EFFECT OF HEAVY METALS ON SELF-PURIFICATION PROCESSES IN RIVERS

J. MALA* – J. MALY

Brno University of Technology, Faculty of Civil Engineering, Department of Chemistry
17 Zizkova Street, 662 37 Brno, Czech Republic
(Phone: +420-541147637; fax: +420-541147667)

**Corresponding author*
e-mail: mala.j@fce.vutbr.cz

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Abstract. The inhibitory effects of heavy metals on self-purification processes in surface water were studied. Toxicities of the metals were evaluated on the grounds of the results of the Biochemical Oxygen Demand (BOD) test in river water. The BOD inhibitions depended on the concentrations of metals and on the incubation time. After one day's incubation, a full inhibition was found to be caused by Ag at all tested concentrations above 0.02 $mg.l^{-1}$ for Hg, Cd, Pb, and Cu above 1 $mg.l^{-1}$, for Be above 2 $mg.l^{-1}$, and for Co, Ni, and Sn^{II} above 5 $mg.l^{-1}$. A strong inhibitory effect ranging from 50 to 80% was observed for Cd (from 0.2 to 0.7 $mg.l^{-1}$), Cu (from 0.4 to 1.0 $mg.l^{-1}$), Be (from 0.5 to 2.0 $mg.l^{-1}$), Zn (from 1.2 to 5.0 $mg.l^{-1}$), Sn^{II} (from 2.2 to 4.2 $mg.l^{-1}$), and Cr^{VI} (above 4 $mg.l^{-1}$). As^{III}, As^{V}, Mn^{II}, Se^{IV} and Se^{VI} did not cause any BOD inhibition to 2 $mg.l^{-1}$ (As) res. 5 $mg.l^{-1}$ (other metals). The inhibitory effect of metals decreased significantly or disappeared with increasing incubation time. Being observed with all metals with toxic effects except for Be, Sn^{II}, and Cr^{III}, the decrease was more intense at the beginning of the incubation period.
Keywords: *self-purification, biochemical oxygen demand, heavy metals, toxicity*

Introduction

Heavy metals are toxic to the mixed culture of microorganisms responsible for the decomposition of organic compounds in surface waters. As aerobic biological processes are an essential part of the self-purification process occurring in surface water, heavy metals diminish the ability of such waters to purify themselves.

Our investigation focused on the evaluation of the toxic effect of heavy metals on the Biochemical Oxygen Demand (BOD) in surface water under the conditions of analytical BOD determination. The course of the BOD was evaluated during several days. The pollution with organic compounds made it possible to carry out the tests without a typical dilution of tested water with dilution water and without seeding or enrichment by other compounds except salts of metals.

Review of Literature

The toxic effects of heavy metals have been studied mostly with respect to the activated sludge [2, 10, 11].

The influence of metals on wastewater treatment evaluated by BOD or COD is, in comparison with the effect on BOD in surface water, considerably lower with respect to a higher concentration of biomass in the environment of a biological reactor. For example, concentrations not exerting any depression on the wastewater treatment efficiency are 0.1 mg.l^{-1} for Hg, 1 mg.l^{-1} for Cu, 2 mg.l^{-1} for Cd, Ni, Cr^{III}, and Cr^{VI}, 5 mg.l^{-1} for Pb, and 10 mg.l^{-1} for Zn and Sn [5].

Since the 1970s, several authors have investigated the inhibitory effect of metals on the BOD under diverse conditions and with the use of various substrates.

[7] chose incubation periods of 5 and 14 days, [9] measured the BOD course for 10 days, and [1, 6, 8] did so for 5 days. The first two investigators used sewage as the substrate, while [8] used a peptone solution, [6] a milk waste, and [1] a river water. [4] tested the influence of Cd on the BOD in solutions of glucose/glutamic acid and glucose/phenol. Sewage was usually used for seeding; only [8] used a culture of *A. hydrophila* strain and [1] tested unseeded samples. The results presented by various authors are often very different (*Table 1*), a fact that can be explained by different test conditions.

Table 1. *Inhibitory effects of selected heavy metals on BOD_5 (concentrations in mg.l^{-1})*

Metal	**Mowat (1976)**			**Stones (1991)**	**Schubert (1980)**			**Mittal *et al.* (2000)**			**Berkun (2005)**		
	BOD inhibition, %												
	20	**50**	**80**	**50**	**20**	**50**	**80**	**20**	**50**	**80**	**20**	**50**	**80**
Ag	0.025	0.18	>10	>0.25	0.01	0.05	1						
Cd	1.2	4.5	>20	> 5	1.0	8	>10						
Co	1.2	5.0	>20					0.5	15				
Cr^{III}	1.6	6.0	14	2.5							0.4	0.6	0.9
Cr^{VI}											0.08	0.14	0.2
Cu	0.2	3.0	18.5	2.5	1.0	3	>10	2.0			0.3	0.7	1.3
Hg	0.07	0.4	0.6	>0 .5	0.1	1	5			0.1	0.05	0.15	0.3
Ni	0.9	7	>20	> 5	1.4	2.6	5	0.5	1.0	10	0.3	0.5	0.8
Pb				5	>10			5.0					
Sn^{II}	12	24	>40										
Zn	6	>40		>25	2.6	5	10	5			0.3	0.8	2.8

Materials and Methods

The evaluation of the metal influence was based on the results of the BOD test [3].

The measurements were carried out in river water taken from the Svratka River about 1 km downstream from the Brno (Czech Republic) water reservoir. Suspended impurities were separated by filtration through gauze. The average water composition is given in *Table 2*.

Table 2. *Average composition of river water*

Basic components		**Heavy metals**	
pH	7.9	Al, mg.l^{-1}	0.06
Total residue, mg.l^{-1}	246	As, mg.l^{-1}	0.00
Loss on ignition, mg.l^{-1}	89	Cd, mg.l^{-1}	0.00
COD_{Cr}, mg.l^{-1}	5.4	Cr, mg.l^{-1}	<0.03
$N\text{-}NH_4$, mg.l^{-1}	0.47	Cu, mg.l^{-1}	<0.01
$N\text{-}NO_2$, mg.l^{-1}	0.05	Fe, mg.l^{-1}	0.06
$N\text{-}NO_3$, mg.l^{-1}	3.25	Mn, mg.l^{-1}	0.06
N_{tot}, mg.l^{-1}	3.77	Ni, mg.l^{-1}	<0.02
BOD_5, mg.l^{-1}	3.29 ± 1.00	Pb, mg.l^{-1}	<0.05
O_2, mg.l^{-1}	8.0 ± 0.7	Zn, mg.l^{-1}	0.06

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The BOD tests were conducted immediately after the collection of samples and the temperature adjustment. Oxygen bottles with volumes of about 300 ml were filled with the samples and incubated at a temperature of 20 ± 1 °C in the thermostat. The concentration of dissolved oxygen was measured at the beginning and then after 1, 2, 3, 4, and 7 days of incubation by means of a WTW Oxi3000 microprocessor oximeter. The sensitivity of the oxygen determination was 0.01 mg.l – 1. Curves showing the dependence of the BOD on the incubation time and the inhibition of the BOD on the incubation time were plotted.

Three to four parallel blank tests (water without addition of metals) were carried out for each metal or group of metals. The standard deviations of the average BOD values after several representative incubation times are presented in *Table 3.*

Table 3. *Standard deviations of the average BOD values*

Incubation time, days	Average BOD, $mg.l^{-1}$	Std. deviation, %	Std. deviation, $mg.l^{-1}$
1	0.99	10 ± 8	0.10 ± 0.08
4	2.76	5 ± 3	0.14 ± 0.11
7	3.90	4 ± 3	0.16 ± 0.12

Metallic compounds were fed into river water as aqueous solutions prepared from chemicals specified in *Table 4* and distilled water. The accurate concentrations of metals in the stock solutions and in river water were determined by AAS. Three parallel tests with one to three different compounds of each metal were usually carried out. The average results are presented in this paper.

Table 4. *Overview of chemicals used to prepare the test solutions*

Metal	Compound
Ag	$AgNO_3$
As^{III}	$NaAsO_2$
As^{V}	$Na_2HAsO_4.7H_2O$
Be	$BeSO_4.4H_2O$
Cd	$CdCl_2.H_2O$, $Cd(NO_3)_2.4H_2O$, $CdSO_4.H_2O$
Co	$CoCl_2.6H_2O$
Cr^{III}	$Cr(NO_3)_3.9H_2O$, $KCr(SO_4)_2.12H_2O$
Cr^{VI}	$K_2Cr_2O_7$
Cu	$CuCl_2.2H_2O$, $Cu(NO_3)_2.3H_2O$, $CuSO_4.5H_2O$
Hg	$HgCl_2$
Ni	$NiCl_2.6H_2O$, $Ni(NO_3)_2.6H_2O$, $NiSO_4.6H_2O$
Pb	$PbCl_2$, $Pb(NO_3)_2$
Se^{IV}	Na_2SeO_3
Se^{VI}	$Na_2SeO_4.10H_2O$
Sn^{II}	$SnCl_2.2H_2O$
Zn	$ZnCl_2$, $ZnSO_4.7H_2O$
Ag	$AgNO_3$

Results

The results obtained after one day's incubation, representing a stage of a shock charge, are presented in *Table 5*. The interpolated results obtained after five days' incubation, representing a stage of bacterial adaptation, are summarised in the same table.

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Table 5. *Inhibition effects of metals on the BOD*

Metal, mg.l^{-1}	**0.02**	**0.1**	**0.2**	**0.4**	**1.0**	**2.0**	**5.0**
	BOD inhibition, %						
Incubation time of 1 day							
Ag	89	100	100	100	100		
As^{III}			–14	–4	–2	–2	
As^{V}			–7	–7	1	19	
Be		41	49	49	55	74	
Cd		37	50	67	88	91	98
Co		13	13	13	47	63	83
Cr^{III}		–8	–4	11	17	24	33
Cr^{VI}		0	1	8	24	36	68
Cu		27	40	63	78	100	100
Hg^{II}	–5	3	9	32	100	100	
Mn^{II}			0	–4	1	–1	8
Ni		–7	2	19	42	69	87
Pb			0	0	88	100	100
Se^{IV}		–17	–11	–9	–12	–2	9
Se^{VI}		–16	–12	–14	–12	–8	–10
Sn^{II}		–5	5	1	25	45	92
Zn		0	17	32	48	57	73
Incubation time of 5 days							
Ag	15	82			83		
As^{III}			–14	–4	–3	–2	
As^{V}			–6	–3	–7	9	
Be		24	35	39	65	90	
Cd		2	3	14	22	33	46
Co		8	15	5	36	36	48
Cr^{III}		–6	0	16	20	28	40
Cr^{VI}		4	7	9	16	19	34
Cu		–2	18	35	46	63	100
Hg^{II}	–5	6	4	44	55	66	
Mn^{II}			–2	–5	–8	4	6
Ni		0	4	17	30	43	52
Pb		0	0	19	24	43	60
Se^{IV}		–5	–5	–2	–3	5	9
Se^{VI}		–7	–3	–5	–6	–2	1
Sn^{II}		3	9	5	32	45	83
Zn		–11	–1	5	15	34	45

no effect
inhibition effect
strong inhibition effect
total inhibition – toxicity

Dependences of the BOD and of the BOD inhibition related to the blank samples on the incubation time are similar for all tested metals. Typical curves relating to Ni are exemplified in *Fig. 1* and *Fig. 2*.

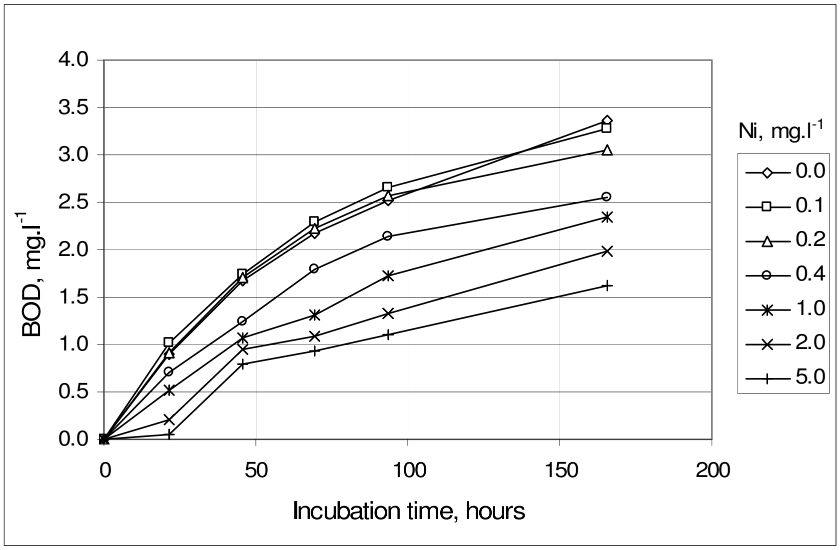

Figure 1. *Dependence of the BOD on the incubation time for Ni*

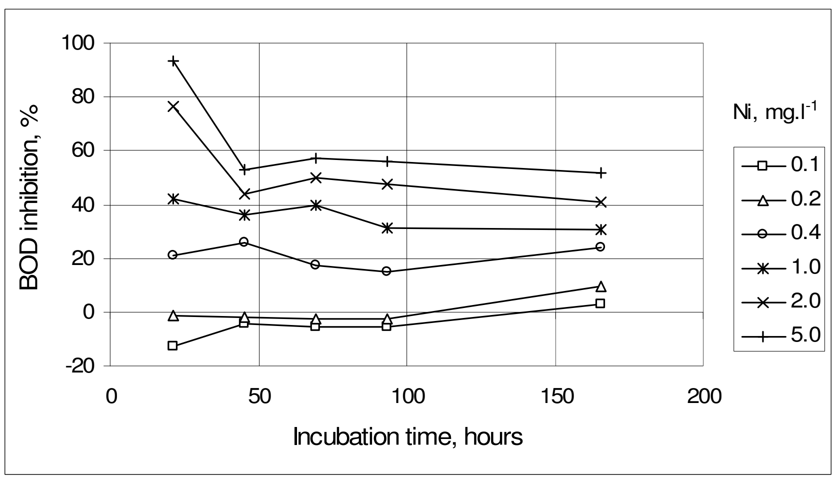

Figure 2. *Dependence of the BOD inhibition on the incubation time for Ni*

Discussion

The concentration of ammonia-nitrogen in river water was low: 0.47 mg.l^{-1} on average (*Table 2*). It was proved that the growth of nitrification bacteria was not significant and that the BOD in the tested samples was caused by the decomposition of organic matter by microorganisms.

The inhibition effects of metals on BOD values depended on their concentrations and on the incubation time. They decreased significantly or disappeared with the increasing incubation time (*Figs. 1* and *2*). The decrease was more intense at the beginning of the incubation time. It was observed with all metals with toxic effects except Be, Sn^{II}, and Cr^{III}. This phenomenon can be primarily explained by a partial adaptation of the mixed culture of microorganisms and by the fact that the more resistant organisms overgrew

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the sensitive ones. Physical and chemical processes (precipitation, complex formation, sorption, etc.) also cannot be excluded.

In the case of some heavy metals, after exceeding a certain concentration in the tested medium, the inhibition effect ranged between 80 and 100%, where the decomposition of organic matter is supposed to be fully inhibited (*Table 5*).

After one day's incubation, the full inhibition was found for Ag at concentrations above 0.02 $mg.l^{-1}$, for Hg, Cd, and Pb above 1 $mg.l^{-1}$, for Cu above 2 $mg.l^{-1}$, and for Co, Ni, and Sn^{II} above 5 $mg.l^{-1}$. A strong inhibition effect ranging between 50 and 80% was reported for Cd (from 0.2 to 0.7 $mg.l^{-1}$), Cu (from 0.4 to 1.0 $mg.l^{-1}$), Be (from 0.5 to 2.0 $mg.l^{-1}$), Zn (from 1.2 to 5.0 $mg.l^{-1}$), Sn^{II} (from 2.2 to 4.2 $mg.l^{-1}$), and Cr^{VI} (above 4 $mg.l^{-1}$). As^{III}, As^{V}, Mn^{II}, Se^{IV}, and Se^{VI} did not cause BOD inhibition in the concentrations up to 2 $mg.l^{-1}$ (for As) and 5 $mg.l^{-1}$ (for other metals).

After five days' incubation, a full inhibition occurred with the additions of Ag (above 0.1 $mg.l^{-1}$), Be (above 1.5 $mg.l^{-1}$), and Sn^{II} and Cu (above 5.0 $mg.l^{-1}$). A strong inhibition was observed with the additions of Hg at about 1.0–2.0 $mg.l^{-1}$ and with additions of Pb and Ni at 2.5–5.0 $mg.l^{-1}$.

Differences were found between Cr^{III} and Cr^{VI}. The inhibition effect of Cr^{VI} on the BOD after one day's incubation was greater than that of Cr^{III}, while after a prolonged period of incubation the converse was true (*Table 5*). The compounds of As^{III} - As^{V} and Se^{IV} - Se^{VI} were not inhibiting in the tested concentration range and therefore their potentially variant influence could not be determined.

Negative values of inhibition observed in some tests mean a stimulation effect (*Table 5*). As these values did not exceed 20%, they were supposed to lie in the range of measurement deviations. The stimulating effect of zinc at concentrations of about 0.05 $mg.l^{-1}$ (about 10%) is in agreement with the statements made by [6].

They reported a stimulating effect of zinc typically less than 20% for concentrations from 0.1 to 1.0 $mg.l^{-1}$. The data are not yet sufficient to unambiguously prove the stimulating effect of Zn.

The increase in the inhibition effect with the increase in the concentration of a metal is not identical for all metals at a given incubation time. It implies that the row of metals sequenced according to their decreasing inhibition effect changes in some cases with the concentration of the particular metals and with the incubation time. The sequence of metals according to the concentration at which the inhibition effect exceeded 50% after one day's incubation was, for example, Ag – Cd – Cu – Be – Hg – Pb – Zn – Co – Ni – Sn^{II} – Cr^{VI}, while for the 80% inhibition effect it was Ag – Cd – Hg – Pb – Cu – Ni – Sn^{II} – Co. The sequence of metals according to the rising concentration at which a 50% inhibition was reached after five day's incubation was Ag – Be – Hg – Cu – Sn^{II} – Pb – Ni. Metals not included in the sequences did not cause 50% or 80% inhibition in the tested concentration range.

The BOD course of the blank samples suited the kinetics of the first order. The addition of a metallic salt caused a deformation of the BOD-curve. Lag-phases could be observed for the following heavy metals: one day for Ag (at 0.01 $mg.l^{-1}$), Pb (at 0.6 $mg.l^{-1}$), and Ni (at 2 $mg.l^{-1}$), and two days for Ag (at 0.4 $mg.l^{-1}$), Cd (at 2 $mg.l^{-1}$), and Sn^{II} (at 5 $mg.l^{-1}$). After the lag period, oxidation started rapidly but then decreased again so that the resulting oxidation curve had a sigmoidal shape (*Fig. 1*).

The inhibition effects of the different salts (sulphates, chlorides, nitrates; *Table 4*) of the tested metals were evaluated. Standard deviations of the data relating to a certain metal, concentration, and incubation time were calculated. The standard deviations decreased slightly with increasing metal concentrations and incubation time. However, the standard deviation was small: 3.2% on average and 10.7% maximum. Thus, it can be stated that in the cases of Cd, Cr^{III}, Cu, Ni, Pb, and Zn the use of different salts of the tested metals did not develop different BOD inhibitions.

In some cases, a comparison of our results with those found by other authors showed a good agreement for certain concentrations of metals but not for the whole concentration range. In exceptional cases, considerable differences were found in the whole concentration range.

Our results for five-day incubation time are comparable with those reported by [7]. The inhibitions by a great number of metals (Ag, Cd, Cu, Ni, Co, Cr^{III}) were very similar. Our experiments documented a somewhat lower toxicity of mercury, while greater differences were found for zinc and tin. While [7] reported 20% inhibition at concentrations of 6 $mg.l^{-1}$ Zn or 12 $mg.l^{-1}$ Sn^{II}, our experiments reported the same inhibition already at a concentration of about 1 $mg.l^{-1}$.

[4] reported a considerably smaller effect of cadmium in comparison with our and Mowat's results. For example, at the concentration of 6.1 $mg.l^{-1}$ Cd determined for two different substrates, the BOD inhibitions were only 14.9 and 26.5%. The differences can be explained by the use of easily degradable substrates by [4].

In comparison with our experiments, [6] reported inhibition effects that were similar for nickel and cobalt but higher for mercury after incubation for five days. These authors reported a quite anomalous behaviour for copper. They found a BOD inhibition ranging from 20 to 50% for concentrations of copper between 2 and 10 $mg.l^{-1}$. Above this concentration, the inhibitory effect decreased. In our experiments, the inhibitory effect of copper was determined at concentrations from 0.4 to 1.0 $mg.l^{-1}$ Cu. These results indicate among others also the influence of the substrate quality, which affects the composition of the mixed bacterial population.

Berkun's experimental conditions [1] were similar to ours. After five days' incubation, he reported similar concentrations causing 20% BOD inhibitions for Cu and Ni. However, in the cases of other metals, Berkun found greater inhibitory effects and less steep dependance of the BOD inhibition effect on metal concentration. He also found a greater inhibition effect of Cr^{VI} than of Cr^{III} after five days' incubation. The BOD of river water used in Berkun's experiments was higher than in ours (5.0 to 14.1 $mg.l^{-1}$). Other data on the river water composition ($N\text{-}NH_4$, metals, other toxic compounds, etc.) were not available.

In comparison with concentrations of metals in surface waters, the concentrations of metals that have an inhibitory effect on the BOD are considerably higher. This indicates that heavy metals in natural waters with no extreme pollution do not influence the rate of microbial decomposition of organic matter, thus having no impact on self-purification processes. But the accumulation of metals in biomass and the hazards for the end element of the food chain (humans) cannot be omitted.

Conclusions

- It was found that the inhibitory effects of heavy metals on the self-purification process started at much higher concentrations of metals than those typically found in surface water.
- The inhibitory effect of BOD depends on the concentration of metals and on the incubation time.

- The toxic effects of heavy metals typically decreased with the increasing incubation time. This effect was not observed with Be, Sn^{II}, or Cr^{III}.
- Metals that showed high toxicity (above 50% inhibition at concentrations below 1.0 $mg.l^{-1}$) after incubation for one day included Ag, Be, Cd, Cu, Hg, and Pb, while Co, Ni, and Zn caused an inhibition of about 40–50% at this concentration, and inhibitions by Sn^{II}, Cr^{III}, and Cr^{VI} were low.
- Ag and Be showed high inhibition effects even after five days' incubation. The effects of Hg and Cu were also considerable.
- As, Se, and Mn did not show any inhibitory effect up to concentrations of 5.0 $mg.l^{-1}$.
- A lag-phase appeared in the BOD-curves affected by Ag, Cd, Ni, Pb, and Sn^{II}.
- None of the tested heavy metals had an evidently stimulating effect.
- It was found that the inhibition effect depended considerably on the quality of the medium in which the test was performed. The toxicity measured in river water is mostly higher than that measured in wastewater. Thus, the study would seem to propose the use of surface water for the evaluation of the effects of toxic compounds on the self-purification processes.

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