

University of Belgrade  
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# PROCEEDINGS

*XVIII International Scientific and Professional Meeting*

## *Ecological Truth*

# *EcoIst '10*

Edited by  
Zoran S. Marković

Spa Junaković, Apatin  
Serbia

01 - 04 June 2010

**University of Belgrade – Technical faculty Bor**



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**MICROBIOLOGICAL PROPERTIES OF DEPOSOL IN THE LOCATION OF  
BANATSKO KARADJORDJEVO**

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**ABSTRACT**

Disposal of drilling mud is a considerable problem in the process of crude oil pumping and transportation. Microorganisms are the most important component of the soil biological phase as their enzymatic systems take part in degradation and synthesis of soil compounds. Soil samples were taken from the location of Banatsko Karadjordjevo. The results show high values of total numbers of microorganisms and azotobacters in indigenous soil than in deposol.

The numbers of ammonifiers, oligonitrophiles, actinomycetes and fungi were higher in deposols than in the local soil. The density of the studied groups of microorganisms and DHA decreased with depth, with the exception of actinomycetes.

**Keywords:** microorganisms, soil, deposol, dehydrogenase

**INTRODUCTION**

Presence of contaminants in the soil, depending on their chemical properties and amounts, causes stress in soil organisms. Microorganisms as the most numerous group of soil organisms [3] provide preliminary information on soil health/quality [6] and the presence of ecotoxic substances [7, 2].

During the pumping and transport of crude oil, a serious problem arises regarding the storage/disposal of drilling fluid and mud. Construction of permanent dumps causes significant changes the physical, chemical and biological properties of indigenous soils.

A legal obligation of drilling contractors is to keep operative wells filled with drilling fluid while a corresponding volume of drilling fluid must be kept nearby in open pools, for backup purposes. On completion of works, drilling fluid in the well is replaced by another fluid and the drilling mud, as technological waste, is temporarily deposited in the abovementioned open pools, wherefrom it is transported to the nearest permanent dump.

Microorganisms as the most important component of the soil biological phase are an important indicator of soil degradation processes (reduction of biodiversity,

accumulation of pollutants, disruption of food cycles and redox state). Microbial activity is influenced by the physical and chemical soil properties, climatic conditions, pesticides level, heavy metals and other pollutants as well as mutual relations in the microbial population [7]. Studies of microbial activity conducted within a project of soil reclamation at oil drilling dumps from several locations in the Vojvodina Province have shown that the number of microorganisms and dehydrogenase activity are reliable indicators of soil quality/health status [6]. In the location of Mokrin [5], chemical properties of deposol (at a depth of 50 cm) reduced the total number of microorganisms, but significantly increased the numbers of actinomycetes and fungi as well as dehydrogenase activity.

Considering the importance of preservation of soil as the most important natural resource of any country, the objective of our research was to monitor microbial numbers and dehydrogenase activity in the soil of the oil drilling dump in the location of Banatsko Karadjordjevo and the soils surrounding the dump.

## **MATERIAL AND METHODS**

Samples for chemical and microbiological analyses were taken from the indigenous hydromorphic black soil and from the oil drilling dump (deposol). Samples were taken from several depths of the soil profile in the location of Banatsko Karadjordjevo.

Soil biological activity was estimated on the basis of microbial numbers (total number of microorganisms, azotobacters, oligonitrophiles, ammonifiers, actinomycetes and fungi) by the method of dilution on appropriate mediums. Total number of microorganisms was determined in agarized soil extract. Ammonifiers were determined on MPA [8]. Oligonitrophiles and azotobacters were determined on a N-free medium. Actinomycetes were determined on a synthetic medium, fungi on Chapek's medium. Dehydrogenase activity (DHA) was determined by a modified method of Thalmann [9], which is based on the measurement of extinction of triphenylformazan (TPF), formed by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC).

## **RESULTS AND DISCUSSION**

Soil is an ecological system and it is the habitat for diverse and numerous microorganisms whose enzymes play a key role in the metabolic activity of soil. Microorganisms are important for the development and maintenance of fertility and they may serve as indicators of the adverse impact of heavy metals and pollutants, as well as of changes in physical and chemical soil properties. Carbon, hydrogen and nitrogen are constituent elements that allow the growth and development of microorganisms. Certain groups of microorganisms have high requirements ( $10^{-3}$  to  $10^{-4}$  M) for phosphorus, potassium, sulphur, magnesium and iron, and the requirement for microelements (Mn, Cu, Co, Zn, and Mo) ranging from  $10^{-6}$  do  $10^{-8}$  M [7].

Disturbances of physical and chemical soil properties of, high concentrations of heavy metals, pesticides and other pollutants are stress factors that may inhibit the activity of microorganisms. On the other hand, microorganisms have the ability to

survive unfavorable soil conditions in the quiescent state, while resuming their activity when good conditions are restored. Poor and/or degraded soils host a narrow range of microbial genera and species.

By storing drilling fluid (lignosulfonic type, basic reaction) in permanent dumps, there develops a tehnogetic soil of deposol type, with has horizons I, II, III (Table 1).

The indigenous soil was a humogley type (Table 1), which is pH neutral to slightly acidic. The content of available phosphorus in the surface horizon is extremely high, even toxic (75.00 mg/100g), while the content of available potassium is high (23.5 mg/100g). The soil in the dump is alkaline and with heterogeneous properties along the profile, which is a consequence of the origin and composition of the deposited waste. The conducted analyses [4] indicates that the content of heavy metals was within the MAC, except for high amounts of chromium (117-218 mg/kg soil, profil 3) and nickel (54-58 mg/kg soil, profil 2).

Physical and chemical characteristics are most important soil features that affect the activity of microorganisms [7].

**Table 1.** Main chemical properties of humogley and deposol

Soil type	Horizon	Depth (cm)	pH		CaCO <sub>3</sub> (%)	Total N (%)	Total C (%)	AL-P <sub>2</sub> O <sub>5</sub> mg/100g	AL-K <sub>2</sub> O mg/100g
			in KCl	in H <sub>2</sub> O					
Indigenous soil (profile 1)	Aor	0-21	6.09	6.84	0.00	0.367	4.116	23.5	75.0
	A	21-70	6.33	7.23	0.17	0.287	3.243	13.3	34.5
	ACGso	70-108	7.64	8.33	35.95	0.043	4.890	7.2	22.7
	CGso,r	108-180	7.92	8.39	41.18	0.038	4.928	8.3	18.2
Deposol (profile 2)	(A)	0-15	7.32	7.88	14.16	0.231	3.921	28.3	102.5
	I	15-62	7.40	8.23	10.72	0.077	1.871	9.1	82.0
	II	62-90	7.43	8.54	9.44	0.064	1.339	6.7	36.4
	III	90-145	7.35	8.22	6.86	0.059	1.171	5.5	30.0
	Gr	145-170	7.17	8.24	11.15	0.066	0.462	10.5	32.3
Deposol (profile 3)	(A)	0-14	7.44	8.10	17.16	0.137	4.381	8.2	50.0
	I	14-132	7.52	8.54	20.59	0.092	2.967	23.6	26.4
	Gr	132-180	7.53	8.53	10.30	0.063	1.530	7.1	29.1

Microbial activity is a good indicator of the presence of pollutants in the soil [1,7].

The microbiological analysis showed high biological values of the surface layers of the soil profiles; however, the number and diversity of microorganisms change significantly with depth (Tables 2 and 3). The surface layers of both indigenous soil (humogley) and deposol contained high numbers of all examined groups of microorganisms. Table 2 shows that in the surface layer of deposols had higher numbers of ammonifiers than the indigenous soil, which is probably influenced by the presence other waste materials in the dumps. Actinomycetes were found in all three profiles in the densities of  $\times 10^4 \text{ g}^{-1}$  soil. It was observed (Table 2) that the number of actinomycetes was high in the deep layers of the deposol (with the exception of the Gr layers of deposol). The highest number of fungi was registered in the surface layer of the dump. Fungi were not found at a depth below 15 cm in profile 2, while profile 3 had a large number of fungi, up to  $16 \times 10^4$  per gram of soil on average.

Azotobacter, an indicator of N fixation balance in the soil, was not observed at greater depths in any of the profiles (Table 3). The oligonitrophilous bacteria are a heterogeneous group of microorganisms and their number was higher in deposol than in the indigenous soil. Although the numbers of this group of microorganisms decreased with depth, they were found in all soil layers. According to Wyszowska and Kucharski [10] soil contamination with crude oil increased the numbers of oligotrophs, copiotrophs and actinomycetes but inhibited the numbers of azotobacters and cellulolytic bacteria.

Dehydrogenase activity (DHA), as an indicator of redox processes, was highest in the surface layer of the indigenous soil. The lowest dehydrogenase activity was observed in deposol, profile 3. DHA decreased with depth in all profiles. In profile 1, DHA was registered in all layers. In deposols, redox processes were not observed at greater depths - below 62 cm in profile 2, and below 132 cm in profile 3.

**Table 2.** The occurrence of microorganisms

Sample	Depth (cm)	Total no. (x 10 <sup>7</sup> g <sup>-1</sup> soil)	Ammonifiers (x 10 <sup>7</sup> g <sup>-1</sup> soil)	Actinomycetes (x 10 <sup>4</sup> g <sup>-1</sup> soil)	Fungi (x 10 <sup>4</sup> g <sup>-1</sup> soil)
Indigenous soil (profile 1)	0-21	63.41	9.70	3.32	4.08
	21-70	61.72	4.78	2.23	2.09
	70-108	12.52	2.09	4.78	1.25
	108-180	0.00	0.00	5.00	0.00
Deposol (profile 2)	0-15	43.95	33.68	5.10	6.77
	15-62	7.90	2.29	4.80	0.00
	62-90	8.92	5.58	9.05	0.00
	90-145	12.86	5.51	9.87	0.00
	145-170	10.88	0.00	0.00	0.00
Deposol (profile 3)	0-14	42.07	39.00	7.37	15.81
	14-132	23.73	18.17	15.85	13.25
	132-180	37.92	7.60	2.80	0.67

The presence of microorganisms in large numbers, their large diversity and high activity are indications of good soil properties. Low values of these indicators hint at unfavorable physical, chemical and toxicological soil properties. Poor soils have a narrow range of microbial species and strains. There are cases when there is a numerous and diverse microbial population which is inactive [2]. Namely, a number of microbial species can survive adverse soil conditions in the quiescent state and resume their activity when conditions improve. Enzymatic activity is a better indicator of soil biological activity than the number of microorganisms.

**Table 3.** The occurrence of N-fixing bacteria and dehydrogenase activity

Sample	Depth (cm)	<i>Azotobacter spp.</i> (x 10 <sup>2</sup> g <sup>-1</sup> soil)	Oligonitrofilii (x 10 <sup>6</sup> g <sup>-1</sup> soil)	DHA (ug TPFg <sup>-1</sup> soil)
Indigenous soil (profile 1)	0-21	27.98	35.75	271
	21-70	3.12	21.78	51
	70-108	0.00	10.02	42
	108-180	0.00	4.05	17
Deposol (profile 2)	0-15	24.23	62.23	171
	15-62	12.00	12.75	28
	62-90	1.80	4.65	0
	90-145	0.00	8.76	0
	145-170	0.00	3.89	0
Deposol (profile 3)	0-14	18.01	75.89	137
	14-132	3.67	12.33	143
	132-180	0.00	3.78	0

Fungi are efficient decomposers of resistant components, and their small numbers indicate a low rate of biodegradation of cellulose, starch, glutens and lignin. As they take part in the forming of macroaggregates, fungi affect the soil structure which is a key to high soil fertility.

The density of microorganisms, their relationships and enzymatic activity reflect the rate of biological activity of a particular ecosystem. Considering the important role of microbes in the overall soil metabolism, biological activity is an indicator of potential and effective soil fertility. Microbiological studies confirm that the number of microorganisms and dehydrogenase activity are important indicators of soil quality.

### CONCLUSION

The study showed that the total number of microorganisms and the number of azotobacters were higher in the indigenous soil than in deposol. The numbers of ammonifiers, oligonitrophiles, actinomycetes and fungi were higher in deposols than in the indigenous soil. The density of the studied groups of microorganisms and DHA decreased with depth, with the exception of actinomycetes.

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