

**IMPACT OF DIESEL ON THE GROWTH, SURVIVAL AND MORPHOLOGY OF  
*EICHHORNIA* CRASSIPES**

**BY**

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**MATRICULATION NUMBER**

**LSC1605695**

**DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY  
FACULTY OF LIFE SCIENCES  
UNIVERSITY OF BENIN,  
BENIN CITY**

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**A THESIS SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND  
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THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF  
SCIENCE (HONOURS), UNIVERSITY OF BENIN, BENIN CITY, EDO STATE,  
NIGERIA.**

**DATE**

**MAY, 2021**

## **CERTIFICATION**

This is to certify that this work was carried out by Anaesthatus Esiokhala AMEDU of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria.

**Mr. Otasowie Micheal Eguagie**

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Head of Department

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Sign and Date

**External Examiner**

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Sign and Date

## **DEDICATION**

This work is dedicated to God and my family

## **ACKNOWLEDGEMENTS**

My acknowledgement goes to God for His infinite mercies, guidance and strength throughout this study. I appreciate my supervisor Mr. O. M. Eguagie for his tireless guidance and perpetual support, I could not have asked for a better supervisor. My appreciation also goes to the HOD of the department plant biology and biotechnology Prof. (MRS) F. I. Okungbowa, the project coordinator, Prof. E. O. Akpaja and the other esteemed lecturers in the department. I acknowledge my family especially Mr. Romanus Amedu, Mr. John Amedu and Mr. Phillip Amedu for their financial assistance as well as moral support. I also appreciate my friends for standing by me.

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## ABSTRACT

This study was aimed at assessing the effects of diesel on the growth and morphology of *Eichhornia crassipes*. Samples of *Eichhornia crassipes* were collected from Ologbo pond, Edo State, Nigeria. Diesel was purchased at the Faculty of Agriculture Cooperative gas station, University of Benin, Benin city. The plants were then exposed to different concentration of 2%, 4%, 6% and 8% (v/v ) and 0% control. Parameters used in the study include plant height, root length, stolon length, number of leaves, chlorophyll content, stolon girth, leaf diameter and determination of fresh and dry weight. Using three replicates per treatment, the plants were exposed to all concentration for 8 days. The results showed that all morphological parameters had a low mean value as compared to control, which shows that diesel was detrimental to the plant. The effects were concentration dependent. The leaves of the test plants also showed changes in morphology. Chlorotic and necrotic lesions were noticed on the adaxial and abaxial surface. Further research should be carried to better understand the effects of diesel on *E. crassipes* and other aquatic macrophytes. Also adequate measures should be put in place to avoid pollution of the environment with diesel. Early response should be ensured in the case of diesel oil spillage in order to reduce the duration of exposure of ecosystems to oil pollution.

## **CHAPTER ONE: INTRODUCTION**

### **1.0 DIESEL FUEL POLLUTION**

Pollution is an anthropogenic process which causes lethal effects to organisms or humans. (Ricardo, 2018). The main types of pollution include land pollution, air pollution or water (freshwater or marine) pollution.

Aquatic pollution is the introduction of substances into the aquatic environment which has lethal effects like harm to living resources, hazards to human health, interference with aquatic activities like fishing, diminishing quality of sea water and reduction of amenities. (Ricardo, 2018). The 1982 United Nations Convention on the law of the sea defined aquatic pollution as the introduction by man, directly or indirectly of substances or energy into the aquatic environment which results or is likely to result in such lethal effects as harm to living resources and aquatic life. The freshwater environment is exposed to contaminants of different forms which can likely change the physical, chemical and biological characteristics of the water. This contamination can occur as a result of direct discharge of effluents and solid wastes from land or human activities on the freshwater bodies like run-offs mainly from land, atmosphere and shipping which can result in oil spills. The freshwater environment can serve as a sink for these contaminants. Freshwater pollution affects the quality of the ecosystem and is a potential threat to the freshwater organisms and biodiversity.

Oil fractions are among the main causes of aquatic pollution (Nunes and Benville, 1979). When crude oil or its fractions are present in the water, some of its constituents especially the hydrocarbons may dissolve in water and these dissolved components when in high concentrations may become toxic to the freshwater ecosystem (Neweay and Seed, 1995).

Petroleum hydrocarbons are natural pollutants which are of major concern because of their wide distribution, complex composition and toxicity (Chirwa and Bazza, 2015). Petroleum hydrocarbons contain oil and products refined from oil which include gasoline and diesel. Diesel is one among the most commonly used petroleum hydrocarbons and it is usually used as fuel for ships. Diesel fuel in general is any liquid fuel used in diesel engines. The most common type of diesel fuel is a specific fractional distillate of petroleum fuel oil. It can be refined traditionally from petroleum and other materials.

Diesel pollution is the direct or indirect introduction of diesel into the environment as a result of human activity. The physical environment of Nigeria has been negatively impacted by diesel pollution due to the activities of oil companies. Diesel pollution most commonly arises from oil spills, and these spills are mostly detrimental to the biodiversity.

Almost all activities involved in the exploration and exploitation of crude oil result in the discharge of crude oil into the environment. Recently, there has been a significant increase in the volume of crude oil being spilled into the environment, especially now that oil has taken the center stage as the major source of energy to mankind. Crude oil-induced pollution is dependent on the nature and type of crude oil, the level of oil contamination, type of environment, and selective degree of sensitivity of the individual organisms (Sadek *et al*, 2017).

When oil is spilled into an aquatic environment, it causes harm to organisms that live on or around the water surface and those that live under water. Spilled oil can also damage parts of the food chain, food resources for humans not excluded. The severity of the impact of an oil spill depends on a variety of factors, including characteristics of the oil itself. Natural conditions, such as water temperature and weather, also influence the behavior of oil in aquatic environments. Various types of habitats have differing sensitivities to oil spills as well.

Since the discovery of oil in Nigeria in 1956, the country has been suffering the negative environmental consequences of oil exploration and exploitation. Between 1976 and 2005, Nigeria recorded 9,107 oil spill incidents, which led to the spill of 3, 121, 909.8 barrels of oil. Several oil-spill related laws and policies have been put in place to manage oil spill incidents in the country (Egberongbe *et al*, 2006).

Nigeria is a country rich in crude oil, and the Niger Delta region of the country is commonly associated with crude oil. The incidents of oil spill cause serious environmental problems throughout the country. Vandalism of oil pipes and storage facilities is one of the major factors responsible for the incidents of oil spill in the country. Oil spillage has led to pollution of drinkable water, destruction of the ecosystem, death of marine fishes and animals in the Niger Delta and Nigeria as a whole. Oil spill incidents have occurred at different times along the Nigerian coast. Between 1976 and 1996, a total of 4647 incidents resulted in the spilling of approximately 2,369,470 barrels of oil into the environment. Of this quantity, an estimated 1,820,410.5 barrels (77%) were not recovered. Available records for this period indicate that approximately 6%, 25%, and 69% respectively, of total oil spilled in the Niger Delta area, were in land, swamp and offshore environments. (Nwilo *et al*, 2005).

**Table 1:** Record of Oil Spill Incidents in Nigeria

S/ N	YR	NO.	(%) OF	QUANTIT	(%) OF	QUANTITY	PERCENTAG
		OF OIL SPIL L	NO. OF OIL SPILLE D	Y OF OIL SPILLED (BARREL S)	QUANTIT Y OF OIL SPILLED	RECOVERE D (BARRELS)	E (%) OF QUANTITY RECOVERED
1.	1976	128	1.41	26,157.00	0.84	7,135.00	27.28
2.	1977	104	1.14	32,879.00	1.05	1,703.01	5.18
3.	1978	154	1.69	489,294.00	15.67	391,445.00	80.00
4.	1979	157	1.72	694,170.00	22.24	63,481.20	9.14
5.	1980	241	2.65	600,511.00	19.24	42,416.83	7.06
6.	1981	238	2.61	42,722.00	1.37	5,470.20	12.80
7.	1982	252	2.77	42,841.00	1.37	2,171.40	5.07
8.	1983	173	1.90	48,351.30	1.55	6,355.90	13.15
9.	1984	151	1.66	40,209.00	1.29	1,644.80	4.09

10.	1985	187	2.05	11,876.60	0.38	1,719.30	14.48
11.	1986	155	1.70	12,905.00	0.41	552	4.28
12.	1987	129	1.42	31,866.00	1.02	6,109.00	19.17
13.	1988	208	2.28	9,172.00	0.29	1,955.00	21.31
14.	1989	195	2.14	7,628.16	0.24	2,153.00	28.22
15.	1990	160	1.76	14,940.82	0.48	2,092.55	14.01
16.	1991	201	2.21	106,827.98	3.42	2,785.96	2.61
17.	1992	378	4.15	51,187.96	1.64	1,476.70	2.88
18.	1993	428	4.70	9,752.22	0.31	2,937.08	30.12
19.	1994	515	5.65	30,282.67	0.97	2,335.93	7.71
20.	1995	417	4.58	63,677.17	2.04	3,110.02	4.88
21.	1996	430	4.72	46,353.12	1.48	1,183.02	2.55
22.	1997	339	3.72	81,727.85	2.62	-	-
23.	1998	399	4.38	99,885.35	3.20	-	-
24.	1999	225	2.47	16,903.96	0.54	-	-
25.	2000	637	6.99	84,071.91	2.69	-	-
26.	2001	412	4.52	120,976.16	3.88	-	-
27.	2002	446	4.90	241,617.55	7.74	-	-
28.	2003	609	6.69	35,284.43	1.13	-	-
29.	2004	543	5.96	17,104.00	0.55	-	-
30.	2005	496	5.45	10,734.59	0.34	-	-
<b>31.</b>	<b>Tota</b>	<b>9,107</b>		<b>3,121,909.8</b>		<b>550,232.9</b>	<b>22.80</b>

Source: Egberongbe *et al*, 2006.

### **1.0.1 Causes of oil pollution in Nigeria**

Some of the causes of oil pollution in Nigeria are outlined below:

1. Socio-political causes such as vandalism and sabotage.
2. Use of outdated and inefficient technological equipment.
3. Poor state of oil transport and storage facilities such as corrosion of oil pipelines.
4. Leakage from shore wells.

### **1.0.2 Consequences of oil pollution**

Oil spilled on land does not spread quickly unlike on water, and the effects remain localized. Most types of oil will penetrate the soil and contaminate organisms in the soil. A full coating of diesel fuel will kill most plants and small trees on contact. Oil pollution in water bodies can be very catastrophic, resulting in genetic modification of aquatic organisms, morphological deformities, alteration in growth and development, reduction in body mass, inhibited swimming abilities and even death from irritating the tissues of organisms. Since oil is insoluble in water, it forms a thin layer on the surface of water bodies, thereby preventing animals and plants living in the water from breathing, the oil coats the feathers of water birds, preventing it from foraging or escaping from predators and also making them lose the ability to thermo regulate in cold water and the fur of animals that swim in the water, causing them to

become sick and, if there is a great amount of oil on their bodies, to die by irritating their digestive tracts, altering liver function, and causing kidney damage even the insects that live on the surface of the water are badly affected (Fingas, 2016).

Aquatic plants and animals are may be affected by floating oil which forms a film on the surface of the water. This film can reduce the rate of transpiration, photosynthesis and feeding (Mason, 1991). This is so because the layer of oil formed on aquatic habitats as a result of oil pollution prevents or reduces the amount of light available to aquatic organisms; this is harmful to photosynthetic organisms and also reduces the food supply in the water bodies. Directly, oil pollution affects aquatic plants variably. It may result in death in some species while it may spur growth of some populations of algae (Fingas, 2016).

### **1.0.3 Effects of diesel on plants**

Diesel is known to impact plants in many ways, ranging from stimulation to hampering of growth. Diesel pollution is also very complex and involves both direct (contact toxicity) and cascading (bottom-up) effects. Diesel is easily absorbed and, since they act at the cellular level, are immediately toxic to the plants. Diesel may also cover the plants thereby leading to asphyxia and hindering gaseous exchange (Pezeshki *et al.*, 2000; Lopes and Piedade, 2009). The effect of diesel pollution on plant growth and germination also varies according to the degradation time and how its components alter over the period when it remains in contact with the vegetation, the degree of its weathering, the time of year and the species, the parts of the plants involved, the age of the plant or plants concerned, the growth and phenological stages, the environmental

conditions, such as sediment granulometry, season of the year, inundation level and characteristics of the climate (Lin and Mendelsohn, 1996; DeLaune *et al.*, 2003). Most plants have differing susceptibilities and rates of recovery from oil pollution (Chang *et al.*, 2014; FiriAppah *et al.*, 2014), in some cases, oil has been shown to cause growth stimulation. Chronic pollution may completely eliminate germination of plants, hence leading to no vegetation (FiriAppah *et al.*, 2014).

Generally, when diesel spills occur in a terrestrial environment, it directly affects the ecosystem through soil contamination (Pezeshki *et al.*, 2000). Soil contaminated with diesel has deleterious effects such as: killing of plant on contact, or causing retarded growth as well as inhibition of germination, this is due to the fact that it enhances the inhibition of activities of the soil micro-organisms by delimiting free water supply and aeration (McGill, 1977). When plants are exposed to diesel, it penetrates into the plant tissues, through the sensitive stomata, the thin cuticle and also through the epidermis. These penetrations are made possible by its transfer through the vascular system of the plant, and these vascular tissues could become clogged up with oil (Turrel, 1947). Cascading effects of oil pollution can be in the form of oil spilled on land, thereby making it unsatisfactory for the growth of plants. This is due to insufficient aeration of the soil because the oil displaces air from the pore spaces between the soil particles (De Jong, 1980). Waste oil causes a break down in the texture of the soil, followed by soil dispersion. However, the presence of diesel also causes the soil particles to coagulate, binding the soil particles into a water impregnable soil block, this seriously impairs water drainage and diffusion of oxygen. Seeds sown in such soil often fail to germinate (Atuanya, 1987). In aquatic ecosystems, diesel affects the various plants in numerous ways. Diesel floating on the surface of

the water is dispersed through the action of wind and wave action in the littoral region, thus affecting the terrestrial environment and vegetation (Pezeshki *et al.*, 2000). The introduction of diesel into aquatic environments leads a series of alterations in the ecosystem such as death and decomposition of aquatic plants in most cases which results in a substantial increase of organic materials in the water body (Akinluyi and Odeyemi, 1987), buildup of heavy metals, such as nickel and mercury in the plants (Ekundayo and Obuekwe, 2000). These alterations in the ecosystem, coupled with difference in sensitivity of each plant species to heavy metals, result in modification of the composition of the community of aquatic plants (Burk, 1977; Pezeshki *et al.*, 2000). Heavy metals also contaminate the dead organic matter (Osuji and Adesiyi, 2005) and when decomposition takes place, toxic elements may be incorporated into the trophic chains through the ingestion of plant material by invertebrates, which are the preferential food items of several fish species (Lopes and Piedade, 2009). Phytoplankton and zooplankton which are small organisms floating freely on the water body are affected by relatively low concentrations of diesel. A dynamic change in plankton population can influence the biomass of other marine animals like fish by bottom-up effects (Beaugrand *et al.*, 2003). Some plankton directly ingest droplets of diesel, the ingestion of diesel by these organisms often causes mortality, while surviving organisms often show developmental and reproductive abnormalities (Jiang *et al.*, 2010).

#### 1.0.4 Methods of cleansing oil spills

1. **Evaporation:** This occurs mostly in light oils such as gasoline, kerosene and other refined oil products due to the fact that they contain flammable components which evaporate immediately after a few hours. Oil weather removal is largely due to the contribution of evaporation, and it depends on the soil type, area of spill, thickness of oil slick, vapour pressure and mass transport coefficient. These in turn are composition of the oil, wind speed and temperature (Egberongbe *et al*, 2006).
2. **Biodegradation:** This is the slow loss of oil due to the ingesting and metabolizing of oil by various organisms (Egberongbe *et al*, 2006). It is the process whereby most of the complex and toxic components of spilled oil are broken down into harmless forms such as fatty acids. The rate at which spilled oil undergoes biodegradation is dependent on the type of oil, concentration of the oil and the type of hydrocarbons contained in it. The natural process of biodegradation can be facilitated by the addition of nutrients such as nitrogen and phosphorous. These nutrients stimulate growth of the microorganisms concerned (Lee *et al*, 1989a: Lee *et al*, 1989b).
3. **Emulsification:** This is the formation of emulsions. Emulsions are stable immiscible droplets of oil and water caused by wave actions. Oil and water emulsions cause oil to sink and disappear from the surface of the water.
4. **Weathering:** This involves a series of physical and chemical changes that leads to the oil becoming heavier than water. Tidal waves, wind and water current may result in natural dispersion, thereby breaking a slithery in blobs which are scattered throughout the water. (Abowei and Njobuenwu, 2008).

## **1.1 AQUATIC MACROPHYTES**

Aquatic macrophytes are plants that are adapted to the aquatic environment. These plants are large enough to be seen with the naked eyes. Aquatic macrophytes can also be defined as plants that grow permanently or periodically submerged below, floating on, or growing up through the water surface. These groups of plants are found predominantly in wetlands, streams and shallow lakes. They include bryophytes, pteridophytes and aquatic angiosperms (Sullivan and Wood, 2012).

### **1.1.1 Classification of aquatic macrophytes**

Aquatic macrophytes are usually classified among three main groups according to their roots and leaves. They include:

- Floating macrophytes
- Submerged macrophytes
- Emergent macrophytes

#### **Floating macrophytes**

Floating macrophytes are plants that float freely on the water surface. These are self-growing plants which are found commonly in the shallow stagnant waters of tropical and sub-tropical countries. They help in controlling algae by competing with them for nutrients. The most commonly found floating macrophytes are water spinach (*Ipomoea aquatica*), water lettuce

(*Pistia stratiotes*), water fern (*Salvinia* sp.), water hyacinth (*Eichhornia crassipes*) and water chestnut (*Trapa* sp.) (Hussner, 2012).

### **Submerged macrophytes**

Submerged macrophytes are plants that are usually rooted in the bottom soil with the vegetative parts predominantly submerged. Submerged macrophytes are distributed all over the world except some very deep and cold water lakes in polar countries. They are found more commonly in shallow stagnant waters. Submerged macrophytes occur in various types of water bodies, such as estuaries, rivers, lakes, ponds, ditches, swamps and natural depressions. They help in improving water transparency, stabilizing the sediment or substratum it grows on and enhancing the aquatic biodiversity in shallow waters. Some examples include *Blyxa lancifolia*, *Cabomba caroliniana*, *Ceratophyllum demersum* and *Elodea canadensis*.

### **Emergent macrophytes**

Emergent macrophytes are plants that possess roots that are attached to a substratum in the shallow part of the water, with the vegetative parts of the plant emerging above the surface of the water. Emergent macrophytes are believed to be the most particularly productive of all aquatic macrophytes, being that they utilize all of three possible states—with their roots being attached to sediments beneath water and their photosynthetic parts in the air (Westlake, 1963). A large variety of emergent macrophytes can be found in the tropical and sub-tropical regions of the world. Examples are *Alisma plantago*, *Alternanthera philoxeroides*, *Cabomba aquatic*, *Colocasia esculenta* and *Eleocharis ochrostachys* (Ahmad *et al*, 2014).

### **1.1.2 Importance of aquatic macrophytes**

Aquatic macrophytes comprises lots of plants with various morphological features, these plants play very important roles in natural ecosystems. They also have a wide variety of uses. Some of the important roles and uses of aquatic macrophytes are listed below:

#### **As fish feed**

Some species of aquatic macrophytes are used as feed for fish; these plants have high nutritive content, making them suitable food for edible fishes. Usually, they are incorporated into ponds as the main meal or as supplements mixed with other food materials. In natural ecosystems, herbivorous fishes like *Tilapia* Sp. feed on aquatic macrophytes. Dried sedge/Chinese water chestnut (*Eleocharis ochrostachys*) leaf meal has been used as feed in the form of pellets for Nile tilapia.

#### **As food for man**

Humans have also utilized aquatic plants as a food source. Cattails (*Typha latifolia*) possess edible shoots and roots and even the pollen has been used in making biscuits. Arrowheads (*Sagittaria* Sp.) form large edible tubers at the root ends, called duck potatoes, which were consumed by Native Americans. Watercress (*Rorippa nasturtium-aquaticum*) has many historic medicinal uses and its spicy vegetation continues to be used in salads and garnishes. Water lily roots are a common source of food in many parts of the world and have historic medicinal value. Even coontail (*Ceratophyllum demersum*) has been used for medicinal purposes. In Asia, macro-algae has served as a vegetable since ancient times (Burtin, 2003). Besides nutritional benefits, macro-algae are used for their antibiotic, antiviral, antifouling, anti-inflammatory, cytotoxic, and

antimitotic activities; some of which have been pursued in pharmaceutical industries (Chen and Jiang, 2001).

### **As feed for animals**

Aquatic plants, both marine and freshwater, are used extensively worldwide as livestock fodder. In a review by Little (1987) on the utilization of aquatic plants, it was reported that many aquatic plants contain as much or more crude protein, crude fat and mineral matter as many conventional forage crops on a dry weight basis. Aquatic macrophytes also provide important food for many animals. Ducks and geese eat the seeds, leafy parts, and tubers of plants such as pondweeds (*Potamogeton* sp.), watershield (*Brasenia schreberi*), arrowhead (*Sagittaria latifolia*), water pepper (*Polygonum* sp.), and duckweed (*Lemna* sp.). Songbirds use fluff from cattails (*Typha* sp.) as nest material and eat the seeds of many emergent plants.

### **Macrophytes improve the quality of water**

Nutrient accumulation in an aquatic ecosystem leads to eutrophication resulting in massive growth of the macrophytes and weeds. Accumulation of nutrients is attributed to rapid urbanization, anthropogenic pressure as well as death of aquatic plants. Storm water runoff and the discharge of sewage into the lakes are two common ways that various nutrients enter the aquatic ecosystem, which leads to the death of those systems (Ahmad *et al*, 2014). Eutrophication indicates aging of a water body. It is caused by the accumulation of nutrients, sediments, silt and organic matter in the lake from the surrounding watershed. Aquatic macrophytes play important roles in balancing the ecosystem of lakes and other freshwater

bodies. They have the capacity to improve the water quality by absorbing nutrients with their effective root system. Macrophytic vegetation plays an important role in maintaining the ecosystem of a lake. Self-purification of wetlands can be largely attributed to the presence of aquatic macrophytes. This ability of macrophytes to absorb nutrient in large quantities is often applied in the treatment of water bodies (Dhote, 2007).

## **1.2 WATER HYACINTH (*EICHHORNIA CRASSIPES* Mart.)**

Water hyacinth (*Eichhornia crassipes* Mart.) is a free floating perennial aquatic plant that is native to the tropical and sub-tropical regions of South America. It is made up of broad, thick, glossy, ovate leaves. It can rise above the surface of the water as much as 1 meter in height. The leaves are 10 – 20 cm across on a stem which is floating by means of buoyant bulb-like nodules at its base above the water surface (Hussner, 2012).

### **1.2.1 Scientific classification of *E. crassipes***

Kingdom:                      Plantae

Clade: Tracheophyta  
Clade: Angiosperms  
Clade: Monocots  
Clade: Commelinids  
Order: Commelinales  
Family: Ponderiaceae  
Genus: *Eichhornia*  
Species: *E. crassipes* Mart.

Source: GISD (2021).



**Plate 1:** Water hyacinth (*Eichhornia crassipes*)

**Source:** Sullivan and Wood (2012)

## **1.2.2 Botanical Description of *E. crassipes***

### **Leaves**

It is made up of broad, thick, glossy, ovate leaves. It can rise above the surface of the water as much as 1 meter in height. The leaves are 10 – 20 cm across on a stem which is floating by means of buoyant bulb-like nodules at its base above the water surface. They have long, spongy and bulbous stalks.

### **Flowers**

The flowers are supported by a single spike of 8 – 15 conspicuously attractive flowers, mostly lavender to pink in colour with six (6) petals. When not in bloom, water hyacinth may be mistaken for frog's-bit (*Limnobiium spongia*) or the Amazon frogbit (*Limnobiium laevigatum*). In their native range, the flowers are pollinated by long-tongued bees and can reproduce both sexually and clonally. Water hyacinth has three (3) flower morphs and is termed “tristylous”. The flower morphs are named for the short length of their pistil: long, medium and short (Barrett, 1977).

### **Reproduction**

*E. crassipes* is one the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. Each plant additionally can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years (Sullivan and Wood, 2012).

## **Habitat and Ecology**

Its habitat ranges from tropical desert to subtropical or warm temperate desert rain forest zones. It grows in a wide variety of aquatic habitats; it appears to be a weed that grows on stagnant water or areas of minimal flow of water. It mostly occurs in lakes and rivers. It can survive terrestrial conditions by anchoring to the hydro-soil for a few weeks when the water level recedes. The temperature tolerance of the water hyacinth is the following: its minimum growth temperature is 12 °C; its optimum growth temperature is 25 – 30 °C; its maximum temperature range is between 33 – 35 °C, and its pH tolerance is estimated at 5.0 – 7.5. The leaves of water hyacinth are killed by frost and do not tolerate temperatures above 34 °C, they do not grow where the water salinity is greater than 15%. In brackish water, its leaves show epinasty and chlorosis and eventually die (Sullivan and Wood, 2012).

## **Invasiveness**

Water hyacinth breeds quickly, is easy to float and spread, and can quickly cover the water body, resulting in poor water transparency. Therefore in natural waters, water hyacinth competes with other aquatic (floating and submerged) plants and algae for mineral nutrition, sunlight, etc. resources, thereby inhibiting the growth of other aquatic and algal organisms. In 2011, Li *et al.* tracked the results of Yunnan Dianchi Lake and also showed that water hyacinth could affect the photosynthesis of phytoplankton, submerged plants and algae by altering water environment quality and inhibit its growth. The invasion of water hyacinth can have socioeconomic

consequences. Since the plant is comprised of 95% water, the evapotranspiration rate is high. As such small lakes that have been covered by with the species can dry out leaving communities that depend on such lakes without adequate water and food. In addition, the plant could impede transportation of both humans and cargo by proliferating on the surface of water ways. The dense mats of water hyacinth formed on water surface poses a problem for fishermen and often destroy their fishing gear; the surface of this plant is often a breeding place for mosquitoes and harmful pathogens, posing a potential threat to the health of local residents (Kong *et al.*, 2011; Xia and Ma, 2006)

## **Control**

Control of water hyacinth depends on the specific conditions of each affected location such as extent of water hyacinth infestation, regional climate, and proximity to human and wildlife. The three commonly used methods of control are:

- Physical control
- Biological control and
- Chemical control

### **Physical control**

Physical control is performed by land-based machines such as bucket cranes, draglines, or bloom or by water based machinery such as aquatic weed harvesters, dredges, or vegetation shredder. The manual or physical method of harvesting is one of the most species-specific control methods available, provided the weed grows in monocultures (Hussner *et al.*, 2017), but is limited to small infestations, as *E. crassipes* forms dense stands with biomass accumulation of up to 100

tons per ha (Gettys *et al.*, 2014). It can cost between 6 – 20 million dollars annually to physically eradicate water hyacinth and this is considered a short term solution to a long term problem because the machinery usually leaves fragments of the plant on the water and these fragments can easily reproduce asexually and cause another infestation (Malik, 2007).

### **Biological control**

Biological control involves the use of natural enemies from their original ecosystem as prime target (De Groote *et al.*, 2003). As chemical and mechanical removal is often too expensive, polluting, and ineffective, researchers have turned to biological control agents to deal with water hyacinth. There are a number of phytophagous insects which were reported to be feeding on *E. crassipes*, 11 of which were assumed as monophagous (Neuenschwander *et al.*, 2009). Out of these, *Megamelus scutellaris* is one of the most important biological control agents, which has been successfully used in Africa, Australia and the U.S (Coetzee *et al.*, 2011). These control agents regulate water hyacinth by limiting its size, its vegetative propagation, and seed production. They also carry microorganisms that can be pathological to the plant. The insects eat the stem tissue, which results in a loss of buoyancy for the plant, which will eventually sink (Jimenez, 2014).

### **Chemical control**

This method involves the use of herbicides. It is the least used method because of its impacts on the environment and the health of humans. Herbicides, in general terms, can be used to control aquatic plants in various types of water bodies, including lakes, channels, irrigation systems, and

ponds (De Winton *et al.*, 2013; Gettys *et al.*, 2014; Hussner *et al.*, 2017). Some herbicides used for the control of *P. stratiotes*, include glyphosate and diquat (Martins *et al.*, 2002). However, the most successful use of herbicide is when it is used for smaller areas of infestation of water hyacinth. This is because in larger areas, more mats of water hyacinth are likely to survive the herbicide and can fragment to further propagate a large area of water hyacinth mats. In addition, it is more cost-effective and less labourious than mechanical control, yet, it can lead to environmental effects as it can penetrate into the ground water system and can affect not only the hydrological cycle within an ecosystem but also negatively affect the local water system and human health. Another important thing to note is that the use of non-specific herbicides can also kill useful aquatic organisms and microalgae and this can disrupt fragile food webs.

### **1.3 AIM AND OBJECTIVES OF RESEARCH**

The study was carried out with the aim of assessing the effects of diesel on the growth and morphology of *Eichhornia crassipes*. The project was done with the following objectives:

- To ascertain the concentration and duration of diesel that is lethal to the survival of *Eichhornia crassipes*.
- To determine the extent of tolerance of *Eichhornia crassipes* when exposed to diesel pollution.
- To determine the shoot to root ratio of heavy metal accumulation in *Eichhornia crassipes*.

## **CHAPTER TWO: MATERIALS AND METHODS**

### **2.1 STUDY SITE**

The study was carried out at the screen house of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

### **2.2 COLLECTION OF PLANTS**

The plant used in the study was *Eichhornia crassipes*. Fresh samples of the plant were collected from Ologbo pond, Edo State, Nigeria. The plants were collected with care to avoid damage to the roots. The plants were placed in a plastic bucket and the roots were covered with water from the pond in order to avoid dehydration before getting to the screen house. The test plants were identified at the Department of Plant Biology and Biotechnology, University of Benin.

### **2.3 SOURCE OF BOWLS**

The bowls used to contain the plants during the course of the experiment were purchased at the plastic market along Mission Road, Benin City, Edo State.

### **2.4 SOURCE OF DIESEL**

Diesel was purchased at the Faculty of Agriculture Cooperative gas station, University of Benin, Benin city.

## **2.5 EXPERIMENTAL SET UP**

The test plants were thoroughly rinsed with tap water to wash off any particles on the leaves and roots. They were then treated with different amounts of 0%, 2%, 4%, 6% and 8% (v/v) of diesel oil. The bowls were categorized into five places as listed below:

- 0%v/v containing 1000ml deionized water used as control.
- 2%v/v containing 995% deionized water and 5ml diesel.
- 4%v/v containing 990ml deionized water and 10% diesel.
- 6%v/v containing 985ml deionized water and 15% diesel.
- 8%v/v containing 980ml deionized water and 20% diesel.

Each category had three replicates and was labeled accordingly. The experimental set up spanned eight (8) days and readings were taken every day. The materials used during the experiment were placed under the same environmental conditions in order to ensure data accuracy.

## **2.6 RECORDING OF DATA**

During the experiment, morphological changes in the plants were observed. Data were also recorded to ascertain other changes in the plant morphology. They include:

### **2.6.1 Measurement of the plant height**

The heights of the plants were measured using a meter rule. This procedure was carried out before (day 0) and after treatment (day 8).

### **2.6.2 Measurement of the root length**

The length of the root was measured using a meter rule. This procedure was carried out before (day 0) and after treatment (day 8).

### **2.6.3 Measurement of the stolon girth**

The girth of the stolon was measured using a digital vernier caliper. This procedure was carried out before (day 0) and after treatment (day 8).

### **2.6.4 Measurement of the leaf diameter**

The diameter of the plant leaves were measured with a meter rule. This procedure was carried out daily.

### **2.6.5 Measurement of stolon length**

The length of the stolon was measured using a meter rule. The procedure was done before (day 0) and after treatment (day 8).

### **2.6.6 Determination of the chlorophyll content index**

The chlorophyll content index of the plant was taken with the Apogee™ chlorophyll meter. The chlorophyll content was taken by holding the leaves directly under the arm of the device until it made a beep. The average reading of three leaves per plant was taken as the chlorophyll content index.

### **2.6.7 Determination of the fresh and dry weight**

The fresh and dry weights of the plants were determined after eight (8) days of treatment. All observations were recorded on the eight (8) day. The plants were carefully packaged in newspapers and labeled accordingly, after which they were weighed individually. The fresh weight was then obtained by making use of an electronic sensitive weighing balance. The dry weight of the plants were obtained by packaging the plants in newspapers and sun drying for about 5 days, after which the dry weight was measured on an electronic sensitive weighing balance.

### **2.6.8 Phytochemical analysis of plants**

Heavy metals: plant parts were air dried for seven (7) days. They were thereafter oven dried at 60<sup>0</sup>c temperature to a constant weight before grinding to powder. One gram of the ground powder was then weighed into a 250ml conical flask. Ten millimeters of mixture of perchloric acid, nitric acid and sulphuric acid in the ration 1:2:2 was added to the flask containing the ground powder and placed in the fume chamber. The mixture was heated for twenty minutes on a hot plate until white fumes were noticed. The digestion was stopped and the mixture cooled, before 20ml of distilled water was added and boiled gently to bring the metals into solution. The solution was aspirated and analyzed with atomic absorption spectrophotometer (AAS). (APHA, 1998).

## **2.7 STATISTICAL ANALYSIS**

The results are represented as the means  $\pm$  S.E. of three independent replicates for each treatment.

The data obtained were subjected to statistical analysis using Statistical Package for Social Science (SPSS) version 20.0. Analysis of variance (ANOVA) was performed appropriate to the experimental design used. Duncan Multiple Range Test was the post-hoc procedure used.



**Plate 2:** Experimental setup with *Eichhornia crassipes* in bowls filled with various concentrations of diesel



**Plate 3:** Measurement of leaf diameter



**Plate 4:** Determination of chlorophyll content



**Plate 5:** Measurement of fresh weight



**Plate 6:** Measurement of fresh weight



**Plate 7:** Determination of dry weight of stem



**Plate 8:** Determination of dry weight of roots

### CHAPTER THREE: RESULTS

The effect of diesel on the leaf diameter of *Eichhornia crassipes* is represented in Table 2. There was significant difference between all treatments. For 8% (v/v), at day 6 and beyond, there was notable shrinkage and even falling off of leaves with diameter of  $0.00\pm 0.00$  cm (dead leaves). Across all treatments, there was observable decrease in leaf diameter of *E. crassipes*. Among the plants subjected to diesel treatment, the leaves of *E. crassipes* at treatment 2% v/v had the least reduction with leaf diameter as  $4.70\pm 2.40$  cm. *E. crassipes* with control treatment 0% grew normally and no shrinkage or leaf discoloration was observed.

**Table 2:** Effects of diesel on the leaf diameter of *Eichhornia crassipes*

Treatment	Days after treatment								
	0	1	2	3	4	5	6	7	8
0	9.17±0.49 <sup>c</sup>	8.90±0.30 <sup>c</sup>	9.00±0.35 <sup>c</sup>	9.10±0.31 <sup>b</sup>	9.30±0.42 <sup>b</sup>	9.37±0.35 <sup>b</sup>	9.33±0.33 <sup>c</sup>	9.30±0.30 <sup>c</sup>	9.30±0.30 <sup>b</sup>
2	7.47±0.53 <sup>b</sup>	7.23±0.50 <sup>b</sup>	7.53±0.57 <sup>b</sup>	7.20±0.42 <sup>a</sup>	7.37±0.60 <sup>ab</sup>	7.33±0.57 <sup>b</sup>	7.43±0.57 <sup>bc</sup>	7.88±0.18 <sup>bc</sup>	4.70±2.40 <sup>ab</sup>
4	6.67±0.42 <sup>ab</sup>	6.63±0.38 <sup>ab</sup>	6.63±0.34 <sup>ab</sup>	6.77±0.27 <sup>a</sup>	4.57±2.31 <sup>ab</sup>	4.50±2.27 <sup>ab</sup>	4.43±2.23 <sup>ab</sup>	4.40±2.21 <sup>ab</sup>	4.47±2.24 <sup>ab</sup>
6	6.13±0.48 <sup>ab</sup>	6.07±0.47 <sup>ab</sup>	6.40±0.58 <sup>ab</sup>	6.80±0.70 <sup>a</sup>	4.53±2.27 <sup>ab</sup>	4.37±2.25 <sup>ab</sup>	4.40±2.21 <sup>ab</sup>	2.03±2.03 <sup>a</sup>	2.03±2.02 <sup>a</sup>
8	5.57±0.53 <sup>c</sup>	5.50±0.55 <sup>a</sup>	5.90±0.36 <sup>a</sup>	5.80±0.42 <sup>a</sup>	3.23±1.63 <sup>a</sup>	1.67±1.67 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
	*	*	*	*	*	*	*	*	*

**Key:** N.S = Non significant (P > 0.05)      \* = Significant (P < 0.05)

The values are means and standard errors of 3 replicates. Means in the column having same superscript alphabet are not significantly different from one another at P > 0.05. Means in the column sharing different superscript alphabets are significantly different from each other at P < 0.05.

The effects of diesel on the stem girth of *Eichhornia crassipes* is shown in Table 3. There was significant difference between the stem girth before and after treatment with diesel. Except the control, all treatments showed a reduction in stem girth after treatment with diesel. Among all samples exposed to diesel, 2% v/v showed little or reduction ( $3.59 \pm 0.45$  cm) while 8% v/v showed the greatest reduction in stem girth ( $3.04 \pm 0.60$ ).

**Table 3:** Effects of diesel on the stem girth of *Eichhonia crassipes*

Treatment % (v/v)	Before treatment (cm)	After treatment (cm)
<b>0</b>	4.53±0.74 <sup>ab</sup>	5.90±0.49 <sup>c</sup>
<b>2</b>	3.83±0.60 <sup>a</sup>	3.59±0.45 <sup>a</sup>
<b>4</b>	4.87±0.15 <sup>ab</sup>	2.33±0.33 <sup>a</sup>
<b>6</b>	6.07±0.29 <sup>c</sup>	3.63±0.40 <sup>a</sup>
<b>8</b>	4.57±0.78 <sup>ab</sup>	3.04±0.60 <sup>a</sup>
	*	*

**Key:** N.S = Non significant (P > 0.05)      \* = Significant (P < 0.05)

The values are means and standard errors of 3 replicates. Means in the column having same superscript alphabet are not significantly different from each other at P > 0.05. Means in the column sharing different superscript alphabets are significantly different from each other at P < 0.05.

The data displayed on Table 4 shows the effect of diesel on the stem length of *E. crassipes*. There was no significant difference in the stolon length. At day 0, 2% (v/v) had the highest stolon length as  $11.57 \pm 2.02$  cm, while 8% (v/v) had the least stolon length as  $11.33 \pm 0.73$  cm. After the experiment, the stem length of the plants subjected to diesel treatment showed a considerable reduction in the length of the stem. The stem of the control plants showed a considerable increase in length as  $12.33 \pm 2.34$  cm, while the 8% (v/v) had the least stem length as  $8.55 \pm 1.73$  cm.

**Table 4:** Effects of diesel on the stem length of *Eichhornia crassipes*

Treatment % (v/v)	Before treatment (cm)	After treatment (cm)
<b>0</b>	10.97±2.07 <sup>a</sup>	12.33±2.34 <sup>a</sup>
<b>2</b>	11.57±2.02 <sup>a</sup>	9.80±2.10 <sup>a</sup>
<b>4</b>	9.13±0.58 <sup>a</sup>	7.06±0.78 <sup>a</sup>
<b>6</b>	9.50±0.87 <sup>a</sup>	7.22±0.28 <sup>a</sup>
<b>8</b>	11.33±0.73 <sup>a</sup>	8.55±1.73 <sup>a</sup>
	<b>N.S</b>	<b>N.S</b>

**Key:** N.S = Non significant (P > 0.05)      \* = Significant (P < 0.05)

The values are means and standard errors of 3 replicates. Means in the column having same superscript alphabet are not significantly different at P > 0.05. The means in the column sharing different superscript alphabets are significantly different from each other at P < 0.05.

The data represented in Figure 1 indicates the effects of diesel on the number of leaves of *Eichhornia crassipes* before and after being subjected to diesel treatment. There was significant difference between the treatments before and after treating with diesel. After treatment with diesel, there was a progressive reduction in number of leaves of *E. crassipes*, with the control having the highest value ( $11.00 \pm 0.58$  cm) and 8% v/v having the least value ( $6.33 \pm 0.33$  cm).

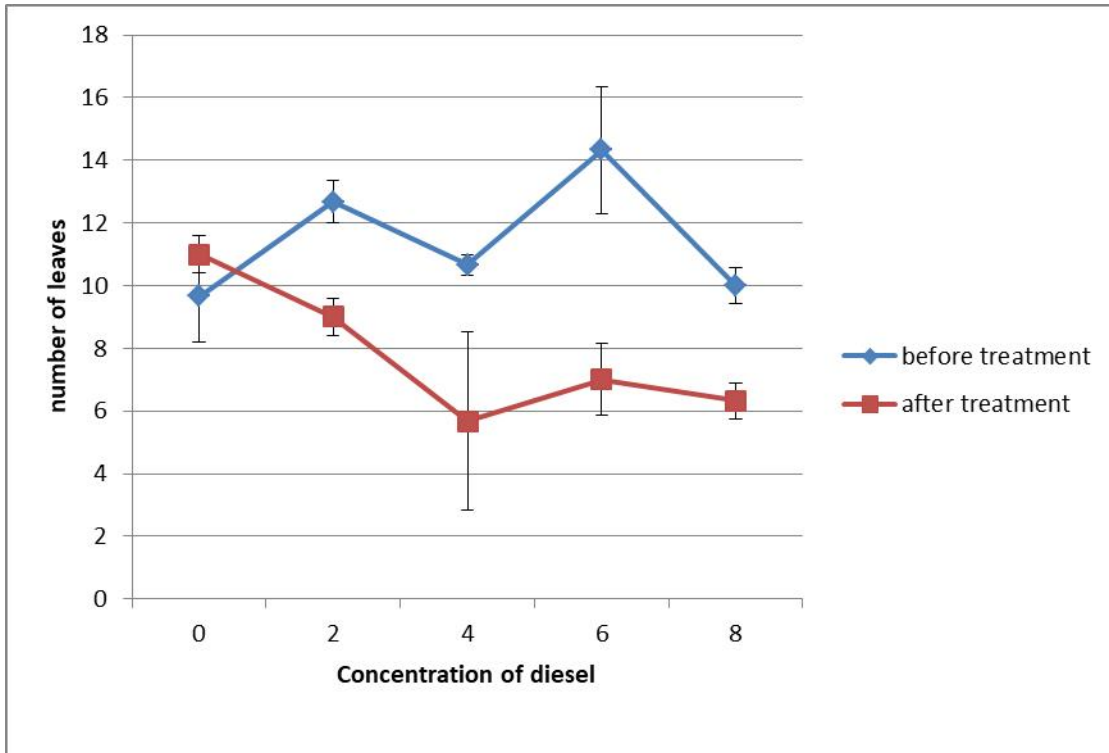


Figure 1: Line graph showing the effect of diesel on the number of leaves of *Eichhornia crassipes*.

The chlorophyll content index of *Eichhornia crassipes* before and after being treated with diesel is shown in Figure 2. There was no significant difference in the treatments before and after subjection to diesel. There was observable decrease in the chlorophyll content index of *E.crassipes* after treatment with diesel with the control having the highest value ( $36.33\pm 4.01$ ) and 8% v/v having the least ( $5.40\pm 3.52$ ).

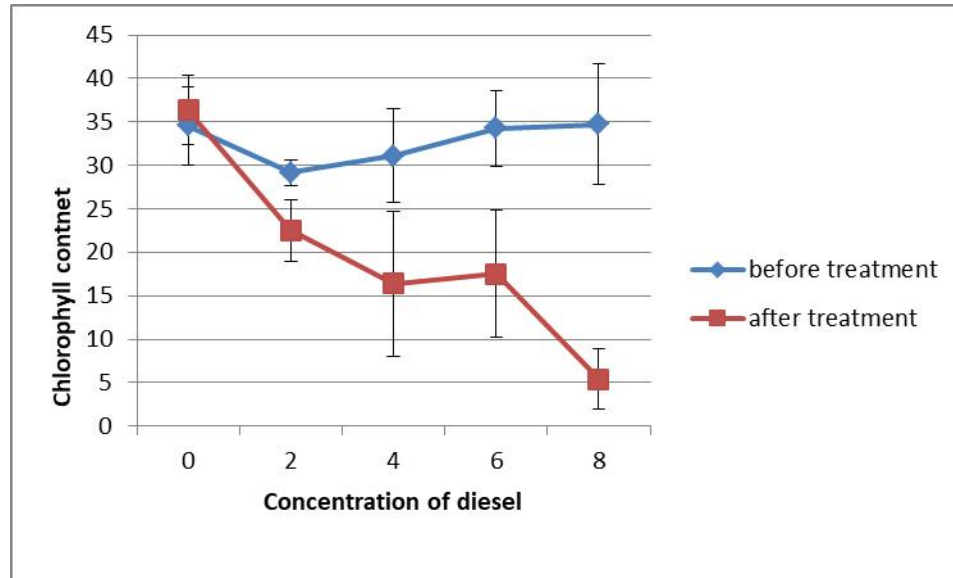
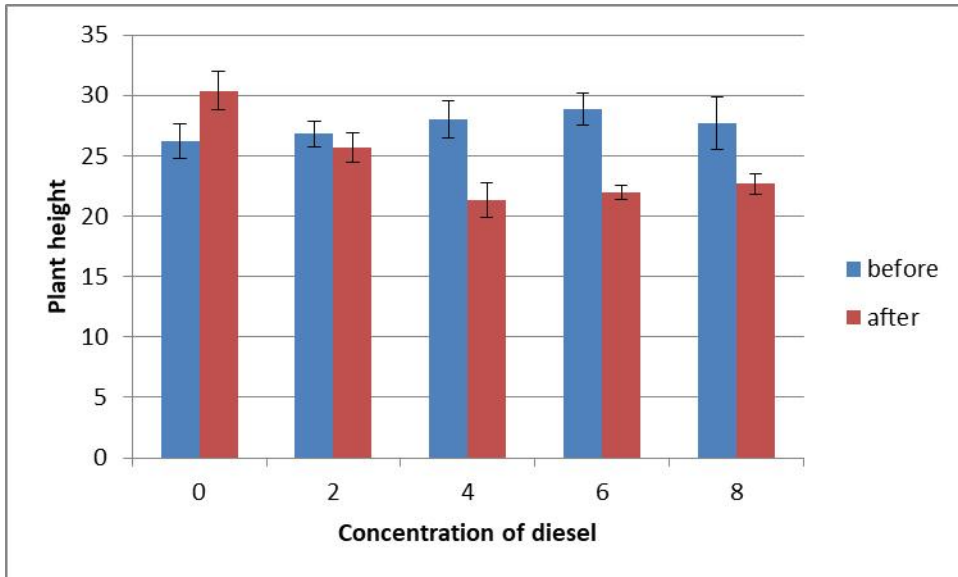


Figure 2: A line graph showing the effects of diesel on the chlorophyll content of *Eichhornia crassipes*.

Figure 3 is a representation of the effect of diesel on the height of *Eichhornia crassipes*. There was significant difference in the samples after treatment with diesel. Conspicuous stunting was observed from 4% v/v ( $21.33 \pm 1.45$  cm) through to 8% v/v ( $22.67 \pm 0.88$  cm). The control showed an increase in height after treatment with diesel and the least value was observed in 4% v/v.



**Figure 3: A bar chart showing the effects of diesel on the height of *Eichhornia crassipes*.**

The root length of *Eichhornia crassipes* before and after being treated with diesel is shown in Figure 4. There was no significant difference in the treatments before and after subjection to diesel. There was little or difference in the root length of *E.crassipes* after treatment with diesel with the control having the highest value ( $33.67\pm 10.17$ ) and 8% v/v having the least ( $26.33\pm 2.24$ ).

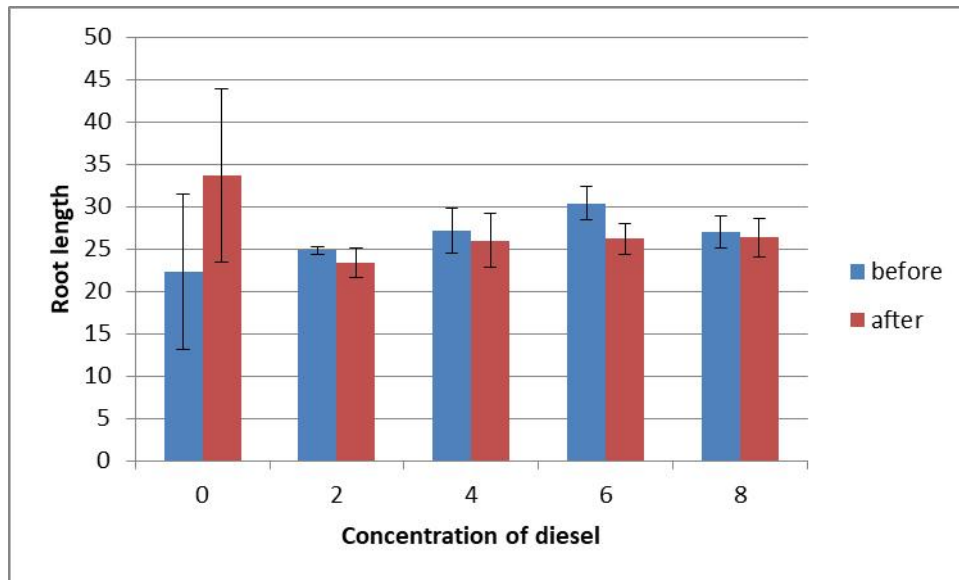
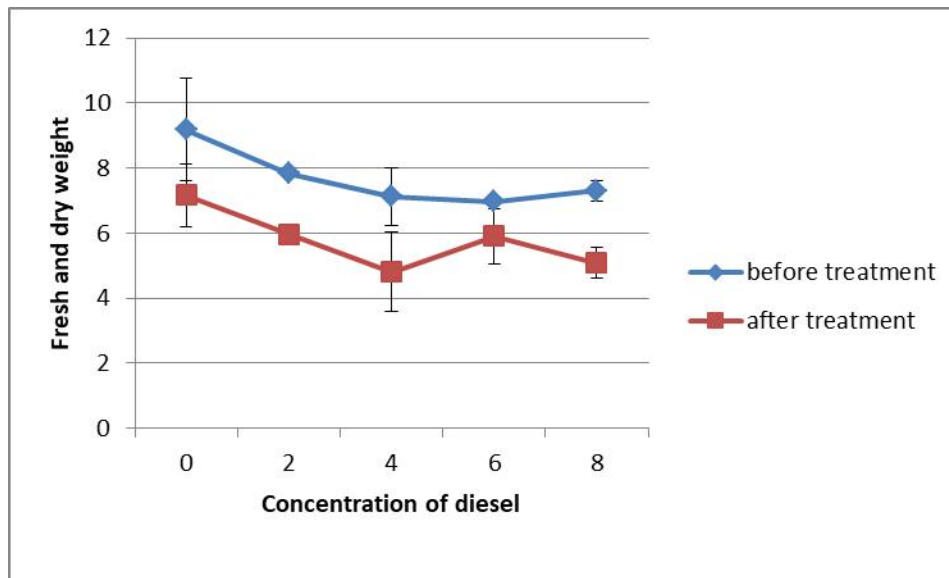


Figure 4: A bar chart showing the effects of diesel on the root length of *Eichhornia crassipes*.

The data displayed on Figure 5 indicates the fresh and dry weight of *P. stratiotes* after being subjected to treatment with diesel. The highest fresh weight was observed in the control as  $9.18 \pm 1.58$  grams, while the lowest was recorded in the 8% (v/v) treatment as  $7.30 \pm 0.30$  grams. The dry weight was observed to be highest at control  $7.16 \pm 0.98$  grams and was least at 8% (v/v) as  $5.08 \pm 0.48$  grams.



**Figure 5:** A line graph showing the effects of diesel on the fresh and dry weight of *Eichhornia crassipes*.

## CHAPTER FOUR: DISCUSSION

The introduction of diesel into aquatic ecosystems causes physical, chemical and biological changes in the environment. This study demonstrated that the growth of *Eichhornia crassipes* is retrogressively affected by diesel, and the effect is dependent on the concentration of diesel in environment. All physical growth parameters measured such as root length, leaf diameter, plant height decreased during growth in the presence of diesel. Eguagie and Orji (2015) also reported a significant decrease in the leaf diameter, chlorophyll content and fresh and dry weight of *Eichhornia crassipes* after being subjected to gasoline and diesel treatments for 15 days. The decrease was shown to be dependent on the concentration of diesel and there was no recovery of growth during the period in the plants treated with diesel (Akapo *et al*, 2011). Upon exposure to different concentration of diesel (2%, 4%, 6% and 8%), the leaves, stem and roots of *E. crassipes* were reduced considerably both in length and girth as opposed to the control. At the end of the experiment, there was a remarkable change in the chlorophyll content of the test plants. The control plants showed a slight increase in chlorophyll content as  $34.53 \pm 4.47$  before the experiment and  $36.33 \pm 4.01$  after the experiment. The chlorophyll content in the various treatments (2%, 4%, 6% and 8%) kept declining and was lowest in the 8% treatment as  $31.73 \pm 6.95$  before treatment and  $5.40 \pm 3.52$  after treatment. This is similar to the report of Bamidele *et al*, (2016). They reported wilting and reduced chlorophyll content and leaf area in the leaves of *S. Africana* exposed to diesel and gasoline treatments.

The leaf diameter was highly affected by the diesel as shown in Table 8. The leaf diameter of treatment 8 at day 0 was  $5.57 \pm 0.53$ , but it remained stable until day 4 where there was a sharp

decrease in leaf diameter; and at day 8, the leaves had died off and were recorded as  $0.00\pm 0.00$ . A steady reduction was also observed in the plant height as  $27.70\pm 2.15$  for treatment 8 at the start of the experiment, but decreased in size to  $22.67\pm 0.88$  at day 13. Ogboghodo *et al.* (2004) also observed that the dose of crude oil from 10 ppm was inhibitory to the growth of *E. crassipes*. This result on plant height also agrees with previous findings of Ikhajagbe and Anoliefo (2011) on the significant decline in the growth of plants caused by oil pollution. The report of Amadi *et al.* (1996) explains that immediately after an oil spill, there is usually a horizontal migrating of oil into soil horizons. Oily scum on surface of the soil would impede oxygen and water. It may also cause some toxic elements to be more available to plants thereby causing reduction in plant growth. Therefore the general depression in growth is due to the adverse effect of diesel. At the end of the experiment, it was observed that the roots of the plants treated with diesel showed little difference before and after treatment. From Figure 4, the root length recorded for 8% concentration before treatment was  $27.00\pm 1.89$ , but after treatment, the root length was  $26.33\pm 2.24$ . However, the control increased in size, with the root length before treatment as  $22.23\pm 9.18$  and  $33.67\pm 10.17$  after treatment. Akapo *et al.* (2011) reported deformity in the cellular anatomy and root tip structure in *P. stratiotes* when exposed to crude oil which may disturb the uptake of water and nutrients.

After some days of being exposed to diesel treatment, it was discovered that there was a reduction in the number of leaves in the plants treated with 2%, 4%, 6%, and 8%. This is not contradictory to the report of Lopes and Piedade (2009) who observed that in *E. crassipes* treated with petroleum, the leaves decreased slightly. Osuagwu *et al.*, (2013) attributed this decrease in the number of leaves to a host of factors including blockage of conducting tissues

which leads to prevention of water and nutrients from getting into the plants and limiting their ability to produce more leaves. The characteristics of the leaves of *E. crassipes* allow it to absorb oil through the surface of the leaves. The presence of oil on the surface of the leaves impedes gaseous exchange, and this affects photosynthesis and plant growth (Pezeshki and DeLaune, 1993). Oil can also alter cell-membrane permeability, thus endangering plant metabolic processes (DeLaune et al., 2003). Okoloko and Berley (1982) also reported that oil pollution impaired membrane integrity, enzymes system especially membrane bound enzymes and affects the metabolic system of the plant.

After a few days of exposure to diesel, chlorosis was observed in the plants treated with 2%, 4% and 6% (v/v) diesel; wilting and necrosis was observed in the plants treated with 8% (v/v) diesel. This is similar to the report of Edema *et al* (2007), who observed the presence of chlorotic and necrotic leaves in *E. crassipes* when exposed to Water Soluble Fraction (WSF) of Ogini well-head crude oil. It also complies with the work of Udo and Fayemi (1975) who showed that plants growing in soils polluted with oil were generally retarded and showed chlorosis of leaves. Silva and Camargo (2007) revealed that *Pistia stratiotes* is extremely sensitive to Urucu petroleum at relatively low doses (0.1, 0.2 and 0.3 L.m<sup>-2</sup> concentrations) and caused death in plants. This observation is also in compliance with that of Lopes and Piedade (2009) who reported that higher concentrations of crude oil from Urucu caused a high mortality to *E. crassipes* and *E. polystachya*. It also agrees with the work of Akapo *et al*, (2011) who reported that at all concentrations, crude oil was toxic to *P. stratiotes*. According to this study, the bioaccumulation of heavy metals from diesel was toxic to the plants, and this led to the reduction in growth parameters of the plants.

## CONCLUSION

Fresh water plants perform a lot of crucial roles in the ecosystem, including phytoremediation of minute amounts of oil and being used as bioindicators for oil pollution. The result of this study shows that diesel is detrimental to the survival of *E. crassipes* and the effects were dependent on the concentration of the diesel. *E. crassipes* is a very sensitive species, because its leaves inevitably come into contact with diesel, and are not resistant to high concentrations of diesel. Therefore, adequate measures should be put in place to avoid pollution of the environment with diesel. It is the responsibility of oil companies to ensure that adequate measures are put in place to reduce the incidences of oil spillage by engaging in exploration and drilling best practices as well as better transportation channels.

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