THE ROLE OF NATURAL ORGANIC MATTER (HUMIC SUBSTANCES) ON ADSORPTION OF PESTICIDES POSSESSING ENDOCRINE DISRUPTOR ACTIVITY

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Abstract: In this paper an overview is provided of adsorption processes of representative persistent organic pollutants (POPs), in particular atrazine, alachlor and *cis*- and *trans*-chlordane pesticides with endocrine disruptor activity, by humic acids (HAs), the most abundant and chemically and biochemically active fractions of humified natural organic matter in soil. Endocrine disruptor compounds (EDCs) are hormone-like substances able to alter, i.e., disrupt, the normal endocrine functions in animals and humans. The estrogenic risk of EDCs is generally related to their distribution and speciation in the various soil phases, in which adsorption processes play a very important role. Adsorption kinetics of atrazine and adsorption isotherms of atrazine, alachlor and *cis*- and *trans*-chlordane onto HA samples isolated from various soil sets were determined using a batch equilibrium method and the HPLC technique with various detectors. Adsorption of atrazine onto HAs occurs rapidly within the first 4 h of contact. Experimental adsorption data were best fitted in a linear isotherm or a nonlinear, L-shaped Freundlich isotherm for atrazine, a linear isotherm for alachlor, and a nonlinear, S-shaped isotherm for *cis*- and *trans*-chlordane. Thus, no limiting adsorption is observed for any pesticide adsorbate onto any HA sorbent examined over the concentration range tested. In general, the adsorption capacity of HAs for chlordane is much larger than that for atrazine and alachlor, which are adsorbed at almost the same extent by HAs. The carboxylic and phenolic hydroxyl group content, the organic free radical concentration, and the aromaticity and humification degrees appear to be the most important chemical properties of HAs affecting atrazine adsorption, whereas it is not clear which compositional, structural and functional features of HAs may affect adsorption of alachlor and chlordane.

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1. Introduction

1.1. PERSISTENT ORGANIC POLLUTANTS POSSESSING ENDOCRINE DISRUPTOR ACTIVITY

An “organic pollutant” may be defined as “any organic chemical that is foreign to the natural ecosystem and may adversely affect, either directly or indirectly, the natural physical, chemical, and biological equilibria and processes in the global environment or a portion of it,” e.g., the soil. A persistent organic pollutant (POP) is an organic chemical particularly resistant to chemical and photochemical decomposition and biodegradation, which can persist unaltered in the environment (and soil) for long time spans.

Several POPs, including various classes of pesticides, are known or suspected to act as “endocrine disruptor compounds” (EDCs), i.e., to interfere either directly or indirectly with the normal functioning of the endocrine system of animals and humans by acting as hormone-like substances (Colborn et al., 1993; Kavlock et al., 1996; Lintelmann et al., 2003). Exposure to potential EDCs may interfere with human reproductive function and success (Nolan, 1998), and can lead, especially during certain stages of life, such as development, pregnancy, and lactation, to serious and long-lasting disturbances of endocrine functions (Colborn and Clement, 1992). Despite the high inputs and potential toxicity of EDCs, and the increasing social concern for a reliable risk assessment of the actual exposure of animals and humans to EDCs, until now the attention on this issue has mainly focused on water contamination, whereas relatively few data are available on soil contamination by EDCs.

Potential POPs can reach the soil: (i) by purpose, e.g., various types of pesticides, including herbicides, fungicides, insecticides, and nematicides, currently used in large amounts in common agricultural practices, and belonging to various classes of organic compounds of extremely variable physical and chemical properties; or (ii) incidentally, e.g., chemicals of widely differing classes and properties, including polynuclear aromatic hydrocarbons, phtalic acid diesters, and several organic solvents, detergents and surfactants (Senesi, 1993a, b).

Once on soil surface, the parent POPs and their degradation products may be subjected to various fates, e.g., they can be transported by soil runoff and/or erosion to surface waters, or undergo volatilization and/or photodecomposition, can enter the soil and be leached, eventually reaching the groundwater table, or be subjected to partial or total chemical decomposition and/or biological degradation, uptake by plant roots, and physical and chemical interactions with soil inorganic and organic solid and colloidal components (Senesi, 1993a, b; Senesi and Miano, 1995).

The behavior and performance of POPs in soil is controlled by several factors including the physical and chemical properties of the POP, the thickness, hydrological status and nature of soil, and the type and extent of interactions of the POP with the various inorganic, organic and biological soil components. Most studies have suggested that POPs show affinity for soil organic colloids greater than that for mineral colloids. Thus, the content and nature of soil organic matter, and especially its humified fractions, play a major role in determining the fate of POPs in soil (e.g., Stevenson, 1994; Senesi and Miano, 1995).

The risk assessment of potential environmental hazards of POPs in soil and the definition of possible remediation measures require an accurate evaluation and quantification of the soil response to these compounds. In particular, adsorption of POPs onto soil organic and inorganic components represents a key factor in their fate in soil.

1.2. SOIL HUMIC SUBSTANCES

Approximately 60–70% of the total soil organic carbon occurs as humic substances (HS), which represent the most ubiquitous and widespread natural nonliving organic materials in soil, as well as in all terrestrial and aquatic environments. HS consist of a physically and chemically heterogeneous mixture of relatively high-molecular mass, yellow-to-black-colored organic compounds of mixed aliphatic and aromatic nature, formed by secondary synthesis reactions, i.e., humification, of products of microbial and chemical decay and transformation with recalcitrant residues of biomolecules originated from organisms during life and after death (e.g., Stevenson, 1994). HS are exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass.

On the basis of their solubility in water solutions at various pH, HS are divided into two main fractions, that are: (i) humic acids (HAs), which are soluble in dilute alkaline solution and are precipitated upon acidification to pH 1–2; and (ii) fulvic acids (FAs), which are soluble at any pH values, even below 1–2 (e.g., Stevenson, 1994). HAs and FAs cannot be regarded as single chemical individuals described by unique, chemically defined molecular formulas, but they can be operationally described by model structures constructed on the basis of available chemical and physicochemical data (e.g., Stevenson, 1994; Senesi and Loffredo, 1999).

A "typical" model macromolecule of soil HA basically consists of aromatic, phenolic, quinonic and heterocyclic "building blocks" that are randomly condensed or linked by aliphatic, oxygen, nitrogen, or sulphur bridges, and bear aliphatic, glucidic, aminoacidic, and lipidic surface chains and chemically reactive functional groups of various nature (mainly acidic, e.g., carboxylic and phenolic, but also alcoholic hydroxyls, carbonyls, etc.) (e.g., Senesi and Loffredo, 1999). FAs generally feature structure and composition less complex than those of HAs, have a lower molecular mass and aromaticity and higher solubility, aliphatic character and content of O-containing functional groups. HA and FA are rich in hydrophilic and hydrophobic sites, exhibit a polydispersed and polyelectrolitic character, possess surface activity, present a relatively open, flexible, sponge-like structure rich of holes (Schnitzer, 1978; Hayes and Swift, 1978), and contain a variable amount of highly reactive organic free radical moieties of prevalent semiquinonic nature (Senesi and Loffredo, 1999). All the above described properties qualify HS, and especially HAs, as privileged natural organic compounds in the interaction with POPs.

1.3. INTERACTIONS BETWEEN HUMIC SUBSTANCES AND PERSISTENT ORGANIC POLLUTANTS

HS may interact with POPs in several ways and thus influence their behavior, performance and fate in soil with important implications in their persistence, immobilization and accumulation, mobility and transport, bioavailability and biotoxicity, degradability, volatilization and leaching, and residue monitoring in soil. In particular, HS are shown to be able to modify water solubility of POPs, exert catalytic activity on some POP transformations, act as photosensitizers in promoting POP photodegradation, and, especially, adsorb and partition POPs (e.g., reviews by Senesi and Chen, 1989; Senesi, 1992, 1993a, b; Senesi and Miano, 1995).

Adsorption represents probably the most important mode of interaction of POPs with soil components, and especially with HS. POPs can be adsorbed to various extents by HS through specific physical and chemical binding mechanisms and forces with varying degrees and strengths. These include ionic, hydrogen and covalent bonding, charge-transfer or electron donor–acceptor mechanisms, dipole–dipole and Van der Waals forces, ligand exchange, and cation and water bridging (e.g., reviews by Senesi and Chen, 1989; Senesi, 1992, 1993a, b; Senesi and Miano, 1995). However, adsorption of nonpolar (hydrophobic) POPs can be better described in terms of nonspecific, hydrophobic, or partitioning processes between soil water and the HS organic phase.

The mechanism and extent of adsorption will depend on the amount and the physical and chemical nature and properties of both the POP and the HS. These include: the molecular structure, the number and type of functional groups, the size, shape and configuration, the polarity, polarizability and charge distribution, and solubility of both HS and POP, and the acidic or basic or neutral, ionic or nonionic, polar or nonpolar nature of POP. The conditions of the medium, such as pH, ionic strength, redox potential, amount of water, will also greatly influence adsorption of POPs onto soil HS. Further, for any given POP, several possible adsorption mechanisms may operate simultaneously and/or in sequence.

The main objective of this paper is to discuss adsorption kinetics and adsorption isotherms of some representative POPs behaving as EDCs onto some selected soil HAs. However, a comprehensive study of adsorption processes of POPs onto HS should include, beside the evaluation of quantitative aspects, the qualitative and molecular aspects and the determination of adsorption mechanisms.

2. Materials and Methods

2.1. CHEMICALS

The POPs with EDC behavior (Keith, 1998) used in this study are the following: (i) atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,2,5-triazine), a nonionizable, slightly basic, polar, low water soluble, widely and abundantly used preemergence and postemergence herbicide for the control of broadleaf and grassy weeds in major crops, especially corn; (ii) alachlor (2-chloro-2′-6′-diethyl-*N*-(methoxymethyl) acetanilide), a nonionizable, moderately water-soluble, extensively used preplant or preemergence herbicide for weed control in several important agricultural crops including corn, soybean and peanut; and (iii) *cis*- and *trans*-chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-indene), a nonpolar, low water soluble, high lipophilic, broad-spectrum, nonsystemic insecticide used in land applications against ants, coleopters, cutworms, and termites.

2.2. SOILS AND HUMIC ACIDS

The HAs used in this study were isolated from the surface horizons (0–30 cm deep) of the three following sets of soils: (i) five reference Eurosoils of the first generation, a Vertic Cambisol (E1) from Sicily, Italy, an Orthic Rendzina (E2) from Peloponnesus, Greece, a Dystric Cambisol (E3) from Wales, UK, an Orthic Luvisol (E4) from Normandy, France, and an Orthic

Podzol (E5) from Schleswig-Holstein, Germany, and an alluvial soil (AS) from Northern Germany (Senesi et al., 2003); (ii) two loamy carbonatic Terra Rossa soils (SO1 and SO2) sampled in Puglia, Southern Italy (Senesi et al., 1993); and (c) two sandy soils provided by the Rothamsted Exp. Station, Harpenden, UK, and the Universidade Nova de Lisboa, Lisbon, Portugal (UK1 and PO1, respectively) (Loffredo et al., 1999).

A conventional procedure (Schnitzer, 1982) was used to isolate the HAs. Briefly, each soil sample was extracted three successive times by a 0.5 M NaOH + $Na_4P_2O_7$ solution. After centrifugation, the supernatants were unified and acidified with 6 M HCl to pH 1–2. The precipitated HA was separated by centrifugation, then purified by three alternate alkaline dissolution and acid precipitation treatments. The HA was then washed and recovered with distilled water, dialyzed until free of chloride ions, and finally freeze dried to obtain a fine-powdered HA sample. More details on the isolation procedure of HAs can be found in Senesi et al. (2003).

The principal chemical and physicochemical properties of the HA samples used, which include moisture and ash contents, elemental composition, total acidity, carboxyl and phenolic hydroxyl group contents, the E_4/E_6 ratio, the organic free radical (OFR) content, and Fourier transform infrared and fluorescence spectra have been described in detail previously (Senesi et al., 1993, 2003; Loffredo et al., 1999).

2.3. ADSORPTION KINETICS

Adsorption kinetics were measured only for atrazine on HAs isolated from the first set of five Eurosoils (EU1–EU5) and sample AS. Aliquots of 10 mg of each HA were suspended in 5 mL of a 10 mg L^{-1} aqueous solution of atrazine. The mixtures were magnetically stirred for 0.5, 1, 2, 3, 4, 8, and 16 h, and centrifuged. The supernatant solutions were then analyzed by high-performance liquid chromatography (HPLC) to determine the concentrations of atrazine using the same procedure as for determining atrazine adsorption isotherms as described below. All experiments were conducted in triplicate at a temperature of 20 ± 2°C.

2.4. ADSORPTION ISOTHERMS

Adsorption isotherms of each POP-EDC onto each HA were obtained using a batch equilibrium method in glass centrifuge tubes. Volumes of 5 mL of aqueous solutions at concentrations ranging from 0.5 to 30 mg L^{-1} for atrazine and from 25 to 200 mg L^{-1} for alachlor were added to 10 mg of each HA, whereas aliquots of 30 mL of 7.5% ethanol–water solutions of an equimolar mixture of *trans-* and *cis-*chlordane at concentrations ranging from 0.01 to 1 mg L^{-1} for each isomer were added to 5 mg of each HA. Equilibration was achieved by magnetic stirring of mixtures for 16 h at 20 ± 2°C in the dark. All experiments were conducted in triplicate. The supernatant solutions were then removed by centrifugation at 14,600 or 17,600 *g* for 25 or 20 min, and the equilibrium concentrations, C_e, of free POP-EDC were measured by HPLC using: either (i) a Perkin Elmer Model Series 2 Liquid Chromatograph equipped with an ultraviolet (UV) detector at 254 nm and a Supelcosil LC-8-DB column for atrazine; or (ii) a Thermo Separation Products Liquid Chromatograph equipped with an ultraviolet (UV) detector at 265 nm and Supelco C-18-reverse phase column for alachlor. The mobile phase used was an isocratic solution of: (i) 60% (v/v) acetonitrile and 40% water for atrazine; and (ii) 70% (v/v) acetonitrile and 30% of a 5% (v/v) water solution of acetic acid for alachlor. No measurable effect of wall adsorption and/or degradation of POP-EDCs was observed in control blank experiments run in parallel at each concentration used for each molecule.

In the case of chlordane, the molecule was extracted from adequate aliquots of the supernatant solutions using solid phase extraction (SPE) with Supelclean Envi 18, 3 mL (0.5 g) cartridges, and the Merck Lichrolut Extraction Unit, according to a previously described procedure (Loffredo et al., 1997). Chlordane analyses were then performed with a Fisons HRGC Mega 2 Series Gas Chromatograph equipped with a GC Mega SE-52 column and an electron capture detector (ECD) containing a Ni^{63} beta-emitting radioactive source at operating conditions described previously (Loffredo et al., 1997).

The amount of each POP-EDC adsorbed by each HA was calculated as the difference between the initial and the equilibrium amounts of POP-EDC in solution. Adsorption isotherms were constructed by fitting tentatively experimental adsorption data to a linear model:

$$x/m = K\,C_e \tag{1}$$

a nonlinear Freundlich equation:

$$x/m = K\,C_e^{1/n} \tag{2}$$

and a Langmuir equation:

$$x/m = (KbC_e)/(1 + KC_e) \tag{3}$$

where x/m is the amount of POP-EDC adsorbed in μg g^{-1} or μmol kg^{-1}; C_e is the equilibrium concentration of POP-EDC in solution in μg mL^{-1} or μmol L^{-1}; the constant K is a measure of the magnitude of adsorption, i.e., the adsorption capacity of the HA substrate; the constant 1/n indicates the degree of nonlinearity between solution concentration and amount adsorbed; and b represents the Langmuir adsorption maximum (Giles et al., 1960, 1974; Hamaker and Thompson, 1972).

The magnitude of adsorption was also evaluated by using the distribution coefficient, K_d, which is defined as the mean value of the amount of POP-EDC sorbed, x/m, at each equilibrium concentration, C_e, and can be calculated by:

$$K_d\ (\mathrm{L/kg^{-1}}) = \frac{x/m\ (\mu\mathrm{mol\ kg^{-1}})}{C_e\ (\mu\mathrm{mol\ L^{-1}})} \qquad (4)$$

3. Results and Discussion

3.1. ADSORPTION KINETICS

Figure 1 shows a representative example of adsorption kinetics curves of atrazine onto AS-HA. In any case, about 100% adsorption occurs in the first 4 h of contact, which is followed by attainment of an apparent steady-state equilibrium. The initial rapid adsorption phase (about the first hour) would occur on the most reactive and/or accessible sites of HAs, whereas the subsequent adsorption would reflect the involvement of less reactive and/or more sterically hindered sites.

3.2. ADSORPTION ISOTHERMS

On the basis of the calculated correlation coefficients (r) values over the whole concentration range tested, experimental adsorption data of atrazine fit best in a linear, C-type isotherm for samples E1-HA, E2-HA, and E3-HA, and in a Freundlich nonlinear L-shaped ($1/n < 1$) isotherm for samples E4-HA, E5-HA, and AS-HA (Figures 2 and 3, respectively). According to Giles et al. (1960, 1974) a linear, C-type isotherm indicates that a constant partition of the herbicide occurs between the solution and the HA substrate, that is, adsorption is directly proportional to the herbicide concentration in solution and no saturation occurs over the whole concentration range examined. A nonlinear, L-shaped isotherm indicates that the sorbent HA has a moderately high affinity for atrazine in the initial stages of adsorption, which occurs with increasing difficulty as adsorption sites are filled, but never reaches saturation up to the liquid-phase solubility (Giles et al., 1960, 1974).

The correlation coefficients, r, the constants 1/n, and the adsorption and distribution coefficients, K and K_d, of atrazine onto the six HAs examined are listed in Table 1. The greatest extent of adsorption for atrazine is apparently shown by AS-HA whose K and K_d values are from about 2–15

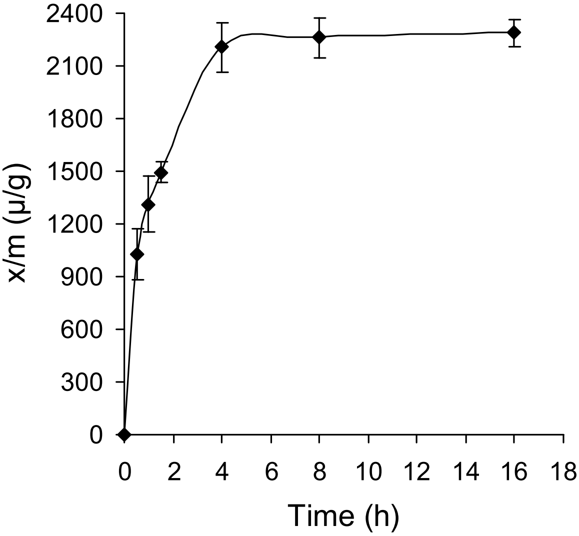

Figure 1. Adsorption kinetics curve of atrazine at 10 mg L^{-1} onto AS-HA. The vertical line on each marker point indicates the standard error ($n = 3$)

times higher than that of any other HA examined. According to the K values, the adsorption capacity of Eurosoil HAs would follow the order: E5-HA > E4-HA >> E1-HA > E3-HA > E2-HA, with the K value of E5-HA about one order of magnitude higher than that of E2-HA. However, the K_d values and their trend are different from those of K values, thus suggesting that the use of K values or K_d values, alone, as an index of adsorption capacity of an adsorbate to a substrate, may be misleading. In general, the adsorption capacity of HAs for atrazine appears to be related positively to carboxylic and phenolic hydroxyl group contents, organic free radical concentration, aromaticity, and humification degree of sorbent HA.

Adsorption data of alachlor onto the two HAs examined, SO1-HA and SO2-HA, fit best in a linear (C-type) model (Figure 4). Thus, also for alachlor no limiting adsorption (saturation) is observed over the concentration range measured. The correlation coefficients, r, and the adsorption and distribution coefficients, K and K_d, of alachlor onto the two HAs examined are shown in Table 2. Both K and K_d values show that the extent of alachlor adsorption is similar onto SO1-HA and SO2-HA, as it can be expected by their similar composition and structural and functional properties. Further, both K and K_d values fall in the range of those measured for atrazine adsorption onto the other set of HAs examined.

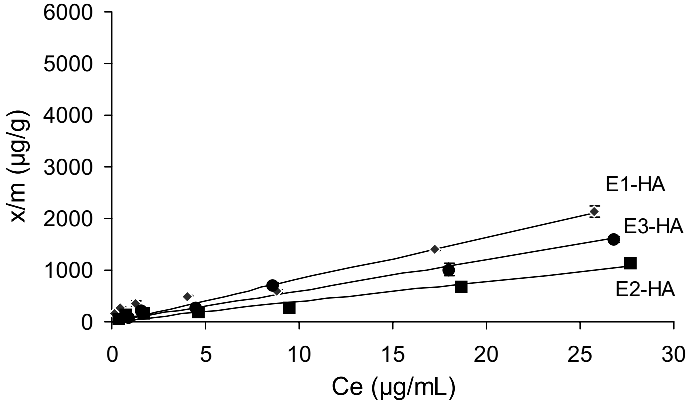

Figure 2. Linear (C-type) adsorption isotherms of atrazine onto the three HA indicated. The vertical line on each marker point indicates the standard error ($n = 3$)

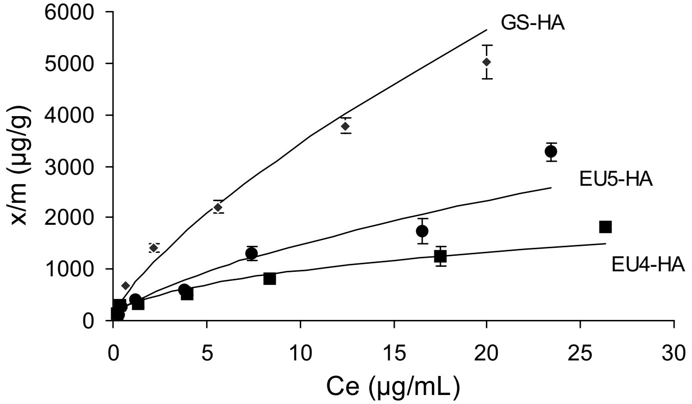

Figure 3. Freundlich (L-type) adsorption isotherms of atrazine onto the three HA indicated. The vertical line on each marker point indicates the standard error ($n = 3$)

TABLE 1. Correlation coefficients, r, adsorption coefficients, K and 1/n, and distribution coefficients, K_d for atrazine onto the six humic acids examined

Origin of HA	r	K (L kg^{-1})	1/n	K_d (L kg^{-1})
E1	0.970	82.4		321.8
E2	0.982	39.2		80.4
E3	0.985	60.4		78.3
E4	0.989	257.8	0.56	122.9
E5	0.980	335.4	0.65	279.5
AS	0.988	646.1	0.72	592.9

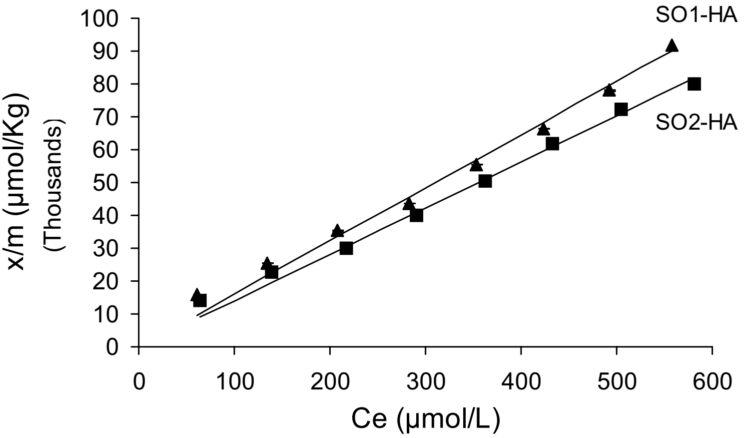

Figure 4. Linear (C-type) adsorption isotherms of alachlor onto the HAs examined

TABLE 2. Adsorption and distribution coefficients, K and K_d, for alachlor onto the humic acids examined

Origin of HA	r	K (L kg^{-1})	K_d (L kg^{-1})
SO1	0.992	161.2	177.4
SO2	0.995	140.8	152.4

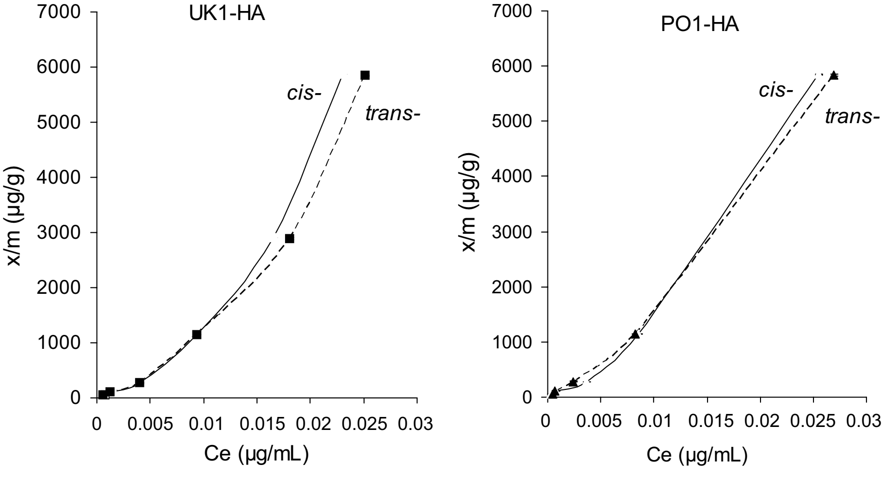

Figure 5. Freundlich (S-type) adsorption isotherms of *cis*- and *trans*-chlordane onto the HA examined

In all cases, adsorption data of *cis*- and *trans*-chlordane onto UK1-HA and PO1-HA best fit a nonlinear, S-shaped (1/n > 1) isotherm (Figure 5). This type of isotherms suggests that the adsorbent has a higher affinity for the solvent than for the adsorbate at low concentration, while the opposite effect occurs at high adsorbate concentration when multimolecular layers may be forming on the sorbent surface (Giles et al., 1960, 1974).

The correlation coefficients, r, the constants 1/n, and the adsorption and distribution coefficients, K and K_d, of *cis*- and *trans*-chlordane onto the two HAs examined are shown in Table 3. According to K values, *cis*- and *trans*-chlordane are both much more adsorbed by UK1-HA than by PO1-HA, whereas K_d values suggest the opposite trend, although the two HAs feature similar chemical and physicochemical properties. Further, K values indicate that UK1-HA adsorbs more *cis*-chlordane than *trans*-chlordane, and PO1-HA adsorbs a little more *trans*-chlordane than *cis*-chlordane, whereas very similar K_d values are measured for UK1-HA or PO1-HA for both chlordane isomers. Thus, similar to atrazine, also in the case of chlordane the trend of K values differ from that of K_d values, and the use of K values or K_d values, alone, may lead to uncorrect evaluation of the adsorption capacity of the substrate for the adsorbate. As a whole, adsorption and distribution coefficients of both *cis*- and *trans*-chlordane onto the two HAs examined are much greater (about three orders of magnitude) than those of atrazine and alachlor onto the correspondent HAs. Thus, even if K and K_d values obtained for different HA substrates cannot be quantitatively compared rigorously, the adsorption capacity of HAs for chlordane is generally very much higher than that for atrazine and alachlor.

TABLE 3. Correlation coefficients, r, Freundlich adsorption coefficients, K and 1/n, and distribution coefficients for *cis*- and *trans*-chlordane onto the two humic acids examined

Origin of HA	r	K ($L\ kg^{-1}$)	1/n	K_d ($L\ kg^{-1}$)
		cis-chlordane		
UK1	0.978	562,031	1.32	119,356
PO1	0.975	155,919	1.01	154,608
		trans-chlordane		
UK1	0.976	298,638	1.19	119,932
PO1	0.989	174,736	1.10	154,381

4. Conclusions

Adsorption data onto HAs fit best either in a linear or a nonlinear Freundlich isotherm for atrazine, in a linear isotherm for alachlor, and a nonlinear Freundlich isotherm for chlordane. In general, the adsorption capacity of HAs for chlordane is much larger than that for atrazine and alachlor, which are adsorbed at almost the same extent by HAs.

The soil HAs examined are slightly different one from another in their compositional, structural and functional properties, on dependence of soil origin and horizon. These differences are shown to affect variously the adsorption processes of POPs onto HAs. In particular, the carboxylic and phenolic hydroxyl group content, the organic free radical concentration, and the aromaticity and humification degrees appear to be the most important chemical properties of HAs affecting atrazine adsorption, whereas it is not clear which compositional, structural and functional features of HAs may affect adsorption of alachlor and chlordane.

The K_d values and their trend are often different from those of K values, which suggests that the use of K values or K_d values, alone, as an index of adsorption capacity of an adsorbate to a substrate, may be misleading. Thus, in general it is advisable to use both parameters for a better evaluation of adsorption capacity of POPs onto HAs.

The type and extent of interaction may change with time and may ultimately result in the immobilization or reversible retention of the POP which may result in a time-delayed risk of contamination. A better knowledge of the chemical nature and reactivity of HS and a better understanding of the mechanisms of their interactions with POPs are expected to help in the progress of the description of POP fate in soil.

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