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ABSTRACT

The purpose of this study was to evaluate the remediation potential and disturbance response indicators of *Impatiens walleriana* exposed to benzene and chromium. Numerous studies over the years have found abundant evidence of the carcinogenicity of benzene and chromium (VI) in humans. Benzene and chromium are two toxic industrial chemicals commonly found together at contaminated sites, and one of the most common management strategies employed in the recovery of sites contaminated by petroleum products and trace metals is *in situ* remediation. Given that increasing interest has focused on the use of plants as depollution agents, direct injection tests and benzene misting were performed on *I. walleriana* to evaluate the remediation potential of this species. *I. walleriana* accumulated hexavalent chromium, mainly in the root system (164.23 mg kg^{-1}), to the detriment of the aerial part (39.72 mg kg^{-1}), and presented visible damage only at the highest concentration (30 mg L^{-1}). Unlike chromium (VI), chromium (III) was retained almost entirely by the soil, leaving it available for removal by phytotechnology. However, after the contamination stopped, *I. walleriana* responded positively to the detoxification process, recovering its stem stiffness and leaf color. *I. walleriana* showed visible changes such as leaf chlorosis during the ten days of benzene contamination. When benzene is absorbed by the roots, it is translocated to and accumulated in the plant's aerial part. This mechanism the plant uses ensures its tolerance to the organic compound, enabling the species to survive and reproduce after treatment with benzene. Although *I. walleriana* accumulates minor amounts of hexavalent chromium in the aerial part, this amount suffices to induce greater oxidative stress and to increase the amount of hydrogen peroxide when compared to that of benzene. It was therefore concluded that *I. walleriana* is a species that possesses desirable characteristics for phytotechnology.

Introduction

Increases in chromium are due to leather, textile, and steel manufacturing, such as electro painting and chemical manufacturing. Groundwater contamination may occur due to seepage from chromate mines. However, these two substances can also be found in the petrochemical industry, one of the sectors in which large number of workers are exposed to chemical agents. Environmental and occupational exposure to benzene has been the object of control at the global level, given its characteristics as a universal contaminant and its potential effects on health. Nadal *et al.* (2004) measured the concentrations of arsenic, cadmium, chromium, mercury, manganese, lead, and vanadium in soil and chard samples collected at various industrial sites in Tarragona County (Spain), a region that is home to numerous petrochemical plants. The authors concluded that, in terms of carcinogenic risks, only the ingestion of arsenic and the inhalation of chromium in the industrial zone might potentially cause an increase in cancer rates.

Benzene is the most toxic compound among the benzene, toluene, ethylbenzene and xylenes (BTEX) hydrocarbons

and is therefore considered of public health concern. According to the International Agency for Research on Cancer – IARC, benzene is classified in Group I, as a provenly carcinogenic. It is widely accepted that benzene can cause hematological diseases, such as acute myeloid leukemia, acute and chronic lymphocytic leukemia, non-Hodgkin's lymphoma, multiple myeloma and aplastic anemia (Collins et al. (2003; Snyder (2012). Hexavalent chromium, in turn, affects people living in the proximity of natural and anthropogenic sources, as well as workers in occupational settings (ATSDR 2000; OSHA 2006). In the late 19th century, evidence was uncovered that workers exposed to chromium, *e.g.*, in the production of stainless steel and leather tanning, were more susceptible to the development of cancer (IARC 1990). Recent studies have found sufficient evidence about the carcinogenicity of chromium (VI) in humans that can cause lung cancer, and a positive relationship has also been found between hexavalent chromium and nasal cavity and paranasal sinus cancer (IARC 2012). Chromium (VI) is classified in Group 1 as carcinogenic to humans (IARC 1990; IARC 2015) and is classified by the

US Environmental Protection Agency as Group A, known to be carcinogenic by exposure via inhalation, and its carcinogenicity via oral exposure is classified as Group D (USEPA 1998).

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 petroleum-based nonaqueous phase liquid (NAPL), and can thus influence the solubility of toxic hydrocarbons (cosolvent effect) in an environment contaminated with petroleum derivatives. Moreover, studies have shown that the biodegradation of ethanol rapidly consumes all the electron acceptors available in the medium, thus drastically affecting the biodegradation of BTEX. Due to these facts, the search for new alternatives for the remediation of contaminated water is absolutely essential (Tiburtius and Peralta-Zamora 2005).

The works of Farr et al. (1990) and Lenhard and Parker (1990) showed that light nonaqueous phase liquid (LNAPL) does not occur in the form of a As LNAPL migrates through the vadose zone toward the capillary fringe, it displaces air, but generally not water, from the pore spaces. The LNAPL-filled pores drain slowly and can leave behind LNAPL globules trapped by capillary forces. An important risk factor associated with the presence of LNAPL in porous media is the potential migration to a receptor. When a leak begins, LNAPL is drawn down by gravity into the unsaturated medium, displacing air and permeating its empty pores. The oil continues to be trapped in the soil as it migrates vertically while its mobility decreases. According to Parcher et al. (1995), the residual LNAPL phase resulting from the vertical movement of the water level is smaller in the unsaturated portion, where the residual phase varies from 3 to 7%, and in the saturated zone, from 5 to 25%. Mercer and Cohen (1990) state that the residual saturation of LNAPL in the vadose zone ranges from 10 to 20%. The presence of LNAPL in the form of a separate phase at the subsurface acts as an active source of groundwater contamination (Marinelli and Dunford 1996). Therefore, it is evident that the presence of residual oil in the porous medium requires remediation methods aimed at removing the mass of residual LNAPL phase.

Hexavalent chromium generally predominates under oxidizing conditions, while Cr(III) predominates under reducing conditions (Saleh et al. 1989; Stanin 2005). The properties of the two forms of chromium differ considerably, including their mobility and toxicity in the environment. Cr(VI) compounds are generally more soluble and thus have greater subsurface mobility than trivalent chromium compounds. The conventional techniques for treating soil and aquatic environments contaminated with hexavalent chromium are based mainly on the excavation or pumping of the contaminated material, the addition of chemical reducing agents, precipitation followed by sedimentation, and ion exchange and adsorption (Hawley et al. 2004). However, given the toxicity of Cr(VI) and its geochemical behavior, knowledge about plant species with a removal potential can help in decision-making for sustainable technologies.

Another important factor for the development of phytoremediation strategies is the choice of plant species. The phytotoxicity of chemical substances depends on various factors such as the species under study, the stage of development, and the concentration of the contaminant (Pita-Barbosa et al. 2009). Impatiens walleriana was chosen for this study because it is an ornamental herbaceous plant (Maciel 2011) found in various regions of the world, grows easily, and is present in urban gardens and natural forests (Yuan et al. 2011; Schenato et al. 2008). This plant, which reproduces through the germination of seeds contained in a capsule-type fruit (Armitage 1994), propagates easily because it has the ability to take root readily when a seed comes into contact with the ground (Carpanezzi 2007). It is found in abundance in shady locations, where the understory has undergone changes (removal or reduction of native species) and in areas of rainforests (Pastore et al. 2012). Its leaves are hypostomatous, i.e., its stomata are located only on the abaxial side.

I. walleriana was effective in removing metals from soils contaminated by waste, showing bioaccumulation of metals such as copper, zinc, chromium, and nickel (Schenato et al. 2008). This species was also able to accumulate mercury, with a higher concentration in the leaves than in its flowers and stems (Pant et al. 2011), zinc (Torrecilha et al. 2013), and cadmium (Lin et al. 2010; Wei et al. 2012). As for cadmium, Lai (2015) found a positive linear relationship between leaf area, transpiration rate, and cadmium accumulation in I. walleriana. According to the author, most of the cadmium accumulated in the roots and leaves of I. walleriana was compartmentalized in the cell's soluble fraction and the cell wall.

Therefore, in parallel with the benzene assays, I. walleriana was treated with nutrient solutions containing chromium, in order to discover its potential to absorb two substances, one organic and the other inorganic. The absorption potential of chemical substances through the root is the initial step to check for adverse or harmful effects to the vegetal species. Toxic effects may include both lethal and sublethal effects, particularly changes in the plant's growth, development, production of dry matter, and physiological processes, among others.

Materials and methods

To ensure consistent results, the dose–response curves were analyzed and the reactions indicating disturbance were checked, such as the occurrence of visible symptoms and the mortality rate. Histochemical tests were performed to determine the accumulation of pigments, hydrogen peroxide (H_2O_2) accumulation, and cell death.

Soil samples were previously characterized by Ramos (2015) and were collected with a trowel from the A horizon of a Red Latosol (0–12 cm depth) in an uncontaminated area in the municipality of Piracicaba, Sao Paulo. The soil was characterized based on the work of Camargo et al. (2009), in which particle size was determined by the pipette method; OM content was determined by oxidation with potassium dichromate and sulfuric acid; soil pH (active acidity) was determined in 1:2.5 soil:$CaCl_2$ (0.01 mol L^{-1}) using a glass electrode; Fe and

Zn were determined by diethylenetriaminepentaacetic acid (DTPA) extraction; Ca, Mg, K, and Na were determined by ammonium acetate extraction; potential acidity (Al + H) was estimated by the SMP pH buffer method; cation exchange capacity (CEC) was determined by the sum of bases (Ca + Mg + K) and potential acidy; and lastly, the base saturation was obtained by the sum of bases (Ca + Mg + K) plus 100 and divided by the CEC value. The chemical analysis was performed by X-ray fluorescence spectroscopy (PANalytical, Axios Advanced), and the mineralogical composition was determined by X-ray diffraction (Bruker AXS D8 Advance) with CuKα radiation and λ equal to 1.5405 Å. The angular range of 2θ was 2–65°, with a step of 0.020° and a count time of 28 second/step.

I. walleriana is an ornamental plant, which is widely available in gardening shops in the form of seedlings. Its propagation was performed using cuttings, since this species takes root easily. The cuttings were taken from the lateral branches grow (5 cm) without flowers, cut at a slant, excess leaves were removed, and were treated with indolebutyric acid as needed. Six cuttings per pot were planted, using commercial substrate Plantmax® and washed sand (1:1). The pots were placed in a shady place and covered with transparent plastic to preserve the soil moisture until the roots emerged. After the cuttings reached their vegetative growth and presented the first leaves, the plants were transplanted into Red Latosol (RL) soil and subjected to the treatments with chemical compounds.

The purpose of the adsorption test was to determine the soil's retention capacity of Cr(III) and Cr(VI) in solution. Solutions were prepared with Cr(III) and Cr(VI) from Cr $(NO_3)_3 \cdot 9H_2O$ and $K_2Cr_2O_7$ salts, both of analytical grade, in known concentrations of 15 and 30 mg L^{-1}, respectively. A sample of 1 g of RL was weighed and mixed with 50 mL of the solutions prepared in polypropylene tubes. The mixtures were placed in a rotary shaker for 24 hours, after which they were centrifuged and filtered. The Cr(VI) was determined by the US EPA Method 7196A (1992), which is based on the use of diphenylcarbazide as a complexing agent and absorbance reading at 540 nm, using a Thermo Scientific Genesys 20 spectrophotometer. Total chromium was determined by flame atomic absorption spectrometry (Analytik Jena Vario 6), according to APHA Method 3111B (2005).

The organic extract of the plant sample exposed to benzene was extracted with ultrapure dichloromethane on shaker table for 60 minutes. The extract was then concentrated to a volume of 1 mL in an evaporator. The final concentrate was injected, without a flow divider, into a stationary phase HP-1 column coupled to a flame ionization detector. The Gas Chromatography (GC) was set to inject at 300°C with the initial column temperature of 60°C. A heating rate of 9°C minute^{-1} was applied for 13 minutes until the system reached an isothermal temperature of 310°C. Helium (99.999% purity) was used as carrier gas at a constant flow rate of 1.0 mL minute^{-1}. To avoid errors in the preparation of the solutions and to correct the percentage of purity, the stock solutions were also quantified by Gas Chromatography-mass spectrometry (GC7/MS), using an Agilent 5975C mass spectrometer coupled to an Agilent 7693A automatic liquid sampler. The water used in the experiments was ultrapure, and all the systems were prepared in triplicate. In the experiments with benzene, the photoperiod was maintained for 16 hours a day, with lighting provided by 40 W fluorescent lamps simulating daylight (Electrolab EL 202 BOD incubator).

Experimental design

Injection of benzene and chromium in soil

The first experimental series consisted of tolerance testing of the plant species, through the direct injection of benzene and chromium into the soil. For this phase, an entirely random experimental design was used, with three replications for each contaminant, as well as the control series.

The direct absorption experiments were carried out using young adult plants of the same age and the same photoperiod. The main purpose of these experiments was to trigger the symptoms of soil pollution in order to understand the accumulation and translocation, as well as histochemical alterations and biomass reduction. After applying the benzene and chromium treatments, the leaves were subjected to histochemical analysis and the seedling growth was assessed.

Trials were performed by direct injection of a benzene solution (20 mg L^{-1}) and a chromium (VI) solution (15 mg L^{-1}) into the soil. H_2O_2 accumulation and indications of cell death were examined to determine the histochemical changes in leaf tissues caused by benzene and hexavalent chromium. After the benzene and chromium (VI) contamination series in soil, leaves were collected from the 4th and 5th nodes and analyzed histochemically to detect the presence of H_2O_2 and cell death. There is evidence in the literature of the participation of H_2O_2 in response to environmental stress (Soares and Machado 2007). To examine H_2O_2 accumulation, four leaves per individual were collected in the three treatments. A fragment of about 1 cm^2 was removed from each leaf, making a total of twelve fragments per treatment. The fragments were immersed in a solution of 1 mg mL^{-1} of 3,3'-diaminobenzidine (DAB)–HCl, (pH 5.6 adjusted with sodium hydroxide) and incubated in a darkroom for eight hours. They were then cleared in 95% alcohol (Faoro *et al.* 2001) and mounted in 50% glycerin. Cells presenting H_2O_2 accumulation showed a brownish color.

To determine cell death, four leaves per individual were collected, in the three replications, from which a fragment of about 1 cm^2 was removed, resulting in twelve fragments from each treatment. The fragments were boiled for 1 minute in a mixture of lactic acid, phenol, glycerin, and water containing 20 mg mL^{-1} of Evans blue (1:1:1:1) (Iriti *et al.* 2003). Immediately thereafter, they were cleared for 24 hours in an aqueous solution of 2.5 g mL^{-1} of chloral hydrate (Iriti *et al.* 2003) and mounted in 50% glycerin. Dead cells were identified by blue staining, in contrast to healthy cells that were transparent.

The samples from both tests were analyzed by bright field microscopy. A count was made of cells with positive reaction in the fragment test, with a total of twelve counts per treatment. The size of the count area was approximately 0.2 mm^2. The number of cells that reacted positively in the tests was divided

into four classes: class 1 (1–5 cells), class 2 (6–10 cells), class 3 (11–15 cells), and class 4 (16–20 cells), using the methodology proposed by Pedroso (2009).

Dose–response curve

In the dose–response experiment, the plant was initially exposed to a low concentration of the toxic agent, followed by a continuous increase for a period of 60 days. The frequency of exposure also affects the toxicity of the chemical compounds. The dose–response relationship was established for different concentrations of benzene and chromium. The dose–response assessment implies considering that plants often differ in susceptibility to the same pollutant, i.e., that exist individual variability accounts for the different responses in the same types of organisms exposed to the same dose of a chemical. The dose at which there is no visible effect was also checked. The data were analyzed for homogeneity of variance and normality. The dose–response curves were built using SigmaPlot® and Origin 9.0.

The data of the dose–response curves were adjusted to the nonlinear logistic regression model. The mathematical equation that correlates the plant's response with the dose–response curve for the contaminant was established by Seefeldt et al. (1995). To adjust Equation (1) and obtain the statistical parameters, the data were subjected to nonlinear regression analysis. Based on the equation, a logarithmic graph was built for the benzene and chromium dose.

The experimental design was a randomized block design with three replications and seven doses of benzene varying from 0 to 1600 mg L^{-1} applied directly on the soil. Different doses of benzene were applied for 60 days, using a

precision pipette to add the solutions to the soil surface. The Cr(VI) concentrations varied from 0 to 300 mg L^{-1}, and were applied for the same period of time. After 60 days of contact, an evaluation was made of the dry matter content obtained by weighing the collected material dried in a forced air drying oven (70°C) for 72 hours.

The symptoms of phytotoxicity or their absence were evaluated visually, assigning scores between 0 and 100%, as follows: zero for the absence of symptoms and 100% for plant death. The percentage scale was used to meet the requirement of the log-logistic model proposed by Seefeldt et al. (1995):

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^c\right]} \quad (1)$$

where y = percentage control; x = benzene dose; a, b, and c = curve parameters, with a corresponding to the difference between the maximum and minimum points of the curve. Parameter b describes the slope of the curve at around C_{50}, and c is the slope of the curve.

Results and discussion

In the natural environment, the half-life of benzene is 0.02–2 years; therefore, combined processes should be adopted, in different environmental compartments, to trigger biological degradation or even to contain benzene to vertically penetrate subsurface mobility and minimize negative impacts by means of various low-cost techniques (ASTM 1995). Thus, the use of plants as depollution agents has aroused increasing interest and has been evaluated mainly in soils contaminated with trace metals (Chowdhury et al. 2015; Houda et al. 2016; Kaewtubtim et al. 2016), crude oil and its derivatives (Fatima et al. 2016; Liao et al. 2016), and other organic compounds (Ignatowicz 2016; Lafleur et al. 2016). The use of plants that can tolerate and simultaneously extract toxic substances may offer an interesting alternative for in situ decontamination (Campos et al. 2014).

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}. Some metals have only one oxidation number, for example, Pb^{2+}, Cd^{2+} and Zn^{2+}, while other metals have more oxidation numbers, for example, Fe^{2+} and Fe^{3+}, As^{3+} and As^{5+}, Cr^{3+} and Cr^{6+}, etc. The mobility of chromium, for example, as well as its accumulation in soil, is due to various types of mechanisms that involve chemical reactions, such as redox potential, precipitation, dissolution, sorption, and desorption. The main factor that controls metal concentrations in groundwater is adsorption on ferric hydroxide. Because iron is one of the most common elements in the earth's crust, and because this metal also presents fast kinetics in the precipitation/dissolution process, the formation of insoluble mixed hydroxide (Cr, Fe)(OH)$_3$ is crucial in order to control the soluble fraction of Cr(III) species in natural environments. In this case, Red Latosol can perform the function of retaining metal in soil, which opens a space for the study

Table 1. Physical and chemical characterization of A Horizon of Red Latosol (modified from Ramos 2015).

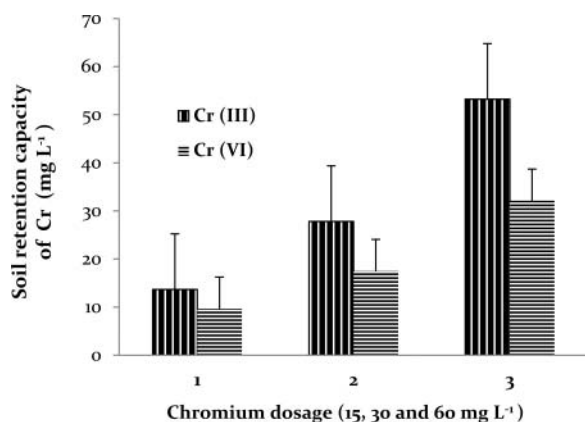| Particle size	Unit	Result
clay	%	49.5
silt	%	16.6
sand	%	33.9
texture	—	Clay
Chemical attributes	Unit	Result
pH in CaCl$_2$ (0.01 mol L^{-1})	—	4.3
OM	g dm^{-3}	46
P	mg dm^{-3}	12
K$^+$	mmol$_c$ dm^{-3}	1.6
Ca^{2+}	mmol$_c$ dm^{-3}	14
Mg^{2+}	mmol$_c$ dm^{-3}	7
H + Al	mmol$_c$ dm^{-3}	64
CEC	mmol$_c$ dm^{-3}	86.8
BS	—	26
Chemical composition		Result (% wt)
SiO$_2$		41.3
Al$_2$O$_3$		26.0
Fe$_2$O$_3$		15.2
P$_2$O$_5$		0.18
K$_2$O		0.17
CaO		0.1
MgO		0.11
TiO$_2$		2.96
SO$_3$		0.13
Cr$_2$O$_3$		0.03
LOI		13.5

Figure 1. 24-hour chromium adsorption test in soil at different concentrations (15, 30, and 60 mg L^{-1}, respectively) and chemical species (hexavalent and trivalent) (error bars = 3).

of organic pollutants—in this case, aromatic hydrocarbons (BTEX). In groundwater with values of pH > 4, Cr(III) precipitates in the presence of Fe(III), resulting in a mixed insoluble compound with a nominal composition of $Cr_xFe_{1-x}(OH)_3$ (Rai *et al.* 1988; Rai *et al.* 1987; Sass and Rai 1987), as already mentioned.

The physicochemical and chemical parameters of soil (Table 1) revealed a clayey texture, strongly acidic pH (4.3), large quantities of organic matter (46 g dm^{-3}) and high CEC (86.6 mmol$_c$ dm^{-3}), significant presence of silicon and aluminum, as well as iron oxides and a low concentration of trivalent chromium (Cr_2O_3) in the original composition of this soil (Ramos 2015). The X-ray diffraction analysis reveals the presence of quartz (SiO_2), kaolinite ($Al_2Si_2O_5(OH)_4$), goethite (α-FeO·OH), hematite (Fe_2O_3), and gibbsite ($Al(OH)_3$) in the composition of the RL soil.

Initially, a retention test with trivalent chromium and hexavalent species was performed to determine the maximum soil retention capacity of Cr(III) (Figure 1), which revealed a higher soil adsorption capacity of the trivalent form of chromium. Based on these results, the experiments with seedlings were carried out using only hexavalent chromium.

Histochemical test

One of the functions of H$_2$O$_2$ is to signal cell death (Levine *et al.* 1994) because this substance accumulates primarily in the
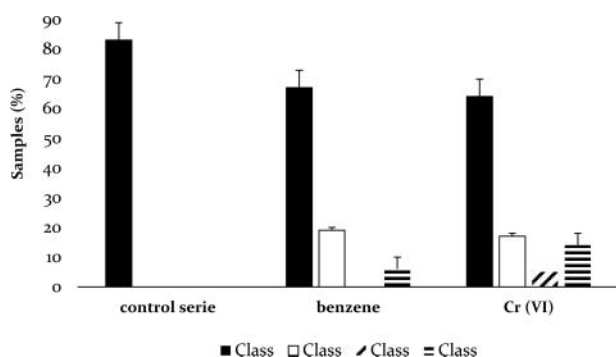

Figure 2. Percentage of samples classified in each class of cells presenting cell death (class 1 = 1–5 cells; class 2 = 6–10 cells; class 3 = 11–15 cells; and class 4 = 16–20 cells) in the benzene, chromium, and control treatments (error bars = 3).
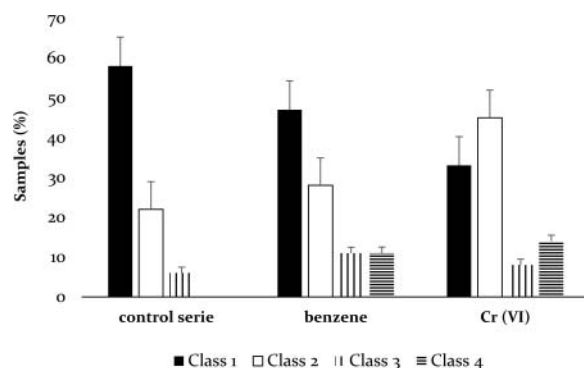

Figure 3. Percentage of samples classified in each class of cells presenting accumulation of hydrogen peroxide (class 1 = 1–5 cells; class 2 = 6–10 cells; class 3 = 11–15 cells; and class 4 = 16–20 cells) in the benzene, chromium, and control treatments (error bars = 3).

cell wall and plasma membrane, after which it reaches the cytoplasm and organelles, causing the cell to collapse (Faoro et at. 2001; Iriti *et al.* 2003). To identify cell death in leaf tissues before the onset of visible symptoms, we used Evans blue dye, which produces an intense blue stain in the dead cells, as indicated in Figure 2.

Cell death was visibly more intense in the treatment with 20 mg L^{-1} of benzene and 15 mg L^{-1} of hexavalent chromium, with the species showing cell death in some areas of the leaf tissue (Figure 2). When *I. walleriana* was exposed to the contaminants, H$_2$O$_2$ production was intensified since the samples were allocated to higher classes (Figure 4). Upon exposure to benzene, the number of samples in class 1 (47%) declined, while those in classes 2 (28%) and 3 (11%) increased when compared with that of the control treatment, in addition to the appearance of samples in class 4 (11%). The plants exposed to benzene showed a lower percentage of samples in class 1 (67%) than in the control treatment, and some samples fell into the class 2 (19%) and class 4 (6%) categories. Compared to the treatment with benzene, the plants exposed to Cr(VI) had a larger number of cells with positive reaction for cell death, and the samples were classified as follows: class 1 (64%), class 2 (17%), class 3 (5%), and class 4 (14%). The percentage of classes 3 and 4 was higher than the percentage of these same classes in the benzene treatment, indicating that cell death in plants exposed to Cr
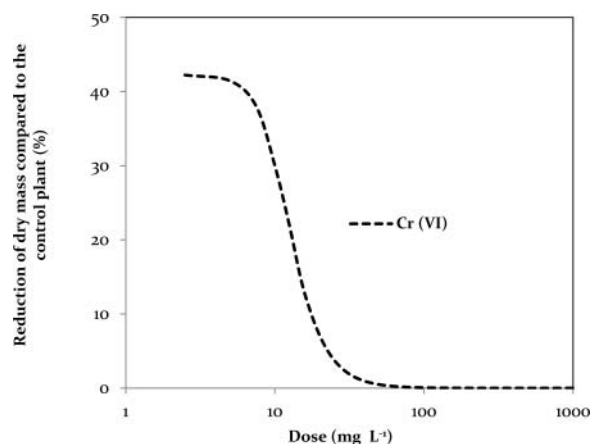

Figure 4. Dose–response curve of *Impatiens walleriana* in contact with chromium, assessed by mass reduction of dry matter after 60 days of contact with the contaminant. Dose in (mg L^{-1}) and reduction of dry mass compared to the control plant, in (%).

(VI) was more intense. In the control treatment, the *I. walleriana* samples presented a number of cells with positive reaction to cell death that fell solely in class 1 (83%).

I. walleriana presented different intensities of H_2O_2 accumulation in the leaf tissues. The presence of H_2O_2 in the control treatment is explained by the regular metabolism of these plants, since reactive oxygen species (ROS) are the result of the decrease in molecular oxygen from the respiratory chain electrons, and the main points of production of these species are mitochondria and chloroplasts (Bray *et al.* 2000; Apel and Hirt 2004). In normal conditions, the production of ROS in the cell is low, but when the plant is subjected to an environmental stress, the generation of ROS increases. The accumulation of H_2O_2, with reduction of other ROS, is a defense mechanism of plants as H_2O_2 is less reactive than the superoxide radical and hydroxyl radical and thus less harmful to the plant reducing damage and increasing the chances of recovery (Ferreira and Matsubara 1997).

In the control treatment, the samples were classified mainly as class 1 (58%), indicating that the fragments contained accumulated H_2O_2 in 1–5 cells, although some samples were classified as class 2 (22%) and class 3 (6%). Medeiros (2015) suggests that, in the control treatment, the production of H_2O_2 in *I. walleriana* originates from the plant's natural metabolism. Low levels of ROS suggest adaptive responses, whereas high concentrations of these species cause severe damage, triggering cell death (Benavides *et al.* 2005). In this study, since only the presence of H_2O_2 was established but not of the other ROS, it can be inferred that benzene and hexavalent chromium induced the production of superoxide radicals (O_2), and that these radicals were converted into H_2O_2 through the enzyme superoxide dismutase (Maiti *et al.* 2012). The accumulation of H_2O_2, with reduction of other ROS, is a defense mechanism of plants as H_2O_2 is less reactive than the superoxide radical and hydroxyl radical and thus less harmful to the plant reducing damage and increasing the chances of recovery (Ferreira and Matsubara 1997).

I. walleriana exposed to Cr(VI) presented more intense accumulation of H_2O_2 than those exposed to benzene. The samples were mostly in the class 2 (45%), with a decrease in classes 1 and 3 and an increase in class 4 (14%) when compared with the results of the benzene treatment.

At the cellular level, absorption of excess chromium will lead to oxidative stress because it generates ROS and induces oxidative damage to lipids, proteins, and DNA biomolecules (Shanker *et al.* 2004; Wang *et al.* 2010), in addition to causing changes in antioxidant enzyme activity (Pandey and Sharma 2003). In normal physiological conditions, plants can accommodate ROS in different cell compartments such as cell wall, plasma membrane, apoplastic space, chloroplasts, and mitochondria (Dhir *et al.* 2009).

Although the amount of hexavalent chromium accumulated in the aerial portion of I. *walleriana* was small, this amount sufficed to induce oxidative stress and increase the H_2O_2 content. According to Soares and Machado (2007), H_2O_2 is harmful when it accumulates, which explains the large number of dead cells, since, according to Levine *et al.* (1994), H_2O_2 is an indicator of cell death, and Van-Breusegem *et al.* (2001) state that at high concentrations, ROS lead to apoptosis, *i.e.*, genetically programmed cell death.
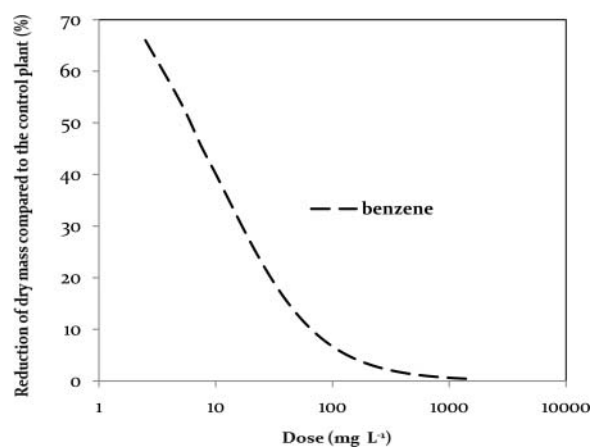

Figure 5. Dose–response curve of *Impatiens walleriana* in contact with benzene, assessed by mass reduction of dry matter after 60 days of contact with the contaminant. Dose in (mg L^{-1}) and reduction of dry mass compared to the control plant, in (%).

According to Campos *et al.* (2014), *I. walleriana* absorbs benzene through the roots, and is translocated to and accumulated mainly in its aerial portion and then volatilized. This mechanism of the plant may have prevented the large amount of contaminant in the leaf tissues from triggering an intense process of ROS production, which would lead to protein and lipid peroxidation. Medeiros *et al.* (2015) state that *I. walleriana* probably has an efficient antioxidant system, which can help to reduce the amount of ROS through asynchronous action of its enzymes, thus diminishing damage and cell death.

Dose–response curve

The analysis of the dose–response curve revealed a significant delay in plant growth as the concentration of hexavalent chromium increased. In general, there was a decrease in root biomass; at high concentrations, breakdown and consequent inability of the root to absorb water were observed (Figure 5). The concentrations of 15 and 30 mg L^{-1} did not affect the plant height growth.

According to Panda *et al.* (2003), chromium may induce oxidative stress in plants, causing lipid peroxidation, thus promoting severe damage to cell membranes, which is triggered by the degradation of photosynthetic pigments, leading to decreased growth. For Vazquez *et al.* (1986), injuries caused to the plasma membrane can be considered the primary mechanism of chromium toxicity, and high concentrations can cause disruption of the chloroplast ultrastructure, thereby impairing photosynthesis. In addition, Mei *et al.* (2002) state that because hexavalent chromium is a strong oxidizing agent with relative mobility, it can cause greater damage to the plasma membrane than trivalent chromium.

The maximum amount of Cr(VI) was 164.23 mg kg^{-1} accumulated in roots and 39.72 mg kg^{-1} in the aerial portion, indicating that about four times more chromium was accumulated in the roots and relatively little was transported to the aerial portion of *I. walleriana*. Authors such as Schenato *et al.* (2008) and Shanker *et al.* (2005) reported higher chromium accumulation in the root system than in the aerial portion of the plants they studied. They stated that the absence of specific transport mechanisms for chromium from the roots to the aerial portion

is due to the fact that this metallic element is highly toxic and nonessential for the growth and development of the studied plants.

It was concluded that increasing concentrations of hexavalent chromium can affect I. walleriana growth. I. walleriana presented potential rhizofiltration, absorbing and concentrating chromium in the root system and presenting injury only when exposed to the maximum concentration of hexavalent chromium.

With regard to benzene, *I. walleriana* exhibited desirable characteristics as a phytoremediation species. In other words, increasing the dose did not cause a significant decrease in biomass, since the species volatilizes absorbed benzene, preventing irreversible damage and recovering its apparent state, visibly demonstrating its tolerance to the product (Figure 6). Tolerance is an innate characteristic related to the natural genetic variability of a species that is able to survive and reproduce after treatment with the substance, despite suffering damage (Silva *et al.* 2007). This selectivity is due to the fact that plant tissues are able to absorb, metabolize, compartmentalize, and/or translocate organic compounds, which are subsequently volatilized, and which may also be partially or completely degraded or even undergo transformations, giving rise to less toxic and especially less phytotoxic compounds (Scramin *et al.* 2001).

I. walleriana responded differently to the benzene and chromium concentrations. When this species was analyzed based on its chromium tolerance level, a more significant effect was observed in terms of the reduction in dry matter, *i.e.*, a 50% reduction, with a susceptibility factor of 2.3. With regard to hexavalent chromium, there was a marked decrease in root biomass (0.77 ± 0.18) compared to the control (1.35 ± 0.27), mainly at the concentration above 30 mg L^{-1}, showing high toxicity of Cr (VI) in comparison to the tests with benzene.

I. walleriana showed high tolerance to benzene, withstanding doses higher than 1000 mg L^{-1}, with a susceptibility factor of 19.7. Therefore, this species can be tested for its remediation potential before being employed in phytoremediation programs of soils contaminated with benzene.

Conclusions

Determining the potential of plant species to absorb chemicals through their roots is the first step in the study of phytotechnology. Moreover, species should be sought that have mechanisms that render them less toxic. In addition, it is necessary to look for species that present mechanisms to reduce the toxicity of contaminants. The results demonstrated that relatively little of the hexavalent chromium absorbed by the root system was transported to the aerial portion of the plant. Chromium uptake by roots caused the root and shoot biomass to decrease, and because Cr(VI) is more mobile than trivalent chromium it was transported throughout the plant, although a higher accumulation of chromium was found in the root system. The small amount of chromium translocated to aerial part has already been able to generate histochemical changes more intense than the plants exposed to benzene. Chromium (VI) caused the greatest oxidative stress in I. walleriana, which can be verified through increased accumulation of H$_2$O$_2$ and cell death. Compared to benzene, chromium has a higher potential to generate

ROS because even a small amount of this contaminant in the aerial portion of the plant caused higher oxidative stress in cells.

I. walleriana absorbs and translocates benzene, accumulating it mainly in the aerial portion, and eliminating the contaminant and reducing ROS through volatilization and its efficient antioxidant system. This explains the smaller number of cells containing H$_2$O$_2$ and the lower cell death rate when compared with Cr(VI). It was concluded that hexavalent chromium affects the growth of *I. walleriana* and is lethal to this plant; hence, it is more toxic than benzene.

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