TITLE PAGE

EFFECT OF ETHYLENEDIAMINETETRACETIC ACID ON LEAD AMONG OCCUPATION LEAD HANDLERS IN ENUGU URBAN NIGERIA

BY

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DEDICATION

To God who in His infinite mercy has made it possible for this work to be completed in spite of all the challenges and set back encountered. To Him be praise and glory forever and ever Amen.

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ABSTRACT

The effect of Ethylenedianinetetraacetic acid (EDTA) on lead content of blood has not been worked on in vitro. This led to carrying out this research. The aims of this research is to ascertain if EDTA has any effect on blood lead levels if the sample is collected using an EDTA tube and also to find out if there is any significant difference in the blood lead levels of the same sample when collected with an EDTA tube and a non-EDTA tube. Hundred (100) males who are commercial painters and who have painted for more than 5 years were recruited for the research. Fifty (50) males who had nothing to do with lead and lead products were also recruited to serve as the control group. The blood lead levels of the test group were significantly higher ($p < 0.05$) than that of the control group. The haematological indices were significantly higher ($p < 0.05$) in the blood in plainon-EDTA tubes than that in the tubes containing EDTA. In the haemoglobin concentration, there was a significant difference ($p < 0.05$) between that of the fresh blood and the sequestered blood. The lead concentration was significantly higher ($p < 0.05$) in the native blood than the sequestered blood. From the investigations the EDTA in the tubes had an effect on the lead already in the blood and the effect was noticed on the haematological parameters.

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CHAPTER ONE

1.1 INTRODUCTION

The heavy metal lead is ubiquitous, and its deposits are scattered throughout the world. “The most common ore is galena” (lead sulphide-87% lead). “It has been mined and disseminated throughout the environment from where it has gradually become incorporated into the structural tissue of plants, animals and humans” *(Pracheta et al., 2009).* Lead is a soft, silvery gray metal, which rapidly becomes dull in air, and is soft, malleable and ductile, melting at 327.5 °C. It is highly resistant to Corrosion, but is soluble in nitric and hot sulphuric acids. Lead (Pb), with atomic number of 82 and atomic weight of 207.19 has a specific gravity of 11.34. It has four naturally occurring isotopes with atomic weights 208, 206, 207, and 204, in decreasing order of abundance. “The usual valence state in inorganic lead compounds is + 2” *(Lead, 2001).*

The common occurrences of lead include naturally occurring ores. Lead ores comprise 0.002% (15g/t) of the earth’s crust. They include galena (lead sulfide), anglesite (lead sulfate), cerussite (lead carbonate), mimetite (lead chloroarsenate) and pyromorphite (lead chlorophosphate).

1.11 INORGANIC LEAD

This is the form of lead found in old paint, soil, dust and various consumer products. The color varies, depending on the chemical form, and the most common forms are white lead (a lead carbonate

compound), yellow lead (lead chromate, lead monoxide) or red lead (lead tetra oxide). Lead acetate has a sweetish taste.

1.12 ORGANIC LEAD

Tetra-ethyl lead is the form of lead used in leaded gasoline. Organic forms of lead are extremely dangerous, as they are absorbed through the skin and are highly toxic to the brain and central nervous system, much more so than inorganic lead. The combustion of organic lead – when it is added to petrol as a fuel additive – results in the release of lead into the atmosphere.

Lead is a metal of antiquity and has been used for many purposes for thousands of years. A variety of forms of lead are used industrially, the most common being lead oxide (PbO) and red lead oxide (lead tetra oxide; Pb3O4). “Lead oxides are used in the plates of electric batteries and accumulators and in other substances” *(Stellman and Osinsky, 1997*). About 40 to 50% of all lead production is used to make lead-acid batteries (both lead metal and lead oxides are used). A further 20% of production is used as the metal, for example, cable sheathing, solder, ammunition, alloys, weights, ballast, and low-melting alloys. “Lead is a major component of many alloys such as bronze and solders” *(Massaro, 1997*). It is used for tank linings, piping, and building construction. Lead-based pigments, such as white lead, have a long tradition of being used in paints, although they have virtually all been substituted in many countries by other pigments in the last 20 years for obvious toxicological reasons. Lead compounds are used in glassware and ceramics and as stabilizer in plastics. Red lead is used to make

television tubes. “About 10% is converted into alkyl-lead compounds and used as antiknock additives in gasoline, although this use is in decline as more and more countries move to limit the concentration of such additives in gasoline” (*Massaro, 1997*). Some of the uses for lead in ancient times (such as lead sheet for lining roofs) remain today.

“Even though worldwide lead exposure has decreased tremendously in the past decades” *(Pirkle, 1994; Hwang, 2004; Gulson, 2006*), “lead is still ubiquitous and often found in flaking lead-based paints” *(Mathee, 2009*), candies *(CDC 2002; Medlin 2004*), cosmetics (*Al-Ashban, 2004*), traditional lead-glazed ceramics used in the preparation and serving of food (*Sabouraud, 2009*), herbal remedies *(Obi, 2006*), canned foods and beverages (lead solder) *(Maduabuchi*, *Nzegwu et al. 2006*), toys *(Greenway and Gerstenberger 2010*), and “can affect communities residing near smelters” *(Hegde, 2010*) and “battery recycling plants” (*Fuller 2009*).

1.2 JUSTIFICATION

This research was designed to investigate the effect of anticoagulant on lead activity in the blood. Bearing in mind that one of the treatment for lead toxicity is by administering a drug edentate disodium calcium intramuscularly, of which part of which is an anticoagulant. It becomes necessary to carry out this research in order to determine if collecting blood samples in anticoagulant for lead investigation inhibits the activity of the lead already in the blood.

1.3 AIMS AND OBJECTIVES.

The aims of the research are to investigate

1. Blood levels of lead and its effect on hematological parameters among workers that work with lead in Enugu.
2. To determine by comparison if the anticoagulant has any effect on the lead activity of the lead already in the blood.
3. To determine whether any effect is significant or not.

CHAPTER TWO

LITERATURE REVIEW

"Environmental lead exposure remains an important issue worldwide" *(Tong, 2000; Gordon, 2004; Rossi 2008)*. Lead is not bio-degradable and only accumulates where it is deposited. "Due to this reason lead levels have increased enormously from the ancient times" *(Flegal and Smith, 1995*). "The magnitude of increase has resulted in adverse health effects" (*Budd et al., 1988*). Lead is a highly toxic metal with no known physiological benefits and is a ubiquitous pollutant in the ecosystem as a result of its natural occurrence and its industrial use. "Mankind has used lead for over 6000 years" *(IPCS, 1995*). Lead's toxicity was recognized and recorded as early as 2000 BC. "There is no such thing as normal or safe levels of lead for its toxicity to humans" *(George, 1999)*. "The burden of lead toxicity is greatly underestimated, because most of the lead poisoning is clinically overt". *(Ukaejiofo, et al 2009*).

2.1 OCCUPATIONAL LEAD EXPOSURE

Lead primarily enters the body through ingestion (eating and drinking) and inhalation (breathing in air). It can also pass through the skin. Lead is absorbed, distributed throughout the body, and excreted. "The rate of lead absorption into the body depends on the chemical and physical properties of the form of lead and the physiological characteristics of the exposed person such as age and nutritional status" *(Lead, 2001*). For example, when inhaled, factors such as the lead particle size and shape and the individual's ventilation rate

influence how lead will be deposited in and absorbed by the respiratory tract. Large particles, which may be encountered in an occupational setting, tend to be deposited in the upper airways and may be directly absorbed by swallowing and absorption in the stomach. Smaller particles tend to be deposited in the bronchial region of the lung, and particles less than one micron, which is typical for urban air, reach the lower respiratory tract where they can be directly absorbed across the thin walls of the alveolar sacs and enter the blood. “Ventilation rate is important because altering the inhalation rate may increase or decrease the amount of the lead ultimately absorbed by the lung” (*Casarett and Doull's, 2001; Lead, 2001).* The absorption rate of lead deposited in the lower respiratory tract is governed by the deposition rate and 30-50% of

Inhaled lead is completely absorbed. A respiratory deposition/ absorption rate of 25-45% has been estimated for children. Absorption via the gastrointestinal tract is highly dependent on the presence of the levels of calcium, iron, fats and proteins. “Fasting and diets with low levels of calcium, vitamin D and iron have been shown to increase lead absorption. 20-70% of ingested lead is absorbed” *(Lead, 2001*). According to *George (1999*), gastrointestinal tract absorption of lead in young children is 42%-48% versus 8%-10% in adults.

When lead reaches the blood, it is distributed primarily among the three compartments: blood; soft tissue such as kidney, bone marrow, liver and brain; and in the mineralizing tissues such as bones and teeth. About 95% of the lead body burden in adults is located in the bones, compared with about 70% in children, acting as a reservoir, where it is in continuous exchange with the soft tissue pools. Some

99% of the lead in the bloodstream is bound to erythrocytes. *Casarett and Doull's (2001)* states that the biological half-life of lead in blood can be as short as 20-40 days, and a steady state is thus achieved in about 6 months, although longer half-time values have been reported in lead workers and may depend on the lead body burden. Lead concentration in bones increases with age and this increase is more noticeable in males in the denser tibia bones. *Casarett and Doull's,(2001)* stipulated that the half-life for lead in bone is approximately 20 to 30 years and lead may be released from the bones in decalcification processes related to elderly people, pregnancy, acidosis, thyrotoxicosis or active remodeling processes in the bones of children. *IPCS (1995)* suggested that lead may be released from bone tissue after the menopause, and clearly higher blood lead levels have indeed been found in postmenopausal than in pre-menopausal women. Its distribution in the body, its affinity for various binding sites, and differences in cellular composition and structure within tissues and organs modulate toxic effects of lead. "As a result there is no single well-defined mechanism that explains the toxicological activity of lead in all tissues" *(Lead, 2001*).

Non-absorbed lead passes through the gastrointestinal tract (GIT) and is excreted in the faeces. In adult subjects, of the absorbed fraction, 50-60% is removed by renal and biliary excretion. Intestinal clearance is about 50% of the renal clearance. Some data indicate that children, particularly infants, retain a higher amount of lead. Lead also is excreted in milk and sweat and is deposited in hair and nails. "Placental transfer of lead also is known to occur" (*Klaassen, 1996).*

2.2 MECHANISMS OF TOXICITY

Lead is a very dynamic element with a wide spectrum of biochemical effects in humans. Lead is a poison that affects virtually every system in the body. Children are more vulnerable to lead exposure than adults because of the frequency of pica, hand-to-mouth activity, and a higher rate of intestinal absorption and retention. “The most deleterious effects of lead are on erythropoiesis, soft tissues, kidney function, and the central nervous system*” (ATSDR, 1993*).

Lead is a divalent cation, and it binds strongly to sulfhydryl groups on proteins rendering them nonfunctional and further contributing to impairment in oxidative balance. “Levels of two specific sulfhydryl containing enzymes that are inhibited by lead – deltaaminolevulinic acid dehydrogenase (ALAD) and glutathione reductase (GR) – have been demonstrated to be depressed in both animal and human lead-exposure studies” *(Brown et al 1983; Holtzman et. al. 1984; Pentschew 1965)*

Of the many organs affected by lead, the most important is the central nervous system (CNS). Much of lead’s toxicity can be attributed to distortion of enzymes and structural proteins, but this versatile toxicant has many other targets. According to World Health Organization (2008) lead interferes with the development of the endogenous opiate system. It efficiently cleaves the ribophosphate backbone of tRNA catalytically at specific sites, with no evidence of a threshold. “Many of lead’s toxic properties are due to its ability to mimic or compete with calcium. At picomolar concentrations, lead competes successfully with calcium for binding sites on cerebellar phosphokinase C and thereby affects neuronal signaling” *(Halterman*

2001). "It inhibits calcium entry into cells" *(Grantham-McGregor et al 2001).* Lead is picked up by mitochondria and produces swelling and distortion of mitochondrial cristae. Uncoupled energy metabolism, inhibited cellular respiration, and altered calcium kinetics follow. "Lead has a binary impact on neurotransmitter release: Spontaneous neurotransmitter release is enhanced, whereas stimulated release is inhibited" *(eMedcine 2006).*

Attention has also focused on the haem synthetic pathway, where many sites for lead activity are found. Delta aminolevulinic acid dehydratase is extremely sensitive to lead. Inhibition of this enzyme results in increased circulating aminolevulinic acid (ALA). ALA is a weak gamma-amino butyric acid (GABA) agonist that decreases GAB release by presynaptic inhibition. Increased circulating ALA may account for some of the behavioral disorders seen in patients with porphyria and perhaps in lead toxicity.

"Lead has diverse impacts on the CNS. Immature astrocytes are sensitive to lead, and lead interferes with myelin formation and the integrity of the blood-brain barrier" *(Krigman 1978*). Lead interferes with the synthesis of collagen and affects vascular permeability. *Pentschew (1965*) states that at high enough doses, this result in brain edema and hemorrhage.

"At lower doses, lead given to lactating rats interferes with synaptogenesis in their pups" *(Averill and Needleman 1980)*. "Lead's interference with brain development has been demonstrated using the rodent barrel field cortex as a model" (*Wilson et. al. 2000).*

Behavioral alterations secondary to lead exposure in rodents and primates are analogous to changes in humans. In one study, monkeys

received lead acetate in their food from birth to 200 days of age and achieved blood lead levels ranging from 3 ¹g/dl to 25 ¹g/dl. At 7 to 8 years, they were given a delayed alternation test, in which the critical positive stimulus was alternated. “Treated monkeys showed impaired ability to learn, particularly at longer intervals of delay” *(Rice 1992*). *Bushnell and Bowman (1979*) showed in their study that lead-exposed primates also demonstrate impaired social function and also that rodent given lead show deficits in learning mediated by dopaminergic and glutamatergic systems. “In one interesting report, untreated rats found 15% solutions of alcohol aversive in a free-choice situation, but when their blood lead levels were raised to 61 ¹g/dl, they increased their alcohol intake in both free- and forced-choice paradigms” *(Nation , Baker and Taylor 1986).* The author speculated that lead increased the irritability of the rats and that they sought alcohol as a tranquilizer.

2.3 EFFECTS OF LEAD ON HAEM BIOSYNTHESIS AND ERYTHROPOIESIS

Hematological indices are very important parameters for the evaluation of physiological state. As an overview, porphyrin biosynthesis begins with the condensation of glycine and succinyl CoA, a tri-cyclic acid (TCA) cycle intermediate, to form amino ketoadipic acid in the presence of the enzyme aminolevulinic acid synthase and vitamin B6. The complex undergoes decarboxylation to form ALA. This reaction occurs in the mitochondria. Subsequently, the compound goes to cytoplasm where two molecules of ALA condense to form porphobilinogen via the dehydratase enzyme. This enzyme is also a zinc-requiring enzyme. “The porphyrin haem ring is formed essentially

by condensation of four monopyrroles synthesized from the Porphobilinogen" *(IPCS,*
1995).

Lead is known to affect several enzymatic reactions critical in haem synthesis, causing abnormal concentrations of haem precursors in blood and urine. Essentially, lead interferes with the activity of three enzymes:

1. It indirectly stimulates the mitochondrial enzyme Aminolevulinic acid synthetase (ALAS);
2. It directly inhibits the activity of the cytoplasmic enzyme Aminolevulinic acid dehydratase (ALAD); and
3. "It interferes with the normal functioning of intramitochondrial ferrochelatase, which is responsible for the insertion of iron (II) into the protoporphyrin ring" *(Timbrell, 2000; Casarett and Doull's, 2001).*

The ALAD enzyme is involved in synthesis of the porphyrin units. *Roels et al (1976)* reported that ALAD inhibition in school-age children first appeared with blood lead concentrations of about 100g/L. Interference by lead with the formation of haem from protoporphyrin is apparent from increased blood and urine levels of free erythrocyte protoporphyrin (FEP) and coproporphyrin III. The FEP combines with Zinc in the blood to form Zinc protoporphyrin (ZPP), which is the moiety assayed. *Hathaway et al., (1991); Gosselin et al., (1984); Marcus and Schwartz (1987)* reported a threshold of 200 g/L on 264 children surveyed by the National Health and Nutrition Examination.

Effects of lead on erythropoiesis and erythrocyte physiology represent more direct signs of damage to the haemopoietic system than haem precursors in blood or urine. Anaemia is a frequent outcome of chronic lead intoxication. “The increases in ALA and FEP are earlier indicators of anemia, which is generally not observed until the blood concentration reaches 40g/L” (*Massaro, 1997*).

Lead often leads to anemia by several mechanisms:

Competing for absorption with the ferrous iron form, inhibiting haem synthesis*;(MedcineNet.com,2000*) and altering the relative composition of cell membranes, including red blood cells, that make them more fragile and likely to haemolyse when passing through tiny capillary spaces *(eMedcine 2009). Bashir et al. (1995*) investigated whether there is correlation between lead in blood, exposure and anemia. These researchers concluded that chronic lead exposure causes normocytic normochromic anemia and showed dose-response relationship between lead levels and severity of anemia. Contrary to this, *Froom et al. (1999*) found that blood samples obtained from 94 workers in lead – acid battery plant in Israel between 1980 and 1993 exceeded 60 g/dL in 14% of the blood samples. They found no correlation between hemoglobin and blood lead levels. These authors suggested that, a diagnosis of anemia in a person with blood lead levels up to 80g/dL should be considered to be due to lead toxicity only after other causes for anemia are ruled out. According *to Hodgkin’s et al (1991), Gittleman et al (1994),Chuang et al (1999), Nuwayhid et al (2001)* some other lead exposure studies in lead acid storage battery workers measured different blood lead levels varied with the nature of the industry (battery manufacturing or recycling units). They also found

that variations in blood levels are influenced by the employment duration, job category, age, gender, smoking habits and ethnicity. “A threshold effect for occupationally exposed adults was estimated at 500 g/L” (*IPCS, 1995*). “Impairment of the erythropoietic system to regenerate from phlebotomy was found in lead–exposed workers with an average blood lead of 445 g/L relative to controls” *(Grandjean et al., 1989)*. The sensitivity of the haem synthesis pathway to increased lead exposure was in the order: children, women, men. On balance it would appear that lead has discernible effects on the urine level of ALA at a blood lead level of around 1.68mol/litre (35 g/dL).

“Lead may be rapidly absorbed and reached considerable amount in the blood” *(Haque et al., 2006).* “Once absorbed, 99% of blood lead is transported to the erythrocytes as lead diphosphate” *(Freeman, 1970).* “Increment of blood lead level -following lead acetate and lead nitrate administration was demonstrated in the experimental animals” *(Ferguson et al., 1998; Sharma et al., 2011d).* Some reports by *Moussa et al (2001*) suggested that this element is strongly bound to macromolecules in the intracellular compartment because lead binding proteins have been isolated from kidney, liver, blood and brain. The half- life of lead differs for each of the compartment, ranging from 25 to 40 days in erythrocytes, 40 days in soft tissues and as many as 28 years in bone.

Erythrocytes have a high affinity for lead, binding 99 percent of the lead in the bloodstream. Lead has a destabilizing effect on cellular membranes, and in red blood cells (RBC) the effect decreases cell membrane fluidity and increases the rate of erythrocyte hemolysis. Hemolysis appears to be the end result of ROS-generated lipid

peroxidation in the RBC membrane*. Lawton, Donaldson (1991). Shafiq-ur-Rehman and Abdulla (1993)* reported that lead can also bind directly to phosphatidylcholine in the RBC membrane, leading to a decrease in phospholipid levels. “Lipid peroxidation of cellular membranes has also been identified in tissue from various regions of the brain of lead-exposed rats” (*Flora and Seth 2000*)

“Hypochromic or normochromic anemia is a hallmark of lead exposure; it results from ROS generation and subsequent erythrocyte hemolysis” *(Gurer and Ercal 2000*). “Lead is considered, along with silver, mercury, and copper, to be a strong hemolytic agent, able to cause erythrocyte destruction through the formation of lipid peroxides in cell membranes” (*Ribarov and Benov 1981*). In addition to membrane peroxidation, lead exposure causes hemoglobin oxidation, which can also cause RBC hemolysis. The mechanism responsible for this reaction is lead-induced inhibition of ALAD. ALAD is the enzyme most sensitive to lead’s toxic effects – depressed heme formation. “As a result, elevated levels of the substrate ALA are found in both the blood and urine of lead-exposed subjects” (*Farant and Wigfield 1982)*. “These elevated levels of ALA generate hydrogen peroxide (H2O2) and superoxide radical (O2 -), and also interact with oxyhemoglobin, resulting in the generation of hydroxyl radicals (OH), the most reactive of the free radicals” *(Bechara 1996*). As ALA is further oxidized, it becomes 4,5-dioxovaleric acid.

“The exact rate of clearance of lead from the blood of an exposed individual varies among species and among exposure levels, but follows a generally predictable pattern” (*Todorovic et al. 2008, Hunt et al. 2009)*. At lower levels of exposure (2-5 g/dL in blood), lead

decreases below detectable levels in the blood within roughly a week. “After moderate to high levels of initial exposure (10-70 g/dL in blood), blood lead levels (BLLs) decline quickly over the first two weeks to one month” (*Miranda et al. 2006, Craighead and Bedrosian 2008*). “This is followed by a decrease in the rate of decline as lead deposited in bone and soft tissues begins to leach back into the bloodstream” *(Gwiazda et al. 2005, Miranda et al. 2006)*. “As BLLs begin to decrease after initial exposure, lead begins to seep into soft tissues, particularly the liver and kidneys, and is present and detectable at elevated levels for 3-6 months after initial exposure” (*Dinius et al. 1973, Sharma et al. 1982, Todorovic et al. 2008).*

2.4 EFFECTS OF LEAD ON THE NERVOUS SYSTEM

Gluckman and Hanson (2004) stated that many adult neurological diseases and degenerative disorders arise as a result of fetal and infantile exposure to environmental toxins. “Therefore, because lead most directly affects the developing nervous system for both pre- and post-natal offspring” *(Schnaas et al. 2006*), lead exposure during youth has a long-term negative consequence. “In humans, lead causes damage to a variety of organs, particularly the central nervous system” (*Massaro, 1997)*. The mechanisms for lead neurotoxicity are not well understood. One of the mechanisms by which lead may affect brain physiology and biochemistry could be due to either a direct influence upon nerve endings or by influence on neurotransmitter release in some fashion. “Low lead concentrations enhance the release of neurotransmitter from presynaptic endings” (*Cooper et al., 1984*). “Lead has been shown to stimulate the release of dopamine,

acetylcholine, and gammaaminobutyric acid (GABA)" *(Minnema et al., 1988*). Some of these effects may be due to the ability of lead to alter calcium entry into nerve cell or by an increase in the intracellular calcium levels. Lead may enter cells through calcium channels. Calcium may also competitively inhibit lead uptake in non-excitatory cells such as the adrenal medulla and cannot be discounted as the potential mechanism in nerve cells. "Calcium channel blockers likewise may inhibit lead uptake in the same cells" *(Simons and Pocock, 1987*.)

Lead is known to substitute for calcium as a second messenger and can bind to calmodulin. In fact, calmodulin has a greater affinity for lead than for calcium. The calcium calmodulin complex may activate a kinase referred to as calmodulin dependent protein kinase, which is high in nervous tissue and may regulate neurotransmitter release. Synapsin I when phosphorylated is believed to have a role in neurotransmitter release. "If lead acts as calcium in the activation of this kinase, this may explain the ability of lead to result in neurotransmitter release" (*Massaro, 1997*). Theoretically, these effects are reversible if lead is removed from the synapse. However, exposure to lead for a long time may result in permanent alteration in cellular responsiveness at pre-synaptic levels. For over a decade, the hippocampus has been considered to be the principal target of lead in the brain because:

1. The hippocampus contains relatively high concentrations of zinc, and zinc-dependent functions may be sensitive to lead as lead is divalent metal and often competes with divalent ions such as zinc with respect to biochemical processes,

2. The hippocampus contains a dense plexus of cholinergic fibers that are affected by lead exposure, and
3. "The hippocampus functions in memory and learning" *(IPCS, 1995).*

"Lead appears to impair hippocampal voltage potentials through protein kinase C. Protein kinase C, which is very sensitive to lead, modulates receptor currents affecting long-term potentiation and other forms of synaptic response that may underlie learning and memory" *(Massaro, 1997*). Lead may serve to activate this kinase and consequently inhibit this potential, thereby impairing memory and learning. Repeated lead exposure of moderate to high levels can cause encephalopathy (a progressive degeneration of certain parts of the brain). Early symptoms of encephalopathy include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations.

"More severe symptoms occur at very high exposures and include delirium, lack of coordination, convulsions, paralysis, coma and death" *(Lead, 2001).* The encephalopathy induced by lead toxicity is most likely due to a compromise in blood brain barrier. Brain edema occurs in the interstitial area and appears due to compromised blood vessel integrity. "The brain capillaries and blood vessels have endothelial cells that contain tight junctions and act as a seal or barrier that excludes many plasma proteins and organic molecules and impedes Na+ and K+ exchange" (*Bradbury, 1984*). Elevated lead levels disrupt these vessels, and plasma proteins such as albumin enter the interstitial spaces, as do some ions. This increases osmotic pressure, and water accumulates in response. The lack of lymphatic structures

within the central nervous system means that the fluid flows into the cerebrospinal fluid. “This edema causes an increased intracranial pressure and restricts blood flow to the brain, resulting in ischemia” *(Goldstein et al., 1974).*

The direct mechanism by which the blood brain barrier and blood vessels that compose the barrier are compromised may be due to the astrocytes appearing to be vulnerable to the toxic effects of lead. The astrocytes cover the vascular walls of the brain vessels, and the permanent neuropsychological deficits seen after exposure to high lead concentrations may be due to impairment of astrocytes function especially in their capacity to regulate the ionic and amino acid concentrations in the extracellular milieu, brain energy metabolism and cell volume.

Encephalopathy has been observed in adults at blood lead levels exceeding 1200 g/L, in children at levels of 800-1000g/L. “The outcome is frequently fatal in children and those who survive often present with irreversible neurological and neuropsychological sequelae*” (Perlstein and Attala, 1966*).

“Consistent neurobehavioral effects, which are to be considered as ‘adverse’ include recurrent seizures, confusion, dizziness, lethargy, headache, disorientation and other CNS symptoms appear in a multiplicity of studies at lead blood levels of 40 g/dL*” (Haenninen et al., 1979; Fischbein et al., 1980; Awad El Karim et al., 1986*). The dose dependency of impairments in performance in neurobehavioral tests, in relation to blood lead levels, may also be drawn from the studies *of Mantere et al. (1982) and Stollery et al. (1991).* Lead induces degeneration of the protective Schwann cells in the motor neurons of

the peripheral nervous system (nerves of the arms and legs). This causes segmental loss of the myelin covering of the neuron, which leads to decreased nerve conduction velocities and possible neuron degeneration. Lead induces a breakdown in the blood-nerve barrier, allowing lead and fluid to enter the endoneurium and disrupt the myelin membranes. The degeneration of sciatic and tibia nerve roots is also possible. Sensory nerves are less sensitive to lead than motor nerves. Peripheral neuropathy is usually, present only after prolonged high exposure to inorganic lead compounds. “This disorder is often referred to as "lead palsy" and symptoms include weakness of the arms and legs (foot drop) and weakness and paralysis of the wrist (wrist drop), fingers and ankles” *(Lead, 1991*). “A study of occupationally exposed workers indicates that motor nerve dysfunction can occur at blood-lead levels below 70 g/dL, possibly as low as 30g/dL” *(Seppalainen et al., 1983*), when assessed clinically by the electrophysiological measurement of nerve conduction velocities. There is some evidence that these effects may be reversible following a 5-month exposure. “However, only partial recovery may occur, particularly if lead exposure continues or treatment is not carried out” (*IPCS, 1995*).

Circulating and excreted levels of Aminolevulinic acid (ALA) are likely to be best described as a continuum of effect. “Elevated levels of this compound are of importance since neurological features of lead exposure have been ascribed in part to increased circulating levels of ALA” *(IPCS, 1995).*

“Lead interferes with the release of neurotransmitters, chemicals used by neurons to send signals to other cells” (Dart *et. al.* 2004). It

interferes with the release of glutamate, a neurotransmitter important in many functions including learning, by blocking NMDA receptors. “The targeting of NMDA receptors is thought to be one of the main causes of lead toxicity to neurons*” (Xu, et. al (2009*). A John Hopkins report found that in addition to inhibiting the NMDA receptor, lead exposure decreased the amount of gene for the receptor in part of the brain. “In addition, lead has been found in animal studies to cause programmed cell death in brain cells” *(Needleman 2004).*

2.5 TOXICITY OF OTHER BODY ORGANS

Lead is known to cause proximal renal tubular damage, characterized by generalized aminoaciduria, hypophosphataemia with relative hyperphosphaturia and glycosuria accompanied by nuclear inclusion bodies, mitochondrial changes and cytomegaly of the proximal tubular epithelial cells*. Goyer and Ryne (1973)* reported that the proximal tubules of the nephron are lead sensitive. It is manifested clinically by decrease in energy dependent transport functions, including aminoaciduria, hypophosphataemia, glycosuria, and ion transport. “Tubular effects are noted after relatively short-term exposures and are generally reversible, whereas sclerotic changes and interstitial fibrosis, resulting in decreased kidney function and possible renal failure, require chronic exposure to high lead levels” *(Lin et al. 2003)*. The renal proximal tubules are rich in mitochondria and swelling of mitochondria can be observed at a relatively low lead concentrations. The functional changes are thought to be related to a lead effect on mitochondrial respiration and phosphorylation. In

exposed workers distortions of the proximal tubules have been noted after a couple of months of exposure. Typical measures of renal failure, e.g. blood urea nitrogen (BUN) and creatinine are elevated as a consequence of lead-induced renal failure.

Lead toxicity is known to influence male and female reproductive organs in humans. At very high blood lead levels, lead is a powerful abortifacient. “At lower levels, it has been associated with miscarriages and low birth weights of infants” *(Nordstrom et. al. 1979)*. “Increased incidences of spontaneous abortions have been documented in female lead workers and also in the wives of male lead workers*” (Landrigan, 1991)*. A few epidemiological studies have been performed on the association between paternal exposure to lead and adverse reproductive outcome. “The results suggest an increased risk of spontaneous abortion, prenatal death and low birth weight following paternal occupational lead exposure*” (Lindbohm et al., 1991; Kristensen et al., 1993*). “In a Finnish study, a significant increase was observed in the risk of spontaneous abortion among the wives of men whose blood lead was 30 g/dL or higher during spermatogenesis” *(Lindbohm et al, 1991).* “Reduced fertility has also been reported for men with a long duration of lead exposure*” (Lin e t al.1996). Coste et al. (1991)* conducted a cohort study in a French battery factory from 1977-1982 to explore the relationship between occupational exposure to lead and fertility. Findings of this study were that lead exposure at any level of absorption did not appear significantly associated with a reduction in fertility after controlling confounding factors such as age, French origin, and educational level, number of children at start of the period of work, cigarette smoking and exposure to heat. “Current

thinking is that significant effects on reproductive capacity are not seen below a blood lead level of ≥50 g/100 ml, but blood lead concentrations of >40 g/100 ml may affect sperm morphology and function" *(Apostoli.et. al.1998).*

Lead poisoning occurs as a result of ingestion or inhalation of inorganic lead particles or through transdermal absorption of organic alkyl lead. The respiratory tract provides the most effective route of absorption as it only depends on the size of lead particles and on the metabolic activity of the body. Airborne lead particles that are less than 0.5 to 1 microns in diameters are generally completely absorbed by the alveoli. "General population studies did not find associations between blood lead levels and cardiovascular morbidity and mortality" *(Pocock et al., 1988)*. The effect of lead on the heart is indirect and occurs via the autonomic nervous system; it has no direct effect on the myocardium. The collective evidence from population studies in adults indicates very weak associations between Pb blood concentration and systolic or diastolic blood pressure. Given the difficulties of allowing for relevant confounding factors, a causal relationship cannot be established from these studies. There is no evidence to suggest that any association of Pb blood concentration with blood pressure is of major health importance*. Al-Saleh et al. (2003), Chen et al. (2005), Wu et al. (1996)* assessed the relationship between occupational lead exposure and elevated blood pressure with consideration of a possible confounding effect of noise exposure. It should be noted that studies done by these authors showed no relation or correlation between lead exposure and blood pressure*. Gerr et al. (2002)* in their study that investigated the association between bone lead concentration and

blood pressure among young adults concluded that substantial lead exposure during childhood could increase blood pressure during young adulthood.

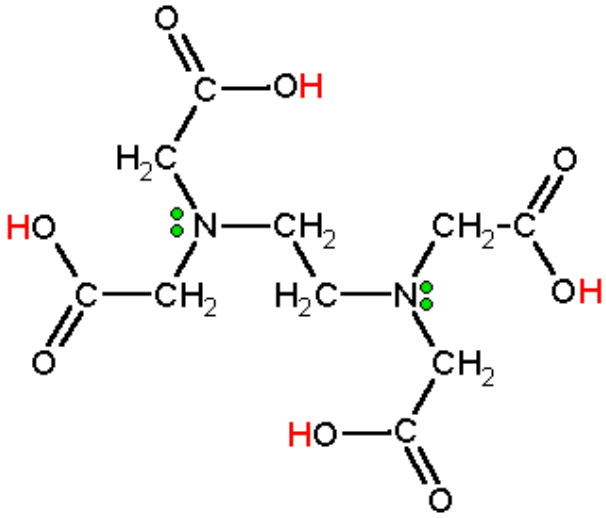
2.6 ETHYLENEDIAMINE TETRAACETIC ACID (EDTA)

EDTA is the anticoagulant of choice in haematology. Anticoagulants are substances that prevent blood from clotting. An ethylenediamine tetraacetic acid (EDTA) anticoagulant, therefore, uses this type of acid to stop the clotting process. The EDTA anticoagulant is often used in laboratory diagnostic tests, such as the full blood count (FBC), because it retains the original shape and size of the cells. This is because the EDTA anticoagulant can bind with the calcium present in blood, thus preventing the start of the coagulation cascade, which is the process by which blood clot formation occurs. Depending on the type of test to be done, the tube of blood collected for a test, may come with an anticoagulant inside. The tube that comes with an EDTA anticoagulant is commonly seen having a lavender cover or cap. EDTA often is used in the FBC tests for haemoglobin, packed cell volume, red blood cell (RBC) count, white blood cell (WBC) count, platelet count, and lymphocyte differential count.

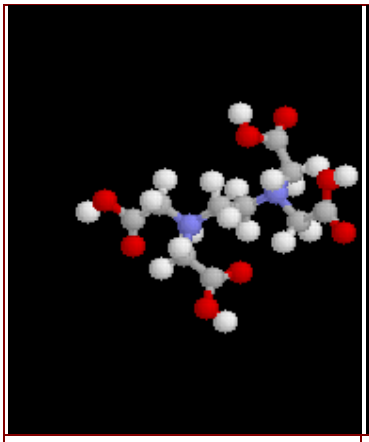
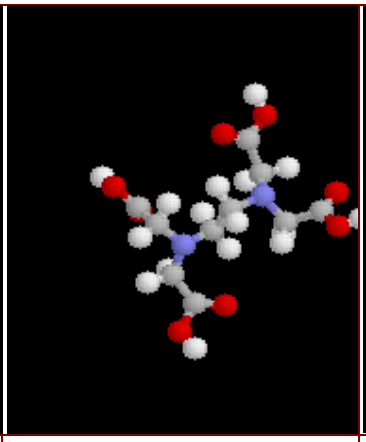
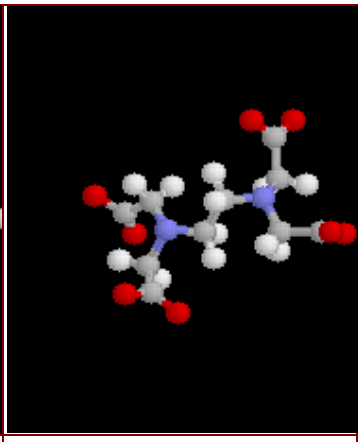
2.7 THE EDTA MOLECULE

EDTA or ethylenediaminetetraacetic acid is a novel molecule for complexing metal ions. It is a polyprotic acid containing four carboxylic acid groups (acidic hydrogens are red) and two amine groups with lone pair electrons (green dots). The classic structural formula is given below. EDTA is synthesized on an industrial scale from

ethylenediamine, formaldehyde, and a source of cyanide (HCN or NaCN).



Besides the four carboxylic group hydrogens, EDTA can add two more hydrogens onto the amine groups. The structures of the fully protonated form (left), the typical form found in many textbook (center, matching the 2D structure above), and the fully deprotonated (all acidic H's removed) form (right) are given below.

		
forms at very low pH or very acidic condition (fully protonated) H_6Y^{+2}	classic form H_4Y	forms at very high pH or alkaline conditions (fully deprotonated) Y^{-4}

The unusual property of EDTA is its ability to chelate or complex metal ions in 1:1 metal-to-EDTA complexes. The fully deprotonated form (all acidic hydrogens removed) of EDTA binds to the metal ion. The equilibrium or formation constants for most metals, especially the transition metals, are very large; hence the reactions are shifted to the complex. Many of the reactions are pH dependent, especially the weaker forming complexes with Ca^{+2} or Mg^{+2}.

2.71 USES OF EDTA

EDTA is a prescription medicine, given by injection into the vein (intravenously) or into the muscle (intramuscularly). EDTA has strong complexing ability for most metal ions and because of this it is used in the food industry as a sequestering agent. The complexing of the metal ion may prevent further reactions, such as binding metals that are cofactors for enzymes, or just remove a metallic taste, such as metal contamination added during processing. Some typical examples are given below. Recent studies have shown that NaFeEDTA and Na_2EDTA added to typical iron fortification compounds in cereals increased the absorption of iron in adult humans. This same property allows EDTA use for incidents of lead poisoning by the medical profession. The formation constant for Pb-EDTA complex is 10^{18}. Intravenous injection of Na_2CaEDTA solution is given at 25 mg/kg body mass/day over 6 hours for 5 days when blood lead levels go over 45 mg/dL. The Pb^{+2} ions replace the Ca^{+2} ions in the complex because the formation constant for the lead complex is greater than the calcium complex. EDTA is added to many commercial beers to stabilize foaming, taking advantage of the surfactant properties of

EDTA, and used to remove scale by complexing calcium from calcium carbonate that forms on the processing equipment. Calcium di sodium EDTA is used to protect quality of the product in HELLMANN'S real mayonnaise. EDTA is sometimes used as an ointment for skin irritations produced by metals such as chromium, nickel, and copper. Eye drops containing EDTA are used to treat calcium deposits in the eye. In manufacturing, EDTA is used in calcium and sodium compounds to improve stability in pharmaceutical products, detergents, liquid soaps, shampoos, agricultural chemical sprays, oil emulsion devices, contact lens cleaners and cosmetics. It is also used in certain blood collection tubes used by medical laboratories.

2.72 EDTA AS A CHELATING AGENT

Chelation therapy is a mainstream treatment used to treat heavy metal poisoning. It can also be defined as a treatment that involves repeated intravenous (IV) administration of a chemical solution of ethylenediaminetetraacetic acid, or EDTA. It is used to treat acute and chronic lead poisoning by pulling toxins (including heavy metals such as lead, cadmium, and mercury) from the bloodstream It most often involves the injection of ethylene diamine tetraacetic acid (EDTA), a chemical that binds, or chelates, heavy metals, including iron, lead, mercury, cadmium, and zinc. The term "chelation" comes from the Greek word chele, which means "claw," referring to the way the chemical grabs onto metals. ." EDTA has a claw like molecular structure that binds to heavy metals and other toxins.Chelation therapy is one of several effective treatments for lead poisoning. . Chelation therapy can be toxic and has the potential to cause kidney damage, irregular

heartbeat, and even death. Chelation therapy using EDTA has been approved by the U.S. Food and Drug Administration (FDA) as a treatment for lead poisoning for more than forty years. The human body cannot break down heavy metals, which can build up to toxic levels in the body and interfere with normal functioning. EDTA and other chelating drugs lower the blood levels of metals such as lead, mercury, cadmium, and zinc by attaching to the heavy metal molecules, which helps the body remove them through urination.

2.721 HISTORY OF EDTA AS A CHELATING AGENT

Chelating agents were introduced into medicine as a result of the use of poison gas in World War I. The first widely used chelating agent, the organic dithiol compound dimercaprol (also named British anti-lewisite or BAL), was used as an antidote to the arsenic-based poison gas, lewisite. The sulphur atoms in BAL's mercaptan groups strongly bind to the arsenic in lewisite, forming a water-soluble compound that entered the bloodstream, allowing it to be removed from the body by the kidneys and liver. BAL had severe side-effects. After World War II, a large number of navy personnel suffered from lead poisoning as a result of their jobs repainting the hulls of ships. The medical use of ethylenediaminetetraacetic acid (EDTA) as a lead chelating agent was introduced. Unlike BAL, it is a synthetic amino acid and contains no mercaptans. EDTA side effects were not considered as severe as BAL.“In the 1960s, BAL was modified into DMSA, a related dithiol with far fewer side effects*.” (Linang et al 1993*). DMSA quickly replaced both BAL and EDTA, becoming the US standard of care for the treatment of lead, arsenic, and mercury poisoning, which it remains today. “More

recently, esters of DMSA have been developed which are reportedly more effective; for example, the monoisoamyl ester (MiADMSA) is reportedly more effective than DMSA at clearing mercury and cadmium.' *(Linang et al 1993).*Research in the former Soviet Union led to the introduction of DMPS, another dithiol, as a mercury-chelating agent. The Soviets also introduced ALA, which is transformed by the body into the dithiol dihydrolipoic acid, a mercury- and arsenic-chelating agent. DMPS has experimental status in the US FDA, while ALA is a common nutritional supplement. "Since the 1970s, iron chelation therapy has been used as an alternative to regular phlebotomy to treat excess iron stores in people with haemochromatosis*." (Vimy et al 1985).*Other chelating agents have been discovered. They all function by making several chemical bonds with metal ions, thus rendering them much less chemically reactive. The resulting complex is water-soluble, allowing it to enter the bloodstream and be excreted harmlessly. "Calcium-disodium EDTA chelation is approved by the U.S. Food and Drug Administration (FDA) for treating lead poisoning and heavy metal toxicity*." (Vimy et al 1985).*The chemical solution most often used in chelation therapy, EDTA, was first made in Germany in the 1930s. It is now widely accepted as an effective treatment for heavy metal poisoning.

In the 1950s, some scientists theorized that EDTA could remove calcium from the body. Calcium can build up on artery walls, eventually causing heart disease, and it was theorized that use of EDTA could unclog blocked arteries. In some early studies, researchers reported positive results among patients with heart disease who received EDTA. Some said that chelation therapy relieved chest pain caused by blocked

arteries. These first observations have not been confirmed by larger, more rigorous studies. Chelation therapy is a proven treatment for lead poisoning and poisoning from other heavy metals. However, available scientific evidence does not support claims that the treatment benefits patients with cancer, heart disease, or any medical problems other than heavy-metal poisoning.

2.722 USES

Chelation therapy using EDTA is the medically accepted treatment for lead poisoning. Injected intravenously and once in the bloodstream, EDTA traps lead and other metals, forming a compound that the body can get rid of in the urine. The process generally takes 1 - 3 hours. Other heavy metal poisonings treated with chelation include mercury, arsenic, aluminum, chromium, cobalt, manganese, nickel, selenium, zinc, tin, and thallium. Chelating agents other than EDTA are also used to clear several of these substances from the bloodstream. EDTA is a synthetic chemical and not found naturally. Because there is concern that EDTA may deplete important vitamins and minerals, EDTA chelation therapy is often given with essential nutrients (including calcium, B vitamins, vitamin C, and magnesium).

2.723 DOSAGE

Pediatric

For the treatment of lead poisoning: A doctor may give EDTA intravenously (IV) in a clinic or hospital. The dose depends on the amount of lead in the child's blood, as well as the child's height and weight. Daily treatment for up to 5 days may be required.

Adult

For heavy metal toxicity: EDTA chelation therapy is often given intravenously with calcium, magnesium, and vitamins B and C over a period of 1 - 3 hours. The recommended adult dosage varies depending on the size of the person and the amount of lead or other metal in the body. For an average sized person, the amount may range from 700 - 3,500 mg every 12 hours until the substance is significantly reduced in the bloodstream. The amount used would be determined in a hospital setting.

Precautions:

The most common side effect is a burning sensation at the site of the injection. In addition, some people may have an allergic reaction to EDTA. Other serious side effects that have been reported include low blood sugar, diminished calcium levels, headache, nausea, dangerously low blood pressure, kidney failure, organ damage, irregular heartbeat, seizures, or even death. According to the Centers for Disease Control and Prevention (CDC), there have been deaths associated with hypocalcemia (low levels of calcium) from intravenous chelation therapy.A qualified health care provider will monitor blood pressure, blood glucose, cholesterol, organ function, and other vital statistics during treatment with EDTA. EDTA may lower levels of nutrients such as calcium, zinc, and potassium. Your health care professional will perform blood tests to monitor vitamin and mineral levels before, during, and after EDTA chelation therapy. Supplements of vitamins and minerals, either orally or intravenously, may be given when needed.

CHAPTER THREE

MATERIALS AND METHODS

The study was conducted with paint workers in Enugu City. One Hundred (100) paint workers who had been on the job for more than one year were recruited from Abakpa and Uwani Enugu.

3.1 SAMPLE COLLECTION

Questionnaires were used to collect personal information about each worker including name, age, sex, duration of exposure, place of domicile and persisting health problems.

Medical Laboratory Scientists were used to collect 5ml samples of blood from each worker by venepunture using vaccutainer tubes and needles. 2.5ml of the sample was allowed into a plain vaccutainer tube and immediately the capillary tube for PCV was filled, the dilution for total white cell count was performed, the dispette for ESR estimation was also filled, the hemoglobin was estimated using the heamocue machine and thin blood film prepared immediately, while the second sample was collected into an EDTA vaccutainer tubes. The second sample was analyzed after the blood collection in the laboratory for PCV, HB, ESR, total white cell count and thin blood film was also made. After analysis the serum and plasma from both the plain and EDTA tubes were separated and analyzed for lead using the atomic spectrophotometer. Care was taken to avoid contamination during sampling by cleaning the skin area with methanol swab before collection and the use of clean, sterile vaccutainer tubes and needles was ensured.

The same procedure was performed for the control group who were office workers whose offices were not near the factories that use lead. They had never worked in an industry or organization that uses lead or any heavy metal in their work place. All the samples were analyzed using standard operating procedures.

3.2 METHOD

3.21 PACKED CELL VOLUME:

The method used for the PCV estimation is micro method which uses capillary tubes as stated in Dacie and Lewis (2012).

PRINCIPLE:

Anti coagulated whole blood is centrifuged and the volume occupied by the erythrocyte is expressed as a percentage of the total volume.

PROCEDURE:

1. Blood was collected into the capillary tubes through capillary action to about three quarter full.
2. One end of each capillary tube was sealed using a sealant to avoid spilling during centrifugation.
3. The filled capillary tubes were spun according to the manufacturer's instruction for five minutes with adequate precaution to prevent breakage.
4. After the centrifuging, the capillary tubes were read using the graph PCV reader.
5. The normal African ranges are, males-40-54%, females-36-46%.

3.22 ERYHROCYTE SEDIMENTATION RATE

The westergren method was used as stated in Dacie and Lewis (2012). It is a common hematology test, and is a non-specific measure of inflammation.

PRINCIPLE:

The Erythrocyte Sedimentation Rate (ESR) is a nonspecific assay used to screen for the presence or absence of active disease. The settling of red corpuscles (red blood cells - RBCs) is due to the differential densities of the RBCs and their medium. Most often, an increased ESR is due to an increased amount of plasma proteins (i.e., acute phase globulins and less commonly to inherent characteristics of RBCs (Wintrobe 30). ESR is measured in mm/hr using the Modified Westergren Method.

PROCEDURE:

1. Blood was filled into the plastic ESR cups already containing 0.4mls of sodium citrate anticoagulant till the indicated mark.
2. The cover was replaced and the test set up according to the manufacturer's instructions.
3. The tubes were placed in the ESR rack and left to stand for 1 hour undisturbed in a flat surface away from sunlight and vibration and adequate precautionary measures taken
4. The normal /reference values are male-0-10 mm /1st hr, females-0-15 mm /1st hr.

3.23 TOTAL WHITE CELL COUNT

The manual visual method is used as stated in Dacie and Lewis (2012).

PRINCIPLE:

Free-flowing capillary or well-mixed anticoagulated (EDTA) venous blood is added to a diluent (ammonium oxalate) at a specific volume in the Unopette reservoir. The diluent lyses the erythrocytes but preserves leukocytes and platelets. The diluted blood is added to the hemacytometer chamber. Cells are allowed to settle for 10 minutes before leukocytes and platelets are counted.

PROCEDURE:

1. 0.38mls of Turks solution was measured and placed into clean glass tubes
2. 0.02mls of blood was added using an automatic pipette and mixed.
3. The counting chamber was assembled (new improved neubauer chamber with bright lines), making sure that the central grid areas of the chamber and the special haemocytometer cover glass are completely clean and dry.
4. The cover glass was in position over the ruled areas and pressed down until rainbow colors (Newton's rings) were seen.
5. The diluted sample was remixed and with a Pasteur pipette at an angle of 45^{o} the neubauer chamber was filled. Care was taken not to overfill the chamber.

6. The chamber was left undisturbed for about 2 minutes in a Petri dish containing a dampened tissue to prevent drying so that the cells will settle.
7. The cells were counted in the four corners of the chamber containing the big squares.
8. The numbers of white cells counted per liter of blood were reported.
9. The normal range is adults 4.0-10.0x10^9/l, for Africans 2.6-8.3x10^9/l.

3.24 DIFFERENTIAL WHITE CELL COUNT

This was done using thin blood films that were made and stained with leishman stain as stated in *Dacie and Lewis (2012).*

PRINCIPLE:

A stained smear is examined in order to determine the percentage of each type of leukocyte present and assess the erythrocyte and platelet morphology. Increases in any of the normal leukocyte types or the presence of immature leukocytes or erythrocytes in peripheral blood are important diagnostically in a wide variety of inflammatory disorders and leukemia

PROCEDURE:

1. A thin blood film was made using a spreader, allowed to dry in air and stained in leishman.
2. The stain was washed off with buffered distilled water and dried in air.
3. The film was inspected using X100 oil immersion lens.

3.25 HAEMOGLOBIN CONCENTRATION

This was determined using the azidemethemoglobin method for hemoglobin determination in blood*. (J. Lab. Clin. Med. 67, 116-26 1966).*

PRINCIPLE:

The reaction in the microcuvette is a modified azidemethemoglobin reaction. The erythrocyte membranes are disintegrated by sodium deoxycholate, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combines with azide to form azidmethemoglobin. The photometer uses a double wavelength measuring method, 570 nm and 880 nm, for compensation of turbidity.

PROCEDURE:

1. The tip of the Haemocue microcuvette which is yellow in color is dipped in the blood and the excess blood wiped off.
2. The microcuvette is then placed inside the haemocue and the port closed.
3. The result is displayed within 60 seconds on the screen.

3.26 LEAD ASSAY

The Kench method (1940) was used.

PRINCIPLE:

It uses wet oxidation of organic matter followed by estimation of the lead as a complex with dithizone after extraction with sodium diethyldithiocarbamate.

3.3 ANALYSIS

The hematological parameters of the samples were estimated at the hematology section of the department of laboratory services National Orthopedic Hospital Enugu, while the plasma and serum were extracted after the analysis of the hematological parameters and sent to biological sciences department of the University of Nigeria Nsukka where the lead concentration was estimated using the wet oxidation technique of the Kench method.

3.4 STATISTICAL ANALYSIS

All findings were tabulated and analyzed. Statistical analysis involved the use of the statistical package for social sciences (SPSS 16.0 version). Data are expressed as the mean± standard deviation (SD). The mean value (χ) and standard deviation (SD) were calculated for each variable measured and were analyzed statistically using one sample T-TEST to determine significant differences between groups at $p<0.05$. The analysis and comparison were evaluated for significance at 5% ($\alpha=0.05$). *(Hinton 2004).*

CHAPTER FOUR

RESULTS

Table 4.1 compares the values of the hematological parameters of the test group and control group. From this table we can see that the values of the test group for both the anti coagulated blood and direct blood was higher than that of the control group. This could be attributed to the age difference between the test group and control group. All the values from the direct samples were significantly higher than those from the anti coagulated samples. This raises a question why?

The quantity of EDTA in the tubes was minute and the equivalent quantity of blood was added, this rules out the issue of over dilution. The two samples were investigated simultaneously, this also rules out the effect of long storage. No literature was found to explain this.

Table 4.1: Comparison of lead concentration and hematological parameters in test group and control group.

PARAMETERS	TEST SUBJECTS n=100	CONTROL SUBJECTS n=50	CALCULATED t-VALUE	CRITICAL t-VALUE	P VALUE
PCV EDTA %	41.17±4.69	37.67±4.21	51.136	1.98	<0.05
PCV DIRECT%	44.03±3.99	39.67±4.23	63.258	1.98	<0.05
WBC EDTA /MM³	5613.9±1413.65	5183.3±1616.49	23.494	1.98	<0.05
WBC DIRECT /MM³	6727.8±1749.07	5658.3±1759.95	22.756	1.98	<0.05
ESR EDTA /1ST HR	11.91±10.18	11.50±9.87	7.229	1.98	<0.05
ESR DIRECT /1ST HR	12.30±11.26	11.62±10.23	7.111	1.98	<0.05
HB EDTA g/dl	13.63±1.45	12.48±1.24	17.045	1.98	<0.05
HB DIRECT g/dl	14.48±1.31	13.30±1.18	21.086	1.98	<0.05
LEAD EDTA µg/100ml	6.52±2.09	3.41±1.22	18.53	1.98	<0.05
LEAD DIRECT µg/100ml	7.31±2.31	3.49±1.25	18.66	1.98	<0.05

Table 4.2 compares PCV, WBC, and ESR values in lead handlers classified based on lead concentration alongside controls using a one way ANOVA for the analysis. In this table the lead handlers were divided into two classes, class 1 with a lead concentration of greater than eight (<8) and class II with a lead concentration of less than eight (>8). Here we can see that as the concentra□on of lead increases its effects on the hematological parameters is reduced. This is also dependent on the duration of exposure and length of time of work

Table 4.4: Comparison of PCV, WBC, and ESR in lead handlers classified based on lead concentration alongside controls using a one way ANOVA for the analysis.

PARAMETERS	LEAD CONC CLASS 1 n=60 (conc.>8)	LEAD CONC CLASS II n=40 (conc.<8)	CONTROL n=50	CRITICAL F-VALUE	CALCULATED F-VALUE
PCV	39.70±5.07	45.62±4.05	36.50±3.82	2.45	7.95
WBC	5520.00±1461.29	6031.25±1436.90	5250.33±1605.51	2.45	7.95
ESR	10.55±7.87	15.69±11.47	8.55±6.61	2.45	7.95
HB	13.23±1.69	15.21±1.35	12.17±1.27	2.45	7.95

CHAPTER FIVE

5.1 DISCUSSION

A lot of work has been done on lead in the human system before now but no work has been done to ascertain the effect of the anticoagulant especially EDTA on the lead content already in the blood. This informed the decision to carry out this research, effect of EDTA on lead among occupational lead handlers in Enugu urban, Nigeria.

In this research, we first estimated the lead content of both the serum and plasma of the occupational lead workers, with the aim to find out if collecting blood in EDTA containers will affect the lead content. We went further to analyze both the samples collected in EDTA and plain tubes (direct samples) to find out what their hematological parameters were and to know if the blood lead content had any effect. The same was done for the control group who were office workers that were not exposed to lead.

From the results we found out that the lead content of those occupational lead workers were almost all in the class 1 group according to CDC classification of lead poisoning which have a lead level of <10µg/dl. The lead content of the serum for each patient was found to be higher than that of the plasma. This is due to the chelation that occurred in the EDTA tubes as the hematological parameters of the blood were being estimated. The difference between the lead content of the serum and the plasma though significant was not in excess because of the small quantity of EDTA in the tubes and the length of time the blood stood before the plasma was separated.

Research has shown that the drug of choice for lead poisoning, in fact heavy metal poisoning, is intravenous injection of EDTA. The EDTA works by chelating the lead in the blood by binding with it and forming complexes that can be excreted in urine.

In the control group, the lead content of the serum was also higher than that of the plasma but was much lower than that of the occupational lead workers. This raised a question: why was lead in the sample of the control group at all?

Due to the fact that in Nigeria we don't have a functional Environmental Protection Agency, a lot of toxic materials are used and we are continually being exposed to them. This is the case with the lead assimilation. We use leaded paints on our buildings and this is more common in the low income areas where the evidence is seen in the peeling paints on the walls of the buildings. Again the petroleum we use here in the country is not free of lead and as the vehicles use it and combustion is taking place, a lot of lead fumes are released into the air and we unknowingly breathe it in. Most of the pencils used in the most recent past were made with lead, and we unknowingly chew on the pencil tips especially when deep in thoughts and our children also chew their pencil tips. These are all ways through which lead gets assimilated into the system of those not occupationally exposed to lead.

The hematological parameters estimated showed that the values obtained from the fresh blood were significantly higher than that derived from the anti coagulated blood. This also occurred in the result of the control group.

Table 4.1 compares the values of the hematological parameters of the test group and control group. From this table we can see that the values of the test group for both the anti coagulated blood and fresh blood was higher than that of the control group. This could be attributed to the age difference between the test group and control group.

All the values from the direct samples were significantly higher than those from the anti coagulated samples both of the test group. This pattern is also seen in the control group. This raises a question why?

The quantity of EDTA in the tubes was minute and the equivalent quantity of blood was added, this rules out the issue of over dilution. The two samples were investigated simultaneously, this also rules out the effect of long storage. No literature was found to explain this.

Dividing the lead handlers into two classes, class 1 with a lead concentration of greater than eight (<8) and class II with a lead concentration of less than eight (>8), it was observed that as the concentration of lead increases its effects on the hematological parameters becomes glaring. The packed cell volume and the hemoglobin were reduced, the erythrocyte sedimentation rate was increased and the total white cell count fluctuated. This is also dependent on the duration of exposure and length of time of work.

The correlation graph shows a correlation between lead concentration and duration of exposure. We can see from the line of the graph that there is a correlation. This shows that the increase in

lead concentration is dependent on the duration of exposure. The longer one is exposed to lead and its products, the higher the concentration of lead assimilated.

Fig 4.1 shows the bar chart of the lead values obtained from the serum and plasma samples of the test group. The first two bars show that of the entire test group with the lead in the serum higher than that in the plasma. The second two bars show that of the test group in class 1 (conc. <8) with the lead in the plasma slightly higher than that in the serum. The last two bars show that of the test group in class II (conc. > 8), with that of the serum very significantly higher than that of the plasma.

In fig 4.2, there are two groups (a) and (b). The PCV in group (a) is higher than that in group (b) which has a higher lead content. The ESR in group (b) is higher than that in group (a). As the lead concentration increases due to duration of exposure, the PCV decreases and the ESR increase. The WBC increases as the lead concentration increases.

5.2 CONCLUSION

From the test results obtained and then statistical analysis performed, EDTA affects the lead content already in the blood by chelating the much it can, thereby lowering the lead content in the plasma samples. This is evident both in the test and control groups. There is a latent assimilation of lead from the environment even among people not exposed to lead in their work places, or who have never come in contact with lead. This is why lead was isolated from both the plasma and serum of the control group. The lead content affected the

values of the hematological parameters; this was obvious when the test group was grouped based on the CDC classification into class 1 and class II. The PCV and HB values were decreasing with the increase in lead concentration, while the ESR and WBC were increasing with the increase in lead concentration. This is due to the lowered immunity caused by the increase in lead concentration. Also the lead concentration increases appropriately with the increase in duration of exposure. The longer a person is exposed to lead the more the assimilation and therefore the more the lead concentration.

Finally the test results showed that the result of the hematological parameters obtained in the direct samples were higher than that of the anti coagulated blood. No literature was found to explain this. It is concluded that lead levels is higher in the direct samples than in the EDTA samples, PCV, HB were decreased with increase in lead concentration and ESR is increased with increase in lead concentration.

5.3 RECOMMENDATION

It is recommended that regular medical checkups be carried out on occupational lead handlers. They should be equipped with protective gears to reduce the rate of assimilation of lead. Further research work be carried should be carried out to find out what causes the hematological values obtained from the fresh samples to be higher than those obtained from anti coagulated blood from the same person.

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APPENDIX 1

Table 4.1: Comparison of lead concentration and hematological parameters in test group and control group.

PARAMETERS	TEST SUBJECTS n=100	CONTROL SUBJECTS n=50	CALCULATED t-VALUE	CRITICAL t-VALUE	P VALUE
PCV EDTA %	41.17±4.69	37.67±4.21	51.136	1.98	<0.05
PCV PLAIN%	44.03±3.99	39.67±4.23	63.258	1.98	<0.05
WBC EDTA /MM³	5613.9±1413.65	5183.3±1616.49	23.494	1.98	<0.05
WBC PLAIN /MM³	6727.8±1749.07	5658.3±1759.95	22.756	1.98	<0.05
ESR EDTA /1ST HR	11.91±10.18	11.50±9.87	7.229	1.98	<0.05
ESR PLAIN /1ST HR	12.30±11.26	11.62±10.23	7.111	1.98	<0.05
HB EDTA g/dl	13.63±1.45	12.48±1.24	17.045	1.98	<0.05
HB PLAIN g/dl	14.48±1.31	13.30±1.18	21.086	1.98	<0.05
LEAD EDTA µg/100ml	6.52±2.09	3.41±1.22	18.53	1.98	<0.05
LEAD PLAIN µg/100ml	7.31±2.31	3.49±1.25	18.66	1.98	<0.05

Table 4.2: Comparison of lead concentration, PCV, WBC, HB, ESR, in EDTA and plain containers of test group.

PARAMETERS	EDTA CONTAINER	PLAIN CONTAINER	CALCULATED t-VALUE	CRITICAL t- VALUE
PCV %	41.17±4.69	44.03±3.99	51.136	1.98
WBC/MM³	5613.9±1413.65	6727.8±1749.07	23.494	1.98
ESR MM/1ST HR	11.91±10.18	12.30±11.26	7.229	1.98
HB g/dl	13.63±1.45	14.48±1.31	17.046	1.98
LEAD µg/100ml	6.52±2.09	7.31±2.31	18.66	1.98

Table 4.3: Comparison of lead concentration, PCV, WBC, HB, in EDTA and plain containers of control group.

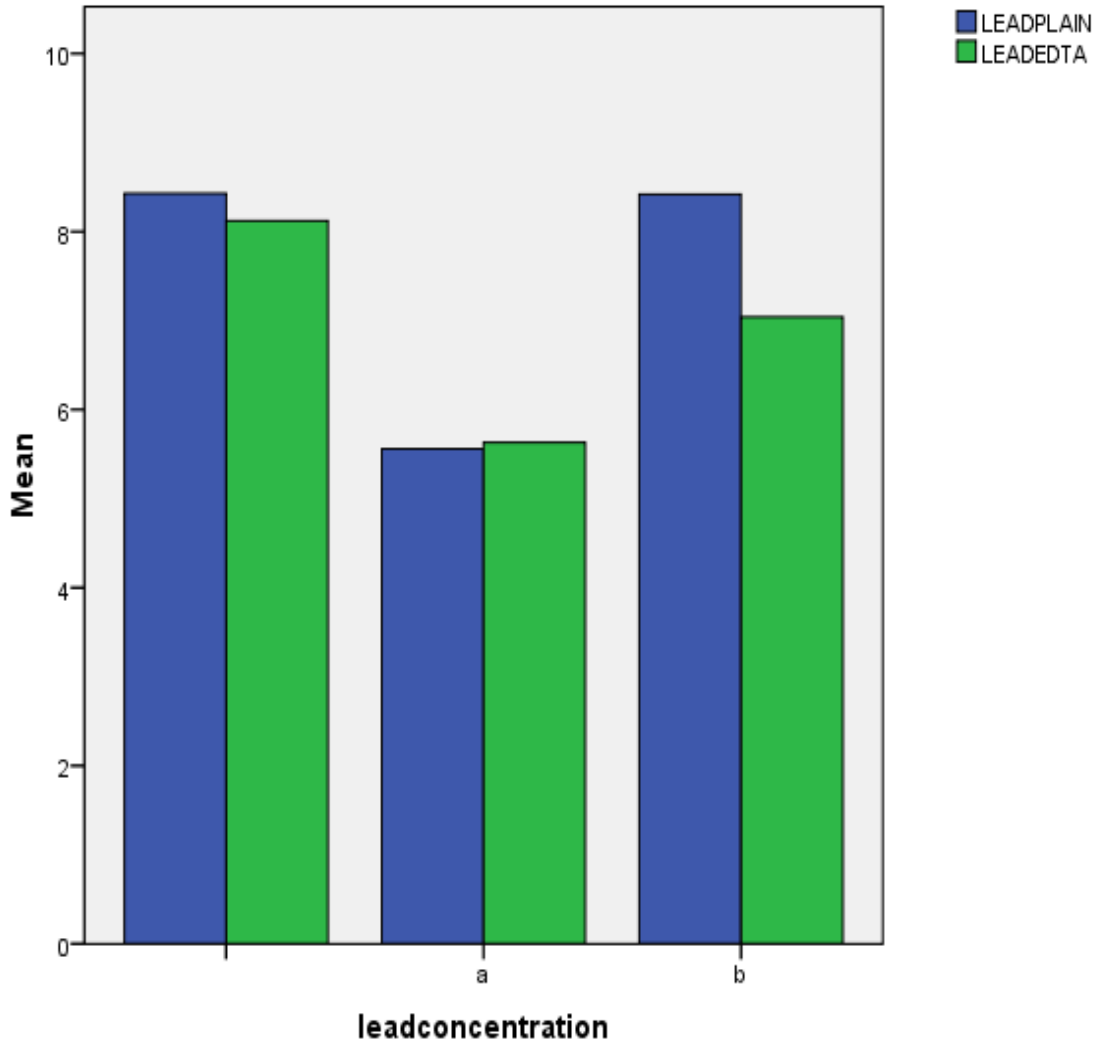
PARAMETERS	EDTA CONTAINER	PLAIN CONTAINER	CALCULATED t-VALUE	CRITICAL t-VALUE
PCV%	37.67±4.21	39.67±4.23	29.625	1.98
WBC/MM³	5183.3±1616.49	5658.3±1759.95	10.635	1.98
ESRMM/1ST HR	11.50±9.87	11.62±10.23	7.229	1.98
HB g/dl	12.48±1.24	13.30±1.18	13.225	1.98
LEAD µg/100ml	3.49±1.25	3.41±1.22	18.53	1.98

Table 4.4: Comparison of PCV, WBC, and ESR in lead handlers classified based on lead concentration alongside controls using a one way ANOVA for the analysis.

PARAMETERS	LEAD CONC CLASS 1 n=60 (conc.>8)	LEAD CONC CLASS II n=40 (conc.<8)	CONTROL n=50	CRITICAL F-VALUE	CALCULATED F-VALUE
PCV	39.70±5.07	45.62±4.05	36.50±3.82	2.45	7.95
WBC	5520.00±1461.29	6031.25±1436.90	5250.33±1605.51	2.45	7.95
ESR	10.55±7.87	15.69±11.47	8.55±6.61	2.45	7.95
HB	13.23±1.69	15.21±1.35	12.17±1.27	2.45	7.95

APPENDIX 2

Fig 4.1: Bar charts showing the mean of the values of the lead in the serum and that in the plasma.

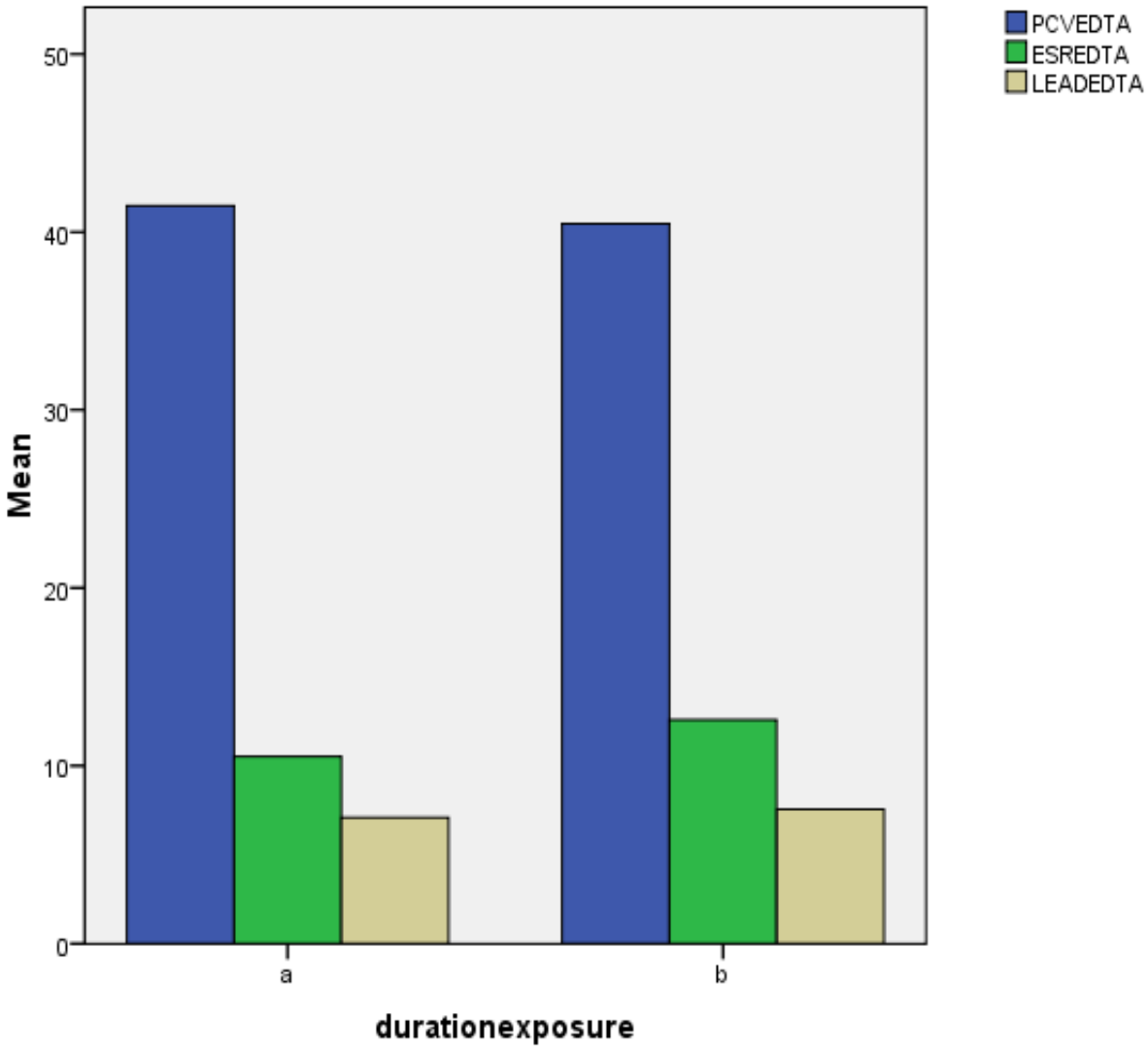


KEY:

a - class 1 classification of lead concentration (conc. < 8)

b - class II classification of lead concentration (conc. >8)

Fig 4.2 bar charts showing the effect of the duration of exposure on the means of the PCV, ESR, and lead in the EDTA containers

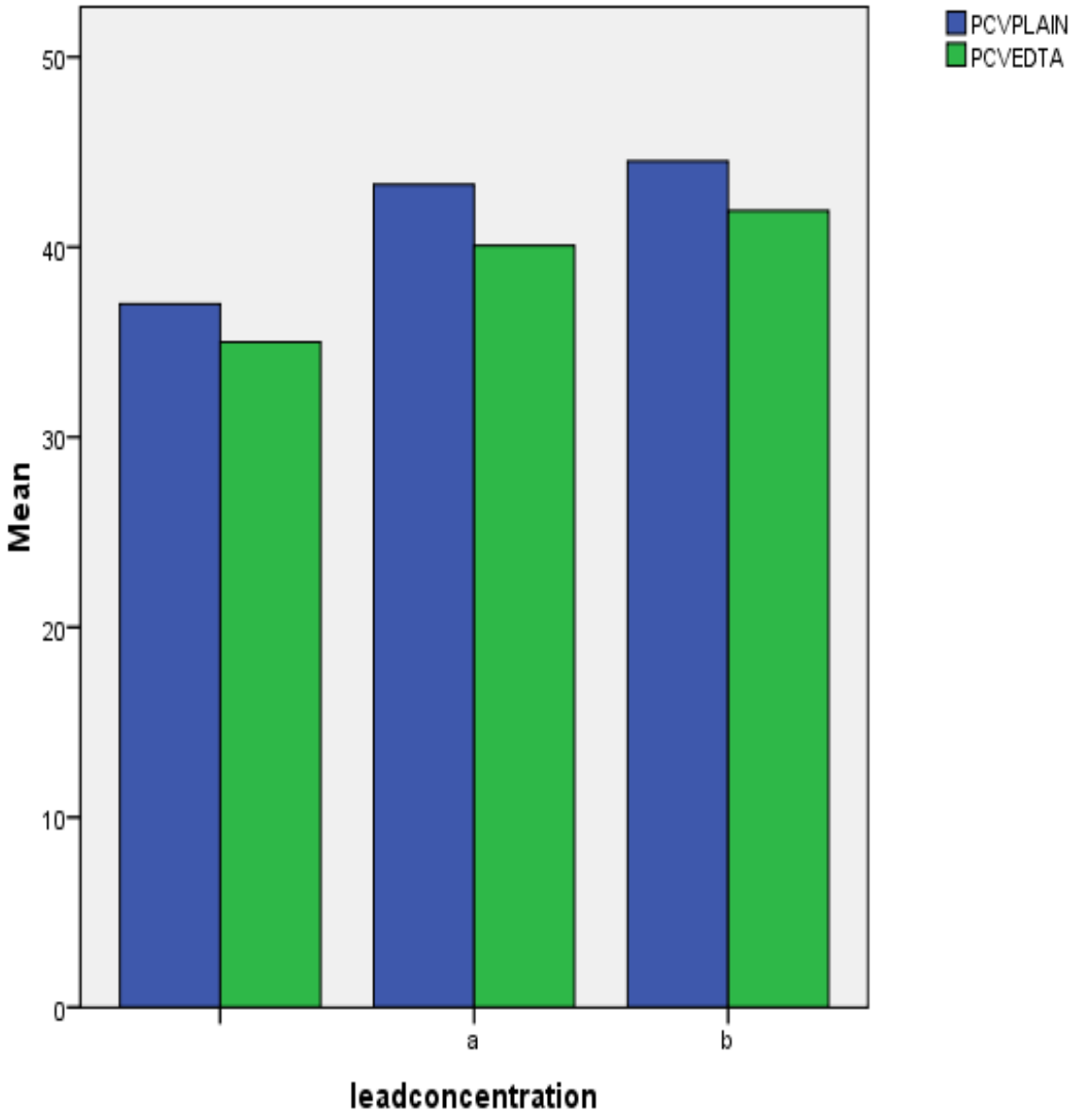


KEY:

a - class 1 classification of lead concentration (conc. <8)

b - class II classification of lead concentration (conc. >8)

Fig 4.3 bar charts showing the means of PCV results gotten from both the fresh blood and the anti coagulated blood

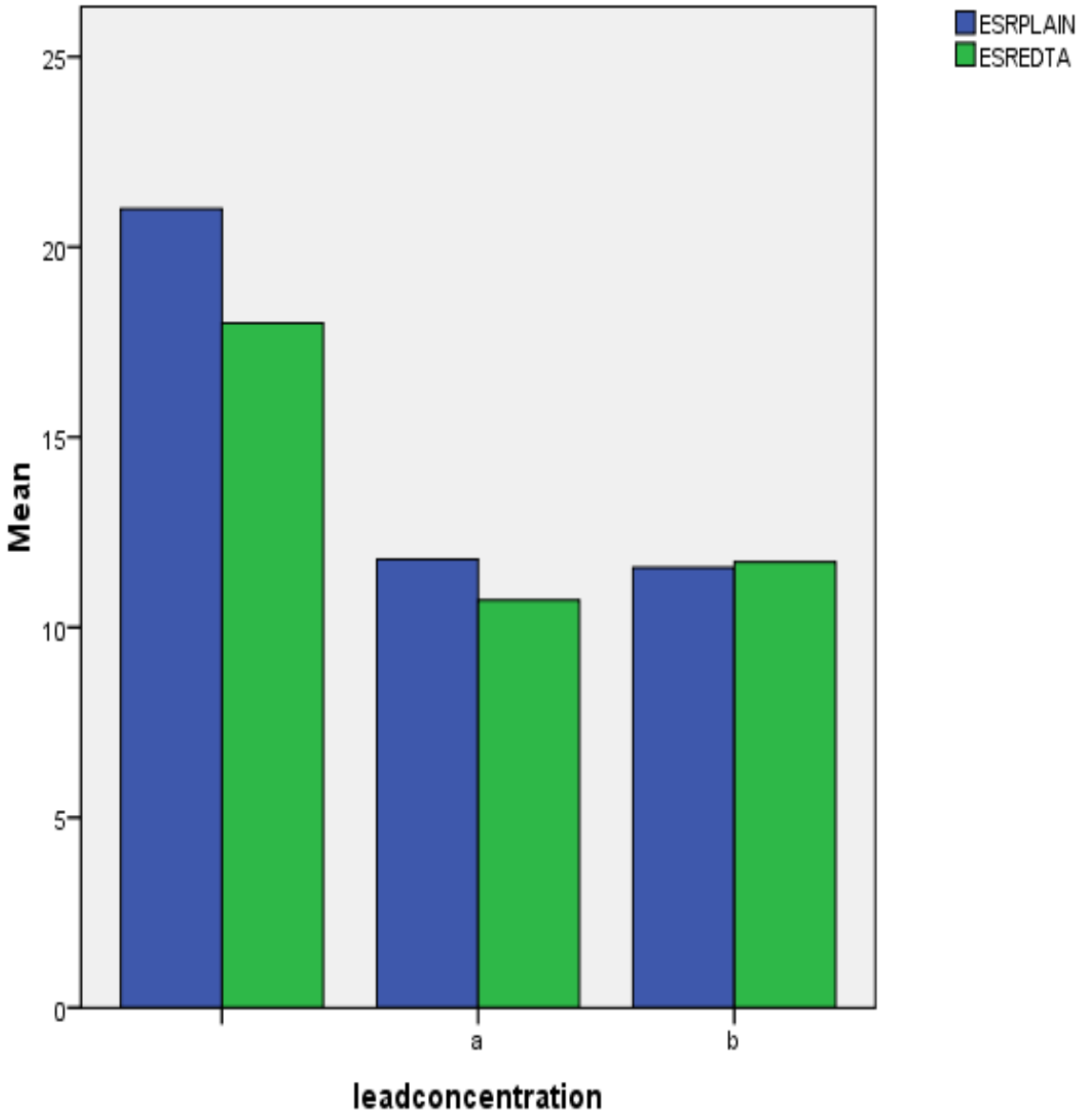


KEYS:

a -class 1 classification of lead concentration (conc. <8)

b -class II classification of lead concentration (conc. >8)

FIG 4.4 bar charts showing the means of the results of the ESR obtained in the fresh sample and anti coagulated sample.



KEY:

a - class 1 classification of lead concentration (conc. <8)

b -class II classification of lead concentration (conc. >8)

Fig 4.5bar charts showing the mean of the results of the WBC obtained in the fresh and anti coagulated sample.

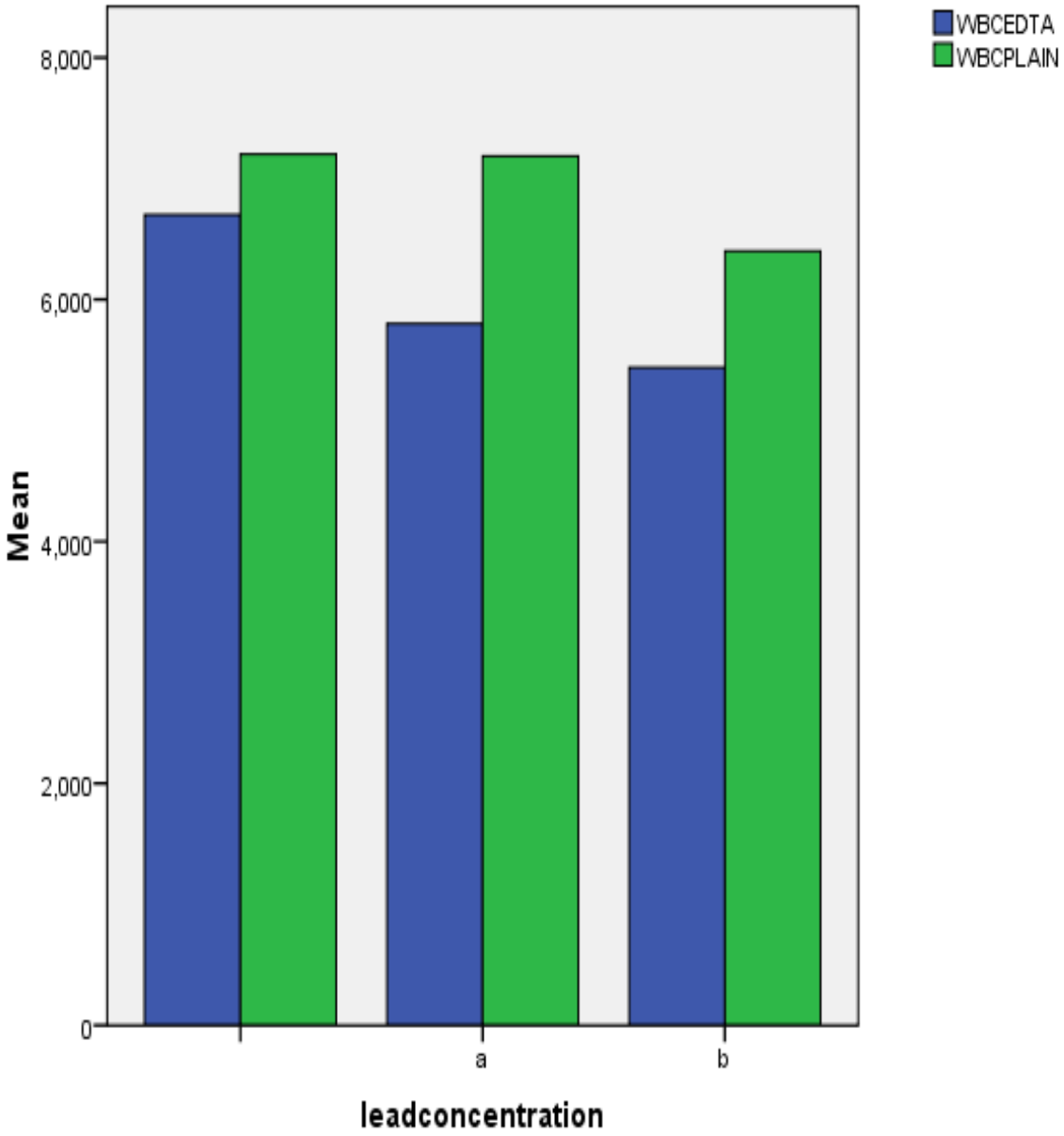
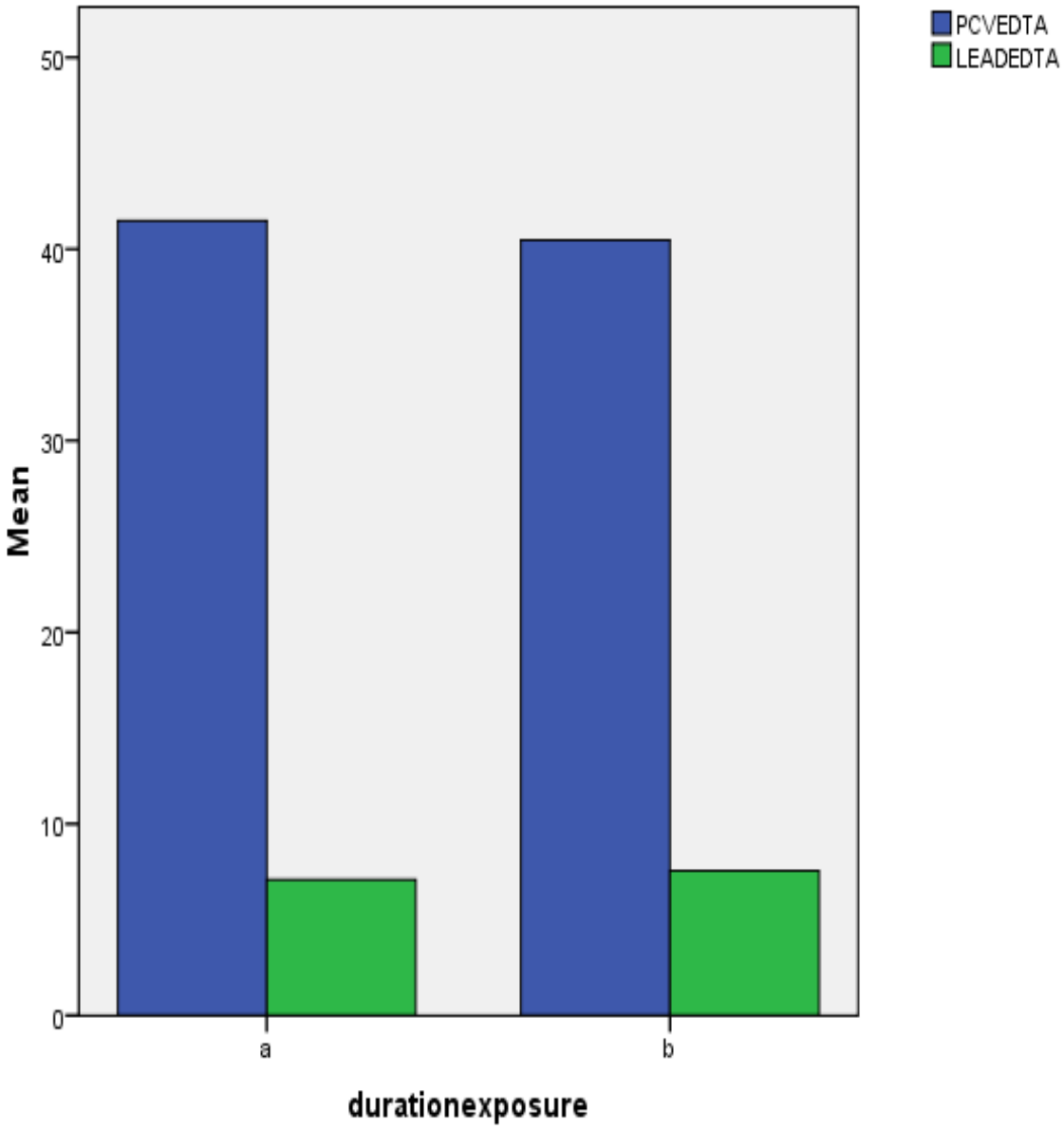


Fig 4.7 bar charts showing the means of the values obtained from the PCV and lead estimation of the anti coagulated blood.



KEY:

a - class 1 classification of lead concentration (conc. <8)

b class II classification of lead concentration (conc. >8)

APPENDIX 3

3.1 REAGENTS

Reagents used are listed below:

Turks solution

Leishman stain

Sodium citrate

Diethyldithiocarbamate

3.2 EQUIPMENTS

The equipments used for the experiment include

The microhematocrit centrifuge

The haemocue machine

The microscope using the X 10 and X 100 objectives