UNIVERSITY POLITEHNICA OF BUCHAREST
FACULTATY OF POWER ENGINEERING

Integrated solutions for the remediation of the polluted sites with petroleum products

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"Man is a piece of land in which God has put sin and love"

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INTRODUCTION

Globally, one of the most polluted resources is the soil. This resource has been and still is polluted as a result of industrial activities, such as mining, production, storage and use of petroleum products, gas production and others. If certain hazardous substances are not used properly and further they are not stored or disposed as the law requires, the soil can become contaminated and people can be exposed to soil contaminants in various ways.

Over the years, Europe has faced the problem of contaminated soils, and this problem still persists not only in Europe but throughout the world.

The European Environment Agency (EEA) mentions that soil is a source without which many industries would not exist [1]. It is indispensable for 90% of the total production of food, feed, fuel, etc. [1]. The soil provides raw material for various activities, including the construction branch. Soil is also needed to maintain the health of the ecosystem, being a global absorber of carbon, playing an important role in the potential slowdown of climate change and its possible effects.

A contaminated site, as defined in a European Commission report, is a confirmed and well-defined area that poses a potential risk to humans, animals and the environment [1]. The number of contaminated sites at National, European and International level has been high in the past, and this number has continued to grow in many areas, where certain specific measures have not been considered and implemented.

The oil industry is among the most polluting industries globally. As a result of oil spills, the soil is contaminated and thus, depending on the number of pollutants, it is very likely that that soil can no longer be used in either the agricultural or non-agricultural sectors.

Competent authorities in many countries are trying to implement legislative measures to prevent the contamination of other new sites and at the same time trying to act as effectively as possible to treat currently contaminated sites. However, even though many countries at the international level have managed to slow down the increase in the number of contaminated sites and / or the treatment of already contaminated sites, this has not been possible everywhere. There are still many countries where decontamination or prevention methods have not been implemented or, at the same time, there are countries where specific legislation for contaminated / potentially contaminated sites is not defined.

Internationally, numerous studies and research have been done to find the best treatment methods, when the prevention of soil contamination has not been possible. Many methods, economically and ecologically viable, have been considered. These methods include: heat treatment, electrochemical treatment, bioremediation, dichlorination, heat desorption, washing, solvent extraction and others. These methods have been applied as needed and at the same time considering different factors. Over time, however, studies have shown that when choosing a decontamination method, the following factors should be considered: time, costs, level of contamination and whether the method is environmentally friendly. In the present doctoral research, the factors mentioned above were considered in the selection of decontamination methods tested in case of remediation of a site historically contaminated with petroleum products, namely, the electrochemical method and bioremediation. The two methods were tested by applying them both separately and sequentially.

Therefore, during the thesis are presented different remediation scenarios applying the two methods of decontamination consecutively, as well as each separately. The thesis includes 5 chapters that are framed by the introductory part and the final conclusions.

The **first chapter** presents the management and issue of contaminated sites at National, European and International level, the main causes of contamination of these sites, soil contamination with petroleum products and, at the same time, soil protection legislation in different countries.

The **second chapter** provides information on remediation methods used in remediation of soils contaminated with petroleum products. The two methods tested in the doctoral research, the electrochemical treatment method and the biological one (bioremediation) are specifically described.

Chapter three contains information such as the description of the context for the experimental study, the physical and chemical characteristics of the investigated soil, the interpretation of the results obtained after determining the characteristics for contaminated soil, considering the specific legislation in force.

The **fourth chapter** presents in detail the experimental research and the results obtained with the application of the two methods of treating historically polluted soil with petroleum products (electrochemical and biological method), methods applied both individually and individually. hybrid (coupling the electrochemical method with the biological one). Therefore, in the second part of the doctoral research, the experimental study focused on the remediation of a historically polluted soil with crude oil by combining an electrochemical treatment with a variety of bioremediation approaches. Priority in sequential treatment was given to electrochemical remediation, considering that the application of natural attenuation (NA), bio stimulation and bio-augmentation led to low degradation performance for total petroleum hydrocarbons (TPHs) and hydrocarbons. polycyclic aromatics (PAHs).

The **fifth chapter** presents an analysis of the costs involved in applying the treatment methods experienced during the research, an assessment that relates primarily to the energy consumption required to apply the proposed remedial solutions.

In the conclusion of the paper are formulated conclusions based on the results obtained in the doctoral research being also highlighted certain aspects of interest that can be considered by decision makers involved in making certain economic and social decisions in the management of contaminated sites.

The experimental framework for the doctoral research was provided by the Laboratory of Soil Analysis, Control and Depollution within the Center for Advanced Research for Innovative Materials, Products and Processes (CAMPUS) of POLITEHNICA University of Bucharest and the Department of Biological Systems Innovation, Food and Forestry DIBAF from the University of Tuscia, Viterbo, Italy, the experimental activity being funded by the research project Substantiation of the decision to REMediate sites polluted with oil products using the source-pathway-receiver model and cost-benefit analysis / REMPET, PNII-RU-TE2014-4 - 2348, UEFISCDI, contract no. 354 / 01.10.2015.

CHAPTER 1

MANAGEMENT OF CONTAMINATED SITES

1.1. MANAGEMENT PROBLEMS OF CONTAMINATED SITES

Over the years, several sources have led to the contamination of the planet's resources, such as: contamination of water, air and finally, soil. To manage these issues, various solutions have been sought and continue to be sought to remedy already contaminated resources or to prevent pollution.

Regarding site contamination, various studies have been carried out to determine the number of contaminated and potentially contaminated sites, both nationally and internationally.

A contaminated site, as defined in a European Commission report, is a confirmed and well-defined area that poses a potential risk to humans, animals and the environment [1]. The potentially contaminated site, on the other hand, is the area suspected of soil contamination that requires more detailed investigations to confirm whether it is indeed a real risk [1].

1.1.1. Management of contaminated sites at European level

According to the European Environment Agency and the European Commission, around 2.5 million **potentially contaminated sites (Figure 1.1)** were estimated at the level of **2011** in the EAA-39 (39 countries of the European Economic Area). Of these, 1,170,000 sites were already identified [1], [2].

As regards the situation of **contaminated sites,** their total number in the SEE-39 was estimated at 342,000 of which the identified ones represented only one third (approximately 127,480 sites), as shown in Figure 1.2 [1], [2]. According to the European Environment Agency, of the estimated total number of contaminated sites, only 15% has been remedied (Figure 1.2) [1].

1.1.2. Management of contaminated sites at the national level

In Romania, between 2006 and 2011 there was no steps to decontaminate polluted soils [1], [2].

In **2016** another study was carried out for the inventory of contaminated and potentially contaminated sites at European level [3].

At the level of 2018 when the second study was written, a study carried out at the level of 2016, there were more than 65,500 already remedied or in the process of remediation, representing for the last 5 years an additional 8,500 new sites remedied [4].

According to Government Decision HG 683/2015 on the approval of the National Strategy and the National Plan for the Management of Contaminated Sites in Romania, in the period 2007-2008, the total number of potentially **contaminated sites** was 1183 [5].

At the national level, also at the level of 2013, the National Environment Agency reported a total of 210 contaminated sites [5].

1.1.3. Management of internationally contaminated sites

Australia

In a 2016 report by the Environmental Protection Agency of Southern Australia, it was mentioned that there were about 160,000 contaminated sites in Australia [6]. In South Australia, about 2,200 contaminated sites were listed in the Public Register, according to the same report [6].

USA

Within the US EPA is defined the term "Superfund" which is the informal name of CERCLA. In 1980, Congress established the Comprehensive Environmental Response and Liability Act [7]. CERCLA (informally referred to as "Superfund") allows the United States Environmental Protection Agency (US EPA) to clean up contaminated sites and forces those responsible for contamination to clean up contaminated sites or even reimburse the government for these clean-ups (for those run by the government) [7].

1.2. SITES POLLUTED WITH PETROLIER PRODUCTS

The main causes of soil contamination are the disposal and treatment of waste and also the oil industry, where the whole cycle is covered, from production to disposal [2], [5]. Accidental oil spills are also added here.

During accidental oil spills, such as oil spills, for example, basic soil components (minerals, water, air, organic matter, microorganisms) may be affected. Due to the chemicals present in the fuel, soil quality is impaired or damaged and subsequent soil use (e.g. when used for cultivation) may be compromised.

At the same time, leakage cases can be repeated, and for this reason the environmental hazards can be even greater [8]. Another factor which may pose a danger to the environment and which must be considered is the ageing of pollution [8].

Other types of site contamination may be: abandoned mines, mining sites, industrial installations, waste management sites, nuclear, biological, chemical plants and traditional weapons production facilities, underground storage tanks/underground storage tanks leaking, contamination due to natural disasters or terrorist activities, etc. [2], [9].

1.2.1. Exposure paths

As a result of soil pollution, humans and animals may be exposed to different types of contaminants present in the soil.

The ways in which humans are exposed to soil contaminants are as follows [10]:

1. **Ingestion** of soil;
2. **Inhalation -** Breathing of volatiles and dust;
3. **Skin contact**;
4. **Diet as a result of ingestion of products that have been obtained from crops that have been grown** in **contaminated soil**.

1.3. CONTAMINATION OF SOILS WITH PETROLIER PRODUCTS

There are different types of contaminants, but in terms of soil contamination with petroleum products, the most common contaminants are **TPH, PAH, BTEX, heavy metals** and others.

Once in the soil, depending on the concentration of pollutants, these substances can lead to the contamination of the soil concerned. A very small amount of contaminants cannot affect the soil in any way, but once certain limits are exceeded, the soil can be considered contaminated.

1.3.1. Oil hydrocarbons

The class of petroleum hydrocarbons is the most widespread and the most problematic, demonstrating over time that these compounds are neurotoxic for both humans and animals [11]. The chemicals that make up the group of petroleum hydrocarbons are classified as aliphatic and aromatic compounds.

Many chemical compounds that reach the soil through different paths originate in unrefined oil (oil).

Substances found in the TPH group may include: hexane, jet fuels, mineral oils, benzene, toluene, xylene, naphthalene and fluorene, as well as other petroleum products and petrol components [12].

1.3.2. Polycyclic Aromatic Hydrocarbons

PAHs are organic compounds that are really difficult to degrade and it must be taken into account that their persistence increases depending on their molecular weight [13].

1.4. LEGISLATION ON THE PROTECTION OF THE SOIL TO THE NATIONAL AND INTERNATIONAL LEVEL

Legislation referring to the assessment of environmental pollution is specific to countries in Europe and around the world. However, each individual law has the same purpose, namely to prevent pollution and to remedy it when it has already taken place.

1.4.1. Legislation on contaminated sites at European level

At European level, only some Member States have specific legislation on soil protection [14]. The first Soil Protection Strategy was the 2006 Soil Framework Directive [14], [15]. This was adopted precisely because the soils were used unsustainable, and many others were contaminated.

1.4.2. Legislation on contaminated sites at national level

At national level, Order 135 of 10 February 2010 is the legislative framework for nature protection, namely the Order on the approval of the "Methodology for applying the environmental impact assessment for public and private projects" [16].

Also at national level, Order no. 756/1997, defines the following two thresholds: alert and intervention. If the level of contamination for a pollutant of concern is above the alert threshold, then additional monitoring should be performed. If the level of contamination of a pollutant exceeds the intervention threshold, the competent authorities must request a reduction in the concentration of pollutants in the soil until the normal values in the current Regulation are reached [17].

The most recent legislative framework covering potentially contaminated and contaminated soils is represented by “Law no. 74 of April 25, 2019 on the management of potentially contaminated and contaminated sites”.

Law no. 74/2019 aims to protect by regulating various measures human health and the environment from the effects that soil contamination can produce. These measures aim to improve the quality of environmental factors that are affected by the existence of contaminants in quantities that exceed legal values and that pose a risk to humans and the environment [18].

1.4.3. Legislation on contaminated sites internationally

In the United States, in 1980 Congress enacted the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) informally known as the "Superfund" [7].

In Canada, the Canadian Environmental Protection Act dates back to 1999 and aims to prevent pollution and protect the environment and human health and aims to contribute to sustainable development [19].

As far as Australia is concerned, the existing environmental laws in each state are quite old, but in 2014, the Institute for Sustainable Future produced the report entitled "Contaminated soil wastes in Australia" [19].

CHAPTER 2

METHODS OF REMEDIATION OF CONTAMINATED SOILS

2.1. CLASSIFICATION OF THE REMEDIATION METHODS OF THE POLLUTED SOILS WITH PETROLEUM PRODUCTS

Over the years, various methods which are more or less economically and ecologically viable, have been used to treat contaminated soils. Among these methods we can list: heat treatment, electrochemical treatment, bioremediation, dechlorination, heat desorption, washing, solvent extraction and others [21], [22], [23].

One of the methods of decontamination of contaminated sites, **bioremediation**, is a commonly used method, cost-effective, but which can take longer compared to applying other remediation methods to achieve the desired results. For this reason, current research is aimed at coupling other existing remediation methods with biological ones, but methods that do not affect the microbial activity in the soil. One such method is the **electrochemical** one. This is effective over a much shorter period of time, but using the method over a longer period of time, will increase energy consumption.

During the doctoral research, the two methods were studied (electrochemical treatment and bioremediation) and subsequently applied to the historically contaminated soil. These methods and the results obtained, will be presented in the following chapters.

2.2. ELECTROCHEMICAL METHOD FOR REMEDIATON OF CONTAMINATED SOILS WITH PETROLEUM PRODUCTS

The electrochemical method used for soil's decontamination, also called electrokinetic decontamination process (EKD), can be applied both in-situ and ex-situ [24].

These electrochemical remediation methods have a common denominator, namely that they are based on the transport processes that occur when an electric field is applied to the ground [25].

Electrochemical treatment also has **advantages** compared to other methods, including the following:

- the method can be applied both in-situ and ex-situ;
- the ecosystem is not affected after the electrochemical method is applied;
- the duration of electrochemical method application is not long and can be easily integrated with other methods for an optimization of the final result.

At the same time, the method has **disadvantages**, and one of the disadvantages is that it depends on many variables such as:

- soil characteristics;
- humidity amount;
- the organic matter that is present.

2.3. BIOLOGICAL METHODS FOR REMEDIATON OF CONTAMINATED SOILS WITH PETROLEUM PRODUCTS

The bioremediation process, which in the recent decades has made great progress, aims to restore polluted environments in an efficient and low-cost way.

During the bioremediation process, the metabolic diversity of some microorganisms is practically used in order to degrade and reduce the concentrations of contaminants [26]. In this case, environmental conditions are very important for the growth and metabolism of indigenous microorganisms [27].

Bioremediation has various advantages and disadvantages. Among the many **advantages** of the method, we can mention:

- The best quality-price ratio;
- Low costs;
- It can treat both soils and waters;
- Natural attenuation process;
- Can be applied both in-situ and ex-situ.

The method also has some **disadvantages**, such as:

- A longer time required when the contamination is high;
- Difficulties in real-time monitoring;
- In case of ex-situ treatment, the soil requires excavation;
- The need for a large space where the process can be applied.

CHAPTER 3

CASE STUDY

3.1. CONTEXT DESCRIPTION OF THE CARRIED OUT STUDY

The experimental framework for the doctoral research was provided by the “Laboratory of Soil Analysis, Control and Depollution within the Center for Advanced Research for Innovative Materials, Products and Processes (CAMPUS) of POLITEHNICA University of Bucharest and the Department of Biological Systems Innovation, Food and Forestry - DIBAF from Tuscia University, Viterbo, Italy ”. The laboratory analyzes that could not be performed in the mentioned laboratories were performed by the laboratory "Givaroli Impex S.R.L." and financed by the implementation of the project “Substantiation of the decision to REMediate the sites polluted with oil products using the source-pathway-receiver model and cost-benefit analysis / REMPET, PNII-RU-TE2014-4 - 2348, UEFISCDI, contract no. 354 / 01.10.2015, coordinator of the POLITEHNICA University of Bucharest ”.

The sample of contaminated soil used to test the remedial solutions proposed in the experimental research is from the territory of a Romanian company, whose main activities are focused on exploration and production of oil and gas, refining and sale of petroleum products. Therefore, the soil samples used for the experiments were collected from an oil-polluted area located in the historical region of Muntenia, in Romania, which has a surface of 1000 m^2.

3.2. DETERMINATION OF THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE INVESTIGATED SOIL

3.2.1. Standard methods used for determination different amounts of contaminants

Using different standard methods (shown in Table 3.1) depending on each class of contaminant analyzed, the were determined the concentrations of total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (ΣPAH), BTEX group, namely benzene, toluene, ethylbenzene and xylene, heavy metals, zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), nickel (Ni), chromium (Cr), arsenic (As) and cadmium (Cd).

The soil characteristics were also determined: pH, potassium - K, phosphorus - P, hexavalent chromium (CrVI), total phosphorus, total potassium, total nitrogen, total organic carbon (OCD), humus, chlorine and humidity.

3.2.2. Contaminated soil characteristics analysis

During the laboratory analyzes, the characteristics of the contaminated soil were determined, among which, pH (having a value of 7.25), humidity (8.61%) and others (Potassium - K, Phosphorus - P, Total Nitrogen, etc.). At the same time, within the laboratory analyzes, the concentrations of

3.2.3. Level of soil pollutant concentrations determination

Chemical analyzes of the concentration of contaminants in the soil sample had the following results:

- TPH/C10 - C40 - 15.967,4 mg/kg d.w.;
- ΣHAP - 57,322 mg/kg d.w.;

heavy metals were determined.

3.3. RESULTS INTERPRETATION

The results regarding the level of concentrations of pollutants identified in the investigated soil were compared with the regulations in force in Romania (Order 756 of 1997 - which defines the alert and intervention thresholds) and Italy (Legislative Decree 152/2006 - which establishes the level allowed by concentration of pollutants in the soil, taking into account the type of land use [28]).

The TPH determined value from the initial sample of contaminated soil is 15 times higher than the maximum allowed value of the alert threshold found in the legislation in force in Romania, and approximately 7.5 times higher than the maximum allowed value of the intervention threshold, for less sensitive land use.

Regarding the comparison of TPH concentration value in the initial soil with the maximum values allowed by the Italian Decree in force, an excess of almost 60 times higher was calculated.

In the case of total PAHs, the concentration determined from the initial soil sample is between the alert and intervention thresholds of the Romanian legislation in force (Order 756 of 1997). The concentration of total PAHs determined from the soil sample does not exceed the intervention threshold of the Italian decree (Legislative Decree 152/2006).

Regarding the BTEX compounds, their values are below the limit of determination and thus, these compounds are not of interest in the context of the present research.

As a result of the complex contamination that exists in the polluted soil, in order to reduce energy consumption on the one hand, but also the remediation time on the other hand, the sequential application of biological and electrochemical methods was evaluated.

CHAPTER 4

TESTING OF ELECTROCHEMICAL AND BIOLOGICAL METHODS FOR THE REMEDIATION OF ORGANIC COMPOUNDS IN THE SOIL

4.1. ELECTRIC REMEDIATION OF THE POLLUTED SOILS WITH PETROLEUM PRODUCTS

The electrochemical experiments were performed in the laboratory "Laboratory of Soil Analysis, Control and Depollution" (LACDS) of the CAMPUS research center belonging to the POLITEHNICA University of Bucharest.

For the testing of the electrochemical method of the polluted soil, an experimental installation comprising 3 cells of 150 mm length was made. For the present experiment, it was used a single electrochemical cell as indicated in **Figure 4.1** and **Figure 4.2**.

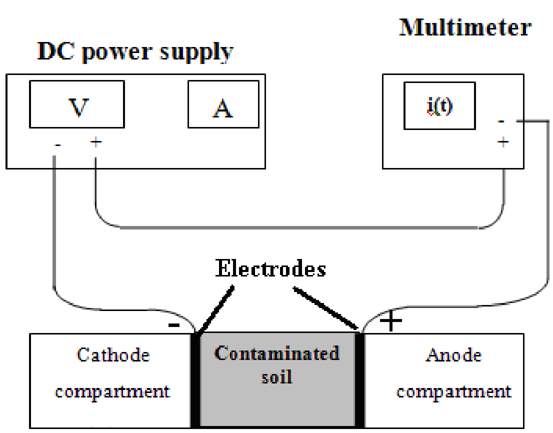


Fig. 4.1. Experimental installation design

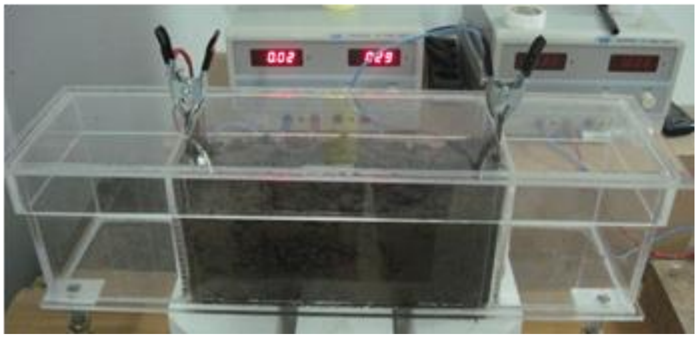


Fig. 4.2. Experimental installation used for electrochemical treatment application

The main characteristics for the application of the electrochemical method are:

- specific voltage: 1V/cm;
- voltage applied:15 V;
- remediation time: 20 days.

During the application of the electrochemical treatment, different parameters were monitored, parameters such as: pH, current intensity, redox potential, humidity, energy consumption, etc.

Consequently, soil samples were taken and analyzed in the following experimental phases:

- ❖ In the initial phase - polluted soil taken from the investigated land;
- ❖ EC10 (soil treated for 10 days by electro-remediation);
- ❖ EC20 (soil treated for 20 days by electro-remediation).

4.1.2. Interpretation of the results obtained after applying the electro-remediation process

After appying the electroremediation process for the EC10 and EC20 soil samples, the following concentrations for TPH, PAHs and BTEX compounds were obtained.

Table 4.1 Results of the chemical analysis of TPH, Total PAHs and BTEX from all the three soil samples (initial, EC10 and EC20)

Nr.	Parameter	U.M.	Initial soil values	EC10	EC20
1.	TPH - Total Petroleum Hydrocarbons /C10 - C40	mg/kg d.w.	15346.2	11933	8104.25
2.	Total PAH (Polycyclic Aromatic Hydrocarbons)	mg/kg d.w.	103.161	89.156	89.524
3.	Benzen	mg/kg d.w.	0,435	<0.25	<0.25
4.	Toluen	mg/kg d.w.	0,383	0.541	<0.25
5.	Ethillbenzen	mg/kg d.w.	<0,25	<0.25	<0.25
6.	O-Xilene	mg/kg d.w.	0,329	<0.25	<0.25

7.	M+P-Xilene	mg/kg d.w.	0,286	<0.25	<0.25

The **humidity** obtained from the processes indicated a value of **31%** in the case of the EC10 sample, respectively **27.93%** in the case of the EC20 sample.

4.1.2.1. *Assessing the evolution of the soil pollutant concentrations during the application of the electrochemical remediation method*

For assessing the time change of the level of soil pollutant concentrations in experimental research, the values of contaminant concentrations in EC10 and EC20 soils were analyzed compared to the initial soil values.

In the case of TPH compounds, their concentration decreased after the first 10 days of EC treatment to the value of 11933 mg / kg d.w., representing a decrease of approximately **25%**. After another 10 days, the amount of TPH decreased until at 8104.25 mg / kg d.w., meaning a decrease of about **49%**, compared to the concentration present in the initial soil (Figure 4.1).

Regarding the amount of total PAHs, a reduction of the concentration level was obtained as well after the application of the electrochemical treatment process, this representing a 13% decrease compared to the concentration present in the initial soil. But the reduction of the concentration level was not a significant.

The level of BTEX compounds identified in the initial soil was below the legislative limit. Although a reduction has been identified following the application of the electrochemical method, this reduction cannot be associated with the application of the proposed remedial solution.

From the results obtained after the application of the electrochemical method, a reduction of soil contaminants is observed in a relatively short time, but in order to reach results that correspond from a legislative point of view for certain types of use, the EC process should be extended by another 10 days. This would involve higher costs due to the required energy consumption.

Consequently, the combination of remediation methods can be a solution for obtaining improved results, both in terms of reducing the level of soil pollution and in relation to the remediation time or costs associated with their application.

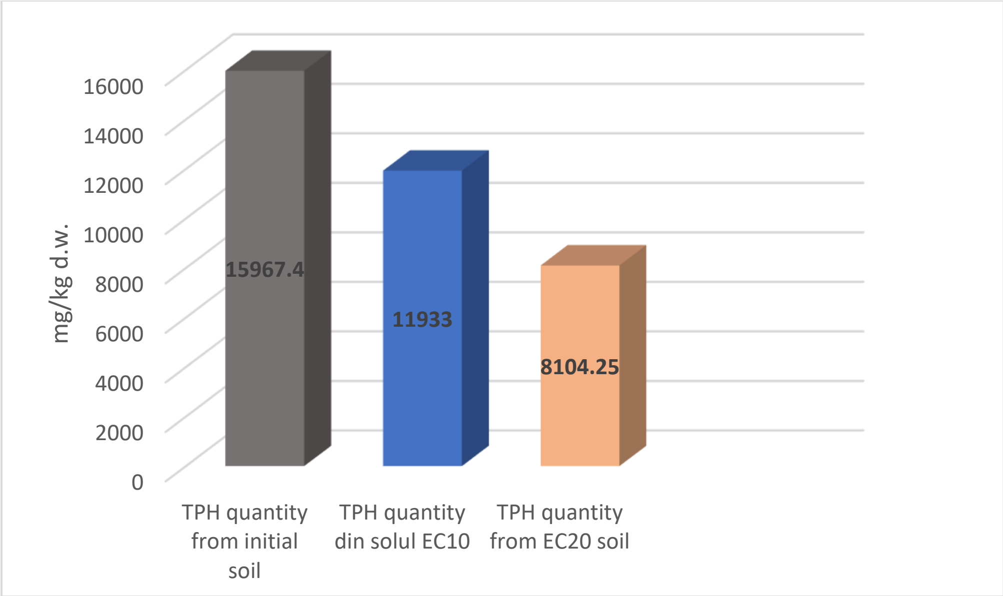


Fig. 4.3. Comparison between the TPH determined values in the initial soil with those determined from EC10 and EC20 soils

4.1.3 Soil biological properties characterization

In order to integrate the electrochemical method with the biological one, it was necessary to identify the biological properties of the soil, both in terms of the initial soil (before applying the electrochemical remediation method) and after using the electroremediation process (soil samples EC10 and EC20).

The number of Colony Forming Units in the case of EC10 soil was equal to $6.7 * 10^7$ CFU/g and in the case of EC20 soil was equal to $6.24 * 10^4$ CFU / g.

4.2 Bioremediation of the site polluted with petroleum products

4.2.1 Experimental activities performed for the application of bioremediation to the polluted soil

4.2.1.1. *Strains isolation*

The contaminated soil (homogeneous sample) was analyzed in order to determine the colonies of bacteria and the number of fungi and yeasts.

Bacteria, **yeast** and **fungi** were isolated from the cell suspension obtained, and subsequently, the dispersion plate method was used. Plate Count Agar (PCA) and 0.1% Chloramphenicol Rose Bengal Agar (RBA) plates were used for the dispersion plate method.

4.2.1.2. *Isolation of bacterial strains in the present experiment*

In the case of bacterial strains, the total number of CFU per gram of soil dry matter was determined, 12846666.67 (CFU / g). The determination was made from the original inoculum (undiluted cell suspension) spread on PCA plates.

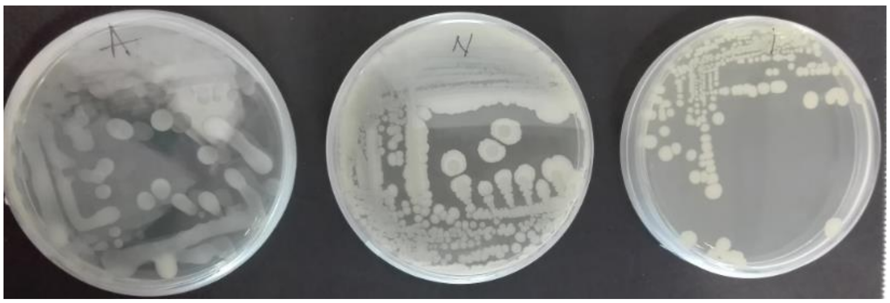
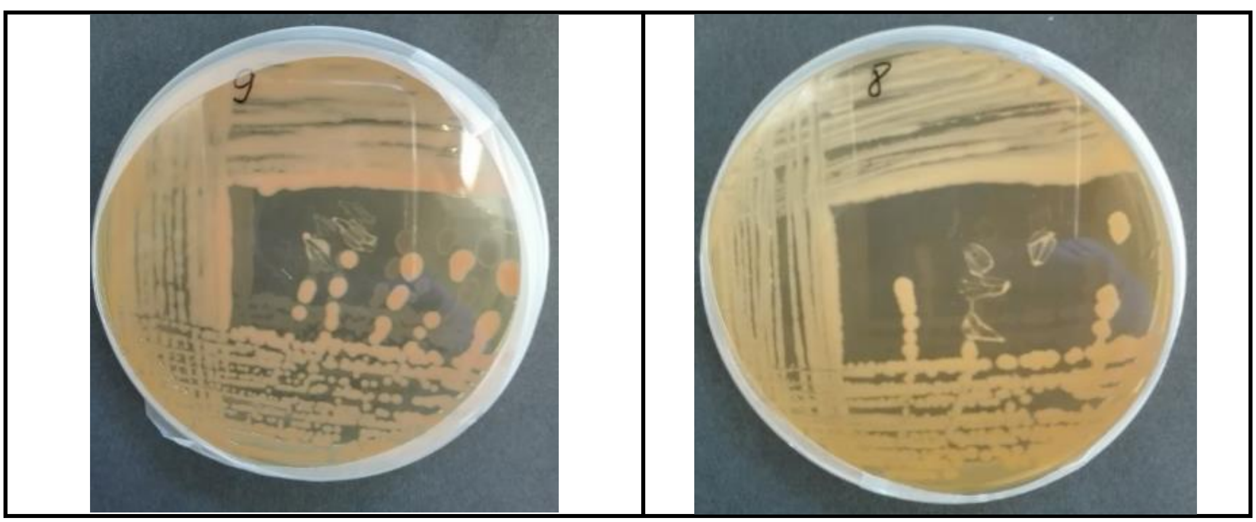


Fig. 4.4. Some of the first pure isolated crops

4.2.1.3. *Isolation of the yeast and fungi strains*

In the second case, for the isolation of yeasts and fungi, the aliquots of the cell suspension were spread on Petri dishes containing Rose Bengal Agar and Chloramphenicol (RBCA). A total of 4 yeast strains and 8 different fungi strains were isolated.



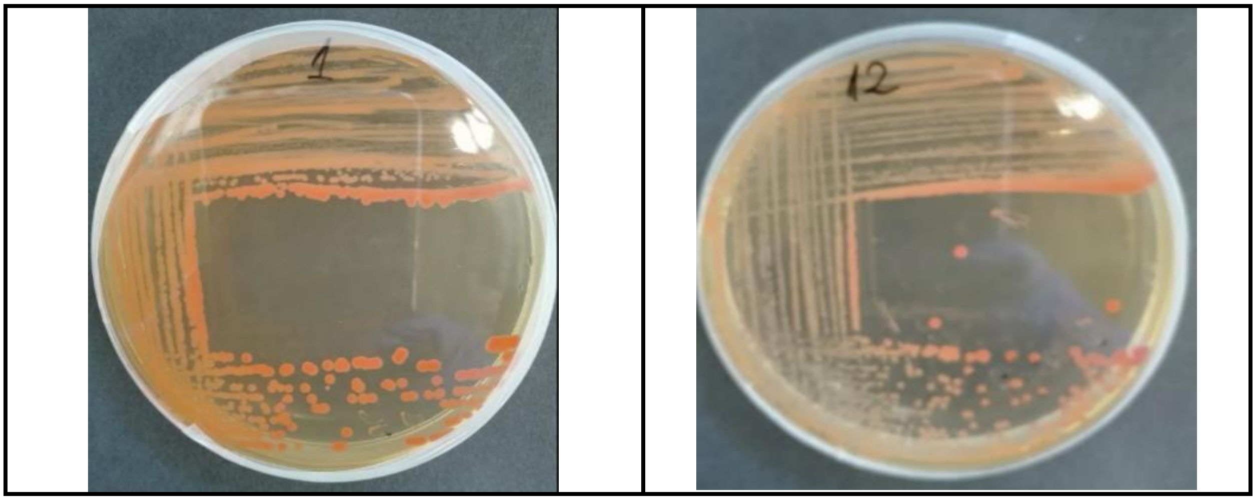
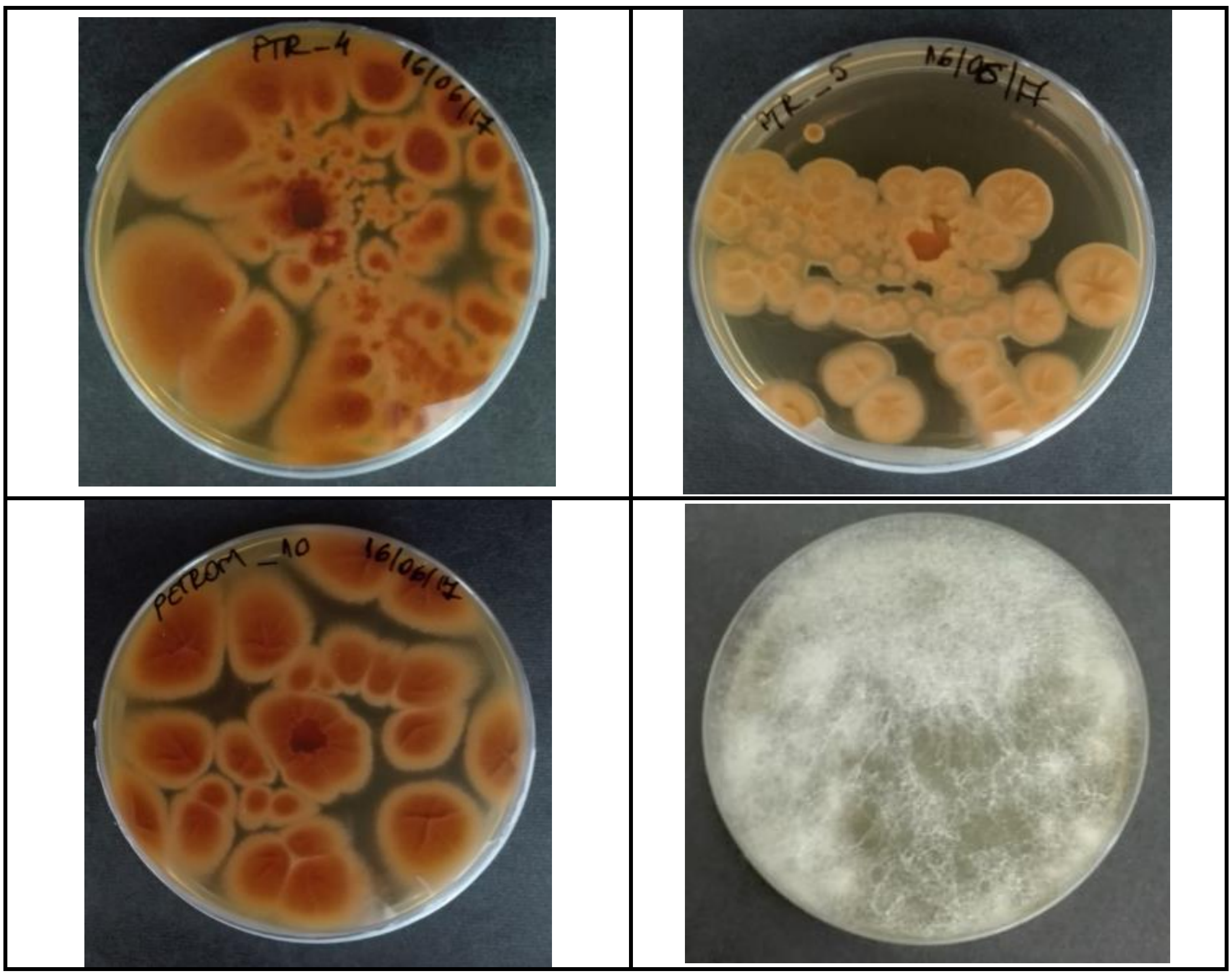


Fig. 4.5. The four pure stems obtained from yeast



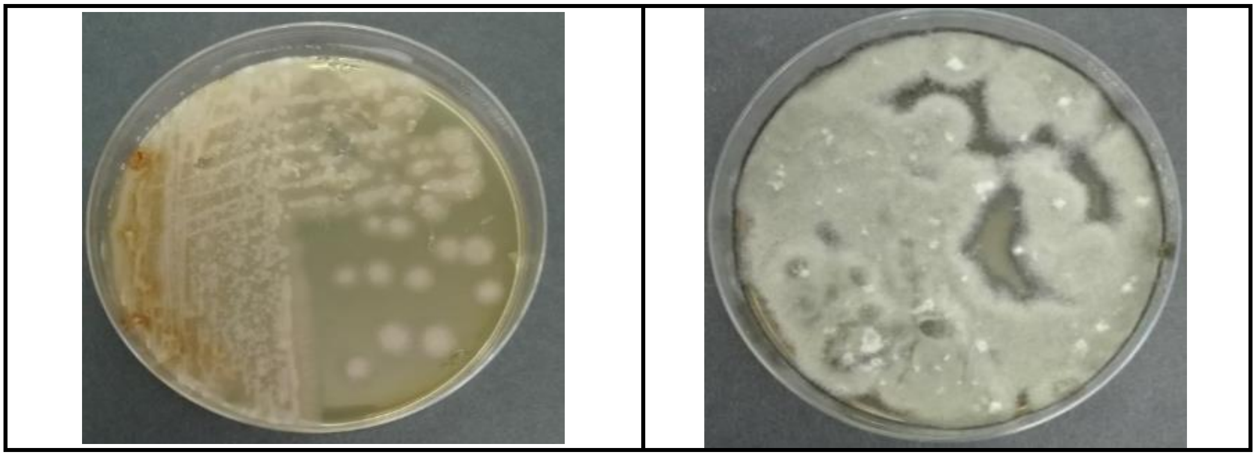


Fig. 4.6. Various pure fungi stems obtained

4.2.2 Determination of alkaline degrading microbial strains (for degradation of the TPH component)

In order to initiate the remediation process, various screening tests of bacteria, fungi and yeast were performed in order to see which of the microorganisms present in the contaminated soil sample are those that can degrade toxic compounds, namely TPHs and PAHs. Various media (both liquid and solid media) were used to perform the tests to isolate various microorganisms from hydrocarbon degradation.

4.2.2.1. *Screening test for bacteria that degrade TPH in liquid culture*

In order to identify the colony that "consumes" the best the TPH contaminant, in 15 sterile tubes were added the final solution of M9 (salt solution) and dodecane (C12). Each **bacterial** isolate was then placed in a tube and then incubated. Bacterial growth was monitored every 12 hours using a turbidimeter for a period of 96 hours.

Two microorganisms (PTROM_H and PTROM_E) have proven their ability to degrade dodecane and will continue to be used for experiments.

4.2.2.2. *Screening test for yeast that degrade TPH in liquid culture*

For the screening of **yeast** cultures, in order to investigate the ability of yeast microorganisms present in the soil to grow in dodecan as a single carbon source, 4 tubes containing final solution of M9 and dodecane (C12) were prepared, as in the case of bacterial screening. The tubes were inserted into the orbital shaker, and the absorbance was measured daily for 10 days using a spectrophotometer, such as OD600.

Following absorbance monitoring, 2 demonstrated dodecan growth capabilities, namely PTROM_1 and PTROM_8.

4.2.2.3. *Screening test for fungi that degrade TPH in liquid culture*

In the case of **fungi**, screening was performed in flasks with M9 final solution over which dodecane (C12) was added. The flasks were then placed in the orbital shaker to stimulate growth and, after 48 hours, each inoculum of mushrooms was taken from each flask with a mushroom inoculum and placed in a new flask.

Of the nine filamentous morphotypes tested, only two, PTROM_11 and PTROM_13, showed a slight development of biomass.

4.2.3 Determination of microbial strains that degrade PAHs

4.2.3.1. *Screening test for bacteria that degrade PAHs in solid culture*

To find the best microorganisms or the microorganisms that can degrade one of the most toxic components present in PAHs, namely phenanthrene, all 15 microorganisms (bacteria) were tested. Bacteria screening tests to identify those microorganisms that degrade phenanthrene were performed in a solid medium, on square plates containing final M9 solution and Agar.

In this case as well, the 2 microorganisms, PTROM_E and PTROM_H, showed degradation capabilities of phenanthrene.

4.2.3.2. *Screening test for yeasts and fungi that degrade PAHs in solid culture*

Screening of fungi and yeasts in solid culture was performed as in the case of bacteria on square plates containing final M9 and Agar on which phenanthrene dissolved in acetone solution was sprayed.

Following the tests, 3 fungi were found to be positive and good phenanthrene degradants, namely: PTROM_2, PTROM_7 and PTROM_13.

4.2.3.3. *Screening test on PDA with POLY R-478*

In recent years it has been shown that several fungal species have been able to degrade PAHs, and therefore fungal isolates have been tested for the degradation of aromatic hydrocarbons using the method of discoloration plates of Poly R-478 dye on plates containing Poly- R478. The aim was to see which fungal isolate is able to discolor the polyaromatic anthraquinone Poly-R478, a model substrate to evaluate the degradation capacity of PAHs.

After performing the tests, 3 fungal isolates gave good results, namely: PTROM_4, PTROM_5 and PTROM_10.

4.2.4. Identification of strains and their taxonomic affiliation

Screening tests have shown some positive strains that can degrade TPH and PAH compounds. These positive strains were selected for molecular identification. To do this, the DNA was extracted using the DNeasy UltraClean Microbial Kit (Qiagen).

63F(5'CAGGCCTAACACATGCAAGTC3')1389R(5'ACGGGCGGTGTGTGTACAAG 3') were used to amplify the almost complete length of the 16S rRNA gene for bacterial identification. ITS1 (5'-TCCGTTGGTGAACCAGCGG-3 ') and ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify ITS1 5.8S rDNA and ITS2 regions [29].

The amplifications were performed in a Primus PCR system (MWGBiotech, Germany).

Bacterial isolate results:

The identification tests showed that both bacterial strains PTROM_E and PTROM_H belong to the genus Pseudomonas. According to the literature, the genus Pseudomonas is represented by several species, both n-alkane and PAH degrading, as well as bio-surfactant producers [30].

Results of fungal isolates:

In the case of fungi, two of the 3 identified strains, PTROM_4 and PTROM_10 were assigned as Aspergillus terreus. The third representative of the fungal class, PTROM_5, was assigned as Penicillium sp. The strains belonging to the regnum of Aspergillus terreus and Penicillium citreonigrum have a high bioremediation potential in preliminary crude oil degradation experiments [31].

Yeast isolate results:

Two strains were isolated in the case of yeasts, PTROM_1, assigned as Rhodotriorula toruloides and PTROM_8, assigned as Yarrowia lypolitica [29]. Both yeast species mentioned are known to be good degradants for both alkanes and PAHs [31].

4.2.5. Consortium pre-assembly tests

The degradation capacity of hydrocarbons was evaluated together with other interaction activities such as: antagonism, biosurfactant production, production of quorum and anti-quorum detection signal molecules [29].

4.2.5.1. *Antagonistic test*

Antagonistic tests performed on PCA (Plate Count Agar) media aimed to verify whether there is an inhibition of the growth of each isolate in the presence of other strains. Tests performed between previously identified strains (bacteria, fungi and yeast) and demonstrating degradation capabilities of dodecan and phenanthrene compounds showed that no strain exhibited antagonistic behavior to another strain.

Analyzing the results, it was assumed that when grown together in a consortium of two or more strains, those selected strains would not exert any inhibitory effect by releasing antibiotics or antistatic substances against each other [28].

4.2.5.2. *Biosurfactant production test*

For the biosurfactant production test, the oil spreading technique was performed according to the literature [32].

A bacterial strain (PTROM_H - Pseudomonas fluorescens) and a yeast strain (PTROM_8 - Yarrowia lipolytica) demonstrated surfactant release capabilities in the environment. The facilitatation of the initial stage of interaction between oil pollutants and microorganisms has been demonstrated following this test [29].

4.2.6. Preparation of cultures for biostimulation and bioaugmentation experiments

This subchapter presents the preparations for the bioattenuation, biostimulation and bioaugmentation processes.

- Preparation of the final M9 environment required in the biostimulation process
- Enrichment culture consortia necessary in the bioaugmentation process
- Preparation of isolated microorganisms (PTROM_H and PTROM_E - Pseudomonas Fluorescens and Pseudomonas sp.) required in the bioaugmentation process

4.2.6.1. Microcosms preparation

14 sterile bottles of 500 ml each were used to simulate microcosms. Of these, 4 were used for the initial soil microcosms, 4 for EC10 soil and 8 for EC20 soil, as can be seen in Table 4.2.

Humidity, being a key factor, was adjusted to 25% to have the same humidity in all 14 bottles.

The **first process** prepared with the help of microcosms was the initial soil **bioattenuation**, in which case nothing else was added to the microcosm bottles other than the soil samples.

The **second process** is the **bioaugmentation** with consortia, in which the mixture of consortia prepared according to the recipe from the previous subchapter was used.

The **third case** is **bioaugmentation** with isolated microorganisms (PTROM_E and PTROM_H).

A **fourth process** is **biostimulation** for which a final M9 solution has been added.

Tabelul 4.2 Microcosms prepared for the bioremediation process using the initial contaminated soil

	Sample	Process	Period
Initial soil			
1.	Soil	Bioattenuation	50 days
2.	Soil + M9	Biostimulation	50 days
3.	Soil + H + E	Bioaugmentation	50 days
4.	Soil + Alkanes mixtures (C_{10} – C_{40}) + Phenanthrene	Bioaugmentation	50 days

In Figure 4.7 a part of the microcosms prepared for the experiment can be seen.

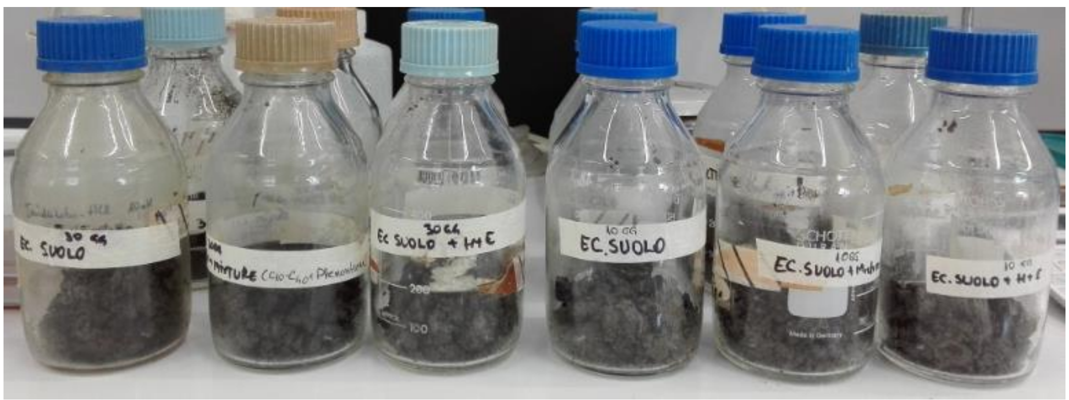


Fig. 4.7. Prepared microcosms – moment t = 0, before starting the experiment

4.2.8 Interpretation of the results obtained from the bioremediation process applied to the initially contaminated soil

After 50 days, soil samples from microcosms were chemically analyzed to observe the percentage reduction in contaminants from those soil sample initial contaminated (Table 4.3). Percentage reductions are calculated for the values of contaminants of interest, namely TPH and PAHs.

Tablr 4.3 Percentage reductions of TPH and PAH from the values of the initial soil subjected to bioremediation processes compared to the measured values from the initial soil

Parameter	U.M.	Det. values Initial soil	% Reduction of the sample of			
			Soil	Soil + M9	Soil + H + E	Soil + Mixture
			After BIO50			
			From initial soil values			

TPH/C10 - C40	mg/kg d.w.	15346.2	10.14%	37.41%	9.87%	3.73%
Total HAPs	mg/kg d.w.	103.161	18.559%	14.617%	-4.511%	20.975%

The following figures will present the differences between the initial values of TPH and PAHs and those obtained after applying the bioremediation process to the initial soil.

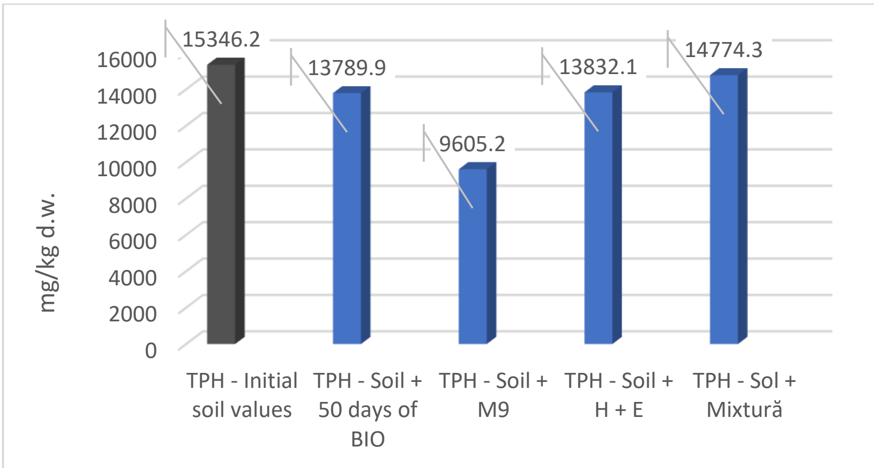


Fig. 4.8. Evaluation of the trend regarding the level of **TPH** concentrations in the initial soil for all the experimental variants tested

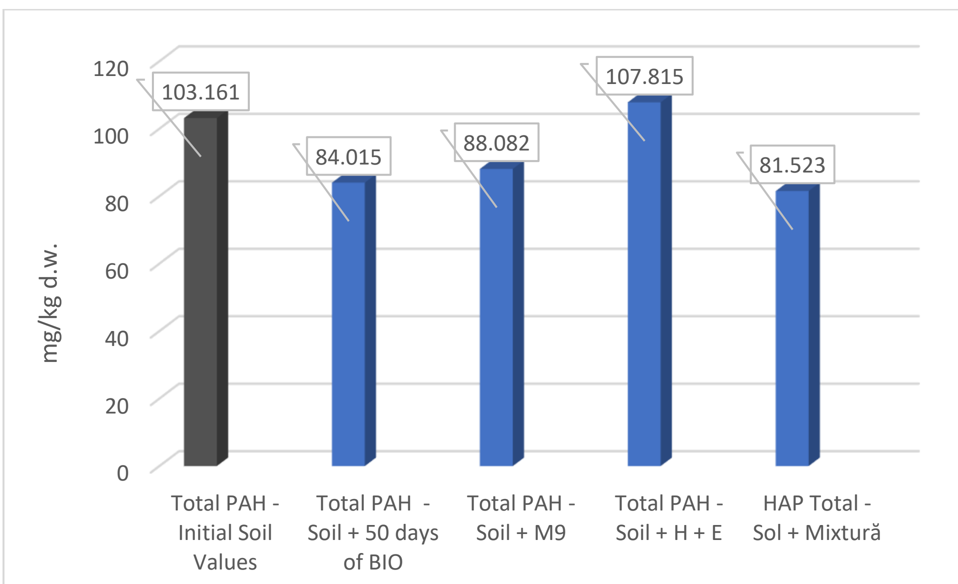


Fig. 4.9. . Evaluation of the trend regarding the level of Total **PAHs** concentrations in the initial soil for all the experimental variants tested

4.2.7 CONCLUSION

As it was observed in the results presented in the last subchapter, the application over a period of 50 days of the bioremediation process only to the initially contaminated soil, did not lead to a huge decrease in the amounts of contaminants.

However, noting the microbial activity in the soil and already previously testing a decontamination method that would not affect the microbial activity, the research was designed to experiment with a hybrid method of coupling two remedial processes (EC + BIO) to see what results can be obtained.

The next sub-chapter presents the experiment of coupling the electroremediation and bioremediation methods.

4.3 Coupling electroremediation and bioremediation processes

The decision-maker to choose a suitable method of decontamination of a historically contaminated soil must take into account several aspects in order to make a decision, namely: the time required for the process, the degree of decontamination, the costs involved and others.

In the case of complex pollution, the idea of coupling two treatment methods was considered to be the most appropriate. The treatment process with which the bioremediation process could be coupled is the electrochemical treatment because it does not affect the microbial activity, activity that must be supported and not combated, as already previously specified.

In this regard, microcosms were created with soil samples obtained from the electroremediation process, namely with soil samples EC10, obtained after applying the electroremediation process for 10 days to the initial soil sample and EC20 obtained after applying the same process. for a period of 20 days.

4.3.1 Electro-remediation and bioremediation processes applied to the EC10 soil sample

In Table 4.4. are presented the types of microcosms created for the EC10 soil sample.

Table 4.4 Microcosms prepared for the bioremediation process using the EC10 soil sample

	Sample	Process	Period
EC10 Soil			
1.	EC10	Bioattenuation	50 days
2.	EC10 + M9	Biostimulation	50 days
3.	EC10 + H + E	Bioaugmentare	50 days

4.	EC10 + Alkane Mixture ($C_{10} – C_{40}$) + Phenantrene	Bioaugmentation	50 days

The results obtained for the compounds of interest (TPH and PAH) following the application of the bioremediation process over a period of 50 days to the EC10 soil sample can be seen in the following table.

Table 4.5 Assessment of the concentrations level of TPH and PAH pollutants in the EC10 soil subjected to the bioremediation process compared to the initial value

No.	Parametru	U.M.	Det. Values – Initial soil	Det. Values – EC soil	EC10	EC10 + M9	EC10 + H + E	EC10 + Mixture
					+ 50 zile BIO			
1	TPH/C10 - C40	mg/kg d.w.	15346.2	11933	9282.8	6971.4	9299.4	7350.5
2	TOTAL PAHs	mg/kg d.w.	103.161	89.156	43.11	45.157	43.296	34.308

Table 4.6 Percentage reduction of the level of total TPH and PAH concentrations in EC10 soil subjected to the remediation process compared to the value of EC10 soil before bioremediation

Param.	U.M.	Det. values - Initial soil	Det. values - Soil EC10	% Reduction of the sample of			
				EC10	EC10 + M9	EC10 + H + E	EC10 + Mixture
				After BIO50			
				From EC10 initial value			
TPH/C10 - C40	mg/kg s.u.	15346.2	11933	22.21%	41.58%	22.07%	38.40%
Total PAHs	mg/kg s.u.	103.161	89.156	51.65%	49.35%	51.44%	61.52%

Table 4.7 Percentage reduction of the level of TPH and Total PAH concentrations from EC10 soil values subjected to the remediation process compared to the initial soil value

Param.	U.M.	Det. values - Initial soil	Det. values - Soil EC10	% Reduction of the sample of			
				EC10	EC10 + M9	EC10 + H + E	EC10 + Mixture
				After BIO50			
				From initial soil value			
TPH/C10 - C40	mg/kg d.w.	15346.2	11933	39.51%	54.57%	39.40%	52.10%

Total HAP	mg/kg d.w.	103.161	89.156	58.21%	56.23%	58.03%	66.74%

The following figures show the differences between the initial values of TPH and PAHs and those obtained from the application of the EC10 soil bioremediation process.

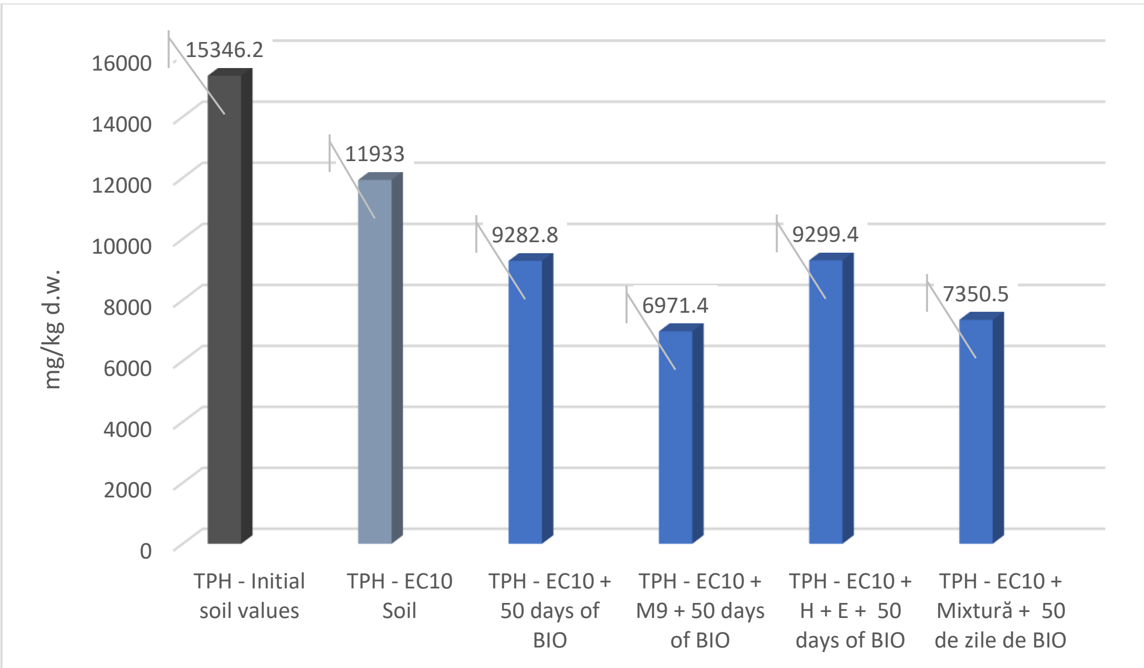


Fig. 4.10. Evaluation of the trend regarding the level of TPH concentrations in the EC10 soil for all experimental variants tested

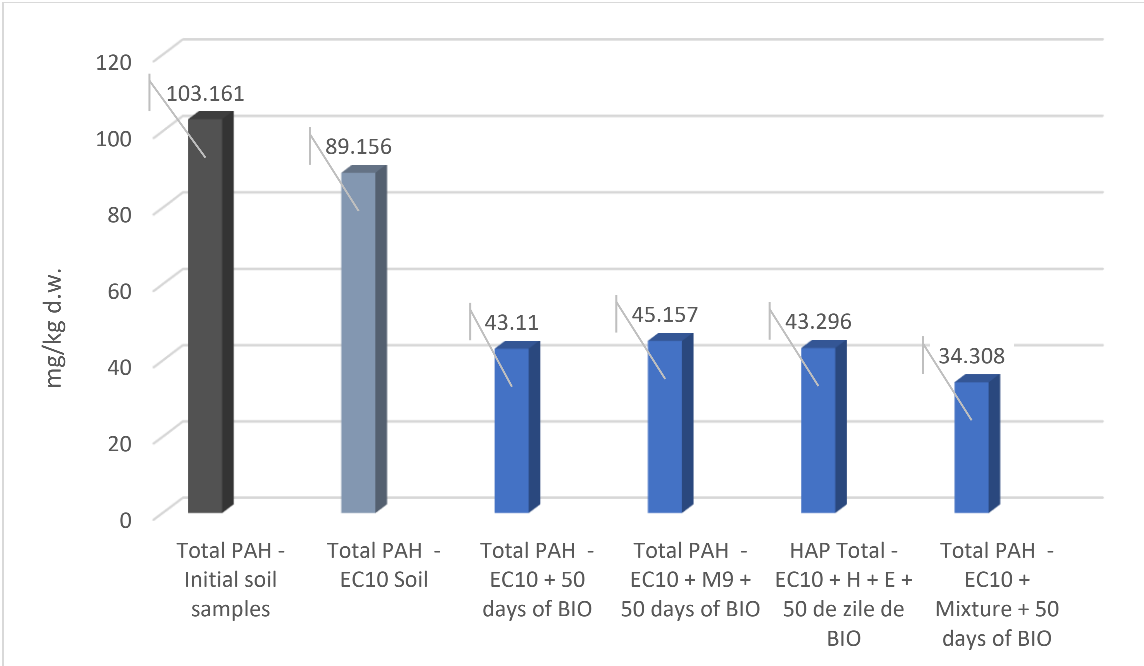


Fig. 4.11. Evaluation of the trend regarding the level of Total PAH concentrations in the EC10 soil for all experimental variants tested

4.3.2. Electro-remediation and bioremediation processes applied to the EC20 soil sample

In the case of EC20 soil (obtained after applying the EC process to the soil initiated over a period of 20 days) several types of microcosms were designed to see which type of process achieves the best results (Table 4.8).

Table 4.8 Microcosms prepared for the bioremediation process using the EC20 soil sample

	Sample	Process	Period
Soil EC20			
1.	EC20	Bioattenuation	50 days
2.	EC20 + H + E	Biostimulation	50 days
3.	EC20 + Alkane Mixture (C_{10} – C_{40}) + Phenantren	Bioaugmentation	50 days
4.	EC20 + M9	Biostimulation	50 days
5.	EC20 + H + E + M9	Biostimulation	50 days
6.	EC20 + Alkane Mixture (C_{10} – C_{40}) + Phenantren + M9	Bioaugmentation	50 days

The microcosms were included in the bioremediation process for a period of 50 days and the results can be viewed in the following tables.

Table 4.9 Assessment of the level of TPH and PAH pollutants concentrations in the EC20 soil subjected to the bioremediation process compared to the initial value

Nr.	Parameter	U.M.	Det. values-Initial soil	Det. Values-EC20 soil	EC20	EC20 + H + E	EC20 + Mixture	EC20 + M9	EC20 +H + E + M9	EC20 + Mixture + M9
					+ 50 days of BIO					
1	TPH/C10 - C40	mg/kg d.w.	15346.2	8104.25	5504.9	4265.5	5794.3	6000.8	4735.2	3683.4
2	Total PAH	mg/kg d.w.	103.161	89.524	45.221	49.00	52.688	53.571	52.39	42.612

Table 4.10 Percentage reduction of TPH and Total PAH concentrations level in EC20 soil subjected to the remediation process compared to the value of EC20 soil before bioremediation

Param.	U.M.	Det. values-Initial soil	Det. Values-EC20 soil	% Reduction of the sample of					
				EC20	EC20 + H + E	EC20 + Mixture	EC20 + M9	EC20 + M9 + H + E	EC20 + M9 + Mixture
				After BIO50					
				From EC20 initial value					
TPH/C10 - C40	mg/kg d.w.	15346.2	8104.25	32.07%	47.37%	28.50%	25.95%	41.57%	54.55%
Total PAH	mg/kg d.w.	103.161	89.524	49.49%	45.27%	41.15%	40.16%	41.48%	52.40%

Table 4.11 Percentage reduction of the TPH and Total PAH concentrations level in the EC20 soil values subjected to the remediation process compared to the initial soil value

Param.	U.M.	Det. values-Initial soil	Det. Values-EC20 soil	% Reduction of the sample of					
				EC20	EC20 + H + E	EC20 + Mixture	EC20 + M9	EC20 + M9 + H + E	EC20 + M9 + Mixture
				After BIO50					
				From inial soil value					
TPH/C10 - C40	mg/kg d.w.	15346.2	8104.25	64.13%	72.20%	62.24%	60.90%	69.14%	76.00%
Total PAH	mg/kg d.w.	103.161	89.524	56.16%	52.50%	48.93%	48.07%	49.22%	58.69%

The following figures show the differences between the values of the initial concentrations of TPH and PAHs and those obtained following the application of the EC bioremediation process to the soil.

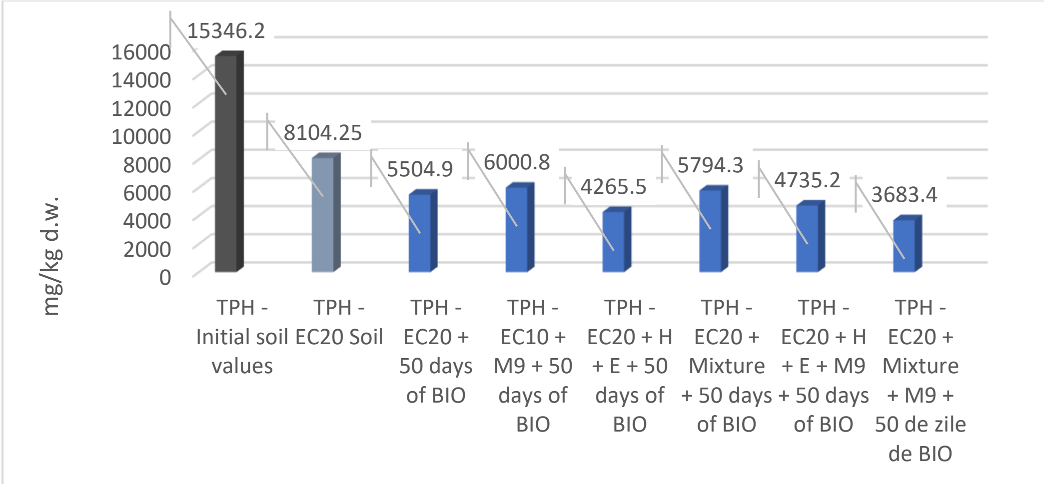


Fig. 4.12 Evaluation of the trend regarding the level of TPH concentrations in the EC20 soil for all experimental variants tested

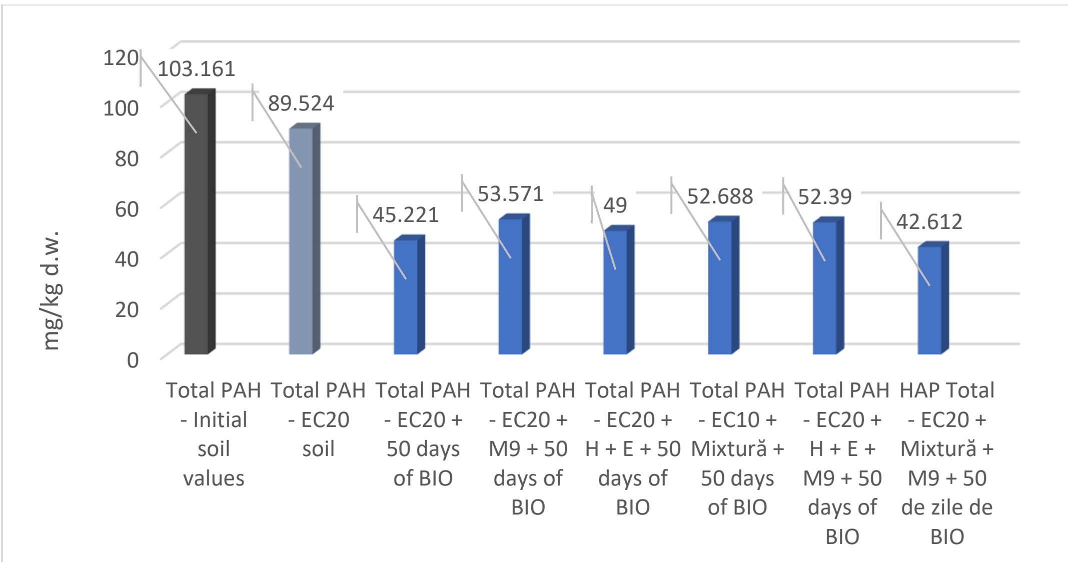


Fig. 4.13 Evaluation of the trend regarding the level of HAP concentrations in the EC20 soil for all experimental variants tested

As can be seen in Table 4.9, the best result in terms of reducing the concentration of TPH was obtained in the case of the **EC20 microcosm + Mixture + M9**. Following the tests, the TPH value decreased from 15346.2 mg / kg d.w. (the value of TPH in the initial soil), up to 3683.4 mg / kg d.w., the percentage decrease being 76%.

The final value of the TPH concentration, 3683.4 mg / kg d.w. did not fall below any value of the alert and intervention thresholds (for both types of use, sensitive, respectively less sensitive) in the Romanian legislation. However, it must be taken into account that the value of TPH in EC20 soil is equal to 8104.25 mg / kg d.w. and in 50 days of bioremediation it decreased by 54%. This decrease leads to the conclusion that the application of the bioremediation process over a longer period would certainly lead to the desired result.

At the same time, in terms of the costs involved in a longer bioremediation process, they would be insignificant because no additional cost is needed.

CHAPTER 5

EVALUATION OF THE INTEGRATED REMEDIATION SOLUTION IN TERMS OF ENERGY CONSUMPTION AND ECONOMIC COSTS

The costs associated with energy consumption differ from one remedial method to another. At the same time, they also depend on certain factors such as: the amount of contaminated soil that is subject to remediation, the degree of contamination, the time in which it is desired to complete the decontamination. Depending on the above, and depending on the treatment method chosen in each case of decontamination, these remediation costs can vary significantly.

In the present experimental research, the two chosen treatment methods, the electrochemical method and the biological method, were applied to a historically polluted soil with a high level of contamination, as presented in previous chapters. This aspect must be taken into account because a low percentage of contamination would have involved both a lower time required for decontamination and low costs.

Specifically, the costs assessed in the current chapter are costs generated as a result of using the method of electrochemical treatment of contaminated soil. This choice is due to the fact that this method requires the use of electricity in order to use it for the decontamination of polluted soil, which generates additional costs. Unlike the electrochemical method, the second decontamination method studied in the research (bioremediation), does not require energy consumption in order to apply it. The only energy consumption can be associated with the preparation of the bioremediation process, when using different equipment such as orbital shaker, turbidimeter, centrifuge, laminar flow hood where working under sterile medium, in order not to contaminate samples

5.1. Energy consumption

During the 20 days of electrochemical treatment of the contaminated soil sample, the current intensity was monitored daily.

At the same time, energy consumption was monitored during the experiments. An electricity meter, model KGS02-01 / 1109, was used to measure energy consumption.

The electricity consumption measured at the end of the EC10 process was 9.93 kWh, and that at the end of the EC20 process was 14.7 kWh.

5.2. Calculation of energy consumption in relation to the amount of pollutant removed

Into the experimental installation, 6 kg of initially contaminated soil were introduced. To treat this amount of soil, as mentioned above, 9.93 kWh were consumed over a period of 10 days of treatment and 14.7 kWh over a period of 20 days of treatment.

In order to calculate the energy consumption required for each process (EC10 and EC20) in relation to the total amount of pollutant removed, Table 5.1 shows the results obtained for the amount of TPH in the soil that was removed using the two processes (EC10 and EC20), compared to the initial value.

Table 5.1. Total amount of pollutant removed at the end of the EC10 and EC20 processes

TPH quantity from the initial contaminated soil	TPH quantity from the EC10 soil	TPH quantity removed from the initial contaminated soil using EC10 process	TPH quantity from the EC20 soil	TPH quantity removed from the initial contaminated soil using EC10 process
mg/kg d.w..				
15346,2	**11933**	***3413,2***	**8104,25**	***7241,95***

Further is shown how to calculate the energy consumption related to the application of the electrochemical process, considering the remediation time of 10 and 20 days, respectively (EC10 and EC20, respectively).

EC10

9,93 kWh : 6 kg (initial soil in electrochemical cell) = 1,655 kWh/kg sol1,655 kWh/kg soil : 0.0034132 kg polutant = 484,88 kWh / kg

EC20

14,7 kWh : 6 kg (initial soil in electrochemical cell) = 2,45 kWh/kg soil
2,45 kWh/kg soil : 0.00724195 kg polutant = 338,30 kWh / kg

As previously highlighted, following the use of the electroremediation process for a period of 10 days (EC10), 1,655 kWh / kg of soil were consumed. For the amount of TPH pollutant eliminated during the 10 days, 0.0034132 kilograms of pollutant, the energy consumption was 484.88 kWh / kg.

Continuing the process for another 10 days, 2.45 kWh / kg of soil were consumed. During the 20 days of treatment, 0.00724195 kilograms of TPH pollutant were removed, and, in this case an energy consumption of 338.30 kWh / kg was required.

5.3. Costs involved in energy consumption during the application of electrochemical treatment

According to Eurostat, in the first half of 2020, the price in Euro for electricity consumption expressed in kWh in Romania, for non-household consumers is 0.0890 Euro / kWh, without taxes included, respectively 0.1265 Euro / kWh, with taxes included [33].

The costs associated with the energy consumption required to apply the EC10 process, during **10 days** of treatment, are 0.209 EUR / kg soil, respectively 209.3 EUR / tonne soil (249.10 USD / tonne soil).

In the case of electrochemical treatment for **20 days**, EC20, the energy consumption costs are 0.309 EUR / kg soil, respectively 309.9 EUR / tonne soil (368.83 USD / tonne).

According to the literature, the costs generated by other treatment methods expressed in USD / ton are [34]:

- Thermal desorption - between 46 and 99 USD / ton;
- Incineration - between 150 - 2900 USD / ton;
- Vitrification - between 486 - 2900 USD / ton;
- Radio frequency heating - between 400 - 7500 USD / ton;
- Steam injection - between 37 - 380 USD / ton;

Figure 5.3 shows the comparison between the average costs of each process presented above, and the costs involved in energy consumption during the application of electrochemical treatment in this thesis.

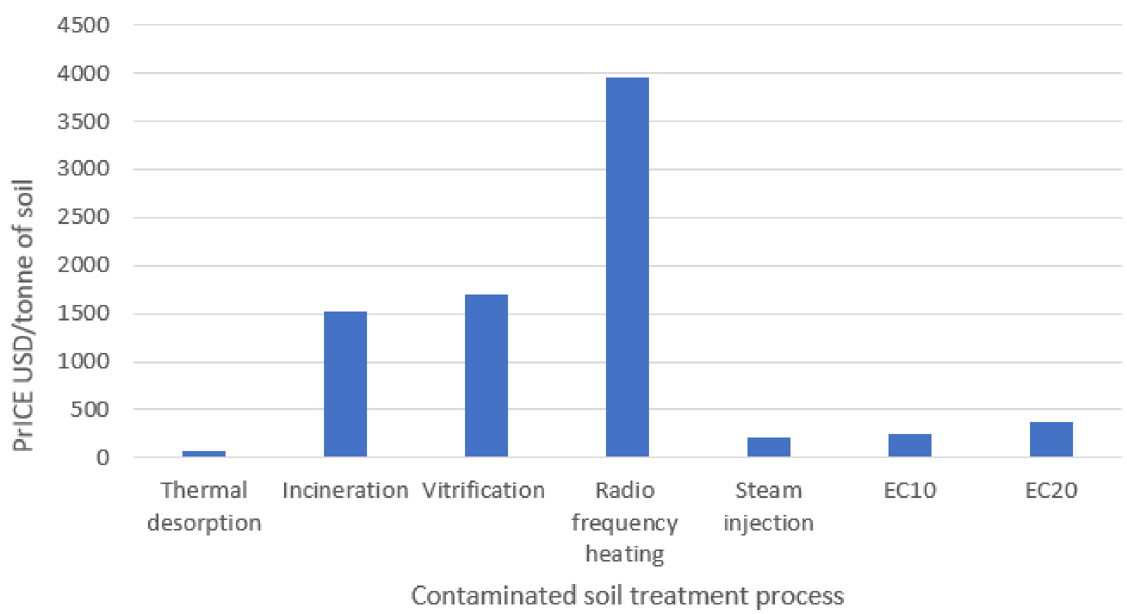


Fig. 5.3. Comparison of the average costs of different contaminated soil treatment processes with the costs involved in energy consumption during the application of electrochemical treatment

The costs of energy consumption generated by the use of the electroremediation process, as demonstrated in this thesis, are lower than those generated by other processes such as incineration, vitrification, radio frequency heating. However, as both the studies in the literature and the present paper have shown, following the application of only the electrochemical treatment method in order to decontaminate a historically polluted soil, higher costs can be generated because higher energy consumption is involved. However, combining this method with a biological and environmentally friendly method (bioremediation), the costs can be greatly reduced and energy consumption is reduced.

CONCLUSIONS

C.1. General conclusions

The main purpose of the study was to identify a method of depollution of a soil historically contaminated with petroleum products that takes into account both the degree of remediation of the proposed solution and the costs associated with it. Given the high concentration of TPH present in the soil, several decontamination strategies were considered. The doctoral research was initiated using the electroremediation process in order to decontaminate the polluted soil. This type of process has been used in many studies over the years and has proven to be successful, especially since, once contaminants are removed, the soil can still be used for certain uses (agricultural or non-agricultural). Following the use of the electrochemical process tested in the paper, it was observed that the level of concentrations of TPH contaminants in the soil began to decrease significantly after 10 days from the start of the experiment. The concentration of TPH in the initial soil was 15967.4 mg / kg d.w. and, after only 10 days in which the electrochemical process was applied, it decreased to 11933 mg / kg d.w., thus registering a percentage decrease of 25% of soil contaminants. Next, another purpose of the research was to evaluate the degree of remediation obtained after applying the electrochemical process over a longer period of time. Thus, the soil sample was subjected to the electroremediation process for another 10 days (a total of 20 days). At the end of the 20 days of application of the electrochemical process (EC20), the percentage decrease in TPH in the soil was 49%, compared to the initial value of the pollutant concentration in the contaminated soil. The TPH concentration was practically halved after the electroremediation process was applied for a total period of 20 days, reaching a value of 8104.25 mg / kg d.w.. It results the fact that the value of the TPH concentration from EC20 soil still exceeds the values of the intervention threshold established by Order 756/1997 in Romania. However, there was a significant decrease in the concentration of contaminants in the soil. Moreover, the initial concentration of PAHs present in the soil was evaluated as well as the value obtained following the application of the electrochemical method: from 103.161 mg / kgs.u. (initial value) reached a concentration of 89.159 mg / kg d.w. after application of the process for 10 days, and respectively 89.524 mg / kg d.w. after application of the process for 20 days.

A second method considered in the research was bioremediation. In order to determine if the bioremediation process can be applied to the researched soil, the soil characteristics were identified, such as: pH, potassium, phosphorus, etc. The soil was further analyzed to determine the colonies of bacteria, the number of fungi and the number of yeasts. Although many studies focus on bacteria that degrade contaminants in the soil, the presence of fungi and filamentous yeasts in environments polluted with hydrocarbons is quite well known and they play an important role in the degradation process of the aforementioned contaminants. In order to successfully apply the bioremediation method, a very important step is to choose the appropriate microbial strains in terms of soil quality, pollution characterization and microbial interactions. Tests performed on the soil sample showed that the microbial load of the soil is high. This indicated that the bioremediation process can be successfully applied.

In order to identify the best methods for decontamination of polluted soil, several factors were taken into account, such as: cost, treatment time and whether or not the method is environmentally friendly. The main treatment methods that have been considered are the two methods mentioned above, the electrochemical process and bioremediation. The two methods were also studied in terms of advantages and disadvantages. Each method applied separately has both advantages and disadvantages for the main purpose that was intended to reach the end of the present research. On the one hand, the electrochemical process has a shorter duration in which the concentration of pollutants decreases promisingly, but a high energy consumption is required to achieve this goal. On the other hand, by applying only bioremediation as a treatment method, the concentrations of contaminants can be reduced at a low cost and with an energy consumption of almost zero, but with a fairly long duration. Also in the case of the application of the bioremediation process, it can be mentioned that, when the initial concentration of pollutants in the soil is higher, as in the case of current research, the decontamination time is significantly extended.

Knowing all these advantages and disadvantages and taking into account the fact that the soil is a historically contaminated soil with a complex contamination, it was considered that a (hybrid) strategy of coupling two remedial processes could provide the desired results in a shorter time and with low costs. At the same time, specialized studies have shown that the application of the electrochemical process does not harm the microbial activity in the soil, this being a great advantage of this hybrid mode (coupling the electrochemical method with the biological one).

In this regard, microcosms were created with soil samples that were obtained after the electroremediation process, namely with the soil samples EC10 (obtained after applying the electroremediation process for 10 days to the initial soil sample) and EC20 (obtained following the application of the same process for a period of 20 days).

The bioremediation process was applied to the two soil samples, EC10 and EC20 over a period of 50 days (bioattenuation). At the same time, in order to obtain optimal results, each soil sample mentioned above was subjected to other types of tests using the bioremediation process such as:

- Biostimulation - where nutrients have been added to encourage the growth of microorganisms present that degrade chemicals
- Bioaugmentation - where specific competent species or consortia of microorganisms have been practically introduced into the contaminated soil.

Following the use of the strategy of coupling two remediation processes, the best result in terms of reducing the concentration of TPH was obtained in the case of the EC20 microcosm + Mixture + M9. Following tests with this combination, the TPH value decreased from 15346.2 mg / kg s.u. (initial value of TPH concentration in the soil), up to 3683.4 mg / kg s.u., the percentage reduction being 76%. This represents a significant percentage that was obtained after treating the contaminated soil with the help of electroremediation for a period of 20 days. The test was continued with the application of the bioremediation process adding the mixture of alkanes and final M9) for a period of 50 days. This value did not fall below the values of the alert and intervention thresholds in the Romanian legislation. However, the value of TPH in the EC20 soil is equal to 8104.25 mg / kg s.u., and following the application of the bioremediation process over a period of 50 days, it decreased by 54%. From this it can be concluded that the application of the bioremediation process over a longer period would certainly lead to the desired result, without additional costs.

In terms of costs, using only the electrochemical treatment process can generate higher costs than if it were combined with another low-cost process. In the present paper, the EC10 process generated energy consumption costs of USD 249.10 / tonne of soil, and the EC20 process of USD 368.83 / tonne of soil. The costs are lower than other treatment processes, such as heat. Moreover, another advantage of this remedial solution is that the soil can be used after electrochemical treatment, for example, unlike the soil which is subjected to thermal processes. However, the fact that the use of only the electrochemical treatment method in order to decontaminate a historically polluted soil can generate higher costs due to the involvement of higher energy consumption, in the current research was proposed the strategy of coupling the electrochemical method with the biological one. Moreover, regarding the method of biological treatment (bioremediation), it is highlighted that this solution has many options for creating different microcosms. Depending on the degradation capacity, several types of microorganisms can be used to treat contaminated soil. Microorganisms such as bacteria, yeast or fungi can be used both together and separately. Using an initially electrochemically treated soil for a shorter period of time, different types of microcosms can be created with the help of microorganisms, nutrients, etc.

Thus, in order to identify an environmentally friendly method for treating soils contaminated with petroleum products, and also to select a remediation strategy that does not generate high energy consumption and high costs, respectively, the coupling of the electrochemical method with the biological one. , remains a viable and future solution.

C.2. Personal contributions

The personal contributions from this research include the experimental activities carried out both in the country, within the Laboratory of Soil Analysis, Control and Depollution within the Center for Advanced Research for Innovative Materials, Products and Processes (CAMPUS) of POLITEHNICA University of Bucharest within POLITEHNICA University of Bucharest, as well as those realized within the Department of innovation of biological systems, food and forestry DIBAF within the University of Tuscia, Viterbo, Italy.

In the doctoral research, I managed to apply the remediation strategy for the decontamination of a historically contaminated soil, successfully combining the two treatment methods, namely the electrochemical and biological treatment method (bioremediation). The methods were applied both individually and together, to observe, in each scenario, the value of the degree of decontamination (this being an essential point of the research) but also to ensure that the purpose for which we chose the research and the application of this combined electrochemical-bioremediation method, is observed. The main purpose is to protect the environment taking into account several aspects: the time of application of each method, the preservation of microbial activity and also ensuring that the treatment methods are environmentally friendly.

In the tests and experiments performed in the laboratory I applied the knowledge acquired both from my mentors and from the literature. At the same time, another important point that must be mentioned is that I learned to work in a field completely new to me, that of microbiology and that I successfully implemented everything I learned.

C.3. Future perspectives

- Application of the bioremediation method to a soil with a low initial concentration of pollutants in the soil to observe the time required for the degradation of contaminants to values lower than the reference values in the current legislation; comparing the time required to degrade contaminants in lightly polluted soil with the time required to degrade contaminants in a soil with more complex pollution;
- Use of the mixture and the final M9 solution for a microcosm containing EC10 soil sample;
- Application of the bioremediation process to the microcosm EC20 + Mixture + M9 for a period longer than 50 days to observe how long the values of contaminants will fall below those of the intervention threshold of Order 756/1997 in Romania;
- Use of yeast and fungal microorganisms in different scenarios of microcosms.

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