

Original Research Paper

Influence of Ethanol and Methyl Groups on the Degradation of Xylenes in Soil

¹Irajá Nascimento, ²Ricardo Baldasso and ²Kira Manfredini

¹Center of Exact Sciences and Technology, University of Caxias do Sul, Caxais do Sul, Brazil

²Post-Graduation Program on Engineering and Environmental Sciences, University of Caxias do Sul, Caxais do Sul, Brazil

Article history

Received: 08-02-2015

Revised: 17-06-2015

Accepted: 16-07-2015

Corresponding Author:

Irajá Nascimento

Center of Exact Sciences and Technology, University of Caxais do Sul, Caxais do Sul, Brazil

Email: inascimf@ucs.br

Abstract: The main objective of the present study was to assess the influence of ethanol on the evaporation profile of xylenes (*ortho* + *meta* and *para* isomers) in active (with microorganisms) and inert (without microorganisms) soils. The vapors from four sealed flasks containing inert soil + neat xylenes, active soil + neat xylenes, inert soil + xylenes + ethanol and active soil + xylenes + ethanol were monitored during 20 days by Gas Chromatography using a Flame Ionization Detector (GC/FID). No statistical differences were observed comparing the concentrations of the *meta* and *para* isomers in all samples. The differences among the average concentrations of *meta*, *para* and *ortho* isomers were relevant only in the active soil samples without ethanol (95% confidence interval). Ethanol may enhance the *ortho* isomer volatilization and delay the degradations of *meta* and *para* isomers.

Keywords: Xylenes, Gas Chromatography, Volatilization, Biodegradation, Soil

Introduction

After the first oil crisis in the 1970 s, with the increasing pollution levels and the need for clean energy production, several nations around the world developed programs to support the use of alternative fuels, including ethanol and gasohol (a gasoline-ethanol blend), as automobile fuels. The main advantages of the ethanol-blend fuels are the reduction of greenhouse gas emissions and the higher octane number of gasoline (Fedrizzi *et al.*, 2013).

Despite the benefits provided by the mixture, the presence of ethanol in gasoline may affect the BTEX (benzene, toluene, ethylbenzene and xylenes) volatilization and these compounds may evaporate from spills of underground storage tanks or by accidental releases (Fedrizzi *et al.*, 2013). The gas transport through the vadose zone can be influenced by moisture content due to variations in gaseous permeability, phase partitioning and aerobic biodegradation (Nerantzis and Dyer, 2010).

The main target organ for acute and chronic exposures to xylenes are the central nervous system (Manuela *et al.*, 2012), the respiratory and cardiovascular systems and kidneys (Sarigiannis and Gotti, 2008).

In the case of soil contamination, remediation procedures are required, such as stabilization, containment or decontamination (Balseiro-Romero and Monteroso, 2012). The most common methods for

decontamination are: bioremediation employing special cultures (Cozzarelli *et al.*, 2010; Jahn *et al.*, 2005; Meckenstock *et al.*, 2012; Morasch *et al.*, 2004), phytoremediation (Boonsaner *et al.*, 2011) and bioaugmentation (Fantroussi and Agathos, 2005). However, despite the recent study published by Chemlal *et al.* (2012), there is a lack of studies evaluating the degradation/volatilization of xylenes isomers in natural soil (without introduction of exogenous cultures) and in presence of ethanol.

The purpose of the present study is to determine the influence of ethanol on the degradation/volatilization of the xylene isomers in crude soil.

Materials and Methods

Soil Samples

The method of soil collection was modified from Cardoso *et al.* (2011). The sample was composed by 100g of soil collected near the Center of Exact Sciences and technology of the University of Caxias do Sul, from a depth of 10 cm and placed in a plastic bag. The soil was dried at room temperature and passed through a 2 mm sieve.

The physical-chemical characteristics of the soil were determined based on Cachada *et al.* (2012). The measured parameters were: Moisture (by gravimetry);

Total Organic Carbon (TOC, by Walkley method, external heat); Total Nitrogen (TN, by Kjeldahl method); Total Phosphorus (TP, by acidic double extractor, Mehlich-1) and Total Volatile Solids (TVS, by gravimetric method).

Microcosm's Preparation

The microcosms were prepared in transparent glass serum bottles (50 mL capacity, base diameter = 3.3 cm and high = 4.8 cm) as follows:

- Inert soil: Ten grams of soil and a serum bottle were treated in an autoclave (121 °C for 15 min). One milliliter of the xylenes standard solution (750 mg L⁻¹ in *n*-hexane) was added to the sample that was homogenized with a glass stick, closed with rubber caps and aluminium rings and left to rest overnight
- Active soil: The same procedures (item a) were applied, except by the autoclave step
- Inert soil + ethanol: The same procedures (item a) were applied, plus the sample contamination with the mixture of xylenes standard solution (750 mg L⁻¹ in *n*-hexane) and ethanol (25% (v/v))
- Active soil + ethanol: The same procedures (item a) were applied, except by the autoclave step, plus the mixture of xylenes standard solution (750 mg L⁻¹ in *n*-hexane) and ethanol (25% (v/v)). After the solvent evaporation the theoretical concentration of xylenes was 0.75 mg g⁻¹

Figure 1 illustrates the microcosm's preparation.

Vapors Collection

The vapours in the microcosms were sampled using a gas-tight syringe (3 mL). Two mL of the vapours were transferred to autoinjector vials (1.5 mL of capacity with a screw top). The autoinjector vials were previously evacuated by flushing 3 mL of the inner air. The vapours were injected (2.0 µL in triplicate) in the injection port of a gas chromatograph. After each sampling, 20 mL of the headspace vapours were flushed with a gas-tight syringe to force the system to reach a new equilibrium state by mass transfer of xylenes to the headspace. In this way, the environment inside the microcosms was gradually oxygen depleted. The interval between each sampling was 24 h. Even though phase equilibrium should be within in few hours for NAPL (Non-Aqueous Phase Liquids) such as xylenes, the interval of 24 h was necessary due to the GC availability for the instrumental analysis. The experimental period was of 20 days.

Instrumental Analysis

Instrumental analysis was performed with a gas chromatograph (Agilent 7820) equipped with autoinjector (7693A) and flame ionization detector.

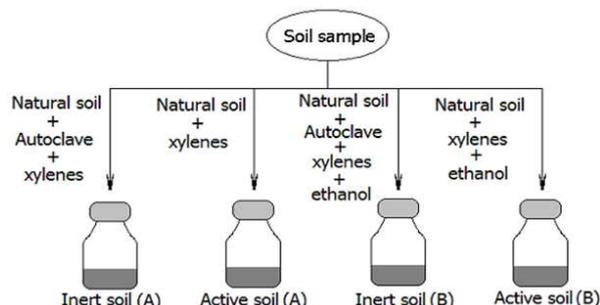


Fig. 1. Microcosm's preparation

A 30 m column (HP-5, film thickness 0.32 mm, internal diameter 0.25 mm) was temperature programmed from 40°C (held for 10 min) to 220°C (held for 0 min) at 5°C min⁻¹. Helium was the carrier gas with a flow rate of 1.0 mL min⁻¹. The Limit of Quantitation (LOQ) was evaluated by injection of the headspace vapours of standard solutions at 0.3, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 mg L⁻¹ obtained by successive dilutions of a xylenes standard solution in *n*-hexane (50 mg L⁻¹).

In the present work the LOD is defined as the lowest amount of an analyte in a sample that can be quantified with acceptable precision and accuracy (Shrivastava and Gupta, 2011). The LOQ was calculated from the signal-to-noise ratio of 10 (Shrivastava and Gupta, 2011).

Kanai *et al.* (2005) used the intermolecular forces theory to explain the chromatographic separation of *meta* and *para* isomers of xylenes. However, in the present study we refer to these compounds as *m+p*-xylenes since their retention times were very close in the gas chromatographic analysis. This approach seems to be usual, according to the publications of (Eshaghi *et al.*, 2011; Simantiraki *et al.*, 2013).

For the LOQ calculations, the relation between the cylinder volume in the headspace ($\pi R^2 h$) and the chromatographic peak area were used. The results were expressed in mg/cm³.

Results

For the *m+p*-xylenes, the LOQ was 0.8115 mg/cm³ and the detector response was linear between 0.8115 and 3.246 mg/cm³ ($R^2 = 0.9972$; equation: $y = 4419.1x - 1546.5$). For the *o*-xylene, the LOQ was 0.4058 mg/cm³ and the detector response was linear between 0.4058 and 6.492 mg/cm³ ($R^2 = 0.9103$; equation: $y = 1637.5x - 2741.8$). For the soil samples without ethanol no differences between the concentrations of the *m+p*-xylenes isomers in the headspace of the inert and active soil were observed (this is the null hypothesis and the *p*-value was > 0.05). On the other hand, considering the final and initial collection days, the concentration of *o*-xylene in the active soil's headspace decreases by 31.95% (Fig. 2) (null hypothesis: No decrease of the concentration of *o*-xylene during the experimental time, *p*-value < 0.05). This difference was relevant in a 95% confidence interval.

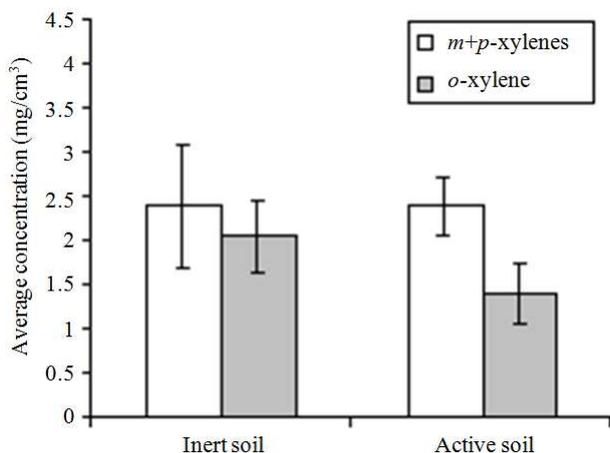


Fig. 2. Degradation of *m+p*- and *o*-xylene in inert and active soils whitout ethanol. Error bars represent standard deviations

Discussion

The results suggest that polarity may exert a key role on the xylenes degradation. The Table 1 shows that the *m+p*-xylenes isomers are by far less polar than the *ortho*-xylene. This may explain the lower concentration of *ortho*-xylene in the active soil's headspace.

According to Sun and Cupples (2012), the initial step of the biodegradation depends on electron transfer reactions. In this way, the most polar isomer may suffer higher degradation.

This hypothesis is supported by several studies focused on the degradation of xylenes. Early reports of anaerobic xylenes degradation under nitrate-reducing conditions indicated that both *para* and *meta* (but not *ortho*) xylene were degraded. In some cases the presence of *meta*-xylene inhibited concomitant *ortho* and *para* xylene degradation (Jahn *et al.*, 2005; Meckenstock *et al.*, 2012; Morasch *et al.*, 2004).

Cozzarelli *et al.* (2010) have used an *in situ* microcosm in a crude-oil contaminated aquifer to measure the biodegradation of benzene, toluene, ethylbenzene, *ortho*, *meta* and *para*-xylenes and four pairs of C₃- and C₄-benzenes over a 3-year period. According to the authors the apparent order of persistence is ethylbenzene > benzene > *meta*, *para*-xylenes > *ortho*-xylene ≥ toluene.

However, none of the studies cited above were performed with crude soil (without chemical modification or introduction special cultures). Also there is a lack of publications evaluating the specific influence of ethanol on the biodegradation of the xylene isomers. Furthermore, no explanations were found about the differences of the degradation kinetics of those compounds. In this way, the results obtained in the present study cannot be directly compared.

Table 1. Dipole moments of ethanol and xylenes (Fedrizzi *et al.*, 2013)

Compound	Dipole moment (D)
Ethanol	1.69
<i>o</i> -xylene	0.59
<i>m</i> -xylene	0.30
<i>p</i> -xylene	0.02

Another reason for the higher degradation of *ortho*-xylene may be the spatial hindering caused by the methyl groups (Fig. 3). According to Balseiro-Romero and Monteroso (2012), the presence of a methyl group at the *meta* or *para* positions of the benzene ring would impart a greater sorptive affinity in soil than a methyl group at the *ortho* position in the ring. A possible steric repulsion between the two adjacent methyl groups in *ortho*-xylene could lower its sorption compared with *meta* and *para*-xylene. In this way, the methyl groups in the *ortho* position may be more available for biodegradation than the *meta* and *para* methyl groups. Kube *et al.* (2004) proposed that the initial step of the toluene biodegradation, at anaerobic conditions, is the oxidation of the methyl group.

Figure 4 shows the effect of ethanol on the volatilization of xylenes in the active soil with ethanol.

The concentrations of the *m+p*-xylenes in the active soil samples were not different in a 95% confidence interval (this is the null hypothesis and the *p*-value was > 0.05). On the other hand, the highest increase of the *ortho* isomer concentration in the ethanol sample was relevant (null hypothesis: No increase of the of the *ortho* isomer concentration, *p*-value < 0.05).

Due to its higher polarity (Table 1) the *ortho* isomer may be preferentially soluble in ethanol, reducing the mixture boiling point. This effect is called cosolvency and may increase the *o*-xylene volatilization.

Many works has been published regarding the cosolvency promoted by gasoline-ethanol mixtures. According to Yu *et al.* (2009), the proportions of BTX (benzene, toluene and xylenes) in ethanol-amended gasoline are changed by the cosolvent behavior of ethanol. Cataluña and Silva (2006) described the development of a device to determine the vapor pressure and the vaporization enthalpy of formulations containing volumes of 5, 15 and 25% of ethanol in four base gasolines. Their results showed that the addition of ethanol to the gasoline hydrocarbons generates a mixture with a boiling point smaller than the original.

Ethanol and *n*-hexane showed very close retention times at the instrumental analysis conditions adopted in the present study. This means that complete chromatographic separation was not achieved. The variation of ethanol concentration was obtained by differences between the peak solvent areas of the samples with and without ethanol, at each sampling. Because of this, the following discussion will be developed in relation to the percent chromatographic peak areas that were calculated by comparison of the initial (100%) and final absolute peak areas.

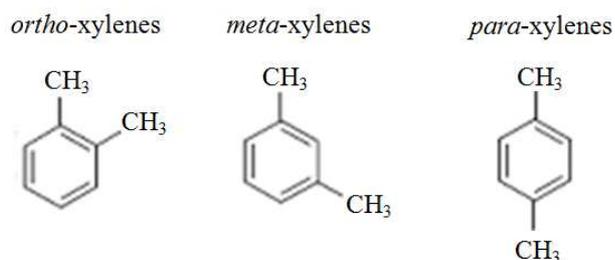


Fig. 3. Chemical structures of the xylene isomers

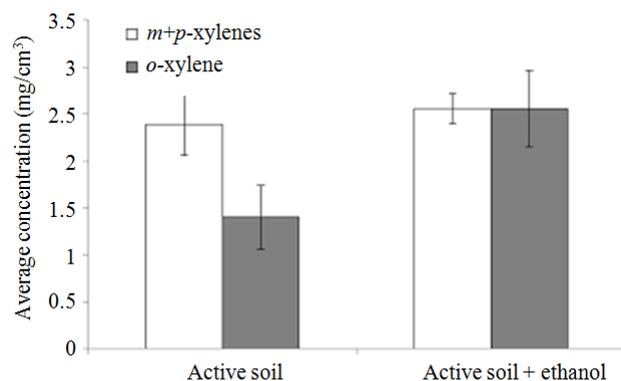


Fig. 4. Effect of ethanol over the degradation and volatilization of xylenes in active soil. Error bars represent standard deviations

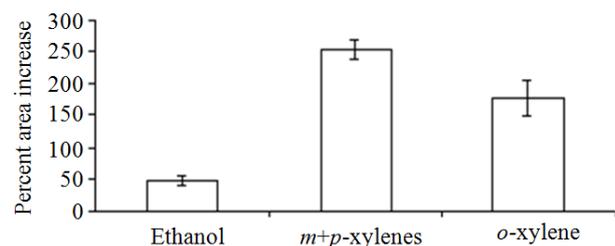


Fig. 5. Percentual area increase of ethanol, *m+p* and *o*-xylenes, in the samples of active soil + ethanol. Error bars represent standard deviations

As can be seen in Fig. 5, ethanol presents the lowest percent area increase in the active soil + ethanol samples.

These results may be explained by the preferential degradation of ethanol. According to Österreicher-Cunha *et al.* (2009), ethanol enhances BTEX retention in soil, boosts microbial activity but delays BTEX biodegradation.

Conclusion

In the active soil with ethanol occurs the preferential degradation of ethanol and the xylenes degradation may be delayed. In this way, considering a spill of gasoline/ethanol the xylenes may be retained in soil and may reach surface and underground water sources. The

volatilization of the *ortho* isomer is favored by ethanol (cosolvency effect).

The degradation of the *meta* + *para* isomers was not affected by ethanol. Comparing the inert and active soil samples, without ethanol, the degradation of the *meta* + *para* isomers was lower than the *ortho*-isomer, suggesting that the compound polarity may exert an important influence over the degradation rate.

Acknowledgement

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

Author's Contributions

Irajá Nascimento: Conceived this study and wrote the paper.

Ricardo Baldasso: Performed GC investigation, interpreted the results and critically reviewed the manuscript.

Kira Manfredini: Performed GC investigation, interpreted the results and critically reviewed the manuscript.

All authors read and approved the final manuscript.

Ethics

No ethical issues may arise after the publication of this manuscript.

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