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# EFFECTS OF COLD WATER LEAF EXTRACT OF AZADIRACHTA INDICA (NEEM) ON CONGESTION OF THE CENTRAL VEIN OF THE LIVER AND SINUSOIDS IN ALBINO RATS EXPOSED TO HIGH VOLUMES OF BONNY LIGHT CRUDE OIL WAS INVESTIGATED

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**ABSTRACT**

Effects of cold water leaf extract of Azadirachta indica (neem) on congestion of the central vein of the liver and sinusoids in albino rats exposed to high volumes of Bonny light crude oil was investigated.Male and female rats weighing 200g were placed in control and test groups consisting of five(5) rats in four(4) cages and used to test the effects of crude oil on the liver (hepatic) tissues. Control group rats were fed with normal (non crude oil polluted) feed in the ratio of 3kg feed mixed with 500ml bonny light crude oil and water for 21days. Histological examination on samples given crude oil feed and water presented observable congestion of the central vein of the liver and sinusoids. Evidence from previous crude oil toxicity tests indicate the congestion was probably due to the harmful effects of crude oil on the liver (hepatic) tissue.

More studies were carried out incorporating Azadirachta indica (neem) into feed and water of the experimental animals in the ratio of 3kg feed mixed with 450ml water and 50ml Azadirachta indica solution. Experimental animals not "sacrificed" in the first experiment were used in this second study. On day 21 of the second experiment, test and control animals were "sacrificed" and histological studies being carried out once again on the hepatic tissues. Findings indicate normal hepatic tissue with no central vein congestion or sinusoids in both control and test animals. Informing the conclusion that cold water leaf extracts of Azadirachta indica: neem resolved the congestion of the central vein of the liver and sinusoids.

**KEYWORDS:**

(a) Ad libitum (b) Azadirachta indica (c) Bonny light crude (d) Congestion of central vein

(e) Hepatic tissue

# 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 2,300cubic meters with an average of 300 individuals 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 7,400km$^2$ 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 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 $38.8^0$ 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 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 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 CH4
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 $C_{10}H_{22}$
10. Tetradecanes $C_{14}H_{30}$
11. Hexadecanes $C_{16}H_{34}$
12. Triacontane $C_{30}H_{62}$
13. Tetracontane $C_{40}H_{82}$
14. Asphaltane $C_{80}H_{162}$

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 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. Bezene 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 16$^{th}$ 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.

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 $SO_2$. 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.

**AZADIRACHTA INDICA (NEEM)**

SCIENTIFIC CLASSIFICATION

Kingdom Plantae

Clade Tracheophytes

Clade Angiosperms

Clade Eudicots

Clade Rosids

Family Meliaceae

Genus Azadirachta

Species indica

Binomial name: Azadirachta Indica

**BACKGROUND INFORMATION**

Neem, Nim tree or Margosa is a tree in the mahogany family Meliaceae and is one of the two species in the genus Azadirachta. Neem is popularly referred to in Hausa language as Dogon-yaro. It is extensively used by

humans in the management and treatments of different ailments. Its latinized name neem-Azadirachta indica is derived from the Persian word Azad which means "free", dirakt which means 'tree' . I-Hind meaning to be of Indian origin. It therefore literally means the tree of India.

It is known as an omnipotent plant and sacred gift of nature and has been declared the tree of the 21st century by the United Nations.

## USES

Neem bark, leaves and seeds are useful in the production of medicines. Not too commonly, the flowers, roots and fruits are also used in the production of medicines.

Neem leaf is used for treating leprosy, eye disorders, intestinal worms eradication, stomach upset, skin ulcer, cardiovascular diseases, diabetes, gingivitis(gum diseases) and liver problems.

The leaves are also used in birth control and as abortifacients.

The bark is used for treating malaria, skin diseases, pain and fever.

The flower is used in ameliorating haemorrhoid discomfort controlling phlegm and in eradication of intestinal worms.

The fruit is useful in the treatment of cough, asthma, urinary disorder and diabetes.

The seed and seed oil are used in treating leprosy and infestation of intestinal worms. It is also used as birth control and abortifacient.

The stem, roots, barks and fruits are used as tonics and astringents. It is applied directly to the skin in the treatment of head lice, skin ulcers, skin diseases and wounds. It is also useful as a repellant for mosquitoes.

## CHEMICAL COMPOSITION

Azadirachta indica (neem) has complex of various constituents including nimbin, nimbidin, nimbolide and limonoids and such types of ingredients play various roles in disease management through modulation of different genetic pathways and other activities.

Quercetin and B-sitosterol were the first polyphenolic flavonoids purified from fresh leaves of neem and are confirmed to possess antifungal and antibacterial functions. Other pharmacological activities such as anti-infflammatory functions have also been reported.

There are also confirmations on their roles in anti-arthritic, antipyretic and anti-gastric ulcer, anti-tumour and hypoglycemic functions.

The neem tree is also called the village pharmacy reason being that it cures diseases and disorders ranging from bad teeth, bed bug infestation to ulcers and malaria (John 2001).

The health promoting effect of neem is attributed to its rich source of anti-oxidants. Earlier findings confirmed that neem and its constituents play a major role in the scavenging of free radicals generation and disease pathogenesis prevention.

Neem has recently been reported as anti cancer and is also used for protective functions of the liver and the kidneys.

Its chief constituents play a vital role in cancer management through the modulation of different molecular pathways.

It is known to modulate various molecular processes without adverse effects.

It has also been found to have contraceptive properties and is also effective in controlling sexually transmitted diseases.

## OBJECTIVES OF THE STUDY

On August 17th 2016, an oil spill traced to crude oil trunk line from the pipeline and products marketing company (PPMC) the products marketing and distribution subsidiary of the Nigerian national Petroleum Coporation (NNPC)

Ten (10) communities which are Tebujor/Okpele-Ama, Ikpokpo Okerenkoko-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 inhabitants example man, aquatic animals and plants of Gbaramatu Kingdom.

Subsequently, in our laboratory in Sagbama, Bayelsa State, Nigeria, rats were fed with crude oil at concentrations bellow that at the spill sites.

Experimental animals (albino rats) used for this study showed signs and symptoms similar to those found in humans with crude oil toxicity. The signs and symptoms include :- loss of appetite, reduced ability to respond to external stimuli, internal organ malfunction and damage and later death.

21days after the crude oil exposure to the rats, leaf extracts of Azardirachta indica (neem) was incorporated into the food and water of experimental animals and it was observed that the rats recovered from organ damage.

Follow up studies were carried out into the pathology of the affected organs which resulted in the malfunction of organs and eventual death during repeated exposure to Nigerian Crude oil.

## HISTORY 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. Ehrlich’s Haematoxylin and Eosin blue were used in the staining with application of the Lillie’s method.

## METODOLOGY

Material I

Twenty albino rats were obtained from Nnamdi Igwe’s farm in Rumuosi, Rivers State Nigeria. The rats were fed as desired (ad libitum). They were acclimatized to the environment in which the test was carried out for seven(7) days. There were four (4) cages each with five(5) rats. Petroleum crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Rivers State Nigeria.

### Preparation of food and water

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

Method 1- male and female

Albino rats weighing 200g were used for the study and the study consisted of two groups (i) The control group (ii) The test group.

The control group received normal (unpolluted) food and water ad libitum (as desired) for 21day. The test group received crude oil polluted food and water as libitum (as desired) also for 21days. Weights of animals

were taken at interval of 7days, appetite was scored on a scale of 0-4 depending on the quantity of food consumed.

0 connotes absence of eating

1 connotes very little food consumed

2 connotes half of the food consumed

3 connotes 3/4 of the food consumed

4 connotes all the food is consumed

Strength was measured by animals ability to cross grade bars, from observations made there was visible congestion of the central vein of the liver and sinusoids.

## METHOLOGY II

The experimental (test) animals that were administered crude oil polluted food and water were used.

Leaves of Azadirachta indica (neem)

Manual blender

Plastic bowls

Cloth sieve

Water

### Preparation of Azadirachta indica (neem) leaf extract

Fresh neem leaves were purchased from Swali market in Yenagoa, Bayelsa State, Nigeria.The leaves were confirmed as neem by a botanist in the department of botany Niger Delta University (NDU) Wilberforce Island.

The leaves were washed and placed inside the manual blender, blended into a pulpy mass. The blended neem leaves were placed in a plastic bowl with addition of 3litres of cold water.

After 2(two) hours, the neem mixture was poured into the cloths sieve and the extract collected. The extract was kept as water for the test animals. 3kg of the feed was mixed with 450ml water and 50ml neem solution. The control and test animals were again “sacrificed “ employing the same methods and histological procedures.

After tissue processing was done and the slides viewed under the microscope, it was observed that there was no longer congestion of the central vein of the liver and sinusoids in both control and test animals.

## EFFECTS OF NIGERIAN CRUDE OIL

Effects of Nigerian crude oil (Bonny Light) on hepatic tissues was reported. The first experiment showed congestion of the central vein of the liver and sinusoids.

The control group animals had normal liver tissue with absence of central vein congestion and sinusoids.

The second experiment incorporating Azadirachta indica (neem) showed test animals having no signs of the previous congestion of the central vein and sinusoids . Just as is found in the control, the test animals had normal hepatic tissues.

What this simply implies is that incorporation of Azadirachta indica (neem leaf) extract restored the liver to its normal state.

## DISCUSSION

The study provides evidence that exposure of animals to Nigerian crude oil as is often seen during heavy crude oil spills is highly toxic to experimental animals (rats). This finding is in agreement with previous findings. (Nwankwoala, 2000: Effects of exposure of Nigerian crude oil on rats and guinea pigs).

On the hepatic tissues, studies showed that animals (rats) had congestion of the central vein of the liver and sinusoids once they were placed on crude oil polluted food and water. The toxic effects on the hepatic tissues are similar to those observed on the effects of crude oil spillage on inhabitants of the affected crude oil spillage sites. The results of this study are also in accordance with other reports of crude oil toxicity.

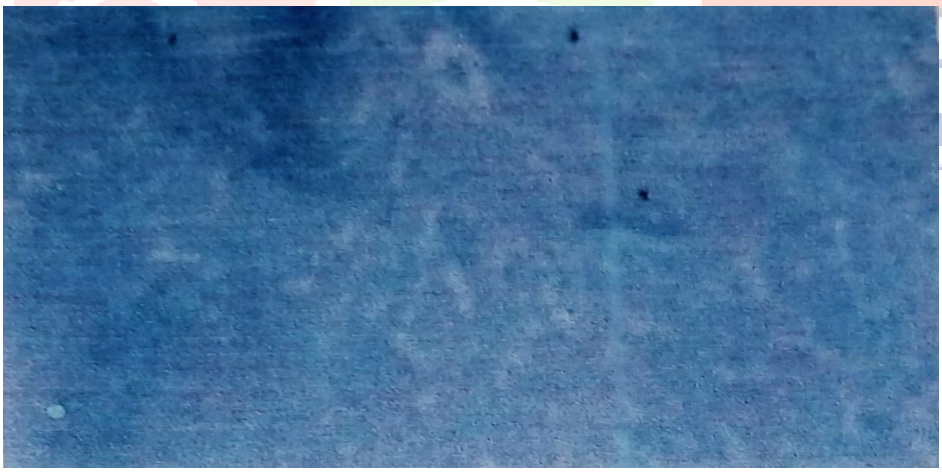
Ziworitin B, O.A Georgewill and R.N.P Nwankwoala; Histopathological effects of Nigerian Crude oil on rats. In the second experiment, it was observed that incorporation of Azadirachta indica (neem) in the food and water of the test group animals resulted in stopping the hepatic cells from being congested. This effect is possibly as a result of the bio-active properties of Azadirachta indica.

Azadirachta indica contains polyphenolic flavonoids such as quercetin and B-sitosterol. The various constituents in neem include nimbin, nimbidin, nimbolide and other ingredients that play a great role in disease management. Though the exact molecular mechanism in neems prevention of pathogenesis is not understood completely, it is considered that Azadirachta indica shows therapeutic roles due to its rich source of antioxidants and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salanin and quercetin.

The anti-congestive effect of the hepatic central vein by Azadirachta indica may be due to its protective functions of hepatic cells.

In conclusion, the congestion of the central vein of the liver due to exposure of rats to crude oil was resolved by incorporating cold water leaf extracts of Azadirachta indica (neem).

**CONGESTION OF CENTRAL VEIN/SINUDOIDS**



**Liver - Normal**

Figure 1a

Rats were fed with 3kg of food per 500ml water ad libitum for 21days . 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.

**Liver - Congestion of central Vein and Sinusoids** Figure 1b

Rats were fed with crude oil polluted food and water in the ration of 3kg food per 500ml crude oil ad libitum for 21days. 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.

**Liver - Normal** Figure 1c

Rats were fed with 3kg feed per 450ml water and 50ml Azadirachta indica (Neem) solution ad libitum for 21days. Organ 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 method.

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