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RESEARCH ARTICLE

BIOSTIMULATORY EFFECT OF CATTLE DUNG ON LEAD DECONTAMINATION POTENTIAL OF INDIGENOUS FUNGAL POPULATION ISOLATED FROM SPENT ENGINE OIL-POLLUTED SOIL

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ABSTRACT

This study assessed the effect of cattle dung (CD) enhanced with fungi isolated from spent engine oil (SEO)-polluted soil on Lead (Pb) decontamination. Twenty plastic bottles containing 20 g of soil each were prepared with varying weights of CD and volumes of fungal isolates in potato dextrose broth each. The mixture was incubated at room temperature. It was a 4 x 2 factorial experiment. Atomic absorption spectrophotometer was employed to determine Pb decontamination of the bio-enhanced fungal consortium in the 2nd, 4th and 6th week of incubation. At the 2nd week, there was no significant ($p > 0.05$) difference between the addition of 10 g of CD (0.1750 mg/kg) and 15 g of CD (0.1750 mg/kg). At the 6th week, the lowest concentration (0.0400 mg/kg) of Pb was recorded with the addition of 20 g of CD and inoculation with fungal isolate (15 mL). Fungi bio-enhanced with CD influenced decontamination of Pb in SEO-contaminated soils in this study.

KEYWORDS

Bioremediation, Bioenhancement, Fungal consortium, Soil, Lead pollution

1. INTRODUCTION

The earth's crust contains trace amounts of Lead (Pb), an element that is naturally occurring and bluish-gray in colour (Tchounwou et al., 2012). The authors reported further that although Pb naturally occurs in the environment, human activities like burning fossil fuels, mining, and manufacturing cause significant concentrations to be released. Many industrial, agricultural, and domestic uses exist for Pb. Pb poisoning's most vulnerable victim is the nervous system coupled with early signs; headache, poor attention span, irritability, drowsiness, and memory loss [Centers for Disease Control and Prevention (CDC, 2001). Pb is a highly poisonous heavy metal that interferes with a number of physiological processes in plants but, unlike other metals like Zinc, Copper, and Manganese, has no biological roles that it plays (Jaishankar et al., 2014).

Due to the piston blow-by of leaded gasoline, detergent additives containing Calcium salt and Zinc, and anti-corrosion/anti-oxidation additives containing Zinc, spent engine oil (SEO) has relatively high concentrations of Pb, Calcium, and Zinc (Stout et al., 2018). If these additives were to enter soil, they could be harmful to human health or the environment. Due to their high incidence as contaminants, low biota solubility, and classification as carcinogens and mutagens, heavy metals pose a serious threat to human health and environmental issues (Brooks, 1998). When consumed in quantities too concentrated for the body to handle, heavy metals can have detrimental consequences on human health. These metals also persist in the environment indefinitely because they cannot be converted into harmless compounds (Jones et al., 2000). Because of their toxicity and threat to human life and the environment, heavy metal pollution is a major concern on a global scale (Van Deuren et al., 2002).

With contributions from a range of industrial and home sources, heavy metal concentrations in all habitats, including the air, water, and soil, are sometimes rising to dangerous levels (Koning et al., 2000). The health and ecosystems are seriously threatened by surroundings that are contaminated with heavy metals. An innovative strategy to improve and increase the remediation efficacy of heavy metals is the direct employment of microorganisms with distinctive properties of catabolic potential and/or their products, such as enzymes and biosurfactants (Schenk et al., 2012; Le et al., 2017). Various options have also been expected to expand the use of microbiological approaches for heavy metal cleanup (Igiri et al., 2018). Due to the toxic nature of Pb, it should be expunged from the environment to avert its associated deleterious effects. This research adopted a locally available material in Cattle Dung (CD) with a view to facilitating the indigenous fungal consortium in its decontamination. Owing to the availability of CD coupled with its sustainability for bio-enhancement of indigenous fungal population, this study was conducted based on the null hypothesis that stated that CD has no biostimulatory effect on the ability of indigenous fungal population to decontaminate Pb in SEO-polluted soil.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the Biotechnology Laboratory at Federal University Dutse, Jigawa state, Nigeria. In the northern Nigerian city of Dutse is where Federal University Dutse is situated. It is located longitudinally at 9.2875E and latitudinally at 11.7333N. It serves as Jigawa State's capital. The Federal University of Dutse opened its doors in November 2011.

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2.2 Collection of Soil Samples

The soil sample wherein indigenous fungi were isolated and employed for bioaugmentation in this study was randomly collected following the procedure described from four (4) different regions at Mechanic Village, Dutse with the aid of a sterilized soil auger. This was done by collecting about 3 g of SEO-polluted soil at a depth of 15 cm. However, the soil sample (3 kg) used for heavy metal decontamination assay was collected with the aid of a sterilized soil auger from four (4) spots at Mechanic Village, Dutse by (Adeleye et al., 2020a).

2.3 Collection and Processing of Cattle Dung

About three (3) kg of CD was collected from the liarage of Dutse Abattoir, Jigawa State. All extraneous materials were removed from the CD. It was sun-dried and crushed into powder to expand the surface area using pestle and mortar. It was sieved into fine form in order to obtain a homogeneous mixture and then sterilized by autoclaving at 121°C for 15 minutes as described by (Ezekoye et al., 2017; Adeleye et al., 2022a). The CD was subsequently stored in an air tight container and labeled appropriately.

2.4 Determination of the Physicochemical Properties of SEO-Polluted Soil and Cattle Dung

Samples of the SEO-polluted soil and CD were analyzed for pH values and electrical conductivity (EC) in deionized water (1: 2.5 w/v for soil, and 1: 5 w/v for CD). Organic carbon was determined by the modified Walkley-Black procedure, whereas, the cation exchange capacity (CEC) was estimated by the summation method of (Chapman, 1965; Nelson and Sommers, 1996). The total nitrogen and phosphorous contents of the SEO-polluted soil and CD were determined by the Kjeldhal and Bray-1 method in reference to (Reeuwijk, 1993; Bremmer, 1996). The soil mechanical analysis of the SEO-polluted soil was equally estimated by the hydrometer method of (Bouyoucos, 1962).

2.5 Media Preparation

All the media were obtained from the Microbiology and Biotechnology Laboratory domiciled at the Faculty of Science, Federal University Dutse. The agar was prepared by suspending 8 g of potato dextrose agar (PDA) in 235 mL of distilled following the manufacturer's guide. This mixture was stirred in order to obtain a homogeneous form. The agar mixture was autoclaved at 121 °C for 15 minutes. The procedure described was employed in the preparation of chloramphenicol added to the mixture with a view to averting possible growth of bacteria by (Heller and Spence, 2019). The mixture was then poured aseptically into 24 petri dishes. Thereafter, the poured agar in the plates was allowed to solidify.

2.6 Preparation of Mineral Salt Medium

According to 0.1 g NaCl, 1.8g KH_2PO_4, 0.2g $MgSO_4.7H_2SO_7$, 0.01 g $FeSO_4$. 7H2O, and 4.0g NH_4Cl were dissolved in one (1) Liter of distilled water to create a medium that contained the essential nutrients (potassium, magnesium, sodium, phosphorus, and ammonium salts) needed by the targeted fungi for their optimum growth. The mineral salt medium (MSM) was then sterilized for fifteen (15) minutes at 121 °C in the autoclave. It was later aseptically dispensed into sterilized test tubes for onward usage (Mukred et al., 2008).

2.7 Isolation of Lead Utilizing Fungi in the Spent Engine Oil-Polluted Soil

The methods described were altered with the goal of isolating Pb using fungi from the SEO-polluted soil by (Mailafia et al., 2017; Adeleye et al., 2022a). To do this, 1 g of the soil that had been polluted with SEO was weighed into three (3) test tubes that each held 9 mL of distilled water. Shaking the mixture briskly allowed space for the production of supernatant. After that, 0.5 mL of SEO was added to each test tube of MSM before 1 mL of the supernatant was added. The test tube mixture was then forcefully mixed and given time to settle. After five (5) days of room temperature incubation, fungal growth in the form of turbidity was visible in the test tubes. A sterile wire loop was used to inoculate the observed growth into tubes of potato dextrose broth (PDB). The PDB tubes were then kept at room temperature for five (5) days on the bench. Following incubation, fungal growth was streaked aseptically on PDA as described and subsequently cultured at 28 °C for five (5) days. Sub-culturing each of the many colonies that emerged onto sterilized PDA plates and incubation at 28 °C for 5 days allowed for the creation and maintenance of a pure culture by (Tudararao-Aherobo and Mesogboriwon, 2022).

2.8 Identification of Lead Utilizing Fungi in the Spent Engine Oil-Polluted Soil

Using cultural and morphological characteristics such as colony development pattern, conidial morphology, and pigmentation, the fungal isolates were identified. Cotton blue in Lactophenol stain was used to identify the isolated fungi by employing the method described by (Oyeleke and Manga., 2008). Using a mounting needle and a little piece of the aerial mycelia from the representative fungal cultures, the stain was applied to a clean slide to make the identification. This was followed by adding a drop of Lactophenol. On the slide with the needle, the mycelium was evenly distributed. To remove air bubbles, a cover slip was put delicately and lightly. After mounting, the slide was examined using a light microscope equipped with x10 and x40 objective lenses. The procedure described was employed to identify the physical attributes and appearance of the observed fungal species by (Onuorah et al., 2015).

2.9 Preparation of Soil Sample for Heavy Metal Decontamination Assay

The soil was air dried, sieved with 2 mm mesh and then sterilized by autoclaving at 121 °C for 15 minutes (Adeleye et al., 2022a). Chloramphenicol was added to the SEO-polluted soil in each experimental pot after sterilization in order to inhibit any bacterial growth and survival. One hundred and fifty grams (150 g) of SEO-polluted soil was placed in twenty four (24) experimental pots and labeled appropriately. Fifteen milliliters (15 mL) of sterilized PDB prepared according to the instructions of the manufacturer and had been inoculated with all the isolated fungi was seeded into twenty (20) plastic bottles containing the SEO-polluted soil. Four (4) plastic bottles containing the SEO-polluted soil did not receive such bioaugmentation, thus, served as the controls. The incubation study was conducted at room temperature in the Department of Microbiology laboratory, Federal University Dutse, Nigeria.

2.10 Experimental Design

The experimental design was completely randomized design (CRD). It was a 4 x 2 factorial experiment with three (3) replicates. There were eight (8) treatment combinations and a total of twenty four (24) experimental units. The factors are listed as follows;

CD at four levels	Fungal isolates at two levels
0 g/pot (C_0)	0 mL (F_0)
10 g/pot (C_1)	15 mL (F_1)
15 g/pot (C_2)	
20 g/pot (C_3)	

2.11 Estimation of Lead in the Spent Engine Oil-Polluted Soil

The procedure reported by was used to estimate Pb concentrations in the SEO-polluted soil at the second week, fourth week and sixth week of bio-degradation assay using Perkin Elmer Atomic Absorption Spectrophotometer Analyst 400 American Public Health Association (APHA, 2012).

2.12 Experimental Scheme

Numerous processes involved in the experimentation ranging from collection of SEO-polluted soils and CD, determination of the physico-chemical properties of SEO-polluted soils and CD, processing of SEO-polluted soils and CD, isolation of indigenous fungal population in the SEO-polluted soils, seeding of SEO-polluted soils with CD, inoculation of SEO-polluted soils with fungal isolates, preparation of SEO-polluted soil for biodegradation assay to the periodic collection of soil samples for the estimation of Pb using AAS are depicted in Figure 1.

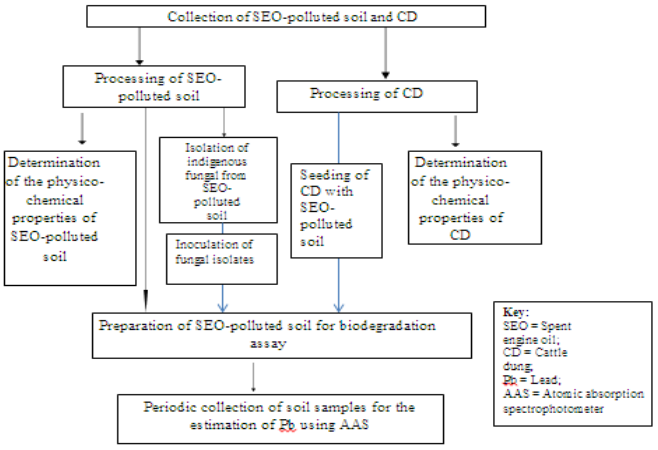

Figure 1: An experimental scheme depicting the processes of experimentation

2.13 Data Analysis

All data collected were subjected to Analysis of Variance (ANOVA) using Proc. GLM of GenStat version 17 and significant means were separated using Duncan's Multiple Range Test.

3. Results and Discussion

Table 1: Physicochemical Properties of Spent Engine Oil Polluted Soil and Cattle Dung

Parameters	SEO-Polluted Soil	Cattle Dung
Moisture Content (%)	0.6	6.9
Ash Content (%)	-	63.2
pH (water)	5.9	8.12
Organic Carbon (%)	0.49	40.02
Total Nitrogen (%)	0.06	2.55
Available Phosphorus (mg/kg)	9.29	1.0
EC (dS/cm)	1.0	8.0
Exchangeable Bases (cmol/kg)		
Potassium	0.05	75
Calcium	0.60	0.2
Magnesium	0.15	1.4
Sodium	0.14	0.3
CEC	1.02	81.1
Particle Size (g/kg)		-
Clay + Silt	190	-
Clay	110	-
Silt	70	-
Sand	785	-
Textural class	Loamy Sand	-

CEC = Cation Exchange Capacity, EC = Electrical Conductivity; SEO = Spent Engine Oil

Results obtained from the physicochemical properties of the spent engine oil polluted soil and cattle dung are presented in Table 1. Higher values of the following parameters were obtained from the SEO polluted soil; Available phosphorus (9.29 mg/kg) and pH (5.9). For the CD, higher values were obtained for the following parameters; Ash content (63.2%) and Organic Carbon (40.02%). The Cation Exchange Capacity (CEC) for SEO-polluted soil and CD were recorded to be 1.02 and 81.1 cmol/kg respectively (Table 1).

The results in Tables 2 and 3 depict the colonial and morphological features of the fungal isolates on PDA in terms of colour, texture, reverse, and growth rate. It can be seen that the isolation of the fungal inoculants from the SEO-polluted soil on PDA led to the identification of five fungal species; *Aspergillus* sp., *Penicillium* sp., *Talaromyces* sp., *Rhodosporidium* sp., and *Trichoderma* sp. (Table 3). Analogous fungi isolated in this study have been reported in their study of soil mycoflora with root rot disease in Malaysia rubber plantation. Similarly, did isolate *Aspergillus glaucus*, *Trichoderma polysporum* and *Talaromyces flavus* from a typical SEO-polluted soil in Delta State, Nigeria by (Go et al., 2015; Tudararao-Aherobo and Mesogboriwon, 2020).

Table 2: Colonial Characteristics of Fungal Isolates on Potato Dextrose Agar

Fungal Isolate ID	Colour	Texture	Reverse	Growth Rate
C_0F_0 (a)	Greyish white	Cotton	White	Fast
C_0F_0 (b)	Greyish white	Cotton	White	Fast
C_0F_0 (b)	White to purple	Moist	Creamy	Medium
C_0F_1 (a)	White	Fluffy	Red pigment	Very fast
C_0F_1 (b)	White to pinkish	Moist, Effuse	Red pigment	Fast
C_0F_1 (c)	Greyish white	Powdery	Pinkish	Fast
C_1F_0(a)	Greyish yellow	Filamentous	Pale Yellow	Fast
C_1F_0 (b)	Dark green	Powdery	Creamy	Medium
C_1F_0 (c)	Greyish Yellow	Fluffy	Yellow	Very fast
C_1F_1 (b)	Dark green	Filamentous	Pale green	Medium
C_1F_1 (c)	Yellowish white	Effuse, moist	Creamy	Slow
C_2F_0 (a)	Pink	Watery	Pinkish white	Fast
C_2F_0 (b)	White	Moist	Creamy	Very Fast
C_2F_0 (c)	Pink	Moist	Creamy	Fast
C_2F_1 (a)	Greyish Yellow	Cotton	Creamy	Fast
C_2F_1 (b)	Dark green	Fluffy	Creamy	Medium
C_2F_1 (c)	Cotton	Fluffy	Pale Yellow	Medium
C_3F_0 (a)	Pink	Cotton	Creamy	Fast
C_3F_0 (b)	Yellow	Watery	Pale green	Very Fast
C_3F_0 (c)	White	Filamentous	White	Medium
C_3F_1 (a)	Dark green	Filamentous	Creamy	Fast
C_3F_1 (b)	Yellowish white	Fluffy	Pale yellow	Medium
C_3F_1 (c)	Pink	Moist	White	Fast

(a)= first replicate; (b) = second replicate; (c) = third replicate

Table 3: Microscopic Characteristics of Fungal Isolates on Potato Dextrose Agar		
Fungal Isolates ID	**Microscopic Characteristics**	**Identity**
C_0F_1 (c), C_2F_1 (a), C_1F_0 (b), C_0F_1 (b), C_3F_1 (c), C_3F_0 (a), C_2F_1 (a), C_2F_1 (b).	Hyphae septate; Conidiophores well-developed, hyaline, spore aggregate in a row; Conidia in chain, 1-celled, globose; Penicillus well developed.	*Penicillium* sp.
C_0F_0 (a), C_0F_1 (b), C_2F_1 (a), C_2F_0 (c), C_3F_0 (c), C_2F_0 (b).	Conidial head biseriate, radiate, conidia in chains or detached and dispersed. Single or paired conidia may resemble yeast cells.	*Aspergillus* sp.
C_3F_1 (a),C_2F_1 (c), C_3F_0(b)	Asomata without distinct walls or covered with hyphal networks; borne in chains; Conidial state Penicillium, but belonging to other series, green, greyish or olive-brown	*Talaromyces* sp.
C_2F_0 (a), C_3F_0 (b)	Short chains of budding cells unbranched.	*Rhodosporidium* sp.
C_2F_1 (c), C_3F_1(b)	Conidiophores hyaline, regularly branched.	*Trichoderma* sp.

(a)= first replicate; (b) = second replicate; (c) = third replicate

The concentrations of Pb in the soil samples were analyzed to check for changes at the second, fourth and sixth weeks after inoculation with CD, all of which are listed in Tables 4-9. Findings from this study have shown that the Pb content of the SEO-polluted soil was significantly ($p< 0.05$) influenced by its inoculation with fungal isolates, addition of CD, and the combinatorial effect of the two factors.

Table 4: Effect of Cattle Dung and Fungal Isolates on Pb Content of Spent Engine Oil Polluted Soil at two weeks after Inoculation	
Treatments	Pb content (mg/kg)
Cattle Dung (g/pot)	
C_0	0.2400a
C_{10}	0.1750b
C_{15}	0.1750b
C_{20}	0.1050c
SE (±)	0.0145
Fungal isolate (mL/pot)	
F_0	0.1825
F_{15}	0.1650 NS
SE (±)	0.0103

Means with the same letter(s) are not significantly different from each other at $p> 0.05$ using Duncan's multiple range test, C_0, C_{10}, C_{15} and C_{20} = cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = fungal isolate at 0 and 15 mL/pot, NS = not significant, SE = standard error.

Table 5: Interaction of Cattle Dung with Fungal Isolates on Pb Content of Spent Engine Oil-Polluted Soil at two weeks after Inoculation		
Cattle dung (g/pot)	Fungal isolate (mL/pot)	Pb content (mg/kg)
C_0	F_0	0.3000a
C_0	F_{15}	0.1800b
C_{10}	F_0	0.1700bc
C_{10}	F_{15}	0.1800b
C_{15}	F_0	0.1500bcd
C_{15}	F_{15}	0.2000b
C_{20}	F_0	0.1100cd
C_{20}	F_{15}	0.1000d

Means with the same letter(s) are not significantly different from each other at $p> 0.05$ using Duncan's multiple range test, C_0, C_{10}, C_{15} and C_{20} = Cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = fungal isolate at 0 and 15 mL/pot.

Specifically, at the second week, there was no significant ($p> 0.05$) difference between the addition of 10 g of CD (0.1750 mg/kg) and 15 g of CD (0.1750 mg/kg) (Table 5). However, addition of 20 g of CD (0.1050 mg/kg) resulted in a significant ($p< 0.05$) reduction in the concentration of Pb (Table 5). The combinatorial effect of the addition of 20 g of CD (C_{20}) and inoculation with fungal isolate at 15 mL (F_{15}) resulted in the least concentration (0.1000 mg/kg) of Pb at the second week (Table 6). The reductions in Pb concentrations recorded at two weeks are in line with microbial decontamination ability reported by Dixit *et al*. (2015). At the fourth week, there was no significant ($p>0.05$) difference between the addition of CD at 10 g (C_{10}), 15 g (C_{15}) and 20g (C_{20}) (Table 7). Again, there was no significant ($p> 0.05$) difference between inoculation with fungal isolates at 0 m/L (F_0) and 15 m/L (F_{15}) at the fourth week (Table 8). The combinatorial effect of CD at 20 g (C_{20}) and 15 m/L (F_{15}) of fungal isolate resulted in a significant ($p< 0.05$) reduction in Pb concentration (0.0900 mg/kg) at the fourth week (Table 8). Significant ($p< 0.05$) difference can be seen in the combinatorial effect of C_0F_0 and other treatments that were not significantly ($p> 0.05$) different from each other (Table 9).

Table 6: Effect of Cattle Dung and Fungal Isolates on Pb Content of Spent Engine Oil Polluted Soil at four weeks after Inoculation	
Treatments	Pb content (mg/kg)
Cattle dung (g/pot)	
C_0	0.2300a
C_{10}	0.1400b
C_{15}	0.1350b
C_{20}	0.0950b
SE (±)	0.0151
Fungal isolate (mL/pot)	
F_0	0.1650
F_{15}	0.1350
LSD (0.05)	0.0324
SE (±)	0.0107

Means with the same letter(s) are not significantly different from each other at $p> 0.05$ using Duncan's multiple range test, C_0, C_{10}, C_{15} and C_{20} = Cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = fungal isolate at 0 and 15 mL/pot, SE = standard error, LSD = least significant difference.

Table 7: Interaction of Cattle Dung with Fungal Isolates on Pb Content of Spent Engine Oil Polluted Soil at four weeks after Inoculation		
Cattle dung (g/pot)	Fungal isolate (mL/pot)	Pb content (mg/kg)
C_0	F_0	0.3000a
C_0	F_{15}	0.1600bc
C_{10}	F_0	0.1500bc
C_{10}	F_{15}	0.1200bc
C_{15}	F_0	0.1100bc
C_{15}	F_{15}	0.1700b
C_{20}	F_0	0.1000bc
C_{20}	F_{15}	0.0900c

Means with the same letter(s) are not significantly different from each other at $p> 0.05$ using Duncan's multiple range test. C_0, C_{10}, C_{15} and C_{20} = Cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = Fungal isolate at 0 and 15 mL/pot

Table 8: Effect of Cattle Dung and Fungal Isolates on Pb Content of Spent Engine Oil-Polluted Soil at six weeks after Inoculation	
Treatments	Pb content (mg/kg)
Cattle dung (g/pot)	
C_0	0.2000a
C_{10}	0.0850b
C_{15}	0.0650b
C_{20}	0.0550b
SE (±)	0.0137
Fungal isolate (mL/pot)	
F_0	0.1300
F_{15}	0.0725
LSD (0.05)	0.0294
SE (±)	0.0097

Means with the same letter(s) are not significantly different from each other at p< 0.05 using Duncan's multiple range test.C_0, C_{10}, C_{15} and C_{20} = Cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = Fungal isolate at 0 and 15 mL/pot, SE = standard error, LSD = least significant difference

Table 9: Interaction of Cattle Dung with Fungal Isolates on Pb Content of Spent Engine Oil Polluted Soil at six weeks after Inoculation		
Cattle dung	Fungal isolate	Pb content (mg/kg)
C_0	F_0	0.2900a
C_0	F_{15}	0.1100b
C_{10}	F_0	0.0900bc
C_{10}	F_{15}	0.0800bc
C_{15}	F_0	0.0700bc
C_{15}	F_{15}	0.0600bc
C_{20}	F_0	0.0700bc
C_{20}	F_{15}	0.0400c

Means with the same letter(s) are not significantly different from each other at p< 0.05 using Duncan's multiple range test. C_0, C_{10}, C_{15} and C_{20} = Cattle dung at 0, 10, 15 and 20 g/pot. F_0 and F_{15} = Fungal isolate at 0 and 15 mL/pot.

These results are in concord with the findings of who reported that the success of any bioremediation project depends on the ability to establish and maintain conditions that favour enhanced biodegradation rates in the contaminated environment (Das and Chandran, 2011). At the sixth week, the lowest concentration (0.0400 mg/kg) of Pb was recorded with the addition of 20 g (C_{20}) of CD and inoculation with 15 m/L (F_{15}) of fungal isolate. The overall results from this study indicate that indigenous fungal population, bio-enhanced with CD had the ability to influence decontamination of Pb in SEO-polluted soils. These results further support the submission of who reported that microorganisms when aided with nutrients are able to decontaminate heavy metals in environmental media (Das and Chandran, 2011; Adeleye et al., 2022b). The results also corroborate the study of which found out that microbial population when aided with nutrients are able to bio-remediate hydrocarbon impacted environment as a result of increased microbial activity (Azubuike et al., 2016). Therefore, the reduction in Pb concentrations in this study may be attributable to the addition of the nutrients in the CD, which significantly enhanced Pb decontamination capability of the indigenous fungal population seeded with the SEO-polluted soil.

4. Conclusion

This study concludes that indigenous fungal population bio-enhanced with CD had the ability to decontaminate Pb in SEO-polluted soil. Significant reductions in Pb concentrations were recorded in the SEO-polluted soil samples bioaugmented with fungal isolates and biostimulated with CD during this study. Therefore, the null hypothesis earlier stated is rejected and the alternative hypothesis which stated that CD has biostimulatory effect on the ability of indigenous fungal population to decontaminate Pb in the SEO-polluted soil is accepted. However, the key shortcoming of this study has to do with the sole biodegradation assay of Pb in the SEO-polluted soil as other heavy metals of environmental concern were not assayed. This shortcoming can be a research focus in the nearest future with a view to assessing the decontamination prowess of the isolated fungi on many heavy metals impacting the environment.

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