

Microbial Activity in Soil During Gasoline-Ethanol Degradation

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ABSTRACT

In Brazil, ethanol has been mixed to gasoline for many years, both compounds entering the environment when leaks occur. In the aquifer, ethanol seems to be preferentially degraded, depleting soil of electron acceptors and consequently delaying BTEX degradation. However, processes in the vadose zone are still poorly understood. Previous studies showed a decrease in culturable bacterial populations in unsaturated residual soils with gasoline and gasoline-ethanol mixture. Recovery of gasoline-contaminated soils happened sooner than for ethanol-containing ones, suggesting a similar situation to the one observed in saturated soil. This study monitored the biodegradation of BTEX-ethanol mixture in unsaturated soil and the influence of ethanol on BTEX degradation. Bioventing was applied to contaminated soils; measures of fluorescein-diacetate hydrolysis ascertained microbial activity, and Time Domain Reflectometry monitored soil dielectric constant shifts. Contaminant residues were analysed by Gas Chromatography. Results show the effect of contamination and bioventing on microbial activity during BTEX degradation in the presence of ethanol.

INTRODUCTION

Ground water contamination by petroleum hydrocarbons (PHC) is mainly originated by leaking underground storage tanks. Two important aspects distinguish PHC leaks in Brazil from those in other countries. The first one is associated to the gasoline used in Brazil, which normally contains 20 to 26% w/w ethanol. The second factor is related to the tropical characteristics of Brazilian soils; residual soils of different origins impart very different structure, physical-chemical and geotechnical properties from sedimentary soils found in industrialised countries where most of the research has been conducted to date

Most studies have focused on the saturated zone while processes occurring in the vadose zone are far less understood and investigated. This study focuses on those processes that take place in the unsaturated zone, specifically analysing biodegradation mechanisms in tropical soils. This study integrates a multi-disciplinary research conducted at the Civil Engineering Department of the Pontifical Catholic University of Rio de Janeiro (PUC-Rio) that aims to unravel the effects of chemical contamination on tropical soils, as well as evaluate monitoring and remediation techniques. Young residual soils of gneissic origin, frequently found in the South-Eastern region of Brazil, have been particularly investigated. The soil in this study contains iron oxides, significant in PHC degradation processes, and small amounts of clay. Low enzymatic activity and culturable heterotrophic bacterial population densities were detected in soil samples as well as the presence of gasoline-degrading microorganisms (1).

The persistence of BTEX (benzene, toluene, ethyl-benzene and xylenes, the most recalcitrant and problematic of gasoline compounds) in soil is well documented: retained in the soil matrix, they establish a residual source of contamination (2). In the aquifer, ethanol preferential degradation seems cause BTEX slower degradation because of a depletion of electron acceptors in soil (3). Ethanol would also enhance BTEX movement and distribution (4-5) and longer plumes are expected (6). Studies in progress at PUC-Rio indicate that, in the unsaturated zone, more gasoline remains in soil when mixed to ethanol (7). The vadose zone could thus be critical when gasoline-ethanol contamination occurs.

PHC are easily degraded by soil native microbiota and are frequently treated by bioremediation (8-10). Bioventing, addition of air or oxygen into the subsoil, stimulates contaminants biodegradation (2)(11). Previous studies evaluated the effect of ethanol and of bioventing on gasoline compounds degradation (1): chemical analysis showed a slightly slower decrease of gasoline compounds in gasoline-ethanol-contaminated soils, while the smallest decrease was detected in non-ventilated ethanol-containing soil. Culturable bacterial populations were practically eliminated from contaminated soils; resuming of growth was delayed in ethanol-contaminated soils, suggesting a similar preferential degradation of ethanol as in the aquifer. Bioventing seemed to have a stimulating effect on the recovery of ethanol-contaminated soils but not on gasoline-contaminated ones. Culturable populations recovered rapidly in ventilated ethanol-containing soils while in non-ventilated ones they remained low. Shifts in soils electro-magnetic properties, followed by Ground Penetrating Radar, could be attributed neither to microbial activity nor to soil water content changes (1).

This study investigates microbial degrading activities during BTEX-ethanol mixture degradation in the same residual tropical soil. Soil dielectric constant and water content shifts were monitored using gravimetric measures and Time Domain Reflectometry. Microbial enzymatic activity was evaluated measuring Fluorescein Diacetate (FDA) hydrolysis (12) in soil samples. Shifts in culturable populations contingents indicate the probable selection of more adapted microbial strains, unable to grow under laboratory conditions; they may also show a stimulation of metabolic processes, but do not evaluate the effectiveness of microbial degrading activities. Furthermore, given the non-culturability of most environmental microorganisms, population quantifying is not sufficient. Integrating microbiological and geo-physical data allows for a better understanding of degradation mechanisms (13-15).

MATERIAL AND METHODS

Study Design

Biodegradation of BTEX and BTEX-ethanol in undisturbed soil was monitored for 20 days. Monitoring of the weight of soil columns determined loss of contaminants by volatilisation and biodegradation; microbiological parameters evaluated biodegradation activities while measures of soil dielectric constant meant to show fluctuations in soil water content and microbial activity.

Table 1 shows the contamination and ventilation treatments; controls consisted of two non-contaminated columns (one ventilated and one not ventilated) and two non-ventilated sterilized columns (one BTEX-contaminated the other BTEX-ethanol-contaminated); five sets of four columns were BTEX-contaminated (two columns) and BTEX-ethanol-contaminated (the two other columns).

Soil

The material used in this study is a natural, unsaturated residual soil of gneissic rocks from the metropolitan area of Rio de Janeiro (1)(16).

Soil Columns

Undisturbed soil columns were collected in PVC rings (10 cm high, 15 cm i.d.).

Contamination

According to average amounts found in Brazilian gasoline (17), the BTEX mixture used contained 20 % benzene w/w, 40 % toluene w/w, 35 % ethyl-benzene w/w and 100 % of a mixture of o-, p- and m-xylene w/w, in n-heptane (Quimex) as solvent. To that mixture, 20% w/w ethanol was added to compose the BTEX-ethanol mixture. 200 mL of contaminants were poured on the soil columns surface; columns were closed at the bottom when percolation stopped.

Bioventing

To provide constant oxygenation to the soil, an airflow of 0.5 psi was injected into the columns by a compressor from the bottom of the rings through a perforated disk. Bioventing started right after contamination.

Sampling

Soil samples were collected from the columns and analysed before contamination. Thereafter, sampling was performed 2, 5, 10 and 20 days after contamination and the beginning of bioventing. At each sampling, one battery of four contaminated soil columns was opened and composite soil samples were taken for analysis. From non-contaminated controls, three sub-samples were taken at different depths and combined in a composite sample. Sterile controls were not sampled during the assay.

Gravimetric Monitoring

To follow loss of contaminants as well as loss or gain of water by soil, columns were weighed before each sampling during the assay. Two sterile controls provided information on volatilisation of contaminants.

Enzymatic Activity Measurements

Microbial degrading activity was determined measuring Fluorescein Diacetate (FDA) hydrolysis, following the modified methodology described by Adam and Duncan (12).

Dielectric Constant Measurements

Soil dielectric constants were determined with a Theta Probe™ (Delta Instruments) inserted into the soil surface.

Chemical monitoring

Gas chromatography analyses were performed at the Fuel Laboratory at the Chemistry Department of the Pontifical Catholic University of Rio de Janeiro. Contaminants were extracted from soil samples by orbital shaking in methanol. BTEX and ethanol amounts in soil were determined using a Shimadzu chromatographer equipped with a flame ionisation detector.

RESULTS AND DISCUSSION

Soil weights remained constant during the assay for most columns, as shown in table 2. Some of them presented low water content at the beginning of the assays and suffered an increase in weight probably due to capture of humidity from air. Ventilated soils showed a decrease at the end of the assay probably due to some loss of water, despite water reposition, and of contaminant.

The non-ventilated control maintained a constant weight during the 20-day experiment, while the ventilated control showed a slight and progressive weight loss due to water loss by the soil. Sterile dry controls showed a slow and progressive loss of contaminant by volatilisation, showing no significant differences between them. Dry sterile soils showed that loss of contaminants by volatilisation is not significant under the experimental conditions adopted.

The decrease in weight was more important in ventilated contaminated columns than in non-ventilated ones, which was expected because of the drying effects of bioventing and the stimulation of degradation. Ventilated ethanol-contaminated soils showed the most important decrease in weight, while non-ventilated ones lost the least weight. Those weight shifts may correspond to the decrease of contaminants in ventilated ethanol-contaminated soils as observed in previous studies.

In non-ventilated soils, weight loss was higher in BTEX-contaminated than in BTEX-ethanol-contaminated columns. In ventilated soils, there was no significant difference between weight loss of BTEX and BTEX-ethanol containing soil. These results, similar to previous ones (16) suggest a slower degradation of BTEX when ethanol is present, as well as a more effective degradation of contaminants when bioventing is applied.

Dielectric constants varied constantly during the assay (figure 2). As for non-contaminated controls, the non-ventilated one showed a fairly constant pattern; the ventilated control showed an important decrease in K_a values at day 20. BTEX-contaminated soils showed a similar behaviour for ventilated and non-ventilated columns. In ventilated soils, K_a values were slightly higher in BTEX- than in BTEX-ethanol-contaminated soils; the opposite was observed in non-ventilated soils for most measures. Ethanol has a higher dielectric constant (24,3) than BTEX (2,0 – 2,6, close to those of soil components), so its presence in soil may alter K_a values.

Measures of FDA hydrolysis showed an increase in enzymatic activity right after contamination and the beginning of bioventing. The non-ventilated contaminated control soil showed a rather constant activity throughout the assay, while the ventilated one also presented a constant behaviour after an initial increase, probably due to the stimulation introduced by oxygenation of the soil. Metabolic activity increased more in ventilated soils than in non-ventilated ones, and it was

higher in ethanol-containing soils (fig. 3). The presence of ethanol seems to have stimulated enzymatic activity right after contamination. These results are compatible with the fact that ethanol is constitutively degraded while BTEX degradation requires the activation of specific enzymes.

In non-contaminated soils, K_a values followed variations in soil weight and responded to water-content shifts but not to heterotrophic activity values (Fig. 4 and 5). In BTEX-contaminated soils, K_a values also followed weight shifts closely (fig. 6 and 7). In all contaminated soils, K_a did not respond to the important increase in microbial metabolism that followed contamination and bioventing (fig. 6, 7, 8 and 9).

Chemical analysis could not detect BTEX nor ethanol in ventilated soils. In non-ventilated ethanol-containing ones, only xylenes and ethyl-benzene (0,003 g/g soil) and heptane (0,011 g/g soil) were found on day 2; no contaminants were detected in all the other soils. This rapid disappearance of contaminants could be due to volatilisation but also to biodegradation, given the high levels of degrading activity observed on day 2 in all contaminated soils. In previous studies, ethanol-containing soils showed the slowest degradation of gasoline compounds, the non-ventilated one presenting the highest amounts of contamination (16). Further studies are necessary to evaluate the mechanisms that determine this fast loss of contaminants.

Retention of contaminants by soil was similar in both BTEX and BTEX-ethanol contamination (6.15 and 6.24 % of soil initial weight respectively).

Metabolism may be higher in BTEX-ethanol-containing soils because of constitutive degradation of ethanol. Once ethanol is no longer present (eliminated by volatilisation and/or degradation), activity may decrease again. In the presence of BTEX, microbiota raises its metabolic activity once more in order to degrade the latter. In ventilated soils ethanol may have volatilised / been degraded sooner, leaving only BTEX, which could explain weight and enzymatic activity shifts.

CONCLUSIONS

- * The presence of PHCs in soil enhanced microbial enzymatic activity suggesting that FDA hydrolysis is an adequate tool to evaluate degrading activities of microbiota.
- * Microbial metabolism is higher in soils contaminated with BTEX-ethanol than with BTEX only.
- * Bioventing stimulated microbial activity.
- * Soil dielectric constants did not respond to higher microbial activity in contaminated soils.

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TABLES

Table 1 – Study design.

ID	Description	Contaminants	Bioventing
C	Non-contaminated control	None	No
CV	Non-contaminated control	None	Yes
SB	Sterile control	BTEX	No
SE	Sterile control	BTEX-ethanol	No
B	Contaminated columns	BTEX	No
BV	Contaminated columns	BTEX	Yes
E	Contaminated columns	BTEX-ethanol	No
EV	Contaminated columns	BTEX-ethanol	Yes

Table 2 – Weight progression during the assay (% of initial weight)

days	0	2	5	10	20
C	100	101,87	101,85	101,13	100,54
CV	100	114,01	114,39	112,43	104,53
B	100	97,10	94,56	94,39	94,47
BV	100	95,33	94,53	91,07	89,42
E	100	96,12	96,61	76,45	94,18
EV	100	91,99	88,15	94,92	90,68
SB	100	112,67	110,80	110,03	109,91
SE	100	103,38	101,95	101,07	100,94

FIGURES

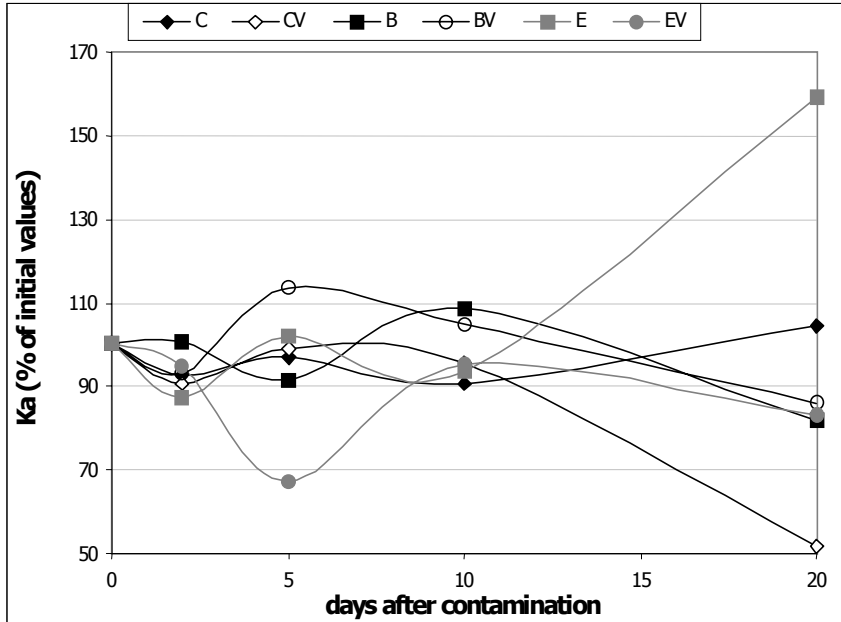


Figure 1 – Dielectric constant shifts during the assay (% of initial Ka values)

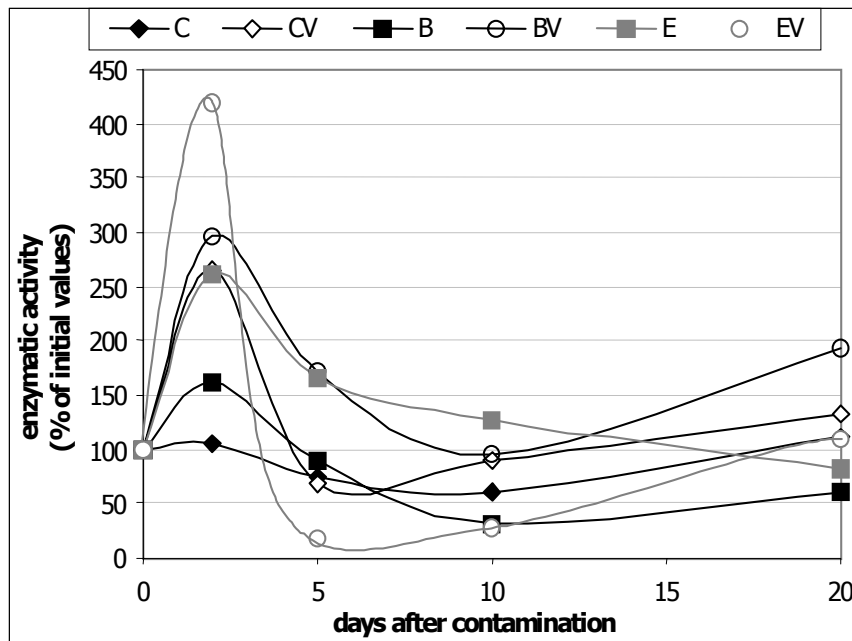


Figure 2 - Enzymatic activity during the assay (% of initial mg fluorescein/ g soil/min)

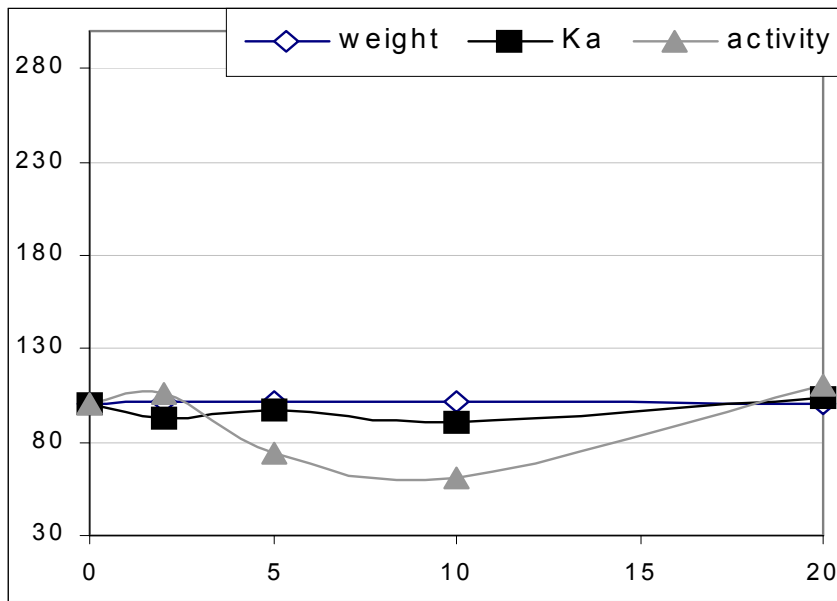


Figure 3 – Soil weight, Ka and enzymatic activity in non-contaminated non-ventilated soil (% of initial values)

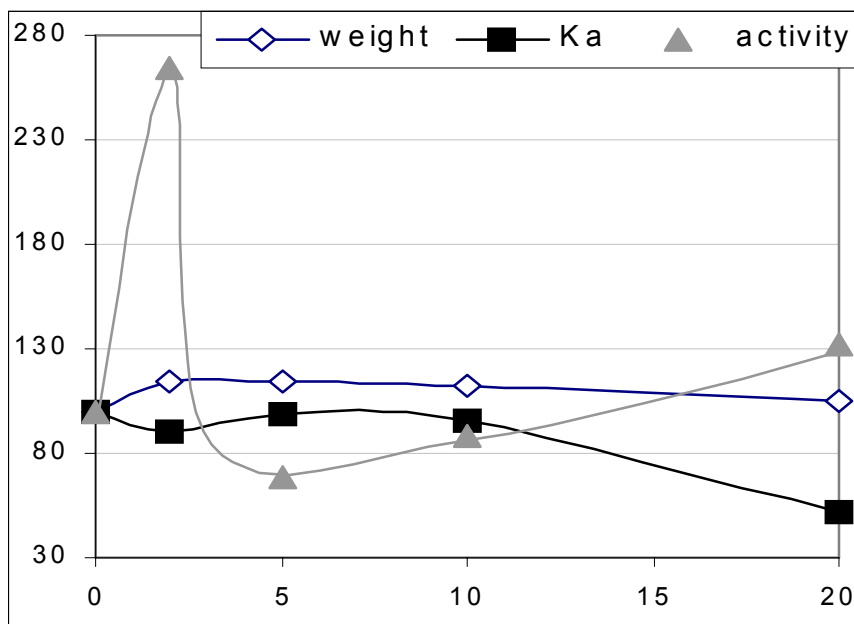


Figure 4 – Soil weight, Ka and enzymatic activity in non-contaminated ventilated soil (% of initial values)

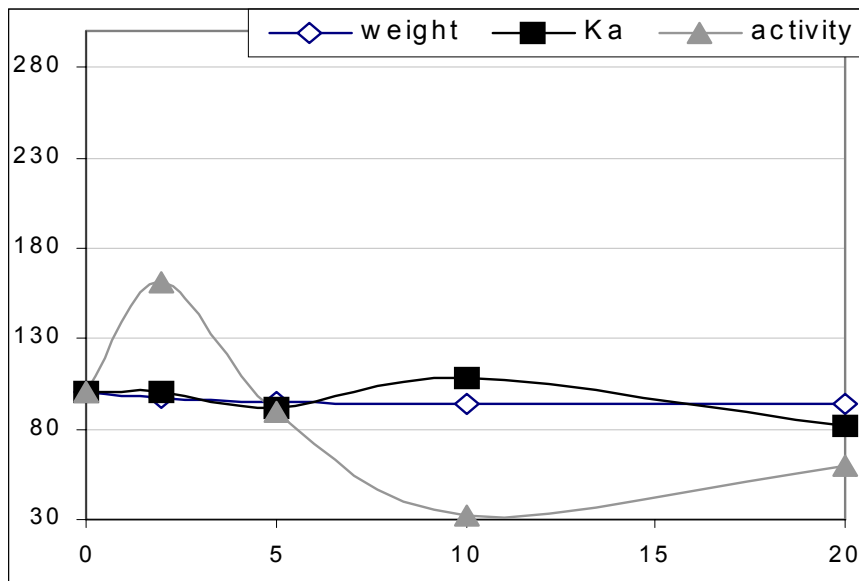


FIGURE 5 - Soil weight, Ka and enzymatic activity in BTEX-contaminated non-ventilated soil (% of initial values)

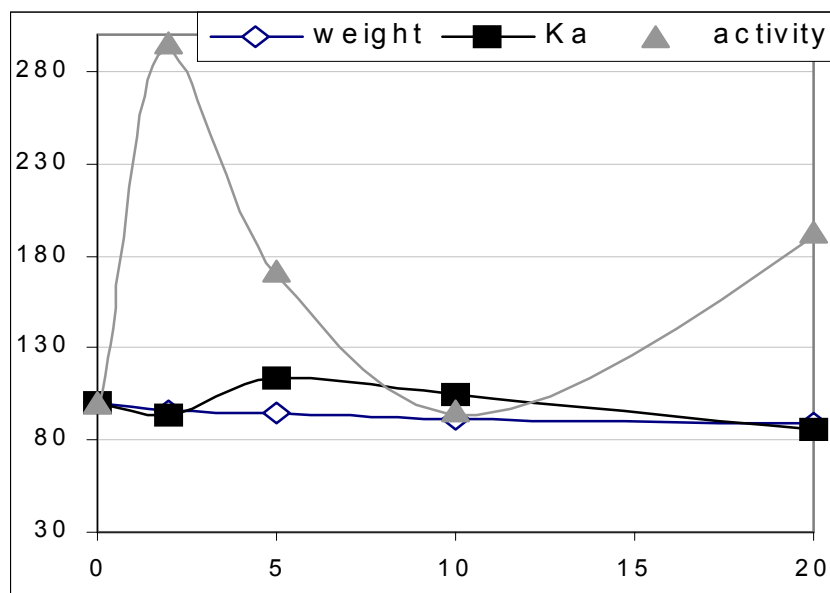


Figure 6 – Soil weight, Ka and enzymatic activity in BTEX-contaminated ventilated soil (% of initial values)

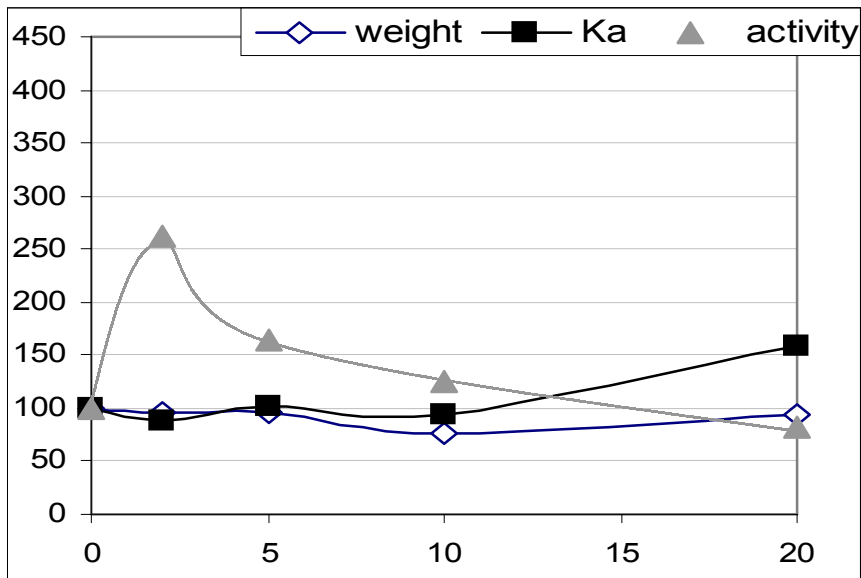


Figure 7 – Soil weight, Ka and enzymatic activity in BTEX-ethanol-contaminated non-ventilated soil (% of initial values)

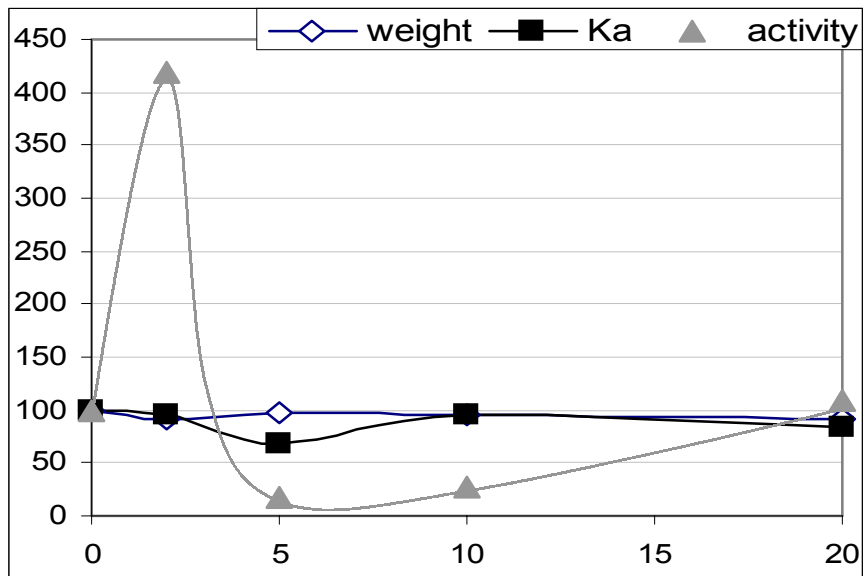


Figure 8 – Soil weight, Ka and activity in BTEX-ethanol--contaminated ventilated soil (% of initial values)