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INFLUENCE OF HEAVY METALS ON SOIL MICROFLORA

1. Introduction

The development of technical civilization is constantly connected with degradation and pollution of environment. One of them is pollution by heavy metals. The toxic heavy metals are common in our natural environment. However, currently together with natural circulation of these elements in nature the effects of human activity occur, causing creation of the huge amount of wastes as sewers being directed to the surface waters and solids being collected on landfills. The heavy metals being directed in this way to the environment may be chemically and biochemically transformed causing the biological degradation of the soil properties and pollution of water [1–10, 12, 13].

Heavy metals are very specific group of pollutants being presented in soil. They occur in every soil, even in those presumed as free of pollution. In most of soils these amount are low, not exceeding several mg/kg and in some cases even decimal or centesimal parts of it. However, soils of natural high concentrations of heavy metals [1, 11] also exist.

The important environmental problem is also deposing of wastes on not properly prepared grounds. Many of such areas occur in Krakow and its neighborhood, influencing badly not only on the soil environment in the closest area, but also being transferred to the surface and underground waters. That causes even more dangerous pollution effect on larger scale.

The purpose of the research presented in the paper was to examine the heavy metals contents in the area of tanning plant and determine their influence on the soil microflora.

2. Methodology of research

2.1. Soil sampling

The investigation was conducted on the soil samples taken from the area located near the Mateczny traffic-circle in Krakow. This is a small right-sided fragment of Wilga river.

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In the past it was the area surrounded by the industrial plants buildings from one side (tan, galvanic and chemistry ones) and by Wilga river and two small pools of local names „Bańka" and „Gnilnik" from the other ones, where the effluents were directed.

The soil samples were taken from three various points according to the norm PN-ISO 10381-6 and PN-R-04031. The depth of sampling was no bigger than 20 cm and plant cover was removed in the purpose of lowering the amount of organic coal in soil.

The first sample was the material from the sideline of pool „Bańka". The second one was collected in the distance of about 10 m from the pool and the third one of about 1 m from the Wilga river-bed.

2.2. Chemical analysis of samples

Determination of pH

The pH was determined according to the norm PN-ISO 10390.

Determination of humidity

The humidity of the sample was determined according to the norm PN-ISO 11465.

2.3. Determination of chromium(VI) contents by culometric method

Before the measurements the water sample was prepared according to the norm PN-Z-15009.

The measurements of chromium contents was conducted on culometric analyzer EcaFlow 150GLP. Before the start, the calibration by the method of calibrative curve was done. The standards were prepared by the dilution of chromium standard by R-011a solution (0,1 M HCl). Next, the measurement of chromium contents in analytical samples of soil was conducted. These were the mixtures of 10ml of R-009 solution (1 M HCl), 5 ml of 0,01 mol/dm^3 EDTA and 35 ml of the water sample prepared before.

2.4. Determination of Pb, Cu and Cd contents by culometric method

Determination of Pb, Cu and Pb was done by the standard addition method, applying the standards prepared by dilution of 0,1 M HCl standards. Preparation of analytical sample in this case was based on dilution of researched sample from 10 to 100 times by the main electrolyte solution R-001 (0,1 M Na_2SO_4, 0,01 M CH_3COOH, 0,01 M CH_3COONa).

2.5. Microbiological qualitative and quantitative analysis

The analyzes were performed by the laminated Koch's method of dilutions. The samples of 10 g of soil were prepared, which were transferred to Erlenmayer cobs with 90 ml of physiological liquid and then the samples were shaken during 15 min in purpose of transferring

microorganisms to solution. Such prepared solutions were diluted in ratio 1:10, from which next dilutions were performed till 1:1 000 000.

In purpose of determining amounts of mesophil and psychrophil bacteria, the inoculation by the method of cast-iron plates. To the sterilized Petri plates, the samples of 1 cm^3 of suspension from each prepared dilution were transferred and were covered with invigorating agar MPA. The incubation was conducted in normal temperature during 72 h and in the temperature of 37°C during 24 h.

The investigation of the nitrificative bacteria titre were conducted by transferring the samples of 1 cm^3 each of inoculation from individual dilutions to the bed according to Winogradzki and incubation in time of 7 days in temperature 28°C. Next, the readings were performed by standard agents. To determine the presence of nitrates(III), the Griess agent was added in phase I of nitrification and to determine the presence of nitrates(V), the two-fenyloamina solution was added in phase II of nitrification.

In purpose of determining the denitrificative bacteria titre, the procedure was the same as for nitrificative ones. The pink (carmin) colour was the prove of the presence of nitrates(III) and the lack of colour proved the presence of nitrates(V).

To conduct the ferrous bacteria titres, the inoculation to the liquid bed containing sulphate(VI) of iron(II) was performed. To this purpose, the samples of 1 cm^3 from each dilution were transferred to the tubes containing adequate selective nourishment. After the time of incubation in temperature of 25°C during 14 days, the observation of culture was performed determining the name of these bacteria.

To determine the titre of sulphur bacteria, the samples of 1 cm^3 from each dilution were transferred to the tubes containing nourishment according to Collins. The incubation was conducted in temperature of 32°C during 7 days.

To determine microscopic fungi, the inoculation by the method of grated plates was performed. To this purpose, the samples of 1 cm^3 of prepared dilutions were transferred on the solid bed of Czapek-Dox. Next, the suspension was regularly distributed by the glass stroker and incubated in time of 72 h in temperature 28°C.

To the purpose of diagnostics, the specimens were coloured by the simple method applying as the pigment fuxine as well coloured by the Gram's complex method. Then they were then observed „by immersion" in magnification of 1000 times.

In case of fungi, the specimens prepared from the mushroom spawn fragments sunken in Lugol liquid and covered by glass were observed in magnification of 400 times.

3. Results

3.1. Chemical analysis of samples

Determination of pH

The results of measurements were presented in Table 1.

TABLE 1
Values of pH for samples

Sample	Value pH
1	8,96
2	8,26
3	9,06

Determination of humidity

The results are presented in Table 2.

TABLE 2
The contents of dry mass and water in ratio to dry mass in researched samples

Sample	Dry mass contents [%] (W_{dm})	Water contents in ratio to dry mass [%] (W_{H2O})
1	86,24	15,94
2	88,90	12,48
3	84,98	17,66

3.2. Determination of chromium contents by culometric method

The chromium concentrations in all researched samples were presented in Table 3.

TABLE 3
Chromium contents in samples

Sample	Concentration Cr, [mg/l]
1	73,7
2	28,7
3	15,2

The given results were re-calculated in ratio to 1 kg of soil dry mass according to the equation (1)

$$q = \frac{V_0 \cdot c}{m} \tag{1}$$

where:

V_0 — volume of water added to leaching, l,

c — concentration of researched component, mg/l,

m — mass of dry sample taken to research, g.

The results were presented in Table 4.

TABLE 4

The contents of chromium in ratio to soil dry mass

Sample	q, [g/kg]
1	0,76
2	0,28
3	0,16

3.3. Determination of Cu, Pb and Cd contents by culometric method

The total concentrations of researched metals were presented in Table 5.

TABLE 5

Total concentrations Pb, Cu, Cd

Sample	Concentration Pb, [mg/l]	Concentration Cu, [mg/l]	Concentration Cd, [µg/l]
1	7,5	4,6	<0,5
2	3,8	2,5	<0,5
3	2,3	2,1	<0,5

The given results were re-calculated in ratio to on kg of soil dry mass according to the equation (1) and presented in Table 6.

TABLE 6

Metal contents in ratio to soil dry mass

Sample	q_{Pb}, [g/kg]	q_{Cu}, [g/kg]	q_{Cd}, [g/kg]
1	0,078	0,048	<0,005
2	0,037	0,025	<0,005
3	0,023	0,022	<0,005

3.4. Microbiological quantitative and qualitative analysis

The results of microbiological qualitative and quantitative analysis were presented in Tables 7–12.

TABLE 7

Amount of bacteria and fungi in individual samples and dilutions

Sample	Amount of mesophil bacteria	Amount of psychrophil bacteria	Amount of fungi	Amount of leaven
1	172 500	511 460	1 530	14 000
2	175 460	402 300	1 560	20 000
3	230 660	857 660	830	88 870

TABLE 8

Results of nitrificative bacteria titre determination

Sample	Dilution						Bacteria titre
	1:10	1:100	1:1000	1:10 000	1:100 000	1: 1000000	
1	+	+	+	+	–	–	0,0001
2	+	+	+	+	+	–	0,00001
3	+	+	+	+	+	–	0,00001

TABLE 9

Results of denitrificative bacteria titre determination

Sample	Dilution						Bacteria titre
	1:10	1:100	1:1000	1:10 000	1:100 000	1: 1000000	
1	+	+	+	+	+	+	0,000001
2	–	–	+	+	+	+	0,000001
3	–	+	+	+	+	+	0,000001

TABLE 10

Results of ferrous bacteria titre determination

Sample	Dilution				Bacteria titre
	1:10	1:100	1:1000	1:10 000	
1	+	+	+	+	0,0001
2	+	+	+	+	0,0001
3	+	+	+	+	0,0001

TABLE 11

Results of sulphur bacteria titre determination

Sample	Dilution				Bacteria titre
	1:10	1:100	1:1000	1:10 000	
1	–	–	–	–	0
2	–	–	–	–	0
3	–	–	–	–	0

TABLE 12

Results of qualitative microbiological analysis

Sample	Mesophil bacteria	Psychrophil bacteria	Fungi
1	*Bacillus sp.*	*Staphylococcus sp.* *Bacterium sp.*	*Aspergillus sp.* *Trichoderma sp.* *Penicillium sp.* *Geotrichum sp.*
2	*Bacillus sp.*	*Staphylococcus sp.* *Bacterium sp.*	*Aspergillus sp.* *Trichoderma sp.* *Geotrichum sp.*
3	*Bacillus sp.*	*Staphylococcus sp.* *Bacterium sp.*	*Trichoderma sp.* *Geotrichum sp.*

4. Conclusions

The subject of investigation was the area located on the right side of Wilga river near Mateczny traffic-circle in Krakow. On the basis of conducted analyzes it was said that the soil from the researched area was polluted by heavy metals. Especially, the chromium contents in each sample was high. Such high chromium concentration may indicate the presence of tan wastes in the area of sampling location. Furthermore, the heavy metal concentration in researched soil is strictly correlated with location — the closer is the distance from the pool, the higher is chromium concentration, what also may indicate the probable wastes storage location.

The high metal concentrations in samples, especially of chromium, influences highly on soil microflora:

— in case of mesophil and psychrophil bacteria the amount of microorganisms was reduced with the growth of metal concentration;
— as in case of bacteria, the amount of fungi was reduced with the growth of metal concentration;

— lack of sulphur bacteria may be connected with presence of metals and may be also caused by high value of pH of selected soils, which may be classified as alkaline soils (pH in range 8,1–9,1);
— the presence of heavy metals does not influence on presence of ferrous bacteria and processes of nitrification and denitrification occurring in soil.

It was proved that various sorts of microorganisms features by various tolerance on high metal concentrations. The most resistant were ferrous, nitrificative and denitrificative bacteria, the weakest were mezo- and psychrophil bacteria and fungi.

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