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XVIII International Scientific and Professional Meeting

Ecological Truth

EcoIst '10

Edited by
Zoran S. Marković

Spa Junaković, Apatin
Serbia

01 - 04 June 2010

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MICROBIOLOGICAL PROPERTIES OF SOIL IN OIL REFINERIES

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ABSTRACT

Certain microbial groups have the ability to utilize the components of petroleum as a source of energy and carbon, which enables them to live in contaminated soils. The objective of this investigation was to assess microbiological properties of soils in the vicinity of Pančevo and Novi Sad oil refineries. Microbiological analyses included the determination of the total microbial number, copiotrophic and oligotrophic bacteria number, azotobacters number and dehydrogenase activity. High concentrations of total hydrocarbons and polycyclic aromatic hydrocarbons adversely affected the microbiological properties of the tested soils. Low concentrations of total hydrocarbons and polycyclic aromatic hydrocarbons tended to stimulate the number and activity of microorganisms in the contaminated soils. The obtained results indicated the importance of monitoring the microbiological properties of soil contaminated with petroleum.

Keywords: microbial activity, petroleum, soil

INTRODUCTION

The expansion of oil and petrochemical industries in the course of the 20th century resulted in an intense contamination of the environment (air, water and land) with petroleum and petroleum products. Contamination with petroleum causes significant changes in the physical, chemical and biological properties of soil [1]. In the process of production and processing of petroleum and petroleum products, various byproducts such as hydrocarbons and other toxic substances are produced, which have a deleterious effect on the biota, while soils contaminated with petroleum lose its fertility. Soil microorganisms, which are a heterogeneous group of organisms, and their enzymatic systems comprise up to 90% of the overall metabolic activity of soil [2]. Petroleum refining products affect the microbial soil activity and cause changes in the quantitative and qualitative composition of the microbial population [3]. Certain microbial groups have the ability to utilize the components of petroleum as a source of energy and carbon, which enables them to live in contaminated soils. High microbial activity in contaminated soils is primarily a result of the activity of indigenous microbial populations adapted to living in areas with high concentrations of hydrocarbons originating from petroleum [4]. Certain groups of microorganisms can break down only some petroleum compounds and petroleum products, but combined microbial

populations can achieve a high level of oil degradation, since some oil compounds can only be decomposed by cometabolic activity.

The objective of this investigation was to assess microbiological properties of soils in the vicinity of Pančevo and Novi Sad oil refineries.

MATERIAL AND METHODS

Sampling of contaminated soils was carried out in the yards of Pančevo and Novi Sad oil refineries in the course of January 2009. Simultaneously, samples were taken of non-contaminated soils from sites in the vicinity of the refineries. The collected samples were analyzed for basic chemical properties and the contents of total hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). Microbiological analyses included the determination of the total microbial number, copiotrophic bacteria number, oligotrophic bacteria number, azotobacters number and dehydrogenase activity. The microbial number was determined by the indirect method of dilution on appropriate nutritive media. The total microbial number was determined in agarized soil extract. The number of azotobacters determined on a N-free surface, by the fertile drops method [5]. The numbers of copiotrophic and oligotrophic bacteria was determined by a media with different carbon concentrations [6]. Dehydrogenase activity in the soil was determined spectrophotometrically, by a modified method of Thalmann [7], which is based on the measurement of 2,3,5-triphenyltetrazolium chloride (TTC) reduction in triphenylformazan (TPF).

RESULTS AND DISCUSSION

Microbial distribution and activity in a soil contaminated with petroleum are affected by physical and chemical soil properties, environmental conditions, chemical structure of petroleum products, their concentration and composition of the microbial population in the soil [8] (Milošević et al., 2010).

Table 1. Basic chemical soil properties and contents of total hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) in the location of Pančevo oil refinery

Sample description	pH		CaCO ₃	N%	AL-P ₂ O ₅ mg/100g	AL-K ₂ O mg/100g	Total hydrocarbons (g/kg)	PAH (mg/kg na a.s.z.)
	u KCl	u H ₂ O						
Pančevo-non-contaminated	6.73	7.86	9.22	0.223	119.50	57.00	0.181	0.0027
Pančevo-contaminated	6.86	7.73	11.32	0.183	17.20	25.90	2.101	0.1751

Soil contamination by byproducts of Pančevo oil refinery, (total hydrocarbon content - 2.101 g/kg, Table 1), stimulated the soil microbial activity as compared with the surrounding (non-contaminated) soil. The total number of microorganisms and the number of oligotrophic bacteria were higher in the contaminated soil than in the surrounding non-contaminated soil (Table 2). The increased hydrocarbon content

promoted the development copiotrophic bacteria whose numbers increased by 121%. However, the contamination had a negative impact on the number of azotobacters, which were not found at this site at all. The increased numbers of microorganisms and the resulting higher microbial activity brought about a higher dehydrogenase activity in the contaminated soil (Table 2).

In a study of Tynybaeva [9], which dealt with soil contaminated with petroleum (100-600 mg/kg of total hydrocarbons), the numbers of oligotrophic and copiotrophic bacteria were higher in the contaminated than in non-contaminated soil. Results of Xu and Johansson [10] showed that increased concentrations of hydrocarbons tended to increase the numbers of soil microorganisms and their enzymatic activity. A research of Wyszowska [11] showed that the numbers of azotobacters, oligotrophic and copiotrophic bacteria, as well as dehydrogenase activity were increased in soils contaminated with petroleum.

Table 2. Mikrobiological properties of the soil at the site of Pančevo oil refinery

Sample description	Total no. of microorganism ms (x10 ⁶)	Oligotrophic bacteria (x10 ⁵)	Copiotrophic bakteria (x10 ⁵)	<i>Azotobacter spp.</i> (x10 ¹)	DHA mg TPF g ⁻¹ soil
	In g ⁻¹ of absolutely dry soil				
Pančevo-contaminated	312.56	313.78	356.34	0.00	1647
Pančevo-non-contaminated	278.20	279.51	161.41	19.68	838

In the contaminated soil in Novi Sad oil refinery (Table3), high concentrations of total hydrocarbons (4,244 g/kg) and polycyclic aromatic hydrocarbons (0.9322 mg/kg), which are most difficult for microorganisms to decompose, reduced the numbers of the investigated microbial groups. The low contents of nitrogen (0.042%), phosphorus (5.6 mg/100g) and potassium (4.50 mg/100g) in the contaminated soil definitely contributed to the reduction in the number of microorganisms. Nitrogen and phosphorus deficits in the soil may be limiting factors in the microbial degradation of petroleum, as indicated by results of numerous authors [12,13,14,15].

Table 3. Basic chemical soil properties and contents of total hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) in the location of Novi Sad oil refinery

Sample description	pH		CaCO ₃	N%	AL-P ₂ O ₅ mg/100g	AL-K ₂ O mg/100g	Total hydrocarbons (g/kg)	PAH (mg/kg a.d.s.)
	in KCl	in H ₂ O						
Novi Sad - non-contaminated	7.11	7.92	9.22	0.139	18.40	9.50	1.055	0.6788
Novi Sad - contaminated	7.79	8.08	11.32	0.042	5.60	4.50	4.244	0.9322

In the contaminated soil at the site of Novi Sad oil refinery, the total number of microorganisms and the number of copiotrophic bacteria were six times lower than in non-contaminated soil (Table 4). The number of oligotrophic bacteria in the contaminated soil was also lower, but the difference in favor of the non-contaminated

soil was not large (Table 4). Results of Wyszowska and Kucharski [3] showed that oligotrophic bacteria are more resistant to soil contamination with petroleum byproducts than the copiotrophic bacteria. Azotobacters were found in the contaminated soil, but their number was low (2.81×10^1) when compared with that in the non-contaminated soil (112.98×10^1) (Table 4).

Studies of Iwanow [16] have shown that azotobakter and other N-fixing bacteria are extremely sensitive to soil contamination with petroleum and its products. In contrast to these results, Bieszkiewicz [17] and Wyszowska and Kucharski [3] claimed that oil refinery products stimulated the development of azotobacters in the soil. Reduced numbers of all examined microbial groups caused a lower microbiological activity in the soil and so was lower the dehydrogenase activity in contaminated soil. Results of Garcia [18] and Sorensen [19] showed that the increased concentration of hydrocarbons reduced the microbial number and enzymatic activity in the soil.

Table 4. Mikrobiological properties of the soil at the site of Novi Sad oil refinery

Sample description	Total no. of microorganisms ($\times 10^6$)	Oligotrophic bacteria ($\times 10^5$)	Copiotrophic bacteria ($\times 10^5$)	<i>Azotobacter</i> spp. ($\times 10^1$)	DHA mg TPF g ⁻¹ soil
	In g ⁻¹ of absolutely dry soil				
Novi Sad - contaminated	56.94	137.32	68.10	2.81	753
Novi Sad - non-contaminated	299.24	299.24	357.66	112.98	2079

The results of our experiments showed that the contamination of soil with petroleum products affected differently the number and enzymatic activity of microorganisms in the oil refineries in Pančevo and Novi Sad. The contradictory results may be explained by the fact that microbial activity is significantly affected by chemical structure of the components of diverse petroleum products. Results of numerous authors [20-24] indicated that the gasoline components released during petroleum processing reduce the microbial number and inactivate the enzymatic activity of soil, while petroleum may stimulate microbial development and the activities of dehydrogenase, urease and phosphatase. Studies of Bosset and Bartha [25] indicated that the microbial number in petroleum-polluted soil was also influenced by the degree of contamination.

CONCLUSION

High concentrations of total hydrocarbons and polycyclic aromatic hydrocarbons adversely affected the microbiological properties of the tested soils.

Low concentrations of total hydrocarbons and polycyclic aromatic hydrocarbons tended to stimulate the number and activity of microorganisms in the contaminated soils.

The obtained results indicated the importance of monitoring the microbiological properties of soil contaminated with petroleum.

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