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BIOCUMULATION OF HEAVY METALS BY MICROFLORA OF THE RIVER OŁAWA**

The ability of accumulating heavy metals such as chromium, manganese, iron, nickel, copper, zinc and lead by microflora of the river Oława water was examined. It was found that all of the metals tested, except chromium, were subject to biocumulation. The growth of the microflora depended on metal concentration in the culture media. Generally, when metal concentration increased, the amount of biomass decreased. Besides, it was established that the highest concentration of chromium, nickel, manganese and iron in the experiment had toxic effect on the microflora inhibiting completely its growth.

1. INTRODUCTION

Some heavy metals can be accumulated in large quantities in cellular mass of microorganisms (CHMIELOWSKI [2], [1]). This process is rapid in the biotypes where concentration of these elements is high. The live cells of microorganisms absorb metals by means of some mechanisms used for their specific and non-specific transportation from external environment (TYNECKA et al. [7]–[9]). Dead cells of microorganisms can also bind metals on their surface by means of chelate formation or sorption (CHMIELOWSKI [1]).

The aim of the present study was to establish the accumulation of seven heavy metals: chromium, manganese, iron, nickel, zinc and lead by the microflora of the river Oława water. The attempts were also made to find out the relationship between metal concentration in the medium and the intensity of growth of the population of microorganisms. The studies performed earlier showing that microflora of this river is not much susceptible to high concentrations of heavy metals (KOŁWZAN [4]) gave grounds to conduct the experiment of this type.

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2. EXPERIMENTAL

The site of water sampling was chosen where water is taken for the city of Wrocław. The samples were collected in spring months: March, April and May, because at this time the contents of heavy metals in the river are increased due to vast infiltration from soil and surface flow.

Table 1

Range of concentrations of the heavy metals used in culture medium

Metal	Compound	Metal concentration in medium (mg/dm^3)
Cr	$K_2Cr_2O_7$	0.0
		5.00
		500.00
Mn	$MnCl_2 \cdot 6H_2O$	1.50
		150.00
		15 000.00
Fe	$FeCl_3$	1.50
		150.00
		15 000.00
Ni	$NiCl_2$	0.05
		5.00
		500.00
Cu	$CuCl_2 \cdot 2H_2O$	0.01
		1.00
		100.00
Zn	$ZnCl_2$	0.05
		5.00
		500.00
Pb	$Pb(CH_3CO)_2 \cdot 3H_2O$	0.05
		5.00
		500.00

Microflora of the surface water and overdeposit water of the river Oława was analysed. There were two media used for microorganisms culture. One was a liquid medium "LB" prepared according to SCHAEFLER [6], which was rich in organic compounds. The other was the water from the river Oława enriched with 0.5% glucose only. Natural microflora of

the river Oława was inoculum. The metals were introduced into the medium in the form of aqueous solutions of their compounds. Each of the metals was tested in three concentrations (table 1). The concentrations corresponded to those formerly established for the river Oława (KOŁWZAN [4]). The first had the same value as the water of the river, whereas the others were higher by 2 orders.

The culture was grown in 250 cm^3 Erlenmayer flasks to which 50 cm^3 of medium and 50 cm^3 of the water with microflora were introduced. Incubation had lasted for 7 days at a temperature of 25°C. At the same time control cultures were grown without any metals being introduced to them. After the incubation the biomass was centrifuged, the deposit was washed 3 times with 100 cm^3 of demineralized water (JONES et al. [3]). The residue was dried up to a constant weight and mineralized in concentrated nitric acid (PINTA [5]).

The content of metals in the samples and in controls were determined by the method of atomic absorption making use of atomic spectroscope Atomspeck manufactured by Hügler and Walts.

The quantities of the accumulated metals are given as a difference between their contents in the samples and controls.

3. RESULTS

A reaction of the microflora in the river Oława to the addition of heavy metals to the medium was observed on the basis of the population growth expressed as the dry mass of microflora determined at the beginning and at the end of the experiment, as well as the quantity of the bound metal converted to the weight unit of dry bacteria mass.

The results (tables 1–4) indicate that the influence of metals depended first of all on the type of metal and its concentration and also on the time of taking water samples. For instance, chromium ($K_2Cr_2O_7$) introduced into the culture media inhibited the growth of microflora, which was proved by the fact that all numbers, and thus biomass, decreased as concentration of the metal increased. After addition of chromium in the concentration of 500 mg/dm^3, no microflora increase was observed in any of the cultures. This element was not detected in the microorganism cells, irrespective of the type of the medium applied. On one hand, we may conclude that hexavalent chromium was not accumulated by microorganisms. On the other, we may also assume that in the course of sample incubation it changed the valency number and it was not taken up in this form by microorganisms.

In the presence of manganese, the growth of microorganism biomass decreased with an increase of metal concentration in the medium. At its greatest concentration manganese had toxic effect on microflora, decreasing the growth of biomass by about 50%. Nevertheless, manganese accumulation took place when it was used in all its other concentrations. It occurred in greater concentrations in the cells grown on "LB" medium than in the cultures grown in the water from the river enriched with glucose.

Microflora of the river Oława reached the maximum increase of biomass at the iron concentration of 1.5 mg/dm^3 and when "LB" medium was used as opposed to the microflora cultivated in the water with glucose where the biomass increase was bigger at concentration of iron amounting to 150 mg/dm^3. However, in both cases, the concentration of iron of 15 g/dm^3 inhibited entirely the growth of microflora. The greatest amount of metal

Table 2

Accumulation of heavy metals by microflora of overdeposit water in the river Oława examined in the "LB" medium according to Schaffer

Metal	Metal concentration in medium (mg/dm^3)	March		April		May	
		Dry weight of cells (g/dm^3)	Accumulation (mg/g d.w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)
Cr	0.05	6.61	0	5.19	0	6.28	0
	5.00	5.91	0	4.37	0	5.97	0
	500.00	0.00	0	0.00	0	0.00	0
Mn	1.50	6.35	1.06	5.40	0.67	5.75	0.34
	150.00	5.77	9.38	6.23	11.33	5.07	9.49
	15 000.00	2.38	10.15	0.95	14.23	1.03	10.01
Fe	1.50	6.36	0	5.05	0	5.79	0
	150.00	7.80	5.67	3.94	31.93	2.93	72.46
	15 000.00	0.08	0	0.10	0	0.52	0
Ni	0.05	6.64	0	5.72	1.15	6.51	0
	5.00	6.74	0	4.86	12.31	5.54	0.27
	500.00	2.36	0	1.99	0	1.36	0
Cu	0.01	5.88	0	5.56	0	6.03	0.11
	1.00	5.59	0.03	5.24	0.25	4.52	0.18
	100.00	6.95	1.86	5.25	1.12	4.50	1.91
Zn	0.05	6.16	0	6.04	0	5.71	0.04
	5.00	5.96	0.81	3.43	0.16	5.26	0.25
	500.00	5.89	10.52	3.22	10.01	5.13	15.52
Pb	0.05	6.27	0	4.30	0.08	3.88	0.84
	5.00	6.52	0	4.98	0.73	5.41	0.92
	500.00	6.69	0	5.22	1.97	4.21	1.98

Table 3

Accumulation of heavy metals by microflora of surface water of the river Oława examined in the "LB" medium according to Schaeffer

Metal	Metal concentration in medium (mg/dm^3)	March		April		May	
		Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)
Cr	0.05	6.40	0.00	5.46	0.00	6.70	0.00
	5.00	5.61	0.00	4.87	0.00	3.93	0.00
	500.00	0.00	0.00	0.00	0.00	0.00	0.00
Mn	1.50	5.31	0.15	5.52	0.18	5.79	0.67
	150.00	4.54	18.41	5.20	9.48	3.98	16.23
	15 000.00	1.98	19.01	0.26	12.07	3.55	16.48
Fe	1.50	5.14	0.00	5.39	0.66	4.87	0.00
	150.00	2.36	49.16	1.53	82.09	2.54	60.14
	15 000.00	0.17	0.00	0.07	0.00	0.49	0.00
Ni	0.05	7.04	0.00	5.92	0.00	5.01	0.00
	5.00	7.20	0.00	6.22	1.44	5.65	0.26
	500.00	2.60	0.00	1.71	0.00	1.38	0.00
Cu	0.01	4.99	0.06	6.15	0.34	5.95	0.13
	1.00	4.90	0.02	5.50	0.04	5.40	0.12
	100.00	6.37	1.05	5.37	0.55	4.92	0.48
Zn	0.05	5.77	0.06	5.01	0.00	5.17	0.05
	5.00	5.14	0.78	5.36	0.00	4.97	0.31
	500.00	4.94	9.13	6.08	5.12	4.08	8.93
Pb	0.05	6.31	0.006	5.54	0.00	5.06	0.32
	5.00	6.90	0.02	4.98	0.63	4.83	1.70
	500.00	6.94	0.15	4.90	0.95	4.13	1.95

Table 4

Accumulation of heavy metals by microflora of the river Oława examined in the overdeposit water enriched with glucose and metals

Metal	Metal concentration in medium (mg/dm^3)	March		April		May	
		Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)
Cr	0.05	0.12	0.00	0.15	0.00	0.28	0.00
	5.00	0.07	0.00	0.10	0.00	0.15	0.00
	500.00	0.00	0.00	0.00	0.00	0.00	0.00
Mn	1.50	0.03	0.81	0.08	1.44	0.31	0.37
	150.00	0.11	1.50	0.23	3.50	0.64	1.77
	15 000.00	0.08	2.01	0.10	4.11	0.11	2.15
Fe	1.50	0.16	0.00	0.50	8.01	0.28	10.66
	150.00	0.31	0.00	0.72	0.11	1.18	0.00
	15 000.00	0.00	0.00	0.00	0.00	0.00	0.00
Ni	0.05	0.12	5.33	0.14	0.00	0.22	0.00
	5.00	0.00	0.00	0.14	5.11	0.29	3.12
	500.00	0.00	0.00	0.03	0.00	0.10	0.03
Cu	0.01	0.13	0.00	0.17	0.11	0.94	0.00
	1.00	0.17	3.91	0.16	4.82	0.62	0.43
	100.00	0.25	4.11	0.23	4.90	0.60	3.13
Zn	0.05	0.13	1.10	0.14	4.45	0.24	0.07
	5.00	0.12	15.78	0.08	5.31	0.22	0.16
	500.00	0.10	16.81	0.07	6.14	0.20	1.48
Pb	0.05	0.08	0.00	0.33	0.00	0.31	0.00
	5.00	0.06	0.00	0.38	0.97	0.82	0.00
	500.00	0.20	0.85	0.41	1.05	0.81	0.00

Table 5

Accumulation of heavy metals by microflora of the river Oława examined in the surface water enriched with glucose and metals

Metal	Metal concentration in medium (mg/dm^3)	March		April		May	
		Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)	Dry weight of cells (g/dm^3)	Accumulation (mg/g d. w.)
Cr	0.05	0.08	0.00	0.33	0.00	0.18	0.00
	5.00	0.02	0.00	0.07	0.00	0.03	0.00
	500.00	0.00	0.00	0.01	0.00	0.03	0.00
Mn	1.50	0.03	0.91	0.09	0.37	0.09	1.97
	150.00	0.003	1.38	0.07	9.09	0.13	4.88
	15 000.00	0.00	0.00	0.05	10.11	0.10	3.18
Fe	1.50	0.08	0.00	0.76	7.85	1.33	1.71
	150.00	0.18	0.00	0.85	1.15	1.99	0.82
	15 000.00	0.00	0.00	0.00	0.00	0.04	0.00
Ni	0.05	0.10	5.58	0.10	11.11	0.07	0.00
	5.00	0.04	0.00	0.07	0.00	0.11	16.60
	500.00	0.00	0.00	0.05	0.00	0.05	0.00
Cu	0.01	0.11	0.00	0.24	0.55	0.09	0.00
	1.00	0.26	5.39	0.34	13.34	0.29	0.40
	100.00	0.36	5.91	0.48	15.04	0.35	4.28
Zn	0.05	0.15	8.03	0.12	6.04	0.07	0.00
	5.00	0.02	18.68	0.10	23.85	0.06	0.73
	500.00	0.01	19.13	0.09	22.13	0.05	1.13
Pb	0.05	0.16	0.00	0.38	0.00	0.22	0.00
	5.00	0.18	0.00	0.29	0.00	0.26	0.00
	5500.00	0.25	0.00	0.28	0.00	0.41	0.00

bound with microorganism cells was found in the media where concentration of iron was 150 mg/dm^3.

It was observed that the growth of microorganism biomass slowed down as concentration of nickel in the culture medium increased. The quantity of the biomass was larger in the "LB" medium than that obtained in the water with glucose. It could be easily noticed that nickel produced toxic effect on microflora when its concentration was 500 mg/dm^3. The quantity of nickel bound with the microorganism cells varied and it did not depend on its concentration in the medium.

Copper, occurring in any concentration, had in general no toxic effect on microflora, and no such effect was seen in the river Oława. A huge increase of biomass was noticed on the "LB" medium, in particular. The quantity of the accumulated copper depended mainly on its concentration in the medium and the type of the medium itself. Accumulation was not observed in bacteria cells at the copper concentration of 0.01 mg/dm^3; it occurred only at greater concentrations. Maximum values of biocumulation were obtained for copper concentration of 100 mg/dm^3. Microorganisms growing in water from the river bound more copper.

With the smallest concentration of zinc an increase of microorganism biomass was generally not limited and no accumulation of this metal was observed. However, an increase of zinc concentration brought about a limitation to the growth of microflora and increased the quantity of accumulated zinc. It was found that in the biomass of microflora cultivated in the presence of zinc in the "LB" medium there was less metal than in the cells growing in the water taken from Oława and enriched with glucose.

Neither lead nor copper limited the growth of microorganisms, no matter how big the concentration was. An increase of biomass was more noticeable in the case of the "LB" medium, compared to the case where water from the river served as a medium. The quantity of the bound metal increased with its concentration in a given medium. In most instances, when culture was grown in water taken from the river, no accumulation of lead by microflora was observed.

4. DISCUSSION

On the basis of the results obtained it can be stated that microflora of the river Oława shows the ability to grow in the presence of heavy metals whose concentrations in the culture medium are high. The influence of individual metals on microflora growth varied with their concentration. In the case of nickel and manganese, the microflora biomass increased only slightly with the highest concentration of these metals. On the other hand, the highest concentrations of zinc, copper and lead usually stimulated the growth of microflora. Similar observations were made in the case of using both culture media, but the increase of biomass obtained from the water of the river enriched only with glucose was much smaller than that recorded for the "LB" medium.

Microflora of the river Oława had the ability to accumulate all of the heavy metals tested, except chromium. The accumulation of metals depended, to a certain degree, on the culture medium applied. The cells grown in the water enriched with glucose usually

contained a greater amount of metal. It is likely that organic substances, being the components of the "LB" medium, bound the metals, this way making them less toxic.

The quantity of the metal accumulated by microorganisms usually increased with its concentration in the medium. When the lowest concentration was applied, metal accumulation was not observed or it was very small.

The quantity of the bound metal depended also on the type of water. Microflora of the overdeposit water was characterized in many cases by a higher ability of biocumulation of metal than the microflora of surface water. In a number of cases, the bounding of metals by microorganism cells was not observed, despite big increase of microorganism mass and high concentration of metal in the medium. The results obtained were also influenced by physical-chemical factors which are characteristic of surface waters taken in a given period.

From our investigation it is clear that metal biocumulation is a very complex phenomenon, which depends on both biological and physical-chemical factors. The fact that microorganism cells, being the components of the microflora of surface water, accumulate large quantities of metals is of particular significance in a situation where water is taken for domestic supply. There is a real danger of their liberation from microorganism cells which can happen during water treatment and may bring about its secondary pollution.

5. CONCLUSIONS

1. The microflora of the river Oława accumulated heavy metals such as manganese, iron, nickel, copper, zinc and lead.

2. Chromium added to the culture medium in the form of potassium dichromate was not accumulated by microorganisms.

3. The growth of microflora depended on metal concentration in the medium. Usually, with the increase of concentration the quantity of the biomass decreased.

4. High concentrations of chromium, nickel (500 mg/dm^3) and manganese and iron (15 g/dm^3) had toxic effect on microflora.

5. The increase of microorganism biomass was more significant in the "LB" medium, compared to the culture grown in the water from the river Oława.

6. In the majority of cases, microflora of the overdeposit water showed greater ability to accumulate heavy metals than microflora of surface water.

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BIOKUMULACJA WYBRANYCH METALI CIĘŻKICH PRZEZ NATURALNĄ MIKROFLORĘ WODY OŁAWY

Zbadano zdolność mikroflory Oławy do kumulacji metali ciężkich, takich jak chrom, mangan, żelazo, nikiel, miedź, cynk i ołów. Stwierdzono, że wszystkie badane metale, z wyjątkiem chromu, podlegają biokumulacji. Rozwój mikroflory zależy od stężenia metalu w podłożu hodowlanym. Zwykle w miarę wzrostu jego stężenia malała objętość uzyskiwanej biomasy. Najwyższe zastosowane stężenia chromu, niklu, manganu i żelaza działały toksycznie na mikroflorę, hamując całkowicie jej wzrost.

БИОКУМУЛЯЦИЯ ИЗБРАННЫХ ТАЖЕЛЫХ МЕТАЛЛОВ НАТУРАЛЬНОЙ ВОДЯНОЙ МИКРОФЛОРОЙ

Исследована способность микрофлоры Олавы к кумуляции тажелых металлов, таких как: хром, марганец, железо, никель, медь, цинк и свинец. Было установлено, что все исследуемые металлы, за исключением хрома, подвергаются биокумуляции. Развитие микрофлоры зависит от концентрации металла в питательной среде. Обычно, по мере роста его концентрации, уменьшался обзем получаемой биомассы. Самые высокие примененные концентрации хрома, никеля, марганца и железа влияли отрицательно на микрофлору, останавливая совсем ее развитие.