective weight of earthworms were observed. The highest increase in the entire experiment occurred in the 6th week at substrate level T_1 (75g of untreated sawdust and 25g of CCA-treated sawdust) which did not differ from the one occurring at substrate level T_0 (100g of untreated sawdust). The other substrate levels also witnessed maximum increases at the same week (6^{th} week) of the experiment. However, the 12th week of the experiment experienced a sudden decrease in earthworms collective weight along all substrate levels with the substantial ones occurring at substrate levels with high amount of CC-treated sawdust in the order of T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) > T_3 (75g of untreated sawdust and 25g of CCA-treated sawdust) > T_4 (100g of CCA-treated sawdust) as shown in figure (16).

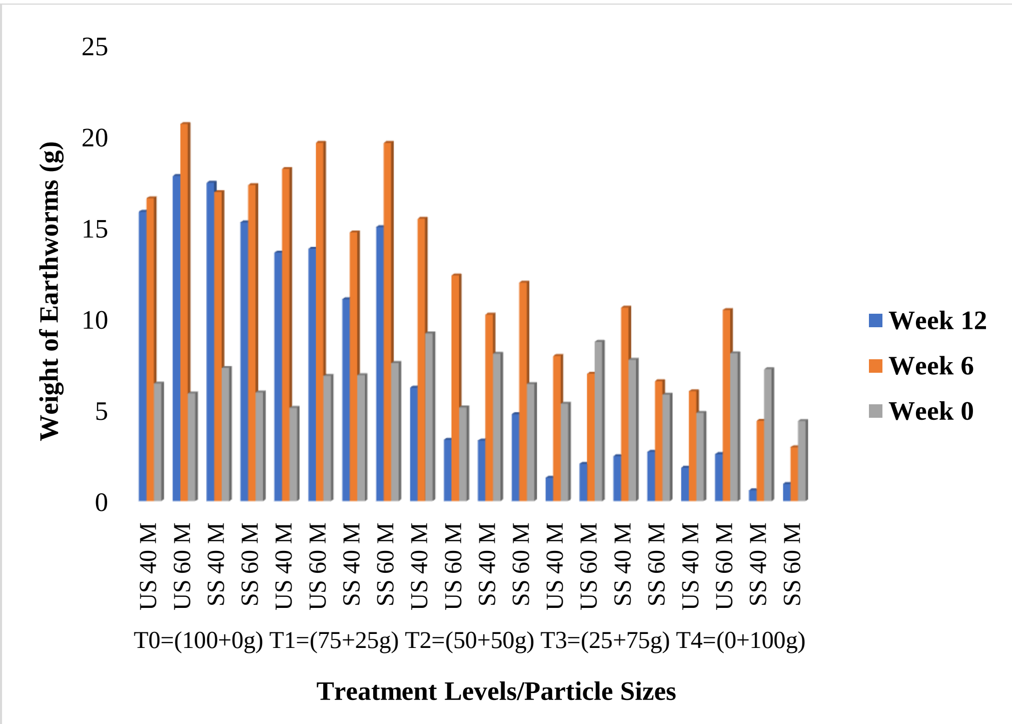

Figure 16. Collective Weight of earthworms at different treatment levels

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh)

The general drop in earthworms' biomass and weight can be as a result of the commencement of cocoon production and subsequently the increase in earthworms' population growth. The population growth in earthworms did not really reflect in their collective weight gained. This could also be owing to the fact that larger quantities of energy are needed for copulation and cocoon production as evidenced in the 12^{th} week of the experiment where the highest mean number of earthworms population was recorded (Figure 15). According to Jesikha and Lekeshmanaswamy (2013), earthworms utilize more of their energy in reproduction activities such as mating, eggs laying and cocoon formation than other growth developmental activities which in relation to the above findings.

The progressive reduction in weight towards the later phase of the experiment could also be due to continued reproduction and aging of earthworms as this had been confirmed in studies alike (Edwards and Bohlen, 1996; Monroy *et al.,* 2007; Degefe and Tamire, 2017) that once earthworms reach their sexual maturity stage, there is a point of growth reduction and dynamic biomass in their life cycle. The results from the present study also confirmed the aging effects on weight reduction in earthworms thus the number of surviving earthworms during and after the experiment were low as the same declining weight effect was reported on *Eisenia eugeniae* by Viljeon and Reinecke (1994).

It is widely known that the biomass development of earthworms is as a result of certain characteristics of the medium serving as their feeding substrate. These characteristics could include edibility of the substrate, content of microorganisms and physico-chemical composition in the substrate (Suthar, 2007; Prasanthrajan and Kannan, 2011). There are evidences in the current study showing the performance and reactions of earthworms' growth rate in substrate made of different organic materials. The obvious one among them was the observed reduction in earthworms collective weights between weeks 6 and 12 (Figure 16). This could among others be ascribed to the amount of CCA-treated sawdust in substrate levels such as T_3 (25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4 (100g of CCA-treated sawdust) These substrate levels might have not been palatable enough for earthworms' consumption or probably because of the lethal properties of the heavy metals involved. However, a substantial collective weight increase was observed at all substrate levels from the beginning till the 6th week of the experiment.

The quality of substrate is said to influence the productive potential and biomass increment of earthworm species. The results from the present study did not demonstrate a favorable growth rate of earthworms in the substrate used as compared to what was reported in a study by Degefe and Tamire (2017). It can be clearly deduced that the constituents of the substrate used in this study did not support earthworms' growth in weight and biomass development unlike what was reported from a study where *Eisenia fetida* gained biomass throughout the period it was fed with complete cattle manure (Venter and Reinecke, 1988).

The collective weight of earthworms in the experiment was maximum at substrate level T_0 (100g of untreated sawdust) and minimum at T_4 (100g of CCA-treated sawdust). The time-dependent change in total collective weight of earthworms showed a steady increased in weight, a peak attainment in weight and then declined as time of exposure prolonged. However, the results reported by Sivasankari (2016) showed a continuous rise in biomass of adult earthworm in different time interval without stoppage. This result may have differed from that of the current study probably because only cow dung was used as food source to the earthworms as compared to a mixture of cow dung and both CCA-treated sawdust and untreated sawdust in the current study.

As mentioned above, earthworms collective weight measured prior to their introduction into the various substrate levels were similar to each other. However, the statistical analysis of variance (ANOVA) for collective weights of earthworms with regards to the independent variables time of exposure and treatment levels of substrates revealed highly significant differences among them (p=0.000). The test of effects between the two independent variables in relation to the collective weights of earthworms equally showed highly significant difference between them (p=0.000) as displayed in Table (8). The post hoc test of homogeneity using Duncan test displayed different groups among the independent variables as shown in table (9)

Table 8. Table 8. Analysis of variance on the collective weight of earthworms

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Sig.
Corrected Model	4949.98	14	353.57	83.68	***
Intercept	14316.24	1	14316.24	3388.17	***
Time of Exposure	1173.25	2	586.63	138.83	***
Treatment Levels	2401.72	4	600.43	142.10	***
Time of Exposure & Treatment Levels	1375.01	8	171.88	40.68	***
Error	697.19	165	4.22		
Total	19963.410	180			
Corrected Total	5647.17	179			

***means highly significant difference ($p = 0.000$)

Table 9. Results on Duncan test of homogeneity of the collective weight of earthworms

Time of Exposure	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Initial Week	60	6.66	A
Week Twelve	60	7.60	B
Week Six	60	12.49	C
Treatment Levels			
T_4=(0+100g)	36	4.5342	A
T_3=(25+75g)	36	5.6953	B
T_2=(50+50g)	36	8.0506	C
T_1=(75+25g)	36	12.6842	D
T_0=(100+0g)	36	13.6269	D

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

4.3. Effects of Particle Size on the Total Number and Weight of Earthworms

The current study begun with a total number of 30 specimens of earthworms in each experimental box. The population growth for this number was noticed to have increased and decreased at the same time for different particle sizes of sawdust used for the substrate. At the end of the 6th week of exposure, earthworms found in substrate with particle size 60 mesh of unsoaked sawdust had the highest (53) population growth with soaked sawdust of 60 mesh recording the lowest (15). After 12 weeks of exposure, earthworms' population growth showed a change in pattern which differed from the one observed at the end of week six. All the particle sizes of substrate witnessed substantial increment in population growth of earthworms as compared to the previous weeks. The maximum (100) population growth of earthworms was recorded in substrate with particle size 40 mesh of unsoaked sawdust. However, the minimum (5) growth was jointly recorded by both soaked sawdust of particle sizes 40 and 60 mesh (Figure 17).

The statistical analysis of variance on the total number of earthworms with respect to the time of exposure, treatment levels and particle sizes of substrates showed highly significant difference within the various independent variables ($p=0.000$). The comparison test of effect between time of exposure and treatment levels on the total number of earthworms also showed highly significant difference between the independent variables ($p=0.000$). However, the pair comparison test of effect between time of exposure and particle sizes, treatment levels and particle sizes were all significantly different ($0.04 \leq p \geq 0.018$) but comparison between all three independent variables was not significant (0.054) as shown in Table (10). The post hoc test of homogeneity with Duncan test displayed 3 different groups for time of exposure, 4 distinct groups for the treatment levels and 2 groups for particle sizes (Table 11).

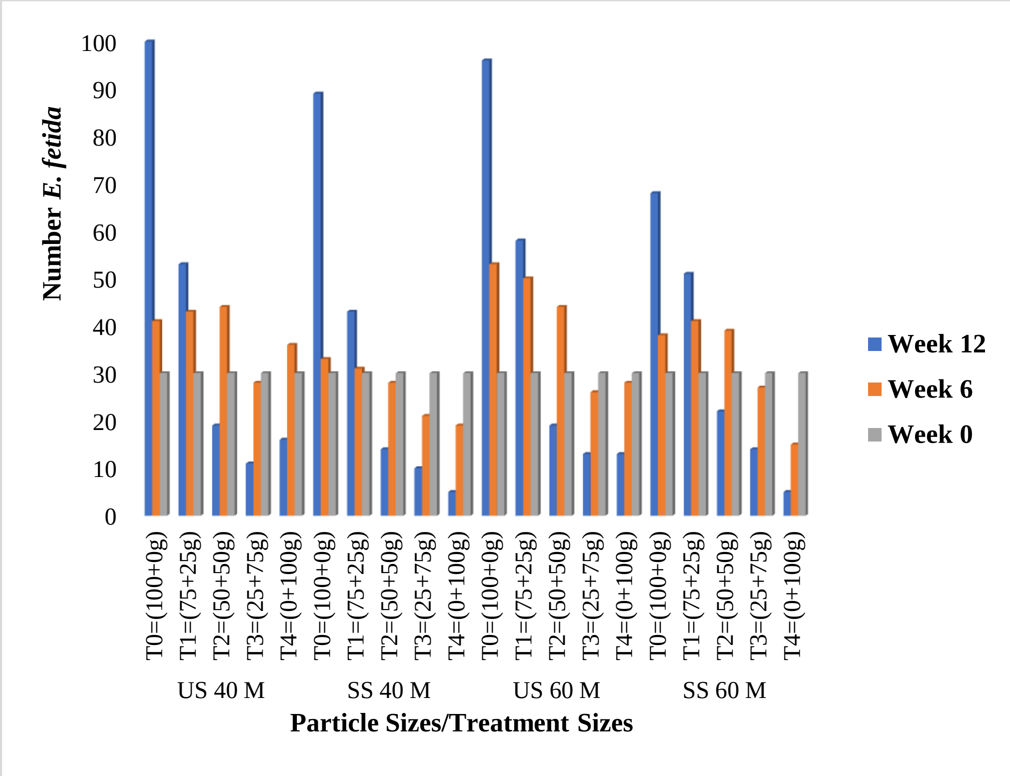

Figure 17. Total number of earthworms with respect to particle size

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA- treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh)

Table 10. Analysis of variance on the total number of earthworms

Source Variance	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Sig.
Corrected Model	63412.19	59	1074.78	24.25	***
Intercept	199666.81	1	199666.81	4505.46	***
Time of Exposure	1215.81	2	607.91	13.72	***
Treatment Levels	26351.22	4	6587.81	148.65	***
Particle Sizes	1508.06	3	502.69	11.34	***

Continuation of Table 10

Time of Exposure & Treatment Levels	30591.08	8	3823.89	86.29	***
Time of Exposure & Particle Sizes	910.19	6	151.70	3.42	**
Treatment Levels & Particle Sizes	1144.47	12	95.37	2.15	**
Time of Exposure & Treatment Levels & Particle Sizes	1691.37	24	70.47	1.59	NS
Error	5318.00	120	44.32		
Total	268397.00	180			
Corrected Total	68730.19	179			

***means highly significant difference ($p = 0.000$), **means significant difference ($0.04 \leq p \geq 0.018$), NS = not significant (0.054)

Table 11. Results of Duncan test of homogeneity of the total number of earthworms

Time of Exposure	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Initial Week	60	30.00	A
Week Twelve	60	33.57	B
Week Six	60	35.36	C
Treatment Levels			
T_4=(0+100g)	36	21.42	A
T_3=(25+75g)	36	22.56	A
T_2=(50+50g)	36	28.56	B
T_1=(75+25g)	36	40.78	C
T_0=(100+0g)	36	53.22	D
Particle Sizes			
Soaked Sawdust of 40 Mesh	45	29.62	A
Unsoaked Sawdust of 60 Mesh	45	31.33	A
Unsoaked Sawdust of 40 Mesh	45	35.96	B
Soaked Sawdust of 60 Mesh	45	36.31	B

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

Earthworms are generally known to have preferential behavior with regards to the type of food they consume as organisms. And this is due to the difference in depth of their location in the soil. *Eisenia fetida* is a typical earthworm species that is not found deep in the soil. For this reason, it is referred to as the ultra epigeic earthworm species. It only prefers foods rich in organic materials (Dominguez, 2004; Edwards and Arancon, 2004). Their preferential behavior with food could be directly connected to the particle size of the food they consume. Many studies have confirmed the important relationship between earthworms and their preference to food. Neuhauser *et al*., (1980) reported that there is a germane link between earthworms total growth rate development and the size of the food they consume thus tiny particle size of food leads higher growth rate of earthworm weight. This may also be fairly referenced to the nutritional quality of the growth media.

The outcome from the present study showed that earthworms population growth and weight development to certain extent was affected by different particle sizes of the sawdust employed in the experiment. The earthworm species used in the study showed preference to substrates with particle size 60 mesh of unsoaked sawdust in terms of weight gain than the particle sizes because of the smaller nature of it. Also, earthworms seemed to have had an appreciable growth in substrate of smaller particle size because less energy was required to decompose sawdust with particle size of 60 mesh. However, earthworms showed some level of lower rate of growth in both soaked sawdust of 40 and 60 mesh probably because the sawdust of those substrates might have reached higher moisture content from the soaking it underwent prior to the preparation of the substrates. And owning to the fact that moisture content is one of the crucial factors of the environment favorable for the survival of earthworm species in the ecosystem. This might have made it difficult for earthworms to feed on the soggy sawdust. With regards to this, literatures on the moisture content requirement of earthworms have been investigated and reported (Evans and Guild, 1948; Reinecke and Venter, 1987).

The nature of substrate and the total earthworms population density were considered by Nath *et al*., (2009) to be the major factors influencing differences in weight gained per gram of substrate in their study. In spite of the necessity for further research, peculiarity of the medium used in this study could also be said to be the major influence for the differences observed in collective weight gained by *Eisenia fetida* relative to the particle sizes of the substrate used. This can also be associated to the fact that the preferred particle size of substrate by earthworms is smaller and can be easily decomposed by earthworms. The growth rate earthworms in terms of weight gained showed three phases of growth with respect to the particle sizes of the substrate used. There was a progressive growth in collective weight gained from the first week of exposure. A maximum threshold was reached at week 6 and then towards the end of week 12 an abrupt reduction which at some level was lesser than the initial collective weight was displayed. The weight gained by *Eisenia fetida* earthworms was higher at unsoaked sawdust of 60 mesh and minimum at soaked 40 mesh (Figure 18).

The abrupt losses in collective weight of earthworms towards the end of the study could be related to the depletion of food as similarly reported by Degefe and Tamire (2017). Because there was no point in time during the study where feed in the form of substrate was added to the experimental substrate. And this could be that earthworms' food in the form of the experimental substrate had run out before the last week of the experiment. This was also in conformity with what was reported by Neuhauser *et al*., (1980), *Eisenia fetida* loses its weight when it's nourished below its maintenance level.

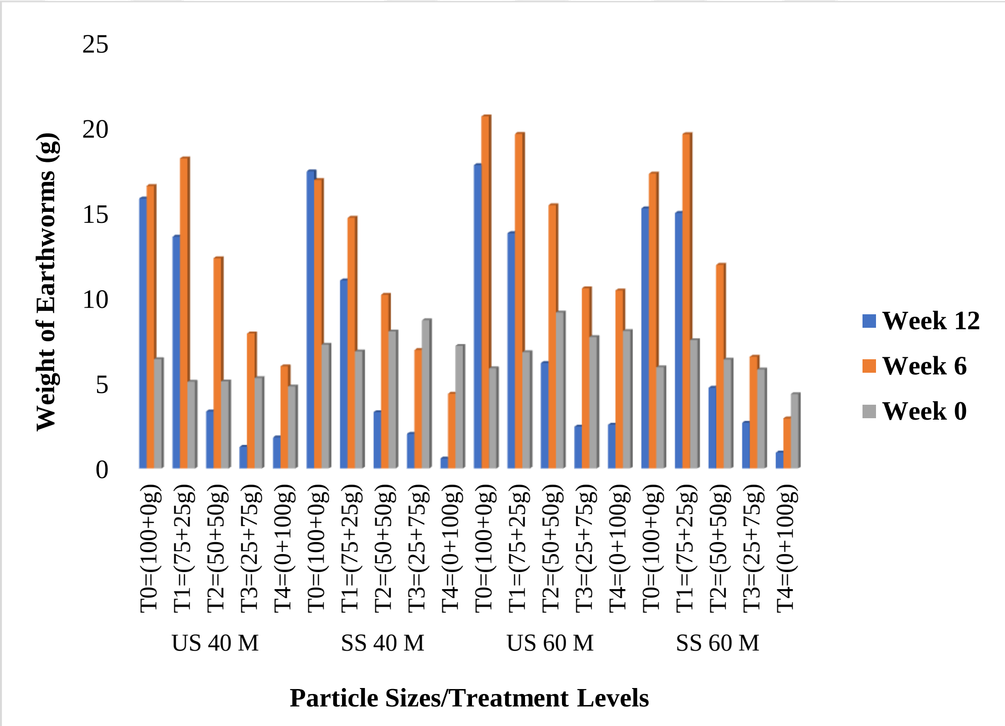

Figure 18. Weight of Eisenia fetida with respect to particle size

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of treated sawdust), T_2=(50g of untreated sawdust and 50g of treated sawdust), T_3=(25g of untreated sawdust and 75g of treated sawdust) and T_4=(100g of treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh).

The statistical analysis of variance on the collective weight of earthworms with respect to the time of exposure, treatment levels and particle sizes of substrates showed highly significant difference within all the independent variables (p=0.000). The comparison test of effect between all three independent variables (time of exposure, treatment levels and particle sizes) on the collective weight of earthworms also showed highly significant difference between them (p=0.000). However, the pair comparison test of effect between all three independent variables was not significant (0.790) as shown in Table (12). The post hoc test of homogeneity with Duncan test revealed 3 different groups for time of exposure, 5 distinct groups for the treatment levels and 2 groups for particle sizes (Table 13).

Table 12. Analysis of variance on the collective weight of earthworms

Source Variance	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Sig.
Corrected Model	5358.86	59	90.83	37.805	***
Intercept	14316.24	1	14316.24	5958.751	***
Time of Exposure	1173.25	2	586.63	244.167	***
Treatment Level	2401.72	4	600.43	249.913	***
Particle Size	152.71	3	50.90	21.187	***
Time of Exposure & Treatment Level	1375.01	8	171.88	71.539	***
Time of Exposure & Particle Size	114.89	6	19.15	7.970	***
Treatment Level & Particle Size	98.02	12	8.17	3.400	***
Time of Exposure & Treatment Level & Particle Size	43.25	24	1.80	.750	NS
Error	288.31	120	2.40		
Total	19963.41	180			
Corrected Total	5647.17	179			

***means highly significant difference (p = 0.000), NS = not significant (0.790),

Table 13. Results of Duncan test of homogeneity of the collective weight of earthworms

Time of Exposure	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Initial Week	60	6.66	A
Week Twelve	60	7.60	B
Week Six	60	12.49	C
Treatment Levels			
T_4=(0+100g)	36	4.53	A
T_3=(25+75g)	36	5.70	B
T_2=(50+50g)	36	8.05	C
T_1=(75+25g)	36	12.68	D
T_0=(100+0g)	36	13.63	E
Particle Sizes			
Unsoaked Sawdust of 40 mesh	45	8.27	A
Soaked Sawdust of 40 mesh	45	840	A
Unsoaked Sawdust of 60 mesh	45	8.49	A
Soaked Sawdust of 60 mesh	45	10.50	B

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

4.4. CCA Concentrations in Substrates after Weeks of Exposure to Earthworm

Prior to ICP-MS chemical analysis of substrates and earthworms species, the background control of arsenic, copper and chromium concentrations of the sawdust used in the experiment was ascertained. Chromium was identified to have had the highest mean concentration of 2144ppm, followed by 1534ppm of Arsenic and then Copper with the least content of 1463.62ppm. All these concentrations were observed at treatment level T_4 which was a mixture of 100g of CCA-treated sawdust and cow dung. However, with respect to the particle sizes the concentration of As and Cu all occurred in soaked sawdust of 40 mesh while that of Cr was observed in unsoaked sawdust of 60 mesh. There was a general increase in arsenic, chromium and copper content for all treatment levels as the amount of CCA-treated sawdust in substrates increases.

Heavy metal concentrations of arsenic, chromium and copper in substrate showed a general reduction for all treatment levels after exposure to earthworms for a period of twelve weeks. The heavy metal content of As, Cr and Cu in substrates progressively reduced as the experiment continued from the initial stage to the end of week six. The trend continued through to the final week of the experiment. The mean decreases in As, Cr and Cu contents in substrates for all treatment levels had a significant range of reduction over the initial concentrations.

Chromium decreased significantly from the initial concentration at various treatment levels with a maximum decrease from 934 to 600ppm at treatment level T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) in the respective particle size of 40 mesh unsoaked sawdust and a minimum decrease from 641 to 623ppm at treatment levels T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) in soaked sawdust of 40 mesh. Copper decreased from 435 to 255ppm and from 423 to 421ppm as maximum and minimum respectively. The minimum decrease of copper was the lowest decrease for the entire period of exposure with respect to all the treatment levels. This was observed at treatment level T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) in soaked sawdust of 40 mesh. Arsenic concentration decreased at treatment level T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) from 719 to 507ppm as the maximum reduction and from 280 to 265ppm as the minimum decrease at treatment level T_1 (75g of untreated sawdust and 25g of CCA-treated sawdust). The maximum and minimum decrease in arsenic concentration was observed in unsoaked sawdust of 40 and 60 mesh respectively (Table 16).

The statistical analysis of variance on the CCA accumulation in substrates with respect to the treatment levels, time of exposure, category of heavy metals and particle sizes of substrates showed highly significant difference within all the independent variables (p=0.000). The comparison test of effect between all the independent variables (treatment levels, time of exposure, category of heavy metals and particle sizes) on CCA accumulation in substrates also showed highly significant difference (p=0.000) except for comparisons between some of the independent variables as shown in Table (14). The post hoc test of homogeneity with Duncan test revealed 3 distinct groups for heavy metals category and time of exposure, 4 for both treatment levels and particle sizes (Table 15).

Table 14. Analysis of variance on the CCA concentration in substrates

Source of Variance	Sum of Squares	Degree of Freedom	Mean Square	F-Value	Sig.
Corrected Model	73097652.034	143	511172.392	43.535	.000
Intercept	236046097.093	1	236046097.093	20103.380	.000
Time of Exposure	2445672.262	2	1222836.131	104.146	.000
Treatment Levels	49208689.475	3	16402896.492	1396.988	.000
Particle Sizes	2378684.599	3	792894.866	67.529	.000
Heavy Metals	10379275.051	2	5189637.526	441.987	.000
Time of Exposure & Treatment Levels	526885.305	6	87814.217	7.479	.000
Time of Exposure & Particle Sizes	1006025.643	6	167670.941	14.280	.000
Time of Exposure & Heavy Metals	170425.697	4	42606.424	3.629	.007
Treatment Levels & Particle Size	1882590.064	9	209176.674	17.815	.000
Treatment Levels & Heavy Metal	2208534.122	6	368089.020	31.349	.000
Particle Size & Heavy Metal	360845.991	6	60140.998	5.122	.000
Time of Exposure & Treatment Levels & Particle Sizes	1113754.380	18	61875.243	5.270	.000
Time of Exposure & Treatment Level & Heavy Metals	202463.100	12	16871.925	1.437	.148
Time of Exposure & Particle Sizes & Heavy Metal	413267.803	12	34438.984	2.933	.001
Treatment Levels & Particle Sizes & Heavy Metal	287823.727	18	15990.207	1.362	.150
Time of Exposure & Treatment Levels &Particle Sizes & Heavy Metal	512714.816	36	14242.078	1.213	.196
Error	3381584.340	288	11741.612		
Total	312525333.467	432			
Corrected Total	76479236.374	431			

***=highly significant difference ($p = 0.000$), **=significant different ($0.001 \leq p \geq 0.007$), NS=not significant ($0.148 \leq p \geq 0.198$),

Table 15. Results of Duncan test of homogeneity of the collective weight of earthworms

Category of Heavy Metals	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Copper concentration	144	604.48	A
Chromium concentration	144	956.31	C
Arsenic concentration	144	656.78	B
Time of Exposure			
Week Twelve	144	632.79	A
Week Six	144	791.18	B
Initial Week	144	793.60	C
Treatment Levels			
T_1=(75+25g)	108	310.85	A
T_2=(50+50g)	108	565.31	B
T_3=(25+75g)	108	866.24	C
T_4=(0+100g)	108	1214.36	D
Particle Sizes			
Unsoaked Sawdust of 40 mesh	108	697.21	A
Soaked Sawdust of 40 mesh	108	835.25	D
Unsoaked Sawdust of 60 mesh	108	781.34	C
Soaked Sawdust of 60 mesh	108	642.97	B

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

Table 16. Chromium, copper and arsenic concentration levels in substrates

Substrate Levels/Particle Sizes			$T_{1=(75+25g)}$				$T_{2=(50+50g)}$				$T_{3=(25+75g)}$				$T_{4=(100g)}$			
			US 40M	**US 60 M**	**SS 40M**	**SS 60M**	**US 40 M**	**US 60M**	**SS 40M**	**SS 60M**	**US 40M**	**US 60M**	**SS 40M**	**SS 60M**	**US 40 M**	**US 60M**	**SS 40M**	**SS 60M**
Control	**As**	**Mean**	306	280	283	222	719	705	534	475	1120	1073	1061	654	1501	1370	1534	1036
		SD	38	79	50	48	41	47	25	47	52	46	101	52	129	26	124	55
	Cr	**Mean**	385	536	633	520	934	1115	641	971	1547	1400	1322	1102	1896	2144	1856	1536
		SD	76	188	48	51	61	48	59	48	50	48	7	47	147	44	48	46
	Cu	**Mean**	267	451	435	325	638	712	423	647	981	897	798	731	1138	1290	1463	1036
		SD	30	53	53	54	29	43	7	46	15	53	40	54	86	96	59	38
Week Six	**As**	**Mean**	221	265	241	171	507	640	441	420	931	835	909	584	1239	1267	1354	905
		SD	61	48	28	33	54	10	32	73	57	49	52	68	115	40	46	316
	Cr	**Mean**	328	420	506	391	600	916	623	763	1286	1118	1284	935	1633	1614	1771	1319
		SD	64	73	2	107	110	13	191	101	19	99	17	97	46	11	121	429
	Cu	**Mean**	233	280	255	264	498	586	421	510	780	719	675	606	993	952	1048	854
		SD	36	39	8	88	54	9	5	65	33	31	38	52	23	33	43	257
Week Twelve	**As**	**Mean**	194	230	198	122	346	573	368	363	842	594	760	512	877	910	1317	776
		SD	43	51	47	14	16	78	5	60	57	34	144	29	60	61	69	52
	Cr	**Mean**	310	407	376	262	566	717	505	553	1125	833	1068	771	1270	1475	1687	1099
		SD	78	61	40	29	47	29	14	17	71	83	181	40	30	70	43	80
	Cu	**Mean**	194	257	222	200	358	454	305	370	679	544	647	479	748	827	971	663
		SD	38	34	51	39	52	1	2	56	46	66	74	18	27	3	45	53

T0=(100g of untreated sawdust), T1=(75g of untreated sawdust and 25g of treated sawdust), T2=(50g of untreated sawdust and 50g of treated sawdust), T3=(25g of untreated sawdust and 75g of treated sawdust) and T4=(100g of treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh).

4.4.1. Percentage Removal of CCA after Weeks of Exposure to *Eisenia fetida*

The percentage decrease of heavy metals in substrate was calculated after the period of exposure. Substantial reduction ranges were realized for all heavy metals in relation to the various treatment levels employed. After six weeks of exposure copper recorded the highest percentage decrease of 41% at T_1 (75g of untreated sawdust and 25g of CCA-treated sawdust) in soaked sawdust of 40 mesh followed by 36% of chromium at treatment level of T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) in unsoaked sawdust of particle size 40 mesh and arsenic witnessed the least percentage reduction over the initial concentration with 29% at T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust) in 40 mesh of unsoaked sawdust.

The removal of heavy metals from substrates by earthworms further witnessed substantial percentage removal of Cr, Cu and As at the end of the exposure. Arsenic had the highest percentage removal of 52% followed by 50% and 49% respectively for chromium and copper at the end of the final week of exposure. The removal of heavy metals witnessed a strong inclination towards 60 mesh of soaked sawdust for the removal of chromium and copper but arsenic was removed more from substrates with unsoaked sawdust of 40 mesh. There was also a general higher removal of CCA from substrates with high amount of untreated sawdust although significant percentages were removed from substrates with high amount of CCA-treated sawdust especially for As and Cr. The order of percentage removal in heavy metals was As > Cr > Cu. The detail information on the percentage removal of Cr, Cu and As with respect to the time of interval for the exposure. The values on the tables are the 3 analytical replications of all treatment levels and particle sizes substrate (Table 17). The percentage removal of each of the heavy metals considered in this study can also be found on the figures numbered from 19 to 30 with respect to each of the treatment levels.

Table 17. Percentage removal of CCA from substrates after weeks of exposure

Substrate Levels/Particle Sizes		Percentage Removed at Week 6 (%)			Percentage Removed at Week 12 (%)		
		As	Cr	Cu	As	Cr	Cu
$T_{1=(75+25g)}$	US 40 M	28	15	13	37	19	27
	US 60 M	5	22	38	18	24	43
	SS 40 M	15	20	41	30	41	49
	SS 60 M	23	25	19	45	50	38
$T_{2=(50+50g)}$	US 40 M	29	36	22	52	39	44
	US 60 M	9	18	18	19	36	36
	SS 40 M	17	3	0	31	21	28
	SS 60 M	12	21	21	24	43	43
$T_{3=(25+75g)}$	US 40 M	17	17	20	25	27	31
	US 60 M	22	20	20	45	41	39
	SS 40 M	14	3	15	28	19	19
	SS 60 M	11	15	17	22	30	34
$T_{4=(100g)}$	US 40 M	17	14	13	42	33	34
	US 60 M	8	25	26	34	31	36
	SS 40 M	12	5	28	14	9	34
	SS 60 M	13	14	18	25	28	36

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh).

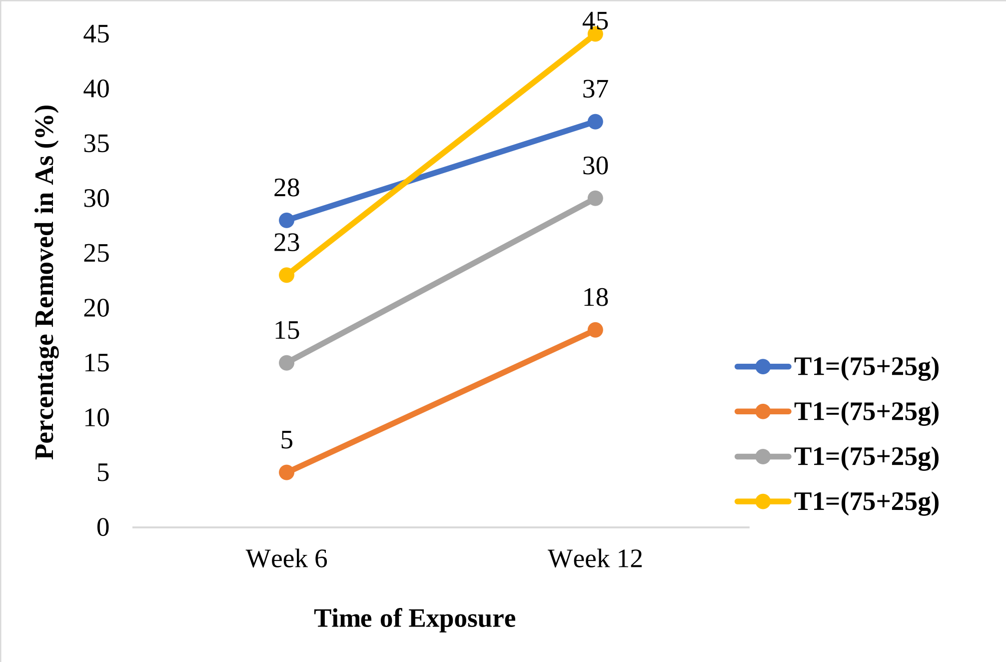

Figure 19. Percentage removed of Arsenic (As) at treatment levels T1

T1=(75g of untreated sawdust and 25g of CCA-treated sawdust)

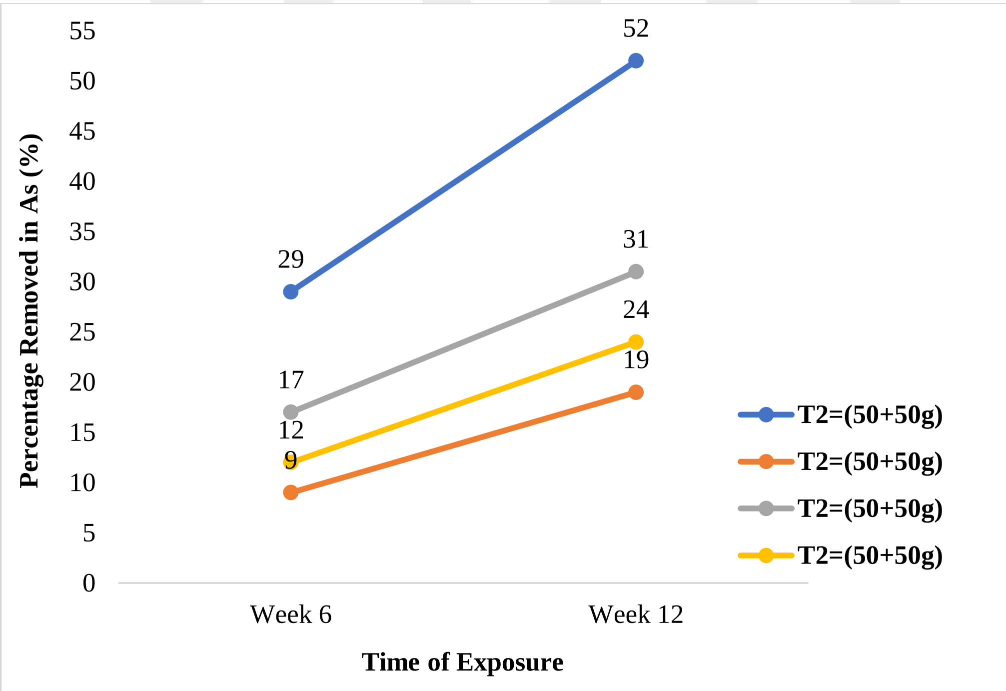

Figure 20. Percentage removed of Arsenic (As) at treatment levels T2

T2=(50g of untreated sawdust and 50g of CCA-treated sawdust)

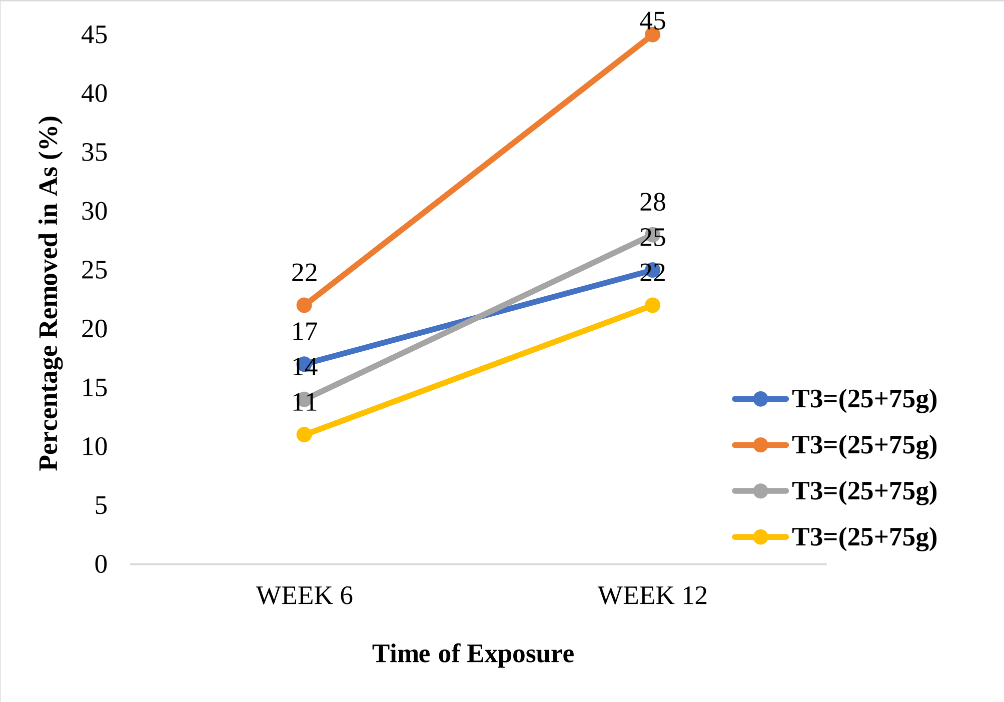

Figure 21. Percentage removed of Arsenic (As) at treatment levels T3

T3=(25g of untreated sawdust and 75g of CCA-treated sawdust)

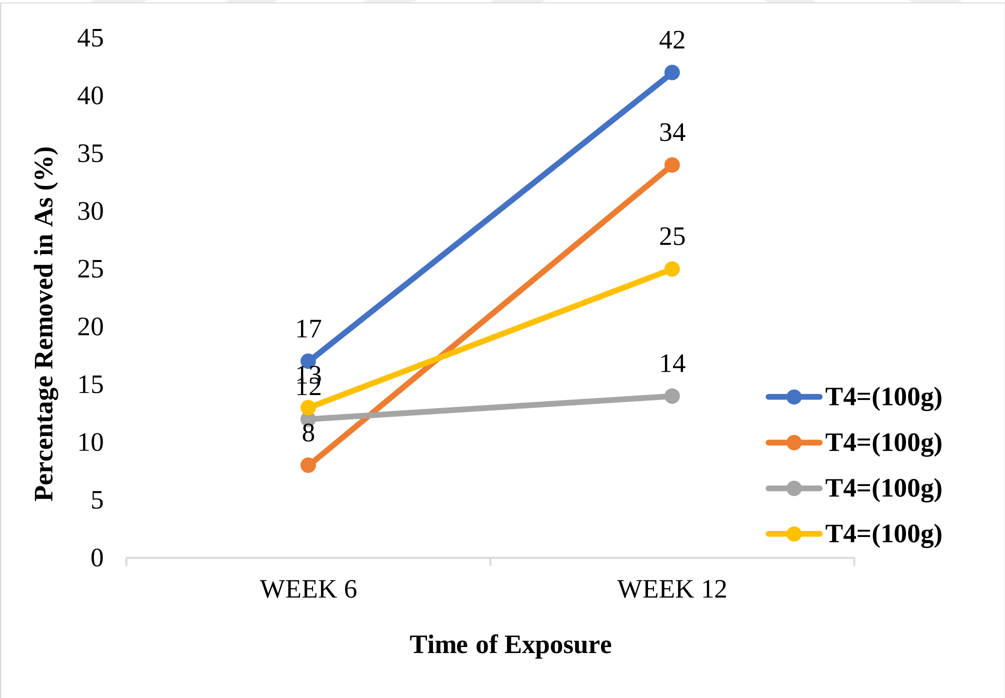

Figure 22. Percentage removed of Arsenic (As) at treatment levels T4

T4=(100g of CCA-treated sawdust)

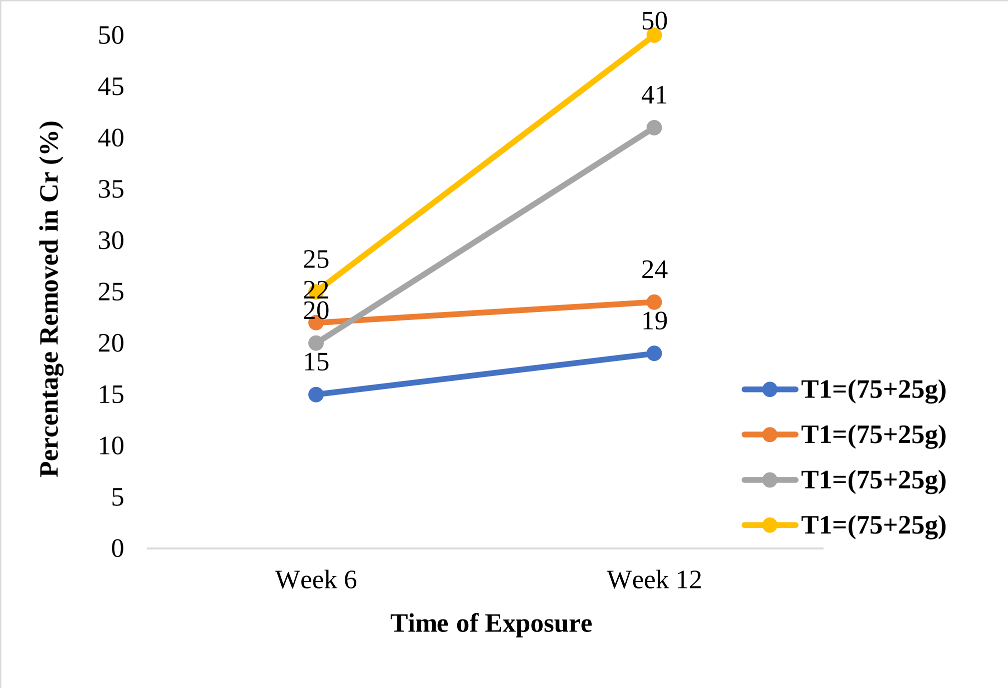

Figure 23. Percentage removed of Chromium (Cr) at treatment levels T1

T1=(75g of untreated sawdust and 25g of CCA-treated sawdust)

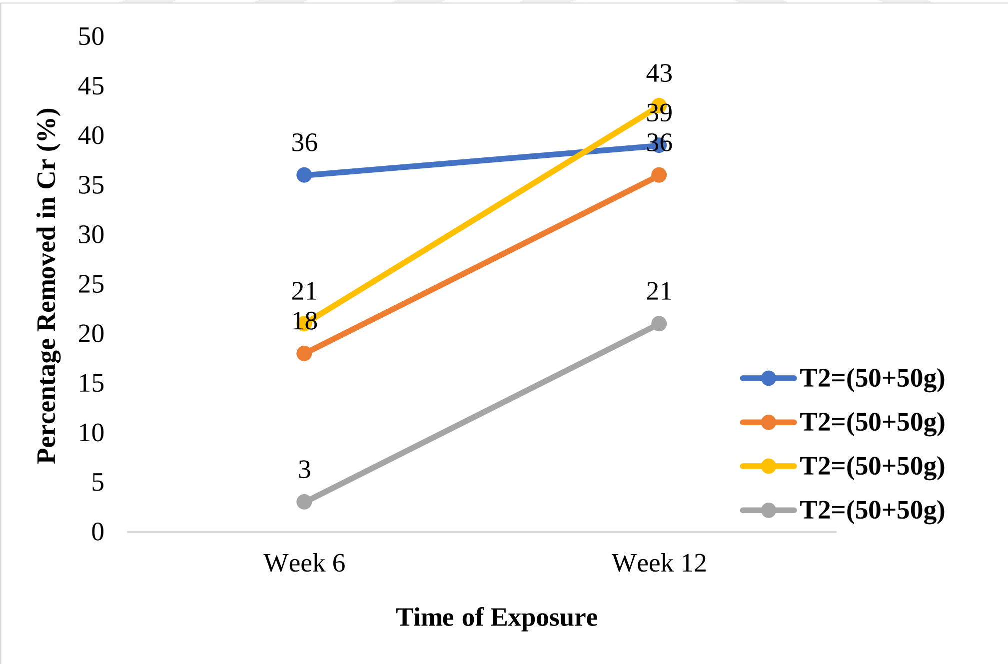

Figure 24. Percentage removed of Chromium (Cr) at treatment levels T2

T2=(50g of untreated sawdust and 50g of CCA-treated sawdust)

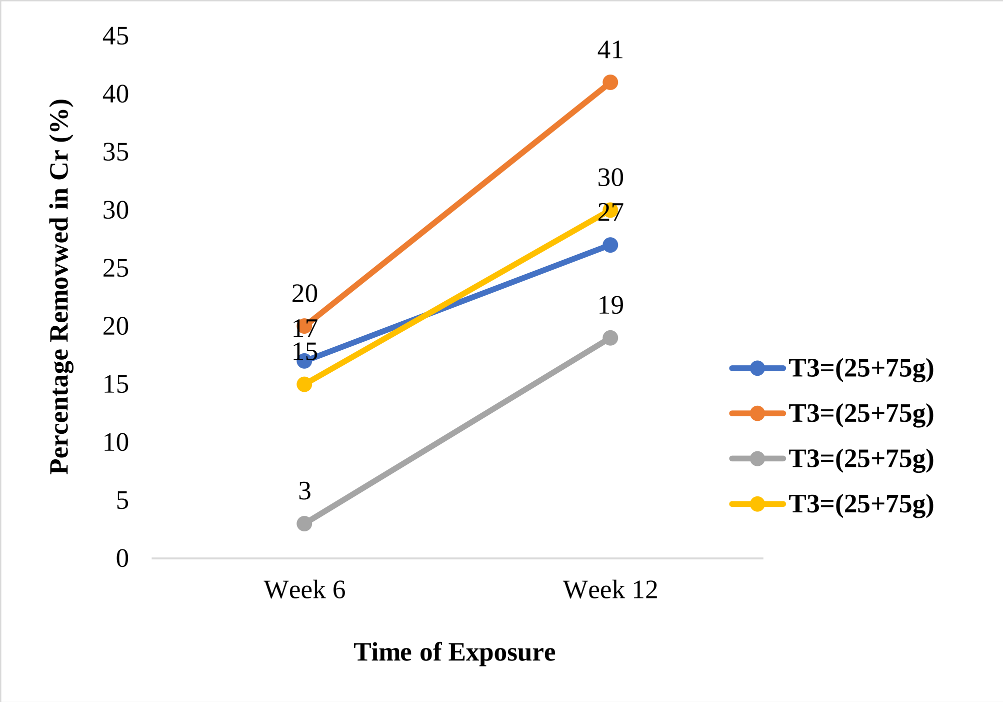

Figure 25. Percentage removed of Chromium (Cr) at treatment levels T3

T3=(25g of untreated sawdust and 75g of CCA-treated sawdust)

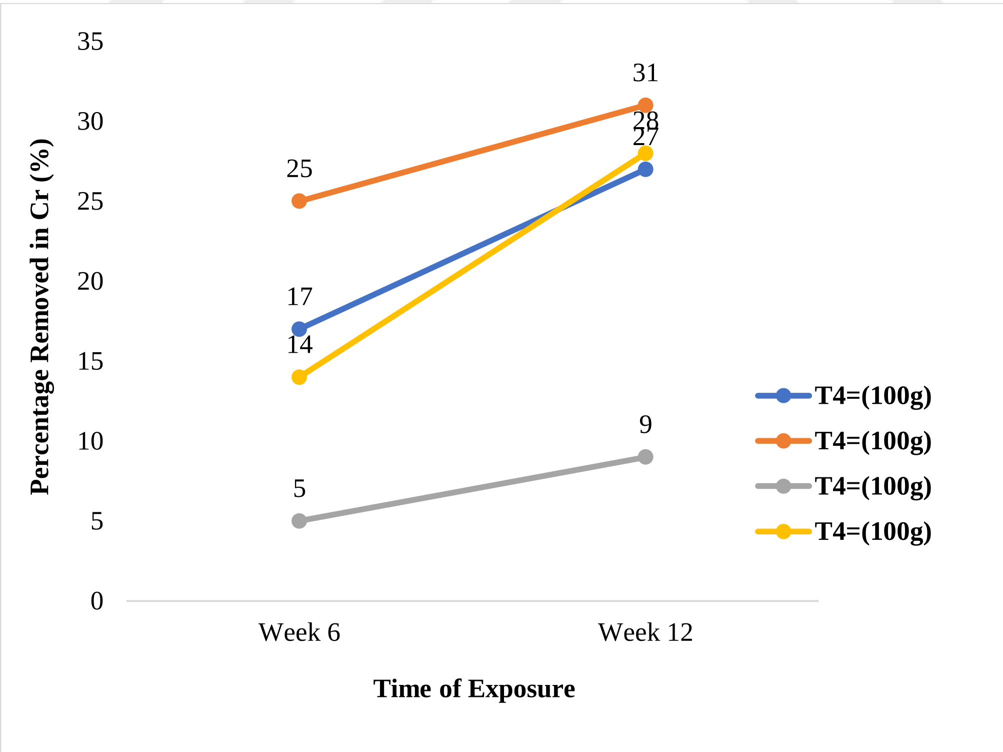

Figure 26. Percentage removed of Chromium (Cr) at treatment levels T4

T4=(100g of CCA-treated sawdust)

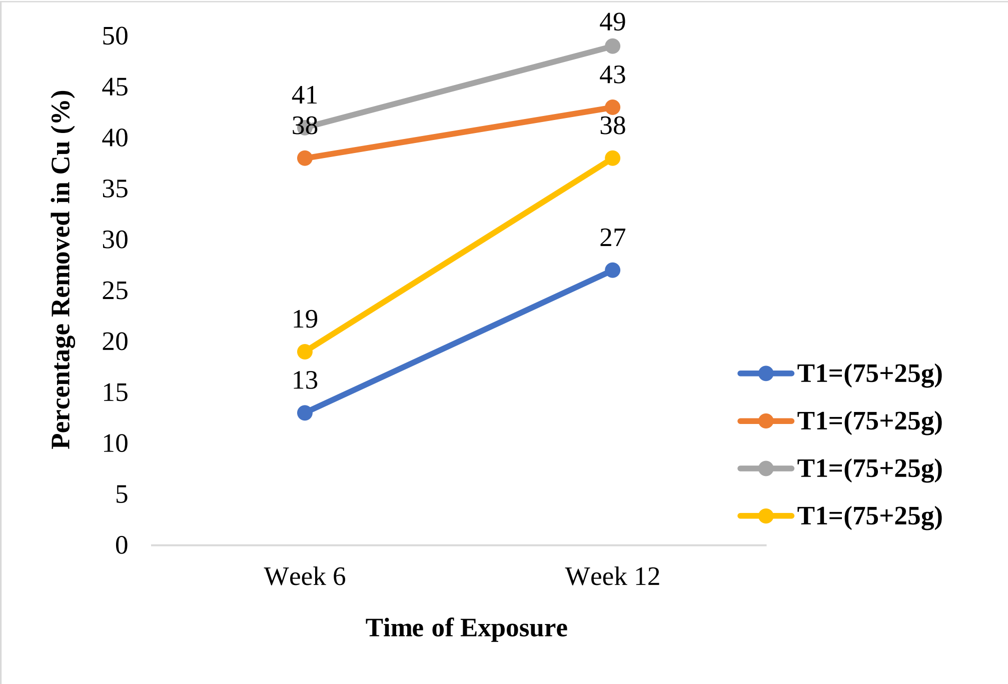

Figure 27. Percentage removed of Copper (Cu) at treatment levels T1

T1=(75g of untreated sawdust and 25g of CCA-treated sawdust)

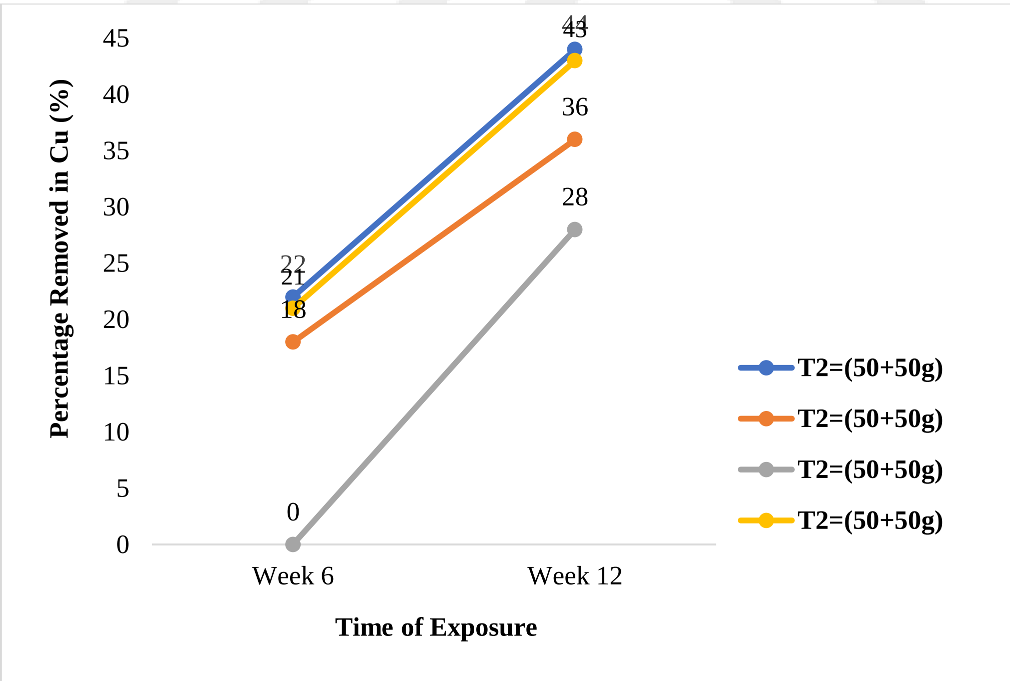

Figure 28. Percentage removed of Copper (Cu) at treatment levels T2

T2=(50g of untreated sawdust and 50g of CCA-treated sawdust)

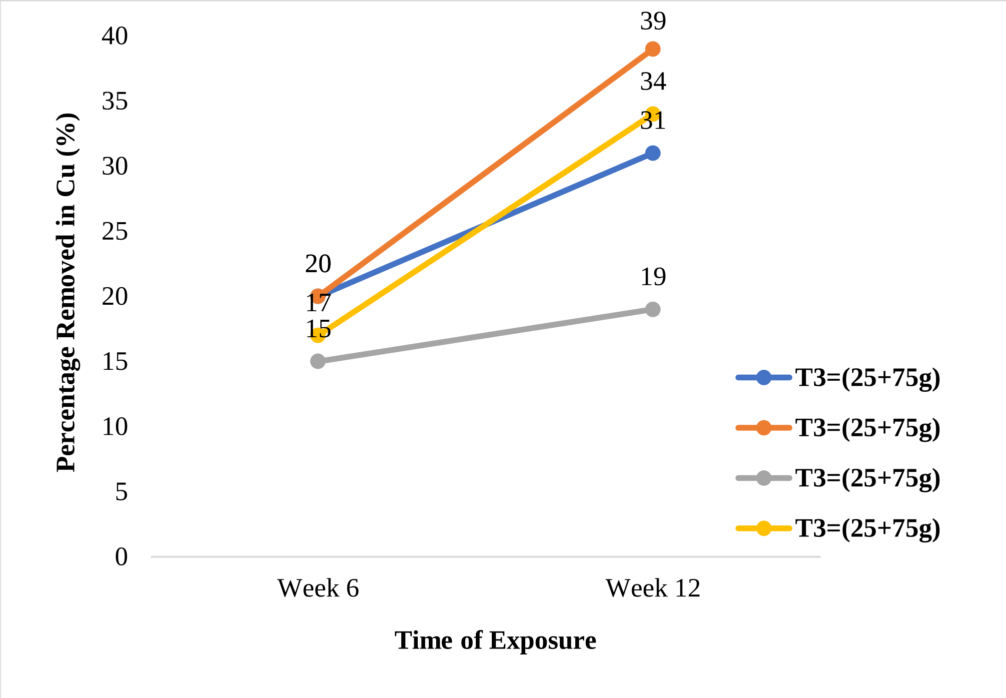

Figure 29. Percentage removed of Copper (Cu) at treatment levels T3

T3=(25g of untreated sawdust and 75g of CCA-treated sawdust)

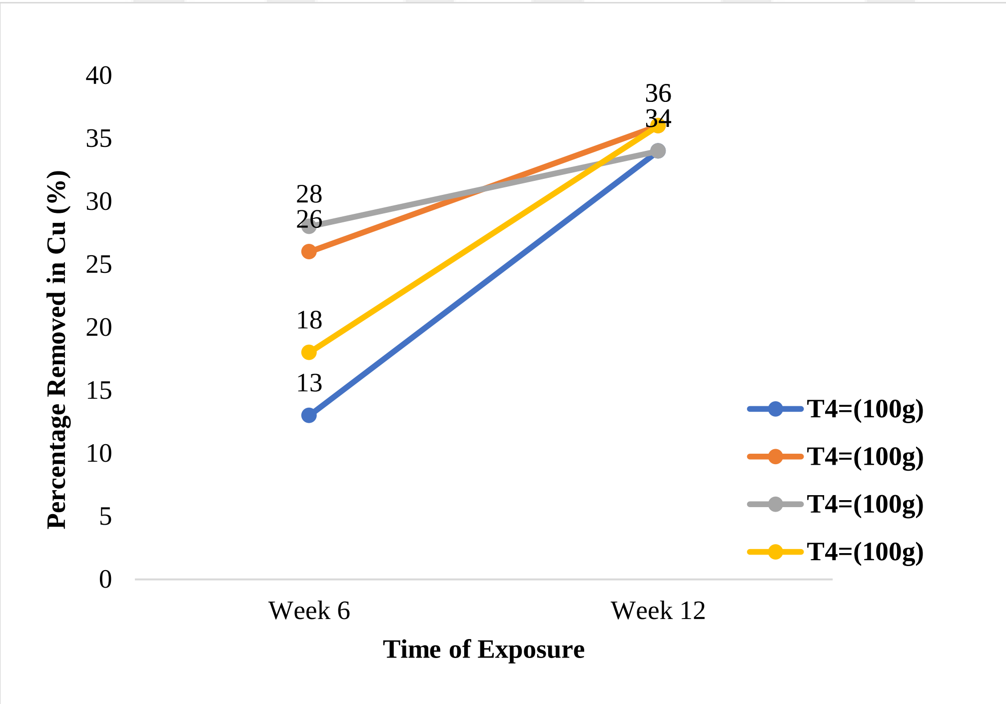

Figure 30. Percentage removed of Copper (Cu) at treatment levels T4

T4=(100g of CCA-treated sawdust).

4.5. CCA Accumulation in Earthworms Tissues after Weeks of Exposure

The accumulation of arsenic, chromium and copper in tissues of earthworm species (*Eisenia fetida)* revealed a significant variation from that of the substrate with respect to the substrate levels employed in the experiment. The accumulation of As, Cr and Cu in earthworms tissue after the period of exposure increases along the treatment levels from T_1 (75g of untreated sawdust and 25g of CCA-treated sawdust) to T_4 (100g of CCA-treated sawdust). There was a general higher accumulation of Arsenic as compared to Chromium and Copper in the tissue of earthworms over the substrates. Arsenic had the highest accumulation of 2351ppm with chromium and copper being 87 and 72ppm respectively. All these accumulations were observed at treatment level T_3 (25g of untreated sawdust and 75g of CCA-treated sawdust) for all the heavy metals. The accumulation of chromium and copper in earthworms tissue were observed in substrate with soaked sawdust of 60 mesh particle size while arsenic was also observed in substrate with soaked sawdust of 40 mesh particle size (Table 18).

The statistical analysis of variance on the CCA accumulation in tissue of worms earthworms with respect to the treatment levels, heavy metals and particle sizes of substrates showed highly significant difference within all the independent variables (p=0.000). The comparison test of effect between all the three independent variables (treatment levels, heavy metals and particle sizes) on CCA accumulation in tissue of earthworms also showed highly significant difference between all of them (p=0.000) as shown in Table (19). The post hoc test of homogeneity with Duncan test revealed 3 distinct groups for heavy metals, 4 for both treatment levels and particle sizes (Table 20).

Table 18. CCA concentration in tissues of earthworms after twelve weeks of exposure

Treatment Levels/Particle Sizes		Initial Mean Concentration in Substrates (Control)			Final Mean Concentration in Earthworms (*Eisenia fetida*)		
		As	Cr	Cu	As	Cr	Cu
T_1	US 40M	306	385	267	9	3	20
	US 60M	280	536	451	1159	14	31
	SS 40M	283	633	435	2141	54	54
	SS 60M	222	520	325	1226	15	38
T_2	US 40M	719	934	638	27	5	13
	US 60M	705	1115	712	1500	30	36
	SS 40M	534	641	423	2045	62	57
	SS 60M	475	971	647	1604	65	60
T_3	US 40M	1120	1547	981	16	5	22
	US 60M	1073	1400	897	1503	27	39
	SS 40M	1061	1322	798	2351	48	54
	SS 60M	654	1102	731	1913	87	72
T_4	US 40M	1501	1896	1138	56	4	21
	US 60M	1370	2144	1290	1481	47	46
	SS 40M	1534	1856	1463	2022	81	70
	SS 60M	1036	1536	1036	1907	48	54

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh)

Table 19. Analysis of variance on the CCA accumulation in tissues of earthworms

Source of variance	Sum of Squares	Degree Freedom	Mean Square	F-Value	Sig.
Corrected Model	82659595.60	47	1758714.80	86576962.67	.000
Intercept	30920402.10	1	30920402.11	1522131103.34	.000
Treatment Levels	10815849.59	3	3605283.20	177478729.77	.000
Heavy Metals (CCA)	51584658.96	2	25792329.47	1269689403.81	.000
Particle Sizes	261791.30	3	87263.77	4295768.62	.000
Treatment Levels & CCA	18834767.17	6	3139127.86	154531113.10	.000
Treatment Levels & Particle Sizes	285108.02	9	31678.67	1559458.58	.000
CCA & Particle Size	379516.31	6	63252.72	3113767.04	.000
Treatment Levels & CCA & Particle Sizes	497904.28	18	27661.35	1361696.38	.000
Error	1.95	96	.020		
Total	113579999.66	144			
Corrected Total	82659597.55	143			

***means highly significant difference ($p = 0.000$)

Table 20. Results of Duncan test of homogeneity of CCA accumulation in tissues of earthworms

Heavy Metals (CCA)	Harmonic Mean Sample Size	Homogeneous Group Means	Homogeneous Subsets
Chromium (Cr)	48	37.32	A
Copper (Cu)	48	43.01	B
Arsenic (As)	48	1309.81	C
Treatment Levels			
T_1=(75+25g)	36	16.86	A
T_2=(50+50g)	36	492.78	B
T_4=(100+0g)	36	590.71	C
T_3=(25+75g)	36	753.18	D
Particle Sizes			
Unsoaked Sawdust of 40 Mesh	36	396.95	A
Unsoaked Sawdust of 60 Mesh	36	458.74	B
Soaked Sawdust of 60 Mesh	36	486.45	C
Soaked Sawdust of 40 Mesh	36	511.39	D

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust).

4.5.1. Bioaccumulation Factors of CCA after Weeks of Exposure to Earthworms

The bioaccumulation factor, BAF calculated in this study was the concentration of the heavy metals in the tissues of earthworms relative to the concentration in the substrates at different treatment levels after twelve weeks of exposure. The BAF values calculated with respect to the treatment levels showed a significant variation among heavy metals accumulated in earthworms tissues. The BAF value for Arsenic was more than one at treatment levels T_2 (50g of untreated sawdust and 50g of CCA-treated sawdust), T_3 (25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4 (100g of CCA-treated sawdust). However, the BAF values for Chromium and Copper were less than one at all treatment levels. They showed BAF values between the ranges of 0.01 to 0.10. Chromium and copper did not show any significant value of BAF in all particle sizes at all the treatment levels. However, bioaccumulation factor values for arsenic were significant in substrates with soaked sawdust of 60 mesh particle size at various treatment levels. They were all more than one.

The higher the BAF value of a specific heavy metal the higher its uptake by earthworms. Arsenic had the highest value of BAF which indicated the high level of arsenic uptake by earthworms while chromium and copper had the lowest values of BAF. The BAF values of CCA were in the order of As > Cu > Cr. Below is the chromium, copper and arsenic accumulation in tissues of earthworms with their corresponding BAF values at various treatment levels (Table 21)

Table 21. Bioaccumulation Factors (BAFs) of Chromium, Copper and Arsenic

Substrate Levels/Particle Sizes		As			Cr			Cu		
		Conc. Substrate	Conc. *E. fetida*	BAF	Conc. Substrate	Conc. *E. fetida*	BAF	Conc. Substrate	Conc. *E. fetida*	BAF
T_1	US 40 M	306	9	0.03	385	3	0.01	267	20	0.08
	US 60 M	280	27	0.10	536	5	0.01	451	13	0.03
	SS 40 M	283	16	0.06	633	5	0.01	435	22	0.05
	SS 60 M	222	56	0.25	520	4	0.01	325	21	0.07
T_2	US 40 M	719	1159	1.61	934	14	0.02	638	31	0.05
	US 60 M	705	1500	2.13	1115	30	0.03	712	36	0.05
	SS 40 M	534	1503	2.81	641	27	0.04	423	39	0.09
	SS 60 M	475	1481	3.12	971	47	0.05	647	46	0.07
T_3	US 40 M	1120	2141	1.91	1547	54	0.03	981	54	0.06
	US 60 M	1073	2045	1.91	1400	62	0.04	897	57	0.06
	SS 40 M	1061	2351	2.22	1322	48	0.04	798	54	0.07
	SS 60 M	654	2022	3.09	1102	81	0.07	731	70	0.10
T_4	US 40 M	1501	1226	0.82	1896	15	0.01	1138	38	0.03
	US 60 M	1370	1604	1.17	2144	65	0.03	1290	60	0.05
	SS 40 M	1534	1913	1.25	1856	87	0.05	1463	72	0.05
	SS 60 M	1036	1907	1.84	1536	48	0.03	1036	54	0.05

T_0=(100g of untreated sawdust), T_1=(75g of untreated sawdust and 25g of CCA-treated sawdust), T_2=(50g of untreated sawdust and 50g of CCA-treated sawdust), T_3=(25g of untreated sawdust and 75g of CCA-treated sawdust) and T_4=(100g of CCA-treated sawdust). US 40 M=(Unsoaked Sawdust of 40 Mesh), US 60 M=(Unsoaked Sawdust of 60 Mesh), SS 40 M=(Soaked Sawdust of 40 Mesh) and SS 60 M=(Soaked Sawdust of 60 Mesh).

4.6. Heavy Metals

4.6.1. Effects of Heavy metals on Total Growth Development of Eisenia *fetida*

The results and findings from the current study revealed that California red worms *Eisenia fetida* have the capacity to accumulate heavy metals in their tissues. The metals under consideration as far as this study was concern included arsenic (As), chromium (Cr) and copper (Cu). The results of bioaccumulation of heavy metals especially for As was high as compared to Cr and Cu (Figures 20 and 21). The level at which earthworms bioaccumulated these metals in their tissues resulted in the slow growth of their population, weight loss (Figures 15 and 16) and subsequently the rate of mortality realized during the early part of the period of exposure. This outcome is parallel to the findings made by Meharg *et al.*, (1998), in an arsenic-dosed soil exposure study where earthworms mortality within 10 days of exposure was high. Coincidentally the effects of CCA accumulation in earthworms was observed at the same treatment levels where the maximum bioaccumulation occurred. These treatment levels were made up of high amount of CCA-treated sawdust. All these effects on earthworms growth and reproduction was also proved by Ludec *et al.,* (2008), in a study where the same species of earthworms but different form of substrate was used.

However, the toxicity of heavy metals to earthworms is said to be dependent upon bioavailability of heavy metals which is also regulated by certain features of substrate like organic matter and pH (Daoust *et al.,* 2006). Although the distribution of heavy metals over different ion phases (metal speciation) in earthworms tissues and substrate was not considered in the present study it could be suggested that the pH (9.30) of the substrate contributed to bioavailability of heavy metals and eventually caused mortality in earthworms. The avoidance of *Eisenia fetida* from a CCA-contaminated substrate was reported to have been as a result of high amount of Cu contained in substrate (Mench and Bes, 2009). Similarly, the high mortality rate of *Eisenia fetida* in the present study in substrate at some substrate levels could also be related to the concentration of Cu in the substrate used for the study especially at substrate levels with high amount of CCA-treated wood sawdust (T_3 and T_4).

4.6.2. Bioaccumulation of Heavy Metals by *Eisenia fetida*

Many studies have reported variations in heavy metals accumulation by different species of earthworms. To date there are records of increases, decreases and no changes in heavy metals accumulated in earthworms tissues. The three heavy metals, arsenic (As), chromium (Cr) and copper (Cu) investigated under this study revealed varying results of bioaccumulation in California red worm (*Eisenia fetida)* after 12 weeks of exposure. There are sufficient evidences on earthworms high capacity to accrue lethal chemicals from variety of medium substrate such as metal contaminated soils, sludges, kitchen waste, farm waste and thermal power plant fly ash.

Nonetheless, the extent and scale of accrual depends on the type of metal or chemical and physiognomies of the substrate (Langdon *et al.,* 2003). In this study arsenic showed the highest and significant bioaccumulation in earthworms tissues at all treatment levels and particle sizes (Table 19). This might be that earthworms bioaccumulated arsenic in their tissues and sequestrated it in a form that could not be easily eradicated from their system as suggested by Meharg *et al.*, (1998). Langdon *et al.*, (2003), also reported that the closeness of earthworms to organic and mineral matters allows it to easily accumulate arsenic in both solid and aqueous state because organic and mineral matters are arsenic bound. Also, the effect of organic matter was reported by Ezemonye *et al*., (2015) to have increased copper bioaccumulation in soil amended with organic substrates like poultry, pig and cow manure. Although cow dung was used in this study a significant bioaccumulation of copper was not achieved as in a study by Iordache and Borza, (2012). Relatively the results on high arsenic bioaccumulation by *Eisenia fetida* in the present study could be associated to the feeding of preference of earthworms on arsenic bound sawdust and cow dung thereby accumulating more arsenic via their feeding habit and fragile skin. This also attested to the fact that earthworms are prone to specific contaminants based on features such as ecological properties, location, mobility behavior and food preference (Tomlin, 1992).

Higher initial concentrations for both Cr and Cu than As was observed in the current study. Heavy metals percentage removal in substrate after weeks of exposure also showed a slightly high percentage values for Cr and Cu at all treatment levels and particle sizes than for As (Tables 15). However, computation of bioaccumulation factors in earthworms tissues showed higher values for arsenic as compared to Cr and Cu (Tables 19) which contrasted the suggestion that high level of Cr and Cu contaminants affect the absorption and metabolism of As (Meharg *et al*., 1998). This could be an indication that substantial amount of heavy metals is still in the substrate after the period of exposure. This can be that earthworms had reached their threshold for heavy metals bioaccumulation or the time of exposure was not enough to absorb all the heavy metals from the substrate. Also, if bioaccumulation dependence upon the degree of contamination and the characteristics of substrate is anything to be considered then the low BAF values for Cr and Cu could be associated to the pH and the content of heavy metals in the substrate used. Heavy metals are known to be mobile under acidic conditions (Ekperusi *et al*., 2016). The pH value of the substrate used in this study was 9.30 which indicated an alkaline nature of the substrate before the exposure and this may have contributed to the mobility of some heavy metals thereby making the metals bioavailable for uptake by *Eisenia fetida.* Earlier studies like Leduc *et al*., (2008), have also confirmed influence of pH values specifically on Cu bioaccumulation in earthworms tissues. They said higher pH values led to lower Cu bioaccumulation in *Eisenia fetida* which was same in this study.

On the other hand, findings from the current study supported the account of Langdon *et al.,* (2003); detection of metals in earthworms tissues may necessarily not insinuate metal biomagnification in tissues of earthworm species. Numerous studies have shown varying values of bioaccumulation factor in earthworm species. In all these studies alike the same category (epigeic) of earthworms but different species (*Aporrectodea rosea, Eisenia fetida and Lumbricus rubellus*) of earthworms were used in heavy metal accumulation test. Studies involving species like *Aporrectodea rosea* and *Lumbricus rubellus,* showed no biomagnification of arsenic in their tissues (Yeates *et al.,* 1994; Geizinger *et al*., 1998) but studies with species like *Eisenia fetida* showed higher bioaccumulation for arsenic in earthworms tissues (Fischer and Koszorus, 1992). And this was verified in the outcome of the current study. In spite of the fact that same category of earthworm species was used in different studies for heavy metals accumulation test, it can be deduced from the above findings that bioaccumulation for Cr, Cu and As in this study were species-driven (Suthar *et al.,* 2008). The lower values of bioaccumulation factor and the level of absorptions of Cu and Cr in tissues of earthworms in relation to the current study also confirmed the fact alluded by Hopkin (1989) that earthworms might have the latitude to control metals in their bodies but the mechanism and bioaccumulation for metals like Cu may be species-specific. A metal accumulation and toxicity study in different earthworm species under the same exposure concentrations also revealed *Eisenia fetida* accumulation of Cu and Ni was lower as compared to *Lumbricus rubellus* (Qiu *et al.*, 2013).

The span and duration of exposure in heavy metals bioremediation studies have proved to be a major factor that influenced differences in heavy metals concentrations in earthworms (Hopkin, 1989; Pattnaik and Reddy, 2011). Variations in bioaccumulation of As, Cr and, Cu in tissues of earthworms were noticed in the present study and this differences in metal accumulation in *Eisenia fetida* tissues could among others be ascribed to the duration of the study as described in other studies of heavy metal toxicity test by earthworms (Jamaludin and Mahmood, 2010; and Pattnaik and Reddy, 2011). Even though the continuous bioaccumulation factor of heavy metals in this study was not monitored. The consistency in the observed decrease in As, Cr and Cu concentration in substrates within the experimental time intervals could equally be predicted for BAF values of heavy metals in earthworms tissues along the same time intervals. However, BAF values for As increased along all the substrate levels except at substrate level T_4 where the trend was decreased while fluctuations were observed for Cr and Cu. The higher value of bioaccumulation factor for As in most of the substrate levels and particle sizes signified that concentrations of As in the tissues of *Eisenia fetida* surpassed the concentrations in the substrate on many occurrences.

The reduction of metal availability to earthworms due to metal attachment to organic matter has been reported by Lukkari (2006). In the current study Cr and Cu were the least accumulated heavy metals in earthworms tissues according to the values of BAF relative to both treatment levels and particle sizes. The low bioaccumulation of Cr and Cu in earthworms tissues may be that Cr and Cu were cinched to organic matter in the experimental substrates and were not bioavailable in an absorbable form for earthworms uptake. Furthermore, findings from the present study showed an independent relationship between metal concentrations in substrate used for the experiment and metal concentrations in earthworms tissues as compared to the findings by Pattnaik and Reddy (2011). The heavy metals concentrations in substrate especially for Cr and Cu manifested not in the tissues of *Eisenia fetida* after 12 weeks of exposure. However, Gupta *et al.*, (2005); Suthar *et al.*, (2008) and Pattnaik and Reddy, (2011) reported direct dependent relationship between metal concentrations in substrate and metal concentrations in earthworms tissues. If the above evidence is anything to go by then results and findings from the current study differed because Cr and Cu had the highest concentration in substrate as compared to As but metal bioaccumulation in earthworms was higher for As than Cr and Cu. This further defies the hypothesis that metal availability in substrate influences tissue-metal levels. The metals of interest and of great concern in this study followed a similar bioaccumulation pattern for other studies especially for the results of As and Cr which are related in terms of metals accumulation in remediation studies.

5. CONCLUSIONS AND RECOMMENDATIONS

The findings from the exposure of a mixture of sawdust (both CCA-treated and untreated) and cow dung as substrate to California red worm (*Eisenia fetida)* over a period of 12 weeks were concluded as follows;

1. The poor trend of growth rate exhibited during the study by California red worm (*Eisenia fetida)* at substrate levels with higher amount of CCA-treated sawdust could be concluded that Cr, Cu and As are growth retarding chemical in earthworms although substantial growth of earthworms was observed at substrate level T_2 in the early stage of the experiment. Meanwhile earthworms growth development in substrate levels with higher amount of untreated sawdust or no amount of treated wood sawdust witnessed an uninterrupted growth rate till the end of the experiment.
2. Earthworms (*Eisenia fetida)* showed and displayed more preference towards substrate with unsoaked sawdust as compared to substrate with soaked sawdust in their population growth and collective weight gained during the study.
3. In terms of the particle sizes of the wood sawdust used in the experiment, substrates of 60 mesh particle size exhibited a significant population build-up and collective weight gained as compared to the substrates with 40 mesh particle size wood sawdust.
4. The time-dependent change in total population growth of earthworms revealed that the number of earthworms decreased in substrate levels with high amount of CCA treated wood sawdust but increased in substrate levels with low or no amount of CCA treated wood sawdust with increase in time (weeks) of exposure.
5. The collective weight of earthworms showed a rise and fall pattern for all treatment levels with regards to time-dependence change in biomass of earthworms. There was a steady increase in collective weight from the initial weeks to the mid weeks and then a decrease in collective weight towards the final weeks at all treatment levels.
6. The observed population growth in earthworms did not necessarily caused increase in earthworms collective weight with regards to both treatment levels and particle sizes of substrate.

The following conclusions were also drawn from results of heavy metals accumulations by California red earthworms (*Eisenia fetida)* in the study;

7. In terms of treatment levels and particle sizes there was a general high amount of Arsenic (As) bioaccumulation than Chromium (Cr) and Copper (Cu) in tissues of *Eisenia fetida* after the period of exposure.

8. The maximum volume of As, Cr and Cu accumulated in earthworms tissues were observed at treatment level T_3 (25g of untreated sawdust and 75g of CCA-treated sawdust) in the order As > Cr > Cu.

9. The values for bioaccumulation factor (BAF) of Cr, Cu and As for heavy metals at all treatment levels revealed biomagnification of As at all treatment levels except for substrate level T_1 (75g of untreated sawdust and 25g of CCA-treated sawdust) but biomagnification for Cr and Cu was not observed because BAFs values were less than one at all the treatment levels.

10. The BAF values of As also showed biomagnification for all particle sizes of substrates but that of Cr and Cu were less than one for all particle sizes of substrates which meant no biomagnification was observed.

11. Heavy metals bioaccumulations by *Eisenia fetida* in the present study was also species driven especially for arsenic.

12. There were significant variations between individual heavy metals accumulated in earthworms tissues with respect to particle sizes of substrates but the maximum accumulation for As was observed in substrate with soaked sawdust of 40 mesh whereas the maximum accumulation for Cr and Cu was observed in substrate with soaked sawdust of 60 mesh.

13. Although the quality of the substrate that served as food source per the preference of earthworms species (*Eisenia fetida)* was not favorable and did not entirely supported their growth and development, *Eisenia fetida* earthworms were able to bioaccumulated substantial amount of Cr, Cu and As heavy metals from the substrate. However, there was significant growth improvement at specific treatment levels.

14. Although earthworms *Eisenia fetida* found at treatment levels with high amount of CCA-treated sawdust showed signs of discomfort in the substrate by escaping from the experimental boxes and subsequently dying, they were able to remove and contain considerable percentages of Cr, Cu and As from the substrate especially at treatment levels T_3 and T_4.

15. Finally, arsenic from CCA-treated wood products is a known heavy metal of concern in most ecotoxicity test. In this study earthworms *Eisenia fetida* showed a significant bioaccumulation of As in their tissues which presents a clear indication of the importance of *Eisenia fetida* in bioremediation of arsenic-contaminated environments. The findings from this study is also suitable for the wide-range of various results from similar topics with different kinds of substrates and methodologies.

The following recommendations were also drawn to aid further studies of the topic;

16. The efficacy of CCA-free substrate (vermicompost) should be tested on its support for agricultural purposes because earthworms have been reported to produce quality vermicompost to support the growth of agricultural crops although the nutrients content of the one from this study was not assessed.

17. The feasibility of metals accumulation by other species of earthworms should be tested by exposing them to the same substrate as in the current study.

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