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Research Article

BIOREMEDIATION OF AN OIL REFINERY EFFLUENT POLLUTED SOIL WITH *GLOMUS HOI* AND *PSEUDOMONAS AERUGINOSA* USING *AMARANTHUS CRUENTUS* AS THE TEST PLANT

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ABSTRACT

This study investigated the effect of *Glomus hoi* and *Pseudomonas aeruginosa* on the degradation of petrochemical effluent polluted soil using *Amaranthus cruentus* as the test plant. This was with a view to determining the degrading potential of the microorganisms on soil polluted with effluent and its effect on the growth of the test plant.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 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 before *A. cruentus*were transplanted to them. The residual twenty four (24) pots served as the control. Thereafter, pot preparation was arranged in the screenhouse in a randomized block design. The *A. cruentus* seedlings were raised in nursery for a period of one week before they were transplanted to the pots; seedlings were left for 3 days to overcome transplanting shock before contaminating the soil with Petrochemical 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. Plant Heightwas recorded weekly. The results obtained were analyzed using Duncan Multiple Range Test (DMRT) and other descriptive statistics.The pre planting analysis of soil showed that heavy metals analyses (chromium and cadmium) of sterilized soil had a lower concentration compared to the unsterilized soil. Total Petroleum Hydrocarbon (TPH) analyses revealed that dual inoculation of *G. hoi* and *P. aeruginosa* was effective in reducing TPH compared to when the microorganism was administered singly. Treatment with *P. aeruginosa* showed high TPH reduction potential compared to *G. hoi*. However, treatment without any inoculation of microorganism had the lowest rate of TPH reduction of 33% at 6% effluent concentration, the TPH in the soilreduced without inoculation with microorganisms but in a slower rate compared to those with single or dual inoculations. This study opined that the combineduse of *G. hoi* and *P. aeruginosa* was more effective in degrading petrochemical effluent polluted soil than when either is used singly.

INTRODUCTION

Industrialization during 19th century changed mankind's life style while new technology raised man's standard of living and made life more comfortable but with increasing industrial development. Also, safe disposal of industrial waste water has becomean ecological challenge with the attendant environmental degradation which has now become a global problem. Thomas *et al*. (1992) reported that industrialization, like other human activities that has impact on the environment, often results in pollution and degradation. It carries inevitable costs and problems in terms of pollution of the air, water resources and general degradation of the natural environment. Oladunjoye *et al*. (2013) also noted that various industries such as food and beverage industries, paint industries, soap

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industries, paper and pulp industries, textile industries, petroleum and petrochemical industries and cosmetic industries, provide a lot of amenities but they do more harm than good. One of these is the petroleum and petrochemical industries which constitute a reasonable amount of pollution source that is prevalent in Nigeria, being one of the major oil-producing countries. Wang *et al.* (2001) noted that crude oil and petrochemical hydrocarbon interferes with the structures or functions of the various organs of plants and animals and may directly kill the organism that comes in contact with it. Oil contaminated soil adversely affects plant growth and other living organism. This is due to poor water retaining ability and aeration of the soil, loss of seed viability etc. These stress conditions interfere with water uptake and gaseous exchange which may create a condition of physiological drought. Hence, there is need for this recalcitrant pollutant to be degraded. Bioremediation is a process that uses naturally occurring microorganisms to transform harmful substances to nontoxic compounds. Bioremediation processes which take advantage of microbial degradation of organic and inorganic substances can be defined as the use of microorganisms to remove environmental pollutants of soils, water and sediments (Pala *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 thatvarious 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* in petrochemical effluent polluted soil.

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 131^0c and left to cool for 4 days. Three hundred grams of soil inoculum containing four hundred spores of *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 *Gliricidiasepium* 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 5mins. 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 petridishes 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 10^8 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

Plant samples were analyzed for the presence of heavy metals such as chromium and cadmium using Atomic Absorption Spectrophotometer (AAS). Heavy metals (chromium and cadmium) of the soil were analyzed both before and after the experiment using soil test and AAS respectively. Total Petroleum Hydrocarbon (TPH) analysis was also carried out on the soil samples after the harvest of *A. cruentus*. Data was 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.

RESULTS

The physicochemical properties of sterilized and unsterilized soil before planting were found to show that heavy metals analyses (chromium and cadmium) of sterilized soil had a lower concentration compare to the unsterilized soil (Table 1).

Table 1 Heavy Metals of Sterilized and Unsterilized Soil before Planting

Parameters	Sterilized	Unsterilized
Cr (ppm)	23.15	31.55
Cd (ppm)	5.69	7.46

Plant Height

The plant height at p level ≤ 0.05 showed that at 0% Petrochemical effluent concentration, treatment 4 with dual inoculation of *Glomus hoi* and *Pseudomonas aeruginosa,* treatment 5 (unsterilized soil with no inoculation of microorganism), treatment 6 (unsterilized soil with *Glomus hoi* inoculation), treatment 7 (unsterilized soil with *P. aeruginosa* inoculation) and treatment 8 (unsterilized soil with dual inoculation of microorganism) all showed insignificance difference from 3 Week After Planting (WAP) to 4 WAP (Table 2). At 5 WAP, treatment 8 had the highest value of plant height of 16.10cm followed by treatment 4 with 15.00cm plant height but the reverse was the case after 5 WAP till the harvest time (Table 2). The plant height of all the treatments was significantly different from 5 WAP to 10 WAP. For 2% effluent concentration, the weekly raw data subjected to DMRT showed that treatments 2, 4, 7 and 8 were insignificantly different from 3 WAP to 4 WAP. There was sharp increase in plant height from the 8WAP to 9 WAP across all treatments (Table 3). At 10 WAP, the plant height in treatment 4 was found highest and lowest at treatment 5.

Means with the same subscript along the columns are not significantly different at (P ≤ 0.05) according to Duncan multiple range test (DMRT).

Legend

SS: Sterilised Soil
US: Unsterilised Soil
TP: Test plant
GH: *Glomus hoi*
PA: *Pseudomonas aeruginosa*

Means with the same subscript along the columns are not significantly different at (P ≤ 0.05) according to Duncan multiple range test (DMRT).

Legend

SS: Sterilised Soil
US: Unsterilised Soil
TP: Test plant
GH: *Glomus hoi*
PA: *Pseudomonas aeruginosa*

For 4% effluent concentration, treatment 1 which was sterilized soil without inoculation showed insignificant difference from 3 WAP to 7 WAP at p level ≤ 0.05 but at 10 WAP, mortality was recorded for the same treatment (Table 4). Treatment 5 at 3 – 6 WAP had insignificant difference in plant height but the plant did not survive beyond 9 WAP. Treatments 2, 3, 4, 6, 7 and 8 which had either single or dual inoculation showed an increase in plant height from 8 WAP to 10 WAP, at the termination of experiment, treatment 4 had highest plant height of 37.83cm followed by treatment 8 with 36.87cm and the lowest was treatment 6 with 30.5cm (Table 4).

Table 2 Mean Height of Test Plant (cm) under Different Treatment with 0 % Effluent Concentration

	Weeks after planting							
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1-SS + TP	9.83c	10.45b	11.50c	12.67c	14.05b	15.11b	24.07c	37.73b
2-SS + GH	12.00b	13.55a	13.33b	15.73b	16.73a	17.57a	36.66b	45.00a
3-SS + PA	11.97b	12.27b	12.83b	13.57b	14.33b	17.40a	35.83b	43.33a
4-SS + GH + PA	14.33a	14.87a	15.00a	17.43a	18.10a	19.89a	41.27a	46.50a
5-US + TP	11.37b	11.54b	12.33b	14.06b	15.57a	16.03a	27.34c	34.67b
6-US + GH	13.00a	13.00a	14.00a	15.13b	15.73a	15.97b	35.87b	43.87a
7-US + PA	12.03b	12.00b	13.33b	13.83c	15.13a	16.10a	35.17b	41.83a
8-US + GH + PA	15.27a	15.43a	16.10a	17.03a	17.50a	18.97a	41.12a	46.03a

Table 3 Mean Height of Test Plant (cm) under Different Treatment with 2 % Effluent Concentration

	Weeks after planting							
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1-SS + TP	7.83c	8.37c	9.02c	9.00c	10.40c	12.77c	24.27c	29.60c
2-SS + GH	10.23b	10.83b	11.27b	13.97b	15.47a	16.37b	30.33b	39.00a
3-SS + PA	9.83b	10.47b	11.37b	13.17b	12.27b	13.70c	33.00b	38.33a
4-SS + GH + PA	13.11a	13.67a	14.89a	15.87a	16.90a	20.63a	37.17a	44.03a
5-US + TP	7.33c	8.11c	8.83c	9.67c	10.83c	11.30c	22.77c	27.03c
6-US + GH	10.27b	11.00b	12.33a	12.77b	13.37b	13.83c	32.80b	37.17b
7-US + PA	11.27b	11.67b	12.43a	13.13b	14.73a	16.63b	30.00b	37.10a
8-US + GH + PA	14.13a	14.33a	14.05a	15.93a	16.33a	19.67a	36.77a	42.17a

At 6% effluent contamination, the plant height of treatments 1, 2, 4, 5 and 6 all showed insignificant difference at 3 WAP to 4 WAP, the treatment with single or dual inoculations of microorganisms (treatments 2, 3, 4, 6, 7 and 8) all showed sharp increase in plant height from 8 WAP to 10 WAP. Treatments 1 and 5 without inoculation of microorganism did not survive beyond 8 WAP. At 10 WAP, treatment 4 had the highest plant height followed by treatment 8 while treatment 2 had the lowest (Table 5).

Means with the same subscript along the columns are not significantly different at ($P \leq 0.05$) according to Duncan multiple range test (DMRT).

Legend

SS: Sterilised Soil
US: Unsterilised Soil
TP: Test plant
GH: *Glomus hoi*

Table 4 Mean Height of Test Plant (cm) under Different Treatment with 4 % Effluent Concentration

	Weeks after planting							
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1-SS + TP	5.23b	5.67c	5.63c	5.87c	6.43c	7.63c	7.57c	*
2-SS + GH	6.83b	7.33b	8.67b	10.13b	10.89b	11.53b	28.67b	32.67a
3-SS + PA	7.37b	8.23a	8.67b	9.11b	9.97b	10.37b	29.00b	32.99a
4-SS + GH + PA	10.67a	10.23a	11.00a	13.67a	14.67a	17.09a	34.83a	37.83a
5-US + TP	4.10c	4.67c	5.00c	5.60c	5.67c	7.8c	6.11c	*
6-US + GH	8.267b	8.43a	8.33b	11.11a	11.77b	12.03b	29.00b	30.50b
7-US + PA	8.33b	8.67a	10.23a	10.83b	11.93b	15a	29.53b	31.57b
8-US + GH + PA	9.63a	10.27a	10.83a	13.2a	15.17a	17.57a	34.33a	36.87a

Table 5 Mean Height of Test Plant (cm) under Different Treatment with 6 % Effluent Concentration

	Weeks after planting							
Treatment	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1-SS + TP	3.57c	3.73b	4.40b	3.78c	4.10c	4.17c	*	*
2-SS + GH	4.50a	4.60b	5.00a	6.27b	7.13b	7.70b	26.50a	27.53b
3-SS + PA	4.20b	4.90b	5.48a	6.67b	6.67b	6.76b	24.83b	27.67b
4-SS + GH + PA	6.00a	6.13a	6.93a	7.97a	10.27a	13.10a	29.83a	32.63a
5-US + TP	2.27c	2.53c	3.00c	3.67c	4.00c	3.43c	*	*
6-US + GH	5.27b	5.67a	5.55b	7.67a	8.47b	8.83b	26.67a	27.67b
7-US + PA	4.67b	5.27a	5.67b	8.33a	10.17a	13.33a	26.17a	28.50b
8-US + GH + PA	7.66a	7.80a	8.78a	8.27a	9.83a	12.57a	29.63a	31.60a

Means with the same subscript along the columns are not significantly different at ($P \leq 0.05$) according to Duncan multiple range test (DMRT).

Legend

SS: Sterilised Soil
US: Unsterilised Soil
TP: Test plant
GH: *Glomus hoi*
PA: *Pseudomonas aeruginosa*
*--Mortality

Heavy Metal Content of Soil After Planting

The heavy metal analyses of the soil were found to show that cadmium increased as effluent increased in all the treatments without any inoculation of the microorganisms (Fig. 1). Treatments 3 and 7 inoculated with *P. aeruginosa* were lower in concentration of cadmium compared to treatments 2 and 6 which were inoculated with *G. hoi*. Treatment 1 without

inoculation of any microorganism at 6% effluent concentration had the highest cadmium concentration followed by treatment 5 which was unsterilized soil in the same condition. The order of cadmium concentration across the treatments with 2% and 6% effluent concentration was 5 > 1 > 6 > 2 > 7 > 8 > 3 > 4 and 1 > 5 > 2 > 3 > 6 > 7 > 4 >8 respectively (Fig. 1). Chromium had the highest concentration in treatment 1 at 6% effluent concentration followed by treatment 5 at the same 6% while treatment 8 at 0% was the lowest, sample treated with one or two microorganisms had lowest value in chromium concentration compared to the soil polluted with effluent without any treatment with microorganisms (Fig. 2).

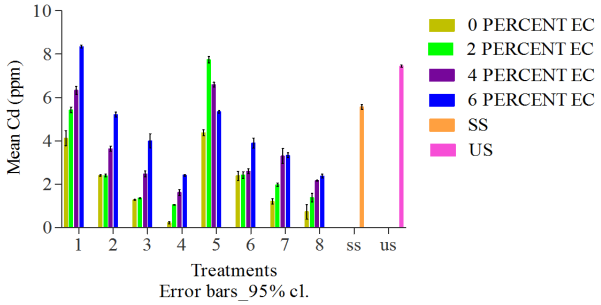

Fig. 1 Cadmium (ppm) content of Pre and Post Planting Soil Samples

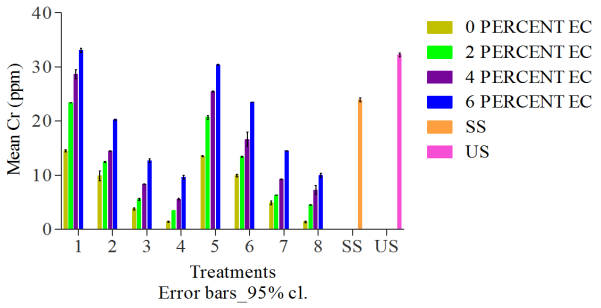

Fig. 2 Chromium (ppm) content of Pre and Post Planting Soil Samples

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

Plant Heavy Metals Build Up After Planting

The analyses of the heavy metals of the test plant showed that treatment 1 had the highest heavy metal concentration in plant tissue at 6% petrochemical effluent concentration followed by treatment 5 at same 6% while treatment 8 at 6% had the lowest value. The order of concentration in cadmium at 6% was treatment 1 > 5 > 2 > 4 > 3 > 6 > 7 > 8. At 2 %, it was treatment 5 > 1 > 6 > 2 > 3 > 7 > 4 >8 (Fig. 3). Chromium at 6% effluent concentration had the highest concentration in treatment 1 and the lowest at treatment 8 at same 6%. The order of chromium concentration at 6% was 1 > 5 > 7 > 6 > 2 > 3 > 4 > 8 while 2% was 5 > 1 > 2 > 6 > 3 > 7 > 4 > 8 (Fig. 4).

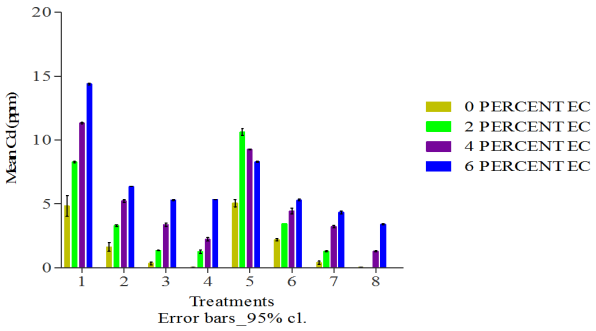

Fig. 3 Cadmium (PPM) content of *A. cruentus* across all the treatments

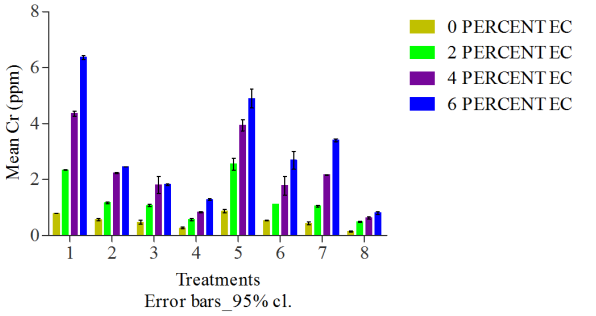

Fig. 4 Chromium (PPM) content of *A. cruentus* across all the treatments

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

Total Petroleum Hydrocarbon of Soil After Planting

As the effluent concentration increased, it was found that there was decrease in % reduction in TPH. Treatment 4 which was dual inoculation had the highest % reduction at 2% effluent concentration followed by treatment 8 at the same 2% (Fig. 5). Treatments 4 and 8 with dual inoculation had higher % reduction in TPH at all levels of concentration in comparison with treatments 2, 3, 6 and 7 with single inoculation. Treatments 3 and 7 inoculated with *P. aeruginosa* had a better and higher % reduction in TPH compared to treatments 2 and 6 inoculated with *G. hoi* (Fig.5). The order of % reduction in TPH at 2% and 4% was 4 > 8 > 3 > 7 > 6 > 2 > 5 > 1 and 4 > 3 > 8 > 7 > 6 > 2 > 5 > 1 respectively while that of 6% was 8 > 4 > 7 > 3 > 6 > 2 > 5 > 1 (Fig. 5).

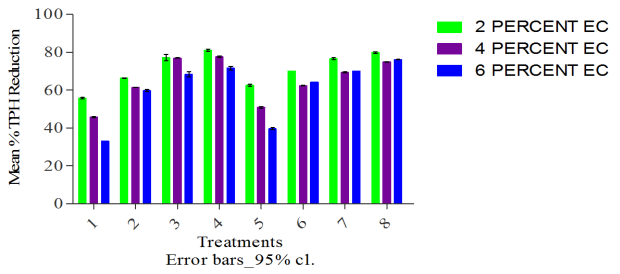

Fig. 5 Mean % TPH reduction of Soil Samples

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

DISCUSSIONS

The physicochemical properties of sterilized and unsterilized soil before planting were found to show that heavy metals analyses (chromium and cadmium) of sterilized soil had a lower concentration compare to the unsterilized soil.

In this study, plant height analyses in treatments with sterilized and unsterilized soil without inoculation of microorganisms were found to have lowest plant height. This is due to the fact that the plants depended mainly on the intrinsic soil fertility which could not have sustained the plant up to 6 WAP. This could also be due to toxicological stress as a result of petroleum hydrocarbon and heavy metals from the effluent. This is in line with the report of Gulfraz *et al*. (2003). This showed that effluent contamination inhibits plant growth. The effect of waste disposal on plant which is generated from the refinery limits its growth; this is partly because the liquid waste product which comes from the refinery in form of oil, blocks air and water passages to the soil which is one of the conditions necessary for plant germination and growth. This disallows the rhizosphere to have access to soil air and water. Samples treated with *P. aeruginosa* in this study had higher mean values in plant height compared to samples treated with *G. hoi*. It was also discovered that treatments with dual inoculations of *Glomus hoi* and *Pseudomonas aeruginosa* in sterilized and unsterilized soil (treatments 4 and 8) across all concentrations had highest values in plant heightat 10 WAP. This is significantly different from sample treated with single inoculation of microorganism and sample treated without any inoculation of microorganism. This result showed that there is positive and productive interaction between *Glomus hoi* and *Pseudomonas aeruginosa* in bioremediation of petrochemical effluent polluted soil and alleviation of toxicology stress in petrochemical effluent polluted soil. This result showed that there is positive and productive interaction between *Glomus hoi* and *Pseudomonas aeruginosa* in bioremediation of petrochemical effluent polluted soil and alleviation of toxicology stress in petrochemical effluent polluted soil.

Also, Total Petroleum Hydrocarbon (TPH) is used to measure the efficiency of bioremediation as a tool for cleaning up hydrocarbon contaminated soils (Edwin-wosu and Albert, 2010). This has also been observed in this study that treatments without any inoculation showed a very low % reduction in TPH. Also, treatments with *P. aeruginosa* showed a higher % reduction in TPH compared to treatments inoculated with *G. hoi*. This may indicate that *P. aeruginosa* had a better reduction potential in the TPH of polluted soil compared to *G. hoi*. However, treatments which had dual inoculation showed the highest % reduction in TPH compared to treatments with single inoculation. This may indicate that the arbuscular mycorrhiza which is *G. hoi* used for this study can perfectly work with *P. aeruginosa* in reducing TPH in petrochemical effluent polluted soil. This result confirmed the report of Toljander*et al*. (2007) which revealed that some of the carbon in the AMF hyphae may be exuded or secreted, which would encourage bacterial growth in a similar way to the well-studied 'rhizosphere' effect. This increased rate of bioremediation with dual inoculation was also in agreement with Jelena in (2001) which states that a single microorganism can degrade only certain types of petroleum compounds, but a mixed population of microbial community enables a higher level of degradation. Several previous published works were also in agreement with this increased bioremediation (Hrynkiewicz *et al.,* (2009); Zimmer *et al.,* (2009)). It was demonstrated in those reports that interactions of mycorrhizal fungus and bacterium can be growth promoting even in situations when the microorganisms used as inoculum does not originate from the same host plant and site. This interaction was also supported by the report of Perotto and Bonfante in (1997) which noted that Mycorrhizal fungus and bacterium in the rhizosphere can interact with each other at different levels of cellular integration, ranging from apparently simple associations, through surface attachment, to intimate an obligatory symbiosis. This synergy may not only be important in promoting plant growth and health, but may also be significant to rhizosphere ecology Baum *et al*., (2006). He also noted that mycorrhizal fungi and rhizobacteria were demonstrated to promote plant growth and degradation of pollutants in soils with increased pollutant concentrations.

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. This is due to the contained heavy and trace metals like Cr, Mn, Fe, Cu, Co, Zn, Ni, As, Cd and Pb that are discharged continuously into water source (streams/ nullahs, canals and rivers). These are allowed to spread on agricultural lands. The unplanned disposal of these effluents has increased the threat of environmental pollution (Gulfraz *et al.,* 2003). According to Gulfraz *et al.,* (2003) some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are 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 heavy metals) 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 (Opaoluwa, 2010). 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 height 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 microorganism. 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). However, it was discovered in this study that treatments inoculated with *P. aeruginosa* showed a lower concentration of cadmium compared to treatments inoculated with *G. hoi.* This result is in line with the findings of Wang *et al*., (1997) which noted the use of *P. aeruginosa* for the removal of cadmium. Roane and Pepper (2000) also reported the detoxification of Cd by *Arthrobacter, Bacillus or Pseudomonas spp*. 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 microorganism. 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.

The heavy metals that are available for plant uptake are those that are present as soluble components in the soil solution or those that are easily solubilized by root exudates. Plant has a lot of consequences from heavy metal pollution in soil (Guo 1994, Liao 1993, Su *et al.,* 2014, Wu *et al,*. 1998), plants were also seen to be polluted by heavy metals (Yin *et al*., 1999), which consequently threatens the health of animals and human beings via the food chain (Wang *et al*., 2001). Heavy metals such as Cd and Pb are non-essential elements for plants. If plentiful amounts are accumulated in the plants, heavy metals will adversely affect the absorption and transport of essential elements, disturb the metabolism, and have an impact on growth and reproduction (Xu and Shi 2000). This account for stunted growth and reduction in other growth parameters of *A.cruentus*in the polluted soil in this study, this study were found to show reduction in growth parameters as heavy metals increased which is brought by increase in petrochemical effluent concentration. In this study, treatments without any inoculation with microorganisms had the highest concentration of heavy metal build up in their plant tissue, this was seen to affect the growth of the plants, the plants in these samples died at 8 week after planting at 6% and at 9 week after planting at 4%, this may be due to the heavy metal build up in the plant which had reached its threshold, hence the plants succumbed to toxicological stress, this scenario is also illustrated by the findings of Su *et al.* (2014) which reported that that a plant's heavy metal concentration exceeding its tolerance threshold would lead to the plant being poisoned and eventually death of the plant.

CONCLUSION

Bioremediation continues to be the favoured 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. Microorganisms used for this study have the ability to degrade petrochemical effluent polluted soil as this is evident in the result of the study.

The arbuscular mycorrhiza Fungus, *Glomus hoi* was found to have greater ability for making plant to survive in petrochemical effluent polluted soil. The AM fungi enable the plants to absorb essential nutrient from the polluted soil. *Pseudomonas aeruginosa* was found to have higher significant potential than *Glomus hoi* in biodegradation of petrochemical effluent polluted soil. The combination of the two microorganisms showed a better improvement in the overall biodegradation of the polluted soil than when they were inoculated singly hence *G. hoi* and *P. aeruginosa* can enhance crop production in petrochemical effluent polluted soil. It was also found in this study that both micro-organisms enhance crop production in soil without effluent pollution. The micro-organisms also worked better in sterilized soil than the unsterilized soil, this may be due to the less competition between native micro-organisms in the sterilized soil with the inoculated micro-organism. This is unlike the unsterilized soil where there is competition between the native micro-organisms in soil and the inoculated micro-organisms.

Recommendation

Government, oil refineries, and other concerned individuals should embrace the use of bioremediation to degrade petrochemical effluent pollution in the environment. This is because bioremediation is a less technical approach to treat petrochemical effluent polluted soil without any negative consequence or whatsoever. However, there should be adequate analysis of the polluted site before and after bioremediation to enhance proper treatment of the soil. Further work should be carried out especially by the government on how to package these micro-organisms and make them readily available for sale at cheap price. Plant products harvested from petrochemical effluent polluted soil should not be sold as the plant contains heavy metals that are poisonous for human consumption.

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