EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND ANTI-TERMITE PROPERTIES OF SOME WOODS, CHARCOALS AND COAL OF NASARAWA STATE, NIGERIA

BY

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B.Sc. PURE CHEMISTRY (NSUK)
(NSU/NAS/M.Sc./CHM/011/14/15)

M.Sc. INDUSTRIAL CHEMISTRY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE IN M.Sc. INDUSTRIAL CHEMISTRY OF THE SCHOOL OF POSTGRADUATE STUDIES OF NASARAWA STATE UNIVERSITY, KEFFI.

JUNE, 2017

DECLARATION

I hereby declare that this project has been written by me and it is a report of my research work. It has not been presented in any previous application for a Master's Degree in Industrial Chemistry. All quotations are indicated and sources of information specifically acknowledged by means of references.

Sign.................................... Date...................
Ndijiekwu, Moses Ebenezer

CERTIFICATION

The dissertation (Evaluation of Polycyclic Aromatic Hydrocarbons and Anti-Termite Properties of Some Woods, Charcoals and Coals of Nasarawa State), meets the regulations governing the award of M.Sc. Industrial Chemistry School of Postgraduate Studies, Nasarawa State University, Keffi, and is approved for its contribution to knowledge.

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ABSTRACT

Lafia-Obi coals, woods and charcoals from Keffi, Marke and Shamagay in Nasarawa State were evaluated for the presence of PAHs using standard methods. 200-mesh size pulverized and saw milled samples were solvent extracted using n-hexane and dichloromethane at 80^o and 30^o for 48 h and fractionated in alumina column. Both fractions were characterized by gas chromatography and mass spectroscopy (GC-MS) that showed a total of 16 PAHs in the samples. Naphthalene was the highest in amount (0.583mg/kg) in the dichloromethane extract of Lafia-Obi Coal while benzo(g,h,i)perylene was the least in amount with approximately (0.000mg/kg) in n-hexane extract for all sample analyzed except Lafia- Obi Coal (0.001mg/kg) for n-hexane extract. In all the samples, the amounts of PAHs were below their toxic levels. Anti-termite activities of wood samples were investigated by treating the wood extractives from the Shanjini (*Pterocapus erinaceus*) and Marke (*Anogessus leocarpa*) on softwoods that are prone to termite attack on the field for 12weeks. The results showed that the efficacy of wood extractives to control termite was higher in Shanjini (*Pterocapus erinaceus*) than Marke (*Anogessus leocarpa*) in terms of resistance to termite attack.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The environment is the life support system given by the creator to mankind. Sometimes in the past, the three components of the environment-air, soil and water – were pure, virgin, undisturbed, uncontaminated and basically most hospitable. But, the reverse is the case today because progress in science and technology is also leading to environmental degradation and serious ecological imbalance, which in the long run, may prove disastrous for mankind (Sharma, 2002).

In quest for economic development, which seeks to increase the quantum of economic output without caring about the short- and long-term short-changes of human and material resources arising from the process, the activities of people and nations conquer and wreck the world, rather than sustain it for the present and future generations (Bhasin, 1991). Progress in agriculture, industry, transportation and technology is usually the barometer of economic development of any nation (Bhasin, 1991). Such activities of man have created adverse effects on all living organisms in the biosphere. Rapid industrialization has left us with polluted rivers, contaminated soil, depleted wild life and exhausted natural resources (Bhasin, 1991). As a result, the environment of today has become foul, contaminated and harmful for the health of living organisms, including man. The unlimited rapacious exploitation of nature by man has disturbed the heritage of ecological balance existing between living and non-living components on the planet earth. This undesirable situation created by man has threatened the survival of man himself and other biota on the earth (Bhasin, 1991)

Most horrible ecological crises result from urban-industrial technological revolution and speedy exploitation of every bit of natural resources. Globally, man-made pollutants from combustion, construction, mining, agriculture and warfare are increasingly significant in the air pollution. Motor vehicle emissions are one of the leading causes of air pollution (Van Loon & Duffy, 2000).

1.2 Environmental Pollutants

These are waste materials that pollute air, water or soil. Three factors determine the severity of a pollutant: its chemical nature, the concentration and the persistence. Some pollutants are biodegradable and therefore will not persist in the environment in a long term, while others non-biodegradable, will persist in the environment for long term (Tietenberg, 2006).

1.2.1 Types of pollutants

1. Fund pollutant
2. Stock pollutant (Tietenberg, 2006).

1.2.1.1 Fund Pollutants

These are pollutants which have some absorptive capacity. Fund pollutant does not cause damage to the environment unless the emission rate exceeds the receiving environment's absorptive capacity. Example of such is carbon dioxide, which is absorbed by plants and oceans. These pollutants are not destroyed, but rather are converted into less harmful substances or diluted/dispersed to non-harmful concentration.

1.2.1.2 Stock Pollutant

This is also known as notable pollutant; these are pollutants that the environment has little or no absorptive capacity for which are also referred to as non-biodegradable. Stock pollutants accumulate in the environment over time. The damage they cause increase as more pollutant is emitted, and persists as the pollutant accumulates. This kind of pollutant can create a burden for future generation by passing on damage that persists while the benefits received from incurring that damage has been forgotten. Examples of such pollutants are

i) Heavy metals
ii) Persistent organic pollutants (POP)
iii) Environmental persistent pharmaceutical pollutants (EPPP)
iv) Polycyclic aromatic hydrocarbons
v) Environmental xenobiotics

1.2.2 Heavy Metals

These are those metals having a specific density of more than 5 g/cm^3. They are noted for potential toxicity, especially in environmental contexts. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury, chromium, nickel, selenium and arsenic (arsenic is a metalloid, but is usually classified as a heavy metal) (Ritter *et al.*, 2007).

1.2.3 Persistent Organic Pollutants

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological and photolytic processes (Ritter, 2007). Because of their persistence, POPs bioaccumulate with potential significant impacts on human health and the environment. Examples of such pollutants are aldrin, dieldrin, chlordane, Endrin, heptachlor and hexachlorobenzene (HCB) which are all pesticides (Ritter *et al.*, 2007).

1.2.4 Environment Persistent Pharmaceutical Pollutants (EPPP)

These are groups of pollutants which can lead to water pollution, ground water pollution or soil contamination (Koller, 1987). Examples, high levels of antibiotics in the water can lead to some highly effective antibiotics becoming ineffective. Ethinyl estradiol a chemical that is used in the production of contraceptive pill and to treat menopause, was found present in many locations downstream of sewage treatment plants, which was responsible for male fish being feminized and lead to reduced sperm production in the male fish.

1.2.5 Environmental Xenobiotics

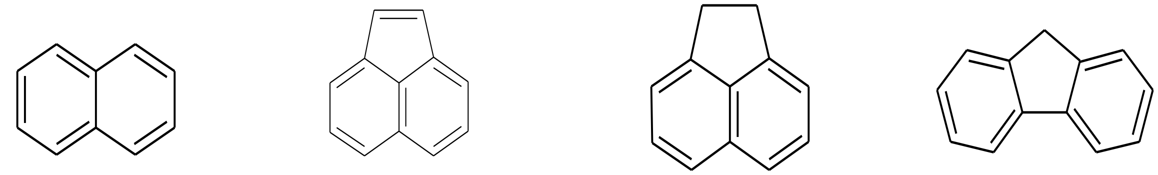
These are substances which do not exist in nature before their synthesis by human but are harmful to the environment. Xenobiotics are chemicals which are found in an organism but is not normally produced or expected to be present in it (Koller, 1987). Examples are Volatile hydrocarbons , dioxins, toxic metals, pesticides, herbicides, insecticides, fungicides, chemical fertilizers, chlorine and fluoride

1.2.6 Polycyclic Aromatic Hydrocarbons (PAHs)

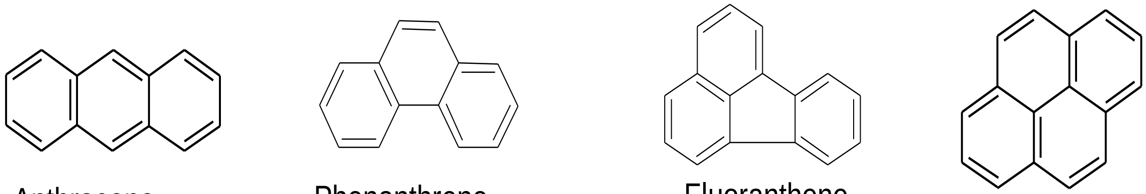
These are a group of organic compounds consisting of two or more fused aromatic rings. PAHs originated mainly from anthropogenic processes, such as volcanic eruptions and forest fires. PAHs can be present in both particulate and gaseous phases, depending upon their volatility. Light molecular weight PAHs (LMW PAHs) that have two or three aromatic rings are emitted in the gaseous phase, while high molecular weight PAHs (HMW PAHs), with five or more rings are emitted in the particulate phase (IARC, 2010).

Tomoaki *et al.*(2011) published a research work on characterization of polycyclic aromatic hydrocarbons (PAHs) present in smoked fish from Ghana and classified PAHs into two, the high molecular weight (HMW) with 228-279 g/mol and the low molecular weight (LMW) with (152-178 g/mol). In the atmosphere, PAHs can undergo photo-degradation and react with other pollutants such as sulfur dioxide, nitrogen oxides and ozone.

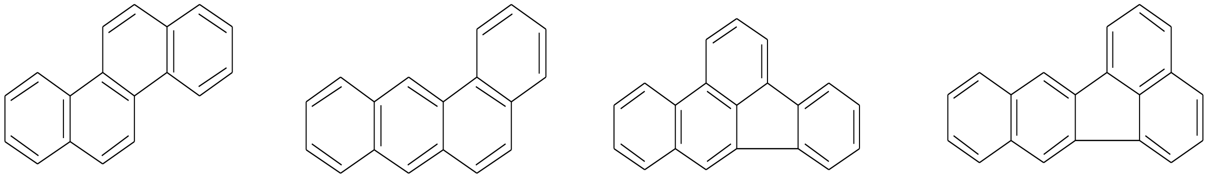
Examples of Some PAH:



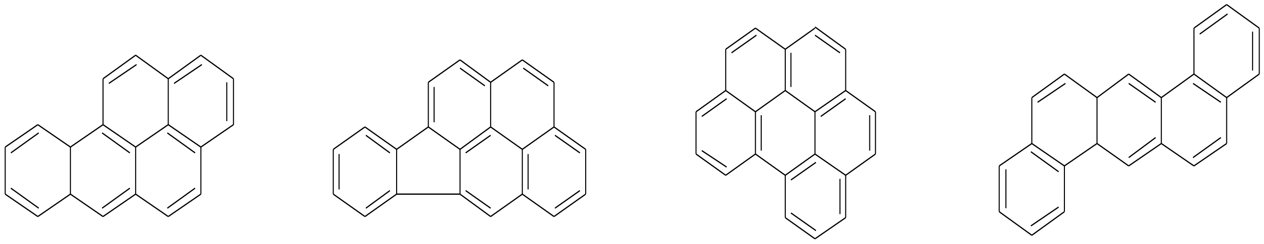
Naphthalene Acenaphthylene Acenaphthene Fluorene



Anthracene Phenanthrene Fluoranthene Pyrene



Chrysene Benzo(*a*)anthracene Benzo(*b*)fluoranthene Benzo(*k*)fluoranthene



Benzo(*a*) pyrene Indeno (1,2,3-*cd*)pyrene Benzo(*g,h,i*) perylene Dibenzo(a,h)anthracene

Figure.1.1: Some Polycyclic Aromatic Hydrocarbons (IAOEH, 2012).

1.3 Statement of the Problem

PAHs content in coals, charcoals and woods are known to be toxic environmental pollutants and can affect or threaten the health of humans as a result of their accumulation and persistence in the food chain through cultivation, preparation, and repeated long-term exposures (Shulte *et al.*,1993). This may lead to cancer, decreased immune function, cataracts, kidney and liver damage (e.g. jaundice), breathing problems, (asthma-like symptoms, and lung function abnormalities) (Shulte *et al.*,1993). Repeatedly, skin contact may induce redness and skin inflammation. Naphthalene, for example, can cause the breakdown of red blood cells if inhaled or ingested in large amounts (Shulte *et al.*,1993).Termites pose serious threats to agriculture, forestry and buildings, where they are found. The commonest method of controlling termite infestation is the application of Synthetically produced chemicals which are the principal termiticides used for this purpose. However, problems associated with the synthetic termiticides are toxicity to non-target organisms, development of resistance to pesticides and environmental contamination which may affect the entire food chain (Fendick *et al.*, 1990).

1.4 Aim and Objectives of the Study

The aim of this work is to investigate the presence of PAHs in coal from Lafia-Obi, Shanjinni (*Pterocapus erinaceus*) and Marke (*Anogessus leocarpa*) woods sourced from Keffi and Marke in Kokona LGA, charcoal from Marke and Shamagay in Nasarawa State and to ascertain the anti-termite properties of the woods.

The Objectives:

The Objectives of this research work are to:

i) Investigate the presence of PAHs in some coals, woods and charcoals from Nasarawa State.

ii) Determine the amount of PAHs in the samples relative to regulated limits.

iii) Determine the physicochemical properties of the woods as anti-termite agents.

iv) To ascertain the anti-termite properties of the woods.

1.5 Significance of the Study

The literature review process revealed that, to our knowledge, no study has been done to investigate and determine the presence of PAHs in coals, woods and charcoals in Nasarawa State. Due to the health challenge PAHs pose on humans, this research work is set to investigate and quantify the amount of PAHs in the samples so as to discourage the indiscriminate exposure or otherwise of coal, charcoal and wood to human health. Shanjini (*Pterocapus erinaceus*) and Marke (*Anogessus leocarpa*) are hardwoods that have been consistently used for roofing for their high resistance to termite attack. In this study, the anti-termite properties of these woods shall be elucidated.

1.6 Scope of the Study

This study is limited to investigating and determining the concentration of PAHs and anti-termite properties of some coals, woods and charcoals found in Nasarawa State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sources of PAHs

Nigeria is an agrarian country. About 80 % of its population are peasant and depend on firewood, charcoal and coal which contain PAHs as domestic sources of fuel for cooking their food (Edwards, 1983). PAHs are produced in incomplete combustion of organic matter such as wood, coal or oil. As a general rule, the lower the temperature of the fire and the less oxygen available, the more incomplete do these materials burn and the PAHs are produced (IARC, 1983).

2.1.1 Natural Sources of PAHs

Natural sources of PAHs include:

i) Forest and grass fire
ii) Oil seeps
iii) Volcanoes (IARC, 1983)

2.1.2 Anthropogenic Sources of PAHs

Anthropogenic sources of PAHs include:

i) Petroleum product
ii) Electric power generation
iii) Refuse incineration
iv) Home heating
v) Production of coke, carbon black, coal tar, and asphalt
vi) Internal combustion engines (Aleksondra, 2010)

Tracking the sources of PAHs based on the presence of LMW or HMW PAHs also becomes questionable as the PAHs are released into the environment and undergo chemical, physical and biological changes (Berko, 1999). Physical changes, such as evaporation, or physical transport of PAHs by air or water from one location to other; chemical changes such as photo transformation of PAH to daughter products; and biological changes such as biotransformation of the PAHs, changes their profile in the environment. Differentiating the sources of PAHs based on observed PAHs molecular weights may be a useful tool if the samples analyzed for PAHs are assumed not to be affected by any of these changes (Berko, 1999).

2.2 Wood as a Natural Source of PAHs

Wood is a porous and fibrous structural tissue found in the stems and roots of trees and other woody plants (Armstrong, 2010).

2.2.1 Types of Wood

i) Hardwood

ii) Softwood (Armstrong, 2010).

Hardwood (angiosperms): it is characterized by broad-leafed foliage, and tends to be deciduous—that is, they lose their leaves in the autumn. (However, many tropical hardwood species exist which are evergreen—they maintain their leaves year-round.) Additionally, hardwood trees tend to have a branched or divided trunk, referred to as a dendritic form (Armstrong, 2010). They are woods with high density and rich heartwood color, which make hardwoods suitable as roofing wood, furniture and interior woodwork.

Softwoods (conifers): These are wood with needle or scale-like foliage, though in some uncommon instances, they can have rather broad, flat leaves. Most softwood trees are evergreen (Jane, 1970). This research work will involve findings in Marke (*Anogeissus* *leiocarpus*) and Shanjini *(pterocapus erinaceus)* wood as part of the samples to be analyzed.

2.2.2 Chemical Composition of Wood

i) Cellulose

ii) Hemicelluloses

iii) Lignin

iv) Extractives (Isabela, 2009).

Cellulose is a long chain of linked sugar molecules that gives wood its remarkable strength. It’s the main component of plant cell wall.

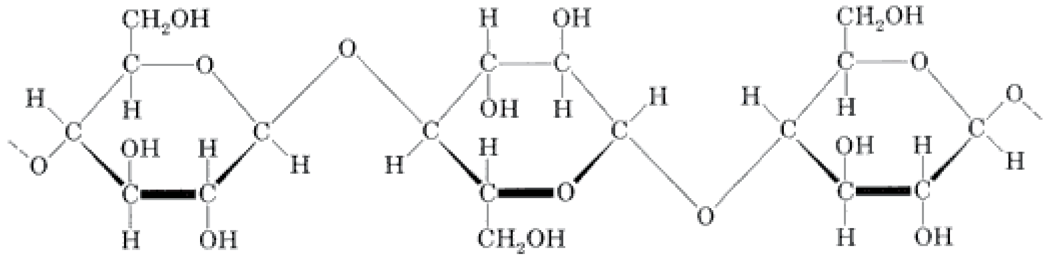


Fig. 2.1: Structure of Cellulose (Klemm, 2005).

Hemicelluloses: These are polysaccharides present in the cellulose of plants.

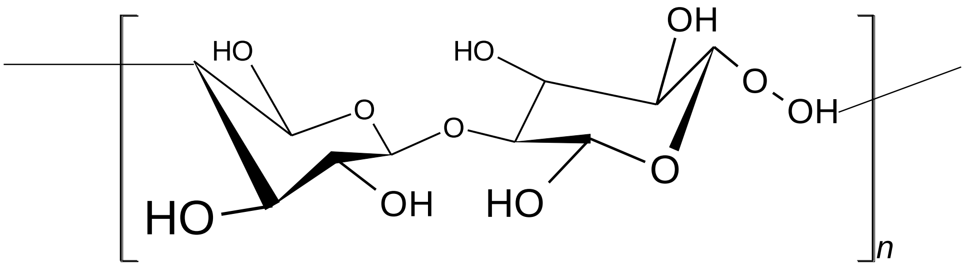


Figure. 2.2: Structure of Hemi-cellulose (Gibson, 2013).

Lignin: This is the third component of the wood that helps in the formation of cell wall.

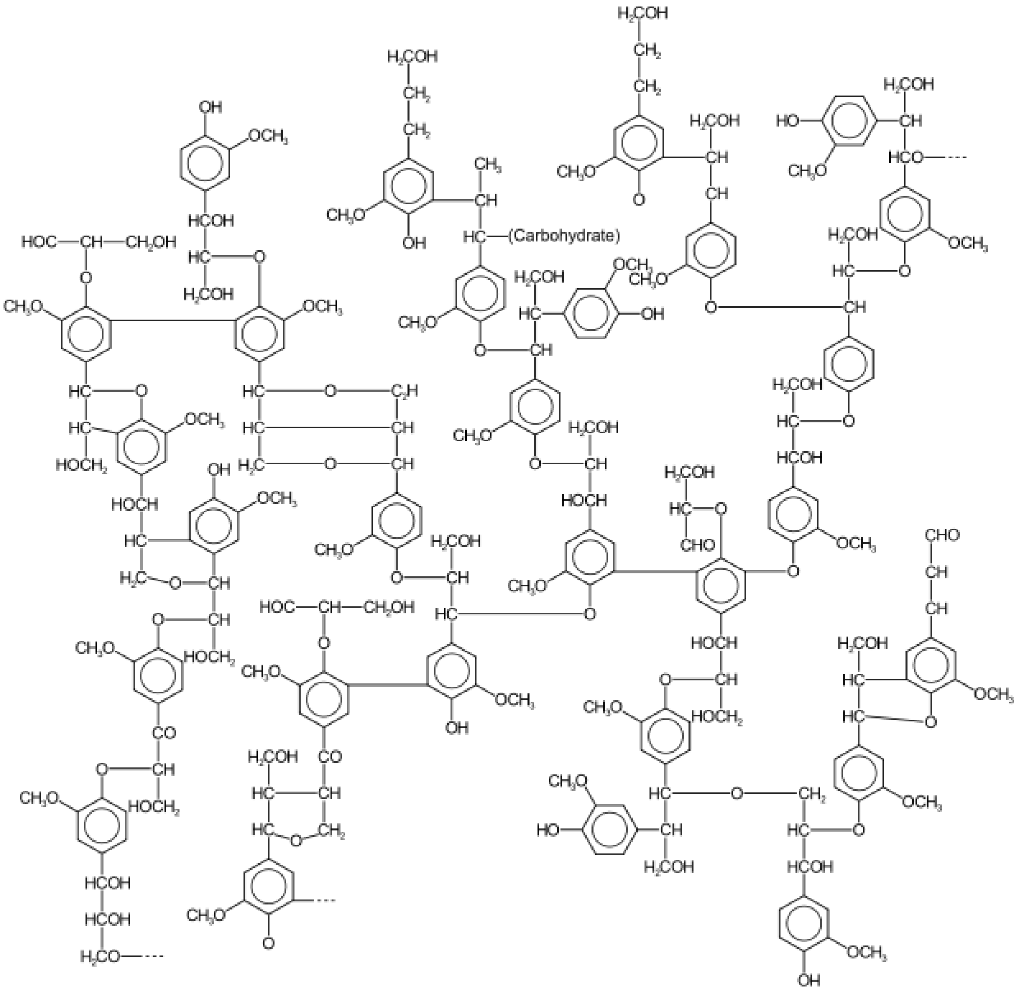


Figure. 2.3: Structure of Lignin (Martone *et al.,* 2009)

Extractives:

Wood extractives are polyphenols found in the heartwood of some tree species (FAO, 1986). Polyphenols are formed during heartwood formation. During the process of evolution, plants and trees developed a mechanism of producing their own line of chemical defense known as extractives (Arango *et al*., 2005).

The relationship between extractives and natural durability of wood was first reported by Arango *et al*., 2005. The natural durability of wood is often related with its toxic extractive components (Scheffer, 1998). Heartwood extractives retard wood decay and can protect the wood against decay organisms (Walker, 1993). Several studies have shown that the removal of extractives makes durable wood to lose its natural resistance and makes them more susceptible to decay (Nascimento *et al*., 2013).

Kityo & Plumptre (1997) reported that resistance is mainly due to the accumulation of extractives in the heartwood some of which are decay inhibitors.

Joao *et al.* (2011) reported that lower content of phenolic compounds and flavonoids is responsible for lower durability of woods.

2.3 Component of Wood Extractives

The following components of wood extractives confers natural resistance against bio-deteriorating agents

i) Flavonoids

ii) Tannins

iii) Quinones

iv) Stilbene (Isabela, 2009).

2.3.1 Flavonoids

Flavonoids are chemical compounds in plants that provides protection against ultraviolet radiation, pathogens, and herbivores in plants (Harbone & Williams, 1992). The general structure includes a C_{15} (C_6-C_3-C_6) skeleton joined to a chroman ring (benzopyran moiety), classified into flavanones, flavones, chalcones, dihydroflavonols, flavonols, aurones, flavan-3-ols, flavan-3,4-diols, anthocyanidins, isoflavonoids, and neoflavonoids.

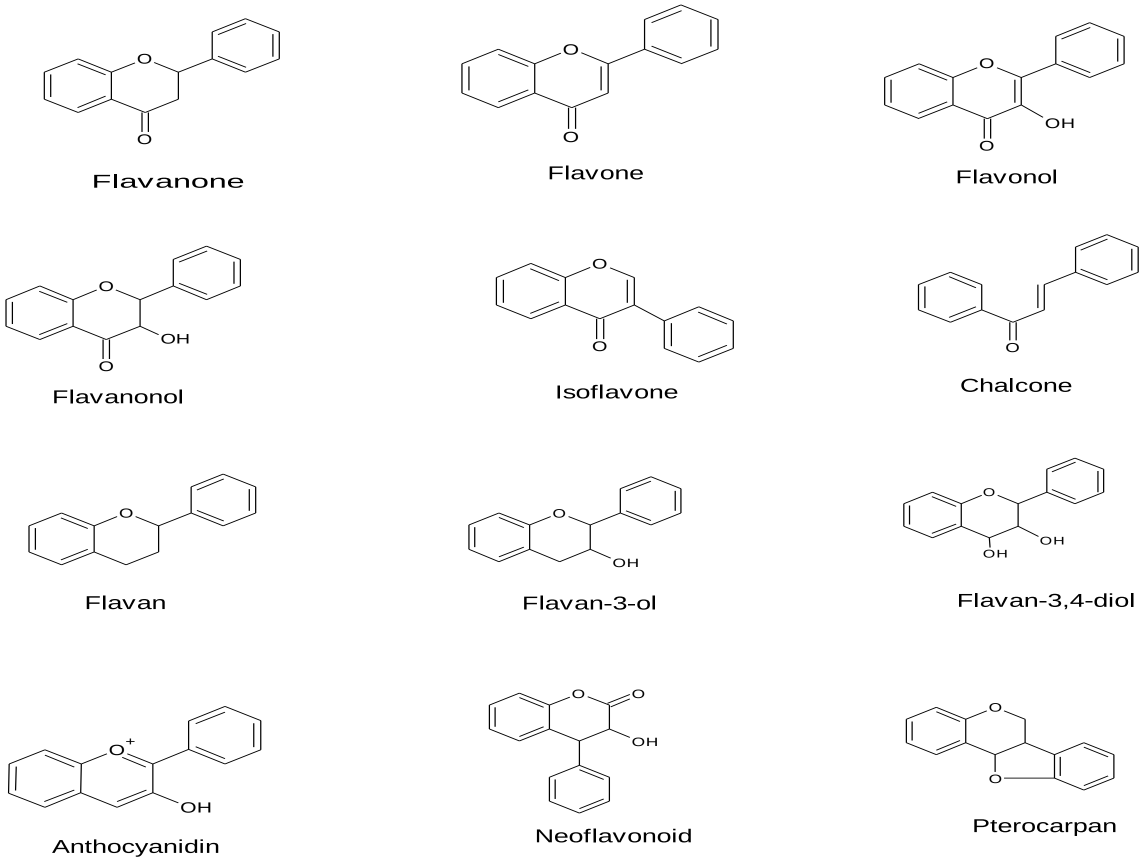


Figure. 2.4: Some Examples of Flavonoids (Harbone & Williams, 1992).

2.3.2 Tannin

Tannins are found in bud and foliage tissues, seeds, bark, roots, heartwood and sapwood. The highest levels are in heartwood and bark (Porter, 1989). It consists of two types namely:

i) Hydrolysable tannins

ii) Condensed tannins

Hydrolysable Tannins: These are molecules with a polyol (D-glucose) as a central core. The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins). Hydrolysable tannins are usually present in low amounts in plants (Porter, 1989).

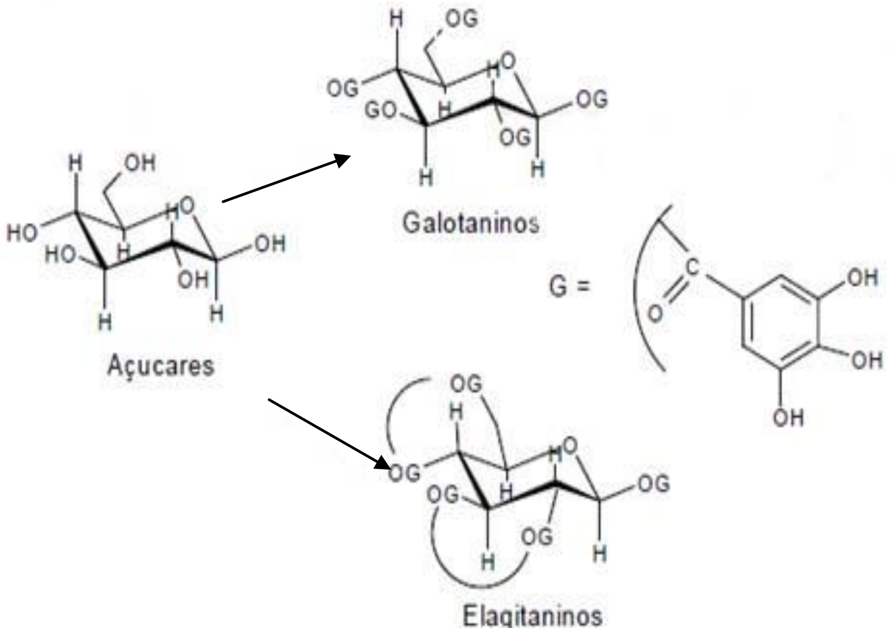


Figure.2.5: Diagram of Hydrolysable tannins

Condensed Tannins: These are natural preservatives and antifungal agents, found in high concentrations in the bark of wood of some tree species (Zucker, 1983). Most plant-pathogenic fungi excrete extracellular enzymes such as cellulases and lignases, involved in the invasion and spread of the pathogen. Condensed tannins most likely act as inhibitors of these enzymes by complexing, blocking their action (Pettersen, 1984). For this reason, extract from various woods and barks rich in tannin have been used as adhesives and wood preservatives for a long time (Hemingway and Karchesy, 1989)).

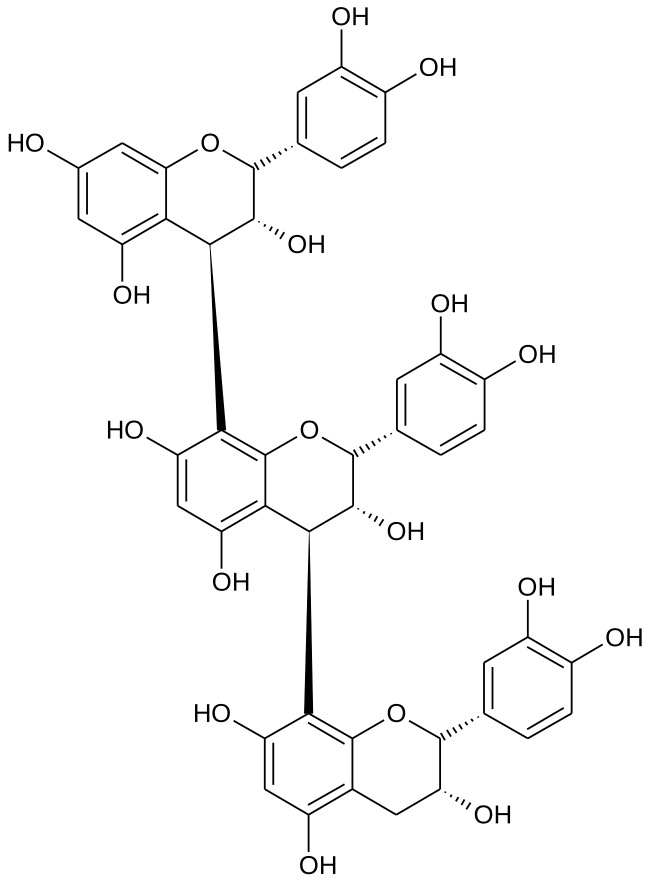
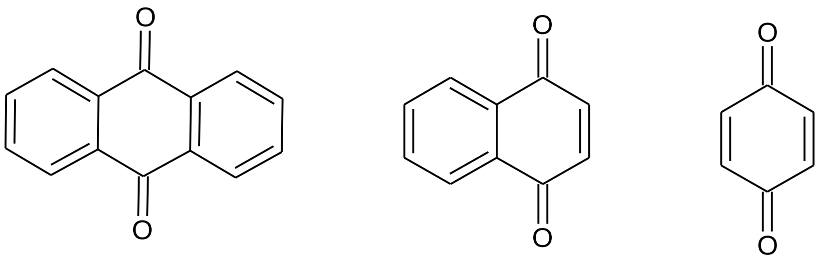


Figure.2.6: Structure of Condensed Tannins (Creasy & Swain 1965).

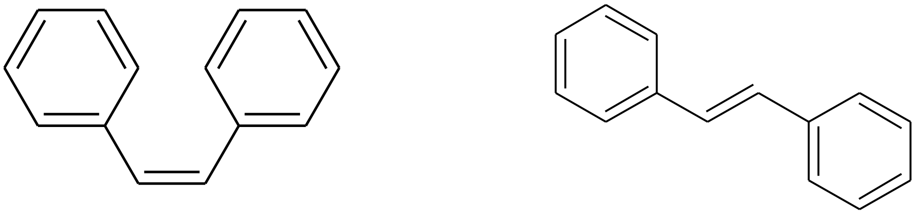
Quinone: This is a chemical present in wood that possess natural repellant and toxic properties, mainly against termites (Dungani *et al.*, 2012).



Anthraquinone Naphtoquinone Benzoquinone

Figure.2.7: Some Structure of Quinones (Toshiaki, 2001).

Stilbenes: Stilbenes are compounds possessing the 1,2-diphenylethene structure, as well as bibenzyls and phenanthrenes, which are composed of C_6-C_2-C_6 skeleton. It plays an important role in durability of wood, especially resistance to fungal decay (Nascimento *et al.*, 2013). There are two isomeric forms of stilbenes, which are 1,2-diphenylethylene: *trans*-stilbene and *cis*-stilbene, and the chemical structure of these two stilbenes.



cis- stilbene

trans- stilbene

Figure. 2.8: Structures of Some Stilbenes Stilbenes (Schröder *et al.,* 1993)

2.4 Components of Wood Burning

The smoke released from wood burning and cigarette smoke are quite similar, and many components of both are carcinogenic. Wood smoke contains fine particulate matter, carbon monoxide, formaldehyde, sulfur dioxide, nitrogen oxides that can scar the lungs. Wood smoke also contains chemicals known or suspected to be carcinogens, such as polycyclic aromatic hydrocarbons (PAHs) and dioxin (EHHI, 2005).

A traditional method to preserve wood in Asian countries such as Indonesia is by smoking samples, which is an indirect way to apply wood condensates into the samples. Wood burning produces smoke that contains a variety of toxic polycyclic aromatic hydrocarbons (PAHs), as well as phenols, aldehydes, ketones, organic acids, alcohols, esters, hydrocarbons and several heterocyclic compounds (Stołyhwo & Sikorski, 2005). When treating wood with smoke, moisture content is reduced and PAHs are condensed and diffused inside wood structure. PAHs have been shown to provide some resistance to biological deterioration driven by termite attack (Hadi *et al*., 2010),

2.4.1 Implications of Wood Burning:

Ozone Layer Depletion: The ozone layer is the protective covering between the sun's rays and the earth. The gases released from the activities of many industries and the domestic burning of fossil fuels, such as wood leads to the depletion of the ozone layer (WMO, 1994).

Green House Effect: It has been discovered by scientists that the release of gases from wood such as carbon (IV) oxide and methane contributes to global warming. Basically, the earth has to be warm for living things to survive but excess heat is developed from the emission of these gases. There is, therefore fear that this will greatly affect man in the near future (WMO, 1994).

Respiratory disorder:-Burning wood/fossil fuel causes a release of harmful bio gas into the atmosphere. When these gases are inhaled, it deteriorates the respiratory organs. Sometimes this can lead to different sicknesses like asthma.

Chronic obstructive pulmonary disease:- This disease is caused by particles in the air. When the particles are inhaled, an abnormal inflammation of the respiratory tract impedes respiration.

Cancer of the bladder: -Particles inhaled from the pollution of the atmosphere are inhaled and settled in the bladder. This causes the feeling of pains around the bladder.

Lung/heart diseases:-Prolonged exposure to bio-gas and particles of wood in the air causes difficulty in breathing. This often leads to heart diseases and possibly the death of the victim if symptoms are neglected.

2.5 Shajinni (*Pterocarpus erinaceus)* Wood

This wood belongs to the family of *Fabaceae* and is a small medium perennial legume tree of Africa savannah and dry forests. It is commonly called Africa rose tree, Africa gum or Africa kino in English. In Nigeria, it is known as "*Modobiya*" or *Shajinni* in Northern Nigeria," *Apepe*" or "*Osun dudu*" in Western Nigeria (Ezeja *et al.,* 2012)

2.6 Marke (*Anogeissus leiocarpus)* Wood

This is also known as axle-wood tree in English, *Marke* in (Hausa), *Kojoli* in (Fulani), *Ayin* or *Orin-odan Ainy* in (Yoruba) and *Atara* in (Igbo). It is a medicinal plant used for dyeing and tanning. *Anogeissus leiocarpus* is a plant that is widely used in Northern Nigeria in ethno medicine. It belongs to the Family: *Combretaceae (combretoideae)* (Amali, 2007). It is a tropical tree which grows up to 28 m and occurs in most of the savannah areas. Those growing in the driest areas tend to have smaller leaves and more hairy flowers than those growing under wetter conditions, but both differences are not sufficiently marked to create distinct varieties. Axle-wood tree is one of the major plants commonly used as chewing stick in Nigeria, which is believed to kill harmful bacteria in the oral cavity. The antibacterial properties of chewing stick have been documented scientifically as the tree contains tannins and polyphenols, which are potent bactericidal agents (Sacande & Sanogo 2007). The use of *Marke* tree in the treatment of oral disease, such as thrush and black tongue has been reported in many studies. *Anogeissus leiocarpus* has many applications; leaves, roots and trunk bark of the tree are used by traditional practitioners for the treatment of helminthiasis (infection of the human body with a parasitic worm such as roundworms and pinworms), trypanosomiasis (sleeping sickness), malaria and dysenteric syndrome (Saeedhalilu, 2014). It is also used in traditional medicine as a remedy for many ailments of livestock and man, which includes schistosomiasis (disease caused by parasitic worms), leprosy, diarrhea and psoriasis. Some members of the *Combretaceae* have high concentrations of bioactive substances, such as flavonoids, terpenoids, tannins or polyphenolic compounds (Saeedhalilu, 2014). The *Marke* tree leaves are rich in tannin, and are used as eye wash for certain complaints (Amali, 2007). In some regions of Upper Guinea, the bark is used as a febrifuge (reduction of fever) in hot lotion sand infusions, and in Ivory Coast prescriptions of leaves and bark with other drug plants are taken for leprosy. It is widely used to dye textiles and leather. The decoction and maceration of the stem bark are used against anorexia, constipation, malaria, jaundice, itching, wounds, eczema, psoriasis, carbuncles, boils and various forms of ulcers. The decoction of the leaves is used in the treatment of jaundice, various forms of hepatitis (liver inflammation) and amoebic dysentery (Saeedhalilu, 2014). *A. leiocarpus* is also used medically for the treatment of roundworms, gonorrhea, general body pain, blood clots, asthma, coughing and tuberculosis. The leaves of the plant are used externally as a decoction in eastern part of Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, and diabetic ulcers with good results. Results of ethno-botanical survey carried out by researchers at Kpando municipality in the Volta Region of Ghana, revealed that many of the indigenous people use the fresh leaves of *A. leiocarpus* to heal wounds. The wood of *A. leiocarpa* is highly appreciated for construction and as firewood. Moreover, bark and leaves of *A. leiocarpa* are used for dyeing and tanning (Amali, 2007).

2.7 Some Uses of PAHs

They are mostly used as intermediaries in pharmaceuticals, agricultural products, photographic products, thermosetting plastics, lubricating materials and other chemical industries (Blumer *et al.,*1975). Examples of some PAHs and their uses are:

i) Arcenaphlthene: it is used in the manufacture of dyes, plastics, pigments, pharmaceuticals and pesticides.

ii) Anthracene: it is used for manufacturing dyes and pigments, diluents for wood preservatives.

iii) Fluoranthane: is used for the manufacture of dyes, pharmaceuticals and agrochemicals.

iv) Fluorene: is used in the manufacture of dyes, pigments, pesticides, thermoset plastic and pharmaceuticals.

v) Phenanthrene: used for the manufacturing of pesticides and resins

vi) Pyrene: used for the production of pigments (Blumer *et al.,*1975)

2.8 Chemical Characteristics of PAHs

Polycyclic aromatic hydrocarbons have two or more single or fused aromatic rings with a pair of carbon atoms shared between rings in their molecules. The term “PAH” refers to compounds consisting of only carbon and hydrogen atoms. PAHs containing up to six fused aromatic rings are often known as “small” PAHs and those containing more than six aromatic rings are called “large” PAHs. The majority of research on PAHs has been conducted on small PAHs due to the availability of small samples of various small PAHs.

Simko (2002) reported that the general characteristics of PAHs are high melting and boiling points (therefore they are solid), low vapour pressure, and very low aqueous solubility, which both tend to decrease with increasing molecular weight, whereas resistance to oxidation and reduction increases. PAHs are highly lipophillic: (have the ability to dissolve in fats, oils), i.e. they are fat loving, meaning it can dissolve in fats, oil liquid and non polar solvents such as hexane or toluene, and therefore are very soluble in organic solvents (Blumer *et al.,*1975).

The simplest PAHs, as defined by the International Union of Pure and Applied Chemists (IUPAC) are phenantrene and anthracene, which contain three fused aromatic rings. Smaller molecules, such as benzene, are not PAHs. Naphthalene, which consists of two coplanar six-member rings sharing an edge, is another aromatic hydrocarbon. By formal convention, it is not a true PAH though is referred to as a bicyclic aromatic hydrocarbon (Aleksandran, 2004).

Table 2.1: Physical Properties of PAHs

S/N	PAHs	Molecular Formula	Molecular Weight
1.	Naphthalene	$C_{10}H_8$	128.16
2.	Fluorene	$C_{13}H_{10}$	166.20
3.	Phenanthrene	$C_{14}H_{10}$	178.2
4.	Anthracene	$C_{14}H_{10}$	178.20
5.	Fluranthene	$C_{16}H_{10}$	202.26
6.	Pyrene	$C_{16}H_{10}$	202.3
7.	Benzo(a)anthracene	$C_{18}H_{12}$	228.29
8.	Chrysene	$C_{18}H_{12}$	228.3
9.	Benzo(b)flouranthrene	$C_{20}H_{12}$	252.3
10.	Benzo(a)pyrene	$C_{20}H_{12}$	252.3
11.	Indeno(1,2,3-cd)pyrene	$C_{22}H_{12}$	276.30
12.	Dibenzo(a,h)anthracene	$C_{22}H_{14}$	278.35
13.	Benz(ghi)perylene	$C_{22}H_{12}$	276.34

Source: Jejal (2007)

2.9 Classification of PAHs

PAHs are classified into two groups, based on their physical and biological properties.

i) High molecular weight (HMW) PAHs

ii) Low molecular weight (LMW) PAHs

The HMW PAHs consist of 4 or more aromatic rings and are less readily bio-degraded by indigenous microorganisms, hence can persist in the aqueous environment by bioaccumulating in aquatic organisms like fish and mussels and they are more carcinogenic (Brown & Peake, 2006).

The LMW PAHs consists of 2–3 aromatic rings and although less carcinogenic also pose toxic effect to many aquatic organisms (Brown & Peake, 2006).

The PAHs composition of water and sediments can give some information about their sources and how they were derived. Larger concentration of LMW PAHs (e.g acenaphthene, fluorene) most often occur in sample matrices contaminated with naturally occurring PAHs (petrogenic and biogenic origins) while the PAHs from combustion processes (pyrolytic origin) often contain elevated concentrations of HMW (e.g. phenanthrene, fluoranthene, pyrene) and fewer LMW PAHs (Kristin & Maria, 2007).

2.10 Metabolism of the PAHs

Aleksandran (2010) reported that since exposure to PAHs is never to a single PAH, understanding what differences may occur in mixtures of PAHs gives an accurate assessment of the dangers of PAHs. Understanding the dynamics of complex metabolism vis-a-vis single metabolism of PAHs and possible effects on the toxicity expression of PAHs is a necessary advancement to accurately impact and guide remediation strategies.

Studies were carried out comparing the metabolism of the PAHs phenanthrene (PHE), flouranthene (FLA) and benzo (a) pyrene (BAP) in single, binary, and ternary mixtures by monitoring the disappearance of the parent compound. It was observed that PAH metabolism in the single PAH experiment differed from metabolism in both binary and ternary mixtures. Enzymes competition was evident in the metabolism of mixtures and the possible creation of toxicity effects during mixture metabolism. PAH concentration changed over time with faster change during single metabolism followed by ternary mixture metabolism and finally binary metabolism. These results affirm that substrate interactions must be considered in the risk assessment approaches to the dangers posed by exposure to PAHs. Due to the high lipophilicity i.e. (its fat soluble or fat loving) of this class of compounds, their bioactivity after ingestion and inhalation is significant (Katarina, 2011). Scientific investigations have shown that detectable levels of PAH occurred in almost all internal organs, particularly in organs that are rich in adipose tissues. These organs can serve as storage depots from which the hydrocarbons can be gradually released. Once they enter the organism, polycyclic aromatic hydrocarbons require a multistep metabolic activation by specific enzymes. The enzyme system primarily is responsible for PAH metabolism (Katarina, 2011).

2.11 Effects of PAHs

2.11.1 Environmental Effects

PAHs are usually released into the air, or evaporated into the air when they are released to soil or water (INCHEM, 2010). PAHs often adsorb onto dust particles in the atmosphere, where they undergo photo-oxidation in the presence of sunlight, especially when they are adsorbed on particles. This oxidation process can break down the chemical over a period of days to week (Santodonato, 1981). Since PAHs are generally insoluble in water, they are generally found to adsorb on particulate matter and precipitated in the bottom of lakes and rivers, or solubilized in any oily matter which may contaminate water. Sediments and soil mixed microbial population in water system may degrade some PAHs over a period of weeks to months (ATSDR, 2010).

The toxicity of PAHs is affected by metabolism and photo-oxidation, and they are generally more toxic in the presence of ultraviolet light. PAHs have moderate to high acute toxicity to aquatic life and birds. PAHs in soil are unlikely to exert toxic effect on terrestrial invertebrates, except when soil is contaminated (Peter, 2003).

Adverse effects on these organisms include tumors, adverse effects on reproduction, development and immunity. Mammals can absorb PAHs by various routes e.g. inhalation, dermal contact and ingestion (ATSDR, 1993). Plant can absorb PAHs from soils through their roots and trans-locate them to other plant parts. Uptake rates are generally governed by concentration, water solubility and their physicochemical state as well as soil type (ATSDR, 2010).

2.11.2 Health Effects

i) Acute or Short-Term Health Effects.

The effects on human health will depend mainly on the length and extend of exposure, the amount or concentration of PAHs one is exposed to (Collins *et al.*,1998). The ability of PAHs to induce short-term health effects in human is not clear. Intake of PAHs from contaminated soil occurs via ingestion, inhalation or dermal (skin) exposure to contaminated soil/dust and from inhalation of PAH vapours. Tilling the dry soil can result in ingestion of small but measurable amounts of soil. Occupational exposure to high level of pollutant mixture containing PAH has resulted in symptoms such as eye irritation, nausea, vomiting, diarrhea and confusion (Collins *et al.,* 1998).

i) Chronic or long-term health effects

Health effect from chronic or long-term exposure to PAHs may include decreased immune function, cataract, kidney and liver damage (e.g. jaundice), breathing problems, asthma like symptoms and lung function abnormalities and repeated contact with skin may induce redness and skin inflammation. Naphthalene can cause the breakdown of red blood cell if inhaled or ingested in large amounts (Collins *et al.*, 1998). If exposed to PAHs, the harmful effect that may occur largely depends on length of period of exposure (BBC News, 2001).

ii) Carcinogenicity

PAHs can have toxic effect; a major concern is the ability of the reactive metabolities, such as epoxides and dihydrodiols, of some PAHs to bind to cellular proteins and DNA. The resulting biochemical disruption and cell damage leads to mutations, developmental malformation, tumors and cancer. Evidence indicates that mixtures of PAHs are carcinogenic to humans (Grimmer *et al.*, 1988). The evidence came primarily from occupational studies of workers exposed to mixtures containing PAHs and those long-term studies have shown an increase in risk of predominantly skin and lung, but as well as bladder and gastro - intestinal cancers (ATSDR, 2010). However, it is not clear from these studies whether exposure to PAHs was the main cause as workers were simultaneously exposed to other cancer – causing agents (e.g. aromatic amines) (Grimmer *et al.*, 1988).

Animals exposed to some PAHs over long periods in laboratory studies have developed lung cancer from inhalation, stomach cancer from ingesting PAHs in food and skin contact. Benzo(a)pyrene is the most common PAH that causes cancer in animals, and is notable to be the first chemical carcinogen to be discovered (IARC, 1983).

ATSDR (1993) classified a number of PAHs as carcinogenic to animal and some PAH – rich mixture as carcinogenic to human. The EPA has classified seven PAH compounds as probable human carcinogens: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(ah)anthracene, and indeno(1,2,3-cd)pyrene.

iii) Teratogenicity

Embroyotoxic effect of PAHs have been described in experimental animals exposed to PAH such as benzo(a)anthracene, benzo (a) pyrene and naphthalene. Laboratory studies conducted on mice have demonstrated that ingestion of high level of benzo (a) pyrene during pregnancy resulted in birth defects and decreased body weight in the offspring. It is not known whether these effects can occur in human (Katarina, 2011). However, the center for children's Environmental Health reports studies demonstrate that exposure of PAH pollution during pregnancy is related to adverse birth outcomes including low birth weight, premature delivery (Katarina, 2011). High prenatal exposure to PAH is also associated with lower IQ at age three, increased behavioral problems at ages of six and eight and childhood asthma. Cord blood of exposed babies shows DNA damage that has been linked to cancer (Nielsen, 1988).

iv) Genotoxicity

This describes the property of chemical agents that damage the genetic information within a cell causing mutation which may lead to cancer. Genotoxic effects for some PAHs have been demonstrated both in rodents and in vitro tests using mammalian (including human) cell lines. Most of the PAHs are not genotoxic by themselves and they need to be metabolized to the diol exposed which react with DNA, thus inducing genotoxic damage. Genotoxicity plays important role in the carcinogenity process and maybe in some forms of developmental toxicity as well (ATSDR, 2010).

A wide range of PAH-induced ecotoxicological effects in a divers suite of biota, including microorganisms, terrestrial plants, aquatic biota, amphibians, reptiles, birds and terrestrial mammals have been reported (Cerniglia, 1992).

However, the primary focus of the toxicological research on PAHs is on genotoxicity and carcinogenicity. In these studies, several PAHs have been shown to damage DNA and cause mutations, which in some cases may result in cancer (Staffan, 2003). However, for the unsubstituted PAHs it is not the original compound that reacts with DNA. The PAHs require metabolic activation and conversion to display their genotoxic and carcinogenic properties. This happens as the PAHs are metabolized in higher organisms (Staffan, 2003). PAHs do not accumulate in the same manner as some other lipophilic organic compounds, e.g. polychlorinated biphenyls (PCBs). Instead, they are converted to more water soluble forms, which facilities their subsequent excretion from the organism (Cerniglia, 1992). Unfortunately, this may also lead to the formation of reactive intermediates that may react with DNA to form adducts, preventing the gene involved from functioning normally (Staffan, 2003). The DNA-damage may be repaired, but if the repair fails, a mutation, i.e. an irreparable genetic damage, would have occurred. Mutation may affect many different functions of a cell, but above all they may induce cancer (Phillips, 1999). The metabolic activation of benzo(a) pyrene is shown below. This compound is probably the most thoroughly studied PAH, and is also one of the most carcinogenic compounds ever known. The initial step in the metabolism of PAHs involves the multifunctional P-450 enzyme system forming different epoxides through the addition of one atom of oxygen across a double bond. The epoxides are short-lived compounds and may rearrange spontaneously to phenols or undergo hydrolysis of dihydrodiols. These products may then be conjugated with glutathione, glucuronic acid or sulphuric acid, to form products that can be excreted by the organism (Fetzer, 2000). This conjugation process is, thus, regarded as the true detoxification and excretion process. However, the dihydrodiols may also act as a substrate for cytochrome P-450 once again to form new dihydrodiol epoxides e.g. trans-7,8-

dihydroxy-7,8 dihydrobenzo(a) pyrene-9,10-oxide, which unfortunately are poor substrates for further hydrolysis. These dihydrodiolepoxides may instead react with proteins, RNA and most seriously, DNA, thus causing mutations and possibly cancer (Kisselev *et al.*,2002).

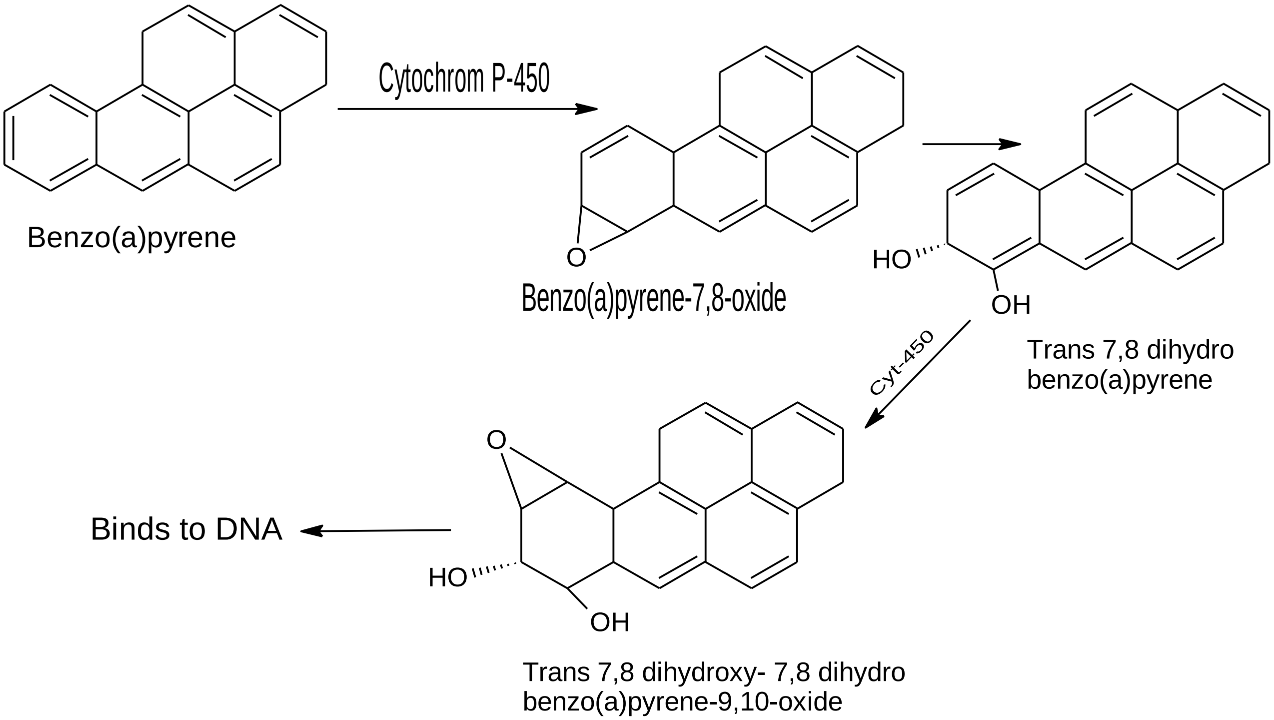


Figure.2.9: Metabolic activation of Benzo[a]pyrene (Kisselev *et al.*,2002)

2.12 Route of Exposure to PAHs

The major route of exposure to PAHs in the general population is from breathing contaminated air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fire places. Tobacco smoke contains a variety of PAHs such as benzo(a) pyrene and more than 40 known or suspected human carcinogens (Aleksandra, 2010).

For non-smokers, the main route of exposure is through the food chain. PAHs concentration in food stuffs varies. Charring meat or barbecuing food over charcoal, wood or other types of fire greatly increase the concentration of PAHs. Some crops such as wheat, rye, and lentils, may synthesize PAHs or absorb them via eating, air or soil. Water can also contain a substantial amount of PAHs since those chemicals can enter water from industrial effluents and accidental spills during oil shipment at sea. Soil also contains PAHs, primarily from airborne fallout (Aleksandra, 2010).

Therefore, PAH exposure occurs on a regular basis for most people. Occupational exposure may also occur in workers breathing exhaust fumes, such as those involved in metal working or oil refining. Routes of exposure include ingestion, inhalation and dermal contact in both occupational and non-occupational settings. Some exposure may involve more than one route simultaneously, affecting the total absorbed dose (such as dermal and inhalation exposures from contaminated air) (Aleksandra, 2010)

Mutumbi *et al.* (2003) reported that levels of PAHs in smoke depend on heat source (coal, wood, gas etc), temperature, flame intensity in flame combustion, particulate material generated during combustion, etc. Silva (2011) conducted a research on the level of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria and found that in *A. heudeloti* (cat fish), the total concentration of the sum of PAHs in the oven-dried method was the least, having a concentration of 332.8 µg/kg. Most of individual PAHs were not detected in oven-dried fish samples. This could be as a result of the method not involving the use of smoke. Some PAHs were not detected in both the over-dried fishes. Benzo(a) pyrene, for example, was not detected in both the over-dried and smoked fishes. The concentrations of PAHs in the fishes were 2,058.1 µg/kg, 1,320.9 µg/kg. 1,136.5 µg/kg and 332.8 µg/kg for saw dust smoked, firewood smoked, charcoal smoked and oven dried methods respectively (IARC, 1983). The level of PAHs in the smoked fishes was higher than those in the oven dried fishes. The PAHs levels were found to vary with heat sources.

The regulation for setting maximum levels of certain contaminants in foodstuff (IARC, 1983) stipulated the highest level of specific contaminants in foodstuffs; benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoroanthene, and chrysene are used as lead substances for PAHs, for the sum total of which limits between one microgram per kilogram for infant formula and follow-on formula and thirty five micrograms per kilogram for smoked mussels have been determined **(**Jejal, 2007). The benzo(a)pyrene limit for drinking water set in the drinking water ordinance is 10 ng/L (1ng = 10^{-6}mg).

ATSDR (2009) stated that the US Federal Government has set regulations to protect people from the possible health effects of eating, drinking, or breathing PAHs. Environmental protection Agency (EPA) has suggested that taking into your body each day the following amounts of individual PAHs is not likely to cause any harmful health effects: 0.3 mg of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per (kg) of your body weight (one kilogram is equal to 2.2 pounds). Actual exposure for most of the United States population occurs from active or passive inhalation of the compounds in tobacco smoke, wood smoke, and contaminated air, and from eating the compounds in foods. Skin contact with contaminated water, soot, tar, and soil may also occur (ACGIH, 1976). Estimates for total exposure in the United States population have been listed as 3mg/day (ACGIH, 1976). From what is currently known about benzo[a]pyrene, the Federal Government has developed regulatory standards and guidelines to protect people from the potential health effects of PAHs in drinking water. EPA has provided estimates of levels of total cancer-causing PAHs in lakes and streams associated with a risk of human cancer development. If the following amounts of individual PAHs are released to the environment within a 24-hour period, EPA must be notifed: 1pound of benzo(b)fluoranthene, benzo[a]pyrene, or dibenz(a.h)anthr- -acene; 10 pounds of benz(a)anthracene; 100 pounds of acenaphthene, chrysene, fluoran5thene, or indeno[1,2,3-c,d]pyrene; or 5,000 pounds of acenaphthylene, anthracene, benzo[k]fluoranthene, benzo[g,h,i]perylene, fluorene, phenanthrene, or pyrene. PAHs are generally not produced commercially in the United States except as research chemicals. However, PAHs are found in coal, coal tar, and in the creosote oils, oil mists, and pitches formed from the distillation of coal tars. The National Institute for Occupational Safety and Health (NIOSH, 2014) concluded that occupational exposure to coal products can increase the risk of lung and skin cancer in workers. NIOSH established a recommended occupational exposure limit, time weighted average (REL-TWA) for coal tar products of 0.1 milligram of PAHs per cubic meter of air (0.1 mg/m) for a10-hour workday, within a 40-hour workweek. The American Conference of Governmental Industrial Hygienists (ACGIH, 1976) recommends an occupational exposure limit for coal tar products of 0.2 mg/m for an 8-hour workday, within a 40-hour workweek. The Occupational Safety and Health Administration (OSHA) has established a legally enforceable limit of 0.2 mg/m averaged over an 8-hour exposure period. Mineral oil mists have been given an IARC classification of 1 (sufficient evidence of carcinogenicity). The (OSHA, 1980) Permissible Exposure Limit (PEL) for mineral oil mist is 5 mg/m averaged over an 8-hour exposure period. NIOSH has concurred with this limit, and has established a recommended occupational exposure limit (RELTWA) for mineral oil mists of 5 mg/m for a 10- hour work day, 40-hour work week, with a 10 mg/m Short Term Exposure Limit (STEL).

Processing of food at high temperatures (grilling, roasting, frying and smoking) are major sources generating PAHs. Levels as high as 200 μg/kg have been found for individual PAH in smoked fish and meat samples. For instance, in barbecued meat, 130 μg/kg has been reported whereas the average background values are usually in the range of 0.01 μg/kg to

1µg/kg in uncooked foods (Guillén *et al.,* 2009). Open burning of brushwood, straw, moorland heather, and stubble are agricultural sources of PAHs. All of these activities involve burning organic materials under suboptimum combustion conditions. Thus, it is expected that a significant amount of PAHs are produced from the open burning of biomass. Emission factors of PAHs from wood combustion ranged from 16.4 to 1,282 mg/kg wood (Jenkins *et al.,* 1996; Oanh *et al.,* 1999; Schauer *et al.,* 2001). PAH concentrations released from wood combustion depend on wood type, kiln type, and combustion temperature. 80-90 % of PAHs emitted from biomass burning are LMW PAHs, including naphthalene, acenaphthylene, phenanthrene, flouranthene and pyrene (Jenkins *et al.*, 1996).

Lui *et al.* (2010) reported that PAHs emitted from the open burning of rice and bean straw is influenced by combustion parameters. Total emissions of 16 PAHs from the burning of rice and bean straw varied from 9.29 to 23.6 µg/g and from 3.13 to 49.9 µg/g, respectively. PAH emissions increased with increasing temperatures from 200 to 700 ^{o}C. Maximum emissions of PAHs were observed at 40 % oxygen content in supplied air. However, emissions of PAHs released from the open burning of rice straw negatively correlate with the moisture content in the straw (Okenyi & Ubani 2014).

Table 2.2: Maximum Level of PAHs (as benzo(a)pyrene on wet weight)

S/NO	Food Stuff	Maximum Level of PAHs as (benzo(a)pyrene on wet weight)
1.	Oils and fats intended for direct human consumption or use as an ingredient in foods	2.0 µg/kg
2.	Baby foods and processed cereal-based foods for infants and young children	1.0 µg/kg
3.	Infant formulae and follow-on formulae, including infant milk and follow-on milk	1.0 µg/kg
4.	Dietary foods for special medical purposes intended specifically for infants	1.0 µg/kg
5.	Smoked meats and smoked meat products	5.0 µg/kg
6.	Muscle meat of smoked fish and smoked fishery products	5.0 µg/kg
7.	Muscle meat of fish, other than smoked fish	2.0 µg/kg
8.	Crustaceans, cephalopods, other than smoked	5.0 µg/kg

OSHA (1980)

Etonihu (2010) conducted a research on pristane, phytane and polycyclic aromatic hydrocarbon distributions in Nigerian coals. The result showed that PAHs present in some coals are naphthalene, methylnaphthalene, acephthene, phenanthrene. Anthracene and pyrene were detected in all the samples in minuscule amounts. The amount of anthracene ranged from $2.44x10^{-1}$ to $8.45x10^{-2}$ mg/kg, while pyrene ranged from$4.05x10^{-1}$ to $1.26x10^{-1}$mg/kg. The amount of acenaphthylene is insignificant in the samples. Carcinogenic PAHs such as benzo(a)pyrene, benzo(a)anthracene, benzo(k)fluoranthrene, benzo(j)flourantheren, dibenz(a,h)acridine and dibenz(a,h)anthracene were not detected among the Nigerian coals (Etonihu, 2010). Benzo (a) pyrene is a particularly potent carcinogen, being oxidized in the liver to an epoxy diol; (7,8-dihdroxy-9,10 epoxy7,8,9,10tetrahydrobenzen(a)pyrene) that can induce mutation leading to cancer (IARC, 1983).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Samples

3.1.1 Coal samples

The coal sample was collected from Lafia-Obi in Lafia LGA area of Nasarawa State, Nigeria.

3.1.2 Charcoal Samples

The charcoal samples were collected from Marke in Kokona LGA and Shamagay area in Nasarawa LGA of Nasarawa State.

3.1.3 Wood Samples

Shanjini (*Pterocarpus erinaceus*) and *Marke* (*Anogeissus leocarpa*) woods were collected from Keffi and Marke in Kokona LGA area of Nasarawa State. The samples were authenticated at the Biological Sciences Department of Nasarawa State University, Keffi.

3.2 Treatment of Samples

3.2.1 Coal samples

The coal sample was reduced by quartering to a laboratory sample, air dried and crushed, ground and sieved with a 200 mics mesh as described by Etonihu, (2010).

3.2.2 Charcoal samples

The charcoal samples were reduced by pounding with mortar and pestle, air dried and sieved with a 200 mics mesh.

3.2.3 Wood samples

The wood samples were reduced by means of a Wiley mill to sawdust, air dried and sieved with 200 mics mesh according to American Society for Testing Materials D1105-96 (ASTM, 2012).

3.3 Solvent extraction and fractionation of extracts

Each of the prepared samples was extracted in a Soxhlet extractor using *n*-hexane and dichloromethane, respectively at 80 °C for 48 h. The extract was concentrated using a rotary evaporator, and fractionated by column chromatography with alumina as adsorbent, which will be activated in an oven at 120 °C for 1 h. Slurry of alumina with the two solvents (n-hexane and dichloromethane) was packed respectively with care in a glass column of 10 cm and 1.5 internal diameter (id). The extract (2 g) was dissolved in 2 cm^3 of *n*-hexane and dichloromethane respectively, poured on the top of the column and allowed to drain. The extract was fractionated successively using *n*-hexane and dichloromethane. Fractions from the respective solvent was concentrated by removing the solvent using rotary evaporator, and dried at room temperature.

3.4 Determination of polycyclic aromatic hydrocarbons (PAHs)

The samples was subjected to GC-MS analysis using the instrument Trace GC Ultra Thermo Scientific DSQII, equipped with TR-5ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm film thickness. Electron ionization system with ionization energy of 70eV was used for GC-MS detection. Nitrogen gas (99.99 %) was used as a carrier gas at a constant flow rate of 1.5 ml/min. The sample injector temperature was maintained at 250 °C, using split less mode and volume injected was 2 cm^3.

The column temperature was programmed as follows; hold at 68 °C for 2 min; 68-260 °C at 12 °C/min; 250-320 °C at 15 °C/min and maintained at 320 °C for 8 minutes (Olabemiwo *et al.,* 2011). At the end of run-time, integrated chromatogram results was displayed on a computer and printed.

3.5 Solvent extraction of anti- termite agent

The powdered wood sample (saw dust) was extracted in 250 cm^3 of dichloromethane and n-hexane extract respectively in a soxhlet extractor at 80 ^{o}C for 6 h. The extract was used in treatment of softwood.

3.6 Preparation of wood sample for anti-termite testing

The woods used for this study were plane cut into small test specimens with dimensions of 7 x 3 x 3 cm. The samples were labeled for ease of identification. Four wood samples were used for these study consisting of two hardwoods (*Shanjini* and *Marke*) and two softwoods. One of the softwoods was treated with the wood extractive while the other softwood served as the control.

3.7 Treatment of samples with extractives

The softwood was collected from a sawmill in Keffi while *Shanjini* (*Pterocarpus erinaceus)* and *Marke* (*Anogeissus leocarpa*) woods were collected from Keffi and *Marke* in Kokona LGA area of Nasarawa State. Treatment of the wood sample involved immersion of the softwood that is susceptible to termite degradation into a bath of wood extract for 5 days until a constant weight was achieved as described by Ibach (1999) and Roll (2003).

After treatment, the samples were air dried, then oven dried at 60 ^{o}C for 10 hours until constant weight. Samples were labeled, conditioned to equilibrium moisture content then weighed to determine initial weight W_1 and then exposed to *Microcerotermesbeesonii* termites in the field (Bultman & Southwell, 1976).

The field site was in Nasarawa State University, Keffi where *Microcerotermesbeesonii* termites were detected and the samples were exposed to termites. Field trial was preferred to laboratory studies because they allow the collective and cumulative effects of all kinds of abiotic and biotic deterioration factors to be evaluated (Peralta *et al.*, 2003).

They also give reliable data regarding natural resistance of wood (Bultman & Southwell, 1976). The samples were laid down rando mly at the site about 12 metres away from the termite mounds but in an area with termite tunnels (Koehler &Tucker, 2003). The samples were covered with grass, and tree branches to prevent grazing animals from destroying them and increasing their susceptibility to termite attack (Figure.3.1)

Figure. 3.1: Layout of the wood samples in the field

The wood samples in the field were inspected every day in order to ascertain when the termites start to act on the samples, after which the samples were removed, cleaned and oven dried for 18 hours at 60 ^{o}C. After conditioning to equilibrium moisture content (EMC), samples were again cleaned of all soil and weighed to determine their final weight W_2 . The percentage weight loss was determined according to method D1413 (ASTM, 2003):

$$\text{Percentage weight loss} = 100\,\frac{(W1 - W2)}{W1}$$

Where W_1 = Weight of test specimen after treatment but before exposure to termites.

W_2 = Weight of test specimens after exposure to termites.

3.8 Instrumentation

GC/MS is an analytical instrument that has separation power of Gas Chromatography (GC), with the detection power of Mass spectrometry. Mass Spectrometry is a wide-ranging analytical technique which involves the production and subsequent separation and identification of charged species according to their mass to charge (m/z) ratio (McLafferty *et al.,*1974). The gas chromatograph uses a capillary column which depends on the column's dimensions (length, diameter, film thickness). The difference in the chemical properties between different molecules in a mixture are separated as the molecules traveled the length of the column. The molecules take different time (called the retention time) to come out of (elute from) the gas chromatograph. This allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio (McLafferty *et al.*, 1974). The most common type of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are hit with free electrons emitted from a filament. The electrons hit the molecules, causing the molecule to fragment in a characteristic way that can be repeated. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z). EI has few, if any, fragments having a mass that is near the mass of the original molecule. Chemists consider hard ionization to be shooting electrons into the sample molecules. In contrast, "soft ionization" is placing a charge on the sample molecule by hitting it with an introduced gas. The molecular fragmentation pattern depends on the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV helps to compare the spectra generated from the test sample against known library spectra.

CHAPTER FOUR

RESULTS AND DISCUSSION

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Thanks……….

Anti-Termite Effects of the Wood Extractives

The percentage weight loss of termite resistance on wood that is treated and untreated with Shanjini (*Pterocarpus erinaceus*) extract was determined mathematically and the result is shown in Table 4.7.

Table 4.7: Anti- Termite Activities of the Wood Samples

S/No	Wood Samples	Length (cm)	W_1 (g)	W_2 (g)	% Weight Loss
1.	Treated wood(SF)	7	4.3	4.1	4.65
2.	Un-treated wood (SF)	7	3.9	2.2	43.59
3.	Shanjini wood (HW)	7	19.5	19.3	1.03
4.	Marke wood (HW)	7	17.7	16.3	7.91

Where W_1 = Weight of samples after treatment but before exposure to termites.

W_2 = Weight of samples after exposure to termites.

(SF) = Softwood

(HW) = Hard wood

Table 4.7 relates the values of the percentage weight loss and weight of the treated wood, control, and two hard woods. *Shanjini* (*Pterocarpus erinaceus*) wood was found to have great resistance to termite attack with percentage weight loss of (1 %) while the soft wood that served as the control was destroyed by termite with weight loss of (40 %). The softwood treated with the wood extractive rather gained weight of (-5 %) and *Marke* (*Anogeissus leocarpa*) wood had a percentage weight loss of (8 %).

Figure.4.1: Residual of the wood samples after termite attack

Discussion

Polycyclic aromatic hydrocarbons

A total of 16 PAHs was present in some woods, charcoals and coal samples evaluated in Nasarawa State as shown in Tables 4.1-4.6. Amongst the PAHs, naphthalene was the highest in amount (0.583 mg/kg) in dichloromethane (DCM) of the Lafia-Obi Coals, while Benzo(g,h,i)Perylene was the least in amount (0.001 mg/kg) for both n-hexane and DCM extracts in Table 4.1. This could be as a result of high temperatures in sedimentary environment resulting from igneous activity (Murchinson & Raymond, 1989). The presence of the PAHs analyzed in the Lafia-Obi Coal will have no much effect on human except when exposed to it for long period of time which is in agreement with Etonihu (2010). From Tables 4.2 and 4.3 Benzo(a)pyrene was highest in amount (0.337 mg/kg) and (0.410 mg/kg) of PAHs in charcoals sourced from *Shamagey* and *Marke* for DCM and n-hexane extracts, while (0.000 mg/kg) benzo(g,h,i)perylene was approximately the least in amount of PAHs for both n-hexane extracts of the charcoal samples. The reason for high concentration of the PAHs in charcoal sourced from Shamagey when compared with charcoal sourced from Marke, could depend on the wood type and temperature as reported by Lui *et al.*(2010). From Tables 4.4 and 4.5 benzo(a)pyrene was the highest in amount (0.326 mg/kg) and (0.300 mg/kg) for both DCM extracts for Shanjini and Marke, while benzo(g,h,i)perylene was the least in amount with (0.000 mg/kg) for n-hexane. The reasons for higher concentration in amount of PAHs in Shanjini wood could be as a result of the type of wood. The high intensity of the color from the wood extract is characteristic of the presence of more phenolic compounds. Comparing *n*-hexane extract and DCM extract of the PAHs in all the Tables, it was found that DCM solvent extracted more than n-hexane solvent. This could be due to difference in polarity. Dichloromethane is a more polar solvent than *n*-hexane. Coal was found to contain more of the PAHs than the woods and charcoal samples. This is expected due to the compact mass of the coal as a result of high temperature, pressure and the less oxygen available beneath the earth crust for its formation. This is in agreement with IARC (1983), which states that PAHs are produced by incomplete combustion of organic matter such as wood, coal or oil. As a general rule, the lower the temperature of the fire and the less oxygen available, the more incomplete do these materials burn and the PAHs are produced. Table 4.6 showed the two classes of PAHs in the samples. The high molecular weights (HMW) PAHs (228-278 g/mol) include benz(a)anthracene ,benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(j)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, benzo(ghi)perylene, dibezo(a,h)- anthracene, Chrysene, cyclopenta(cd)pyrene, indeno (1,2,3-cd)pyrene, and anthranthrene. The Low molecular weights (LMW) PAHs (152-178 g/mol) include naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, flouranthene, pyrene and phenanthrene. As can be observed from Table 4.6, the total concentration of the high molecular weights (HMW) polycyclic aromatic hydrocarbons was higher than the low molecular weights (LMW) in all the samples analyzed. Most of the carcinogenic polycyclic aromatic hydrocarbons fall within the group of the high molecular weights (EFSA, 2002). From the result, we could infer that there are higher concentrations of these polycyclic aromatic hydrocarbons in coal than woods and charcoals, and it could be due to the reflex input from combustion of organic matter and fossil fuels. Suitably high temperatures for the formation of the PAHs can occasionally arise in sedimentary environment as a result of igneous activity (Murchinson & Raymond, 1989). Kwaka & Simoneit (1990), reported the detection of pyrolytic PAHs in oil seeps in the Guyamas Basin of California (USA) as a result of PAH generation by igneous activity. They attributed their formation to the *in situ* pyrolysis of contemporary organic matter (with significant terrestrial component) at temperatures in excess of 300 ^{o}C and the heat was supplied hydrothermally by activity associated with spreading center in the basin. Tables 4.1- 4.6 have shown that PAHs were detected but in a miniscule amount when compared to ATSDR (2009) which stated that the US Federal Government has set regulations to protect people from the possible health effects of eating, drinking, or breathing PAHs. Environmental protection Agency (EPA) of US has suggested that taking into the body each day the following amounts of individual PAHs is not likely to cause any harmful health effects: 0.3 mg of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per (kg) of your body weight (one kilogram is equal to 2.2 pounds). REACH (2006) placed a maximum limit on eight PAHs classified as carcinogenic in products not to be above a concentration of 0.2 milligrams per kilogram. International Agency for Research on Cancer (IARC, 1987) has classified seven PAHs, namely: (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a.h) anthracene as probable (2A) and Napthalene, acenaphthylene, fluorine and anthracene as possible (2B) carcinogens expectant.

Anti Termite Activity

The percentage weight loss of some selected wood sample analyzed for anti-termite activity is shown in Table 4.7. The results revealed that *Shanjini* had the highest anti-termite activity with percentage loss of 1.03 % while the untreated softwood had the least anti-termite activity with percentage loss of 43.59 %. The treated softwood recorded percentage weight loss of 4.7 % and 7.91 % for *Marke* wood after 12 weeks of exposure to termites, respectively. The anti-termite test on the wood samples showed that the wood extractives provided resistance to termite attack in softwood treated with wood extract from *Shanjini* (*Pterocarpus erinaceus*). Similar results were reported by Goktas *et al.* (2007) who evaluated the wood preservative potentials of *Sternbergia candidum.* Extracts where by weight losses for all treated samples decreased significantly confirming the effectiveness of extract solution in enhancing decay resistance. Onuorah (2000) found that heartwood extract of *Erythrophleumsuaveolens* and *Miliciaexcelsa* when applied to less durable sapwoods were effective in suppressing attack of either *Lenzitestrabea* or *Polyporousvericolour* fungal species. In their study on the effect of wood extracts on growth and cellulose production strains of *Bacillus subtilis,* addition of wood extract significantly inhibited the growth of *Bacillus* strains (Femi-Ola & Aderibigbe, 2008) confirming their effectiveness.

The comparative high loss in weight of *Marke* wood (*Anogeissus leocarpa*), even though it is a hardwood, could be due to the it's low content of the extractives making the wood prone to termite attack when compared to *Shanjini* (*Pterocarpus erinaceus*). Stołyhwo & Sikorski (2005), conducted a research on polycyclic aromatic hydrocarbons in smoked fish. The traditional method to preserve fish in countries such as Indonesia is by smoking, which is an indirect way to apply wood condensates into the fish. Wood burning produces smoke which contains a variety of toxic polycyclic aromatic hydrocarbons (PAHs), as well as phenols, aldehydes, ketones, organic acids, alcohols, esters, hydrocarbons and several heterocyclic compounds. When treating wood with smoke, moisture content is reduced and PAHs are condensed and diffused inside wood structure. PAHs have been shown to provide some resistance to biological deterioration driven by termite attack (Hadi *et al*., 2010), which is also in agreement with the findings in this study whereby, wood extractives containing chemical components such as Polycyclic aromatic hydrocarbons (PAHs), phenols, aldehydes, ketones, organic acids, alcohols, ester, hydrocarbons, flavonoids, tannins, quinines, stilbene were used as anti-termite treatment for woods. The resistance of the treated wood to termites could be due to the wood extractives' repellent characteristics. The repellent characteristics could be due to the toxic chemical composition of the wood extractive and the durability of the heartwood of the tree species from which they were extracted. This is in agreement with Taylor *et al*. (2006) who in their study suggested that the methanol-soluble extractives of *Thujaplicata* and *Chamaecyparisnootkatensis* played an important role in heartwood resistance to attack by *Coptotermesformosanus* and *Postiaplacenta.* They further noted that methanol soluble extractives of the heartwood of those tree species were positively correlated with both termite and decay resistance.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATION

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds that consist of two or more fused aromatic rings. These compounds are ubiquitous environmental pollutants generated primarily during the incomplete combustion of organic materials such as woods and fossil fuels. Lafia-Obi coals, woods and charcoals from Keffi, Marke and Shamagay in Nasarawa State were evaluated for the presence of PAHs using standard methods. 200-mesh size pulverized and saw milled samples were solvent extracted using n-hexane and dichloromethane at 80^o and 30^o for 48 h and fractionated in alumina column. Both fractions were characterized by gas chromatography and mass spectroscopy (GC-MS) that showed a total of 16 PAHs in the samples. Naphthalene was the highest in amount (0.583mg/kg) in the dichloromethane extract of Lafia-Obi Coal while benzo(g,h,i)perylene was the least in amount with approximately (0.000mg/kg) in n-hexane extract for all sample analyzed except Lafia- Obi Coal (0.001mg/kg) for n-hexane extract. In all the samples, the amounts of PAHs were below their toxic levels. Anti-termite activities of wood samples were investigated by treating the wood extractives from the Shanjini (*Pterocapus erinaceus*) and Marke (*Anogessus leocarpa*) on softwoods that are prone to termite attack on the field for 12weeks. The results showed that the efficacy of wood extractives to control termite was higher in Shanjini (*Pterocapus erinaceus*) than Marke (*Anogessus leocarpa*) in terms of resistance to termite attack.

Conclusion

Evaluation of PAHs in Lafia-Obi coals, woods and charcoals from Keffi and Marke in Nasarawa State showed the presence of naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, floranthene, pyrenebenzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a)pyrene, indeno (1,2,3-cd)pyrene, dibenzo (a,h) anthracene and benzo (g,h,i) perylene below their toxic levels as stipulated by REACH (2006).Wood resistance of less durable softwood increased after treatment with extract from Shanjini wood. The results of this study showed the efficacy of wood extractives to control termites, and that Shanjini is better hardwood than Marke in terms of resistance to termite attack. This can be a very promising biocide and an alternative for synthetic pesticides for woods protection because of its resistance, abundance and annual renewability.

Recommendations

i) Adequate attention should be paid on the usage of coals, charcoals and woods as sources of energy due to the hazard they could pose by PAH production and emission during usage.

ii) Further research can be done on the use of wood extractives as insecticide.

iii) Government should encourage the production of anti-termite chemicals from wood by providing the enabling environment in form of provision of finance for the cultivation of high breed of trees that would be able to produce wood extractives for companies that would want to go into the production of anti-termite chemicals.

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