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Phytoremediation Using Willow in Industrial Contaminated Soil

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Abstract: In our previous work, we used *Salix viminalis* in the field to decontaminate agricultural soils containing cadmium. Our aim in the current study was to determine whether *S. viminalis* could decrease the levels of heavy metals, arsenic, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in industrial soil at a former workshop site. The site was planted with *S. viminalis* cuttings in July 2003. Soil samples were collected yearly from 2005 to 2015 and analysed for heavy metals, arsenic, PCBs and PAHs. The results showed that 21% of chromium, 30% of arsenic, 54% of cadmium, 61% of zinc, 62% of copper, 63% of lead, 87% of nickel, 53% of PCBs and up to 73% of PAHs were removed from the soil after 10 years of *S. viminalis* treatment. After just 1 year of *Salix* cultivation, a significant decrease was observed in most of the contaminants in the soil. The reduction in contaminants was linear at first but slowed down after a few years. The number of years prior to a slow-down in rate of removal differed between the contaminants. This study concludes that *S. viminalis* can be used for the phytoremediation of contaminated industrial soil and that the rate of decontamination differs between substances.

Keywords: arsenic; metals; PAH; PCB; phytoremediation; *Salix*

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1. Introduction

An enormous number of sites around the world are contaminated to a lesser or greater degree, in part because many industries have left sites with untreated contaminated soil after ending their activities. Such sites are very costly to remediate; a common technique involves excavating the contaminated soil, removing it, and placing it in a landfill, in the so-called 'dig and dump'. Other methods such as thermal and chemical remediation can also be applied. The remediation of sites with a high level of contamination is prioritized. However, many other sites with a medium or relatively low degree of contamination have been left untreated for decades. Thus, there is a need for a cheap and environmentally friendly method for remediating such sites, such as phytoremediation.

Although it takes a long time for phytoremediation to clean up a contaminated site, this method is outstanding from an economic perspective, especially when used for large areas [1,2]. Phytoremediation is defined as a method "using green plants to remove, contain or render environmental contaminants harmless" [3]. Only a few plant species or cultivars of a species have the ability to take up, stabilize or degrade certain contaminants, and thus be useful in phytoremediation [1,4]. Moreover, the plants used in phytoremediation should be domestic in order to ensure that they will thrive in a given climate and to avoid invasion by alien species.

Most polluted sites contain a mixture of pollutants. Inorganic pollutants—such as heavy metals and arsenic—and organics—including polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)—are common contaminants in many polluted soils. Specific plant species and varieties can be selected for the phytoremediation of various pollutants: some plants degrade organics well, while others show good performance in removing heavy metals [1,5]. Plants can degrade organic pollutants by exuding degrading enzymes into the soil, enabling degrading microbes to degrade organics in the rhizosphere, or taking up organics and transforming them within plant cells [6]. Plants can take up inorganics—such as metals and metalloids, which cannot be degraded—from the soil by their roots and accumulate them in plant tissue [7]. Plants may also stabilize contaminants in the soil by binding them to soil particles, thereby decreasing their availability and toxicity; however, the contaminants are not removed from the soil in such cases [8].

Willow, which is cultivated for bioenergy production, has been investigated as a possible phytoremediation crop since the 1990s [9–17]. This species has been shown to take up and accumulate large quantities of zinc (Zn) and cadmium (Cd) [18]; it also has high biomass production [19]. In comparison with species such as poplar, sunflower and tobacco, willow shows the highest uptake capacity in the field [4]. Some clones of willow (Salix pp.) used in short-rotation coppicing can extract more heavy metals from soil than other clones [9,10,12,20–22]. It has been shown that the soil concentration of Cd, Zn and copper (Cu) decreases after cultivation with *S. viminalis* [12,16] and *S. caprea* [23]. The ability of Salix clones to remove PAH from gas work soil has been shown to vary [24]. Vervaeke et al. [25] demonstrated mineral oil reduction and PAH degradation in contaminated sediment after 1.5 years of treatment with willow in the field. Faubert et al. [26] showed that *S. miyabeana* cultivation facilitated the migration of heavy metals, PAHs and PCBs towards the plant roots due to the plants' high transpiration rate.

In our earlier work, we found that *S. viminalis* decreased the total Cd concentration in agricultural soil by up to 25% after 4 years of Salix cultivation [16]. The aim of the present study was therefore to investigate whether *S. viminalis* could decrease the total levels of PCBs, PAHs, heavy metals and arsenic (As) in the contaminated soil left at a site on which a mechanics workshop was previously situated for many decades. However, our intention in this study was not to achieve certain limit values or to remediate the whole surface.

The decrease in each of the contaminants at the site was followed over a period of 10 years to determine whether the rate of removal varied among the contaminants. The concentrations of the contaminants in the soil were analysed each year in a reference location with no *S. viminalis* and in a location with *S. viminalis*. An unpolluted location was chosen as the reference location in order to analyse the rate of removal of the contaminants each year without the presence of *S. viminalis*. In addition, to analyse the decrease in contaminants without the presence of *S. viminalis*, three contaminated locations without *S. viminalis* were monitored, starting at the beginning of the study and continuing for 7 years after the end of the study.

2. Materials and Methods

2.1. The Site

This work was performed during the period 2002–2016 on a 2466 m^2 area of land on which a mechanics workshop was previously situated. The land has also been used for boat storage and the repair of leisure boats. The site is situated in Sunnersta, a village south of Uppsala in Sweden (59°47′14.2″ N, 17°39′39.4″ E). There was a building on the site (Figure 1), which was removed in 2003.

Prior to the plantation of *S. viminalis*, soil samples were collected during 2002 to gain knowledge of the contamination situation at the site. From these samples, a contamination map was drawn (Figure 1), which was used to plan the plantation. The analysis of the samples revealed elevated levels of heavy metals, arsenic, PAHs and PCBs at the site (Table 1). The contamination was not spread homogeneously; rather, it was accumulated in hotspots with varying concentrations (Figure 1; Table 1). High levels of PAHs and PCBs were found in Areas A and C, while high heavy metal concentrations were found in Areas A and B (Figure 1). The contamination was shallow, at <0.6 m depth. The soil was sandy with low water- and nutrient-holding capacity and a neutral pH of 7.2 ± 0.5 (SD).

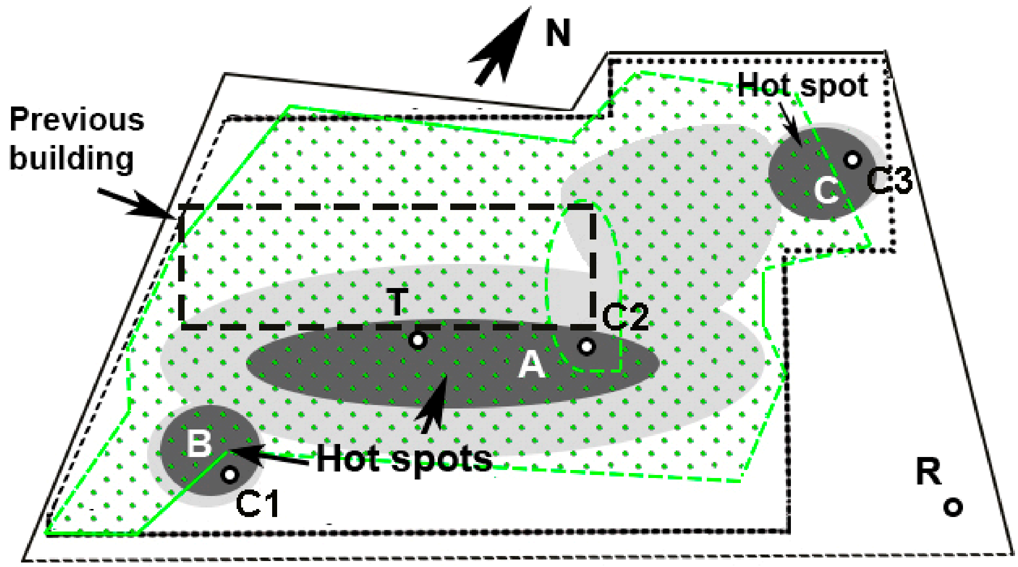

Figure 1. Map of the site. The black dashed line near the centre outlines the building location prior to its removal. The letters A, B and C indicate hotspot areas, where A and B contain high levels of heavy metals, and A and C contain high levels of PAH and PCB. The dark grey colour indicates high contamination, while the light grey colour indicates lower contamination. Sampling points: T, contaminated point; R, non-contaminated reference point; C1, C2 and C3, control points. The black dotted line delimits the area planted with *S. viminalis*; the green area shows the established *S. viminalis* plantation.

Table 1. Total concentration range of various contaminants in soil samples 30 cm deep collected in 2002, prior to phytoremediation, compared with guideline limits for sensitive land use (KM) and less sensitive land use (MKM) according to the Swedish Environmental Protection Agency (EPA).

Site	Pb	Cd	Cu	As	PAH	PCB
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Total conc. range	2.3–2600	<0.2–4.5	3.7–290	<1.9–8.9	<0.3–150	<0.01–0.05
KM	50	0.8	80	10	1.0	0.008
MKM	400	12	200	25	10	0.2

2.2. Plant Materials

Two clones of *S. viminalis* were used for phytoremediation. Clone 78,198 has been previously shown to remove metals from the soil well [16], while clone 78,183 has exhibited a high level of removal of PAHs [24]. Cuttings comprising 20 cm-long pieces of 1-year-old stems of the two clones were collected from a nearby *S. viminalis* cultivation site and used for planting.

2.3. Plantation and Cultivation

The surface was prepared in 2003 by harrowing before planting. The hotspot areas were then manually planted with nine cuttings per square metre. Clone 78,198 was planted in the hotspot areas A and B, while clone 78,183 was planted in the hotspot areas A and C (Figure 1). Thus, a mixture of the clones was planted in Area A.

Planting was performed at the end of June 2003. Unfortunately, July was very dry that year, causing 50% of the cuttings to die in 2003. Therefore, the site was replanted with cuttings in May 2004. Soil sample collection started in 2005, once the plants had been established. Even then, some of the *S. viminalis* cuttings did not survive, for unknown reasons, leaving some areas within the *S. viminalis* plantation site uncovered (Figure 1).

During the first 3 years—and later, when necessary—plants were irrigated with tap water due to the low water-holding capacity of the soil. Long-acting granules of complete organic fertilizer (Algomin, Algomin AB) were used to fertilize yearly at a ratio of 30 kg N ha^{-1}. Until the *S. viminalis* cuttings grew to be large, weeding was performed by hand.

2.4. Sample Collection

Once the *S. viminalis* cultivation had been established, soil sampling was performed in November each year, from 2005 to 2015. Sampling was performed at two locations: one at Area A (marked *T* in Figure 1), which had a high level of contamination and most of the contaminants, and was planted with *S. viminalis*; the other location was a less contaminated reference site (marked *R* in Figure 1) outside of Areas A, B and C, and it was not planted with *S. viminalis*. At each of the two sampling points, six samples with a 1 m diameter were taken with a garden shovel at a depth of 25–30 cm. The six samples were pooled together to make a single pooled sample. To analyse the decrease in the contaminants in the contaminated soil with no *S. viminalis*, samples were taken at three control sampling sites (C1, C2, C3) within the polluted sites and were sampled in 2005 and 2022 (Figure 1).

The stem biomass at the site in the year 2015 was estimated to be 10 tons per ha. This value was calculated based on the number of plants per square metre, the stem thickness, the number of stems per plant, including branches, and the dry biomass density (550 kg/m^3). The root biomass was estimated to be about 30% (not measured) of the stem biomass, according to Telenius [27].

2.5. Analysed Contaminants

The following contaminants were analysed:

- Metals and metalloids: Arsenic, cadmium, chromium, copper, lead, nickel and zinc.
- PAHs: Naphthalene, acenaphthene, acenaphthylene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(k)fluoranthene, benzo(g,h,i)perylene, benzo(a)pyrene, indenol(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene (the last six of these are carcinogenic).
- PCBs: PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180.

Analyses of the total concentrations of the various contaminants were performed by the accredited laboratory ALS Scandinavia AB.

2.6. Calculations and Statistics

Each year, six soil samples were taken at each of the two locations (*T* and *R*), and the six samples were pooled into one sample. The first samples taken in 2005 at the two locations were used as reference material each year and were analysed at the same time as the yearly collected samples for a better comparison.

When testing whether the contaminant concentration in the soil changed over time, a standard linear regression was applied for all compounds (using the linear model $y = \beta 0 + \beta 1 * x$). Thus, the regression is based on a total of 11 data points, one for each year (i.e., one soil sample per year of the experiment). The significance of the change over time in years, as the independent predictor, was determined by testing whether the coefficient $\beta 1$ (i.e., the slope) differed from zero. Significant levels were set at $p < 0.05$. The statistical packages used were SPSS 21 and R. A one-sample t-test was used to test if the contaminant removal differed significantly between the soils cultivated with *S. viminalis* and the control soils without *S. viminalis*.

3. Results and Discussion

The total concentrations of the various contaminants in the soil before and after treatment with or without *S. viminalis* are shown in Table 2. After 10 years, all contaminants in the soil decreased significantly in the location planted with *S. viminalis* (Table 2). In contrast, the reference spot with low contamination and no *S. viminalis* showed no significant changes ($p > 0.05$) in the total concentrations of any of the contaminants analysed. Furthermore, the concentrations of the contaminants in the contaminated locations in the absence of *S. viminalis* did not change significantly in the period 2005–2022 (Table 2). These findings show that *S. viminalis* influenced the decrease in the contaminants in the soil.

During the 10 years of treatment with *S. viminalis*, the total concentrations of the various metals and metalloids in the soil decreased by 21–87% (Table 2). The percentage of each contaminant that was removed after 10 years of treatment was as follows: Cr, 21%; As, 30%; Cd, 54%; Zn, 61%; Cu, 62%; Pb, 63%; and Ni, 87%. The lowest decrease was found for As and chromium (Cr). These elements are commonly found in complexes with oxygen; in the available soil fractions, they occurred in the forms of arsenate and chromate anions, which can be taken up by plants. A greater decrease was found for Cd, Zn, Cu and lead (Pb), all of which were removed to a similar extent by the plants. Nickel (Ni) was removed to an even greater extent from the soil by *S. viminalis*. In the mobile phase, these elements exist in cationic forms, which can be taken up by the plant. Thus, the data indicate that it may be more difficult to remove anions than cations from the soil with *S. viminalis*.

No good reason has been found to explain the low removal of Cr and As, in comparison with the other metals. However, one possible explanation is that, at high pH, Cr and As anions have higher mobility in the soil solution than at lower pH, while the opposite holds true for cations. The plant root exudate may influence the soil pH and thereby affect the availability of metals to plants in the rhizosphere soil [28,29]. *S. viminalis* may have acidified the soil and thus increased the solubility of the metal cations [30], while simultaneously precipitating the Cr and As anions [31,32].

Although this was not the focus of this work, the decrease in the total concentration of the metals caused the concentrations to reach under the limit values (Table 1) in some cases. For example, the concentration of Cu decreased from 294 to 111 mg kg^{-1} during 10 years of treatment with *S. viminalis* (Table 2), and reached the less sensitive land-use limit value (see Table 1) after just 2 years.

The analysed organic substances also decreased in the soil under *S. viminalis* cultivation (Table 2). During the 10 years of treatment, PAHs decreased by 20–73%, depending on the substance. It has been shown that PAHs with more rings and more complex ring structures are less able to degrade and be removed from the soil by plants [33]. However, in this work, 47% of a PAH with two aromatic rings—namely, naphthalene—was removed. Even more of phenanthrene—a PAH with a three-ring structure—was removed, at 73%. Regarding PAHs with four rings—namely, chrysene and pyrene—25% and 54% were removed, respectively (Table 2, Figure 2). In comparison with chrysene, pyrene has a more complex structure with more bindings to carbon (Figure 2). Thus, it could be expected that pyrene would be more difficult to degrade and remove than chrysene; however, that was not the case in this study.

The sum of the PCBs decreased by 53% (Table 2). PCBs consist of two aromatic rings; unlike the PAH naphthalene, the rings are connected with just one C–C binding (Figure 2). Therefore, based on C-bindings alone, PCBs ought to be easier to degrade than naphthalene. However, no pattern was observed in terms of the differences in the removal of the two substances. On the other hand, PCBs differ from PAHs in terms of their chloride content, with various PCB congeners having different numbers of chloride and chloride binding sites (Figure 2). Chlorine atoms bind to carbon with bindings that have only a few natural degraders [34]. Nevertheless, this study showed that chloride-containing molecules can be removed from soil (Table 2). Based on the data collected in this study, it is not possible to know whether the PAHs or PCBs were degraded or taken up by the plants, as such information lies beyond the scope of this research. The data show no signs of transformation between different types of PCBs or PAHs.

Table 2. Concentration of various contaminants in the soil at the start (2005) and after 10 years (2015) of treatment without (reference location) or with *S. viminalis* cultivation, and at the start (2005) and after 17 years (2022) in contaminated control locations (C1–3) not planted with *S. viminalis*, along with the percentage of each substance and element removed.

Contaminants	Reference Location			Location with *S. viminalis*			Control Location, C1			Control Location, C2			Control Location, C3		
	2005	2015	Removal	2005	2015	Removal	2005	2022	Removal	2005	2022	Removal	2005	2022	Removal
	(mg kg^{-1})		(%)	(mg kg^{-1})		(%)	(mg kg^{-1})		(%)	(mg kg^{-1})		(%)	(mg kg^{-1})		(%)
Metals and metalloids															
Cr	0.25	0.26	0	9.0	7.1	21.1 **	7.96	8.23	0	7.72	7.54	2.3	5.68	6.11	0
As	0.15	0.15	0	5.3	3.7	30.2 **	1.28	1.21	5.4	3.77	3.63	3.7	1.84	1.97	0
Cd	0.13	0.13	0	4.4	2.1	54.5 ***	0.76	0.74	2.6	0.38	0.39	0	0.34	0.35	0
Zn	52	52	0	64	25	60.9 ***	62	60	3.2	60	58	3.3	50	49	2
Cu	32	33	0	294	111	62.2 ***	4.3	4.4	0	—	2.1	0	3.7	3.5	5.4
Pb	25	24	0	2350	879	62.6 ***	130	132	0	42	44.5	0	56	55.2	1.4
Ni	5.5	5.5	0	15.3	2.0	86.9 ***	11.9	12.3	0	11.3	10.7	5.3	9.61	9.73	0
PAHs															
Chrysene	0.09	0.09	0	0.36	0.27	25.0 *	0.32	0.31	3.1	0.24	0.22	8.3	0.41	0.41	0
Sum of carcinogenic PAHs	0.31	0.31	0	0.96	0.77	19.8	0.83	0.79	4.8	0.79	0.77	2.5	1.25	1.29	0
Naphthalene	0.25	0.24	0	0.92	0.49	46.7 ***	0.30	0.31	0	0.45	0.47	0	0.91	0.89	2.2
Phenanthrene	0.08	0.08	0	0.37	0.10	73.0 ***	—	—	—	—	—	—	—	—	—
Pyrene	0.18	0.18	0	0.77	0.32	54.3 ***	0.31	0.29	6.5	0.55	0.54	1.8	0.75	0.76	0
Sum of other PAHs	0.86	0.82	0	2.67	1.73	35.2 ***	1.20	1.22	0	1.79	1.83	0	3.28	3.32	0
PCBs															
PCB 153	—	—		0.01	0.009 ^{Y}	10.0 ^{Y,***}	—	—	—	—	—	—	—	—	—
PCB 180	—	—		0.01	0.008 ^{Y}	20.0 ^{Y,*}	—	—	—	—	—	—	—	—	—
Sum of PCBs	—	—		0.03	0.014	53.3 ***	0.03	0.03	0	0.03	0.03	0	0.03	0.03	0

Benzoanthracene, benzofluoranthene, benzopyrene, indenolpyrene, dibenzoanthracene, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene and benzoperylene were analyzed, but the data were under the detection limit. ^{Y} Year 2011. Significance levels for contaminant removal during the time: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. — indicates a value under the detection limit.

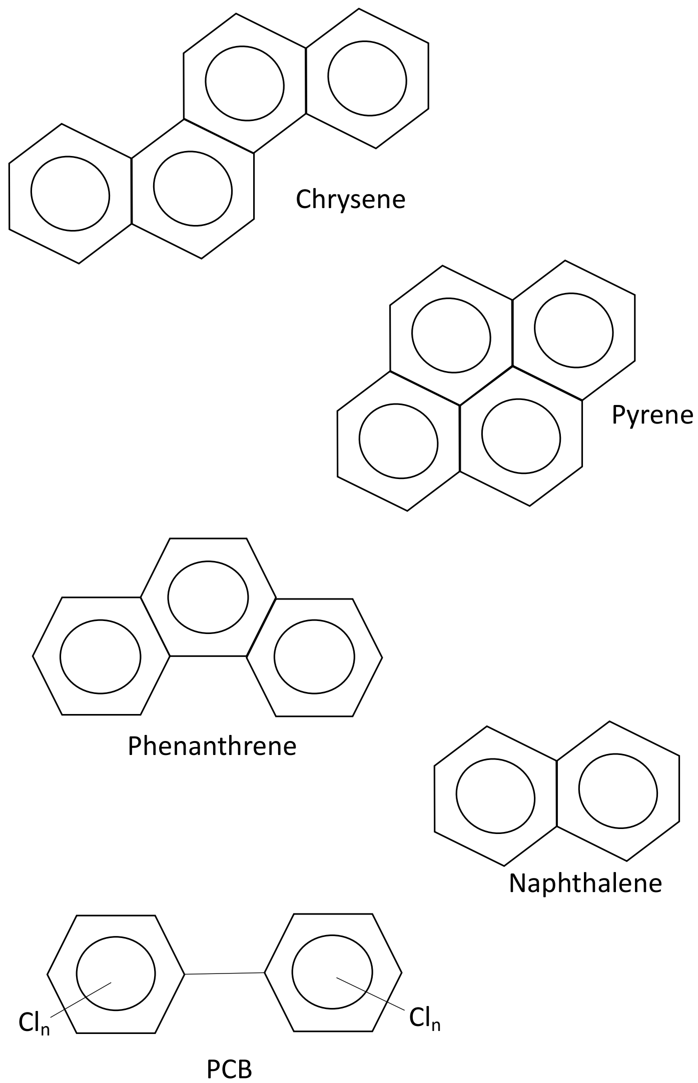

Figure 2. Basic structures of the PAHs and PCBs analysed in this study.

The rate of removal differed among the contaminants. Most of the elements showed a decrease in their removal, with a plateau in the curve after 5 years of treatment with *S. viminalis* (Figure 3). However, Cr was an exception; even after 10 years, its concentration in the soil seemed to decrease at the same rate. Regarding the organics (Figure 4), no plateau in the curve was observed for the sum of PCBs; however, specific substances such as PCB-153 and PCB-180 did exhibit a plateau after 2 and 4 years, respectively. Among some of the PAHs (i.e., naphthalene, phenanthrene and pyrene; Figure 4), there was a slight decrease in the removal after 5 years of *S. viminalis* cultivation. The removal of chrysene, the sum of the carcinogenic PAHs and the other PAHs decreased drastically after 2 years. This decrease in the removal after a couple of years may be caused by root growth and by roots interfering with each other during uptake, the bioremediation rate of bacteria decreasing, the contaminant reaching a depletion zone of removal, or biomass growth slowing down due to the depletion of nutrients.

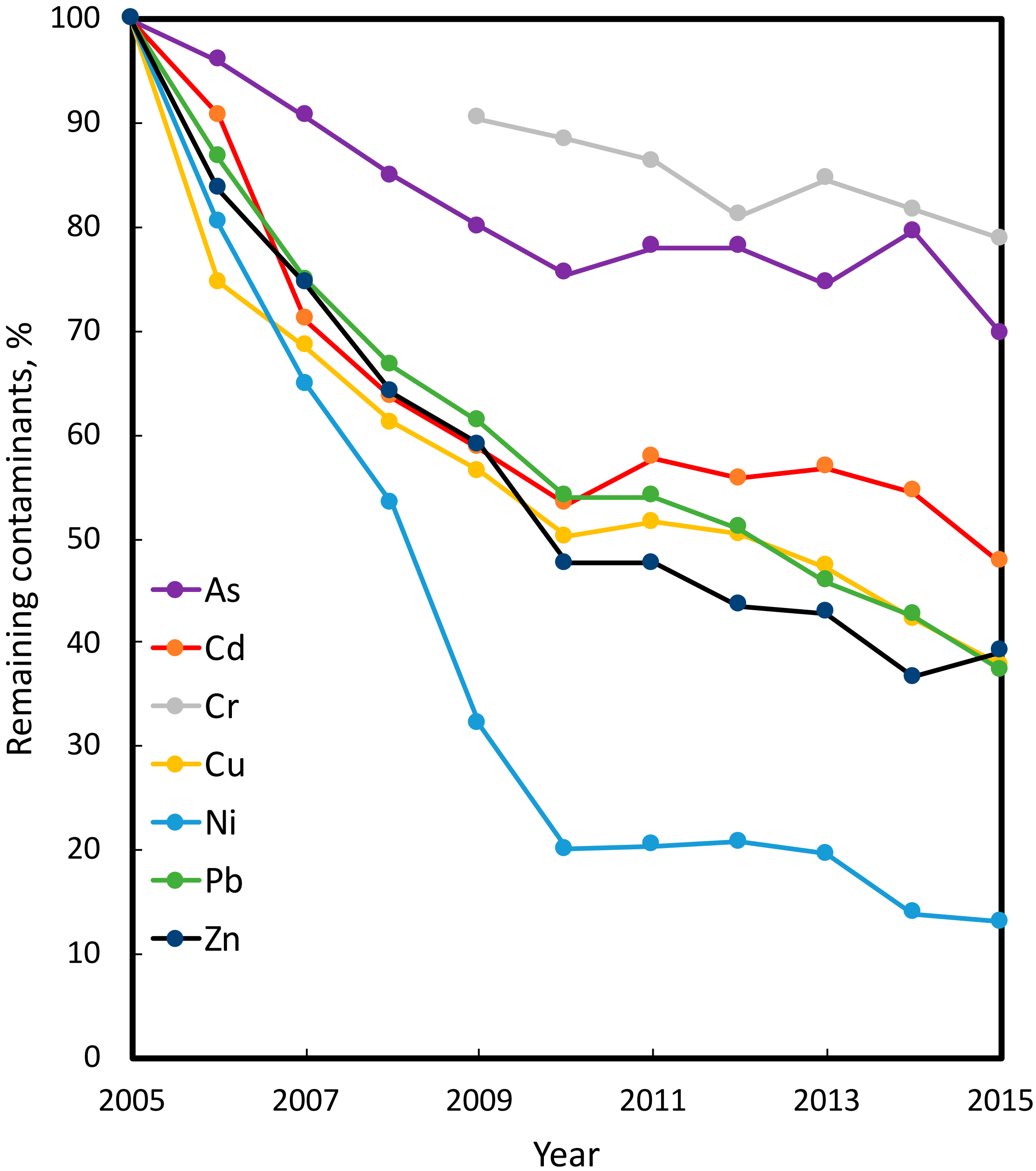

Figure 3. Remaining percentage of heavy metals and arsenic each year in the soil at the contaminated location, with *S. viminalis* cultivation.

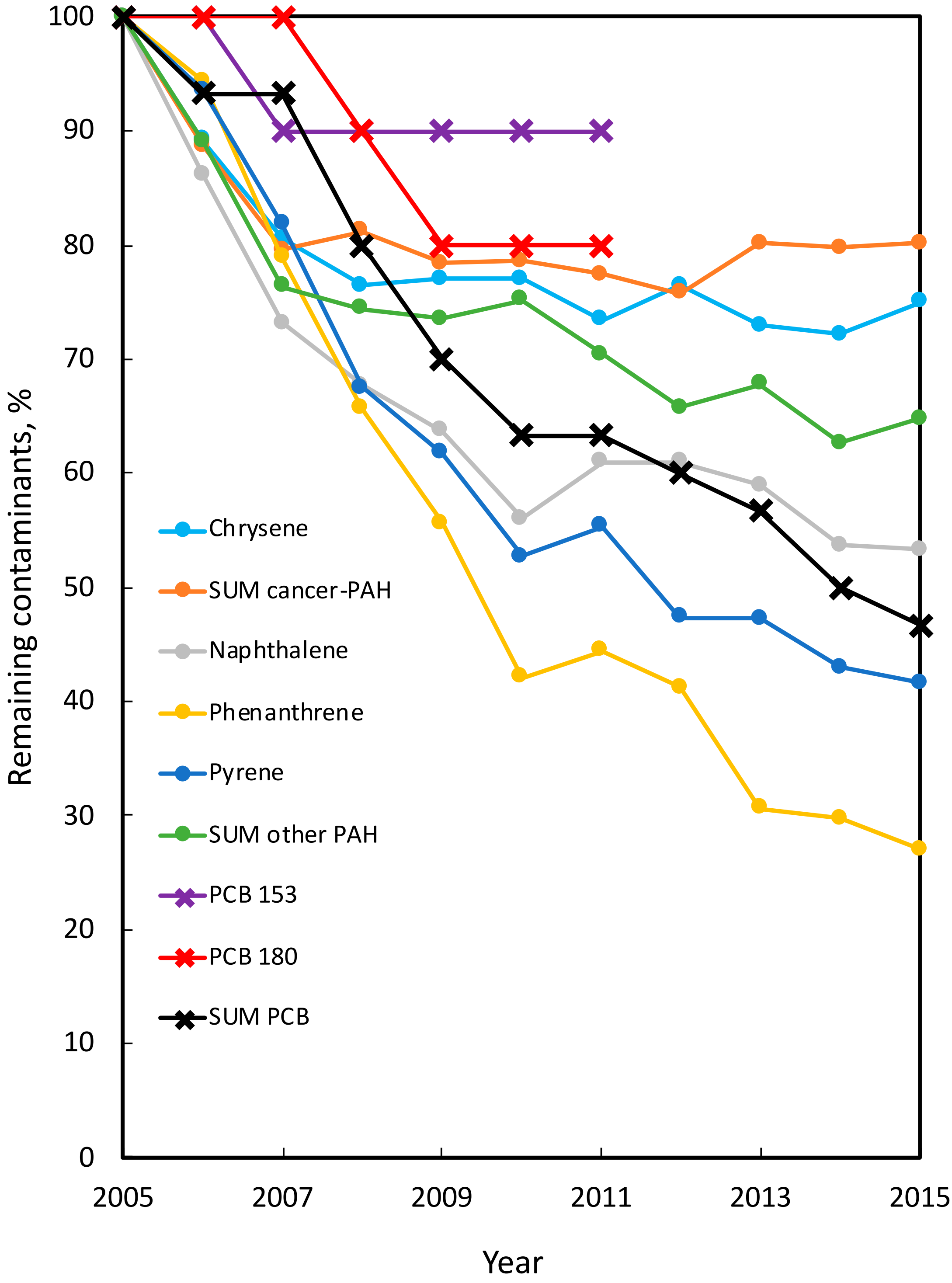

Figure 4. Remaining percentages of PAHs and PCBs each year in soil at the contaminated location, with *S. viminalis* cultivation.

4. Conclusions

This work shows that *S. viminalis* can be used for the phytoremediation of contaminated soil at industrial sites and can remove metals, metalloids and organic pollutants from the soil. It also shows that the capacity, rate of removal and time for removal varies between contaminants. Therefore, when using *S. viminalis* for phytoremediation, it may be possible to increase the removal of some contaminants by increasing the time of phytoremediation; however, doing so is impossible for other contaminants, whose removal ends in an earlier phase.

Author Contributions: Both authors designed the research. The data was collected by T.L. Analysis of data was performed by both M.G. and T.L. The paper was written by both M.G. and T.L. All authors have read and agreed to the published version of the manuscript.

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