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Survival of Earth Worm in Crude Oil Contaminated Soil

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Abstract: ***Crude oil contains a wide range of compounds which are largely toxic to humans and the environment.Bioremediation is a waste management technique that consists the use of organisms to eliminate or neutralize pollutants from a contaminated site. Eiseniafetida, the most widely used earthworm for vermicomposting and waste reduction.The physical parameters pH, EC and biochemical parameters and total TPH were analysed in the normal and crude oil contaminated soil.The soil samples were contaminated with various concentrations of crude oil (10, 20, 30, 40, 50,100 and150 g per kg of soil).Ten earthworm (Eiseniafetida) was introduced into the containers and the weight of each test container was recorded. At intervals of time (after every 5 days), the number of living earthworms in each container was recorded. As the result the increased survival was observed in 10,20,30,40 g of crude oil contaminated soil than 50,100,150 g contaminated soil. The result showed that the Eiseniafetida has the ability to survive at the concentration of 10, 20, 30, 40gm of crude oil concentration.***

Keywords: Bioremediation, Crude Oil, Earthworm

1. Introduction

Environmental Pollution

Environmental pollution is a major global concern of the day. Environmental pollution is contamination of earth's biological and physical components which include water, air and land. This hints to their reduction to an extent that normal environmental processes are extremely affected. Common public in general and young generation in particular should come forward to reduce this hot issue of pollution and protect our environment.

Land Pollution

Land pollution, the accumulation of solid or liquid waste materials on land or underground during a manner that may contaminate the soil and groundwater, threaten public health, and cause unpleasant conditions and troubles.

Hazardous wastes include injurious and risky substances generated primarily as liquids but also as solids, sludge's, or fumes by numerous chemical producing companies, crude oil refineries, paper mills, smelters, machine outlets, dry cleaners, automobile repair outlets, and lot of different industries or commercial facilities.

In mechanic workshops, there are coincidental or conscious discharges or release of petrol, diesel, solvents, grease, and lubricants on the land and the environment. A large number of these Petroleum items are natural synthetic substances that can be exceptionally dangerous and risky to soil fauna and man. The utilization of autos has also led to trace element and heavy metals-contaminated soil, which have grave consequences for soil dwelling organisms. [1].

Oil Contamination

Oil discharged into the surroundings may be a well recognized drawback in today's world. Oil spills have an effect on several species of plants and animals within the surroundings, similarly as humans. Oil contaminated soil in and around exploration and spillage areas causes noticeable injury to the surroundings and it's believed that this changes the chemical, physical and biological standing of the soil within the locality. It's reported that the agricultural land close to the oil exploration sites are typically contaminated by hydrocarbons and land become unfit for cultivation [2]. So, it's necessary to develop new and trendy technology to revive the soil health because of contamination of organic compound.

The properties of crude oil pollution on the soil have been the subjects of many studies. Crude oil pollution rise the organic carbon content of soil, decreases the nitrogen and phosphorous content of soil, destruction soil flora and fauna and reduces soil aeration [3].Bioremediation technique, among alternative treatment choices is the most cost effective and environmental friendly manner of restoring contaminated soils [4].

Crude oil contains a large vary of compounds which are largely toxic to humans and the environment. Some authors have instructed that soil remediation standards ought to be based on the BTEX elements in crude oil and oil products (fuels)-impacted soils. Each of these compounds or their combination poses a serious concern to human health, living organisms and the environment.Crude oil bioremediation technology is the most significant that can avoid oil spills pollution and damages to the ecosystem and environment. Crude oil contamination create a threat to the environment and the remediation is being a major challenge for environment.

Bioremediation

Bioremediation is a waste organization technique that involves the use of organisms to get rid of or neutralize pollutants from a contaminated site. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to interrupt down dangerous substances into less toxic or nontoxic substances". Bioremediation Technologies are commonly classified as *in situ*or*exsitu*. *In situ* bioremediation involves treating the contaminated material

at the location, where as*ex situ* involves the removal of the contaminated material to be treated elsewhere. Some samples of bioremediation connected technologies are phytoremediation, bioventing, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.Soil is the material basis for the sustainable economic and social development, and is one among the foremost valuable natural resources in every country, particularly for our country. Beside, the use of chemicals and mechanical methods for cleaning oil in the environment, one of the most promising bioremediation technologies is the use of earthworms specifically known as vermin remediation. Earthworms are terrestrial annelids with bilateral symmetry. They are important factors in the development of soil structure, organic matter breakdown and nutrient sport. Generally, the activities of earthworm are considered to enhance soil health and in theory will translate to improved bioremediation.

Earthworm Selection for this Study

Eiseniafetida

Eiseniafetida, popularly known as red wriggler, red worm, tiger worm etc is perhaps the most widely used earthworm for vermicomposting and waste reduction. The Earthworm species is commonly used in the U.S., Europe and Australia. The species is active in a wide temperature and moisture ranges. It can process large amounts of organic matter and, under ideal conditions, can consume proportional to its body weight each day. The species has also been in wide usages for various toxicological studies as test worm. The worms are tough, readily handled, and survive in mixed species cultures. It also reproduces rapidly, and is very tolerant against variation in growing conditions.

Figure 1: Eiseniafetida

2. Materials and Methods

Collection of Soil Sample

The Normal soil sample used in the experiment were collected from SriAdi Chunchanagiri Women's college, Cumbum, Theni (DT).The contaminated soil sample and crude oil used in the experiment were collected from workshop at Cumbum, Theni (DT).Contaminated soil sample was collected by hand digging to a depth of 30 cm, it was mixed thoroughly sieved through screens to remove stones, wood particles and other debris.

Figure 2: Crude oil

Collection of Earthworm

The earthworm species (*Eiseniafetida)* was collected from vermicompost Unit, C. Pudupatti. The following test was carried out inTamil Nadu Agricultural University, Department of Soil science and Environment, Agricultural College and Research Institute, Madurai.

Analysis of contaminated soil

Physical Test

The colour and odour of the samples were tested. The following test was carried out in Tamil Nadu Agricultural University, Department of Soil science and Environment, Agricultural College and Research Institute, Madurai.

- pH
- Electrical Conductivity (EC)

Determination of pH

The pH of the given Normal and Crude oil contaminated soil were determined with the help of a pH meter. Before determination, the pH meter was standardized using buffer solution.

Procedure [5]

10g of soil was weighed in 50ml beaker and 25ml of distilled water was added to the soil sample. The solution was stirred with a glass rod occasionally for half an hour. After half an hour, the pH electrodes were dipped in the solution. Once the value shown in the digital pH meter it was stabilized, the pH was recorded for each sample.

Electrical Conductivity

The EC of the given Normal and Crude oil contaminated soil samples were determined with the help of Electrical conductivity meter.

Procedure [6] [7]

Prepared a 1:5 Soil: Water suspension by weighing 10g air-dry soil into a bottle. Add 50ml deionized water. Mechanically shake at 15rpm for 1 hr to dissolve soluble salts.

Calibrated the conductivity meter according to the manufacturers instruction using the KCl reference solution to obtain the cell constant.

Rinsed the cell thoroughly. Measure the electrical conductivity of the 0.01M KCl at the same temperature as the soil suspensions.

Rinsed the conductivity cell with the soil suspension. Refill the conductivity cell without disturbing the settled soil. Recorded the value indicated on the conductivity meter. Rinsed the cell with deionized water between samples.

Biochemical Test

- Macronutrients
- Micronutrients
- Soil Fertility (Organic carbon).

Major Elements

Major elements are the important nutrients required by any agricultural crop.

Estimation of Nitrogen [8]
Materials required

- 0.32 % KMnO4
- 2% Boric Acid
- 2.5% NaOH
- N/50 H_2SO_4
- Liquid Paraffin or Glass Beads

Procedure
20 gm of the sample was taken in distillation flask and then add 30 ml of distilled water and liquid paraffin 2 ml or glass beads were added to prevent pumping.

Then added 100 ml of 0.32% $KMnO_4$and 2.5% NaOH, closed it and delivery end into250ml beaker.

25 ml of 2% Boric Acid was taken in 250 ml beaker and it was titrated against N/50 H_2SO_4. The end point was dark green to brown color.

Estimation of Phosphorus [9]
Materials required

- 0.5N $NaHCO_3$
- Charcoal G60
- Reagent A (H_2SO_4)
- Reagent B (Ascorbic Acid and $H_2SO_{4)}$.

Procedure
5 gm of sample was taken in a shaking bottle. To this added 50 ml of 0.5N $NaHCO_3$then added a pinch of charcoal G60 shake it for 30 minutes by mechanical shaker. After shaking filtered it in 50 ml volumetric flask.

5ml of the filterable sample was taken in the 20ml of volumetric flask. Added 2-3 drops of P.Nitrophenol (yellow colour).Added 4 ml of Reagent B (Ascorbic Acid) then add 5N H_2SO_4drop by drop. The yellow color was disappeared. Then it was make up with distilled water upto 25 ml. Shake it and stand for half on hour. Find the reading of Phosphorus using colorimeter (620, 660nm).

Estimation of Potassium [10]

Materials required
1 N Ammonium Acetate

Procedure
5 gm of sample was taken in Shaking bottle to this add 25 ml of 1N Ammonium Acetate. Shake it for 5 minutes using mechanical Shaker. After shaking it was filtered in 25ml of conical flask. Then the concentration of potassium was read using flame photometer. Then calculate the available Potassium.

Micronutrients [11]
Micro elements are the important nutrients required by any agricultural crop.

Estimation of Copper and Iron
Materials required

- Standard copper solution
- Standard iron solution
- Standard Manganese solution
- Standard ferrous solution
- DTPA (DiethyleneTriaminePenta Acetic acid).

Procedure
15gm of the sample was taken and to this add 30ml of DTPA solution. Then it was shaken for 2 hours. After shaking the sample was filtered. Then the concentration of the micro element in the sample was read using AAS.

Determination of Total Petroleum Hydrocarbon[12]
One gram of each soil samples were dissolved in 10ml of normal hexane and shaken for 10 minutes using a mechanical shaker. The solutions were filtered using whatman No:2 filter paper and the filtrate was diluted by 1ml of the extract into 5ml of the hexane. Test were carried out at three days interval over 18 days period. Residual hydrocarbon was determined using spectrophotometric analysis.

Determination of Organic Carbon
Organic Carbon[13]
Materials required

- 1NPotassium dichromate
- 0.5N Ferrous ammonium Sulphate
- Concentrated sulphuric acid, Orthophosphoric acid
- Diphenolamine

Procedure
0.5gm of soil was taken in a 500ml conical flask. Added 10ml of 1N potassium Dichromate and then added 20ml of Conc.H_2SO_4. Kept it for 30 minutes. After that added 200ml of distilled water and then added 10ml of orthophosphoric acid. Then added 1or 2 drops of Diphenolamine indicator. Then it was titrated against 0.5N Ferrous Ammonium Sulphate. The end point was Black or blue colour change to Green colour.

Earthworm Survival Assessment
The soil sample collected was sieved to remove coarse stones and to homogenize. 1 kg of soilsample was weighed into plastic test containers. The soil moistened with deionised water. The soil samples were contaminated with various concentrations of crude oil (10, 20, 30, 40, 50,100 and150 g per kg of soil). The mixture was thoroughly mixed manually, (it is worth noting that all samples were prepared in duplicates). Ten earthworm (*Eiseniafetida*) was introduced into the containers and the weight of each test container was recorded. At intervals of time (after every 5 days), the number of living earthworms in each container was recorded. The moisture content was maintained throughout the test period by adding deionised water when necessary. The samples were monitored for a total of 30 days(5,10,15,20,25,30). This procedure was repeated to find out the survival rate of earthworms.

3. Results and Discussion

Analysis
Nutrient content of normal Soil Sample
Soil was collected and subjected to nutrients analysis (Table: 1). the pH 7.8 was suitable for proper growth and efficient

uptake of nutrients and compounds from soil. Macro-nutrients including metals were also present in substantial amount[14].

Table 1: Nutrient Content of Normal Soil Sample

	Parameter	Unit	Value
Sample Details	p^H	-	7.8
	EC	Ds m^{-1}	0.33
	Micro nutrient		
	Nitrogen	%	74
	Phosphorus	%	49
Soil	Potassium	%	350
	Macro nutrient		
	Iron	%	4.9
	Manganese	%	2.4
	Zinc	%	0.89
	Copper	%	0.92

Nutrient Content of Crude Oil Contaminated Soil Sample

Contaminated Soil was collected and subjected to nutrients analysis (Table: 2). The results showed that the soil contains higher level of hydrocarbon and heavy metals.Crude oil is rich in carbon and a small amount of nitrogen compounds, so it can change the composition and structure of soil organic matter and impact the C/N, C/P, salinity, pH, and conductivity of soil. The heavy metals in oil mixtures and high concentrations of salt in oilfield output water can also damage the soil environment.

Crude oil polluted soil pore spaces might be clogged which reduces soil aeration, infiltration of water into the soil, increased bulk density of the soil which may affect plant growth. Crude oil which is thicker than water may decrease and restrict permeability. Some of the effects of crude oil may have adverse effects on soil physical properties include decreased pore spaces, saturated hydraulic conductivity and increased bulk density.

Table 2: Nutrient Content of Crude Oil Contaminated Soil Sample

Sample Details	Parameter	Unit	Value
Oil Contaminated Soil (workshop soil)	pH	-	8.2
	EC	dSm^{-1}	0.63
	KMnO4-N	$Kgha^{-1}$	302
	Olsen-P	$Kgha^{-1}$	40
	NNNH4-AcK	$Kgha^{-1}$	505
	Organic carbon	%	5.37
	DTPA Fe	Ppm	39.8
	DTPA -Mn	Ppm	15.2
	DTPA -Zn	Ppm	1.1
	DTPA cu	Ppm	1.6
	Hydro carbon	%	76.5

Survival Rate of *Eisenia fetida* in Crude Oil Contaminated Soil

The survival rate of *Eiseniafetida* was shown in Table3. 100% of survival was observed in 10g concentration on 20 days incubation.70%,60%,50% survival was observed in 20g,30g,40g of concentration on 30 days incubation.30% survival was observed in 50g concentration on 15days incubation.20% of survival was observed in 100g concentration and 0% survival was observed in 150g concentration in the 5days incubation.

Earthworms were unable to survive in soil for 2 weeks after contamination with 5% crude oil[15]. 0.5% crude oil contaminated soil was not harmful to the survival of earthworms for 7 days but an oil concentration of 1.5% reduced survival to less than 40% also, bioremediated soil containing 1.2% oil did not reduce survival of earthworm for 10 days [16].

Table 3: Survival Rate of Eisenia fetida in Crude Oil Contaminated Soil

gm Crude Oil/kg soil	After 5days %	After 10 days %	After 15 days %	After 20 days %	After 25 days %	After 30 days %
10	100	100	100	100	90	90
20	80	80	70	70	70	70
30	70	70	60	60	60	60
40	70	60	60	50	50	50
50	50	40	30	0	0	0
100	20	0	0	0	0	0
150	0	0	0	0	0	0

Table 4: Body Weight of Eisenia fetidain Crude Oil Contaminated Soil

Body weight of Earthworm	After 5days (gm)	After 10 days (gm)	After 15 days (gm)	After 20 days (gm)	After 25 days (gm)	After 30 days (gm)
10	8.2	7.6	7.0	6.5	6.2	5.9
20	7.6	7.1	6.5	5.7	5.2	4.8
30	6.8	6.1	5.8	5.3	4.7	4.1
40	6.7	5.3	4.8	4.3	3.8	3.3
50	4.2	2.1	0.8	0	0	0
100	2.0	0	0	0	0	0
150	0	0	0	0	0	0

The body weight of *Eiseniafetida* was shown in Table 4. 71.95% reduction of body weight was observed in 10g concentration after 30 days of incubation.58.53 %, 50%,40.24% of body weight reduction were observed in 20g, 30g, 40g of concentration after 30 days incubation. 9.75% reduction was observed in 50g concentration after15days incubation.24.39% of reduction was observed in 100g concentration after 5days and no earthworm was found in 150g concentration after 5days of incubation.

4. Summary and Conclusion

Crude oil bioremediation technology is the most important that can avoid oil spills pollution and damages to the ecosystem and environment. Crude oil contamination poses a threat to the environment and the remediation is being a major challenge for environment.

Earthworms are aptly called ‘Cinderella of organic farming’, due to their vital role in sustainable organic farming. Earthworms help to solve some of the problems facing civilization today in rebuilding the soil and restoring the earth to immediate usable form for safe healthy food production and clean environment.

Beside, the use of chemicals and mechanical methods for cleaning oil in the environment, one of the most promising bioremediation technologies is the use of earthworms in a processed specifically known as vermin remediation.

The present investigation was carried out with earthworm *Eiseniafetida* with oil contaminated soil. The Survival rate of earthworm was observed in 5days interval upto 30days (5,10,15,20,25,and 30). As the result the increased survival was observed in 10,20,30,40 g of crude oil contaminated soil than 50,100,150 g contaminated soil. The result showed that the *Eiseniafetida* has the ability to survive at the concentration of 10,20, 30,40gm of crude oil concentration.

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