

Effects of sodium azide on the limnological and phytoremediation potential of water hyacinth in aquatic environments contaminated with crude oil

Jim Jonathan Eze*, Umar I. D., and Solomon, R. J.

Department of Biological Sciences, Faculty of Science, University of Abuja, Nigeria.

Corresponding author Email: jim_4jesus@yahoo.com

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ABSTRACT: This study assessed the phytoremediation of aquatic habitats contaminated with crude oil in University of Abuja. These habitats were simulated in twelve (12) white containers in the Biological Garden of the Department of Biological Sciences. Some of the water hyacinth plants used in this research were treated with sodium azide, a chemical mutagen to induce beneficial mutation in the genes of the plants that can result in an improvement in the rate of phytoremediation by the plant. The result from this study showed a significant decline in BOD, DO, TDS, EC and pH through the weeks of the studies for each month both for the plants that were treated with sodium azide and those that were not treated with sodium azide. The survival rate of the sodium azide treated water hyacinth plants was also higher, almost one hundred percent in some cases compared to the ones that were not treated with sodium azide. The plants that were treated with sodium azide identified as C, C1, C2 and D, D1 and D2 also gave a better result in terms of uptake of heavy metals like Fe^{2+} , Pb^{2+} and SO^{+4} ions. The study recommends that phytoremediation by plants, especially aquatic plants like water hyacinths can be improved upon using chemical mutagens like sodium azide that can induce beneficial mutation which will bring about an improvement of the plant's natural ability to clean-up or reclaim contaminated habitats through phytoremediation.

Keywords: Sodium azide, limnological, phytoremediation, water hyacinth

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INTRODUCTION

Globally speaking the problem of oil spillage remains a problem that is yet to be solved. Many nations of the world especially oil producing nations like Nigeria have continued to be puzzled by the menace of oil spillage. In Nigeria there has been incessant oil spill within the oil fields in the Niger-Delta region from old pipelines and illegal oil bunkering. According to UNEP report (2011), there are 116 oil wells, 5 flow stations, 12 manifolds and over 122km of pipeline rights of way. It has been reported that a family was burnt to death because they were fetching refined products instead of water from a well dug inside their compound that is about 10 meters from a refined product pipeline (www.thecable.ng/ogoni-cleanup).

There is therefore a need for oil companies and local communities to partner or collaborate with research experts to map out the entire network of oil facilities and carry out an assessment of areas prone to corrosion and leakage and find a way to stop it (Abraham and Ekpensi, 2017). As a result of the menace of oil spillage in the Niger-Delta, water is polluted leaving the people with unclean and contaminated water for drinking and domestic purposes (Abraham and Ekpensi, 2017). The biodiversities living in water or land in such areas affected by oil spillage are destroyed even as some species have gone into extinction as a result of the scourge caused by oil spillage (Abraham and Ekpensi, 2017). The crude oil

cannot be cleaned easily except by the use of conventional methods like chemical dispersants so it creates another complex problem for future generations, because these chemicals are toxic to aquatic and terrestrial life. The most prominent chemicals used by Exxon and the United States Environmental Protection Agency for the Exxon Valdez cleanup, though judged to be successful to a certain degree, were controversial due to effects on the environment and public health (www.history.com/amp/Exxon-valdez-laid-to-rest). According to Material and Safety Data Sheet (MSDS) for EAP22, skin contact and inhalation are the primary routes of occupational exposure; it can be moderately toxic if absorbed through the skin and eyes (Abraham and Ekpeni, 2017). High vapor concentrations may irritate the eyes and respiratory tract and may result in Central Nervous System (CNS) effects including headaches, dizziness, nausea and drowsiness. Also, prolonged or repeated contact may remove natural oils from the skin, dry the skin, and cause irritation, redness, and rash (Abraham and Ekpeni, 2017).

Methods for phytoremediation of contaminated lands and water bodies such as lakes, rivers, seas, etc. have been described in many researches (Salt et al., 1998). Plants such as water hyacinths (*Eichornia crassipes*), onion (*Allium cepa*), Poplar trees (*Populus* spp.), American pondweed (*Potamogeton nodosus*), Forage Kochia (*Kochia* spp.), Alfalfa (*Medicago sativa*), etc. have been used in phytoremediation practices, etc. (Fresh Water Management Series No. 2, 2 000). Algae has been extensively used in recent times to carry out phytoremediation and has proved to be effective in hyperaccumulation of heavy metals as well as degradation of xenobiotics (Suresh and Ravishankar, 2004). Worldwide trees, grasses, herbs, algae as well as other microorganisms such as phytoplanktons are being used increasingly for cleaning polluted sites.

To make phytoremediation an ecofriendly technology, plants should have characteristics such as quick growth rate, high biomass yield, the uptake of a large amount of pollutants, the capacity to transport pollutants in aboveground parts of plant, and a mechanism to tolerate pollutants toxicity (Ali et al., 2013; Arslan et al., 2017; Burges et al., 2018; Rezaia et al., 2015b). Other factors such as pH, availability of nutrient and salinity have a very great effect on the phytoremediation potential and plant growth (Rezaia et al., 2015c; Reeves et al., 2018 and Tewes et al., 2018; Yadav et al., 2018). Pollutants bind to specific points on the surface of the root. Phytoremediation is further enhanced by the accumulation of pollutants in the roots and the transfer to other parts of plant (Burges et al., 2018). Water hyacinth (*Eichornia crassipes*) is preferred among several plants for phytoremediation because of its capability to grow in highly polluted water (Mahalakshmi et al., 2019; Sarkar, 2017; Ebel et al., 2007; Roy and Hanninen, 1994). Water

hyacinth can have significant variation in nutrients, pH levels (optimum growth at 6 - 8 pH) and temperatures (from 1 to 40 °C, optimum growth at 25–30 °C) (Hounkpe et al., 2022).

Techniques in genetics can be used to improve the phytoremediation property of plants. Chemical mutagens like sodium azide can be used to cause beneficial mutation in plants such as the ability to accumulate and degrade harmful pollutants especially crude oil (Das and Chandran, 2011; Subashandrabose et al., 2013). Phytoremediation can be done *insitu* that is directly on the site of contamination and *exsitu* by moving the contaminated material to a controlled environment where the technique of phytoremediation can be easily applied (Khan et al., 2020).

This technology has become more popular as there has been more interest in recent times in conservation of the ecosystem, especially with the recent Global Climate Action that was presented at the 26th Session of the Conference of the Parties to the United Nations Framework Convention on Climate Change (COP26) in Glasgow, United Kingdom (www.uk.cop26.org). World leaders committed themselves to the huge and urgent task of saving the planet by tackling the great menace of climate change which poses a great threat to the survival of our planet and every biodiversity represented in it (www.uk.cop26.org). Curbing the menace of pollution is very vital to this task and phytoremediation remains the most viable option to salvaging our polluted habitats (Yan et al., 2020).

LITERATURE REVIEW ON PHYTOREMEDIATION AND GENE MUTATION USING SODIUM AZIDE

Phytoremediation is from the ancient Greek word “phyto” which means plant, and the Latin word “remedium” which means to restore balance. It refers to the technologies that use living plants to clean up soil, air, and water contaminated with hazardous chemicals (Huang et al., 2010). Phytoremediation is a biological process that has been used for some inorganic materials like metals, to lower radioactivity and to remediate organic contaminants (Nwe et al., 2020). With metal contamination, the usual challenge is to accumulate the metal into harvestable plant parts which must then be disposed-off in a hazardous waste land fill before or after incineration to reduce the plant ash (Gupta et al., 2019). Two exceptions are mercury and selenium, which can be released as volatile elements directly from plants to atmosphere (Yan et al., 2020).

Phytoremediation is a cost-effective plant-based approach of remediation that takes advantage of the ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues (Gupta et al., 2019). It refers to

the natural ability of certain plants called hyperaccumulators to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air (Saha et al., 2017). Toxic heavy metals and organic pollutants are the major targets for phytoremediation (Saha et al., 2017). Knowledge of the physiological and molecular mechanisms of phytoremediation began to emerge in recent years together with biological and engineering strategies designed to optimize and improve phytoremediation (Burgess et al., 2017). In addition, several field trials have confirmed the feasibility of using plants for environmental cleanup (Campos et al., 2008). Phytoremediation is a natural process that has been recognized and documented by man for over 300 years ago (Lasat, 2000). Ever since, man has utilized the abilities of plants to survive in contaminated areas to help in the removal of contaminants from soil or water (Gupta et al., 2019). Studies of the development of these plants' unique qualities were not conducted until the early 1980's (Pas-Alberto and Sigua, 2013). It was discovered that certain species of plants could accumulate high amount of heavy metals from the soil while it continues to grow and proliferate (Pas-Alberto and Sigua, 2013).

Research in phytoremediation has been slow and difficult due to the scientists' insufficient understanding of the cellular mechanisms of plants. Nevertheless, the rise of new genetic technology has enabled scientists to determine the genetic basis for high rates of accumulation of harmful substances in plants (Yan et al., 2020; Moffat, 1995). Scientists may soon be able to exploit plants' characteristics that can provide faster and more efficient means of removing contaminants from soil using genetic engineering (Yan et al., 2020). Genetic engineering will also play a vital role in the creation of transgenic plants that will combine the natural agronomic abilities of plants (ease of harvest and rapid, expansive growth) with the remediation capabilities of bacteria-a traditional organism used in bioremediation (Yan et al., 2020; Huang and Cunningham, 1996).

Analysts estimate that it will cost \$600,000 to \$3,000,000 to clean up one hectare of land that is highly contaminated at a depth of one meter depending on the extent of the pollutants (Pas-Alberto et al., 2013; Huang and Cunningham, 1996). The cost of phytoremediation could be as much as 20 times less expensive, making this practice far less prohibitive than conventional methods (Lasat, 2000). The ideal type of phytoremediator is a species that creates a large biomass, grows quickly, extensive root system, and can be easily cultivated and harvested (Moffat, 1995). The only problem is that natural phytoremediators often lack most of the qualities described above. Therefore, scientists have been forced to become very creative in developing effective transgenic phytoremediators (Yan et al., 2020).

There are many human diseases that result from the building of toxic metals in the soil, making remediation to

be very important in protecting man's health. One of the most difficult contaminants to be removed from the soil is lead and it is one of the most dangerous. The presence of lead in the environment may have a very damaging effect on plant growth and can result in serious side effects-such as seizures and mental retardation if ingested by man or animals (Paz-Alberto et al., 2013). Most of the cases of the global lead contamination occurred due to mining and iron smelting activities (Cortez, 2005). The phytoremediation of lead in the soil involves two processes; Phyto-stabilization and phytoextraction. Scientists believe that the ability of plants to phytoextract certain metals is a consequence of its dependence upon the absorption of many metals such as Zn, Mn, Ni, and Cu (Paz-Alberto et al., 2013). Phytoremediation of heavy metals from the environment serves as an excellent example of plant-facilitated bioremediation process and is useful in removing environmental stress (Yan et al., 2020).

Application of Phytoremediation

Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully include the restoration of abandoned metal mine workings, and sites where polychlorinated biphenyls have been dumped during manufacture and mitigation of ongoing coal mine discharges reducing the impact of contaminants in soils, water, or air. Contaminants such as metals, pesticides, solvents, explosives, and crude oil and its derivatives, have been mitigated in phytoremediation projects worldwide. Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites (Huang, 2010).

Phytoremediation process

The following are a range of processes utilized by plants in treating environmental pollution using phytoremediation (Figure 1):

- *Phytosequestration* – Phytochemical complexation in the root zone, reduce the fraction of the contaminant that is bioavailable. Transport protein inhibition on the root membrane-preventing contaminants from entering the plant. Vacuolar storage in the root cells: contaminants can be sequestered into the vacuoles of root cells (Moffat, 1995).
- *Phytoextraction* – uptake and concentration of substances from the environment into the plant biomass (Lasat, 2000).
- *Phytostabilization* – reducing the mobility of substances

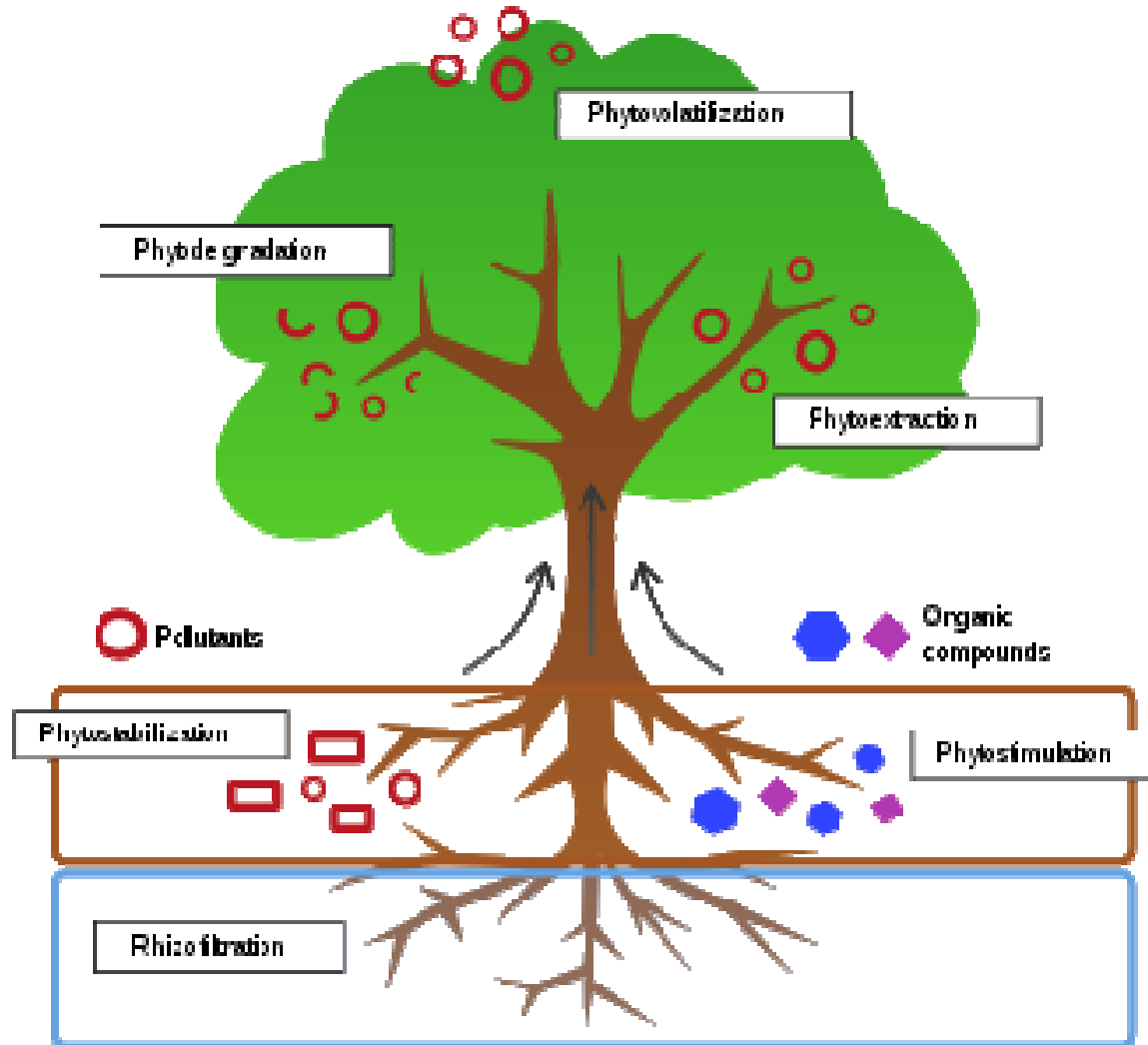


Figure 1: Phytoremediation process

SOURCE: http://en.wikipedia.org/wiki/file:phytoremediation_process (2017)

in the environment, for example, by limiting the leaching of substances from the soil (Lasat, 2000).

- **Phytotransformation** – chemical modification of environmental substances as a direct result of plant metabolism, often resulting in their inactivation, degradation (phytodegradation), or immobilization (phytostabilization) (Pilon-smith, 2008).

- **Phytostimulation** – enhancement of soil microbial activity for the degradation of contaminants, typically by organisms that associate with roots. This process is also known as *rhizosphere degradation*. Phytostimulation can also involve aquatic plants supporting active populations of microbial degraders, as in the stimulation of atrazine-degradation by hornwort (McIntyre and Lewis, 1997).

- **Phytovolatilization** – removal of substances from soil or

water with release into the air, sometimes as a result of phyto-transformation to more volatile and/or less polluting substances. Plants extract pollutants from the soil and then convert them into a gas that can be safely released into the atmosphere (Salt *et al.*, 1998).

- **Rhizofiltration**– filtering water through a mass of roots to remove toxic substances or excess nutrients. The pollutants are either absorbed in or adsorbed to the roots of plants. Rhizofiltration is a similar concept to phytoextraction, but mainly used for the remediation of contaminated groundwater rather than the remediation of polluted soils. Plants used for rhizofiltration are not planted directly in situ, but are acclimated with the pollutant first. Until a large root system has developed, plants are hydroponically grown in clean water rather

than in soil. Once a large root system is in place, the water supply is substituted for polluted water supply to acclimate the plant. After the plants become acclimatized, they are planted in the polluted area. As the roots become saturated, they are harvested and disposed of safely (Pilon-smith, 2008).

•Biological hydraulic containment– Some plants, like poplars, draws water upwards through the soil into the roots and out through the plant decreases the movement of soluble contaminants downwards, deeper into the site and into the groundwater (Huang *et al.*, 2010).

Phytoremediation of petroleum hydrocarbons

The accumulation of petroleum hydrocarbons in the environment can cause serious problems, affecting negatively the stability of many aquatic ecosystems and can also cause difficulties for animals and human health. The amount of natural crude-oil seepage is currently estimated to be 600,000 metric tons per year, with a range of uncertainty of 200,000 to 2,000,000 metric tons per year (Kvenvolden and Cooper, 2003). The phytoremediation technology offers a promising tool to treat aerobically oil-contaminated shorelines (Atlas, 1991, Rosenberg *et al.*, 1992, Venosa *et al.*, 1992). This technique of decontamination of polluted areas gives much hope on the restoration of polluted mangrove swamps and is being utilized for the degradation of crude oil in soil matrix by using microalgae to transform the petroleum hydrocarbons into less toxic compounds (Davies and Westlake, 1979, and Orji *et al.*, 2012a). This is achieved by the help of bacteria, fungi, algae that produce enzymes capable of degrading harmful organic compounds (Davies and Westlake, 1979). In the presence of abundant hydrocarbon in the environment after a spill, microalgae cannot effectively attack and utilize the hydrocarbon unless limiting nutrients such as nitrate, phosphate, even microelements are incorporated into the polluted medium (Van Hamme *et al.*, 2003).

Microalgae and protozoa are the important members of the microbial community in both aquatic and terrestrial ecosystems however only few reports are available regarding their involvement in hydrocarbon biodegradation (Jain and Bajpai, 2012). On the other hand, introduction of hydrocarbon degrading bacteria in oil contaminated sites does not guarantee the removal of all components of crude oil because some components still remain difficult to degrade, such as alkanes of shorter and longer chains (<C10 and C20-C40) and polycyclic aromatic hydrocarbons (PAHs) (Yuste *et al.*, 2000). Several microorganisms can metabolise the nonchlorinated aliphatic and aromatic hydrocarbons as sources of carbon, but due to their hydrophobicity they pass very slowly to the aqueous phase liquid where microorganisms are active (Brusseau, 1998).

Recently Orji *et al.* (2012b) reported that the Remediation by Enhanced Natural Attenuation (RENA) is currently being used as a cleanup technology in polluted environments with petroleum hydrocarbon in the Niger-Delta including mangrove ecosystems. RENA is a full-scale bioremediation technology in which contaminated soils, sediments and sludge's, are periodically turned over or tilled into the soil to aerate the waste. This technique involves factors such as evaporation, dispersion, spreading, dissolution, oxidation, emulsification and spreading. The asphaltene's, the most complex hydrocarbons of the petroleum, contain nitrogen, sulphur and oxygen (Mitra-Kirtley *et al.*, 1993), and are very resistant to the microbial degradation (Guiliano *et al.*, 2000).

Chemical mutagens that induce beneficial mutation

There are chemical substances that are capable of inducing changes in the genetic composition of organisms. The mutation caused by these substances can be negative, but can sometimes be beneficial (Ismaila and Umaru, 2005). Chemical mutagens induce point mutation. It does this by changing a base that is in the DNA and changes its hydrogen bonding specificity (Ismaila and Umaru, 2005). A covalent bond is formed between the electrophilic part of the molecule and a nucleophilic part of the DNA. This will change the shape of the DNA and may lead to mispairing of bases on the strand or to errors in the DNA replication, typically base pair substitutions (Vivian *et al.*, 2004). Other molecules may react with DNA by intercalating between the two strands of DNA. There are grooves in the double helical structure of the DNA and certain planar molecules fit within these grooves. The presence of these intercalated molecules may cause errors in DNA replication or DNA repair, which will result in the mutation of the DNA (Vivian *et al.*, 2004).

The advantage of using chemical agents is the possibility of improving one or more traits of the organism without causing undesirable changes (Jeng *et al.*, 2011). A list of some of the most common chemical mutagens are; ethyl methane-sulfonate (EMS), methyl methane sulfonate (MMS), hydrogen fluoride (HF), sodium azide (SA), N-methyl-N-nitrosourea (MNU), and hydroxylamine (H3NO) are the chemicals most frequently used in plants (Parry *et al.*, 2009). The effect of treatments with these chemical mutagens can be silent, missense (50%) or nonsense (5%)(Nawaz and Shu, 2014).

Inducing beneficial mutation using sodium azide in plants

Sodium azide is relatively safe to handle, inexpensive and non-carcinogenic as compared to other mutagens (Eze and Dambo, 2015). It has been reported to be the

least dangerous and the most efficient mutagen which is mutagenic in several crop species (Adamu and Aliyu, 2007; Mostafa, 2011). It is an ionic compound and its mutagenicity is mediated through an organic metabolite (analogous to L. azidoalanine) of the azide compound generated by acetylserine sulfhydrylase enzyme (Gruszka *et al.*, 2012). This metabolite enters the nucleus of the cell and interacts with the plants DNA, creating point mutations in the plant genome. The mutagenic effect of sodium azide depends on the pH of the solution and can be increased when germinated seeds are treated (Gruszka *et al.*, 2012). It has been found to have potential in tissue culture mutagenesis for inducing biochemical mutants because it is a point mutagen and is known to be highly mutagenic in mammals (Eze and Dambo, 2015). Being a strong mutagen in plant, it affects the different parts of the plants and their growth developmental phenomena by disturbing the metabolic activities. Treatments with sodium azide resulted in increased rate of maturity in soybean plant (*Glycine max*) when higher doses were administered (Archarya *et al.*, 2004). Kiruki *et al.*, 2006 observed that mutant maize plants became resistant to *Striga hermonthica* and *Striga asiatica*. Mensah and Obadomi (2007) have also reported that barley plants became resistant to mildew disease. Sodium azide has also been shown to significantly influence the growth of and germination of wheat plant (Srivastava *et al.*, 2011).

Water hyacinth and its use in phytoremediation

Water hyacinth is a perennial fresh water plant that is free-floating or rooted in some cases (Moyo *et al.*, 2013). It belongs to the family, Pontederiaceae that has proven to be a significant economic and ecological burden to many sub-tropical and tropical regions of the world (Mapira, 2011). Water hyacinth is listed as one of the most important productive plants on earth that shows logistic growth like other floating plants (Moyo *et al.*, 2013). It is known to be an invasive plant across the world which acts as a nuisance weed which invades rivers, lakes and dams that have been polluted (Nazir *et al.*, 2011). The IUCN lists it as the most dangerous invasive species. It has invaded freshwater bodies in over 50 countries on five different continents and is present in Southeast Asia, Southeastern United States, Central and Western Africa, and Central America (Lu *et al.*, 2007; Martinez and Gomez, 2007). Water hyacinth has a very fast reproduction rate and can easily cover the surface of water bodies, forming a thick "carpet". Water hyacinth has been found to be very efficient in removal of pollutants from contaminated water (Shahabaldin *et al.*, 2015). It forms the central unit of a recycling engine driven by photosynthesis and therefore the process is sustainable, energy efficient and cost efficient under a wide variety of rural and urban conditions (Swain and Mohanty, 2014). It can be

established and survived in wastewater (Sooknah and Wilkie, 2004). The enormous biomass production rate, its high tolerance to pollution, and its heavy metal and nutrient absorption capacities (Singhal and Rai, 2003; Ingole and Bhole, 2003; Liao and Chang, 2004; Jayaweera and Kasturiarachchi, 2004; Swarnalatha *et al.*, 2015) qualify it for use in wastewater treatment ponds. For example, studies have shown that about 1 million L/ day of domestic sewage could be treated over an area of 1 ha through water hyacinths, reducing the biological oxygen demand (BOD) and chemical oxygen demand (COD) by 89% and 71%, respectively (Zayed and Terry 2003; Mohanty *et al.* 2005a, 2009, 2010).

MATERIALS AND METHODS

Study area

This study was conducted in the Biological Garden in the Department of Biological Sciences, University of Abuja, FCT, Nigeria. It lies between latitude 8°58' north of the equator and longitude 7°10' east of the Greenwich meridian. University of Abuja main campus is planned in phases over an area of 118sq km. It has an annual rainfall of 1650mm (Google earth, 2018) and an average temperature of 21°C to 26°C. The months of July, August and September has approximately 60% of the annual rainfall. There are two main seasons, namely the dry season with maximum temperature of 30°C and 35°C, as well as 25°C during the rainy season.

Plant collection

Mature water hyacinth (*Eichornia crassipes*) plants were obtained from Jabi Lake in Abuja at the commencement of the studies for each month.

Crude oil collection

Twenty (20) litres of raw crude oil was collected from Warri Refining & Petrochemicals Company limited (WRPC) in Ekpan – Warri, Delta State, Nigeria. It was used to contaminate the simulated aquatic habitats in the Biological Science Department of University of Abuja, where this research was carried out.

Experimental design

Experimental Set-Up

Mature water hyacinth plants (*Eichornia crassipes*) were brought from Jabi Lake and put into white transparent

Table 1: Nutrient composition of individual salts.

Nutrient Chemicals	Weight in grams
1. Potassium dihydrogen phosphate	131.5
2. Potassium nitrate	291.5
3. Calcium nitrate	501.5
4. Magnesium sulphate	256.5
5. EDTA iron	39.5
6. Manganous sulphate	2.03
7. Boric acid	0.85
8. Copper sulphate	0.195
9. Ammonium molybdate	0.185
10. Zinc sulphate	0.22

water containers which was used to simulate aquatic environments. Twenty litres of water was poured into each container which was further enriched by coopers solution, a hydroponic nutrient used for growing plants hydroponically.

Preparation of hydroponic solution

Individual constituent salt for cooper hydroponic solution were obtained from Biology Laboratory, Microbiology Laboratory and Steve Moore Chemicals Co in Zaria (Table 1). The amount of the constituent salts obtained for Coopers hydroponic solution was the right amount required to be dissolved in 500 litres of water. The constituent salts and their amounts in (Table 1). They amount of the constituent salt stated is half of the amount of the salt that should have been dissolved in 1000 litres of water. Calcium nitrate and EDTA iron was first dissolved in 5 litres of water and labeled, "A" and the rest of the constituent salts dissolved at once in another 5 litres of water labeled B. the hydroponic solution is then added to the main aquatic habitats with the solution labeled "A" added first before the solution labeled "B" (aquafrica.co.za). Coopers solution was prepared in the biology laboratory of the department of biological sciences monthly before the commencement of each month's studies. 200 millilitres of Cooper solution were added to each container of water to make up a volume of 20 litres for the first month of the study in the rainy season in May. This was increased to 400 millilitres in the second month of June and further increased to 600 millilitres in July. This is repeated in the same manner from August to October for dry season studies (aquafrica.co.za).

Plant setting

Ten mature water hyacinth plants with well-developed shoot and root system were introduced into each container at the commencement of each month's studies. The plants were introduced first into the aquatic habitats before they were contaminated with crude oil.

Crude oil contamination

Solution of crude oil will be prepared with five different concentrations and a control. A stock solution of sodium azide was also prepared in the biology laboratory of the department. Some of the water hyacinth plants were isolated and treated with sodium azide solution for four hours. This is to induce mutation of the genes of the water hyacinth plant, so that compares can be made between its rate of phytoremediation of the natural plants with the genetically modified plants. The concentration of the sodium azide used in treatment of the water hyacinth to induce mutation was also altered from 0.05 mol/dm³ in May to 0.03 mol/ dm³ in June and 0.01 mol/ dm³ in July. This is repeated from August to October in the same order. This is to be able to determine the lethal dose of the sodium azide solution that is deleterious to the survival of the water hyacinth or that can give optimal results. The set-up was divided into two sections. The first section is the section for plants that were not treated sodium azide to induce mutation. The second section is for the plants that were treated with sodium azide to induce beneficial mutation. The containers were labelled A - D. Each treatment is replicated twice. That means containers labeled "A" includes A, A1 A2 and D contain D1 and D2, plants that were not treated with sodium azide were labeled B and C with two replications each. This means containers labeled B are B, B1 and B2 while those labeled "C" are C, C1 and C2. Those labeled B and C were contaminated with crude oil together with their replications. Those labeled A and D together with their replications, were not contaminated with crude oil. All together there were 12 containers in the set up with 6 containers contaminated with crude oil while the other six were not contaminated with crude oil. The control for this experiment were those labeled A, A1 and A2. The planting layout that will be used is completely randomized design.

New emerging plants

The new emerging plants were studied for each week to

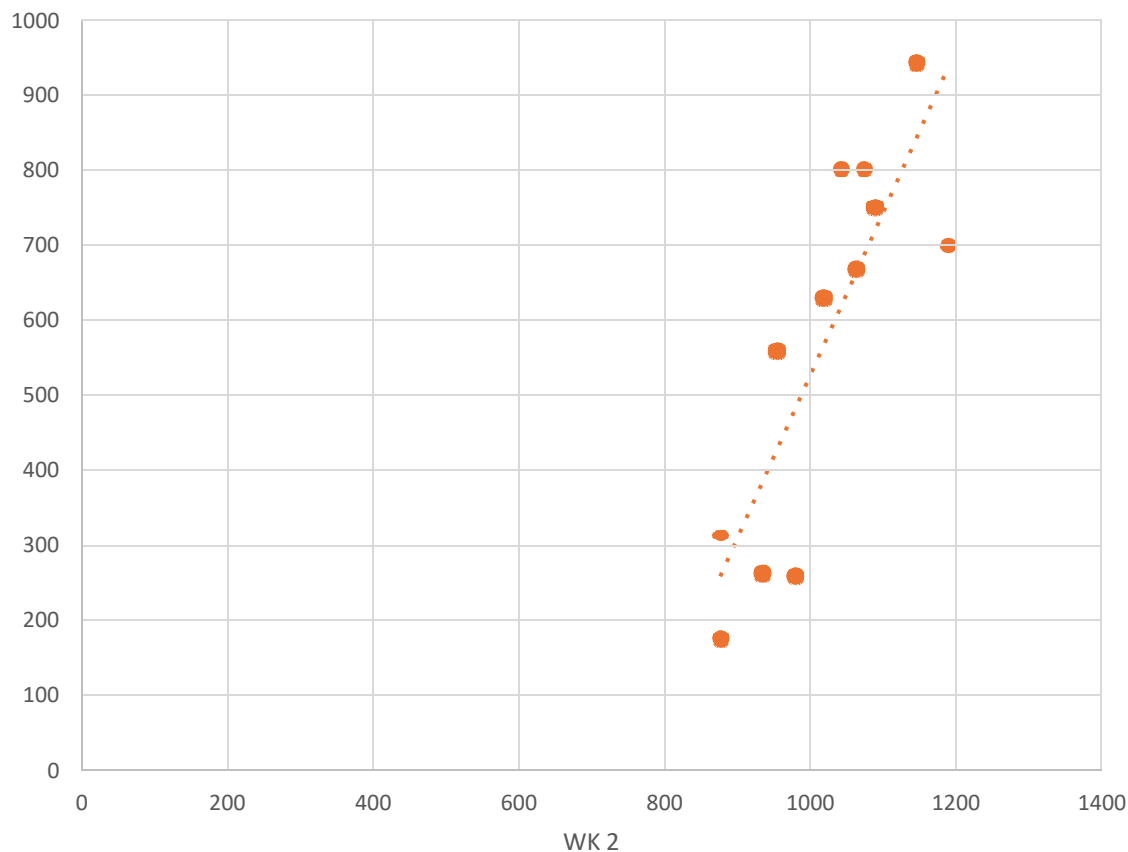


Figure 3: Correlation of new plants in week 1 and 2

chelating agents can also be a factor that has led to a higher uptake of Fe^{2+} by the genetically modified plants than Pb^{2+} (Yan *et al.*, 2020). These chelating agents may have been brought about through the Cooper solution that was used in the experiment to grow the plants hydroponically. Abdullah *et al.* (2011) reported that in chelate- assisted phytoremediation studies, synthetic chelating agents such as NT and EDTA are added to phytoextraction of soil polluting heavy metals. This research showed that the genetically modified plant could store more than 130.5770mg/100g of Fe^{2+} with treatment A4 which is for the month of August recording upto 231.312mg/100g of Fe^{2+} . This is quite higher compared to results by Abdullah *et al.* (2011) which showed an uptake of more than 50mg/g dry weight of plant. The result of this study showed a very high uptake of sulphate ions by the leaves of the genetically modified water hyacinth plants compared to the results studies by Oleivera *et al.* (2009) who observed an inhibition in the absorption of sulphate by water hyacinth due to the

presence of inhibitors such as cadmium. This is might be due to the change in the gene of the plants that could have been induced by the chemical mutagen, sodium azide that was used in the genetic modification of the plant. This study also contradicts the results of Lin *et al.* (2009) who isolated a gene conferring low-sulphur tolerance to water hyacinth. The result from this study showed a very high uptake and absorption of SO_4^{2-} ion by the plant leaves. This can be as a result of the induced mutation on the water hyacinth plants by sodium azide. This may have brought about an over-expression of the sulphur tolerance gene making it to become very high. Studies by Ali *et al.* (2020) showed that water hyacinth had more ability to absorb heavy metals than non-metallic ions like sulphate, but the result of this study showed that the genetically modified samples showed a very high rate of uptake of sulphate than that of the heavy metals studied (Pb^{2+} and Fe^{2+}). This is as a result of the genetic modification of the sodium azide plant that was induced after treatment with sodium azide.

Table 2: Result of elemental analysis of Fe²⁺ AND Pb²⁺

Treatments	Pb (Mg/100g)	Fe (Mg/100g)
A1	-	189.0370
B1	0.083	163.1455
C1	1.375	161.805
A2	2.0315	144.158
B2	3.155	193.9655
C2	—	196.331
D2	3.765	201.7965
A3	4.7880	193.6555
B3	1.7185	165.5260
C3	1.1405	191.7360
D3	3.8590	226.1890
A4	2.1815	231.3120
B4	0.2735	218.2020
C4	0.5875	
D4	1.2150	69.6850 142.6850

Conclusion

Through the use of sodium azide, we have been able to induce beneficial mutation in the phytoremediation ability of water hyacinth which is commonly used in phytoremediation, especially with regards to the uptake of the pollutants. We can therefore conclude that technics in genetics remains a viable tool to be able to improve the quality or ability of plants to absorb or take up metals and other contaminants like crude oil that poses a great threat to the environment. The result from this study also shows that contrary to the long term rate of plants to phytoremediate contaminated environments especially those contaminated with crude oil, ideas in genetics like the use of chemical mutagens like sodium azide can be used to speedy up the rate of phytoremediation. This is clearly depicted in the amount of Fe²⁺, Pb²⁺ that was absorbed by the leaves of the plant. The study conclude that chemical mutagen such as sodium azide is effective in improving the survival rate of plants, especially their ability to cope with stress factors like the pollutants in their habitats. The result of this research showed that the highest survival rate in the contaminated habitats were recorded in plants that were treated with sodium azide.

Recommendations

More support should be given to researchers carrying out researches on phytoremediation by the government and private partners. At the commencement of this research, difficulties were experienced in sourcing of materials such as raw crude oil for ex-situ studies. The government and the ministry of environment should give more support to researchers carrying out phytoremediation studies, especially those that have to deal with the cleaning up of aquatic habitats. The study also recommend that the

government should make more funds available to sponsor scientific research in universities, especially at MSc. And Ph.D Levels, because such research usually incur very high costs and their results will be of direct benefit to the government in its efforts towards remediating or cleaning-up contaminated habitats.

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