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Bioremediation of chlorinated pesticides in field-contaminated soils and suitability of Tenax solid-phase extraction as a predictor of its effectiveness.

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Abstract

Results of desorption kinetics study using consecutive Tenax TA solid phase extraction were tested as predictors of 3-week anaerobic soil bioremediation effectiveness for chlorinated pesticides γ-HCH, DDT and methoxychlor. Field-contaminated samples were used in these experiments. Estimation of rapidly desorbing fractions using two-compartment model of desorption kinetics has shown that amounts of pesticides removed during bioremediation tests (43-98% of initial concentrations) were considerably larger (1.37 ± 0.45 times) than these fractions. However, both values were correlated to some extent (R^2 = 0.6355). In all, determination of rapidly desorbing fractions was considered rather a poor indicator of soil bioremediation efficiency for chlorinated pesticides. The total amounts of pesticides desorbed

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by Tenax in 72 h performed better in this respect (R^2 = 0.7274, fraction removed/desorbed = 1.10 ± 0.20). Disappearance of DDT during bioremediation was accompanied by DDD formation but the latter was considerably lower than results expected from stoichiometry.

1 Introduction

Chlorinated pesticides, with the most known being DDT, are almost all counted among persistent organic pollutants presenting a special threat to the environment. Although banned in most countries of the world, they still remain in considerable quantities in obsolete pesticide stocks [1]. Moreover, they occur as heavy soil contamination at many sites. These places include areas of former production, waste storage, pesticide distribution, etc. Also sediments in water bodies used to receive discharges from these places are often highly polluted.

Previously reported experiments [2] have demonstrated the potential of methanogenic granular sludge to effectively remove chlorinated pesticide, like γ-hexachlorocyclohexane (HCH), 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane (p,p'-DDT) and 1,1,1-trichloro-2,2-bis-(4-methoxyphenyl)ethane (methoxychlor), from contaminated soil. p,p'-DDT was partially converted to 1,1-dichloro-2,2-bis-(4-chlorophenyl)ethane (p,p'-DDD), and partially underwent more advanced transformation, which was confirmed by formation of 4,4'-dichlorobenzophenone (p,p'-DBP), the terminal product of anaerobic DDT degradation [3]. Removal of parent compounds (γ-HCH, p,p'-DDT, methoxychlor) appeared to be controlled by their desorbability from the soil. Easily desorbing fractions of these pesticides were removed first, while their desorption-resistant fractions largely persisted. Such residuals were not removable even in a very long incubation time.

The relationship between degradability of hydrophobic organic contaminants (HOCs) in soil on their desorbability is a commonly accepted concept [4]. This led to development of several methods aimed at prediction of HOCs bioremediation efficiency, based on results of desorption studies. These tests employ extraction with solid phase, like Tenax TA [5], XAD-2 [6], or with an aqueous solution of hydroxypropyl-β-cyclodextrin [7, 8]. However, all these methods were validated almost only for one group of HOCs, namely polycyclic aromatic hydrocarbons (PAHs). For chlorinated pesticides such experimental evidence is missing

(investigations in this area were concentrated rather on relation between desorbability and bioaccumulation), so the applicability of such methods is in fact only a speculation based on similarity of hydrophobic character. It is therefore interesting to check practically if such assays will also work for this group of compounds.

The method chosen for the presented research was consecutive solid phase extraction (SPE) by Tenax TA polymer, as proposed by Cornelissen et al. [9], due to possibility of precise study of desorption kinetics. The Tenax SPE was applied to pesticide field-contaminated soil samples of different characteristics, and its results were compared against effects of separately run bioremediation tests using methanogenic granular sludge as an inoculum. Particular attention was paid to suitability of rapidly desorbing fraction estimate [5] to predict bioremediation efficiency. A secondary goal of this investigation was also to examine the ability of methanogenic granular sludge to remove chlorinated pesticides in a wide range of concentrations and in different types of soil. For that reason, bioremediation tests included additional soil samples with high and low concentrations of pesticides, which were not tested in Tenax SPE.

2 Materials and methods

2.1 Chemicals

Hexane and acetone (Picograde quality) as well as 99% decachlorobiphenyl (PCB209) standard were purchased from LGC Standards (Lomianki, Poland). Anhydrous sodium sulfate (12-60 mesh ultra-resi analyzed) and sodium lactate (60% syrup) were purchased from J.T. Baker (Lodz, Poland). Tenax TA, 20-35 mesh, was obtained from Alltech (State College PA, US). Standards of chlorinated pesticides and their metabolites (purity 99.6-99.8%): γ-HCH; p,p'-DDE (1,1-dichloro-2,2-bis-(4-chlorophenyl)ethene), -DDD and -DDT; methoxychlor were purchased from the Institute of Industrial Organic Chemistry (Warszawa, Poland).

2.2 Granular sludge

The granular sludge originated from an anaerobic reactor treating wastewater from the soft drink factory "Hellena" in Kalisz, Poland. Before use, the sludge was thoroughly rinsed on a 0.25 mm sieve with tap water and mineral medium (the same as in bioremediation tests), to remove fine particles and products of decay.

2.3 Soil samples

Soil samples used in this study were collected at three pesticide "tombs" in central Poland: Bogomilow and Sepno-Radonia (Lodz voivodship): samples BOG4, BOG5, SEP2 and SEP5; Mlynow (Wielkopolskie voivodship): samples MLY2 and MLY4. Sampling points were located at or below concrete storage wells forming the "tombs" structure. BOG samples were characterized as sand (sand 90-95%, silt 4-8%, clay 1-2%; 0.4-0.7% organic matter as weight loss at 550 °C; pH 6.3-6.6 using 0.01 M $CaCl_2$), similarly as MLY samples (sand 90-94%, silt 3-6%, clay 3-4%; 0.3-0.5% organic matter; pH 5.6-6.0). SEP2 soil represents sandy clay loam (sand 57%, silt 17%, clay 26%; 2.3% organic matter, pH 3.8) whereas SEP5 was clay (sand 31%, silt 28%, clay 41%; 2.7% organic matter; pH 4.1). All the samples were air dried and sieved (2 mm), then refrigerated until used. Contamination by chlorinated pesticide varied, from slight (BOG4) to very heavy (MLY4) - see tab. 1. The pollution was dominated by γ-HCH, technical DDT (o,p'- and p,p'- isomers) and methoxychlor. Primary DDT metabolites, DDE and DDD were present in relatively small quantities comparing to parent compound, with the exception of the MLY2 sample.

2.4 Remediation experiments

3 g of soil (dry mass) and 1 g of granular sludge (wet mass) were added to 120 ml serum bottles, and then slurried with 30 ml of mineral medium (after Holliger et al. [10]; medium contained carbonate and phosphate buffers, macro-, micronutrients and vitamins; originally applied yeast extract was omitted). 20 mM of sodium lactate was added as an electron donor. Bottles were sealed with Viton stoppers and secured with aluminum crimp caps. Headspace was replaced with a mixture of N_2/CO_2 (80/20%, overpressure 0.7 bar). The bottles were incubated in the dark statically, apart from being shaken by hand once a week. Incubation at 30 ºC lasted for 3 weeks. Previous experiments [2] have shown that longer incubation in such conditions contributes little to further pesticide removal. Triplicate sacrificial analyses were performed at the start and after incubation.

2.5 Desorption kinetics study by SPE

The SPE procedure was based on the method described by Cornelissen et al. [9]. 1g of soil (dry mass) was weighted into a 100-ml separatory funnel. Next, 30 ml of 0.01 M $CaCl_2$ solution together with 15 mg of sodium azide (serving as an inhibitor of microbial activity) and 0.5 g of Tenax TA beads were added. The stoppered funnel was then shaken horizontally at about 120 cycles per minute. At set times (1, 3, 6, 10, 24, 48 and 72 h) the slurry was

drained into another separatory funnel and then shaken again with a fresh portion of Tenax. After 72 h, the drained slurry was analyzed for non-desorbed pesticide. The grains of Tenax remaining on the walls of the used funnel in each step were extracted by 15 min shaking with 16 ml of hexane. The collected extract was spiked with surrogate standard solution (PCB209 in toluene) and then diluted with hexane to fit the GC calibration range, if necessary. Blank samples of appropriately diluted surrogate standard were analyzed concurrently and these results were used for correction of determined concentration of pesticides, together with recovery factors. The initial concentration of pesticide S_0 was calculated as sum of compound non-desorbed (remaining in slurry) and cumulative concentration desorbed in all steps of 72 h SPE. Tests of pesticide recovery in Tenax sorption and hexane re-extraction produced the following results (average±standard deviation): γ-HCH 97±10%; p,p'-DDT 94±7%; methoxychlor 83±6%. Desorption tests were performed at least in triplicate. Two-compartment model of first-order desorption kinetics [9] was fitted by non-linear regression to results of individual runs (fraction of pesticide remaining in given soil after extraction time t = 1, 3...72 h relative to initial concentration, S_t/S_0):

$$S_t/S_0 = F_{rapid} * \exp(-k_{d,rapid} * t) + F_{slow} * \exp(-k_{d,slow} * t)$$

where F_{rapid} and F_{slow} are respectively rapidly and slowly desorbing fraction of pesticide ($F_{rapid} + F_{slow} = 1$), $k_{d,rapid}$ and $k_{d,slow}$ are rate constants for rapid and slow desorption (h^{-1}), t is desorption time (h). The fractions were estimated for dominant original pollutants: γ-HCH; p,p'-DDT and methoxychlor.

This study was performed for BOG5, SEP2, SEP5 and MLY2 soils only. Very high levels of contamination in the MLY4 sample would require excessive dilution to fit the GC calibration range, whereas low concentrations in BOG4 soil was supposed to produce very small concentrations in extracts, difficult to measure accurately, especially in later steps of Tenax SPE. Therefore, both these samples were considered not suitable for a reliable assessment of rapidly and slowly desorbing fractions.

2.6 Soil slurry extraction procedure

Soil slurry, both in remediation and SPE tests, was filtered through paper filter and the solids were left to dry, usually until the next day. The collected filtrate was spiked with surrogate standard solution (PCB209 in toluene) and then extracted by 15-minute shaking with 16-ml

volume of hexane, previously used for rinsing the sample bottle. Solids were extracted using modified method by Wang et al. [11] described elsewhere in details [2]. Briefly, dried solids, spiked with PCB209 surrogate standard, were extracted by heating for 4 h at 70 ºC in 40 ml tightly closed amber vials with 16 ml of hexane/acetone. After cooling, extract was clarified by centrifuging and dried using a small addition of anhydrous sodium sulfate. If necessary, extracts were diluted to fit the gas chromatography (GC) calibration range. No extra clean-up was used. Liquid and solid extracts were analyzed separately. The results were corrected using PCB209 recovery factor using blank samples with appropriately diluted surrogate standard.

2.7 GC analyses

Analyses were performed on MEGA (Carlo Erba) GC with ECD detector and capillary column Stx-500 (30 m × 0.25 mm ID, 0.15 µm film). This column was selected due to its excellent ability to separate o,p'-DDT and p,p'-DDD, which could be a problem for more commonly used phases. Temperature program: 65°C, raised by 15 °C/min to 300 °C, held for 13 min., carrier gas: hydrogen at 80 kPa. Injection of 1 µl extract was splitless (Uniliner, Restek) at 210 °C. Measurements included: γ-HCH; p,p'-DDE, -DDD, -DDT; methoxychlor for remediation experiments and γ-HCH; p,p'-DDT and methoxychlor for Tenax desorption studies.

Calibrations were made daily, using external standards of different concentrations. DDT thermal breakdown was checked periodically by injecting p,p'-DDT standard, according to EPA 8081B guidelines. Method detection limits (MDL) for GC analysis were estimated at 0.2 ppb for γ-HCH and p,p'-DDT; 0.4 ppb for p,p'-DDE and -DDD; and 0.8 ppb for methoxychlor. For individual soil samples they depend on dilution of extract. Tests of the whole analytical procedure (extraction+GC), using spiked soil, produced 101 ± 3% recovery (average±standard deviation) for γ-HCH, 105 ± 3% for p,p'-DDE, 100 ± 1% for p,p'-DDD, 103 ± 7% for p,p'-DDT and 105 ± 9% for methoxychlor.

3 Results and Discussion

3.1 Remediation experiments

In all tests a noticeable decrease in pesticide concentration occurred over the incubation period, however, removal varied both for individual compounds and among soil samples (tab. 1). γ-HCH was removed most efficiently, over 90% in all cases with measurable concentration (above detection limit). Methoxychlor was the next, removed from 64% in BOG5 to 97% in SEP2 sample. p,p'-DDT was the most recalcitrant chemical, with only 43% disappearance in MLY4 soil (perhaps as a result of very high initial concentration of this pesticide) to 96% in MLY2.

Literature data indicate that primary mechanism of γ-HCH and methoxychlor removal in applied experimental conditions should be biodegradation (e.g. [12, 13]). Reductive dechlorination of p,p'-DDT was confirmed by formation of p,p'-DDD in all samples, except for MLY2. In BOG5, there was also an increase of p,p'-DDE concentrations. As DDE is likely to be formed in aerobic conditions [3] it is supposed that this measurement could be rather an effect of interfering unidentified compounds of similar GC retention time.

An increase of p,p'-DDD concentration was in all cases smaller than stoichiometrically corresponding amounts of removed p,p'-DDT. This further suggests more advanced metabolism. Previous experiments [2] confirmed this by appearance of p,p'-DBP, considered the final product of anaerobic degradation of DDT. Consequently, in the presented study total concentration of DDT and its primary metabolites, DDD and DDE, was reduced by 25% to 76% (on molar basis). Decrease of this parameter is a necessary condition for effective DDT-contaminated soil remediation [3].

3.2 SPE experiments

The results of these experiments are given in tab. 2. In all samples desorption of contaminants followed a distinctive two-phased pattern. Comparison of the F_{rapid} estimates to the cumulative amounts of pesticides extracted in consecutive steps has shown that the time necessary to near complete extraction of this fraction was 6h, without any noticeable difference among compounds. Average ratio of F_{rapid} to the amount extracted in 6h was 0.99 ± 0.03 (standard deviation). In Tenax SPE of p,p'- and o,p'-DDT and their primary metabolites from lab aged sediment, de la Cal et al. [14] found this relationship quite close, as 0.76 ± 0.04. This little difference is presumably a result of using a three-compartment model in their study, with an additional very slowly desorbing fraction. With such an approach, the relative estimate of a rapidly desorbing fraction could be smaller than determined using a model with only two compartments. However, Yang et al. [15] reported that rapidly desorbing fractions

(three-compartment model) of p,p'-DDT and p,p'-DDE were twice larger than their fractions extracted by Tenax in 6 h. Presumably, the cause might have been use of smaller amounts of sorbent (only 0.2 g of Tenax for 1 g of soil).

Noticeable deviations of rapidly desorbing fractions of p,p-DDT and methoxychlor in SEP2 and SEP5 samples is probably a result of some heterogeneity in soil from this site - partial presence of these compounds in a form of randomly distributed hardly soluble microaggregates [2].

Considering the relationships between desorption kinetics parameters and properties of investigated soils and compounds, the only markedly important factor appears pesticides hydrophobicity. The highest rapid desorption constants and largest rapidly desorbing fractions mostly occurred for the least hydrophobic contaminant, γ-HCH (log K_{OW} 3.90), whereas the lowest values were mostly noted for p,p'-DDT (log K_{OW} 6.19; log K_{OW} for methoxychlor 4.83). A similar observation was made by de la Cal et al. [14], who found inversely linear correlations between rapidly desorbing fractions and hydrophobicity of different polybrominated biphenyl ethers. Small rapidly desorbing fractions of p,p'-DDT and methoxychlor in BOG5 sample could be a result of long contact time (up to 37 years comparing to 26-28 years for other tombs, elapsed from termination of their operation) or low concentration of these pesticides. Chung and Alexander [16] observed that percentage of sequestered PAHs declines with their increasing concentration.

3.3 Bioremediation efficiency versus pesticide desorbability

The removal efficiencies of γ-HCH, methoxychlor and p,p'-DDT in bioremediation tests, for BOG5, SEP2, SEP5 and MLY2 soils (the result for γ-HCH for MLY2 has been omitted as not reliable enough – below detection limit) exhibit observable correlation (Fig. 1, $R^2 = 0.6355$), statistically significant (t-test, $p < 0.01$), with their rapidly desorbing fractions. However, the fraction removed was almost always notably higher than rapidly desorbed, with the average ratio and standard deviation of 1.37 ± 0.45. Quite similar results were reported by Cornelissen et al. [5], who compared percentages of degraded PAHs and their rapidly desorbing fractions in contaminated sediments. They found a ratio of degraded/rapidly desorbed as 1.4 ± 0.5. The authors concluded that during bioremediation not only the rapidly desorbing fraction is removed but also a part of the slowly desorbing one, given that bioremediation time is long enough. This should be in fact expected. If in the later phase biodegradation is controlled mainly by contaminant desorption rate (as stated by Huesemann et al. [17]), then for slowly

desorbing fraction with desorption rate constant in the range of 10^{-2} - 10^{-3} h^{-1}, a considerable part would be degraded within several days, or at worst weeks. It should be noted as well that underestimation of bioremediation efficiency by rapidly desorbing fraction in the presented study could be partially a result of a difference in temperature during the tests: 30°C for bioremediation and room temperature (~22°C) for SPE. Temperature affects desorption rates [18] and, to some extent, may influence end-point pesticide concentration after bioremediation [2].

These findings indicate that the rapidly desorbing fraction was rather a poor indicator of potential pesticide bioremediation efficiency. It seems that determination of very slowly desorbing fraction (three-phased desorption) would probably better suit to this purpose, as an indicator of practically non-removable residue. With desorption rate constants in the range of 10^{-4} h^{-1} desorption/degradation of such strongly bound compounds should take several months, or even years, thus being outside acceptable limits of bioremediation. However, determination of slowly desorbing fraction requires a very long time (>300 h) and/or use of elevated desorption temperature, according to Cornelissen et al. [18]. Also Cuypers et al. [19] reported that residual PAHs fraction after bioremediation could be related only to results of long, 264 h Tenax SPE. Such a long extraction time, comparable to that required for proper bioremediation tests, would question the practical applicability of such a procedure. A compromise solution could be a Tenax SPE for a set time, long enough to remove all of rapidly and a considerable part of slowly desorbing fractions. In fact, in the reported investigation the amounts of pesticide extracted in 72 h gave quite reasonable results as a prediction of bioremediation effectiveness. The ratio of pesticide desorbed in 72 h to removed in bioremediation tests was 1.10 ± 0.20 (average ± standard deviation, $R^2 = 0.7274$).

Certainly, the conclusions of this study should be taken with some care, given the limited set and specific characteristics of the samples. On the other hand, its advantage is using real field-contaminated soils, as opposed to other studies made often with lab aged samples. Verification with a larger number of soils of different types is necessary. Also testing the similar procedure, using cyclodextrin instead of Tenax TA, would be interesting as it is reported to be a good estimate of the extent of contaminant biodegradation [8]. Nevertheless, the obtained results, together with previously published findings [2] point to the fact that pesticide remained after bioremediation are hardly desorbable, thus presenting a considerably reduced risk to the environment [15, 20, 21].

4 Concluding remarks

The ability of methanogenic granular sludge to remove γ-HCH, DDT and methoxychlor was confirmed over a wide range of concentrations and in soils of different characteristics. It seems that only a very high level of contamination, hundreds mg/kg of DDT and methoxychlor, partially inhibit this process. The compounds removal effectiveness was correlated with their desorbability, however, the amounts removed were usually much larger than the rapidly desorbing fractions. Therefore, estimation of rapidly desorbing fraction by consecutive Tenax SPE is considered not suitable as a predictor of chlorinated pesticide degradability in contaminated soils. Such measurement should also include part of slowly desorbing fraction prone to desorption and degradation within timeframe of bioremediation process. The fraction of pesticide desorbed in 72 h was quite finely correlated (1.10 ± 0.20) with removal efficiency, and thus may be useful as a rough indicator of bioremediation effect.

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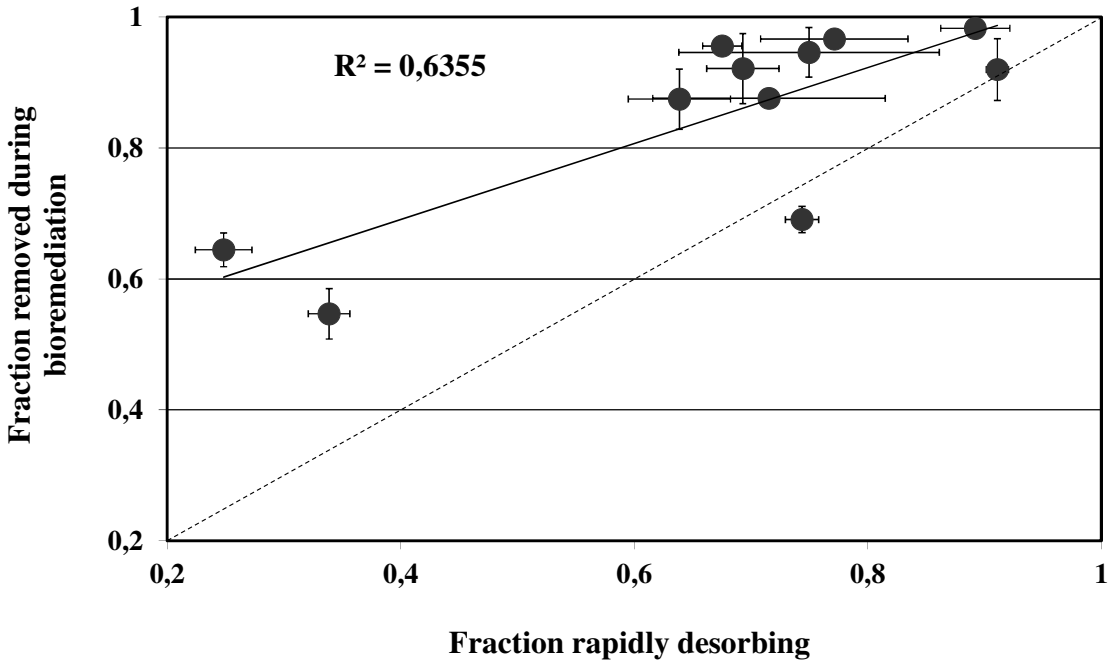

Figure 1. Rapidly desorbing fractions and corresponding removal efficiency for γ-HCH, p,p'-DDT and methoxychlor, averages and standard deviations (bars). Marked are 1:1 line (dotted) and regression line (solid).

Table 1. Concentration of individual pesticides in bioremediated soil samples. Figures are given as μmol kg^{-1} dry soil; average (standard deviation) of at least triplicate analysis.

sample	BOG4	BOG5	SEP2	SEP5	MLY2	MLY4
start of incubation						
γ-HCH	0.99 (0.12)	1.35 (0.07)	19.6 (0.68)	2.20 (0.05)	0.32 (0.00)	39 (5)
p,p'-DDE	0.13 (0.02)	0.18 (0.03)	0.69 (0.07)	< 0.06	< 0.15	< 25
p,p'-DDD	0.09 (0.02)	0.42 (0.11)	3.53 (0.79)	< 0.06	4.52 (0.31)	123 (8)
p,p'-DDT	0.98 (0.14)	3.82 (0.45)	97.2 (7.15)	8.21 (0.95)	11.1 (2.20)	4130 (337)
methoxychlor	0.96 (0.18)	1.71 (0.14)	17.4 (0.81)	3.31 (0.33)	1.59 (0.17)	371 (18)
end of incubation						
γ-HCH	0.04 (0.00)	0.11 (0.06)	0.34 (0.01)	0.17 (0.12)	< 0.08	< 13
p,p'-DDE	0.06 (0.01)	0.51 (0.40)	0.43 (0.17)	< 0.06	< 0.15	< 25
p,p'-DDD	0.21 (0.03)	1.06 (0.19)	19.2 (1.71)	2.15 (0.15)	3.13 (0.36)	232 (13)
p,p'-DDT	0.20 (0.05)	1.73 (0.15)	12.1 (1.15)	1.03 (0.38)	0.49 (0.05)	2370 (299)
methoxychlor	0.17 (0.03)	0.61 (0.04)	0.58 (0.18)	0.18 (0.13)	0.49 (0.03)	68 (8)

"< X" denotes concentration lower than detection limit "X" estimated for the sample

Table 2. Results of Tenax SPE: rapidly desorbing fractions, constants of rapid and slow desorption (h^{-1}). Average and (standard deviation).

Compound	γ-HCH			p,p'-DDT			methoxychlor		
	F_{rapid}	$k_{d,rapid}$	$k_{d,slow}$	F_{rapid}	$k_{d,rapid}$	$k_{d,slow}$	F_{rapid}	$k_{d,rapid}$	$k_{d,slow}$
BOG5	0.911	2.899	0.0074	0.339	0.593	0.0040	0.248	0.672	0.0032
	(0.010)	(0.232)	(0.0020)	(0.018)	(0.035)	(0.0009)	(0.024)	(0.114)	(0.0002)
SEP2	0.892	2.508	0.0012	0.716	1.343	0.0062	0.771	1.299	0.0087
	(0.030)	(0.468)	(0.0007)	(0.100)	(0.072)	(0.0023)	(0.063)	(0.181)	(0.0014)
SEP5	0.693	2.332	0.0185	0.639	1.271	0.0169	0.750	1.834	0.0156
	(0.031)	(0.108)	(0.0053)	(0.044)	(0.036)	(0.0060)	(0.102)	(0.183)	(0.0115)
MLY2	0.971	3.672	0.0125	0.675	0.794	0.0113	0.744	1.386	0.0055
	(0.008)	(0.086)	(0.0069)	(0.017)	(0.129)	(0.0008)	(0.014)	(0.283)	(0.0019)