Microbiological and Biochemical Indicators for Anthropogenically Polluted Soils of the City Mednogorsk, Russia

Ngun T. Clement[*1], Pleshakova, Ye[1], Reshetnikov M.V.[1]

[1]Department of Biochemistry and Biophysics
Laboratory of Geoecology and Ecological Geochemistry
Saratov State University, PMB 410012, Saratov, Russia.
*clementngun@yahoo.com

Abstract: This study is a bio-indicative evaluation of anthropogenically-polluted soils of the city Mednogorsk in orenburg region, Russia. This work evaluated – the total number of heterotrophic microorganisms, the number of iron- and manganese-oxidizing bacteria in the polluted soil samples, the activity of soil enzymes (dehydrogenase, catalase, invertase), and also the magnetic susceptibility of these soils (K_{mag}) – an index which shows the concentration of iron (Fe) in soil. 10 samples were analysed which showed the highest coefficient of magnetism (K_{mag} > 3) and also a reduced content in heterotrophic microorganisms compared to the control soil samples with K_{mag} <1, which indicates the inhibitory effect of heavy metals on soil bacteria. It was discovered that soil samples with extremely high significance of magnetic susceptibility possessed high amount of iron-oxidizing bacteria in their soil microbial community. Also, based on the sensitivity to metallic pollution, the studied enzymes formed a decreasing order: dehydrogenase>invertase>catalase. This study reveals the possible use of these indicators as diagnostic tools for monitoring soils polluted with heavy metals.

***Key words*:** Heavy metals, Coefficient of magnetism, iron- and manganese oxidizing bacteria, Heterotrophic microorganisms, Dehydrogenase, Catalase, Invertase.

Introduction

As a result of anthropogenic pollution, significant amount of different xenobiotics are released into the environment among which the most dangerous are heavy metals (HM) [1]. Heavy metals accumulating in soils reduce its biological potential: changes the number, species composition, biomass and productivity of soil microorganisms, represses the activity of soils enzymes, leads to the proliferation of phyto-pathogenic microorganisms and inhibits the growth of plants [2]. Soil contamination by HM need to be strictly controlled, since these toxicants can have long and dangerous impacts on living

organisms. As essential components of any ecological community, soil microorganisms can serve as indicators of changes in the state of the environment. The index of the fermentative activity of soils provides information of the biochemical processes, which occur in soil, and also provides information of the state of the microbial community during cases of anthropogenic disturbances [3,4].

This study is a bio-indicative evaluation of anthropogenically-polluted soils of the city Mednogorsk. The work evaluated: the total number of heterotrophic microorganisms, the number of iron- and manganese-oxidizing bacteria in soil samples, the activity of soil enzymes (dehydrogenase, catalase, invertase), and also the magnetic susceptibility of soils (K_{mag}) – an index which shows the concentration of iron (Fe) in soil.

The Objects for this research were soil samples obtained from a copper - sulphuric plant in the city Mednogorsk located at the region called Orenburg (*Fig.1*), which is among the five most difficult cities to live in based on environmental and sanitary living conditions in Russia and the major pollutants are copper, iron, manganese and sulfur compounds.

Fig. 1. Copper-Sulphuric plant in the city Mednogorsk.

Materials and Methods

From the 70 samples obtained from the city Mednogorsk, 10 samples, which were characterized by an extremely high level of K_{mag} (>3) were selected for microbiological analysis. And 3 samples (No К1, К2 и К3) with low levels of K_{mag} (<1) served as control samples.

An estimation of the total number of heterotrophic microorganisms was carried out using a 10-fold serial dilution and subsequently plating dilutions 10^{-3}, 10^{-4}, 10^{-5} on nutrient

agar while observing conventional bacteriological methods [6]. Total number of iron- and manganese-oxidizing bacteria, was carried out by plating dilutions 10^{-1} , 10^{-2} , 10^{-3}, 10^{-4} on selective media having the following composition, g/l: $(NH_4)_2SO_4$ – 0.5; $NaNO_3$ – 0.5; K_2HPO_4 – 0.5; $MgSO_4 \times 7H_2O$ – 0.5, citric acid – 10, glucose – 2, peptone – 1, agar – 20. To determine the iron-oxidizing bacterial content in the soil we added to the medium: 5.9 g/l of $FeSO_4 \times 7H_2O$; and for manganese-oxidizing bacteria – 4.72 g/l of $MnSO_4 \times 5H_2O$. Given that the number of neutrophilic bacteria was being analyzed, the pH of the media before sterilization was adjusted to 7.0 by titrating with 30% aqueous NaOH.

The plates were incubated at temperature (28±2oC) and counts were recorded from duplicate plates after 2-3 days for total heterotrophic bacteria and 5-7 days for iron- and manganese-oxidizing bacteria. The surfaces of the selective media showed characteristic colonies whose growth was accompanied by the accumulation of yellow-orange oxides of iron or brownish oxides of manganese.

Dehydrogenase activity in the soil was determined calorimetrically based on substrate recovery, and the substrate used was a 2.3.5-triphenyl-tetrazoliumchloride, which accepting mobilized hydrogen dehydrogenase is converted to 2.3.5-triphenylformazan having a red color [7]. Catalase activity in the soil was measured by titration method of R.S. Katznelson and V.V. Yershov [7], based on the measurement of the rate of decay of hydrogen peroxide when reacting with the soil and by the number of undecomposed peroxide determined by permanganometric titration. Invertase activity in soil was determined by the method of F.H. Khaziyev, Y.M. Agafarovoy and A.E. Gulko. 5% of sugar solution, incubation time – 3 hours incubation temperature – 30°C, reducing sugars were detected in the filtrate by using a 0.2% alkaline solution of ferricyanide, their contents were calculated based on the standard scale prepared for glucose [7].

All data on the number of microorganisms and enzyme activity of soil were calculated for air-dry samples.

Results and Discussion

In soil samples with high levels of magnetic susceptibility total number of heterotrophic microorganisms averaged from 18.4 to 71.5×10^5 CFU g^{-1} of soil. A low content of heterotrophic microorganisms was observed in sample No 1 and 6, these samples were characterized by a maximum significance of magnetic susceptibility. It was observed that sample (No 2) was characterized by high numbers of heterotrophic microorganisms, and this may be associated with another type of soil pollution, for example, a high organic content. In control samples

of soil total number of heterotrophic microorganisms varied from 1.9 to 34.6×10^5 CFU g^{-1} of soil (Table 1).

The number of cultured iron-oxidizing bacteria in soils investigated was at an average of 0.8 to 32.0×105 CFU g-1 of soil. Some samples had higher values as compared with other samples (No 3, 4, 9 and 10). The soil sample No 2 was observed to have the highest number of iron-oxidizing bacteria, as well as heterotrophic bacteria. In the control soil samples, the content of iron- oxidizing

bacteria was low, accounting for 0.6 to 4.8×10^5 CFU g^{-1} of soil (Table 1). This is consistent with a number of published data on the decrease in the number of prokaryotic microorganisms in different soil types under the influence of pollution with heavy metals [5]. Manganese- oxidizing bacteria content in soils compared with iron bacteria was much less in the two samples (No 1 and 10) – less than 100 CFU g^{-1} of soil and in four samples it ranged from 0.90 to 2.30×10^4 CFU g^{-1} of soil. Sample No 2 was characterized by very high amount of manganese-oxidizing bacteria (14.20×10^5 CFU g^{-1} of soil). In the control soil samples, the content of the manganese-oxidizing bacteria was 0.13 to 2.40×10^5 CFU g^{-1} of soil (Table 1).

Table 1. Researched Indices of Soil Samples from the City Mednogorsk

Parameters	№ of soil sample												
	1	2	3	4	5	6	7	8	9	10	K1	K2	K3
Index (K_{mng}) χ	6.49	4.10	3.82	3.15	4.97	5.60	3.16	4.64	4.02	3.18	0.33	0.57	0.37
THM, CFU g^{-1} of soil ($\times10^5$)	6.2	325.0	71.5	24.7	26.5	0.2	26.5	18.4	44.5	68.5	14.9	1.9	34.6
№ of iron oxidizing bacteria, CFU g^{-1} of soil ($\times10^5$)	2.1	74.2	32.0	10.9	1.1	<0.1	0.8	3.3	19.9	17.8	4.8	2.4	0.6
№ of manganese oxidizing bacteria CFU g^{-1} of soil ($\times10^5$)	0.01	14.15	0.12	2.30	0.88	0.01	0.31	0.001	0.67	<0.001	2.08	0.13	2.89
Dehydrogenase activity µl H_2 g^{-1} of soil h^{-1}	0.176	0.352	0.380	0.260	0.210	0.187	0.380	0.230	0.420	0.493	0.610	0.587	0.751
Catalase activity, ml of 0.1 N $KMnO_4$ h^{-1}	12.5	13.4	2.5	11.0	14.6	9.8	8.3	6.9	8.2	7.6	4.7	2.9	3.4
Invertase activity, mg of glucose g^{-1} of soil	0.9	2.3	2.5	2.9	1.4	1.7	2.1	3.0	2.1	2.7	2.1	2.6	2.4

The activity of dehydrogenase varied between 0.176 to 0.493 μl H_2 g^{-1} of soil h^{-1}. This was a low index for dehydrogenase activity, which could justify the presence of soil agents (most likely HM) inhibiting these enzymes. Minimal activity was observed in the samples No 1, 5, 6 and 8, which had the highest values of magnetic susceptibility justifying the very dangerous level of iron in the soil. In control soil samples where the index of magnetic susceptibility was within acceptable limits, dehydrogenase activity varied between 0.610-0.751 μl H_2 g^{-1} of soil h^{-1}, i.e it was1.5 to 4 times higher than in anthropogenically-damaged soils.

The activity of catalase in most of the researched samples was higher, than in control samples, varying from 6.9 to 14.6 ml of 0.1 N $KMnO_4$ h^{-1} (Table 1). The index of catalase activity in control soil samples varied from 2.9 to 4.7 ml of 0.1 N $KMnO_4$ h^{-1}. Increased activity of catalase in anthropogenically disturbed soils perhaps could be as a result of exposure to contaminants and the accumulation of peroxides in soils, which served as substrates for catalase (Table 1). Based on our results, there was no significant negative effect of HM on the activity of invertase in the polluted soil samples (Table 1). In the experimental samples with increased magnetic susceptibility was observed a high, and also a low significance in the activity of invertase when compared with the control samples. The activity of invertase in the researched samples varied from 0.9 to 3.0 mg of glucose g^{-1} of soil. Also some samples precisely No 1, 5 and 6 with very high values of magnetic susceptibility 6.49; 5.60, and 4.97 respectively, were characterized by very low significance of invertase activity- 0.9; 1.4 and 1.7 mg of glucose g^{-1} of soil respectively (Table 1).

Conclusion

Thus, from the researched samples of the anthropogenically polluted soils of the city Mednogorsk, two samples were identified which showed the highest coefficient of magnetism and a reduced content in heterotrophic microorganisms, which indicates the inhibitory effect of HM on soil bacteria. The results of the microbiological analysis showed also that the content of manganese-oxidizing bacteria in the soil samples was lower than iron- oxidizing bacteria and it varied irrespective of high or low significance of magnetic susceptibility in the soil. It was discovered that soil samples with extremely high significance of magnetic susceptibility possessed high amount of iron-oxidizing bacteria in their soil microbial community. The results of our study helps suggest that the index of the number of this physiological group of bacteria can be used for monitoring soils polluted with HM.

Also, based on the sensitivity to

metallic pollution, the studied enzymes form a decreasing order: dehydrogenase>invertase>catalase. These results justifies that the activity of dehydrogenases most significantly reflects the influence and impact of HM on the biochemical activity of soils and serves as a sensitive monitoring index for diagnosing soils polluted with heavy metals.

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