Science of the Total Environment 599–600 (2017) 572–580

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Preliminary study of phytoremediation of brownfield soil contaminated by PAHs

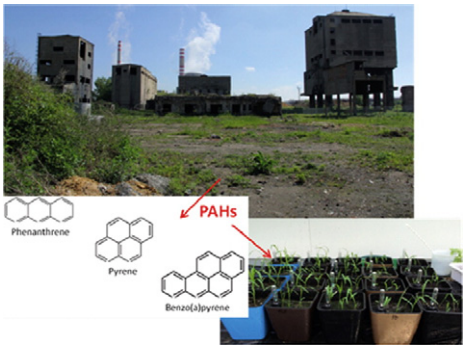
Šárka Petrová, Jan Rezek, Petr Soudek, Tomáš Vaněk *

Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, Prague, Czech Republic

HIGHLIGHTS

- Organic pollution on site is very high (TPH 13 g/kg).
- PAHs were accumulated in the shoots of both tested plant species.
- *Brassica napus* plants showed high resistance and ability of PAH accumulation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form 19 April 2017
Accepted 21 April 2017
Available online 7 May 2017

Keywords:
Brownfield
Polyaromatic hydrocarbons
Phytoremediation
Energy plants

ABSTRACT

Our project was aimed at improving a brownfield in the city of Kladno, where an old steel producing facility used to be in operation. Ecological risk is mainly caused by the processing of co-products during coal production (tars, oils). Knowledge of toxicology and environmental aspects can help us protect human health and the environment.

Primarily, we focused on soil sampling and identification of pollutants. Results showed that organic contamination on the site is very high. Average concentration of total petroleum carbon in the soil was about 13 g/kg DW, which is much more than the maximum allowed concentration. For selection of suitable plant species for phytoremediation at the site, experiments were conducted in a greenhouse. Biomass growth, root morphology, and pigment content in the leaves of *Brassica napus* var. Opus-C1 and *Sorghum* × *drummondii* var. Honey Graze BMR plants were studied. Plant analysis confirmed that polyaromatic hydrocarbons (PAHs) accumulated in the shoots of both plant species. *B. napus* plants grown on Poldi soil in a greenhouse were able to survive the toxicity of PAHs in soil, and their ability to accumulate PAHs from soil was evident. However, more studies are needed to decide if the plants are usable for phytoremediation of this brownfield.

1. Introduction

Multi-contaminated soils near inhabited areas might cause the death of plants or reduce their productivity, in addition to endangering the health of local human population. Sources of such contamination are mostly anthropogenic, especially industrial activities (i.e., mining, processing and treatment of ores, industrial production) (Page and Berger, 2006). Even after an industrial activity has ended, the production site often remains contaminated. These locations are usually called brownfields and their recovery is very costly and time-consuming (Zhu et al., 2015). For cases that meet the requirements for in-situ recovery

* Corresponding author.
E-mail address: vanek@ueb.cas.cz (T. Vaněk).

http://dx.doi.org/10.1016/j.scitotenv.2017.04.163
0048-9697/

processes, phytoremediation is the most commonly used technique (Kovacs and Szemmelveisz, 2017). Each industrial brownfield represents specific challenges for the environment and the adjacent community; owing to the previous operations at the site, it might have real or perceived contamination problems. Non-degradable pollutants from past activities like smelting and the associated waste heaps could still act as a source of pollution. The main contaminant categories in Europe are heavy metals, and organic pollutants such as mineral oil, benzene, toluene, ethylene, xylene, chlorinated, and polyaromatic hydrocarbons (Panagos et al., 2013). Examples include, Cd, Cu, Pb, and Zn contamination in the Lower Swansea Valley (Davies, 1997; Gallego et al., 2015), and polyaromatic hydrocarbons (PAHs) pollution resulting from coal-mining activities in China (Liu et al., 2012). A co-occurrence of PAHs and heavy metal(loid)s in brownfield sites is also common (Gallego et al., 2015; Thavamani et al., 2011). PAH pollution is a worldwide problem (Abdel-Shafy and Mansour, 2016; Cébron et al., 2009; Pelaez et al., 2013). Due to their high persistence time, their concentrations increase in both the abiota and biota (Kayal and Connell, 1995; Srogi, 2007; Tolosa et al., 2005). The estimated range of half-lives for PAHs in soil is quite wide. It is dependent on the compound, and studies have reported the range as 2 months to 2 years (Mackay et al., 1991), as well as and 8 to 28 years (Wild et al., 1990). A natural attenuation study in wasteland soil showed that PAH concentrations decreased very slowly in time (Ouvrard et al., 2011). However, plants can effectively reduce the contamination in brownfields by immobilizing the contaminants in the soil, by accumulating them in their tissues (Baker et al., 1994) or by promoting the growth of soil bacteria via root exudates (Joner et al., 2002). Under the influence of phenanthrene, the population of phenanthrene-degrading microorganisms in the sorghum root zone increased, but the secretion of root exudates stayed the same (Muratova et al., 2009). Other studies have widely dealt with grasses and trees. For instance, inoculation with endophytic bacterial strain *Achromobacter xylosoxidans* F3B during remediation has been shown to protect vetiver grass plants against toluene stress (Ho et al., 2013). Another study reported that the establishment of *Festuca rubra* plant cover on fly ash deposits stabilized the ash, prevented wind erosion, and reduced dispersion of toxic chemical elements into the environment (Gajić et al., 2016). It has been shown that trees can effectively reduce the contamination of the soil, in addition to their economic benefits (French et al., 2006). It has previously been reported that trees can immobilize contaminants in the soil or accumulate them in their tissues (Dickinson, 2000; Pulford and Watson, 2003; Vangronsveld and Cunningham, 1998). Woody plants, *Terminalia arjuna*, *Prosopis juliflora*, and *Dendrocalamus strictus*, growing on tannery sludge dumps accumulated appreciable amounts of metals in their tissues, which led to a reduction in the contamination levels in the tannery sludge (Shukla et al., 2011). Moreover, other study showed that after 60 weeks of treatment, the concentration of total PAHs decreased by 73% in planted sediments, but only by 25% in unplanted controls (Huesemann et al., 2009). A field study, which targeted the way organic pollutants dissipate in the substrate, showed a significant decrease in the concentrations of the sediment planted with willow (Vervaeke et al., 2003). Moreover, by using high-biomass plants that can provide biofuel, the costs involved in the operation of phytoremediation techniques, maintenance of areas, and monitoring of contamination, can be saved (Dickinson, 2000; Pulford and Watson, 2003).

Unfortunately, the accumulated PAHs in soil have toxic effects on plants. PAHs enter the plants either directly via stomata, or indirectly via the root system (Kuhn et al., 2004). It has been shown that they negatively affect the photosynthetic processes (Duxbury et al., 1997) and seed germination (Sverdrup et al., 2003), and cause an increased production of reactive oxygen species (Babula et al., 2012). Plants metabolize xenobiotics through a two-stage system of reactions, commonly designated as Phase I and Phase II. Phase I transformations are oxidation, reduction, and hydrolysis reactions. Conjugation, the process by which metabolites react with additional substrates to form larger macromolecules and bound residues, is designated as a Phase II reaction (Coleman et al., 1997; Shimabukuro, 1985; Shimabukuro et al., 1982). Phase I reactions, which usually reduce the toxicity of a xenobiotic, might also activate the compound for Phase II conjugation (Shimabukuro et al., 1982). However, in plants, the lack of a well-developed excretory system precludes the removal of metabolites, which occurs routinely in animals. Alternatively, Phase II conjugation in plants is often followed by immobilization of the metabolite through compartmentalization into storage vacuoles, formation of insoluble salts, complexation with plant constituents, or binding to structural polymers (Hatzios and Penner, 1982; Sandermann, 1992).

In the Czech Republic, there are many sites with old ecological burdens, and therefore, there is a grave need for cheaper and effective methods of remediation. One such site is an old steel-producing facility in the city of Kladno (about 500 ha). The history of this area dates back to the mid-19th century. The town's coking plant was built during the Second World War. It went into operation in 1944, and then it expanded. Downturn of coke production during the 80's resulted in the end of the production in 1988. Ecological burdens are caused mainly by the processing of the by-products of coke production, especially tars or oils. Knowledge of toxicology and environmental aspects would help improve the safety of human health and the environment. Moreover, contaminated lands are not suitable for agricultural production but can be used for biofuel crop cultivation. This study can contribute toward the development of a cleaning-up solution for contaminated lands, integrated with bioenergy demand fulfillment. We carried out experiments under controlled conditions in a greenhouse to observe the behavior of plants grown on soils with different concentrations of pollutants. After assessing the results from experiments such as ours, open field trials can be undertaken. Our research is an important first step toward friendly remediation of the site.

2. Material and methods

Experiment was set up on the base of legislative method for detection of toxicity effect of pollution (International Organization for Standardization, 2012). The test measures an emergence and early growth of at least two terrestrial plants species. For control, an artificial soil was prepared by mixing 30% agricultural substrate with clay (Gramoflor GmbH & Co. KG, Germany) and 70% quartz sand (with >50% particles of the size 0.05 to 0.2 mm). Polluted soil (Poldi soil) was sampled from the site of the old steel producing facility at Poldi, Kladno, near the tar waste pipelines (50°09′05.0″N 14°07′19.8″E). To determine the basic soil properties (organic carbon, exchangeable pH, available nutrients) of polluted soil from brownfield, the samples were analyzed in commercial laboratory.

The contaminated soil was air dried, sieved through a 4-mm sieve, mixed with artificial soil in ratios 0, 12.5, 25, 50, and 100% of contaminated soil, and placed in plastic self-watering pots (13 cm × 13 cm × 13 cm) with inner dimensions of dimension 10 cm × 10 cm × 10 cm. After five days of watering, the soil was planted with five seeds of *Brassica napus* var. Opus-C1 (Forestina s.r.o., Czech Republic) or *Sorghum bicolor* × *drummondii* var. Honey Graze BMR (Seed Service s.r.o., Czech Republic) per pot. The experiment was carried out in controlled conditions in a greenhouse (23 °C, humidity about 60%). Plants were irradiated for four weeks using sodium discharge lamps (400 W, ZG Lighting Czech Republic s.r.o.) with daily light phase of 16 h and an average irradiation of 72 μmol/m^2 s at the plants' surfaces, and the horizontal differences in irradiation were <20%. Growth of plants' biomass was calculated from shoots and was expressed as a percentage growth rates.

2.1. Chemicals and reagents

Ethyl acetate LiChrosolv®, and Supelclean™ PSA SPE were purchased from Sigma Aldrich, Germany. Magnesium sulfate anhydrous

p.a. and sodium chloride p.a. were purchased from Chempur SA, Poland. Standard EPA 525 PAH Mix-A (containing 16 compounds: acenaphtheneacenaphtene (Ace), acenaphthylene (Acy), anthracene (Ant), benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*ghi*]perylene (BghiP), chrysen (Chr), dibenzo[*a*,*h*]anthracene (DahA), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-*cd*]pyrene (IcdP), phenanthrene (Phe), naphthalene (Nap), pyrene (Pyr), and acenaphthene-D8 and anthracene-d10 were obtained from Supelco, USA. Deionised water (18 MΩ) was produced using a Milli-Q system (Millipore, USA).

2.2. QuEChERS extraction and clean-up

Sample preparation procedure was based on QuEChERS extraction and clean-up (Drabova et al., 2012). Freeze-dry samples were ground in liquid nitrogen, and 100-mg aliquots were spiked with acenaphthene-D8 and flouranthene-D10 to reach 1 μg/g DW. After re-hydration with 1 mL of ultrapure water for 10 min in ultrasonic bath, the samples were extracted with 1 mL of ethyl acetate using a combination of anhydrous $MgSO_4$ and NaCl, shaken, and centrifuged (5000 RPM). Upper organic layer was clean-up of with $MgSO_4$, Supelclean™ PSA SPE Bulk, shaken, centrifuged (5000 RPM), and pipette to an insert in a glass vial.

2.3. PAH analysis

Agilent 7890 Gas Chromatograph, equipped with a LECO dual-jet thermal modulator between the primary and secondary columns, and LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) system with Gerstel multipurpose sampler and temperature programmed CIS4 inlet were used to perform the GC × GC-TOFMS analyses. Column setup was 60 m Rxi-PAH (0.25-mm ID, 0.1-μm film) in 1st dimension and 1 m Rxi-5HT (0.25-mm ID, 0.1-μm film) in 2nd dimension. The quantification of the compounds was done by normalizing the peak areas with the areas of the respective internal standards, followed by comparison with a matrix-matched calibration curve. Tests were carried out in quintuplicate (five aliquots of the same sample individually extracted and injected). In every batch of 6 samples, a procedural blank was included.

To estimate the accumulation rates of PAHs in plants, shoot concentration factor (SCF) was used. It was expressed as the ratio of the concentration of PAH in the plant shoot to the concentration of PAH in the soil.

2.4. Seed germination

Growth inhibition test examines the effect of contaminants in a soil extract on the seed germination of white mustard (*Sinapis alba*), as well as the root growth in early stages of development (Fargašová, 2012; Leitgib et al., 2007; Wang et al., 2001). The Poldi soil (0.5, 1, or 2 g) or Poldi soil extract (10 g of soil/1 L of distilled water supplemented with nutrients) were tested. A control treatment was performed with nutrient solution or artificial soil in same weight as for Poldi soil. The soil and 5 mL of nutrient solution or 5 mL of leachate with nutrient solution were added to a Petri dish in quadruplicates. Seventeen seeds per dish were used, and after 72 h in a dark room, the root length was measured and inhibition was calculated according to the following equation:

$$I = 100 \times (r_c - r_s)/r_c$$

where r_c is the mean value of root length of the control, and r_s is the mean value of root length for each concentration.

2.5. Chlorophyll analysis

Chlorophyll was extracted overnight in dark at 4 °C by placing a third fully expanded leaf from apex (approximately 0.5 g) into 10 mL pure methanol. Extracted pigment was determined spectrophotometrically at the following wavelengths: 665.2, 652.4, and 470 nm (Lichtenthaler, 1987), and calculated on dry weight basis.

2.6. Statistical analysis

Relative responses were expressed as mean ± standard deviation. Data from all experiments were analyzed using the software STATISTICA (StatSoft, USA). The differences between the groups were assessed using a one-way ANOVA followed by Duncan's test with $p < 0.05$.

3. Results and discussion

3.1. Soil characteristic

Analysis of Poldi soil parameters indicated a soil pH ($CaCl_2$) of 5.2 to 8.0 (Table 1). Exchangeable soil reaction (pH/KCl), defined as the ability of soil to change the pH of mineral salt solutions (electrolytes), was very slightly acidic, typical of the value for contamination-free soils. pH values in this range provide optimum conditions for most agricultural plants. However, element analysis indicated several problems. Low phosphorus (P), and high iron (Fe), calcium (Ca), and organic concentrations can have negative effects on plant growth (Table 1). In plants suffering from P deficiency, reductions in leaf expansion, leaf surface area, and leaf number are the most striking effects. Moreover, the excess of Ca, and Mg can slow down plant growth, increase the elongation of plant, and lead to chlorosis of leaves (Marschner, 1995b). The excess of Fe, however, does not necessarily lead to problems. The oxidized Fe(III) has a very low solubility at basic pH, resulting in its limited uptake by plant roots as it cannot be absorbed by root cells (Lucena et al., 2007). Non-graminaceous plants, such as *B. napus*, release protons into the rhizosphere to acidify the soil to increase the solubility of Fe through the formation of ferric ions Fe(III). These are reduced to ferrous ions Fe(II) on the root surface by a ferric reductase-oxidase (FRO) enzyme. Fe(II) ions are carried across the root plasma membrane into the root cells by an Fe^{2+} transporter (IRT1), which is an Fe-regulated member of the large ZIP family (Sinclair and Krämer, 2012). Graminaceous plants, such as *S. bicolor*, also use another mechanism that involves the synthesis and secretion of phytosiderophores), which are high-affinity chelating compounds. Chelated Fe(III) is translocated into the root cells by YS like proteins (Curie and Briat, 2003). Free, unchelated iron in plants can be damaging to the cells, because its interaction with oxygen can generate superoxides. Plants have two mechanisms of storing iron in non-toxic form: first, by chelation with amino or organic acids, and second, by complexation with the

Table 1
Poldi soil agrochemical parameters.

Parameter	POLDI soil
pH $CaCl_2$	7.29
pH KCl	6.46
$P_{available}$ [mg/kg]	8.04
K [mg/kg]	248
Mg [mg/kg]	346
Mn [mg/kg]	1040
Ca [mg/kg]	12,100
Fe [mg/kg]	131,000
Na [mg/kg]	1540
TOC [%]	26
$C_{exchangeable}$ [%]	26.40
N_{total} [%]	0.48
TPH [mg/kg]	12,900

Table 2
Toxic characteristics: Inhibition of root growth in mustard (*S. alba*); zero value represents the Poldi soil leachate.

m_{soil} (g)	I_{soil} (%)
0	−32.98
0.5	61.36
1	43.40
2	43.91

protein, phytoferritin (Seckback, 1982). Two uptake mechanisms in sorghum plants might led to excess of iron in tissues which resulted in slower growth.

Toxic characteristics of contaminated soil from Poldi area were investigated using a germination test. Test with the leachate of Poldi soil did not show any inhibition of seed growth (Table 2). When Poldi soil itself was used, it inhibited the root growth in all treatments compared with the artificial soil. Inhibition was calculated for 0.5, 1, and 2 g of Poldi Soil as 61.36, 43.40, and 43.91%, respectively (table 2). Higher content of soil in Petri dish lead to seeds dryness which can mislead the test. The inhibitions were compared with artificial soil of the same weight to avoid the inadequacy. A decrease in root growth is in agreement with the literature. At the concentration of 1 mg/g PAH (naphthalene 98%, pyrene 98%, fluorene 98%, phenanthrene 98%,% and anthracene 98%), the germination level of *Lepidium sativum* was 16% (Maila and Cloete, 2002).

Average organic pollution in the soil was quite high. Total Petroleum Hydrocarbon (TPH) concentration in Poldi soil was about 13 g/kg (Table 1). TPH includes many substances, and this parameter is usually selected to conduct an exploratory investigation (Pinedo et al., 2012). A detailed analysis of PAH content in the soil showed that the highest concentration was detected for fluoranthene (706 mg/kg) and pyrene (610 mg/kg) (Table 3). High concentrations of animal carcinogens (BaA, BbF, BaP, DahA, and IcdP) were also detected in the soil. Average PAH concentrations in the soil were above the maximum permitted levels for hazardous substances for all soil usage types (from agricultural to industrial) (CR, 1994, 2013). The highest limit for industrial soil was exceeded for benz[*a*]anthracene (339 mg/kg), benzo[*a*]pyrene (353 mg/kg), benzo[*b*]fluoranthene (188 mg/kg), benzo[*k*]fluoranthene (359 mg/kg), chrysene (365 mg/g), and indeno[1,2,3-*cd*]pyrene (138 mg/kg). Moreover, polychlorinated biphenyls (heptachlorobiphenyls - 11 isomers, hexachlorobiphenyls - 10 isomers, octachlorobiphenyls - 2 isomers, pentachlorbiphenyls - 5 isomers, and tetrachlorobiphyenyl - 1 isomerisomer) were detected in the samples (data not shown). The PAH content of over 3700 mg/kg of dry Poldi soil indicated a heavy contamination (Maliszewska-Kordybach, 1996). It corresponded with the data from other brownfields worldwide (Bakker et al., 2000). Moreover, some urban soils in Europe and China have showed similar PAH concentrations (Morillo et al., 2007; Wang et al., 2013).

3.2. Physiology

Two plant species were chosen for the experiment, *B. napus* and *S. bicolor*. Plants grew for a month in pots with contaminated or clean soil and their various combinations. The aboveground biomass of *B. napus* was not significantly affected by polluted Poldi soil. Its growth rate decreased slightly but it was comparable with the control (Fig. 1). On the other hand, the aboveground biomass of *S. bicolor* showed slower growth in polluted soil. Plants from 100% (Fig. 1) Poldi soil treatment showed retarded growth. Such morphological symptoms might be caused by stress. The excess of calcium, iron, and PAH might have slowed down the plant growth (Alkio et al., 2005; Marschner, 1995c).

Our analysis of chlorophyll amounts in shoots and morphology of roots supported the toxicity of soil toward *S. bicolor* plants. Chlorophyll a and b concentrations in sorghum shoots increased with an increase in pollutant levels (in SM Figs. 1 and 2). Chlorophyll levels in *B. napus* leaves were not significantly affected; their concentrations were comparable with those in control plants from non-polluted soil. *S. bicolor* plants were more sensitive to soil conditions, and the increase of chlorophyll *b* in plants indicated stress, whereas chlorophylls in *B. napus* leaves did not react to the stress conditions - plants were more resistant. These findings supported also chlorophyll *a* to b ratios. High Poldi soil content (50, and 100%) led to a decrease of ratio in *S. bicolor* plants; whereas no significant changes for *B. napus* plant were detected (Fig. 2). It is known that photosystem II electron transport is one of the most sensitive indicators of damage in the photosynthetic apparatus (Krause and Weis, 1991). Plant species show different sensitivity to the presence of PAHs in the environment. A negative correlation between phenanthrene accumulation and total chlorophyll in *Triticum aestivum* was reported (Shen et al., 2017). The damage of PSII by Flt has been reported as an increase in basal chlorophyll fluorescence in *Pisum sativum* leaves (Kummerová et al., 2006). It has been shown that PAH contamination results in adverse effects such as damage of photosynthetic function and acceleration of shoot senescence (Li et al., 2011). It is known that increases in the relative proportion of chlorophyll *b* to a under reduced illumination is an adaptive response to maximize the light harvesting capability of the chloroplasts (Dale and Causton, 1992). A decline in chlorophyll *a* to b ratio is due to more rapid degradation of the reaction center complexes than light-

Table 3
PAHs concentrations in Poldi soil; maximum permitted levels for hazardous substances in agricultural soil (CR, 1994), industrial areas, and other soils (CR, 2013); log K_{ow} indicates the potential of an organic compound to partition from water into lipids; log K_{oc} indicates a compound potential to bind to organic carbon in soil (Alagić et al., 2015).

PAHs	Log K_{ow}/log K_{oc}	Poldi soil c (mg/kg DW)	Limits c (mg/kg DW)		
			Agricultural soil	Industrial area	Other soil
Ace	3.98/3.66	32.34 ± 3.615	–	33,000	3400
Acy	4.07/1.40	57.20 ± 4.635	–	–	–
Ant	4.45/4.15	109.1 ± 2.671	0.01	170,000	17,000
BaA	5.61/5.30	339.4 ± 30.41	1	2.1	0.15
BaP	6.06/6.74	352.5 ± 28.58	0.1	0.21	0.015
BbF	6.04/5.74	188.3 ± 21.64	–	2.1	0.15
BkF	6.06/5.74	359.3 ± 8.539	–	21	1.5
BghiP	6.50/6.20	119.9 ± 45.04	–	–	–
DahA	6.84/6.52	110.9 ± 10.65	–	0.21	0.015
Flt	4.90/4.58	706.5 ± 47.78	0.1	22,000	2300
Flu	4.18/3.86	53.40 ± 1.039	–	22,000	2300
Chr	5.16/5.30	365.1 ± 9.247	0.01	210	15
IcdP	6.58/6.20	138.5 ± 7.989	–	2.1	0.15
Nap	3.36/2.97	16.56 ± 0.580	0.1	18	3.6
Phe	4.45/4.15	169.1 ± 11.86	0.1	–	–
Pyr	4.88/4.58	610.6 ± 69.52	–	17,000	1700

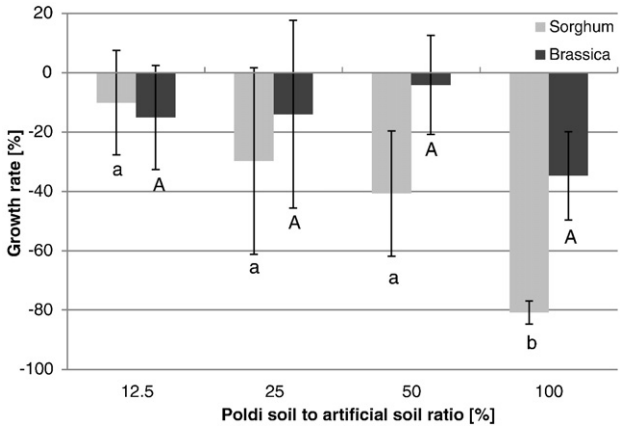


Fig. 1. Growth rate of *B. napus* and *S. bicolor* shoots after 4 weeks of growth in the contaminated soil mixed with sand in the ratio 0; 12.5; 25; 50; 100%.

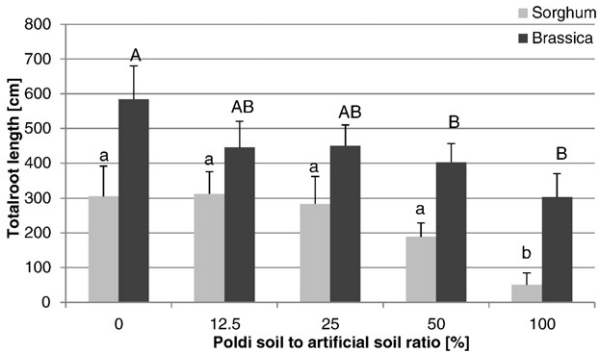


Fig. 3. Total root length of sorghum and rape plants after 4 weeks of growth in the contaminated soil mixed with sand in the ratio 0; 12.5; 25; 50; 100%.

harvesting chlorophyll *a* to b proteins of photosystem II and that the photosystem I reaction center disappears in parallel with the inactivation of photosynthesis (Kura-Hotta et al., 1987). Such changes obviously lead to a decrease in efficiency of energy transfer from light harvesting chlorophyll-protein complex (LHCs) to reaction centers of PSII. C4 plant sorghum have demonstrated that changing the light environment of mature leaves in the lower canopy not only affects the structure of newly developed leaves of the upper canopy, but also affects their photosynthetic function (Jiang et al., 2011). Different plant metabolisms (rape: C3, sorghum: C4) might play a role in the reduction of sorghum growth and increase in the chlorophyll levels. Theoretically, C4 plants should be more productive than C3 plants because there is no loss of carbon via photorespiration, which is an advantage in conditions with high temperature and water stress. However, carbon dioxide assimilation via C4 mechanism requires a greater input of energy. Moreover, a substantial portion of the total respiratory energy cost represents the ion influx across plasma membrane in root cells, which increases with increasing number of ions in the rhizosphere (Marschner, 1995a).

Root analysis supported the toxicity of soil. Both plants root systems showed a decreased growth with increasing levels of polluted soil in the pot, but again, there were differences between the two species. The non-diluted Poldi soil mainly affected sorghum roots. As mentioned above, sorghum plants did not grow well in polluted soil. Total root length and toot surface area of rape plants was less affected by pollution in comparison with sorghum plants (Figs. 3 and 4). On the other hand, total root volumes of both species were similar at the end of the experiment (in SM Fig. 3).

Generally, it is known that sorghum plants can grow in stress conditions. PAHs are taken up into the plants via the transpiration stream or by penetrating leaves, hydroxylated, and translocated together with the more hydrophilic pollutants to other plant tissues. They are then either volatilized, or excreted into the extracellular cell wall or the vacuole as glucosyl or glutathione conjugates (Harvey et al., 2002). Ecotoxicity test based on seedling growth showed that PAH toxicity was highly affected by plant species. PAHs had little impact on the shoot fresh weight or chlorophyll content of *B. napus* plants, but are known to markedly inhibit the root fresh weight (Ren et al., 1996). EC20 ranges from 37 to 140 mg/kg for *Trifolium pratense*, 300 to >1000 mg/kg for *Lolium perenne*, and, 77 to 650 mg/kg for *Sinapis alba* (Sverdrup et al., 2003). The lowest observed level of soil contamination with PAH significantly inhibiting (EC20) plant growth is about 20 mg/kg (*Lycopersicon esculentum*), while EC20 values for most other plants (*Tanacetum vulgare*, *Avena sativa* L., *Zea mays* L., *Phaseolus vulgaris* L., and *Helianthus annus* L.) exceeds 100 mg/kg (Maliszewska-Kordybach and Smreczak, 2000). Morphological symptoms of PAH-stress might appear as growth reduction in the root and shoot, chlorosis, trichome malformations, reduction in root hairs, late flowering, and the formation of white spots, which later turn into necrotic lesions (Alkio et al., 2005).

Sorghum spp. are able to take up Fe upon the release of phytosiderophores (PSs), which are organic compounds with a strong chelation affinity for Fe(III). The Fe-PS complex is then transported into root cells through a high affinity uptake system (Curie and Briat, 2003). Once it enters the cells, iron is compartmentalized for usage as well as avoidance of excessive accumulation, which can lead to cytotoxicity. The highest concentration of Fe is in chloroplasts, for photosynthetic purposes, and in mitochondria, for carrying out cellular respiration (Mimmo et al., 2014). While our results showed that chlorophyll levels change in sorghum shoots, it can be deduced that excess iron might cause stress to the plants. Rape shoots grew well and changes in chlorophyll levels were not significant. On the other hand, the growth of the root system of plants grown in non-diluted Poldi soil was stunted. The decrease in root biomass of both plant species could

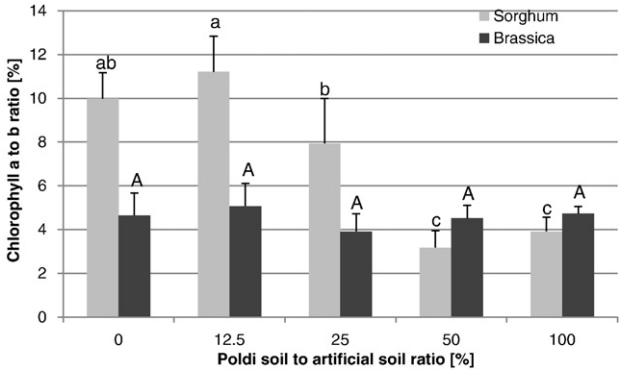


Fig. 2. Chlorophyll a to b ratio in fresh leaves of sorghum and rape plants after 4 weeks of growth in the contaminated soil mixed with sand in the ratio 0; 12.5; 25; 50; 100%.

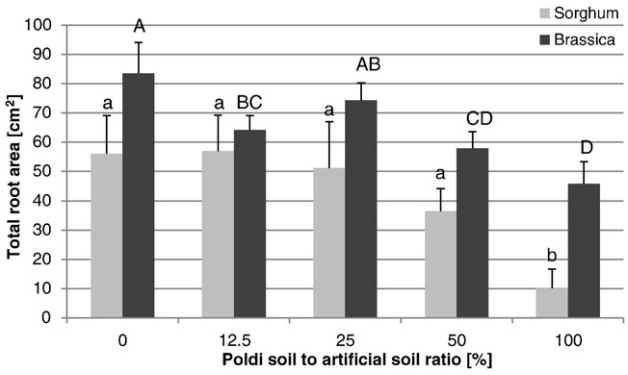


Fig. 4. Total root surface area of sorghum and rape plants after 4 weeks of growth in the contaminated soil mixed with sand in the ratio 0; 12.5; 25; 50; 100%.

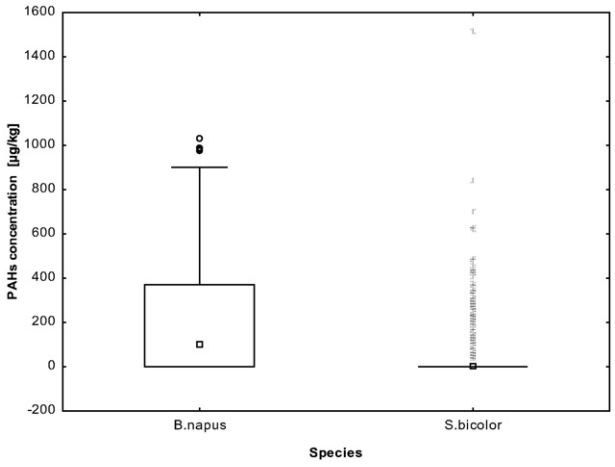


Fig. 5. Comparison of PAHs accumulations ranges in *B. napus* and *S. bicolor* shoots from all soil treatments. Factorial ANOVA, the median values with vertical columns 0.95 confidence interval ($p < 0.05$). The box plot data were visualized as median + 1.96 SE, where 1.96 is the 0.975 quantile of the normal distribution; * - extreme value; ° - outlier.

be caused by PAH accumulation. Current knowledge about PAH uptake by roots and shoots is ambiguous. Several studies have indicated that many higher plants take up only the PAHs with low molecular weights, whereas heavier PAHs have the tendency to sorb onto the surface of roots (Fismes et al., 2004; Gao and Ling, 2006; Oleszczuk and Baran, 2005).

3.3. Concentration of PAHs in plants

The variation in plant PAH concentrations was large, arising from growth differences between the species. Data of total PAHs concentrations in all soil treatments (Fig. 5) showed that values in *S. bicolor* plants were shifted toward lower values (the median moving down to the bottom quartile). *B. napus* plants grew well and accumulated quite high amounts of PAHs in their shoots. In general, the concentrations of PAHs in rape shoots increased with the proportion of Poldi soil in the mixture (in SM Table 1). The highest concentrations in the shoots (800 to 1000 µg/kg) were detected for BkF, Flt, and Pyr (in SM Fig. 4). Relatively high PAH concentrations (400 to 640 mg/kg) were recorded for BaA, BaP, BbF, BghiP, Chr, IcdP, and Nap (Fig. 6). Analysis of *S. bicolor* shoots confirmed that PAHs accumulated in the shoots, leading to even the reduction of shoot biomass, but the total PAH concentration was much lower compared with rape shoots (Fig. 5). The highest concentrations of PAHs were detected in the sorghum plants grown in 50% Poldi soil (in SM Table 2, and Fig. 5). Plants in 100% contaminated soil hardly survived, which might be the reason for lower PAHs concentrations in the shoots. On the other hand, some contaminants (BaA, BaP, BbF, Chr, Flt, IcdP, and Pyr) in *S. bicolor* shoots reached concentrations similar to *B. napus* shoot concentrations (above 400 µg/kg) (Fig. 7). It was notable that, even in the plants grown in unspiked control soil, accumulation of PAHs in the shoots was clearly detectable (In SM Tables 1 and 2). PAHs detected in control plants should result only from shoot uptake and accumulation from atmosphere, probably through the retention of vapor phase of PAHs on the waxy leaf cuticle (Trapp et al., 1990). Atmospheric accumulation mostly through the retention of vapor phase PAHs on the waxy leaf cuticle has been widely shown in previous studies (Gao and Zhu, 2004a; Gao and Zhu, 2004b; Trapp et al., 1990). Total average PAH concentration in the leaves of *Quercus ilex* L. has been reported as 297 µg/kg (De Nicola et al., 2015). Plants are capable of showing a physiological adaptation to soils with varying PAH contamination levels (26 to 364 mg/kg) in both laboratory and field conditions (Techer et al., 2012). Previous studies have indicated that PAHs enter plants via stomata in leaves, and then through cuticles into the epidermis to form a residue on the cell walls (Kvesitadze et al., 2009; Wild et al., 2006). Fundamental principles regarding the uptake of organic compounds by plant roots and the tendency of vascular plants for its metabolic degradation have been investigated in several studies (Fismes et al., 2002; Gao and Zhu, 2004a; Oleszczuk and Baran, 2005; Simonich and Hites, 1995). For instance, it has been shown that PAH uptake is positively correlated with the n-octanol/water partition coefficient (K_{ow}) (Paterson et al., 1990). Studies have indicated that only low and medium molecular weight PAHs are translocated to the aboveground plant parts (Kipopoulou et al., 1999; Trapp et al., 1990). High molecular weight PAHs are considered incapable of translocation

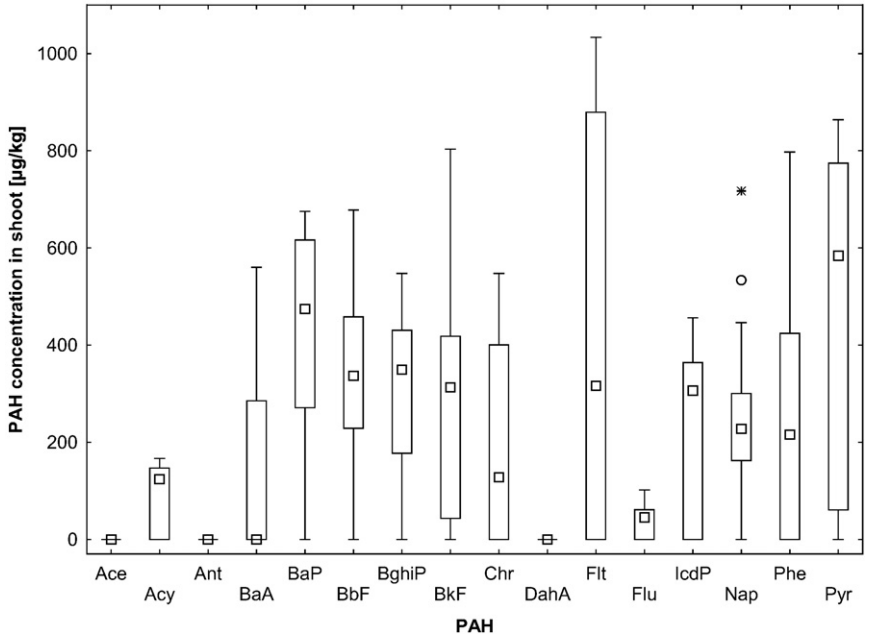


Fig. 6. PAH concentrations range in shoots of *B. napus* plants grown in the contaminated soil mixtures (data from ratios 0; 12.5; 25; 50; 100%); Factorial ANOVA, the median values with vertical columns 0.95 confidence interval ($p < 0.05$). The box plot data were visualized as median + 1.96 SE, where 1.96 is the 0.975 quantile of the normal distribution; * - extreme value; ° - outlier.

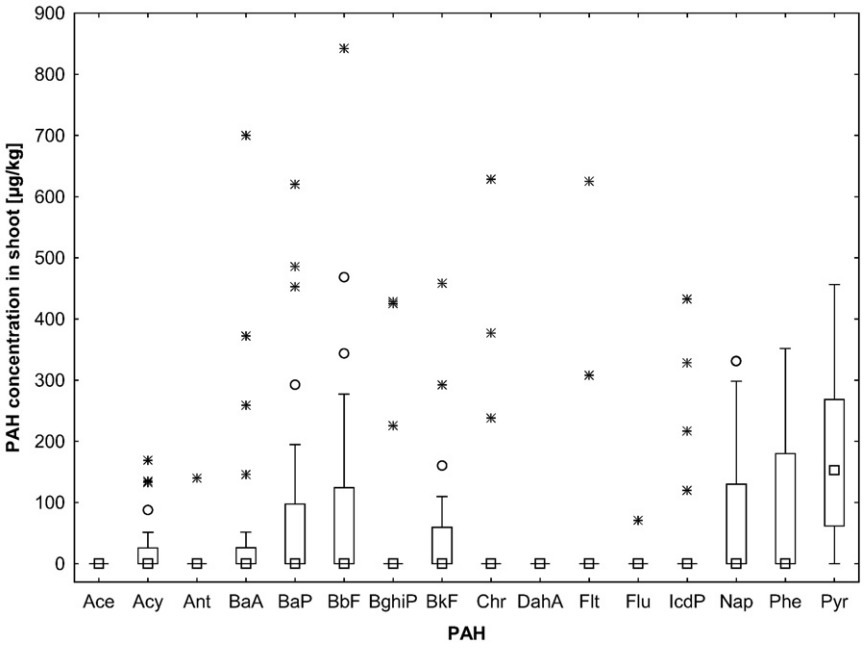


Fig. 7. PAH concentrations in shoots of *S. bicolor* plants grown in the contaminated soil mixtures (data from ratios 0; 12.5; 25; 50; 100%); Factorial ANOVA, the median values with vertical columns 0.95 confidence interval ($p < 0.05$). The box plot data were visualized as median + 1.96 SE, where 1.96 is the 0.975 quantile of the normal distribution; * - extreme value; ° - outlier.

to the aboveground plant parts because they are weakly water soluble with high values of Henry's constant and a K_{ow}, and they are strongly bound to the roots (Kang et al., 2010). Since uptake and translocation are two separate processes, compounds of high lipophilicity such as PAHs might be absorbed by the roots but their further movement might be limited owing to low water solubility. Thus, root tissue concentrations increase with increasing lipophilicity of the compounds (higher log[K_{ow}]). Translocation to aboveground plant tissues has been positively correlated with aqueous solubility, and is most efficient for compounds of intermediate polarity (Briggs et al., 1982). Moreover, several studies suggest that the root uptake of lipophilic organic compounds might be correlated with root composition such as lipid contents (Chiou et al., 2001; Simonich and Hites, 1995).

Shoot concentration factors (SCFs), defined as the ratio of PAH concentration in shoot and in soil, were calculated in relation to the dry weight (in SM Table 3). They tended to decrease with the increase in PAHs' soil concentrations but the values were negligible for phytoremediation purposes. SCF values reached a maximum 0.077 and 0.046 for Nap, and 0.066 and 0.021 for Phe (*B. napus* and *S. bicolor*, respectively) at the treatment with 12.5% Poldi soil. Values for other PAHs were applicable only for *B. napus* plants and were below 0.016. Low transfer factor values corresponded with the results of numerous studies. The shoot concentration factors for Phe and Pyr, with initial concentrations of 133 and 172 mg/kg, respectively, were in the range of 0.004 to 0.12 (Gao and Zhu, 2004a). Bioconcentration factor (ratio of PAH concentrations in plant and in soil) ranged from 0.001 to 0.4, although in the cases of extreme soil saturation, their values can reach up to 2 (Kipopoulou et al., 1999; Reichenauer and Germida, 2008; Tao et al., 2004). Even if the accumulation is low, studies have shown that plants can contribute significantly to the microbial degradation of PAHs (Binet et al., 2000; Somtrakoon et al., 2014). Root secondary metabolites (quercetin, apigenin, catechin, and gallic acid) are involved in the biostimulation of PAH-utilizing soil bacteria, leading to an enhancement in the bacterial growth and degradation activity (Techer et al., 2012). To facilitate the PAH removal from soil, combinations of phytoremediation with other in situ methods could be useful, including soil cultivation, fertilization, irrigation, composting, surfactant addition, and bioaugmentation (Alagić et al., 2015). The critical management decision for these combinations is the selection of plant species with an effective protective mechanism.

4. Conclusions

The present results indicated that organic contamination at the brownfield is very high. Average concentrations of organic pollutants in the soil were higher than the maximum permitted levels. Soil analysis showed that detected concentrations of BaA, BaP, Bbf, BkF, BkF, Chr, and IcdP exceeded the recommended limits for corresponding soil categories. Plant defense against these toxic pollutants can be realized through the transformation of parental PAH compounds into metabolites of lower toxicity as well as by limiting their movement through sequestration (compartmentation). Plant analysis confirmed that PAHs accumulated in the shoots of both plant species. Plants of *B. napus* grown on Poldi soil showed a better resistance to the toxicity of soil and their ability of PAH accumulation from the soil was evident. However, additional studies are needed to decide if the plants are usable for phytoremediation at this brownfield. Future monitoring of plant cultivation and soil remediation in situ may pave the way for sustainable phytoremediation with the production of non-food crops.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.04.163.

References

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123.
Alagić, S.Č., Maluckov, B.S., Radojičić, V.B., 2015. How can plants manage polycyclic aromatic hydrocarbons? May these effects represent a useful tool for an effective soil remediation? A review. Clean Techn. Environ. Policy 17, 597–614.
Alkio, M., Tabuchi, T.M., Wang, X., Colón-Carmona, A., 2005. Stress responses to polycyclic aromatic hydrocarbons in *Arabidopsis* include growth inhibition and hypersensitive response-like symptoms. J. Exp. Bot. 56, 2983–2994.
Babula, P., Vodicka, O., Adam, V., Kummerova, M., Havel, L., Hosek, J., et al., 2012. Effect of fluoranthene on plant cell model: tobacco BY-2 suspension culture. Environ. Exp. Bot. 78, 117–126.

Baker, A.J.M., McGrath, S.P., Sidoli, C.M.D., Reeves, R.D., 1994. The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour. Conserv. Recycl. 11, 41–49.

Bakker, M.I., Casado, B., Koerselman, J.W., Tolls, J., Kollöffel, C., 2000. Polycyclic aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery. Sci. Total Environ. 263, 91–100.

Binet, P., Portal, J.M., Leyval, C., 2000. Dissipation of 3–6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass. Soil Biol. Biochem. 32, 2011–2017.

Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic. Sci. 13, 495–504.

Cébron, A., Beguiristain, T., Faure, P., Norini, M.-P., Masfaraud, J.-F., Leyval, C., 2009. Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. Appl. Environ. Microbiol. 75, 6322–6330.

Chiou, C.T., Sheng, G., Manes, M., 2001. A partition-limited model for the plant uptake of organic contaminants from soil and water. Environ. Sci. Technol. 35, 1437–1444.

Coleman, J., Blake-Kalff, M., Davies, E., 1997. Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. Trends Plant Sci. 2, 144–151.

CR Mote, 1994. Indicators of Pollution. 3. Ministry of the Environment of the Czech Republic, Prague, Czech Republic.

CR Mote, 2013. Overview of Indicator Values Contamination of Soil, Air and Soil Groundwater. 3.

Curie, C., Briat, J.-F., 2003. Iron transport and signaling in plants. Annu. Rev. Plant Biol. 54, 183–206.

Dale, M.P., Causton, D.R., 1992. Use of the chlorophyll a/b ratio as a bioassay for the light environment of a plant. Funct. Ecol. 6, 190–196.

Davies, B.E., 1997. Heavy metal contaminated soils in an old industrial area of Wales, Great Britain: source identification through statistical data interpretation. Water Air Soil Pollut. 94, 85–98.

De Nicola, F., Baldantoni, D., Maisto, G., Alfani, A., 2015. Heavy metal and polycyclic aromatic hydrocarbon concentrations in *Quercus ilex* L. leaves fit an a priori subdivision in site typologies based on human management. Environ. Sci. Pollut. Res. 1–8.

Dickinson, N.M., 2000. Strategies for sustainable woodland on contaminated soils. Chemosphere 41, 259–263.

Drabova, L., Pulkrabova, J., Kalachova, K., Tomaniova, M., Kocourek, V., Hajslova, J., 2012. Rapid determination of polycyclic aromatic hydrocarbons (PAHs) in tea using two-dimensional gas chromatography coupled with time of flight mass spectrometry. Talanta 100, 207–216.

Duxbury, C.L., Dixon, D.G., Greenberg, B.M., 1997. Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*. Environ. Toxicol. Chem. 16, 1739–1748.

Fargašová, A., 2012. Plants as models for chromium and nickel risk assessment. Ecotoxicology 21, 1476–1483.

Fismes, J., Perrin-Ganier, C., Empereur-Bissonnet, P., Morel, J.L., 2002. Soil-to-root transfer and translocation of polycyclic aromatic hydrocarbons by vegetables grown on industrial contaminated soils. J. Environ. Qual. 31, 1649–1656.

Fismes, J., Schwartz, C., Perrin-Ganier, C., Morel, J.L., Charissou, A.-M., Jourdain, M.-J., 2004. Risk of contamination for edible vegetables growing on soils polluted by polycyclic aromatic hydrocarbons. Polycycl. Aromat. Compd. 24, 827–836.

French, C.J., Dickinson, N.M., Putwain, P.D., 2006. Woody biomass phytoremediation of contaminated brownfield land. Environ. Pollut. 141, 387–395.

Gajić, G., Djurdjević, L., Kostić, O., Jarić, S., Mitrović, M., Stevanović, B., et al., 2016. Assessment of the phytoremediation potential and an adaptive response of *Festuca rubra* L. sown on fly ash deposits: native grass has a pivotal role in ecorestoration management. Ecol. Eng. 93, 250–261.

Gallego, J.R., Esquinas, N., Rodríguez-Valdés, E., Menéndez-Aguado, J.M., Sierra, C., 2015. Comprehensive waste characterization and organic pollution co-occurrence in a Hg and As mining and metallurgy brownfield. J. Hazard. Mater. 300, 561–571.

Gao, Y., Ling, W., 2006. Comparison for plant uptake of phenanthrene and pyrene from soil and water. Biol. Fertil. Soils 42, 387–394.

Gao, Y., Zhu, L., 2004a. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55, 1169–1178.

Gao, Y.Z., Zhu, L.Z., 2004b. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55.

Harvey, P.J., Campanella, B.F., Castro, P.M.L., Harms, H., Lichtfouse, E., Schäffner, A.R., et al., 2002. Phytoremediation of polyaromatic hydrocarbons, anilines and phenols. Environ. Sci. Pollut. Res. 9, 29–47.

Hatzios, K.K., Penner, D., 1982. Metabolism of Herbicides in Higher Plants. CEPCO Division, Burgess Pub. Co.

Ho, Y.-N., Hsieh, J.-L., Huang, C.-C., 2013. Construction of a plant–microbe phytoremediation system: combination of vetiver grass with a functional endophytic bacterium, *Achromobacter xylosoxidans* F3B, for aromatic pollutants removal. Bioresour. Technol. 145, 43–47.

Huesemann, M.H., Hausmann, T.S., Fortman, T.J., Thom, R.M., Cullinan, V., 2009. In situ phytoremediation of PAH- and PCB-contaminated marine sediments with eelgrass (*Zostera marina*). Ecol. Eng. 35, 1395–1404.

International Organization for Standardization, 2012. Soil Quality - Determination of the Effects of Pollutants on Soil Flora - Part 2: Effects of Contaminated Soil on the Emergence and Early Growth of Higher Plants. EN ISO 11269-2. third ed.

Jiang, C.D., Wang, X., Gao, H.Y., Shi, L., Chow, W.S., 2011. Systemic regulation of leaf anatomical structure, photosynthetic performance, and high-light tolerance in sorghum. Plant Physiol. 155, 1416–1424.

Joner, E.J., Corgié, S.C., Amellal, N., Leyval, C., 2002. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. Soil Biol. Biochem. 34, 859–864.

Kang, F., Chen, D., Gao, Y., Zhang, Y., 2010. Distribution of polycyclic aromatic hydrocarbons in subcellular root tissues of ryegrass (*Lolium multiflorum* Lam.). BMC Plant Biol. 10, 210.

Kayal, S., Connell, D.W., 1995. Polycyclic aromatic hydrocarbons in biota from the Brisbane River Estuary, Australia. Estuar. Coast. Shelf Sci. 40, 475–493.

Kipopoulou, A.M., Manoli, E., Samara, C., 1999. Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environ. Pollut. 106, 369–380.

Kovacs, H., Szemmelveisz, K., 2017. Disposal options for polluted plants grown on heavy metal contaminated brownfield lands – a review. Chemosphere 166, 8–20.

Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349.

Kuhn, A., Ballach, H.-J., Wittig, R., 2004. Studies in the biodegradation of 5 PAHs (phenanthrene, pyrene, fluoranthene, chrysene und benzo(a)pyrene) in the presence of rooted poplar cuttings. Environ. Sci. Pollut. Res. 11, 22–32.

Kummerová, M., Krulová, J., Zezulka, Š., Tříska, J., 2006. Evaluation of fluoranthene phytotoxicity in pea plants by Hill reaction and chlorophyll fluorescence. Chemosphere 65, 489–496.

Kura-Hotta, M., Satoh, K., Katoh, S., 1987. Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol. 28, 1321–1329.

Kvesitadze, E., Sadunishvili, T., Kvesitadze, G., 2009. Mechanisms of organic contaminants uptake and degradation in plants. World Acad. Sci. Eng. Technol. 55, 458–468.

Leitgib, L., Kálmán, J., Gruiz, K., 2007. Comparison of bioassays by testing whole soil and their water extract from contaminated sites. Chemosphere 66, 428–434.

Li, J.H., Yu, X.Z., Wu, S.C., Wang, X.R., Wang, S.H., Tam, N.F.Y., et al., 2011. Responses of bioaugmented ryegrass to PAH soil contamination. International Journal of Phytoremediation 13, 441–455.

Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology vol. 148. Academic Press, pp. 350–382.

Liu, J., Liu, G., Zhang, J., Yin, H., Wang, R., 2012. Occurrence and risk assessment of polycyclic aromatic hydrocarbons in soil from the Tiefa coal mine district, Liaoning, China. J. Environ. Monit. 14, 2634–2642.

Lucena, C., Romera, F.J., Rojas, C.L., García, M.J., Alcántara, E., Pérez-Vicente, R., 2007. Bicarbonate blocks the expression of several genes involved in the physiological responses to Fe deficiency of Strategy I plants. Funct. Plant Biol. 34, 1002–1009.

Mackay, D., Shiu, W.Y., Ma, K.C., 1991. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals: Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins, and Dibenzofurans. Lewis Publishers.

Maila, M.P., Cloete, T.E., 2002. Germination of *Lepidium sativum* as a method to evaluate polycyclic aromatic hydrocarbons (PAHs) removal from contaminated soil. Int. Biodeterior. Biodegrad. 50, 107–113.

Maliszewska-Kordybach, B., 1996. Polycyclic aromatic hydrocarbons in agricultural soils in Poland: preliminary proposals for criteria to evaluate the level of soil contamination. Appl. Geochem. 11, 121–127.

Maliszewska-Kordybach, B., Smreczak, B., 2000. Ecotoxicological activity of soils polluted with polycyclic aromatic hydrocarbons (PAHs) - effect on plants. Environ. Technol. 21, 1099–1110.

Marschner, H., 1995a. 2 - ion uptake mechanisms of individual cells and roots: short-distance transport. Mineral Nutrition of Higher Plants, (second edition) Academic Press, London, pp. 6–78.

Marschner, H., 1995b. 8 - functions of mineral nutrients: macronutrients. Mineral Nutrition of Higher Plants. Academic Press, London, pp. 229–312 (second edition).

Marschner, H., 1995c. 12 - diagnosis of deficiency and toxicity of mineral nutrients. Mineral Nutrition of Higher Plants. Academic Press, London, pp. 461–479 (second edition).

Mimmo, T., Del Buono, D., Terzano, R., Tomasi, N., Vigani, G., Crecchio, C., et al., 2014. Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. Eur. J. Soil Sci. 65, 629–642.

Morillo, E., Romero, A.S., Maqueda, C., Madrid, L., Ajmone-Marsan, F., Grcman, H., et al., 2007. Soil pollution by PAHs in urban soils: a comparison of three European cities. J. Environ. Monit. 9, 1001–1008.

Muratova, A., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A., Hirche, F., et al., 2009. Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of *Sorghum bicolor* (L.) Moench. Environ. Exp. Bot. 66, 514–521.

Oleszczuk, P., Baran, S., 2005. Polycyclic aromatic hydrocarbons content in shoots and leaves of willow (*Salix viminalis*) cultivated on the sewage sludge-amended soil. Water Air Soil Pollut. 168, 91–111.

Ouvrard, S., Barnier, C., Bauda, P., Beguiristain, T., Biache, C., Bonnard, M., et al., 2011. In situ assessment of phytotechnologies for multicontaminated soil management. International Journal of Phytoremediation 13, 245–263.

Page, G.W., Berger, R.S., 2006. Characteristics and land use of contaminated brownfield properties in voluntary cleanup agreement programs. Land Use Policy 23, 551–559.

Panagos, P., Van Liedekerke, M., Yigini, Y., Montanarella, L., 2013. Contaminated sites in Europe: review of the current situation based on data collected through a European network. J. Environ. Public Health 2013, 11.

Paterson, S., Mackay, D., Tam, D., Shiu, W.Y., 1990. Uptake of organic chemicals by plants: a review of processes, correlations and models. Chemosphere 21, 297–331.

Pelaez, A.I., Lores, I., Sotres, A., Mendez-Garcia, C., Fernandez-Velarde, C., Santos, J.A., et al., 2013. Design and field-scale implementation of an "on site" bioremediation treatment in PAH-polluted soil. Environ. Pollut. 181, 190–199.

Pinedo, J., Ibáñez, R., Primo, Ó., Irabien, Á., 2012. Hydrocarbons analysis for risk assessment in polluted soils. Chem. Eng. Trans. 28, 79–84.

Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees—a review. Environ. Int. 29, 529–540.

Reichenauer, T.G., Germida, J.J., 2008. Phytoremediation of organic contaminants in soil and groundwater. ChemSusChem 1, 708–717.

Ren, L., Zeiler, L.F., Dixon, D.G., Greenberg, B.M., 1996. Photoinduced effects of polycyclic aromatic hydrocarbons on *Brassica napus* (canola) during germination and early seedling development. Ecotoxicol. Environ. Saf. 33, 73–80.

Sandermann Jr., H., 1992. Plant metabolism of xenobiotics. Trends Biochem. Sci. 17, 82–84.

Seckback, J., 1982. Ferreting out the secrets of plant ferritin - a review. J. Plant Nutr. 5, 369–394.

Shen, Y., Li, J., Gu, R., Yue, L., Zhan, X., Xing, B., 2017. Phenanthrene-triggered Chlorosis is caused by elevated Chlorophyll degradation and leaf moisture. Environ. Pollut. 220 (Part B), 1311–1321.
Shimabukuro, R.H., 1985. Detoxification of herbicides. In: S.O. D. (Ed.), Weed Physiology.
Shimabukuro, R.H., Lamoureux, G.L., Frear, D.S., 1982. Pesticide metabolism in plants reactions and mechanisms. In: Matsumura, F., Murti, C.R.K. (Eds.), Biodegradation of Pesticides. Springer US, Boston, MA, pp. 21–66.
Shukla, O.P., Juwarkar, A.A., Singh, S.K., Khan, S., Rai, U.N., 2011. Growth responses and metal accumulation capabilities of woody plants during the phytoremediation of tannery sludge. Waste Manag. 31, 115–123.
Simonich, S.L., Hites, R.A., 1995. Organic pollutant accumulation in vegetation. Environ. Sci. Technol. 29, 2905–2914.
Sinclair, S.A., Krämer, U., 2012. The zinc homeostasis network of land plants. Biochim. Biophys. Acta, Mol. Cell Res. 1823, 1553–1567.
Somtrakoon, K., Chouychai, W., Lee, H., 2014. Comparing anthracene and fluorene degradation in anthracene and fluorene-contaminated soil by single and mixed plant cultivation. Int. J. Phytoremediation 16, 415–428.
Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ. Chem. Lett. 5, 169–195.
Sverdrup, L.E., Krogh, P.H., Nielsen, T., Kjær, C., Stenersen, J., 2003. Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pratense*), ryegrass (*Lolium perenne*), and mustard (*Sinapsis alba*). Chemosphere 53, 993–1003.
Tao, S., Cui, Y.H., Xu, F.L., Li, B.G., Cao, J., Liu, W.X., et al., 2004. Polycyclic aromatic hydrocarbons (PAHs) in agricultural soil and vegetables from Tianjin. Sci. Total Environ. 320, 11–24.
Techer, D., Martinez-Chois, C., Laval-Gilly, P., Henry, S., Bennasroune, A., D'Innocenzo, M., et al., 2012. Assessment of *Miscanthus* × *giganteus* for rhizoremediation of long term PAH contaminated soils. Appl. Soil Ecol. 62, 42–49.
Thavamani, P., Megharaj, M., Krishnamurti, G.S.R., McFarland, R., Naidu, R., 2011. Finger printing of mixed contaminants from former manufactured gas plant (MGP) site soils: implications to bioremediation. Environ. Int. 37, 184–189.
Tolosa, I., de Mora, S.J., Fowler, S.W., Villeneuve, J.-P., Bartocci, J., Cattini, C., 2005. Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman. Mar. Pollut. Bull. 50, 1619–1633.
Trapp, S., Matthies, M., Scheunert, I., Topp, E.M., 1990. Modeling the bioconcentration of organic chemicals in plants. Environ. Sci. Technol. 24, 1246–1252.
Vangronsveld, J., Cunningham, S.D., 1998. Metal-contaminated Soils: In Situ Inactivation and Phytorestoration. Springer.
Vervaeke, P., Luyssaert, S., Mertens, J., Meers, E., Tack, F.M.G., Lust, N., 2003. Phytoremediation prospects of willow stands on contaminated sediment: a field trial. Environ. Pollut. 126, 275–282.
Wang, X., Sun, C., Gao, S., Wang, L., Shuokui, H., 2001. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chemosphere 44, 1711–1721.
Wang, X.-T., Miao, Y., Zhang, Y., Li, Y.-C., Wu, M.-H., Yu, G., 2013. Polycyclic aromatic hydrocarbons (PAHs) in urban soils of the megacity Shanghai: occurrence, source apportionment and potential human health risk. Sci. Total Environ. 447, 80–89.
Wild, S.R., Waterhouse, K.S., McGrath, S.P., Jones, K.C., 1990. Organic contaminants in an agricultural soil with a known history of sewage sludge amendments: polynuclear aromatic hydrocarbons. Environ. Sci. Technol. 24, 1706–1711.
Wild, E., Dent, J., Thomas, G.O., Jones, K.C., 2006. Visualizing the air-to-leaf transfer and within-leaf movement and distribution of phenanthrene: further studies utilizing two-photon excitation microscopy. Environ. Sci. Technol. 40, 907–916.
Zhu, Y., Hipel, K.W., Ke, G.Y., Chen, Y., 2015. Establishment and optimization of an evaluation index system for brownfield redevelopment projects: an empirical study. Environ. Model. Softw. 74, 173–182.