Investigating the integration of physical-electrochemical-biological processes in the rehabilitation of dead soils contaminated with lead and phenanthrene

Running title: rehabilitation of dead soils

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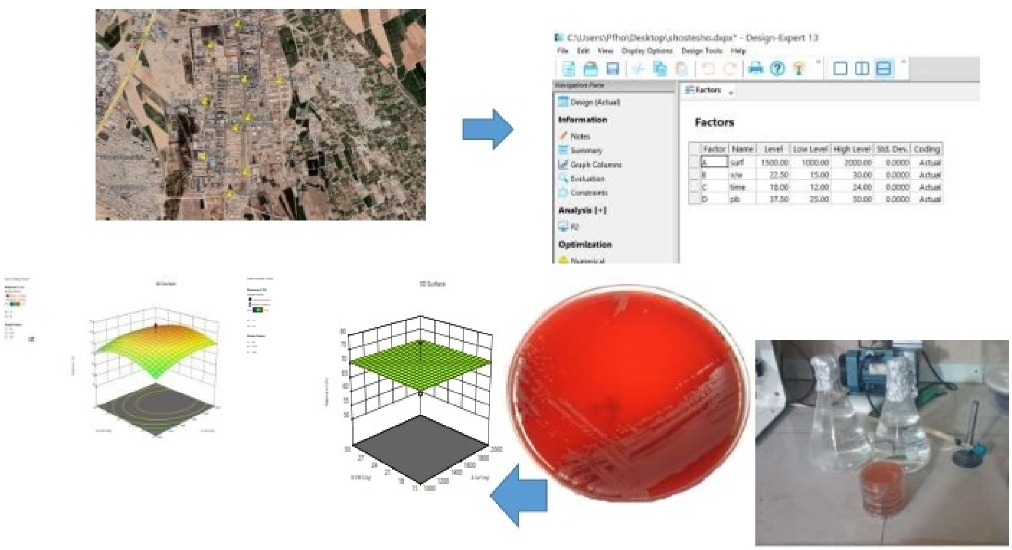
GRAPHICAL ABSTRACT



Abstract

This study was carried out with the aim of using three processes of soil washing, electrofenton and biological leaching method in order to rehabilitate the soil contaminated with lead and phenanthrene around the south Tehran oil refinery. The variables examined in this study are the

amount of soil (500, 1000, 1500, 2000 mg), the ratio of surfactant to soil (7.50, 15, 22.50, 30, 37.50 L/Kg), time (6, 12, 18, 24, 30 hours), lead concentration (25, 50, 37.50, 62.50 mg/kg) and phenanthrene concentration (50, 100, 15, 200 mg/kg). In the washing process, the highest percentage of lead removal is 78.23% at the contact surface of 2000 mg, the surfactant-to-soil ratio is 30 ml/kg, the time is 24 hours, the concentration of lead and phenanthrene is 50 and 100 mg/kg and the highest removal percentage of phenanthrene (73.4%) in the soil amount of 1000 mg, the ratio of soil to surfactant equal to 30 liters/kg, in 12 hours, the amount of lead and phenanthrene were obtained equal to 25 and 100 mg/kg. Next, the soil was placed in a discontinuous reactor with graphene electrode as cathode and iron electrode as anode to conduct electrofenton. The investigated variables are solution pH (2, 4, 6, 8, 10), reaction time (12.5, 25, 37.5, 50, 62.5 minutes), hydrogen peroxide concentration (0.5, 1, 1.5, 2, 2.5 w/w) and direct electric current (0.75, 1.5, 2.25, 3, 3.75 A). The results indicated that in the electrofenton pH process, hydrogen peroxide concentration and then voltage had the greatest effect and time had the least effect on the removal of lead and phenanthrene. In this way, the highest percentage of lead removal was 85.4% at pH equal to 8, hydrogen peroxide concentration equal to 2 W/W, current equal to 1.5 A and 50 minutes and the highest percentage of phenanthrene removal (85.9%) at pH equal to 6, hydrogen peroxide concentration equal to 1.5W/W, current equal to 2.25 A and 37.5 minutes were obtained. In the biological leaching method, the purified strain (Pseudomonas aeruginosa) was isolated from the soils around the refinery. Environmental pH (3, 5, 7, 9, 11), pollutant to biomass ratio (7.50, 15, 22.50, 30, 37.5 mg/g) and retention time (1, 2, 3, 4, 5 hour) were considered as main variables. pH was the most important parameter influencing the removal of lead and phenanthrene from soil in biological method. The highest percentage of lead removal with 85.4% was obtained at pH equal to 8, hydrogen peroxide concentration equal to 2W/W, current 1.5 A and 50 minutes time. In the case of phenanthrene, the highest removal percentage of phenanthrene (85.9%) was measured at pH equal to 6, hydrogen peroxide concentration of 1.5 W/W, current of 2.25 A and in 37.5 minutes. Comparing the results of the third stage of soil cleaning with the control sample without preparation steps with the removal percentage of 0.67 and 0.42 for lead and phenanthrene, shows the effect of the soil preparation process in order to improve the ability of bacteria to function and shows that by combining three methods of washing, electrofenton and biologic, it is possible to rehabilitate contaminated soils resistant to biological decomposition.

Key words: electrofenton, biological leaching, lead, phenanthrene, soil remediation, response surface method.

1.Introduction

Soil pollution is important from an environmental point of view because soil is a protective source and a natural filter for underground water, plant growth, etc. (Nuralykyzy et al., 2021). Wastewater from factories that deal with metal compounds, or from crude oil refineries, or from factories that manufacture plastic and food products, lead to soil pollution (Li et al. al., 2020). Although some elements in wastewater are essential for cell metabolism in very low concentrations, for many such as cadmium and lead, their positive effect on specific biological activity is not yet known (Rodrigues et al., 2018). The presence of these factors in the environment in the long term has led to a decrease in the growth, survival and reproduction of plants and herbivores, and at the same time, due to the accumulation of these substances in the body (Biomagnification) and their transfer to the next consumers, including humans can cause irreparable complications. Most heavy metals interfere with the biological reactions of the cells of living organisms and even prevent some reactions. The presence of heavy metals in urban wastewater with disruption in the treatment system reduces efficiency and in severe cases causes the biological activities of treatment systems to stop (Qayyum et al., 2019). A large group of soil organic pollutants are polycyclic aromatic hydrocarbons. Petroleum substances are composed of linear and cyclic hydrocarbons (Han et al., 2018). The mutagenic and teratogenic carcinogenic effects of polycyclic aromatic hydrocarbons are more than their toxic effects. Due to the hydrophobic nature of aromatic hydrocarbons, after combustion, some of them are absorbed on dust and small particles and are spread in the environment by air circulation and precipitate as a result of washing (Naseri et al., 2015). One of the most important polycyclic organic compounds is phenanthrene. This compound is an angular three-ring aromatic compound. These compounds are allergenic and under certain conditions have mutagenic properties for the bacterial system (McQuirter et al., 2001). In many industries, the simultaneous use of heavy metals and PAHs has been reported, where we can refer to petrochemical and refinery complexes. According to the use of these compounds in the production process, these pollutants are released in the environment and cause environmental pollution. So far, various methods have been reported in soil regeneration and removal of heavy metals and volatile organic compounds, and soil washing is one of the first ways to remove pollution from soil. Chemical surfactants are effective for increasing the separation of pollutants from the soil during soil washing, but they are usually toxic to the activity of microbes at high concentrations (Babayigit et al., 2018). We can refer to the study of Chen et al. (2015) who have recovered heavy metals and organic compounds by Bioremediation method using the method of composting contaminated soil. In a study, Atagana et al. (2009) rehabilitated soil contaminated with chromium, nickel, and phenanthrene. These studies, along with other studies, inform about the importance of soil purification from heavy metals and dangerous organic compounds. Advanced electrochemical oxidation processes are processes that use direct electric current to eventually cause direct and indirect oxidation of pollutants in the liquid phase (Zhai et al., 2018). One of the used electrochemical processes that is of interest to many researchers today is the chemical oxidation process in the presence of hydrogen peroxide. The simultaneous use of electric current with iron electrodes and hydrogen peroxide eventually leads to the formation of hydroxyl radicals. This process is called the electrofenton process in many authoritative sources and articles (Babayigit et al., 2018). In 2006, during a study, Zarrindoost et al investigated the effect of microbial activity in increasing the efficiency of removing lead and petroleum substances from contaminated soils by electrokinetic process. In 2019, Tao et al. conducted a study aiming at the degradation of phenanthrene using Fe (III)-DED photoactivation under simulated sunlight. In 2019, Zhao et al. conducted a study to remove PAH in contaminated soils by Fenton oxidation.

Bioabsorption is a simple and low-cost method that extracts metals from aqueous solutions during equilibrium reactions and by binding and absorbing metals on functional groups on the cell surface, especially when heavy metals are present in small amounts (Dell Anno et al., 2021). Using biological methods can be very powerful. The ability of biological methods to purify heavy metals and the use of bacteria resistant to these pollutants depends on the level of resistance and the ability to reduce the toxic effects of these toxic compounds and the compatibility of bacteria, hence the use of bacteria compatible with the environment can be effective in increasing bioremediation ability (Kamika and Momba, 2013). Because different types of bacteria show different thresholds of resistance to heavy metals, which depends not only on the type of bacteria, but also on the concentration of the metal in the environment and in contact with the microbial body. Biosorption of heavy metals has been introduced as a potential method for the recovery of heavy metals from wastewater and polluted environments (Koolivand et al., 2022), which includes the studies of Kamika and Momba (2013), on the resistance and bioremediation ability of bacteria and protozoa from wastewater containing heavy metals. The study of Swati et al. (2020), refers to the ability of Pseudomonas sp bacteria to remove and reduce pyrene contamination from soil and the study of Hentati et al. (2021), focuses on the use of Staphylococcus sp. mentioned in the removal of petroleum hydrocarbons from saline soil. In general, due to the high cost, the production of secondary waste and even the high resistance of the soil against each of these methods individually, the need for a new method in treating this type of contaminated soil is felt (Jamshidi-Zanjani and Khodadadi, 2017). Based on the explanations provided, the purpose of this study is to use the electrofenton process as an electrochemical oxidation process for the destruction, decomposition and mineralization of organic compounds along with soil washing processes and biological methods in order to rehabilitate the contaminated soil around the south Tehran oil refinery to heavy metal of lead and phenanthrene from soil.

Materials and methods

Soil preparation

Due to the lack of access to different parts of the refinery and due to the fact that the soil pollution in the refinery is spotty, sampling is done in a composite manner and from 10 points of the refinery (considering the size of the refinery which includes administrative and executive departments, parts of the soil that are continuously exposed to petroleum pollutants) were selected (Figure 1). The soil was collected from a depth of about 0-30 cm from the surface of the earth (surface soil (soil sampling instructions LSW-10-00)) and from a 20 x 20 cm surface in an amount of one kilogram. In the laboratory, soil from different places was mixed together. In order to prepare, the soil sample was dried in room temperature and passed through a 2 mm sieve to obtain a uniform soil, and the desired compounds in this study were determined in the soil using the EPA-1311 analysis method (EPA, 1983) (Table 1). To remove organic matter, the soil was washed several times with industrial acetone and in the last step with pure acetone (Merck, Germany) and then with distilled water to remove acetone (Wu et al., 2021). In this study, pollutants were removed in three steps of washing, electrofenton and biological leaching.

Table 1. Measurement of heavy metals in the studied soil (mg/kg)

Factor	Iron	Nickel	Cupper	Zinc	Cadmium	Lead	TPH	Phenantrene
Amount	354.6	11.75	14.67	241	100.3	466	11500	586

Measurement method	HPLC) 1290Infinity II LC, Agilent, USA(

Figure 1. Sampling points in Tehran refinery

First step: soil washing process to remove phenanthrene and lead

In various studies, Tween 80 is used more compared to other surfactants due to its high solvent capacity, lower toxicity, higher economic quality, and biodegradability (Strbak, 2000; Svab et al., 2009). The usefulness of EDTA (ethylene diamine tetraacetic acid) is due to the role of six-band ligand, a chelating agent that actually provides the conditions to bind to heavy metals and remove them from the environment (Voglar and Lestan, 2013; Zhao et al., 2016). Also, different studies (Cheng and Wong, 2006; Lestan et al., 2008; Fonseca et al., 2011; Wan et al., 2011) showed that the simultaneous use of two surfactants, the simultaneous and separate removal efficiency increase the removal of organic hydrocarbons and heavy metals. Therefore, in this study, the combination of these two substances was used for washing. To specific amounts of contaminated soil (500, 1000, 1500, 2000, 25000 mg), surfactant solution Tween80 (Merck, Germany) and EDTA (Merck, Germany) with specific ratios of the volume of solution, surfactant (7.5, 15, 22.5, 30, 37.5 V/W) was added to soil to obtain the best conditions for phenanthrene and lead absorption, separately and in concentrations of 12.50, 25, 37.50, 50 mg/kg for lead and was added in concentrations of 50, 100, 150, 200 mg/kg for phenanthrene. Then the samples were placed on a shaker at a speed of 180 rounds per minute at room temperature for a certain period of time (12, 18, 24 hours). After certain times, the samples were centrifuged for 20 minutes at a speed of 5000 rounds per minute. The samples were filtered by a PTFE filter with a pore diameter of 0.45 micron and for measurement (1290 Infinity II LC, Agilent, USA) (analysis column C_18 4.1 x 250 mm ID, flow rate 1 mL/min, injection volume 20 µ detector wavelength 220mm) was injected to the HPLC device. In order to continue the pollutant removal process, the washed soil was entered into the reactor by the electrofenton process. The soil washing process was optimized to remove phenanthrene and lead in 50 experiments.

Second step: Electrofenton process to remove phenanthrene and lead from soil

To perform this test, the soil was passed through a sieve of 0.95 mm. Then it was mixed with acetone solution at a ratio of 1 to 20 into a tumbler container and mixed for 18 hours at a speed of 30 rpm and then transferred to the reactor. The reactor designed by 2022 SPO Solid Wordcs software is of discontinuous type with a useful volume of 500 cc and equipped with iron electrodes (as anode) and graphene (as cathode) with dimensions of 2×10 cm and thickness of 3 mm was used. The type of reactor was pyrex and dark in color (Qayyum et al., 2019; Li et al., 2020). The variables studied in this section include solution pH (2, 4, 6, 8, 10), reaction time (12.5, 25, 37.5, 50, 62.5 minutes), hydrogen peroxide concentration (0.5, 1, 1.5, 2, 2.5 w/w) and direct electric current (0.75, 1.5, 2.25, 3, 3.75 A). Before designing the experiment, a range of variables was obtained by conducting preliminary experiments. The electrofenton process was optimized in the removal of phenanthrene and lead in 30 experiments. After separating the solid and liquid phase, the concentration of pollution in the solution was determined by performing an absorption test in an HPLC device (1290 Infinity II LC, Agilent, USA) with the specifications of the analysis column C_{18} 4.1×250 mm ID, flow rate of 1 mL/min, injection volume of 20 μ, the wavelength of the detector was determined to be 220mm.

Third step: biological process to remove phenanthrene and lead from soil

In this biological method, which is the final stage of the integrated process, the remaining pollutants were purified in the previous stages. In this process, first, the dominant bacterial strain in the contaminated soil around the petrochemical complex was isolated and concentrated in the laboratory environment and was used after biocompatibility and enrichment. The steps of conducting tests at this stage are as follows:

Sampling, isolation and identification of bacterial strains resistant to lead and phenanthrene

In order to carry out this step, samples were taken from 6 points (zero to 20 cm) of the soil around the refinery. The soil sample was transferred to sterile zip-pack bags with a sterile shovel. In the laboratory, homogenized soil samples were diluted 1-10 times with physiological serum and cultured on mechanical agar medium. Bacteria grown in the culture medium were identified by biochemical tests and gram staining.

Cunducting experiment

In this section, the pH of the environment (3, 5, 7, 9, 11), the pollutant to biomass ratio (3, 5, 7, 9, 11 mg/g) and the retention time (1, 2, 3, 4 and 5 hours) were considered as main variables. To perform this operation, specified amounts of nutrient broth containing solution containing metals (left over from the previous step) were mixed in a 100 ml Erlenmeyer flask and shaken on an incubator shaker at 200 rpm at a temperature of 24 degrees Celsius. After separating the solid and liquid phase, the concentration of pollution in the solution was determined by performing an absorption test in an HPLC device (1290 Infinity II LC, Agilent, USA) with the specifications of the analysis column C_{18} 4.1×250 mm ID, flow rate of 1 mL/min, injection volume of 20 μ, the wavelength of the detector to be 220mm. The efficiency of the biological leaching process in pollutant absorption was determined by conducting 20 tests.

Control

In order to investigate the effect of the two processes of electrofenton and washing on soil preparation for biological process, the control sample without electrofenton and washing, in the same conditions with the highest percentage of lead removal in biological conditions (pH equal to 8, the pollutant ratio to the living organism equals to 30W/W and 2 hours) was placed in the test samples.

Results

The results of the application of the soil washing process in the rehabilitation of contaminated soil in the refinery

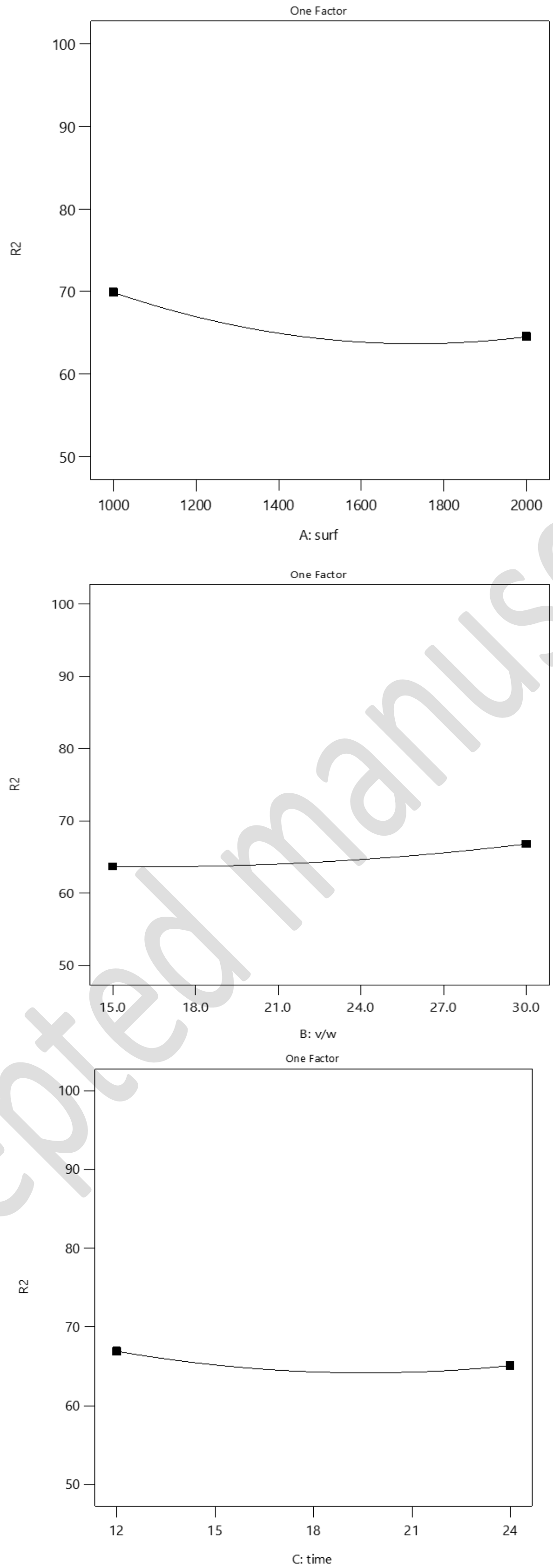
Lead

According to the results of Table 2, in the removal of lead, the ratio of soil to surfactant with a P-Value of 0.0258 and the amount of lead with a P-Value of >0.0001 were the only significant and effective factors on the removal of lead ($P<0.05$). The improved model of lead removal under the influence of the washing process was a quadratic equation type (Equation 1).

Equation 1 $$\text{Lead removal formula} = 202.55-0.048A+0.0199D^2$$

A-Surf, D-Pb

In the case of individual investigation of each parameter, the process of lead removal decreased with the increase of the amount of soil (Figure 2-A) and the highest percentage of lead removal was measured at the level of 1000 mg. Such a trend was also observed regarding time (Figure 2-C), lead concentration (Figure 2-D) and the highest percentage of removal was in 12 hours and lead concentrations were 25 mg, respectively. By increasing the ratio of surfactant to soil to 30 liters per kilogram of soil, the highest percentage of lead removal was measured.


One Factor
R2
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A: surf
One Factor
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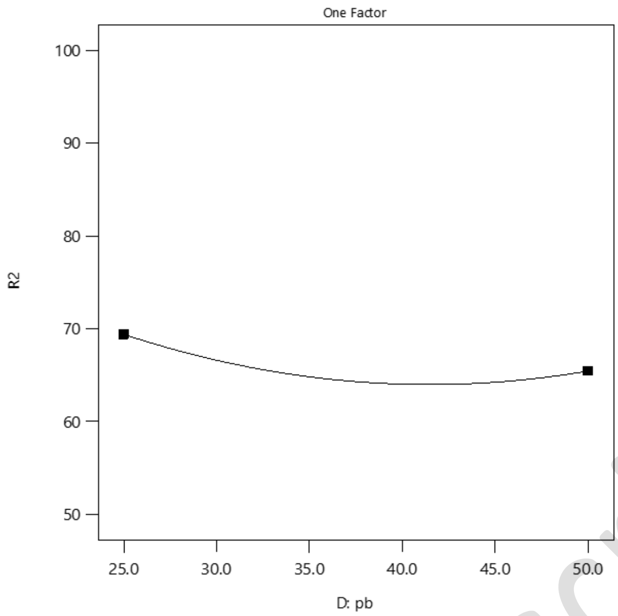


Figure 2. The influence curve of independent variables on the percentage of lead removal A-surface, B- ratio of soil to surfactant, C- time D- amount of lead

The highest percentage of lead removal was obtained with the amount of 78.23% at the level of 2000, the ratio of soil to surfactant was equal to 30 liters/kg, 24 hours and the amount of lead was equal to 50 mg/kg (Figure 3).

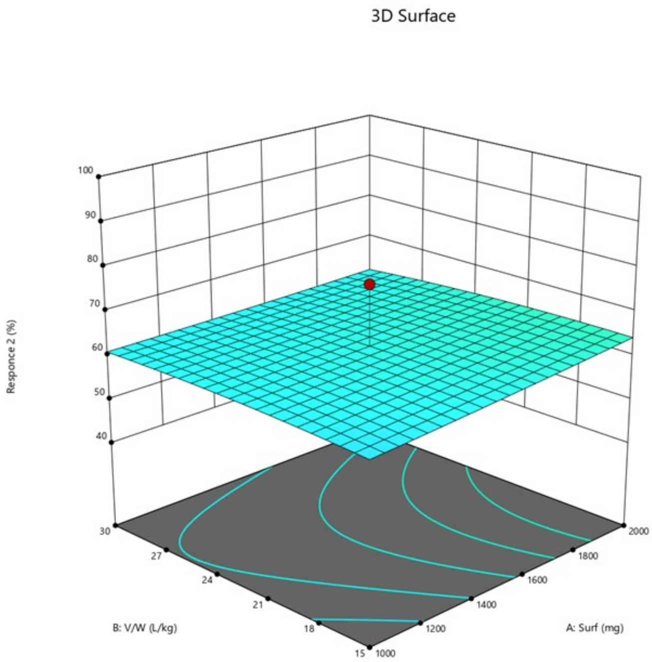


Figure 3. Interaction effect of ratio of soil to surfactant and surface on lead removal using soil washing process

Phenanthrene

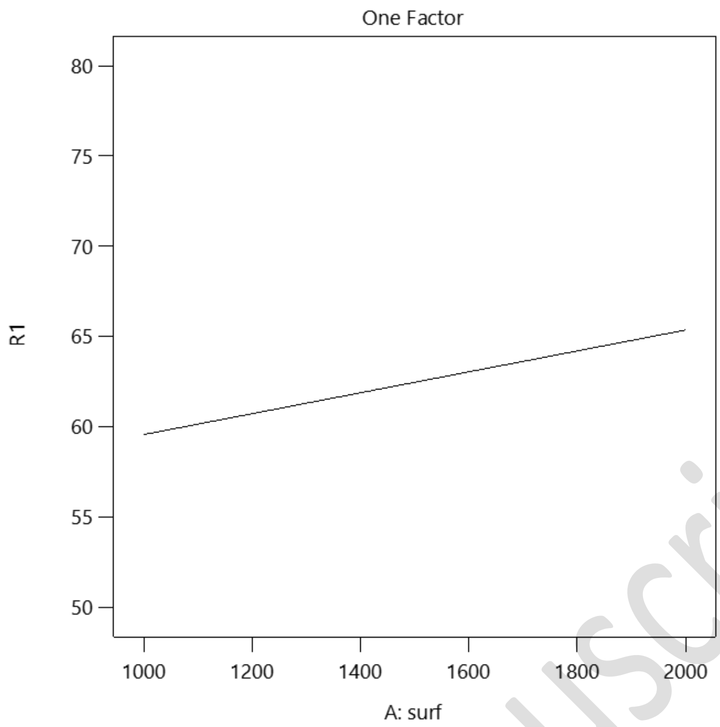
In removing phenanthrene (Table 2), time, amount of phenanthrene were not significant factors ($P>0.05$), but the contact surface and time were effective ($P<0.05$), which according to the F-value, the contact surface was the most important parameter affecting the removal of phenanthrene from the soil during the washing process. The improved model of phenanthrene removal using the washing process is of the quadratic relationship type presented in equation 2.

Equation 2

$$\text{Phenanthrene removal formula} = 47.46+0.0057A+0.0205C-0.004\,E^2$$

A-Surf, C- Time, E-Phenantren

In the case of individual investigation of each parameter, the percentage of lead removal increased with the increase of the contact surface (Figure 4-A) and the highest percentage of phenanthrene removal was measured at the level of 2000 mg. Such a trend was also observed regarding time (Figure 4-C) and the highest removal percentage was measured in 24 hours. Increasing the ratio of surfactant to the amount of soil (4-B) and phenanthrene (Figure 4-E) had no effect on the percentage of phenanthrene. However, the amount of lead and phenanthrene had a non-linear effect.


One Factor
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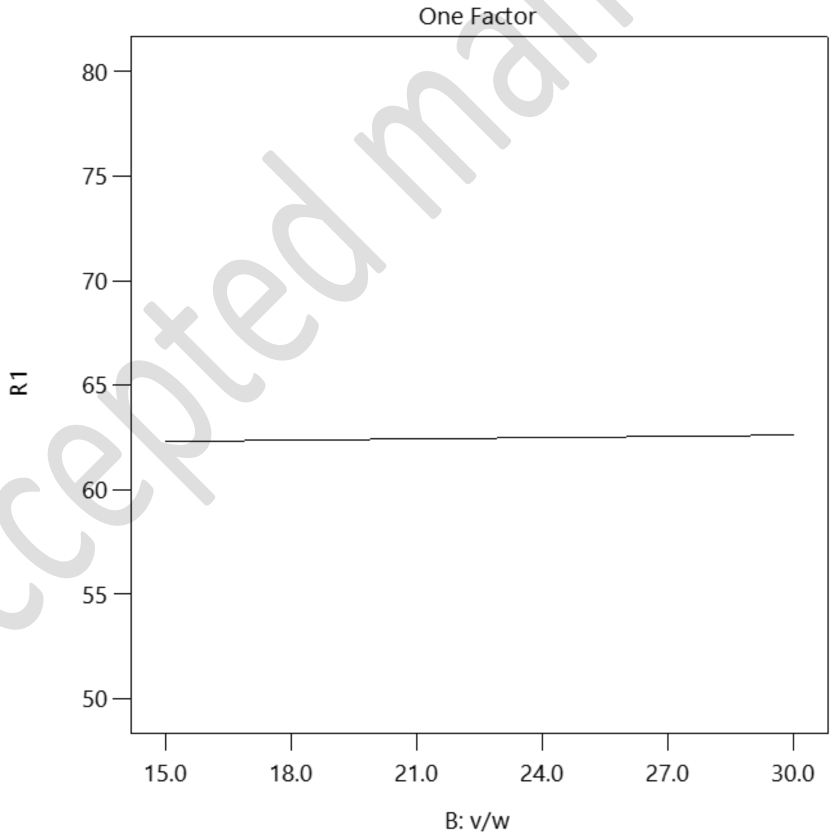
A: surf


One Factor
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21.0
24.0
27.0
30.0
B: v/w

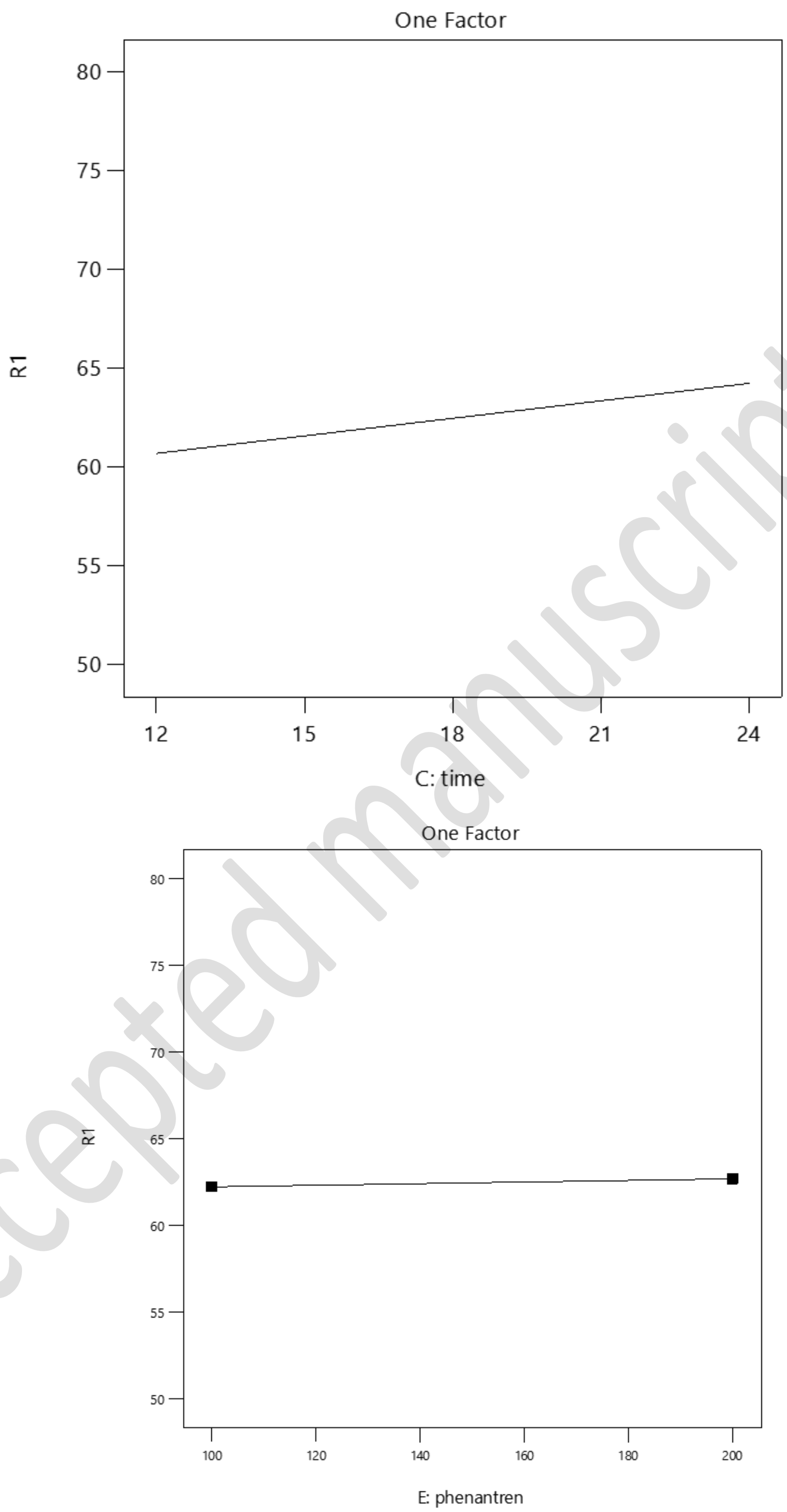


Figure 4. The influence curve of independent variables on the removal percentage of phenanthrene A surface, B- ratio of soil to surfactant, C- time E- amount of Phenanthrene

In this way, the best conditions for the highest removal percentage of phenanthrene (73.4) were obtained at the level of 1000, the ratio of soil to surfactant equal to 30 L/kg, time 12, the amount phenanthrene equal 100 mg/kg (Fig. 5).

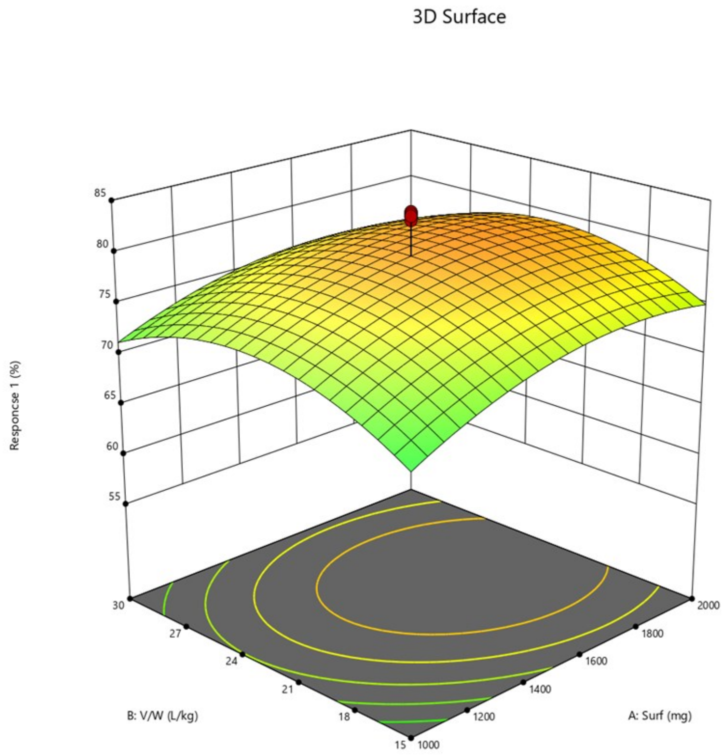


Figure 5. Interaction effect of ratio of soil to surfactant and surface on phenanthrene removal using washing process

The values of the corrected regression coefficient or Adj R^2 and the values of the predicted regression coefficient or Pred R^2 for the predicted quadratic model were 0.9123 and 0.9458 in lead removal and 0.9554 and 0.9233 in phenanthrene removal (Table 2) which indicates that the experimentally obtained values have a high agreement with the predicted value of the model.

Table 2. Analysis of variance for the removal of phenanthrene and lead resistant to biological degradation using the washing process

Source	df	phenanthrene		lead	
		F-value	p-value	F-value	p-value
Model	20	2.05	0.0383	2.15	0.0291

A-Surf	1	360.104	0.0125	104.6	0.0258
B-V/W	1	254.59	0.0578	0.0916	0.07643
C- Time	1	0.3407	0.0048	3.6	0.0678
D-Pb	1	0.-	-	0.7698	0.3875
E-Phenantren	1	1.91	0.1776	-	-
AB	1	0.1376	0.7134	0.6064	0.4425
AC	1	1.56	0.2217	1.56	0.0221
AD	1	0.00	0.9973	0.2152	0.6462
AE	1	1.57	0.2200	0.2582	0.6152
BC	1	0.0071	0.9332	0.0285	0.8672
BD	1	1.79	0.1913	0.1410	0.7100
BE	1	0.3445	0.5618	0.0062	0.9380
CD	1	0.1630	0.6893	1.25	0.2674
CE	1	0.8995	0.6893	0.7458	0.3989
DE	1	0.0446	0.3507	1.19	0.2840
A^2	1	4.05	0.8341	0.0224	0.8820
B^2	1	10.44	0.0534	0.4216	0.5213
C^2	1	3.16	0.3107	0.1733	0.6803
D^2	1	4.45	0.0436	21.97	0.0001>
E^2	1	5.97	0.0209	4.12	0.0517
Lack of Fit	22	0.7045	0.7525	1.51	0.2990
R^2-Squared			0.9554		0.9123
Adj R^2-Squared			0.9233		0.9458
Pred R^2-Squared			0.9227		0.9635
Adeq Precision			45.58		41.39

The results of the application of the Electrofenton process in the rehabilitation of contaminated soil in the refinery

Lead

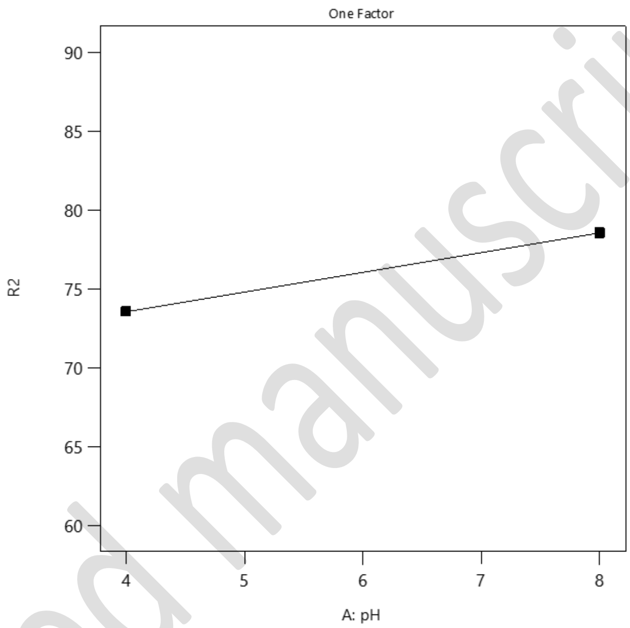
According to Table 3, in lead removal, pH with F-Value of 587.18 and H_2O_2 concentration with F-Value 66.84 were the most important influencing parameters on lead removal from soil, while time and electric current were not significant and effective factors on lead removal ($P>0.05$). The improved model of lead removal under the influence of the electrofenton removal process was of the quadratic equation type, which is presented in equation 3.

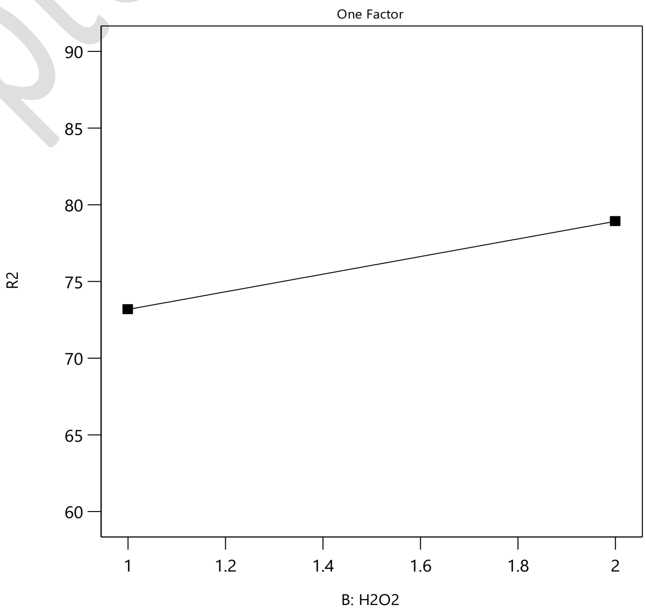
Equation 3

$$\text{Lead removal formula} = 76.05 + 2.5A + 2.87B + 62.25AB$$

A-pH, B-H_2O_2

In the case of individual investigation of each parameter, the percentage of lead removal increased with increasing pH (Figure 6-A) and reached its highest level at pH 8. Such trend was also observed regarding H_2O_2 concentration (Figure 6-B) and time (Figure 6-D) and the highest removal percentage was measured at 2 ml/l and 50 minutes. Regarding the electric current, the change trend was limited, but the increasing trend of removal percentage with the electric current was visible.





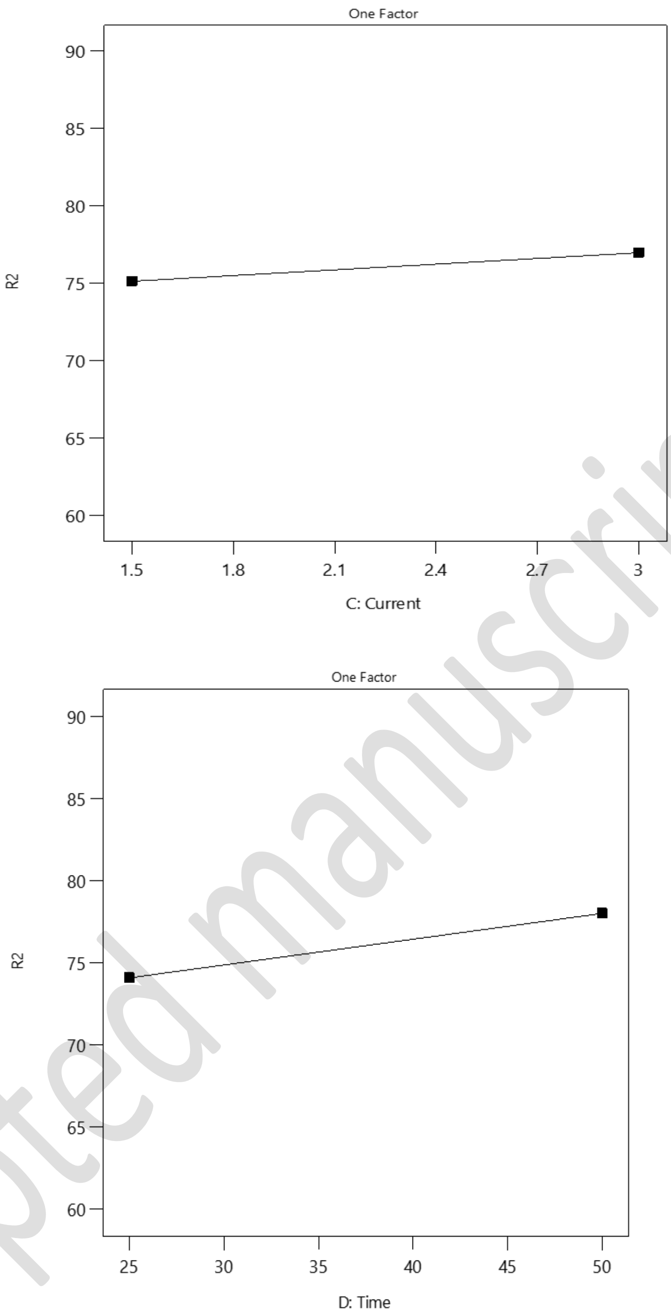


Figure 6. The influence curve of independent variables on the percentage of lead removal A- pH, B- H_2O_2 concentration, C- Electric current D- Time

The highest percentage of lead removal was obtained with 85.4% at pH equal to 8, H_2O_2 concentration equal to 2W/W, current 1.5 A and 50 minutes (Figure 7).

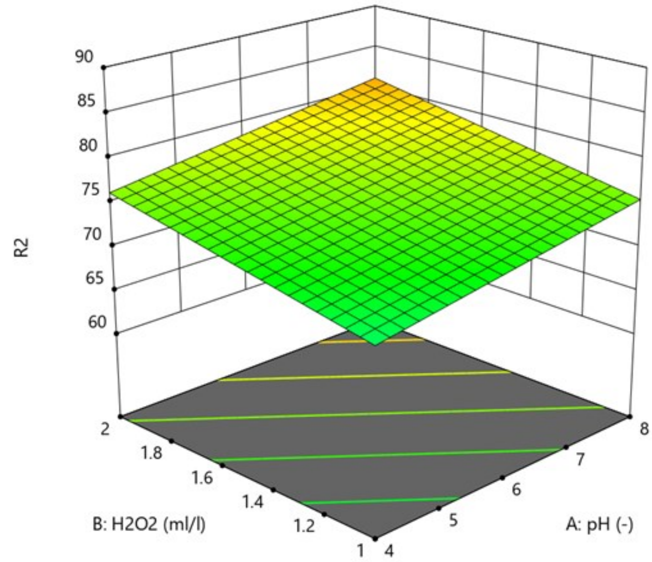


Figure 7. Interaction of pH and H_2O_2 on lead removal using electrofenton process

Phenanthrene

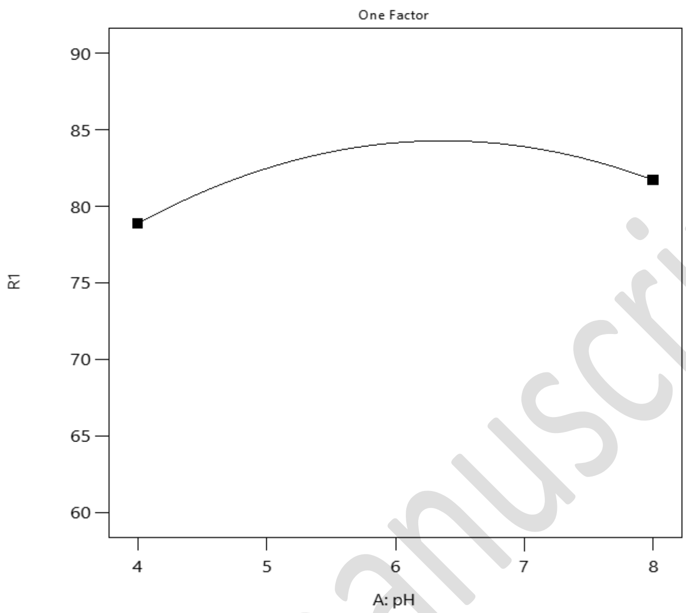
In removing phenanthrene (Table 3), time and electric current were not significant factors ($P>0.05$), but pH and H_2O_2 concentration were effective factors ($P<0.05$). The pH level was the most important parameter affecting the removal of phenanthrene from soil during the electrofenton process. The improved model of phenanthrene removal using the electrofenton process is of the quadratic relation type presented in Equation 4. Also, based on this relationship, pH × H_2O_2 concentration had an interference effect.

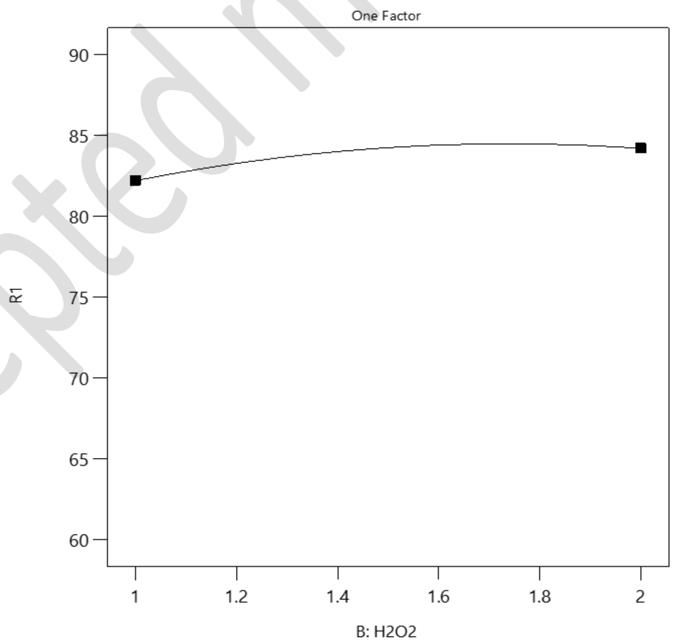
Equation 4

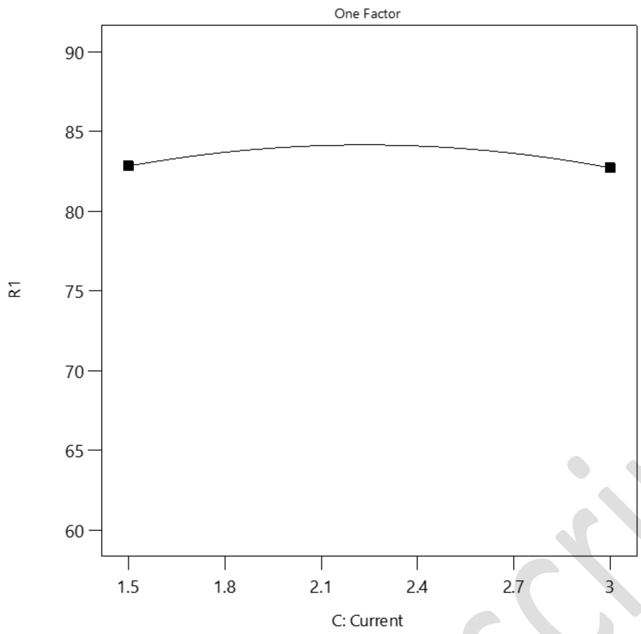
$$\text{Phenanthrene removal formula} = 84.27 + 1.47A + 0.99B - 3.86A^2 - 1.05B^2 + 32AB$$

$$A\text{-pH, } B\text{-}H_2O_2$$

According to Figure 8-A and 8-B, pH and H_2O_2 concentration had a higher curve slope compared to electric current and time, in this way, with the increase in pH, the removal increased up to the range of 6%, and then reached its decreasing trend. According to Figure 8-B regarding the concentration of H_2O_2 , the trend of increasing the percentage of removal with the increase of this index can be seen with a gentle slope. In Figure 8-C, the electric current was measured in the range of 2.1 and 2.4 A regarding the time of the highest percentage of phenanthrene removal in nearly 40 minutes.







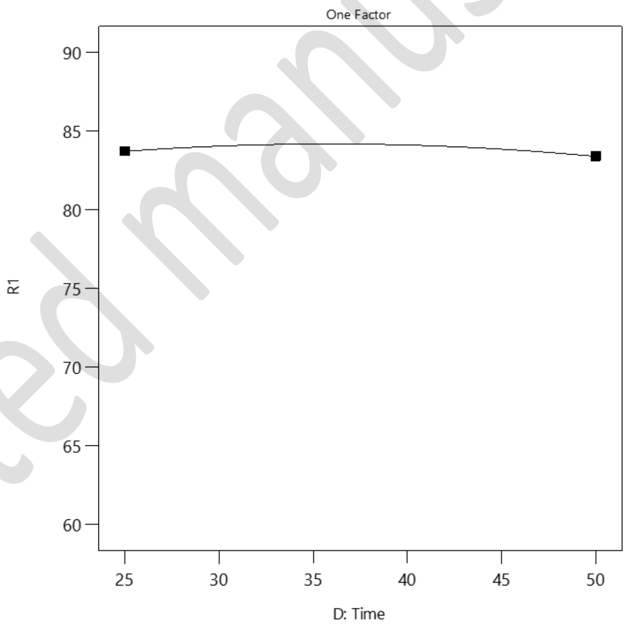


Figure 8. The influence curve of independent variables on the percent removal of phenanthrene A- pH, B- H_2O_2, C- Electric current D- Time

In this way, the best conditions for the highest percentage of phenanthrene removal (85.9%) were obtained at pH equal to 6, H_2O_2 concentration equal to 1.5 W/W, flow 2.25 A and 37.5 minutes (Figure 9).

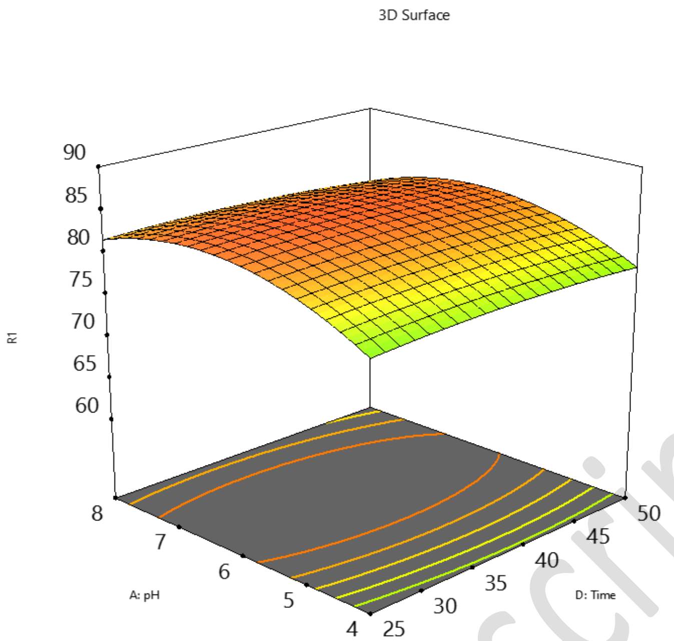


Figure 9. Interaction effect of pH and H_2O_2, electric current and time on phenanthrene removal using electrofenton process

According to Table 3, the values of the regression coefficient or R^2 and the corrected regression coefficient or Adj R^2 for the predicted quadratic model were 0.8223 and 0.8111 in case of lead and 0.9810 and 0.9460 in the removal of phenanthrene. The regression coefficient predicted by the model or Pred R^2 in case of lead and phenanthrene were 0.8085 and 0.9460, respectively, which indicates that the experimentally obtained values are in high agreement with the predicted value of the model.

Table 3. Analysis of variance for the removal of phenanthrene and lead resistant to biological degradation using the electrofenton process

Source	Df	phenanthrene		lead	
		F-value	p-value	F-value	p-value
Model	4	37.97	8.50	3.98	0.0124
A-pH	1	51.80	11.60	587.18	0.0316
B-H_2O_2	1	23.80	5.33	66.84	0.0149
C-Current	1	0.3504	0.0784	0.7047	0.4092
D-Time	1	0.5704	0.1277	3.20	0.0857
AB	1	0.016	0.1524	49.54	0.413
AC	1	13.88	3.11	11.13	2.58
AD	1	1.38	0.3091	1.05	1.77
BC	1	3.71	0.8295	1.62	0.98
BD	1	0.6806	0.1524	0.3508	0.31

CD	1	3.15	0.7053	2.65	6.47
A^2	1	407.66	91.26	32.64	3.55
B^2	1	26.96	6.71	14.22	0.80
Residual	25	52.44	11.74	52.98	0.94
Lack of Fit	20	10.55	2.36	4.18	0.0598
Pure Error	5	4.47		2.13	
Cor Total	29				
R^2-Squared		0.9810		0.8223	
Adj R^2-Squared		0.9678		0.8111	
Pred R^2-Squared		0.9460		0.8085	
Adeq Precision		44.40		30.71	

Application of biological process in the rehabilitation of polluted soil of the refinery

In soil samples, Pseudomonas aeruginosa bacteria was identified by isolation and laboratory diagnosis. This aerobic bacterium is obligatory and was isolated on blood agar and eosin methylthionin blue agar. The grown colonies were smooth and round with white color. Identification of this bacterium was based on warm staining, no spores and flagellated, positive catalase, motile, inability to ferment lactose (a positive oxidase reaction), odor (grape) and ability to grow at 42 degrees Celsius. Fluorescence property under ultraviolet light was also used for immediate detection of Pseudomonas aeruginosa colonies.

Lead

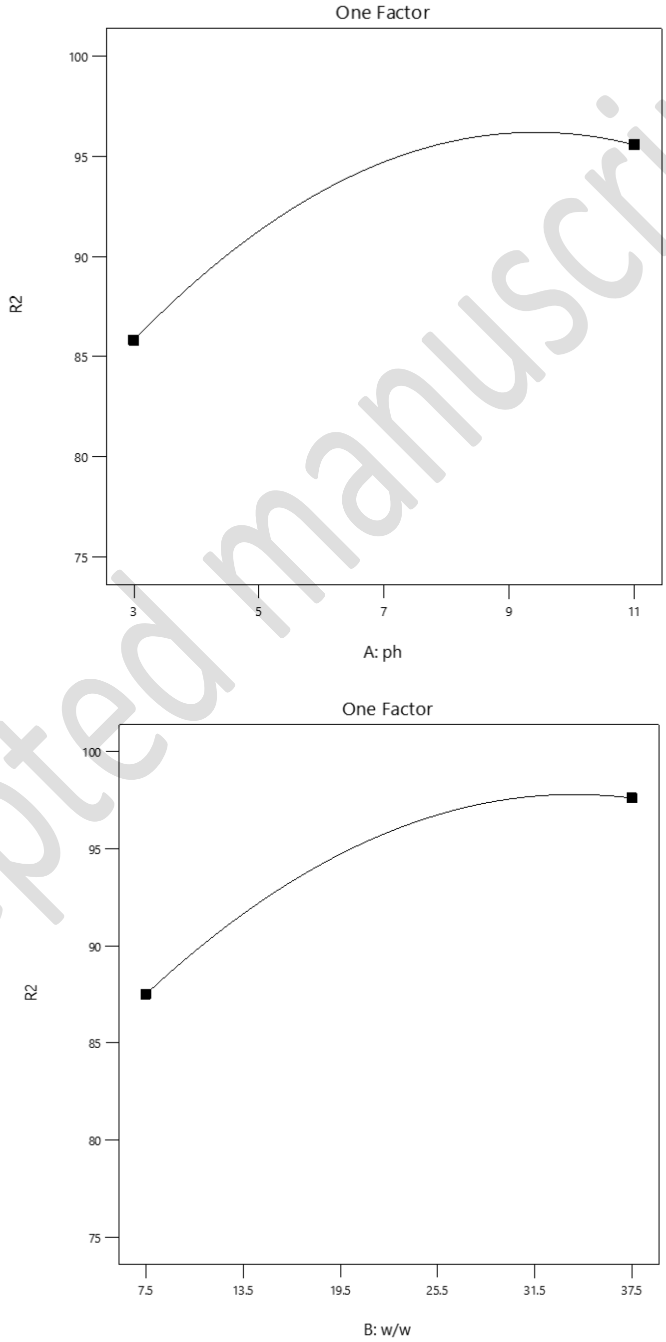
According to the results of Table 4, in lead removal, time was a significant and influential factor on lead removal with a P-Value of 0.0458 ($P<0.05$). Also, pH with P-Value 0.0081 and pollutant-to-living organism ratio with P-Value 0.250 were respectively the most important influencing parameters on the removal of lead from soil during the biological process. The improved model of lead removal under the influence of the biological process according to equation 5 was of the quadratic equation type.

Equation 5

$$\text{Lead removal formula} = 95.87 + 4.21A + 5.05B + 8.365C + 4.85AC - 4.02A^2$$

A-pH, B- W/W, C- Time

In the case of individual investigation of each parameter, the percentage of lead removal increased with the increase in pH (Figure 10-A), and at pH 8, the percentage of lead removal was the highest. Such a trend was observed regarding the ratio of pollutant to living organism (Figure 10-B) and time (Figure 10-D) and the highest removal percentage was measured at 37.5 W/W and 3 hours.



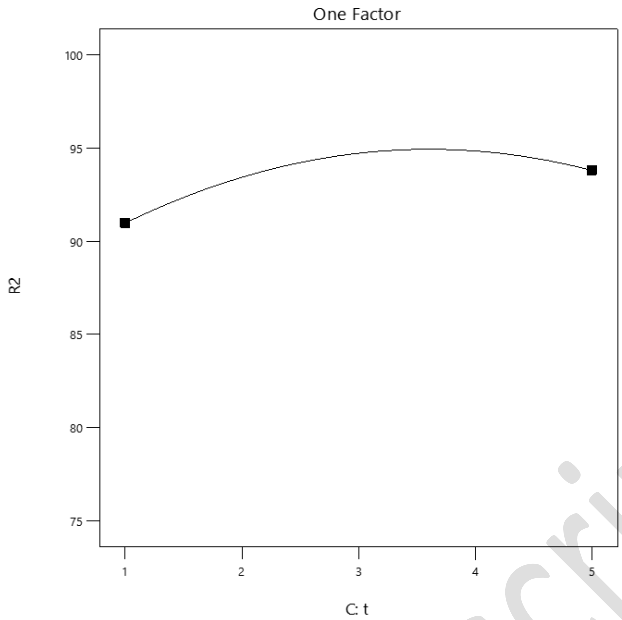


Figure 10 . The influence curve of independent variables on the percentage of lead removal A- pH, B- ratio of pollutant to living organism, C- time

The highest percentage of lead removal was obtained with 96.79% in the following conditions: pH equal to 8, the ratio of pollutant to living organism equal to 30W/W and a time of 2 hours (Figure 11). In the control sample, the highest percentage of lead removal was measured at 0.67%.

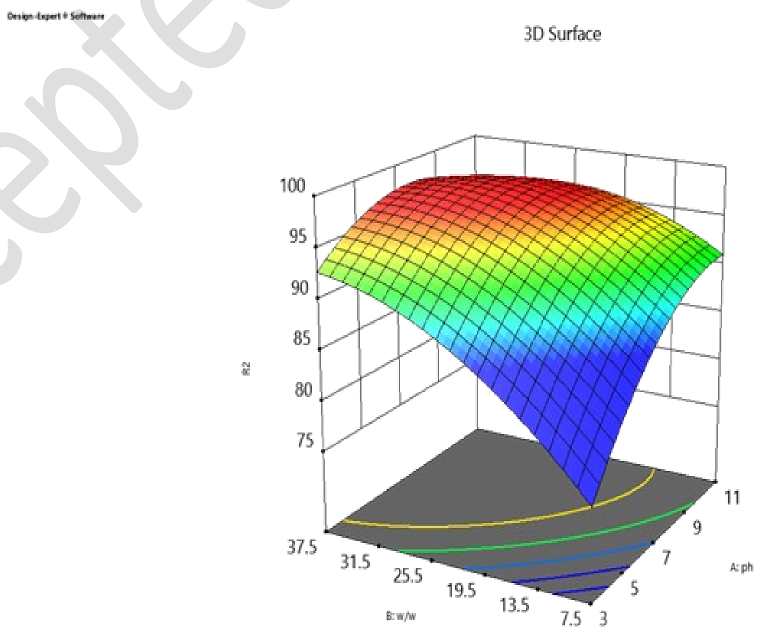


Figure 11. Reaction effect of pH, time and pollutant-to-living organism ratio on lead removal using biological process

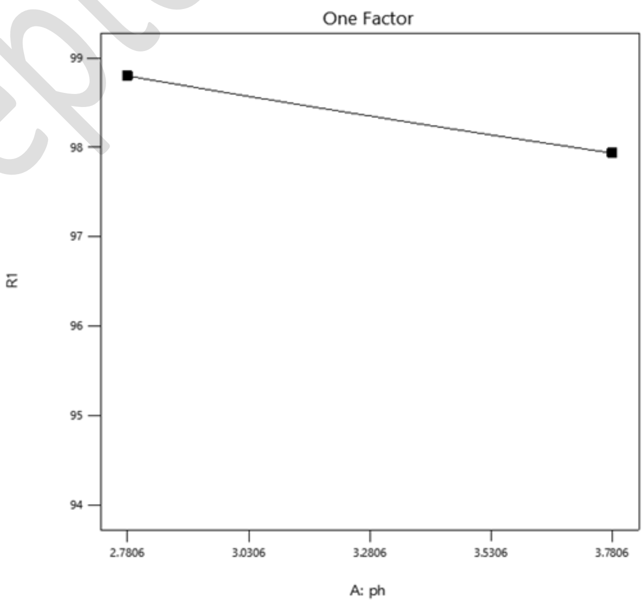
Phenanthrene

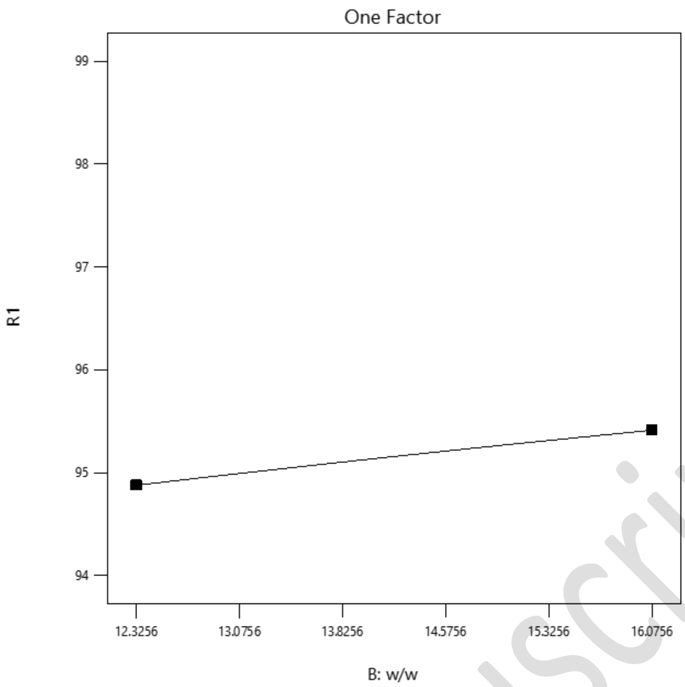
In the removal of phenanthrene (Table 4), time was not a significant factor ($P>0.05$), but pH and the ratio of pollutant to living organisms were effective factors ($P<0.05$) that according to the F-value, the pH level was the most important parameter affecting the removal of phenanthrene from the soil during the biological process. The improved model of phenanthrene removal using biological process was of quadratic relationship type which is presented in equation 6. Also, based on this time relationship, the ratio of pollutant to living organisms had an interfering effect.

Equation 6 $$\text{Phenanthrene removal formula} = -96.11 - 1.38A + 1.258B - 2.56BC - 1.40\,C^2$$

A-pH, B- W/W, C- Time

According to Figure 12, with increasing pH from 3, the removal percentage increased and then decreased. According to Figure 12-B regarding the ratio of pollutant to living organisms, the trend of increasing the percentage of removal with the increase of this index was visible with a gentle slope. In Figure 12-C, the removal process of phenanthrene increased up to 3 hours and then decreased.





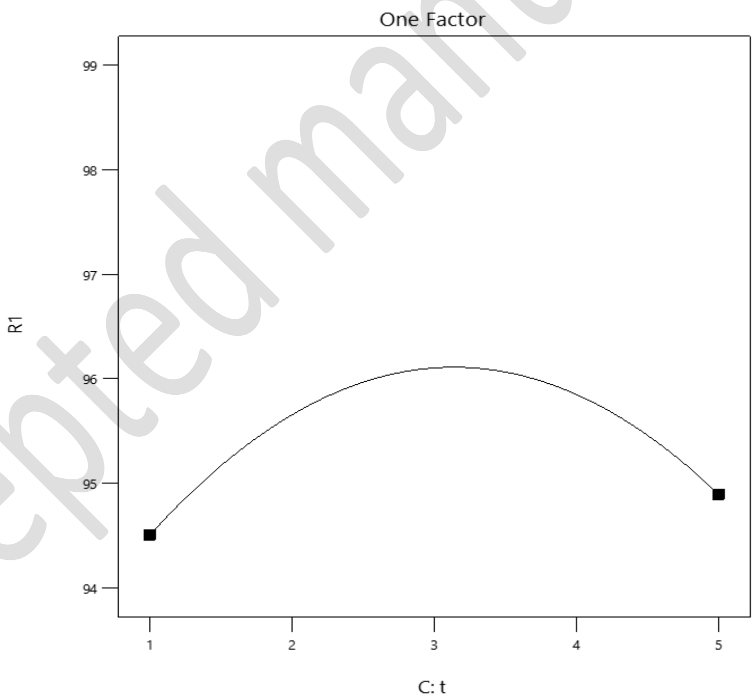


Figure 12. The influence curve of independent variables on the percent removal of phenanthrene A- pH, B- the ratio of pollutant to living organisms, C- time

In this way, the best conditions for the highest removal percentage of phenanthrene (97.4%) were obtained at a pH of 7, a pollutant-to-living organism ratio of 22.5 W/W and a time of 1 hour (Figure 13). In the control sample, the highest removal percentage of phenanthrene was measured as 0.42%.

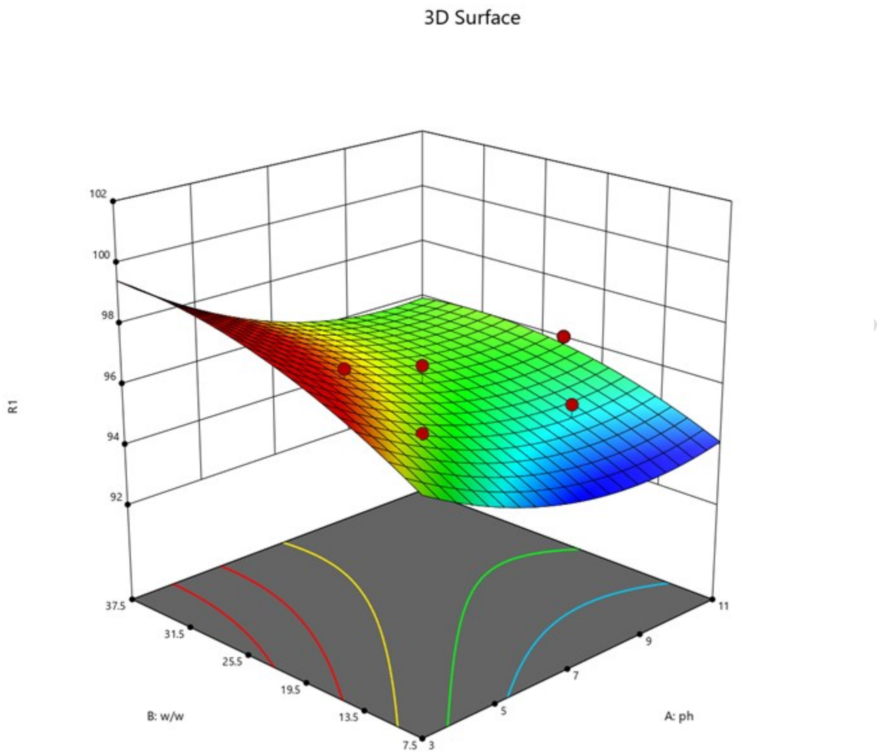


Figure 13. The mutual effect of pH, time and the ratio of pollutants to living organisms on the removal of phenanthrene using a biological process

The values of the regression coefficient (R^2) and the corrected regression coefficient or Adj R^2 for the predicted quadratic model were 0.8311 and 0.9267 for lead and 0.9801 and 0.9568 for phenanthrene. The regression coefficient predicted by the Pred R^2 model in case of lead and phenanthrene were 0.9455 and 0.9758, which indicates that the experimentally obtained values are in high agreement with the predicted value of the model (Table 4).

Table 4. Analysis of variance for removal of phenanthrene and lead resistant to biological degradation using biological process

Source	df	phenanthrene		lead	
		F-value	p-value	F-value	p-value
Model	9	3.66	0.0277	5.47	0.0069

A-pH	1	12.95	0.0049	110.85	0.0081
B- W/W	1	4.97	0.0498	86.94	0.0250
C- Time	1	0.6392	0.4426	59.15	0.0457
AB	1	0.1044	0.7532	0.8111	0.3890
AC	1	0.0525	0.8234	7.49	0.0210
BC	1	9.68	0.0110	4.01	0.0730
A^2	1	4.84	0.0524	5.66	0.0386
B^2	1	1.89	0.1994	3.09	0.1093
C^2	1	14.15	0.0037	3.51	0.0905
Residual	10				
Lack of Fit	5	0.5971	0.7074	1.86	0.2558
Pure Error	5				
Cor Total	19				
R^2-Squared		0.9801			0.8311
Adj R^2-Squared		0.9568			0.9267
Pred R^2-Squared		0.9758			0.9455
Adeq Precision		71.85			73.23

Discuss

The results of using the washing process in removing pollutants from the refinery soil

In the soil washing method, the concentration of lead and the contact surface were among the parameters affecting the removal of lead, and the contact surface and the ratio of surfactant and EDTA to soil were among the parameters affecting the removal of phenanthrene. In high concentration, chelating materials are better attached to heavy metal and more easily bind to metal ions as ligands and form complexes, while the competitive effects that exist between heavy metals can reduce heavy metal removal especially in high concentration. (Wu et al., 2015). According to the results, increasing the time and increasing the concentration of lead and phenanthrene had a negative effect on the percentage of lead removal in such a way that the highest percentage of lead removal was at the level of 1000 mg, in 12 hours and the concentration of 25 and 100 mg of lead and phenanthrene. However, by increasing the ratio of surfactant and chelant to soil to 30 liters per kilogram of soil, the percentage of lead removal increased, which is due to the reduction of the surface tension between the pollutant and soil particles by the surfactant and the increase of pollutant removal from the soil. Peng et al. (2011) observed a significant effect of increasing the volume ratio of surfactant solution to soil in increasing the extraction of petroleum hydrocarbons from soil, which is consistent with the findings of the present study. Voglar et al. (2013) in the study of washing contaminated soil to remove cadmium and lead using EDTA stated that with increasing the ratio of surfactant to soil, the absorption rate increased by 64 and 71%. The chelating agent is similar in appearance to a claw or fork, which by changing the electronic charge, changes the metal ions from negative to positive and causes the removal of metals from the soil. Also, EDTA greatly increases the mobility of lead metal and, as a result, its extraction (United States Environmental Protection Agency, 2001). With increasing time, a significant amount of the chelating agent is absorbed by the soil and the release of metals and organic substances decreases because the amount of surfactant and chelating agent available for the solubility of pollutants is reduced and its mobility within the applied environment decreases (Eweis et al., 2017; Dermont et al., 2018), which justifies the decrease in efficiency with increasing contact time. Trellu et al. (2016) reported that over time, a significant amount of surfactant is absorbed by the soil, which reduces the effectiveness of the surfactant because the amount of surfactant increases the hydrophobicity of the soil, as a result, the dissolved organic matter is reabsorbed into the soil.

In the single investigation of the effective parameters in the removal of phenanthrene in the soil washing process, except for time and contact surface, the straight line graph indicates the low influence of the single situation of the ratio of surfactant to soil and the concentration of lead and phenanthrene in the removal of phenanthrene (Dermont et al., 2018). In case of phenanthrene, which is a hydrocarbon with a hydrophobic nature and has little tendency to dissolve in water, although the hydrophobic tails of the surfactant molecules penetrate into the pollutant and their hydrophilic heads cause them to separate from the soil grain by pulling the pollutant towards water, but it seems that this issue has less effect on hydrocarbons. In case of lead and phenanthrene, the trend of changes in the effect of the contact surface and the ratio of surfactant and chelant to soil was a similar trend, in the sense that with the increase of the contact surface, the ratio of surfactant to soil decreased, or vice versa, which can be justified with the behavior of surfactant and chelant. At the beginning of surfactant addition, molecules exist as monomers. But as the concentration increases, these molecules take on a spherical or layered structure, which causes a decrease in surface and interfacial tensions (Wu et al., 2021). In the study of Herati and Rezaei Kalantari (2020) in the optimization of the soil washing process in the presence of Tween80 and EDTA compounds, the efficiency of the process for separating phenanthrene was reported as 76%, which is lower compared to 93.4% in the present study and can be related to the interference of pH and the presence of bacteria.

The results of using the Electrofenton process in the removal of soil pollutants

According to the obtained results, among the parameters of pH, amount of H_2O_2, electric current and time, the two parameters of pH and amount of H_2O_2, had the greatest effect on the removal percentage of lead metal and phenanthrene. The basis of the electrofenton process is based on the advanced oxidation process based on the production of very strong hydroxyl radicals due to the decomposition of hydrogen peroxide in the presence of a divalent iron catalyst based on the relationship number 2 (Ruiz et al., 2011). In the electrofenton process, this radical causes the non-selective destruction of pollutants that conventional purification methods such as biological methods are unable to remove the pollutant (Erick et al., 2019). The iron anode electrode releases Fe^{2+} ions and at the same time water molecules are reduced on the surface of the cathode electrode. Meanwhile, by introducing H_2O_2 into the reactor, the Fenton reaction occurs, and the continuous regeneration of ferric ions on the surface of the cathode causes the regeneration of ferro ions. Such a trend can be seen in relation 2 (Bagheri et al., 2012).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^+$$

In this process and other electrochemical processes, pH is the most influential parameter in the process, and in this study also in case of lead and phenanthrene, pH is the most influential parameter. The reason for this can be found in the direct effect of this parameter on the stability of H_2O_2, the amount of hydroxyl ions produced, and the state of iron in the solution (Britto et al., 2006). In case of lead (R2=76.05+2.5A+2.87B) and phenanthrene (R1=84.27+1.47A+0.99B-3.86A^2 -1.05B^2-1.38 C2) the relationship of the response level lacked sentences with two factors, which indicates the lack of effect of the interfering effect in this process, which can be caused by the strong effect of pH on the removal percentage of these compounds. Also, the obtained equations show the nonlinear behavior of the presented model in the case of phenanthrene in relation to the lead agent/ H_2O_2 or phenanthrene/H_2O_2. The mechanism of lead removal from soil using hydrogen peroxide is shown in relation 3.

$$H_2O_2 + pb(OH)_2 \ \rightarrow H_2O + pbO_2$$

The highest removal percentage of lead and phenanthrene was 85.4 and 85.9, respectively, both of which occurred at pH 8 and 6, and this issue could be one of the reasons for the inability to completely remove lead and phenanthrene in the electrofenton process, because the electrofenton process generally happens in acidic solutions with a pH of 2 to 4 (Masomboon et al., 2010; Nidheesh et al., 2012) and in higher limits, especially above 5, the efficiency of this process is due to its instability and ability to quickly convert H_2O_2 into water and carbon dioxide and the reduction of hydroxyl ion oxidation potential increases (Babuponnusami et al., 2012). Also, at a pH higher than 4, divalent iron ions are precipitated in colloidal form ($Fe_2O_3H_2O$) and are removed from the flow of the electrofenton process (Masomboon et al., 2010). In the study of el Álamo et al. (2007) using electrofenton in the removal of methyl red from electromechanical systems and Panizza et al. (2019) have introduced pH as one of the effective parameters in the removal efficiency in using the electrofenton process in the degradation of synthetic color and in acidic pH, the efficiency of electrofenton process was higher than other pHs.

Examining the effective parameters on the removal of phenanthrene and lead (Equations 1 and 2) shows that pH and H_2O_2 had an interference or interaction effect, which means that if the effect of one factor on the response at a certain level at different levels of another factor are not the same, the two factors interact with each other. Thus, in case of lead, when the pH is in the range of 6 to 8 (removal percentage of 82 to 84), increasing the level of H_2O_2 in the range of 1.6 to 2 ml/liter can affect the percentage of lead removal and in case of phenanthrene, the range of pH change from 5 to 7 (removal percentage 78 to 80) increased the effectiveness of H_2O_2 on the percentage of phenanthrene removal and increased the efficiency, but outside these ranges, the increase in the level H_2O_2 had no significant effect on the percentage of phenanthrene.

The results of this study indicated that in case of combining the tested factors, increasing the voltage up to the range of 2 times W/W that of lead and 1.5 W/W times phenanthrene, decreased by performing side reactions the removal efficiency of lead and phenanthrene decreased. As a result of voltage increase, the release of oxygen and hydrogen gases from the surface of anode and cathode increases, which is the factor of reducing the efficiency of the electrofenton process. On the other hand, due to the increase in voltage, hydrogen peroxide turns into water and leaves the decomposition cycle (Relation 4). At lower currents, there is not enough iron ion for the reaction (Nidheesh et al., 2012).

Relationship 4 $$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

The reduction of hydroxyl radicals, which the efficiency of the electrofenton process depends on, has also been reported in high currents, we can refer to the research of Nasr Esfahani et al. (2015) in the field of investigating the performance of the electrofenton process in reducing the pollution load of caustic wastewater used in Isfahan Refinery which introduces the intensity of current more than 215 mA/cm as the reason for the reduction of hydroxyl radicals. In the study of Samarkandi et al. (2013), they reported that the increase in the amount of oxidized iron under high voltage conditions and the increase in hydroxide radical production due to the decomposition of hydrogen peroxide is the reason for the increase in cyanide removal during the electrofenton process at high voltage. Time did not have a significant effect on the performance of electrofenton (P>0.05) in case of both pollutants, but in the individual examination of each factor in case of phenanthrene, the trend was almost smooth linear (5-D) and in case of lead, with increasing time, the percentage of removal also increased, which shows that the decomposition of phenanthrene is more economical and faster compared to lead, from the point of view of energy and the short duration of time. In the study of Bedlians, Gholi Kandi et al. (2014) introduced the short retention time as a positive factor in the utilization of the system and reduction of energy consumption.

In the present study, H_2O_2 was introduced at the levels of 0.5, 1, 2 and 2.5% in the electrofenton process, where it had the highest efficiency in case of lead and phenanthrene at levels of 1.5 and 2 W/W, and at a higher level it will play the role of a radical scavenger and will cause the consumption of radicals, the process of which is shown in equations 5 and 6, that reaction 5 is the direct effect of increasing H_2O_2 and reaction 6 is the indirect effect of this increase in reducing the removal percentage as a result of consumption of the hydroxide ion (Masomboon et al., 2010).

Relationship 5

$$\mathrm{H_2O_2} + 2\mathrm{H}^o \rightarrow 2\mathrm{HO}_2^o + \mathrm{H_2O}$$

Relationship 6

$$\mathrm{HO}_2^o + \mathrm{HO}^o \rightarrow 2\mathrm{H_2O} + \mathrm{O_2}$$

One of the objectives of the response surface method is to determine the optimal conditions for carrying out processes such as removing lead and phenanthrene from the soil of Tehran Refinery as a soil with low biological degradability. The value of R^2 for lead and phenanthrene removal data was obtained as 0.82 and 0.98, respectively, which shows a good match between the experimental data and the data obtained from the experiment and predicted by the software.

The results of using biological leaching process in removing soil pollutants

Bioremediation is a sustainable method and by breaking down pollutants, it prevents them from spreading in the environment. Researchers have developed and modeled various bioremediation methods due to their compatibility with the environment and low costs of bioremediation methods. However, due to the variety of environmental pollutants, there is no bioremediation method that can be used alone as a single method to restore polluted environments, therefore native microorganisms present in polluted environments are the best way to decompose pollutants. After two stages of washing and electrofenton, the contaminated soil was exposed to bacteria isolated from the environment that are compatible with the soil of the area. Three parameters of time, pH and ratio of pollutant to living organism were effective on the percentage of lead removal and two parameters of pH and ratio of pollutant to organism were effective in removing phenanthrene. pH is one of the most important parameters in the purification of metal species in the form of biological absorption processes. Because the pH of the solution affects the behavior of metal types due to its role in the possibility of binding them to exopolysaccharides. In fact, the charge of functional groups in exopolysaccharides is changed due to protonation and deprotonation, and in this way, it affects the absorption and removal of metal types (Silva et al., 2008).

Regarding the percentage of phenanthrene removal, with increasing pH from a limit of 3, there was a decreasing trend, and in case of lead, it increased up to a limit of 8 and then decreased. However, in the combined case where the effect of all conditions is examined together, at pH equal to 7 in the case of phenanthrene and at pH equal to 8 in case of lead, they had the best absorption percentage, which can be related to the ability of bacteria to live in the neutral pH range (Wirasnita et al., 2016). At high pHs, a net negative charge is present on the adsorbent cell wall components. As a result of this negative charge, metal cations are easily attracted to the binding points on the adsorbent (Vijayaraghavan and Yun, 2008). Pawar et al. (2015) investigated the effect of soil pH on the biological reduction of polycyclic aromatic hydrocarbons and concluded that a pH of 7.5 is suitable for the biological reduction of all PAH compounds. At pHs higher than 5.5, due to the increase in the concentration of OH ions in the solution, lead precipitates as $Pb(OH)_2$ (Norton and Amore, 1994). It is also possible that due to the increase in pH and alkalinity of the environment and calcium deposition in the soil, the activity of calcium ions decreases and as a result, the removal of heavy metal by EDTA from the soil increases (Wang et al., 2019). Also, in acidic conditions, metals are mainly connected to stable lattice structures and do not react (Chen et al., 2006), which can be seen in case of lead, but in case of phenanthrene, it seems that high pH values cause precipitation of complex metals and their removal have become unavailable (Volesky, 1990). In both pollutants, the amount of absorption increased at the beginning of the experiment and decreased as time passed, which can be due to the effect of competition with other ions in the solution due to the increase in the solubility of the solid phases of the soil with the passage of time (Wang et al., 2019).

Also, according to the graph of the removal percentage of lead and phenanthrene, with the increase in the ratio of pollutant to living organism, the removal percentage increased, which is due to the surrounding of the active sites of bacteria by metal ions or hydrocarbons, which increases absorption. Of course, by further increasing this ratio to 22.5 and 30 W/W for lead and phenanthrene, it seems that bacteria lose their ability, which indicates the direct effect of concentration on the structure and metabolism of bacteria. Edward raja et al. (2006) by exposing P.aerugionsa bacteria to different concentrations of lead metals and examining its growth, found that with the increase in the heavy metal concentration, the growth rate decreased and the bacteria began to grow with a delay which justifies the decrease in removal percentage with the increase of this ratio. The presence of a delay phase in the beginning of bacterial growth in high concentrations of metal may be due to the repair of damages caused by exposure to lead metal and due to adaptation to new environmental conditions, it spends time in the form of a delay phase. Comparing the results of the third stage of soil cleaning with the control sample without preparation steps with the removal percentage of 0.67 and 0.42 for lead and phenanthrene, the findings show the effect of the soil preparation process in order to improve the functioning ability of bacteria.

Conclusion

The aim of the present study is to prepare dead soils (lacking biological activity) contaminated with lead and phenanthrene using washing and electrofenton methods in order to enable the use of bacteria present in the same soil and return the soil to a living situation. The findings of this study showed that soil washing as a method with the intervention of chemicals is able to remove 78.23% of lead and 73.4% of phenanthrene, in the washing process, the highest percentage of phenanthrene removal with 85.9% and the highest percentage of lead removal was recorded in 85.4%. After reducing the pollution level and improving the soil quality, the resistant bacteria found in the soil of the region were used. The percentage of lead removal in this process was 96.79% and in case of phenanthrene it was 97.4%, on the other hand, in the control sample without soil preparation, the percentage of lead removal was 0.67% and 0.42%, which indicates an increase in the ability of soil bacteria in removing lead and phenanthrene through biological process were due to soil preparation through washing and electrofenton process.

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