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REMEDIATION POTENTIAL OF (*Pleurotus ostreatus* AND *Eisenia fetida)* ON SOME HEAVY METALS CONTENTS OF CRUDE OIL POLLUTED SOIL IN RIVERS STATE

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ABSTRACT

This study evaluated the effectiveness of *Eisenia fetida* (earthworm) and *Pleurotus ostreatus* (mushroom) in remediating heavy metals in soil polluted with crude oil at concentrations of 0%, 5%, 7.5%, and 10% over six months. Standard chemical methods measured heavy metal contents, revealing that levels of Lead, Iron, Chromium, and Cadmium increased with higher crude oil concentrations. Lead levels rose from 0.39 mg/kg at 0% to 19.6 mg/kg at 10%, while Iron ranged from 26.1 to 3.27 mg/kg. After three months, mushrooms reduced heavy metals more effectively than earthworms, which showed minimal reduction at both three and six months. At 10% crude oil pollution over six months, mushrooms reduced Lead by 90%, Iron by 95%, Chromium by 75%, and Cadmium by 64%. Earthworms reduced lead by 70%, Iron by 60%, Chromium by 68%, and Cadmium by 54%. The study concluded that while both *P. ostreatus* and *E. fetida* supported soil remediation, *P. ostreatus* was more effective.

Keywords: Remediation, *Pleurotus ostreatus, Eisenia fetida*, heavy metals, crude oil

INTRODUCTION

Illegal bunkering activities and industrial revolution in Niger Delta region has resulted to environmental pollution and eventually low crop production in the affected areas (Ozogu *et al.,* 2023). Several remediation techniques are available, but the most preferred are the environmentally safe and cost efficient natural control techniques (Ugrina, and Jurić, 2023). Wasewar *et al.,* (2019) noted that a wide variety of harmful organic and inorganic substances are routinely discharged into the environment, either intentionally or accidently, causing public concern. Common example of toxic chemicals that constantly pollute the environment in large quantities through numerous channels is petroleum (Truskewycz *et al.,* 2019). Oil pollution has the greatest immediate and economic impact it has adverse effects to a great level on the ecosystem not only on an isolated environment (Ugwu *et al.,* 2020). Manisalidis *et al.* (2020) emphasized that the problem with environmental pollution is enormous and interferes with the balance of the ecosystem. Kumar and Chandra (2020) highlighted some species of fungi that have been reported to have the potential to break crude oil by secreting extracellular enzymes required for the oxidation of complex aromatic compounds such as lignin degrading enzymes. Some other fungal species have also been shown to help plants in the uptake of nutrients and water like phosphorus and micronutrients from the soil (Hestrin *et al.*, 2019). The use of fungi as hyper accumulators in heavy metal polluted soils have been documented (Tovar-Sánchez *et al.,* 2023). Vermiremediation (use of earthworm for remediation) which aims to improve the fertility of the soil, is a suitable, environmentally friendly and low-cost technique for tackling soil pollution, for a reason mentioned above this technology is currently gaining ground (Mishra *et al.,* 2022). Generally, earthworms can adapt to wide range of chemical pollutants in soil as well as organic pollutants and heavy metals and have been documented to accumulate part of them in their tissues (Mishra *et al.,* 2022). The use of selected fungi and earthworm species in remediation of crude oil contaminated sites in relation to comparison of their biodegradation potentials of heavy metals contents in soil have not been extensively investigated (Zhang *et al.,* 2020). Therefore, this present study was conducted to examine the remediation potential of *Pleurotus ostreatus* and *Eisenia fitida* on some heavy metals contents of crude oil polluted soil.

MATERIALS AND METHODS

Study Area

This research was conducted at the Arboretum of Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria (4.7958° N, 7.0246° E). The study area is characterized by a tropical wet climate with a long rainy season, a short dry season, an average annual temperature of 26.4°C, and an average annual rainfall of 2629 mm.

Sterilization of Materials for the Experiment

This experiment was conducted under controlled conditions. All the glassware was washed with detergent, rinsed with distilled water, wrapped in aluminum foil, and then sterilized in an oven and autoclaved at 121°C for 15 minutes.

Source of Culture and Spawn Preparation

Pleurotus ostreatus was obtained from Dilomat and propagated at the mushroom center of Rivers State University, Nkpolu Oroworukwo, where pure mycelia culture was aseptically isolated. Sorghum grains were used to make the spawn by rinsing and soaking them in tap water for 24 hours, then boiling them for 15 minutes in a 1:1 ratio (sorghum grains to water). Following Chukunda and Simbi-Wellington (2019), 4% calcium carbonate ($CaCO_3$) was added to adjust the pH, and 2% calcium sulfate ($CaSO_4$) was added to prevent grain clumping. The sorghum grains were then filtered, placed in glass bottles, sealed with aluminum foil, and sterilized at 121°C for 30 minutes. After cooling, *Pleurotus ostreatus* mycelia were aseptically inoculated into the sorghum and cultured in the dark at 27±2°C for 10-15 days to ensure complete colonization by the fungal mycelia.

Source and Identification of Earthworm Species

Earthworms were collected from moist forest soil using the hand sorting method (Edwards, 2004), placed into jars with some natural soil, and transported to the laboratory. There, they were washed and stored in a 10% formalin solution. Identification was carried out using the morphological method of Yousefi *et al.,* (2009), the internal anatomical method of Ismail (2005), and the setae form and organization method of Malek (2007). *Eisenia fetida* was chosen for its resilience to environmental stress (Contreras-Ramos et al., 2008). This species is widely available, easy to cultivate in laboratory conditions, and is an epigeic earthworm that spends most of its life on or near the soil surface.

Multiplication of Earthworms for Experiment

An appropriate quantity of identified earthworm species was grown and replicated for three months using a mix of cow dung, banana roots, and garden soil in a 3:1:1 ratio. A shaded area was cleared, and a shallow 3 cm excavation was made. A polyethylene bag was placed in the excavation, covered by an old motor tire to prevent earthworm escape. Layers were added in the following order: clay soil to absorb moisture, garden soil to mimic a natural environment, cow dung to support microbial degradation of banana roots, banana root fragments to provide nutrients, and garden soil to protect the surface. A 5 cm shallow well was created at the center, where earthworms were placed, moistened with water, and covered with polyethylene to retain moisture and prevent escape. The setup was watered twice weekly for three to six months (Anon *et al.,* 2017).

Preparation of Soil for Screening for the Bioremediative Potential of *P. ostreatus* and *Eisenia fetida* on Crude Oil

The technique used for the screening of the bioremediation properties of *P. ostreatus* and *Eisenia fetida* was done by using Purnomo *et al.,* (2010) method. Three Kilogram of agricultural soil was weighed using a laboratory weighing balance into a rectangular basket of 9.31m^2, laid with a cloth sack bag to absorb and retain moisture, and excess crude oil and also prevent escape of earthworms but allow adequate ventilation for both earthworm and mushroom. The soil polluted with crude oil in varying concentrations via (0,5, 7.5, and 10% were properly labelled. The experiment used 60 baskets (12 baskets in five replicates for each treatment) and was conducted as a 3x4 factorial experiment in a completely randomized design with 5 replications. The factors considered were soil treatments (soil only, soil with earthworms, and soil with mushrooms) and crude oil concentrations (0%, 5%, 7.5%, and 10%). The setup included three soil treatments and four pollution levels.

In a Forestry Laboratory at Rivers State University, artificially polluted soil was ventilated for two weeks to eliminate toxic volatile components, and simulate natural crude weathering. After this period, 1.5 kg of coconut coir was spread over the soil and moistened with sterile distilled water to create a favorable environment for earthworms and mushrooms. Ten grams of actively growing mycelium of *P. ostreatus* were aseptically weighed and inoculated into each appropriately labeled basket containing the soil and coconut coir mixture. On the 15^{th} day, 20 grams of clitellated earthworms (*Eisenia fetida*) were weighed using a laboratory balance and added to the soil. The earthworms were fed weekly with 100 grams of a carrot-cabbage mixture in a 3:1 ratio. Two control sets were maintained: one with artificial soil and earthworms but no crude oil to test for background mortality in uncontaminated soil, and another with artificial soil and crude oil but no earthworms to assess the natural rate of crude oil degradation without earthworms.

Analyses of Heavy Metals on different treated Soil Samples

Heavy metals analyses were performed on each treated soil samples treated with crude oil, earthworms and mushrooms. For each treatment, the mean and standard error of five replicates were calculated and presented. Heavy metal concentrations were analysed using a Shimadzu Atomic Absorption Spectrophotometer (model 6650F) following APHA 3111B guidelines. Acidified samples (pH < 2) were directly aspirated into the AAS, and heavy metal concentrations were quantified using calibration curves from individual metal standards. Soil samples were digested using the Addis and Abebaw (2017) wet digestion method. Air-dried, ground and sieved soil samples (0.5g each) were measured in a digestion tube. Aqua regia (6ml) and H_2O_2 (1.5ml) were precisely measured in the digestion tube and gently swirled to homogenize the mixture. The digestion tubes were heated at 180°C for 3 hours in an oven, then cooled, filtered through Whatman No. 42 filter paper, and diluted to 50 mL with distilled water. The samples were stored in acid washed glass vials for metal analysis. Calibration was done using a blank and standard solutions for each metal. A flame atomic absorption spectrometer measured the concentrations of Fe, Cr, Cd, and Pb in the digested samples, with final metal concentrations calculated accordingly using the formula:

$$concentration\ {}^{mg}/_{kg} = \frac{concentration\ \left({}^{mg}/_{l}\right)}{W}\ x\ \frac{V}{1}$$

Where V= final volume (50 mL) of solution, and W = initial weight (0.5g) of sample measurement. The mean and standard error of the five replicates of each sample were determined. Heavy metals concentrations in the test samples were analyzed using standard methods (AOAC, 2000). The Statistical Package for the Social Sciences (SPSS) was used to perform an analysis of variance (ANOVA) on all the collected data. The Duncan Multiple Range Test (DMRT) was then applied to separate and compare the means at a 5 percent significance level.

RESULTS

Composition of Heavy Metals in the Soil Sample

The results of the heavy metal composition in the soil sample show the following: Lead at 0.396 mg/kg, Iron at 26.1 mg/kg, Chromium at 0.562 mg/kg, and Cadmium at less than 0.002 mg/kg.

Table 1: Results of Preliminary Findings on Heavy Metal Composition in the Soil

Parameters	Standard Method	Result (mg/kg)	DPR Intervention Limit
Lead	ASTM D 1971/4691	0.396	530
Iron	ASTM D 1971/4691	26.1	NA
Chromium	ASTM D 1971/4691	0.562	380
Cadmium	ASTM D 1971/4691	<0.002	12

Determination of the Effects of Different Concentrations of Crude Oil Pollution on Heavy Metal Content in the Soil

The results in Table 1 indicate the composition of heavy metals in the soil with 0%, 5%, 7%, and 10% crude oil mixtures significantly ($P < 0.05$) affected the heavy metal content. The data showed that an increase in the concentration of crude oil significantly raised the heavy metal content in the soil. Cadmium was the least abundant in the soil at 0 percent crude oil content, with values of 0.002 mg/Kg, while iron was the most abundant, with a value of 26.1 mg/Kg. At 0% crude oil content, 0.396 mg/Kg of lead was found, followed by 5.96 mg/Kg at 5%, 9.96 mg/Kg at 7.5 %, and 19.6 mg/Kg at 10%. At 0 % to 10% crude oil concentration, iron levels ranged from 26.1mg/Kg to 3.27mg/Kg, while chromium levels ranged from 0.562mg/Kg to 5.78mg/Kg. Cadmium concentrations ranged from 0.002 mg/Kg to 5.72 mg/Kg at 0% to 10% crude oil concentrations, respectively.

Table 2: Composition of Heavy Metals in the Soil with 0%, 5%, 7%, and 10% Crude Oil Mixtures

Heavy Metals (mg/kg)	Concentration of crude oil (%)			
	0	5	7.5	10
Lead	0.396 ± 0.00^{d}	5.94 ± 0.01^{c}	9.60 ± 0.01^{b}	19.4 ± 0.01^{a}
Iron	26.1 ± 1.15^{d}	35.6 ± 1.44^{c}	43.4 ± 0.35^{b}	53.167 ± 1.59^{a}
Chromium	0.562 ± 0.01^{d}	1.62 ± 0.01^{c}	4.11 ± 0.02^{b}	5.76 ± 0.01^{a}
Cadmium	0.002 ± 0.00^{d}	0.98 ± 0.01^{c}	3.59 ± 0.01^{b}	5.70 ± 0.01^{a}

DMRT shows that the means with various letters differ considerably ($P<0.05$).

Quantification and Comparison of the Effects of Bioremediators (Mushrooms and Earthworms) on Heavy Metal Composition in 0% Crude Oil Polluted Soil Over a Period of Three to Six Months

Table 2 shows a significant decrease ($P < 0.05$) in the proportion of heavy metals (lead, iron, chromium, and cadmium) as a result of introducing bioremediators (mushrooms and earthworms). The longer these bioremediators were present in the soil, the greater the reduction in heavy metals, observed over a period from three to six months. Specifically, after six months, mushrooms achieved a more substantial reduction in heavy metal composition compared to earthworms. From three to six months, mushrooms reduced lead by 65%, iron by 14%, and chromium by 60%, while earthworms reduced lead by 45%, iron

Table 3: Quantification and Comparison of the Effects of Bioremediators (Mushrooms and Earthworms) on Heavy Metal Composition in 0% Crude Oil Polluted Soil Over a Period of Three to Six Months

Heavy metals(mg/kg)	Bioremediators			
	Mushroom 3months	Earthworm 3 months	Mushroom 6 months	Earthworm 6 months
Lead	0.183 ± 0.001^{b}	0.273 ± 0.001^{a}	0.064 ± 0.001^{d}	0.156 ± 0.002^{c}
Iron	24.18 ± 0.001^{b}	0.183 ± 0.001^{b}	0.183 ± 0.001^{b}	0.183 ± 0.001^{b}
Chromium	0.389 ± 0.001^{b}	0.183 ± 0.001^{b}	0.183 ± 0.001^{b}	0.183 ± 0.001^{b}
Cadmium	0.00 ± 0.001^{a}	0.00 ± 0.001^{a}	0.00 ± 0.001^{a}	0.00 ± 0.001^{a}

DMRT shows that the means with various letters differ considerably ($P<0.05$).

Determination and Comparison of the Effects of Bioremediators on Lead Composition of Crude Oil Polluted Soil at a Three to Six Month Interval

Tables 4-7 present the effect of bioremediators on the heavy metal composition in crude oil-polluted soil over a three to six month interval. The results showed a significant decrease (P < 0.05) in heavy metal concentrations when using either mushrooms or earthworms. The overall result shows that at a maximum crude oil pollution level of 10%, mushrooms reduced lead content by 90%, iron by 95%, chromium by 75%, and cadmium by 64%. In comparison, earthworms reduced lead content by 70%, iron by 60%, chromium by 68%, and cadmium by 54%.

Table 4: Determination and Comparison of the Effects of Bioremediators on Lead Composition of Crude Oil Polluted Soil at a Three to Six Month Interval

Crude conc. (%)	Polluted Soil	Bioremediators Mushroom 3Month	Mushroom 6Month	Earthworm 3Month	Earthworm 6Month
5	5.96 ± 1.154^{a}	3.27 ± 1.154^{ab}	1.47 ± 0.88^{b}	5.14 ± 1.154^{a}	5.23 ± 1.154^{a}
7.5	9.96 ± 1.154^{a}	5.14 ± 1.154^{bc}	2.02 ± 1.154^{c}	6.14 ± 1.154^{b}	5.7 ± 1.154^{bc}
10	19.6 ± 1.154^{a}	12.5 ± 1.154^{bc}	5.84 ± 1.154^{d}	15.14 ± 1.154^{b}	11.2 ± 1.154^{c}

DMRT shows that the means with various letters differ considerably (P<0.05).

Table 5: Determination and Comparison of the Effects of Bioremediators on the Composition Iron in Crude Oil Polluted Soil at a Three to Six Month Interval

Crude conc. (%)	Polluted Soil	Bioremediators Mushroom 3Month	Earthworm 3Month	Mushroom 6Month	Earthworm 6Month
5	86.1 ± 1.154^{a}	52.1 ± 1.154^{d}	80.5 ± 1.154^{b}	20.5 ± 1.154^{e}	66.3 ± 1.154^{c}
7.5	281 ± 1.154^{a}	164 ± 1.154^{d}	228 ± 1.154^{b}	72.8 ± 1.154^{e}	174 ± 1.154^{c}
10	327 ± 1.154^{a}	248 ± 1.154^{b}	225 ± 1.154^{c}	115 ± 1.154^{e}	198 ± 1.154^{d}

DMRT shows that the means with various letters differ considerably (P<0.05).

Table 6: Determination and Comparison of the Effects of Bioremediators on Composition of Chromium in Crude Oil Polluted Soil at a Three to Six Month Interval

Crude conc. (%)	Polluted Soil	Bioremediators Mushroom 3Months	Earthworm 3Months	Mushroom 6Months	Earthworm 6Months
5	1.74 ± 0.477^{a}	1.52 ± 0.395^{a}	1.53 ± 0.577^{a}	1.25 ± 0.188^{a}	1.45 ± 0.115^{a}
7.5	4.12 ± 0.011^{a}	2.29 ± 0.015^{d}	3.52 ± 0.011^{b}	1.3 ± 0.011^{e}	2.45 ± 0.115^{c}
10	5.78 ± 0.11^{a}	2.69 ± 0.008^{d}	5.27 ± 0.11^{b}	2.3 ± 0.008^{e}	5.123 ± 0.037^{c}

DMRT shows that the means with various letters differ considerably (P<0.05).

Table 7: Determination and Comparison of the Effects of Bioremediators on Cadmium Composition of Crude Oil Polluted Soil at a Three to Six Month Interval

		Bioremediators			
Crude conc. (%)	Polluted Soil	Mushroom 3Month	Earthworm 3Month	Mushroom 6Month	Earthworm 6Month
5	2.06 ± 0.07^{a}	1.62 ± 0.12^{d}	1.88 ± 0.12^{b}	1.33 ± 0.12^{e}	1.73 ± 0.12^{c}
7.5	3.61 ± 0.12^{a}	2.56 ± 0.12^{d}	3.38 ± 0.12^{b}	1.88 ± 0.12^{e}	3.18 ± 0.12^{c}
10	5.72 ± 0.12^{a}	3.44 ± 0.12^{d}	5.21 ± 0.12^{b}	2.73 ± 0.12^{e}	5.06 ± 0.07^{c}

DMRT shows that the means with various letters differ considerably (P<0.05).

DISCUSSION

This study found that crude oil contamination significantly increased the levels of lead, iron, chromium, and cadmium in the soil. As the amount of crude oil increased, so did the concentration of these heavy metals. This finding is consistent with Adesina and Adelasoye (2014), noted that crude oil spills predominantly cause rises in heavy metal concentrations in soil, and Iwegbue (2011), observed much higher metal levels in polluted soils compared to controls. Heavy metal contamination negatively impacts soil quality, plant performance, and microbiological activity (Chibuike and Obiora, 2014). At 5% crude oil pollution, iron reached a maximum concentration of 73.8 mg/kg, with other metals also increasing significantly. While some metals like iron are essential in small amounts, others like lead and cadmium are harmful. Plants' ability to absorb necessary metals also leads them to take in non-essential elements. High concentrations of these metals cannot be broken down and harm plants both internally and externally. Even low levels of crude oil pollution (5%) can significantly increase heavy metal content in soil, adversely affecting the environment and plant health (Adesina and Adelasoye, 2014). Crude oil contamination alters soil fertility and nutrient status (Osu *et al.,* 2021).

This study found that soils polluted with crude oil experienced a greater reduction in heavy metal content when mushrooms were used for bioremediation compared to earthworms. Mushrooms reduced heavy metals by 59-96%, while earthworms achieved reductions of 2-68% at 10% crude oil pollution over six months. Mushrooms were more effective in absorbing heavy metals than earthworms at 5-10% crude oil pollution over three to six months. This aligns with Kapahi and Sachdeva (2017), who reported significant decreases in heavy metal content with mushroom treatment. Earthworms facilitate pollutant biodegradation by enhancing interactions between soil microbes and toxins (Khaldoon *et al.,* 2022). Studies, such as Kavehei *et al.,* (2018), have explored the relationship between earthworms and metal pollutants, suggesting that earthworms use various techniques to clean polluted soil. Tagliabue *et al.,* (2022) proposed that earthworms enhance pollutant removal through soil amendments and microbial activity in their gastrointestinal system. This study supports the idea that earthworms boost microbial activities, aiding in the reduction of heavy metals (Martinkosky *et al.,* 2016).

White-rot fungi have successfully addressed soil contamination through processes such as heavy metal accumulation, pollutant biodegradation, mineralization, biodeterioration, bioremediation, transformation, and co-metabolism (Chen *et al.,* 2022). Echezonachi, (2021) proved that White-rot fungi are increasingly used in bioremediation due to their ability to digest a wide range of persistent or toxic environmental pollutants. Mushrooms, capable of thriving in diverse environments, are ideal for soil remediation as they can break down, absorb, and mineralize contaminants, rendering them harmless (Kulshreshtha *et al.,* 2014). It was found that the presence of heavy metals and other toxins promotes mushroom growth, which leads to the clearance of these toxic metals from the environment (Mohamadhasani and Rahimi, 2022). The purifying properties of mushrooms allow them to scavenge metals from polluted environments (Ogbomida *et al.,* 2018). According to Mohammadkazem and Rahimi (2022), mushrooms produce enzymes that detoxify heavy metals and other pollutants. Wang *et al.,* (2021) noted that fungi, including mushrooms, decompose metal-contaminated soil. Rhaman *et al.,* (2021) explained that mushrooms transport heavy metals to their fruiting bodies by first denaturing the toxins and then absorbing the metals from the soil and environment. Mushrooms are highly effective at absorbing heavy metals, including radioactive ones, thus removing them from the environment.

CONCLUSION

Soil pollution is primarily caused by the release of petroleum oil into the environment, leading to harmful effects on both biotic and abiotic components of the ecosystem. Bioremediation is a highly effective and environmentally friendly method for removing contaminants from various polluted sites. This study revealed that mushrooms are more effective than earthworms in biodegrading and absorbing pollutants, offering a low-cost solution to the problem of increasing heavy metal content in crude oil-polluted soils. Mushrooms demonstrated higher resistance and greater remediation potential at various levels of crude oil pollution. Results showed that mushrooms consistently outperformed earthworms in remediating polluted soils across all properties investigated, regardless of the concentration of crude oil.

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CONFLICT OF INTEREST

There is no conflict of interest

AUTHORS CONTRIBUTIONS

All authors contributed to the planning, conduct and reporting of the work described in the article.

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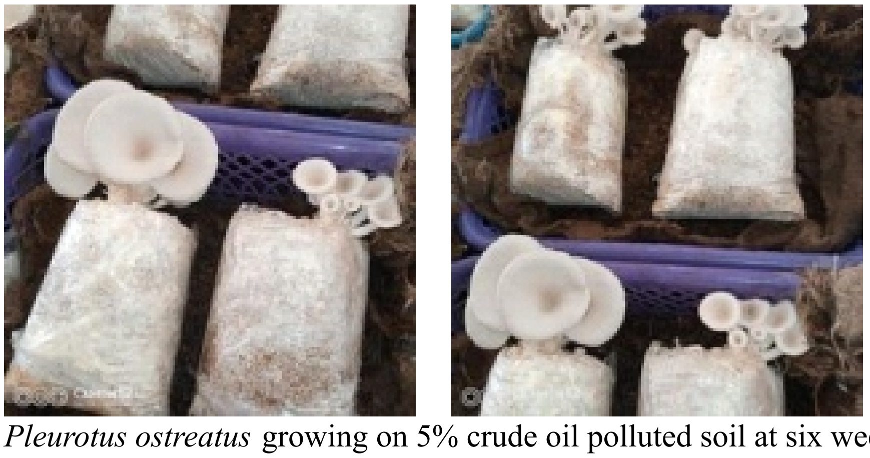

Pleurotus ostreatus growing on 5% crude oil polluted soil at six weeks

Pleurotus ostreatus growing on 10% crude oil polluted soil at six weeks

Pleurotus ostreatus growing on 7.5% crude oil polluted soil

Eisenia fetida in crude oil polluted soil

Eisenia fetida on 5% crude oil polluted soil at 6months

Eisenia fetida on 10% crude oil polluted soil at 6months


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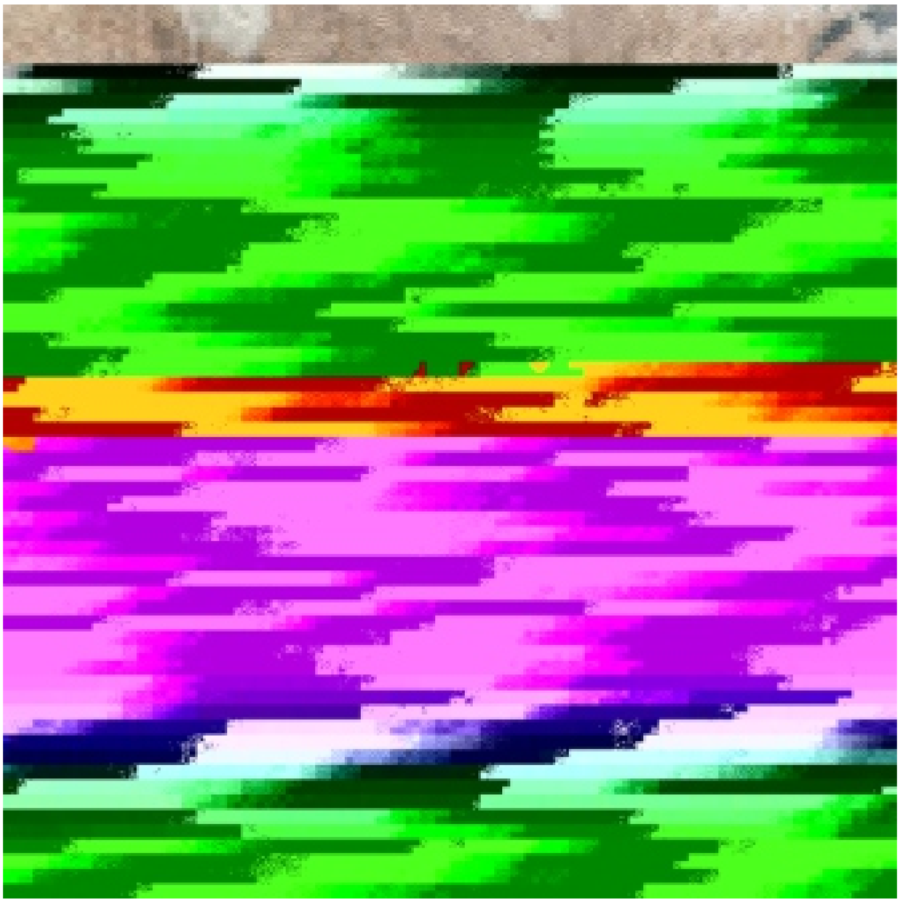


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