EFFECTIVENESS OF REMOVING HEAVY METALS BY BACTERIA FROM WATER SOLUTIONS

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The research on the effectiveness of removing heavy metals from water solutions by bacteria was carried out at the laboratory of the Institute of Environmental Engineering of the University of Zielona Góra in 2007-2008. The research covered bacterial strains isolated from sludge, soil and water. The tests concerned the effect of 3 heavy metals: Pb (0,1%), Cu (0,1%) and Zn (0,1%). The results prove that due to the presence of heavy metals, the size of colonies was much lowered in comparison with the control pan. Some of the strains, reader doesnt know numbers in the 0,1% concentration of Cu and Zn in the culture scale. It turns out that this concentration was completely hazardous with respect to the growth of those bacterial strains. Considering all the heavy metals under analysis, it was found that Cu had the most hazardous effect on the growth of 4 bacterial strains, then Zn and Pb. Under the influence of 4 bacterial strains, the content of the metals that after 7-day long incubation remained is different depending on metal and bacterial strain. Removing Heavy Metals by Bacteria from Water solutions Heavy Metals by Bacteria from Water solutions: Pb (34,38%), then Cu (29,69%) and Zn (21,88%). The content of the heavy metals removing by 4 strains was from 65,62% to 78,12%. The biggest amount removing was for Zn (78,12%) and the smallest for Zn (65,62%).

Keywords: bacteria, heavy metals

1. INTRODUCTION

Influence of metals on biomechanical processes of organisms is versatile and specific. Depending on their chemical properties, some heavy metals contribute significantly to the metabolism of microorganisms, for example bacteria, taking part in regulation of biomechanical processes, stabilization of cell structures or enzymatic reaction catalysis. Toxicity of heavy metals results not only from a degree of environmental pollution, but also from a biomechanical role they play

in live animals. Bacteria that exist in water or in waste waters are saprophytes in the majority. They are typically water or soil species. However, there also may exist species for which human or animal organism is a natural environment, and they have been disposed of to water together with household waste waters or waters. Water contamination – it is an unfavourable change in physical, chemical and bacteriological properties, which limit or make it impossible for water to use environmentally-isolated bacteria for absorbing heavy metals in water solutions. Aim of the study were the qualifications of removing heavy metals by bacteria from water solutions.

2. MATERIALS AND METHODS

When dealing with any microorganism in a laboratory, it is important to behave as if a particular organism was potentially dangerous. Therefore, it was crucial that the methods applied ensured no contact or that the contact with a microbe was indirect or minimum.

2.1. Sterilization of various materials used for tests

Selection of the method to be applied, namely physical or chemical, depends on type of the materials sterilized, for example, medium, various types of glass and other laboratory equipment [Nicklin et.al, 2004]: Preparation of the medium, preparation of agar slants and bacterial broth.

2.2. Preparation of the medium

The following components were used for preparing the medium: meat broth – 3 g, peptone – 5 g, agar – 20 g, distilled water – 1000 ml. The mediums were prepared in two flasks 500 ml of each and then sterilized following the tyndallization procedure, then they were left in a refrigerator in order to be used for identification and inoculation of bacterial strains [Rodina, 1968].

2.3. Preparation of agar slants and bacterial broth

Before preparing the agar slants, all the test tubes were sterilized at the temperature of $180^{o}C$ for 2 hours. After having been sterilized and cooled down, the test tubes were taken to the inoculation room. Then they were filled with the medium to the 1/3 of the tube volume and put on a slant board until they set. The set medium in the test tubes was then inoculated with clear bacterial strains and put into an incubator at the temp. of $30^{o}C$ for 48 hours. Then a broth solution of the bacterial strains under analysis was made following the procedure: 1 bacterial strain cultured in a test tube – was taken away from the tube and put into a sterilized solution containing 2 g of the meat broth, 2 g of yeast broth, 5 g of peptone – in 1000 ml of distilled water, then cultured for 24 hours at the

temp. 30°C, pH$_{KCl}$ - 7,5, [Microbiological mediums – catalogue, 2008, Nowak and et.al. 1995].

2.4. Preparation of scales containing the medium and concentrations of heavy metals and inoculating the bacterial culture

Inoculation samples were made following the rules of sterility, that means they was made in a room designed specifically for this purpose. All the equipment used for the inoculation procedure were sterilized. The sterile scale was filled with 1ml of the bacterial strain broth, 3-5 ml of the sterilized medium from the flask and 0,1% concentration of heavy metals (repeated twice for each bacterial strain). Then the contents were stirred thoroughly and left for setting, then put into a thermostat at the temperature of 30°C for 48 hours. After culturing it was necessary to count colonies growing on scales; this was to be done twice on the apparatus of bacterial colony meter, type: LKB-2002 and then the next procedure was to calculate the average number of colonies growing on scales for each bacterial strain under analysis [Little et al., 1988].

2.5. Bacterial strains

There were 4 bacterial strains used for the tests. Morphological characteristics of the cells and colonies of the bacterial strains under analysis are presented in table 1.

Table 1. Morphology of the cells and colonies of the bacterial strains

| Features of bacterial strains	Bacterial strains			
	1	2	3	4
Features of the colonies:				
- colony shape	Irregular	Irregular	Irregular	Sphecical
- profile	Flat	Flat	Flat	Protuberant
- edge	Serrate	Even	Corrugated	Corrugated
- colour	Creamy	White	Creamy	Yellow
- transparency	Non-transparent	Non-transparent	Non-transparent	Non-transparent
- structure	Non-ductile	Non-ductile	Non-ductile	Non-ductile
- diameter(mm)	20	14	15,5	16
Features of cells:				
- cell shape (simple method of colouring).	Rods	Rods	Bacilli	Spherical
- cell structure	Single (linked)	Single (linked into small chains)	Single	Single
- endospores	Gram + endospores are not clearly visible	pear-like (terminal)	Spherical (central)	Not visible
- Gram positive/negative		Gram negative	Gram positive	Gram positive

Explanation:

   (a): colonie of bacteria   (b): cells of bacteria
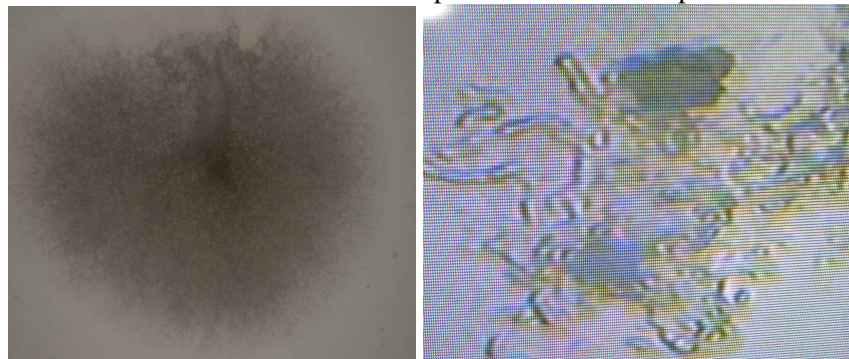
1. Bacterial rods isolated from the soil at the experimental station Lipki in Szczecin.
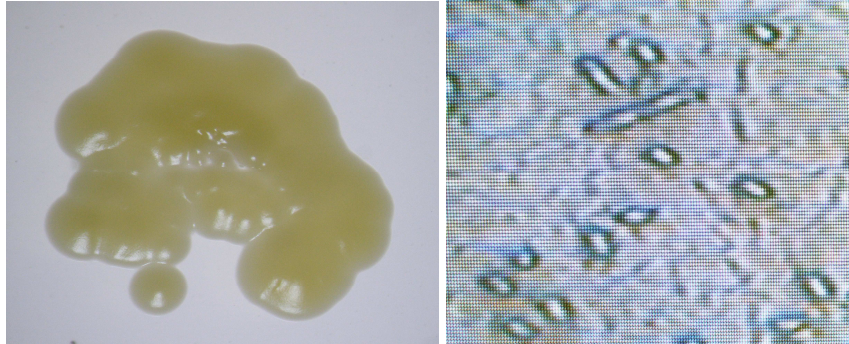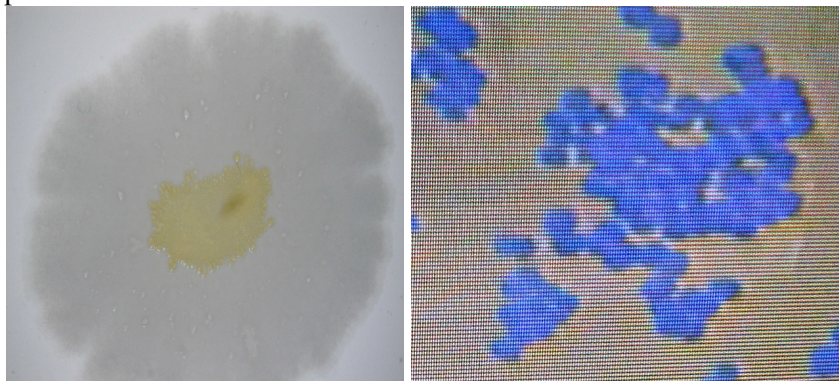

2. Bacterial rods isolated from the sludge taken from the waste water treatment plant in Łężyca.
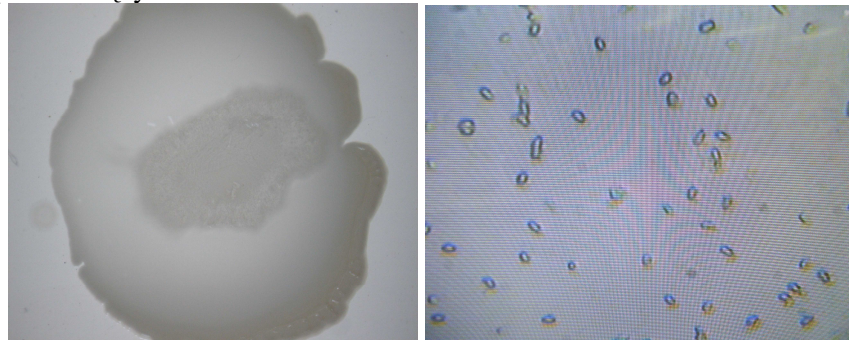
3. Bacilli isolated from the soil at the experimental station Lipki in Szczecin.

4. Spherical bacteria isolated from the water of the Rudziński Lake West Poland

2.6. Preparation of heavy metal concentrations and a liquid medium for the bacterial culture

For preparation of heavy metal concentrations (lead, copper and zinc) distilled water was used along with the following:
- lead nitrate $Pb(NO_3)_2 – 0,1\%$ (0,1 g in 100 ml of the distilled water),
- copper sulphate: $CuSO_4.5H_2O$ - 0,1%,
- zinc sulphate: $ZnSO_4 – 0,1$ %.

The liquid medium was prepared in cone flasks in the amount of 100 ml (repeated twice) for each bacterial strain. After having been sterilized and cooled down 1 ml of a bacterial strain broth was added to the flask with the medium along with 10ml of 0,1% concentration of heavy metals and then the flasks were put into the thermostat at the temperature of 30^oC for 7 days. After incubation it was necessary to determine the heavy metals concentrations in all the flasks. The procedure was carried out in an atomic Spectrophotometer SPECTRAA-10, Varian.

2.7. In concentration of heavy metals the flasks after the performance of the bacteria

Metal concentrations in solution is the direct result of AAS determination, which were calculated for all the flasks under analysis. The results were compared with the bacterial strains and control test (with no bacterial broth added). Basing on the results of the control test, the concentrations of the heavy metals left in the flasks were calculated. The results were carried out by calculating the confidence interval (the lowest significant difference) for the number of colonies and concentrations of heavy metals (two-factor laboratory tests [Drab, 2007]).

3. TEST RESULTS

3.1.   The results of tests on the influence of heavy metals on the size of bacterial colonies on scales

The tests concerned 3 heavy metals: Pb (0,1%): scales were labeled with Pb-1, Pb-2; Cu (0,1%): scales Cu-1 and Cu-2 and Zn (0,1%): scales Zn-1 and Zn-2 on 4 bacterial strains (table 1). There were two repeats made (the results are shown in table 2).

Table 2. Size of the colony of particular bacterial strains under the influence of heavy metals (colonies on scales)

Experimental trials	Bacterial strains				Comparison with the test control (100%)			
	1	2	3	4	1	2	3	4
Scales K-1 Scales K-2	197 210	180 149	176 280	210 181	-	-	-	-
Average K	**203,5**	**164,5**	**228,0**	**195,5**	100 %	100 %	100 %	100 %
Scales Pb -1 Scales Pb-2	7 9	10 7	9 11	13 10	-	-	-	-
Average Pb	**8,0**	**8,5**	**10,0**	**11,5**	**4**	**5,2**	**4,4**	**5,9**
Scales Cu-1 Scales Cu-2	3 2	0 0	0 0	2 1	-	-		-
Average Cu	**2,5**	**0**	**0**	**1,5**	**1,2**	**0**	**0**	**0,9**
Scales Zn -1 Scales Zn-2	2 4	1 1	0 0	2 3	-	-	-	-
Average Zn	**3,0**	**1,0**	**0**	**2,5**	**1,5**	**0,6**	**0**	**1,3**
LSD 0,5	**1,0**	**1,0**	**1,0**	**1,0**	-	-	-	-

Abbreviations: Scales K-1, Scales K-2, Petri control scales for 4 bacterial strains (1,2,3,4) in two repeats 1 and 2.

Scales Pb-1,scales Pb-2; experimental scales with addition of 0,1% of Pb in two repeats 1 and 2.

Scales Cu-1, scales Cu-2; experimental scales with addition of 0,1% of Cu in two repeats 1 and 2.

Scales Zn-1, scales Zn-2; experimental scales with addition of 0,1% of Zn in two repeats 1 and 2.

Average Pb, Cu and Zn: average size of the bacterial colony for the test on bacterial culture with addition of particular concentrations of heavy metals.

The results in table 2 show, that as affected by heavy metals in 0,1% concentrations (Pb, Cu and Zn) the sizes of the colonies of the four bacterial strains under analysis growing on the scales were much lower in comparison with the size of the colonies on the control test pans. There was no growth bacteria in the size of the colonies of the two strains 2 and 3 in the Cu concentrations of 0,1%. It turns out that this concentration had a hazardous

effect onto the growth of those bacterial strains. Zinc had a definitely hazardous effect onto the development of only the bacterial strain 3. Considering three heavy metals, it turned out that the most hazardous effect onto the growth of the four bacterial strains was first illustrated by Cu, then Zn and Pb as the least hazardous.

3.2. Determining heavy metal concentrations in the test flasks

The results in table 3 show, that the concentration of heavy metals in the flasks containing cultures of particular bacterial strains were lowered than those on the control test flasks. This depends on the strains of bacteria and the type of heavy metal, the concentration remaining in the solution can be to some degree differentiated. In the flask – Pb the lowest amount of the heavy metal was determined (0,015 g in 100 ml of the solution) with the bacterial strain 1. In the flask containing Cu the lowest amount of the heavy metals was in the flask with the culture of strain 2 (0,020 g in 100 ml of the solution). The concentration of Zinc was small in all the flasks containing the culture of bacteria 4 (from 0,015 g to 0,02 g in 100 ml of the solution).

Table 3. Concentration of the heavy metals that remained in the solutions after the period of the bacterial strain culturing (g·100 ml^{-1} of the solution)

Flasks of the bacteria under culture	Bacterial strains			
	1	2	3	4
Flask K-1	0,08	0,08	0,08	0,08
Flask K-2	0,08	0,08	0,08	0,08
(control without bacteria) **Average:**	**0,080**	**0,080**	**0,080**	**0,080**
Flask Pb-1	0,01	0,03	0,05	0,03
Flasks Pb-2	0,02	0,02	0,03	0,03
Average:	**0,015**	**0,025**	**0,040**	**0,030**
Flask Cu-1	0,02	0,01	0,02	0,03
Flasks Cu-2	0,03	0,03	0,05	0,02
Average:	**0,025**	**0,020**	**0,025**	**0,025**
Flask Zn-1	0,02	0,03	0,01	0,02
Flask Zn-2	0,02	0,01	0,02	0,01
Average:	**0,020**	**0,020**	**0,015**	**0,015**
LSD $_{0,05}$	**0,001**	**0,002**	**0,001**	**0,001**

Basing on the control test (with no addition of the bacterial strains), but under the same conditions as in case of the test with bacteria added, the contents of heavy metals remaining in the solution after the performance of the four bacterial strains were calculated (table 4).

Table 4. Contents of the heavy metals remained in the solutions

Bacterial strains	Contents of the heavy metals in the solutions (g/100 ml)			Ralative content as compared with the control test (%)		
	Pb	Cu	Zn	Pb	Cu	Zn
Control test	0,080	0,080	0,080	100%	100%	100%
1	0,015	0,025	0,020	18,75	31,25	25,00
2	0,025	0,020	0,020	31,25	25,00	25,00
3	0,040	0,025	0,015	50,00	31,25	18,75
4	0,030	0,025	0,015	37,50	31,25	18,75
Average content of the heavy metals remained in the solution (%)				**34,38**	**29,69**	**21,88**
Contents of the heavy metals absorbed by bacteria (%)				65,62	70,31	78,12

Under the influence of 4 bacterial strains in the solutions containing 3 heavy metals (Pb, Cu, Zn) in the initial 0,1% concentration, the contents of the heavy metals lowered depending on the bacterial strain. Considering 3 heavy metals under analysis the highest content was in the solution: Pb – 34,38%, then Cu - 29,69% and the last Zn – 21,88% of initial concentration. The amount of the heavy metals absorbed by bacteria is within the range of from 65,62% to 78,12% in comparison with the control test.

4. DISCUSSION

The amount of metal absorbed by microorganisms depends on its type, degree of oxidation, the size of atom radius, coordination number and the ability to bind in an organic compound structure. High concentrations can inhibit microorganism growth (Klimiuk and et.al., 2004). In practice, treatment of industrial waters requires adapting the conditions of industrial waters flow to the pace of microorganism growth in order to prevent the biomass to be flown away from the reactor. Bacterial strains in the treatment were used under aerobic conditions at the temperature of 30o C, thus they are mesophilic bacteria, aerobes (Vandenbergh et al., 1987).

The research work concerned the of 3 heavy metals: Pb (0,1%): scales designated as Pb-1, Pb-2; Pb (0,1%) : scales designated as Cu-1, Cu-2; Cu and Zn (0,1%): scales as Zn-1, Zn-2 4 bacterial strains (table 1). The test results show that the 0,1% concentration of Pb, Cu and Zn had an unfavourable effect onto the growth of the bacterial strains. The size of the colonies of the four strains were lowered significantly in comparison with the test scale. It turns out, that this concentration could have been too high and hazardous to the growth of those bacterial strains. In practice, we must ensure adequate medium for the processes of microorganism growth, due to the necessity of disposing of the metabolism products, because they may form complexes with the metals remaining in the solution which was causes that the quality of the products of

microorganism metabolism deteriorates. Thus they have a hazardous effect on their growth.

Effectiveness of removing metals under environmental conditions (for example in water solutions, on waste waters) is the basis for the research work on synthetic waste waters containing various heavy metals such as: copper, nickel, zinc, lead, chrome (Morper, 1986, Groenestun and et al, 1989). As a result of oxidation of reduced sulfuric compounds, bacteria produce huge amounts of sulfuric acid and show a peculiar adaptation to growth in the surroundings that have acid reaction. The research work analyzed single strains of bacteria for removal/disposal of a single heavy metal. In practice, on industrial scale, some mixes of different bacterial strains can be used for disposal of metals in waste waters. The results of the author's own research show, that depending on a particular strain of bacteria and the type of heavy metal, concentrations of the other metals that remained in the solution can vary. The results of the author's own research work presented in table 4 show that under the performance of 4 bacterial strains (1,2,3,4) in solutions containing 3 heavy metals (Pb, Cu, Zn) in the concentration of 0,1 % the content of the metals that remained in the solutions after 7 days of the test was from 21,88 % to 34,4% depending on the heavy metal. (from 0,015 g to 0,04 g in 100 ml of solution).

The mechanism of soliciting heavy metals, such as zinc or nickel is based on biotic oxidation Fe(II) to Fe(III), and then on abiotic-chemical oxidation of insoluble metal salts (sulfides) into soluble salts (sulfates) and elementary sulfur by iron (III) formed by bacteria (Ostrowski et al, 1996, Cheremisinoff, 1996). The bacteria oxidizing iron and sulfur are psychrophiles, mesophiles and thermophiles. In case of thermophiles the optimum temperature for the microorganism growth falls between 45-60oC. Use of thermophiles is most profitable due to the possibility of shortening the reaction time. In the research work the author used 4 strains of mesophiles. The process of the bacteria reaction lasts 7 days in culture solutions at the control temperature of 30oC. The content of the heavy metals absorbed by bacteria was from 65,6% to 78,12%. The calculation results show that the research was done at a high degree of confidence. The differences of the content of the heavy metals remained in the solutions were significant according of used strains of bacteria.

5. CONCLUSIONS

1. 0,1% concentration of heavy metals (Pb, Cu and Zn), causes the size of the colonies of 4 bacterial strains on the scales: 0,0-5,9%) in comparison with the amount on the control pans.
2. The most hazardous effect on the growth of 4 bacterial strains in test conditions was shown by Cu, then Zn and Pb.
3. The lowest concentration of the metals was found in the test probe with the bacterial strain 1 with addition of Pb and strains 3 and 4 with Zn (0,015 g in 100 ml of the solution).

4. The highest content (0,04 g in 100 ml of the solution) was in the probe with the bacterial strain 3 with addition of Pb.

5. The content of the heavy metals absorbed by 4 strains was from 65,62% to 78,12%. The biggest amount absorbed was for Zn (78,12%) and the smallest for Zn (65,62%), depending on the performance of particular bacterial strains in test trials.

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