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EFFECT OF CRUDE OIL CONTAMINATION ON MICROBIAL COMMUNITY STRUCTURE AND UREASE ACTIVITY IN COASTAL PLAIN SANDS OF UYO, AKWA IBOM STATE, NIGERIA.

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ABSTRACT: *Petroleum pollution of soils is a major environmental problem. Soil microorganisms can decompose a significant fraction of petroleum hydrocarbons in soils. This research was conducted to investigate the effect of crude oil contamination on microbial community structure and Urease activity. This experiment was conducted from 2020 to 2021 at the Department of Soil Science and Land Resources Management, University of Uyo. The experiment was a pot experiment. Garden soil weighing 1.6kg was placed into five different plastic pots and the following quantities of crude oil were dispensed into them: 0 (control), 4. 8, 16 and 24 ml representing 0, 0.25, 0.5, 1.0 and 1.5 per cent pollution levels. This was repeated in triplicates giving a total of 15 pots and placed in a screen house. Soil samples were collected from each pot on the 2, 4, 6 and 8 weeks after contamination (WAC). Soil samples were analysed for bacterial, fungal population, and urease activity. The results revealed that bacterial cell density decreased by one log order of magnitude from 2.67 x 10^7 cfu/g to 9.13 x 10^6 cfu/g soil in week 8. At 0.25 percent population level bacterial cells decreased from 2.38 x 10^7 in (week 2) to 4.02 x 10^6 cfu/g soil (week 8). At 0.5 percent pollution level, bacterial cell density decreased from 2.37 x 10^7 (week 2) to 1.46 x 10^7 cfu/g soil (week 8). At 1.0 per cent pollution level bacterial cell density decreased from 2.30 x 10^7 (week 2) to 9.56 x 10^6 cfu/g soil (week 8) and at 1.50 per cent pollution level bacterial cell density decreased from 2.21 x 10^7 (week 2) to 1.22 x 10^7 cfu/g soil (week 4), and thereafter rose to 3.16 x 10^7 (week 6) and then decreased to 8.0 x 10^6 cfu/g soil (week 8). A total of 16 bacterial genera were identified the most abundant was Bacillus subtilis (23.53%) followed by Micrococcus albus (18.83%), and Bacillus cereus. Unlike bacteria, fungal cells are seen to increase rather than decrease. The unpolluted soils had fungal cell density increased from 1.8 x 10^6 cfu/g soil (week 2) to 6.3 x 10^6 cfu/g (week 8). 0.25 percent pollution level had 1.17 x 10^6 (week 2) to 6.0 x 10^6 cfu/g soil. At 0.5 percent pollution level fungal cell density increased from 1.10 x 10^6 cfu/g to 7.03 x 10^6 cfu/g (week 6), and a slight decrease (6.0 x 10^6 cfu/g soil) week 8 and in 1.0 percent pollution level it increases from 9.33 x 10^5 cfu/g to 9.06 x 10^6 cfu/g. At the 1.5 per cent pollution, an increase by one log order of magnitude was observed (from 1.13 x 10^5 (week 2) to 6.03 x 10^6 cfu/g soil week 8). In this study, 16 fungal genera were identified, the most abundance was candida sp 20% followed by Mucor muccedo, 16.97%, and Penicillium notatium 12.73% Urease activity in all the treatments was not affected significantly. To degrade crude oil pollution in the soil, fungi should be used prominently for better results.*

KEYWORDS: Crude Oil, Contamination, Microbial Community, Coastal Plain Sands.

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INTRODUCTION

Petroleum pollution in soils is currently considered one of the most serious environmental problems. This type of pollution decreases or fully destroys soil fertility changes the elemental composition of soil and water cycles, leads to losses in the aesthetic value of ecosystems, causes secondary pollution of groundwater and air, and inhibits or eliminates soil organisms (Koshat & Ball 2017). There is a growing concern about the rate of soil degradation throughout the world, arising from the production and use of fossil fuels (FAO, 1994). Crude oil contains more than 30 parent polyaromatic hydrocarbons (PAHs), and it contains hundreds of different hydrocarbon compounds such as paraffin, naphthene, aromatic as well as organic sulfur compounds and oxygen-containing hydrocarbons (phenols). The leakage of crude oil into the soil damages the biological systems residing in the soil, including microorganisms and plants (Dariush *et al*., 2007). Some fractions of crude oil are toxic to living organisms. Microbial breakdown of hydrocarbon pollutants is generally a very slow process but biodegradation could be optimized only if the right environmental conditions such as pH, temperature, nutrients and relevant microbial consortia are present (Akpoveta *et al*., 2011), as well as the pollution of petroleum hydrocarbons caused major changes in physical and chemical properties of the soil, it is an environmental concern because contaminated soils may be unsuitable for agricultural, industrial or recreational use and also potential sources for surface and groundwater contamination (Chainaeall *et al*., 2000). Soil microbes and enzymes provide the basis for ecological processes such as biogeochemical cycles and food chains as well as maintaining vital relationships among these cycles and with superior organisms (Bardgett & Vander Putten, 2014).

Soil microorganisms predominantly bacteria and fungi play a major role in the decomposition of soil organic matter, synthesis of humus cycling of nutrients and promotion of plant growth. After their introduction into soils, hydrocarbons affect soil microorganisms directly or indirectly. Indirectly petroleum leads to increased soil surface temperatures, changes in the content of soil organic matter, disturbance in the oxygen and water supply and a decrease in nutrient availability. These changes in turn alter the structure and function of soil microbial communities (Fan et al., 2024).

Directly, petroleum, hydrocarbons may inhibit soil community members, causing non-specific membrane disturbances, damage to membrane functions, growth inhibition, and cell lysis (Lazaroaie 2010; Sirkema et al., 1995). Another direct effect of petroleum hydrocarbon introduced into the soil is the growth stimulation of microbial populations that can decompose or tolerate hydrocarbon. These organisms are ubiquitous in soils, but their abundance and decomposition patterns are found to be dependent on soil physicochemical characteristics and soil genesis (Chikere et al., 2019; Cho et al., 2015). Microorganisms use hydrocarbons as a sole carbon source or alter them to decrease their toxicity. The main pathways occur aerobically; however, bacteria can also utilize hydrocarbons anaerobically (Koshlaf & Ball 2017). Interestingly, bacteria have been demonstrated to decompose aliphatic and aromatic compounds while fungi can also degrade polycyclic aromatics. Additionally, bacteria use specific metabolic pathways such as those of alkane monooxygenase and dioxygenase to decompose hydrocarbon, while fungi utilize different hydrocarbons in nonspecific enzyme composes (e.g. cytochrome P450, lignin peroxidase, manganese peroxidase and laccase) that enable them to decompose lignin and cellulose (Llodo et al., 2013; Hans et al., 2011), considering the contribution of microorganisms to the soil ecosystem, the potential effects of crude oil contamination on soil microbiology are important, soil enzymes fulfil a critical role

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in many biochemical processes and may serve as process level indicators of soil quality. Dehydrogenase is a widely studied oxidoreductase based on evidence including correlation with oxygen consumption and an assay of dehydrogenase activity may serve as a measure of the general metabolic activity of the soil microbial community (Friedel et al., 1994). This research was therefore designed to assess the effect of Crude Oil Pollution on soil microbial community and enzyme activity in coastal plain sand in Uyo, Nigeria.

MATERIALS AND METHODS

Samples of Crude oil

Petroleum Hydrocarbon was sampled by Agrip Nigeria Limited. It was transferred to the laboratory in a plastic container tightly closed.

Collection of soil samples

Non-contaminated soil samples were obtained from a cultivated area of the University of Uyo teaching and Research farm. The soil inoculation was carried out by weighing 1.6kg of wet samples into five plastic planting pots. The five plastic pots were contaminated with the following millilitres of crude oil: 0, 4, 4, 8, 16, and 24 ml, representing 0 (control), 0.25, 0.50, 1.00 and 1.5%. These were replicated three times and incubated for eight weeks. Samples from each treatment were taken at 2 weeks, 4 weeks, and 8 weeks for microbial and enzymatic analyses.

Microbiological Analysis

Serial dilution

Ten-fold serial dilution of the contaminated soil samples was made as in Collins and Lyre (1976).

Inoculation and Incubation

One millilitre of appropriate ten-fold serial dilutions of the contaminated soil samples from each treatment was inoculated onto Nutrients agar and Sabouraud Dextrose Agar plates in triplicates using pour plate methods (Collins & Lyre, 1976). The inoculated plates were incubated at 28±2ºC for 18-24 hours and 48-72 hours for the enumerations of total heterotrophic bacteria and fungi respectively. Visible discrete colonies in incubated plates were counted and expressed as colony-forming units per gram (cfu/g) of the soil sample.

Maintenance of pure culture

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on Nutrient agar slants and at an ambient temperature of 28ºC for further tests.

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Characterisation and Identification of Microbial Isolates

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques and biochemical tests including carbohydrate utilization. Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of the known taxa as in (Holt *et al*., 1994) characterisation and identification of fungi isolates were carried out (Domch *et al*., 1980).

Determination of Enzyme activity

Determination of Urease

Urease activity was determined as described by Pancholy and Rice (1973). Briefly, one millilitre of toluene was thoroughly mixed with a 10g field moist soil sample in a 100ml Erlenmeyer flask. After 15 mins, 20 ml of phosphate buffer (pH 6.5), and 20 ml of 10% urea solution were added to the flask. The reactants were incubated at 37ºC for 24hrs followed by shaking for 15 mins with 30 ml Kcl solution. The contents were filtered with Whatman No 41 filter paper and the filtrates made up to 100 ml with deionized water. Aliquots (15ml) were analysed for the $NH_4 - Ng^{-1}$ soil.

RESULTS AND DISCUSSION

An estimate of the size of the microbial community was based on total heterotrophic bacterial and fungal counts. The unpolluted soils at the beginning of the experiment (week 2) had a bacterial cell density of 2.67×10^7 and 9.13×10^6 cfu/g soil at the end of the experiment (week 8). At 0.25% level of pollution bacterial cell density decreased from 2.38×10^7 in week 2 to 4.02×10^6 bacterial cells. At 0.5% pollution level bacterial cell density decreased from 2.37×10^7 (week 2) to 1.47×10^7 (week 4) and thereafter, rose again in week 6 to 3.75×10^7 and a further decrease in bacterial density was observed at the end of the experiment (week 8) to 8.53×10^6 cfu/g soil. At 1.0% pollution level, bacterial cell density decreased from 2.3×10^7 (week 2) to 9.567×10^6 bacterial cells at the end of the experiment (week 8) and at 1.50% pollution bacterial cell density decreased from 2.21×10^7 (week 2) to 1.22×10^7 (week 4) and then rose to 3.12×10^7 (week 6) and thereafter decreased to 8.0×10^6 cfu/g soil in (week 8). In the results, we observed in the unpolluted soil, one would have expected exponential or steady growth of bacterial cells from the beginning of the experiment to the end (week 8) but this was not so. The reasons may be because the soil microbes are taken from their natural soil environment to containers where there are changes in moisture, temperature and nutrient contents. These may lead to the essential nutrients in the containers being used up or the waste products of the organisms accumulating in the containers and inhibiting the continuous growth of bacterial cells. In the polluted soils, oil pollution led to a slight decrease in the number of bacterial cells in all the polluted soils, in particular, spike with high concentrations of oil and as the week increased. This could be explained by the toxic effect of oil on soil microorganisms, as well as by indirect effects connected with changes in the physico-chemical properties of the soil (Fig 1).

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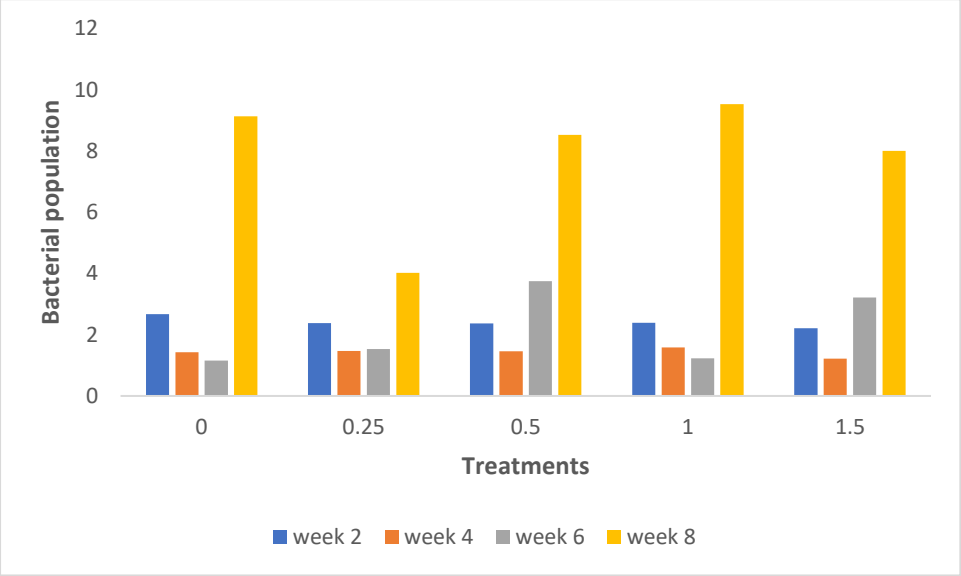

Fig 1: Effect of crude oil on Bacterial population

Oil pollution led to significant changes in the bacterial communities of all treatments. However, these changes were observed with some delay, despite the short life cycle of bacteria. This could be due to the slow rate of hydrocarbon decomposition and bacteria have to adapt to the new carbon source and start growth. These observations agreed with the results presented previously (Polina *et al*., 2021) working on the response of bacterial and fungal communities to high petroleum pollution in different soils. The unpolluted soils at the beginning of the experiment had fungal cells from 1.87 x 10^6 (week 2) to 6.13 x 10^6, the significant increase in fungal density in a confined environment such as a pot, the cells must have developed a complete complement of enzymes for synthesis of the essential metabolites not present that soil environment. At the 0.25% pollution level fungal cell density increased significantly from 1.17 x 10^6 (week 2) to 6.0 x 10^6 at the end of the experiment (week 8). At the 0.5% pollution level, fungal cell density increased from 1.10 x 10^6 cfu/g soil at the beginning of the experiment (week 2) up to 7.03 x 10^6 cfu/g soil in (week 6) and a slight decrease was observed in week 2 (6.0 x 10^6 cfu/g soil). Similarly, at the 1.0% pollution level, fungal cell density increased from 9.33 x 10^5 to 9.0 x 10^6 cfu/g soil in week 6 and thereafter decreased to 6.90 x 10^6 cfu/g soil at the end of the experiment (week 8). It was also observed at the 1.50% pollution level increase in fungal cell density at the beginning of the experiment (week 2) by one log order of magnitude from 1.13 x 10^5 to 6.03 x 10^6 cfu/g soil at the end of the experiment (week 8).

Despite the decrease in bacterial cell density as a result of oil pollution, the fungal population was not however affected significantly as observed in week 8 of 0.5%, and 1.50% respectively. This was likely due to the ability of fungi to decompose hydrocarbon using non-specific pathways normally used for the decomposition of lignin and consequent higher tolerance to hydrocarbons (Fig 2).

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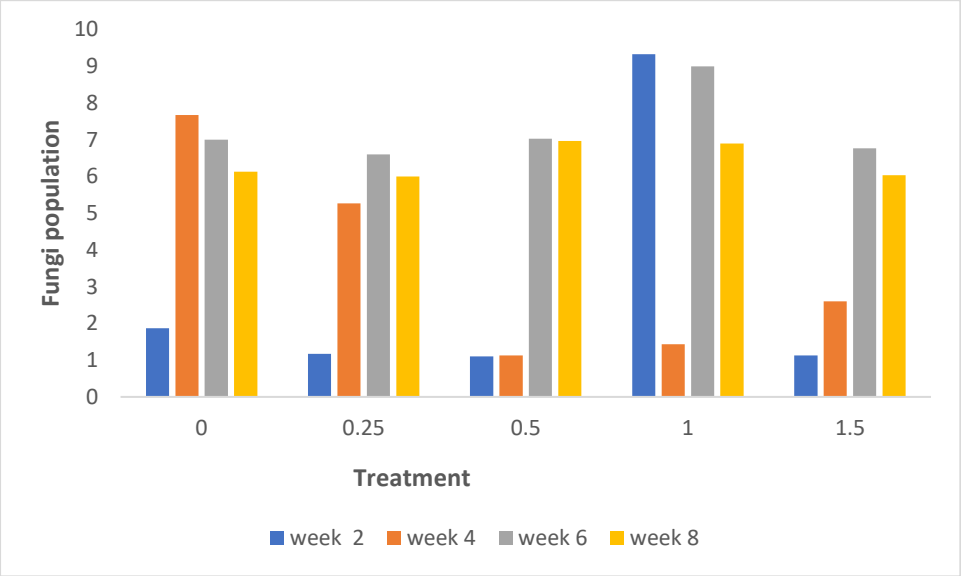

Fig 2: Effect of crude oil on the Fungi population

The observation in this report corroborates with previous reports by Varramic and Uposanic (2017) working on a new look at factors affecting microbial decomposition of petroleum hydrocarbon pollutants. Bacteria communities in oil-polluted soils. The bacterial composition in the polluted soils was similar to that in the unpolluted soils in all the treatments. Oil pollution led to an increased relative abundance of *Micrococcus roseus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* sp, *Serratia* sp, *and Klebsiella aerogens.* Relatively high relative abundance was found for *Bacillus subtilis*, *Micrococcus roseus*, and *Bacillus cereus*. The abundance represents 23.53, 18.82 and 15.29% respectively.

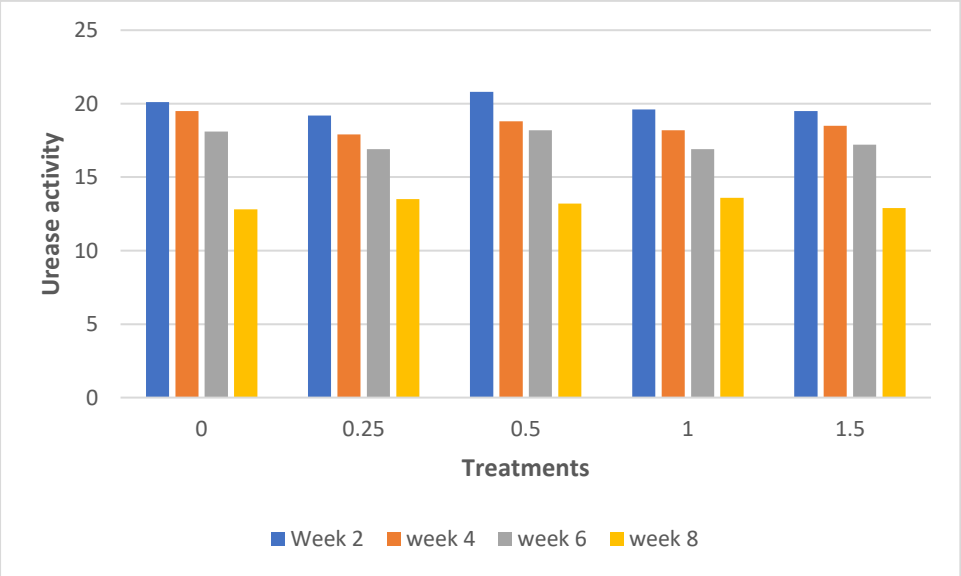

Fig 3: Effect of crude oil on Urease activity

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Table 1: Bacteria species and abundance in the soils.

SN	Species	0	0.25	0.50	1.0	1.5	Total	%
1	*Micrococcus roseus*	11	8	6	5	2	32	18.82
2	*Staphyloccocus albus*	2	4	1	1	9	8	4.71
3	*Bacillus subtilis*	6	13	10	7	4	40	23.53
4	*Staphyloccocus aureus*	3	2	5	3	3	16	9.41
5	*Pseudomonas aeroginosa*	4	3	0	0	9	7	4.12
6	*Bacillus cerus*	5	9	7	3	2	26	15.29
7	*Proteus sp*	1	1	3	1	4	10	5.88
8	*Serratia sp*	4	1	1	0	0	6	3.53
9	*Klebsiella aerogenes*	2	2	1	0	1	6	3.53
10	*Citrobacter sp*	1	0	1	0	0	2	1.18
11	*Clostridium sp*	0	1	0	1	1	3	1.76
12	*Pediococcus sp*	0	1	0	0	2	3	1.76
13	*Entrobacter sp*	0	0	1	1	1	3	1.76
14	*Lactobacillus sp*	0	0	1	2	2	5	2.94
15	*Streptococus faecalis*	0	0	0	1	0	1	0.59
16	*Chrometium sp*	0	0	0	2	0	2	1.18
	Total	**39**	**45**	**37**	**27**	**22**	**170**	**100.0**
	%	22.94	26.47	21.76	15.88	12.94	100	

Other representatives of bacteria in the polluted soil ranged between 1.18 to 9.41%. Indigenous microbial communities can restore soil quality after petroleum pollution by biodegrading hydrocarbons. However, impactful biodegradation occurs at relatively low petroleum concentrations, say 1-5% pollution levels as observed in our experiment. Additionally, some gram-negative bacteria were identified but were not able to persist up to week 8 of the experiment, for example, Pseudomonas which can synthesise the enzyme dioxygenase are reported to be active hydrocarbons decomposers that can attack not only alkanes but also aromatic compounds (Koshla & Ball 2017). Although the main dominant bacteria in the oil-polluted soils detected in this experiment (Micrococcus roseus, Bacillus subtilis, and Bacillus cereus) are gram-positive and are known to be active in hydrocarbon degradation. However, we observed that they were decreasing from 0.5% concentration to .5%. the most abundance fungal communities were Candiela sp (20.0%) followed by Mucor muceada (16.97%) and Penicillium notatium (12.73%). They showed a steady increase from unpolluted soils up to 1.0% pollution level before decreasing to 1.5% pollution level. This was most likely due to the ability of fungi to decompose hydrocarbons using nonspecific pathways normally used for decomposition of lignin and also have higher tolerance to hydrocarbons (Table 2)

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Table 2: Fungi species and abundance in the soils.

SN	Species	0	0.25	0.50	1.0	1.5	Total	%
1	*Aspergillus glaucus*	1	1	1	0	0	3	1.83
2	*Geotricum sp*	3	1	0	1	1	6	3.64
3	*Aspergillus niger*	5	4	1	0	2	12	7.27
4	*Candida sp*	3	5	7	10	8	33	20.00
5	*Rhyzopus oryzae*	5	6	4	2	3	20	12.12
6	*Mucor muccedo*	2	7	9	6	4	28	16.97
7	*Penicicilium notatium*	3	4	7	5	2	21	12.73
8	*Clasdosponium sp*	1	0	1	0	0	2	1.21
9	*Fusarium sp*	1	1	2	1	0	5	3.03
10	*Aspergilus fumigates*	2	3	1	1	1	8	4.85
11	*Aspergilus flavus*	1	0	2	2	4	9	5.45
12	*Trichoderma sp*	2	1	1	0	0	4	2.42
13	*Aspergillus terreus*	1	1	1	4	3	10	6.06
14	*Verticullum sp*	0	1	0	0	0	1	0.61
15	*Euroticum sp*	0	1	0	1	0	2	1.21
16	*Absidia sp*	0	0	0	1	0	1	0.61
	Total	**30**	**36**	**37**	**34**	**28**	**165**	100.0
	%	**18.18**	**21.82**	**22.42**	**20.16**	**16.97**	**100.0**	

CONCLUSION

Despite the high concentration of petroleum in soil and the insignificant rate of decomposition, the soil microbial community not only sustained its quality but also adapted to the new quality of the environment. The effect of crude oil on bacteria, fungi communities, and urease activity showed a common pattern in all the treatments, especially at the higher concentration levels. Bacteria possess specific enzyme complexes that can degrade hydrocarbons as they drastically increase their ability to complete this degradation. We observed successions in the bacterial community structure, including alteration in dominant composition and decreased biodiversity. In fungal communities, the species then can degrade lignin, hemicellulose, and cellulose still maintaining their dominating positions because they possess nonspecific enzymes enabling them to degrade aromatic compounds. Interestingly, changes in fungal communities were not as significant as those in bacterial communities. There was also a study decrease in cellulose activity from week 2 to week 8 across the pollution levels.

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