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Determination of Chlorophenols and Phenoxyacid Herbicides in the Gulf of Gdansk, Southern Baltic Sea

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Chlorophenols belong to the priority pollutants, which enter to the aquatic environment mainly as industrial wastes, intermediates of many industrial processes and pesticides (Venigerova et al. 1998). Non-persistent phenoxyacid herbicides, which are widely used in agriculture and forestry (Rompa et al. 2003), in the aquatic environment undergo degradation processes, which result in formation of chlorinated phenols (Dąbrowska et al. 2002; Juhler at al. 2001).

Chlorophenols even in low ppb concentrations are hazardous to the living organisms. This is also true for phenoxyacid herbicides, although none belongs to the group of very toxic pollutants. Depending on the concentration levels in the environment both groups of pollutants are mutagenic, teratogranic, cancerogenic and have detrimental effects on mammals as well as other living organisms (Kremer et al. 2003; Erne K 1996).

Due to the toxicity of both chlorophenols and phenoxyacids, their presence and concentration levels should be monitored. In consequence, it is necessary to apply highly sensitive analytical techniques and sample enrichment and clean-up procedures for trace level determination. In principle, analytes preconcentration and clean-up is based on solid phase extraction (SPE), solid phase micro extraction (SPME), liquid-liquid extraction (LLE) followed by determination using high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) (Kot-Wasik et al. 2004; Wells and Yu 2000; Rompa M et al. 2002, Llompart et al. 2002, Frébortová J 1995)

At present, data available in literature on the presence of phenoxyacid herbicides and phenols in Gulf of Gdansk is limited (Kot-Wasik et al. 2003).

The aim of this study was acquire data on the presence of selected phenoxyacid herbicides and chlorophenols and their seasonal variation in the Gulf of Gdańsk and the Vistula River. In this study the HPLC with diode array detection (DAD) method for the simultaneous determination of selected chlorophenols and phenoxyacid herbicides was evaluated.

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MATERIALS AND METHODS

Methanol and acetonitrile of HPLC-gradient grade were obtained from Merck (Darmstadt, Germany), deionised water was provided by Milli-Q water purification system (Millipore, Bedford, MA, USA). Standards of purity 99.0%: phenol (Ph), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,6-dichlorophenol (2,6-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TCP), pentachlorophenol (PCP), 4-chloro-3-methylphenol (4-C-3-MP) were obtained from Riedel-de Haën (Seelze, Germany), dichlorprop, mecoprop, 2,4-D, MCPA and dinoseb were obtained from Promochem (Warsaw, Poland). Stock solution of each standard were prepared in methanol and stored at 4°C. Working diluted solutions of standards were prepared in ACN:MeOH (1:1) / H_2O 75:25 v/v.

Samples from the selected sampling sites were collected twice a year in autumn and in spring starting form October 2001 to October 2003, at the seven sampling sites along the Gulf of Gdańsk and Vistula River. The sites were: Hel (H), Władysławo (W), Gdynia Bulwar(G), Orłowo Klif (O), Brzeźno Molo (B), Wisła Ujście (U), Kiezamark (K). Samples (1 L) were collected in amber glass bottles. In the laboratory they were acidified with ortophosphoric acid to pH = 2 and were subjected to analytical procedure.

The development of the analytical procedure has been described elsewhere (Kot-Wasik et al. 2004). In brief, the target analytes (phenoxyacid herbicides and chlorophenols) were isolated and enriched from the water sample using solid phase extraction over EN cartridges (200mg - Merck, Darmstadt, Germany); before usage, each SPE column was conditioned twice with: acetonitrile (2.5mL), methanol (2.5mL), deionised water (2.5mL) followed by deionised water (2.5mL) acidified with ortophosphoric acid to pH=2. In each case up to 300 mL seawater or surface water sample was passed through the column. Afterwards, columns were washed twice with deionised water (2.5mL) and dried in stream of nitrogen for few seconds. Analytes were eluted to glass vials with two portion of acetonitrile, 2.5 mL each. Then 1.0 mL of acidified water was added and organic solvent was evaporated in a gentle stream of nitrogen up to 1.5 mL. Next, the extract was subjected to HPLC analysis.

HPLC analyses were carried out using a liquid chromatograph (Merck-Hitachi, Darmstadt, Germany) equipped with a diode array detector L-7450, a column oven L-7350, a gradient pump L-7100. A Lichrospher RP-18e (5 μm, 250 x 4.0 mm I.D.) (Merck, Darmstadt, Germany) chromatographic column was used. All analyses (injection volume of 100 μL) were performed at 30°C at a flow rate of 1.0 mL/min using the following gradient: solvent A: H_2O + 0.1 % v/v acetic acid, solvent B: ACN:MeOH (1:1 v/v) + 0.01% v/v acetic acid; 0–10.5 min 25-57 %B, 10.5–15.0 min 57-80 %B, 15–17 min 80-100 %B, then 100 %B was maintained for 7 min. The analytical wavelength for each compound was as follows: 230 nm – dichlorprop, mecoprop, MCPA, 2,4-D; 270 nm – Ph,280 nm – 2-CP, 4-CP, 2,4-DCP, 2,6 - DCP, 4-C-3-MP, 2,4,6-TCP, 300 nm – 2,4,5,6-TCP, PCP and dinoseb.

RESULTS AND DISCUSSION

Figure 1 presents the chromatograms of seawater sample (B) and sample spiked with standards of chlorophenols and phenoxyacid herbicides. The chromatogram clearly shows that all analytes can be baseline separated, and there are no interferences from the matrix.

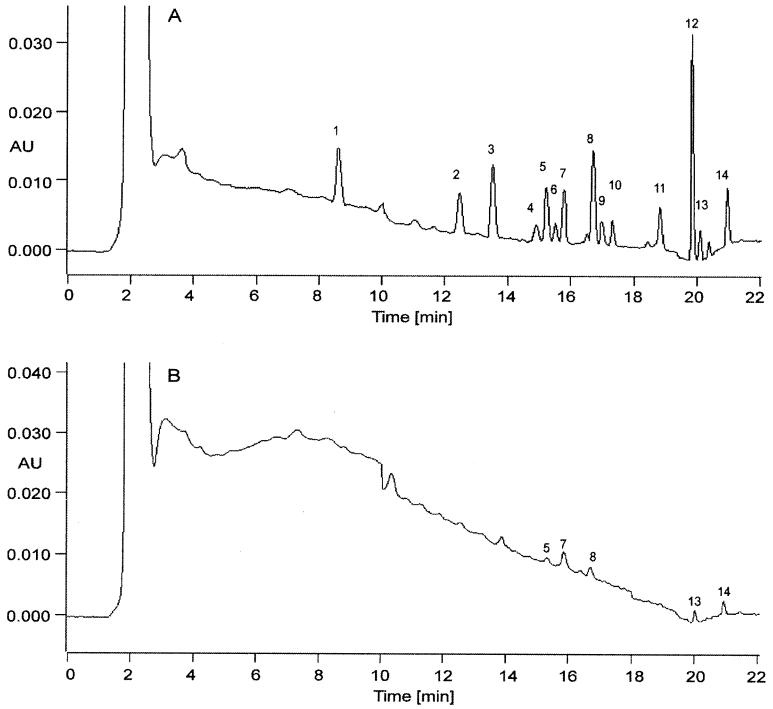

Figure 1. Chromatograms of the extracts after SPE of the seawater sample of 300 mL volume: (A) spiked with a standard solution of phenoxyacids and phenolic compounds at 5 μg/L, (B) non-spiked seawater sample. Peaks: 1) Ph; 2) 2-CP; 3) 4-CP; 4) 2,4-D; 5) 2,6-DCP; 6) MCPA; 7) 2,4-DCP; 8) 4-C-3-MP; 9) dichlorprop; 10) mecoprop; 11) 2,4,6-TCP; 12) dinoseb; 13) 2,3,4,6-TCP; 14) PCP. Chromatographic conditions as described in materials and methods.

The limits of quatitation (LOQ), recoveries, precision of independent determinations after the SPE preconcentration for the analyzed compounds are given in table 1.

The LOQs obtained are low enough to meet the requirements of EU directives concerning the maximum concentrations of phenols and phenoxyacids in surface waters (Peruzzi et al. 2000). Using the SPE preconcentration procedure, in this simple and relatively fast way the quantitation limit could be lowered *ca* 100 - 200 times, depending on the analyte.

Table 1. LOQs, mean recoveries and precision of independent determinations for the analyzed compounds after SPE preconcentration.

Compound	LOQ [μg/L]	Recovery [%]	R.S.D. [%]
Ph	0.25	98	1.6
2-CP	0.20	86	9.1
4-CP	0.15	91	3.8
2,4 – D	0.25	65	5.4
2,6-DCP	0.20	109	3.2
MCPA	0.25	72	9.4
2,4-DCP	0.20	107	5.2
4-C-3-MP	0.10	91	3.3
dichloroprop	0.25	62	8.1
mecoprop	0.25	62	7.7
2,4,6-TCP	0.25	94	3.2
dinoseb	0.15	93	5.4
2,4,5,6-TCP	0.25	89	3.6
PCP	0.25	71	5.1

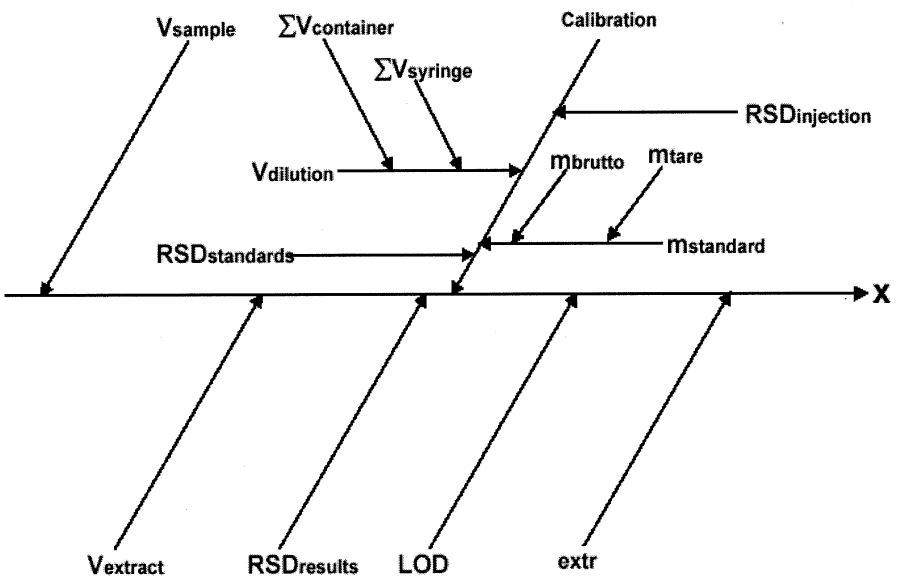

Figure 2. Ishikawa diagram presented uncertainty contributions for determination of analytes: V_{sample} - volume of water sample; $RSD_{results}$- RSD of results; $RSD_{injection}$ - RSD of injections of standards; $m_{standard}$ - mass of standard; m_{tare} – net weight; m_{brutto}- brutto weight; $V_{dilution}$ - dilution of standard; $V_{container}$ - volume of container(s) used for dilution; $V_{syringe}$- volume of syringe(s) used for dilution; $V_{injection}$- volume of standard(s) injected; $V_{extract}$ - final volume of extract; LOD - limit of detection; extr - recovery of extraction; $RSD_{standards}$ - RSD of determinations of analytes (results).

Even higher enrichment factor may be obtained in case of less contaminated water samples (e.g. drinking water, underground water). The high recoveries of analytes, with a good precision expressed as a relative standard deviation (RSD) in the range 1.6-9.4 % were obtained.
The uncertainty budget of determination of chlorophenols and phenoxyacid herbicides is presented in the Ishikawa diagram in Figure 2.

The expanded uncertainty (in %) was calculated according to GUM (Guide to the Expression of Uncertainty in Measurement) using formula (1).

$$U = k \cdot \sqrt{u(c_{cal})^2 + u(V_{sample})^2 + u(V_{extract})^2 + u(LOD)^2 + u(extr)^2 + \frac{(RSD_{results})^2}{n}} \quad (1)$$

where: U – expanded uncertainty; k – coverage factor (usually 2); u(ccal) – uncertainty of calibration step; u(Vsample) – uncertainty of water sample volume; u(Vextract) – uncertainty of final extract volume; u(LOD) – uncertainty of limit of detection; u(extr) – uncertainty of recovery of extraction; RSDresults – RSD of results; n – number of independent determinations;
For calculation of every standard uncertainty the GUM Workbench version 1.3 (Metrodata GmbH, Germany) software have been used. The obtained values of expanded uncertainty are given in table 2.

Table 2. The values of standard uncertainties and expanded uncertainty.

Compound	*Uncertainty [%]*						
	u ccal	*u Vsample*	*u Vextract*	*RSDresults*	*u LOD*	*u extr*	*U(k=2)*
Ph	1.3	1	0.75	3	3.54	0.92	8.9
2-CP	1.3	1	0.75	3	10.77	5.25	24.5
4-CP	1.3	1	0.75	3	1.86	2.19	7.6
2,4 - D	1.3	1	0.75	3	3.77	3.12	11.0
2,6-DCP	1.3	1	0.75	3	4.49	1.85	10.9
MCPA	1.3	1	0.75	3	11.94	5.43	26.7
2,4-DCP	1.3	1	0.75	3	6.25	3.00	14.7
4-C-3-MP	1.3	1	0.75	3	3.95	1.91	10.1
dichloroprop	1.3	1	0.75	3	3.70	4.68	12.9
mecoprop	1.3	1	0.75	3	16.00	4.45	33.6
2,4,6-TCP	1.3	1	0.75	3	10.00	1.85	20.9
dinoseb	1.3	1	0.75	3	33.33	3.12	67.1
2,4,5,6-TCP	1.3	1	0.75	3	20.00	2.08	40.5
PCP	1.3	1	0.75	3	5.13	2.94	12.8

The obtained values of uncertainty are in the range from 7.6 to 67.0 %. This is mainly due to the fact that the concentration of chlorophenols and phenoxyacid herbicides are close to the detection limit of the method (see table 3). Therefore, the uncertainty of LOD (*uLOD*) determinates the overall uncertainty of the analysis.

The presented method has been successfully applied for the determination of organic pollutants in seawater samples and surface water samples collected during spring 2002, 2003 and autumn season 2001, 2002. The results are presented in Table 3.

Table 3. Concentrations of chlorophenols and phenoxyacid herbicides determined in the samples collected from the Gulf of Gdansk and the Vistula River in the period from 2001 to 2003.

Compounds	Autumn 2001							Spring 2002						
	H	W	O	B	G	K	U	H	W	O	B	G	K	U
Ph	nd	nd	nd	2.3	nd	nd	nd	0.5	0.8	0.5	0.6	0.8	0.4	0.5
2-CP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6	nd	nd
2,4-DCP	nd	nd	nd	0.5	nd	nd	nd	0.3	0.3	0.4	0.6	0.8	6.0	3.3
4-C-3-MP	nd	nd	nd	0.7	nd	0.6	nd	0.5	0.5	nd	d	nd	nd	0.4
PCP	nd	nd	d	1.6	nd	0.4	nd	0.4	0.7	0.4	0.7	0.3	0.9	0.6
Σ phenols	-	-	**0.1**	**5.1**	-	**1.0**	-	**1.7**	**2.3**	**1.3**	**2.1**	**2.5**	**7.3**	**4.8**
2,4-D	d	d	nd	d	nd	nd	nd	d	0.6	nd	nd	0.2	nd	nd
MCPA	nd	nd	d	nd	nd	nd	nd	0.4	0.2	nd	nd	nd	0.2	nd
dichlorprop	d	nd	d	0.6	nd	nd	0.4	nd	2.2	nd	d	nd	0.2	nd
mecoprop	nd	nd	nd	d	nd	nd	0.4	nd	nd	nd	nd	nd	0.2	0.5
dinoseb	nd	d	nd	nd	nd	d	d	nd	nd	nd	d	d	nd	d
Σ phenoxyacids	-	-	-	**0.6**	-	-	**0.8**	**0.4**	**3.0**	-	-	**0.2**	**0.6**	**0.5**
	Autumn 2002							Spring 2003						
Ph	nd	nd	nd	nd	nd	nd	nd	0.6	0.4	d	nd	d	nd	0.3
2-CP	nd	0.3	nd	0.3	0.3	nd	0.4	nd	nd	0.6	nd	0.3	nd	nd
4-CP	d	nd	nd	nd	nd	nd	nd	nd	2.7	nd	nd	0.5	0.9	0.8
2,4-DCP	0.3	nd	nd	nd	nd	nd	1.0	nd	nd	nd	nd	nd	0.9	1.1
2,6-DCP	nd	nd	nd	nd	nd	nd	nd	nd	1.6	0.7	nd	0.4	1.6	1.6
2,4,6-TCP	d	0.3	nd	0.2	0.2	0.4	nd	nd	nd	nd	0.8	nd	nd	nd
2,3,4,6-TCP	nd	nd	0.3	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd
4-C-3-MP	0.3	0.4	nd	nd	0.2	nd	0.4	nd	nd	nd	nd	nd	nd	nd
PCP	nd	nd	nd	nd	nd	0.4	nd	nd	nd	nd	0.6	nd	0.2	nd
Σ phenols	**0.6**	**1.0**	**0.3**	**0.5**	**0.7**	**1.2**	**1.8**	**0.6**	**4.7**	**1.3**	**1.4**	**1.2**	**3.6**	**3.8**
2,4-D	0.2	0.2	0.3	0.3	0.2	nd	nd	nd	0.9	1.0	1.0	0.9	2.1	0.5
MCPA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.7
dichlorprop	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd
mecoprop	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	d	nd
dinoseb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ phenoxyacids	**0.2**	**0.2**	**0.3**	**0.3**	**0.2**	-	-	-	**0.9**	**1.0**	**1.0**	**0.9**	**2.5**	**1.2**

nd – not detected, d – detected; below LOQ, (values in the table expressed as μg/L)

The average concentrations of the chlorophenols and phenoxyacids ranged between 0.2 – 6.0 and 0.2 – 2.2 μg/L, respectively. Outstandingly high concentration of 2,4-dichlorophenol (6 μg/L) was monitored in samples collected from the Vistula River, which carry pollutants from the large part of our country.

Hence this is not a shocking result in contrary to a relatively low content of phenols and phenoxyacids found in samples collected from the pier in Gdansk, where typical recreation areas and beaches are located.

In general higher concentrations of both chlorophenols and phenoxyacid herbicide are determined in spring. This is most likely due to the leaching processes from surrounding agricultural areas and the degradation processes of phenoxyacidic herbicides that are released to the environment in spring. It is apparent that low concentrations of phenol are determined in samples collected in springtime. In this case, phenol may be of non-anthropogenic origin. It can be formed due to the degradation processes of organic matter.

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