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STATISTICAL APPROACH TO ASSESSING GROUNDWATER POLLUTION FROM GASWORKS

The primary objective of this work was to ascertain the effectiveness of the Microtox system in assessing the quality of groundwater polluted with by-products of coal pyrolysis. Another major objective was to investigate how biological treatment contributes to the change in water toxicity. Making use of the results of toxicological and instrumental analyses, as well as of statistical methods, attempts were also made to specify which particular compound is the main contributing factor in the toxicity of water. When used for assessing the progress of the treatment process, the Microtox test proved very useful for application in the treatment of groundwater polluted by gasworks. The results obtained with this test have provided a reliable description of the course of the technological process, which can be efficiently corrected owing to a quick availability of the results of toxicological analysis. Another benefit offered by the Microtox test is that the use of statistical methods makes it possible to decide which of the compounds being components of the mixture is responsible for the toxicity of an environmental sample.

1. INTRODUCTION

The pollution of an aquatic environment derives from a wide diversity of polluting species, whose composition differs noticeably both in quantitative and qualitative terms. Those differences, as well as the interactions among the pollutants, make it difficult to assess explicitly the imminent threats and potential implications. A key issue is the choice of adequate analytical methods in order to detect and quantify the polluting substances occurring in the environment [1–4]. Analytical techniques for the control of chemical pollutants are costly, time-consuming, and in most instances apply to laboratory tests only. The costs involved can be reduced, when the test is targeted at the quantitative determination of a known pollutant. But they may increase considerably in the case of quantitative and qualitative analyses of multicomponent samples. It is essential to note, however, that the characteristics in terms of the quantitative and

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qualitative compositions of the polluting species fails to adequately describe their threats to living organisms [5–7].

Unbiased information on the variety of threats to which the aquatic environment is exposed can only be obtained by toxicity tests. Among various methods enabling qualitative assessments, the set of Microtox tests using the luminescent bacteria *Vibrio fisheri* bioindicators deserves particular attention. In the electron transport system of these bacteria, luciferase catalyzes the oxidation of the reduced substrate (reduced flavin mononucleotide, riboflavin phosphate or flavin adenin dinucleotide) [8]. The luminescence that occurs during the process is recorded with a photometer [9]. Other substrates participating in the reaction are oxygen and long-chain aldehyde. Under normal conditions, the test bacteria utilize approximately 10% of their energy for luminescence; in the presence of substances exerting a negative effect on cell metabolism, they react quickly and a drop in luminescence occurs. The straightforward structure of the test permits examination of diverse environmental samples (water, wastewater, soil). With the Microtox test it is possible to quickly assess the toxicity of environmental samples and the progress of the treatment process. As yet, little information is available on the problem of how the results of instrumental analyses compare with those of toxicological analyses. Some of the experiments were carried out by McDonald et al. [10] and Ntziachristos et al. [11]). Having such information at hand would promote a wider use of toxicological methods, such as screening or supplementary analyses, in physicochemical studies.

The aim of this study was to ascertain the applicability of the Microtox test in assessing the quality of groundwater polluted by gasworks.

2. MATERIALS AND METHODS

The natural groundwater samples, containing by-products of coal pyrolysis, were used for the toxicity tests. The tests were performed using the Microtox (Strategic Diagnostics Inc., Newark, USA) system, with luminescent bacteria *Vibrio fisheri* as the bioindicator. Lyophilized bacteria can be stored at –20 °C for one year, but they may be used at any time upon suspension in deionized water. When placed in a diluent (2% NaCl), the bacteria emit light with a constant intensity for 1–1.5 h. The measure of toxicity is the decrease in luminescence after 15-min incubation of the test bacteria in the presence of the compound being examined. Tests were performed with an M 500 analyzer and lyophilized bacteria, according to the standard procedure specified by the manufacturer (SDI). The results were calculated using the manufacturer's software MicrotoxOmni. The results of analyses are expressed in toxicity units (TU) calculated in terms of EC_{50} (sample concentration reducing luminescence by 50%), which was determined for particular groundwater samples according to the formula $TU = 1/EC_{50} \times 100$.

Statistical analysis was carried out using Statgraphics packets; in regression analysis use was made of linear, exponential, reciprocal and multiplicative models [12].

3. RESULTS AND DISCUSSION

The polluted groundwater samples were subject to toxicological analyses before and after biological treatment (activated sludge process – pilot plant). The results revealed a direct influence of the treatment process on the toxicity of the sample. After treatment in the bioreactor, toxicity level was reduced noticeably as compared with raw groundwater samples (Tables 1, 2). However, the extent of reduction varied from one sample to another, and in most instances did not depend on the toxicity level of the raw sample. Thus, on 26.04.2010, after passage through the bioreactor, the toxicity of $TU = 400$ which was measured in the raw sample decreased to $TU = 47.6$, whereas one month later it decreased from $TU = 153.8$ to $TU = 66.7$. This should probably be attributed to some other physicochemical and biological factors which affected the treatment process at that time.

Table 1

Chemical contaminations of water recorded from 2009.11.23 to 2010.04.19 [mg/m^3]

Parameter	2009				2010				
	11.23	12.07	12.07	12.21	01.04	01.04	02.02	04.12	04.19
Measuring point	Raw water		Bioreactor	Raw water		Bioreactor		Raw water	
Toxicity [TU]	31.7	33.0	43.5	45.4	83.3	37.0	43.5	200	153.8
TOC	36.8	35.2	64.6	38.9	40.9	61.4	60.4	169	148
MAH	12 399	10 333	5006	10 868	11 379	7379.1	1912.8	20 304	10 191
Phenol	110	90	80	82	92	130	53	195	140
2-Methylphenol	180	190	150	160	170	23	22	2079	2500
3-Methylphenol	150	140	37	130	140	61	12	3000	3700
4-Methylphenol	240	240	38	210	230	23	57	5012	6700
2-Ethylphenol	140	140	100	130	150	130	61	1160	1200
3-Ethylphenol	250	230	190	220	270	520	62	4773	5200
4-Ethylphenol	270	230	330	340	810	690	690	2666	1400
2,3-Dimethylphenol	2300	2300	6200	2700	2700	6900	7300	17220	17500
2,4-Dimethylphenol	4200	4200	7200	3800	4100	9800	5800	21128	22500
2,5-Dimethylphenol	1100	1300	2900	1100	1200	3100	2400	10454	11200
2,6-Dimethylphenol	1200	1200	2900	1100	1100	2600	3100	4802	5100
3,4-Dimethylphenol	490	530	710	500	550	1400	490	4209	4600
3,5-Dimethylphenol	320	410	870	380	400	1100	690	2686	3300
2,3,5-Trimethylphenol	270	270	700	260	280	650	800	1414	1500
2,3,6-Trimethylphenol	150	170	350	140	150	340	410	967	930
2,4,6-Trimethylphenol	560	590	1500	540	520	1400	1600	3290	3300
3,4,5-Trimethylphenol	20	24	47	22	22	51	59	91	98
1-Naphthol	37	40	56	46	53	82	110	53	71
2-Naphthol	37	38	62	43	51	80	90	83	1300
Phenols	12 024	12 332	24 420	11 903	12 988	29 080	23 806	85 283	92 239

Table 2

Chemical contaminations of water recorded from 2010.04.26 to 2010.06.14 [mg/m^3]

Parameter	04.26 Raw water	04.26 Bioreactor	05.10 Raw water	05.10 Bioreactor	05.27 Raw water	05.27 Bioreactor	06.07 Raw water	06.07 Bioreactor	06.14 Raw water
Toxicity [TU]	400	47.6	153.8	66.7	125.0	15.2	111.1	62.5	133.3
TOC	144	33.0	167	33.3	143	31.9	142	33.5	145
MAH	10836	607.1	8555	128.4	6184	228.7	5274	244	9928
Phenol	190	12	330	13	84	60	86	65	<1.0
2-Methylphenol	2500	3.5	3400	6,6	2400	3.7	2448	3.9	1300
3-Methylphenol	4500	2.5	5100	1.7	3800	2.1	3876	2.2	2600
4-Methylphenol	7200	4.6	9000	4.7	6400	6.6	6528	7.1	3800
2-Ethylphenol	1400	3.8	1400	5.2	1600	6.6	1632	7.0	1200
3-Ethylphenol	6000	<1.0	7100	<1.0	6600	<1.0	6732	<1.0	4700
4-Ethylphenol	4000	32	8700	65	5500	219	5610	233	3300
2,3-Dimethylphenol	23300	560	24100	580	17400	2200	17748	2347	13800
2,4-Dimethylphenol	25700	5.4	28400	19	27200	14	27744	15	19800
2,5-Dimethylphenol	14100	14	12900	39	12000	150	12240	160	10000
2,6-Dimethylphenol	5700	1300	5500	1200	5700	1600	5814	1707	4400
3,4-Dimethylphenol	5700	4	6300	7,4	5500	8,6	5610	9.2	4100
3,5-Dimethylphenol	3900	13	3400	12	3800	34	3876	36	2900
2,3,5-Trimethylphenol	1700	150	1100	132	1700	350	1734	373	1400
2,3,6-Trimethylphenol	1100	150	690	150	1200	180	1224	192	970
2,4,6-Trimethylphenol	3500	630	2200	590	4000	790	4080	843	3300
3,4,5-Trimethylphenol	110	15	91	12	120	32	122	34	88
1-Naphthol	53	1.6	15	3.1	22	7.3	22	7.8	<1.0
2-Naphthol	84	<1.0	42	4.1	150	24	153	25	<1.0
Phenols	110 737	2901	119 768	2843	105 176	5687	107 280	6068	77 658

To identify the group of compounds or a single compound being responsible for the toxicity of the groundwater, use was made of statistical analysis, which entailed the results of toxicological and instrumental analyses (Tables 1, 2). The aim was to define the correlation between the results of toxicological analyses and the concentrations of particular polluting species occurring in the sample. The study was carried out in two stages. The former one was targeted at the correlations between the values obtained from toxicological analyses and the concentrations of main pollutants, i.e. total organic carbon (TOC), monoaromatic hydrocarbons (MAH) and phenols.

Use was made of four statistical models: linear, exponential, reciprocal and multiplicative, because a great number of biological processes are characterized by such relations (Table 3). Furthermore, models of that type justify the use of the least squares method, and consequently (owing to the available statistical tests) enable qualitative verification of the econometric model obtained. The regression models

(Table 3) clearly indicate that the relation between *TU*, TOC and phenols follows an evidently nonlinear trend, and that the type of this nonlinearity is closest to that of the multiplicative trend (as can be inferred from the values of the coefficients of determination R^2). The number of statistical samples was insufficient to clearly define the trend for the relationship between *TU* and MAH.

Table 3

Regression models for the relation between toxicity [TU] and concentrations of TOC, MAH and phenols in groundwater

Variable	Model			
	Linear $y = a + bx$	Exponential $y = e^{a + bx}$	Reciprocal $1/y = a + bx$	Multiplicative $y = ax^b$
TU/TOC	$y = -1.78 + 1.19x$ $R^2 = 52.3\%$ $(r = 0.723)$	$y = e^{3.25 + 0.01x}$ $R^2 = 70\%$ $(r = 0.83)$	$1/y = 0.034–0.0002x$ $R^2 = 45\%$ $(r = -0.67)$	$y = 1.68x^{0.9604}$ $R^2 = 66.5\%$ $(r = 0.81)$
TU/MAH	$y = 45 + 0.0074x$ $R^2 = 20\%$ $r = 0.43$	$y = e^{(3.78 + 0.000068x)}$ $R^2 = 21\%$ $r = 0.46$	$1/y = 0.026–0.00000107x$ $R^2 = 15\%$ $(r = -0.39)$	$y = 295x^{0.22}$ $R^2 = 18.5\%$ $(r = 0.43)$
TU/Phenols	$y = 28051 + 0.0015x$ $R^2 = 55.57\%$ $(r = 0.745)$	$y = e^{3.59 + 0.0000148x}$ $R^2 = 68\%$ $(r = 0.82)$	$1/y = 0.028–0.000000216x$ $R^2 = 42\%$ $(r = -0.65)$	$y = 0.55x^{0.449}$ $R^2 = 53\%$ $(r = 0.72)$

Tables 4 and 5 show the values of the coefficient sof correlation between *TU*, TOC, MAH and phenols. Based on those values, it can be assumed that the correlation coefficients for *TU* and TOC, as well as for *TU* and phenols, take significant values and thus characterize a significant trend for a linear dependence. However, the data in Table 3 make it clear that the exponential trend is more adequate because of a higher coefficient of correlation and, also, R^2. Hence, if the coefficient of correlation takes a high value, this does not exclude the possibility of obtaining a higher one. The key issue consists in tracing the right trend. The research conducted by other authors [13] proved, that statistical analysis can be efficiently used in tracing such trend for aims of toxicology.

Table 4

Significance levels for the correlation coefficients

x_i	y_i	
	Toxicity [TU]	Significance level
TOC	0.7232	0.0007
MAH	0.4379	0.0691
Phenols	0.7454	0.0004

Table 5

Significance levels for the correlation coefficients

Parameter	TOC	MAH	Phenols
TOC	1 (18) $p = 0$	0.48 (18) $p = 0.044$	0.97 (18) $p = 0$
MAH	0.48 (18) $p = 0.044$	1 (18) $p = 0$	0.40 (18) $p = 0.098$
Phenols	0.97 (18) $p = 0$	0.40 (18) $p = 0.098$	1 (18) $p = 0$

Analysis of the relationship between the parameters characterizing the concentrations of the chemical compounds occurring in the water sample used in this study has revealed a close correlation between the result for TOC and the result for phenols (Table 5). The results of statistical analysis indicate that phenols were responsible for the toxicity of the groundwater polluted by gasworks.

Table 6

Regression analysis: exponential model $Y = \exp(a + bX)$

Dependent variable – toxicity, independent variable – 2,3-dimethylphenol				
Parameter	Estimate	Standard error	t-value	Probability level
Intercept	3.52538	0.166789	21.1357	0.00
Slope	0.00008	0.000013	5.98636	0.00002
Correlation coefficient – 0.831467 Standard error of estimation – 0.462556 $R^2 = 69.13\%$				

The aim of the latter stage was to establish which of the compounds belonging to the group of phenols was directly responsible for the toxicity of the groundwater being tested. Analysis was carried out for 15 compounds detected in the sample (phenol, methylphenols, dimethylphenols, trimethylphenols and naphthols) (Tables 1, 2). The calculated results indicate that 2,3-dimethylphenol was responsible for the toxicity of the samples; the rise in the concentration of this compound was best correlated with the toxicity of the groundwater (Table 6). This dependence was established when the linear regression model was constructed using the stepwise variable selection forward method. The stepwise variable selection backward method produced the same result. Both the methods are widely used for this purpose and have been verified in engineering applications. In this study, the most satisfactory equation was obtained for the exponential relation.

4. SUMMARY

The Microtox test was found to be a highly effective tool in assessing not only the extent of groundwater pollution from gasworks, but also the progress of the treatment process. The results obtained provide reliable information on the course of technological process of water treatment. Quick availability of the results of toxicological analyses makes it possible to correct the treatment process within a short time, and thus achieve the treatment effects desired. Furthermore, it has been demonstrated that in the case of environmental samples, when use is made of statistical methods, it is possible to make a decision which compound being a component of the mixture is the main contributor to the toxicity of the sample examined. Any other analyses, even those of high accuracy, fail to offer such possibilities. Toxicity tests have the capacity for providing them, because they incorporate the interaction between particular components of the mixture of compounds that pollute the groundwater sample.

Owing to its wide use, the Microtox test has become not only an effective tool for assessing the concentrations of model substances, but also an excellent screening method. Such method may prove useful when quick decisions are to be made about the technological process or the environment. It is essential to note that for reasons resulting from the environmental regulations being currently in force, as well as for technical and economic reasons, standard assessments of water and wastewater quality do not include many groups of pollutants whose concentrations are low. However, these concentrations may increase sporadically – and then a quick toxicity test becomes invaluable in minimizing the environmental risk.

5. CONCLUSIONS

The Microtox system is a useful tool for assessing the progress of the biological treatment process for groundwater polluted by gasworks.

Phenols, and specifically 2,3-dimethylphenol whose concentration increases in best correlation with water toxicity, were found to be responsible for the toxicity of the groundwater sample.

When use is made of statistical methods, it is possible not only to determine the relation between toxicity and the concentrations of the compounds being components of the pollutants, but also to identify the compound responsible for the toxicity water.

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