

Biodegradation of Toluene Under Seasonal and Diurnal Fluctuations of Soil-Water Temperature

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Abstract An increasing interest in bioremediation of hydrocarbon polluted sites raises the question of the influence of seasonal and diurnal changes on soil-water temperature on biodegradation of BTEX, a widespread group of (sub)-surface contaminants. Therefore, we investigated the impact of a wide range of varying soil-water temperature on biodegradation of toluene under aerobic conditions. To see the seasonal impact of temperature, three sets of batch experiments were conducted at three different constant temperatures: 10°C, 21°C, and 30°C. These conditions were considered to represent (1) winter, (2) spring and/or autumn, and (3) summer seasons, respectively, at many polluted sites. Three additional sets of batch experiments were performed under fluctuating soil-water temperature cases (21 > 10°C, 30 > 21°C, and 10 < 30°C) to mimic the day–night temperature patterns expected during the year. The batches were put at two different temperatures alternatively to represent the day (high-temperature) and night (low-temperature)

times. The results of constant- and fluctuating-temperature experiments show that toluene degradation is strongly dependent on soil-water temperature level. An almost two-fold increase in toluene degradation time was observed for every 10°C decrease in temperature for constant-temperature cases. Under fluctuating-temperature conditions, toluene degraders were able to overcome the temperature stress and continued thriving during all considered weather scenarios. However, a slightly longer time was taken compared to the corresponding time at daily mean temperature conditions. The findings of this study are directly useful for bioremediation of hydrocarbon-polluted sites having significant diurnal and seasonal variations of soil-water temperature.

Keywords Biodegradation · Toluene · BTEX · Varying temperature · Soil-water pollution

1 Introduction

Pollution of soil-water due to the release of hydrocarbons is a major public health concern, and therefore, remediation of these natural resources is needed to eliminate risk to human and/or to the environment. Monoaromatic hydrocarbons, such as benzene, toluene, ethylbenzene, and mixture of xylenes (BTEX), are of particular concern because of their high water solubility which enables them to spread in the (sub)-surface widely (Margesin et al. 2003). Contamination

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typically occurs near petroleum and natural gas production sites, petrol stations, and other areas with above-ground storage tanks (AST) or underground storage tanks (UST) containing gasoline or other petroleum products.

Biological treatment of soil-water polluted by such organic contaminants is receiving increasing interests and, where applicable, can serve as a cost-effective soil remediation alternative. The success of bioremediation greatly depends on the prevailing environmental variables, and hence, it requires a sound understanding of their effects on pollutants fate under site-specific conditions. Most of the problematic sites are characterized by environmental parameters like variable temperatures, high/low pH, water table dynamics, and fluctuating soil moisture content. Among these, soil-water temperature plays the most crucial role in the bioremediation process (Sims et al. 1993). The diurnal and seasonal fluctuations of shallow soil-water temperature, expected at most polluted sites, may influence hydrocarbon's properties, rate of degradation, mass transfer rate, and the activity or survival of degraders (Chablain et al. 1997).

At low temperatures, usually, there is reduced volatilization and decreased water solubility of BTEX and thus delayed onset of biodegradation process (Margesin and Schinner 2001). On the other hand, solubility and thus bioavailability of BTEX compounds are enhanced at elevated temperatures. Rising temperature also decreases adsorption by soil solids, which makes more organic material available for microorganisms to degrade (JRB 1984). Moreover, temperature plays a significant role in controlling nature and extent of the microbial population, responsible for degradation of hydrocarbons (JRB 1984). Microbial metabolism accelerates with increasing soil temperatures up to an optimum value at which growth is maximal. On the other hand, low soil temperatures reduce the fluidity and permeability of the microbial cellular membrane, which hinders nutrient and contaminant uptake (Corseuil and Weber 1994). Though most of the bacteria present in subsurface environments operate most effectively in the range 20–40°C (Chapelle 2001), a wide range of hydrocarbons has been shown to be biodegraded under extreme (low or elevated) temperature conditions (Muller et al. 1998; Margesin and Schinner 1999).

Based on temperature, soil microorganisms are mainly divided into three groups: (1) psychrophiles,

(2) mesophiles, and (3) thermophiles (Chablain et al. 1997; Margesin and Schinner 2001). The psychrophiles grow at temperatures below 20°C, the mesophiles grow at temperatures between 20°C and 44°C, and the thermophiles require growth temperatures above 45°C (Stetter 1998). Most hydrocarbon degraders are mesophiles which metabolize optimally in the temperature range of 20–35°C (Chambers et al. 1991). In general, higher temperatures are associated with higher enzymatic activity and faster biodegradation rates, up to an optimum value that is species specific. In this range, degradation rates of hydrocarbons can double or triple due to a temperature increase of 10°C (Corseuil and Weber 1994). If the temperature rises far above the optimum value, proteins, enzymes, and nucleic acids become denatured and inactive, leading to the inhibition of biodegradation. On the other hand, decrease in temperature can slow down degradation, but it will not stop it.

The biodegradation of monoaromatic hydrocarbons in cold environments has been reported extensively in various soil-water ecosystems (Braddock et al. 1997; Margesin 2000; Aislabie et al. 1998; Siron et al. 1995; Delille and Delille 2000). Conclusions arrived from these studies were reviewed by Margesin and Schinner (1999), Stempvoort and Biggar (2008), and Yang et al. (2009). These studies show that psychrophiles play a significant role in the degradation of pollutants in cold regions. Similarly, thermophilic bacteria possess substantial potential for degrading the soil-water hydrocarbons, including BTEX (Muller et al. 1998). Sorkhoh et al. (1993) analyzed several soil samples of hydrocarbon-polluted Kuwaiti desert a couple of years after the Gulf War. This was in a semi-arid zone where the soil temperature frequently exceeds 50°C in summers (Khan and Al-Ajmi 1998). They found a population density of thermophilic bacteria from 3×10^3 to 1×10^7 per gram of soil, which shows the potential effectiveness of bioremediation in hot soil-water environments.

A mixture of thermophilic aerobic bacteria, mainly comprising *Pseudomonas* species, was successfully used by Lugowski et al. (1997) to degrade various components of BTEX containing wastewater at a temperature range of 40–42°C. Furthermore, the aerobic bacteria used by Lugowski et al. (1997) were used along with other thermophiles by Taylor et al. (1998) for thermally enhanced in-situ remediation of a polluted soil. The authors coupled the thermal treatment (using dynamic underground stripping heating) with

in-situ bioremediation of the soil to target BTEX contaminants. The targeted soil volume was first heated to vaporize a part of the trapped pollutants, followed by vacuum extraction. The soil temperature was kept between 50 and 70°C for two months after the thermal treatment. This enabled thermophilic bacteria to metabolize lower-concentration BTEX compounds in the soil.

Furthermore, Deeb and Cohen (1999) studied the temperature effects and substrate interactions during the aerobic biotransformation of BTEX mixtures by toluene-enriched consortia and *Rhodococcus rhodochrous*. They found out that cell growth on toluene increased with temperature from 7°C to 35°C, decreased sharply at 36°C to 40°C, and was almost inhibited above 45°C. Also, cell growth on toluene increased four-fold from 20°C to 35°C.

The aforesaid bioremediation studies performed at low and elevated temperature environments on various hydrocarbon-contaminated soils indicate that soil-water temperature plays a crucial role in the process. However, the impacts of temperature were often studied either at a constant temperature or on long-term basis without considering the biological stress of seasonal and diurnal changes in temperature on degradation of pollutants. Recently, Yadav and Hassanizadeh (2010) emphasized the need for considering the impact of cyclic short-term variability in shallow soil-water system on the rate of biodegradation in order to obtain a more realistic and widely applicable estimate of biodegradation. Therefore, in this study, we investigated the effect of soil-water temperature on biodegradation of toluene. We have performed experiments at different constant temperatures pertaining to different seasons. Moreover, we have considered diurnal changes in temperature under cold, warm, and extreme weather conditions. The aerobic degradation of toluene with and without temperature fluctuating conditions is also studied. We have chosen toluene as a representative component of BTEX. Toluene is widely used as an industrial feedstock and as a solvent. It is a representative component in several surrogate mixtures for gasoline, jet fuel, and diesel (Edwards and Maurice 2001). Like other solvents, toluene is sometimes also used as an inhalant drug for its intoxicating properties (Violi et al. 2002). Our research on toluene is of generic nature. Our findings on its biodegradation under a range of environmental conditions will be qualitatively valid for other BTEX components.

2 Materials and Methods

2.1 Materials

Natural groundwater containing organic pollutants was collected from a pump-and-treat site (A&G Milieutechniek) in Haarlem, Netherlands. The collected groundwater was left open under a ventilated hood to remove all volatile components and was later used as a primary source of toluene degraders. Clean sand (Quartz sand H31) used in the experiments had grain sizes between 0.1 and 1.0 mm and was obtained from Sibelco, Belgium. A stock was prepared by dissolving toluene (Merck) with 99.9% purity in distilled water to its solubility limit. Batches of 120-ml capacity, provided by Alltech, were assembled by adding appropriate volume of stock solution to get a required initial concentration of 1 mg/l. A sufficient headspace, ~80 ml, was provided to maintain aerobic conditions throughout the experimental period. Viton stoppers (Rubber B.V., Hilversum, Netherlands) together with crimp seals (Wheaton, Netherlands) were used to prevent any leakage of toluene. In each batch, 10 g of sand was mixed with 15 ml of groundwater. Sterile batches were prepared by adding 1 ml of 10-g/l $HgCl_2$ in order to check whether there were any abiotic losses. After closing the batches airtightly, 40 μ l of toluene stock was injected through the stopper with a gastight syringe (SGE). They were then thoroughly shaken and incubated in dark at required temperature according to the experiment type. During incubation, the batches were placed on an orbital platform shaker with 150 rpm.

2.2 Biodegradation Experiments

Preliminary batch experiments, with and without soil, were run first at room temperature to see the toluene degradability of the microorganisms present in the groundwater. The amount of $HgCl_2$ for sterilization purposes and the sampling interval of pore-water and headspace were also determined by the preliminary batch experiments. Subsequently, two sets of batch experiments were conducted: one at three different constant temperatures and the other under three different fluctuating-temperature conditions.

The constant-temperature batches were performed at room temperature (21°C) along with low (10°C) and high (30°C) extremes representing

spring/autumn, winter, and summer conditions at most of polluted sites in semi-arid regions, respectively. The batches at 10°C were put in a refrigerator with required temperature, together with a shaker installed inside. The 21°C batches were performed in a constant-temperature room. The batches for 30°C were incubated in a water-bath having a built-in shaker.

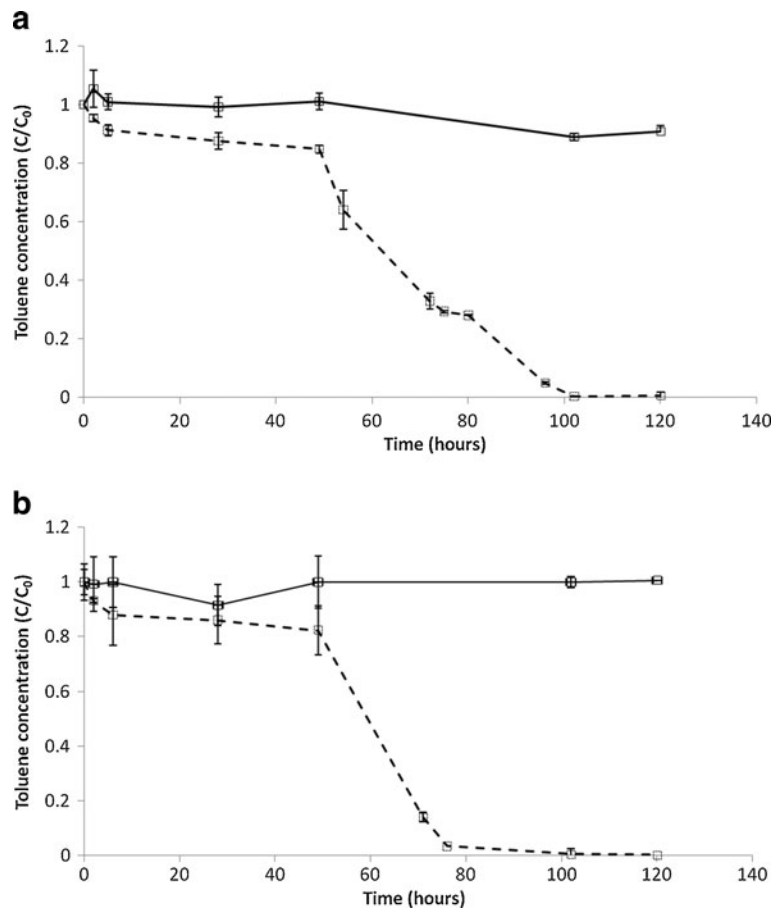
For the fluctuating-temperature cases, batches were moved between fridge, constant-temperature room, and the waterbath according to the temperature requirement. The first case was for the cold weather during which day–night temperatures were maintained at 21°C and 10°C for 14 and 10 h, respectively, for a total period of 5 days. A day–night temperature of 30–21°C was considered for 14 and 10 h for a period of three days during the second case, representing warm conditions. The third experiment was performed to see the effect of weather extremes on toluene degradation, during

which the batches were put at 30°C and 10°C for 14 and 10 h, respectively, for a period of four days. The batches were run in triplicates for both live and sterile sets.

2.3 Sample Analysis

For the constant-temperature batches, liquid samples were taken out periodically using 1-ml syringes and 0.6-mm disposable needles (Terumo). Gas samples were taken out with airtight syringes (luer lock syringes, SGE) and measured by hand injections in Varian Star 3600CX GC. Only liquid concentrations were analyzed in case of fluctuating-temperature batches. Liquid samples from all batch experiments were put in 1.5-ml vials (Grace Discovery Science) with 5 μ l of 1-g/l HgCl_2 to prevent further degradation in the vials. The vials were then placed on an auto-sampler (Varian 8200

Fig. 1 Attenuation of toluene over time in **a** groundwater and **b** headspace during winter season (constant temperature of 10°C). *Dotted lines* are for live batches, and *solid lines* show the toluene concentration in sterile batches. *Error bars* represent \pm SE ($n=3$)

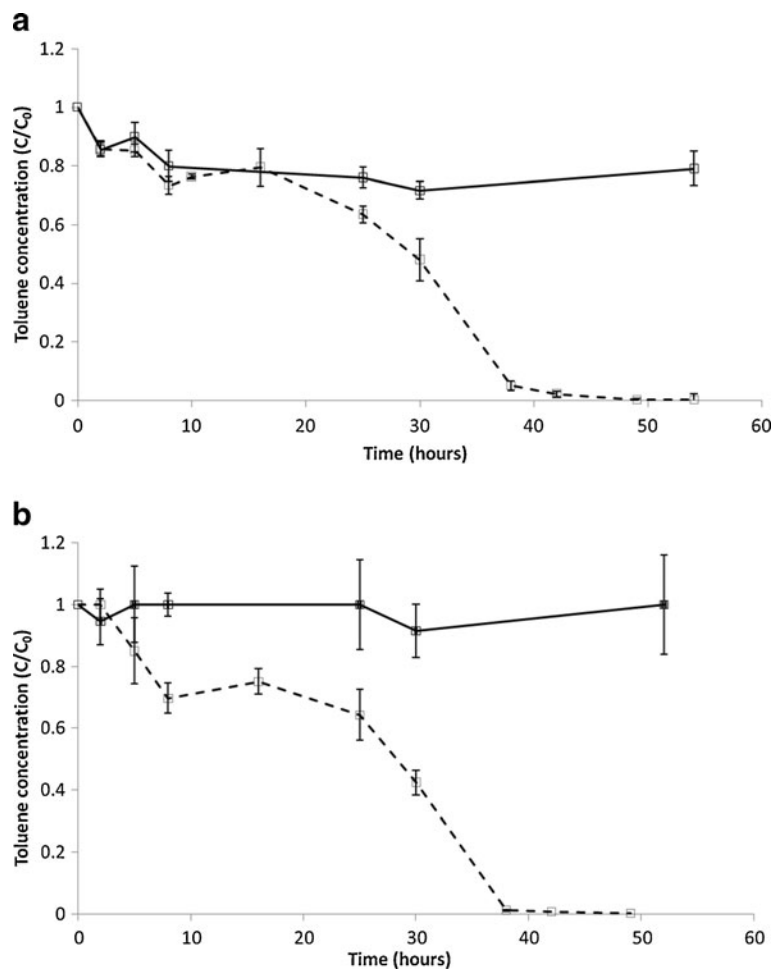


CX) attached to the GC for analysis. The GC has a Stabilwax DB column of 0.32 mm diameter, 30 m length, and film thickness of 1 μm , with a UV lamp and a PID detector (200°C). The air-water partitioning coefficient, or the dimensionless Henry's constant, for toluene at the considered temperatures is calculated using a mass balance.

3 Results and Discussions

A significant amount of biodegradation (98.4%) of toluene was observed during initial two days of the experiments. This emphasizes the quick acclimatization and metabolic capabilities of microbes to decontaminate the considered BTEX component. The results of constant- and fluctuating-temperature conditions are presented next.

Fig. 2 Attenuation of toluene over time in **a** groundwater and **b** headspace during spring and/or autumn season (constant temperature of 21°C). The live and sterile batches are represented by *dotted* and *solid* lines, respectively. Error bars represent \pm SE ($n=3$)

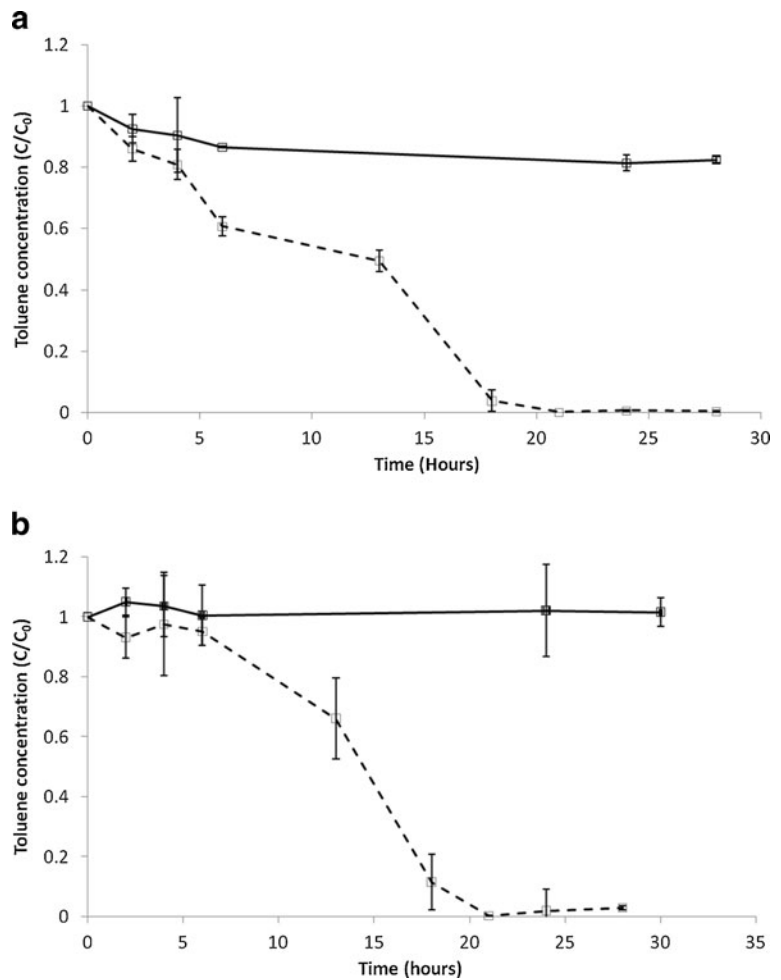


3.1 Degradation at Constant Temperatures

Variations in the toluene concentration in liquid and headspace at three constant soil-water temperatures (10°C, 21°C, and 30°C) are presented in Figs. 1, 2 and 3. The toluene was degraded in all live batches at the selected soil-water temperatures, while sterile batches showed relatively small losses of toluene compared to biodegradation assays over the entire experimental time. This small reduction in toluene concentration in sterile batches seems to be due to the abiotic depletion of the substrate. At 10°C (Fig. 1), most of the toluene was degraded within 80–90 h of incubation. However, the same amount of degradation occurred in 35–45 h of incubation in 21°C batches (Fig. 2) and in only 20–25 h at 30°C (Fig. 3).

These results show that toluene degradation rate is increased by almost two-fold for every 10° increase in

Fig. 3 Change in toluene concentration with time in **a** groundwater and **b** headspace during spring and/or autumn season (constant temperature of 30°C). The live and sterile batches are represented by *dotted* and *solid lines*, respectively. *Error bars* represent \pm SE ($n=3$)



temperature. This increase of the selected hydrocarbon's degradation rate with increased soil-water temperature agrees well with results of Corseuil and Weber (1994), who suggested that degradation rates of monoaromatic hydrocarbons can double or triple due to a temperature increase of 10°C. As seen in Figs. 1, 2 and 3, headspace and liquid concentrations have almost similar trends of degradation. Table 1 shows that the observed value of Henry's constant is qualitatively and quantitatively close to the simulated values obtained by Washington 1996 and by the Office of Solid Waste and Emergency Response (OSWER) method (USEPA 2002). Similar experimental observations are reported by Staudinger and Roberts (1996).

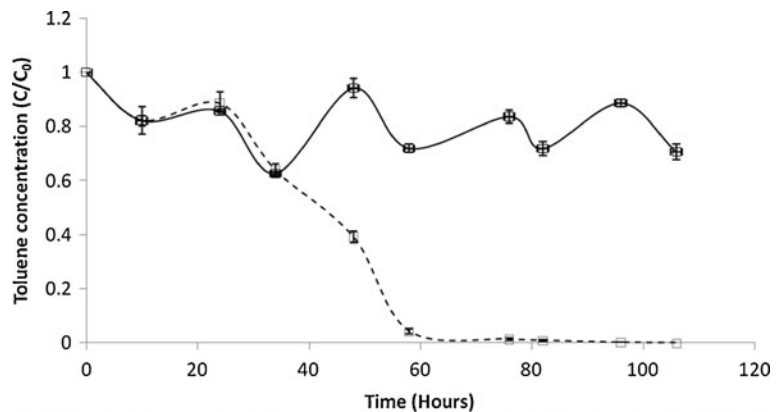
Room temperature (21°C) and low- (10°C) and high-temperature (30°C) extremes were chosen here for characteristically representing the spring and/or

autumn, winter, and summer seasons, respectively. Degradation time for polluted sites having different temperatures than the considered one can be interpolated and/or extrapolated from these findings.

Table 1 The observed and simulated air-water partitioning coefficient (dimensionless Henry's constant) for toluene during winter, spring/autumn, and summer seasons

Season	Temperature (°C)	Observed	Washington (1996) method	OSWER (2002) method
Winter	10	0.122±0.011	0.127	0.124
Spring/autumn	21	0.205±0.07	0.201	0.222
Summer	30	0.312±0.013	0.284	0.343

Fig. 4 Time progression of toluene concentration observed in groundwater for cold weather case. The soil-water temperature was maintained at 21°C and 10°C for 14 and 10 h alternatively. Measurements are taken right before changing the temperature. The live and sterile batches are represented by *dotted* and *solid* lines, respectively. *Error bars* represent \pm SE ($n=3$)



3.2 Degradation Under Fluctuating Temperatures

Results of experiments on degradation of toluene under three different varying soil-water temperature conditions are presented in Figs. 4, 5 and 6. A distinct trend of fluctuations in the toluene concentration in groundwater is seen in all sterile batches. However, the average concentration of toluene remained around 75–80% of the original concentration. The observed undulations in toluene concentration can be attributed to the temperature dependency of the Henry's law constant as reported in Table 1. For instance, at high temperature, the air-water partitioning coefficient increases, and more toluene can be transferred from liquid phase to the headspace.

For the cold weather case ($21 > 10^\circ\text{C}$), it took 70–75 h for the toluene to degrade in live batches (Fig. 4). The time taken is much shorter when compared to 10°C batches and longer when compared to 21°C batches. For the warm weather case ($30 > 21^\circ\text{C}$), it took 35–45 h for

the toluene to degrade (Fig. 5). The time taken is longer when compared to 30°C batches and almost similar to 21°C batches. The result of the extreme case (10°C and 30°C) shows that 45–55 h are required for the degradation of the same amount of toluene (Fig. 6). This is less than half of the degradation time in 10°C batches and more than twice the time in 30°C batches. Thus, this set of batches neither followed the lower nor the higher temperature degradation curves.

A summary of total degradation time along with a lag phase rating for all batch experiments is given in Table 2. In addition to the measured values, the expected degradation times for the fluctuating-temperature experiments based on constant temperature were also calculated. To do so, for each of the three fluctuating-temperature cases, daily mean temperature was calculated. Those are reported in the last three columns of Table 2. Then, the mean degradation time at any of these constant temperatures was interpolated from the degradation times of constant-

Fig. 5 Variation in toluene concentration of groundwater for extreme weather case. The soil-water temperature was maintained at 21°C and 30°C for 14 and 10 h alternatively. Measurements are taken right before changing the temperature. The live and sterile batches are represented by *dotted* and *solid* lines, respectively. *Error bars* represent \pm SE ($n=3$)

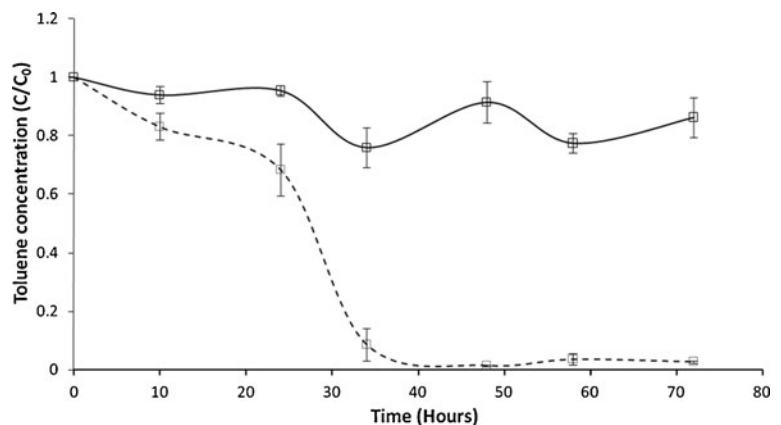
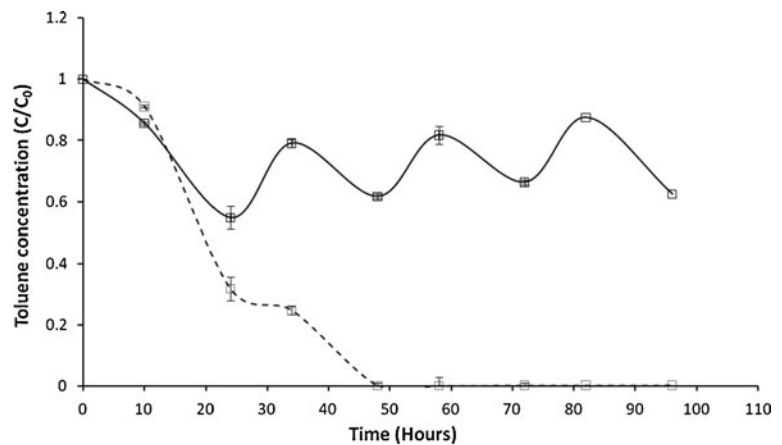


Fig. 6 Variation in toluene concentration of groundwater for extreme weather case. The soil-water temperature was maintained at 10°C and 30°C for 14 and 10 h alternatively. Measurements are taken right before changing the temperature. The live and sterile batches are represented by *dotted* and *solid* lines, respectively. Error bars represent \pm SE ($n=3$)



temperature cases. We found that the degradation times for fluctuating cases are considerably longer than the corresponding calculated mean degradation times. Note that time taken for variable cases are considerably more than the constant-temperature (arithmetic average of day–night temperature) batches. This time difference can be attributed to the biological stress faced by the toluene degraders due to the changing day–night soil–water temperatures.

Our results suggest that for achieving highest efficiency in a bioremediation project, it is important to keep the soil temperature constant as much as possible. Though controlling the soil–water temperature in the field is quite difficult, it can be partially achieved by regulating the incoming and outgoing radiation or by changing the thermal properties of the land surface. Barren surface becomes very warm during the hottest part of the day, but also loses its heat rapidly at night. But, vegetation plays a significant role in controlling soil–water temperature because of insulation properties of the plant cover (Radwan et al. 1998). In the winter,

the vegetation acts as an insulator to reduce heat loss from the soil. On the other hand, during summer times, a well-vegetated surface does not become as warm as a bare surface. Moreover, plants can be used to promote microbial restoration in rhizosphere of BTEX-contaminated sites (Aprill and Sims 1990; Narayanan et al. 1995; Mathur and Yadav 2009).

4 Summary and Conclusions

The focus of this study was to investigate the impact of diurnal as well as seasonal change of soil–water temperature on biodegradation of toluene using lab experiments. To achieve this, two sets of batch experiments were performed for a sandy soil and natural groundwater having dissolved toluene. The first set was performed under three constant soil–water temperature conditions. Room temperature (21°C) and low- (10°C) and high-temperature (30°C) extremes were chosen to represent spring and/or autumn, winter,

Table 2 Toluene degradation time taken in constant, arithmetically deduced constant, and variable soil–water temperature batches

Temperature (°C)	Constant temperature			Fluctuating temperature			Daily mean temperature		
	10 (winter season)	21 (spring/autumn)	30 (summer season)	21 \diamond 10 (cold weather)	21 \diamond 30 (warm weather)	10 \diamond 30 (extreme weather)	16.4	26.25	21.67
Total degradation Time ^a (h)	80–90	35–45	20–25	70–75	35–45	45–55	65–70	25–30	30–35
Lag phase	Long	Medium	Short	Medium	Low	Low	–	–	–

^a Total degradation time for arithmetically deduced constant temperatures is interpolated from the degradation times of constant temperature (10°C, 21°C, and 30°C) batches

and summer seasons, respectively, expected at most of the polluted sites. The second set of experiments was performed under diurnal change in soil-water temperature, and the batches were put at two different temperatures alternatively to mimic day (high) and night (low) temperature patterns. The typical day–night temperature conditions during three weathers, i.e., cold (21–10°C), warm (21–30°C), and an extreme scenario (10–30°C), were considered for this purpose.

The results show that both seasonal and diurnal fluctuations of soil-water temperature strongly influence the time taken for degradation of toluene. The degradation time is increased almost two-fold while moving from winter to spring/autumn and again two-fold from spring/autumn to summer. On average, the degradation is increased by a factor of 2 for every 10°C rise in soil-water temperature. The lag phase period was lowest at 30°C while highest at 10°C. Toluene degradation observed in all fluctuating-temperature batches was found to be slower than the rate at a corresponding constant mean temperature. This indicates the vulnerability of BTEX degrading microorganisms to frequent temperature stresses. Therefore, it is important to take into account the diurnal fluctuations in temperature of the polluted shallow soil-water resources for a better estimation of time required for a bioremediation project. The results of these lab experiments can be used for predicting the process of biodegradation and for determining time frame needed to remove BTEX or other organic pollutants from shallow soil-water resources. Investigating biodegradation of BTEX under a combination of other environmental factors relevant to a polluted site along with the associated temperature conditions would further improve the site-specific remediation forecast.

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