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Vegetal layer restoration of contaminated sites from petroleum industry using sewage sludge

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Abstract
Soil is a non-renewable natural resource, extremely valuable for maintaining life on Earth. Large areas of soil are often polluted with petroleum products as a result of various human activities. In this case, it is necessary to remediate the contaminated soils from the oil industry and return them to the natural and economic circuit. In addition, large quantities of sewage sludge are generated daily by sewage treatment plants. The purpose of this study was to use the soil subjected to a bioremediation experiment with sewage sludge as support for the development of a new vegetal layer to restore the natural balance. The grass was sown in the land, which had previously been treated with sewage to monitor its development. Through microbiological analysis, we observed that soil and sewage sludge mixtures have a high content of heterotrophic bacteria and filamentous fungi that could play an important role in seed germination. IR (infrared spectroscopy) analysis revealed a decrease in hydrocarbon concentrations in the petroleum- contaminated soil treated with sewage sludge. By using the combined methods SEM-EDX (scanning electron microscopy – energy dispersive X-ray), we also analyzed the soil microstructure and the distribution of chemical elements to highlight the plants' evolution. The present study considers soil regeneration in areas contaminated with petroleum products and opens new perspectives for the use of sewage sludge in the ecological reconstruction of petroleum-contaminated sites.

Keywords: soil, sewage sludge, bioremediation, vegetal layer

1. INTRODUCTION

Soil is a non-renewable natural resource, in decline due to anthropogenic processes (industrial development, infrastructure development, pollution, etc.) and natural phenomena (erosion, flooding, landslides, etc.). Physico-chemical and microbiological processes, which ensure terrestrial ecosystems, biodiversity, and human life, take place in the soil . Soil fertility represents its property to provide conditions for plant development, by providing micro and macronutrients [1]. Oil hydrocarbon pollution affects soil fertility and represents a threat to all forms of terrestrial life. Petroleum hydrocarbons change the physico-chemical properties and produce imbalances in the microbial activity of the soil. Soils contaminated with petroleum hydrocarbons present risks for the environment and human health, and require interventions for decontamination. Various methods of soil decontamination (physical, chemical, biological) have been developed. Depending on the concentrations and nature of the pollutants, biotechnologies based on the use of microorganisms are used more and more, being environmentally friendly and economically profitable [2]. However, it is not enough only to eliminate pollutants from the environment, but also to restore the topsoil for the preservation of the ecosystem, and for the resumption of economic and landscape functions [3]. For this, natural resources are used by excavating the soil, intervening in other areas and affecting the vegetation layer.

A source of micronutrients, macronutrients and microorganisms is the sludge, resulting from the purification of municipal wastewater [4]. The quantities of generated sludge increase with the increase in the number of treatment stations. The sludge resulting from the purification processes requires an adequate disposal due to the content of organic, inorganic and microbiological pollutants [5]. From previous studies, it was found that a solution for valorizing sewage sludge is its use in the bioremediation of soils contaminated with petroleum hydrocarbons [6].

The present study is a continuation of the research that considers the possibility of using the contaminated soil subjected to the bioremediation experiment with sewage sludge as support for the development of a new plant layer. It is extremely important to restore the vegetation layer, without resorting to natural resources external to the contaminated site. This solution could answer a small part of the soil security problem [1]. The revitalization of contaminated sites with the help of sewage sludge would return large areas of land to the economic and natural circuit, significantly replacing the consumption of natural resources of topsoil with significant quantities of sewage sludge.

2. EXPERIMENTAL

Sampling.

In the bioremediation experiment, three mixtures of soil and sludge (S:N) were made in the following volume proportions: S1:N1 (1:1 v/v), S1:N2 (1:2 v/v), and S2 :N1 (2:1 v/v), respectively. At the end of the bioremediation process, samples were taken from each mixture before sowing with lawn (Fig. 1). The soil mixtures were introduced separately into the pots and sown with several types of plants to observe their development (lawn, clover, alfalfa, perennial grass mixture), but the study was carried out on the pots seeded with lawn. The lawn was sown in pots on the last day of January 2024, at a time of the year when seed germination cannot occur under natural conditions. The pots were kept in the free atmosphere of the laboratory, at a temperature of approximately 22 - 24^0C.

Fig. 1. Schematic representation of the stages carried out for the restauration of petroleum contaminated soil
a. Stages of preparing the vegetable layer, b. Plants development, c. Analysis method

Determination of total petroleum hydrocarbons (TPH) in soil and sewage sludge mixtures.

The TPH is a parameter that indicates the contamination with petroleum hydrocarbons of a contaminated site. This parameter was determined for the three initial samples, and after a period of 3 months after seeding with lawn. The petroleum hydrocarbons in the samples were extracted with S-316 solvent and then analyzed through infrared detection (IR) on with an Infra Cal 2 device. The samples were dried in the free atmosphere of the laboratory for 48 hours, then they were plastered. The organic compounds were extracted using S316 solvent, and the residual moisture of the samples was removed with silica gel.

After extraction (5 min vigorously stirred by hand) the organic phase was recovered from the samples by filtration. The device was calibrated using S-316 solvent. After calibration, the samples were introduced one by one into the device, recording the TPH values.

Microbiological analysis of soil and sewage sludge mixtures.

The microbiological analysis of the samples was carried out using the classical plate count agar method. Serial dilutions of each sample (10^{-1}-10^{-6}) were inoculated on LB agar [7] for heterotrophic bacteria enumeration and on Czapek agar and EMB agar [8] for filamentous fungi enumeration. Petri dishes were incubated for 1-5 days at 30°C, and then the number of bacteria and filamentous fungi present per g of sample was determined (cfu g^{-1}).

Scanning electron microscopy – energy dispersive X-ray spectroscopy.

Surface morphology and elemental content of the soil-sludge/plants systems were investigated by means of the Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX) combined methods. The imagistic results were performed using TESCAN VEGA apparatus (Brno, Czech Republic), within CC-ITI Research Centre platform from “Dunarea de Jos” University of Galati (DJUG), in low-vacuum environment. In order to enhance the image resolution, and due to the low sample’s conductivity, a metallization procedure is required. Prior to examination, the powdered and grass samples were fixed onto stubs using carbon adhesive tape, than coated with a thin alloy layer using a sputter coater system (SPI Supplies, West Chester, USA). EDX is a quantitative method for determining the distribution of major and trace elements in the scanned area.

3. RESULTS AND DISCUSSION

After the bioremediation process, the mixtures were microbiologically and chemically tested. Through combined SEM-EDX analysis, the microstructure of the mixtures and the distribution of nutrients were determined, in order to assess whether the conditions for use as a vegetable layer were met. The initial values of TPH concentrations in the three mixture samples are presented in Table 1 and it can be seen that they vary from one sample to another. The highest value (1060 mg Kg^{-1} d.s.) was recorded in the S2:N1 mixture, followed in descending order by the THP concentration in S1:N2 (930 mg Kg^{-1} d.s.), and the lowest value was recorded in the S1 mixture: N1 (840 mg Kg^{-1} d.s.). These values fall within the limit values for ecological reconstruction of oil industry contaminated sites.

After 3 months from the initiation of the experiment, large decreases in TPH concentration were observed in the three soil samples in which the plants grew. The decrease in TPH concentrations varies from one mixture to another, with different decreases being recorded. The most important decrease was recorded in the mixture S2:N1 (880 mg Kg^{-1} d.s.), followed by S1:N2 (800 mg Kg^{-1} d.s.), and S1:N1 (730 mg Kg^{-1} d.s.). It is thus observed that the most important decrease is recorded in the mixtures with the highest initial concentrations.

Although the THP parameter concentration values in the soil were high, seed germination and plant development took place (Fig. 1b). The decrease of the THP parameter can be due to both the presence of microorganisms and the development of plants.

Table 1. Total petroleum hidrocarbons content in the mixtures of soil and sewage sludge

Parameter (mg Kg^{-1} d.s.)	Period (months)	Samples		
		Soil and sludge mixture (v/v)		
		S1:N1	S1:N2	S2:N1
Petroleum hydrocarbons	P1	840	930	1060
	P2	110	130	180

Through microbiological analysis (Table 2, Fig. 2), we observed that the soil and sewage sludge mixtures have a higher number of heterotrophic bacteria (10^8 cfu g^{-1}), as compared to the number of filamentous fungi (10^6 cfu g^{-1}). The number of these microorganisms slightly differs from one sample to another (i.e., S1:N1, S1:N2, S2:N1).

As it can be observed in Table 2, the number of heterotrophic bacteria was between 1.2×10^8 and 1.4×10^8 cfu g^{-1}, and the number of filamentous fungi was between 4.3×10^6 and 4.6×10^6 cfu g^{-1}. Both these microorganisms could play a significant role in seed germination in soil treated with sewage sludge. Bacteria and fungi have the potential to restore the fertility of degraded soil by various processes, such as nitrogen fixation and mobilization of key nutrients (phosphorus, potassium, iron) [9].

Table 2. Microbiological analysis of soil and sewage sludge mixtures

Number of bacteria or fungi (cfu g^{-1})	Soil and sludge mixtures (v/v)		
	S1:N1	S1:N2	S2:N1
Heterotrophic bacteria	1.2×10^8	1.3×10^8	1.4×10^8
Filamentous fungi	4.4×10^6	4.3×10^6	4.6×10^6

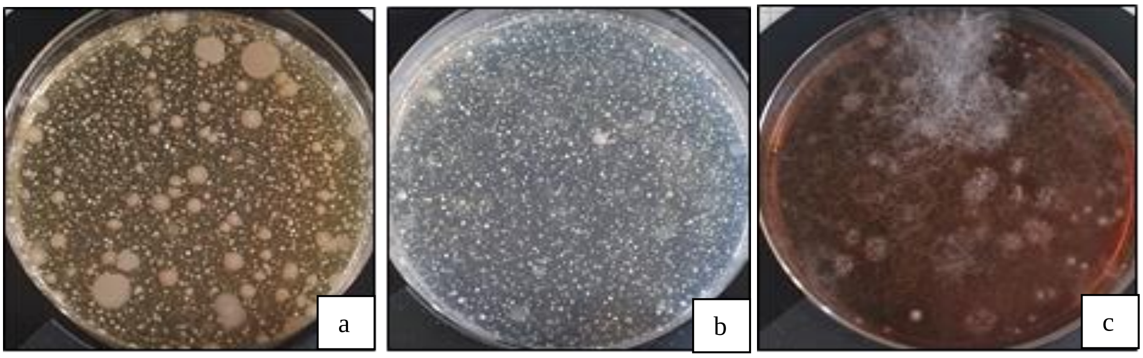

Fig. 2. Microbiological analysis of soil and sewage sludge mixtures
a. LB agar, b. Czapek agar, c. EMB agar

Secondary electron micrographs at two scales (500 μm – an overview and 10 μm – a detail), EDX spectra and spatial distribution of elements showed the effect of soil bioremediation treatment on grassy vegetal layer (Fig. 3). Primarily, we must specify that bioremediation will be discussed in terms of macro/micro-elements and nutrients.

The morphological characteristics of the control soil sample show a compact surface with a mixture of larger and fine grain powdered particles (varied shapes and sizes), along with aggregates and low-porosity structure (Fig. 3a). A few vegetal fragments, along with mineral compounds in the untreated soil, are recognised by varied shades of gray (from darkest to brightest). Therefore, clay minerals, calcite, silica, and oxides are highlighted by their specific geometry: spherical-shaped and rod-like particles, well-defined edges, or rounded flat microstructures. EDX chemical analysis of a selected micro-area (1.25 mm^2) revealed the presence of minerals and organic matter (Fig. 3b). The quantitative evaluation indicates that this type of soil is silicon dominated (smooth grains), among other macro-elements (carbon, oxygen, aluminium, magnesium, potassium and calcium). The presence of Al and Si (almost 25 wt.% from soil and 20 wt.% from sewage sludge, respectively) is due to the soil breaking process. Silicon is the second most frequent micro-element necessary for the plants development, influencing the availability and bioaccumulation of macro- and micro-nutrients [10]. It is worth mentioning that this value can be compared to 65 wt.% of SiO_2 in the upper horizon of European soils and 27 wt.% in the Earth's crust [11].

Fig. 3c shows the overall image of the angular and irregular sewage sludge particles, with specific dimensions and geometries such as sphere, polyhedrons and plates [12]. Fig. 3c most likely includes fragments of twigs or roots covered with grains. A microporous structure with different grains (diameter below 50 μm) and aggregated particles can be observed. As seen in Fig. 3d, the similar macro-, micro- and trace of elements found in the soil sample are well mapped in the sewage sludge powder. However, an abrupt decrease in oxygen (at half) in favour of carbon (increased by ten times) can be observed. Higher content of carbon could be due either to the vegetal piece trace or carbon footprint effect. Generally, the sewage sludge has a relatively high content of organic compounds and lower in nutrients. Carbon levels in soil of 2.39 wt% is comparable with the

European average for the soil upper horizon (2.48 wt.%) [13], but double than the carbon average in the Earth's crust (0.18 wt.%) [11]. Instead, the highest carbon value of 22.32 wt.% in sludge is due to the presence of carbonate minerals (calcite). Heavy metals were identified in low concentrations in the sludge, not being relevant to this study.

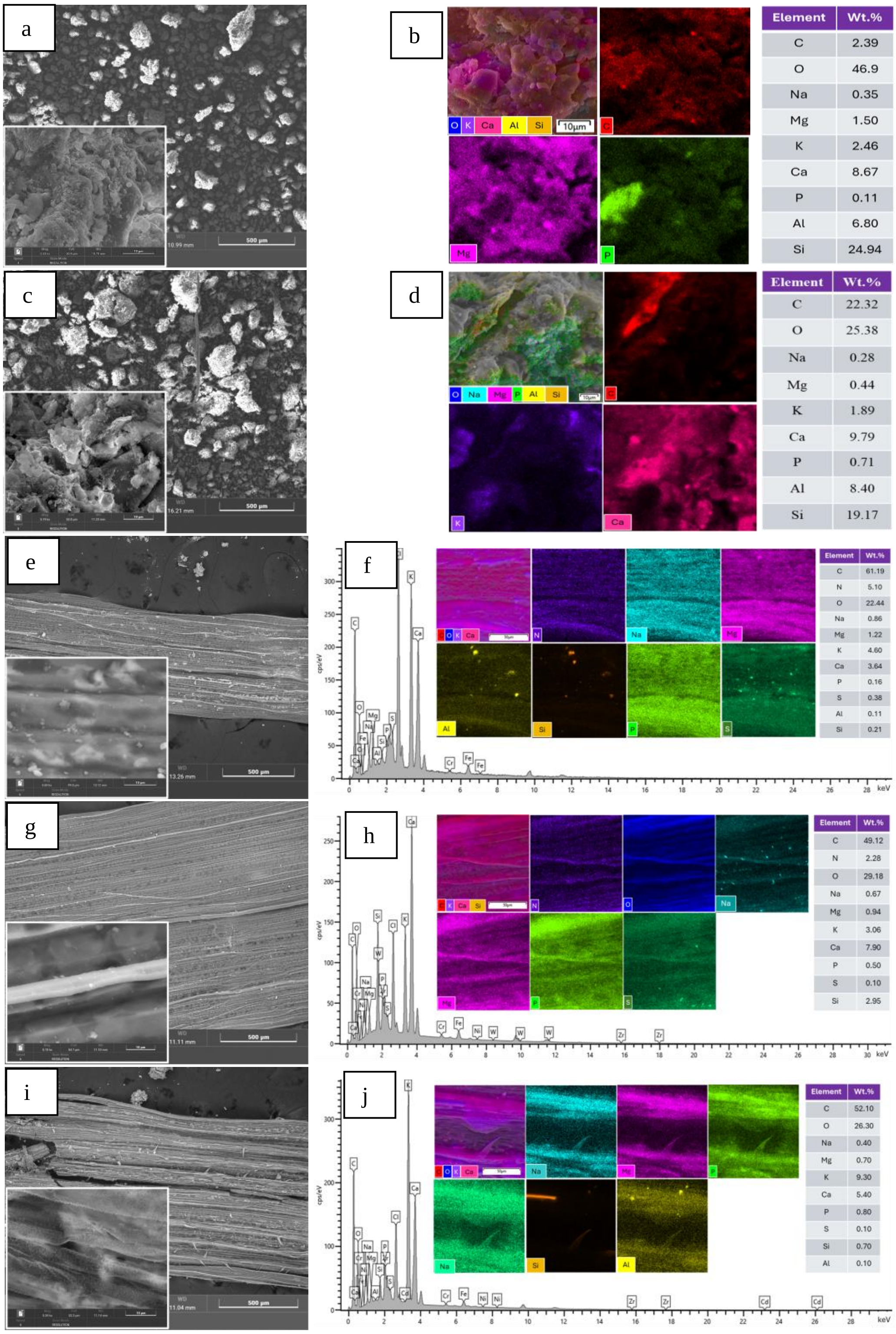

Fig. 3. Secondary electron images, EDX spectra and overlaid elemental maps of the scanned area: a-b. control soil, c-d. sewage sludge, and grass developed from treated soil with sewage sludge (e-f. S1:N1, g-h. S1:N2, i-j. S2:N1)

The imagistic and microstructural studies of dry leaves grass, which are grown in bioremediated soil systems, added in different proportions, are presented in Fig. 3 e-j. Higher content of nutrients (P, N, K, S) and organic elements, in contrast to the individual medium from the new proposed system, can be obviously noted. The optimum medium for plant development in terms of nitrogen (5.10 wt.% for S1:N1; 2.28 wt.% for S1:N2; not determined for S2:N1) and sulphur (0.38 wt.% for S1:N1; 0.10 wt.% for S1:N2 and S2:N1) contents were found to be S1:N1 combination. Instead, the S1:N2 and S2:N1 environments have created favourable conditions for the grass to be rich in phosphorus (0.16 wt.% for S1:N1; 0.50 wt.% for S1:N2; 0.80 wt.% for S2:N1) and potassium (4.60 wt.% for S1:N1; 3.06 wt.% for S1:N2; 9.30 wt.% for S2:N1) nutrients. Despite the nitrogen decrease, the increase of C:N ratio from 61.19:5.10 for S1:N1 to 49.12:2.28 for S1:N2, will recommend the sludge as treatment for nitrogen soil mineralization. While all the 14 nutrients are indispensable for the plants' growth and development, actually we must take into account the synergist effect of nutrients, globally [12]. So, the sludge activated soil could represent promising a candidate as fertilizer in soil bioremediation. In conclusion, the positive effect of sewage enriched sludge depends on the plant type and the chemical characteristics of the environment, being a hopeful source of nutrients.

4. CONCLUSIONS

The results obtained in the present study are preliminary and may constitute a basis for future research for the use of bioremediated soils with the use of sewage sludge in the restoration of the vegetal layer, from the ecological reconstruction of sites contaminated with petroleum products.

Through microbiological analysis, we observed that soil and sewage sludge mixtures have a high content of heterotrophic bacteria and filamentous fungi that could play an important role in seed germination.

Through the IR analysis, a decrease in the concentrations of petroleum hydrocarbons was observed in the soil treated with dehydrated sludge and sown with lawn.

With the help of the combined SEM and EDX methods, we analyzed the microstructure of the soil and the distribution of chemical elements with a role in plant development.

The obtained mixtures could constitute support for the development of a new plant layer in order to restore the natural balance.

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