

Full Length Research Paper

Uptake and compartmentalization of phenanthrene by roots of *Cyperus hermaphroditus* in hydroponic system

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Contaminants enter plants through three major pathways, by the root uptake and subsequent translocation into various plant parts through the transpiration process, by vapour uptake from the surrounding atmosphere and by the deposition of contaminated soils and dusts on plant cuticles and subsequent contaminant diffusion through plant surfaces. The uptake and immobilization of phenanthrene by the radical system of *Cyperus hermaphroditus* was studied by employing a hydroponic system. The plants were exposed to 40, 80, and 120 mg/L of this pollutant for 3 and 12 days. Phenanthrene was extracted and quantified by U.V. spectroscopy and HPLC analysis. The uptake and sorption of phenanthrene in these species increased with plant age and with the exposition time to the contaminant. Due to the greater total root mass with a more surface area as well as the enhanced affinity of the roots for the pollutant, the radical system of *C. hermaphroditus* may thus provide a surface for phenanthrene biosorption. Consequently the retention of contaminants by plant roots could be an important control in stabilizing otherwise immobile organic compounds.

Key words: Hydroponic culture, phenanthrene, radical system, uptake, bioconcentration factor.

INTRODUCTION

Understanding the process of contaminant accumulation by plants is essential in assessing crop contamination and subsequent human exposure. The accumulation of xenobiotics by roots can be the result of active uptake or surface adsorption mechanisms. Both are controlled by chemical and physical compound characteristics and plant roots properties. Experiments involving the uptake on nonionized chemicals from hydroponic solution into plant roots have demonstrated that the uptake process consists of two components, first the equilibration of the aqueous phase in the plant root with the concentration in the surrounding solution and second the sorption of the chemical into lipophilic root solids, include lipids in membranes and cell walls (Shone and Wood, 1974; Briggs et

al., 1982, 1983; Patterson et al., 1991; Schwab et al., 1998; Chiou et al., 2001; Li et al., 2005).

For nonionic organic contaminants, with selective plant seedlings, useful empirical equations have been established for estimating the partition uptake by specific plant-parts from external water (or other media) under near-equilibrium conditions (Briggs et al., 1982, 1983; Trapp, 1995). For the plant partition uptake, in general where the plants and external water may or may not be close to equilibrium, a quasi equilibrium partition model may be applied to related the level in plants (or in a part plant) at a given point of time to the corresponding level in external water (or soil water) (Chiou et al., 2001; Su and Zhu, 2007).

During the last decades, there has been a considerable interest in the uptake of organic chemicals by plants (Topp et al., 1986; Wang and Jones, 1994; Mattina et al., 2002). Plants can be exposed to contaminants in different

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ways, active or passive uptake by the root occurs depending on the contaminant and plant type (Shone and Wood, 1974; Briggs et al., 1982, 1983; Gao et al., 2005).

The passive transport to plants may be treated as a series of contaminant partitions between plant water and plant organic components.

The Chiou et al. (2001), partition limited model for the passive uptake of contaminants either from soil or from external water to plants, taking explicit account of the contaminant level in either soil or water and the plant composition.

The model formulation sets the upper (equilibrium) limit for the level of a contaminant in a plant, with respect to the external source level, it seems best at this time to limit the interest to the passive uptake of the parent contaminant species. The overall plant uptake process is driven by the external-water concentration and is considered to consist essentially of a series of partition uptakes, with the understanding that the contaminant concentrations within the plant may or may not come to full equilibrium with the external water solution. On the other hand, for any given volume element inside the plant, local equilibrium is assumed for a contaminant between sap water and the various organic constituents within that volume element.

Thus, the concentration of a contaminant either in the whole plant or in a specific part of the plant (C_{pt}), expressed as the mass of contaminant per unit wet mass of the plant, can be equated with the contaminant concentration in external water (C_w) at the time of sample analyses (Chiou et al., 2001).

Polycyclic aromatic hydrocarbons (PAH's) are hydrophobic organic compounds with some number of condensed benzene rings. The number and arrangement of the rings dictate the physical and chemical characteristics of these compounds. Although they are found in trace concentrations throughout the environment, elevated levels of PAH's can result from combustion, pyrolysis, and pyrosynthesis of organic materials. Concern arises over these compounds, because of their mutagenic and carcinogenic effects on animals (Qui et al., 1997; Schwab et al., 1998).

There has been some works trying to understand plant uptake and accumulation of PAH's (Briggs et al., 1982; Ryan et al., 1988; Schreiber and Schonherr, 1992; Wild and Jones, 1992; Simonich and Hites 1994a, b, 1995; Burken and Schnoor, 1997; Böhme et al., 1999; Gao and Zhu, 2004; Lin et al., 2006). Numerous studies showed that PAH's were accumulated in both vegetable roots and leaves, however it is still under investigation whether PAH's can be translocated within plants. These compounds are lipophilic and the adsorption to the surfaces of roots may be an important sink for these in soils and the first step in phytoremediation (Schwab et al., 1998).

Phenanthrene is a tricyclic aromatic hydrocarbon widely present in the environment as a result of pyrolytic pro-

cesses. Although phenanthrene is not mutagenic or carcinogenic, it has been shown to be toxic to aquatic organisms and is often used as a model substrate for studies on the metabolism of carcinogenic PAH's (Narro et al., 1992).

The aim of this work was to investigate the phenanthrene uptake and accumulation by the roots of *Cyperus hermaphroditus*, through phenanthrene distribution between the different compartments of the culture system, medium, radical apoplast and roots; considering only the interaction between the roots as a biological surface with sorptive characteristics and a polycyclic aromatic hydrocarbon with special physicochemical properties to establish the knowledge of the capacity of this species to accumulate and stabilizing and organic contaminant.

MATERIALS AND METHODS

Plant material and growth conditions

For purposes of this study, specimens of *C. hermaphroditus* (Jacquin Standley), a sedge species, were collected from the Santa Alejandrina swamp in the state of Veracruz, México. Seeds of *C. hermaphroditus* were obtained from the collected samples, germinated in sterile sand with sterile distilled water and maintained in a humid chamber at 36°C.

Once germinated, the seedlings were transferred for grow in amber glass containers (300 ml) with a plastic cap with 2 holes on its surface, one hole for the plant and the other for a glass tube, connected to an air pump to supply air to the system, filled with 250 ml of nutrient solution that contains, 200 mM NH₄H₂PO₄, 500 mM NH₄NO₃, 1150 mM Ca(NO₃)₂, 260 mM CaCl₂, 200 mM MgCl₂·6H₂O, 200 mM Mg(NO₃)₂·6H₂O, 400 mM MgSO₄·7H₂O, 200 mM KH₂PO₄, 120 mM KNO₃, 500 mM K₂SO₄, 40 mM FeCl₃·6H₂O, 12 mM H₃BO₃, 0.12 mM CuCl₂·H₂O, 2.3 mM ZnCl₂, 0.44 mM MnCl₂·4H₂O, 6 x 10⁻³ mM Na₂MoO₄·H₂O, 7.19 mM EDTA and 7.12 mM FeSO₄·7H₂O (pH 6). All of these components were SIGMA and BAKER Co. analytical grade with a purity of > 98%. The seedlings were maintained during 3 months in a greenhouse with temperatures of about 35 - 37°C in the daytime and 15 - 17°C in the night time and with 56% relative humidity. Previously, all of the materials and solutions employed were sterilized.

Exposition of *C. hermaphroditus* plants to phenanthrene

The plants were transferred to clean amber glass containers with 900 ml of the nutrient solution and phenanthrene (from concentrated ethanol stocks pure grade HPLC, SIGMA Chemical, Co.) was added to the mineral solution of each container at test concentrations of 40, 80 and 120 mg/L, with constant aeration to ensure the distribution of the contaminant in the system. Controls without phenanthrene were considerable. All the containers were cover with aluminium foil to keep the roots in darkness. The hydroponic cultures were maintained in a greenhouse with temperatures of about 35 - 37°C in the daytime and 15 - 17°C in the night time and with 56% relative humidity. The hydroponic systems have a constant aeration to ensure that roots were supplied with nutrients and O₂ and homogeneous distribution of the contaminant in the system. The volume of the containers during the study was reviewed every week, and if lost of solution was present, this was replenished with the same amount of distilled water. In this study,

plant concentrations of phenanthrene were all reported on a fresh weight basis. The plants were exposed for 3 and 12 days to phenanthrene and the experiments were performed by triplicate.

Microscopy analysis

In order to detect the adhesion of phenanthrene to the radical surface, a microscopy analysis was done after the period of exposition to the contaminant. Samples of the radical systems from all of the experiments were analyzed by optical microscope analysis: 1 cm² hand root sections were taken at 10 mm below the root-shoot junction from fourth plants. These were previously rinsed with sterile water and finally mounted using non-colored glycerin gelatine. The root sections were observed and photographed using a Nikon Labophot-2 Microscope employing the DCI technique (Differential contrast of interferences).

Measure of the distribution of phenanthrene in roots of the hydroponic cultures of *C. hermaphroditus*

After the exposition time of 3 and 12 days, the experimental plants were removed from nutrient solutions, and sectioned into roots and shoots and the relative weight fractions of fresh roots and shoots (FW) were determined. The roots were used for the extraction and quantify of phenanthrene sorbed at radical surface. The determination of phenanthrene distribution in the compartments of the hydroponic system of *C. hermaphroditus* was done considering the mass of phenanthrene in solution (out of the roots: M_{sol}), the mass of phenanthrene from the apparent free space of the roots (not adsorbed to the roots: M_{afs}) and the mass of phenanthrene adsorbed to the roots (M_{ads}). The phenanthrene was extracted with benzene (SIGMA Chemical Co.) from the 3 different compartments, the media culture (nutrient solution), the surface and the apparent free space of roots (apoplast) and from the radical extract.

The phenanthrene extraction from the radical surface and the root apoplast was done according to the method of Schwab et al. (1998). The total radical biomass was deposited in a flask with 50 ml of an ionic solution (1 mM CaCl₂, 100 mM MgSO₄, 10 mM sodium acetate, pH 5.0), mixed for 20 min and extracted phenanthrene from the ionic solution with 10 ml of benzene. The root biomass was ground with a mortar and pestle with 15 ml of benzene, recovering the radical extract for the quantification of the contaminant adsorbed at the radical surface and from inside of the roots. The samples of residual phenanthrene extracted with benzene from all of the experiments were analyzed using a Shimadzu U.V. 160 Spectrophotometer and subsequently the extracts were evaporated to dryness by a rotary evaporator (Heidolph) and then dissolved in 2 ml of methanol (HPLC grade).

The methanolic extracts of phenanthrene were quantified and analyzed by a high-performance liquid chromatography (HPLC) system, performed on a Beckman component system, model with a 116 absorbance detector. The samples were fractionated on a HICHRON C-18 hypersil column (10 μm x 4.6 mm x 25 cm) an eluted with a programmed linear methanol-water gradient (50 to 95% methanol, 60 min) at a flow rate of 1 ml/min as the eluting solvent. The phenanthrene was detected reading the absorbance at wavelength of 254 nm.

Determination of the bioconcentration factor (BCF) of phenanthrene in *C. hermaphroditus* roots

Based on the quantify of phenanthrene in the fresh roots (Cpt), concentration of a contaminant in a specific part of the plant (roots)

on a fresh weight basis and the phenanthrene quantified in nutrient solution (Cw), the phenanthrene accumulated in the roots after the 3 and 12 days of exposition of *C. hermaphroditus*, was determined by the bioconcentration factor (BCF = Cpt/Cw ratio).

Uptake time for each sampling was defined as the time from the addition of solution containing phenanthrene to the time of removal by plants for chemical analysis. The phenanthrene accumulated of the radical system was calculated by the determination of the BCF measured as the ratio of the organic chemical sorbed on the root (mg of phenanthrene per kg of fresh root weight) to that in nutrient solution (mg of phenanthrene per liter of nutrient solution) (Shone and Wood, 1974).

Statistical analysis

Results are the means from 3 replicates of the experiments and the significance of differences between mean values of root and shoot fresh weight and phenanthrene concentration from HPLC analysis was determinate by a 2 way analysis of variance (ANOVA) and comparison among means was performed using a Tukey's test. These analyses were done applying the FAUANL ver. 1.4 software for experimental data statistics (Olivares, 1989) and the GraphPad instat ver. 2.03 software for experimental designs (Aceves, 1993).

RESULTS

The phenanthrene mass in the compartments of the hydroponic cultures of plants exposed 3 days, showed that the phenanthrene mass quantified in the nutrient solution ($M_{sol} = 60\%$) was greater than the mass of the apparent free space ($M_{afs} = 20\%$) and the phenanthrene mass adsorbed at the roots ($M_{ads} = 15\%$) was less in all of the phenanthrene concentrations tested (Figure 1a). For 12 days, there was a similar distribution within the different compartments in the system for the 3 experiments, where the mass of phenanthrene quantified in nutrient solution (M_{sol}) was around 38% in the apparent free space (M_{afs}) was 35% and the adsorbed at the roots (M_{ads}) was 25% (Figure 1b).

The phenanthrene delivery in the cultures could be related with the period of exposition to the contaminant, the saturation event at the radical surface and a physicochemical limitation in the interaction between the compound and the molecular constituents of roots. The initial accumulation of phenanthrene for both times of exposition was restricted to mechanisms that facilitate the transfer of the chemical directly from the external non-living compartment, into various plant structures like cell walls in the periphery and cannot enter the cell fluids (Pilon, 2005).

Examples of nonliving structures may include the plant apoplast, and the initial rapid uptake into root free space, where rapid sorption phase is passive and diffusion controlled it. Great majorities of environmental organic compounds exhibits a passive uptake mechanism and therefore, are assumed to follow a process of successive movement into the various plant compartments governed by physicochemical process of partitioning (Ryan et al.,

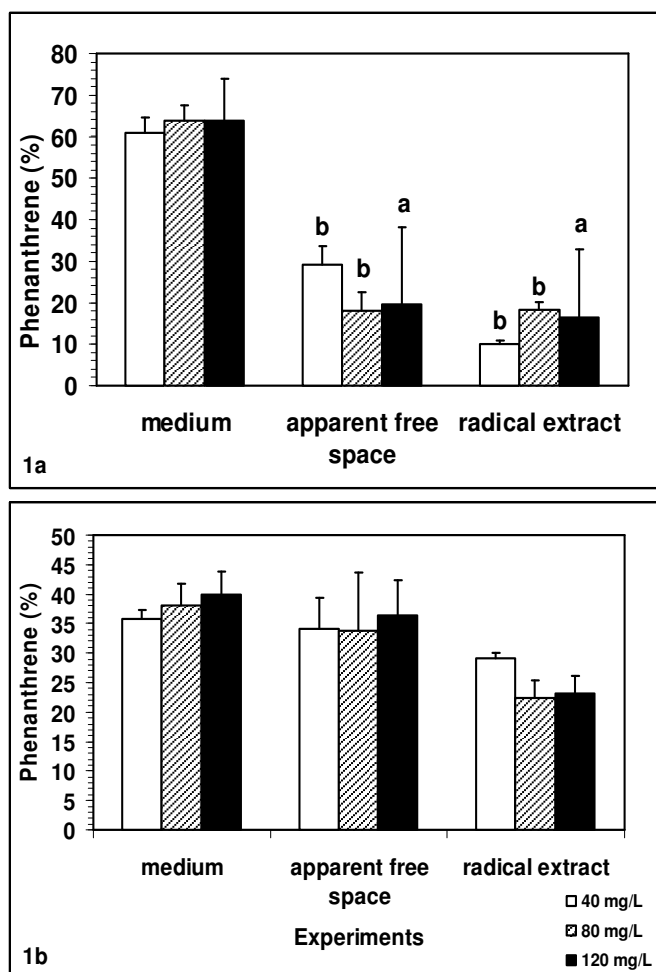


Figure 1. Mass distribution of phenanthrene in the different compartments of the hydroponic culture of *C. hermaphroditus* (medium, AFS and radical extract) after a) 3 days and b) 12 days. For the three concentrations, $n = 3$. Mean values \pm S.D. from three replicates are given. The different lower-case letters shows the significant differences founded only for the experiments of 3 days of exposition to phenanthrene ($p < 0.05$).

1988).

The incorporation of a compound to roots was described through the BCF, which permits understand the correlation between the phenanthrene concentration in the roots and its concentration in the external solution. The BCF was calculated from the results of phenanthrene quantified for both times of exposition and for the 3 concentrations tested (Table 1). In the experiments of 3 days of exposition to phenanthrene, BCF was higher for the 40 mg/L concentration (42.81) compared with the experiments of 80 and 120 mg/L, where the values of BCF ranging from 20 to 30. For 12 days, the BCF values were higher than 3 days for all of the concentrations of phenanthrene tested (between 44 and 83). These values can be associated with the adsorptive capacity presented

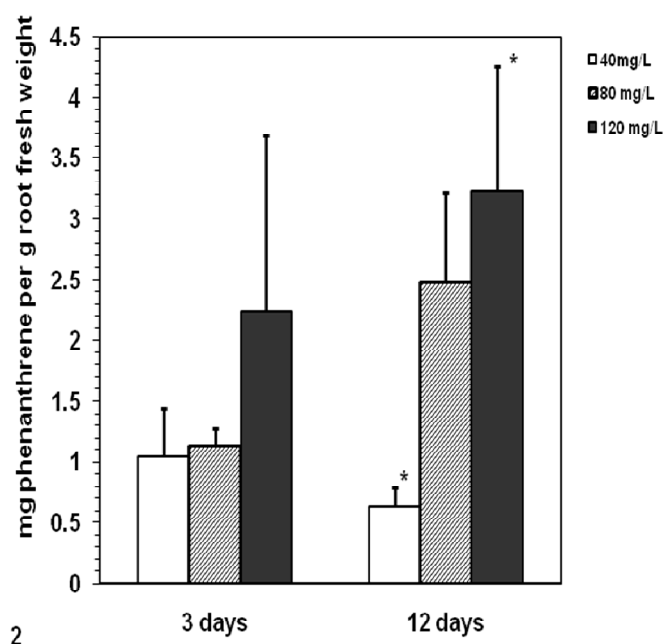


Figure 2. Quantified phenanthrene in the root extracts of *C. hermaphroditus* for the three concentrations ($n = 3$). Mean values \pm S.D. from three replicates are given. The asterisks shows the significant differences among treatments founded only for the experiments of 12 days of exposition to phenanthrene ($p < 0.05$).

in the radical systems of *C. hermaphroditus* related with the compound and the time of exposition; although the BCF values for 3 days decrease as the phenanthrene experimental concentrations were high, the contaminant quantified at the radical surface increased for both times (Figure 2). The results obtained from this study showed that the adsorption degree of the contaminant at the radical surface of this species was related with the development and increment of contact surface of the radical system at the time of exposition ($p < 0.05$), accounting for the enhanced partitioning with phenanthrene (root/shoot ratio, Figure 3).

Microscopy analysis of the roots was performed in order to demonstrate the phenanthrene sorption. Figure 4a and b shows the image of a *C. hermaphroditus* radical fragments exposed to phenanthrene, where the contaminant (white mass) is clearly observed adhered to the radical surface. The lipophylic properties of the membranal components, the cell walls of the roots, the molecular size of the compound and its hydrophobic characteristics may allowed the phenanthrene partition at the radical surface and its diffusion to the apoplast and not its translocation to other parts of the plant (shoots). These events confirm the characteristic patterns of PAH's, which are lipophylic, hydrophobic and with a high tendency to concentrate into the roots (Schwab et al., 1998; Gomez and Pardue, 2001).

Table 1. Comparison of calculated and experimental bioconcentration factor (BCF) of phenanthrene (n = 3).

Phenanthrene experimental concentration (mg/L)	BCF (mg of phenanthrene / kg root fresh tissue / mg of phenanthrene / L of nutrient solution)	
	3 Days	12 Days
40	42.81 ± (13.39)	44.35 ± (12.31)
80	22.33 ± (1.22)	83.49 ± (24.21)
120	29.07 ± (18.24)	67.63 ± (23.01)

Values are means with standard deviation in parentheses, without statistic difference ($p > 0.05$, Tukey's test).

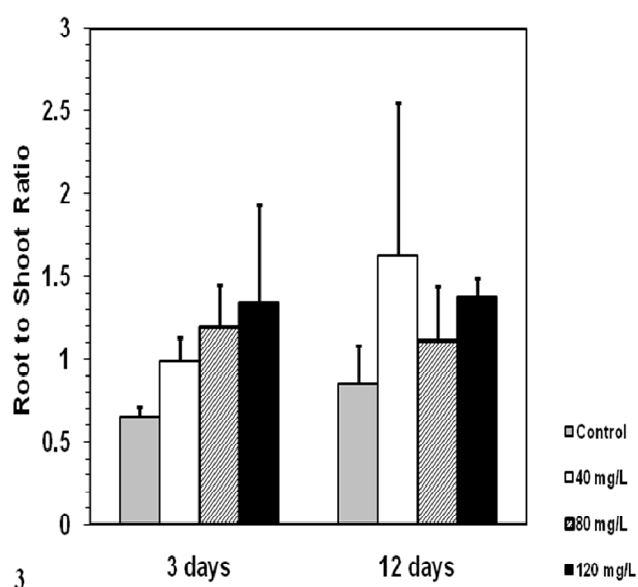


Figure 3. Root / shoot ratio of *C. hermaphroditus* for the three concentrations of phenanthrene at 3 and 12 days of exposition (n = 3). Mean values ± S.D. from three replicates are given. Experiments none significantly different ($p < 0.05$).

DISCUSSION

Organic pollutants like phenanthrene tend to move into and within plant tissues driven by simple diffusion, dependent on their chemical properties. Bioaccumulation in the broadest sense has been defined as the uptake and retention of pollutants from the environment by organisms via any mechanism or pathway or similarly the extent to which a living organism accumulates a compound from its surrounding environment by all processes (Pilon, 2005). For plants, the process of bioaccumulation starts with the transfer of a chemical from the abiotic environment to the plant.

The inherent conditions of hydroponic cultures of *C. hermaphroditus* favored an interaction, between the roots of this species and the compound employed, establishing

and maintaining the conditions where the distribution of phenanthrene was homogeneous to all parts of the roots with aeration in the system. Other factors like root architecture, growth stage of the plants and root surface area were involve in the interaction too. In this case, *C. hermaphroditus* was chosen because it has highly fibrous roots with a high degree of branching. Hydroponic cultures or similar approaches have been used successfully for studying the removal of organic compounds (Durmishidze et al., 1982; Edwards et al., 1982; Narayanan et al., 1995; Flocco et al., 2002).

The distribution of phenanthrene in the hydroponic system between the compartments of the medium and the apparent free space, showed a filling of the root cortex (apoplast) with the medium solution and a rapid entry of solute to the periphery of the cortical cells, which suggests that the uptake process change as the time progress, as the ryegrass uptake of phenanthrene is a function of time (Li et al., 2002; Gao et al., 2005). It maintains almost a constant value after a sharp enhancement after the initial uptake of phenanthrene and there are some possible reasons for this phenomenon. These authors suggested that a portion of parent compound in plant water and partitioned to plants might be metabolized during the uptake and will result in the diminished of concentration of the parent compound measured.

This was evident for the experiments exposed for 12 days, where phenanthrene distribution was similar in the medium and in the apparent free space compartments for the 3 experiments that showed an equilibrium tendency for the nonionic organic contaminant and a phenanthrene adsorption at the roots. In Gao et al. (2006) the results clearly indicate an initial rapid uptake phase of tested PAH's by ryegrass, based on the observed plant concentrations (C_{pt}) and accumulation amounts of these compounds, at about 48 - 96 h of the exposition to phenanthrene. The nature of the Cyperaceae species roots combined with the high octanol-water coefficient and a low water solubility of phenanthrene, may facilitate the affinity of this contaminant toward the roots.

The temporal change of plant concentrations of phenanthrene depended primarily on the rates of plant growth,

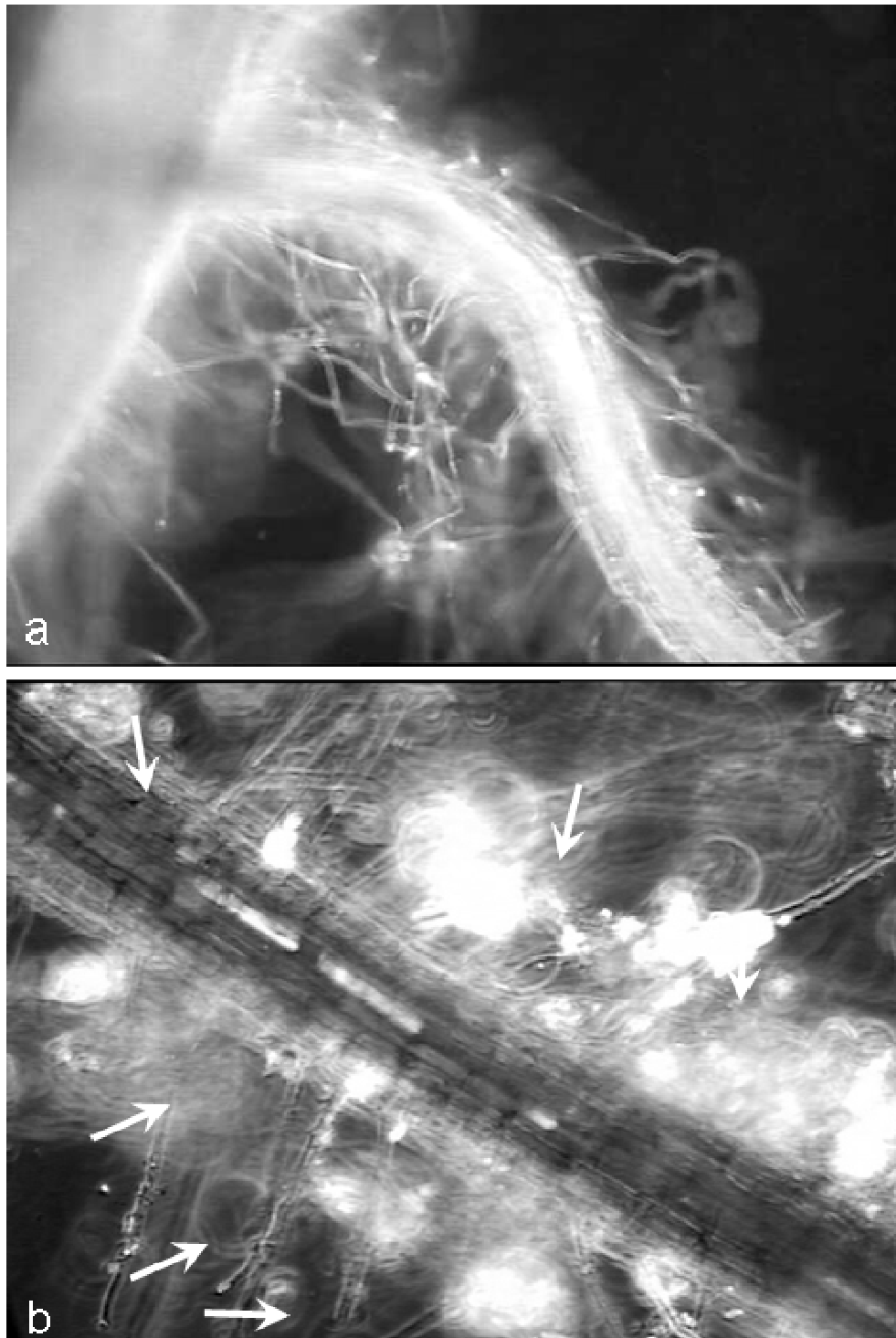


Figure 4. Details of the root fragments of *C. hermaphroditus* using differential contrast of interferences (DCI) microscopy technique: a) control roots, without phenanthrene and b) treatment with 120 mg/L of phenanthrene (200x). The arrows indicate the phenanthrene adhered.

uptake and metabolism of these chemicals. Thus, plant uptake of organic chemicals from solution can be des-

cribed as a series of consecutive partitions including partitions between aqueous solution and plant root, plant

root and transpiration stream, and transpiration stream and plant steam (Ryan et al., 1988; Gao et al, 2005).

For hydrophobic organic chemicals, such as phenanthrene, their uptake by plants could be described as the partition between aqueous solution and plant roots (Simonich and Hites, 1995; Gao et al., 2005). After the maximum uptake, the plant concentrations of phenanthrene decreased gradually with uptake time.

Compared with the results of Wild et al. (2005), the movement of PAH's like phenanthrene and pyrene into the cortex of wheat over a 56 day period was dominated by apoplastic flow through multiple cell walls. Only a small amount of the compound was transferred into the cellular vacuoles by the symplastic movement. Apparently, the partition of PAH's into organic substrates of fresh roots was from equilibrium in a relative short time (16 h) and the uptakes were dominated by sorption to cell walls. For Su and Zhu (2007) their concentration-dependent uptake curves of phenanthrene and pyrene into fresh and dead rice roots are consistent with the conclusion of Wild et al. (2005).

For non polar phenanthrene, the apoplastic pathway appeared to play a more important role in the root-system transport. The movement of water and solutes through the apoplast was dominated by the diffusion into the root cell walls and the motion through cell walls between cells. The cell wall was more permeable than the protoplast to water and its solutes (Marschner, 1995).

The increment of phenanthrene adsorption in 12 days could be attributed to the partitioning of the chemical between the lipophilic root solids and the aqueous phases of the roots (Briggs et al., 1982; Bell 1992; Chang and Corapcioglu, 1998). The uptake of phenanthrene is clearly likely to be linked to the uptake of water, where compound solubility plays a role. Other factors must therefore, also affect the rate of movement, most likely the degree and nature of compound movement interactions with the cell walls and other structures.

In the present study was evaluated the ability of *C. hermaphroditus* radical system to the uptake of phenanthrene. In this species there was an increased with the time of exposition to the contaminant. This fact was due to the greater total root mass with a more surface area as well as the enhanced affinity of phenanthrene to the roots. The radical system of *C. hermaphroditus* may thus provide a surface for phenanthrene biosorption. Consequently the retention of contaminants on the surface of plant roots could be an important control in stabilizing otherwise immobile organic compounds.

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