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Detection of an Aromatic Compound at the Roots of *Cyperus Hermaphroditus* by Photoacoustic Techniques[1]

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Some plants are used to remove organic and inorganic contaminants like chlorinated solvents, petrochemicals, pesticides, and explosives. These contaminants can be captured by the plant roots and then sequestered, degraded, immobilized, or transformed into other less toxic or non-toxic products. *Cyperus hermaphroditus* is a species endemic to the Santa Alejandrina swamp in Veracruz, Mexico, which is a site highly contaminated with industrial and petroleum wastes. The capability of *Cyperus hermaphroditus* to remove phenanthrene is reported in this study. Removal occurs by adsorption of this contaminant on the plant roots and can be assessed by analyzing the evolution of the absorption spectrum of the root system. *Cyperus hermaphroditus* plants of three months were cultivated in a hydroponic culture and exposed to 40, 80, and 120 ppm phenanthrene for twelve days. Photoacoustic spectra of the root system indicate that higher amounts of phenanthrene are adsorbed with increasing phenanthrene concentrations, suggesting the use of *Cyperus hermaphroditus* for phenanthrene removal.

KEY WORDS: *Cyperus hermaphroditus*; phenanthrene removal; photoacoustic spectroscopy; phytoremediation.

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1. INTRODUCTION

It has long been recognized that plants carry out various activities that ultimately modify the surrounding soil. In particular, plant roots provide nutrients and organic matter to soil and thus can support microbial populations in the rhizosphere. Under certain circumstances, adapted rhizosphere communities can sequester, degrade, immobilize, or transform several contaminants, increasing the ability of some plants to tolerate unfavorable environmental conditions and removing pollutants from the environment [1]. It has encouraged the application of phytoremediation and bioremediation methodologies as a promising alternative to soil contaminant removal [2].

Phytoremediation, which specifically involves the use of plants *in situ* to remediate polluted sites, has proven to be successful in the remediation of soils, sediments, and water contaminated with hydrocarbons contained in crude oil and some of its derivatives, such as benzene, toluene, xylene, and the polycyclic aromatic hydrocarbons (PAHs) [2]. Special attention has been paid to PAHs, for these compounds are known to be toxic and, in some cases, they exhibit mutagenic and carcinogenic effects.

The intense activity performed by the petrochemical industry in the southeast of Mexico has been the main source of pollution with PAHs, via accidental leakage from pipelines and improper disposal of petroleum sludge and chemical waste [3]. The Santa Alejandrina swamp, located in the Mexican state of Veracruz near the Minatitlan Oil Refinery, is a site with very high contamination levels. For instance, total petroleum hydrocarbons are found at a mean concentration of 81,640 ppm. In spite of it, endemic plants of this swamp (e.g., *Cyperus hermaphroditus*) can grow under such unfavorable conditions, suggesting their use for phenanthrene removal.

Based upon the conversion of absorbed optical energy into heat, photothermal (PT) science encompasses a wide range of techniques that have been successfully applied in a variety of fields belonging to the life sciences. Current applications in biotechnology [4–6], medicine [7], and the environmental sciences [8] include the thermal and optical characterization of solid, liquid, and gaseous specimens. Among the PT techniques, Photoacoustic spectroscopy (PAS) is well suited for obtaining the optical absorption spectra of highly transparent or opaque materials. In addition, as PAS is insensitive to light scattering, this technique has the capability of analyzing highly light-scattering samples, e.g., biological systems [7, 9].

In this work, the ability of *Cyperus hermaphroditus* to remove phenanthrene from a hydroponic culture was studied by PAS. Phenanthrene removal was evaluated from the absorption spectrum of the root system of plants exposed to different phenanthrene concentrations for twelve days.

2. MATERIALS AND METHODS

2.1. Plant Specimens

Specimens of *Cyperus hermaphroditus* were collected in a swamp near Santa Alejandrina, Veracruz, Mexico. This site is close to a petroleum refinery and shows severe contamination. Seeds of *Cyperus hermaphroditus* were obtained from the collected samples, and once germinated in sterile sand with sterile distilled water, the seedlings were placed in containers with 250 ml of mineral solution and were grown in hydroponic culture for three months under controlled temperature and humidity in a greenhouse humid chamber at 36°C. The plants were then transferred to single containers and exposed to 40, 80, and 120 ppm phenanthrene (SIGMA) for 12 days. The contaminant had been previously added to a flask containing 40 ml benzene (SIGMA). All the experiments were performed in triplicate.

After phenanthrene exposition, samples from the root system were harvested, weighed, and dried at 40°C for two hours, and then were placed in the photoacoustic (PA) cell to obtain the optical absorption spectra.

2.2. Experimental Setup

The optical absorption spectra were obtained in the range 270 to 535 nm with a homemade photoacoustic spectrometer. The experimental setup consists of a 1000 W xenon lamp (Oriel), a variable frequency mechanical chopper set at 17 Hz, a monochromator, and an air-filled brass cell equipped with a condenser microphone. The PA signal from the brass cell provides the input to the signal channel lock-in amplifier (SR-850), which is interfaced to a personal computer that simultaneously displays the wavelength-dependent signal amplitude and phase.

3. RESULTS AND DISCUSSION

In order to detect traces of phenanthrene adsorbed on the root system of *Cyperus hermaphroditus*, we have first analyzed the optical absorption spectrum of this contaminant under different conditions. Figure 1 shows the PA spectra of phenanthrene powder and phenanthrene dissolved in benzene at different concentrations, e.g., 30 ppm and 100 mg/10 ml. The PA spectrum of benzene (a) has also been included. Benzene shows no absorption in the range 285 to 470 nm. The characteristic absorption peaks of phenanthrene are better resolved with increasing concentration. The spectrum for phenanthrene diluted at 30 ppm (b) displays only one

absorption peak centered at 295 nm. For the 100 mg/10 ml solution (c), it is possible to observe the absorption peak at 295 nm and an absorption band from 310 nm to approximately 335 nm. For solid phenanthrene (d), the PA optical absorption spectrum shows two peaks, around 295 and 340 nm, and one shoulder at 380 nm. These absorption peaks agree with those reported in the literature [10].

Figure 2 shows the PA spectra of *Cyperus hermaphroditus* roots exposed to different phenanthrene concentrations for 12 days. The spectra (a), (b), and (c) correspond to roots exposed to 40, 80, and 120 ppm phenanthrene, respectively. For the 40 ppm exposition, none of the expected phenanthrene absorption peaks were well resolved. On the other hand, for the 80 ppm case, a broad absorption band with a maximum value around 343 nm was observed. This corresponds to the main absorption band of phenanthrene powder. When the roots were exposed to 120 ppm phenanthrene, it was possible to detect the three absorption peaks recorded in the solid-phenanthrene spectrum (295, 340, and 380 nm).

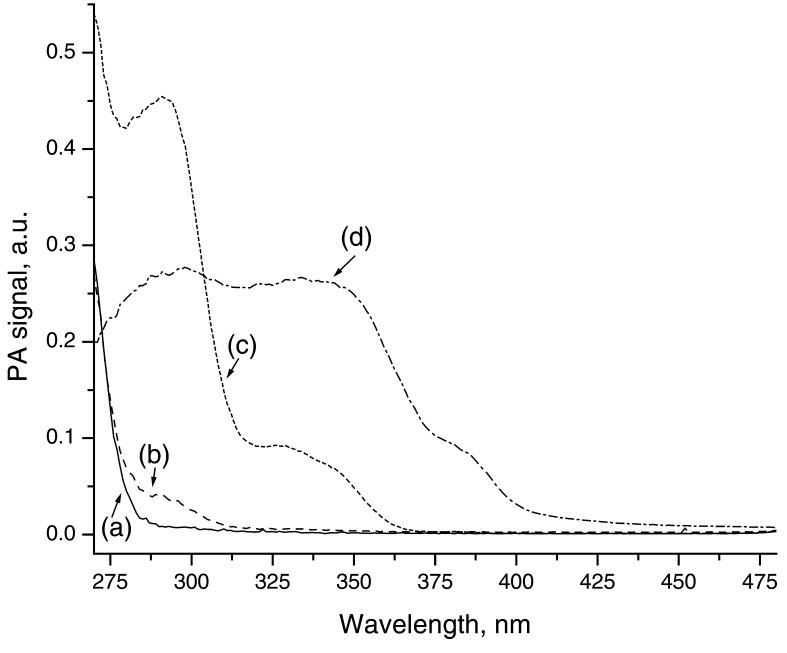

Fig. 1. Optical absorption spectra of phenanthrene, obtained by PAS: (a) the solvent (benzene), (b) phenanthrene dissolved in benzene (30 ppm), (c) phenanthrene dissolved in benzene (100 mg/10 ml), and (d) solid phenanthrene.

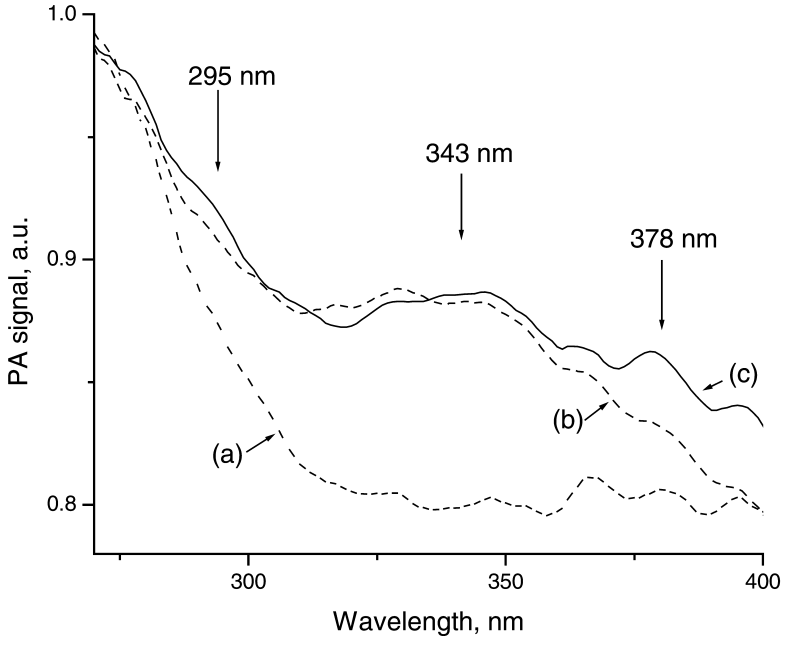

Fig. 2. Optical absorption spectra of *Cyperus hermaphroditus* roots, exposed for 12 days to different phenanthrene concentrations. The curves (a), (b), and (c) correspond to the PA spectra of roots exposed to 40, 80, and 120 ppm phenanthrene, respectively.

An improved resolution of the phenanthrene absorption peaks was achieved by performing differential spectroscopy, namely, the 40 ppm spectrum was subtracted from the 80 and 120 ppm spectra. As shown in Fig. 3, the differential spectrum for the 80 ppm root (curve a) presents an absorption band around 340 nm, which coincides with the main absorption peak of solid phenanthrene. For the 120 ppm case (curve b), we observed peaks at 295, 343, and 378 nm, corresponding to solid phenanthrene, and one peak at 310 nm. The latter was observed in the spectrum of phenanthrene dissolved in benzene at 100 mg/10 ml. As expected, the resolution is enhanced for higher contamination levels, indicating a higher phenanthrene adsorption at the root system.

Finally, a microscopic analysis of the roots was performed in order to know the mechanism of phenanthrene removal. Figure 4a shows the image (D.I.C. 200×) of a typical *Cyperus hermaphroditus* radical fragment exposed to 40 ppm phenanthrene. The primary and secondary roots can be

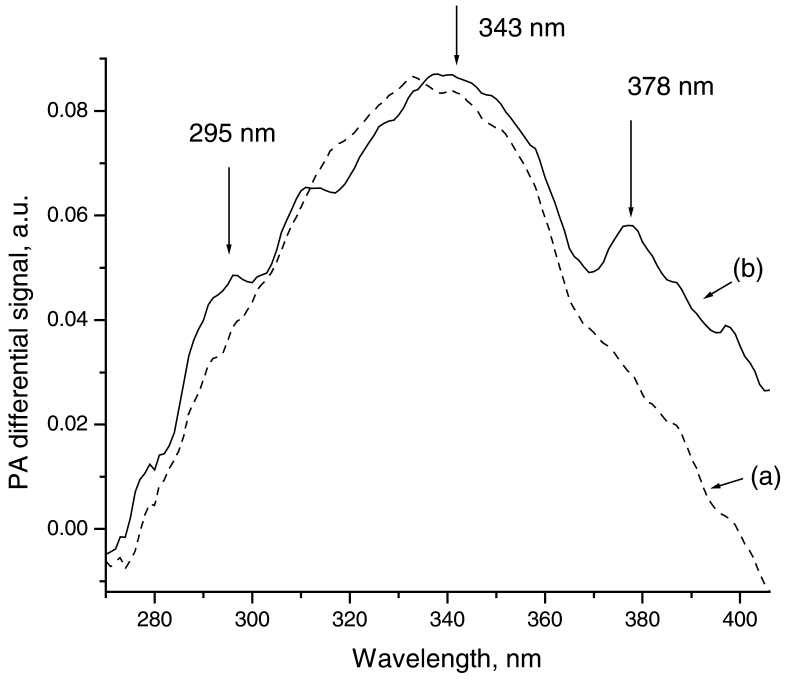

Fig. 3. Differential optical absorption spectra of samples exposed to (a) 80 ppm and (b) 120 ppm phenanthrene. The data show the difference between the spectra of these samples and the spectrum of a root exposed to 40 ppm phenanthrene.

distinguished with negligible presence of phenanthrene. In contrast, a different situation takes place when the roots are exposed to higher phenanthrene concentrations. Particularly, for the 120 ppm exposition (Fig. 4b), phenanthrene, indicated in white, is clearly observed around the radical surface. Images of roots exposed to 80 ppm (not shown) confirmed that adsorption occurs already at this level. These results are in correspondence with the PA analysis and suggest that phenanthrene is immobilized at the root system.

4. CONCLUSIONS

Adsorption of phenanthrene on the roots of *Cyperus hermaphroditus* was confirmed by photoacoustic spectroscopy and optical microscopy. A negligible presence of phenanthrene was found in roots exposed to 40 ppm phenanthrene. For the 80 and 120 ppm expositions, adsorption was detected by PAS.

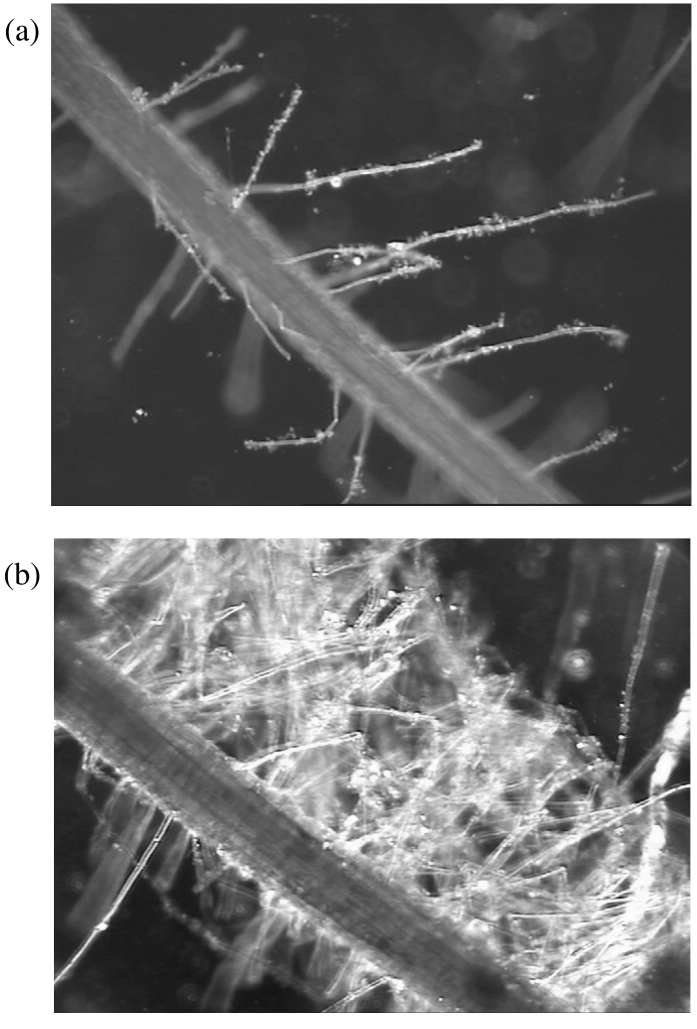

Fig. 4. Microphotographs (D.I.C. 200 ×) of *Cyperus hermaphroditus* radical fragments exposed to different phenanthrene concentrations: (a) 40 ppm and (b) 120 ppm.

The detection of phenanthrene at the *Cyperus hermaphroditus* root system demonstrates the capability of this plant to remove this contaminant.

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