



REVERSAL OF MATURATION ARREST USING ALLIUM SATIVUM

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ABSTRACT

Reversal of maturation arrest using *Allium sativum* in albino rats exposed to high volumes of Bonny light crude oil was investigated. Male and female rats weighing 180-220g were placed in control and test groups consisting of four (4) subgroups with each sub group consisting of five (5) animals and used to test the effect of crude oil on testicular function (arrest of maturation). The animals were divided into control and test groups. Control group rats were administered unpolluted “normal” feed and water ad libitum – as desired. While the test group animals were administered crude oil polluted feed in the ratio of 5kg feed mixed with 500ml Bonny light crude oil and water for 21(twenty one) days. Histological examination on animals given crude oil feed and water showed maturation arrest. From previous crude oil toxicity test, arrest of maturation of sperm cells was possibly as a result of the lethal effects of crude oil on the testicular tissues. Successive studies were carried out incorporating *Allium sativum* (garlic) into the feed and water of experimental animals in the ratio of 5kg feed mixed with 450ml water and 50ml *Allium sativum* (garlic) solution. Experimental animals that were not “sacrificed” from the previous experiment were used in this study. On day 21 of the second experiment, the control and test animals were “sacrificed” and histological studies carried out once again on the testicular organ with observations of a reversal of maturation arrest of the sperm cells in the experimental animals. These findings indicate that extracts of *Allium sativum* reversed maturation arrest of sperm cells.

INTRODUCTION

Pollution

The term pollution refers to any substance that negatively impacts the environment or organisms that live within the affected environment. Addition of the substance is done at rates faster than can be dispersed, diluted, decomposed, recycled or stored in some harmless form

Oil spillage (Pollution)

Oil spillage can be defined as the release of crude oil hydrocarbons into the environment. It is an important environmental disaster of global concern that usually occurs accidentally or intentionally mostly resulting from everyday human activities that release crude oil into coastal waters and land. Environmental pollution arising from oil prospecting and exploration in the Niger Delta regions of Nigeria has definitely impacted negatively on the biodiversity of the affected regions. The major problems arise from leakage of crude oil, gas flaring and the escape of other chemicals used in the production process.

Records of oil spills in Nigeria only became evident in the late 1970's due to occasional spills caused by corrosion and ageing facilities than through sabotage. An estimated 240,000 barrels of crude oil is spilled in the Niger Delta of Nigeria every year polluting waterways, contaminating crops and releasing toxic chemicals.

The Nigerian National Petroleum Corporation places the quantity of crude spilled into the environment yearly at 2,300 cubic meters with an average of 300 individual spills of 300 individual spills annually. Oil spills have become a common event in Nigeria with half of all the spills occurring due to pipeline and tanker accidents.

Other causes include sabotage, oil production operations and inadequate or non-functional production equipments. Sabotage and theft through oil siphoning has become a major issue in the Niger Delta states as well as contributing to degradations.

In December 2006, more than 200 people were killed in Lagos state, Nigeria in an oil-line explosion.

CONSEQUENCES OF SPILLS

Oil spills have a major effect on the ecosystem into which spilled crude is released and may therefore constitute ecocide. Ecocide is criminalized human activity that violates the principles of environmental justice such as causing extensive damage, destroying ecosystems or harming the health and well being of species humans inclusive.

The mangrove forests are highly susceptible to oil. The oil is stored in the soil and released annually during inundations. An estimated 5-10% of Nigerian mangrove ecosystems have been wiped out.

The rainforest that previously occupied about 7,400km² of land has disappeared. In populated areas, spills often spread out over a wide area destroying crops and aquacultures through contamination of the ground water and soil. Reports of death of fish and other aquatic forms that are of great economic importance are high.

The consumption of dissolved oxygen by bacteria feeding on the spilled hydrocarbons also contributes to the death of fish. Agriculture is also affected and some agricultural communities experience losses in food production. The effects of an oil spill will depend on a variety of factors including the quality and type of oil spilled and how it interacts with the marine environment. Prevailing weather conditions also influence the oil's physical characteristics and behaviour.

Other factors include biological and ecological attributes of the area; ecological significance of key species and their sensitivity to oil pollution as well as the time of year.

CRUDE OIL COMPOSITION

Crude oil is a mixture of comparatively volatile liquid hydrocarbon compounds composed mainly of hydrogen and carbon. It also contains some nitrogen, sulphur and oxygen. The elements form diverse groups of complex molecular structures with some not being readily identified. Variations notwithstanding, almost all crude oil range from 82-87% carbon by weight. Customarily, classification is done by the type of hydrocarbon compound that is most prevalent in them.

These are paraffins, naphthenes and aromatics, with paraffin being the most common hydrocarbon found in crude oil. Certain liquid paraffins are the major constituents of gasoline and are therefore of very great importance. Naphthenes are a crucial part of all liquid refinery products and also form some of the heavy asphalt like residues of refinery processes. The hydrocarbon structures found in oil include saturates, aromatics and polar compounds which include resins and asphaltenes.

Resins and asphaltenes are largely refractory in the environment. They evaporate, dissolve and degrade poorly and end up accumulating as residues especially after a crude oil spill. The percentage of saturates and aromatics called the light compounds in comparison to the heavy residue forming resins and asphaltenes vary with type of crude oil.

Aromatics generally constitute only a small percentage of most crude with the most common aromatic in crude oil being benzene, a popular building block in the petrochemical industry. Because crude oil is a mixture of such widely varying constituents and proportions, its physical properties also vary widely. In appearance, crude oil ranges from colourless to "black". The most important physical property probably is specific gravity; which is the ratio of the weight of equal volumes of the crude oil and pure water at standard conditions.

In laboratory measurements of specific gravity, it is customary to assign pure water a measurement of one (1). Substances lighter than water such as crude oil receive a measurement less than one (1). The petrochemical industry however uses the American Petroleum Institute (API) gravity scale in which pure water has been arbitrarily assigned an API gravity of 10^0 .

Liquids lighter than water such as oil have API gravities numerically greater than 10. On the basis of API gravities, crude oil can be classified as heavy, medium and light as follows;

Heavy $10 - 20^0$ API gravity

Medium $20 - 25^0$ API gravity

Light above 25^0 API gravity

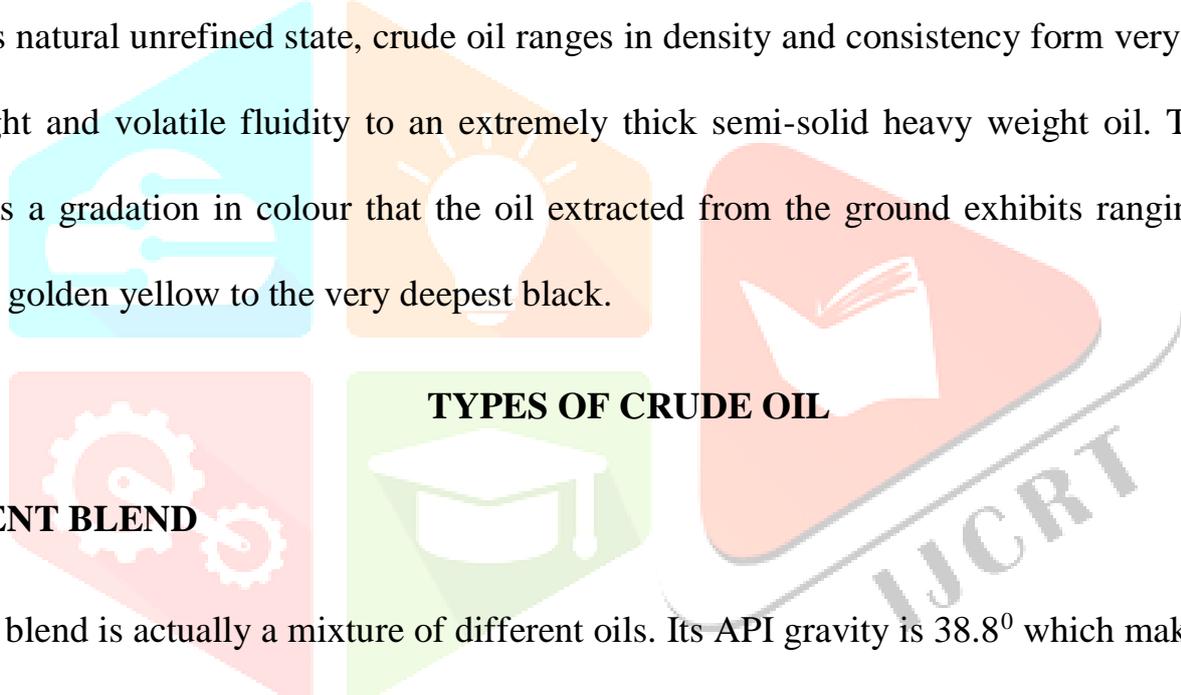
Crude oil is also categorized as “sweet” or “sour” depending on the level of sulphur which occurs whether as elemental sulphur or in compounds such as hydrogen sulphide. Sweet crudes have sulphur contents of 0.5% or less by weight while “sour” crudes have sulphur contents of 1% or more by weight.

Generally, the heavier the crude oil, the more its sulphur content. Excess sulphur is often removed from crude oil during refining because sulphur oxides released to the atmosphere during combustion of oil is a major pollutant. Petroleum in Nigeria is often classified as “light” and “sweet” since the oil is largely free of sulphur.

Most crude oils are named according to export terminals; for example there is Bonny light, Qua iboe, Escravos blend, Brass river, Forcados and Pennington Anfan. The major and minor classes of crude oil come from various export terminals in Nigeria. Bonny light was so named because its export terminal is located in the city of Bonny in Rivers State, South-South Nigeria.

Another classification into light, medium and heavy oil is based on their densities and toxicity levels which also depend on their volatilities. The purification of crude oil focuses first on the use of industrialized fractional distillation to separate crude oil into primary products which is followed by different cracking and refining processes to generate secondary products from the primary products whose purification is outsmarted by their utilization locally or whose utilization by the Nigerian populace is well reduced as compared to their level of production. Crude oil is not a single indistinguishably homogenous substance without differences that are unique. There are many different types of crude oil.

In its natural unrefined state, crude oil ranges in density and consistency from very thin light weight and volatile fluidity to an extremely thick semi-solid heavy weight oil. There also exists a gradation in colour that the oil extracted from the ground exhibits ranging from a light golden yellow to the very deepest black.



TYPES OF CRUDE OIL

BRENT BLEND

This blend is actually a mixture of different oils. Its API gravity is 38.8^0 which makes it light crude oil. It also contains about 0.37% sulphur and this makes it a “sweet” crude oil.

Brent blend is excellent in making gasoline and middle distillates, it remains a major benchmark for other crude oils in Europe and Africa.

OPEC BASKET

The OPEC basket is a collection of seven (7) different crude oils from Algeria, Saudi Arabia, Indonesia, Nigeria, Dubai, Venezuela and the Mexican Isthmus. Because OPEC oil has a much higher percentage of sulphur within its natural make up and therefore is not nearly as “sweet” as Brent blend it is also not naturally as light as Brent blend.

The bulk of the compounds present in crude oil are hydrocarbons. Crude oil generally contains classes of hydrocarbons and other compounds.

HYDROCARBON COMPOUNDS

Alkanes (Paraffins)

Alkanes are straight chain normal alkanes and branched iso-alkanes with general formula C_nH_{2n+2} . The major paraffinic components of most crude oils are in the range $C_1 - C_{35}$, although small quantities of alkanes up to C_{60} or higher may be present. Crude oils vary widely in alkane content.

CYCLOALKANES (Naphthenes)

Cycloalkanes, cycloparaffins or naphthenes are saturated hydrocarbons containing structures with carbon atoms linked in a ring. The cycloalkane composition in crude oil worldwide typically varies from 30-60%.

The predominant monocycloalkanes in crude oil are in the cyclopentane series having 5(five) carbon atoms in the ring and cyclohexane having a six (6) membered ring.

AROMATIC HYDROCARBONS

Aromatic hydrocarbons are those which contain one or more benzene rings. The name of the class comes from the fact that many of them have strong pungent aromas. The most common aromatic compounds in crude oil are benzene. The concentration of benzene in crude oil is estimated to be between 0.01% and 1%, Aromatic hydrocarbons also include benzene active metabolites.

ALIPHATIC HYDROCARBONS

Aliphatic stems from the Greek word meaning fat or oil. It refers to hydrocarbons that are straight chained, branched or non aromatic. Hydrocarbons which do not contain a benzene ring are called aliphatic hydrocarbons. Some of the most common flammable and volatile compounds are aliphatic hydrocarbons. They can either be saturated or unsaturated.

NON HYDROCARBON COMPOUNDS

Vanadium, nickel, nitrogen and sulphur are known to be the most significant non hydrocarbon constituents of crude oil. Non hydrocarbon compounds provide fundamental answers in petroleum geo-chemistry.

They may be small in quantity but some have considerable influence on the quality of products. They have harmful effects in most cases and must be removed or converted to less harmful compounds during the refining process. The most common occurring non-hydrocarbons are sulphur, nitrogen and oxygen.

SULPHUR COMPOUNDS

Sulphur is common in crude oil but varies from 0.2-6% by weight. There are corrosive and non corrosive sulphur compounds. One corrosive compound is hydrogen sulphide (H_2S). Fortunately, sulphides have highly obnoxious smell that gives some warning of their danger.

If one hydrogen atom is replaced by a hydrocarbon group, the compound is called a Mercaptan or thiol. Such compounds are formed during the distillation of crude oils. They can cause severe corrosion of the processing units and the addition of chemicals; proper temperature control and the use of special alloys in refinery equipment are required to control them. If both of the two hydrogen atoms are replaced by hydrocarbon groups, the

compound is called a sulphide or thio-ether. An example is thiophene (C_4H_4S). Thiophenes have a relatively pleasant odour.

NITROGEN COMPOUNDS

Nitrogen compounds in crude oil are complex. They may cause discolouration in gasoline and kerosene or may produce a lacquer quality there by reducing the effectiveness of lubricating oils. Almost all crude oils contain nitrogen usually in quantities less than 0.1% by weight.

OXYGEN COMPOUNDS

Some crude oils contain oxygen compounds. Their structures have not been established but on distillation of the crude, the oxygen compounds decompose to form ring compounds with a carboxylic acid group in the side chain. The oxygen content of crude oil is usually less than 2% in weight with heavier oils containing the most oxygen.

OTHER COMPOUNDS

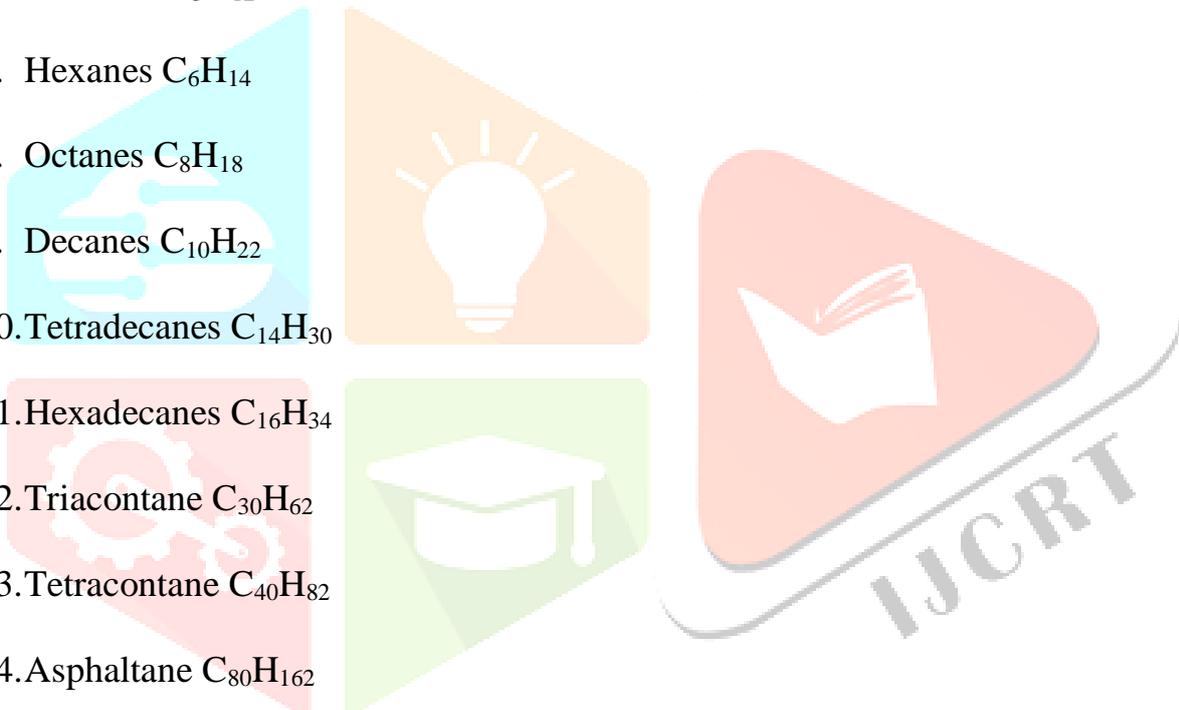
Several other organic and inorganic compounds occur in crude oil. An example is the metal vanadium which is an important component in the manufacture of specialty steels and can be recovered from the residue of the refining process. Many metallic elements are found in crude oils including most of those that occur in sea water probably because of the close association between sea water and the organic forms from which oil is generated.

Vanadium is the most common metallic element in oil followed by nickel. They probably occur in organic combinations as they do in living plants and animals. Crude oil also contains a small amount of decay resistant organic remains such as siliceous skeletal fragments, wood, spores, resins, coal and various other remnants of former life.

CONSTITUENTS OF NIGERIA CRUDE OIL

The following are found in Nigeria crude oil;

1. Methane CH_4
2. Ethane C_2H_6
3. Propane C_3H_8
4. Iso-butane I- C_4H_{10}
5. N-butane N- C_4H_{10}
6. Pentanes C_5H_{12}
7. Hexanes C_6H_{14}
8. Octanes C_8H_{18}
9. Decanes $\text{C}_{10}\text{H}_{22}$
10. Tetradecanes $\text{C}_{14}\text{H}_{30}$
11. Hexadecanes $\text{C}_{16}\text{H}_{34}$
12. Triacontane $\text{C}_{30}\text{H}_{62}$
13. Tetracontane $\text{C}_{40}\text{H}_{82}$
14. Asphaltane $\text{C}_{80}\text{H}_{162}$



There are hydrocarbons of simple, short chains, long chains and branched chains. These hydrocarbons are also called simple paraffins (alkanes), branched paraffins (cycloalkanes), Naphthenes, aromatics, benzene, naphthalene and alkyl derivatives, polynuclear aromatic hydrocarbons (PAHs) which contain two or more fused rings. Essentially, some of these paraffins are gasoline and kerosene.

Crude oil also contains small but significant amounts of impurities such as formation sand and water, sediments, trace metals especially lead (Pb), iron (Fe), copper (Cu), asphalt (As)

and Vanadium (V). Compounds of sulphur(s), chlorine(cl), oxygen(o) and Nitrogen(N) are present as mercaptans, salts, phenols and anilines respectively.

In addition to the constituent compounds that are derived from the crude which result from the interaction of crude oil constituents and living cells also exist when crude oil is spilled or taken into a living cell.

The compounds are;

1. 3, 4 benzo(a) pyrene
2. 20 methylcholanthrene (2methylcholanthrene)
3. 7, 12 dimethyl benzanthracene
4. Polycyclic aromatic hydrocarbons
5. Dibenz (a, b) acridine
6. 5-methyl chrysene
7. Dibenz (a, b) anthracene
8. Toluene

A significant effect resulting from crude oil spillage is the emission into the atmosphere some of the gases of non-metallic origin and discharge into the air of volatile hydrocarbons.

The emission of these gases constitutes air pollution.

The direct consequences of air pollution is the oxidation of the non metallic gases to form gaseous compounds which produce harmful effects in the environment. The gases include;

1. Hydrogen sulphide
2. Nitrogen oxides
3. Carbon monoxide
4. Ozone

5. Sulphur oxides
6. Particulate sulphates

TOXIC EFFECTS OF THE CHEMICALS IN THE NIGERIAN PETROLEUM CRUDE OIL.

1. **ALIPHATIC HYDROCARBONS:** The C_1 - C_4 aliphatic hydrocarbons are simple asphyxiants and their effects are observed when they are highly concentrated in air leading to reduction in the amount of oxygen
2. The C_5 - C_8 aliphatic hydrocarbons depress the central nervous system causing dizziness and in-coordination.

Paraffin, gasoline and Kerosene

These contain aliphatic, aromatic and a variety of branched chain and unsaturated hydrocarbons. Ingestion or exposure of humans to these agents produce unpleasant signs and symptoms which include in-coordination, restlessness, excitement, confusion, disorientation, ataxia, delirium and coma. Paraffinic vapour sensitizes the myocardium such that small amounts of circulating epinephrine may precipitate ventricular fibrillations.

High concentrations of paraffinic vapour may lead to rapid depression of the central nervous system and finally death from respiratory insufficiency. Poisoning from these hydrocarbons result either from inhalation of the vapours or from ingestion of the liquid. Ingestion is known to be more hazardous. Ingestion of the substances can be easily aspirated into the respiratory tract by vomiting or eructation.

Chemical pneumonitis complicated by secondary bacterial pneumonia and pulmonary edema is the most serious sequel to aspiration. Death usually occurs by haemorrhagic pulmonary edema within 16-18 hours.

AROMATIC HYDROCARBONS

Aromatic hydrocarbons example benzene is very toxic. After acute exposure to large amounts to benzene either by ingestion or breathing concentrated vapours, the major toxic effects is on the central nervous system (CNS). Symptoms from mild exposure include headache, nausea, vomiting, tightness in the chest and staggering. With more severe exposures, symptoms progress to blurred vision, tremors, shallow and rapid respiration, ventricular irregularities, paralysis and unconsciousness. Chronic exposure to benzene is usually due to inhalation of vapour.

Signs and symptoms include effects on the central nervous system and the gastrointestinal tract which include nervousness, headache, and loss of appetite, drowsiness and palor. Aplastic anaemia is the major manifestation of toxicity. The most sensitive to benzene are bone marrow cells in early stages of development with arrest of maturation leading to gradual depletion of circulating cells.

GENOTOXICITY OF AROMATIC HYDROCARBON DERIVATIVES

BENZENE

The major effect of benzene from long term exposure is on the blood. Long term exposure could be a year or more. Benzene causes harmful effects on the bone marrow causing a decrease in red blood cells thereby leading to anaemia. Epidemiological studies demonstrate that benzene is a human leukenogen. Benzene has also been shown to be a multi organ carcinogen in animals, high levels of benzene can cause eye, skin and respiratory irritation, difficulty breathing, cardiovascular effects such as ventricular fibrillations, gastritis, kidney congestion and neurological effects such as distal neuropathy, abnormality in nerve conduction velocity, difficulty sleeping and memory loss.

At high levels of exposure, confusion, convulsive movements, paralysis and death can occur.

Intermediate and chronic exposure to benzene cause a variety of pathological states which include cytopenia (anaemia, leukopenia or thrombocytopenia).

Central nervous system effects such as headache, dizziness, fatigue, anorexia, visual disturbances, hearing loss and respiratory irritation evidenced by difficulty in breathing.

Benzene has also been shown to be an immune suppressive agent. Critical effect of chronic exposure to benzene is an increased risk of cancer.

Benzene can also cause chromosomal aberrations in humans. Chromosomal analyses have been used in investigations of benzene exposures.

ARENE OXIDE

Arene oxide is a metabolite of benzene and two important families of arene oxides are benzene oxides and naphthalene oxides which are intermediates in the oxidative degradation of benzene and naphthalene which are two common pollutants.

Arene oxide causes leukemia and its threshold value is set at 1ppm (parts per million). Other metabolites of aromatic hydrocarbons such as benzo (a) pyrene, 3 methycholanthrene, benza anthracene dibenz, anthracene and toilene are carcinogenic.

OZONE AND NITROGEN DIOXIDE

OZONE

Ozone is found in very high amounts during a prolonged spillage of crude petroleum. It is a lung irritant capable of causing death from respiratory edema. Long term exposure to ozone may cause thickening of the terminal respiratory bronchioles, chronic bronchitis, fibrosis and

emphysematous changes are observed in a variety of species exposed to ozone at concentrations slightly above 1ppm.

The ozone layer or ozone shield is a region of the earth's stratosphere that absorbs most of the sun's ultraviolet radiation. It contains high concentration of ozone relative to other parts of the atmosphere.

The ozone layer contains less than 10parts per million of ozone while the average ozone concentration in earth's atmosphere as a whole is only about 0.3 parts per million. The ozone layer absorbs 97-99% of the sun's medium frequency ultra violet light from (about 200nm – 500nm) wave length which otherwise would potentially damage exposed life from near the surface.

The ozone molecule is unstable and is created in the earth's stratosphere by ultra violet light striking ordinary oxygen molecules containing two oxygen atoms (atomic oxygen). It then combines with unbroken oxygen to form ozone, the molecule is unstable and when ultra violet light hits ozone it splits into a molecule of O_2 and an individual atom of O_2 (oxygen) in a process known as the ozone-oxygen cycle.

The ozone-oxygen cycle is the process by which ozone is continually regenerated in earth's stratosphere, converting ultraviolet radiation (UV) into heat. Sydney Chapman resolved the chemistry involved in 1930. The process is commonly called the Chapman cycle. He discovered the photochemical mechanisms that give rise to the ozone layer.

Ozone is also known tri-oxygen is composed of 3(three) oxygen atoms. That is to say one molecule of ozone is made up of 3 oxygen atoms and averages 3 molecules of ozone for every 10million air molecules. The ozone layer was discovered by Charles Faby and Henri Buisson in 1913. The British meteorologist G.M.B Dobson developed a simple

spectrophotometer (the Dobson meter) for use in measuring atmospheric ozone. September 16th has been designated for the preservation of the ozone layer by the United States general assembly.

NITROGEN OXIDE

Nitrogen dioxide is a pollutant that is capable of causing pulmonary edema and is a particular risk to farmers. Chronic exposure results in emphysematous changes. Nitrogen dioxide can irritate the lungs and also lower resistance to respiratory infections such as influenza.

Effects are not clear with short term exposures but frequent exposure to concentrations that are typically much higher than what is found in ambient air may likely cause increased incidence of acute respiratory illness in children.

CARBON MONOXIDE

Carbon monoxide is a major pollutant that results from crude oil spillage because its natural source is atmospheric oxidation of methane, a gas that is found in abundance in petroleum crude oil.

It reduces the oxygen carrying capacity of blood. Signs and symptoms of carbon monoxide poisoning include headache, weakness, dizziness, nausea, vomiting, syncope, increased respiration and pulse, depressed cardiac function, respiratory failure, coma and death.

The pathology of acute carbon monoxide poisoning indicates that the tissues most affected are the brain and heart and lesions are predominantly haemorrhagic. During prolonged and low level exposure to carbon monoxide, the heart is highly susceptible since there is a shift in metabolism from aerobic to anaerobic. Foetuses have been observed to be extremely susceptible to effects of carbon monoxide during prolonged exposure with the gas readily

crossing the placenta. Women that survived short term exposures to high concentrations of the gas while pregnant delivered infants that often displayed neurological sequelae with possibilities of gross damage to the brain.

Polycythemia develops with long term exposures to carbon monoxide. Poisoning occurs after much inhalation of the gas. It is a toxic gas but since it is colourless, odourless and tasteless and initially non-irritating, it is very difficult to detect.

Carbon monoxide is a product of incomplete combustion of organic matter due to insufficient oxygen supply to enable a complete oxidation to carbon dioxide. Carbon monoxide is frequently produced in domestic or industrial settings by motor vehicles that run on gasoline, diesel, methane or other carbon based fuels. Poisoning occurs when carbon monoxide builds up in the blood stream.

When there is too much carbon monoxide in the air, the body replaces the oxygen in the red blood cells with carbon monoxide which can lead to serious tissue damage or death.

SULPHUR DIOXIDE AND SULPHURIC ACID

Because crude oil contains sulphur, oxidation may occur from sulphur oxides and sulphuric acid during a spill of great magnitude. Oxides and sulphates of sulphur primarily cause bronchial constriction and increase in airway resistance which leads to a decrease in pulmonary function particularly with sulphur dioxide. Particulate sulphates also have similar effects to those of sulphur oxide and sulphuric acid. Sulphur dioxide is a gas with a suffocating odour that is similar to a just struck match. It has an acidic taste and is a liquid when under pressure.

Sulphur dioxide is formed when fuels containing sulphur such as coal and oil are burned. The chemical symbol for sulphur dioxide is SO_2 . Most sulphur dioxide in the air comes from

burning of coal and oil at electric power plants. Other sources of sulphur dioxide in the air are industrial facilities that use coal or oil, petroleum refineries, cement manufacturing, metal processing, paper pulp manufacturing and copper smelting, trains, large ships and some equipments burn high sulphur fuel which releases sulphur dioxide into the air. Volcanic eruptions also release sulphur.

Signs and symptoms of poisoning

Breathing difficulties and obstruction of airways especially for patients with lung disease. Long term exposures to persistent levels of sulphur dioxide can cause chronic bronchitis, emphysema and respiratory illness.

It can also exacerbate existing heart disease. Short term exposure can also cause stomach pain, menstrual disorders, watery eyes, inhibition of thyroid function, nausea, vomiting, fever, convulsions and dizziness. Prolonged industrial exposure to sulphur dioxide may decrease fertility in men and women.

Breathing sulphur dioxide can irritate the nose, throat and lungs and cause coughing and shortening of breath.

SULPHURIC ACID

Sulphuric acid is a highly corrosive, strong mineral with a molecular formula H_2SO_4 and molecular weight 98.079g/mol. It is a pungent ethereal colourless to slightly, yellow viscous liquid which is soluble in water at all concentrations. It is sometimes dyed dark brown during production to alert people to its hazards. Sulphuric acid shows different properties depending on its concentration.

It has strong dehydrating and oxidizing properties. At high concentrations, sulphuric acid can cause very serious damage upon contact since not only does it cause chemical burns via

hydrolysis, it also causes secondary thermal burns through dehydration. It can lead to permanent blindness if splashed into the eyes and irreversible damage if swallowed. It is hygroscopic readily absorbing water vapour from the air.

Applications of sulphuric acid include: Domestic acidic drain cleaner, electrolyte in lead acid batteries.

ALLIUM SATIVUM

Garlic known binomially as *Allium sativum* is a species in the onion genus with close relatives that include; leek, Chinese onion, chive and shallot. It is found in abundance in central Asia and the North eastern parts of Iran and has been used for many years in culinary and medical purposes. Approximately 80% of the world's supply of *Allium sativum*; garlic is grown in China.

SCIENTIFIC CLASSIFICATION

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Allioideae
Genus	<i>Allium</i>
Species	<i>A. sativum</i>

Binomial name: *Allium Sativum*

Garlic is derived from Old English garléac which means gar or spear and leek which generally means as a spear shaped leek. *Allium sativum* is a perennial plant which grows up to 1m.

There are two sub species of *Allium sativum*, 10 major groups and hundreds of varieties or cultivars. *Allium sativum* (garlic) is easy to cultivate with growth carried out the year round in climates that are mild. Garlic plants grow better in soils that are rich in high organic matter content and can also be grown in different types of soil conditions and also pH levels.

Different cultivars of garlic are known with the more common being hard neck and soft neck garlic. Latitude also plays a role in the choice of type since there are cultivars that are day-length sensitive, colder climates favour hard neck garlic with production of large cloves. Soft neck garlic is more often grown closer to the equator with small tightly packed cloves. Garlic plants are usually hardy and are therefore not affected by most pests or diseases. Garlic plants may however, suffer from pink root which is a non fatal disease that stunts the roots of the plant and turn them pink.

Garlic possesses a sharp flavour with the phytochemicals (plant chemicals) responsible for this characteristics sharp flavour being produced when there is damage to the plant's cells, if a cell is broken by chewing, crushing and even chopping, enzymes that are stored in the cell vacuoles trigger breakdown of various sulphur containing compounds that are stored in the cytosol (cell fluids) the resultant compounds are implicated in the sharp taste and strong (pungent) smell of garlic.

In comparing other members of the onion family, garlic has the greatest concentration of initial reaction products and this makes garlic stronger than shallot, onion or leek. Crushed garlic yields the sulphur containing compounds allicin, diallyl disulphides, vinylidithins, ajoene, s-allyl cysteine and enzymes, saponins, mallard reaction products and flavonoids.

The compounds are believed to have evolved as a defense against animals from eating the plant. Different sulphur compounds contribute to the smell and taste of garlic and garlic is

sometimes called the “stinking rose”. What is known as “garlic breath” is made less intense by eating fresh parsley.

Allicin is implicated as the major contributor to the smell and taste of garlic (the hot sensation). Allicin is known to open up thermo transient receptor potential channels that are responsible for the burning sense of heat in foods.

Allicin and its decomposition products diallyl disulphide and trisulphide are the primary contributors to the characteristic odour of garlic, with other Allicin derived compounds such as ajoene and vinylidithins. Garlic’s strong smelling compounds of sulphur are metabolized to form allyl methyl sulphide. Allyl methyl sulphide or AMS remains undigested and is passed into the blood, carried to the lungs and the skin where its excretion takes place.

The after effects of eating garlic takes several hours because digestion takes hours and consequently release of allyl methyl sulphide also happens several hours later thereby leaving behind the pungent aroma. The sulphur containing compound Allicin reacts with amino acids to make pyrroles which are molecules composed of four (4) atoms of carbon and one atom of nitrogen. They form rings that can be linked together into polypyrrole molecules. Particular wave lengths of light are absorbed by the ring structures and this makes them appear coloured.

With the two pyrrole molecule, it looks red, blue with the three pyrrole and green with the four pyrrole molecule similar to chlorophyll which is a tetra pyrrole.

Ancient Medicine

Pliny the elder outlined different areas in which garlic was considered useful. In the second century Galen eulogized garlic as the “rustics” theriac or cure all. In the canon of medicine, Avicenna recommended garlic for the treatment of a wide variety of ailments which

included insect bites, persistent cough, as an antibiotic for infectious diseases and many more cures.

Sydenham in the 17th century placed high values on garlic for the treatment of small pox. Garlic was also used as an antiseptic to prevent gangrene during the world wars.

For cooking purposes

Garlic is widely used for its properties as a good seasoning agent in foods. The garlic plants bulb is the most commonly consumed part of the plant. The characteristic pungent flavour is reduced with cooking. The characteristic aroma is primarily due to organo-sulphur compounds which include Allicin present in fresh garlic cloves and ajoene which is formed when garlic is crushed or chopped.

Another metabolite, ally-methyl-sulphide is responsible for garlic breath. Immature scapes also known as “garlic spears”, tops or stems are tender and edible and have a milder taste than the cloves. Garlic leaves are a popular vegetable in many parts of Asia. Oils are also flavoured with garlic cloves and later used to season vegetables, pasta and other types of food.

Medical uses (research)

Clinical research to determine the effects of consuming garlic on hypertension found that consuming garlic can have an effect on blood pressure though more research needs to be done before recommending garlic for use in blood pressure regimens.

Diabetes

Garlic is said to modestly reduce pre-meal blood sugar levels in people with or without diabetes and is at its optimal levels in those suffering from diabetes. It is rather unclear if garlic reduces post meal blood levels.

Prostate cancer

Research shows that eating garlic may be associated with a reduced risk of developing prostate cancer.

Hyperlipidemia

Evidence show that taking garlic may reduce total cholesterol and low density lipoprotein – LDL or bad cholesterol in people with high cholesterol levels. Benefits are probably minimal and taking garlic does not increase high density lipoprotein (HDL, “good cholesterol) or even lower the levels of triglycerides.

Ringworms

Ringworm (Tinea corporis): Ajoene, a chemical found in garlic applied two times daily on tinea corporis seems to be as effective as anti fungal medication in treating the infection.

Athlete’s foot (Tinea pedis)

1% ajoene gel seems to be effective for treating athlete’s foot. It also seems to be about as effective as the medicine Lamisil for treating Tinea pedis.

MAJOR ACTIVE COMPONENTS OF ALLIUM SATIVUM

1. Allicin: Allicin is an oily, slightly yellow liquid that gives garlic its unique odour. It is a thioester of sulfenic acid and is also known as allyl thiosulfinate. Its antioxidant activity and reaction with thiol containing proteins is what brings about the biological activity of Allicin.

Allicin is activated and released when there is damage to garlic cells for example by chopping or cooking garlic there by producing the potent smell characteristic of garlic. For decades, Allicin has been recognized as the compound responsible for the benefits of garlic. Allicin has shown the ability to kill bacteria and fungus in test tubes and raw garlic was used to fight infections at wartime.

Allicin is also known to possess anti-viral and anti parasitic activity.

2. Ajoene: Ajoene is a Organo sulphur compound that is found in extracts of garlic. It is colourless having sulfoxide and disulfide functional groups. With crushing or chopping of garlic cloves Allicin is released with subsequent formation of ajoene. The medical uses of ajoene are multiple, for instance, ajoene functions as an antioxidant by inhibiting the release of superoxide. It also possesses anti clotting (anti thrombotic) properties which help platelets in the blood to stop blood clots from forming thereby minimizing the risks of heart disease and stroke.

Ajoene has viracidal properties against several viruses including human rhinovirus, herpes simplex, vaccinia etc. Ajoene has also been shown to have activity against the human dermatophyte; *trichophyton rubrum* which is the most common cause of *Tinea pedis* otherwise known as the athlete's foot. It has been shown to be effective in inhibiting tumour cell growth by targeting the microtubule cytoskeleton of the cells and through other mechanisms.

Ajoene has also been investigated as a chemotherapeutic agent in treatment of cancer stem cells in glioblastoma multiforme, lung adenocarcinoma and as an anti leukaemia agent for acute myeloid leukaemia treatment. Ajoene also inhibits genes controlled by quorum sensing.

3. **Diallyl disulfides, DADs or 4,5-dithia -1,7-octadiene** is an organo sulphur compound derived from garlic and some other plants of genus *Allium*. Diallyl disulfide, trisulfide and tetrasulfide are the principal components of distilled oil of garlic. Diallyl disulfide apart from having the health benefits of garlic is also an allergen that causes garlic allergy.

Diallyl disulfide decomposes in the human body into other compounds such as allyl methyl sulphide. Diallyl sulphide is non polar making it insoluble in water but soluble in fats, lipids and oils and also in non polar solvents like toluene and hexane.

It is the primary reason for inhibition of mold and bacteria growth by garlic oil. Diallyl disulfide is also potent against the stomach ulcer germ; *Helicobacter pylori*.

Owing to its potency, it is used in combination with tobramycin in preparations that are used for selective decontamination of the gut before surgical operations are done. Clinical studies indicate that the preparations prevent endotoxemia in heart valve operations.

Diallyl disulfide and trisulphide are produced by the decomposition of Allicin which is released upon breaking the cells of the Alliaceae plants.

4. **Flavonoids:** flavonoids are a diverse group of plant chemicals – phytonutrients in most fruits and vegetables and in combination with carotenoids are responsible for the bright colours of fruits and vegetables. They are the largest group of plant chemicals with the most common types being quercetin and kaempferol.

Flavonoids are rich in antioxidants, anti-inflammatory and also immune system functions. It is not yet known if flavonoids are responsible, but what is known is that diets rich in flavonoids containing foods have been implicated in cancer, cardiovascular disease and neurodegenerative disease prevention.

Flavonoids are part of the polyphenol class of phytonutrients and polyphenols have been used in Ayurvedic and Chinese medicine and are linked with blood sugar and blood pressure regulation, brain function, skin protection and anti-inflammatory and anti-oxidant activities. Important flavonoids include: anthocyanidins, flavones, flavonols and isoflavones with yet other groups each with its own separate set of actions, originating foods and benefits.

BENEFITS OF FLAVONOIDS

Consumption of flavonoids accounts for about 25% of the differences observed in mortality rates from cancer and coronary heart disease

- **Weight management**

Flavonoids are associated with inflammation and weight loss.

- **Cardiovascular disease**

Due to their anti-oxidant and anti-inflammatory properties, flavonoids are associated with cardiovascular disease prevention. Several studies have found an association between higher flavonoid intake levels and lowered cardiovascular disease risks.

A study published in 2002 in the American journal of clinical nutrition found that those with higher levels of quercetin had lower rates of ischemic heart disease and those with higher levels of kaempferol, naringenin and hesperetin had lower cerebrovascular disease rates. Quercetin and other flavonoids are effective at preventing platelet aggregation. Because platelet aggregation is a major component in heart disease, it contributes to forming blood clots that can lead to strokes and other heart defects.

- **Diabetes**

A study published in the journal: Diabetic medicine found that among men with type 2 diabetes, addition of a flavonoid rich spice mix to meat improved their vascular function later on.

- **Cancer prevention**

A study published in 2003 in the British journal of cancer found that women with higher flavonoids in-take levels were at lower risk for developing breast cancer.

- **Neurodegenerative disease prevention**

The anti-inflammatory and anti-oxidant effects of flavonoids may help protect against neurodegenerative diseases such as Parkinson's and Alzheimer's disease. This is clearly shown in animal studies where flavonoid levels have been correlated with reduced risks of these diseases.

Human studies are however inconclusive. Flavonoids may also improve cognitive function by increasing blood flow to the brain. A study published in the American journal of epidemiology in 2007 found that elderly men and women with higher intake of flavonoids had a better cognitive performance and less age related cognitive decline over the years than those whose flavonoids intake was low.

OBJECTIVE OF THE STUDY

On August 17th 2016 an oil spill traced to a crude oil trunk line from the pipeline and products marketing company (PPMC) the products marketing and distribution subsidiary of the NNPC. 10(ten) communities which are Tebujor/Okepele-Ama, Ikpokpo, Okerenkoko-Gbere, Opuedebubor, Opuede, Opuendezion, Atanba, Oto-Gbene, Meke Ama communities

in Gbaramatu kingdom along the Escravos river in Warri South-West Local government area of Delta State were affected.

The spill had devastating effects on agricultural capacity and also threatened the local ecosystem. Toxicity was expressed in the inhabitant's example man, aquatic animals and plants of Gbaramatu Kingdom. Subsequently in our laboratory in Bayelsa State, Nigeria, rats were fed with crude oil at concentrations below that at the oil spill sites. Yet again, the rats (experimental animals) showed signs and symptoms of toxicity similar to those in humans. These signs and symptoms include loss of appetite, reduced ability to respond to external stimuli, loss of weight and finally death.

Furthermore, 21 days after the crude oil exposure to the rats; extracts of *Allium sativum* was administered to the experimental animals and recovery of rats was observed.

HISTOLOGY STUDY

The organs obtained from the rats were sliced and dehydrated with a range of concentrations of ethyl alcohol and later cleared with xylene and embedded in molten paraffin wax.

The embedded tissue blocks were sectioned with a Shandon AS325 rotatory microtome and slides were prepared with the sections. The tissues were stained with Ehrlich's haematoxylin and eosin blue using Lillie's method (Lillie 1965).

METHODOLOGY

Materials I

Twenty albino rats were obtained from Daniel Okwudiri's farm in Omoku, Rivers State, Nigeria.

The rats were fed ad libitum (as desired). They were acclimatized to the environment in which the test was carried out for 7days. Five (5) rats were kept in each cage. Petroleum crude oil was obtained from Nigerian National Petroleum Cooperation (NNPC) Port Harcourt.

PREPARATION OF FOOD AND WATER

The crude oil polluted food and water were prepared by adding 250ml of crude oil to 500ml of tap water and stirred vigorously for 2hours. The crude was later decanted and the water used as the animals source of drinking water.

The other part of the decanted crude oil was mixed with the animal feed in the ratio of 3kg of feed per 500ml of decanted crude oil. The feed and crude mixture was done thoroughly and manually.

METHOD 1

Male and Female albino rats weighing 180-200g were used for the experiment. The study consisted of control and test groups. Each test group consisted of two males and 3 female rats, the control group also consisted of two male and 3 females. The control group received unpolluted (normal) food and water ad libitum, while the test group were given crude oil polluted food and water for 21days.

Various parameters were checked in order to relate effects seen at oil spill sites and what happens to the experimental animals so as to enable the researcher extrapolate effects in human subjects. Organs were excised after the experimental period of 21days. The heart, lungs, liver, kidneys, testes and endometrium were analyzed to find out the effects the crude oil had on them.

The focus on this particular experiment was on the testicular tissue and it was observed that there was maturation arrest of the sperm cells of experimental animals (test group rats) with chronic administration of crude oil.

METHODOLOGY II

The rats that were fed crude oil polluted food and water from the first experiment were used for this study.

Materials

Cloves of *Allium sativum*, plastic mortar and pestle, plastic bowls, weighing scale, water and sieve.

PREPARATION OF ALLIUM SATIVUM EXTRACTS

Cloves of garlic was purchased from Swali market in Yenegoa, Bayelsa state Nigeria. Firm cloves were weighed and a total of 2gram weight was peeled, washed and pounded in the plastic bowl and 3 litres of water was used in soaking the *Allium sativum* pulp.

After three (3) hours, the contents were drained using sieve. The product from mixing *Allium sativum* pulp and water after sieving is used for the experimental animals.

5kg of feed was mixed with 450ml water and 50ml *Allium sativum* extract. On day 21 of the second experiment, the animals were “sacrificed” again using the same methods and histological process. On observation, both control and test group animals had normal testicular tissue without maturation arrest of sperm cells.

EFFECTS OF NIGERIAN CRUDE OIL

RESULTS

Effects of Nigerian crude oil (Bonny Light) on testicular tissue was investigated. In the initial (first) experiment, there was maturation arrest due to chronic exposure to crude oil. In the second experiment incorporating *Allium sativum* extracts, the test and control animals had normal testicular tissue with no maturation arrest.

What this simply implies is that incorporating *Allium sativum* restored testicular tissue to normal.

DISCUSSION

The study brings to notice the fact that exposure of animals to Nigerian crude oil as that observed during massive crude oil spillage is highly toxic to animals. This finding is in agreement with earlier findings (Nwankwoala, 2000): Effects of exposure of Nigerian crude oil on rats and guinea pigs.

The study on the effect on testicular tissue/maturation arrest of sperm cells showed that when the experimental animals (rats) were placed on crude oil polluted food and water; there was maturation arrest of sperm cells.

The toxic manifestations observed on the sperm cells are similar to those on the effects of crude oil spillage on inhabitants of the crude oil spillage sites. These results also reflect results of other studies of crude oil toxicity: (Ziwontin B, O.A. Gerogewill and R.N.P Nwankwoala Jan. 2006: Histopathological effects of Nigerian crude oil on rats).

In the second experiment it was observed that incorporation of *Allium sativum* in the food and water of the experimental animals resulted in the reversal of maturation arrest of sperm

cells. This reversal of maturation arrest is possibly due to the phytochemicals, enzymes, saponins, flavonoids, vitamin B6, selenium and other potent products found in *Allium sativum*.

Flavonoids found in *Allium sativum* are reported to be useful in the prevention of cancer, cardiovascular disease, neurodegenerative disease, diabetes and in weight management. Sulphur containing compounds such as Allicin have been shown to possess antiviral, antifungal and anti parasitic activity. Alliin another sulphur containing compound is found to be effective in immune responses. Yet another group of flavonoids; the isoflavones act like the hormone estrogen.

They are also suspected to be beneficial in lowering the risks of hormonal cancers like breast, endometrial and prostate cancers. Isoflavones are also being studied as a way to treat menopausal symptoms. Studies have shown that the body uses the selenium that is obtained from foods like garlic to synthesize proteins known as selenoproteins and selenoproteins are enzymes that aid the thyroid gland, support the health of the immune system and help prevent DNA damage by inhibiting the activity of free radical compounds. It is also known that men who lack adequate selenium may be more likely to suffer from infertility.

The phytochemicals present in *Allium sativum* have high anti oxidant properties. In this study cold water extracts of *Allium sativum* reversed the maturation arrest presented in the first experiment of exposure to Bonny light crude oil.

REFERENCES

- Abbot A; 2005: Animal testing more than a cosmetic change PDF Nature 438 (7065): 144-146
- Amdur M.O; 1989: Health effects of air pollutants; Sulphuric acid, the old and the new environmental health.
- Aroro, Annu, Tripathi, Chitra; Skukla, Yogeshwer 2015; Garlic and its organo sulphides as potential chemopreventive agents, a review.
- Bajan, L; Koulivand, P.H; Gogi A. January, 2014. Garlic A review of potential therapeutic effects Avicenna Journal of phytomedicine (4) (1) : 1 -14
- Block E, Bechand B; Gandala, S; Vaattekatte A; Wang K; Mousa, Shaymaa; Godugu K; Yalcin, M; Mousa Shaker 2017. Fluorinated analogs of organo sulphur compounds from garlic (*Allium Sativum*) synthesis, chemistry, antiangiogenesis and anti thrombotic studies.
- Block, E 2010 Garlic and other Alliums: the lore and the science. Royal Society of chemistry.
- Boyer J; R.H. Liu 2004; Apple phytochemicals and their benefits Nutr J. 3:1-15
- Brown, Deanna G; Wilkerson, Eric C; Love, W. Elliot 2015: a review of traditional and novel oral anti-coagulant and anti platelet therapy for dermatologists and dermatologic surgeons.
- Casarette and Doull's Toxicology (2019) the basic science of poisons 9th edition.
- Croteau R, Kutchan T.M, Lewis N.G 2000 Natural products (secondary metabolites) in: Buchanan B, Grissem W, Jones R (eds) Biochemistry and molecular biology of plants American Society of plant Physiologists. Rockville pp 1250-1252
- George F. Kalf and Carroll A. Snyder; Recent advances in metabolism and toxicity of benzene, pages 141 -143.
- Harvard medical school: Jan 2019, Harvard health publishing carbon-monoxide poisoning.
- John A. Donald; 2016 in: handbook of carbon monoxide. Page 32
- Lillie, R.D; 1965; Histopathologic techniques and practical histochemistry.
- Menzel D.B.: 1984, Ozone: an overview of its toxicity in man and animals; Journal of toxicol. Environ Health, 13; 183-204.
- National research council 2003, Biological effects of oil releases; oil in the sea III

- Nwankwoala R.N.P 2000: effects of Nigerian crude oil on inhabitants of Asitubo Gbanraun.
- Nwankwoala R.N.P and Georg O.A. 2000: Reversibility of crude oil effects to rats.
- Ried, Karin; Frank, Oliver R; Stocks, Nigel P; Fakler, Peter; Sullivan, Thomas; June 16, 2008. Effects of garlic on blood pressure: A systematic review and meta-analysis.
- Robert Snyder, Gisela Witz and Benard D. Goldstein 1993; the toxicology of benzene, environmental health perspectives vol.100, pp. 293-295
- Van der Muellen, J.H 1982: some conclusions regarding long term biological effects of some major oil spills. Phil. Trans. Roy. Soc. London B29, 7:335-337
- Zhang et al 2002: the nature of chromosomal aberrations detected in humans exposed to benzene.
- Zhu, Beibei, Zou, Li; Qi, Lu; Zhong, Rong, Mioco, Xiaoping December 1, 2004 “Allium vegetables and garlic supplements do not reduce risk of colorectal cancer based on meta analysis of prospective studies. Clinical gastroenterology and hepatology.
- Ziworitin B, Georgewill O.A. and Nwankwoala R.N.P 2006: Histopathological effects of prolonged exposure of Nigerian Crude oil to rats. African A.P.C Environ. Biol. 8: 73-79.

