SCAP
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Assisted dissipation of a mix of PAHs in contaminated soils: effect of soil type and availability enhancers

Disipación asistida de un mix de PAHs en suelos contaminados: efecto del tipo de suelo y compuestos que incrementan su disponibilidad

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ABSTRACT

A remediation strategy using three non-toxic availability enhancers (two cyclodextrins and a rhamnolipid biosurfactant) was applied to various soils artificially contaminated with a mix of 14 Polycyclic Aromatic Hydrocarbons (PAHs) considered priority pollutants (from 3 to 6 rings). The high number of experimental factors (soil type, availability enhancers and incubation time) allowed to conduct a multivariate statistical analysis. Principal Component Analysis separated the studied PAHs in 3 groups, in relation with their molecular weight and Kow. The first principal component (PC1) was related with lower molecular weight PAHs, those with lower Kow and that have shown a high dissipation rate with final negligible contents. In this way, PC1 was related with the PAHs dissipated during the remediation process, and accordingly, the effect of incubation time was significantly associated to PC1, with decreasing values whereas time increases. Even more, this component was able to clearly separate the RAMEB (ramdomized methyl-β-cyclodextrin) from the other availability enhancers used. Otherwise, the second principal component was correlated with the higher molecular weight PAHs (5 and 6 rings) and was able to separate soils with different characteristics. The third principal component grouped two PAHs with intermediate molecular weight and more erratic dissipation pattern.

Keywords: soil remediation, biodegradation, principal component analysis, cyclodextrins, rhamnolipid biosurfactant.

RESUMEN

A varios suelos contaminados artificialmente con una mezcla de 14 hidrocarburos aromáticos policíclicos (PAHs) considerados contaminantes prioritarios (de 3 a 6 anillos) se les aplicó una estrategia de recuperación utilizando tres compuestos no tóxicos que aumentan su disponibilidad (dos ciclodextrinas y un biosurfactante ramnolípido). El elevado número de factores experimentales (tipo de suelo, potenciadores de la disponibilidad y tiempo de incubación), y contaminantes permitió realizar un análisis estadístico multivariante. El análisis de componentes principales separó los PAHs estudiados en 3 grupos, en relación con su peso molecular y Kow. El primer componente principal (PC1), se relacionó con los PAHs de menor peso molecular, aquellos con menor Kow y los que habían mostrado una alta tasa de disipación con muy bajos contenidos finales. De este modo, PC1 se relacionó con los PAHs disipados durante el proceso de recuperación, y, en consecuencia, el efecto del tiempo de incubación se asoció significativamente a PC1 con valores decrecientes conforme aumenta el tiempo. Además, este componente fue capaz de separar claramente la RAMEB (β-ciclodextrina aleatoriamene metilada) de los otros compuestos utilizados para aumentar la disponibilidad. Por otra parte, el segundo componente principal se correlacionó con los PAHs de mayor peso molecular (5 y 6 anillos) y fue capaz de separar suelos con características diferentes. El tercer componente principal agrupó a dos PAHs de peso molecular intermedio y con un patrón de disipación más errático.

Palabras clave: recuperación de suelos, biodegradación, análisis de componentes principales, ciclodextrinas, biosurfactante ramnolípido.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are chemical substances characterized by two or more fused benzene rings. The aromatic structure of these rings gives rise to very stable compounds, so, they show a low degradability and high persistence in the environment, being considered priority pollutants.

PAHs can be grouped in Low Molecular Weight PAHs (LMW, 2-3 rings) and High Molecular Weight PAHs (HMW, 4 or more rings), showing different properties. LMW PAHs are easier to be dissipated from soils by volatilization or biodegradation, contrary to HMW PAHs, which are more recalcitrant to microbial attack (Leech *et al*., 2020). This different behavior affects the capacity to remediate PAHs polluted soils. In order to increase their bioavailability for soil microorganisms, extractants and solubilizing agents are used, and non-toxic solubility enhancers such as cyclodextrins (CDs) and biosurfactants (BSs) have been increasingly used in the last years.

In the present study, a 120-days incubation in microcosm experiment was conducted on three soils with different properties, artificially contaminated with 14 PAHs (from 3 to 6 rings) to investigate the effect on their dissipation of: i) the soil properties; ii) three availability enhancers (two CDs and a rhamnolipid BS).

MATERIALS AND METHODS

Soils

Three agricultural soils (CR, CN and TM) of different textures and properties were selected. They were sampled from different areas of Southwest Spain showing a background of several pesticides application. Soils were sampled from the 0–10 cm layer, dried at room temperature, sieved by 2 mm, and stored at 4°C. Physicochemical characteristics are shown in Table 1. The three soils were selected because they showed in preliminary tests a microbial flora capable of provoking the natural attenuation of pyrene when they were artificially contaminated (data not shown).

Incubation assay

Incubation experiments were carried out in glass containers (300 mL) in triplicate for each treatment. The 3 soils were spiked with a mix of 14 PAHs (40 mg kg^{-1} ∑14PAHs from 3 to 6 rings; (acenaphthylene (ACL), acenaphthene (ACE), anthracene (ANT), fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BANT), chrysene (CHR), benzo[a]pyrene (BPYR), benzo[b]fluoranthene (BFLT), dibenzo[a,h]anthracene (DBANT), benzo [g,h,i]perylene (BPER), and indeno[1,2,3-c,d]pyrene (IPYR)). Two CDs (2-hydroxypropyl-β-cyclodextrin (HP) and randomly methylated-β-cyclodextrin (RAMEB)) and a rhamnolipid (RL) were used as availability enhancers at a dose of 1% (dry soil basis). Samples were taken at 1, 15, 30, 60 and 120 days for individual PAHs content analysis.

Statistical analysis

Data of the 14 PAHs sudied contents at every incubation time were statistically analysed using IBM SPSS Statistics v.25. Varimax-rotated principal components analysis (PCA) was used to ascertain the existence of possible associations among variables (PAHs contents) in order to get a dimensionality reduction associated to the evolution of the 14 PAHs studied affected by the 3 experimental factors: soil (CR, CN, TM), availability enhancer (control, HP, RAMEB, RL) and incubation time (1, 15, 30, 60, 120 days)).

Table 1 - Physicochemical characteristics of soils

Soil	pH	CO_3^{2-} (%)	OM (%)	Water field capacity (%)	Sand (%)	Silt (%)	Clay (%)	Textural class
CR	8.70	17.0	1.04	46.5	59.6	19.4	21.0	Sandy Clay Loam
CN	5.5	1.1	1.91	39.3	50.5	39.3	10.2	Loam
TM	7.84	25.4	2.36	41.4	6.8	33.6	59.6	Clay

RESULTS AND DISCUSSION

Natural attenuation of PAHs in all soils showed high degradation capacity for seven of the lower molecular weight PAHs (ACL, ACE, ANT, FLU, PHE, FLT, and PYR), with a final content < 5% of their initial concentration. Conversely, for the rest of the PAHs (high molecular weight PAHs, HMW) biodegradation is considered almost negligible, remaining in the soils (from 61% - 83.5%), with a percentage of abiotic dissipation directly related with the OM content of the soils, due to formation of non-extractable residues.

PCA is a useful tool for treating a large amount of data, as in this assay. For this analysis, individual PAH contents at every incubation time and treatment have been included. PCA grouped the PAHs into three principal components (PCs) that explain 82.0% of the total variance observed (Table 2).

Table 2 - Correlation coefficients between the content of PAHs and the first three principal components resulting from PCA, eigenvalues and percentage of variance accounted for

PAH	Component			
	PC1	PC2	PC3	Communalities
FLU	**0.914**	0.044	0.097	0.846
ACE	**0.889**	0.087	-0.133	0.815
FLT	**0.872**	0.205	0.173	0.833
ANT	**0.858**	-0.020	0.250	0.798
ACL	**0.842**	0.057	-0.104	0.722
PYR	**0.818**	0.168	0.125	0.713
PHE	**0.803**	0.374	-0.212	0.830
IND	0.065	**0.958**	0.033	0.922
DBANT	0.005	**0.890**	0.238	0.848
BPER	0.060	**0.877**	0.049	0.776
BFLT	0.211	**0.775**	0.138	0.664
BPYR	0.556	**0.719**	-0.067	0.831
CHR	-0.100	0.070	**0.959**	0.934
BANT	0.229	0.255	**0.909**	0.944
Eigenvalue	5.567	3.890	2.020	
Variance (%)	39.764	27.784	14.427	
Cumulative	39.764	67.548	81.975	

The first component (PC1) explained 39.8% of the variance and was associated with 3-rings PAHs (FLU, ACE, ANT, ACL, and PHE) and two 4-rings PAHs (FLT and PYR). In this way, among the PAHs studied, PC1 has grouped those 7 with log $K_{ow} \leq 5.2$ which have shown higher dissipation rates (Madrid *et al.*, 2021). This means that, probably, their dissipation processes were affected by similar factors. All other PAHs included in the analysis have log $K_{ow} \geq 5.6$ and were mainly associated to the other PCs. PC2 explained 27.8 % of the variance and grouped the heavier PAHs, those that have 5- (BFLT, BPYR, and DBANT) or 6-rings (IPYR and BPER) and log $K_{ow} \geq 6.2$. This could be related with the high adsorption capacity of these PAHs. In this group of PAHs, BPYR showed a lower correlation value with PC2 and a higher value with PC1 than the others, probably because of the moderate dissipation that this PAH showed in the 3 soils. On the contrary, the other PAHs grouped by PC2 have shown very low dissipation rate after 120 days. Finally, the third Principal Component extracted (PC3) explains 14.4% of the variance and directly groups the other 4-rings PAHs not included in PC1, CHR and BANT. Their behaviors have been more erratic than PYR and FLT, probably related with the higher log K_{ow}. Golobočanin *et al*. (2004) applied PCA analysis to a wide set of PAHs contaminated soils. These authors also found an influence of the number of rings in the distribution of the different PAHs among the extracted components.

To describe the global effect of the availability enhancers used for every soil, Figure 1 shows the mean scores of each treatment plotted on the plane PC1 vs. PC2. It is observed that RAMEB treatments (that retarded the dissipation of PAHs, Madrid *et al.*, 2021) showed the highest values in PC1 for the

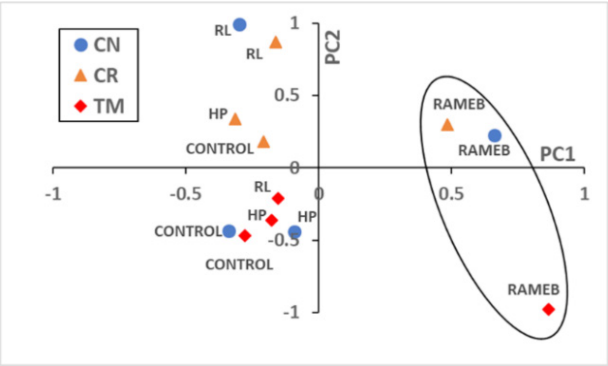

Figure 1 - Scatter of the data obtained in PCA for each soil and treatment on the plane (PC1 vs PC2).

three soils. Other treatments (HP, RL, and control), with higher dissipation rates of the lighter 7-PAHs, show negative mean scores in this component. In summary, lower values in PC1 is related with decreasing PAH content along the incubation period.

It is corroborated in Figure 2 representing the evolution of the centroid values at every incubation time for the three soils and for all treatments simultaneously. Decreasing values can be observed in PC1 of centroids, as incubation time increases, from the highest value at time 1 to negative values in this component at 30, 60 and 120 days.

Otherwise, the effect of soil characteristics is mainly shown in PC2 (Figure 3, mean scores of each treatment plotted on the plane PC2 vs. PC3), where all treatments with soil CR (soil with the lowest OM) showed positive data in this PC2 and were clearly separated from treatments with soil TM (soil with the highest OM), that show negative values in all cases.

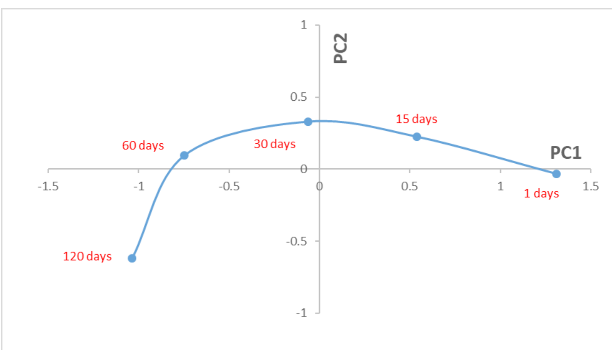

Figure 2 - Evolution of scores corresponding to the whole set of data (centroid value at each incubation time for all the treatments simultaneously) plotted in the plane PC1 versus PC2.

Although OM is the main soil component responsible of increasing adsorption capacity of hydrophobic pollutants, our results (higher dissipation rates in TM) indicate that other soil properties should be considered, such as native degrader

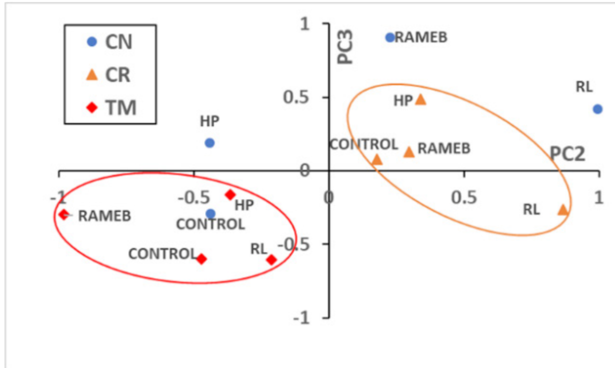

Figure 3 - Scatter of the data obtained in PCA for each soil and treatment on the plane PC2 vs PC3.

microorganisms. The points corresponding to treatments with soil CN (acidic pH, low clay, and medium OM content), have more disperse values and are not separated in PC2 from the other soils. The importance of these two factors (incubation time and soil type) in PAH dissipation agrees with results of Wu *et al.* (2014) that applied conjoint statistical analysis to PAHs amended contaminated soils. They also found these characteristics as the most significant factors in PAHs bioavailability.

CONCLUSIONS

PCA applied to PAHs assisted remediation of polluted soils, identified incubation time as the main factor affecting PAHs dissipation process, whereas the effect of availability enhancer and soil type are also significant.

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