In Situ Evaluation of Benzene and Toluene Biodegradation in a Large Block of Gneissic Residual Soil

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Abstract. Numerous socio-economic benefits are associated with the oil industry. However, problems cannot be neglected, resulting in many accidents that can occur at any production stage of this industry. A serious problem created by this industry is environmental contamination by hydrocarbons. Gasoline in contact with infiltration water, the aromatic constituents, in particular the BTEX group, partially dissolve being the first contaminants to reach the groundwater. Studies published in the literature indicate that biological degradation is the main mechanism responsible for reducing the concentration of these toxic pollutants. Laboratory studies of this kind have been conducted in small scale columns, where the biological field conditions are not well reproduced. However, studies conducted in a larger scale with structured soil, remaining closer to field conditions, evidenced biodegradation processes of BTEX. This paper presents the *in situ* construction, instrumentation and monitoring of a large rectangular block of undisturbed residual gneissic soil, subjected to a horizontal flow of a contaminant solution composed of water, benzene and toluene to evaluate the biodegradation of these compounds in different points of the block over time. During the period of 85 days, samples of the percolating solution were periodically collected from pre-established monitoring points and the concentrations of benzene and toluene were determined using a coupled technique of gas chromatography and mass spectrometry (GC-MS). Based on the monitoring results, decay in the concentrations of the compounds over time and along the length of the block was observed. These results indicated, consistently with the biological characterization of the soil that showed the existence of microbial degrading activity, the occurrence of biodegradation of benzene and toluene, and, by an extraction process, the retention of these compounds in soil was also verified.

Keywords: contamination, soil block, instrumentation, biodegradation, benzene, toluene.

1. Introduction

Since the oil crisis in the seventies, Brazil has experienced a strong economic growth that increased oil demand and required large restructuring of the entire chain of oil production, from the discovery of new oil fields and the formation of various petrochemical plants to the increase in distribution networks. As a consequence of this huge and complex structure for production and commercialization, the oil industry deals daily with environmental accidents caused by leaks and spills that occur during the exploration, refining, transportation and storage operations of oil and its derivatives (Corseuil & Marins, 1997; Silva, 2002).

Benzene, toluene, ethylbenzene and xylenes (BTEX), hydrocarbons present in gasoline, show great mobility in water since they are the most water soluble oil compounds,

and they also are the most recalcitrant. Benzene is the most toxic and high concentrations of toluene may be toxic to microorganisms, hampering biodegradation. Thus, in a possible leakage of gasoline, these compounds will be the first to percolate through the soil and reach the water table or aquifer (Alvarez & Illman, 2006).

A variety of techniques (natural attenuation, bioventilation, etc.) has been used to remove dissolved hydrocarbons from soil and groundwater. However, natural attenuation has been noted for its efficiency and low cost. This technique is based on monitoring the environment decontamination by natural environmental factors (diffusion, dispersion, volatilization, sorption, biodegradation and chemical reactions) arising from physical, chemical and biological processes. The biodegradation technique reduces

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the mass of contaminants by degradation of organic compounds provided by the action of living microorganisms, which may transform these compounds into harmless ones, limiting the transport of petroleum hydrocarbons to groundwater (Corseuil & Alvarez, 1996; Fetter, 1993; Wiedemeier *et al.*, 1996; Jacques *et al.*, 2007; Corseuil & Marins, 1998; Mazzuco, 2004).

Biological activity is capable of mineralizing or biotransforming organic compounds. Biotransformation consists in the partial degradation of compounds in one or more compounds that may or may not be less toxic than the original substance. Mineralization represents the complete degradation of organic molecules on inorganic substances such as carbon dioxide (CO_2) and water (Jacques *et al.*, 2007; Corseuil & Marins, 1997).

The degradation of contaminants in soil by microorganisms occurs if: the appropriate degrading microorganisms are naturally present in the soil; the amount of contaminant is not toxic to the microbial population; the environment is adequate and stable so as to allow the survival of the adapted autochthonous microbial population, that is, pH is ideally not excessively acidic or alkaline, or alternately, does not present important fluctuations that could cause impact to the microbiota; moisture is adequate; and there are available nutrients (Mariano, 2006). Thus, depending on the amount of microorganisms in the soil and the type of contaminant, biological activity may be important in reducing the amounts of contaminants and therefore their transport and dispersion. The bioremediation technique has been widely studied and used as a strategic mechanism of transformation of toxic organic compounds (*e.g.* benzene and toluene) into less toxic products (*e.g.* carbon dioxide and water) and the elimination of these compounds from soil and aquifers (Alvarez & Illman, 2006).

A large number of field studies to evaluate soil and groundwater contamination by petroleum products have been developed in the Northern Hemisphere (Borden, Ontario, Canada, Cape Cod, Massachusetts, United States). In Brazil, the field experiments carried out in *Fazenda Ressacada* (Santa Catarina, Brazil) are noteworthy. However, in all of these cases, the experiments were carried out with sandy soils (Vicente *et al.*, 2009).

Laboratory studies have reported the effects of various environmental factors on the biodegradation of BTEX compounds. Many of these researches have been conducted in micro reactors and soil columns (Mohammed & Allayla, 2000). Studies with bigger microcosms and natural soil give a better idea of real processes as they remain closer to field conditions. Previous research has shown the degradation potential of regular and ethanol-amended gasoline in undisturbed Brazilian soils (Corseuil & Marins, 1998; Österreicher-Cunha *et al.*, 2004, 2007).

As field conditions cannot be fully reproduced in the laboratory, and microbial populations are extremely sensitive and reactive to shifts in their environment, up scaling is crucial to determine whether microcosms data are representative of actual environmental processes; thus the importance of *in situ* tests.

Since *in situ* studies of biodegradation in tropical soils contaminated by petroleum components are scarce, the primary objective of the research reported herein was to build, instrument and monitor an *in situ* experiment carried out in an undisturbed large block of soil isolated from a residual soil mass of gneissic origin in order to assess biodegradation of benzene and toluene under saturated conditions. The paper describes and discusses the experimental issues associated with the experiments and the results obtained.

2. Materials and Methods

The experiment consisted in isolating *in-situ* a large block from a residual soil mass of gneissic origin and subjecting it during a time interval of three months to a permanent flow of water containing dissolved toluene and benzene. During this time, samples of water were collected from the block and concentration of the contaminants measured.

2.1. Materials

2.1.1. Soil

a) Geotechnical characterization

The experiment was constructed at the Waste Mechanics Laboratory of the Federal University of Viçosa. The block was excavated in the C horizon of a gneissic residual soil profile. The stratigraphy and soil texture were defined based on results of standard penetration tests and visual and tactile analyses performed by Jesus (2009).

The soil was characterized by means of geotechnical tests conducted according to ABNT standards NBR-7181/84 and NRB-6459/84, NRB-7180/84, and NRB-6508/84 - clay fraction mineralogical analysis, chemical and physicochemical analyses and scanning electron microscopy (SEM). According to the Unified Soil Classification System (USCS), the soil was classified as clay of high plasticity (CH). Soil geotechnical properties are listed in Tables 1 and 2.

The saturated soil hydraulic conductivity (ksat) was also determined. For permeability determination, three tests were carried out in the laboratory using a variable head permeameter. The average saturated permeability obtained from these samples is 3.78 E-06 ms^{-1}.

b) Clay mineralogy analysis

X-ray analysis was conducted with a Rigaku D-Max diffractometer equipped with a cobalt tube (Co-Kα radiation) and a graphite curved crystal monochromator operated at 45 kV and 30 mA.

For mineralogical analyses, soil samples were obtained from the C horizon of a gneissic residual soil profile. The X-ray analysis of the soil clay fraction was performed

Table 1 - Soil grain size distribution and Atterberg limits.

Grain size distribution (%)				Atterberg limites (%)		
Clay	Silt	Sand	Gravel	[1] w_L	[2] w_P	[3] I_P
58	14	28	0	66	32	34

[1] w_L – liquid limit; [2] w_P – plastic limit and [3] I_P – plasticity index.

Table 2 - Soil physical indexes.

G_s kN m⁻³	γ_n kN m⁻³	γ_d kN m⁻³	n	e	S (%)	w (%)
27.77	15.97	12.87	0.54	1.16	58.42	24.49

G_s- solids unit weight; γ_n– soil natural unit weight; γ_d- soil dry unit weight; n – porosity; w– water content;
e – void ratio; S – saturation degree.

in two different types of samples: (i) prepared with natural clay by the paste method for orientation of the clay minerals; (ii) prepared after treating the clay to remove iron oxides, to improve the identification of clay minerals and/or aluminum oxides, possibly present in the soil sample. This analysis was performed on two soil samples.

Iron oxides were removed with sodium bicarbonate citrate dithionite, according to the methodology described by Mehra & Jackson (1960). The evaluation of the diffractograms resulting from X-ray analysis of the clay fraction before and after the removal of iron oxides allowed defining the clay soil as consisting of kaolinite (Ka) and gibbsite (Gb) with traces of goethite (Gt) and hematite (Hm).

c) Scanning electron microscopy (SEM)

A microphotograph of a deformed soil sample was produced with a scanning electron microscope (SEM) in order to determine the chemical elements present in soil block. Table 3 presents concentration percentages of the chemical elements found in five points of the analyzed soil sample. The results show higher percentages of oxygen (O), aluminum (Al), iron (Fe) and silica (Si).

d) Chemical and physicochemical properties

Chemical and physical-chemical properties of the soil are shown in Table 4.

Table 3 - Concentration percentage of chemical elements found in soil sample.

Point	O	Al	Si	K	Ti	Fe
1	38.54	25.06	28.04	0.46	ND	7.89
2	ND	ND	ND	ND	ND	100.00
3	32.14	19.03	18.63	ND	1.70	28.50
4	55.18	20.50	22.11	ND	ND	2.21
5	41.07	16.80	23.24	ND	1.40	17.50

ND – Not detected.

Table 4 - Chemical and physicochemical analyses of the soil.

P (mg dm⁻³)	K (mg dm⁻³)	Zn (mg dm⁻³)	Fe (mg dm⁻³)	Mn (mg dm⁻³)	Na (mg dm⁻³)	Cu (mg dm⁻³)	Cr (mg dm⁻³)	Ni (mg dm⁻³)	Cd (mg dm⁻³)	Pb (mg dm⁻³)	pH
0.80	37	1.28	22.50	5.00	10.20	0.58	0.32	0.19	0.0	0.06	5.29

Ca^{2+} ($cmol_c$ dm⁻³)	Mg^{2+} ($cmol_c$ dm⁻³)	Al^{3+} ($cmol_c$ dm⁻³)	H+Al ($cmol_c$ dm⁻³)	SB ($cmol_c$ dm⁻³)	$CEC_{(t)}$ ($cmol_c$ dm⁻³)	$CEC_{(T)}$ ($cmol_c$ dm⁻³)	ISNa (%)	OM (dag kg⁻¹)	OC (dag kg⁻¹)
0.80	0.12	0.0	0.50	1.05	1.05	1.55	4.22	0.64	0.37

$CEC_{(T)}$ - Cation Exchange Capacity
$CEC_{(t)}$ - Effective Cation Exchange Capacity
SB - Sum of Exchangeable Bases OM - Organic Matter (OM = OC x 1,724 - Walkley-Black OC - Organic Carbon

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Considering the mineralogical composition of the clay particles (in high percentage) and its low organic matter content, the studied soil should have little capacity of retaining benzene and toluene or, at least, of retarding their migration.

According to the values showed in Table 4, the natural soil presents the following characteristics:

- Acidic pH, which is generally associated with the presence of aluminum and manganese in toxic concentrations, and low levels of cations such as Ca^{2+} and Mg^{2+}. The acidity may decrease the microbial population of organic matter decomposers, depending on soil characteristics and environmental conditions.
- Low percentage of organic carbon. The organic matter content is the soil characteristic that most influences sorption of hydrocarbons (Jacques *et al.*, 2007).
- The amount of inorganic contaminants (zinc, copper, nickel, iron, chromium, cadmium, lead and manganese) is within the thresholds established by the Soil and Groundwater Guiding Values (CETESB, 2009).
- According to calcium and magnesium contents, the soil is classified as a low fertility soil by the Soil Fertility Commission of the State of Minas Gerais - CFSEMG (1999).
- The soil presents low effective cation exchange capacity, according to the chemical classification of the CFSEMG (1999).

e) Microbiological analysis

Microbiological activity in soil was determined on five soil samples collected in an representative area of the experiment, according to the methodology for measuring hydrolysis of fluorescein diacetate (FDA), following a protocol adapted from the Adam and Duncan (2001) and Green *et al.* (2006) methodologies (Österreicher-Cunha *et al.*, 2007).

The results summarized in Table 5 show low microbial degrading activity considering that the samples were retrieved from a superficial soil in area covered by vegetation, and that the soil contains more than 50% of clay in its composition. Activity levels detected (mean of 0.829 mg fluorescein.min^{-1}.g^{-1}) were close to those found in stable undisturbed subsurface Brazilian soils and in stabilized contaminated soil undergoing biodegradation of organic compounds (Österreicher-Cunha *et al.*, 2007, 2012).

Table 5 - Total degrading activity.

Sample	µg fluorescein.g^{-1} soil.min^{-1}	Standard deviation	Depth (m)
A	0.831	0.162	0.40
B	0.836	0.515	0.40
C	0.928	0.299	0.40
D	0.607	0.000	0.50
E	0.943	0.093	0.45

2.1.2. Contaminant solution

An aqueous solution prepared from the mixture of benzene, toluene and water from an artesian well was used in the experiment. The concentration of each compound in solution was set within the limit of solubility of each (benzene: 1800 mg L^{-1} and toluene: 500 mg L^{-1}).

Forty five liters of contaminant solution were required per day to feed the upstream reservoir. This volume was determined based on the water flow in the block for steady state conditions.

The water from the artesian well showed neutral pH (6.94) and a high concentration of dissolved oxygen (5 mg O$_2$ L^{-1}) both favorable to microbial survival; low concentration of potassium, phosphorus and nitrogen; and low concentrations of chemical substances (*e.g.* calcium, magnesium, aluminum, lead, iron, cadmium, silver and sodium). The water was considered appropriate to perform the experiment.

2.2. Methods

2.2.1. The block construction

The block subjected to the experiment is prismatic in shape having dimensions 1.5 m long; 0.60 m high and 1.0 m wide. Features of the block geometry, lay-out, location, instrumentation and constructive details are shown in Figs. 1 to 9.

Figure 1 shows the covered area where the experiment was carried out.

The construction of the undisturbed block started with the excavation of its lateral faces using appropriate tools. The lateral faces (length direction) were sculpted with dimensions 1.0 m high and 2.20 m long. In sequence, the block was involved with paraffin to retain its natural water content (Fig. 4).

The lateral faces (width direction) remained in contact with the ground until the end of the bottom excavation. Later these faces were excavated for the construction of the reservoirs.

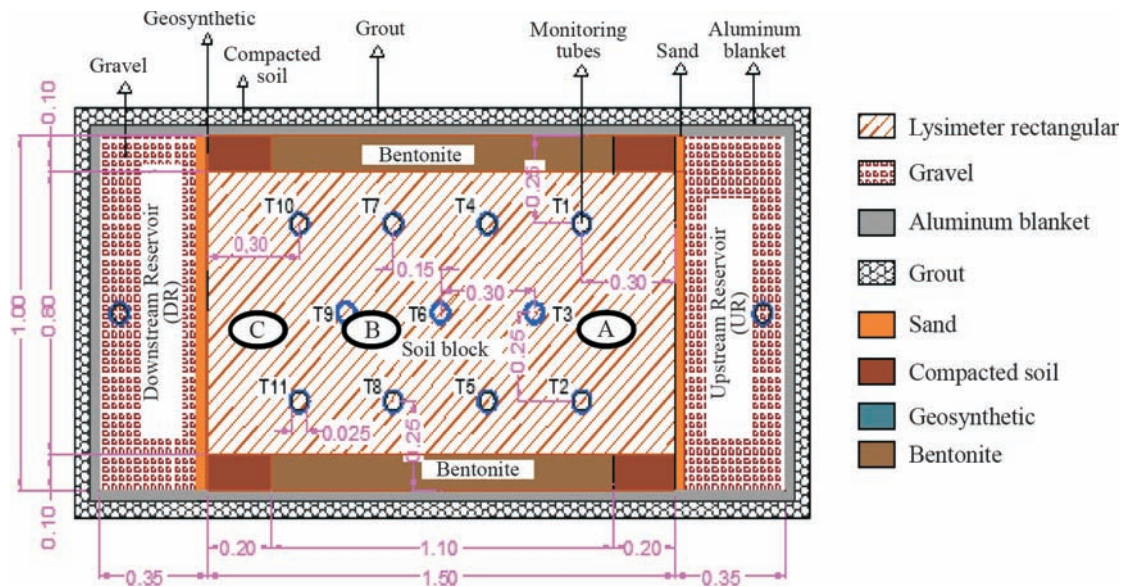**Figure 1** - Covered area where the soil block was constructed.

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Figure 2 - Plan view of the block detailing the arrangement of monitoring tubes in the block and the upstream and downstream reservoirs.
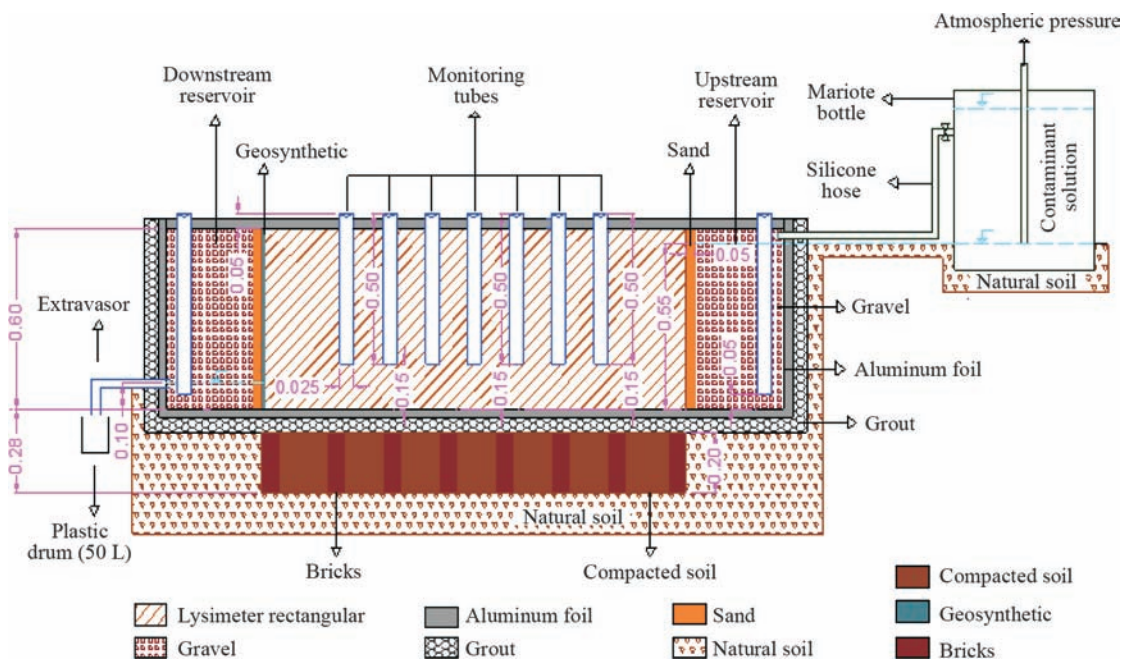

Figure 3 - Longitudinal section showing the arrangement of tubes, tanks and Mariotte bottle.

The bottom of the block was then progressively excavated in two stages. In the first stage 0.40 m of the bottom part of the height was removed, leaving it with a depth of 0.60 m. Using appropriate tools, the base was trimmed as near as possible of a horizontal plan. At each stage, the soil was removed, an aluminum foil was, step by step, positioned at the bottom face, and a wood panel (0.10 m high, 1.10 m wide and 1.10 m long) supported by bricks, was placed below and the empty space left was filled with a cement mass (grout). Once the grout was dry, the pre-fabri-cated wood panel was removed. The cement layer below the bottom of the block was supported by bricks and the void space between the bricks filled with soil as shown in Figs. 3 and 5.

The upstream and downstream reservoirs, 0.35 m long, were dug in the sequence. As a consequence, the block length was reduced to 1.50 m. After the excavation of the upstream and downstream reservoirs the block was partially wrapped with aluminum foil as seen in Fig. 6.

In order to stiffen the block, cement walls, 0.10 m thick, were built around it. These walls were also covered with aluminum foil to avoid sorption of hydrocarbons by the cement, by the time the block was contaminated by the benzene and toluene solution (Fig. 7).
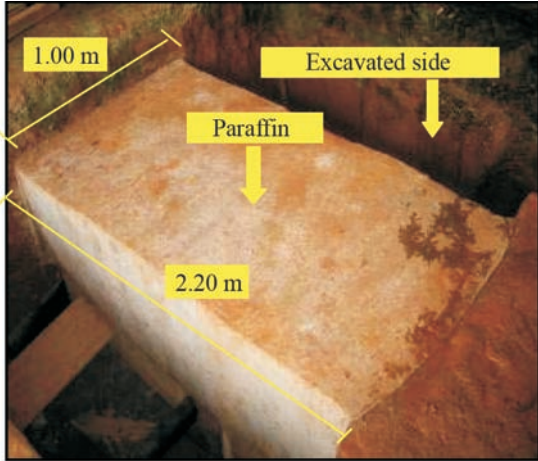
In order to avoid preferential flow between the soil and the aluminum foil on the side walls, a narrow strip of soil, 0.10 m wide, was excavated and filled with soil and bentonite as shown in Fig. 8.

In order to offer extra support to the side walls of the block, the empty space behind them was filled out with compacted soil. In sequence, both reservoirs were filled

Figure 4 - Side excavation and block involved with paraffin.

Figure 6 - Excavation of the upstream (UR) and downstream (DR) reservoirs.
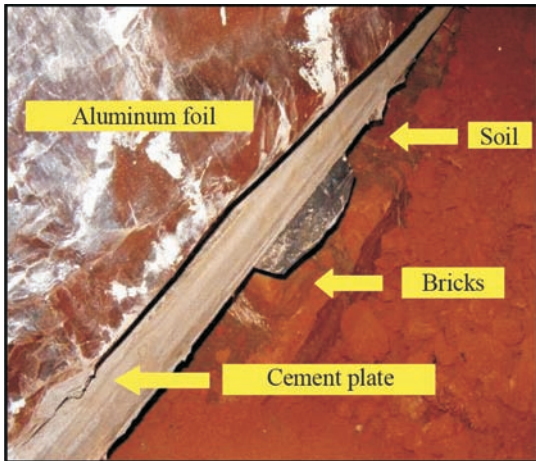

Figure 5 - Arrangement of materials at the base of the block.
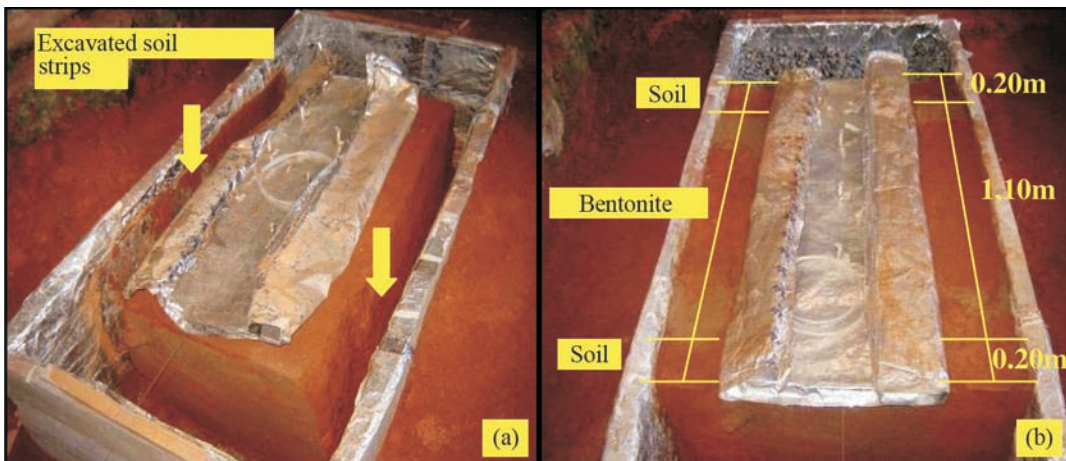

Figure 7 - Side cement walls.

Figure 8 - (a) Excavated soil strips; (b) Compacted soil and bentonite along the length of the block.

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with gravel to avoid disaggregation of soil particles in the block due to moisture. A 0.05 m wide layer of sand was added to avoid soil clogging and damage of the soil surface by the gravel. In the downstream reservoir, between the block face and the sand layer, a geosynthetic was placed to function as a filter, preventing soil particles to enter the reservoir. An overview of the soil block after construction is presented in Fig. 9.

2.2.2. Monitoring tubes

Eleven monitoring tubes, one inch in diameter and fifty centimeters in length, made of aluminum were installed along the length of the soil block, 0.15 m from the base (Fig. 10).
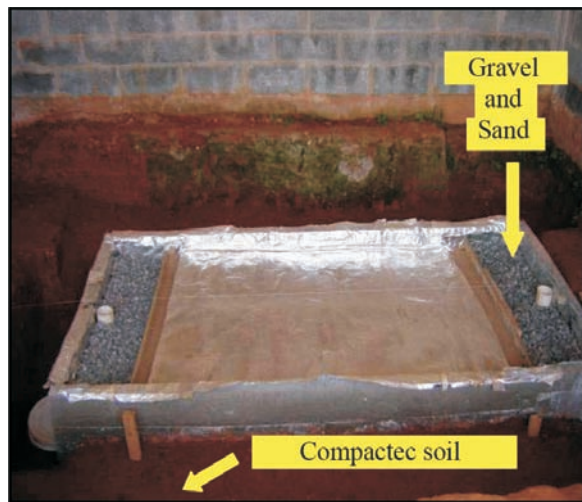

Figure 9 - Overview of soil block showing the final state of both reservoirs.

Each monitoring tube had a threaded lid at the top and an aluminum filter at the base to prevent solid particles entering it. Attached to the lid, there was an aluminum inner rod with an o'ring at its tip that controlled the entrance of the contaminant solution through the base. The effluent was removed from the tubes through a hose which was introduced in a small hole (to minimize volatilization of compounds during sampling), with a screw lid.

A metal auger, 0.7 m long and one inch in diameter, was used to drill holes at the points where the monitoring tubes were installed in the soil block, as illustrated in Fig. 11.

In order to maintain a constant level of the contaminant solution in the upstream reservoir, a 50 L stainless steel container was installed to function as a Mariotte bottle. This bottle was connected to the upstream reservoir through a silicone hose, as shown in Fig. 12. The tests were carried out maintaining constant heads both in the downstream and upstream reservoirs.

Figure 12 shows the tube feeding the upstream reservoir and the tube exiting the downstream reservoir. The positions of the extravasation and feed tubes are shown in Fig. 3. The hydraulic head in the upstream reservoir was maintained at 0.55 m above the bottom surface of the block while in the downstream reservoir it was kept at 0.10 m above the bottom surface of the block, resulting in a hydraulic gradient of 0.30 m m^{-1}.

Amongst the difficulties encountered during the construction of the soil block, those considered most relevant were the construction of a cement "box" around it and also, the interruption of preferential flow along the length of the block, only achieved after the use of a compacted layer of bentonite in a strip of 0.10 m.
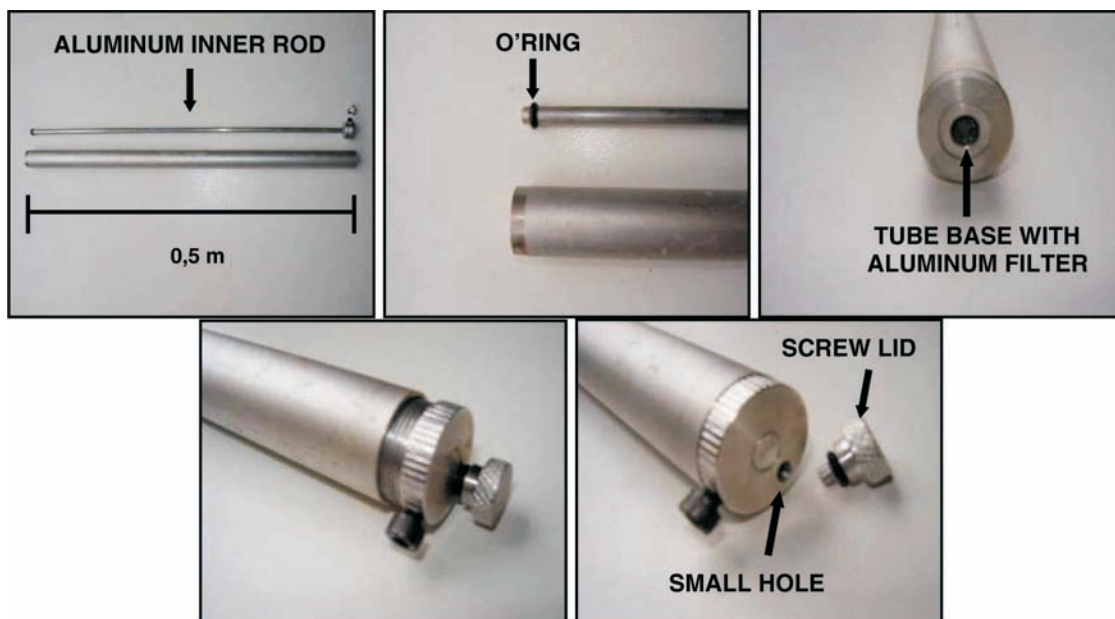

Figure 10 - Details of aluminum tubes.

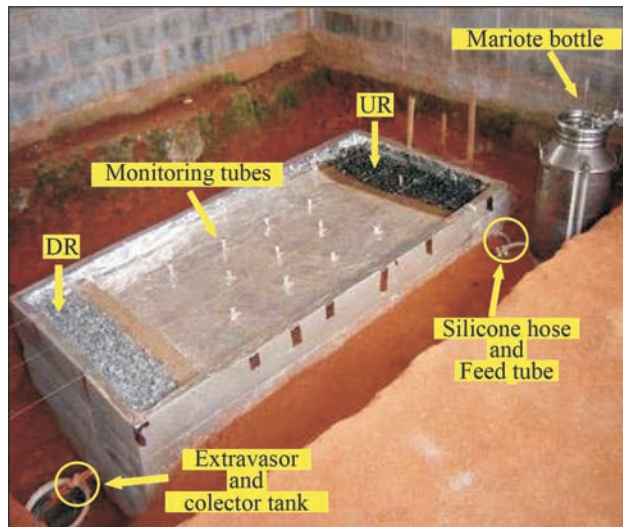**Figure 11** - Installation of monitoring tubes.

Figure 12 - Overall view of the experiment.

2.2.3. Testing procedure

After finishing the construction and instrumentation of the experiment, the upstream reservoir was filled with water, starting the saturation process of the block with pure water (no contaminant at this stage). During a period of three months, the level of water in the monitoring tubes was measured and the water levels in the block were verified.

The measured average flow rate in the block was of 45 L a day. After the saturation period with water, the up-

stream reservoir was filled with the contaminant solution and the Mariotte bottle was fed daily with the contaminant solution. It was guaranteed that the continuous flow of the solution along the block was not interrupted. The experiment was monitored during three months.

The collection of effluent samples from monitoring tubes was performed by applying suction with the aid of a syringe coupled with a hose that was inserted in the tubes. A volume of 15 mL of the contaminant solution was collected and equally distributed in three vials. The samples were then sent to the laboratory to be analyzed by gas chromatography (Fig. 13).

2.2.4. Chemical analysis

The methodology used to extract and quantify the monoaromatic hydrocarbons samples of contaminated soil was developed by Fernando (2010), adapted from Melquíades *et al.* (2006).

The concentrations of benzene and toluene in the contaminated soil were quantified after solvent extraction and static headspace injection into a gas chromatographer equipped with a flame ionization detector (SHIMADZU GC -MS QP 5000), as detailed in Fernando (2010).

Soil samples were collected 83 days after contamination, 0.50 m below the soil block surface, to undergo chemical analyses.

The concentrations of toluene and benzene in the contaminated water were obtained with a SHIMADZU gas chromatographer coupled to a mass spectrometer, using the headspace technique. These analyses were performed at the

Figure 13 - (a) Introduction of the hose in the monitoring tube; (b) Injection of the contaminated solution in the vial; (c) Sealed vials.

Laboratory of the Chemistry Department at of the Viçosa Federal University according to the methodology proposed by method EPA 8260B.

In the field, 5 mL of contaminated water samples were collected in a silicone lid vial. After arriving in the laboratory, the samples were incubated at 70 °C for 30 min to release volatile compounds. Once removed from the incubator and allowed to cool for 1 min, the manual injection of the volatiles present in the headspace was conducted. For the chromatographic separation, 2 mL of sample was injected with a syringe (HAMILTON). The identification of the pick of each compound was accomplished by the comparison with the time of retention of the peaks in the pattern of benzene and toluene. For the integration of the area of the peaks, each chromatogram was integrated individually, through the Software Labquest Chromatography it Dates System (Wang *et al.*, 2002).

2.2.5. Statistical analysis

Relations between variables were analyzed at 5% level of significance by testing of variance (multi-factor ANOVA). Tests were performed with OpenStat®.

3. Results and Discussion

3.1. Effluents analysis

Concentrations of benzene (B) and toluene (T) measured in the contaminant solution of the upstream reservoir remained around 300 mg L^{-1} throughout the experiment. Concentrations in the effluent collected from the tubes in the monitored points are presented in Figs. 14b to 14m. Results show, at first, stable concentrations then a trend of decreasing concentrations of benzene and toluene in time in all wells, which may be attributed to retention in soil, volatilization and biodegradation, as discussed further on. The time values in the horizontal axis correspond to the number of days after contamination started (on September 1st, 2009).

Graphs in Fig. 14 show stable B and T amounts in upstream and downstream reservoirs; as they were filled with sand and gravel, it was not expected that the granular material would retain significant amounts of these compounds, besides the microbial activity being probably low (not measured). In monitoring wells, B and T amounts decreased significantly only after 45 days, which may correspond to the stabilization of biodegrading activities after the lag time required for the microbiota to adapt. Indeed, besides the natural microbial adaptation, microbial cells may be carried with water flow from the upstream half of the block, increasing biomass and thus rates of biodegradation in its downstream half. Also, lower concentrations found in downstream wells may be due to cumulative retention of contaminants in soil with time as well as to higher biodegradation. The lowest amounts are found in wells 10 and 11, corroborating this hypothesis. The greater the dis-

tance between the monitoring tubes and the upstream reservoir, the lower the concentration of each compound, which can be attributed to the processes of biodegradation and soil sorption, and/or volatilization.

Statistical analysis shows that contaminants concentrations vary significantly in time in both reservoirs and in each monitoring well (p < 0.05).

The trend of constant concentration of benzene and toluene in the effluent from the 75th day on was expected, considering that the flow regime became permanent. It is possible that the soil reached its maximum capacity of retention of the compounds. As for biodegradation, according to the kinetic model of Monod (1949), a maximum rate followed by a decrease of substrate utilization by microorganisms is expected. However, biological systems present a more complex behavior. The onset of new environmental conditions, as in this case, saturation then contamination, requires an adaptation of the microbial population in order to first survive the impacts and then resume growth and development, which can be assessed by measuring microbial total activity. In the present experiment, the soil block was first saturated and contamination happened three weeks later, when the microbial population would be expected to have already adapted to saturated conditions. An initial impact on microbial activity is generally observed with BTEX contamination (Fernando, 2010; Österreicher *et al.*, 2007). A lag time before activity levels start to increase again corresponds to the adaptation of the microbiota: new strains are selected which are able to survive and use the contaminant as carbon source for survival. With the establishment and the stabilization of biodegradation, lower amounts of contaminant are expected in downstream monitoring wells.

Also, according to the graphs of Fig. 14, concentrations of benzene and toluene showed a similar behavior over time. In most cases, benzene concentration was slightly lower than that of toluene, which could be related to its greater solubility and, therefore, higher bioavailability to microbial attack. However, statistical analysis of results indicates that B and T concentrations are not significantly different in water (p < 0.05). From the 49th day on, though, a more accentuated fall in the amounts of toluene is observed.

Volatilization was greatly reduced as the block was covered with aluminum foil. Besides, volatilization from saturated soil is also reduced by contaminants dissolution in soil water (Delle Site, 2001).

Figure 15 shows B and T concentrations throughout the experiment along the well lines. At the end of the first week, contaminants are found mostly at the entrance of water flow, in high amounts upstream. From the 21st till the 35th day, contaminants appear more evenly spread in the whole block, already showing some decrease in concentrations. Samplings on the 49th and 56th days present a significant decrease in contaminants amounts, more so downstream where probably aerobic biodegradation is still taking place. Lower concentrations next to the source can
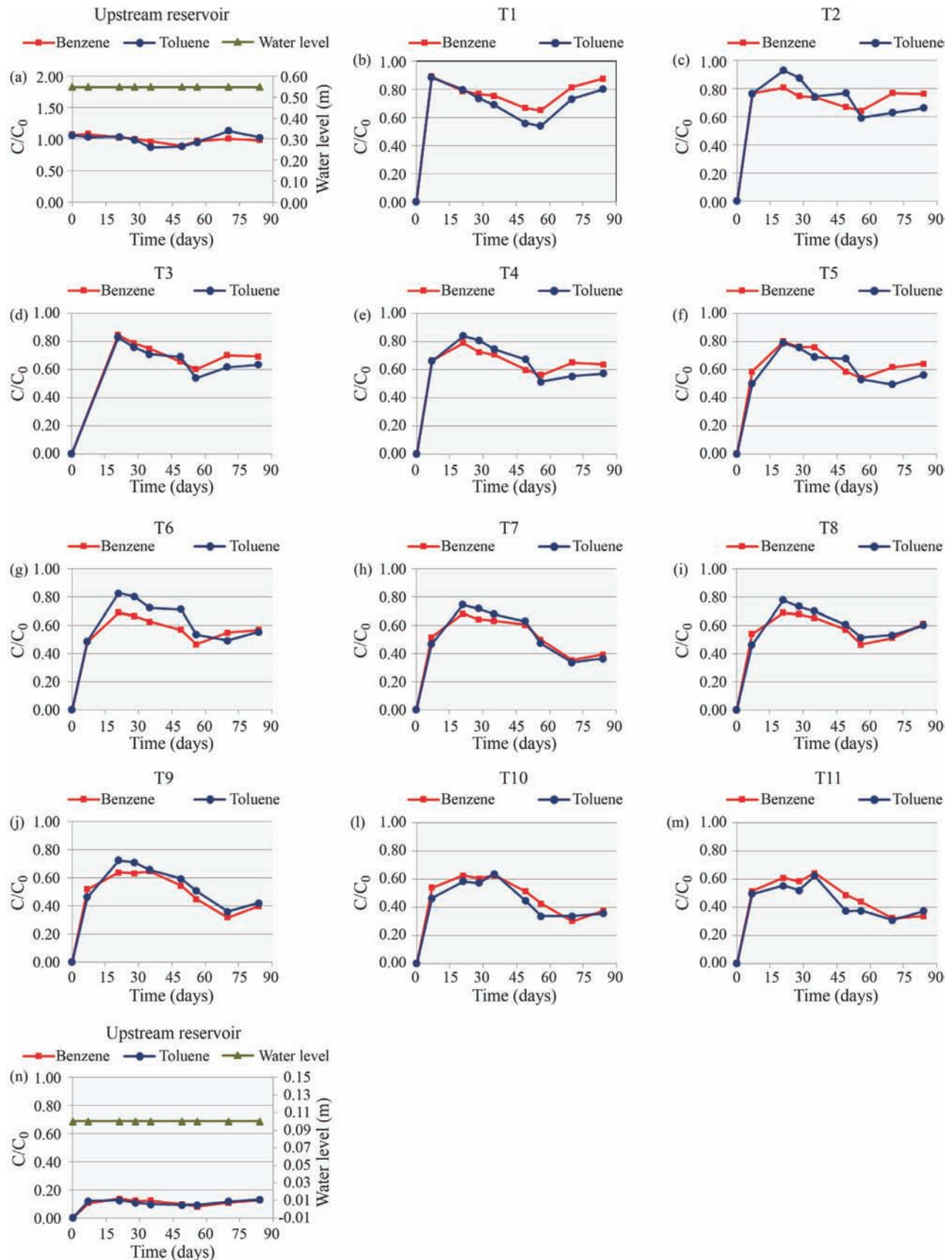
Figure 14 - Benzene and toluene relative concentrations in each monitoring point over time. (a) Upstream reservoir; (b) T1; (c) T2; (d) T3; (e) T4; (f) T5; (g) T6; (h) T7; (i) T8; (j) T9; (l) T10; (m) T11; (n) Downstream reservoir.
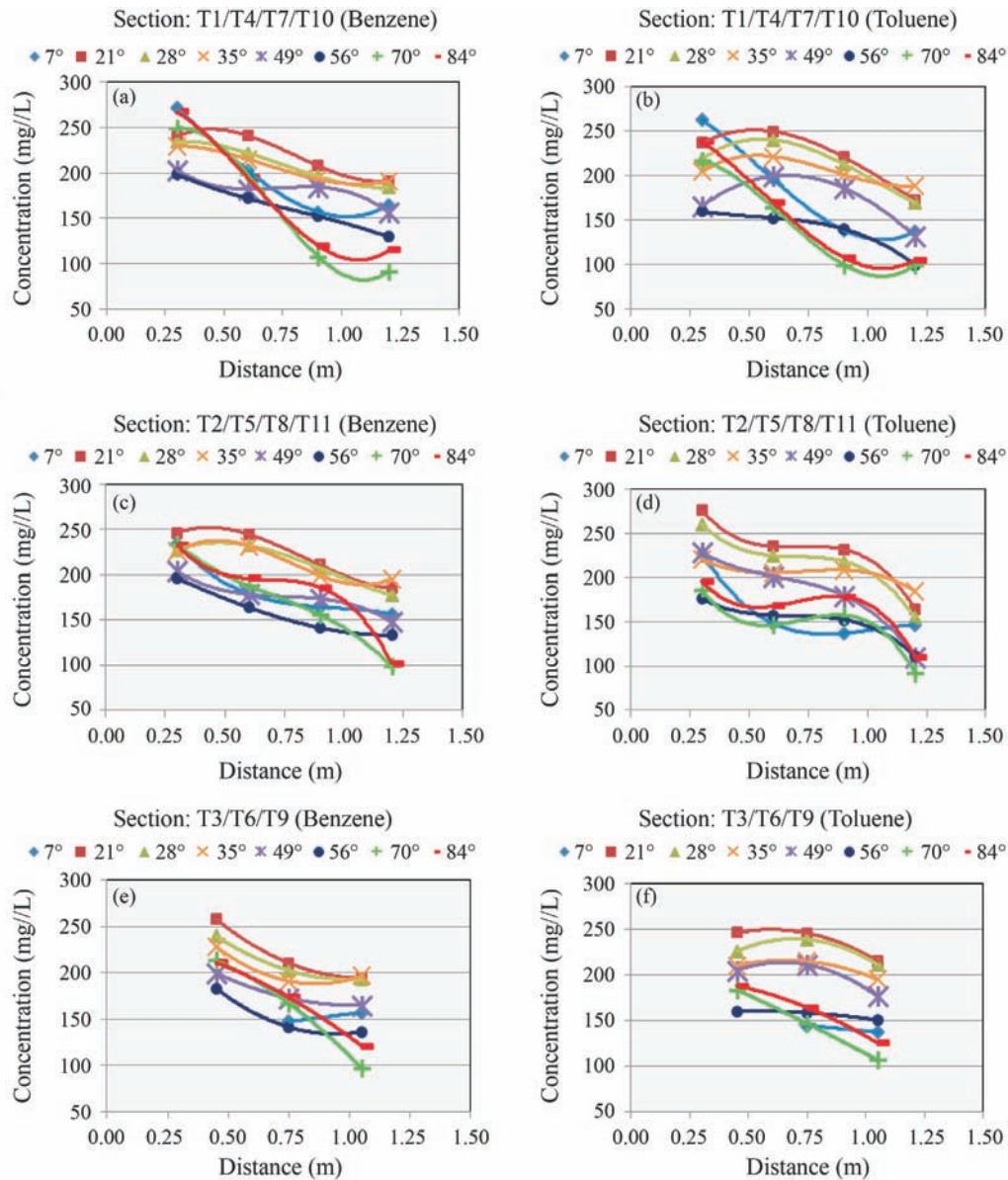
Figure 15 - Variation of Concentration of the compositions benzene and toluene in the monitoring tubes *vs.* Distance of the tubes from upstream reservoir. (a) Section: T1/T4/T7/T10 (Benzene); (b) Section: T1/T4/T7/T10 (Toluene); (c) Section: T2/T5/T8/T11 (Benzene); (d) Section: T2/T5/T8/T11 (Toluene); (e) Section: T3/T6/T9 (Benzene); (f) Section: T3/T6/T9 (Toluene).

be due to biodegradation and/or sorption to soil particles. The last two samplings reveal a situation very similar to the one on the 7th day after contamination, that is, high concentrations upstream, close to the entrance of water flow, and lower concentrations downstream, below the ones at the beginning of the experiment. These data suggest that 70 days after contamination B and T amounts distribution in water along the soil block is similar to the one at the beginning of the experiment, strongly suggesting microbial degradation in the central part of the block.

Higher amounts seen upstream at the end of the experiment could also be due to a saturation of soil particles which could no longer retain the contaminants, and would then remain dissolved in water. Or, to the onset of anaerobic conditions in the soil block in the area close to the contamination source; despite the constant arrival of dissolved oxygen in the water flow, the increased biodegrading activity could be depleting the environment of oxygen in a faster rate than its arrival with the water flow. Much lower amounts seen downstream on the 84th day are most probably due to the action of biodegrading soil microorganisms as well.

3.2. Soil analysis

For chemical and microbiological analysis, three soil samples, A, B, and C (Fig. 2), were collected 0.30 m,

0.90 m and 1.20 m, respectively, from the upstream reservoir, 85 days after contamination, 0.50 m below the soil block surface.

Soil chemical analysis evidenced higher amounts of benzene than of toluene, as shown in Table 6. Data from statistical analysis of soil samples also showed that B and T amounts are significantly different at each sampled location (p = 0.0574). Benzene seems to present higher affinity for the tested soil than toluene, and/or higher degradability, as its amounts decrease significantly in downstream samples, attaining the same levels as toluene. The sampled points are also significantly different among them concerning contaminants amounts (p = 0.197).

The retention of benzene and toluene by soil enables the interaction between bacteria and these hydrocarbons. However, higher retained concentrations may indicate greater difficulty of the compound to be degraded.

Results presented in Table 7 show microbial activity existing in the contaminated soil.

Figure 16 shows microbial activity and amounts of B and T in soil and water samples on the 83rd day after contamination. Microbial activity is still low at the entrance of the block (sample A), even after 2.5 months of contaminant presence in soil. It would be expected that the microbiota would have adapted by then and the degrading activity be higher. This low activity could be due to a decrease in biomass caused by water flow carrying microbial cells downstream, as mentioned previously. The double impact on the microbiota, first the shift from unsaturated to saturated environment, followed by the entrance of contaminated water, could have delayed their adaptation which could still not be completed after 85 days, explaining the low activity. Also, biodegradation may have already taken place and, despite the constant input of dissolved oxygen with the water flow, redox potentials near the upstream reservoir may already be low, tending to the anaerobic degradation of com-
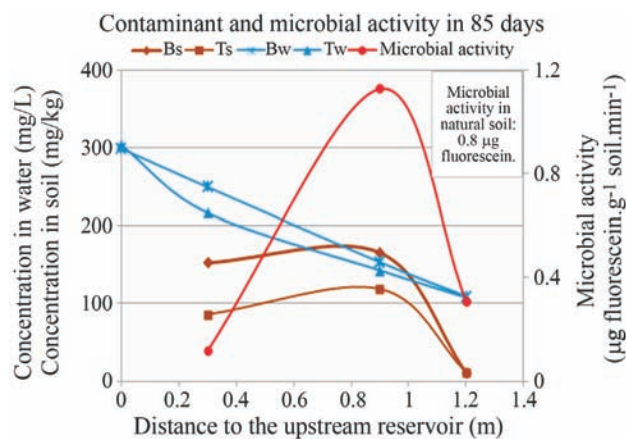

Figure 16 - Microbial activity in soil, B and T amounts in soil (B$_s$ and T$_s$) and water samples (B$_w$ and T$_w$) on the 85th day of experimentation.

pounds while aerobic activity decreased. Biodegradation may have become faster than oxygen input. Besides, determinations of microbial total activity were performed under aerobic conditions and did not measure anaerobic activity. Other studies and analyses would be necessary to better understand and explain these results. Downstream (sample B), microbial activity increases to higher levels than those measured in the natural soil, to then decrease next to the downstream reservoir (sample C). Concomitantly, contaminants amounts in water decrease along the soil block, while those retained in soil samples remain unchanged until the second half of the block, decreasing next to the downstream end. This decrease in B and T concentrations in soil follows the increase in microbial activity. On the other hand, the enhanced microbial activity does seem to affect the rate of contaminants removal from water. These data suggest that biodegradation happens mostly for contaminants adsorbed on soil, while a lesser decrease is seen in water samples. As B and T are transported dissolved in water along the soil block, and degraded, what is retained in the soil matrix is further degraded by soil microbiota, which tends to remain preferentially on soil particles and aggregates rather than in suspension (Ranjard & Richaume, 2001). Differences in B and T amounts seen in soil samples but not in water also corroborate this possibility, because of different biodegradability of both compounds. Thus, a constant small decrease is observed in contaminants concentrations in water, while in soil their amounts seem related to microbial activity.

Sample C shows a very reduced microbial activity, corresponding to almost undetected amounts of contaminants by the methodology adopted. The enhanced biodegrading activity is not maintained as soil contamination decreases.

In soil, there is a significant difference in B and T amounts between the sampling points. Thus, statistical analysis corroborates the conclusion that degradation is

Table 6 - Results of the chromatography analysis of benzene and toluene in the soil.

Samples	Benzene (mg kg^{-1})	Standard deviation	Toluene (mg kg^{-1})	Standard deviation
A	153.00	67.29	86.33	26.41
B	166.33	48.64	119.00	35.17
C	11.67	4.93	12.00	8.19

Table 7 - Microbial activity of the contaminated soil.

Samples	μg fluorescein x g^{-1} soil x min^{-1}	Standard deviation
A	0.12	0.00
B	1.13	0.16
C	0.31	0.08

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probably more accentuated on soil particles and/or inside soil aggregates than in water, and that T concentrations are generally lower in soil samples.

4. Conclusions

The paper presented results obtained from an *in-situ* experiment where water contaminated with both benzene and toluene was allowed to flow through an isolated block composed of residual soil of gneissic origin. A number of conclusions could be drawn regarding both the assembly and validity of the experiment as well as biodegradation properties of both organic compounds inside the residual soil mass.

Regarding the assembly of the experiment, it appears that the methodology used in the sample preparation proved to be satisfactory. A number of experimental issues had to be addressed as for example block excavation/isolation and proper sealing of its faces, installation of monitoring tubes for water samples collection, maintenance along time of hydraulic heads at block boundaries and contaminant concentrations in the upstream reservoir. The obtained results indicate that these issues were properly addressed.

Chemical and microbiological analyses showed decay in concentrations of benzene and toluene in water with time. Both compounds presented similar decay profiles in water, indicating no significant differences in retention and biodegradation of B and T under the conditions of this study.

One single sampling of soil, performed at the end of the experiment, showed a higher concentration of benzene than of toluene in the upstream region of the block, possibly because of its higher retention and/or lower degradation. Both benzene and toluene amounts decreased to the same level in the downstream region, indicating higher degradation at the center portion of the block, where a peak in microbial activity was also measured in the same sampling. These results suggest that biodegradation of these organic compounds may happen preferentially on soil particles and/or in soil aggregates.

Levels of aerobic activity along the block coupled with contaminants concentrations throughout the experiment suggest the onset of anaerobic degradation upstream during the final weeks of the monitoring period.

Acknowledgments

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