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RESEARCH ARTICLE

Enhanced bioremediation of BTEX contaminated groundwater in pot-scale wetlands

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Abstract Pot-scale wetlands were used to investigate the role of plants in enhancing the performance of engineered bioremediation techniques like biostimulation, bioaugmentation, and phytoremediation collectively. *Canna generalis* plants were grown hydroponically in BTEX contaminated groundwater supplied in wetland mesocosms. To quantify the contaminant uptake by the plants, wetlands with and without shoot biomass along with unplanted gravel bed were used under controlled conditions. The residual concentration of the selected BTEX compound, toluene, in the rhizosphere water was measured over the entire period of the experiment along with the water lost by evapotranspiration. The rate of biodegradation in all wetland mesocosms fitted best with the first-order kinetics. The total removal time of the BTEX compound was found to be highest in the unplanted gravel bed mesocosm followed by wetlands without and with shoot biomass. The cumulative uptake of toluene in shoot biomass of the wetland plants initially increased rapidly and started to decrease subsequently till it reached a peak value. Continuity equations integrated with biodegradation and plant uptake sink terms were developed to simulate residual concentration of toluene in rhizospheric water for comparison with the measured data for entire period of the experiments. The results of this research can be used to frame in situ plant-assisted bioremediation techniques for hydrocarbon-contaminated soil-water resources.

Keywords BTEX · Plant uptake · Pot-scale wetland · Plant-assisted bioremediation

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Introduction

Hydrocarbons like BTEX compounds entering the soil-water system through anthropogenic activities can be long lasting sources of groundwater pollution to the down-gradient receptors. Owing to the toxicity of these compounds, it is essential to look for remediation options that are environmentally benign. Various physical, chemical, and biological methods are used to achieve remediation of BTEX at spill sites (Farhadian et al. 2008). A large quantity of pure phase BTEX spills can be removed by methods like physical containment, booming and skimming, pump and treat, and water flushing (Alvarez and Illman 2006; Zhu et al. 2004). The complete removal typically involves methods like hot water application, air sparging, soil vapor extraction, and in situ burning which can lead to destruction of indigenous biota and air pollution. The removal of BTEX from soil-water systems can also be done by chemical methods like use of dispersants, chemical oxidation, photo catalysis, etc. (Mascolo et al. 2007). However, the use of these co-solvents has its disadvantage with respect to its efficacy and toxicity concern over a long period of time (USEPA 1998). The other promising treatment options are through biological processes like bioremediation, phytoremediation, and treatment wetlands (Langwaldt and Puhakka 2000; Wallace and Kadlec 2005; Yadav et al. 2013; Mathur and Yadav 2009). Bioremediation is a promising cost effective technique

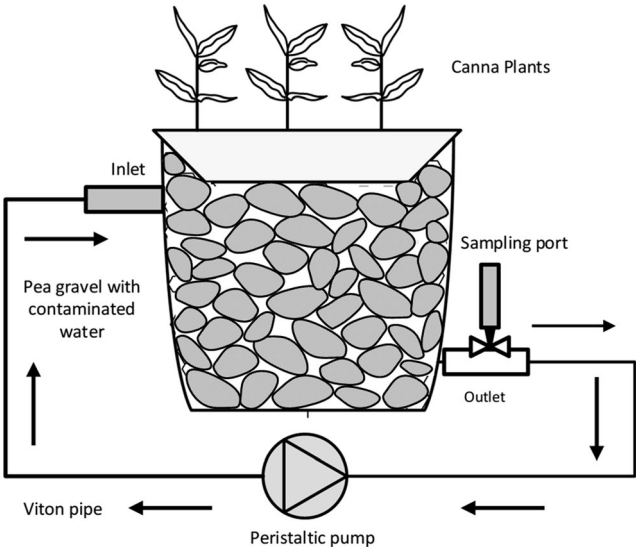

Fig. 1 Schematic diagram of pot-scale wetland mesocosm grown with *Canna generalis* used for investigating the plant assisted bioremediation

causing no harm to the contaminated ecosystem as compared to the traditional chemical and physical methods (Yang et al. 2009; Mercer and Trevors 2011). Various bioremediation techniques have been developed to clean up residual BTEX from polluted soils, marine shorelines, and surface and groundwater systems under a broad range of environmental conditions (Yadav and Hassanizadeh 2011).

BTEX compounds get biodegraded slowly in their aqueous phase by naturally occurring microorganisms (Newell et al. 1995), but the process of their attenuation is quite slow under prevailing environmental conditions. So, in order to enhance the degradation rate, engineered bioremediation is practiced using additives to the polluted environment. This involves the addition of seeded cultures and/or nutrients, popularly known as bioaugmentation and biostimulation. Another key role in the success of bioremediation is played by various site-specific environmental conditions like temperature, moisture content, and oxygen availability. The use of plants may provide a suitable environment (Dzantor 2007) by maintaining optimum conditions favorable for metabolism of microorganisms, subsequently enhancing the rate of biodegradation in the

Table 1 Plant growth characteristics in the wetland mesocosms with and without shoot biomass

Plant characteristics	Values	Units
Wet weight of shoots and leaves	149	g
Dry weight of shoots and leaves	23	g
Total amount of water lost	200	mL
Water lost in evapotranspiration	2.77	m L/h
Wet weight of root biomass	500	g
Dry weight of root biomass	279	g
Water lost in evaporation	1.66	mL/h

contaminated root zone. Paterson et al. (1990), Shimp et al. (1993), Simonich and Hites (1995), Watanabe (1997), and Chang and Corapcioglu (1998) documented various processes involved in phytoremediation for restoring contaminated soil-water systems. The phytoremediation of organic contaminants occurs directly via root uptake and subsequent translocation to shoot biomass, metabolism in biomass (phytodegradation), and indirect attenuation through interactions between the contaminant and root zone termed as rhizodegradation (Dzantor 2007). The rhizospheric zone has been reported as an apt location having significantly large numbers of pollutant degraders than unplanted soils (Yadav et al. 2011). Plant root exudates consisting of a complex mixture of organic acids, sugars, vitamins, purines, nucleosides, and inorganic ions (Dakora and Phillips 2002) act as supplemental substrates which stimulate the microbial activities in the root zone. Further, plants can take up organic compounds from soil-water into their tissues and increase oxygen transfer in the root zone. A phytoremediation technique that combines the remediation potential of plants and their associated microorganisms is the treatment wetlands used for petroleum hydrocarbon removal (Wemple and Hendricks 2000; Ji et al. 2002; Omari et al. 2003; Gessner et al. 2005; Salmon et al. 1998).

A faster biodegradation rate may be achieved by integrating biostimulation and bioaugmentation techniques of engineered bioremediation with phytoremediation in treatment wetlands. Yadav et al. (2013) termed this as 'plant-

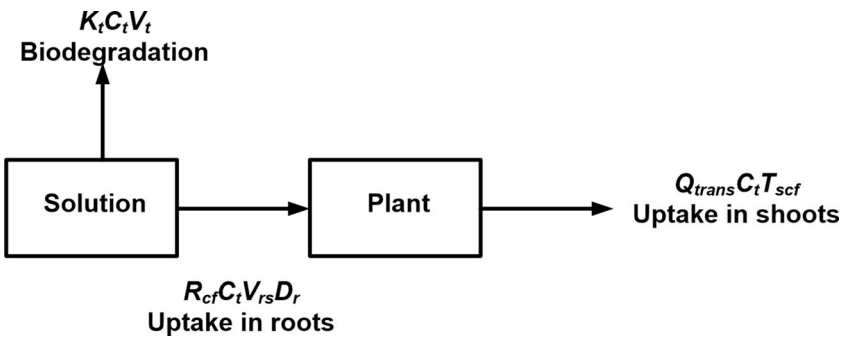

Fig. 2 Block diagram showing all the processes considered for simulating the toluene uptake in wetland mesocosms

Table 2 Mass balance of toluene in wetland mesocosm with shoot biomass

Time (h)	Mass of toluene in root biomass (mg)	Mass of toluene biodegraded (mg)	Mass of residual toluene (mg)	Mass of toluene in shoot biomass (mg)	Mass error (%)
8	54.66	184.92	228.87	4.95	1.04
24	30.06	343.63	91.37	8.34	1.76
32	50.32	362.06	50.32	10.69	2.25
40	27.74	386.37	45.04	14.25	3.01
48	3.62	412.55	40.65	16.57	3.5
65	2.22	426.99	22.84	21.3	4.5
72	1.208	427.16	21.32	23.71	5.0

assisted bioremediation' and studied the role of plants in engineered bioremediation in nutrients supply, electron acceptors and microorganisms in a symbiotic way by conducting completely mixed batch experiments. However, quantification of BTEX uptake by root and shoot biomass with temporal attenuation of the pollutant in rhizospheric water in treatment wetlands was not investigated in their findings. Thus, the main focus of this study is to investigate the major role of plants in enhancing engineered bioremediation using wetland mesocosms.

Materials and methods

Contaminated groundwater was collected from shallow hand pumps near a petroleum refinery in India. In order to remove background concentration of dissolved volatile organic compounds (VOCs), the collected groundwater was left open overnight under a ventilation hood before storing it at 4 °C. Three laboratory-scale simulating wetlands, termed hereafter as mesocosms, were fabricated using viton-coated PVC containers of 28 cm inner diameter with 30 cm height having 4 L measured pore volume packed with pea gravels of ~4 mm diameter (Fig. 1). The *Canna generalis* plants were grown in two sets of mesocosms under sufficient sunlight. Primary treated domestic wastewater, collected from the campus of Indian Institute of Technology (IIT), Roorkee and the collected groundwater were used as a growth media in all mesocosms.

One mesocosm, M_1, with fully grown plants was spiked with 150 mg/L of toluene to acclimatise the rhizospheric microbes with the pollutant. The second mesocosm, M_2, was similar to the M_1 except that the plant shoot biomass was chopped off. The remaining plant stems were sealed by silicone material for blocking the transpiration loss from the xylem. The purpose of using these two setups (M_1 and M_2) was to quantify the BTEX compound translocated from root to shoot biomass of the selected plants. The third mesocosm, M_3, was set up as control and operated in parallel with the mesocosms M_1 and M_2 in order to characterize the natural attenuation of toluene. A schematic of the experimental setup is shown in Fig. 1. The growing media of the mesocosms was recirculated in a closed loop at 500 mL/h by connecting inlets and outlets of the mesocosms with a viton tube of diameter 3 mm using a peristaltic pump. The surfaces of all the three mesocosms were covered with aluminum foils and mulches to minimize the volatilization of toluene and water to atmosphere. The toluene and water loss from the mesocosms were measured regularly after spiking the mesocosms with an initial toluene concentration of 120 mg/L.

Table 3 Mass balance of toluene in wetland mesocosm without shoot biomass

Time (h)	Mass of toluene biodegraded (mg)	Mass of residual toluene (mg)	Mass of toluene in root biomass (mg)	Mass error (%)
8	200.87	284.25	2.43	0.49
24	360.28	122.40	4.87	1.0
32	379.79	101.67	6.1	1.25
40	405.27	73.73	8.56	1.75
48	432.85	44.92	9.79	1.99
65	448.63	25.52	13.41	2.75
72	449.92	23.03	14.60	2.99

Table 4 Mass balance of toluene in unplanted wetland mesocosm

Time (h)	Mass of toluene biodegraded (mg)	Mass of residual toluene (mg)	Mass of toluene adsorbed (mg)	Mass error (%)
8	314.18	367.11	3.42	0.49
24	484.22	193.64	6.86	1.0
32	538.85	137.30	8.56	1.20
40	570.55	103.89	10.27	1.50
48	599.72	73.02	11.98	1.75
56	581.34	87.98	15.41	2.25
65	610.19	57.40	17.12	2.50
72	633.01	32.87	18.83	2.75
82	622.38	40.05	22.28	3.25

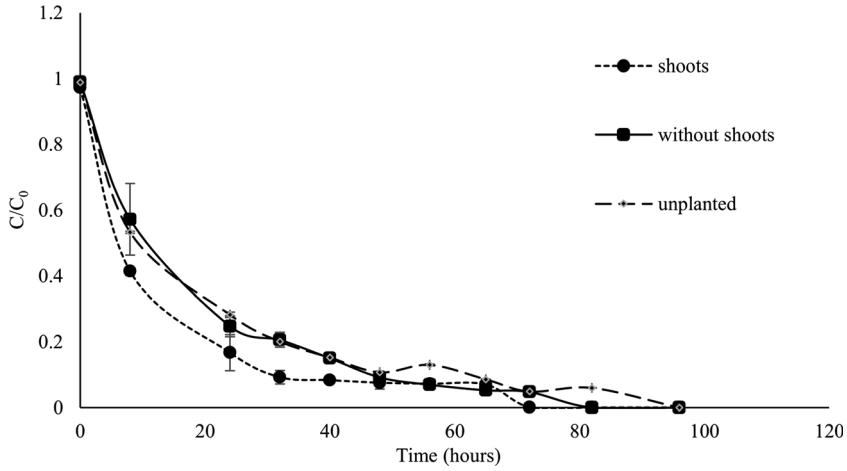

Fig. 3 Biodegradation of toluene with time spiked in three wetland mesocosms. *Error bars* represents±standard error for three replicates

Sample analysis

The water samples from the rhizospheric zones of the mesocosms were collected and filtered using a 0.22 μm syringe filter at fixed time intervals for analysis in a gas chromatograph (Varian GC model CP-3800). The collected water samples (4 μL) were then injected using 10 μL gas-tight syringes (Hamilton) into the GC inlet equipped with flame ionization detector (FID) for detecting the concentration using a calibration curve prepared for toluene. The analytes were separated using a Chrompack capillary column (30 m long, 0.25 mm inner diameter with 0.25 μm film). High purity N_2 was used as the carrier gas at a flow rate of 25 mL/min; the flow rate of H_2 and air into the FID was 20 mL/min, respectively. The temperatures of the GC inlet, detector, and oven are kept isothermal at 161, 100, and 150 °C, respectively, during the analysis. The dry and live shoot and root biomass were weighed after the end of each experiment.

Simulating plant uptake in wetlands

The two broad categories of models to describe the solute uptake by plant root biomass are (1) empirical and (2) mechanistic. The empirical approach correlates the solute concentration in aqueous media with that in plant biomass using plant uptake factor (Yerokun and Christenson 1990; Ross 1981). In such models, the contaminant concentrations in plant biomass and in soil pore water are assumed to be at equilibrium irrespective of the contact time between plant roots and the surrounding soil-water system. However, the amount of solute uptake in plant biomass is a dynamic process which increases initially before reaching to a steady-state level. Further, such a relationship is only valid for a narrow range of contaminant concentration (Carlson and Bazzaz 1977; Jiang and Singh 1994), and thus the empirical approach does not represent contaminant behavior under site-specific conditions (USDE 1998). Mechanistic approaches can simulate solute removal

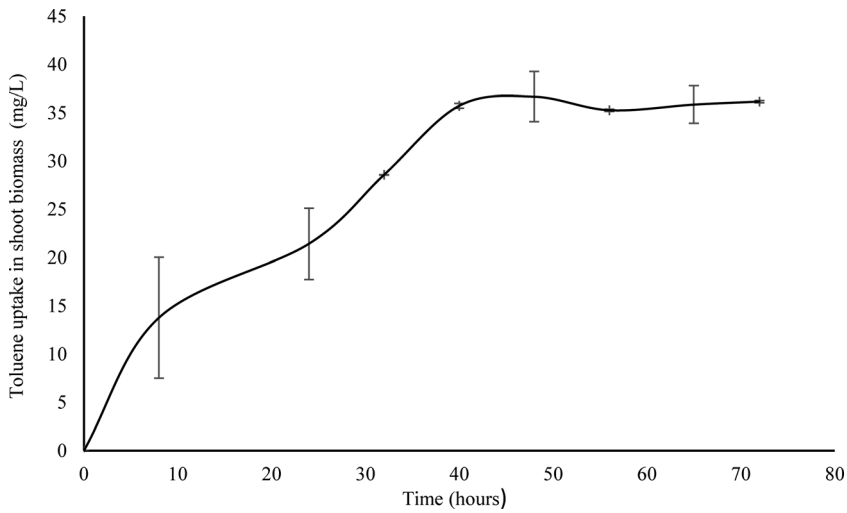

Fig. 4 Progression of total toluene uptake by shoot biomass of *Canna generalis*. *Error bars* represents±standard error for three replicates

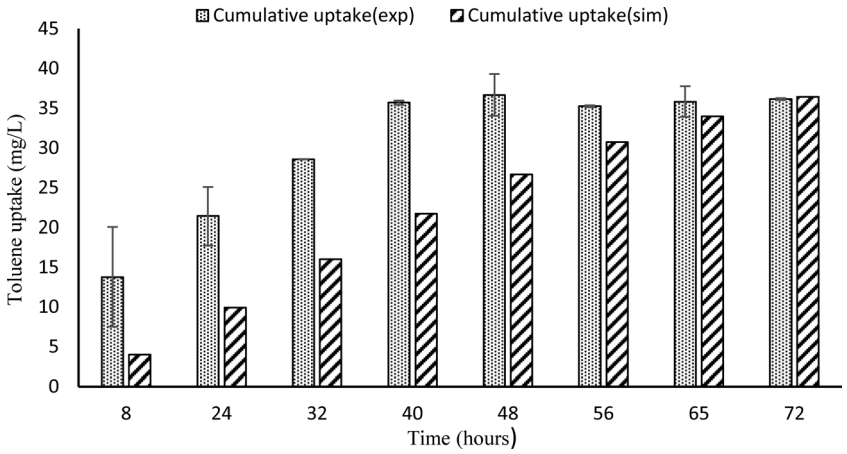

Fig. 5 Comparison of simulated and experimental uptake of toluene by shoot biomass with time. Error bars represents± standard error for three replicates

using uptake kinetics by roots (Claassen and Barber 1976; Cushman 1979; Rao and Mathur 1994; Mathur 2004; Yadav and Junaid 2014). Based on the solute uptake parameters considered, these methods can be further sub-divided into (1) active and (2) passive uptakes (Vogeler et al. 2000; Rengel 1993; Mathur and Yadav 2009). Most of the organic contaminants follow a passive diffusive process at low concentrations (Bromilow and Chamberlain 1995).

The removal of toluene from the rhizospheric pore water of the planted and unplanted mesocosms was simulated using a set of mass balance equations (1 and 2), which were developed to include aqueous diffusion of toluene towards the root surface and its subsequent translocation from root to shoot biomass (Fig. 2). A uniform aqueous phase concentration of toluene (C_t) was considered around the root biomass for formulating the continuity equations for wetland mesocosms without and with shoot biomass:

$$V_t \frac{\partial C_t}{\partial t} = -K_t C_t V_t - R_{cf} C_t V_{rs} D_r \quad (1)$$

$$V_t \frac{\partial C_t}{\partial t} = -K_t C_t V_t - R_{cf} C_t V_{rs} D_r - Q_{\text{trans}} C_t T_{scf} \quad (2)$$

where V_t is the total volume of pore water remaining in the mesocosm after time t in liters, K_t is the mean first-order toluene removal rate constant in hour in the gravel bed without shoot, V_{rs} is the active root surface volume and considered as 8 % volume of the total root biomass (Brennan and Shelley 1999), where 1 g of live root biomass is 0.96 cm^3 (±0.0306), R_{cf} is the plant root concentration factor defined as $R_{cf} = 0.82 + 10\,(0.77 \log (K_{ow}) - 1.52)$ (Davis et al. 1993), T_{scf} is the plant's transpiration stream concentration factor defined as $T_{scf} = 0.784\, e\, (\{-(\log k_{ow} - 1.78)2)/2.44$ (Davis et al. 1993), Q_{trans} is the water uptake rate (mL/h) by plants, and D_r is the effective diffusion rate (h^{-1}) between the aqueous phase toluene concentration (C_t) and the root biomass.

The plant biomass growth and the amount of water lost in evapotranspiration are shown in Table 1. The plant uptake was calculated from the difference of mass balance in the unplanted and planted mesocosms measured during the experimental period. The toluene loss

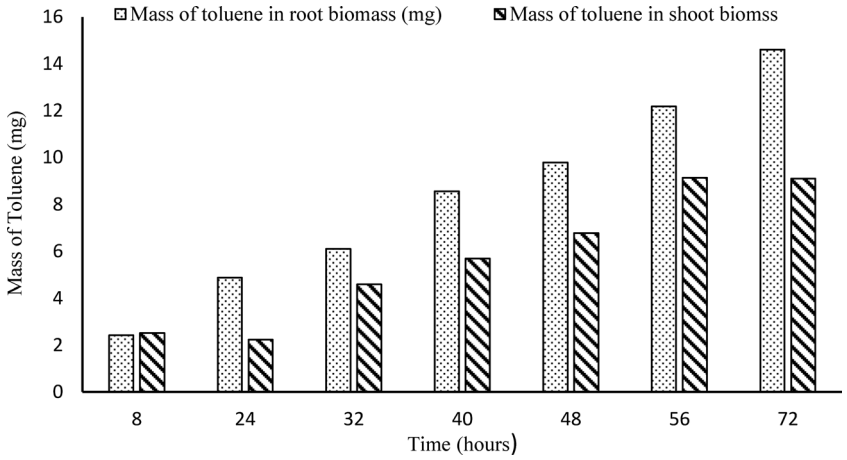

Fig. 6 Mass of toluene accumulated in root and shoot biomass with time

Table 5 List of parameters used for simulating toluene uptake in wetland mesocosm with shoot biomass

Parameters	Values	Unit	Reference
Initial concentration, C_t	118.35	mg/L	Experimental
Total volume, V_t	4.0	L	Experimental
Rate constant, K_t	0.04	h^{-1}	Experimental
Water lost in transpiration, Q_{trans}	2.77	mL/h	Experimental
Root concentration factor, R_{cf}	4.13	dimensionless	Narayanan et al. 1998a, b
Transpiration stream concentration factor, T_{scf}	0.74	dimensionless	Boonsaner et al. 2011
Root surface volume, V_{rs}	0.267	L	Yadav et al. 2011
Diffusion rate constant, D_r	0.05	h^{-1}	Calibrated

in the control mesocosm (M_3) was subtracted from toluene loss in planted mesocosms (M_1 and M_2) to obtain the toluene uptake in shoot and root biomass (Tables 2, 3, and 4). In addition, ambient aqueous concentration of toluene, C_t, in the mesocosm water at a given time t was calculated with and without shoot biomass by solving equations (1) and (2) and compared with the experimental uptake values. Equations 1 and 2 utilize the first-order toluene removal rate constant, K_t, which is taken as an average value for the experiment.

Results and discussion

The relative residual concentration (C/C_0) of toluene in unplanted (control) mesocosm (M_3) filled with pea-gravel and in mesocosms with live plant (M_1) and with clipped shoot (M_2) is plotted versus time since toluene amendment (Fig. 3). The magnitude of standard error is shown using vertical bars. Figure 3 shows that toluene was degraded in all three mesocosms but the rate constant of toluene removal is greater for planted mesocosm with shoots (M_1) in comparison to mesocosms M_2 and M_3. During the early stages (till 50 h), the degradation rate constant was found to be 0.07, 0.05, and 0.05 h^{-1} for M_1, M_2, and M_3, respectively. For the late phase, rate constants were 0.05, 0.03, and 0.04 h^{-1} for M_1, M_2, and M_3, respectively, suggesting a higher rate of degradation in the early stages than towards the end. A faster rate of removal for M_1 as compared to M_2 and M_3 can be attributed to the role of plants in wetlands (Powell et al. 2014). The root exudates, which are known for their capability to enhance the breakdown of organic pollutants (Susarla et al. 2002), could contribute towards reducing the total degradation time.

The natural attenuation of toluene in mesocosm M_3 (gravel bed) shows the degrading capability of indigenous microbiota present in the mesocosm. Though the selected BTEX compound is readily degraded by natural attenuation in all three mesocosms, the time required for biodegradation was reduced up to 25 % in the presence of plants. The attenuation of toluene in mesocosm M_3 (gravel bed) also confirms that sampled groundwater has sufficient nutrients for pollutant degradation as reported by Yadav et al. (2013) for the same site.

The removal of toluene from mesocosm pore water was calculated using mass balance calculations of toluene in the root zone and plant biomass. Figure 4 shows that the total toluene removal increases with time and reaches a steady state towards the end. Similar trend was observed by Yadav et al. (2011), Trevors et al. (1986), and Ting et al. (1989) for metal extraction by plants from aqueous solutions. The initial phase of exponential uptake takes place due to a higher tendency of roots to adsorb the organic compound and a sharp diffusion/concentration gradient with the contamination in the exterior solution. With time, the amount of organic compound accumulated in the shoot biomass decreases indicating a state of saturation in plants with decreasing concentration gradient in the external solution. Also, the cumulative uptake in the shoot

Table 6 List of parameters used for simulating toluene uptake in wetland mesocosm without shoot biomass

Parameters	Values	Unit	Reference
Initial concentration, C_t	121.89	mg/L	Experimental
Total volume, V_t	4.0	L	Experimental
Rate constant, K_t	0.04	h^{-1}	Experimental
Root concentration factor, R_{cf}	4.13	Dimensionless	Narayanan et al. 1998a, b
Root surface volume, V_{rs}	0.267	L	Yadav et al. 2011
Diffusion rate constant, D_r	0.05	h^{-1}	Calibrated

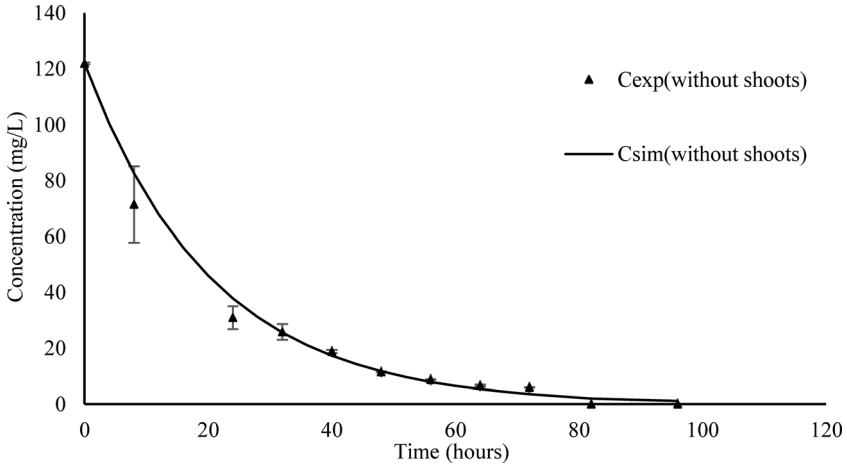

Fig. 7 Simulated and experimental comparison for wetland mesocosm without shoot biomass. *Error bars* represents± standard error for three replicates

biomass is predicted using linear kinetics using a mean value of first-order rate constant which is shown in Fig. 5. Initially, some discrepancy between the measured and the simulated values is observed which is narrowed down during the last phase of the experiments. Here, the slight discrepancy between experimental and simulated values was because of the constant rate assumed for the simulations whereas, in reality, the rate constant may vary slightly due to the changing concentration of the substrate between the two observations.

The extraction of BTEX from root zone occurs via water uptake or transport via diffusion (Mathur and Yadav 2009). Experiments done by Briggs et al. (1982) show that the uptake of non-ionized chemicals taking place at high concentrations into roots from hydroponic solutions consists of two parts (1) aqueous phase equilibration around the plant roots with the concentration of surrounding solution and (2) sorption of the contaminant on lipophilic root solids. Plants may take up, immobilize or translocate, and transform the contaminant during bioremediation (Narayanan et al. 1998a, b). One of the crucial parameters to predict the uptake in plants from soil-water systems is octanol-water partition coefficient, K_{ow}, of the contaminant (Cunningham and Berti 1993). Briggs et al. (1982) gave a linear relationship to predict the uptake of compounds in plants based on its physicochemical properties. Non-ionized chemicals that have log K_{ow}>4 were categorized by Wild and Jones (1992) to have a greater affinity for retention in plant roots. The transpiration concentration stream factor, T_{scf}, is a function of the compound's K_{ow} and it depends on its hydrophobicity (lipophilicity), solubility, polarity, and molecular weight. Hydrophobic compounds with log K_{ow} values between 1.0 and 3.5 can translocate from roots to shoots (Schnoor 1997; Briggs et al. 1982; Ryan et al. 1988). BTEX compounds have log K_{ow} between 2.13 and 3.20, and a study done by Collins et al. (2002) has shown accumulation of BTEX in root and their translocation to shoots.

The translocation of toluene from root to shoot biomass was quantified using mass balance equations. The mass of toluene taken up by the root and shoot biomass is shown in Fig. 6. This clearly suggests that with time the toluene mass in root biomass predominates due to absorbance and immobilization of toluene in root biomass. The mass balance of toluene for all the cases is calculated in Tables 2, 3, and 4 showing a variation of less than 5 %.

Equations 1–2 were solved using the data set in Tables 5 and 6 to simulate the concentration of toluene in mesocosms with and without shoot biomass. The data points calculated

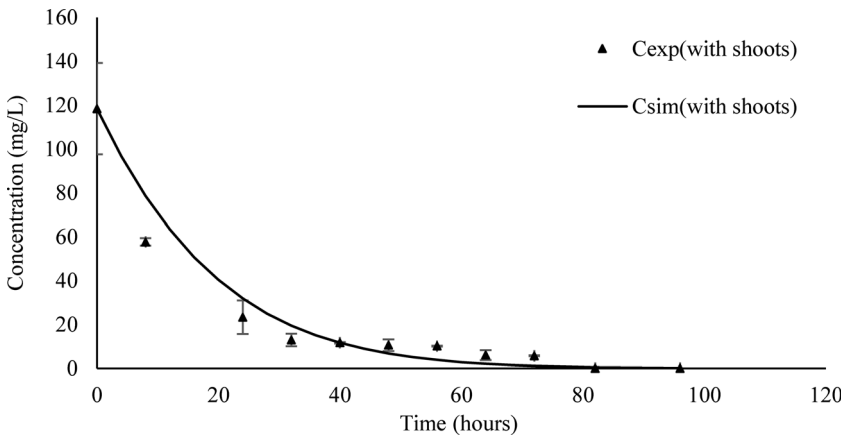

Fig. 8 Simulated and experimental comparison for wetland mesocosm with shoot biomass. *Error bars* represents± standard error for three replicates

from first-order kinetics match very well with those from the experiment for wetland without shoot biomass (Fig. 7). A slight discrepancy in some observed and simulated values for the case of wetland with shoot biomass (Fig. 8) may be due to the adsorption onto gravel bed.

Summary and conclusions

The main objective of this study was to investigate the role of plants in engineered bioremediation and to quantify the uptake of toluene by root and shoot biomass. To achieve this, the water quality in pot-scale wetlands with and without shoot biomass along with unplanted gravel beds was studied under controlled conditions. A high biodegradation rate was achieved by integrating biostimulation and bioaugmentation techniques of engineered bioremediation with phytoremediation in wetland mesocosms. The removal time of toluene was reduced significantly, 25 %, in presence of plants as compared to the unplanted gravel bed. The results presented here demonstrate that the toluene mass gets accumulated in the shoot biomass with time, and its accumulation was higher in the root biomass as compared to shoot biomass. The continuity equations developed to simulate the entire process of biodegradation and uptake matched quite well with the experimental data and hence can be used for forecasting pollutant concentration in root zone under similar environmental conditions. This technology of plant-assisted bioremediation can be useful for planning field scale application of enhanced bioremediation in hydrocarbon polluted lands.

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