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STUDY OF THE TOXICOLOGICAL IMPACT OF DIFFERENT COMPONENTS OF ASH-HEAP WATER (SULPHUR RICH PHENOLIC LEACHATE) USING LUMINESCENT BACTERIA AS TEST ORGANISMS

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The chemical composition and the toxicity of sulphur rich phenolic leachates (ash-heap water; AHW) was determined and impact of its main components (phenolic and sulphuric compounds, heavy metals) and pH to the net toxicity of AHW was estimated. According to the analysis and calculations the toxicity of AHW was mainly caused by its phenolic and sulphuric compounds whereas the main contributors were p-cresol (58 % of the toxicity), sulphide (22 %), 3,4-dimethylphenol (8.5 %) and phenol (5.6 %). The toxicity of AHW and its components was analyzed using Photobacterium phosphoreum - based BioTox™ *test.*

Introduction

Oil shale is among the most important natural resources in Estonia being widely used in the chemical industry as well as in the production of energy. The pollution of the environment caused by the oil-shale industry is one of the major ecological problems for Estonia. Every year approximately 1.35 million tons of solid waste (ash formed in the retorting process of oil-shale) is deposited in big spent shale piles - ash heaps. The leachates from the ash-heaps (so-called ash-heap water) (3000-8000 m^3/day) are discharged without treatment through rivers of Kohtla and Purtse directly to the Gulf of Finland [1-4]. The ash-heap water (AHW) is characterized by high pH (pH = 10-12), high concentration of phenolic (up to 500 mg/L) and sulphuric compounds (mainly in the form of sulphates, up to 1300 mg/L) and its composition varies according to the weather conditions [1]. The AHW is not purified

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in the local biopurification facilities due to its (supposed) high toxicity to the activated sludge. In our previous paper [5] we analyzed the phenolic composition of AHW and determined the toxicity of AHW towards photobacteria (using BioTox™ and Microtox™ tests) and activated sludges, and showed that the biopurification of the AHW could be feasible.

The toxicity of complex mixtures (e.g., AHW) depends on the individual toxicities of its components and their concentration in the mixture. Also, the individual components in the complex mixture can exhibit antagonistic or synergistic effects (i.e. the overall toxicity of the mixture could be lower or higher, respectively, than expected by the summed toxic effects of its individual components).

In this work an attempt was made to take into account all the potential toxicants of the AHW (e.g., phenolic and sulphuric compounds, heavy metals) and to estimate their share in the net toxicity of AHW. Luminescent bacteria (BioTox™) were used for the toxicity testing. For the calculations an assumption was made that the toxic effects of individual components in the mixture were additive.

Materials and Methods

Sampling of AHW

AHW for this study was sampled from the equalization basin of AHW on the territory of "Kiviter" Ltd. (Kohtla-Järve, Estonia) on 07.03.95. AHW (pH = 10) was stored in glass bottles at +4 °C in the dark.

Chemical Analysis of AHW

The HPLC and GC-MS analysis of phenolic composition of AHW was ordered from Central Laboratory of Environmental Research (Tallinn, Estonia). Heavy metals from AHW were kindly determined by Dr. A. Viitak (Tallinn Technical University, Estonia) using atomic adsorption spectroscopy (AAS). Total sulphur content in AHW was determined gravimetrically after oxidation of all sulphur compounds to sulphate and precipitation by barium chloride [6]. Sulphide, sulphite and thiosulphate were determined iodometrically [6] as described by Lure and Rõbnikova [7]. The concentration of sulphate was calculated as a difference between the total sulphur and the sum of sulphide, sulphite and thiosulphate.

Chemicals

Phenol, *o*-, *m*- and *p*-cresol and 3,4-dimethylphenol were purchased from Merck; 2,6-dimethylphenol and 2,3-dimethylphenol from Ferak.

Na_2S, Na_2SO_3, Na_2SO_4 and $Na_2S_2O_3$ were of Russian origin (purity: chemically clean). $Na_2S \cdot 9H_2O$ was semi-aqueous solution and concentration of Na_2S in it was determined iodometrically [6].

Toxicity Testing

The BioTox™ reagent was reconstituted and measurement of toxicity was performed essentially as described by us previously [8-9]. Control samples (i.e., bacterial suspensions to which 2 % NaCl was added instead of a test chemical) were always run parallel to the test sample. Tests were performed at 15 °C. As the pH of all dissolved chemicals and diluted AHW analyzed in this study was 5-8, it was not adjusted. The concentration of the toxicant (mg/L) which caused a 50 % reduction in light (INH % = 50 %) after exposure for 5 minutes was designated as the 5-min EC 50. In the case of AHW the concentration of AHW in the test (%) which caused a 50 % reduction in light (INH % = 50 %) after exposure for 5 minutes was designated as the 5-min EC 50.

Results and Discussion

Table 1 characterizes the ash-heap water (AHW) sampled for this study. The more detailed information on the concentrations of individual phenolic and sulphuric compounds is presented in Table 2.

Table 1 shows that the toxicity of AHW could be caused by high concentration of phenolic and sulphuric compounds, presence of heavy metals and also by high pH. However, the AHW had very low buffering capacity: already after 3-fold dilution with deionized water the pH of the AHW was neutral (pH = 7). Hence, already after minor dilution the toxic effect of pH of the AHW could be excluded.

Table 1. **Characterization of the Ash-Heap Water (AHW)**

Parameter	Method of analysis	Value or characterization
Colour	Visual	Brownish
Smell	Organoleptic	Phenols and sulphur
pH	pH-meter	10
COD	Potassium bichromate	3070 mg O_2/L
Total heavy metals*1	AAS	0.5 mg/L
Total phenolic compounds*2	HPLC	194.9 mg/L
Total sulphur compounds*2	Total sulphur compounds - gravimetrically; Sulphide, thiosulphate and sulphite - iodometrically	2400 mg/L
Toxicity (5-min EC 50)	BioTox™ test	1.5 %

Notes: *1 mg/L: Fe - 0.4; Mn - 0.08; Zn - 0.02; Cu - 0.01; Cr - 0.02; Ni - 0.001; Co - 0.001; Mo - 0.001; Pb - 0.003; Cd < 0.0003 and Hg < 0.001.
*2 See also Table 2.

Table 2. **Concentration and Toxicity of Phenolic and Sulphuric Compounds Found in the Ash-Heap Water Sampled for this Study**

Component of the AHW	Concentration in the AHW		Toxicity according to BioTox™ test		
	mg/L	%	5-min EC 50 of the pure compound, mg/L (BioTox™)	Theoretical impact (TI) of the individual component to the net toxicity of AHW	Relative theoretical impact (RTI), %
	A	A, %	B	A/B	A/B, %
Phenolic compounds					
Phenol	84.1	3.2	107.4	0.8	5.6
p-Cresol	70.4	2.7	8.7	8.1	58.0
m-Cresol	17.2	0.7	108.2	0.2	1.1
o-Cresol	8.5	0.3	63.1	0.1	1.0
3,4-Dimethylphenol	7.7	0.3	6.5	1.2	8.5
2,6- Dimethylphenol	4.4	0.2	41.8	0.1	0.7
2,3- Dimethylphenol	2.8	0.1	25.5	0.1	0.8
Sulphuric compounds					
SO_4^{2-}	1676.0	64.5	11155*1	0.2	1.1
$S_2O_3^{2-}$	705.4	27.1	3777*2	0.2	1.3
S^{2-}	22.2	0.9	7.3*3	3.0	22
SO_3^{2-}	0	0.0	3383*4	0.0	0.0
Total	2599	100		14.0	100

Notes: *1 determined as Na_2SO_4.
*2 determined as $Na_2S_2O_3$.
*3 determined as Na_2S.
*4 determined as Na_2SO_3.

The toxicological impact of a certain chemical could be determined by calculating its TI value (TI = theoretical impact):

$$TI = [C]/EC\ 50$$

where [C] - concentration of the chemical, mg/L
EC 50 - toxicity of this chemical (mg/L), determined using a biotest (in our case, BioTox™ test)

By calculating the TI values for heavy metals (the respective EC 50 values were taken from our previous articles [8-9]) it was shown that the concentration of heavy metals in the AHW was too low to cause toxicity: the sum of the TI values of heavy metals in the AHW was 0.1 (data not shown) that is very small compared by to the sum of TI values of phenolic and sulphuric compounds in the AHW (Total TI = 14.0; Table 2). Hence, also the impact of heavy metals to the overall toxicity of the AHW could be considered negligible.

Table 2 shows that AHW contained altogether 7 different phenolic compounds whereas the most abundant components were phenol (84.1 mg/L) and *p*-cresol (70.4 mg/L). The total amount of phenolic compounds was 194.9 mg/L (Table 1). All the phenols detected were monohydroxyphenols. The absence of dihydroxyphenols in the current sample of AHW (proven also by GC-MS; data not shown) is a bit unusual for AHW that has usually shown to contain also dihydroxyphenols (e.g., resorcinols) [1]. However, the prevalence of monohydroxyphenols over dihydroxyphenols in AHW has also shown by Munter *et al.* [2] and Tuhkanen [10] and, as mentioned above, the chemical composition of the AHW could be dependent on the season and the amount of atmospheric precipitation [1]. Also, it is quite peculiar, that the amount of *p*-cresol in the AHW sampled for this study was higher than that of *m*-cresol (Table 2), as in the oil shale process water (used for compacting of the ash on the spent shale piles) the amount of *m*-cresol exceeds that of *p*-cresol [11].

From the sulphuric compounds (altogether 2400 mg/L; Table 1) the most abundant was sulphate (1676 mg/L) followed by thiosulphate (705 mg/L) and sulphide (22 mg/L). Sulphite was not found (Table 2). If the data on phenolic and sulphuric compounds were summarized then the results were following: the highest impact by weight (%) had sulphate (65 %), followed by thiosulphate (27 %), phenol (3.2 %) and *p*-cresol (2.7 %) (Table 2). Hence, at the first glance it could be supposed that the main ecological problems connected with the AHW are caused by these four chemicals. However, from the point of view of environmental protection the ultimate goal is to avoid/reduce the toxicity. Hence, both, the amount and the toxicity of respective waste should be taken into account.

The most toxic compounds according to the BioTox^{TM} test were 3,4-dimethylphenol (5-min EC 50 = 6.5 mg/L), followed by sulphide (5-min EC 50 = 7.3 mg/L) and *p*-cresol (5-min EC 50 = 8.7 mg/L) (Table 2). In Table 2 the theoretical impact (TI) and relative theoretical impact (RTI) of every phenolic and sulphuric component to the net toxicity of the AHW is calculated. As mentioned above, in our calculations additive toxicity of individual components of the mixture was assumed. According to these calculations (Table 2) the highest toxicological impact to the net toxicity of AHW had *p*-cresol (58 % of the toxicity of AHW), followed by sulphide (22 %), 3.4-dimethylphenol (8.5 %) and phenol (5.6 %). Hence, due to the high toxicity (5-min EC 50 = 6.5 mg/L) the toxic impact of 3,4-dimethylphenol was higher than that of phenol despite of the fact that phenol was present in AHW in 10-times higher concentration than 3,4-DMP. Analogously, concentration of sulphide in AHW was 75-fold lower than that of sulphate, but due to the high toxicity of sulphide compared to sulphate the toxicological impacts of these sulphur compounds were 22 % and 1 %, respectively (Table 2).

Table 3 summarizes the results of this work: 75 % of the ecotoxicological risk of the AHW could be incriminated to phenolic compounds (mainly to *p*-cresol, 3.4-dimethylphenol and phenol) and 25 % to the sulphuric compounds (mainly to sulphide).

Table 3. **The Analysis of Impact of Different Factors to the Toxicity of AHW Sampled for this Study (Data are Summarized from Tables 1 and 2)**

Component that could cause toxicity of AHW	Value or amount	Impact to the net toxicity of AHW (according to BioTox^{TM} test)
pH	10	Negligible
Heavy metals	0.5 mg/L	Negligible
Phenolic compounds:	195 mg/L	75 %
The highest impact on weight basis, mg/L:		The highest impact on toxicity basis, %:
Phenol - 84		*p*-Cresol - 58
p-Cresol - 70		3,4-Dimethylphenol - 8.5
		Phenol - 5.6
Sulphuric compounds:	2400 mg/L	25 %
The highest impact on weight basis, mg/L:		The highest impact on toxicity basis, %:
Sulphate - 1700		
Thiosulphate - 700		
Sulphide - 22		Sulphide - 22

As it was mentioned above, in our calculations it was supposed that the toxicity of the mixture was the sum of the toxicities of its individual components (i.e. additive toxicity was assumed). If the AHW was tested for the toxicity using BioTox^{TM} test its 5-min EC 50 value was 1.5 %. As according to the sum of TI values of phenolic and sulphur components of the AHW (14.0; Table 2) the theoretical toxicity of AHW according to the calculations ought to be 100 %/14 = about 7 % and not 1.5 % obtained by us experimentally. It could be supposed that:

(i) there were some (minor) toxic components in the AHW that were not detected by the chemical methods used in this study, or,

(ii) the toxicities of individual components were not additive, but synergistic effects occurred.

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