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REMEDIATIVE PROPERTIES OF GARCINIA KOLA ON THICKENED ALVEOLAR WALLS OF ALBINO RATS EXPOSED TO HIGH VOLUMES OF BONNY LIGHT CRUDE OIL WAS INVESTIGATED.

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ABSTRACT

Remediative properties of Garcinia Kola on thickened alveolar walls of albino rats exposed to high volumes of Bonny light crude oil was investigated. Male and female rats weighing 200-220g were organized into control and test groups. Control group rats were fed with unpolluted feed and water ad libitum. While test group rats were fed with crude oil polluted feed and water for 28days. Histological examination on samples fed crude oil polluted feed and water exhibited prominent thickened alveolar walls of the lungs. From previous crude oil toxicity test, it was reported that the thickening of the alveolar walls was directly linked to the poisonous effects of the crude oil on the lung tissue. With further studies incorporating Garcinia kola, into the feed and water of experimental animals in the ratio of 3kg of feed mixed with 450ml water and 50ml Garcinia kola solution using experimental rats not sacrificed in the first experiment. The test animals were "sacrificed" on day 28 of the second experiment. Histological study was carried out again on the lung tissue and these samples came back not showing any signs of thickening in the alveolar walls.

INTRODUCTION

ENVIRONMENTAL DEGRADATION:

Environmental degradation is a process through which the natural environment is compromised in some way reducing bio-diversity and the overall health of the environment. The process can either be natural, accelerated or caused by human activities.

Oil spillage is the release of crude oil hydrocarbons into the environment. It is a disaster of global proportions and concern and it usually occurs intentionally or accidentally and results most times from our day to day human activities which release crude oil into out coastal waters and land. Environmental degradation arising from oil prospecting and exploration has negatively impacted on the biological diversity of affected regions in the Niger Delta of Nigeria. Major problems arise from leakage of pipes carrying crude oil, gas flaring and escapee chemicals that are involved in the production process. Since crude oil consists of many toxic compounds that have the potentials of causing grave health challenges, which include immune

chemicals that are involved in the production process. Since crude oil consists of many toxic compounds that have the potentials of causing grave health challenges, which include immune system effects and eventually coma and death, it is important for remediation of the areas spills occur even to surrounding areas miles away because the oil travels long distances and is also known to bio accumulate.

Most records kept on oil spills don't relate the true situation since there always exist a code of silence by Government agencies to enable the major oil companies in the areas to keep exploring and exploiting to the detriment of the inhabitants of the areas. Still, estimated values are being reported because of interference of heavy current that often times "sweep" off the oil to other areas.

The Nigerian National Petroleum Corporation places the quantity of crude oil spilled into the environment on a yearly basis at 2,300 cubic meters with average annual spills of 300. In Nigeria oil spills are becoming more rampant with most spills occurring due to accidents on pipelines and tankers.

One major bottle neck is sabotage, where youths in oil producing communities seek to get rich by all means possible and break these pipelines in order to steal crude oil and other products.

On the 26th of December, 2006 a pipeline explosion in Abule Egba in Lagos State Nigeria killed hundreds of people with number of deaths initially reported at 500. This number was later reported as more than the number of casualties. This occurred due to puncture marks deliberately inflicted on an elevated pipeline carrying petroleum products.

CRUDE OIL (General Feature)

Crude oil is a naturally occurring fossil fuel and this means it originates from the remains of dead organisms. It is made up of a mixture of hydro carbons: compounds composed of hydrogen and carbon. It exists in liquid form underground in tiny spaces that are found within sedimentary rocks (rocks formed from pre-existing rocks).

Crude oil is extracted through drilling and refined to produce products that can be used which include gasoline, different forms of petrochemicals and diesel. It is made up of hydrocarbons.

Approximately 62-97% of crude oil is composed of hydrocarbons, with about 6-10% being nitrogen, sulfur, and oxygen. Metals make up less than 1% of crude oil. Crude oil consists of the remains of algae and planktons which fell to the bottom of the ocean with a mixing of crude with mud which was later covered by layers of sediments. With high pressures, the remains over several millennia become a wax substance known as kerogen which becomes liquid oil with great heat and pressure.

Crude oil is a combination of compounds of varying degrees of volatility. The compounds are mainly composed of hydrogen and carbon with trace amounts of nitrogen, sulfur and oxygen. The difference notwithstanding, almost all crude oil ranges from 82-97% carbon by weight and about 11-15% hydrogen by weight. Characterization of crude oils are considerably done

according to the type of hydrocarbon compound found in greater weight in them. Examples include: paraffins, aromatics and naphthenes.

PARAFFINS

Are waxy crystalline and flammable substances. It is a common name for alkane hydrocarbons which have the formula $C_nH_2n + 2$. The simplest paraffin molecule is methane CH_4 , it is a gas at room temperature. Paraffin is derived from Latin parum meaning barely plus affinis meaning lacking reactivity or affinity the reason for this is that alkanes are non-polar and also lack functional groups which makes them highly unreactive.

Paraffins are major components of petroleum and natural gas. Gaseous paraffins contain fewer than 5 carbon atoms per molecule at room temperature. Liquid paraffins usually have 5-15 atoms with straight chain paraffins having more than 15 carbon atoms per molecule being solids. There is a much higher-octane number rating compared to straight chain paraffins and this makes them better components of gasoline. All paraffins are colourless.

The octane number also known as anti-knock rating is a measure of the ability of a fuel to resist knocking when ignited in a mixture with air in the cylinder of an internal combustion engine. It is the percentage by volume of iso-octane in a mixture called the iso-octane-heptane flow which matches fuel that is tested in an engine that is standard.

NAPHTHENES

Naphthenes are cyclic aliphatic hydrocarbons that are derived from petroleum. The naphthenes have a general formula C_nH_2n and are characterized by possessing one or more rings of saturated hydrocarbons. Naphthenes are essential components of liquid petroleum products. A primary difference between naphthenic crude oil is that it is more readily converted into gasoline than

paraffin-rich crudes. Naphthenes can also be called cycloalkanes or cycloparaffins. Examples include cyclohexane and cyclopropane.

AROMATICS

Aromatics compounds are named based on the number and kind of substituents found on the ring aromatic compounds are ring compounds that have unusual stability because of the pi electron density which is not localized and is shared between all carbon atoms found in the ring. Examples methylbenzene known as toluene and dimethylbenzene called xylene. Aromatic compounds are produced from petroleum and coal tar. Aromatic compounds have a closed ring of alternate single and double bonds with delocalized electrons.

CONSEQUENCES OF OIL SPILLS

Oil spills are detrimental to marine organisms, bird and mammals. Oil destroys the capacity of animals that bear furs to conserve heat and what this directly entails is that the animals are at the mercy of harsh elements.

With the occurrence of spills, thousands of tons of oil find their way into the ecosystem and seep into the soil. These spills are grave causing death of plants and animals, pollute and air and the water, cause a huge upset in salinity values. With spills that occur in large bodies of water like the oceans and fresh water bodies it travels fast, thinning out on the surface of the water and eventually block sunlight from reaching the oceanic environments thereby disabling producers and the whole food chain when this happen, death occurs leading to a complete disruption of the ocean food chain.

Plankton: producers that dwell at the bottom of the food chain are often times killed by an oil spill due to changes in the water and lack of sunlight. Other sea animals like mussels that feed

on the planktons can't get food and die off. If oil cakes the feathers of a bird, it inhibits flight and if the bird is not cleaned of the oil, it may die because in an attempt to clean their feathers, many birds ingest large amounts of oil.

Oil spills also affect human health and these effects depend largely on the kind of oil spilled on the area spilled; land or water, the kind of exposure and extent of exposure. Effects on humans are more common on those that carry out remediation procedures. Most common problems seen are skin and eye irritation, breathing (respiratory insufficiency) and weakness.

Oil spills have a major effect on the ecosystem into which spilled crude is released and may therefore constitute what is known as ecoside. Ecoside is basically a criminalized human activity that violates the principles of environmental justice by causing extensive damage destroying ecosystems or harming the health an wellbeing of a species humans inclusive.

The effects of oil spill basically depend on various factors which include the quality and type of oil spilled and its interactions with the marine environment. Weather conditions at the time also influence the physical characteristics of the oil. Still other factors like biological and ecological attributes of the area, importance of major species and their sensitivity to oil pollution and 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 and 12–15% hydrogen 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° .

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^o 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 form 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° 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 bench mark 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_6O 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.

ALPHATIC 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 13CR sulphur, nitrogen and oxygen.

SULPHUR COMPOUNDS

Sulphur is common in crude oil but varies from 0.2-6% by weight. There are corrosive and noncorrosive sulphur compounds. One corrosive compound is hydrogen sulphide (H₂S). 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 thioether. 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

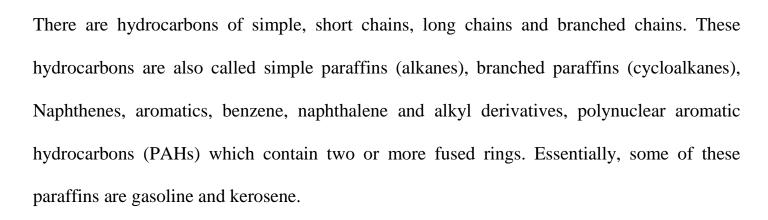
Several other organic and inorganic compounds occur in crude oil. An example is the metal vanadium which is an important component in the manufacture of specialty steels and can be recovered from the residue of the refining process. Many metallic elements are found in crude oils including most of those 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 CH4
- 2. Ethane C₂H₆
- 3. Propane C₃H₈
- 4. Iso-butane I-C₄H₁₀
- 5. N-butane N- C_4H_{10}
- 6. Pentanes C₅H₁₂
- 7. Hexanes C₆H₁₄
- 8. Octanes C₈H₁₈
- 9. Decanes $C_{10}H_{22}$
- 10. Tetradecanes C₁₄H₃₀
- 11. Hexadecanes C₁₆H₃₄
- 12. Triacontane C₃₀H₆₂
- 13. Tetracontane C₄₀H₈₂
- 14. Asphaltane C₈₀H₁₆₂





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 Benz anthracene
- 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 consequence 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

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 C₅-C₈ 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 in 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 Fabey 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 hemorrhagic. 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.

Polycythermia 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 SO₂. 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 equipment 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, and 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 H₂SO₄ 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.

BITTER KOLA (Garcinia kola: Heckel Guttiferae)

Garcinia kola commonly called bitter kola is a nut bearing tropical tree native of Nigeria's coastal rain forest is found useful in the cure of many ailments and also serves as a potent cleansing agent.

Every part of this plant has been proven to be useful. For instance, the bark leaves and nuts are used for treatment of various ailments. The stem is also used as chewing sticks and is also a potent cleanser. Garcinia kola is about 3-5 cm in length, has an ellipsoid shape with a brown to deep brown peel covering the seed. This peel is removed before it is eaten. It leaves a bitter taste in the mouth when it is a species of flowering plants in the or Guttiferae family its natural habitat is sub-tropical or tropical moist low land forests. 1JCR

SCIENTIFIC CLASSIFICATION

KINGDOM	PLANTAE
K I NUTINUN	PLANIAR

Clade Tracheophytes

Clade Angiosperms

Clade **Eudicots**

Rosids Clade

Malpighiales Order

Family Clusiaceae

Genus Garcinia

Species kola

Binomial name: Garcinia Kola

Garcinia kola was traditionally eaten by most elderly people as they believed that it had life prolonging effects. Currently, Garcinia kola (has certain beneficial compounds) is known to have certain beneficial compounds that assist in glycogen break down in the liver thereby promoting longevity Garcinia kola has medicinal benefits:

- 1. Anti-biotic Properties: It is popularly known for having strong anti-biotic effects when ingested in the body and is proposed for the treatment of Human Immune Virus (HIV) due to its cleansing and anti-bacterial detoxification effects. The natural component saponin found in Garcinia Kola offers its cleansing and antibiotic effects. Saponin is extensively used as a liver tonic and is credited with improving both gall bladder and liver function.
- 2. Management / Treatment of Osteoarthritis: Garcinia kola has been productively used in the management and treatments of Osteoarthritis and it provides treatment through reducing swelling and pain which are the major symptoms of Osteoarthritis. It also improves movement of patients thereby ensuring that people with osteoarthritis can live their normal lives again.
- **3. Treatment of bacterial infections:** Garcinia kola (bitter kola) is also potent in treatments of cough, tuberculosis, common cold and various ailments caused by bacteria. In cases of supposed food poisoning chewing Garcinia kola has been known to neutralize the poison and the formation of any bacterial infection. It is also effective in treating measles and mumps in children.
- **4. Health benefits in treatments of malaria:** Experimental studies have found bitter kola to have anti-malarial properties. In a 2010 issue of Journal of medicinal plants research,

kolaviron the potent chemical compound found in bitter kola was reportedly tested on a malaria parasite and found to inhibit parasite activity.

- **5. Treatment of Infertility:** It is also believed to cure infertility and with frequent intake of Garcinia kola there was a boost of sexual activity.
- **6. Treatment of glaucoma:** An increase in ocular (eye) pressure can lead to glaucoma. Researchers at the Lagos State University Teaching Hospital in Nigeria, tested the effectiveness of eye drops which contained 0.5% extract of Garcinia kola, results of their study published in June 2010 issue of middle East African journal of Ophthalmology showed that the Opthalmolic solution that contained the Garcinia kola significantly reduced eye pressure when used twice a day.
- 7. Human Immune Virus (HIV): Garcinia kola is a potent anti-biotic which could be efficacious in the treatment of HIV and AIDS. A researcher with the Nigerian (NNMDA) Chinyere Nwokeke proposed that bitter kola could be used in treating opportunistic infections associated with HIV. It is highly recommended because of its anti-bacterial, detoxification because of its properties. She also proposed that the chemical saponin in bitter kola is responsible for its cleansing effects. Saponin is used as a tonic for the liver and enhances liver and gall bladder functions.

SAPONINS

Saponins are chemical compounds found in particular abundance in various plant species. They are amphipathic glycosides which are phenomenological grouped by their soap forming properties visible when they are shaken in aqueous solutions and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative.

The word saponin is derived from the Latin word "sapo" which means plants that consist of frothing agents when diluted in aqueous solutions. Saponins comprise polycyclic glycones. Basically, the sapogenin or aglycone part is either a triterpene or steroid combination of sapogenin, hydrophobic or fat-soluble sugar part enhances the foaming ability of saponins. Sapotoxins are toxic saponins.

It is widely known that useful topical effects of all saponins have been widely neglected in modern pharmacology. The more pronounced effects appear on the respiratory system by the process of reflex stimulating expectoration there is a huge emetic effect (nausea or vomiting) producing effect when saponins are taken in large amount elimination on the portions of the stomach increases due to their detergent action.

There are also saponins which aid the absorption of important minerals and cause lesser irritating effects on the digestive system. There is a high popularity of saponins in possessing great systemic effects (effects occurring in tissues distant from the site of contact between the body and a medical device) example are: a terrible case of influenza which alters all parts of the body.

Saponin effects are beneficial in the control of blood cholesterol level, cancer and buildup of the immune system. Also beneficial in the treatment of pathogenic organisms that are present in agricultural crops since they control the level of toxic manifestations.

Saponins are used in dietary supplements and provide foam to products such as carbonated beverages, barley drinks, juice, and beer etc. Saponin is a natural aid to help low libido, impotence and male infertility. It is clinically proven in enhancing energy and vitality and helps to build muscles and strength. It lowers blood pressure and is also used in coronary (heart) problems.

POSSIBLE SIDE EFFECTS OF SAPONINS

Saponins come from natural plant sources like yucca, alfalfa, soy and herbs and vegetables. Humans in general do not suffer severe poisoning from saponins since cholesterin in our system inactivates them thereby affecting only our mucous membranes. Saponins derived from alfalfa may cause potential health effects since they reduce serum cholesterol by preventing reabsorption after excretion in bite.

There is not much possibility of humans having an overdose of saponins from a vegetable diet because there is a breakdown of saponins into cholesterol-like compounds and sugars.

There are hardly any known negative side effects from having saponins in dietary supplements.

The majority of saponins cause some degree of bloating. They can also cause nausea and diarrhea in pregnant woman and in people who are anemia or have other blood disorders. Such people mentioned, should restrict their intake of saponin if their doctors are not consulted.

From studies, it is known that saponins found in soy beans slow the growth of cancer cells in humans. According to an article published in the journal of phytochemistry, saponins can cause the death of tumor cells. The exact mechanism of this cell death is different and this depends on the dose and source of the saponins. Most studies on saponins use animals and isolated cells in test-tube with little research done on humans.

ACTIVE INGREDIENTS IN GARCINIA KOLA

Crude protein, Total phenols, Flavonoids and Anthocyanins.

KOLAVIRON: Kolaviron is bi-flavonoids isolated from seeds of Garcinia kola with anti-oxidant, anti-inflammatory and antigenotoxic properties. Beneficial effects of kolaviron are said to be due to its anti-inflammatory and anti-oxidant properties.

The properties of kolaviron have been used beneficially in various disease models which include diabetes mellitus, hepato-toxicity and cardio-toxicity. Kolaviron is known to have cardio protective, hemato protective and hepato protective activities. There are also effects on malaria and reproductive health and this can actually be explored for development of drugs. Kolaviron is used for treating hepatitis, laryngitis, bronchitis and gonorrhea.

PHENOLS: Phenols are any of a family of organic compounds which are characterized by a hydroxyl (-OH) group attached to a carbon atom which is part of an aromatic ring. Phenols are similar to alcohols with the only difference being that they form stronger hydrogen bonds which make them more soluble in water compared to alcohols and also have higher boiling points.

Phenol is used in industry as a starting material to make plastics and drugs such as aspirin.

Phenol is found present in coal tar and creosote and is produced during natural fires, phenolic compounds are secondary metabolites found in cereals, olives, fruits, vegetables and tea leaves.

Phenol is a versatile precursor to a great number of drugs, mostly notably aspirin. It is also a precursor to many pharmaceutical drugs and herbicides. Phenol was previously used as an antiseptic highly concnetrate3d liquid phenol can be used topically in otology procedures like myringotomy and tympanotomy tube placement as a local anesthetic. It is used as an alternative to general anesthesia or other local anesthetics. Phenol is used as first aid treatment for pharyngitis (inflammation of the pharynx, which is in the back of the throat).

ANTHOCYANINS: Anthocyanins also known as anthocyans from the Greek word that means flower. They are water soluble vascular pigments which have different colours at different PH. They may appear purple, blue, black or red. Plant sources of anthocyanin include Soy bean, Blueberry, Raspberry.

Anthocyanins are members of a class of molecules known as flavonoids that are synthesized via the phenylpropanoid pathway. Derivation of anthocyanins can be done from anthocyanidins by adding sugars to it. Anthocyanins are likely PH indicators because of their colour changes.

Anthocyanins possess anti-inflammatory, anti-microbial, anti-obesity, anti-diabetic effects with additional advantage of preventing cardiovascular diseases. This therefore means that anthocyanins extracted from edible plants are potential pharmaceutical complexes.

The natural sources of anthocyanins include leaves, roots of plants, flowers; fruits with the most frequently consumed natural products from anthocyanins being cereals, berries and cherries. They possess likely ingredients that serve as sunscreen that protect the skin from ultra-violet damage. Anthocyanins inhibit or stop oxidation by scavenging free radicals and also reducing oxidative stress. In traditional (herbal) medical practices, foods rich in anthocyanin have been used in the treatments of a vast number of conditions like problems of blood vessel health which

may include diabetic retinopathy, venous insufficiency, and high blood pressure. Other ailments such as urinary tract infections and influenza have also been treated.

FLAVOIDS: These are group of plant metabolites that are presumed to provide health benefits through anti-oxidant effects and cell signaling pathways. They are found in different vegetables and fruits. They are soluble in water, are polyphenolic molecules that contains 115 carbons atoms with high water solubility.

Flavonoids help fight off free radicals that cause oxidative stress and also regulate activities in a cell. They are powerful anti-oxidant agents. Food rich in flavonoids includes: onions, parsley, red wine, and kale, citrus fruits including oranges, tangerines, and grape fruits. Soy beans are the best flavones.

Quite a good number of flavonoids have been shown to have the ability to strengthen blood vessels and also possess anti-inflammatory effects. The ability to strengthen blood vessels and anti-inflammation can be used in the amelioration or treatment of hemorrhoids also known as piles.

CRUDE PROTEIN: This is the amount of protein of specific food or animal feed. This protein quantity is dependent on the nitrogen content of the food protein. Crude protein content is used to calculate the amount of carbohydrate in food.

The name crude is used because the assay used in its determination does not in any way measure protein at all, what is actually measured is nitrogen which is expressed as ADIN (Acid Detergent Insoluble Nitrogen). Proteins are important building blocks of animal tissues; protein is needed for the growth and regene ration of tissues.

OBJECTIVE OF THE STUDY

A major spill in the Niger Delta region of Nigeria occurred on the 1st of May 2010. It was the Exxon Mobil Oil spill which occurred at an Exxon Mobil offshore oil platform. It was approximately 20-25 miles from the shore.

More than a million gallons of crude was spilled into the Delta. This spill contributed to major environmental problems in the Niger Delta region of Akwa Ibom State, Nigeria. The leakage discharged approximately 323 barrels of crude oil into the Atlantic Ocean thereby contaminating the waters and coastal settlements in areas known predominantly for fishing. The consequence of this leak was attested by locals who claimed a huge environmental damage as a result of the leak.

Fish allegedly killed from the Exxon Mobil were brought in from locations about 20km from the shore line. The spill increased the problems of pollution already suffered by the region. Great expanse of the Delta was covered with tar in addition to stagnant lakes of crude oil resulting from oil spills of the past. The effects of this spill are seen as air pollution, soil and water pollution. Toxicity was also expressed in the inhabitants such as man, aquatic animals and plants of Qua Iboe area.

This informed a study into pathological effects of this spill. Laboratory animals (albino rats) were fed with crude oil concentrations below that at the oil spill sites. The rats established signs and symptoms similar to those in human of the Qua Iboe area. The signs and symptoms include reduced ability to run across bars placed in cage, aversion for mating, restlessness before they finally died. Furthermore, 28days after the crude oil exposure to the rats extracts of Garcinia

kola (bitter kola) was administered to the experimental (test) animals with observations of recovery of rats being recorded.

With these observations, it was significant to carryout studies into histopathology of affected organs in the rats which resulted to their deaths due to re-exposure to Nigerian crude oil.

HISTOLOGY STUDY

The organs obtained from the rats were "cut" into sections and dehydrated with a range of concentrations of ethyl alcohol, later cleared with xylem and embedded in molten paraffin wax. The embedded tissue blocks were sectioned with a Shandon As 325 rotatory microtome and slides prepared with the sections. Lillie's method of staining was doing using Ehrlich's Haematoxylin and Eosin blue.

METHODOLOGY

Material I

Twenty albino rats were obtained from the University of Port Harcourt animal house Choba, Rivers State Nigeria. The rats were fed ad libitum (as desired). They were acclimatized to the test environment for seven (7) days. There were four (4) each cages "housing" five (5) rats. Petroleum crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Rivers State Nigeria.

Preparation of food and water

The crude oil polluted food and water were prepared by mixing 250ml of crude oil to 500ml of tap water and stirred vigorously for two (2) hours. The crude was later decanted and the water (decant) used as the animals drinking water. The other part of decanted crude oil was mixed with the animal feed in the ration of 3kg of feed per 500ml of decanted crude oil, the feed and crude were mixed manually.

Method 1

Male and female albino rats weighing 200 - 220g were used.

The study was made up of: (i) The control group (ii) The test group.

Each test group consisted of 2males and 3female. The same applied to the control group. Normal (unpolluted) feed and water was given to the control group rats and as desired (ad libitum). The test (experimental) animals were given crude oil polluted food and water for 28days. Strength was measured by animals' ability to cross graded bars. Appetite was measured by quantity of feed consumed by the animals. Appetite was scored on a daily basis as 0 means absences of feeding 2 which means consumption of 25percent of feed, 3 means consumption of 75percent of feed and 100percent consumption of all feed.

METHODOLOGY II

MATERIALS: Previous rats fed with crude oil polluted feed and water was used.

Seeds of Bitter Kola (Garcinia kola) – 12 medium sized seeds of Garcinia of Kola

Grater, Plastic bowls, water and sieve.

Preparation of Garcinia kola extract

Freshly plucked seeds of Garcinia kola (Guttiferae) were washed, peeled and grated, put in the plastic bowl with addition of 3litres of water used in soaking the grated Garcinia kola. Contents are drained 2hours later and the filtrate used as water for experiment, 3kg feed was mixed with 450ml water and 50ml Garcinia kola extract.

On day 28 of second experiment, the animals were sacrificed again employing the same methods and histological procedures. With observations carried out on samples, it was discovered that the animals had normal lungs with no signs of thickened alveolar walls. There were no changes in the lungs of control group rats as was expected. This simply means the lungs of test and control animals were the same after administration of Garcinia kola extracts.

EFFECTS OF NIGERIAN CRUDE OIL ON LUNGS

Effects of Nigerian Crude Oil on the lungs were investigated. With the first experiment thickened alveoli walls was reported in the lungs of experimental (test) animals. The control animals had normal lungs.

The second experiment incorporating Garcinia kola, the test animals had normal lungs just like the control animals. The results of the test are straight forward and it simply translates to the effectiveness of Garcinia kola in aborting thickness in the alveoli, thereby allowing free flow of gases between respiratory vessels. This is important because though the alveoli are tiny, they collect the incoming oxygen we breathe in and release carbon-dioxide to the exterior. The alveoli have a large surface area which enables the mechanism of breathing in and out to go on smoothly.

DISCUSSION

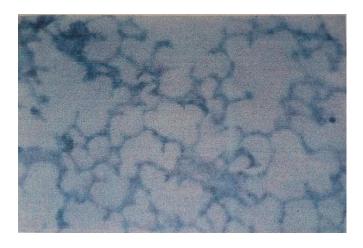
This study has shown that exposure of Nigerian crude oil like is often seen in massive oil spills extremely toxic and deleterious to animals (rats) on the effects on the lung, it clearly showed thickness in the alveoli spaces once they were exposed and re-exposed directly to crude oil polluted feed and water in form of ingestion. The thickened alveoli walls if not treated can lead to reduction in respiratory surface which can lead directly to respiratory insufficiency and consequently death.

These toxic manifestations are observed on the lungs similar to those observed by the inhabitants of Qua Iboe area of Akwa Ibom state, Nigeria. The results on lung tissue are in agreement with other reports related to toxicity to crude oil.

Ziworitin B, O.A Georgewill and R.N.P Nwankwoala; Histopathological effects of Nigerian Crude oil to rats. In subsequent experiment incorporating Garcinia kola, in the feed and water of the test group animals, the thickened alveolar walls were no longer seen. This effect is possibly as a result of the many natural agents such as saponins, flavonoids, anthocyanins and other phytochemical like kolaviron and phenols.

Garcinia kola contains saponins which are steroidal or triterpenoid saponins. Saponins are useful in the treatment of hypercalciuria, it is also a strong antidote against lead poisoning, and they have anti-inflammatory properties and also act as antibiotics.

Almost all parts of Garcinia kola are medicinal; it has antibiotics, anti-inflammatory, anti-oxidant and anti-genotoxic properties. In all, exposure of rats to crude oil led to thickening of the alveolar walls. Garcinia kola extract returned the lungs to their normal state.



Lung - Normal

Figure 1a

Rats were fed with 3kg of food per 500ml water ad libitum for 28days. Organs were excised from the animals and preserved in 10% formaldehyde, sliced and dehydrated with ethyl alcohol and embedded in molten paraffin wax. The embedded tissue blocks were sectioned and stained as described in the methods.

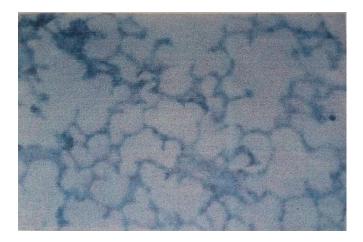


Lung – Thickened Alveolar Walls

Figure 1b

Rats were fed with 3kg of food per 500ml water ad libitum for 28days. Organs were excised from the animals and preserved in 10% formaldehyde, sliced and dehydrated with ethyl alcohol and embedded in molten paraffin wax. The embedded tissue blocks were sectioned and stained as described in the methods.

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Lung - Normal

Figure 1c

Experimental rats were fed with 3kg feed mixed with 450ml water and 50ml Garcinia kola solution ad libitum for 28days. Organs were excised from the animals and preserved in 10% formaldehyde, sliced and dehydrated with ethyl alcohol and embedded in molten paraffin wax. The embedded tissue blocks were sectioned and stained as described in the methods.

REFERENCES

- 1. Abbot A; 2005: Animal testing more than cosmetic change PDF Nature 438(7065): 144-146
- 2. Azubike P.E; Isaac A.A; Owoeye O.; Ebenezer O.F. (2013): Neurotoxicity of Nigerian Bonny light Crude oil on rats, Drug and Chemical toxicology.
- 3. Bayer J; R.H. Liu 2004; Apple phytochemicals and their benefits Nutr J. 3:1-9
- 4. Casarette and Doull's Toxicology (2019) the basic science of poisons 9th edition.
- 5. Cheek M. 2004: Garcinia kola; Red list of threatened species June 27, 2014
- 6. Croteau R, Kutchan T.M, Lewis N.G 2000 Natural products (secondary metabolites) in: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants American Society of plant Physiologists. Rockville pp 1250-1252
- 7. David Thomas (13th November, 1995): Niger Delta oil production reserves; Field sizes assessed, Industry Briefs Oil and gas journal.
- 8. Davis Kevin M. (2004) Plant pigments and their manipulation; Wiley Blackwell
- 9. John A. Donald; 2016 in: handbook of carbon monoxide. Page 32
- 10. Lillie, R.D; 1965; Histopathologic techniques and practical histochemistry.
- 11. Menzel D.B.: 1984, Ozone: an overview of its toxicity in man and animals; Journal of toxicol. Environ Health, 13; 183-204.
- 12. National research council 2003, Biological effects of oil releases; oil in the sea III
- 13. Nwankwoala R.N.P and Georgewill O.A. 2000: Reversibility of crude oil effects to rats.
- 14. Okonkwo, H.O; Kojejo O.A, Osewa S.O and Babalola O.T. (2014): Techniques for improvement of Garcinia kola (Heckel) seeds germination, International journal of International research and technology 3(8): 80-82.
- 15. Olaleye S., Onasanwo S., Ige A., Wuk, Chioc: Anti-inflammatory activities of Kolaviron inhibition of nitric oxide, prostaglandin E2 and tumour necrosis factor-alpha production in activated macrophage like cell line Apri J. Med. Med Sci. 2010, 39:41-42.
- 16. Robert Snyder, Gisela Witz and Benard D. Goldstein 1993; the toxicology of benzene, environmental health perspectives vol.100, pp. 293-295
- 17. Rossberry M Lindle, W Pfleiderer G.; Togel A., Dreher E.L, Langer E; Rasaerts H; Kleinshmdt P; Strack (2006) "Chlorinated Hydrocarbons" Ullman's Encyclopedia of Industrial chemistry
- 18. Van der Muellen, J.H 1982: some conclusions regarding long term biological effects of some major oil spills. Phil. Trams. Roy. Soc. London B29, 7:335-337
- 19. Zhang et al 2002: the nature of chromosomal aberrations detected in humans exposed to benzene.
- 20. 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.