

Bioremediating Effect of *Glomus Hoi* and *Pseudomonas Aeruginosa* on the Organic Content and Heavy Metals of Soil Polluted with Oil Refinery Effluent using *Amaranthus Cruentus* as a Test Plant

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Abstract— This study analyzed the degrading effect of *Glomus hoi* and *Pseudomonas aeruginosa* on the organic content and heavy metals of oil refinery effluent polluted soil using *Amaranthus cruentus* as the test plant. This study was carried out to determine if agricultural activities can be improved using any or both of the microorganisms.

Eight different treatment layouts were used with three replicates for each level of pollution in the treatment layout. Ninety six (96) pots, each containing three kilograms of soil from both sterilized and unsterilized soil were used for the study. Fifty (50) grams of soil inoculum from propagated Arbuscular mycorrhiza was inoculated to a set of twenty four (24) experimental pots containing both sterilized and unsterilized soil before *A. cruentus* seedlings were transplanted to them. Another set of twenty four (24) pots containing both sterilized and unsterilized soil were injected with thirty (30) mL of *P. aeruginosa* inoculum solution before transplanting *A. cruentus* seedlings to them. The third set of twenty four (24) pots received dual inoculation of both fifty (50) grams of soil inoculum containing *G. hoi* and thirty (30) mL of *P. aeruginosa* inoculum solution before *A. cruentus* were transplanted to them. The residual twenty four (24) pots served as the control. Thereafter, pot preparation was arranged in the greenhouse in a randomized block design. The *A. cruentus* seedlings were raised in nursery for a period of two weeks before they were transplanted to the pots, seedlings were left for 3 days to overcome transplanting shock before contaminating the soil with refinery effluent at various concentrations of 0%, 2%, 4% and 6% v/w. The seedlings were allowed to grow for eight weeks before the termination of the experiment.

The pre planting analysis of soil showed that heavy metals analyses (zinc and iron) of sterilized soil had a lower concentration to the unsterilized. The soil pH ranged from 6.3 to 6.8.

It also revealed that organic matter and organic carbon content ranged from 0.8% to 1.3% and 0.4% to 1.7%. However, after the experiment, it was discovered in this study that treatments without any microorganism inoculation in sterilized and unsterilized soil had a higher level of % organic carbon and % organic matter content compared to the other treatments that were inoculated with one or two micro-organisms across all the levels of effluent concentration. Heavy metals of soil in all the soil samples were found to increase as the petrochemical effluent increased in concentration. The results obtained were analyzed using Duncan Multiple Range Test (DMRT) and other descriptive statistics.

This study opined that the combined use of *G. hoi* and *P. aeruginosa* was more effective in improving the organic content and the reduce heavy metals of oil refinery effluent polluted soil than when either is used singly.

Keywords— *Glomus hoi*, *Pseudomonas aeruginosa*, Refinery effluent, *Amaranthus cruentus*, Bioremediation.

I. INTRODUCTION

Pollution of the environment with petroleum substances containing many highly toxic compounds is extremely dangerous to plant and animal lives. Petroleum substances, for example, include aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs which pollutes the soil (Turrio-Baldassarri *et al.*, 2004). All over the world, scientists and environmentalists are faced with

the challenge of overcoming the detrimental effects of the contamination of soil, air and water. Large-scale crude oil spills on soil, leakages from pipelines, underground and surface fuel storage tanks, indiscriminate spills and careless disposal and mismanagement of waste and other petroleum by-products of the society, constitute the major sources of petroleum contamination in our environment. It has become a topic of interest and attracted increasing attention because of the carcinogenic, mutagenic and toxic effects. Various activities in crude oil exploration, exploitation, storage and transportation lead to spillage of oil to the environment (Nicolleti and Eglis, 1998). The spilled oil pollutes soils and the soils become less useful for agricultural activities with soil dependent organisms being adversely affected (Lundstedt, 2003). The effects of crude oil on the growth and performance of plants have been reported in many researches. These effects have been observed to occur due to the interference of the plant uptake of nutrients by crude oil and the unfavourable soil conditions due to pollution with crude oil (McGill and Rowell, 1977).

Bioremediation is a modern method in which the natural ability of microorganisms is employed for the reduction of the concentration and/or toxicity of various chemical substances, such as petroleum derivatives, aliphatic and aromatic hydrocarbons, industrial solvents, pesticides and metals (Korda, 1997). The speed and efficiency of bioremediation of a soil contaminated with petroleum and petroleum products depends on the number of hydrocarbon-degrading microorganisms in the soil (Chen *et al.*, 2006). Bacteria, algae, fungi are some of the microorganisms that can be used to degrade oil polluted soil.

Glomus hoi which is an arbuscular mycorrhiza fungus is used for the treatment of polluted soils (Chen *et al.*, 2007). Mycorrhiza is the symbiotic association between fungi and the roots of vascular plants. The plant supplies the fungi with carbohydrate, while the fungi (mycorrhizal fungi) extends the surface area of the plant's roots and thus, increases their ability to absorb more nutrients (especially phosphorus) and water from the soil. Edwards *et al.* (2006) noted that various bacteria produce surfactants such as *Pseudomonas aeruginosa* that aid in the biodegradation of fuels. The surfactant helps to decrease the surface tension and disperse the oil to allow maximum access to biodegrading microorganisms.

The interactions between the remediating organisms and the environment that leads to the process of bioremediation has not been well explored, hence this study, that sets out to investigate the degrading potentials of *Glomus hoi* and

Pseudomonas aeruginosa on the physicochemical properties of soil polluted with oil refinery effluent.

II. MATERIALS AND METHODS

Experimental site

This study was conducted in the screenhouse of Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife

Collection of Materials

The petrochemical effluent was obtained from Warri Refinery and Petrochemical Company, Ekpan, Delta State. Soil inoculum of *Glomus hoi* and a culture of *Pseudomonas aeruginosa* were collected from the Mycology unit of Department of Crop Protection and Production, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The test plant used for this study was *Amaranthus cruentus*, the seeds "cultivar variety NHAe-3" were obtained from National Horticulture Research Institute, Ibadan.

Propagation of Arbuscular Mycorrhiza

A sieved mixture of top soil and river sand in the ratio of 10 to 1 which was used for the propagation of arbuscular mycorrhiza was sterilized in the screenhouse using an autoclave; it was heated for 5 hours at 131°C and left to cool for 4 days. Three hundred grams of soil inoculum containing *Glomus hoi* was obtained from the Mycology unit, Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The inoculum was propagated using *Zea mays* cultivated variety IZEE-YPOP STRC5 for a period of four months. Chopped leaves of *Gliricidia sepium* were used every 2 weeks to mulch the soil throughout the four month period.

Determination and Estimation of Mycorrhizal Propagules in the Soil

The maize plants were removed after four months of propagation. The soil coupled with the root of the plant was air dried. The air dried soil was packed into sterile brown envelopes and taken to the laboratory for assessment. The population of arbuscular mycorrhizal spores in the soil inoculum collected was estimated using wet and sieving method. The soil (100 grams) was mixed with distilled water, stirred for two minutes and allowed to settle for 5 mins, the soil suspension was then poured into the sieve of various mesh sizes (45 and 53 micrometer) arranged in descending order. A stream of wash bottle was used to wash down the spores into a centrifuge tube. It was then centrifuged at 2000 rpm for 3 minutes and the supernatant was decanted from the tube, the sediment was suspended in 40% sucrose solution and centrifuged again at 2000 rpm for

1 minute. The supernatant which contained the spores was poured into a grid line plate.

Spores Counting

The counting of spores was done in 9cm diameter petri dishes with a grid line of 1cm per slide under a field dissecting microscope (mg. x 35).

Culture of *Pseudomonas aeruginosa*

A crude oil degrading strain of *P. aeruginosa* was isolated by preparing a bacterium culture of *P. aeruginosa* using nutrient agar in petri dishes and kept in the incubator for 48 hours at 37°C. This was followed by flooding it with sterile distilled water in order to harvest it. The inoculum was then added to a medium to which sterile crude oil acting as the sole source of carbon has been added and left at 37°C for 10 days. A pure colony of *P. aeruginosa* was isolated from this broth. The bacterium inoculum was prepared by streaking a single colony of *P. aeruginosa* on nutrient agar plate and then incubated at 37°C for 48 hours. Cells of *P. aeruginosa* was harvested from agar plates by flooding with sterile distilled water and standardized using a colorimeter to 10⁸ CFU/ml.

Preparation of Pot for the Experiment

Sterilized and unsterilized soil was used for this experiment, there were ninety six (96) experimental pots comprising of a set of forty eight (48) pots with sterilized soil and another set of forty eight (48) pots with unsterilized soil. Each pot contained 3 kg of soil.

Planting and Inoculation of Soil with Microorganisms

Fifty (50) grams of soil inoculum from the propagated Arbuscular mycorrhiza containing 150 spores was inoculated to a set of twenty four (24) experimental pots containing both sterilized and unsterilized soil before *A. cruentus* seedlings are transplanted to them. Another set of twenty four (24) pots containing both sterilized and unsterilized soil was injected with thirty (30) ml of *P. aeruginosa* inoculum solution before transplanting *A. cruentus* seedlings to them. The third set of twenty four (24) pots received dual inoculation of both fifty (50) grams of soil inoculum containing *G. hoi* and thirty (30) ml of *P. aeruginosa* inoculum solution before *A. cruentus* seedlings

are transplanted to them. The residual twenty four (24) pots served as the control. Thereafter, pot preparation was arranged in the screenhouse. Seedlings was left for a week to establish and overcome transplanting shock before contaminating the soil with petrochemical effluent at various concentrations of 0, 2, 4 and 6% v/w. Each treatment of the experiment was replicated three times and watered regularly to ensure adequate moisture.

Data Collection and Analyses

After the termination of experiment, %OC and %OM was analysed using soil test. Heavy metals (Zinc and Iron) of the soil were also analyzed both before and after the experiment using Atomic Absorption Spectrophotometer (AAS). Data were analyzed using appropriate descriptive and inferential statistics.

TREATMENT LAYOUT

Sterilized and unsterilized soils were polluted with petrochemical effluent at a calculated percentage using the formula; Percentage soil contamination = (Volume of effluent/Volume of soil) x 100.

The layout of the experiment is as follows;

Treatment 1- sterilized soil + effluent + *A. cruentus*

Treatment 2- sterilized soil + *Glomus hoi* + effluent + *A. cruentus*

Treatment 3- sterilized soil + *Pseudomonas aeruginosa* + effluent + *A. cruentus*

Treatment 4- sterilized soil + *Glomus hoi* + *P. aeruginosa* + effluent + *A. cruentus*

Treatment 5- unsterilized soil + effluent + *A. cruentus*

Treatment 6- unsterilized soil + *Glomus hoi* + effluent + *A. cruentus*

Treatment 7- unsterilized soil + *P. aeruginosa* + effluent + *A. cruentus*

Treatment 8- unsterilized soil + *Glomus hoi* + *P. aeruginosa* + effluent + *A. cruentus*

Each of the layouts contaminated at 0, 2, 4 and 6% (v/w) petrochemical effluent concentration was replicated thrice. The experimental pots were irrigated regularly to ensure adequate moisture for proper growth of the test plant.

III. RESULTS

Table.1: Physicochemical Properties of Sterilized and Unsterilized Soil before Planting

Parameters	Sterilised	Unsterilised
OC (%)	0.4	0.7
OM (%)	0.8	1.3
Zn (ppm)	81.75	97.46
Fe (ppm)	5.89	9.34

The physicochemical properties of sterilized and unsterilized soil before planting were found to show that heavy metals analyses (Zinc and Iron) of sterilized soil had a lower concentration compare to the unsterilized soil (Table 1). Organic matter and organic carbon percentages were also found to be lower in concentration in sterilized soil compared to the unsterilized soil. The textural class of the soil was loamy sand (Table 1).

Physico Chemical Properties of Soil after Planting

After the termination of experiment, the physicochemical properties of soil were analyzed to check for the change that occurred. % Organic carbon and organic matter content had the highest value at 6% effluent concentration in sample 1 and its lowest value was recorded at 0% effluent concentration in sample 7, the order of % organic carbon and organic matter content across the samples at 2% and 6% is $1 > 5 > 2 > 7 > 6 > 3 > 8 > 4$ and $1 > 5 > 2 > 6 > 3 > 4 > 7 > 8$ respectively (Fig. 1 and 2).

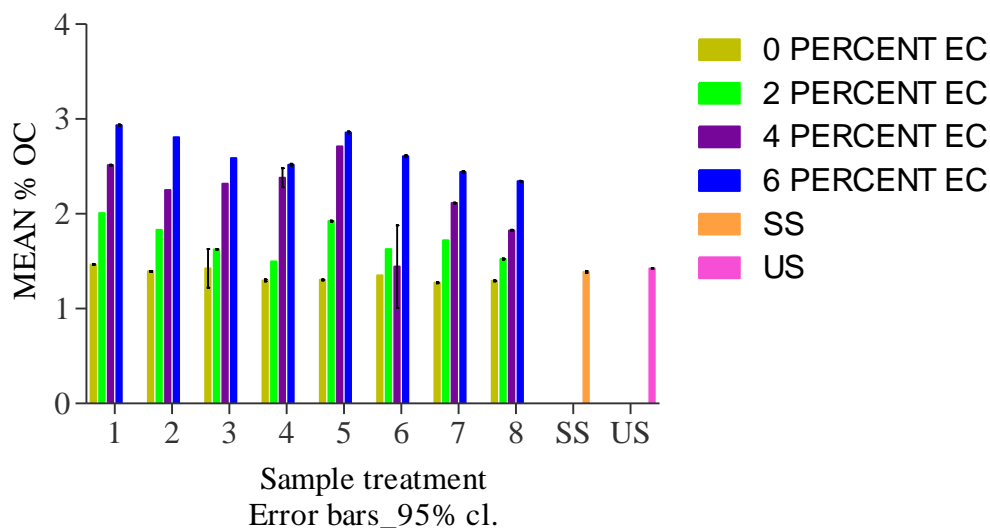


Fig.1: % Organic Carbon content of Pre and Post Planting Soil Samples

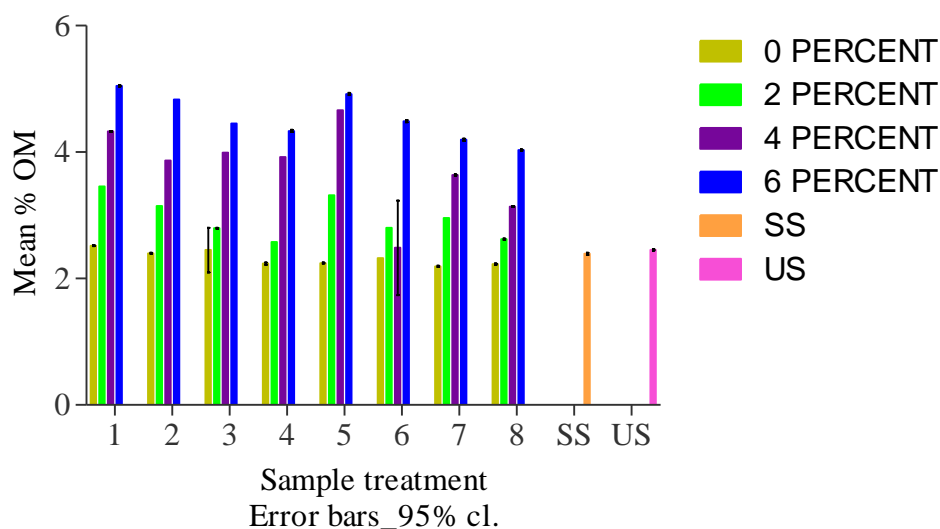


Fig.2: % Organic Matter content of Pre and Post Planting Soil Samples

Legend

- 1 - SS + TP
- 2 - SS + GH + TP
- 3 - SS + PA + TP

- 4 - SS + GH + PA + TP
- 5 - US + TP
- 6 - US + GH + TP
- 7 - US + PA + TP

8 - US + GH + PA + TP

SS - Sterilized Soil before Planting

US - Unsterilized Soil before Planting

GH - *G. hoi*PA - *P. aeruginosa*

TP - Test Plant

EC- Effluent Concentration

HEAVY METAL CONTENT OF SOIL AFTER PLANTING

Zinc analyses in the soil showed that the order of the concentration in 2% and 6% was $5 > 1 > 2 > 3 > 6 > 4 > 7 >$

8 and $5 > 1 > 2 > 3 > 4 > 6 > 7 > 8$, treatment 5 had the highest level of zinc concentration followed by treatment 1 both at 6% effluent concentration while treatment 8 had the lowest at 0% (Fig. 3). The heavy metal analyses of the soil were found to show that iron concentration had highest value in treatment 1 at 6% effluent concentration followed by treatment 5 at 6% while treatment 4 at 0% was the lowest. The order of iron concentration in 2% and 6% was treatment $1 > 5 > 2 > 3 > 6 > 7 > 4 > 8$ and $1 > 5 > 2 > 6 > 7 > 3 > 4 > 8$ respectively (Fig. 4).

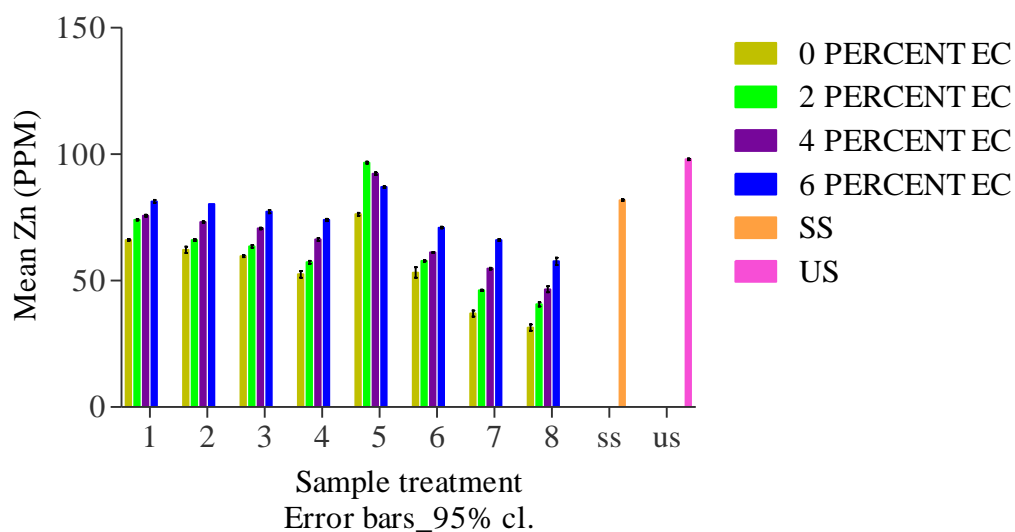


Fig.3: Zinc (PPM) Content of Pre and Post Planting Soil Samples

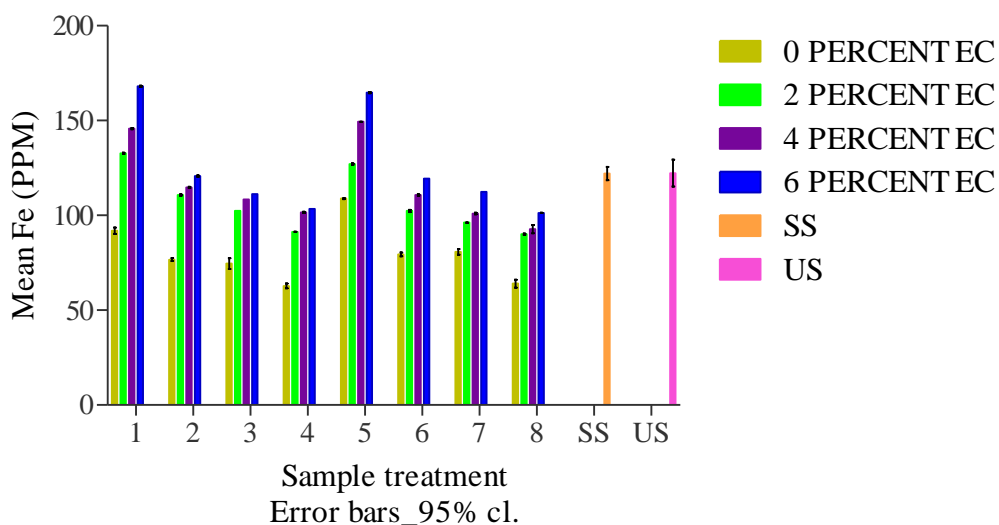


Fig.4: Iron (PPM) content of Pre and Post Planting Soil Samples

Legend

- 1 - SS + TP
- 2 - SS + GH + TP
- 3 - SS + PA + TP
- 4 - SS + GH + PA + TP
- 5 - US + TP
- 6 - US + GH + TP
- 7 - US + PA + TP
- 8 - US + GH + PA + TP

SS - Sterilized Soil before Planting

US - Unsterilized Soil before Planting

GH - *G. hoi*

PA - *P. aeruginosa*

TP - Test Plant

EC- Effluent Concentration

IV. DISCUSSIONS

In this study, % organic carbon and organic matter content were found to increase as petrochemical effluent concentration increased across all the treatments, this may be due to the addition of effluent which increased petroleum hydrocarbon content of the soil thereby resulting into high carbon in the polluted soil. This is in line with the findings of Nwazue (2011) which reported that PHC polluted soil had lower pH value, low moisture content and more organic carbon than the unpolluted soil. It was discovered in this study that treatments without any microorganism inoculation in sterilized and unsterilized soil had a higher level of % organic carbon and % organic matter content compared to the other treatments that were inoculated with one or two microorganisms across all the levels of effluent concentration. This can be as a result of *Glomus hoi* and *Pseudomonas aeruginosa* in the soil which had utilize the carbon in the soil for their growth and metabolism which is one of the reasons for their ability to degrade the effluent.

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity (Opaoluwa, 2010). They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20. Industrial effluents are usually considered as undesirable for arable soil, plants, animals and human health.

According to Gulfraz *et al.* (2003) some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn is required in minute quantities by organisms. However, excessive amounts of these elements can become harmful to organisms. Other heavy metals such as Pb, Cd, Hg, and As (a metalloid but generally referred to as a heavy metal) do not have any

beneficial effect on organisms and are thus regarded as the “main threats” since they are very harmful to both plants and animals. For other metals which are beneficial to plants, “small” concentrations of these metals in the soil could actually improve plant growth and development. However, it was discovered in this study that at higher concentrations of these metals, reductions in plant growth occurred. This may account for the decrease in growth parameters of *A. cruentus* as the effluent concentration increased in this study. However, heavy metals of soil in all the soil samples were found to increase as the petrochemical effluent increased in concentration, but treatments inoculated with *G. hoi* showed lower concentration in heavy metals compared to treatments without inoculation of micro-organism. This low concentration of heavy metals in this inoculated soil may be as a result of *G. hoi* ability to absorb and sequester some heavy metals in to their mycelia which is retained in the roots (Marques *et. al.*, 2009). Due to a change in their oxidation state, heavy metals can be transformed to become either less toxic, easily volatilized, more water soluble (and thus can be removed through leaching), less water soluble (which allows them to precipitate and become easily removed from the environment) or less bioavailable (Marques *et. al.*, 2009). He also noted that mycorrhizal fungi have been used in several remediation studies involving heavy metals pollution and the results obtained showed that mycorrhiza employs different mechanisms for the remediation of heavy metal polluted soils. Soils polluted with various heavy metals including As, Cu, Cd, Pb, U and Zn can be remediated via MAR. The MAR can also help with the transfer of elements such as carbon (Francis and Read, 1984), nitrogen (Haystead *et al.*, 1988, Rogers *et al.*, 2001), and phosphorus (Chiariello *et al.*, 1982).

Treatments inoculated with *G. hoi* showed a lower concentration of zinc in the soil compared to the treatments without inoculation of microorganism, this may be due to the absorption of the zinc in the soil by the AM (*G. hoi*), this result is the same with the findings of Vogel-Mikus *et al.* (2005); Chen *et al.* (2006) which reported that AM fungi absorb N, P, K, Ca, S, Fe, Mn, Cu, and Zn from the soil and then translocate these nutrients to the plants with whose roots they are associated with. This report also confirmed the reason for the lower concentration of iron and copper in the soil samples inoculated with *G. hoi* compared to treatments without inoculation of *G. hoi*. Treatments with dual inoculation in this study however showed lower heavy metals concentrations compared to those treatments with single inoculation and treatments without inoculation of

micro-organism. This revealed that there is positive and productive interaction between *G. hoi* and *P. aeruginosa* in bioremediation of heavy metals in petrochemical effluent polluted soil.

V. CONCLUSION

The arbuscular mycorrhiza Fungus, *Glomus hoi* was found to be able to give way for reduction in heavy metals in the soil which can make plants survive in petrochemical effluent polluted soil. The combination of the two microorganisms showed a better improvement in the overall reduction in heavy metal concentration. They also had a fruitful combination in increasing the organic matter of the soil compared to when they were inoculated singly. Hence, *G. hoi* and *P. aeruginosa* can enhance crop production in oil refinery effluent polluted soil. The microorganisms also worked better in sterilized soil than the unsterilized soil, this may be due to the no competition between native microorganisms in the sterilized soil with the inoculated microorganism.

This informs the reason of bioremediation being an acceptable approach for processing organic and inorganic wastes. The result of this study has shown that bioremediation is an environmental friendly and easy approach to degrade petrochemical effluent polluted soil.

VI. RECOMMENDATION

There should be adequate analysis of the site polluted with refinery effluent or petroleum hydrocarbon before and after bioremediation to enhance proper treatment of the soil.

However, government, oil refineries, farmers and other concerned individuals should embrace the use of bioremediation to treat oil refinery effluent pollution in the environment. This is because bioremediation requires no negative consequence or whatsoever. It is also easy to carry out. It is less skillful and efficient.

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