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The Relationship Between Soil Oil Pollution Levels, Microbial Enzyme Activity, and Bioremediation Strategies

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ABSTRACT

Soil pollution is a serious environmental issue in most industrial and agricultural regions, as human activities such as intensive farming and industrialization result in soil pollution and degradation through the introduction of toxic compounds, including hydrocarbons and heavy metals. Microorganisms, particularly bacteria and fungi, play a crucial role in the degradation of these pollutants by producing enzymes that convert toxic compounds into less toxic forms. This research aims to explore the linkage between enzymatic activity and degrees of pollution within the soil because enzymatic activity is needed for the degradation of organic pollutants such as hydrocarbons. Soil samples were defined as either unpolluted or polluted. The enzymatic activity was measured with spectrophotometric analysis, and the degree of pollution was divided into low, medium, high, and very high. The findings showed that enzymatic activity is proportionally related to levels of pollution; as levels of pollution increased, enzymatic activity also increased, indicating the microbial reaction to such changes. The enzyme activity was minimal in unpolluted soils but grew stronger in polluted soils, particularly in cases of severe pollution. The findings identify the need to measure biological activity in polluted soils for the purpose of guiding remediation processes. Several methods have been suggested to enhance biodegradation, including adding essential nutrients, improving aeration, and introducing pollution-tolerant organisms.

1. INTRODUCTION

Land pollution is a severe environmental hazard that exists in most industrial and agricultural regions of the world. The soil is an essential component of an ecosystem, sustaining life by providing food and water to plants. Nevertheless, practices such as intensive farming, industrialization, and chemical storage reduce the soil quality and contaminate it with various toxic materials, for instance, hydrocarbons and heavy metals [1].

Fungi and soil bacteria are the primary agents for pollutant degradation due to their ability to break down toxic organic molecules into less toxic compounds. Microorganisms' released enzymes are the agents of pollutant degradation, which shows biological activity in the soil and the impact of pollution on the soil's biology. A study of hydrocarbon-degrading enzymes is crucial in determining how pollution affects biological activity in the soil. Soils that have been contaminated with hydrocarbons from industrial activities, oil spills, or the use of petroleum products undergo rigorous biological degradation [2]. Microorganisms, such as bacteria and fungi, enable the degradation of these contaminants through the secretion of some enzymes that break down complex organic chemicals, i.e., hydrocarbons, into simpler and more degradable compounds or broken down further into less toxic chemicals [3].

Earlier research [4] demonstrated that higher soil pollution is responsible for enhanced enzyme activity. The study [5] determined that hydrocarbon-polluted soil contained higher activity of certain enzymes, such as catalase and dehydrogenase. The findings of study [6] indicated that increased aeration and the introduction of nutrients led to increased biological activity in soil, which increases the breakdown of pollutants, and evaluated the effectiveness of the application of pollution-resistant microorganisms in the breakdown of pollution in soil. The findings indicated that the application of specific bacterial strains can improve the efficiency in the treatment of pollution, particularly in highly contaminated situations. Soil enzymes play a significant role in breaking down contaminants and enhancing bioremediation processes by catalyzing chemical reactions that degrade organic compounds and heavy metals into less harmful substances. However, the underlying mechanisms of these processes are not yet fully understood [6]. The reason for such ignorance is the drastic range of microbial activity, the impact of a variety of environmental factors, and the nature of the pollutant content of the soil. There are a variety of enzymes that are responsible for the biodegradation of contaminants, including dehydrogenases, which have been documented as a valuable indicator of microbial activity because they catalyze oxidation-reduction reactions included in organic matter decomposition. Some peroxidases and laccases facilitate the breakdown of recalcitrant organic pollutants, like hydrocarbons and heavy metals, by biological oxidation processes. Phosphatases are some of the enzymes that also play a very crucial role in biogeochemical cycles, but their contamination will disrupt the nutrient balance in the soil. Soil enzyme activity is regulated by chemical and environmental factors. To begin with, the nature and concentration of the pollutant affect the activity of enzymes because high pollution will inhibit the activity of enzymes and, hence, biodegradation processes [6]. Secondly, microbial diversity plays a role in promoting or suppressing the activity of enzymes because the presence of heavy metal-resistant bacteria helps in enhancing the stability and activity of these enzymes [7]. Thirdly, soil properties such as pH, water, and organic matter content dictate the stability and effectiveness of enzymes [8]. Finally, external environmental factors, such as oxygen availability and temperature, regulate the rate of biodegradation [9].

In an attempt to increase soil enzyme participation in biodegradation, some emerging strategies, such as microbial bioaugmentation, are performed, where there is a microbial-based addition of microbes specializing in biodegrading toxins into the soil for a better native enzyme content [10]. Nanotechnology-based immobilization techniques of enzymes are also being used, which give stability and activity to enzymes in extreme conditions [11]. Interactions between plants and microbes are also driving forces that provoke the activity of enzymes because the roots of plants secrete compounds that stimulate the production of enzymes capable of degrading contaminants from the soil [12]. Therefore, this study aimed to explore the relationship between pollution levels and enzyme activity in soil, given the importance of enzyme activity in the degradation of organic pollutants, particularly hydrocarbons.

2. MATERIALS AND METHODS

2.1 Collection and preparation of soil samples

Sampling was chosen 16 sites at the Daura refinery, both in contaminated zones (near pollution sources) and uncontaminated zones (comparison sites) according to Figure 1, and soil samples were collected from a depth of 15-30 cm using clean tools (shovels and bags). Each sample was placed in a separate bag, tagged with the location and date, and stored at a low temperature (in the refrigerator) until analysis, according to the study [13].

In the lab, the samples were sifted to remove pebbles and bigger debris and then moistened with distilled water as needed. The samples were divided into three groups: polluted, uncontaminated, and controlled with additional hydrocarbons according to study [14].

Enzyme activity measurement: Each category had its own set of enzyme analysis solutions (such as hydrocarbon solutions). The soil samples were placed in test tubes, and the solutions were applied. The tubes were then incubated at a suitable temperature (such as 25-30°C) for 24-48 hours according to study [15].

After incubation, the samples were collected, and the optical absorption measuring technique was used to determine enzyme activity levels (low, medium, high, and very high by adding the standard solution. The hydrocarbon solution was added to the tubes containing the soil samples after the experimental procedure according to study [16]. Reagents were introduced into the tubes, the contents were thoroughly mixed, the measuring instrument was turned on, and the proper wavelength was selected based on the reagent employed, which ranged from 400 to 600 nm. The test tubes were placed in the measuring equipment after a predetermined response time had passed, according to study [17].

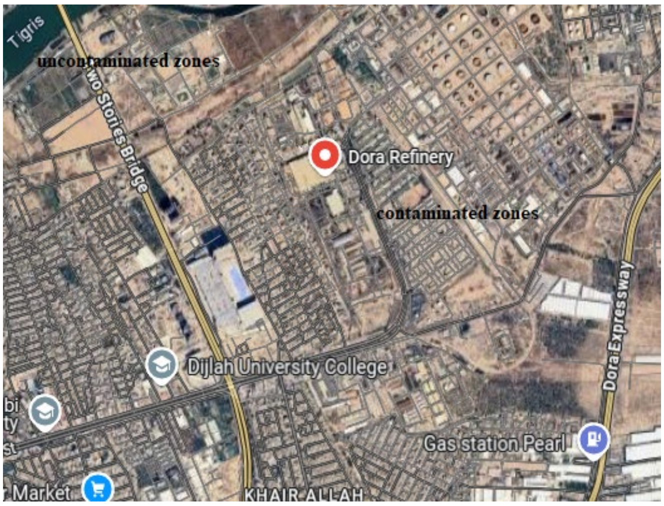

Figure 1. Google Maps sampling sites in the Daura refinery: Contaminated and uncontaminated areas
Longitude Latitude Contaminated and uncontaminated (44.426984° 33.279958°), (44.431183° 33.273127°)

3. RESULTS AND DISCUSSION

The absorbance readings for each sample were recorded in Table 1; the absorbance readings into different contamination levels were based on the average readings for each sample.

Table 1. The absorbance reading for each sample was recorded

Sample Number	Sample Condition	Absorbance Reading (450 nm)
1	Non-polluted (Control Group)	0.10
2	Non-polluted (Control Group)	0.09
3	Non-polluted (Control Group)	0.08
4	Non-polluted (Control Group)	0.10
5	Polluted (Low Hydrocarbon Level)	0.15

6	Polluted (Low Hydrocarbon Level)	0.12
7	Polluted (Low Hydrocarbon Level)	0.14
8	Polluted (Medium Hydrocarbon Level)	0.25
9	Polluted (Medium Hydrocarbon Level)	0.22
10	Polluted (Medium Hydrocarbon Level)	0.23
11	Polluted (High Hydrocarbon Level)	0.35
12	Polluted (High Hydrocarbon Level)	0.30
13	Polluted (High Hydrocarbon Level)	0.32
14	Polluted (Very High Hydrocarbon Level)	0.45
15	Polluted (Very High Hydrocarbon Level)	0.50
16	Polluted (Very High Hydrocarbon Level)	0.48
Standard Solution	Reference	0.20

The bar chart in Figure 2 shows the average absorbance of each sample at 450 nm. The dashed blue line indicates the standard absorbance of the reference solution 0.5.

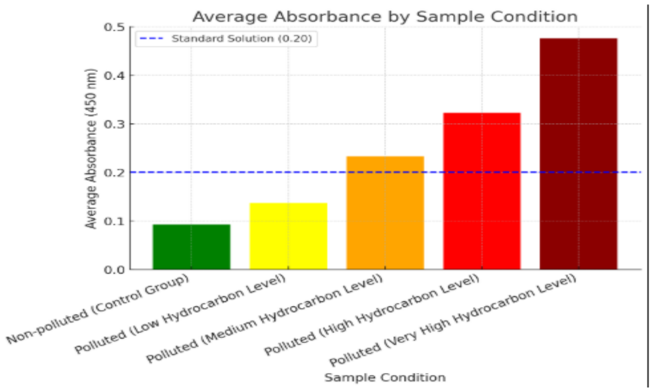

Figure 2. Average absorbance at 450 nm for samples with different contamination conditions

The mean absorbance at 450 nm for samples of different levels of contamination including:

Uncontaminated: The uncontaminated samples recorded the lowest reading of absorbance (0.095), indicating the presence or minimal effect of contaminants.

Low: The absorbance increased to 0.135, which indicates a minimal effect of contamination.

Medium: The absorbance increased to 0.240, which indicates increased absorption of contaminants.

High: The absorbance increased to 0.325, indicating an increase in contaminant concentration.

Very High: The highest absorbance values were observed for the samples (0.490), indicating the highest levels of contamination and the highest impact on absorbance.

According to Table 2, the general trend is that absorbance is rising with increasing levels of contamination, which indicates that contaminated samples absorb light at 450 nm to higher degrees than the non-contaminated samples. The relationship can be applied as a tool for quantifying the degree of environmental contamination by measuring absorbance.

3.1 Creating enzyme activity curves

The absorbance data have been used to create a curve that shows the relationship between the level of contamination and the enzyme activity, according to the study [18].

To create a curve that shows the relationship between the level of contamination and the enzyme activity using the absorbance data, the data was prepared to determine the levels of contamination and the absorbance readings for each level, as shown in Table 2, and the average absorbance for each level.

Table 2. The average absorbance reading for each contamination level

Contamination Level	Absorbance Readings	Average Absorbance
Uncontaminated (Low)	0.10, 0.09, 0.08, 0.10	(0.10 + 0.09 + 0.08 + 0.10) / 4 = 0.095
Low Contamination	0.15, 0.12, 0.14	(0.15 + 0.12 + 0.14) / 3 = 0.135
Medium Contamination	0.25, 0.22, 0.23	(0.25 + 0.22 + 0.23) / 3 = 0.233
High Contamination	0.35, 0.30, 0.32	(0.35 + 0.30 + 0.32) / 3 = 0.323
Very High Contamination	0.45, 0.50, 0.48	(0.45 + 0.50 + 0.48) / 3 = 0.477

3.2 Prepared data for plotting

The prepared data (Table 3) have been utilized to build the enzyme activity curve.

Table 3. The final prepared data for plotting the enzyme activity curve

Contamination Level	Average Absorbance
Uncontaminated	0.095
Low Contamination	0.135
Medium Contamination	0.233
High Contamination	0.323
Very High Contamination	0.477

There is a relationship between pollution levels and enzyme activity average absorbance, enzyme activity increases as pollution levels grow based on the presented data in Figure 3.

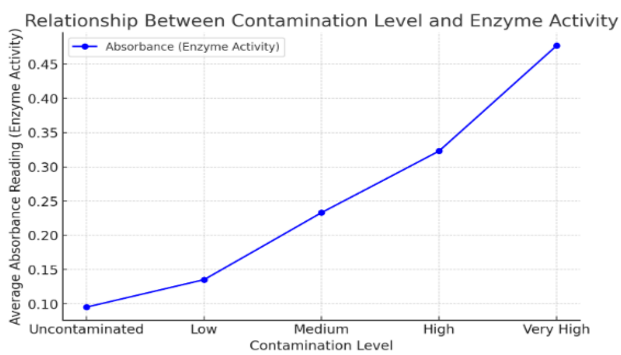

Figure 3. The relationship between contamination level and average absorbance (enzyme activity)

The absorbance in uncontaminated samples was less than 0.1, but at extremely high pollution levels, it climbed to roughly 0.5, and pollution response by organisms, this increase in enzyme activity could be attributed to the interaction of soil organisms with contaminants. Enzymes normally help to break down contaminants like hydrocarbons, which leads to increased activity when such compounds are present [19]. The relationship between contamination level and average absorbance (enzyme activity). The X-axis indicates various levels of pollution, while the Y-axis gives the corresponding average absorbance measurements.

The curve obtained from this data will be linear, indicating a direct relationship between pollution levels and enzyme activity. As pollution levels grow, so does enzyme activity, implying that organisms' interactions will increase as they seek to break down or manage pollutants. With low pollution levels, enzyme activity remains moderate, indicating that there is some reaction to minor pollution. At moderate and high pollution levels, enzyme activity increases significantly, showing that soil organisms are responding to pollution by decomposing dangerous chemicals. In cases of extreme pollution, at this point, we see a peak rise in enzyme activity, indicating that organisms are making a concerted attempt to combat pollution [20].

Several studies have indicated a linear relationship between hydrocarbon pollution levels in soil and increased enzyme activity of microorganisms, which is a direct biological response to attempts to degrade contaminated organic matter. For example, study [21] reported increased activity of soil enzymes, such as dehydrogenase, during the bioremediation processes of oil-contaminated soils. Similarly, study [22] used *Vicia faba* as a bioindicator to assess oil pollution by analyzing both enzymatic and genetic changes. Study [23] confirmed the direct impact of hydrocarbon contamination on microbial communities and enzymatic activities, emphasizing the potential of enzymes as effective biological monitoring tools in remediation efforts. The results of study [24] demonstrated a significant enhancement in soil enzyme activities, particularly dehydrogenase and phosphatase, in response to increasing levels of oil pollution. Furthermore, study [25] showed that Pseudomonas species isolated from polluted soils significantly enhanced the degradation of hydrocarbons by increasing the activity of hydrolytic enzymes. These findings are in agreement with study [26], which highlighted the use of enzymatic and genetic biomarkers in plants to monitor contamination levels and treatment effectiveness.

Evaluating the influence of pollution on biological activity in soil

Enzymatic activity rises as pollution levels increase. The findings indicated a direct link between soil pollution levels and the enzymatic activity of microorganisms. As pollution escalates (regardless of whether it is low, medium, high, or very high), a notable rise in enzymatic activity is observed. This demonstrates the organisms' reaction to environmental pollution, as enzymes help degrade organic pollutants like hydrocarbons [12]. The effect of pollution on biological activity in unpolluted soil exhibited low enzymatic activity, reflecting the stability of microorganisms in a natural, untainted environment. As pollution intensifies, biological activity increases, displaying the microorganisms' effort to adapt to environmental changes and dismantle hazardous substances [21].

Biological activity at enhanced pollution levels at extremely high pollution levels, the absorbance readings peaked at the highest values (0.477), indicating that microorganisms in the soil are exerting maximum effort to tackle pollutants. This heightened activity emphasizes the essential role of hydrocarbon-degrading enzymes in the breakdown of oil pollutants [4]. Because of the effects of pollution on soil biological activity and bioremediation methods, numerous strategic approaches can be suggested for treating pollution and encouraging biological activity in soil. Here are some of the proposed strategies, according to study [22].

Stimulating bioremediation-enhanced remediation involves a process of biostimulation that aims to increase the activity of microorganisms in polluted soil and encourage the production of enzymes involved in the breakdown of pollutants by adding vital nutrients like nitrogen and phosphorus to the soil. The nutrients induce the growth of microorganisms that break down pollutants such as hydrocarbons. The improvement of aeration can also enhance the oxygen level for aerobic microorganisms [26].

The use of pollution-resistant organisms in this method treats contaminated soil with the assistance of pollution-resistant organisms like bacteria and fungi by choosing species that can withstand high pollution levels, particularly those that can efficiently degrade complex organic substances like hydrocarbons. These organisms are inoculated into contaminated soil to increase their activity in degrading pollutants and converting them into less harmful products [8]. Phytoremediation involves planting vegetation capable of removing contaminants from the soil or of promoting organism activity in their roots to degrade contaminants. Some plants, such as buckthorn and linden, can be planted to remove contaminants or promote microorganisms in the soil using root systems. They assist in removing or degrading contaminants in the soil [20].

On combined methods (integrated approaches): Enhancing the effectiveness of cleaning up contaminated soil through the integrated use of biological, chemical, and physical methods makes it possible to utilize biological treatment processes in combination with other strategies, such as increased aeration or chemical additives, to facilitate microbial activity and speed up contaminant degradation. For instance, in areas like the Dora refinery where oil is polluted, aeration techniques may be used along with chemical activators to increase biological activities and maximize pollutant removal rate, according to study [21].

Better aeration is achieved through increasing oxygen levels in polluted ground to support aerobic microbes and utilizing techniques like air tubes or air pumps to maximize oxygen flow to the ground. This underpins the mechanism of aerobic bacteria, which are oxygen-dependent for biodegrading organic contaminants such as hydrocarbons, and the method puts emphasis on enhancing the analytical competence of polluted soil by adding especially selected microorganisms with a high biodegradation capability and giving expert microbial strains with a good capability to biodegrade given contaminants in polluted soil. It is a strategy that can help preserve the potential of biodegradation of the soil and limiting the buildup of pollutants by continuous environmental monitoring of assessment and analysis of the activity of organisms and enzymes within the soil after taking remediation measures, as well as tracking the alterations in biological activity with time using bioindicators and making remediation effective. These techniques may include the measurement of microbial activity and enzyme concentration to assess the advancement of the bioremediation process. These techniques are grounded in understanding the interaction of pollutants with microorganisms in soil and aim to enhance the biological activity that promotes the decomposition of pollutants and enhances soil quality [22].

The most effective technique in accordance with research [23] to address pollution in the samples under study is enhanced biological treatment. This method relies on increasing the biological activity of the microorganisms in the soil by improving environmental conditions, the addition of nutrients, and carrying out an Initial Pollution Assessment. This involves measuring the amount of pollution in the samples using environmental pollution-measuring devices and laboratory testing to ascertain pollutant concentration, categorizing each sample as unpolluted, low, medium, high, or severe. Measuring the enzyme activity in each soil sample is imperative in order to understand how microbes respond to available pollution [24]. This is attained by measuring light absorption to represent the activity of enzymes breaking down pollutants and contrasting enzyme activity in contaminated versus uncontaminated samples to determine inherent biological efficacy. Basic nutrients, nitrogen, and phosphorus are then added, either as organic or industrial fertilizers, to promote microbial growth in certain ratios so that these nutrients are evenly distributed over the contaminated samples to provide accurate results. To facilitate the growth of aerobic microbes that require oxygen to break down contaminants, aeration in polluted samples has to be enhanced through processes like repeated soil turning or inserting aeration tubes into soil to facilitate penetration of oxygen far into the polluted soil [25].

Following the addition of nutrients and aeration optimization, microbial biological activity in samples is checked occasionally by measuring light absorbance against time. This allows for the monitoring of the changes in enzyme activity and comparison with original enzyme activity, ensuring increased microbial ability to degrade pollutants. Following a mutually agreed duration (in terms of the degree of pollution and biological activity), the samples are re-analyzed to determine the remaining levels of pollutants while comparing levels of pollution and biological activity before and after treatment to assess the efficacy of the improved bioremediation method agreed with the study [26].

According to study [27], the findings indicate an important reduction in pollution and ongoing improvement in biological activity, then the treatment has been effective for areas with low pollution levels. However, in high-pollution areas, if pollutants have not been sufficiently degraded, the strategy can be adjusted by adding additional nutrients or refining aeration techniques. The overall effectiveness of the treatment is contingent on the biological quality of the soil and the adaptability of microorganisms to shifting environmental conditions.

4. CONCLUSION

Direct Correlation of Pollution to Absorbance (Enzyme Activity): There was a direct apparent correlation of soil hydrocarbon pollution intensity to the absorbance at the wavelength of 450 nm. Average absorbance rose from 0.095 for clean samples up to 0.477 in heavily polluted samples, demonstrating increasing enzyme activity due to the struggling of microorganisms to degrade pollution.

Enhanced Enzyme Activity as a Biological Reaction to Pollution: High absorbance readings in polluted samples reflect the fact that microorganisms in contaminated soil are actively secreting enzymes that can break down organic pollutants like hydrocarbons, thereby showing a definitive biological interaction with environmental pollution.

Potential for Use of Absorbance as Pollution Bioindicator: Absorbance measurement at 450 nm is a quick, effective way to estimate the level of soil contamination, based on the direct relation between absorbance and concentration of contaminated hydrocarbons.

Potential Efficiency of Enhanced Bioremediation: The results support the importance of using upgraded bioremediation strategies based on microbial activation, adding nutrients (phosphorus and nitrogen), and improving aeration in contaminated soils to improve the efficiency of organic pollutant degradation.

The results demonstrate the utility of early observation of biological activity in soils after treatment through continuous absorbance monitoring. It helps in the assessment of treatment effectiveness and the degree to which decontamination goals have been achieved.

Microorganismal Resilience: The findings reveal the ability of microorganisms to adapt to varying levels of contamination, as rising contamination is associated with increasingly slow-rising enzyme activity, which indicates their essential role in environmental cleansing processes.

Practical Application in Contaminated Industrial Areas: The study demonstrates the potential for applying its findings to actual contaminated locations, such as oil refineries, where biological and physical measurements can be utilized to design and implement effective treatment measures for contaminated soil and improve its environmental quality.

It is also recommended to using optical absorption as a bioindicator for monitoring hydrocarbon soil contamination, in addition to the utilization of enhanced bioremediation with nutrients such as N and P to improve microbial activity, placing filter plants such as *Tilia and Ziziphus* in contaminated areas to promote phytoremediation, inoculating specialized microbial strains to biodegrade petroleum hydrocarbons in heavily contaminated areas.

Using GIS systems to map out contamination distribution and identify remediation priorities, routine monitoring of enzyme activity to verify efficacy of remediation and stability of the ecosystem, along with integrating remediation technologies (chemical, physical, and biological) to maximize purification efficiency and promotion of environmental consciousness and local education on bioremediation techniques.

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