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MYCOREMEDIATION OF DIESEL CONTAMINATED SOIL WITH OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) USING MAIZE (ZEA MAYS) AS THE TEST CROP

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Abstract

The remediation of soil contaminated with petroleum based compounds by oyster mushroom (Pleurotus ostreatus) has been well documented. In this study the ability of diesel contaminated media treated with mushroom to support plant growth was investigated using maize as test crop. The mushroom was grown on different levels of diesel (14ml, 29ml, 58ml, 106ml and control), each to 500g of soil (contaminated media). Each of the five samples (RS 14, RS 29, RS58, RS 106 and RS Control) was replicated five times. Another set of media with various diesel levels (14ml, 29ml, 58ml and 106ml) were formulated as above but they were not remediated by the mushroom spawn (NRS 14, NRS 29, NRS 58, NRS 106 and NRS Control). Data was subjected to analysis of variance (ANOVA) using Genstat statistical package and treatments were compared at 5% probability level. The highest removal of petroleum hydrocarbons by oyster mushroom was most pronounced in the 29ml level of diesel contamination which recorded the highest germination percentage (100%) of the maize seeds. The least reduction in hydrocarbons was in the 58ml and 106ml media, which did not record any germination of maize seeds. There was a reduction in the toxicity of the soils treated with Pleurotus ostreatus, since the mycelium was able to run through all the treatments, even though the maize seeds did not grow on remediated soil of 58ml diesel (RS58) and remediated soil of 106ml diesel (RS106). Germination of the seeds of the test plant in 29ml and 14ml diesel fuel contaminated substrates was higher than in the control. Growth of the maize plant was inhibited on the contaminated soils which however differed significantly ($p < 0.05$) from those of the control.

Keywords: Remediated media, Non remediated media, Mushroom, Maize, Mycoremediation

Introduction

The term 'mycoremediation' was coined by Stamets (2005). Bioremediation is an economical and effective treatment for diesel and other medium distillate fuels because a large portion of these contaminants are easily degradable (Riffaldi *et al*., 2006).

Crude oil is a major contaminant of soil and water in oil producing countries as a result of extraction and processing of the oil. Crude oil spills from pipelines changes the physicochemical and biological properties of the soil because the oil may be toxic to some soil microorganisms and plants.

Depending on the crude oil composition and concentration, its effects on living organisms and the environment vary (Minai-Tehrani and Herfatmanesh, 2007). Under normal conditions, diesel fuel was absorbed in the organic rich surface soil and impedes downward migration (Adam and Duncan, 1999). The extensive use of diesel and other petroleum products has resulted in their intrusion into soil environments by various routes, including leakage from

underground storage tanks (UST) and pipelines, accidental spills, improper waste disposal practices, and leaching landfills. Various fuel constituents may enter the human and animal system by inhalation, ingestion, or dermal contact and may pose hepatic, renal, neurological, and/or respiratory risks (ATSDR, 1999).

These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their natural environment. Germination and root elongation are two critical stages in plant development that are sensitive to environmental contamination (Baud-Grasset *et al*., 1993). Contamination of soil environment by petroleum hydrocarbons is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for environmental health.

Mushrooms have enzymes which are capable of utilizing complex organic compounds which occur as agricultural wastes and industrial by-products. Thus agricultural wastes can also be used as bedding material for mushroom cultivation (Baysal and Peker, 2001). The importance of edible mushrooms has increased due to the advances in cultivation technology, which makes the use of agricultural and industrial residues possible by recycling them as substrates for cultivation, consequently resulting in low cost production and a continuous market (Pandey *et al*., 2000). Moreover, they represent an excellent alternative for discarding several residues, helping in reducing pollution caused by the presence of these materials in the environment (Carvalho, 2010).

Large volumes of lignocellulose agricultural residues (fish waste, vegetable materials) are generated annually through agricultural and food processing industries. These are either disposed of by burning or dumping in landfills, thus posing a hazard to the environment and human health; and which would otherwise be used in the cultivation of edible and medicinal mushrooms (Atipko *et al*., 2008).The oyster mushroom *Pleurotus* sp. is cultivated in many countries both in sub-tropical and temperate regions of the world. Like other edible mushrooms, *Pleurotus* species can be grown on various agricultural waste products without the addition of enrichment materials (Rajapakse, 2007).

The main objective of this research work was to assess mushrooms' (*Pleurotus ostreatus*) ability in the amendment of diesel contaminated soil.

METHODOLOGY

EXPERIMENTAL SITE

The study was conducted at the plant house of the Faculty of Agriculture, University for Development Studies, the area falls within the Guinea savanna agro-ecological zone of Ghana.

EXPERIMENTAL MATERIALS

Experimental containers used were the plastic bugs (rubber bugs) of 20 liters capacity, with a diameter of 22.5x 30 cm and thick black polythene about 5 meters long. The diesel used was purchased from the oil filling station in Tamale. Mushroom (*Pleurotus ostreatus*) spawn was obtained from the Food Research Institute, Ghana. Maize used as the test crop was obtained from the Savanna Agricultural Research Institute (SARI), Ghana.

COMPOSTING

The experiment was laid out in a completely randomized design. Rough sawdust was used as the media with urea and lime as decomposers. 500g urea and 100g of lime was added to 3500g of sawdust and thoroughly mixed with a spade until a uniform mixture was obtained, 16 liters of tap water was sprinkled on the mixture and

thoroughly mixed until a reasonable moisture level was achieved in the media. A thick black rubber (5 meters long) was used to cover the compost and allowed to decompose for 35 days with constant stirring every six days interval, to ensure uniform distribution of temperature in the media.

SAMPLE PREPARATION AND BAGGING

Garden soil obtained from the experimental field of the University for Development Studies was weighed (500g) each into 25 different bowls. To each of the soil samples different quantities of diesel oil (14 mls, 29 mls, 58 mls and 106 mls) was added with a control (no diesel oil). This was repeated five times. Each sample was thoroughly mixed to ensure a homogeneous soil-diesel mixture before it was transferred into the polyethylene rubber bags. 200g of the compost was first laid at the bottom of the polyethylene rubber bags followed with 500g of diesel contaminated soil and 200g of sawdust on top of the soil giving a total of 900g media in each polyethylene rubber bag.

A PVC pipe was placed at the neck of the polyethylene bag, covered with a cotton wool and a paper was then used to cover the cotton and clipped with a rubber band.

Plate 1: Polyethylene rubber bags containing diesel contaminated soil and sawdust arranged in the growth room.

STERILIZATION (PASTEURIZATION AND CONDITIONING)

The media in polyethylene rubber bags were placed in sterilizing tank with water at the bottom, the media was sterilized by steaming at a temperature of 115°C for two hours. The aim was to kill microorganisms that were competitive to the mushrooms growth.

Plate 2: Set up for sterilization of media

INOCULATION OF MUSHROOM SPAWN

The media was cooled and spawned with the *Pleurotus ostreatus* mushroom by broadcasting the spawn over the surface of the polyethylene rubber bags containing the media. Fresh cotton wool and paper were used to cover the bags with the help of the PVC pipes and the rubber bands. This was done under sterile condition and left in the dark room for 30-35days.

SPAWN RUN AND MYCELIUM GROWTH

At 30 days after inoculation the mycelium germinated from the spawn and permeated into the media. The mushroom began to form around the edges of the perforated polyethylene rubber bags. The bags were maintained under optimal growth conditions of temperature (15°C) and pH of 7.0. The humidity was maintained at 70-80% by watering the bags regularly to favor mushroom fruiting. The media containing the samples were then transferred to an air free flow room for the development of the mushroom fruiting bodies.

FRUITING AND HARVESTING OF MUSHROOM

Following the mycelium growth, the PVC pipes were then plugged out including the paper and the cotton wool to allow primordial formation to occur under suitable environmental conditions followed by the production of the fruiting bodies. The mushroom fruiting bodies were then harvested one week after transferring to the production room.

SOIL SAMPLING AND GROWTH OF MAIZE

After harvesting the mushroom the media were sampled separately from the polyethylene rubber bags based on the diesel concentrations of the media. This was used as the growth media for the test crop (maize). Another set of media with various diesel levels (14ml, 29ml, 58ml and 106ml) were formulated as above but they were not remediated by the mushroom spawn.

Plate 3: Polyethylene rubber bags after mushroom fruiting bodies were harvested.

DATA COLLECTION AND ANALYSIS

The experiment was stopped at the five leaf stage which was two weeks after germination. Data collected includes the germination percentage, plant height; shoot length, the number of rootlets, and leaf area index.

Data was subjected to analysis of variance (ANOVA) using Genstat statistical package and treatments were compared at 5% probability level.

RESULTS AND DISCUSSION

GERMINATION PERCENTAGE

Media that was properly remediated were observed to have high germination percentage and hence good growth parameter, but media that were poorly remediated were observed to have low percentage germination.

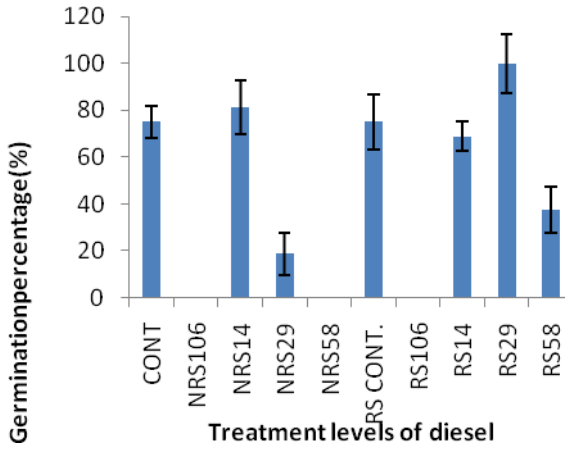

FIGURE 5: Germination percentage of maize grown on each media

The germination percentage of maize (figure 5) varied significantly at p< 0.05 between the various levels of the diesel polluted media, however figure 5 showed that the lowest germination percentage were recorded from; non remediated soil of 106ml diesel (NRS 106ml) and non-remediated Soil of 58ml diesel (RS58), since there was no growth, the non remediated media of 29ml diesel and remediated media of 58ml diesel however have 18.8% and 37.5% respectively, the remediated media of 14ml diesel has a germination percentage (68.8%), the two controls however, had the same germination percentage (75%), non remediated soil (NRS14) with (81.2%), and the highest germination percentage (100%) were found on the remediated media of 29ml of diesel (RS29). Baran *et al.*, 2002 has reported that petroleum derived components on soil leads to severe nitrogen and phosphorus depletion, disruption of water balance and biological equilibrium.

LEAF AREA INDEX

Table 1: The leaf area index of maize on different media

Treatments	Mean leaf area index
Non Remediated soil (NRS) Control	19.40[a]
Non Remediated soil (NRS)106	0.00[d]
Non Remediated soil (NRS)14	13.54[b]
Non Remediated soil (NRS)29	2.15[d]
Non Remediated soil(NRS)58	0.00[d]
Remediated Soil (RS) Control	13.30[b]
Remediated Soil (RS) 106	0.00[d]
Remediated Soil (RS) 14	13.02[b]
Remediated Soil (RS) 29	13.03[b]
Remediated Soil (RS) 58	6.75[c]
P- Value	4.51

a, b, c, and d means in the same column differ significantly at ($P < 0.05$)

The leaf area index of the maize (Table 1) grown on both the remediated media and the non-remediated media varied significantly at p<0.005 (Table 1). The levels; remediated soil control (RS-Control), remediated soil (RS 106), non remediated soil (NRS106), non remediated soil (NRS58), non remediated soil (NRS29), remediated soil (RS58), remediated soil (RS14), and remediated soil (RS29), and have the following mean, 0.00cm, 0.00cm, 0.00cm, 2.15cm, 6.75cm, 13.02cm, and 13.03cm respectively; followed by non-remediated media with 14ml diesel (NRS14) had (13.54cm) and the result from Table 1 indicated that; the non-remediated media (NRS-Control) had the highest leaf area index (19.4cm)

PLANT HEIGHT (cm)

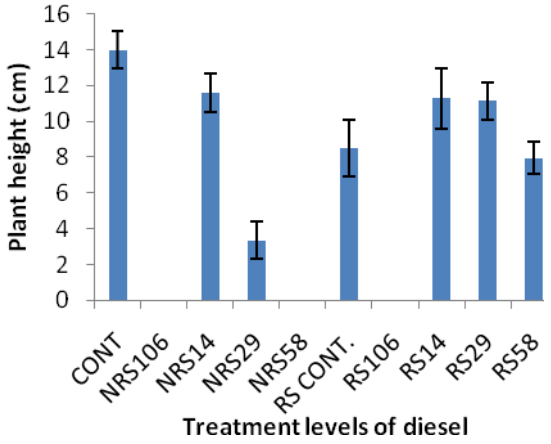

FIGURE 6: Plant height of maize on the different media

Plant height of maize (figure 6) in response to the various diesel contaminated media were significant at $p<0.05$. The mean plant height assessed for NRS-Control, NRS106, NRS14, NRS29, NRS58, RS-Control, RS106, RS14, RS29 and RS58 were 14.00cm, 0.00cm, 11.58cm, 3.35cm, 0.00cm, 8.50cm, 0.00cm, 11.28cm, 11.15cm, and 7.95cm respectively.

ROOT LENGTH (cm)

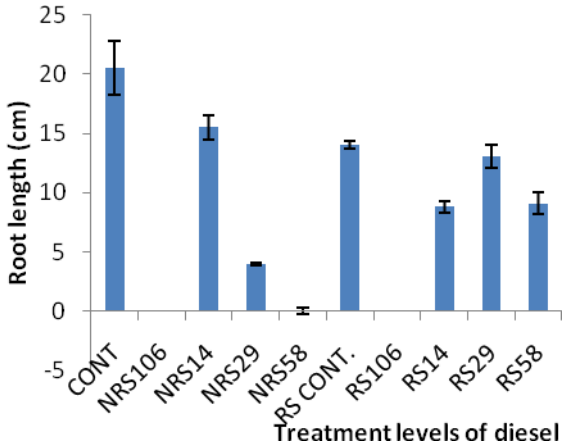

Figure 7: The root length of maize on the different media

The root length (figure 7) of the maize varies significantly at $p<0.05$ in all the contamination levels. From the results obtained, it was estimated that; the NRS-Control has the highest root length (20.50cm), followed by NRS14 with 15.50cm, RS-Control with 14.00cm, RS29 with 13.07cm, RS58 with 9.10cm, RS14 with 8.80cm, NRS29 with 3.97cm and the following, NRS106, NRS58 and RS106 were not having root length (0.00cm). Also it could be due to smearing of plant roots with oil substance forming hydrophobic layer which reduced water absorption and nutrient uptake thereby limiting growth and production of roots. Also Njoku *et al*. (2008) reported that the major effect of petroleum products to the soil is the disruption of the absorption and uptake of essential nutrients by plant roots. However, the ability of plant roots to grow in a polluted soil was reduced by the severe diesel oil pollution level (2.0 litres). This could be due to stress imposed by high diesel oil dose, which limits nutrient availability, water uptake and lack of oxygen for root growth. Hydrocarbon affects plant directly, smearing roots of plants with oily substances and thus reducing or limiting transpiration and respiration by plants, reducing permeability of cell membrane, upsetting metabolic conversions leading to changes in the chemical composition and lastly through the toxic of some hydrocarbon on plants (Pazeshiki *et al.*, 2000).

SHOOT HEIGHT (cm)

Table 2: The shoot length of maize grown on different media

Treatments	Mean shoot heights
Non Remediated soil(NRS) Control	8.50[bc]
Non Remediated soil (NRS) 106	0.00[e]
Non Remediated soil (NRS) 14	13.85[a]
Non Remediated soil (NRS) 29	3.00d
Non Remediated soil (NRS) 58	0.00[e]
Remediated Soil (RS).	10.00[b]
Remediated Soil (RS) 106	0.00[e]
Remediated Soil (RS) 14	10.88[b]
Remediated Soil (RS) 29	13.50[a]
Remediated Soil (RS) 58	7.68[c]
P- Value	2.87

[a, b, bc, c, d and e] means in the same column differ significantly at ($P < 0.05$)

The shoot height (Table 2) of maize in the various levels is significant at $p<0.05$. However the results shows that NRS14 has the highest (13.85cm) shoot height, followed by RS29 with 13.50cm, RS14 with 10.88cm, RS-Control with 10.00cm, NRS- Control with 8.50cm, RS58 with 7.68cm, NRS29 with 3.00cm, and the following levels have 0.00cm shoot height, these are NRS58, NRS106, and RS106.

CONCLUSION

Based on the findings of this research, the highest fruiting bodies of *pleurotus ostreatus* were found on the RS29 media, this same level gave the greatest degree of degradation as it has the highest germination percentage. However, highest leaf area index, plant height, root length and shoot length of maize were found on the NRS-Control. There was no growth of *Zea mays* on some of the media; which indicated that, the use of fungus (*Plearotus ostreatus*) and plants (maize) for both mycoremediation and phytoremediation has a limit.

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