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Stability Studies of Selected Phenoxyacid Herbicides in Water Samples and Determination of Their Transformation Products

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Herbicides as phenoxyacids are commonly used in agriculture (i.e.: for post-emergence control of broadleaf weeds and wood plants) and it is not surprising that many of these compounds have been detected in natural waters. Current understanding of the biological impacts of herbicide use is limited by the fact that most investigations have focused on the active ingredients (mother compounds) without considering their transformation products (Kolpin et al. 2000). The degradation products of many herbicides are relatively stable and persistent, and in same cases they can be more toxic then their parent compounds (Coats 1993; Belfroid et al. 1998).

The most common metabolites of phenoxyacid degradation are the corresponding chlorophenols. The identification of these chlorophenols at phenoxyacid site may therefore be indicative of phenoxyacid degradation. However, this interpretation could be complicated by the presence of chlorophenols as well as other phenoxyacids as impurities in the herbicides due to complicated synthesis reactions and insufficient cleanup procedures in herbicide production (Reitzel et al. 2004).

The need for monitoring acidic herbicides in natural waters is essential for achieving good water quality objectives. 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 (Kot-Wasik et al. 2004; Frébortová 1995).

The main purpose of this work was to recognize the rate of degradation of four selected phenoxyacid herbicides (such as 2,4-D, MCPA, mecoprop, dichlorprop) in natural water samples. The effect of various parameters, including light, temperature and time storage has been investigated.

MATERIALS AND METHODS

Methanol and acetonitrile of HPLC-gradient grade were obtained from Merck (Darmstadt, Germany), deionized water was provided by Milli-Q water

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purification system (Millipore, Bedford, MA, USA). Standards of purity 99.0%: 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 4-chloro-2-methylphenol (4-C-2-MP) were obtained from Riedel-de Haën (Seelze, Germany), dichlorprop, mecoprop, 2,4-D and MCPA were obtained from Promochem (Warsaw, Poland). Stock solution of each standard were prepared in methanol and stored at 4°C.

Different types of water samples were used to carry out the degradation study. All of them were collected from the Gulf of Gdansk region and they consisted of seawater (pH = 7.52, conductivity = 17 mS/cm, TOC = 2.42 mg/L) from Southern Baltic Sea and river water (pH = 7.31, conductivity = 0.75 mS/cm, TOC = 5.50 mg/L) from Vistula River. The natural water samples were collected from the top meter of each water body in 2.5 L precleaned amber glass bottles and stored at 4°C prior use. All water samples were used without previous treatment, filtration or sterilization. The water samples were analyzed to ensure that they were free of interfering compounds.

Because of very good water solubility of phenoxyacids (2,4-D – 620 mg/L, MCPA – 825 mg/L, mecoprop – 734 mg/L, dichlorprop – 350 mg/L (Castillo et al. 1997)) all water samples were spiked at the final concentration of 2 mg/L of each standard, and they were analyzed directly without preconcentration step. The advantage of the simplified sample preparation procedure is the elimination of analytical errors caused by i.e. analyte loss throughout sample preconcentration or evaporation of solvent excess.

All samples were stored in two types of glass bottles: amber glass bottles which are not penetrably by ultraviolet light and glass containers fully penetrable to the light, and they were stored in the laboratory at the room temperatures 20 ± 2°C full exposed to sunlight (during May to June). During this period the weather was sunny and five sunlight hours per day was observed on average.

HPLC analyses were carried out using a liquid chromatograph (Agilent 1100 Series) equipped with a diode array detector, mass spectrometric detector, a column oven, a binary pump and autosampler. A Lichrospher RP-18e (5 μm, 125 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) + 0.1% v/v acetic acid; 0–8 min 50-80 %B, 8–15 min 80-100 %B, then 100 %B was maintained for 5 min. The analytical wavelength was as follows: 230 nm and 280 nm. Quantitation limits for 2,4-D, MCPA, mecoprop and dichlorprop were 0.3; 0.2; 0.2 and 0.25 μg/mL, respectively.

Kinetics of phenoxyacids degradation were calculated using the first-order equation:

$$\ln C/C_0 = -kt \qquad (1)$$

where C_0 and C are the compound concentration at times zero and t, respectively, and k is the rate constant. The results were plotted using the natural logarithm of the compound concentration as a function of time. First-order degradation rate constants were determined by regression analysis. Half-lives $t_{1/2}$ were calculated using Eq. (2) which was derived from Eq. (1) by replacing C with $C_0/2$:

$$t_{1/2} = \ln 2/k \qquad (2)$$

RESULTS AND DISCUSSION

The decrease in phenoxyacids concentration in water samples fitted well the first order reaction kinetics and rate constants and half-lives times are summarized in table 1.

Table 1. Phenoxyacids degradation rate constants (k), correlation coefficients (r) and half-lives ($t_{1/2}$) calculated on the basis of analytical data.

	River water (pH 7.31)					
Compound	samples exposed to sunlight			samples storage in dark		
	r	k [per day]	$t_{1/2}$ [day]	r	k [per day]	$t_{1/2}$ [day]
2,4-D	0.958	0.0349	19.9	0.937	0.0207	33.5
MCPA	0.987	0.0284	24.4	0.965	0.0186	37.3
mecoprop	0.987	0.0355	19.5	0.991	0.0184	37.7
dichlorprop	0.989	0.0273	25.4	0.975	0.0138	50.2
	Seawater (pH 7.52)					
	samples exposed to sunlight			samples storage in dark		
	r	k [per day]	$t_{1/2}$ [day]	r	k [per day]	$t_{1/2}$ [day]
2,4-D	0.960	0.0622	11.1	0.984	0.0222	31.2
MCPA	0.984	0.0722	9.6	0.985	0.0176	39.4
mecoprop	0.974	0.0496	14.0	0.962	0.0106	33.6
dichlorprop	0.988	0.0433	16.0	0.977	0.0176	39.4

Degradation in seawater samples is faster in comparison with river water samples. The presence of mineral salts as well as organic compounds (such as humic substance and fulvic acids) in river water and seawater is responsible for the enhanced degradation rate of phenoxyacids. This is in a good agreement with literature reports (Lagana et al. 2002, Pozo et al. 2001, Sun and Lee 2003), according to which dissolved organic matter is able to absorb the light and thus highly reactive hydroxyl radical (·OH) are formed. Their rapid reactions with substances in water cause degradation. It can be observed that phenoxyacids in natural water samples degraded faster under sunlight than under darkness (samples storage in dark in amber glass bottles).

It can be seen that light intensity affects the k and $t_{1/2}$ in each natural water sample. The rate of degradation together with half-lives time were changed, k

increases and $t_{1/2}$ decreases accordingly when the light intensity increases in the order of darkness and sunlight.

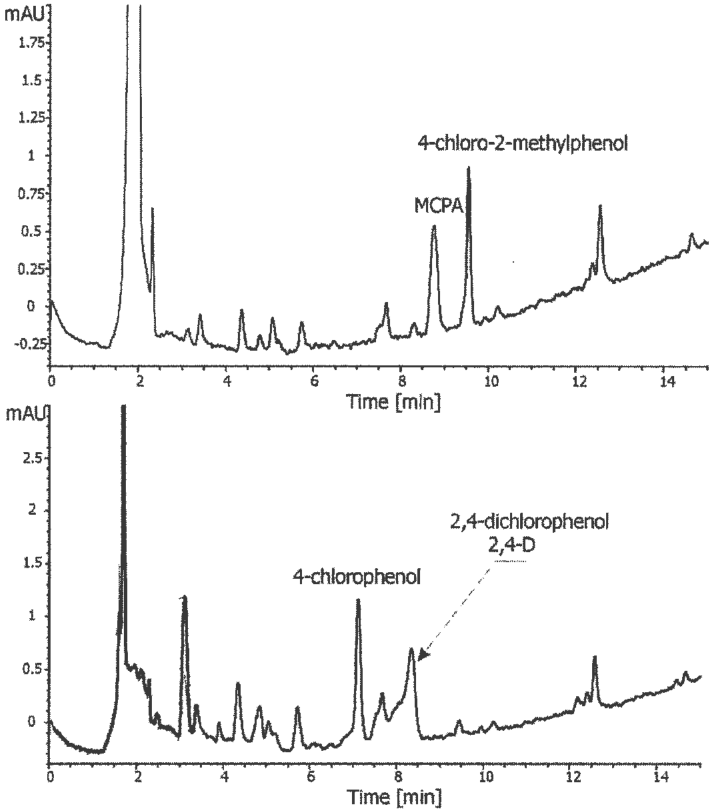

Figure 1. Chromatograms obtained during the analysis (HPLC-DAD) of seawater sample solutions of (A) MCPA and (B) 2,4-D after 39 days of exposition.

The most commonly reported metabolites occurring during phenoxyacids degradation are the corresponding chlorophenols (Reitzel et al. 2004; Zertal et al. 2001; Juhler et al. 2001). In water samples, where both processes (photodegradation and biodegradation) were going simultaneously, main degradation products of phenoxyacids were identified. Identification of them using only HPLC technique coupled with DAD detection makes it quite difficult since absorption spectra of phenoxyacids and their degradation products are quite similar. Spectra of this compounds were recorded in the common range of 200-400 nm. It is well known however, that in real natural water samples co-eluting peaks interfere at low UV wavelengths. Therefore, for all compounds secondary maxima were preferred. For the final confirmation of peak identity, UV spectra are needed, however spectra of some compounds are too similar for unmistakable identification especially, when these compounds co-elute (mecoprop and 2,4-D; MCPA and 2,4-D and 2,4-DCP) (Kot-Wasik et al. 2004). As a result, identification step has been done using HPLC-DAD-MS technique and MS spectra were compared with corresponding standards.

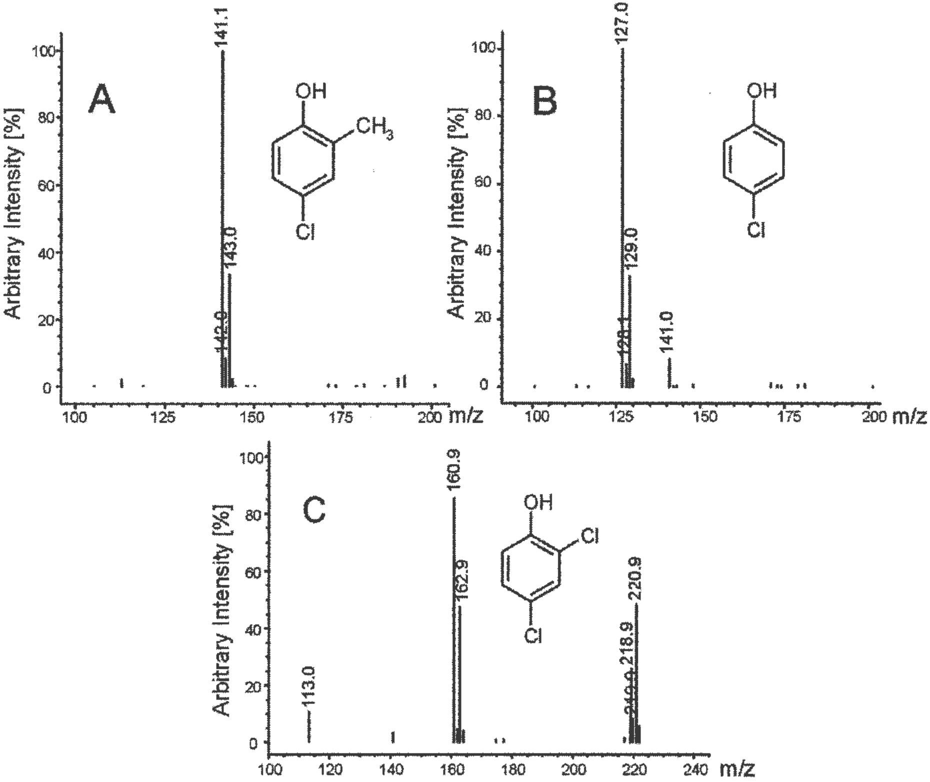

Figure 2. Mass spectra of identified compounds: (A) 4-Cl-2-MP; (B) 4-CP; (C) 2,4-CP.

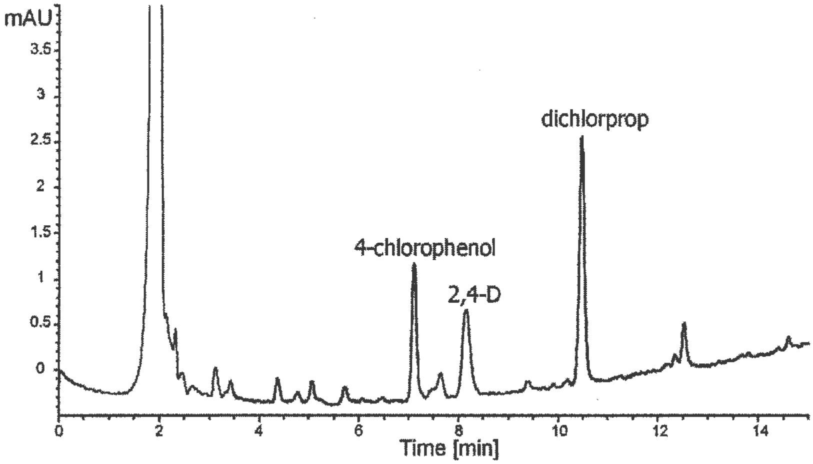

Figure 3. Chromatogram obtained during the analysis of an extract from seawater sample spiked with dichlorprop.

Because of co-elution of 2,4-D with their degradation product which is 2,4-DCP (Figure 1B) mass spectrum presented on Figure 2C contains molecular ions formed by deprotonation of molecules (2,4-D and 2,4-DCP). Chromatographic analyses of samples prepared using seawater and spiked with mecoprop and dichlorprop performed during degradation allowed to distinguish new peaks. This proves that there are products which formed during the exposition.

Degradation of mecoprop resulted in the formation of 4-chloro-2-methylphenol, degradation of dichlorprop lead to the formation of 2,4-D and 4-chlorophenol (Figure 3). The presence of main degradation products of dichlorprop, like 2,4-D and 2,4-dichlorophenol is in a good agreement with literature data (Juhler et al. 2001). However, the presence of 4-chlorophenol can be explained by the degradation of 2,4-D not dichlorprop.

Knowledge concerning the degradation of herbicides and the dissipation of their metabolites in the environment could include consideration of herbicide metabolites as part of the basis for the establishment of health guidelines and water-quality regulations. Phenoxyacids could possibly originate due to the leaching processes from surrounding agricultural area while chlorophenols might be presented because they come from degradation processes of phenoxyacid herbicides as well as industrial activity. This study demonstrates that obtaining data on phenoxyacid herbicides depredates is critical for understanding the fate of this compounds in the hydrologic system.

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