OIL POLLUTION AND MICROBIOLOGICAL QUALITY OF GROUNDWATER AT THE LOCATION OF THE WATER SOURCE *RATNO OSTRVO* NEARBY NOVI SAD

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ABSTRACT

The "Ratno ostrvo" is the biggest drinking water source in wider area of Novi Sad. It is located on the left bank of the river Danube near the Oil Refinery "Novi Sad". One of the consequences of the NATO bombing of the Oil Refinery "Novi Sad" (which is placed in hinterland of the water source) was the uncontrolled spillage of crude oil and oil derivatives. Because of that, microbiological examinations of groundwaters, from both ecological and sanitary aspects were carried out simultaneously during post-war period. Microbiological analyses from sanitary aspects show very low number of coliform bacteria. Determination of numbers of bacteria of the investigated physiological groups, revealed that there was a constant relatively high number of oil-, phenoloxidizing and lipolytic bacteria which are indicators this kind of specific pollution. Microbiological analysis of oil polluted soil show presence relatively high number of different groups of bacteria. In spite of changed ecological situation caused by war effects, microbiological analyses of groundwater quality showed satisfactory results in source "Ratno ostrvo" by now. The presence and potential activity of indigenous microflora could be utilized in recultivation processes in examined area.

Key words: drinking water, groundwater, oil pollution, bombing, microbiological quality, oil refinery

INTRODUCTION

The main drinking water resource of larger area of the city of Novi Sad (Vojvodina, Yugoslavia) – "Ratno Ostrvo" (alluvial aquifers) is located on the left bank of the River Danube, unfortunately the only some hundred meters downstream the oil refinery. This drinking water wells represent the only groundwater resource for the inner city of Novi Sad, large suburbia and industry.

During the NATO aggression the Refinery complex was destroyed and enormous quantities of oil and oil derivatives were spilled on the surrounding ground. Large quantities of crude oil and oil products leaked into the soil and into the River Danube. This is a potential danger for the whole water source. Because of that, microbiological examinations of groundwaters of the water source were carried out during post-ware period.

MATERIAL AND METHODS

From October 1999. till July 2001., 85 water samples (4 from reni wells ,15 from deep and 12 from shalllow piezometers) were analyzed taken only once from the same place or 2-6 times during the investigation period. The sampling sites for the monitoring were chosen according to hydrogeological situation, assumed and possible underground contamination spreading.
Microbiological analysis from both ecological and sanitary aspects were carried out simultaneously. The following microbiologic features were determined: heterotrophic plate count on 37^{o}C, total coliforms and faecal coliforms (sanitary aspect), heterotrophic plate count on 26^{o}C (H), facultative oligotrophs (O), nitrifying, denitrifyng, lipolytic, phenol- and oiloxidizing bacteria (ecological aspect) according to standard procedure which is published eleswhere[6]. The number of hydrocarbon-oxidizing bacteria were determined on MSWYE (low nutrient) and Tauson (pure mineral) medium with oil. The categorization of investigated waters were carried out after Kohl (according to number of heterotrophic bacteria)[6]. Autopurification potential of water were determined acording to O/H ratio[6]. Besides, enzyme (phosphatase) activity of water was determined on the basis of p-nitrophenylphosphate hydrolysis[5].

RESULTS AND DISCUSSION

Results which are given in figures and tables represents mean values of the determined microbiological parameters of all samples from raw wells and piezometers.

RAW WATER WELLS

The quality of raw water wells was satisfied during the examined period from the both of sanitary and ecological aspects (Tab.I and Fig.1). Occurrence of aerobic mozophilic bacteria was extremely low, and the coliformes bacteria (total and fecal) were not presented at all. The number of organotrophs was also very low. According to heterothrophs number the water has been categorized as the I class, after Kohl (Tab.I) The facultative oligothrops was the dominant group. The index O/H indicated very high potential of autopurificaton. Hydrocarbon-oxidizing bacteria were more frequent in BHD-7, BHD-8 and BHD-9 wells. At the same time these bacteria were found on the Tauson's mineral media enriched with oil. These results suggested that investigated waters have been contaminated with fat and/or oil Matura.

PIEZOMETERS

Microbiological analyses indicated satisfactory groundwater quality (deep and shallow piezometers) – from the human health viewpoint (Tab. II, Tab. III, Fig. 2, Fig.3) . Fecal coliforms bacteria were not found, while the number of aerobic mezophilic bacteria and

total coliforms was different in the individual samples. After the Kohl categorization, the waters were classified into the I, I-II or into the II class infrequently (Tab. II, Tab. III). According to water phosphatase activity, the water quality was ranged from clean, satisfactory clean, slightly polluted, moderately polluted to very polluted.

Table I – ROW WATER WELLS - microbiological analysis (number of bacteria per ml)

Groups of bacteria and water classification	BHD-6	BHD-7	BHD-8	BHD-9
Aerobic mesophilic bacteria 37°C	22	7	13	1
TC	N.D. *	N.D.	N.D.	N.D.
FC	N.D.	N.D.	N.D.	N.D.
Organotrophic bacteria (H)	24	13	7	65
Water classes according Kohl	I	I	I	I
Facultative oligotrophs	75	140	57	125
Index O/H	3.1	10.7	8.1	1.9
Lipolytic bacteria	8	3	2	20
Hydrocar.oxid. bact. MS medium	4	14	15	130
Hydrocar.oxid. bact. Tauson medium	N.D.	N.D.	22	9
Phenoloxidat. bacteria	N.D	N.D	7	6
Nitrifying bact.	N.D.	N.D.	N.D.	N.D.
Denitrifying bacteria	N.D.	N.D.	N.D.	N.D.
PAI (phosphatase activity index)	0,50	0,81	0,45	0,08
Categorization according IPA (Matavulj, 1984)	II A satisfactory clean	II B slightly polluted	II A satisfactory clean	I B very clean

* N.D. – no detect ; TC-total coliforms, FC-feacal coliforms;

High occurrence of specific group of bacteria, such as hydrocarbon-oxidizing and lipolytic bacteria, was common for all piezometer examined during the investigated period (Fig. 2, Fig. 3). In accordance with our results, it could be suggested that the presence of specific groups of bacteria has indicated the existence of specific substrates (hydrocarbons). It is confirmed comparing the control piezometer (RO-PZ-12) results (Fig. 4) with the results of all investigated piezometers – especial from piezometers from oil contaminated soil. The appearance and growth of bacteria in the mineral Tauson media (where the oil was the only one source of organic compounds) showed the presence of active oil degradable bacterial strains in piezometers. Results of microbiological analyses corresponds with the chemical one[1,2,3,4]. Characteristics of microflora reflected the ecological features of the ecosystems from which they originated.

Table II – Deep piezometers – microbiological analyses (number of bacteria / ml)

Gropus of bacteria	MB	TC	FC	H	Kohl	O	Ind O/H	LB	HOB MS	HOB TA	POB	NB	DB	PAI	Categ. accordi ng PAI
PJC-1	102	N.D.	N.D.	178	I	698	3.9	71	160	5	57	-	-	0.20	I-II
PJC-2	16	N.D.	N.D.	450	I	670	1.5	120	84	N.D.	35	N.D.	2	0.9	IIB
PJC-3	53	4	N.D.	99	I	403	4.1	91	1190	924	32	trag	trag	0.47	IIA
PJC-5	270	N.D.	N.D.	270	I	800	3.0	130	250	9	130	-	-	0.16	I-II
PJC-6	120	N.D.	N.D.	112	I	270	2.4	77	83	N.D.	18	N.D.	50	0.29	II-A
PJC-8	130	N.D.	N.D.	808	II	950	1.2	159	1200	7	30	N.D.	186	0.67	II-BB
PJC-10	60	N.D.	N.D.	450	I	1015	2.2	60	210	10	300	trag	750	1.18	II-III
PJC-11	27	N.D.	N.D.	60	I	1020	17.0	60	1000	1500	125	N.D.	95	0.02	I-AB
PJC-13	72	N.D.	N.D.	146	I	480	3.3	58	130	6	105	N.D.	10	0.38	II-A
ROPZ-12	**390**	**N.D.**	**N.D.**	**1340**	**II**	**430**	**0.32**	**200**	**35**	**5**	**16**	**-**	**-**	**0.62**	**II--B**
PZ-2	1090	3	N.D.	2220	II	910	0.4	70	320	5	58	N.D.	16	0.20	I-II
PZ-17	160	56	N.D.	1660	II	2070	1.2	110	260	16	170	N.D.	2500	5.45	III-B
PZ-333	440	102	N.D.	610	I-II	2000	3.3	150	140	9	120	4	45	6.61	III-B
SLO-35	275	N.D.	N.D.	65	I	223	3.4	33	25	20	22	-	-	0.17	I-II
SlO-40	80	3	N.D.	460	I	770	1.7	76	630	490	100	3	630	0.33	II-A

MB- mesophilic bacteria (37°C); TC-total coliforms; FC-faecal coliforms; H-heterotrophic bacteria; O-facultative oligotrophs; LB-lipolytic bacteria; HOB-MS- hydrocarbon oxid. bact. on MSWYE medium; HOB-TA - hydrocarbon oxid. bact. on Tauson medium; POB- phenol oxidizing bacteria; NB-nitrfying bacteria; DB- denitrifying bacteria; PAI-phosphatase activity index;
N.D. = no detect
(-) = no investigation ;

Table III – Shallow piezometers – microbiological analysis (number of bacteria / ml)

Groups of bact.	**PPJ C-1**	**PPJ C-2**	**PPJ C-4**	**PPJ C-7**	**PPJ C-10**	**PPJ C-11**	**PPJ C-12**	**PPJ C-13**	**PPJ C-14**	**PPJ C-15**	**PPJ C-16**	**PPJ C-17**
MB	72	180	420	143	46	5	100	40	180	313	1950	1070
TC	N.D.	250	21	N.D.	N.D.	N.D.	2	N.D.	5	20	40	25
FC	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
H	540	370	1010	157	93	90	270	40	730	465	810	595
Classif. Kohl	I-II	I	II	I	I	I	I	I	I-II	I	I-II	I-II
O	870	640	1770	386	875	185	328	260	813	560	180	555
Ind. O/H	1.6	1.7	1.7	2.4	9.4	2.0	1.2	6.5	1.1	1.2	0.2	0.9
LB	81	170	550	33	39	90	37	3	188	240	250	145
HOB-MS	795	130	86	398	256	400	265	26	581	615	1080	635
HOB-TA	238	N.D.	26	N.D.	N.D.	700	55	N.D.	15	15	30	25
POB	515	N.D.	320	27	23	N.D.	17	N.D.	161	160	210	180
NB.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-	N.D.	N.D.	N.D.	N.D.
DB	237	950	250	trag	trag	10	39	-	220	220	450	225
PAI	0.13	0.34	0.15	0.6	0.36	0.10	0.26	-0.19	-0.18	-0.13	1.79	0.1
Cat acc. PAI	I-II clean	II A satisf. clean	I-II clean	II B slight. poll.	II A satisf. clean	I B very clean	II A satisf. clean	IA maxi. clean	IA maxi clean	IA maxi clean	II-III mod poll	I-II clean

MB- mesophilic bacteria (37°C); TC-total coliforms; FC-faecal coliforms; H-heterotrophic bacteria; O-facultative oligotrophs; LB-lipolytic bacteria; HOB-MS- hydrocarbon oxid. bact. on MSWYE medium; HOB-TA - hydrocarbon oxid. bact. on Tauson medium; POB- phenol oxidizing bacteria; NB-nitrfying bacteria; DB- denitrifying bacteria; PAI-phosphatase activity index;

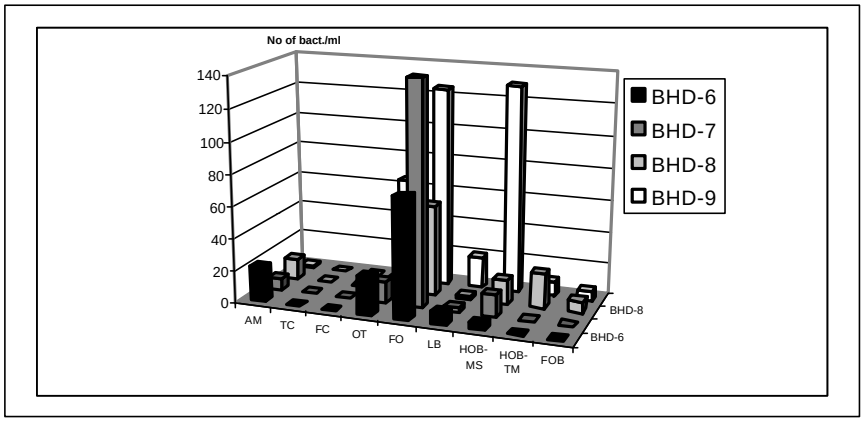

Figure 1 Microbiological quality of raw water wells (average number of bacteria per ml; AM-aerobic mesophiles, TC- total coliforms, FC – faecal coliforms, OT-organotrophs, FO – facultative oligotrophs, LB- lipolytic bacteria, HOB-MS-hydrocarbon oxidative bact. on MS medium, HOB-TA- hydrocarbon oxidative bact. on Tauson's medium, FOB – phenol oxidative bacteria)

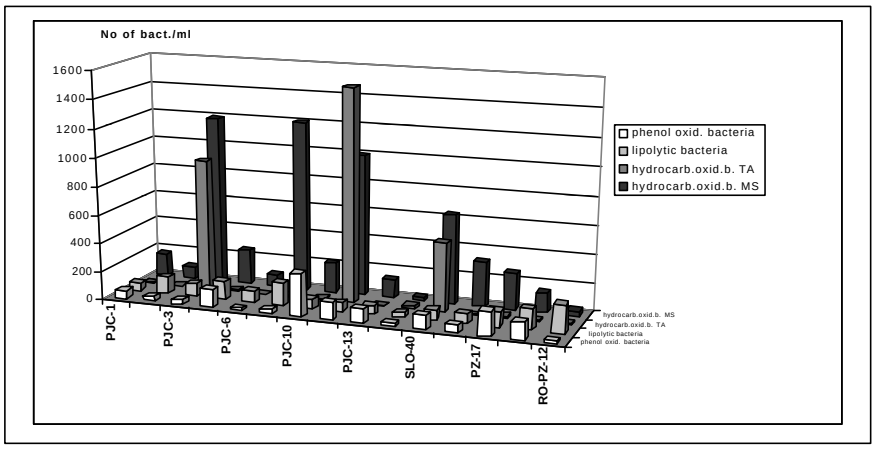

Figure 2 Microbiological water quality of deep piezometers (averaga number of bacteria per ml)

CONTAMINATED SOIL

The experimental demands for analyses of recultivation ability of indigenous bacterial strains were taking the soil samples from polluted refinery area. Isolated and previously isolated strains (from oil refinery wastewater) have been used for bioreactor treatment. Before the treatment, microbiological analyses resulted in very high number of bacteria in contaminated soils. After 30 days of soil treatment in bioreactor the increase of all bacteria groups was registered (Tab. IV). The activity of bacteria could be explained by the aeration effects.

The results referred to the great bioremediation ability showed the decrease of 33-43% of mineral oils after the 30 days of bioreactor treatment (Tab. V)

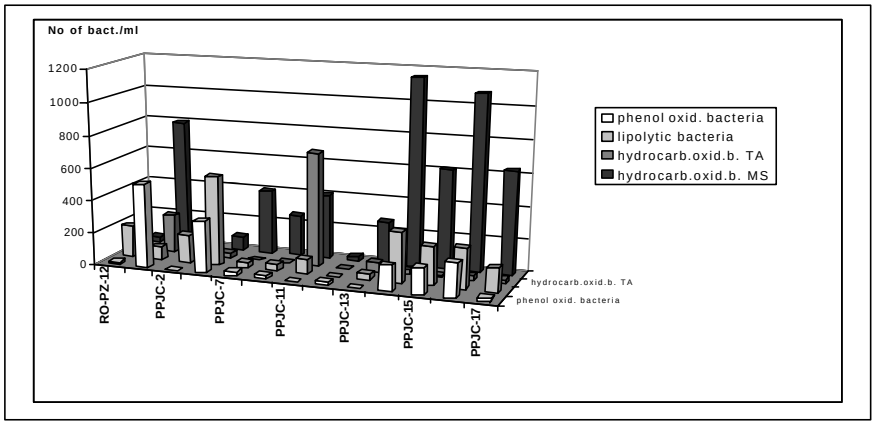

Figure 3 Microbiological water quality of shallow piezometers (average number of bacteria per ml)

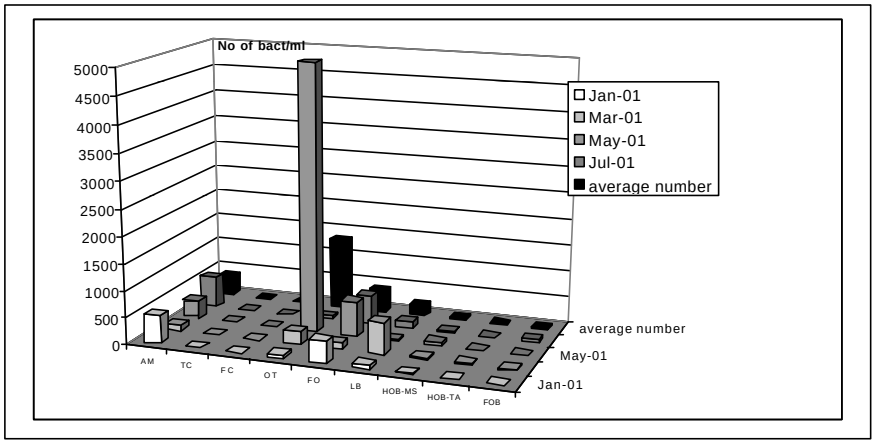

Figure 4 Microbiological water quality of control RO-PZ-12 piezometers from "Ratno ostrvo" wells (average number of bacteria per ml; AM-aerobic mesophiles, TC- total coliforms, FC – faecal coliforms, OT- organotrophs, FO – facultative oligotrophs, LB-lipolytic bacteria, HOB-MS- hydrocarbon oxidative bact. on MS medium, HOB-TA-hydrocarbon oxidative bact. on Tauson's medium, FOB – phenol oxidative bacteria)

Tab. IV- Number of investigated bacterial groups per gramm of soil (10^7 CFU)

Groups of bacteria	Soil before treatment	Soil after treatment (20 days)
Organotrophs	630	4800
Facult. oligotrophs	309	4460
Lipolytic bacteria	150	1470
Hydrocarb.oxid.bact. MSm	120	1010
Hydrocarb.oxid.bact. Tam	0.5	660

Tab. V –Total and mineral oil content in contaminated soil (g/kg)[4]

Parameters	**Soil before treatment**	*Soil after 35 treatment days*			
		Top of columns	**% of decrease**	**Bottom of columns**	**% of decrease**
total oil	**124**	**71**	**42**	**75**	**39**
mineral oil	**101**	**58**	**43**	**67**	**33**

CONCLUSION

- In spite of changed ecological situation caused by war effects, microbiological analyses of groundwater quality showed satisfactory results in source "Ratno ostrvo" by now.
- Microbiological soil analyses from refinery area resulted in high abundance of microorganisms and in presence of indigenous microflora even affected by pollutants.
- The presence and potential activity of indigenous microflora could be utilized in processes of soil and water recultivation in examined area.
- The microbial investigations are highly corresponding with the date of chemical analysis

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