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ISPITIVANJE MIKROBIOLOŠKIH KARAKTERISTIKA SEDIMENTA ZAGADJENOG
UGLJOVODONICIMA NAFTE NA LOKALITETU TOPLANE NOVI BEOGRAD
DETERMINATION OF MICROBIOLOGICAL CHARACTERISTICS OF SEDIMENT POLLUTED
WITH PETROLEUM HYDROCARBONS AT THE SITE OF HEATING PLANT "NOVI BEOGRAD"

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UVOD: Zagadjenje naftom i naftnim derivatima predstavlja visok rizik po čoveka i životnu sredinu. Do zagadjenja može doći tokom eksploatacije, transporta i skladištenja nafte, ali i pri akcidentalnim izlivanjima. Tehnologijom bioremedijacije - upotrebom mikroorganizama kao agensa za uklanjanje zagadjenja sediment i voda se mogu vratiti u prvobitno stanje. Pre procesa bioremedijacije potrebno je utvrditi broj autohtonih mikroorganizama u sedimentu, a zatim ih okarakterisati biohemijskim testovima i molekularnim metodama. Neki rodovi bakterija, kao što su *Pseudomonas*, *Nocardia*, *Rhodococcus*, *Achromobacter*, *Flavobacterium*, *Corynebacterium*, gljiva - *Candida*, *Aureobasidium* i *Aspergillus* sa visokom efikasnošću razlažu naftu i naftne derivate. Preliminarna istraživanja obavljena na lokalitetu Toplane Novi Beograd, potvrdila su postojanje naftnog zagadjenja kako u sedimentu tako i u podzemnim vodama i postojanje potrebe za sanacijom i remedijacijom terena.

CILJEVI: Određivanje različitih fiziološko-biohemijskih grupa mikroorganizama sedimenta na opštim i selektivnim podlogama.

METODE: U radu je korišćen sediment sa tri različite tačke na lokaciji Toplane Novi Beograd. Kao podloge za određivanje broja mikroorganizama korišćeni su: Hranljivi agar (Torlak) za bakterije; Sladni agar (Torlak) za gljive; mineralni agar sa dizelom D2 na 1 L (UG) za mikroorganizme koji razlažu ugljovodonike, *Pseudomonas* isolation agar (PIA) za bakterije roda *Pseudomonas*, M3 za bakterije rodova *Nocardia* i *Rhodococcus*. U uzorcima je pored mikrobiologije praćen i sadržaj ukupnih ugljovodonika nafte gasnom hromatografijom.

REZULTATI: U uzorcima sedimenta odredjen je broj mikroorganizama (CFU/g): ukupne bakterije $1,0-4,0 \times 10^6$; kvasci i plesni $2,5 \times 10^2-1,2 \times 10^3$; bakterije roda *Pseudomonas* $2,2 \times 10^4-5,5 \times 10^4$; mikroorganizmi koji razlažu ugljovodonike $2,2 \times 10^4-1,2 \times 10^5$; bakterije rodova *Nocardia* i *Rhodococcus* $2,3 \times 10^2-3,6 \times 10^3$. Ukupan sadržaj ugljovodonika nafte varira između 2760-28865 mg/kg suve supstance.

ZAKLJUČAK: U ispitivanim uzorcima je prisutan veliki udeo degradera ugljovodonika u ukupnom broju mikroorganizama. Upotreba selektivnih podloga je omogućila izolovanje čistih kultura koje će u narednim koracima biti identifikovane molekularnim metodama. Planirano je da se ove kulture koriste za eksperimente biodegradacije.

KLJUČNE REČI: sediment, naftni zagadjivač, mikroorganizmi degraderi

**DETERMINATION OF MICROBIOLOGICAL CHARACTERISTICS OF SEDIMENT POLLUTED
WITH PETROLEUM HYDROCARBONS AT THE SITE OF HEATING PLANT "NOVI BEOGRAD"**

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INTRODUCTION: Pollution with petroleum and petroleum products poses a high risk to the human environment, thus affecting all life. Pollution may occur during the exploitation, transportation and storage of petroleum, but also in accidental spills. Bioremediation technology-using microorganisms as an agent for removing pollution, sediment and water can be restored to its original state. Before the bioremediation process, it is necessary to determine the number of autochthonous microorganisms in the sediment. Preliminary research carried out at the site of heating plant "Novi Beograd" confirmed the existence of petroleum pollution both in sediment and in groundwater.

OBJECTIVES: Determination of different physiological-biochemical groups of microorganisms from sediment on general and selective medium.

METHODS: In this work we used sediment from three points at the site of heating plant "Novi Beograd". For the determination of number of microorganisms next medium were used: Nutrient agar (Torlak) for bacteria; Malt agar (Torlak) for fungi; mineral base medium containing 2 g of standard D2 diesel fuel in 1 L of medium for microorganisms degraders of hydrocarbons, Pseudomonas isolation agar for bacteria of the genus *Pseudomonas*, M3 for bacteria of the genera *Nocardia* and *Rhodococcus*. In addition to microbiology, we determined content of the total petroleum hydrocarbon.

RESULTS: Number of microorganisms (CFU/g) was determined: total bacteria $1.0-4.0 \times 10^6$; yeasts and molds $2.5 \times 10^2-1.2 \times 10^5$; bacteria of the genus *Pseudomonas* $2.2 \times 10^2-5.5 \times 10^4$; microorganisms that degrade petroleum $2.2 \times 10^4-1.2 \times 10^5$; bacteria of the genera *Nocardia* and *Rhodococcus* $2.3 \times 10^2-3.6 \times 10^4$. The total content of hydrocarbons varies between 2760-28865 mg/kg of dry substance.

CONCLUSION: High number of hydrocarbons degrading microorganisms is present in the tested samples. The use of selective medium has made it possible to isolate pure cultures that will be identified in molecular methods in the next steps. It is planned that these cultures are used for biodegradation experiments.

KEYWORDS: sediment, petroleum pollutant, microorganisms degraders

DETERMINATION OF MICROBIOLOGICAL CHARACTERISTICS OF SEDIMENT POLLUTED WITH PETROLEUM HYDROCARBONS AT THE SITE OF HEATING PLANT "NOVI BEOGRAD"



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Conclusion

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Introduction

Pollution with petroleum and petroleum products poses a high risk to the human environment, thus affecting all life. Pollution may occur during the exploitation, transportation and storage of petroleum, but also in accidental spills. Bioremediation technology- using microorganisms as an agent for removing pollution, sediment and water can be restored to its original state. Before the bioremediation process, it is necessary to determine the number of autochthonous microorganisms in the sediment. Preliminary research carried out at the site of heating plant "Novi Beograd" confirmed the existence of petroleum pollution both in sediment and in groundwater.

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Results and discussion

Number of microorganisms (CFU/g) was determined:

Microorganisms	Number (CFU/g)
Total bacteria	1,0-4,0x10 ⁶
Yeasts and molds	2,5x10 ² -1,2x10 ⁵
Bacteria of the genus <i>Pseudomonas</i>	2,2x10 ³ -5,5x10 ⁴
Microorganisms that degrade petroleum	2,2x10 ⁴ -1,2x10 ⁵
Bacteria of the genera <i>Nocardia</i> and <i>Rhodococcus</i>	2,3x10 ³ -3,6x10 ⁵



Figure 1. Mixed cultures of microorganisms on various media.

The total content of hydrocarbons varies between 2760-28865 mg/kg of dry substance.