*International Research Journal of Environmental Sciences*_____ ISSN 2319–1414
Vol. **8(1),** 16-22, January **(2019)** *Int. Res. J. Environmental Sci.*

Consequences of crude oil on some indigenous microorganisms and edible plants in Kogi State University demonstration farm, Anyigba, Nigeria

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Available online at: www.isca.in, www.isca.me
Received 31st August 2018, revised 20th December 2018, accepted 10th January 2019

Abstract

Different levels of crude oil were added to soil samples: 2%, 4%, 6% and 8% v/w for 4 weeks and observations were made for the effects of the crude on the indigenous bacterial and fungal populations and also on the dry matter of spinach, maize, tomato and beans crops. The microbial count showed that there was a progressive decrease in the microbial counts of the soil treated samples as shown in tables 2 and 3. Lower microbial counts were observed in the treated samples compared to control. The mean bacterial counts decreased at 2% treatment (2.46 X10^6) when compared to the control (0%) (2.60 X10^6) treatment, it slightly increases at 4% treatment to 3.06X10^6 then, the counts decreased all through the 6 and 8% treatments progressively. The mean fungal counts also had an initial decrease of 3.38X10^4 and 1.9 X10^{4}at both 2% and 4% treatments compared to the control which was 4.6 X10^4. The mean fungal count tend to increase at 6% (5.86 X10^4) but it decreased again at 8% (4.08 X10^4). The crude oil made the soil to be acidic compared to the control; it also made the soil to retain moisture content, thereby becoming water logged and anaerobic in nature; at 28 day, it slightly increased the contents of the organic matter in the treated soil samples. Phytochemical properties present in the plants (alkaloid, phenols, tannin and flavonoid) were affected negatively by the crude oil application. Also, it was observed that the stem dry weight matter, leaves dry weight matter and root dry weight matter reduced as the crude oil concentrations in the soil samples increased.

Keywords: Crude oil, microorganism, emergence, germination.

Introduction

To the environment, oil spill is very dangerous because it affects both plants and wildlife negatively, this results into huge economic losses and causes human to suffer. According to the estimates by Environmental Protection Agency (EPA), 200 million gallons of oil that are used are improperly disposed off each year. Apart from the effects it has on animals which already receive great attention from media, its discovered that oil spills also hugely destroy plant life because it possesses the potency to impair a plant's ability to carry out basic functions of life, such as photosynthesis. Refined petroleum product as crude oil, aromatic hydrocarbons is abundantly present in the environments. In food chains, biochemical and physiological activities of many organisms are disrupted by them; consequently, they can cause mutagenesis in the genetic material, cancer of some organs, and impairment of reproductive capacity or causing haemorrhage to exposed population. In soil fertility and crop productivity, one of the most limiting factors is soil pollution caused by oil spills. These terrible consequences make it compulsory having an alternative means of reducing petroleum hydrocarbon pollution in the environment[1]. Oil contamination seeps into ground to contaminate ground water, weakens soil's potential to support plant's growth, and heightens the concentrations of heavy metals which can cause adverse health problems based on available literature[2]. Crude oil can be described as hydrocarbon, liquid mixed in their natural state. It can be grouped as alicyclic, aliphatic and aromatic compounds[3]. In nature, majority of these compounds are widely recognized to be deleterious to different living organisms[4]. A lot of concern has emerged on this topic especially. Enormous quantity is spilled yearly in Nigeria. Between 1976 and 1988 about 2,000 oil spillage occurred in Nigeria with approximately 212 barrels of crude oil being released to soil. Oil pollution is recognized to portray dangerous harm on soil flora and fauna. Spillage of Crude oil on soil generally reduce or stunt the growth of plant[5-7], it also creates a condition of anaerobiosis which lowers the influx of air by closing up air spaces between soil particles[8]. It also reduces leaf growth by causing root stress in plant[9].

Large quantities of microorganisms that are aerobic are taken out of soil polluted with oil. These microorganisms are bacteria (heterotrophs), fungi, actinomyces, phototrophic and. Microorganisms can either cause crude oil break down, or probably metabolize pollutant-oxidizing organisms' intermediate products[10]. *Bacillus, Streptomyces, Arthrobacter, Micrococcus, Flavobacterium, Achromobater, Methanobacterium Thiobacillus, Cloctridium, Pseudomouas,* and fungi such like *Candida, Aspergillus, Cladosparium, Penicillium* are abundant among the genera of microorganisms taken from soil contaminated with petroleum[11]. However, in order to assess the deleterious effects crude oil at various concentrations has on some lithosphere microbes, emergence

and production of dry matter in tomato, beans, maize with spinach plants, this research work was done.

Methodology

Analysis of soil: Soil samples were collected from university demonstration farm land and were pound to disintegrate the soil, thereafter; it was dried at 37^0C for fourteen days in laboratory. After this, the soil was sieved by using a sieve graduated in 2 mm to eliminate stones, bigger soil component and unwanted matter. Organic matter was determined, then organic carbon was also determined via Walkley and Black method[12]. Sodium hexametaphosphate as a dispersing agent was used for the mechanical analysis by the hydrometer method. By using pH meter, soil pH was analysed. Micro-kjeldahl method was used to determine the total nitrogen[13]. Bray's P method was used to determine the available-P in the soil sample. Effective cation exchange capacity (ECEC) was calculated[13].

Soil preparation and planting: Soil samples were collected from the university's demonstration farm and poured into nine different containers with each container containing 1,200grams of soil. They were arranged in three sets (A, B and C). Set B was treated with various levels of crude oil. Set A was without crude oil and no plant and set C was not polluted but contains plant and serves as control of the experiment. Twenty four milliliters of crude oil was introduced into sample container B1 in and that is 2% pollution. The 4, 6 and 8% pollution for sample containers B2, B3 and B4 was prepared through adding 48, 72, 96ml of crude oil unto the group B containing soil sample and were adequately mixed together in soil sample container. The containers containing the soil samples were wet every morning with 15ml sterile water per container for the period of this experiment.

A seed crop (maize, vegetable, bean and tomato) was planted in each of the rubber samples in, group B and C.

Microbial analysis: One gram of soil was collected out of each treated soil and dilutions done serially. With aid of pipette, an ml of the dilution was collected from the fifth dilution. The pour plate method was used for the isolation of microorganisms using Collins and Lyne's[14] method.

Dry matter analysis: Destructive sampling method described by Ekpo and Odu[15], was used for the dry matter analysis. From each treated soil sample, randomly uprooted were two plants which were separated to stem, leaves and root. Initial n weight was determined and penned down as "a" thereafter, it was dried at 180°C for 12 h to a constant weight. Recorded was the final dry weight as "b". Percentage dry weight calculated using the formula:

Dry weight (%) = (b/a) x 100

Where: a = fresh weight of plant part, b = dry weight of plant part.

Phytochemical Analysis: Powdered crude extract of the plants spinach (B1), maize (B2) tomato (B3) and bean (B4) were tested for phytochemical properties to determine the presence of some biomolecules and other metabolites viz; Alkaloids, flavonoids, and Tannins by using the standard qualitative steps[16-18].

Test for Alkaloids: A fraction of the plant was treated with Wagner's test reagent (that is 1.27g of iodine and 2g of potassium iodide were diluted in 100ml of water) and observation was made for reddish brown color.

Test for Flavonoids: Shinoda or magnesium chips test: The plant in powdered form (0.5gm) was extracted in ethanol by boiling on a water bath (5min), filtered and made to cool. Magnesium chips small quantity were added to the filtrate and concentrated HCl was added in few drops in a test tube and colour change was observed.

Test for Tannin: About 0.2g of the extract was mixed with 3ml water and heated on water bath. Filtration was done thereafter and ferric chloride (few drops) mixed in to the solution. The presence of tannin was confirmed by the resultant dark green coloration.

Results and discussion

Germination/Emergence: The emergence/germination of crop species spinach (B1), maize (B2) tomato (B3) and bean (B4) shown (Table-1). The result shows 2% and 4% treated soil samples have 100% germination, while 6% and 8% have 90% and 50% germination rates respectively level of the applied crude oil. Comparison between the growth length of the various plants and changes in colour observed in controls and soil samples treated with various concentrations of crude oil was made. The length was measured using a calibrated meter rule. The result showed that the length of the plants in soil samples that contained lower levels of crude oil (2% and 4% treatments) were higher than ones treated with concentrations of 6% and 8% treatments in all the weeks they were measured. The changes in growth rate were significant different compared to the control. There was no notice of leaves changing from normal plants or folding or dropping in the controls, but different characters were observed in the plants of polluted soil. The changes were more visible as it was observed in the 14^{th} day of germination of plant even with concentration 4% and 6%.

Microbial count: Tables-2 and 3 show the bacterial and fungal counts from treated samples. The microbial count showed that there was a progressive decrease in the counts. There was reduction in microbial counts treated samples compared to control. The mean bacterial counts decreased at 2% treatment ($2.46X10^6$) when compared to the control (0%) ($2.60X10^6$) treatment, it slightly increases at 4% treatment to $3.06X10^6$ then, the counts decreased all through the 6 and 8% treatments progressively. The mean fungal counts also had an initial

decrease of $3.38X10^4$ and $1.9X10^4$ at both 2% and 4% treatments compared to the control which was $4.6X10^4$. The mean fungal count tend to increase at 6% ($5.86X10^4$) but it decreased again at 8 % ($4.08X10^4$).

Dry matter weight: The dry matter weight of B1, B2, B3 and B4 of the roots, leaf and stem in treated samples are in Figure-1, Figure-2 and Figure-3 respectively. Result showed that dry matter content of the root, leaf and stem decrease as the days increase. At 6% and 8% samples, decrease in dry matter of leaf was observed. Result showed broad leaf growth in 2% and 4% treated samples compared to 6% and 8% treated samples. The dry matter of the stem (Figure-3) of the plant B1, B2**,** B3 and B4 all reduced.

Table-1: Plants emergence in control and treated samples.

Name of plant		No of seed plants	Applied conc (%)	Emergence/germination (Days)						Total no of germination	Germination (%)
				5	7	9	11	13	15		
Spinach (B1)		20	2	20	20	20	20	20	20	20	100
Maize (B2)		20	4	20	20	20	20	20	20	20	100
Tomato (B3)		20	6	18	15	15	0	0	0	18	90
Beans (B4)		20	8	0	0	5	10	0	0	10	50
Spinach (B1)	Control	20	0	20	20	20	20	20	20	20	100
Maize (B2)		20	0	20	20	20	20	20	20	20	100
Tomato (B3)		20	0	20	20	20	20	20	20	20	100
Beans (B4)		20	0	20	20	20	20	20	20	20	100

Table-2: Bacterial counts (cfu/g) from crude oil treated soil samples at various concentrations.

Days	Crude oil concentrations ($x10^6$)				
	0% ($x10^6$)	2%	4%	6%	8%
0	1.5	1.6	1.3	1.4	1.3
7	2.9	2.5	1.7	1.1	1.1
14	2.1	1.0	2.4	1.2	1.9
21	3.9	3.8	6.6	2.5	2.4
28	2.6	3.4	3.2	2.0	2.7
mean	2.6	2.4	3.0	1.68	1.60

Table-3: Fungal counts (cfu/g) from crude oil treated soil samples at various concentrations.

Days	Crude oil concentrations				
	0% ($x10^4$)	2% ($x10^4$)	4% ($x10^4$)	6% ($x10^4$)	8%($x10^4$)
0	3.2	2.3	1.3	1.2	1.0
7	6.1	8.8	3.3	4.5	5.5
14	6.5	1.0	1.5	4.2	4.4
21	3.0	3.2	1.4	3.8	4.2
28	4.2	1.6	2.0	4.8	5.3
mean	4.6	3.38	1.9	5.86	4.08

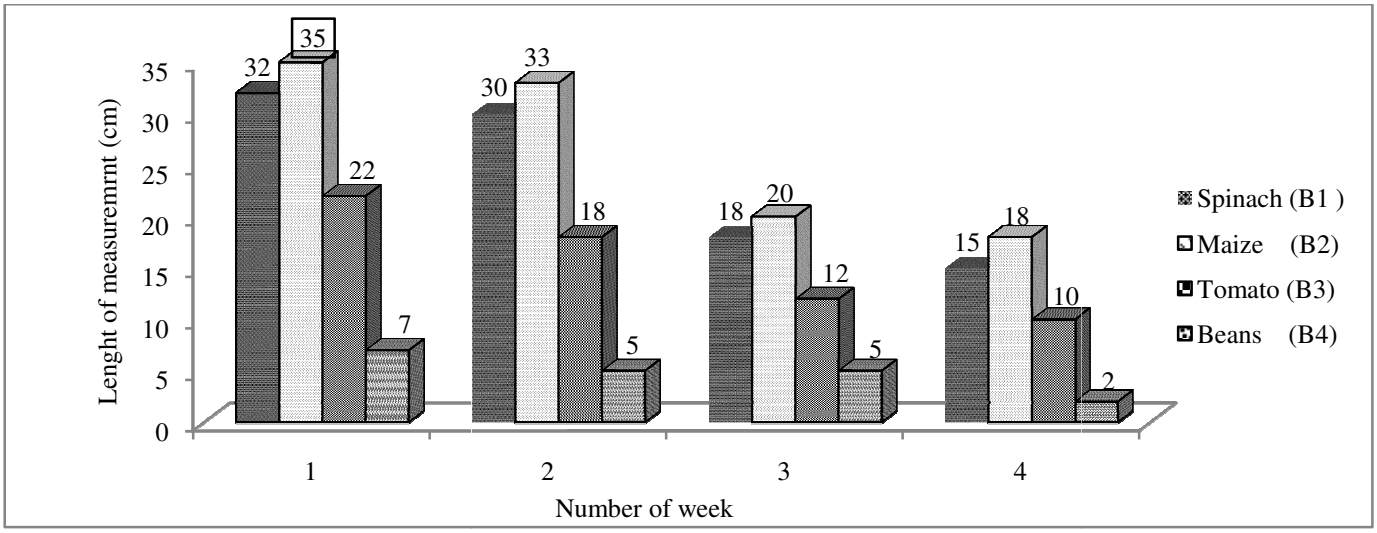

Figure-1: Root dry weight measurement.

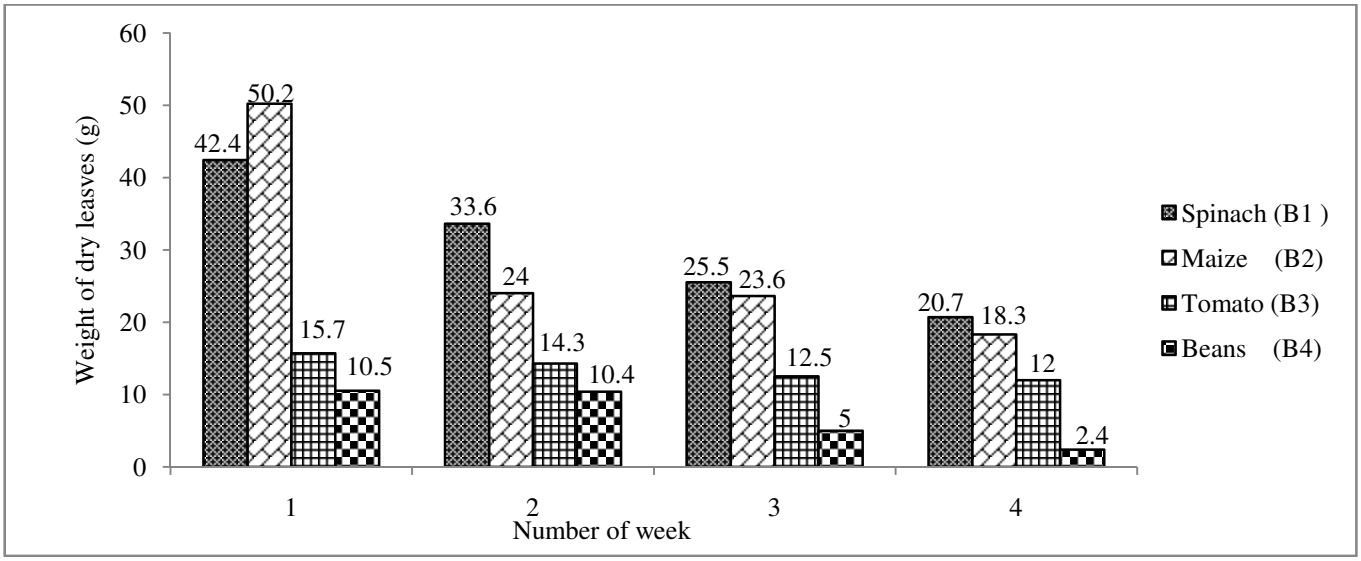

Figure-2: Leaves dry matter.

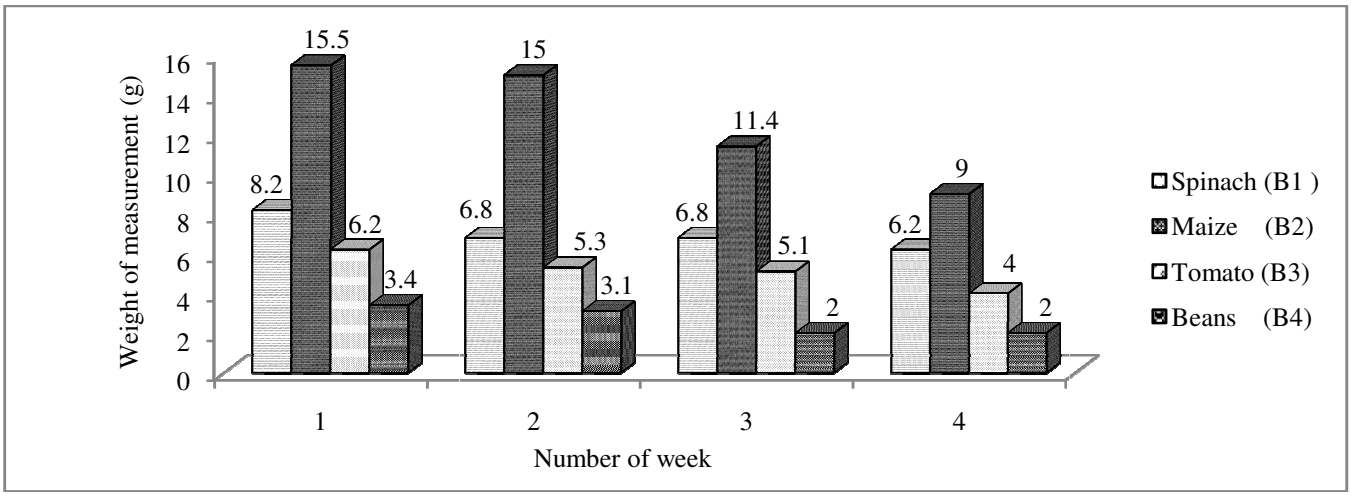

Figure-3: Stem dry weight matter.

Weight for the 6% and 8% treatment compared to the 2% and 4% treated soil samples. The gradual decrease was observed in all the root, stem and leaf dry matter as the days increase.

Phytotoxicity: There was leaf colouration and abscission few weeks after planting in physical observation of bean than in spinach and maize. This is because beans is more sensitive to the toxic hydrocarbon molecules present in the treated soil samples causing a greater inhibition of the germination activities in beans than the other crops planted. This is in consonance with research done by Luhach and Chaudry[19], who also revealed that *V. radiate* with *Z. mays* portray higher ability for diesel contaminated soils' phytoremediaton when laid side by side with *P. glaucum* and *S. vulgare*. The qualitative analysis of bioactive compounds for the four crude extract analyzed in this study are shown in Table-4. The result reveals the presence of all the phytochemicals screened. In spinach, phenol, alkaloid, tannin and flavonoid were all present, they were also all present in maize except for phenol that was absent; in tomato, alkaloid and phenol were absent but tannin and flavonoid were present while in bean, only alkaloid was present while the rest phytochemicals were absent. This is because it is reported that food legumes tannins that occur naturally react with complexes of tannin-protein that form with protein which result to inactivation of some enzymes and protein insolubility as reported by Reddy N.R. et al[20].

Discussion: Reverberations of petroleum pollutions in soil microflora, plant weight (dry), physicochemical effects and phytochemicals/phytotoxicity on some plants was done. At the commencement of this research, the weight of the soil for the treatment was determined and it was 1,200kg each, unrefined crude oil from Warri refinery Nigeria was used, percentage of pollution was determined, the soil pH, moisture content and organic matter content was 6.50, 2.5 and 25.0 respectively.

It was revealed via this research that both the 6% and 8% concentrations in soil samples delayed the plants germination. The delayed emergence of plants observed in soil samples polluted with various concentrations of crude oil was due to poor soil aeration and the seeds taking in the oil, these effects made the seeds to be slimmy and swollen as seen when the non-germinated seeds were pulled out from the treated samples. This observation agree with Ekpo and Etim[21] who worked on crude oil effect on fluted pumpkin at 0%, 1%, 3%, 6% pollution noted a harmful effect on growth and germination at 3% above. Amakin and Onofeghara[22] also worked on the *Capsicum fruitescen* and *Zea mays*' reaction to crude oil. He observed drastic reduction in the plants germination rate and he attached the reduction basically to the nature primarily to the characteristics of the oil, this covers seedlings from getting access to water and oxygen. Similarly, it was said by Proffitt et al[23] *Avicenia germinans* and *Rhizophora mangle* seedlings can be sustained for fourteen days in the presence of crude oil. It was also reported by Ekpo and Nwaankpa[24] that ginger Zingiber officinale germinated well in soil contaminated with 1% crude oil, but soil polluted with 15% totally inhibited its germination.

Table-4: Phytochemical properties observed.

Plants	Phytochemical properties			
	Phenol	Alkaloid	Tannin	Flavonoid
Spinach	+	+	+	+
Maize	-	+	+	+
Tomato	-	-	+	+
Bean	-	+	-	-

Key: += present; - =absent

Results bacterial and fungal counts showed progressive decrease for bacteria, fungi counts in treated soil samples as shown in Tables-2 and 3. There was reduction in the number of microorganisms in the treated soil samples compared to control. The mean bacterial counts decreased at 2% treatment (2.46 $X10^6$) when compared to the control (0%) ($2.60X10^6$) treatment, it slightly increases at 4% treatment to $3.06X10^6$ then, the counts decreased all through the 6 and 8% treatments progressively. The mean fungal counts also had an initial decrease of $3.38X10^4$ and $1.9X10^4$ at both 2% and 4% treatments compared to the control which was $4.6X10^4$. The mean fungal count tend to increase at 6% ($5.86X10^4$) but it decreased again at 8% ($4.08X10^4$). The initial reduction in microbial count can be attributed to the initial cidal or inhibitory effects the crude oil had on the microorganisms. The microorganisms being in their lag phase in the oil treated environment, were killed and their growth inhibited. The crude oil produced an anaerobic condition in the soil samples and this eliminated majority of the organisms that are aerobic. It is in consonance with work of other researchers[25-28] they reported crude oil addition caused huge harm to soil biota. Rise in microbial counts in this work is because bio degraders got adapted to their new environment (oil-treated soil environment), recovered, and then enzymes were produced to break down the oil. In other words, crude oil became substrate for them.

It is shown by this study that the weight of stem, root and leaf dry matter at increased pollution levels were lower compared to those in the 2% and 4% treated soil. Progressive fall of number of leaf alongside rise in concentration of pollution was observed. This result is in agreement with the report of other researchers[26,29,30] showed pollution with crude oil that is less than 4% boost organic matter and soil fertility. High moisture content may cause reduction in microbial activities because of the hindrance it causes air movement and it lowers air penetration to plants and not as a result of the water itself as noticed in the Table-5. Because of the low moisture levels of the polluted soil, less dissolved materials will be available for plant uptake and subsequent metabolism. From this study, the average pH value in each day of analysis ranges from 5.4 to 5.9. All values showing that the soils were all acidic compared to the pH in control for all the days which has the average value of 6.5. According to Ekpo and Etim[21] pH value between 6.5 and 7.5 is considered perfect for the germination of many plants. Furthermore, soil microbes' behaviour is affected by soil pH[28].

Table-5: The physiochemical properties of the polluted soils at different concentration at days interval.

% of conc.	pH Days					Moisture Content Days					Organic Matter Content Days				
	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
2 %	6.50	5.63	5.68	5.45	5.15	2.00	6.00	6.40	4.00	4.50	25.0	15.0	12.0	10.8	9.9
4 %	6.50	5.66	5.91	5.60	5.35	2.00	4.30	7.00	9.00	4.30	25.0	13.0	10.0	10.0	9.2
6 %	6.50	5.68	6.00	5.80	5.60	2.00	5.00	6.80	2.00	4.00	25.0	13.6	9.6	9.3	9.0
8 %	6.50	6.70	6.00	6.30	5.60	2.00	2.60	3.50	5.00	6.00	25.0	22	20	23	24

Conclusion

Considering the result in this work, it be deduced that crude oil in soil at lower quantities (for example, 2% and 4%) are beneficial to both microorganisms and plants, above this concentrations, it has deleterious effects on both the soil micro flora and plants.

Crude oil causes poor plants germination and has negative effects on soil microflora. Everyone should therefore, guard against crude oil pollution in order to protect the soil microflora that increase soil fertility thereby maintaining our quest for sustainable and higher agricultural productivity.

Recommendation: i. Measures should be taken to eradicate indiscriminate oil spilling in any environment. ii. Avoid farming on polluted soil, to reduce accumulation of toxic materials for the plants. iii. Polluting any soil with any form of crude oil will inhibit, kill and eradicate the soil microflora and as well as the fauna.

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