

ORIGINAL ARTICLE

Pesticide sorption in typical Central European soils evaluated using a photometric microplate assay based on acetylcholinesterase inhibition

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Summary

Sorption isotherms were estimated for two model organophosphorus pesticides – methamidophos and paraoxon-ethyl– and four typical Central European soils endangered by these pesticides: haplic Chernozems, Cambisols, Luvisols, and Fluvisols. A photometric microplate assay based on the recognition capability of the enzyme acetylcholinesterase toward organophosphorous pesticides was used for the construction of sorption isotherms. The sorption capacity of each soil was then determined and it was found that the soils with the highest content of humic acid (haplic Chernozems and haplic Luvisols) sorbed pesticides most. Pesticides in concentrations over the sorption capacity were easily removed from soil by water.

Key words: organophosphates; methamidophos; paraoxon; sorption isotherms; capacity

INTRODUCTION

Organophosphates are a large group of compounds well known because of their toxicity pathway based on the inhibition of enzyme acetylcholinesterase (AChE). The inhibition results in a partial or total dysfunction of neurosynapses in the body (Goel and

Aggarwal 2007). Two groups of organophosphates are commonly known: pesticides (e.g. chlorfenvinphos, dichlorvos, paraoxon, parathion, and propoxur) and nerve agents (e.g. sarin, soman, tabun, and VX) (Barthold and Schier 2005).

The sorption of pesticides in soil is influenced by multiple parameters, including factors such as organic carbon, the consistency of the soil (texture), pH and metal content (e.g. iron, aluminium) (Johnson and Sims 1993, Seybold et al. 1994, Fujisu and Urano 2001, Sheng et al. 2005, Jarvis et al. 2007). Humic acids are considered one of the more important fractions of organic carbon influencing the properties of soils (Delgado et al. 2003, Gondar et al. 2005) and an approachable way of presenting sorption characteristics seems to be by means of the distribution coefficient K_d (Coquet 2003). Soils from

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New Zealand (Baskaran et al. 1996) and Brazil (Spadotto and Hornsby 2003), for example, have been investigated in this way.

An estimation of the sorption of pesticides is typically based on chromatographic techniques such as high performance liquid chromatography (HPLC), used, for example by Spadotto and Hornsby (2003). However there are other techniques available, such as the analysis of the ^{14}C labelled pesticide (Baskaran et al. 1996). Our contemporary effort is aimed not only at the investigation of the sorption capacities of selected soils but also at the performance of the assays engaged with the use of AChE. The above-mentioned assay could thus be used in several ways; biosensor (Pohanka et al. 2008a, b, 2009a) and photometric (Pohanka et al. 2008c) assays may be mentioned as examples, but, its use for pedological purposes has not yet been described. We decided to perform a photometric assay using 96-wells microplates for the data amplification and demonstration of the method.

MATERIAL AND METHODS

Soil sampling

Soil samples were sieved through a 5 mm mesh commonly used for fresh soil samples, and were air-dried at room temperature. Sieving through a 2 mm mesh, more typical for analyses of dry soil samples, was performed only to determine a number of physical, physical-chemical and chemical properties of the uppermost soil layer. Four profiles representative of soils in Central Europe and Czech Republic (from 75%) were sampled. Man's influence is widespread, with cultivation over a long period and one soil sample is from a flood-plain forest with its original type of vegetation.

The soils studied are haplic Chernozems (S1), haplic Cambisols (S2), haplic Luvisols (S3), and haplic Fluvisols (S4). The soils were divided according to FAO (2006).

Soils

S1 Locality Prace (N 49° 8.333', E 16° 45.444'): haplic Chernozems (ha-CH), Climatic region by Köppen's Classification is Cfb (temperate broadleaf deciduous forest). Silty-clay loam Soil. **S2** Locality Nové Město na Moravě (N 49° 32.840', E 16° 4.877'): haplic Cambisols (ha-CM), Climatic region by Köppen's Classification is Dfb (boreal climates). Loam Soil. **S3** Locality Želešice (N 49° 7.194', E 16° 35.743'): haplic Luvisols (ha-LV), Climatic region by Köppen's Classification is Cfb (temperate broadleaf

deciduous forest). Silt loam Soil. **S4** Locality Ivan (N 48° 55.533', E 16° 34.033'): haplic Fluvisols (ha-FL), Climatic region by Köppen's Classification is Cfb (temperate broadleaf deciduous forest). Clay loam Soil.

Soil analysis

Air-dried soil samples were analysed. The particle size distribution was measured by the sedimentation method (Day 1965, Franzmeier et al. 1977). Cation exchange capacity (CEC) and Base saturation were measured by the Mehlich method (Mehlich 1948, Deller 1981), solid organic matter (SOM) by a modified version of the Walkley-Black method (Walkley and Black 1934); humic to fluvic acid ratio (HA/FA) were measured according to the Kononova and Belcikova's (1961) method, and pH (in KCl) by ISO 10390:2005.

Pesticides sorption

Paraoxon-ethyl and methamidophos compounds (both of analytical standard, pesticides content over 98%) were obtained from Labor Dr. Ehrenstorfer-Schafers (Augsburg, Germany). The pesticides were diluted in deionized water to obtain the final concentration 10^{-5} – 10^{-10} M. 1 g of soil sample was suspended with 1 ml of the pesticide solution and kept in a fridge for 24 hours of incubation. Deionized water was treated in the same way as the pesticide for the purpose of negative controls. Centrifugation $3.000 \times g$ for 10 minutes was used for obtaining the liquid phase (further sample) from the soil – pesticide mixture.

Acetylcholinesterase-based assay

Measurements were carried out in standard conditions for temperature and pressure (SATP). Buffered lyophilized human recombinant AChE (2.000 U/mg) was purchased from Sigma-Aldrich (Czech Republic branch). AChE was diluted with deionized water up to the final activity 1 U/ml. Polystyrene 96-well microplates (Gama, České Budějovice, Czech Republic) typically used for immunological studies (Pohanka 2009b) were used throughout the AChE-based experiments. The photometric evaluation was carried out according to Pohanka et al. (2008b).

The sample was injected with an amount of 10 μl per well. 40 μl of acetylthiocholine chloride (ATChCl) 1 mM with 1 mM Ellman's reagent: 5,5'-dithiobis(2-nitrobenzoic acid) (further DTBN) in phosphate buffered saline (PBS) per well was injected after thirty minutes pre-incubation of AChE with the sample. Absorbance was measured at 412 nm using a microplate reader MRX (Dynatech Laboratories, Chantilly, USA) after another 10 minute-period of

pre-incubation. ATChCl with DTBN in PBS alone served as a blank.

AChE inhibition by the pesticide from the sample could be described using a percentage scale (I):

$$I = \left(1 - \frac{\Delta A_i}{\Delta A_n}\right) \times 100 \quad (\text{Eq. 1})$$

The ΔA_i indicates absorbance shift (against blank) provided by AChE effected by the pesticide from the samples; ΔA_n is the absorbance shift caused by intact AChE when the negative control is used in the same manner as the pesticide-containing sample.

RESULTS AND DISCUSSIONS

Characterization of soils

The most important parameters of the sampled profiles are summarized in Table 1. The profiles typically occurring in Central Europe were selected, while the exact localities were selected at random. The haplic Chernozems, Cambisols and Luvisols (S1–S3) are typical soils of arable land where the application of pesticides by farmers may be expected. The haplic Fulvisols (S4) represent the soil endangered by contamination after rain and/or floods.

The fine earth was characterized at the start of the experiments (Table 2). The most important parameters for estimation of pesticides sorption by soils were considered to be organic material, and humic and fulvic acids. The correlation between above-mentioned parameters and AChE-based assay is described below.

Performance of AChE-based assay

The photometric assay based on AChE was found to be reliable in pesticide analysis. No significant interference caused by soil particles was observed when pure water was used instead of the pesticide. The proposed assay could be considered very intriguing for pedology because the low consumption of reagents results in low costs per each analysis: an estimation of the material costs per each analysis is lower than ten euro cents. The low cost of the whole device is an undisputed advantage when we consider the price and costs of the more traditional devices such as chromatography or mass spectroscopy. The simultaneous analysis of a large number of samples (one plate includes 96 wells) is another advantage.

Complete graphic presentations of the percentage of inhibition vs. pesticide concentration (sorption isotherm) for samples eluted from soil by water are

shown in Fig. 1. The elected concentration (mol/l) used in the axis description corresponds to molar sorption per one kilogram of soil (mol/kg) for our experimental setup.

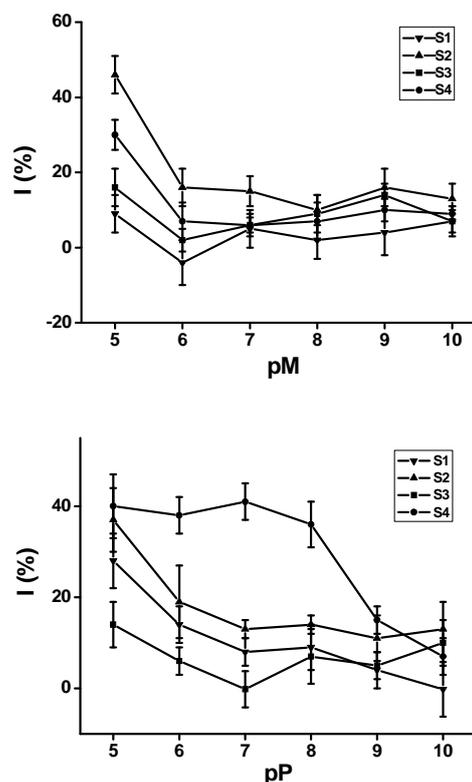


Fig. 1. Sorption isotherms for organophosphorus pesticides and soils (S1–S4). Description of axes is following: (x-axis) inverse logarithm of pesticide paraoxon (pP) or methamidophos (pM) molar concentration; (y-axis) percentage of inhibition. Used soils are marked in the graphs. Means \pm standard deviation are indicated ($n = 4$).

A similarity between the sorption of methamidophos and paraoxon can be observed for soils S1–S3. However, soil S4 provided a different shape of sorption isotherm for paraoxon. Methamidophos and paraoxon sorption amounted to approximately 10^{-6} mol/kg for all tested soils and 10^{-7} mol/kg for soils S1–S3, respectively. A sharp increase in percentage of inhibition occurred when the amount of methamidophos or paraoxon used exceeded this level. The soils S2 and S4 had a lower influence on the percentage of inhibition and probably less affinity of soil particles to pesticides, than the others. S1 and S3 soils remained partially effective in methamidophos and paraoxon retention. We could not

Table 1: General characteristics of the profiles sampled.

Profile	Soil classification ^a	Altitude [m]	Slope [%]	Vegetation or land use	Substratum	Soil climate ^b	
						Precipitation [mm]	Temperature [° C]
S1	haplic Chernozems	240	5	Arable land	Loess	490	8.7
S2	haplic Cambisols	636	5	Arable land	Syenite	581	6.6
S3	haplic Luvisols	210	9	Arable land	Loess	490	8.7
S4	haplic Fluvisols	170	< 1	Flood-plain forest	Fluvial deposits	482	9.2

a – According to FAO [7]

b – Annual quantities (data from the Czech Hydrometeorological Institute), temperature average at 2 m above earth

Table 2: Selected properties of the fine earth.

Sample	Depth [cm]	Particle size distribution [%]			pH (KCl)	CEC [cmol/kg]	Base saturation [%]	SOM [%]	HA/FA
		Clay	Silt	Sand					
S1	5–25	17.6	50.4	32.0	6.7	288.0	87	3.28	1.25
S2	5–25	26.1	42.0	31.9	5.0	165.7	55	2.90	0.51
S3	5–25	21.3	55.1	23.6	7.2	218.2	100	1.46	0.84
S4	5–25	29.2	36.5	34.3	6.0	330.5	87	4.24	0.79

SOM: Soil organic matter (from Soil Organic Carbon * 1.724)

HA/FA: Humic (HA) and fulvic acid (FA)

clearly explain this fact. These soils may be characterized by higher pH and HA/FA in comparison with the last two soils. Although there were some expectations about the parameters effecting the sorption capacity (Seybold et al. 1994), correlation with the SOM and CEC was not clearly confirmed. Because of near neutral or only slightly acidic pH, we may exclude the influence of pH on the stability of pesticides. The most important parameter seems to have been the content of HA. We could see a correlation between HA and C contents and explain the expected influence of the C content in this way, as already proposed in references: Karickhoff et al. 1979, Yaron et al. 1985, Johnson and Sims 1993, but when soil S4 and paraoxon were used, unexpected

data were observed. Sorption of paraoxon on S4 soil was minimal in comparison with any other sorption isotherm. Sorption maximum for S4 soil was less than 10^{-9} mol/kg when paraoxon was used. The data in Fig. 1 point at a sharp increase in the percentage of inhibition – up to 40%; however, this effect is caused by a slow reaction between the pesticide and serine hydroxyl in the enzymatic active centre. Prolongation of the pre-incubation interval up one hour caused a better distribution of inhibition. The percentage of inhibition increased up to 80% (10^{-5} M paraoxon; data not shown). The low sorption of paraoxon is probably caused by its lipophilic characteristics since the S4 soil contains a lot of dissociable organic groups. This idea is supported by the highest CEC and SOM from

applied soils (Table 2). We consider low sorption of paraoxon in S4 soil as a serious menace since paraoxon could be easily eluted into drinking water supplies, especially after floods or rains. The described menace is amplified due to the occurrence of S4 in the tidal lands. The sorption capacities of the other soils are incomparably higher. However, pesticides could be eluted from it under other conditions.

The overall sorption capacity of soils is due to the specific structure of the soil matrix. The surface of soil particles is extensive because of their minute size. Though the average size of silt and clay (Goossens and Buck 2009) is approximately ten to one hundred times greater than typical nanoparticles (Panyala et al. 2008, Tejral et al. 2009), some specific parameters such as sorption on catalyzed oxidation would be expected.

CONCLUSIONS

An AChE-based assay was successfully performed for the evaluation of organophosphates in soils and the estimation of sorption capacity and sorption isotherms in four soils typically found in Central Europe. Two model organophosphates, i.e. paraoxon and methamidophos, were selected as important pesticides widely available on the market. The described assay proved to be very reliable and seems to be suitable for routine applications. The sorption isotherms and sorption capacities were obtained using the AChE-based assay only. Results were comparable with the studies found in available databases.

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