THE ROLE OF HUMIC SUBSTANCES IN THE FATE OF ANTHROPOGENIC ORGANIC POLLUTANTS IN SOIL WITH EMPHASIS ON ENDOCRINE DISRUPTOR COMPOUNDS

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Abstract: In the first part of this paper an overview is provided of the various phenomena that anthropogenic organic pollutants (AOPs) of various nature and origin are subjected in soil and of the specific role exerted by soil humic substances (HS) in these processes. The paper then focuses on adsorption/desorption of an important class of organic pollutants, i.e., endocrine disruptor compounds (EDCs), onto soil humic acids (HAs) that are the most abundant and chemically and biochemically active fractions of HS and soil organic matter. EDCs are hormone-like substances that include natural and synthetic estrogens and several pesticides, industrial chemicals, and plastic and pharmaceutical products and byproducts which are able to alter, i.e., disrupt, the normal endocrine functions in animals and humans. These compounds may enter the soil through current agricultural practices and /or disposal of urban and industrial effluents, sludges and wastes. The estrogenic risk of EDCs is generally related to their distribution and speciation in the various soil phases, in which adsorption/desorption processes play a very important role. Adsorption kinetics and adsorption/desorption isotherms of the EDCs bisphenol A (BPA), octylphenol (OP), 17-alpha-ethynyl estradiol (EED) and 17-beta-estradiol (17ED) onto HA samples isolated from the surface (0-30 cm) and deep (30-90 cm) horizons of two sandy soils were determined using a batch equilibrium method and the HPLC technique. Adsorption of EDCs onto HAs occurs in two phases, a rapid one in the first few hours of contact, which corresponds to more than 90% of total adsorption, and a slow one that needs generally less than 24 h to be completed. Experimental adsorption data were best fitted in a Langmuir isotherm for BPA and 17ED, in a linear isotherm for OP and in a nonlinear, L-shaped Freundlich isotherm for EED. Thus, no limiting adsorption is observed for EED and OP

onto HAs examined over the concentration range tested, whereas a maximum adsorption, i.e., saturation, is reached only by BPA and 17ED. In general, the adsorption capacity for EDCs of HAs from surface horizon soils is much larger than that of HAs from deep horizon soils. The extent of adsorption of BPA is generally smaller than that of the other three EDCs that are adsorbed at almost the same extent. Adsorption of EED and, especially, BPA onto HAs is generally reversible, and desorption occurs quickly and almost completely after few desorption steps. On the contrary, adsorption of OP is mostly irreversible, a partial desorption occurs slowly, and high amounts of OP are retained by most HAs at the end of the experiment. In conclusion, the soil HAs examined in this work are able to adsorb variable amounts of EDCs that tend to accumulate in the soil surface horizon. EDCs quickly and completely desorbed are expected to move easily down the soil profile, and possibly contaminate groundwater, whereas EDCs slowly and only partially desorbed are expected to remain mostly HA-adsorbed, especially on the surface soil layer, with corresponding top soil contamination.

Key words: Anthropogenic organic pollutants; soil; humic substances; endocrine disruptor compounds; adsorptio/desorption isotherms

1.   INTRODUCTION

An "anthropogenic organic pollutant" (AOP) 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.

The purpose of this papers is to firstly provide an introductory overview of the various phenomena that AOPs of various nature and origin are subjected in soils and on the specific role exerted by soil humic substances (HS) on these processes. Thus, the general and succinct concepts and information contained in sections 2 to 4 are meant to be addressed to those readers who are not familiar with AOP behaviour and fate in soil and related processes involving soil HS. For this reason, and due to the limited space available for this part of the text, detailed references are not included but only some fundamental and comprehensive reviews and books are cited to which the interested reader may refer for further details. The paper will then focus on adsorption/desorption of an important class of organic pollutants, i.e., endocrine disruptor compounds (EDCs), onto soil humic acids (HAs) that are the most abundant and chemically and biochemically active fractions of HS and soil organic matter.

Adsorption/desorption of EDCs onto soil HAs is generally considered one of the most important processes that controls EDC behavior, performances, and fate in soil, including mobility, transport, accumulation, bioavailability and toxicity, and the assessment of their potential environmental hazard.

2. ANTHROPOGENIC ORGANIC POLLUTANTS AND THEIR FATE IN SOIL

Substantial amounts of AOPs can reach the soil either by purpose, e.g., pesticides, or incidentally, i.e., deposited from a variety of sources that include wet and dry atmospheric deposition of AOPs originated from waste chemicals and solid waste incineration, introduced into the soil by discharge of municipal and industrial wastes, refuses, sludges and effluents, also used as soil amendement.

Potential AOPs that can reach the soil include chemicals of widely differing classes and properties. The most important and abundant of these are: (a) various types of pesticides, e.g., herbicides, insecticides, fungicides, etc., currently used in large amounts in common agricultural practices and agricultural product conservation, which belong to various classes of organic compounds of extremely variable physical and chemical properties; (b) polynuclear aromatic hydrocarbons (PAH) present in waste streams originated from various industrial processes such as the combustion of fossil fuels, chemical manufacturing, petroleum refining, metallurgical processes, and some coal, oilshale and tar sand conversion systems; (c) phtalic acid diesters (PAE) used mainly as plasticizers, but also as pesticide carriers and insect repellents, in dyes, cosmetics and lubrificants; (d) endocrine disruptor compounds (EDCs) (see below); and (e) several alkanes, organic solvents, detergents, and surfactants (Senesi, 1993a).

Once on soil surface, the parent AOPs and their degradation products may be subjected to various fates (Figure 1). They can be transported by soil runoff and/or erosion to surface waters, or undergo volatilization and/or photodecomposition. They can enter the soil and be leached, eventually reaching the groundwater table, or be subjected to various phenomena including partial or total chemical decomposition and/or biological degradation, uptake by plant roots, and various physical and chemical interactions with soil inorganic and organic solid and colloidal components (Senesi, 1993a; Senesi and Miano, 1995).
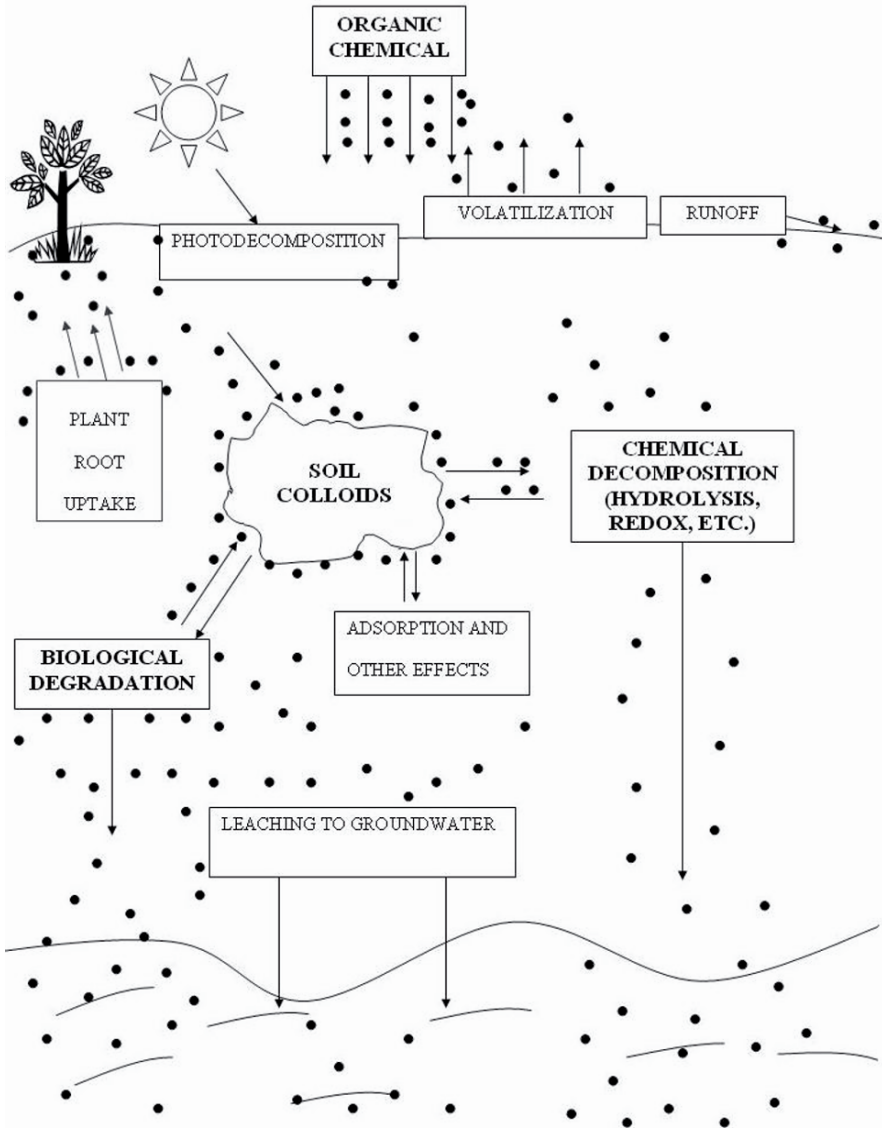
Figure 1. Fate of organic pollutants in soil.

The behaviour and performance of AOPs in soil is controlled by several factors including the physical and chemical properties of the AOP, the thickness, hydrological status and nature of soil, and the type and extent of interactions of the AOP with the various inorganic, organic and biological soil components. Most studies have suggested that AOPs show affinity for soil organic colloids greater than that for mineral colloids. Thus, the content

and nature of soil organic matter, and especially its HS fractions, play a major role in determining the fate of AOPs in soil (e.g., Stevenson, 1994; Senesi and Miano, 1995).

3.   SOIL HUMIC SUBSTANCES

Soil 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 and recalcitrant residues of biomolecules originated from organisms during life and after death (e.g., Stevenson, 1994). These materials are exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass.

Approximately 60-70% of the total soil organic carbon occurs in HS. The estimated level of soil organic carbon on the earth surface occurring as HS is 30×10^{14} Kg (Stevenson, 1994). As such, HS are the most ubiquitous and widespread natural non-living organic materials in soils, as well as in all terrestrial and aquatic environments.

On the basis of their solubility in water solutions at various pH, HS are divided into two main fractions, that are: (a) humic acids (HAs), the portion that is soluble in dilute alkaline solution and is precipitated upon acidification to pH 2; and (b) fulvic acids (FAs), the portion that is soluble at any pH values, even below 2 (e.g., Stevenson, 1994). HAs and FAs cannot be regarded as single chemical individuals described by unique, chemically defined molecular formulas. Rather, they can be operationally described by model structures constructed on the basis of available compositional, structural, functional and behavioral data and containing the same basic structural units and the same type of reactive functional groups that are common to all the single, indefinitely variable and unknown molecules (e.g., Stevenson, 1994).

Figure 2 illustrates one example of a "typical" model macromolecule of soil HA which basically consists of aromatic, phenolic, quinonic and heterocyclic "building blocks" that are randomly condensed or linked by aliphatic, oxygen, nitrogen, or sulphur bridges. The macromolecule bears aliphatic, glucidic, aminoacidic and lipidic surface chains as well as chemically reactive functional groups of various nature (mainly carboxylic and phenolic but also alcoholic hydroxyls, carbonyls, etc.) which render the HA polymer acidic. The structure and composition of FAs are less complex than those of HAs. FAs generally feature a lower molecular mass and

aromaticity and higher solubility, aliphatic character and content of O-containing functional groups.
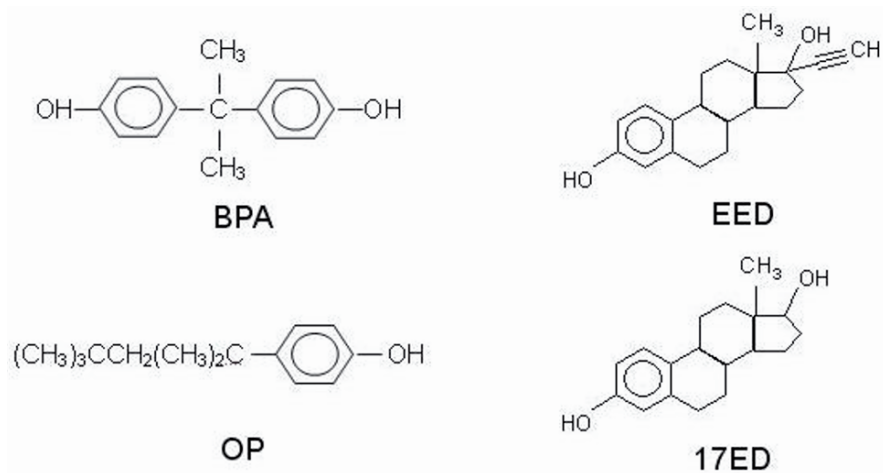

Figure 2. Molecular formulas of endocrine disruptor compounds examined: bisphenol A (BPA), Octylphenol (OP), Ethynilestradiol (EED), and 17 B estradiol (17ED).

HS are rich in hydrophilic and hydrophobic sites, exhibit a polydispersed and polyelectrolitic character, possess surface activity, and present a relatively open, flexible, sponge-like structure rich of holes (Schnitzer, 1978; Hayes and Swift, 1978). An additional typical feature of HS is the presence in their structure of highly reactive organic free radical moieties of prevalent semiquinonic nature (Senesi, 1990).

All the above described properties qualify HS, and especially HAs, as privileged natural organic compounds in the interaction with AOPs.

4.   INTERACTIONS BETWEEN HUMIC SUBSTANCES AND ORGANIC POLLUTANTS

HS may interact with AOPs in several ways and thus influence their behavior, performance and fate in soil with important implications in their persistence, imobilization and accumulation, mobility and transport, biovailability and biotoxicity, degradability, volatilization and leaching, and AOP residue monitoring in soil. In particular, HS have been shown to be able to modify water solubility of AOPs, exert catalytic activity on some AOP transformations, act as photosensitizers promoting AOP photo-degradation, and, especially, adsorb and partition AOPs (e.g., reviews by

Senesi and Chen, 1989; Senesi, 1992, 1993 a, 1993b; Senesi and Miano, 1995).

In the presence of HS, relatively water-insoluble, nonionic AOPs, e.g., PAHs, PAEs, PCBs, DDT, n-alkanes, are mostly easily solubilized, possibly as a result of AOP partitioning into HS or adsorption onto HS, or of an overall increase in solvency. HAs have been shown to enhance water solubility of DDT and PCBs more effectively than FAs, possibly due to their larger molecular mass and nonpolar volume. The magnitude of AOP solubility enhancement was also shown to be dependent on the concentration of HS, pH and temperature. For example, water solubility of DDT and some PCBs increased at low HS concentrations and by a decrease of temperature, whereas it decreased by a raise in pH.

HS, especially in the dissolved phase, were shown to exert a positive or negative catalytic effect in the abiotic hydrolysis of some pesticides. For example, the acid hydrolysis rate of the chloro-s-triazines simazine, atrazine and propazine was enhanced in the presence of FAs or HAs. This effect was possibly attributed to the specific H-bonding interaction occurring between surface carboxylic groups of the HS molecule and the side-chain N atoms of the triazine, which would promote replacement of the Cl atom by water. On the contrary, the alkaline hydrolysis rate of n-alkyl esters of 2,4-D (2,4-DOE) was lowered in the presence of FAs or HAs. Inhibition by HAs of hydrolytic enzymes in soil was indicated as an additional possible mechanism that may operate in certain reactions.

HS are able to adsorb sunlight, and thus behave as initiators of photoreactions, e.g., as photosensitizers, of some AOPs in the top layer of soil by accelerating, increasing or even determining light-induced transformation of AOPs. HS principally act as precursors for the production of greatly reactive, short-lived photoreactants such as the solvated electron, e^-_{aq}, singlet oxygen, $^1O_2$, superoxide anion, O_2^-, peroxy radicals, $RO_2^{\cdot}$, hydrogen peroxide, H_2O_2, and redox-active species including photoexcited parent HS molecules and HS organic radicals. However, HS can also act as scavengers of other phototransients such as the hydroxyl radical, $OH^{\cdot}$. Photodegradation may modify the physical and chemical properties of AOPs, and significantly affect their fate and migration into the bulk soil. For example, photolysis of atrazine was shown to be more extensive in the presence of FA.

Adsorption represents probably the most important mode of interaction of AOPs with soil components, and especially with HS. AOPs can be adsorbed to various extent by HS through specific physical and chemical binding mechanisms and forces with varying degrees and strenghts. 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. However, adsorption of nonpolar (hydrophobic) AOPs can be better described in terms of non-specific, 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 AOP 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 AOP, and the acidic or basic or neutral, ionic or nonionic, polar or nonpolar nature of AOP. The conditions of the medium, such as pH, ionic strength, redox potential, amount of water, will also greatly influence adsorption of AOPs onto soil HS.

The formation of ionic bonds is generally achieved following proton transfer from acidic carboxylic and phenolic OH groups (proton donors) of HS macromolecules and appropriate proton acceptor basic groups of the AOPs such as the pesticides diquat, paraquat, chlordimeform, phosphon, phenacridane chloride, s-triazines and amitrole. Several O- and N-containing sites available on HS macromolecules can be used to form hydrogen bonds with several AOPs containing suitable complementary groups, such as acidic pesticides, including chlorophenoxyalkanoic acids and esters, asulam and dicamba, and several nonionic polar pesticides, including substituted ureas, phenylcarbamates, alachlor, metolachlor, cycloate, malathion and glyphosate. HS macromolecules contain both electron-deficient structural moieties, such as quinones, and electron-rich moieties, such as diphenols, which result in the formation of electron donor-acceptor, or charge-transfer, bonding with AOPs possessing the complementary feature, i.e., electron donor groups, such as s-triazines, substituted ureas, amitrole, or electron acceptor groups, such as paraquat, diquat, chlorodimeform, chloranyl, DDT, PCBs, dioxins. The formation of stable covalent bonds, often mediated by chemical, photochemical and enzymatic catalysts, has been shown to occur between reactive functional groups of HS and suitable functional groups of AOPs such as chlorophenoxyalkanoic acids and esters, and their degradation products such as anilines, catechols and phenols. HS have been shown to be able to adsorb s-triazines and anionic pesticides, such as pichloram, through ligand exchange mechanisms occurring through the displacement of hydration water or other weak ligands partially holding a polyvalent metal ion associated to HS by a suitable functional group ligand of the AOP molecule. Dipole-dipole and Van der Waals forces are considered also to occur in AOP adsorption onto HS, either in addition to stronger binding forces or, more important, in the adsorption of nonionic and nonpolar AOPs. Finally, hydrophobic adsorption, or partitioning, on HS hydrophobic active

sites, such as aliphatic side chains and lipidic and aromatic structural moieties, has been shown to be an important nonspecific mechanism acting for nonionic, nonpolar AOPs that interact weakly with water, such as DDT and other organochlorine insecticides, PAEs, PAHs, PCBs, etc.

For any given AOP, several possible adsorption mechanisms may operate simultaneously and/or in sequence. Initially, the AOP molecule may be adsorbed by HS sites providing the strongest binding, followed by progressively weaker sites as the stronger sites become filled. Once adsorbed, the AOP may be subject to other processes, e.g., may further react becoming either covalently and irreversibly bound or only physically trapped into the HS matrix. Adsorption processes may thus vary from complete reversibility to total irreversibility, i.e., the adsorbed AOP may be easily desorbed, desorbed with various degrees of difficulty, or not at all. Further, the effect of adsorption on AOP migration in soil depends on whether the adsorption occurs on insoluble, immobile HS such HAs, or on dissolved or suspended, mobile fractions such as FAs. Thus, HS can either "attenuate" or "facilitate" AOP movement and transport in soil.

A comprehensive study of adsorption/desorption processes of pesticides onto HS should include the evaluation of quantitative aspects, through the measurement of adsorption/desorption isotherms and coefficients (see below for EDCs), and qualitative and molecular aspects through the determination of adsorption/desorption mechanisms. More detailed information and discussion on the above topics can be found in several recent reviews (e.g., Senesi and Chen, 1989; Senesi, 1992, 1993 a, 1993b; Senesi and Miano, 1995).

5.   ENDOCRINE DISRUPTOR COMPOUNDS

Several organic compounds of natural and anthropogenic origin are suspected or known to interfere with the endocrine system of animals and humans by acting as hormone-like substances in the organism (Lintelmann et al., 2003). These compounds are known as "endocrine disruptor compounds" (EDCs), and possess the property to alter or disrupt the normal functioning of the endocrine system either directly, by blocking or imitating natural hormones, or indirectly, by interfering with the synthesis, storage, secretion, transport, catabolism and activity of various natural hormones (Kavlock et al., 1996). Although the consequences of such interference on animal and human health are not yet completely understood, it is known that small disturbances in endocrine functions, especially during certain stages of life, such as development, pregnancy, and lactation, can lead to serious and long-lasting effects (Colborn and Clement, 1992). Recent progress in both

epidemiological and toxicological research indicates that exposure to potentially endocrine disrupting agents may interfere with human reproductive function and success, and that these agents may have a particularly important role in the aetiology of a variety of endocrine mediated disorders (Nolan, 1998).

Compounds proven or suspected to act as EDCs may be often introduced into the environment through common agricultural practices and by application, discharge and/or disposal of sewage and industrial effluents and sludges, and disposal of plastic and pharmaceutical residues. Different classes of herbicides, fungicides, insecticides and nematicides, several industrial chemicals, including PCBs, PAEs, dioxins, and some pharmaceutical products, like estrogenic compounds, have been identified as potential environmental EDCs (Colborn et al., 1993). 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, relatively few data are available on the types and amounts of EDCs introduced into soil and aquatic systems. Until now, the attention on this issue has mainly focused on water contamination, and very few information is available on soil contamination by EDCs.

The risk assessment of potential environmental hazards associated with the presence of EDCs in soil, as well as the definition of possible remediation measures, require an accurate evaluation and quantification of the soil response to these compounds. In particular, the study of adsorption/desorption processes of EDCs onto soil HAs represent a key factor for the understanding of the fate of EDCs in soil. The objective of this work was thus to determine adsorption kinetics and adsorption and desorption isotherms of some representative EDCs onto some soil HAs.

6.   MATERIALS

The EDCs used in this study were the following: bisphenol A (BPA) [2,2-(4,4-dihydroxydiphenyl)propane], octylphenol (OP) [4-(1,1,3,3-tetrame-thylbutyl)phenol], ethynyl estradiol (EED) [17alpha-ethynil-1,3,5(10)-estratriene-3,17beta-diol], and 17-beta-estradiol (17ED) [1,3,5(10)-estratriene-3,17beta-diol]. The molecular formulae of these EDCs are shown in Figure 3.
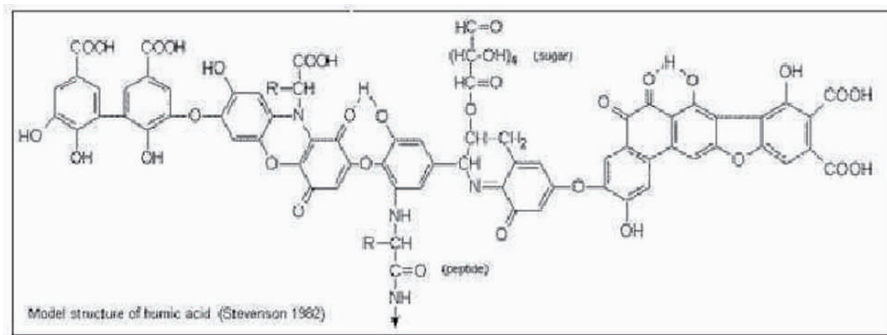
Figure 3. Model structure of humic acid.

The HA fractions of soil organic matter used in this study were isolated by a conventional procedure (Schnitzer, 1982) from two horizons (0-30 cm and 30-90 cm deep) of two different sandy soils sampled near Lisbon (Portugal) and Dresden (Germany), which are abbreviated, respectively, P30 and P90, and G30 and G90. 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 HCl to pH 1-2. The precipitated HA was separated by centrifugation, then purified by two alternate alkaline dissolution and acid precipitation treatments, and finally freeze dried to obtain a fine-powdered HA sample.

The four HA samples were characterized chemically and physico-chemically by current analytical methods and techniques (Senesi and Loffredo, 1999) for moisture and ash contents, yield, elemental composition, total acidity, carboxyl and phenolic hydroxyl group contents, the ratio of absorbances at 460 and 660 nm (E_4/E_6 ratio), organic free radical (OFR) content, and by various spectroscopic techniques.

7. METHODS

7.1 Adsorption Kinetics

Aliquots of 10 mg of each HA were suspended either in 5 mL of a 10 mg/L aqueous solution of BPA, or 25 mL of a 1 mg/L 6% (v/v) ethanol/water solution of OP, or 15 mL of a 10 mg/L 5% (v/v) ethanol/water solution of EED or 17ED. The pH of HA suspensions so obtained ranged from 4.4. to 4.9. The mixtures were mechanicaly shaken for 0.25, 0.5, 1, 2, 4, 8, 16, 24, 48, and 72 h, and centrifuged. The supernatant solutions were

then analyzed by high performance liquid chromatography (HPLC) to determine the concentrations of each EDC using the same procedure as for determining adsorption isotherms as described below. All experiments were conducted in triplicate at a temperature of 20+/-2°C.

7.2   Adsorption Isotherms

Adsorption isotherms of each EDC onto each HA were obtained using a batch equilibrium method. Aliquots of 10 mg HA were added in glass flasks to 5 mL of aqueous solutions of BPA at concentrations of 1, 2, 4, 8, 12, 20 and 40 mg/L, and to 15 mL of 10 % (v/v) ethanol/water solution of OP or 5% (v/v) ethanol/water solution of EED or 17ED at concentrations of 0.1, 0.2, 0.5, 1, 2 and 5 mg/L. All experiments were conducted in triplicate. Equilibration was achieved by mechanical shaking of mixtures for 24 h at 20+/-2°C in the dark. Suspensions were centrifuged at 17,400 g for 15 min, and the supernatant solutions were then analyzed for the equibrium concentrations, C_e, of each free EDC by HPLC. A Thermo Separation Products Liquid Chromatograph was used equipped with a Merk LiChrospher® 60 RP-Select B column for BPA, EED and 17ED, and a SUPELCOSIL™ LC-18 column for OP. In all cases, the mobile phase used was a solution of acetonitrile/water at a ratio of 40/60 (v/v) for BPA, 50/50 (v/v) for EED and 17ED, and 75/25 (v/v) for OP. Ultraviolet (UV) detection at 280 nm was used for BPA, and fluorescence detection at 230 nm excitation and 310 nm emission for OP.

In the case of EED and 17ED, 2-mL aliquots of the supernatant solutions were subjected to a solid phase extraction (SPE) procedure using a Merk Lichrolut Extraction Unit and Merk Lichrolut EN cartridges (200 mg). The cartridges were previously conditioned with 2 mL of methanol and then with 2 mL of 5% (v/v) methanol in water. After the addition of sample solution, the cartridge was dried under vacuum, and the residue eluted twice 1 mL with acetone. Eluates were then analyzed by HPLC using a fluorescence detector operating at excitation and emission wavelengths of 280 and 306 nm, respectively.

The amount of each EDC adsorbed was calculated as the difference between the initial and the equilibrium amounts of EDC in solution. To construct adsorption isotherms, experimental adsorption data of each EDC onto each HA were fitted tentatively to both a linear model and a nonlinear Freundlich equation

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

and a Langmuir equation

$$x/m = (KbC_e)/(1 + KC_e) \qquad (2)$$

where x/m is the amount of EDC adsorbed in $\mu g/g$, and C_e is the equilibrium concentration of EDC in solution in $\mu g/mL$. The magnitude of adsorption, i.e., the adsorption capacity of the HA substrate, was estimated by the values of the Freundlich constant, K_f, and the distribution coefficient, K_d, which was calculated at each equilibrium concentration as the mean value of the ratios of sorbed concentration to the solution-phase concentration at equilibrium.

7.3   Desorption Isotherms

Desorption isotherms of BPA, OP and EED from each HA were obtained by measuring the sequential release of each compound immediately after its adsorption onto 10 mg of HA. This was achieved using either 5 mL of a 20-mg/L aqueous solution of BPA, or 15 mL of a 5 - mg/L of 10 % (v/v) ethanol/water solution of OP, or 15 mL of a 2 - mg/L 5% (v/v) ethanol/water solution of EED. Attempts made to measure desorption of 17ED from substrates examined were unsuccessful, because 17ED apparently degraded during the experiments, as shown by the appearance of not quantifiable degradation products in the HPLC analysis data.

After 24-h equilibration, the mixtures were centrifuged, and the equilibrium solution was carefully removed and replaced with the same volume (4 mL for BPA and 14 mL for OP and EED) of doubly distilled water, in the case of BPA, or 10% (v/v) of ethanol/water solution in the case of OP, or 5% (v/v) ethanol/water solution in the case of EED. The amount of dissolved EDC present in the equilibrium solution that remain entrapped in the substrate was duly calculated and subtracted from the total amount of EDC measured in the supernatant solution after each desorption step. The suspensions were shaken mechanically for 24 h in order to obtain a new equilibrium condition, and then centrifuged. The desorption procedure for BPA was repeated five times or until its concentration in the supernatant solution reached the detection limit of 1 microg/mL, which corresponds to the lower limit of BPA amount detectable in the conditions used, five times for OP, and six times for EED. All experiments were conducted in triplicate.

After each desorption step, the concentration of EDC in the supernatant solutions was measured by HPLC as described above, and the amount of EDC that remained adsorbed was calculated by difference. For comparative purpose, desorption isotherm parameters, K_{fdes} and $1/n_{des}$, were calculated from the Freundlich equation (1) in all cases. The values of K_{fdes} provide an indication of the degree of irreversibility of the adsorption process

(McCall et al., 1981), whereas the values of $1/n_{des}$ account for nonlinearity of the desorption isotherm and is an index of the intensity of desorption (Pignatello and Huang, 1991.

8. RESULTS AND DISCUSSION

8.1 Humic Acids

The ash content, extraction yield, elemental and acidic functional group composition, E_4/E_6 ratio, and concentration of organic free radicals (OFR) of the HAs examined are referred in Table 1. A detailed description and discussion of chemical and physicochemical properties of HAs examined can be found elsewhere (Loffredo and Senesi, 2005).

Briefly, the four HA samples show only some minor differences as a function of the horizon depth and/or the soil type. A larger N, S and OFR content and a slightly smaller aliphaticity characterize the surface horizon HAs, with respect to the deep horizon HAs. In particular, the much larger OFR concentration of both surface soil HAs than that of the corresponding deep soil HAs suggests a larger degree of polymerization and aromatic polycondensation for the former HAs with respect to the latter HAs (Senesi and Loffredo, 1999).

Further, a larger N content, E_4/E_6 ratio and aromatic character, and a slightly smaller C/N ratio, aliphaticity and carbonyl group content are measured for the P-HAs, with respect to the G-HAs. These differences, although limited, may be expected to affect differently the adsorption/desorption processes of HAs for EDCs.

Table 1. Some chemical properties of humic acids (HAs) isolated from soil samples used.

Parameter	P30 - HA	P90 - HA	G30 - HA	G90 - HA
C %	57.12	56.54	58.12	55.07
H %	6.19	5.35	5.80	6.44
N %	4.75	3.94	3.58	3.45
S %	0.41	0.19	0.43	0.37
O %	31.54	33.97	32.00	34.67
C/N atomic ratio	14.0	16.8	18.9	18.6
C/H atomic ratio	0.8	0.9	0.8	0.7
O/C atomic ratio	0.4	0.4	0.4	0.5
Carboxyl groups meq/g	2.63	3.28	3.10	3.22
Phenolic OH meq/g	4.28	3.55	2.63	4.88

Parameter	P30 - HA	P90 - HA	G30 - HA	G90 - HA
Total acidity meq/g	6.91	6.83	5.73	8.10
E4/E6 ratio	6.6	6.8	5.5	5.8
Organic free radicals spins/g x 1017	1.27	0.56	1.73	0.22

8.2   Adsorption Kinetics

Figure 4 shows two representative examples of adsorption kinetics curves of EDCS onto HAs. In any case, most adsorption (>90%) occurs rapidly in the first few hours of contact.
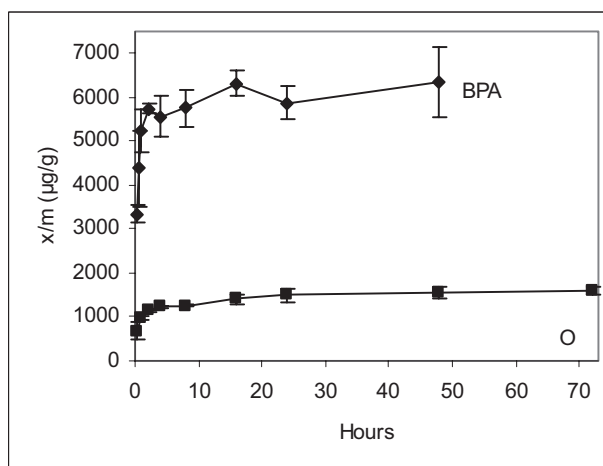

Figure 4. Adsorption kinetics of BPA and OP onto G-30-HA.

This phase is followed by a slow attainment of a steady-state equilibrium, which is reached generally in less than 24 h. BPA appears to be the most quickly adsorbed EDC. The rapid adsorption phase would occur on the most reactive and/or accessible sites of HAs, whereas the slower adsorption may reflect the involvement of less reactive and/or more sterically hindered sites.

8.3   Adsorption Isotherms

On the basis of the calculated correlation coefficients (r) values over the whole concentration range tested, experimental adsorption data of BPA and 17ED onto HAs fit best in a Langmuir model (Figs. 5 and 6). The related

correlation coefficients, r, and the distribution coefficients, K_d, are referred in Table 2.

Table 2. Correlation coefficients, r, adsorption parameters, K_f and $1/n$, and distribution coefficients, K_d, for endocrine disruptors adsorption onto soil humic acids (HAs).

| Sample	r	K_f (L/Kg)	$1/n$	K_d (L/Kg)
		BPA (Langmuir)		
P30-HA	0.986	n.a.	n.a.	273
P90-HA	0.997	n.a.	n.a.	59
G30-HA	0.994	n.a.	n.a.	165
G90-HA	0.986	n.a.	n.a.	87
		17ED (Langmuir)		
P30-HA	0.985	n.a.	n.a.	1397
P90-HA	0.999	n.a.	n.a.	554
G30-HA	1.000	n.a.	n.a.	1405
G90-HA	0.995	n.a.	n.a.	933
		OP (Linear)		
P30-HA	0.988	1158	n.a.	1341
P90-HA	0.992	889	n.a.	905
G30-HA	0.992	1398	n.a.	2326
G90-HA	0.975	351	n.a.	503
		EED (Freundlich)		
P30-HA	0.958	1430	0.97	1586
P90-HA	0.990	780	0.95	828
G30-HA	0.994	1489	0.89	1778
G90-HA	0.961	852	1.03	889

n.a.: not applicable
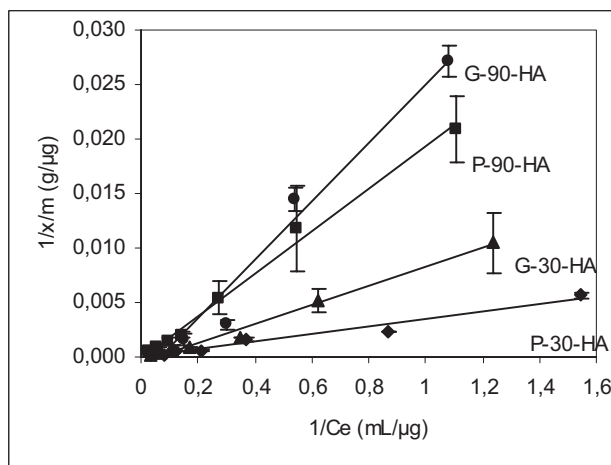
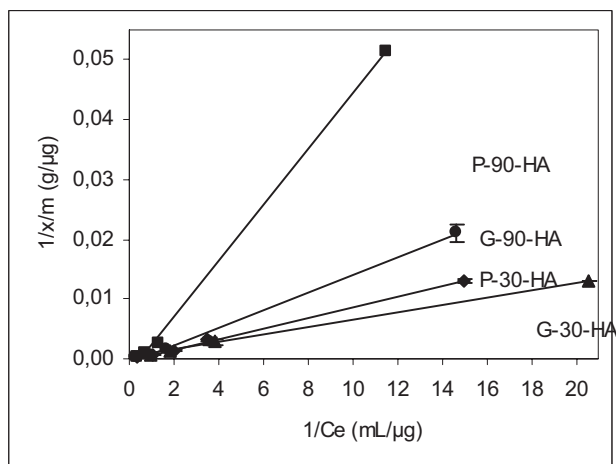*Figure 5.* Langmuir adsorption isotherms of BPA onto soil humic acids (HA).

Figure 6. Langmuir adsorption isotherms of 17ED onto soil humic acids (HA).

A Langmuir isotherm is characterized by linear uptake at low concentrations transitioning to nonlinear (concave-down) uptake as sites are filled, finally leveling off at a maximum adsorption when the sites become saturated. The K_d value, i.e., the adsorption capacity of any HA for 17ED is much larger (about one order of magnitude) than that for BPA. Further, the trend of adsorption capacity of the various HAs for BPA and 17ED is almost similar, showing that surface soil HAs adsorb much more (about two to five times) EDC than deep soil HAs.

Experimental adsorption data of OP onto HAs fit best in a linear, C-type, adsorption isotherms (Figure 7). In all cases, no limiting adsorption (saturation) is observed for OP. The correlation coefficients, r, the Freundlich adsorption coefficient, K_f, and the distribution coefficients, K_d, are referred in Table 2. A linear, C-type isotherm indicates that a constant partition of OP occurs between the solution and the HA substrate, that is, adsorption is directly proportional to the solution concentration and no saturation occurs over the whole concentration range examined. Although slightly different, the K_f and K_d values follow a similar trend with values of surface soil HAs a little larger for P30-HA or much larger (about four times) for G30-HA than those of the corresponding deep soil HAs. Similar to 17 ED, OP is much more adsorbed than BPA.
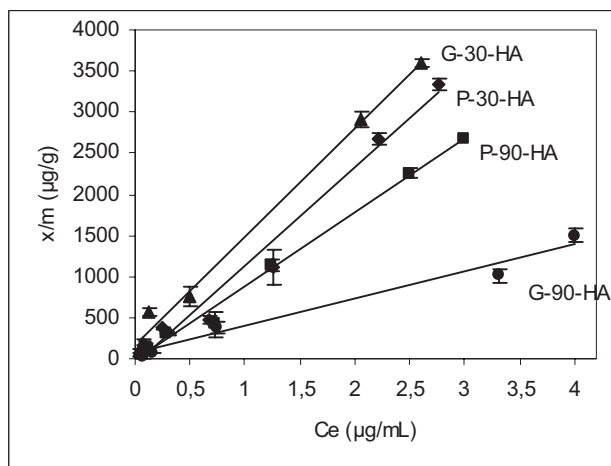
Figure 7. Linear adsorption isotherms of OP onto soil humic acids (HA).

Experimental adsorption data of EED onto HAs are best described by nonlinear, L-shaped ($1/n < 1$) Freundlich isotherms (Figure 8). A nonlinear, L-shaped isotherm indicates that EED sorption occurs with increasing difficulty as adsorption sites are filled, but never reachs saturation up to the liquid-phase solubility. The correlation coefficients, r, the Freundlich adsorption parameters, K_f and $1/n$, and the distribution coefficients, K_d, are referred in Table 2. Calculated values of $1/n$ are generally very close to unity
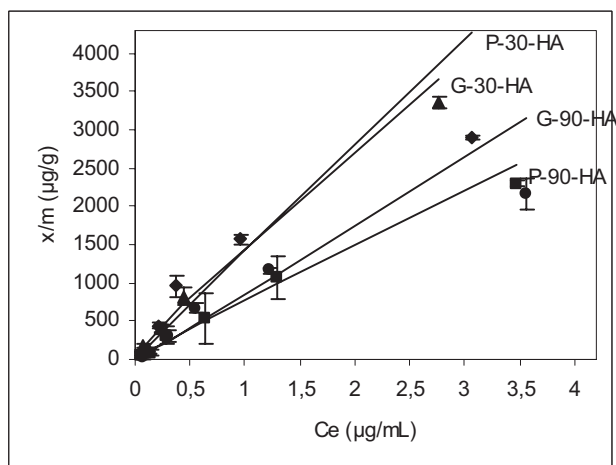

Figure 8. Freundlich non linear adsorption isotherms of EED onto soil humic acids (HA).

thus an almost linear, C-type adsorption model for EED can be considered also valid. Similar to OP, also the corresponding K_f and K_d values of EED are different but follow the same trend, with values of surface soil HAs about twice larger than those of the corresponding deep soil HAs. Similar to OP and 17ED, also EED is much more adsorbed than BPA.

8.4   Adsorption-Desorption Studies

Even if adsorption data of each EDC onto HAs could be best fitted in one of the three equations described above, a nonlinear Freundlich equation was used in all cases to fit both experimental adsorption data and desorption data, in order to obtain adsorption and desorption parameters comparable for any EDC and any substrate examined. This procedure is acceptable on the basis of the generally good correlation coefficients obtained for any EDC and any HA for a nonlinear Freundlich fitting of adsorption and desorption data.

Correlation coefficients, r, Freundlich nonlinear adsorption and desorption parameters, and total amounts (averages of three replicates) of EDCs that are desorbed during the experiment from each HA, expressed as the percentage of the initially adsorbed amount (100%), are shown in Table 3. The percentages of initially adsorbed (100%) EDC that remains adsorbed onto HA after each desorption step are shown graphically in Figs. 9, 10 and 11.

Table 3. Correlation coefficients, r, for Freundlich nonlinear isotherms, Freundlich adsorption and desorption parameters and total amount desorbed after a number of desorption steps indicated between parentheses, for endocrine disruptors onto soil humic acids (HAs).

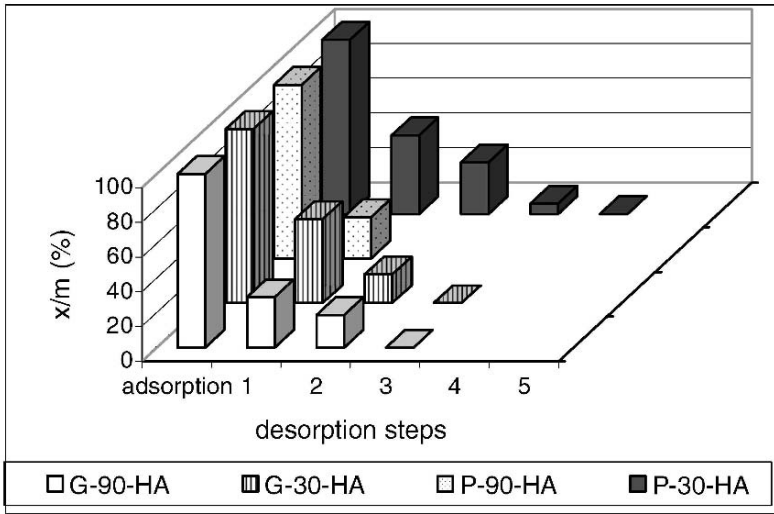| Sample	ADSORPTION			DESORPTION			
	r	K_{ads} (L/Kg)	$1/n_{ads}$	r	K_{ads} (L/Kg)	$1/n_{ads}$	% desorbed
BPA							
P30-HA	0.973	326	0.85	0.932	44.3	1.55	100 (4)
P90-HA	0.996	51	1.08	1.00	2.82	2.14	100 (2)
G30-HA	0.992	131	1.13	0.994	23.8	1.84	100 (3)
G90-HA	0.958	51	1.24	0.948	48.8	1.07	100 (3)
OP							
P30-HA	0.980	1115	0.84	0.983	3028	0.08	26 (5)
P90-HA	0.979	857	0.98	0.995	2400	0.09	31 (5)
G30-HA	0.972	1662	0.80	0.980	3336	0.08	25 (5)
G90-HA	0.967	440	0.91	0.988	968	0.28	70 (5)
EED							
P30-HA	0.958	1430	0.97	0.920	971	0.60	87.2 (6)
P90-HA	0.990	780	0.95	0.909	730	1.08	100 (4)
G30-HA	0.994	1489	0.89	0.984	1102	0.78	88.4 (6)
G90-HA	0.961	852	1.03	0.963	721	0.82	93.3 (6)

Figure 9. Percentages (%) of initially adsorbed (100%) BPA that remain adsorbed onto each humic acid (HA) after each desorption step.
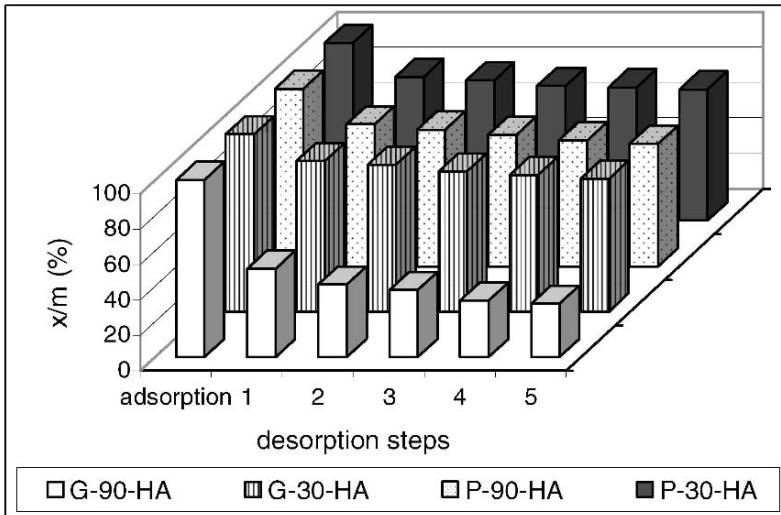

Figure 10. Percentages (%) of initially adsorbed (100%) OP that remain adsorbed onto each humic acid (HA) after each desorption step.
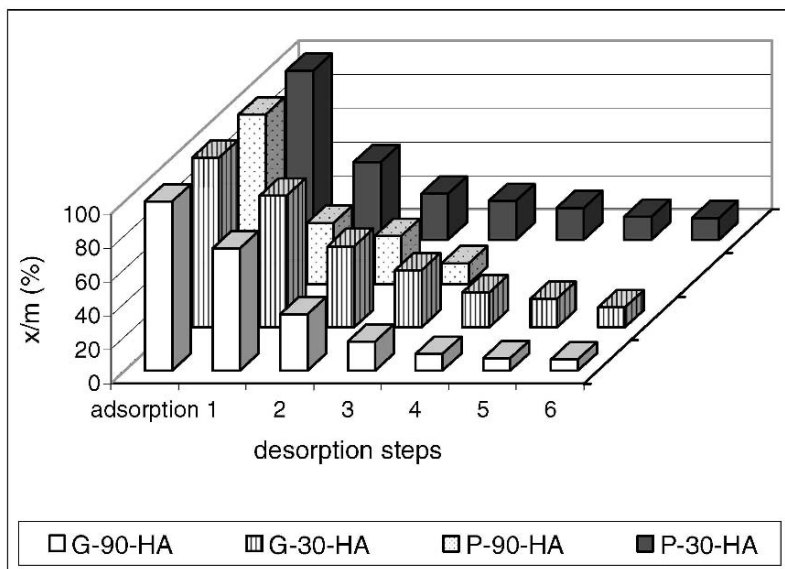
Figure 11. Percentages (%) of initially adsorbed (100%) EED that remain adsorbed onto each humic acid (HA) after each desorption step.

For all HAs the K_{des} values of BPA are smaller or much smaller than the corresponding K_{ads} values and, with the exception of G90-HA, the values of $1/n_{des}$ are larger than those of $1/n_{ads}$. These results suggest that a largely reversible adsorption of BPA occurs onto HAs. Desorption of BPA is generally completed (100%) after two to four desorption steps.

For all HAs the K_{des} values of OP are larger (up to three times) than the corresponding K_{ads} values, and $1/n_{des}$ values are much smaller (up to four orders of magnitude) than the corresponding $1/n_{ads}$ values. These results suggest for all HAs a marked irreversibility of OP adsorption. After five desorption steps, HAs desorb OP at various extents ranging from a maximum of 70% (G90-HA) to a minimum of 25% (G30-HA), thus complete desorption never occurs.

In the case of EED, all HAs show K_{des} values slightly smaller and $1/n_{des}$ values slightly smaller or slightly larger than the corresponding K_{ads} and $1/n_{ads}$ values, respectively. These results suggest the occurrence of a partially reversible adsorption. A total (100%) desorption of initially adsorbed EED occurs only for the sample P90-HA after six desorption steps, whereas the other HAs retain variable but small amounts of EED at the end of the experiment.

9.   CONCLUSIONS

In soil, HS both in the solid and dissolved phases are the privileged components able to interact with AOPs in several ways, thus affecting the behavior, performance and fate of AOPs. In particular, adsorption processes directly or indirectly control all other processes that involve AOPs in soil, i.e., solubility and mobility, degradation and decomposition, etc. Rarely a unique process is involved in the interaction. More often, several processes with different mechanisms occur side by side, one or a few of which may dominate for a given AOP under given conditions. The type and extent of interaction may change with time and may ultimately result in the immobilization or reversible retention of the AOP 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 AOPs are expected to help in the progress of the description of AOP fate in soil. Advanced techniques that are available and show promise for the molecular and mechanistic investigation of HS-AOP interactions include Fourier transform infrared, nuclear magnetic resonance, electron spin resonance and fluorescence spectrocopies.

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, however, are shown to affect variously the adsorption/desorption processes of EDCS onto HAs.

Adsorption kinetics experiments show that the rate of adsorption of any EDC onto any HA is very fast (few hours) in the first phase which corresponds to more than 90% of total adsorption. A much slower phase follows which needs several hours until equilibrium. BPA appears to be the most rapidly adsorbed EDC onto all HAs.

Adsorption of BPA and 17ED is best described by a Langmuir model, which indicates that a saturation adsorption state is reached onto all substrates for these EDCs. Adsorption data of OP are best fitted in a linear, C-type isotherm, and those of EED in a Freundlich non-linear, L-shaped isotherm, thus indicating that no saturation occurs for adsorption of these EDCs onto any HA. The adsorption capacity of surface soil HAs for any EDC is greater (up to four times) than that of deep soil HAs. Further, the extent of adsorption onto HAs is generally smaller for BPA than for the other three EDCs that show a similar extent of adsorption onto HAs.

A comparison of adsorption and desorption data shows that: (a) with the exception of sample G90-HA, BPA adsorption is totally reversible with complete desorption occurring in few steps with a rate faster than that of adsorption; (b) OP adsorption is markedly irreversible with complete desorption never obtained and occurring at a rate much slower than that of

adsorption; and (c) EED adsorption from most HAs is partially reversible, its rate of desorption is similar to that of adsorption, and a complete desorption is obtained only from sample P90-HA, whereas other HAs retain variable, generally low amounts of EED at the end of the experiment.

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