Water Air Soil Pollut (2014) 225:2033
DOI 10.1007/s11270-014-2033-7

Assessment of the Removal Capacity, Tolerance, and Anatomical Adaptation of Different Plant Species to Benzene Contamination

V. Campos · L. S. Souto · T. A. M. Medeiros · S. P. Toledo · I. J. Sayeg · R. L. Ramos · M. C. Shinzato

Received: 13 November 2013 / Accepted: 13 June 2014 / Published online: 2 July 2014

Abstract The medium most directly affected by anthropic contamination is soil and, hence, groundwater (saturated and unsaturated zones). In the phytoremediation process, the direct absorption of soil contaminants through the roots is a surprising pollutant removal mechanism. Plants can act as a natural filter of shallow groundwater contamination, controlling and reducing the vertical percolation of contaminants into the soil, and after reaching the level of the water table, the roots can absorb contaminants dissolved in the water, thus reducing the size of the plume and protecting receptor sites (water supply wells, rivers, lakes) from possible contamination. In the first phase of the research, assays were performed to evaluate the tolerance of plant species to the direct injection of a benzene solution into the roots. Subsequent experiments involved direct absorption and spraying. The aim of this study was to evaluate the potential for tolerance and reaction to high levels of benzene. Three plant species were used, an herbaceous ornamental plant (*Impatiens walleriana*), a fern (*Pteris vittata*), and forage grass (*Brachiaria brizantha*). At the end of the study, the surface changes caused by VOCs (aerial structures) of benzene were evaluated, using an environmental scanning electron microscope (ESEM) to identify possible mechanisms of resistance of the plant to air pollution, i.e., hydrocarbon pollution. The plant material used here was young plant species selected for study. For the analysis by gas chromatography (GC), the plant material was separated into aerial (stem, leaves, and flowers) and underground parts (roots). A comparison of the benzene content in different parts of the plant indicated a higher concentration in the stem+leaves, followed by the roots, which is justified by its translocation inside the plant. *P. vittata* showed low uptake (5.88 %) mainly in the root and (<2 %) in the leaves, which was also observed in the tolerance experiment, in which visual symptoms of toxicity were not observed. *I. walleriana* showed benzene removal rates of approximately 18.7 % (injection into the soil) as a result of direct absorption through the

V. Campos · T. A. M. Medeiros
Campus de Sorocaba, Universidade Estadual Paulista, UNESP, São Paulo, Brazil

V. Campos (✉)
Centro de Capacitação e Pesquisa em Meio Ambiente, CEPEMA, Universidade de São Paulo, São Paulo, Brazil
e-mail: vcampos@sorocaba.unesp.br

V. Campos
e-mail: vcampos@usp.br

L. S. Souto
Campus de Sorocaba, Universidade Federal de São Carlos, UFSCar, São Carlos, Brazil

S. P. Toledo
Instituto de Física, Universidade de São Paulo, USP, São Paulo, Brazil

I. J. Sayeg
Instituto de Geociências, Universidade de São Paulo, USP, São Paulo, Brazil

R. L. Ramos · M. C. Shinzato
Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, São Paulo, Brazil

roots. After the treatment was suspended, *I. walleriana* gradually reacted to the detoxification process by recovering its stem stiffness and normal color. *B. brizantha* showed intermediate behavior and did not react to the detoxification process.

Keywords Three plant species · Benzene · Tolerance · Microscopic injuries

1 Introduction

In a gasoline spill, attention to groundwater contamination focuses on the presence of monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX). In situ remediation is one of the management strategies often employed in the recovery of sites contaminated with petroleum products. In Brazil, the migration of BTEX to the saturated zone has been potentiated due to the interaction of ethanol with the hydrocarbons found in gasoline. The gasoline sold in Brazil differs considerably from that in other countries due to the addition of 20 to 25 % ethanol. This alcohol content is much higher than the maximum allowed in the USA (10 %) and Europe (5 %). Aromatic hydrocarbons are highly mobile in soil-water systems, a feature that can be represented significantly by the lower octanol-water partition coefficient (K_{ow}). A lower partition coefficient implies slow absorption in the soil and, hence, preferred transportation through water and rapid migration to the free aquifer (Log K_{ow} of 2.13 for benzene and of −0.31 for ethanol). Another concern of companies operating in the oil sector is the loss of fuel and oil by evaporation, which represents not only economic loss but also a negative impact on the environment, since tons of products are released into the atmosphere.

Benzene is known to be the most toxic compound in the BTEX group and is therefore considered the most worrisome one in terms of public health. According to the International Agency for Research on Cancer (IARC), an agency of the World Health Organization, benzene is classified in group I, i.e., it is a provenly carcinogenic substance which and can also cause leukemia in humans. Like the IARC, the National Institute for Occupational Safety and Health (NIOSH) and the Environmental Protection Agency (EPA) in the USA also include benzene in their lists of carcinogenic products (EPA 1998; IARC 2006; NIOSH 2006). That is why it is extremely important to monitor these contaminants in cases of contamination.

The use of plants as depollution agents has aroused increasing interest and has been evaluated mainly in soils contaminated with heavy metals (Campos and Pires 2004; Bose and Bhattacharyya 2008; Campos 2009), crude oil and its derivatives (Anderson and Walton 1995; Moreno and Corseuil 2001), and other organic compounds (Newman et al. 1998; Cunningham et al. 1996; Burken and Schnoor 1996). The use of plants that can tolerate and simultaneously extract toxic substances may offer an interesting alternative for in situ decontamination.

Leaves are vegetative organs with a wide variety of morphological and anatomical characteristics, and in general, they express the environmental conditions of their habitat (Hickey and King 2000). Moreover, they exhibit plasticity, showing variations in these characteristics in response to different light intensities (Strauss-Debenedetti and Berlyn 1994; Lindorf 1997; Baruch et al. 2000; Jaakola et al. 2004; Rossatto and Kolb 2010), availability of nutrients in the soil, hydrological regime (Rôças et al. 1997; Wang et al. 2007), and herbivory (Turner 1994). In most species, the anatomical structure of the leaves tends to maximize the food and energy production processes, particularly the capture of photosynthetically active radiation, and also minimizes water loss through evapotranspiration and excessive radiation damage (Brown and Hattersley 1989; Mediavilla et al. 2001).

Symptom assessment, the method most commonly used to assess the sensitivity of plant species to various stressors, often requires additional validation by means of microscopic interpretation (Vollenweider et al. 2003; Reig-Arminaña et al. 2004), and in recent years, many authors have used plant anatomy as a tool to study the effects of pollutants on plants (Fornasiero 2003; Silva et al. 2006).

The objective of this work was to ascertain the adaptation or mortality of three plant species (*Brachiaria brizantha*, *Impatiens walleriana*, and *Pteris vittata*) based on the characterization of morphological changes on the leaf surface when exposed to benzene. Morphologic evaluation and stomatal counts were made by scanning electron microscopy coupled with ImageJ image analysis software. We tested the hypothesis that, upon exposure to benzene (injection and spraying), these plants could accumulate pollutants in their tissues and respond with macroscopic symptoms or through

microscopic injuries in their portions, without apparent damage.

2 Plant Species

2.1 *B. brizantha* (A. Rich.) Stapf

B. brizantha (Fig. 1) is a grass species of the Poaceae family originating from a volcanic region with a tropical climate in Africa. It was introduced in the Americas during the colonial period, probably as bedding for slaves on slave ships (Parsons 1972). Species of the genus *Brachiaria* are the ones most commonly used as forage for cattle. Today, *Brachiaria* grass is grown on 80 million ha, the predominant species being *B. brizantha* and *B. decumbens* (Boddey et al. 2004). Considering the widespread use of *B. brizantha* as forage for cattle, our aim was to evaluate the phytotoxic potential of benzene based on the morphological and anatomical parameters of this species and its ability to accumulate this substance, contributing to the body of knowledge about its sensitivity to the pollutant. This species is characterized by its high forage yield, tolerance to acid soils, heat (drought and fire), and water stress; however, it is intolerant of saturated soils and has little tolerance for shade. *B. brizantha* has a vigorous and deep root system which is responsible for keeping it green for longer. It is a perennial and robust species that can form clumps varying in height from 1.5 to 2.5 m and is therefore considered a cespitose plant. *B. brizantha* has stomata on both sides of its leaves.

2.2 *I. walleriana* Hook. f

I. walleriana (Fig. 2) is one of the 500 species of *Impatiens* of the family Balsaminaceae and an herbaceous ornamental plant of African origin, which can be considered invasive (Maciel 2011). It grows 20 to 60 cm

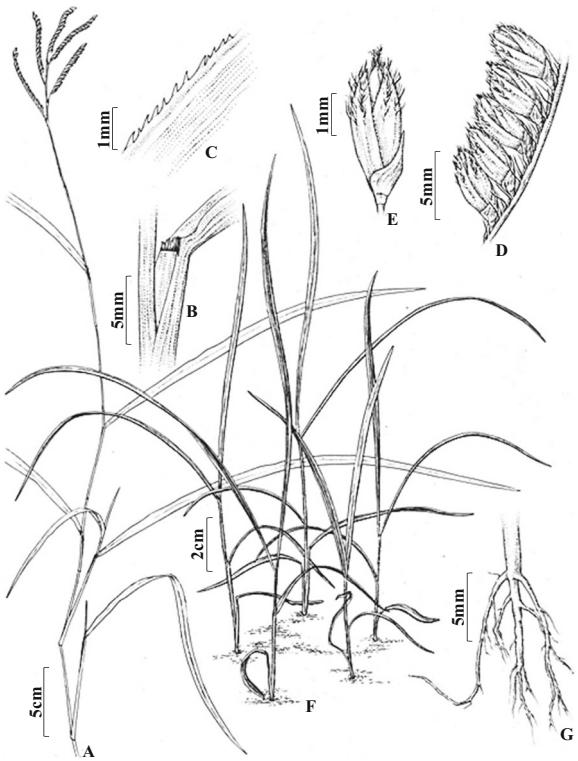

Fig. 1 *Brachiaria brizantha* and its structures. *A* Habit. *B* Base of leaf with ligule and smooth sheath. *C* Limb with serrated edges. *D* Raceme. *E* Spikelet hairy. *F* Stems erect. *G* Fasciculated root

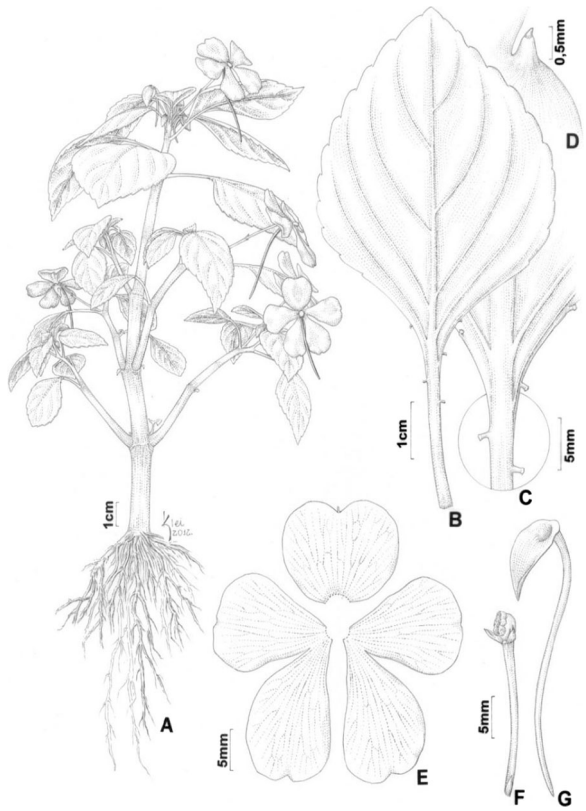

Fig. 2 *Impatiens walleriana* and its structures. *A* Habit. *B* Abaxial face of leaf ovate lanceolate with crenulated-serrated margin and pinnate venation. *C* Petiole with sparse extrafloral nectaries. *D* Slender spur. *E* Petals anterior and lateral. *F* Stamen with visible anther. *G* Sepal posterior

tall and produces flowers with five petals of various colors, the most common ones being red, pink, and white (Armitage 1994). Because it is undemanding, this species reproduces almost anywhere where its seeds fall, and these are stored in capsule. It accepts both sunlight and shade, low or high humidity, and blooms practically year-round. *I. walleriana* (Fig. 2) is a species widely used in gardens. The producers of these cultivars have developed compact plants, well branched and with more colors (Armitage 1994). This species roots easily, even when a node comes in contact with the ground, thus spreading easily not only by the stem but also by its seeds. The stomata on *I. walleriana* are found only on its abaxial surface. It appears in the understory of mixed ombrophilous forest, with a preference for partially shaded sites with plenty of organic matter and moisture.

2.3 *P. vittata* Linnaeus

Pteridophytes are species known as ferns, comprising 32 families with approximately 250 genera and about 10,000 species in forests throughout the world. The best known species in Brazil is *P. vittata* (Fig. 3), which behaves as a ruderal plant, propagating easily in artificial environments. The fronds of Pteridophytes greatly influence their taxonomic characterization. In *P. vittata*, the ramifications of the leaf blade, the vein pattern, the shape of the petiole, the edges of the pinnae, and the indumentum have proved to be the best taxonomic characters for the recognition and characterization of the species. The stomata, which are of identical size, are found only on the abaxial surface of the pinnae between the veins.

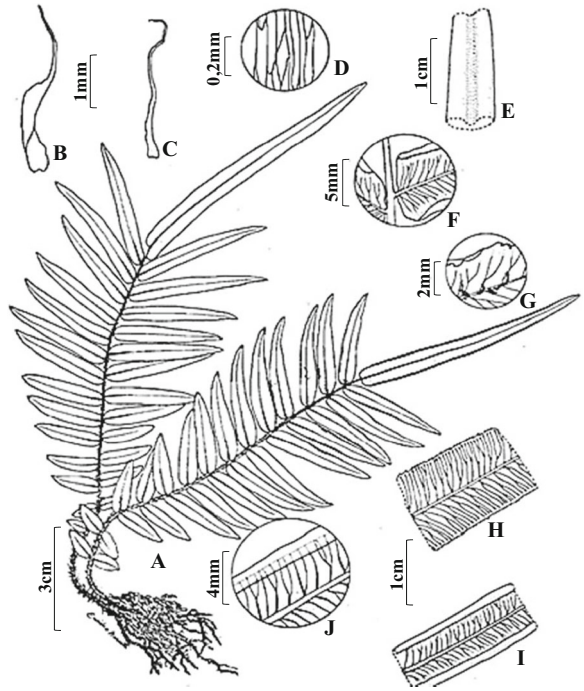

Fig. 3 *Pteris vittata* Linnaeus and its structures (Prado and Windisch 2000). *A* Habit. *B*, *C* Scales of the stem. *D* Detail of the scale cells. *E* Petiole grooved. *F* Detail of pinna rachis insertion. *G* Adaxial view of the edge. *H* Venation pattern of the sterile frond. *I* Venation pattern of the fertile frond. *J* Detail of the edge of the fertile frond

3 Material and Methods

Two experimental series were conducted in a greenhouse, using the forage (*B. brizantha*), ornamental herbaceous (*I. walleriana*), and fern (*P. vittata*) species. Seedlings were obtained by vegetative propagation from a single individual of each species and were planted in plastic and concrete pots, in the case of *P. vittata*. The young plants were given a nutrient solution once a week.

In the first phase of the research, the tolerance of the three species was tested by injecting benzene directly into their roots. The next phase involved spray tests, applying a completely randomized experimental design of benzene contamination with three replications, in addition to a control series.

The direct absorption experiment basically consisted of injecting benzene into soil (20 mg L^{-1}). This experiment lasted for 10 days and was performed in an incubator at a constant temperature of 25 °C. The photoperiod consisted of 16 h of light per day provided by 40-W fluorescent lamps. After 10 days of contact, each species was separated into stems, leaves, and roots, whose benzene content was then quantified. This was followed by evaluations of benzene dose-response curves, with more consistent results obtained by applying increasing concentrations of the pollutant. For the sprinkle experiments, the plants were exposed daily for 30 min to a simulated mist of 4.05 mm day^{-1} with a benzene solution containing 20 mg mL^{-1}. The mist, which was applied inside cages (reactors), lasted for 10 days. The control plants were subjected to simulated mist using only deionized water. At the end of the

simulation, leaf samples were collected for chemical and microscopic analyses.

The translocation index (TI) of the substance in the plant was determined to evaluate the potential tolerance of each species to benzene sorption. In addition, the benzene content in the soil was analyzed to calculate the bioaccumulation factor. The soil used here was a dystrophic red latosol (oxisol) containing 8.74 % of crystalline endogenous iron, and samples were collected from noncompacted areas (0:00 to 0:20 m). The bioaccumulation factor of benzene was determined by dividing the concentration of the substance in the aerial part of the plant by the concentration of the contaminant available in the soil, by shake extraction (Shin and Kwon 2000), according to US EPA method 5030B and 8260B. The transfer rate was obtained by dividing the concentration of benzene in the aerial part by the concentration in the root system.

To evaluate the damage on the leaf surface, the samples were fixed in an aqueous solution of 2.5 % glutaraldehyde and 0.1 M phosphate buffer (pH 7.2), postfixed in 1 % osmium tetroxide, dehydrated in an ethanol series, and subjected to critical point drying. The leaf surface was coated with gold using an Emitech K550 sputter coater and documented by scanning electron microscopy. The analyses were performed at the Institute of Geosciences, University of São Paulo, using a FEI Quanta 250 environmental scanning electron microscope. Image analysis was performed with ImageJ software. The stomatal density, perimeter, and diameter of each stoma were calculated based on the electron micrographs, for comparison with the healthy individuals.

The plant specimens exposed to benzene were extracted with ultrapure dichloromethane in a table shaker for a minimum of 60 min. The organic extract was then reduced to a volume of 1 mL in an evaporator. The final

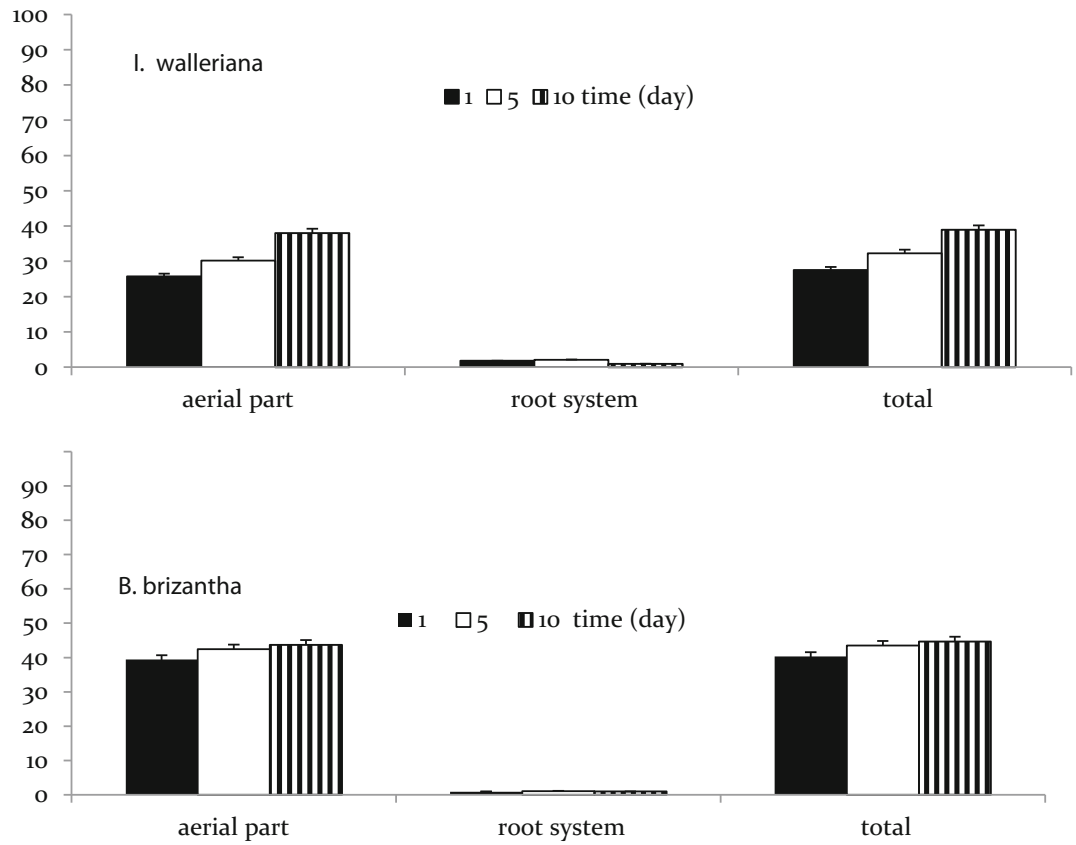

Fig. 4 Content of benzene (20 mg L^{-1}) applied to vegetable samples (*I. walleriana* and *B. brizantha*), separated between aerial part (stem, leaf, and flower) and root system, in relation to the content found in the soil. The experiment was carried out in 10 days and the values represent the average of three replicates. The *error bars* represent the standard deviation for $n=3$

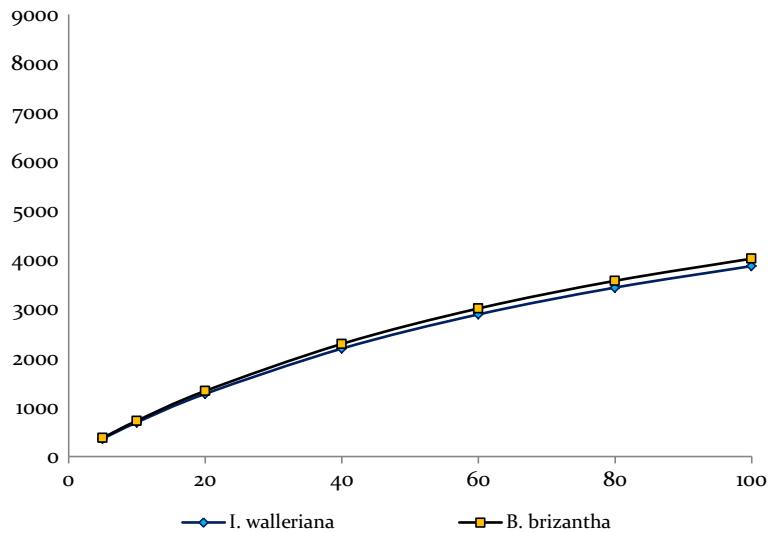

Fig. 5 Absorption of benzene by *I. walleriana* and *B. brizantha* treated at different concentrations of pollutant (mg L^{-1})

concentrate was injected without dividing the flow into an HP-1 stationary phase column coupled to a flame ionization detector. The CG injector temperature was set at 300 °C, with an initial column temperature at 60 °C. The heating rate was 9 °C min^{-1} up to a temperature of 310 °C, with a soaking time of 13 min. The carrier gas used was helium (99.999 % purity) at a constant flow of 1.0 mL min^{-1}.

4 Results and Discussion

The physiological changes in plants in response to contamination by petroleum hydrocarbons usually involve loss or deficiency of photosynthetic pigments (chlorophyll *a*, *b*) and decreased content of water and assimilates such as starch, sugars, fats, and proteins (Griffiths 1975). Morphologically, the plants begin to exhibit lower density and height than plants that have developed without the influence of the contaminant. After benzene was injected, there was a very rapid and remarkable decrease in the chlorophyll content of the leaves. In *B. brizantha* the benzene content at the end of soil treatment led to the death of the plant, probably by interrupting the root respiration or by acidifying the water contained in the soil. *I. walleriana* showed weakened growth of the aerial portion and leaf loss, but the plant survived in the benzene-contaminated soil.

The direct absorption experiments revealed different contents in different parts of the plant (Figs. 4 and 5). In general, the aerial part showed higher concentrations, followed by the root system. The direct absorption of benzene resulting from the injection of the contaminant solution in the soil close to the roots was assessed by gas chromatography of the aerial (stems, leaves, and flowers) and underground parts (rhizome and root). The soil sample (Tables 1 and 2) was dystrophic red latosol (dRL) containing 8.74 % of endogenous crystalline iron collected from an undisturbed area (0.00–0.20 m). The presence of substances in groundwater is controlled by their mobility and persistence in soils and aquifers. Most of the metals in groundwater occur in low concentrations, often less than 1 mg L^{-1}. The main factor that controls metal concentrations in groundwater is adsorption on ferric hydroxide. In this case, dystrophic red latosol can perform the function of retaining metal in soil, which opens a space for the study of

Table 1 Physicochemical analysis of the dystrophic red latosol (dRL)

MO	pH	P	K^+	Ca^{2+}	Mg^{2+}	H+Al	CEC	Texture	CC
17	5.1	13	3.8	10	5	26	46.5	Clayey	64.5

OM organic matter (g dm^{-3}); S and P (mg dm^{-3}); K, Ca, Mg, H+Al and *CEC* cation exchange capacity ($mmol_c$ dm^{-3}); *CC* clay content (%)

Table 2 Goethite and hematite content in the clay fraction of dRL soil

dRL	Goethite (%)	Hematite (%)	Color
	9.56	90.44	2.5 YR

dRL dystrophic red latosol; *YR* yellow-red (munsell soil color)

organic pollutants, in this case, aromatic hydrocarbons (BTEX). Understanding the subsurface behavior of hydrocarbons that are less dense than water, which the international literature calls light nonaqueous phase liquid (LNAPL), together with the hydrogeological characteristics of the medium, allows one to establish the parameters needed for remediating and monitoring an area impacted by hydrocarbons. Hydrocarbons in petroleum are slightly water soluble, but the risk of water contamination in Brazil generally increases in the presence of a simultaneous leak of ethanol and petroleum derivatives or of petroleum mixed with ethanol (oxygenated). Ethanol (CH_3CH_2OH) is an oxygenated compound completely soluble in both water and NAPL and can thus influence the solubility of toxic hydrocarbons (cosolvent effect) in an environment contaminated by petroleum derivatives.

The benzene solution showed moderate mobility in *I. walleriana*, as indicated by the translocation index and bioaccumulation factor. After the treatment ended, *I. walleriana* reacted slowly to the detoxification process, recovering the rigidity of its stem and its normal coloration. This likely indicates that benzene was gradually volatilized by the leaves, the aerial region of the plant where highest levels of benzene were found. Volatilization in this case may be related to the plant's transpiration rate. The high concentration of the substance in the leaf tissues of *I. walleriana* probably did not inhibit photosynthesis and transpiration. *B. brizantha*, on the other hand, showed higher benzene uptake and did not react to the detoxification process. *P. vittata* L. presented low absorption of benzene (5.88 %) concentrated mainly in its roots.

During the experiments of benzene injection into the soil, several visual changes were observed in *I. walleriana*, which exhibited yellowish leaves and considerable depigmentation of its petals shortly after the first day of contamination. On the fourth day, the petals showed more intense depigmentation, and one of the flowers lost two petals. More leaves lost chlorophyll and turned yellowish. After the fifth day of contamination, *I. walleriana* did not appear to change after absorbing a certain amount of contaminant, at which time the petals stopped losing pigment.

On the first day, *B. brizantha* presented small discolored spots at the base of one of the leaves. The plant's green coloration gradually decreased with each passing day until, by the end of the sixth day, its stem was limp and its leaves brownish yellow. *P. vittata* L. showed no visible change during the entire period of soil contamination with benzene. In the case of *I. walleriana*, considering the octanol-water partition coefficient (log K_{ow}), benzene, which is a moderately hydrophobic compound, easily penetrates the endodermal membranes (Corseuil et al. 1998) and reaches the transpiration current and may undergo volatilization from the leaf surface.

To evaluate the potential of each species to extract benzene and identify it as a tolerant species or a

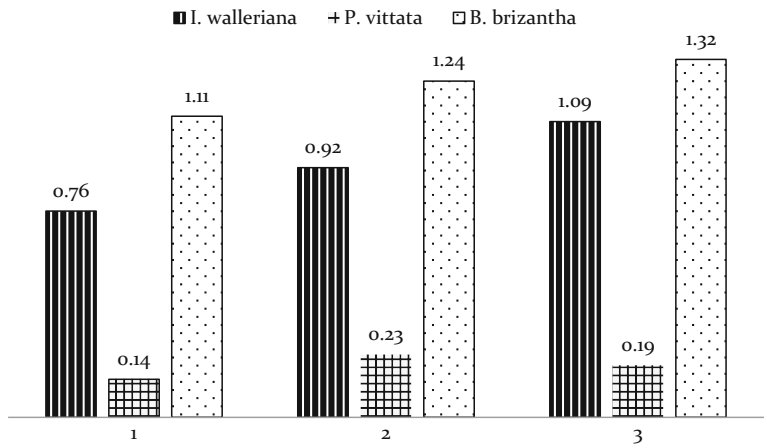

Fig. 6 Average values of the bioaccumulation factor of benzene in *I. walleriana*, *B. brizantha*, and *P. vittata* in soil contaminated artificially with benzene (20, 40, and 60 mg L^{-1})

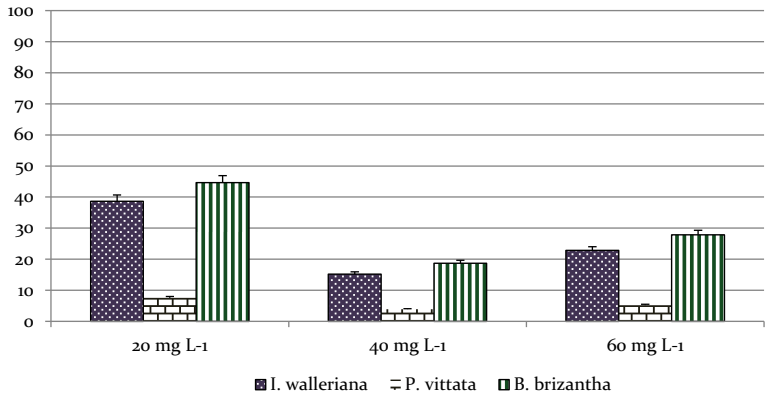

Fig. 7 Average values of the translocation index (%) of benzene in *I. walleriana*, *P. vittata*, and *B. brizantha* in soils contaminated artificially with 20, 40, and 60 mg L^{-1}

hyperaccumulator of benzene, the values of the bioaccumulation factor and translocation index from soils contaminated artificially with benzene were adopted (Figs. 6 and 7). In the case of *P. vittata*, it can be inferred that this species is benzene tolerant by restricting the translocation of benzene from the root to the aerial part and by not absorbing the pollutant in the soil. With regard to the translocation values, little benzene was translocated to the aerial parts (<2 %) of *P. vittata*, since it was retained predominantly in the roots (5.88 %). Evaluating the translocation rates, *I. walleriana* absorbed and actively translocated benzene from the soil. *B. brizantha* cannot be considered tolerant, or a bioaccumulator of benzene, since this species died at the end of the treatment.

Symptom assessment, the method most commonly used to assess the sensitivity of plant species to various stressors, often requires additional validation with microscopic interpretation (Fig. 8). In the experimental series involving benzene mist, *P. vittata* suffered only minor leaf damage, without visual symptoms of toxicity such as chlorosis or necrosis. One fact that should be kept in mind is the ruderal condition of this species, which is highly adapted to urban environments. The morphology of *I. walleriana* differed significantly from that of *P. vittata*. In the sprinkle experiments, the species showed chlorosis of irregular shapes and sizes. Yellow coloration was observed mainly at the leaf margins. White patches appeared on the *I. walleriana* flower on the second day of contact with the benzene mist. In this case, the benzene seems to have formed various complexes which were responsible for the occurrence of uncolored areas on portions of the leaf, probably due to a decline in chlorophyll biosynthesis. Over time, the chlorosis evolved into small necrotic spots located at the edges and tip of the leaf. The most intense lesions appeared after 6 days of exposure to the pollutant. *B. brizantha* did not drop its leaves nor did it display visible leaf symptoms when exposed to benzene but instead showed normal growth.

The leaf micromorphology revealed significant changes in stomatal density. The stressful condition

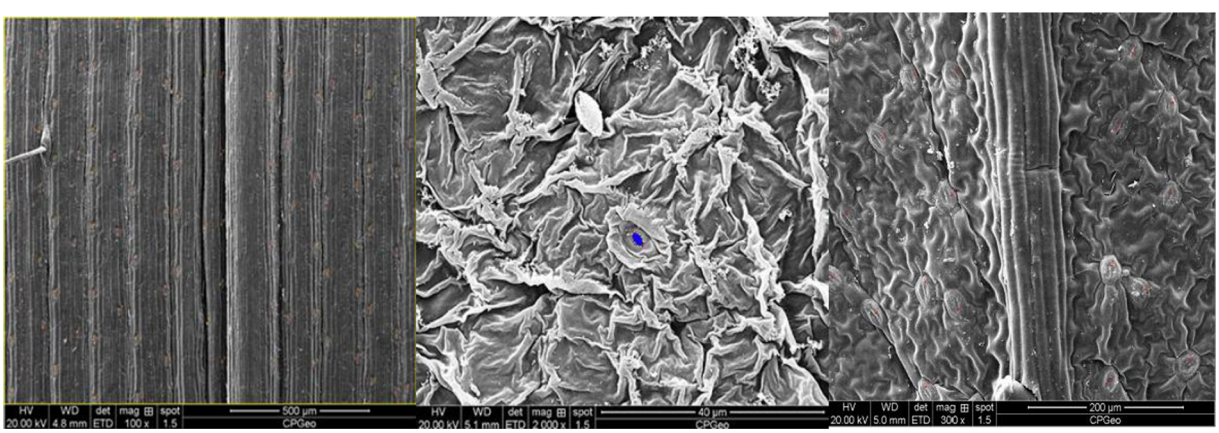

Fig. 8 Leaf micromorphology of the species under study: *Brachiaria brizantha*, *Impatiens walleriana*, and *Pteris vittata*. Control species

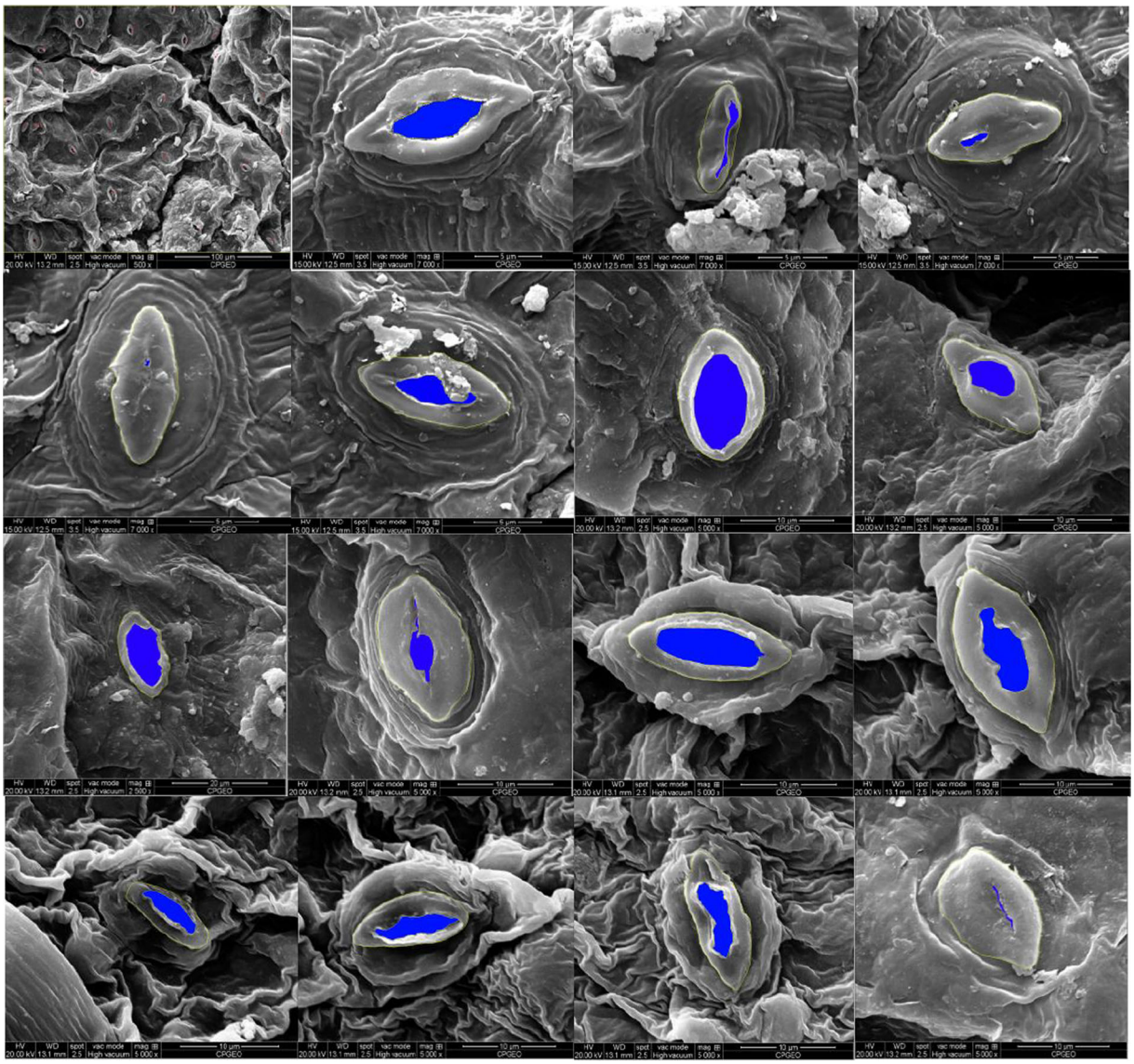

Fig. 9 Leaf micromorphology of *Impatiens walleriana* contaminated with benzene

promoted by contaminated soil led to anatomical changes. After 10 days of experiment, the species were affected differently by the contaminant, as evidenced by the absorption values and overall symptomatology. The stomatal density of *P. vittata* was 91 stomata/mm^2 in the control group and about 132/mm^2 in the

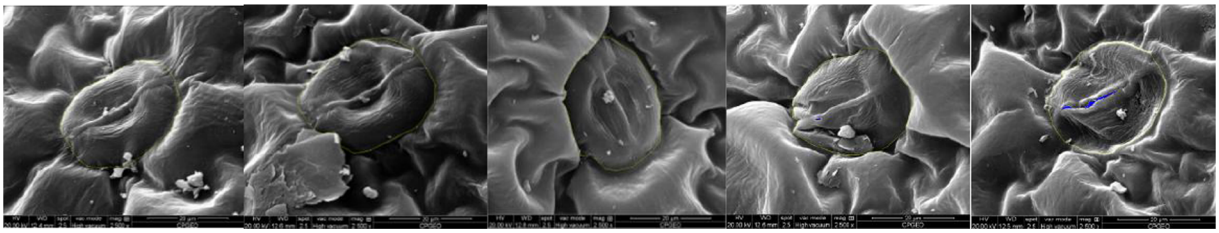

Fig. 10 Stomata of *Pteris vittata* L. after treatment, characterized using ImageJ software

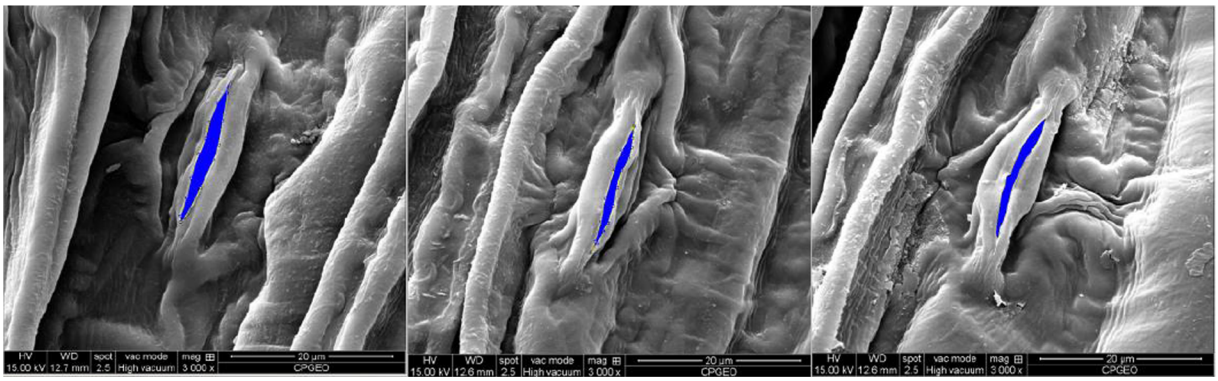

Fig. 11 Stomata of *B. brizantha* after treatment, characterized using ImageJ software (×3,000).

contaminated soil group at 10 days, whereas that of the *B. brizantha* control group was 52/mm^2 and that of the contaminated group was 83/mm^2. *I. walleriana* showed a stomatal density of 277/mm^2 in the control group and of 389/mm^2 in the contaminated soil group. This variation in the stomatal density is attributed to a reduction in leaf area caused by lower water availability. In *B. brizantha*, the sinuosity of the epidermal cell walls was higher in the control group, and this characteristic disappeared in the other groups. This change may also be the result of a condition of water stress. According to Hernandez-Valencia and Mager (2003), when the oil film covers the roots, water and nutrient absorption is altered.

The leaves of *I. walleriana* exposed to benzene mist showed changes in epidermal cell shape and turgor, and deformation and rupture of the cuticular ridge of stomata (Fig. 9). *P. vittata* trichomes appeared flaccid, and the apical cell of secretory trichomes looked shapeless, while the stomata showed changes in cell turgidity (Fig. 10). In *B. brizantha*, the degree of damage was identical on both leaf surfaces, probably due to their similar micromorphological characteristics, such as the presence of stomata and abundant hairs. The main injuries caused by benzene to the leaves were lesions of the stomata's cuticular ridges and loss of turgor of the epidermal cells (Fig. 11). However, it should be noted that the plant did not respond with macroscopic symptoms, i.e., it showed no apparent signs of damage from exposure to benzene. *P. vittata* did not lose any of its pinnae, but it presented macroscopic symptoms by darkening of the rachis, which extended along the leaf blade. Benzene appears to have been absorbed, particularly by the stomata and, to a lesser extent, by the waxy cuticle,

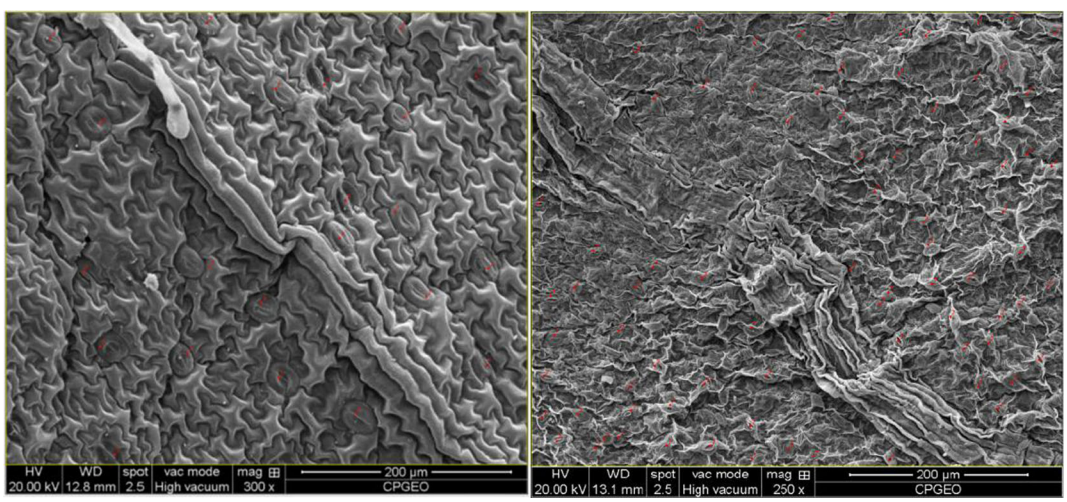

Fig. 12 Stomata count after benzene contamination, using ImageJ software and×250 magnification to identify the stomatal density of *P. vittata* L. Also visible are lesions of leaf veins after sprinkler-induced contamination (10 days). The *left side* of the figure shows the control species (×300 magnification)

reaching the main vein via the apoplast, where it accumulated and caused lesions at some points (Fig. 12).

One of the major limitations in laboratory studies using benzene is the volatility of this substance. This limitation also occurs in the field, in terms of the residence time of the contaminant phase in soil. The free phase is the most mobile one in porous media, thus representing a source of aquifer contamination. For the phytoremediation process, the direct uptake of contaminants from soil or surface and groundwater through the roots is a fascinating and desirable mechanism of pollutant removal. As for the limitations of phytoremediation of volatile organic contaminants, several remarks in the discussions of Moreno and Corseuil (2001) deserve to be mentioned. Technologies currently used in the USA for cleaning contaminated sites not only are less than 100 % effective but also often cause other problems such as the destruction of the soil profile, resulting in serious ecological damage to the site. The current viable alternatives for groundwater remediation, in the case of volatile organic contaminants, involve the use of pumping and soil vapor extraction (SVE), which consist of transferring these compounds to the atmosphere in the form of vapor at a very high cost. The use of plant species for the removal of these contaminants and their subsequent conversion into inactive metabolites in plant tissues has proved to be an environmentally friendly alternative, and more economically feasible.

5 Conclusions

Comparing the values of bioaccumulation at the various concentrations, it cannot be stated that there was hyperaccumulation of benzene in *I. walleriana* or *B. brizantha*, since these values were close to 1. The values of the bioaccumulation factor ranged from 0.76 to 1.32. *P. vittata* was the most tolerant species, with a bioaccumulation factor well below 1. In addition, this species showed low benzene absorption (5.88 %), localized mainly in the root and (<2 %) in the leaves, which was also indicated by the absence of visual symptoms of toxicity.

The study of leaf micromorphology revealed significant changes in stomatal density. The stressful condition caused by contaminated soil gave rise to anatomical alterations. Ten days into the experiment, the species were affected differently by the contaminant, as evidenced by the absorption rates and symptoms. In terms of stomatal density, the control group of *P. vittata* showed 91 stomata/mm^2 while the contaminated soil group showed about 132/mm^2 at 10 days, while the control group of *B. brizantha* showed 52 stomata/mm^2 and the contaminated group showed 83/mm^2, and lastly, *I. walleriana* showed a stomatal density of 277 stomata/mm^2 in the control group and 389/mm^2 in the contaminated group. This variation in stomatal density is related to a reduction in leaf area, which in turn is linked to lower water availability. In *B. brizantha*, the epidermal cell walls of the plants in the control group showed greater sinuosity than in the other groups. This change may also be the consequence of a water deficit condition, which caused the oil film that coated the roots to alter the plant's water and nutrient absorption. The decrease in biomass can be attributed to the reduction in the transport of assimilates.

Acknowledgments This research was financed by the National Council for Scientific and Technological Development (CNPq) (grant 470012/2012-9) and the São Paulo Research Foundation (FAPESP) (grants 2012/05595-0, 2012/05982-3, 2012/08337-1, 2012/24832-2 and 2012/24832-2). The authors acknowledge the Geochronological Research Center (CPGeo) of the Institute of Geosciences, University of São Paulo, for its support of this work. We are also indebted to gardening technician Luiz Alberto de Campos, Lourdes Lossurdo de Campos, and to Amelie Yukie Wagner for their invaluable assistance in this project.

References

Anderson, T. A., & Walton, B. T. (1995). Fate of 14C trichloroethilene in the root zone of plants from a former solvent disposal site. *Environmental Toxicology and Chemistry, 14*(12), 2041–2047.

Armitage, A. M. (1994). *Ornamental bedding plants (p. 175)*. Wallingford: CAB International.

Baruch, Z., Pattison, R. R., & Goldstein, G. (2000). Responses to light and water availability of four invasive Melastomataceae in the Hawaiian Islands. *International Journal of Plant Science, 161*(1), 107–118.

Boddey, R. M., Macedo, R., Tarre, R. M., Ferreira, E., Oliveira, O. C., Rezende, C. P., et al. (2004). Nitrogen cycling in *Brachiaria* pastures: the key to understanding the process of pasture decline. *Agriculture, Ecosystems & Environment, 103*, 389–403.

Bose, S., & Bhattacharyya, A. K. (2008). Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. *Chemosphere, 70*, 1264–1272.

Brown, R. H., & Hattersley, P. W. (1989). Leaf anatomy of C3-C4 species as related to evolution of C4 photosynthesis. *Plant Physiology, 91*, 1543–1550.

Burken, J. G., & Schnoor, J. L. (1996). Phytoremediation: plant uptake of atrazine and role of root exudates. *Journal of Environmental Engineering, 122*, 958–963.

Campos, V. (2009). Influence of redox environments on the solubility of arsenic in soils. *Communications in Soil Science and Plant Analysis, 40*(9–10), 1590–1603.

Campos, V., & Pires, M. A. F. (2004). Phytoremoval of arsenic from soil. *Communications in Soil Science and Plant Analysis, 35*(15–16), 2137–2146.

Corseuil, H. X., Hunt, C. S., Santos, R. C. F., & Alvarez, P. J. J. (1998). The influence of the gasoline oxygenate ethanol on aerobic and anaerobic BTX biodegradation. *Water Research, 7*, 2065–2072.

Cunningham, S. D., Anderson, T. A., & Schwab, A. P. (1996). Phytoremediation of soils contaminated with organic pollutants. *Advances in Agronomy, 56*, 55–114.

Fornasiero, R. B. (2003). Fluorides effects on *Hypericum perforatum* plants: first field observations. *Plant Science, 165*, 507–513.

Griffiths, W. T. (1975). Characterization of the terminal stages of chlorophyll (Ide) synthesis in etioplast membrane preparations. *Biochemical Journal, 152*(3), 623–655.

Hernández-Valencia, I., & Mager, D. (2003). Uso de *Panicum maximum* y *Brachiaria brizantha* para fitorremediar suelos contaminados con un crudo de petróleo liviano. *Bioagro, 15*, 149–155.

Hickey, M., & King, C. (2000). *The Cambridge illustrated glossary of botanical terms*. Cambridge: University Press.

IARC: International Agency for Research on Cancer. (2006). Carcinogenicity evaluation of BTEX, 71, 829. Available at http://www.iarc.fr/. Accessed 14 March 2008.

Jaakola, L., Määttä-Riihinen, K., Kärenlampi, S., & Hohtola, A. (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta, 218*, 721–728.

Lindorf, H. (1997). Wood and leaf anatomy in *Sessea corymbiflora* from an ecological perspective. *IAWA Journal, 18*(2), 157–168.

Maciel, L. A. (2011). *Controle mecânico da herbácea exótica invasora lírio-do-brejo (Hedychium coranarium Koenig) no Parque Estadual Turístico do Alto Ribeira – PETAR, SP.* (p. 99) Dissertação de mestrado. - São Paulo: Universidade de São Paulo. Piracicaba.

Mediavilla, S., Escudero, A., & Heilmeier, H. (2001). Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiology, 21*, 251–259.

Moreno, F. N., & Corseuil, H. X. (2001). Phytoremediation potential of willow trees for aquifers contaminated with ethanol-blended gasoline. *Water Research, 35*(12), 3013–3017.

Newman, L. A., Doty, S. L., Gery, K. L., Heilman, P. E., Muiznieks, I., Shang, T. Q., et al. (1998). Phytoremediation of organic contaminants: a review of phytoremediation research at the University of Washington. *Soil and Sediment Contamin, 7*, 531–542.

NIOSH: National Institute for Occupational Safety and Health. (2006). NIOSH Carcinogen List. Available at http://0-www.cdc.gov.mill1.sjlibrary.org/niosh/homepage.html.

Parsons, J. J. (1972). Spread of African pasture grasses to the American tropics. *Journal of Range Management, 25*(1), 12–17.

Prado, J., & Windisch, P. G. (2000). The genus Pteris L. (Pteridaceae) in Brasil. *Boletim do Instituto Botânico, 13*, 103–199.

Reig-Arminaña, J., Calatayud, V., Cervero, J., Garcia-Breijo, F. J., Ibars, A., & Sanz, M. J. (2004). Effects of ozone on the foliar histology of the mastic plant (*Pistacia lentiscus* L.). *Environment Pollution, 132*, 321–331.

Rôças, G., Barros, C. F., & Scarano, F. R. (1997). Leaf anatomy of *Alchornea triplinervia* (Euphorbiaceae) under distinct light regimes in a Brazilian montane Atlantic rain forest. *Trees, 11*, 469–473.

Rossatto, D. R., & Kolb, R. M. (2010). *Gochnatia polymorpha* (Less.) Cabrera (Asteraceae) changes in leaf structure due to differences in light and edaphic conditions. *Acta Botânica Brasílica, 24*(3), 605–612.

Shin, H., & Kwon, O. (2000). The simultaneous analysis of benzene, toluene, ethylbenzene, o,m,pxylenes and total petroleum hydrocarbons in soil by GC-FID after ultra-Sonication. *Bulletin of the Korean Chemical Society, 21*(11), 1101–1105.

Silva, L. C., Oliva, M. A., Azevedo, A. A., & Araujo, J. M. (2006). Responses of restinga plant species to pollution from an iron pelletization factory. *Water, Air, and Soil Pollution, 175*, 241–256.

Strauss-Debenedetti, S., & Berlyn, G. P. (1994). Leaf anatomical responses to light in five tropical Moraceae of different successional status. *American Journal of Botany, 81*(12), 1582–1591.

Turner, I. M. (1994). Sclerophylly: primarily protective? *Functional Ecology, 8*, 669–675.

U.S. Environmental Protection Agency. 1998. "Carcinogenic effects of benzene: an update. http://www.epa.gov/ncea/pdfs/benzenef.pdf; page 42.

Vollenweider, S., Grassi, G., König, I., & Puhan, Z. (2003). Purification and structural characterization of 3-hydroxypropionaldehyde and its derivatives. *Journal of Agricultural and Food Chemistry, 51*(3), 3287–3293.

Wang, W., Xiao, Y., Chen, L., & Lin, P. (2007). Leaf anatomical responses to periodical waterlogging in simulated semidiurnal tides in mangrove *Bruguiera gymnorrhiza* seedlings. *Aquatic Botany, 86*, 223–228.