



Bioremediation of soil polluted with oil

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ABSTRACT

Microplastics have reached all corners of our planet, including soil and water. Plastic-degrading bacteria are seen as a promising, environmentally friendly tool for the bioremediation of soil polluted with microplastics. The petroleum origin of plastics makes them candidates for bioremediation analogous to the bioremediation of soil polluted with oil and its derivatives. A mud pit, located near the village of Turija, used for mud formation for the lubrication of drill pipes for drilling rigs, ended up polluted with oil and its derivatives. It was bioremediated using the in situ procedure. The content of n-hexane extractable substance, total petroleum hydrocarbon, dry substance, and loss on ignition were analyzed.

Keywords: soil pollution, bioremediation, oil spills, zymogenous consortium of microorganisms, microplastics.

ИЗВОД

Микропластика се може наћи у сваком кутку наше планете, укључујући земљиште и водене површине. Сматра се да су бактерије које разграђују пластику будућност еколошки прихватљиве биоремедијације земљишта загађеног микропластиком. Због нафтног порекла, постоји оправдана сумња да се поступак биоремедијације земљишта загађеног нафтом и дериватима нафте адекватним избором микроорганизама може применити и код биоремедијације земљишта загађеног микропластиком. Испљачна јама у близини села Турија, која се користила за прављење исплаке за цеви за бушење нафтних бушотина, након завршетка бушотине, била је загађена нафтом и дериватима. Извршена је биоремедијација ин ситу поступком. Одређен је садржај супстанци растворних у хексану, укупних нафтних угљоводоника, суве супстанце и губитка при жарењу, непосредно пре и 90 и 180 дана након поступка биоремедијације.

Кључне речи: загађење земљишта, биоремедијација, изливање нафте, конзорцијум зимогених микроорганизама, микропластика.

1. Introduction

Due to the amount of plastic waste that can be found all around the world, the legacy that we leave for the generations to come will inspire them to call this era *plasticene* (Reed, 2015). Plastic products are made from different kinds of polymer materials. During the synthesis of polymers, under special conditions (pressure and temperature), monomers, the smallest building blocks of polymers, consisting of hydrocarbons (originating mostly from the cracking of oil and gas), form long chains, which give them specific properties, including durability. The impact of plastic waste (macroplastics) on the environment has long been the subject of environmental research, due to aesthetic, economic, and environmental concerns (Cole et al., 2011). But, in recent years, there has been

increasing environmental concern about 'microplastics', too (Cole et al., 2011). As defined by Frias and Nash (2019), "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water". Primary microplastics are particles that are directly released into the environment as small particles, and secondary microplastics are obtained from the breakdown of macroplastics (Cole et al., 2011). Microplastics can be released to the environment via several pathways: down-the-drain disposal, municipal solid waste disposal, and direct release (ECHA, 2019). The pathways for the generation of microplastics and their entry into the environment are illustrated in Figure 1.

The presence of microplastics in water and soil poses a great threat to ecosystems (Guo et al., 2020). Due to small size, microplastics are easily ingested by soil organisms and transferred further, along the food chain (Huerta Lwanga et al., 2017). They can adsorb hazardous contaminants from the environment, thus increasing the content of pollutants in soil, and have harmful effects on living organisms, in which they eventually end up (Hodson et al., 2017).

Microplastics can affect plant growth (De Souza Machado et al., 2019) and accumulate and transport in plants (Li et al., 2019). The ingestion of high concentrations of microplastics inhibits the growth and increases the mortality of earthworms (Cao et al., 2017).

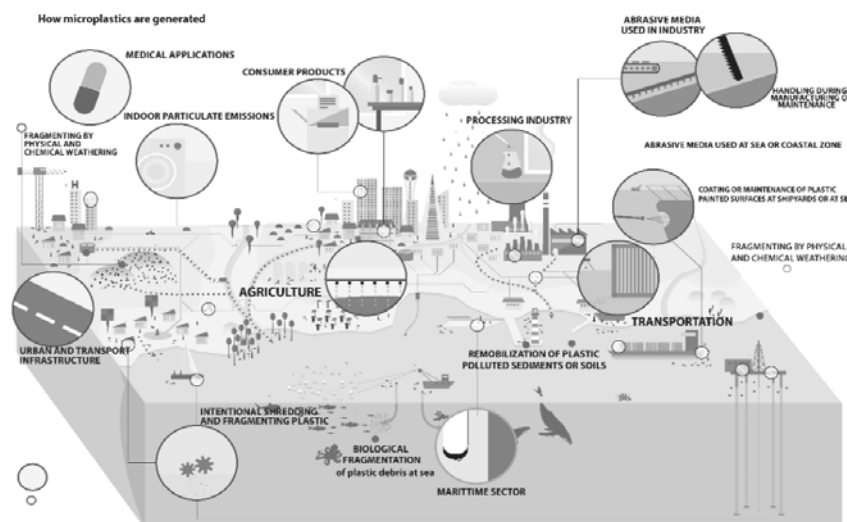


Figure 1. How microplastics are generated (author: Maphoto/Riccardo Pravettoni source: GRID-Arendal <https://www.grida.no/resources/6929>) (Maphoto an Pravettoni)

Based on a comparison of different methods for plastic waste disposal and degradation, Shahnawaz et al. (2019) concluded that “biodegradation is the only method which is significantly valid at both economic and ecological level”. They encouraged the further development of this method to discover more effective microbes for the degradation of different kinds of plastics. Since biodegradation of plastics is less likely to occur naturally in the environment, the further step is to design a bioremediation process for soil polluted with microplastics. Bioremediation involves environmental restoration using microorganisms to either restore or clean-up contaminated sites via oxidizing, immobilizing, or transforming the contaminants, to reduce or bring down pollutant levels to undetectable, nontoxic, or acceptable levels (Crawford, 1996; Vishwakarma et al., 2020). Because plastics are obtained from petroleum derivatives, our hypothesis is that the bioremediation of soil polluted with microplastics should be analogous to the bioremediation of soil polluted with oil and its derivatives. To achieve this, a consortium of zymogenous microorganisms should be isolated directly from the polluted soil.

The food consumption in the world is growing (Fukase and Martin, 2020), therefore the necessity for the arable land is rising. On the other hand, the agricultural region, close to the urban core is transformed into the peri-urban area (Rondhi et al., 2018), which causes declining of the arable land. In the Republic of Serbia, most grown crops are wheat and maize (Grčak et al., 2020). One way to increase amount of arable land is to bioremediate polluted soil and prevent further pollution of the environment.

The goal of this work was to describe how mud pit polluted with oil and its derivatives can be remediated,

and transformed into fertile land. The old technology for drilling oil included the formation of mud pits next to the oil rig. Mud from those pits was used for the lubrication of drill pipes to facilitate their penetration through the ground. After the rig was completed, mud pits remained and were used as disposal pits for all spills during oil handling. Even though mud pits should not contain oil and its derivatives, because mud itself does not consist of such substances, in practice, these pits are substantially polluted with oil and its derivatives. One such pit was located in the area near the village of Turija, on highly fertile land in the municipality of Srbobran (Serbia). This location has been subjected to oil extraction for more than 30 years. Near the oil fields, the Krivaja River flows into the Danube-Tisa-Danube canal. The main focus was to bioremediate this mud pit, remove the remaining oil and derivatives, and transform it into fertile land.

2. Materials and methods

The bioremediation procedure was done *in situ*. Microorganisms that have the ability to degrade the target contaminants were isolated from their natural habitat – contaminated soil in order to avoid the insertion of foreign pathogens and reduce the risk of disrupting the ecological balance between the remediation site and the environment.

Thereafter, in the laboratory, a consortium of zymogenous microorganisms was adapted (without genetic modification) and multiplied, and then applied to the contaminated site. A single nutrient supplement providing optimal conditions for the growth of the selected microbial population was developed. To make an optimal remediation plan, biochemical tests were performed at different points of the locality. Sites for

self-remediation were chosen accordingly. Then, the selected microbial consortium and nutrients were applied on selected sites. Steps in the preparation of the zymogenous microbial consortium, as well as the design of a biopile for the bioremediation of polluted soil are described in detail in our previous work (Beškoski et al., 2011).

To estimate the efficacy of bioremediation, n-hexane extractable substance (HES), total petroleum hydrocarbon (TPH), dry substance (DS), and loss on ignition (LOI) were analyzed before, and 90 and 180 days after the bioremediation. HES was analyzed according to EPA's SW-846 Test Method 9071B: n-Hexane Extractable Material (HEM) for Sludge, Sediment, and Solid Samples (US EPA, n.d.). TPH was analyzed gravimetrically according to the standard BS EN 14345:2004. DS was determined gravimetrically according to the standard ISO 11465:1993 (*ISO -ISO 11465:1993*) by calculating the difference in mass before and after drying at 105°C; the result is expressed in percentages. LOI was determined gravimetrically, by

calculating the difference between 100% of DS and residue after ignition at 550°C (Wilke, 2005).

To determine soil fertility, the field was sown with corn, whose growth was recorded photographically. The entire experiment, including mud pit monitoring, microbial growth, and use of microorganisms until tolerable limits for TPH were reached, lasted from April 2011 until May 2012. Microorganisms were applied in November.

3. Results and discussion

If weather conditions are favorable, it takes up to 90 days for TPH to reach tolerable limits, but in our case, due to cold weather during bioremediation, tolerable limits of TPH were reached after 180 days.

The results of the HES and TPH analysis before and 90 and 180 days after the application of microorganisms into the soil are shown in Figure 2.

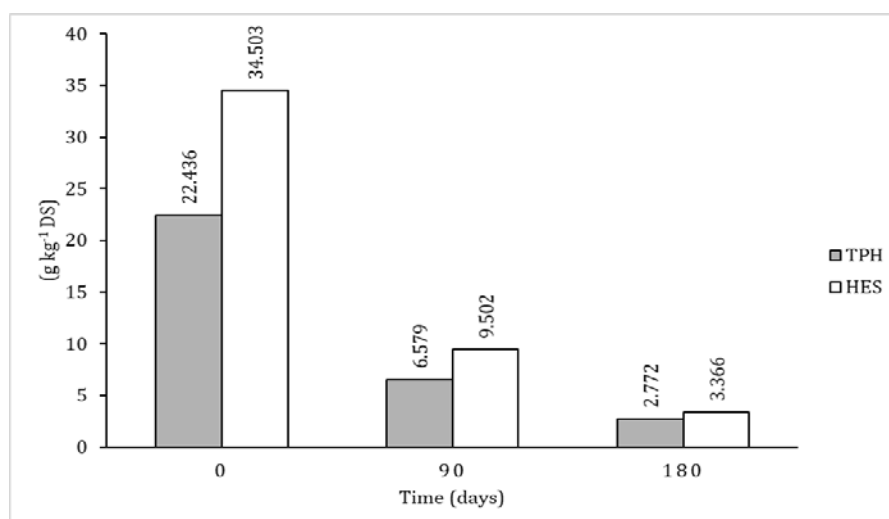


Figure 2. Results of TPH and HES before and 90 and 180 days after the application of microorganisms into polluted soil

The results of the TPH analysis indicate a declining trend in their values over a period of 180 days. The initial value of TPH in untreated soil was 22.436 g kg⁻¹ DS. After the application of microorganisms, the level of TPH decreased to 6.579 g kg⁻¹ after 90 days and 2.772 g kg⁻¹ after 180 days. The value of TPH was reduced by 70% after three months, and by 87% after six months, which indicates that biodegradation is more pronounced at high concentrations of pollutants. These results are comparable with our previous work

(Avdalović et al., 2016), as well as with the study by Chen et al. (2019), who observed that TPH values decreased to below the threshold level after 30 weeks. This trend of declining TPH values is correlated with the declining trend of HES, which decreased by 72% and 90% three and six months after the application of microorganisms, respectively.

The percentage of DS and LOI in the soil before and three months after the application of microorganisms are shown in Table 1.

Table 1.

Percentage of DS and LOI in the soil before, and 90 and 180 after the application of microorganisms

Before application		After 90 days		After 180 days	
DS (105°C) %	LOI (550°C) % of DS	DS (105°C) %	LOI (550°C) % of DS	DS (105°C) %	LOI (550°C) % of DS
78.85	16.54	71.42	13.08	70.26	14.06

The data in Table 1 show that the value of the residue after drying at 105°C decreased and LOI (ignition at 550°C) decreased 90 days after the

application of microorganisms into the polluted soil, but LOI started to increase 180 days after the application of microorganisms. The decrease in the

value of dry substance during the six months' observation indicates that the water content in the soil increased (depending on soil water properties and meteorological conditions). LOI is a parameter that is associated with the content of organic matter in soil (Wilke, 2005). During the first 90 days, while the content of organic pollutants was high, LOI can be described as the sum of soil organic matter and organic pollutants. Therefore, the initial decrease in LOI can be explained by the degradation of most petroleum hydrocarbons. As the level of pollutants dramatically decreased, a further increase in LOI indicated that the

content of soil organic substance increased, which is an indicator of soil humification through the bioremediation process (Wilke, 2005). This is in accordance with HES and TPH results.

Figure 3. shows how the field looked before (a) and after (b) the bioremediation process. The bioremediation process had a significant impact on the environment and aquatic ecosystem. It can be observed that the barren field was transformed into fertile land. Our further research will focus on the possibility of applying a similar bioremediation system to the soil polluted with microplastics.



Figure 3. The field during initial monitoring (a) and 180 days after the application of microorganisms (b)

4. Conclusions

Plastic waste can be found all around the world. There is growing concern about the problem of microplastics, both primary and secondary. Among other places, microplastics often end up in the soil. Due to their long lifecycle, plastics can stay in the soil forever. The optimal way to deteriorate plastics is through biodegradation. Since biodegradation of plastics is less likely to occur naturally in the environment, it should be aided by bioremediation: a process that uses microorganisms isolated from soil to restore natural environmental conditions. A mud pit located in the area around the village of Turija was selected for the bioremediation of soil polluted with oil and its derivatives. The contents of n-hexane extractable substance and total petroleum hydrocarbon were found to be below limits 180 days after the application of microorganisms. After the successful bioremediation process, a barren soil was transformed into fertile land, suitable for growing field crops. It is necessary to restore fertile soil, as well as to prevent further pollution of the environment, primarily fertile arable land and aquatic ecosystems in the immediate vicinity, and hence prevent harmful substances from reaching the food chain.

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Declaration of competing interest

The authors declare that they have no known personal and/or financial relationships with other people or organizations that could inappropriately influence the work reported in this paper.

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