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Uptake, metabolism, and toxicity of methyl *tert*-butyl ether (MTBE) in weeping willows

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Abstract

Methyl *tert*-butyl ether (MTBE) is a high volume production chemical and the most commonly used gasoline oxygenate. Uptake, metabolism and toxicity of MTBE in trees were investigated in this study. Pre-rooted weeping willows (*Salix babylonica* L.) were exposed to hydroponic solution spiked with MTBE and incubated at $25.0 \pm 1\,^{\circ}C$ for 168 h. The normalized relative transpiration (NRT) rate of weeping willows was used to determine toxicity. MTBE and possible intermediate *tert*-butyl alcohol (TBA) in solution, tissues of aerial parts of plants, and air were analyzed. Results from the toxicity test showed that severe signs of toxicity (the reduction of the NRT $\geq$35%) were only found at the treatment group with high doses of MTBE 400 mg L^{-1}. Neither chlorosis of leaves nor large reduction in the NRT was observed at MTBE exposure to weeping willows $\leq$200 mg L^{-1}. Almost all applied MTBE was removed from the hydroponic solution by plants in all treatment groups. Small amounts of MTBE were detected in the plant tissues, but a large fraction of the applied MTBE was found in the air through plant transpiration. Mass balance studies showed that MTBE was assimilated into the plants from hydroponic solution but was not metabolized during transport in the plant. Phytovolatilization was the only relevant removal process for MTBE. Transpiration stream concentration factor (TSCF), an important parameter for design of engineered MTBE phytoremediation systems, was estimated to be 1.12. In conclusion, although this compound is persistent to the attack by plant enzymes, atmospheric MTBE is much more susceptible to photo-oxidation for decomposition. Phytoremediation of MTBE polluted soils and groundwater is an alternative to presently available remediation technologies.

Keywords: Metabolism; Methyl *tert*-butyl ether (MTBE); Phytoremediation; Toxicity; Willows

1. Introduction

The large volume and commercial use of methyl *tert*-butyl ether (MTBE) as a fuel oxygenate, replacing alkyl lead additives, can be traced back to the late 1970s in the United States to deal with air pollution. Over the past several decades MTBE as additives to gasoline intended to either boost ratings of fuel or to reduce air pollution has been accepted worldwide. The annual production of MTBE increased from 1.38 billion lb in 1984 to 24.1 billion lb in 1993, making it the second on the list of organic chemicals being manufactured in the U.S. [1]. In 1998, approximately 24% of all gasoline contained oxygenates in the U.S. [2]. The cause of MTBE leaking into the environment is mainly attributed to gasoline spills and leaks from pipelines, underground- and aboveground-storage tanks, and transport accidents [3]. Due to its high solubility in water and low sorption tendency in soils, MTBE can rapidly penetrate the soil layer and enter the groundwater shortly after the spill. It has become one of the most problematic pollutants in urban soils and groundwater worldwide [4]. A draft lifetime health advisory limit of 20–35 $\mu g\,L^{-1}$ has been issued in the United States [5].

The toxicity of MTBE to animals and humans is well documented. It has been established that MTBE is carcinogenic to animals [6]. Inhalation of MTBE vapors may cause headaches and nausea at higher concentrations (ppm) for people [7]. Respiratory, neurological, cardiac, and allergic symptoms associated with chronic and low level exposure of MTBE were also reported [8]. The U.S. EPA has classified MTBE as a possible human carcinogen [5]. Toxicity of MTBE to algae, invertebrates, and fish has been intensively studied [9–12]. No work, however, has been conducted on MTBE toxicity to terrestrial plants. In the literature review by Nellessen and Fletche [13], the response of vascular

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plants to organic chemicals was grouped into three categories: unique features, common features, and growth parameters. An acute phytotoxicity assay was undertaken in the laboratory to assess the toxicity of MTBE to weeping willows using plant transpiration rate as an end point of determination.

MTBE is quite persistent to abiotic decomposition, e.g., the natural attenuation of MTBE in aquifers is slow and, in some cases, undetectable, with half-life of at least 2 years [14]. A number of aerobic microorganisms were found to be able to degrade MTBE [15–18], however, it is still not feasible for field applications [19]. Although, the detailed degradation pathway of MTBE by microorganisms is understood poorly, one conclusive result was that *tert*-butyl alcohol (TBA) was the first stable intermediate from the degradation process. MTBE is initially attacked by cytochrome P450 (CYP) monooxygenase, with the probable formation of intermediate *tert*-butoxy methanol, and after the release of formaldehyde, with TBA as a product [14]. Human liver is also capable of actively metabolizing MTBE to TBA, and similarly cytochrome P450 enzymes play a critical role in the metabolism [20].

Plant uptake and transpiration of organic compounds is largely dependent upon octanol-water partition coefficient ($\log K_{ow}$), a measurement of a chemical's affinity for water versus lipid or fats. The process is most effective for pollutants with $\log K_{ow}$ in an optimum range of 0.5–3.0 [21,22]. Since the $\log K_{ow}$ for MTBE is 1.14, which is within the range that is readily transpired by plants, phytoremediation may be a feasible remedial technique for removing MTBE from contaminated sites. Plants possess various enzymes that can detoxify or degrade toxic chemicals [23,24]. CYP450s are widely distributed in the plant kingdom and play crucial roles in the plant metabolism, e.g., the hydroxylation of fatty acids, phenylpropanoid biosynthesis (lignin precursors), synthesis of terpenoids and other secondary metabolites, and also the metabolism of xenobiotics [25]. Phytoremediation of MTBE has been studied in two different continents and climatic zones [1,26–28]. However, no information is available concerning the phytoremediation of MTBE by terrestrial plants in China. The objective of this study was to provide quantitative data that can be used for the design of engineered phytoremediation of MTBE.

2. Materials and methods

2.1. Trees and exposure regimes

Weeping willow (*Salix babylonica* L.) was taken from those grown on the campus of Hunan Agricultural University, PR China. Tree cuttings of 40 cm long were removed from mature specimens and all used in this study were obtained from a single tree. They were placed in buckets of tap water at room temperature of 15–18 °C under natural sunlight until new roots and leaves appeared. After a 2-month period of growth, each of the pre-rooted cuttings was transferred into an individual 250 mL Erlenmeyer flask filled with approximately, 250 mL modified ISO 8692 nutrient solution (Table 1). All flasks were sealed with cork stoppers with the cutting in the center and silicon sealant (Dow Chemical Co., Midland, Michigan) to prevent escape of water or chemicals, and wrapped with aluminum foil to prevent algal growth. For each treatment concentration, six replicates were prepared. All flasks were housed in a climate control chamber maintained at a constant temperature of 25.0 ± 1 °C under continuous artificial light. The plants were conditioned for 48 h first to allow adaptation to the new environmental conditions. Then, the weight of the plant-flask system was measured individually. The flasks including the tree cuttings were weighed again after 24 h. By doing this way, the transpiration rate was determined. Trees with similar transpiration rate were selected for the tests. The nutrient solution for these trees was replaced by spiked solution, except for the controls. MTBE used was analytical grade with ≥95%. Six different treatment concentrations of MTBE were applied (0, 10, 25, 50, 100, 200, and 400 mg L^{-1}). The weight of each flask including the tree cutting was measured daily during the incubation.

Table 1
Composition of the modified ISO 8692 nutrient solution for hydroponic growing of tree in this study

Macronutrients (μmol L^{-1})		Micronutrients (nmol L^{-1})	
$NaNO_3$	2823.9	HBO_3	2992.1
$MgCl_2{\cdot}6H_2O$	59.0	$MnCl_2{\cdot}4H_2O$	2097.0
$CaCl_2{\cdot}2H_2O$	122.4	$ZnCl_2$	22.0
$MgSO_4{\cdot}7H_2O$	60.9	$CoCl_2{\cdot}6H_2O$	6.3
KH_2PO_4	246.0	$CuCl_2{\cdot}2H_2O$	0.1
$NaHCO_3$	1785.5	$NaMoO_4{\cdot}2H_2O$	28.9

The presence of MTBE in the aqueous solution, roots, stems, and leaves, was measured at the end of experiments. A mass balance was carried out to assess MTBE loss from hydroponic solution, recovery in different parts of plant materials, and possibly metabolism of MTBE during the transport in plants. The concentration of MTBE in solution was analyzed daily for a duration of 5 days to estimate the TSCF values.

2.2. Normalized relative transpiration

Trapp et al. [29] specially designed an acute toxicity test system for chemicals in water or in soil for willow cuttings. The weight loss of water subjected to MTBE exposure, compared to the initial loss prior to the exposure, was used to assess the toxicity. To compare the toxic effect on cuttings with different initial transpiration (before the toxicant is added), weight loss was expressed as relative transpiration. The transpiration was normalized with respect to the initial transpiration rate (to eliminate the necessity of cuttings with equal or similar initial transpiration) and with respect to the transpiration of the non-contaminated control cuttings (to include the effect of normal growth of the cuttings during the test). The mean normalized relative transpiration (NRT) was calculated by Eq. (1) below:

$$\mathrm{NRT}(C,t) = \frac{1/n\sum_{i=1}^{n} T_i(C,t)/T_i(C,0)}{1/m\sum_{j=1}^{n} T_j(0,t)/T_j(0,0)} \quad (1)$$

where C is the concentration (mg L^{-1}), t time period (h), T absolute transpiration (g h^{-1}), i replicate 1, 2,. . ., n, and j is control 1,

2,. . ., *m*. Controls always have the NRT of 100%; NRT < 100% shows inhibition of the trees' transpiration, which is usually connected with other effects (reduced growth, necrosis of leaves, and in severe cases, death). An inhibition of NRT of about 50% is typically accompanied by a complete inhibition of growth.

2.3. Chemical analysis

MTBE and the major degradation product TBA were analyzed using a purge and trap gas chromatography–mass selective detector (GC–MS) system. The GC was a Hewlett-Packard 6890 equipped with a HP-624 capillary column (25.0 m × 0.2 mm × 1.12 μm film thickness) silicone-coated, fused-silica capillary column (Hewlett-Packard HP-5MS or equivalent) and the MS was an Agilent 5973. Tekmar Heating Mantle was used to maintain the purging chamber at 40 ± 1 °C to improve purging efficiencies because MTBE and TBA did not purge equally. A Hewlett-Packard 6890 Series temperature-programmable GC equipped with pressure programming, split/splitless injection was used. The temperature was programmed at 35 °C for 4 min, then to 150 °C at a rate of 10 °C min^{-1}, and held at 150 °C for 2 min. The carrier gas used was helium. The injection port temperature was 250 °C and samples were injected using split mode at 10:1. A mass range of 28–600 amu was scanned in all electron ionization mass spectroscopy studies where the electron energy was 70 eV. For solution samples, 10–5000 μL water samples were directly injected, depending on the concentration of MTBE in the hydroponic solution.

Additional pre-treatment procedures were carried out prior to analysis of MTBE in plant materials. Willow roots, stems, and leaves were separately at the end of incubation, blot-dried, dipped into liquid nitrogen, pulverized, and placed in bottles for analysis of MTBE in each component of the biomass. All samples were stored at <6 °C and isolated from light until the concentration of MTBE was determined. MTBE in biomass was analyzed using the same techniques as aqueous solution samples. The samples were analyzed within 24 h.

2.4. MTBE transpired by plants

MTBE removed by transpiration was measured using a modified test chamber as illustrated in Fig. 1. Treated plants were prepared as described above and placed into a glass chamber (20 cm × 20 cm × 50 cm) with an air-flow system at 25 °C. Tubing from the outflow of the vessel was connected to a carbon tube that served as a trap for any airborne MTBE. The gas trap tube wrapped with aluminum foil was placed in a vessel and changed daily, after which all gas tubes were analyzed for MTBE. MTBE in the air was analyzed based on NIOSH Method 1615 as described by Rubin and Ramaswami [26]. Three replicates were conducted for each treatment groups. The duration of this test was 120 h.

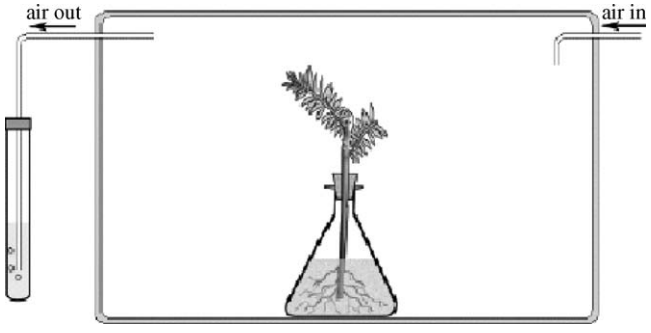

Fig. 1. A schematic diagram of the test chamber system for measuring MTBE transpiration into the air.

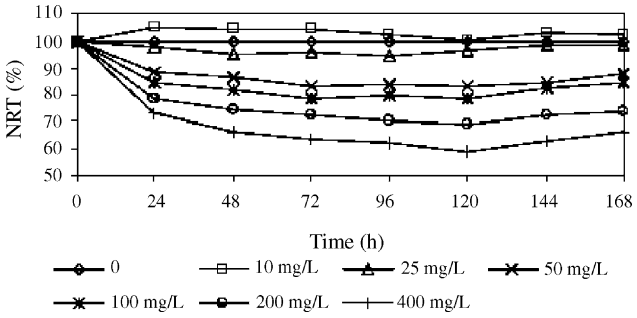

Fig. 2. Normalized relative transpiration NRT (%) of weeping willows (*Salix babylonica* L.) grown in hydroponic solution spiked with MTBE. Values are means of six replicates.

3. Results and discussion

3.1. Phytotoxicity of MTBE to weeping willows in hydroponic solution

Fig. 2 shows the normalized relative transpiration of weeping willows grown in hydroponic solution spiked with various levels of MTBE. In this study, the logarithm of NRT data were used and the correlation between the MTBE dosage and the NRT was very high ($R^2 = 0.9051$) (Fig. 3). At 10 mg L^{-1} MTBE, NRT was slightly higher than that of the non-treated plants. Negligible reduction of NRT was observed for the weeping willows exposed to 25 mg L^{-1} MTBE after 168 h. However, 25% reduction of the NRT was found for the treatment group exposed to 200 mg L^{-1} MTBE. When exposed to higher doses of MTBE (400 mg L^{-1}), weeping willows showed severe toxic symptoms after a short time period (120 h) as shown by a significant reduction in NRT of ≥35%. Chlorosis of leaves was not observed in all treatment groups for the whole duration of the test. It should be noted that

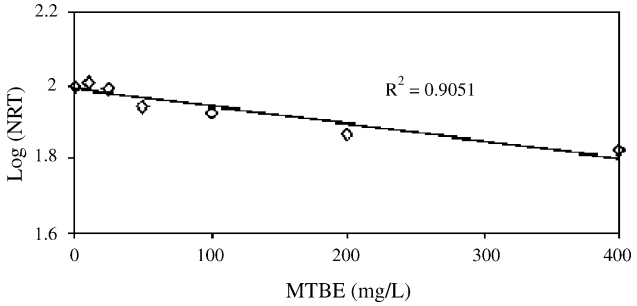

Fig. 3. Toxic effects of MTBE to weeping willows (*Salix babylonica* L.); linear regression with *R*-square values added.

NRT of all treatment groups showed a slightly increase after the initial 120 h of exposure, indicating the fact that large fraction of applied MTBE in hydroponic solution was removed by plants and the physiological functioning of plants was gradually restored afterward when MTBE concentration was lowered.

The effect concentration (EC_{10}) is defined as the concentration of a chemical, which produces 10% of the maximum possible response for a specific chemical. In this study, the response is a 10% inhibition of the maximum NRT observed. The slope of the linear regression of the exposure concentration against $\log_{10}$ (NRT) was used to calculate the EC_{10}-values. EC_{10}-values for weeping willows at 24, 48, 72, 96, and 120 h were 3.34, 1.67, 0.98, 0.62, and 0.53 g L^{-1}, respectively. A wide range of toxicity of MTBE towards bacteria, microalgae, invertebrate, amphibian, and fish was reported in the literature. A typical range of LC_{50} was between 57 and 1000 mg L^{-1} for invertebrate, and 388–2600 mg L^{-1} for vertebrate using mortality as the sensitive end points [11]. In microalgae, a measured EC_{50}-value of 184 mg L^{-1} for *S. capricornutum* was reported [10]. BenKinney et al. [10] and Hockett [30] reported that 96 h LC_{50} values were 681 and 542 mg L^{-1}, respectively, for *Daphnia magna*. Another common zooplankton species, *Ceriodaphnia dubia*, showed similar sensitivities; survival was significantly reduced when animals were exposed to 580 mg L^{-1} MTBE for 5 days. The no-observed-effect-concentration (NOEC) was 342 mg L^{-1} [30]. The lowest-observed-effect-concentration (LOEC) for growth was 388 mg L^{-1} with a NOEC of 234 mg L^{-1}. Toxicity was found to be slightly higher for rainbow trout (*Oncorhynchus mykiss*) [30]. In a study by An et al. [31], the combined LC_{50} and EC_{50}-values for lettuce (*Lactuca sativa*), wild oats (*Avena sativa*), wheat (*Triticum aestivum*), and sweet corn (*Zea mays*) were in the range of 18–91, 362–459, 432–751, and 672–964 mg MTBE kg^{-1}soil (dry wt), respectively, when growth of shoots and roots was used as the sensitive end points. Results from the current study indicated that weeping willows seem to be less sensitive than other species tested before.

3.2. MTBE uptake from hydroponic solution by weeping willows

In the controls without plant, a slight change of MTBE concentrations was detected in the solution over the entire period of exposure (data not shown), probably due to experimental errors from the handling and the headspace removal, but the disappearance of MTBE in aqueous solution can be attributed to the uptake by willows. Results also showed that all applied MTBE was removed from the hydroponic solution in the presence of willows for all treatment groups over a 168 h period of exposure.

The water, root, stems, and leaves were also analyzed for MTBE at the end of experiments. Small amounts of MTBE were detected in plant biomass. The highest concentration of MTBE in plant materials was found in the roots, followed by the stems. The lowest MTBE concentration in the biomass was in the leaves (Figs. 4–6). Concentrations detected in this study were lower than those of other reported tissue concentrations in the literature [28], possibly due to the different species and the physiological stages of plants used. A mass balance assessment of MTBE

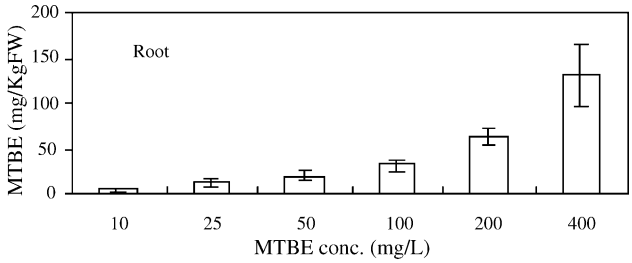

Fig. 4. Measured MTBE (mg CN kg^{-1} FW) in roots of plants exposed to different concentrations of MTBE. Values are means of six replicates. Vertical bars represent standard deviation.

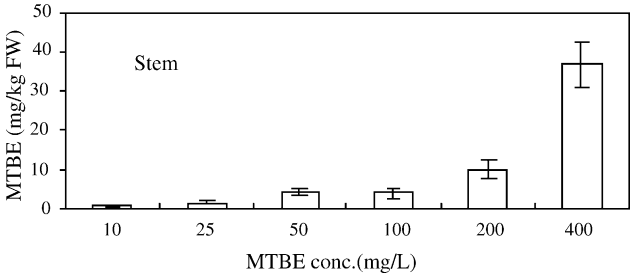

Fig. 5. Measured MTBE (mg CN kg^{-1} FW) in stems of plants exposed to different concentrations of MTBE. Values are means of six replicates. Vertical bars represent standard deviation.

recovered in plant biomass considering the initial MTBE used indicated that only 1.03–1.18% of MTBE were recovered in the plants as MTBE at the time of analysis after over 168 h of exposure. This was slightly lower than the values obtained in the studies by Ma et al. [28] and Hong et al. [1]. However, Newman et al. [32] found that approximately 0.4% of dosed MTBE were incorporated into hybrid poplar and eucalyptus tree tissues when the plants were exposed to 5 mg L^{-1} of MTBE solutions over a 3 day period. In our study, approximately 94% of the applied MTBE were found in the gas trap due to plant transpiration over the 120 h. No MTBE breakdown products were detected in the solution or the plant materials of all treatment groups. The mass balance of the overall experiments showed 3.46–4.89% of applied MTBE were lost due to the headspace removal. The percentage of MTBE in headspace obtained in this study was lower than that by Hong et al. [1], in the latter 5.9% of applied MTBE were detected in the headspace removal. Results from this study indicated that MTBE was quite recalcitrant to the attack by plant enzymes. However, it is susceptible to photo-

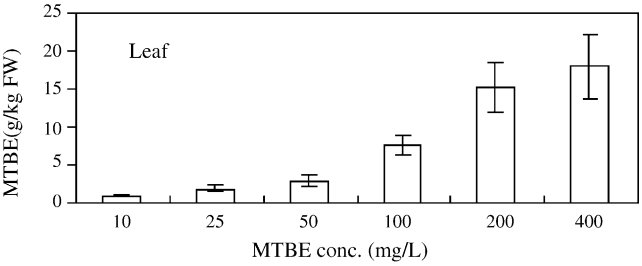

Fig. 6. Measured MTBE (mg CN kg^{-1} FW) in leaves of plants exposed to different concentrations of MTBE. Values are means of six replicates. Vertical bars represent standard deviation.

Table 2
Mass balance of MTBE in this study

Exposed concentration (mg L^{-1})	MTBE in solution (mg)		MTBE in tissue (μg)			MTBE transpired (mg)	MTBE headspace removal* (mg)
	Initial	Final	Root	Leaf	Stem		
10	2.5	ND	5.46 (0.679)	3.75 (0.735)	20.26 (5.964)	2.38 (0.674)	0.09
25	6.25	ND	14.52 (1.672)	13.07 (1.923)	42.29 (10.587)	5.92 (1.245)	0.25
50	12.5	ND	24.5 (6.897)	19.6 (4.321)	98.0 (16.543)	11.75 (3.231)	0.61
100	25	ND	45.13 (8.139)	31.59 (7.921)	192.38 (28.327)	23.76 (4.897)	0.97
200	50	ND	92.15 (13.452)	82.94 (11.972)	392.85 (67.453)	47.43 (8.654)	2.0
400	100	ND	188.02 (21.151)	94 (17.435)	752.12 (98.674)	94.74 (10.654)	4.23

Values are mean of six replicates for samples, except MTBE transpired (three replicates). In parenthesis: standard deviation. ND denotes concentrations below the limit of MTBE detection.

* MTBE in headspace removal = $\text{Mass}_{(\text{initial})} - \text{Mass}_{(\text{final})} - \text{Mass}_{(\text{tissue})} - \text{Mass}_{(\text{transpired})}$.

oxidation through vapor phase reactions with photo-chemically produced hydroxyl radicals with an estimated half-life of 4 days in the atmosphere [1] (Table 2).

3.3. Additional findings

Zhang et al. [33] used a six-channel system with alfalfa plants and found pronounced rhizosphere effects. Two bacterial strains capable of degrading MTBE were added to two channels. The vegetated channels with bacteria showed the lowest amounts of MTBE in the surface soil, which was lower than those with vegetation or bacteria alone. Hong et al. [1] found phytovolatilization was the dominant disappearance process for MTBE from vessels with poplar trees (*Populus deltoides* × *nigra* DN-34 Imperial Carolina). About 2.21% initial mass were detected in the plant biomass and no other metabolite was found by HPLC analysis using radiolabeling techniques. Rubin and Ramaswami [26] also found that 30% MTBE mass in water were removed by small poplar saplings (*populus deltoids* × *nigra*) over a 1-week period, at both high (1600 ppb) and low (300 ppb) MTBE concentrations. MTBE was detected in biomass at 100 ppb level, confirming passage of MTBE through the plant. A mass balance indicated that MTBE was largely untransformed during transport through the small poplar saplings to air. In the study of Trapp et al. [29], leaf cells from 28 Danish plant species of 15 families were all found to be unable to degrade MEBE, although trace amounts of the metabolite TBA was detected in the solution with roots of poplar (*Populus robusta*) and a willow hybrid (*Salix viminalis* × *schwerinii*). It is unlikely to conclude whether the small amount of degradation observed was really originated from plant cells or root-colonizing bacteria.

3.4. Determination of transpiration stream concentration factors (TSCF)

Organic contaminants in the solution phase may reach the root surface by mass transfer, penetrate the root, enter the xylem, and be transported in the transpiration stream. Once in the transpiration stream, chemicals may react with or be partitioned into plant tissues, be metabolized (detoxified) by plant enzymes, or escape by gaseous diffusion through stomata in leaves. Transpiration stream concentration factor, the concentration in the xylem sap with respect to the external solution concentration, can be used to characterize and estimate the ability of plants to extract the pollutant from contaminated media and then translocate it to its shoots.

Burken and Schnoor [22] used a simple mathematical description to estimate TSCF for woody plants (experiments were carried out on poplars).

$$\text{TSCF} = 0.756 \times \exp\left\{\frac{-(\log\ K_{\text{ow}} - 2.50)^2}{2.58}\right\} \quad (2)$$

This approach is only valid under the assumption of no diffusion occurrence into plant roots and no degradation within plants (Trapp, personal communication). The correlation predicted a TSCF value of 0.37 for MTBE.

A higher TSCF of 0.66 for MTBE was also calculated by the Brigg's equation (experiments were done with grass barley) [21]:

$$\text{TSCF} = 0.784 \times \exp\left\{\frac{-(\log\ K_{\text{ow}} - 1.78)^2}{2.44}\right\} \quad (3)$$

In this study, the relationship between percentage of MTBE mass removed from aqueous solution and volume of water transpired by trees was used to determine the TSCF value [26]:

$$\text{TSCF} = \frac{\mathrm{d}M\%}{\mathrm{d}V_{\text{transp}}} \times \frac{V_0}{100} \quad (4)$$

where $\mathrm{d}M\%/\mathrm{d}V_{\text{transp}}$ was the slope of the line plotting percent reduction in MTBE mass in water versus volume of water transpired; V_0 was the initial volume of water in the treated system. The factor of 100 incorporates for the % values plotted in the graph (Fig. 7).

The observed TSCF value for MTBE computed from the graphical method yielded an estimate of 1.12. A similar value of TSCF for MTBE was also reported by Rubin and Ramaswami [26]. However, a lower TSCF value for MTBE was experimentally determined to be approximately 0.5–0.8 by Hong et al. [1]. The calculated TSCF in this study was 1.12, indicating that MTBE was readily taken up by the roots of plants (but did not stay there longer), and then quickly translocated in the xylem to the leaves and evaporated without being metabolized.

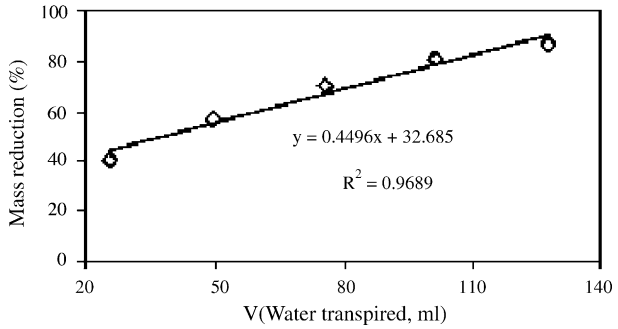

Fig. 7. Percent mass reduction of MTBE (%) vs. volume of water transpired (mL).

4. Conclusions

MTBE pollution in soils and groundwater occurs all over the world. The toxicity tests in hydroponic solution using the normalized relative transpiration as a sensitive end point showed that the short-term acute toxicity of MTBE to weeping willows was related to the dosage during the 168 h exposure period. Severe signs of toxicity were only observed at the treatment groups exposed to high doses of MTBE. Weeping willows were found to be able to extract MTBE from hydroponic solution due to plant transpiration. Small amounts of applied MTBE were detected in the tissues of plants. Mass balance studies showed that MTBE taken into plants from hydroponic solution was not being metabolized during transport and phytovolatilization was the only relevant removal process for MTBE. Although, MTBE is quite persistent to the acttack by plant enzymes, it is very susceptible to photo-oxidation in the atmosphere. These informations collectively suggest that phytoremediation of MTBE polluted soils and groundwater is an alternative to the present available remediation technologies.

Acknowledgement

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