

Research Article

Microorganisms in Cleaning Soil Contaminated by Waste Oils: Effective Microorganisms (EM) Based Treatment

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Abstract

With increasing industrial activities, pollution also increases and the environment is adversely affected. Especially heavy metal wastes maintain their existence in nature for a long time and seriously pollute the environment. Petroleum-based wastes such as waste oil contain heavy metals and pose a major threat to the environment. In addition to the physical and chemical treatment of heavy metals and other pollutions, more economical biotechnological methods have been developed and applied in recent years. In this study, it has been investigated whether soil pollution can be treated by applying effective microorganisms (EM) in the treatment of soil sample contaminated with waste oil. It is thought that EM technology in soil sample containing waste oil containing heavy metals in high concentration is applicable because it is economical. In the study, 80% of the total petroleum hydrocarbons (TPH) in the soil were removed.

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ATIK YAĞLARLA KİRLENMİŞ TOPRAK TEMİZLİĞİNDE MİKROORGANİZMALAR: ETKİN MİKROORGANİZMALAR (EM) TABANLI ARITMA

Özet

Artan endüstriyel faaliyetler ile birlikte kirlilikler de artmakta ve çevrede bundan olumsuz etkilenmektedir. Özellikle ağır metal özellikli atıklar doğada uzun süre varlığını korumakta ve bulunduğu ortamı ciddi düzeyde kirletmektedir. Atık yağ gibi petrol kökenli atıkların içerisinde ağır metaller bulunmakta ve çevre için başlıca tehdit oluşturmaktadır. Ağır metal ve diğer kirliliklerin fiziksel ve kimyasal arıtımının yanında son yıllarda daha ekonomik biyoteknolojik metotlar da geliştirilerek uygulanmaktadır. Bu çalışmada, atık yağ ile kirlenilen toprak numunesinin arıtımında etkin mikroorganizmalar (EM) uygulanarak toprak kirliliğinin arıtılıp arıtılamayacağı araştırılmıştır. Yüksek konsantrasyonda ağır metal içeren atık yağ ihtiva eden toprak numunesinde EM teknolojisi ekonomik olmasından dolayı uygulanabilir olduğu düşünülmektedir. Yapılan çalışmada topraktaki toplam petrol hidrokarbonların (TPH) %80 oranında giderimi gerçekleştirilmiştir.

Anahtar Kelimeler
Biyolojik Arıtım
Etkin Mikroorganizma
Toprak Kirliliği
Atık Yağ

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INTRODUCTION

With the increasing population, the importance of land required for socio-economic living habits is also increasing [1-3]. Rapid, unplanned urbanization and industrialization, increased traffic, mines operated without necessary precautions, improper land use, agricultural land use, increased chemical fertilizer and pesticide applications, and soil erosion cause environmental pollution in our country as well as worldwide [4, 5].

Soils can be contaminated in different ways, such as the unconscious spilling or dumping of hazardous and toxic substances (oil, paint, heavy metals/metalloids, etc.) into the soil, leaks resulting from accidents in oil pipelines, discharge of wastewater into open areas, unconscious dumping or burial of waste, dumping waste oil, and even harmful gases emitted from chimneys can cause soil contamination [6, 7]. These contaminants remain in nature for a long time and can be transported by many different external factors such as air, water, and wind. This displacement puts our clean areas at risk and causes serious problems. Soil can be thought of as the meeting point of all pollutants and can be considered the environment where pollutants permanently settle [8, 9]. If left unaddressed, soil pollution contaminates in different ways depending on the pollutant and its characteristics. These contaminants can remain in the soil for various periods of time, causing a decline in the quality of the contaminated environment, making it unproductive, barren, and leading to soil loss. They also accumulate in the human body and show their effects at different times [10, 11]. With recent technological advances, different treatment methods have been developed for both wastewater treatment and soil contamination remediation. Physical, chemical, and biological treatment methods are constantly evolving, and efforts are being made to make costly treatment methods more economical [12-15].

EM is a mixture of microorganisms that can coexist and positively affect soil properties and plant growth (it contains mixed cultures of beneficial microorganisms such as photosynthetic bacteria, lactobacilli, yeasts, and actinomycetes). EM stimulates the activity of soil microflora that naturally participates in the decomposition processes of organic matter, contributing to the improvement of soil quality and environmental protection [16, 17]. Biopreparations made with EM are recommended to increase soil fertility and productivity, enhance soil biological activity, improve germination, rooting, and flowering processes, and improve fruit quality [17]. EM are an example of biological methods (bioremediation) used to restore the appropriate ecological balance of the ecosystem and the self-purification capacity of water [18]. In addition to their use in increasing soil productivity, EM are also applied in various pollution treatments. According to a study, EM and their consortia, used in commercial biological preparations and formulations for soil revitalization, were investigated for their contribution to the degradation of a mixture of 13 polycyclic aromatic hydrocarbons (PAHs) commonly found in the soil environment, and a reduction of 75.5-95.5% was observed [19]. Another study aimed to evaluate the potential of EM and plant cover as methods to support soil bioremediation processes and achieved productive results [20]. EM can be utilized to address soil problems in terms of improvements in soil texture, electrical conductivity (EC), organic matter, and phosphorus content [21]. It has also been investigated that EM can effectively serve as a bioremediation medium for hydrocarbon-contaminated soils [22]. Microorganisms are the most effective management tool for managing polluted environments and remediating contaminated soil. Therefore, beneficial microorganisms are an innovative technology with the potential to mitigate toxic pollution [23]. The use of microorganisms shows promise in the remediation of soil contaminated with organic compounds, including heavy metals and radionuclides, chlorinated solvents such as TCE (Trikloroetilen); explosives such as TNT (2,4,6-trinitrotoluen) and RDX (heksahidro-1,3,5-trinitro-1,3,5-triazin); petroleum hydrocarbons including (PAHs); PCBs; and pesticides such as atrazine and organophosphates [24]. Previous studies have reported limited research on the ability of EM to remediate soil contamination. Similar microbial and biosorption-based treatment mechanisms have also been successfully demonstrated in aqueous systems [25-26], which provide a conceptual background for understanding microbial interactions in soil remediation. The novelty of effective microorganism technology has raised questions about its potential to effectively remediate contaminated soils.

This study investigated whether soil collected from the garden of a factory in an organized industrial zone and a waste oil mixture considered contaminated with heavy metals could be treated using EM technology. Waste oil with code number 130208 was obtained from a waste oil recovery and refining facility located in Aksaray province, and soil samples were collected from the garden of the same company at the same address. The soil samples obtained were mixed in a laboratory environment and contaminated. An EM culture was added to the contaminated soil mixture, and the results were observed. Here, the goal is to treat contaminated soil using EM technology, which is a biological treatment method. Previous studies have not found much research on EM's ability to remove soil contamination. The novelty of EM technology has raised questions about its ability to remediate soil contamination. The reason for choosing soil contamination in this study is that the spillage of waste oil onto soil as a result of accidents has become a serious threat frequently encountered in road transport. As a result of increasing accidents and pollution, all countries have begun to take ownership of their soils and have started to try new technologies for pollution remediation methods. The novelty of this technology and the fact that it has never been tested on waste oil pollution has made our study unique.

MATERIAL AND METHOD

Soil sample collection and preparation are shown in Figure 1. The soil sample used in this study was collected from the garden of a factory located in the Organized Industrial Zone in Aksaray Province. The samples were taken from a sampling depth of 20 cm, brought to the laboratory, and stored in sample storage bags at room temperature until they were prepared for analysis. To prepare the system, 1 kg of soil was sieved from the soil sample and placed in the test container.



Figure 1. Preparation of soil samples

Mineral oils, group (Group I, Group II, Group III, Group IV, and Group V), base stock (mineral-based lubricants, synthetic lubricants, semi-synthetic lubricants, and bio-based lubricants), product type (engine oil, transmission and hydraulic fluid, metalworking fluid, general industrial oil, gear oil, grease, process oil, and other product types), end-user industry (energy production, automotive and other transportation, construction machinery, food and beverage, metallurgy and metalworking, chemical manufacturing, and other end-user industries), and geography (Asia-Pacific, North America, Europe, South America, Middle East, and Africa) [27]. According to the lubricant classification, the sample studied falls under GROUP 1 [27]. The waste oil sample was taken from waste oil storage tank No. 1, a waste oil recycling and refinery facility operating in Aksaray, and placed in a 3 kg glass sample container. The waste oil is 130208-grade engine oil [28].

Typical diesel oil concentrations in contaminated soil have been reported in the range of 5,000-15,000 mg total petroleum hydrocarbons (TPH) (kg soil-1) [28]. The target contaminant in this study was waste oil added at 10,000 mg/kg in the dry basis to the sample. The added waste oil is mixed homogeneously with the soil. The soil sample contaminated with waste oil is shown in Figure 1.

EM, Microbix plus INICULANT this environmental product containing microorganisms was obtained from Egem Natural and Biotechnology Agricultural Products Production and Marketing Trade Ltd. Co. This mixture contains lactic acid bacteria, yeasts, molds, actinomycetes, aerobic and anaerobic microorganisms. This product is an environmental product belonging to the EM-1 (Microbial Inoculant Soil Amendment) type. Studies have found that soil remediation is achieved by adding EM technology to contaminated soil at a rate of 1 L per hectare or by applying 10 L of microorganism culture and 100 kg of powdered organic matter per acre of land. Additionally, in applications where the mixture is thoroughly mixed until it reaches 30% moisture content [30], the prepared EM mixture is evenly sprayed onto the soil sample contaminated with waste oil. After spraying, the plastic soil sample container is closed and left in a laboratory environment under normal conditions for 2 weeks (Figure 1). Figure 1 shows the condition of the soil sample contaminated with waste oil and treated with EM after 2 weeks.

The names and methods of the analyses performed in this study are summarized in Table 1. After pre-treatment, the prepared samples were measured using a TOC-VCPN Total Organic Carbon Analyzer SHIMADZU and a TNM-1 Total Nitrogen Measuring Unit SHIMADZU device. This analysis examined the soluble forms of carbon and nitrogen. ICP-OES Heavy Metal Determination: After pre-treatment, heavy metals in soil samples were measured using an OPTICAL Emission Spectrometer optima 2100 DV (Perkin Elmer Precisely) device. This analysis examined the soluble forms of heavy metals.

Table 1. Analysis Methods

Analysis	Method
pH	EPA Method 9045C
EC	EPA Method 9045C
Water Content	Standard Methods-AWWA-1981
Organic Matter Content	Standard Methods-AWWA-1981
Unit Volume Weight	ASTM D7263 - 09
Porosity	ASTM F1815 - 11
Specific Gravity	ASTM C 128
Field Capacity	
Oil-Grease Determination	TS 8312
Total Petroleum Hydrocarbons (TPHs)	TS EN 14039
Eluate	Methods 1310

RESULT AND DISCUSSION

This section contains the findings and evaluation of the study. The system was operated for 15 days. We named the initial sample 0, the sample taken at the end of the 7th day 1, and the sample taken at the end of the 15th day 2. They were named accordingly in the tables and figures. The results were obtained by taking the averages of two replicates.

The properties of the raw soil and waste oil samples were determined. The general properties of the soil sample, based on the analysis results, are given in Table 2. The analysis results and analysis methods of the waste oil used are given in Table 3. As a result of the analyses, it was determined that the sample studied falls into the 1st category of waste oil according to the classification table of mineral oils.

Table 2. General properties of the soil

	pH	EC, μS/cm	SM, %	OM, %	BHA, g/cm ³	Porosity, %	G _s , g/cm ³	Field Capacity
Soil	8.48	458	5.37	15.92	1.09	32.14	2.80	57.45

Table 3. Properties of waste oil sample

Parameter	Unit	Analysis Result	Analysis Method
Arsenic (As)	mg/kg	<0.1	EPA 3051 A EPA 6020 B
Cadmium (Cd)	mg/kg	<0.1	EPA 3051 A EPA 6020 B
Lead (Pb)	mg/kg	5.09	EPA 3051 A EPA 6020 B
Chromium (Cr)	mg/kg	1.82	EPA 3051 A EPA 6020 B
Determination of Polychlorinated Biphenyls (PCBs)	mg/kg	<0.1	TS EN 12766-2

As a result of the test period, pH values are shown in Figure 2. Looking at the pH values in the figure, a very slight decrease is observed compared to the beginning. As soon as waste oil mixes with the soil, it causes undesirable results in the ecosystem where it is located and negatively affects soil life. It disrupts the soil texture, reduces aeration, blocks the nutrients used by plants, and causes the soil pH to decrease [31].

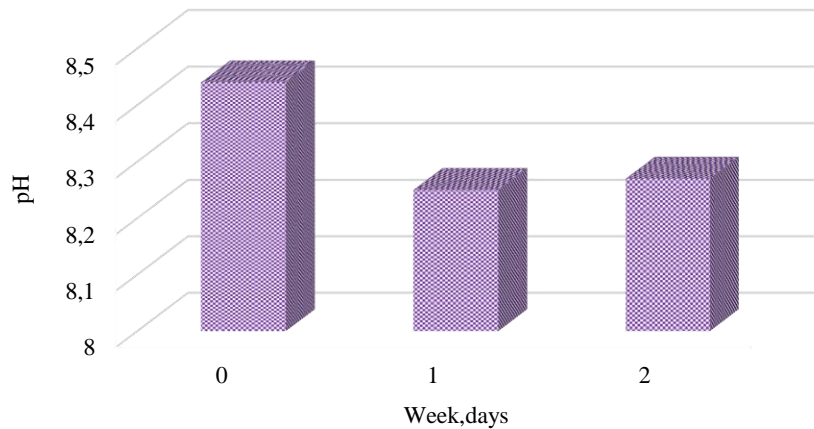


Figure 2. pH results

Looking at the soil pH values, the initial value of 8.44 decreased by 2.2% to 8.25 at the end of the first week and by 2% to 8.27 at the end of the second week. Compared to the initial value, the values for the first and second weeks decreased by approximately 2%, increasing the acidity of the soil. As the pH decreased, the uptake of trace elements in the soil increased, resulting in an increase in iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) values. As the soil pH decreased, there was an increase in organic matter in the soil. It is easier to regulate the soil structure of low pH soils, and this can be done with lime [32].

When examining the EC values, only a slight increase was observed compared to the initial value (Figure 3).

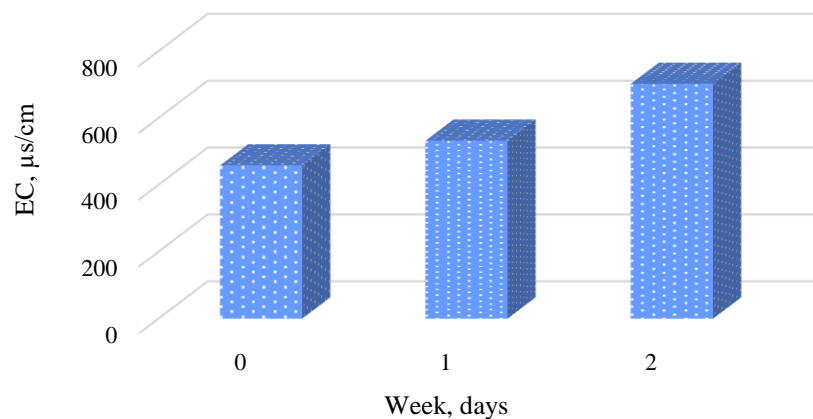


Figure 3. EC results

When looking at the EC values, a very slight increase was observed. The EC values were 532 µs/cm at the end of 7 days and 702 µs/cm at the end of 15 days, respectively. Due to the anions and cations released into the soil as a result of the decomposition of organic matter, an increase in soil conductivity has occurred. Furthermore, due to the decrease in pH, an increase in trace element uptake has been observed, along with a parallel increase in soil conductivity [32].

Figure 4 shows the water content results. Looking at the results of the samples taken at the beginning, at the end of the 1st week, and at the end of the 2nd week, the first thing that stands out is that there was a decrease at the end of the 7th day and at the end of the 15th day, but the value at the end of the 7th day continued to increase at the end of the 15th day.

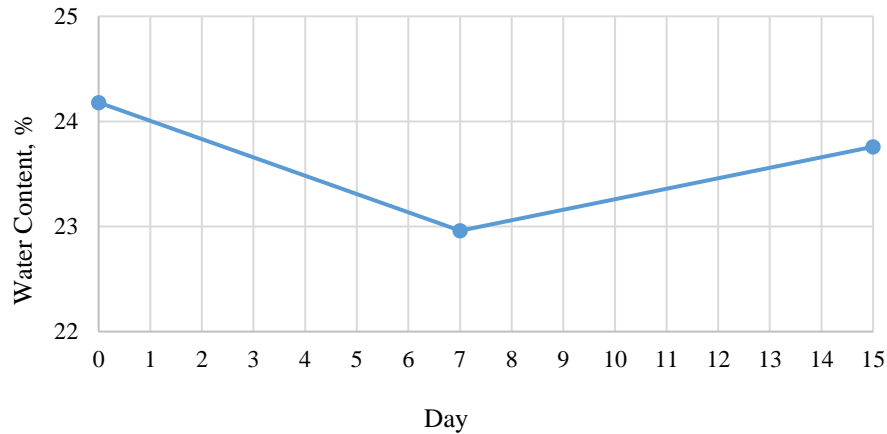


Figure 4. Water content results

Water content varies depending on the region where the soil is located and changes according to the environmental conditions, climatic factors, and soil structure of the region. In temperate and rainy regions, the water retention capacity of the soil is low, while in arid regions with low rainfall, the water retention capacity is high. At the same time, water content and organic matter content are inversely proportional [32].

When looking at organic matter content, it was found to be inversely proportional to water content; organic matter increased when water content decreased, and decreased when water content increased (Figure 5). Looking at the organic matter results, the organic matter content on Day 7 increased by approximately 4% compared to the initial value. Active microorganisms increased the organic matter content immediately after being applied to the soil. The organic matter value on Day 15 decreased by approximately 3% but was still higher than the initial value.

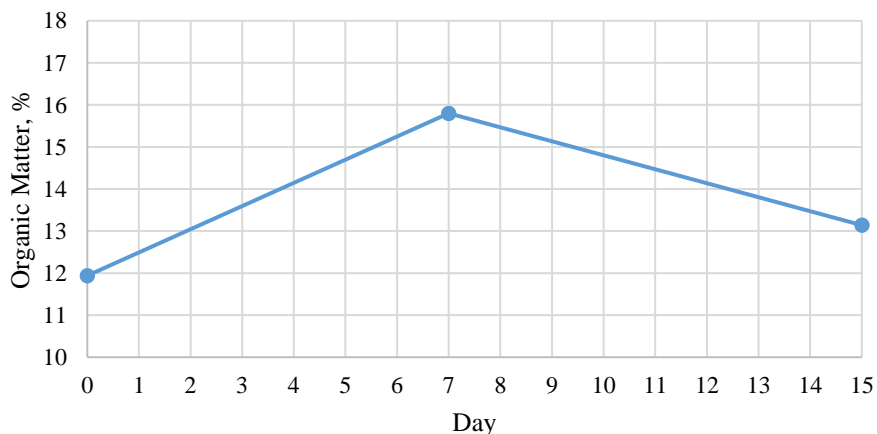


Figure 5. Organic matter results

Soil bacteria, fungi, and actinomycetes contribute to the decomposition of organic matter in the soil into minerals that are beneficial to plants. Enzymes produced by microorganisms, which are generally heterotrophic, convert complex compounds in organic matter, such as proteins, lignin, starch, cellulose, and similar compounds, into a form that can be used by plants and microorganisms. The amount of organic matter in soils is considered an indicator of microbial activity [33].

The results of the TPH analysis are presented in Figure 6. A decrease was observed in the results of the samples taken at the beginning, in the 1st week, and in the 2nd week.

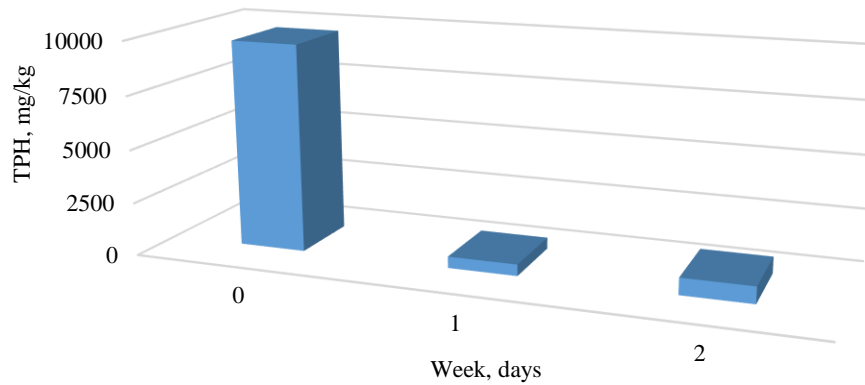


Figure 6. Results of TPH

The presence of TPH in the soil indicates that the soil is rich in petroleum and petroleum gas. Waste oils also cause an increase in the total petroleum hydrocarbon content of the environments they are found in due to their complex petroleum-based structure [34]. A high total petroleum hydrocarbon value indicates the presence of petroleum or petroleum-based contamination in that area [35].

TPH value of the soil sample contaminated with waste oil was initially measured at approximately 10,000 mg/kg, and after 7 days, i.e., in the first week, the value measured after the addition of EM decreased to approximately 2,000 mg/kg. There was an approximate 80% decrease in the total petroleum hydrocarbon amount. This decrease shows us that the EM improved the contaminated soil to a very high degree. The value read in the second week, approximately 2500 mg/kg, indicates that the activity of the microorganisms decreased very little.

The oil-grease results of the samples taken at the beginning, 1st week, and 2nd week are shown in Figure 7.

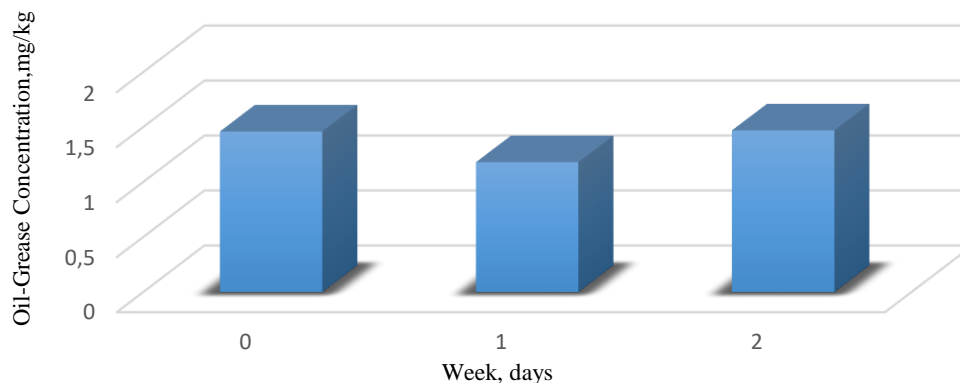


Figure 7. Oil-grease results

Oil layers form at different points in the soil sample mixed with waste oil, mostly in the upper layer. These oil layers block the passage of oxygen from the air, reducing the aeration rate of the soil. At the same time, water passage from the oil layers to the soil is also difficult. Soils with no water transfer can become dry and infertile. Oil and grease can persist in soils for long periods and cause pollutants to remain in soil for many years due to the anaerobic environment they create. Soils are seriously adversely affected because they are unwanted contaminants in the soil for a long time [31]. In 1st week, a decrease was observed in our oil grease ratio compared to the initial value, while in 2nd week, there was a slight increase. The reason for this can be said to be the activities of microorganisms.

The TOC, TC, and IC results of the initial sample, raw soil, and samples taken in the first and second weeks are shown in Figure 8. At the end of the second week, TOC and organic carbon showed a parallel increase. The IC results remained at the same levels in the raw soil, initial sample, and 1st week, while a slight decrease occurred in 2nd week. These differences indicate that they arose as a result of microorganisms exhibiting biological activity working in a discontinuous system.

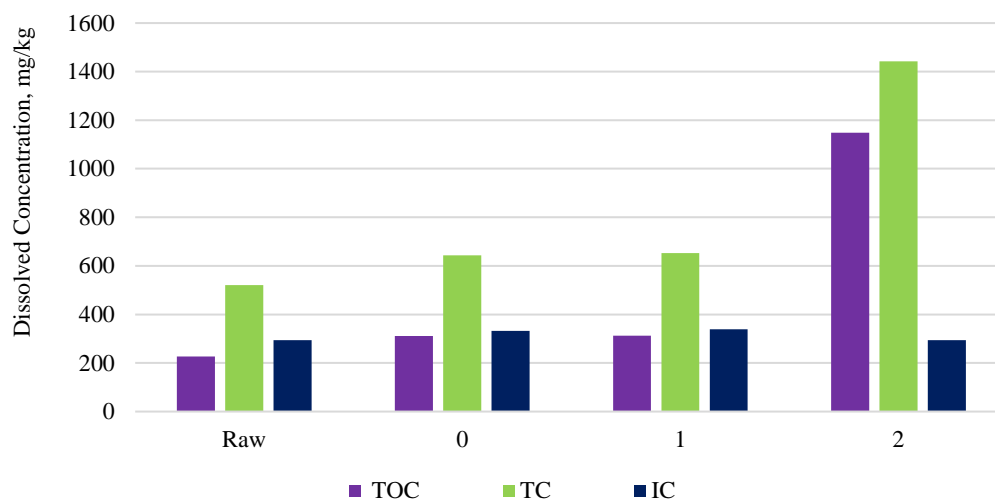


Figure 8. TOC, TC, and IC results

TC, TOC, and TPH provide significant guidance for petroleum contamination in soil and waste oil contamination, which is petroleum-based pollution. A positive relationship was found between TPH and TOC. This relationship is due to the fact that hydrocarbons, which increase from organic matter decomposed by microorganisms in sediments, subsequently prepare the ground for the accumulation of TOC and TC in soil pores and groundwater. The results for B, Ca, K, Mg, and Na in the samples taken from raw soil, at the beginning, after 1st week, and after 2nd week are shown in Table 4, and the heavy metal results are shown in Table 5.

As a result of the values read in the soil sample, increases occurred and their approximate percentages were calculated. There was an increase of approximately 93% in barium (Ba), 16 % in calcium (Ca), 6.5% in potassium (K), 9% in magnesium (Mg), and 21% in sodium (Na).

Table 4. Ba, Ca, K, Mg, and Na results

mg/kg	Raw	0	1	2
Ba	0.394	0.754	1.423	1.457
Ca	332,229	404,229	411,943	471,429
K	200,400	196,800	225,086	209,657
Mg	130,354	157,320	173,486	171,771
Na	153,874	170,537	198,343	207,600

Table 5. Heavy metal results

mg/kg	Raw	0	1	2
Ag	0	0	0	0
Al	0.303086	0.117994	0.179829	5.036571
As	Outside measurement range	Outside measurement range	Outside measurement range	Outside the measurement range
Ba	Outside the measurement range	Outside the measurement range	Outside the measurement range	Outside the measurement range
Be	Outside the measurement range	Outside the measurement range	Outside the measurement range	Outside the measurement range
Bi	0	0	0	0
Cd	Outside measurement range	Outside measurement range	Outside measurement range	Outside the measurement range
Co	Outside the measurement range	Outside the measurement range	Outside the measurement range	Outside the measurement range
Cr	Outside the measurement range	Outside the measurement range	Outside the measurement range	Outside the measurement range
Cu	0.021789	0.051291	0.060891	1.724571
Fe	0.247714	0.182229	0.204857	4.088571
Hg	0.04548	0.071949	0.074829	0.074846

Li	1.131771	1.055143	1.185771	1.151486
Mn	0.044503	0.040491	0.04812	0.135394
Mo	0.259714	0.678514	0.746057	0.598971
Ni	0.147446	0.169149	0.198171	0.198
Pb	0.08496	0.097406	0.124303	1.849714
Sb	57.97714	60.82286	85.21714	87.99429
Se	0.201086	0.206914	0.233657	0.233143
Si	109.32	111.3429	157.92	163.5943
Sn	0	0	0	0
Sr	0	0	0	0
Zn	0.27486	0.350229	1.829143	1.774286

The microbial population in the soil mostly expends energy to break down waste mineral products, but it also causes an increase in certain compounds found in the soil. As a result of soil contamination with waste oil, changes occur in the physicochemical, chemical, and biochemical properties of the soil, along with the processes that cause these changes. Due to its characteristic properties, waste oil contains toxic compounds and metal components. Because of these properties, it is thought that some heavy metals cause inhibition [31].

The values read in the soil sample can be summarized as follows. Ag values were read as 0. The value of Al in the initial sample decreased compared to the value in the raw soil, but the values in 1st week and 2nd week increased compared to the initial value. The 2nd week value increased by 4.856881 mg/kg compared to the 1st week value. The 1st week value increased by 0.061835 mg/kg compared to the initial value. The Al value increased by more than 100% compared to the initial value. As, Ba, Be, Cd, and Cr values were considered outside the measurement range because they were read as negative values. Looking at the 1st and 2nd week values for Co, it is noteworthy that there was an approximate 22% increase compared to the initial value. There was a gradual increase in the Cu value, and the value read at the end of the 2nd week was approximately three times higher than the value read at the beginning. The Fe value in the raw soil continued to increase and reached its highest value at the end of 2nd week. An increase of over 100% occurred, which is thought to be due to the increased uptake of trace elements in the soil as the pH decreased [32]. Parallel to the decrease in pH, there was also an increase in the values of iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). There was a steady increase of approximately 4% in Hg values. The Li value in the raw soil decreased initially but increased by approximately 9% at the end of the 1st and 2nd weeks. The Mn value in the raw soil decreased initially but gradually increased by approximately over 100% during the 1st and 2nd weeks. The Mo value increased initially and in 1st week but decreased by 11% in 2nd week. The Ni value in raw soil increased by approximately 17% initially and in Week 1, remaining stable at the 1st week value at the end of 2nd week. The Pb value increased at the beginning, with a slight increase compared to the initial value in 1st week, but by the end of 2nd week, the Pb value had increased significantly, well above 100% compared to the initial and 1st week values. The Sb value increased steadily by approximately 40% and increased by 30.01715 mg/kg compared to the raw soil value by the second week. The Se value increased by 12% compared to the initial value in the first week, and the second week value decreased compared to the first week value but remained above the initial and raw soil values. The Si value increased by approximately 45%. The second week showed an increase compared to the first week, the first week showed an increase compared to the initial value, and the initial value also showed an increase compared to the raw soil value. The raw soil value increased by 54.2743 mg/kg after 2nd week, reaching 163.5943. Si is known to strengthen plant tissue. Therefore, an increase in Si in the soil can be considered a positive result because it increases soil fertility. Sn and Sr values were read as 0. The initial Zn value increased by 0.075369 mg/kg compared to the Zn value in raw soil. The value in 1st week increased by 1.478914 mg/kg compared to the initial value, and the value in 2nd week decreased by 0.054857 mg/kg compared to the value in 1st week. This analysis examined the soluble forms of heavy metals, carbon, and nitrogen, and the results were recorded as follows.

CONCLUSION

When the contaminated soil sample was observed one week later with the container lid open, a strong oil odor was detected. This odor indicated that the bacteria in the EM culture were active in an anaerobic environment. When we examined our sample two weeks after adding the active microorganism culture to the soil contaminated with waste oil, green sprouts had formed on it. These sprouts gave us a clue that there could be living organisms in the soil sample, and the sprouts supported this. In conclusion, the EM technology as a soil remediation method is a preferable method due to its ease of application, natural structure, environmentally friendly alternative, and economic nature. The fact that it leaves no harmful residues in the environment after remediation and has a natural structure that enriches the soil alongside remediation supports the increase in the productivity of our soils and contributes to the country's economy. However, the use of effective microorganisms is recommended for future soil remediation and pollution prevention efforts.

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