STUDIES OF INTERACTION BETWEEN SOME ORGANOCHLORINE INSECTICIDES AND HUMIC ACID USING SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

Helena Prosen, Andreja Troha, Lucija Zupančič-Kralj

Faculty of Chemistry and Chemical Technology, Aškerčeva 5, SI-1000 Ljubljana, Slovenia

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Abstract

The interaction of three organochlorine insecticides lindane, heptachlor and dieldrin with humic acid (HA) was studied in water suspension by means of gas chromatographic analysis with electron capture detection (GC/ECD) and solid-phase microextraction (SPME). The use of internal standard α-endosulfan to improve repeatability was abandoned as it was also bound to HA. Pesticides were quantified by external standards. SPME-GC/ECD method was linear in the 0.5 - 20.0 μgL^{-1} concentration range with RSD below 10%. Up to 90% of organochlorines was bound to humic acids at contact time of 35 days. Equilibrium between free and bound fraction of pesticides was reached after 10 - 15 days and was not affected by pH, ionic strength or concentration of humic acid in suspension, suggesting the purely hydrophobic interaction between pesticides studied and humic acid. SPME was compared to solvent extraction to test its applicability in HA suspensions. The results for both extraction methods were similar except for lindane because of a coeluting compound arising from degradation of humic acid.

Introduction

Environmental samples, such as soil and surface waters, contain natural organic matter, commonly named humic substances. These are roughly divided into fulvic acids, humic acids and humin on the basis of their average molecular weight and solubility in water. Humic acids (HAs) with molecular weight up to a few ten kDa still remain an analytical problem regarding their structure. While some suggest they are macromolecules, others propose they are associations of smaller molecules forming pseudomicellar structures in solution.[1] Apparently, the conclusions about structure and even molecular weight of humic acids largely depend on the source of HAs, method of isolation and method of analysis.

In spite of this controversy, it is an established fact that humic acids consist of aromatic rings, alkyl chains and polar functional groups: carboxylic, alcoholic, amino and phenolic groups. Thus, in aqueous solution they act as hydrophobic compounds, as well as polyelectrolytes.[2]

Whether in soil or in solution, humic acids interact with both inorganic ions and organic compounds, e.g. micropollutants of human origin. Organochlorine insecticides persist in the environment because of their low degradability. Binding of some compounds from this group to HAs is an established fact.[3-6] These interactions influence the mobility of pesticides in the environment and their degradation.[7,8] They also have to be considered while evaluating results of pesticide analysis in environmental samples, especially soil. Extraction methods employing organic solvents are usually unsuccessful in extracting the bound fraction of pesticides.[3,9]

The extent of pollutant binding to HAs can be measured by different techniques: equilibrium dialysis of C^{14}-labelled pesticide followed by scintillation counting,[5] ultracentrifugation,[6] fluorescence quenching,[10] but the most common method is extraction of unbound residues and analysis by gas chromatography.[3,4,8,9] Extraction methods used are either liquid-liquid extraction[3,4,9] or resin / solid-phase extraction.[3,8]

Now already well established solventless extraction technique solid-phase microextraction (SPME) can be applied to the analysis of organochlorine insecticides in soil, as we have shown in the previous work.[11] SPME has already been used for the studies of interaction between natural organic matter and polycyclic aromatic compounds[10] as well as PAHs, biphenyls and alkanes.[12]

In the present work, we studied the interactions of selected organochlorine insecticides with HA and evaluated the applicability of SPME for this purpose. A comparison was made with the conventional liquid-liquid extraction (LLE).

Experimental

1. Materials

The pesticides lindane - **1** (γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane), heptachlor - **2** (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) and dieldrin - **3** (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphtalene), as well as α-endosulfan - **4** (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide), used as internal standard, were of >99% purity from Serva (Heidelberg, Germany). Sodium salt of humic acid, technical grade, was purchased from Aldrich (Seelze, Germany). Hexane and acetone

were HPLC grade from Rathburn (Walkerburn, Scotland, UK). Other chemicals used were of analytical grade.

Stock solutions of pesticides lindane, heptachlor and dieldrin, as well as internal standard α-endosulfan, were prepared in hexane (1 - 2 gL^{-1}) and stored in refrigerator. They were stable for several months.

2. Chromatographic conditions

The gas chromatographic analysis was performed with a Hewlett-Packard 6890 gas chromatograph equipped with electron capture detector - ECD (Hewlett-Packard, Palo Alto, CA, USA). Compounds were separated on an HP-1 capillary column (Hewlett-Packard), dimensions 25 m x 0.2 mm i.d., stationary phase thickness 0.11 μm. Carrier gas was helium at flow rate 1 $mLmin^{-1}$. The oven temperature program: start at 100 ^oC (1 min), then ramp 30 ^oC/min to 180 ^oC, then ramp 20 ^oC to 280 ^oC and hold for 2 min. Injector was operated in the splitless mode at temperature 250 ^oC. Injection volume of hexane solutions was 1 μL. Detector was heated to 320 ^oC, make-up gas was nitrogen at flow rate 50 $mLmin^{-1}$.

3. Solid-phase microextraction of organochlorine insecticides

The equipment used was a SPME needle holder for manual injection with polydimethylsiloxane (PDMS) fibre, 100 μm phase thickness (both from Supelco, Bellefonte, PA, USA).

SPME was performed by direct sampling from water solutions. 4 mL solution were placed in a vial and SPME fibre was immersed into it for 30 min. No stirring was applied. The fibre was then placed into the injector of gas chromatograph for thermal desorption of adsorbed compounds.

4. Liquid-liquid extraction of organochlorine insecticides

100 mL water solution of pesticides was extracted with 10 mL hexane in a separation funnel, which was shaken for 5 min. The phases were separated and residual water from organic phase was removed by adding sodium sulphate. In some experiments, the aqueous phase was re-extracted with 10 mL hexane for 5 min. The recoveries for hexane extraction of pesticides from tap water solution ranged from 73% to 85%. The limits of detection were

0.4 μgL^{-1} for lindane, 0.2 μgL^{-1} for heptachlor and 0.2 μgL^{-1} for dieldrin calculated from blank chromatogram, that is, extract of tap water.

5. Adsorption experiments

Standard solutions of compounds were prepared by diluting stock solutions with tap water. Humic acid, sodium salt, was weighted into an Erlenmeyer flask and 10 mL of pesticide solution in tap water was added. For LLE, at least 100 mL of pesticide / humic acid suspension was prepared.

The pH of suspension after addition of humic acid, sodium salt, was in the 9-10 range; for adjusting to pH 2-3, sulphuric acid was used. In some experiments, solid NaCl was also added to yield concentration 40 gL^{-1}.

Flasks were sealed to prevent evaporation and stored at room temperature and in daylight for up to 35 days. To establish the initial concentration of pesticides in suspension, SPME and subsequent GC analysis was performed immediately after mixing the pesticide solution with HA. In every experiment, two replicates were analyzed.

Results and discussion

1. Chromatographic procedure

Manual injection of very small volumes in gas chromatography causes well-known problems with repeatability. In order to overcome them, we introduced internal standard α-endosulfan, eluting at the same chromatographic conditions as the compounds of interest. Gas chromatographic analysis was evaluated regarding its linearity and repeatability. The results are shown in Table 1.

Limits of detection (LOD) were estimated as three-fold value of the baseline noise measured by the injection of solvent only (hexane). The comparison of chromatograms of solvent and pesticide solution of very low concentration (1.0 μgL^{-1}) is depicted in Figure 1.

TABLE 1

Parameters for gas chromatographic analysis of selected pesticides. Internal standard α-endosulfan was added at concentration 19.3 μgL^{-1}. Relative standard deviation (RSD) was calculated from 5 replicate analyses. Limit of detection (LOD) was calculated as three-fold value of baseline noise.

compound	linearity range (μgL^{-1})	r	RSD	LOD (μgL^{-1})
lindane	1.0 - 66.0	0.9973	0.05	0.5
heptachlor	1.0 - 65.5	0.9975	0.05	0.3
dieldrin	1.0 - 80.4	0.9989	0.03	0.5

The analysis with use of internal standard was superior to the analysis without it in terms of repeatability and linearity. However, evaluation of results obtained with the use of internal standard became complicated when applied to the GC analysis combined with SPME, and especially when humic acids were added to the extracted solution, resulting in an unknown amount of analyzed compounds and internal standard bound to humics. For these reasons, the use of internal standard was eventually abandoned.

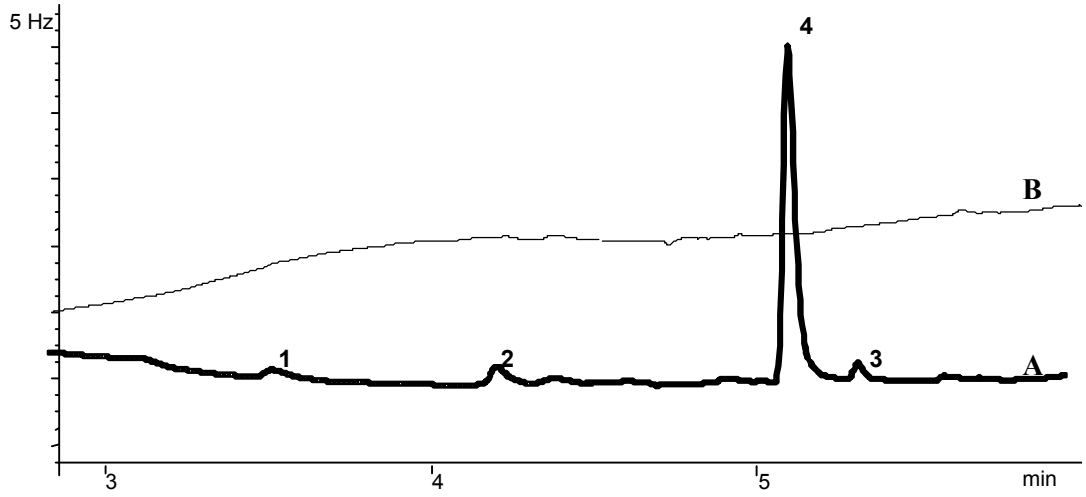

Figure 1: GC/ECD chromatograms of pesticide solution - **A** (1.0 μgL^{-1} in hexane, 1 μL) and hexane - **B** (1 μL). Peak numbering: **1**- lindane, **2**- heptachlor, **3**- dieldrin, **4**- internal standard α-endosulfan (19.3 μgL^{-1})

2. Optimization of the solid-phase microextraction procedure

SPME was performed on pesticide solutions in tap water. In Figure 2, a comparison is made between chromatograms of fibre extracted pesticide solution in tap water and tap water alone. Tap water used was free from interferences except for some baseline distortion at approximately the retention time of heptachlor.

The extraction time was determined by sampling pesticides from solution with SPME fibre for different periods of time, ranging from 10 to 35 minutes. In Figure 3, peak area in GC/ECD analysis is compared with time of sampling for solutions without and with stirring. Obviously, SPME with stirring is capable of extracting a much higher amount of pesticides in the same time due to a better mass transport to the fibre; however, repeatability was not satisfactory under these conditions. We decided to use SPME sampling without stirring, since the limits of detection for pesticides were acceptable for the concentration range used in this study, while the RSD was below 10%, which was comparable to the RSD for the injection of standard solutions in hexane. The sampling time was chosen to be 30 minutes and was the key factor to control in order to obtain reproducible results. The parameters for the SPME-GC/ECD method are shown in Table 2.

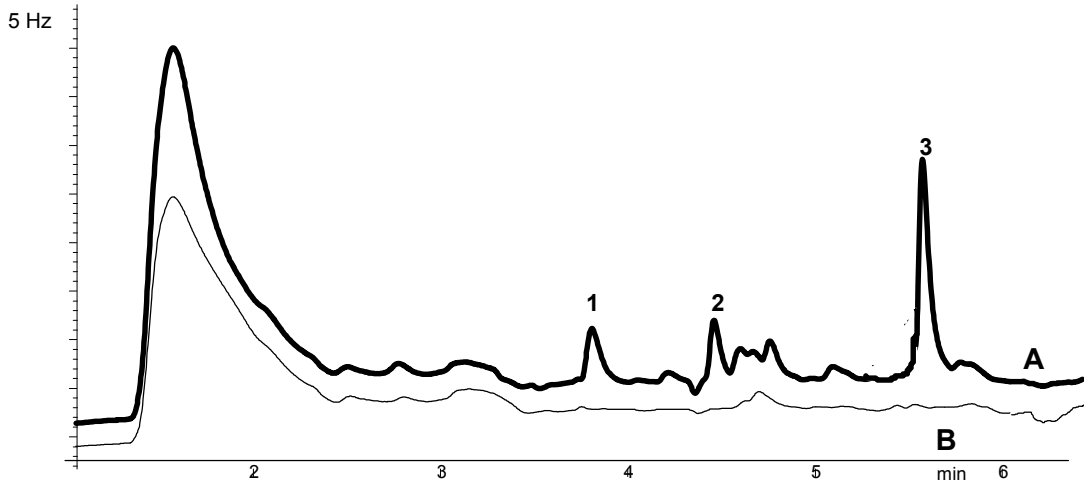

Figure 2: GC/ECD chromatograms after 30 min of SPME of pesticide solution - **A** (1.0 μgL^{-1} in tap water) and tap water - **B**. For peak numbering, see Figure 1.

Humic substances in suspension may influence the rate of pesticide transport to the fibre, e.g. for highly hydrophobic PAHs it is accelerated and for medium hydrophobic PAHs the rate is not affected.[12] Taking this in consideration, we assumed the chosen sampling time would be appropriate also for extraction of highly hydrophobic organochlorine insecticides from humic acid suspensions.

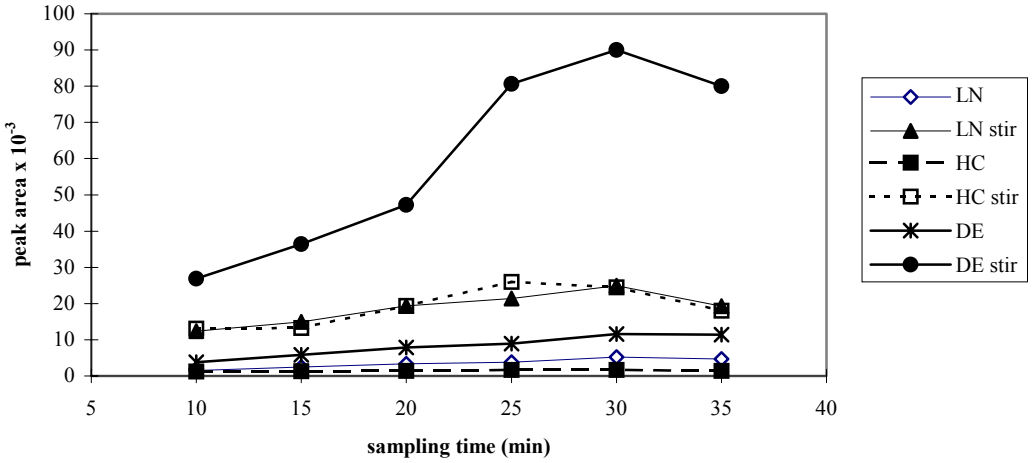

Figure 3: Dependence of peak area in GC/ECD chromatograms from sampling time with and without stirring.

TABLE 2
Parameters of SPME-GC/ECD method for pesticides in tap water solution.

compound	linearity range (μgL^{-1})	r	RSD	LOD (μgL^{-1})
lindane	0.5 - 20.0	0.9983	0.05	0.02
heptachlor	0.5 - 20.0	0.9976	0.09	0.03
dieldrin	0.5 - 20.0	0.9952	0.03	0.01

3. Binding of pesticides to humic acid

Suspensions of humic acid (up to 1 gL^{-1}) in pesticide solution were monitored for up to 35 days. A part of suspension was removed every 5-10 days and extracted by SPME. After suspending humic acid, sodium salt, in pesticide solution, the resulting pH was 9 - 10. Binding of pesticides to HA was monitored either at alkaline (pH 9 - 10) or acidic conditions (pH 2 - 3). Acidic pH was adjusted by addition of sulphuric acid. At acidic conditions, HA

was more extensively degraded during the experiment (up to 35 days), giving rise to some unknown degradation products appearing in the chromatogram approximately at the retention time of lindane as shown in Figures 4 and 5.

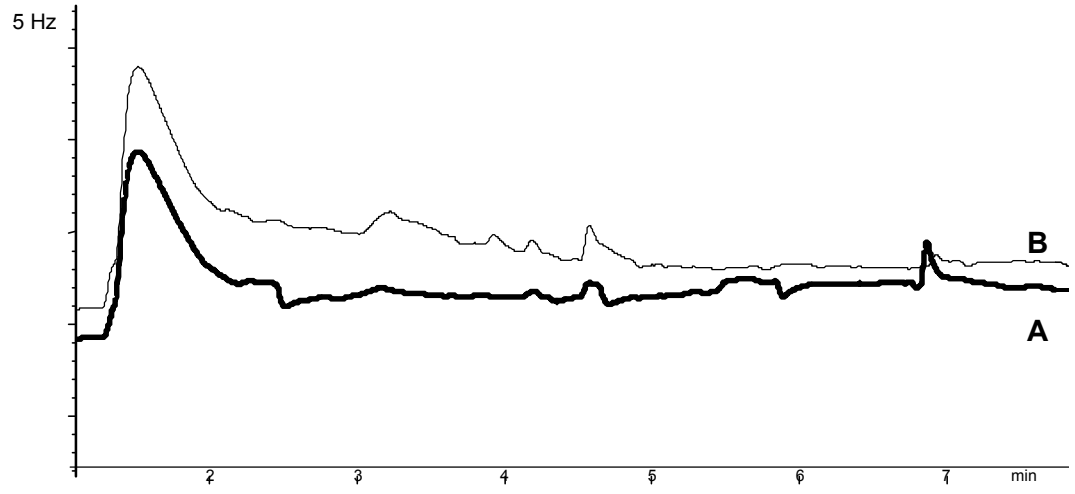

Figure 4: GC/ECD chromatograms after 30 min of SPME of HA suspension in tap water (0.4 gL^{-1}) at pH 9-10 (**A**) or pH 2-3 (**B**) at the beginning of the experiment.

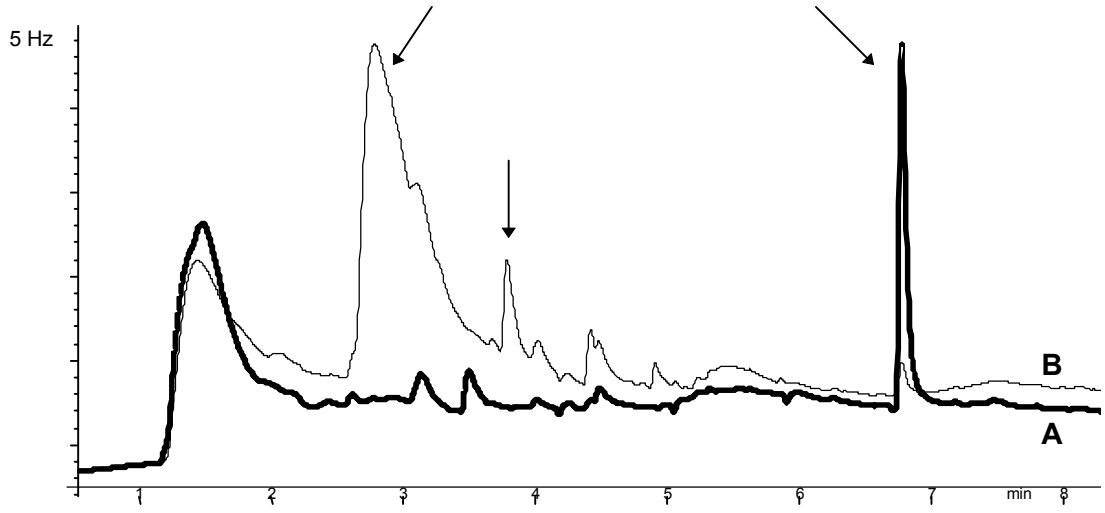

Figure 5: GC/ECD chromatograms after 30 min of SPME of HA suspension in tap water (0.4 gL^{-1}) at pH 9-10 (**A**) or pH 2-3 (**B**) after 30 days of experiment. Prominent peaks appearing as a result of humic acid degradation are marked with an arrow.

However, pH of the suspension had no effect on the amount of pesticides bound to humic acid as it is obvious from Figure 6. Regardless of the suspension pH, only 10 - 15% of the initial amount of pesticides was determined after 35 days of experiment. Therefore, 85 - 90% of pesticides was bound to humic acid in that time. Also, no difference in the extent of binding was noticed when neutral salt (NaCl, 40 gL^{-1}) was added to the suspension.

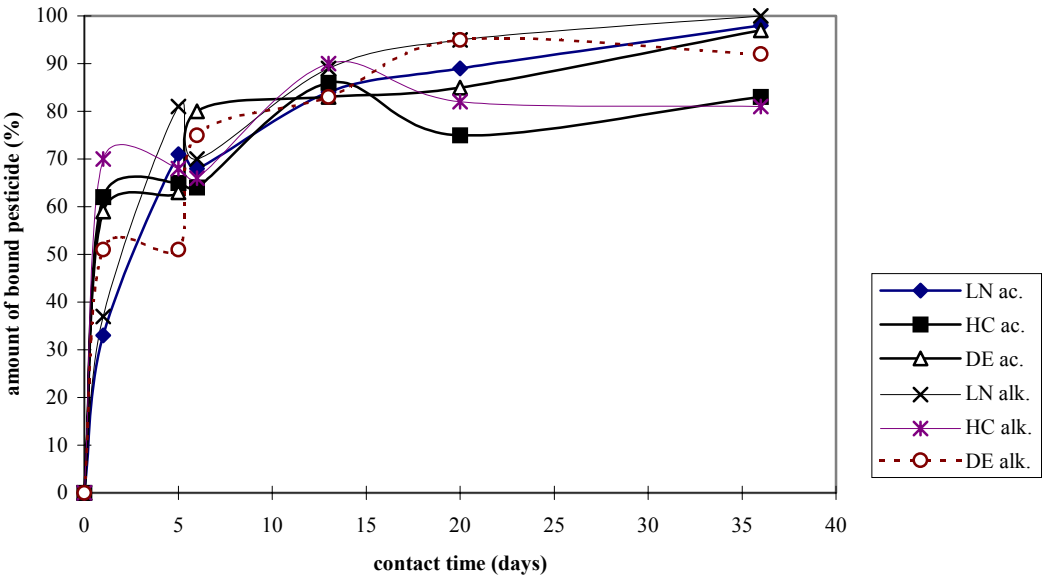

Figure 6: Adsorption of organochlorines (4.2 μgL^{-1}) onto HA (1.0 gL^{-1}) under acidic (pH 2-3) or alkaline conditions (pH 9-10).

The variation of HA content in suspension from 0.1 to 1 gL^{-1} had no effect on binding, as it is depicted in Figure 7. The equilibrium between the fraction of pesticides bound to HA and free pesticides in solution was reached after 10 - 15 days regardless the variations in experimental conditions listed above. Therefore, none of the experimental conditions varied (pH and ionic strength of suspension, HA concentration) influenced the extent of interaction of pesticides and HA. This fact supports the proposed mechanism of organochlorine pesticides partitioning into more hydrophobic humic phase - possibly into pseudomicellar structures already present at the HA concentrations used.[1,4] Lindane, heptachlor and dieldrin would therefore bind to humic acids by hydrophobic interactions only, as was postulated by some authors for p,p'-DDT.[4,5]

The results from these experiments can further be of importance in explaining the behaviour of organochlorines in environmental conditions, e.g. surface waters with higher

content of HAs (usually up to 0.2 gL^{-1}) and pH bracketed by the pH conditions used in our experiments. This conclusion has several implications: firstly, results of organochlorine pesticide analysis in surface waters might lead to underestimation of the real content of these compounds in the sample; secondly, the organochlorines bound to HAs are probably not available for biologic activity (biodegradation), but their photodegradation might be enhanced.[8] They also might concentrate at the bottom of lakes and rivers because of HA precipitation.

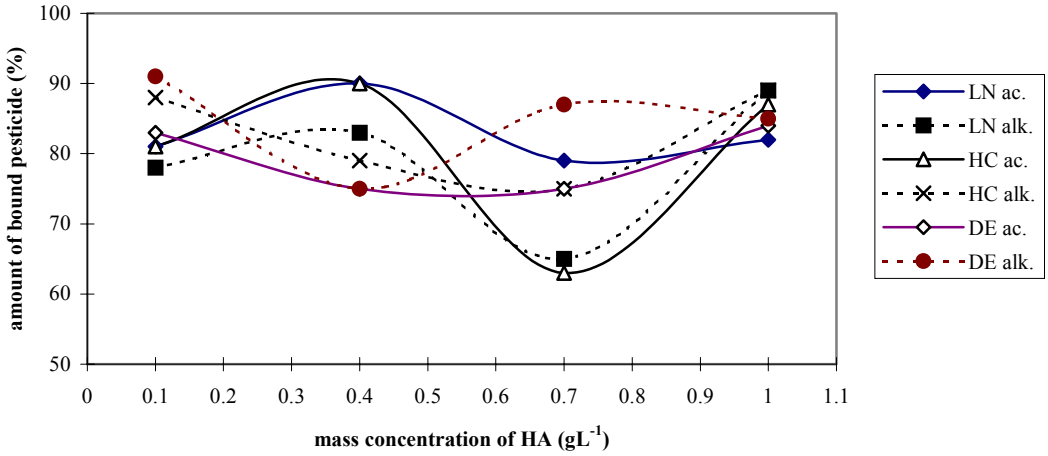

Figure 7: Dependence of the organochlorines (4.2 μgL^{-1}) adsorption from HA concentration (contact time 14 days) under alkaline (pH 9-10) and acidic (pH 2-3) conditions.

4. Evaluation of SPME as an extraction method for determination of unbound pesticides in HA suspension

Humic acids are polyfunctional organic compounds which could possibly adsorb onto the SPME fibre from the solution. In fact, in previous experimental work we noticed blackening of the SPME fibre exposed to HA solutions and its performance gradually deteriorated.[11] Thus, humic acids could compete with pesticides for binding onto the fibre. This would result in underestimation of free pesticide fraction in solution due to a lower amount of extracted compounds.

The simplest way to test this hypothesis was to parallelly determine free pesticides by another method. We have chosen solvent extraction combined with GC/ECD analysis. Solvent extraction has proven before to recover successfully the free fraction of some

organochlorine pesticides, but not the fraction associated with natural organic matter (humic substances).[9]

As was proven with a repeated extraction, unbound fraction of pesticides was entirely removed already by the first extraction. In spite of that, if humic acid was removed from the suspension by filtration prior to extraction, the recoveries were smaller, probably because of pesticide adsorption onto the filter.

The results of free pesticide fraction determination were different from results obtained by SPME, as shown in Table 3.

TABLE 3
Comparison of free pesticide fraction after 35 days of experiment, determined by SPME-GC/ECD and by solvent extraction - GC/ECD

	percentage of free pesticide in suspension			
	SPME		solvent extraction	
compound	acidic pH	alkaline pH	acidic pH	alkaline pH
lindane	10%	10%	50%	55%
heptachlor	10%	15%	7%	5%
dieldrin	15%	15%	10%	8%

In the case of heptachlor and dieldrin, solvent extraction yields somewhat lower free fraction estimates than SPME, which does not support the hypothesis of humic acids competitive adsorption onto the SPME fibre. The difference in results is most pronounced in lindane. Possible cause for that, however, could be the unknown HA degradation product adsorbed to the fibre and eluting near the lindane peak in SPME-GC/ECD chromatogram, as can be seen from Figure 5. Thus, peak areas for lindane are probably underestimated, resulting in too low calculated percentage of free lindane. Another reason for such a difference, that is, less available sites for binding of analyzed compounds to the fibre because of HA adsorption onto it, is highly improbable as the same effect should be seen in the other two compounds. Thus we can conclude the HA in suspension does not interfere with adsorption of pesticides onto the fibre, probably because sufficiently hydrophobic fibre stationary phase, polydimethylsiloxane, was used.

Conclusions

From the comparison of SPME and solvent extraction, we can conclude there are no general objections to application of SPME as an extraction method for pesticides from humic acid suspensions, as it yields similar results as the more established solvent extraction. The appearance of interfering peaks in the chromatogram cannot be predicted and is possible with both methods of extraction, but the interferences can usually be avoided by using appropriate gas chromatographic conditions. Furthermore, a much smaller amount of sample and no organic solvents are needed for SPME, making this method more environment-friendly and also cost-effective.

The three organochlorine insecticides studied bind extensively to the humic acid, but the equilibrium is reached only after 10 - 15 days of contact. From the results obtained by variation of some experimental conditions - pH, ionic strength, HA concentration, we conclude that interaction of these compounds with HA involves the partitioning into hydrophobic phase, but there seem to be no other processes.

Acknowledgments

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Povzetek

Proučevali smo interakcijo treh organoklornih insekticidov lindana, heptaklora in dieldrina s huminskimi kislinami (HK) v vodni suspenziji. Insekticide smo analizirali s plinsko kromatografijo, za ekstrakcijo smo uporabili mikroekstrakcijo na trdni fazi (SPME). Preizkusili smo možnost uporabe internega standarda α-endosulfana, vendar se je le-ta tudi sam vezal na huminske kisline in je bil zato kot interni standard neprimeren. Insekticide smo kvantificirali z eksternimi standardi. Metoda je bila linearna v koncentracijskem območju 0,5-20,0 μgL^{-1}, relativni standardni odmik pa je bil pod 10%. Pri kontaktnem času 35 dni se je na huminske kisline vezalo do 90% organoklornih insekticidov. Do ravnotežja med prosto in vezano frakcijo pesticidov je prišlo po 10-15 dnevih; pH, ionska jakost ali koncentracija HK v suspenziji nanj niso vplivali. Iz tega lahko sklepamo, da je interakcija med izbranimi pesticidi in HK docela hidrofobne narave. Da bi ugotovili, ali je SPME primerna za ekstrakcijo pesticidov iz suspenzij huminskih kislin, smo jo primerjali z ekstrakcijo s heksanom. Rezultati za obe ekstrakcijski metodi so podobni, le za lindan se precej razlikujejo zaradi moteče spojine, ki nastaja pri razpadu huminskih kislin in se v kromatogramu pojavlja hkrati z lindanom.