Experimental Investigation of a Pilot-Scale Concerning Ex-Situ Bioremediation of Petroleum Hydrocarbons Contaminated Soils

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Research Article

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Experimental investigation of a pilot-scale concerning ex-situ bioremediation of petroleum hydrocarbons contaminated soils

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Abstract

The soil samples were taken from the site of a former oil products depot from an industrial area (Romania). The soil samples taken were analyzed from a physical and chemical point of view: texture, ph, soil micronutrient content, metals concentration and petroleum hydrocarbon concentration (PHCs). The soil contaminated with TPH (4280 $mgkg^{-1}$) was disposed in the form of a pile (LxWxH:3000x1400x500 mm). Experiments a pilot-scale were conducted over 12 weeks at constant pH (7.5–8), temperature (22–32ºC), nutrient contents C/N/P ratio 100:10:1, soil aeration time (8 hour/day) and moisture (30%). Samples were taken every two weeks for the monitoring of the TPH and the microorganisms content. During experiment every two weeks were added microoganisms (*Pseudomonas* and *Bacillus*). Results of the analyzes regarding the concentration of PHCs were revelead a linear decrease of the concentration of PHCs after only two weeks of treatment. This decrease in concentration was also achieved in the following weeks. Following the analysis performed on the model at the pilot scale regarding the depollution process, it can be concluded that a soil contaminated with petroleum hydrocarbons can be efficiently depolluted by performing an aeration of 8 h/day, adding microorganisms *Pseudomonas* and *Bacillus* to ensure the conditions for increasing in the total number of germs (colony forming units–CFU) from $151x10^5$ to $213x10^7$ CFU/gram of soil, after 12 weeks of soil treatment - the depollution efficiency achieved is 83%.

Keywords: aeration, bioremediation, moisture, microorganism, petroleum hydrocarbons, soil.

1 Introduction

Currently, petroleum (oil) is the main energy source in the world[1]. Saturated hydrocarbons are the main components of natural gases and oil[2]. Petroleum hydrocarbons are natural chemical substances used by humans for many activities, being a complex mixture of a wide range of chemicals found in crude oil and refined products[1,3].

The development of the oil industry is sometimes accompanied by the appearance of certain side effects, as it pollutes the environment and affects the health of the human population[4]. Environmental pollution with PHC through leaks and spills taking place during production, storage and transport of oil causes water and soil pollution, affecting the safety of ecosystems and human health, thus becoming a global environmental problem[5, 6]. The most disastrous effects occur when crude oil doses exceed 200 g/kg of soil[7].

Studies have shown that bioremediation is a safer and less expensive method for removing dangerous contaminants and producing secondary non-toxic substances[8]. Interest for polluted soil and water bioremediation has increased over the past thirty years[9], primarily due to the fact that bioremediation is based on the ability of some microorganisms (bacteria, fungi) to degrade organic matter and allow the acceleration of natural decomposition of organic pollutants[10, 11, 12]. The success of bioremediation depends on a series of factors: the affected area, type, amount and concentration of pollutants, soil pH, ambient temperature, soil moisture, amount of nutrients, type and amount of microorganisms and oxygen availability, available time and financial resources [13, 14]. Bioremediation has several disadvantages: it requires long remediation time, it is climate dependent and its effect is not fully elucidated[15,16,17,18].

The biopile method can be used for decontamination of soil polluted with 83% aromatic polycyclic hydrocarbons – nine months of treatment, and for the decontamination of a soil polluted with 60–73% mineral oils after seven months of treatment[19].To achieve a maximum level of biodegradation by using the biopile method, the aeration and irrigation systems' designs are important[20]. Natural and forced aeration (blowing or extracting air

through pipes) can be introduced to improve soil ventilation in order to assure the oxygen supply needed for the bio-reactions taking place in the pile of polluted soil[21].

In order to evaluate the use of the biopile method in bioremediation of soils polluted with oil, Iturbe et colab. conducted several studies: 1) in a former station of storage and distribution of oil, after 66 days of treatment they achieved a remediation efficiency of 85.2%[22]; 2) at an oil plant in northern Mexico, after 22 weeks of treatment they achieved a remediation efficiency of 80%[23].

In a study conducted on soil contaminated with hydrocarbons (2000, 4000 and 6000 $mgkg^{-1}$), three biopile piles of 0.6 m^3 were made, they were bio-stimulated with nutritive substances and aerated, obtaining an efficiency of 66–75%[24].

Gogoi et al. (2003) depolluted soil contaminated with HTP (44.000 $mgkg^{-1}$). Soil was placed in cells (500 kg of soil/cell), amended with nutrients and inoculated with a microbial consortium isolated from hydrocarbon-contaminated soils. The system was aerated one hour per day at a rate of 100 m^3/h. At the end of the 365 days of operation, remediation in the cells was 75% with a degradation rate of 90 $mgkg^{-1}$/day[25].

Studies have shown that using indigenous microbial strains is preferred in bioremediation processes[26,27], and adding nutritive substances may increase the efficiency of removal[28,29].

Chemlal et al. (2012) conducted a study in which they achieved a removal efficiency of diesel fuel of 70% in 40 days using a pre-adapted consortium, together with nutrient growth (urea as nitrogen source and K_2HPO_4 as phosphorus source), maintaining moisture content (15–25%) and aeration[30].

Wu et al. (2016) compared treatments with biodisposition and biostimulation achieving a degradation of 60% of PHC by adjusting soil C:N:P to 100:10:1 at a water content of 20%, and a degradation of 34% by adding *Acinetobacter SZ-1* to the soil after 10 weeks of treatment[31].

A pilot scale bioremediation study of soil contaminated with PHC from a sub-arctic site indicated that aeration and moisture addition were sufficient to obtain efficient biodegradation, while supplementation of nitrogen did not influence the efficiency of biodegradation[32].

Hernández-Espriú et al. (2013) research has demonstrated the applicability of natural gums as soil remediation enhancers in diesel-contaminated systems. Ionic surfactants showed removal rates above the control test of about 78.51% (Maranil LAB), 71.27% (Texapon 40), 60.13% (SDS), and 48.19% (Surfacpol G) and Guar gum and locust bean gum showed efficiencies of 54.38% and 53.46%, respectively. An 82% TPH-diesel removal rate was achieved for a very low gum concentration (2 ppm)[33].

Bioremediation is performed using specific strains of oil degradation: *Rhodococcus* with an efficiency of up to 55-59% in the case of crude petroleum hydrocarbons[34].

O'Brien and colleagues aimed at degradation the natural degradation of petroleum hydrocarbons (PHC) from soils contaminated Bakken crude oil (western North Dakota, USA). Initial concentrations of PHC, 1400, 700, 220 and 100 $mgkg^{-1}$). After two seasons of growth under crop management Wheat (*Triticum aestivum L.*) and field peas (*Pisum sativum L.),* PHC concentrations were reduced between 46 and 53%. Continuing a normal crop rotation can be a viable management strategy for low-level soil contamination[35].

In a study performed by Baoune et al. it was pointed out that microorganisms of the type *Streptomyces sp. Hlh1* removed total petroleum hydrocarbons (TPH), they obtained a yield of 40% at an initial concentration of 10% and 55% at an initial concentration of 2%, respectively[36].

The removal of total petroleum hydrocarbons (TPH) can be achieved in 112 days by using a bacterial consortium (bioaugmenatation): 82.6% and by adding endogenous earthworms (*Pontoscolex corethrurus*) the yield increases (86.4%)[37].

Starting from the results obtained previously through experiments at laboratory level[38], the objective of the study was to determine the yield of the ex-situ bioremediation process of soils contaminated with PHC using a pilot scale treatment set that controls soil aeration duration, moisture and microorganism content.

2 Materials and methods

2.1 Soil sample investigation

In order to carry out the pilot scale experiment, soil was collected from the site of a former warehouse of petroleum products from an industrial area (Romania). The taken soil samples were transported into the laboratory where the stones and roots were removed and the soil was mixed to ensure homogeneity of the sample. Soil thus prepared (samples P1, P2 and P3) was quantitatively and qualitatively analyzed:

- The soil's texture was determined using a gravimetric method;

- The soil pH was determined in 1/2.5 (w/v) soil/water extract using a HANNA pH-meter;
- Nitrogen was determined by Kjeldhal[39];
- For determining total potassium and phosphorus content 3 g of soil with 100 μm granulation was used over which was added 7 mL of 12 M HCl and 21 mL of 15.8 M HNO_3 and the mixture was refluxed for 2 hours, filtered and diluted up to 100 mL with 2% (v/v) HNO_3[40];
- Mobile phosphorus and potassium were determined by ICP-OES after extraction of 5 g soil in 100 ml ammonium acetate-lactate mixture (pH=3.75) for 4 hours according to Egnèr-Riehm-Domingo method;
- The organic carbon was determined by Walkley-Black method by oxidising the organic matter from 0.2 g soil with 5–10 ml of 1.6% (w/v) sulfochromic mixture on a hot plate for 20 min. The excess of chromic acid was titrated with 0.2 mol L^{-1} Mohr salt solution in the presence of diphenylamine as an indicator;
- Heavy metals concentration of the collected soil sample was determined through Atomic Absorption Spectrometry (AAS) using a SHIMADZU AA-6800 spectrometer. For determining heavy metals concentration 3 g of soil with 200 μm granulation was used over which was added 7 mL of 12 M HCl and 21 mL of 15.8 M HNO_3 and the mixture was mineralized for 3 hours, filtered and diluted up to 100 mL;
- Hydrophysical indices: withering coefficient, field capacity and useful capacity were determined taking into account soil moisture that was determined by the gravimetric method[39,41];
- The PHC content was determined by Fourier Transformed infrared spectroscopy (FTIR)[5]. The dry soil (5–10 g) was subjected to 2 consecutive extractions with 20 mL tetrachlorethylene (TCE) for 30 minutes/extraction. After extraction, the supernatant was separated and the soil residue. Polar compounds (water, vegetable oils and animal fats) was removed applied by passing the extract through a 10 cm long and 0.6 cm with column packed with 0.150–0.250 mm grain-size magnesium silicate for column chromatography (Florisil). The purified extract was made up to 50 mL with TCE. The FTIR spectrum of the purified extract was recorded between 3150–2750 cm^{-1} at 4 cm^{-1} resolution in 10 mm optical path-length quartz cells by a Spectrum BX II (Perkin Elmer) spectrometer equipped with DTGS detector. The measured absorbance at 2925 cm^{-1} attributed to CH_2- group was converted to TPH using the linear regression model. The TPH content of soil was calculated according to equation[5]:

$$C = \frac{c \cdot D_f \cdot V}{C_f \cdot w}$$

Where: C-is the concentration of TPH in soil ($mgkg^{-1}$); c-is the concentration of TPH in the extract ($mgmL^{-1}$), D_f-is the dilution factor; C_f- is the concentration factor; V is the volume of the extract (mL); w is the weight of the sample (kg).

2.2 Isolation of indigenous microorganisms

The indigenous microorganisms was isolated from the test soil and grown on GPS culture media prepared from sodium L-glutamate–10 gL^{-1}, starch soluble–20 gL^{-1}, potassium dehydrogenate phosphate–2 gL^{-1}, magnesium sulfate–0.5 gL^{-1}, phenol red–0.36 gL^{-1} and agar–12 gL^{-1}. The isolated strains were stored and multiplied in a nutrient broth: meat extract–10 gL^{-1}, peptone–10 gL^{-1}; NaCl–5 gL^{-1}[38].
Bacterial strains were incubated and shaken (120 rpm) at 25°C for 24h. Inoculation was made with 100 μL of culture from liquid culture medium. The development of microorganisms was observed at 600 nm were using a UV spectrophotometer (Lambda 25, Perkin-Elmer). These cultures were morphologically and tinctorially characterized using the Gram staining technique[38].
The amount of soil microflora in the samples taken from the experimental groups was established by the numerical determination of the microorganisms existent in the PHC polluted soils. For the numerical determination of microorganisms existing in the soil, decimal dilutions (saline solution) were made. Incubation was done at 30 °C for 48-72 h. The assessment of the total aerobic microflora is made from plates having 30-300 colonies. The average of the three plates was corrected with the dilution factor to obtain the colony-forming unit UFC g^{-1} soil[38].

2.3 Pilot scale experimental investigation

Soil polluted with petroleum hydrocarbons (4000 kg), after sorting and homogenization was added the microorganisms and placed in a pile (Lxlxh: 3000x1400x500 mm) in order to be subjected to the proposed experiments (fig. 1). The pile of soil was placed on an impermeable surface consisting of a concrete platform over which plastic foil was added. At the bottom of the pile was placed a drainage layer made of gravel with a 4–7 mm diameter, which favors the aeration process. In this layer of gravel was introduced a part of the pipe system through which aeration and wetting are carried out.

The system for introducing water and the nutrients and microorganisms solution (*Pseudomonas* and *Bacillus*) consists of a tank with a capacity of 100 L, a self-priming pump with a flow of 50 L/min, corrugated absorption and discharge hoses and a blower. The discharge hose is connected to the distribution network that consists of an Ø180 mm PVC pipe branched into 5 perforated Ø50 mm PVC pipes placed horizontally in the middle of the pile.

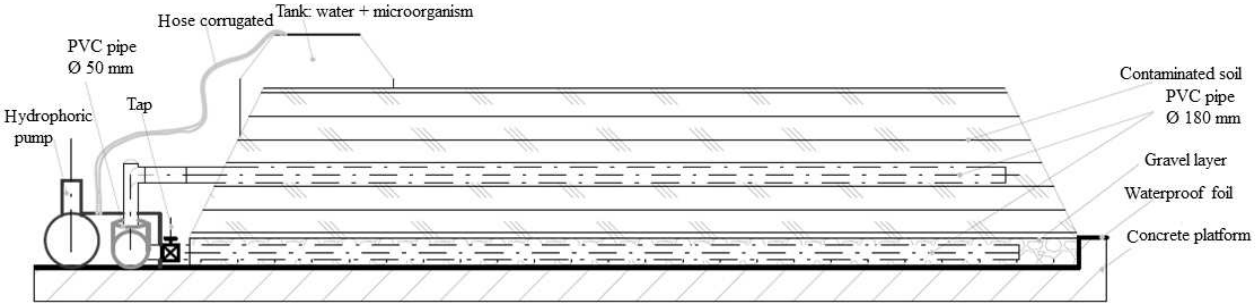


Fig. 1 Pilot scale experiment

Research carried out at pilot scale level lasted 12 weeks at the following parameters: 30% moisture, a temperature of 20–32°C, pH of 7.5–8, 8 h/day aeration duration.

Soil moisture was manually achieved by sprinkling through the top of the biopile cell and through the water supply system inside of the pile, aiming for field capacity moisture of 30%. Moisture was monitored throughout the experiment.

The temperature at which the pilot scale experiment was carried out was between 25–32°C. Temperature was measured and monitored throughout the experiment with the WTW Multiline IDS-3430 Multiparameter meter.

Soil pH was monitored weekly using the HI 3512–02 pH meter.

Soil aeration was achieved through the aeration system consisting of a blower and an air distribution network. The air distribution network consists of 5 perforated PVC pipes with a diameter of 50 mm each. These pipes are distributed inside the pile of soil as follows: three pipes are placed horizontally in the gravel layer at the base of the pile and two pipes are placed in the middle of the pile in order to ensure uniform aeration of the soil. Aeration was performed for 8 hours a day throughout the experiment (12 weeks) by means of the blower, having a flow of 10 m^3min^{-1}.

Quantity of microorganisms. Microorganisms used for bioremediation were isolated from the native micro flora of the polluted soil and grown in the laboratory on culture media.

When making the soil pile for the experiment, after placing each layer of soil with a thickness of 10 cm, a manual sprinkling of the soil was carried out with a solution loaded with microorganisms – a total quantity of 9 L with a concentration of $94x10^3$ CFU. Throughout the experiment microorganisms were added once every 2 weeks (weeks 2, 4, 6, 8, 10) by means of the perforated pipes inside the pile of soil – the quantity of 9 L with a concentration of $94x10^3$ CFU and by manual sprinkling through the upper part of the pile of soil.

Soil sampling. Before adding the microorganism loaded solution, soil samples were taken from the pile of soil every 2 weeks (weeks 2, 4, 6, 8, 10, 12) in order to determine the hydrocarbon concentration (150 g of soil/sample) and to determine the total number of germs (50 g of soil / sample). The determination of the number of microorganisms present in the biopile cell during the experiment can be done according to point 2.2.

Soil samples were taken from 3 points at different depths, according to the diagram in figure 2, with a total of 9 soil samples being taken every week. These soil samples were coded according to their location, sampling depth and week in which they were taken (Table 1).

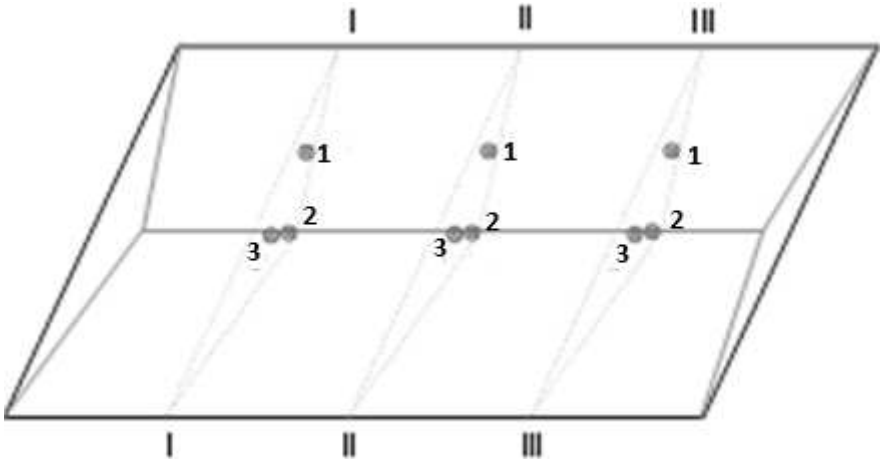


Fig. 2 Soil sampling scheme

Table 1 The code of soil samples according to their sampling location and depth

The sampling section	Sampling depth	The code
I-I	Sample 1: 0–5 cm	I.1
	Sample 2: 15–25 cm	I.2
	Sample 3: 25–35 cm	I.3
II-II	Sample 1: 0–5 cm	II.1
	Sample 2: 15–25 cm	II.2
	Sample 3: 25–35 cm	II.3
III-III	Sample 1: 0–5 cm	III.1
	Sample 2: 15–25 cm	III.2
	Sample 3: 25–35 cm	III.3

2.4 The evaluation of the effectiveness

The evaluation of the effectiveness of the depollution process was performed by determining the yield for each sample with the following Eq.[42]:

$$\eta = \frac{C_i - C_f}{C_i} 100 \ [\%]$$

In which: η is the yield, in %;

C_f –PHC concentration in soil at the end of the treatment time, in $mgkg^{-1}$;

C_i –initial PHC concentration of soil, in $mgkg^{-1}$.

3 Results and discutions

3.1 Soil samples investigation

The soil was classified as clay medium (LL): 41.6% clay, 17.59% silt, 18.7% fine sand and 22.1% coarse sand according to the USDA classification.

The test soil has a weak basic reaction (pH=7.5±0.5), a content of 2.34% total organic carbon and 0.126% total nitrogen. The investigated soil has a moderate humus content (4%). A high content of potassium (K_{AL}=272 $mgkg^{-1}$) and soluble phosphorus (P_{AL}=19.2 mg kg^{-1}) has been registered.

The concentration of Cu (28.1 $mgkg^{-1}$), Ni (33.5 $mgkg^{-1}$) and Pb (76 $mgkg^{-1}$), exceeds the normal value (20 $mgkg^{-1}$), according to the Romanian legislation (Order 756/1997)[43], being below the alert threshold. The

concentration of Zn (174.6 mgkg^{-1}) exceeds the normal value (100 mgkg^{-1}). The Manganese level found in the analyzed soil (698 mgkg^{-1}) presents lower concentrations compared to the normal value (900 mgkg^{-1}).

The investigated soil has the average field capacity (25.5% g/g) that is used together with the withering coefficient (13% g/g) and the useful capacity (15.5% g/g) in order to establish the minimum moisture threshold required for depollution.

The average concentration of PHC in the tested soil was of 4280 ± 400 mgkg^{-1}. This value was considered the initial content of PHC for the amount of TPH in the pile of soil. The initial concentration of PHC exceeded more than 2 times the intervention threshold for less sensitive soil uses (2000 mgkg^{-1}) established by Romanian legislation (Order no. 756/1997)[43], thus requiring remediation.

3.2 Isolation of indigenous microorganisms

In the test soil two bacterial strains with bioremediation potential were isolated using a selective enrichment technique. These cultures are from genus *Pseudomonas sp.* and *Bacillus sp.* (1 mL solution contains 94x10^3 CFU), it two bacterial genus are commonly found in soil.

3.3. Pilot scale experiment investigation

3.3.1 PHC concentration

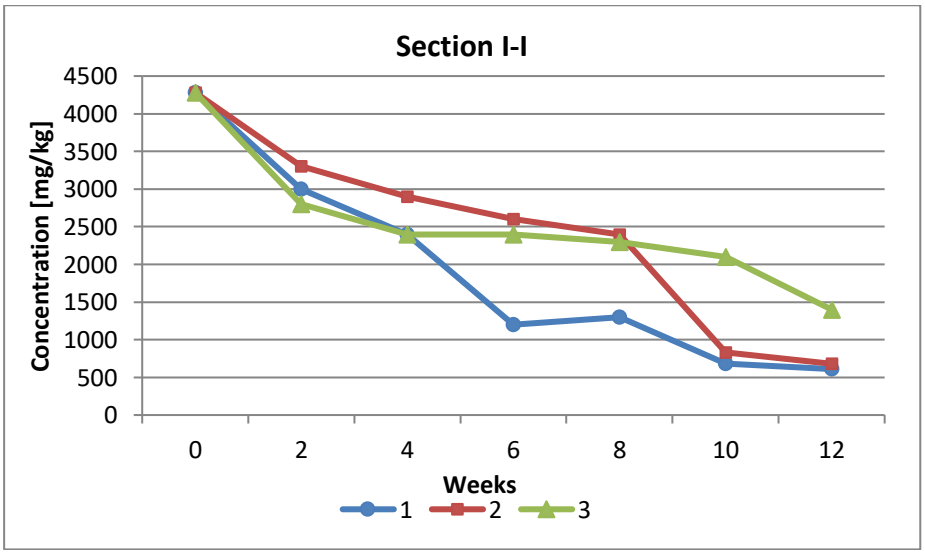
Results on the evolution of hydrocarbon concentration in the soil samples taken during experiments were subjected to an analysis in order to highlight the influence of treatment duration and to determine the efficiency of the bioremediation process.

In figure 3 is presented the evolution of PHC concentration in the 9 soil samples taken from the pile of soil throughout the experiment depending on the sampling section (I–I; II–II; III–III) during the experiment (12 weeks).

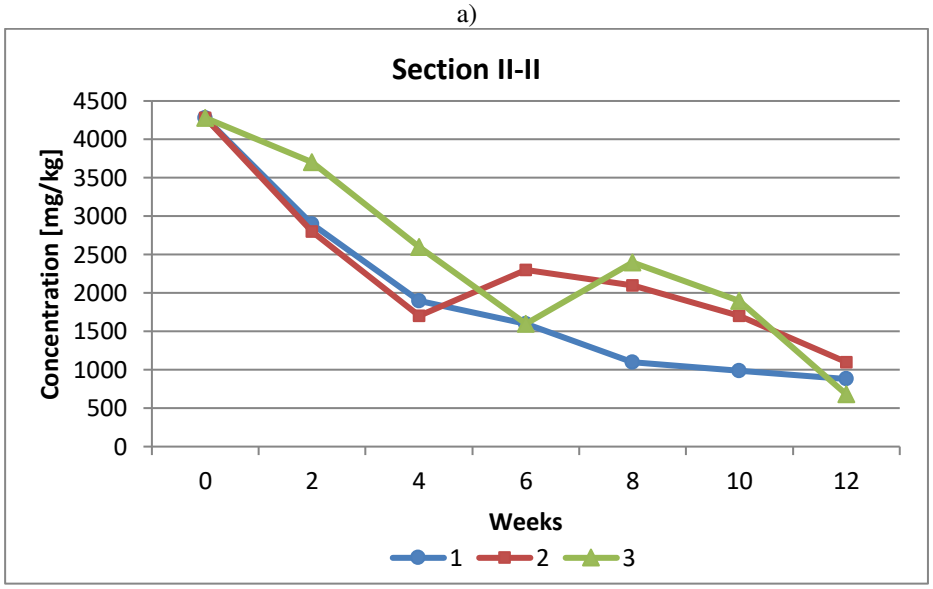
Analyzing figure 3 we observe a pronounced and linear decrease of the PHC concentration in the first 4 weeks for all the 3 sections, regardless of the sampling depth (1; 2 and 3). In the case of section I–I (fig. 3a) the PHC concentration from sample 1 continues with a sharp decrease being followed by stability and starting from week 8 the concentrations have a pronounced decrease. In sample 2 we can observe the same tendency starting with week 8 and the PHC concentration by the end of the experiment (680 mgkg^{-1}) is similar to the one in sample 1 (610 mgkg^{-1}), while sample 3 has slightly higher concentrations (1300 mgkg^{-1}).

In section II-II we can observe a continuous and linear decrease for sample 1 throughout the experiment, while samples 2 and 3 show an increase in week 6 and 8 respectively, after which a linear decrease is recorded until the end of the research period, PHC concentrations reaching around 680–1100 mgkg^{-1}.

Section III-III shows a decrease in the PHC concentration (250-710 mgkg^{-1}). Several stability areas can be observed: sample 3 (weeks 2–4), sample 2 (weeks 4–6) and sample 1 (weeks 6–8), followed by a significant increase (samples 2 and 3) in week 8, except for sample 1 which decreases after the stability period to a value of 250 mgkg^{-1}. After the increase from week 8, samples 2 and 3 show a decrease until the end of the research period (410 mgkg^{-1} and 710 mgkg^{-1} respectively).



a)



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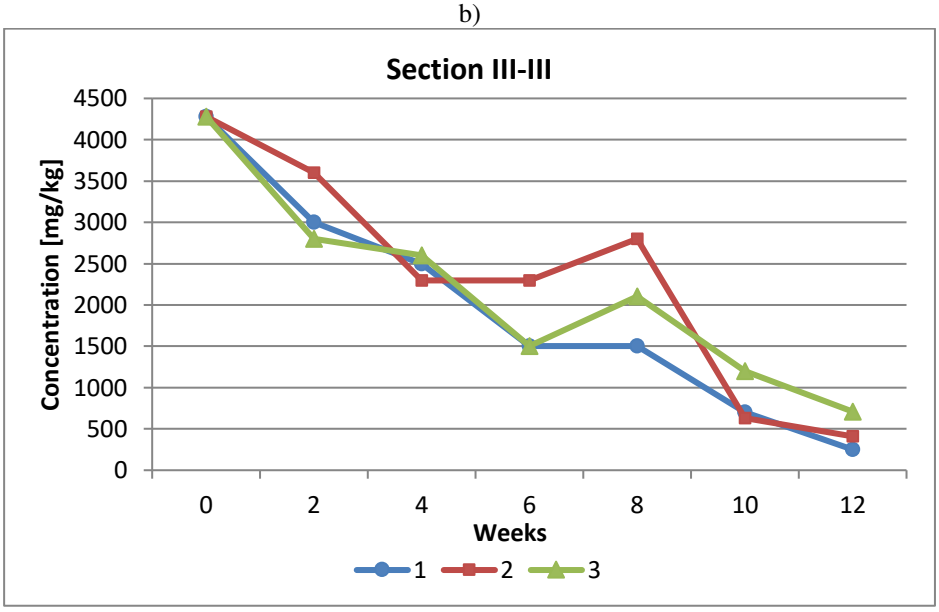

c)

Fig. 3 PHC concentration depending on time and section:
a) section I-I; b) section II-II; c) section III-III

Analyzing figure 3 a linear decrease can be observed for all 9 soil samples. All samples show a pronounced decrease in the PHC concentration after only two weeks of treatment, regardless of sampling point or depth. This decrease of concentration continues but the amount of TPH extracted from the soil is much lower as of week 4. At the end of the experimentation period (12 weeks) it can be observed that the value of the concentration of pollutant decreased below 1000 $mgkg^{-1}$, except for samples I.3 and II.2.

Concerning the concentration of PHC (fig. 3a) in the 3 sampling sections (I, II, III), a linear decrease can be observed throughout the experiment and more pronounced after only 2 weeks. In week 2 the concentrations are close in all the 3 sampling sections, lower concentrations can be observed for section II in weeks 4 and 8 of experiments, and in weeks 6 and 10 they are higher compared to section I and section III. The value of the PHC concentration decreases, being under 1000 $mgkg^{-1}$ in week 12 in the 3 sections at sampling points, the lowest value being in section III (250 $mgkg^{-1}$).

Concernig the concentration of PHC (fig. 3b) in the 3 sampling sections, a linear decrease of the pollutant concentration can be observed throughout the experiment. Section II shows lower values compared to sections I and III in weeks 2, 4 and 8 of experiments. Towards the end of the testing period (week 10) the PHC concentration in section II is much higher than in section I and in section III, where the concentration is similar. In the last week

there is a stability in section I and a decrease in sections II and III. The lowest concentration is reached in week 12 in section III (250 $mgkg^{-1}$).

PHC concentration (fig. 3c) is decreasing during the experiment. In week 2 the concentration of pollutant in section II is much higher than in sections I and III. In weeks 4 and 8 in all 3 sections the concentration has approximately the same value. Low values can be observed at the end of the experiment in section II (680 $mgkg^{-1}$) and section III (710 $mgkg^{-1}$), except for section I (1400 $mgkg^{-1}$).

Analyzing from the treatment duration point of view a linear decrease for all the 3 sampling sections can be observed. All samples show a pronounced decrease in the concentration of PHC after only two weeks of treatment, regardless of the depth at which research is carried out. This decrease in concentration is also achieved in the following weeks, but the amount of TPH extracted from the soil is much lower starting from week 4. At the end of the treatment period values under 1000 $mgkg^{-1}$ were registered.

Following analyses on the concentration of PHCs, a linear decrease of the PCH concentration was revealed after only two weeks of treatment. This decrease in concentration is also achieved in the following weeks, but the amount of TPH extracted from the soil is much lower starting from week 4 and week 6 of treatment, respectively.

Results obtained at the pilot scale level are similar to those obtained in the preliminary study at laboratory scale level[38], with a pronounced decrease in the first weeks of treatment followed by a much slower decrease in the concentration of PHC.

3.3.2 Quantity of microorganisms

When the selected microorganisms are added to the soil on the soil to the experiment, an increase in the total number of germs (NTG) is observed independent of the sampling place (table 2). Concentration of microorganisms in the second week had values between $102x10^5$–$209x10^5$ CFU/g of soil, followed by an increase in weeks 4 and 6 ($106x10^5$–$298x10^6$ CFU/g of soil). This growth trend is also observed in the following weeks, but after week 8 they are not so significant ($127x10^7$–$238x10^7$ CFU/g of soil).

Table 2 Determination of total microflora during the experimental development stage

Total number of germs **(NTG)**									
Week	**SECTION I-I**			**SECTION II-II**			**SECTION III-III**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
0	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$	$94x10^3$
2	$144x10^5$	$175x10^5$	$126x10^5$	$160x10^5$	$121x10^5$	$102x10^5$	$209x10^5$	$184x10^5$	$142x10^5$
4	$125x10^6$	$127x10^6$	$106x10^6$	$158x10^6$	$176x10^6$	$85x10^6$	$183x10^6$	$162x10^6$	$199x10^6$
6	$206x10^6$	$198x10^6$	$238x10^6$	$208x10^6$	$188x10^6$	$124x10^6$	$274x10^6$	$235x10^6$	$298x10^6$
8	$168x10^7$	$144x10^7$	$159x10^7$	$131x10^7$	$127x10^7$	$147x10^7$	$158x10^7$	$141x10^7$	$133x10^7$
10	$170x10^7$	$130x10^7$	$144x10^7$	$165x10^7$	$165x10^7$	$207x10^7$	$171x10^7$	$162x10^7$	$153x10^7$
12	$201x10^7$	$185x10^7$	$190x10^7$	$226x10^7$	$238x10^7$	$259x10^7$	$219x10^7$	$199x10^7$	$197x10^7$

Analyzing the evolution of the number of microorganisms during the ex situ bioremediation process, a significant increase can be observed in the 3 sections. This is due to the fact that once every 2 weeks a new amount of microorganisms was added, but also to the fact that they have the optimal conditions for their development were ensured: soil aeration and temperature.

3.4 The evaluation of the effectiveness

Analyzing figure 4 it can be observed that the depollution efficiency is high, reaching 94% for sample III.1. The lowest efficiency is registered for sample I.3 (67%) and the rest of the samples have efficiency around 80%.

Compared to the results obtained in the preliminary study carried out at a laboratory scale level[38] which highlighted the possibility of obtaining high efficiencies (56–76%), the treatment period being 18 weeks on soil containing 7600±400 $mgkg^{-1}$ petroleum hydrocarbons, results of pilot scale experiments carried out under slightly different conditions indicate a reduction in the PHC concentration (64–94%) in only 12 weeks. These results were partially validated by submitting a patent application[44] to the Official Bulletin of Industrial Property (OSIM). It may also be mentioned that the obtained yields are comparable with yields obtained in other research conducted with similar treatment conditions: 80–85.2%[23] and 66–75%[24].

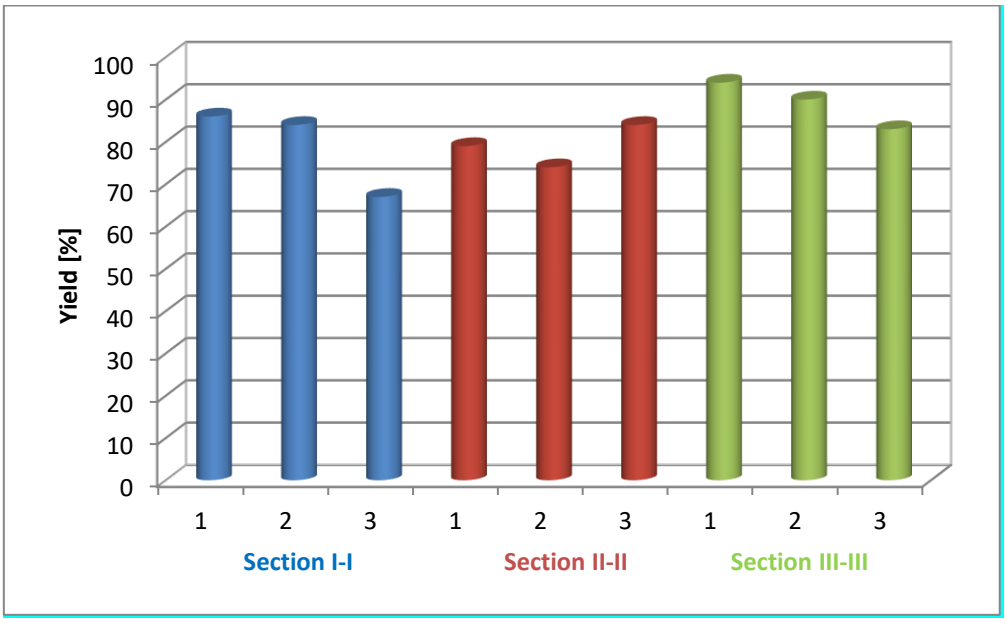

Fig. 4 Depollution yield

Yields obtained in a relatively short time (3 months) are considerably higher than those obtained by Lecomte (1998): 60–73% – 7 months[19], Gogoi et al. (2003): 75 %–12 months[25] and Chemlal et al. (2012): 70 %–40 days[30].

Results can be compared to results obtained by adding natural gums: 82% (Hernández-Espriú et al. 2013) or through combined processes: vermiremediation (*Pontoscolex corethrurus*) + bioaugmentation (86%) and phytoremediation (*Panicum maximum*) + vermiremediation (*Pontoscolex corethrurus*) + bioaugmentation (82.7%)[37].

The results are much better than results obtained by natural degradation using plants: Wheat (*Triticum aestivum L.*) and field peas (*Pisum sativum L.),* (46–53 %)[35] or by adding microorganisms belonging to genera *Rhodococcus*–55–59%[34]; *Streptomyces sp. Hlh1*–40%[36] and *Acinetobacter SZ–1*–34 %[31]. The obtained results support the claims of Cerquira et al. (2011) and Suja et al. (2014) according to which indigenous microbial strains are recommended for use in the bioremediation process[26,27].

4. Conclusions

All samples show a pronounced decrease in the concentration of TPH after only two weeks of treatment, regardless of the sampling point or depth. At the end of the experiment period (12 weeks) it can be observed that the value of pollutant concentration has decreased below 1000 $mgkg^{-1}$.

During the experiment there was an increase in the number of microorganisms from $102x10^5$–$209x10^5$ to $127x10^7$–$238x10^7$ CFU /g of soil, which led to a significant decrease of TPH.

Results obtained on the pilot scale model showed a depollution efficiency between 64–94%, the average efficiency being 83%. Yields obtained in the pilot scale experiment, under shorter treatment time, are comparable or even higher than yields obtained by other researchers.

Following the analysis performed on the model at the pilot scale regarding the depollution process, it can be concluded that a soil contaminated with petroleum hydrocarbons can be efficiently depolluted by performing an aeration of 8 h/day, adding microorganisms *Pseudomonas* and *Bacillus* to ensure the conditions for increasing in the total number of germs (colony forming units–CFU) from $151x10^5$ to $213x10^7$ CFU/gram of soil, after 12 weeks of soil treatment–the depollution efficiency achieved is 83%.

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Figures

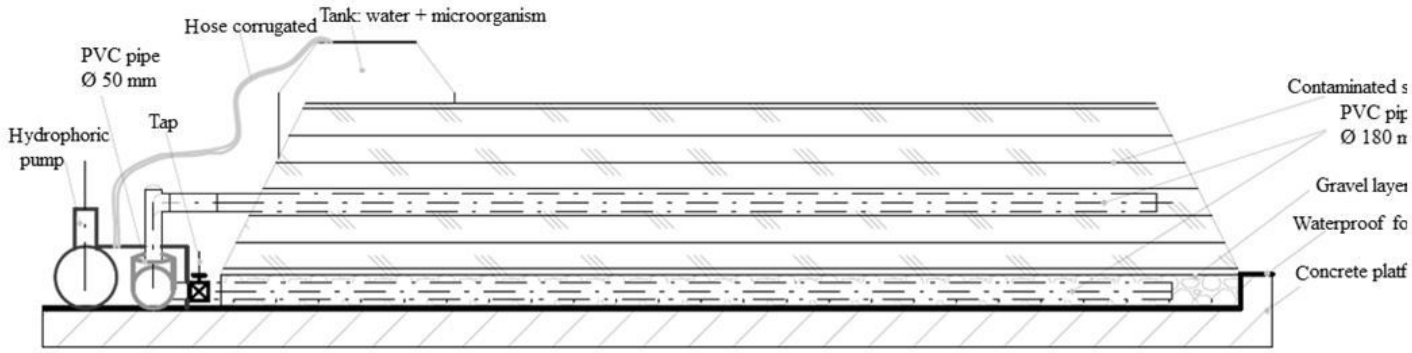

Figure 1

Pilot scale experiment

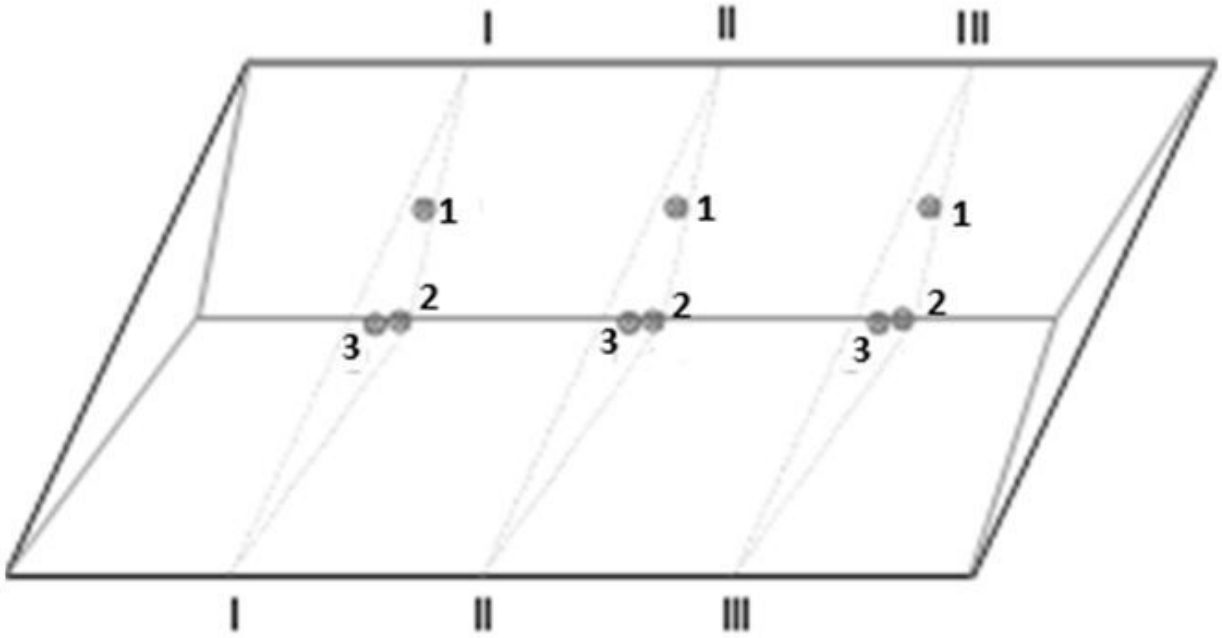

Figure 2

Soil sampling scheme

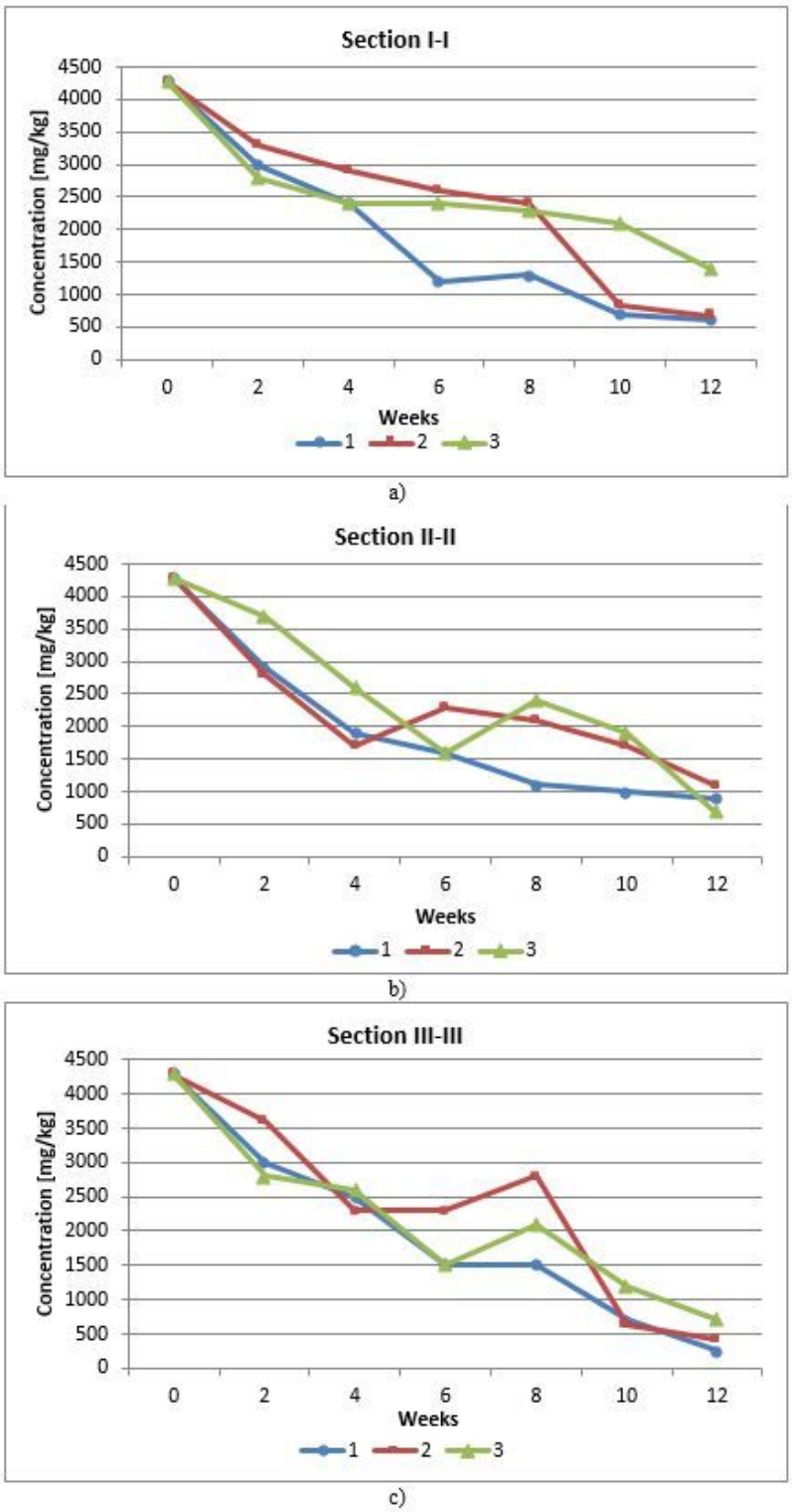

Figure 3

PHC concentration depending on time and section: a) section I-I; b) section II-II; c) section III-III

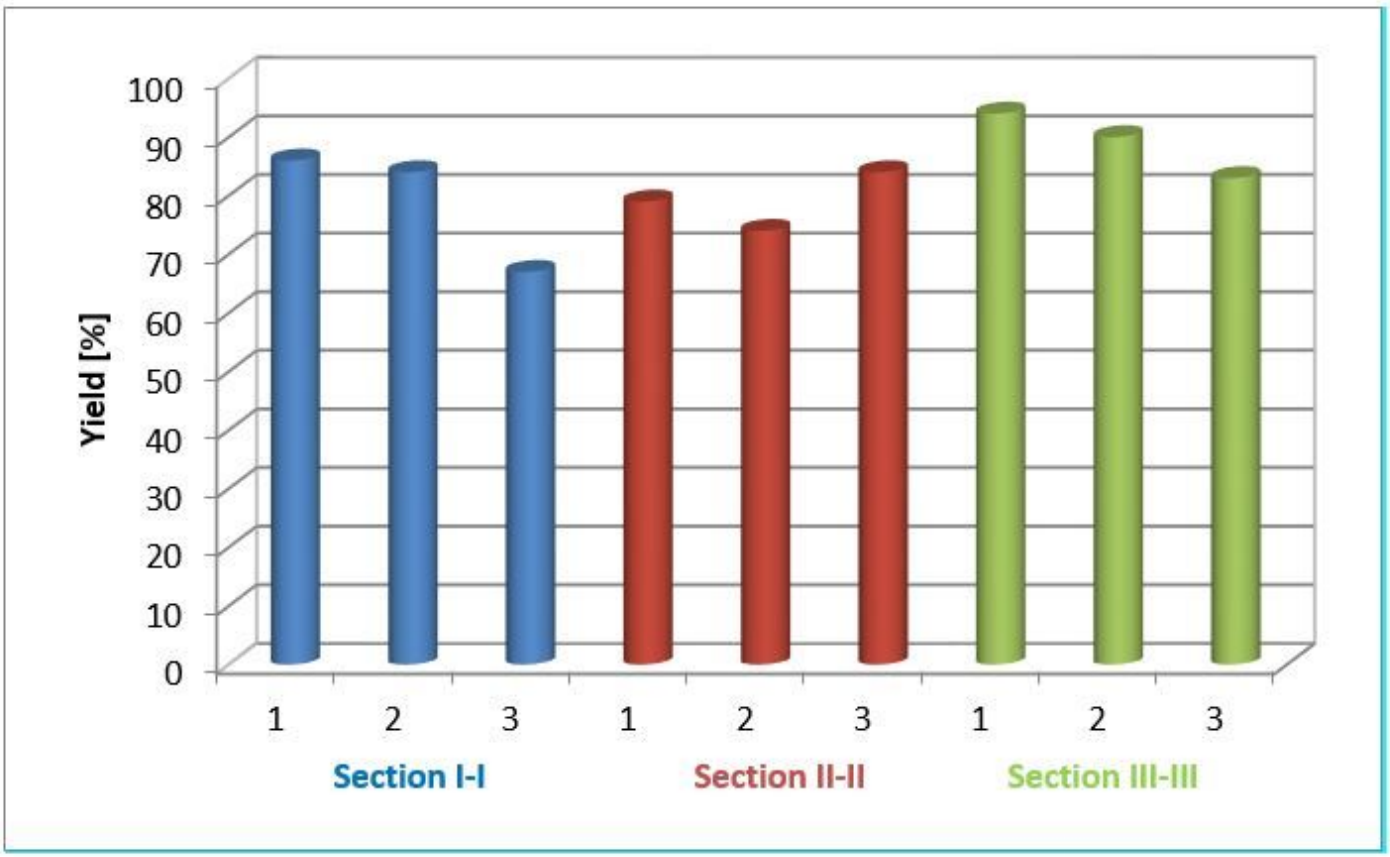

Figure 4

Depollution yield