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**EXTRACTION OF PAHS FROM AN AGED CREOSOTE-POLLUTED SOIL BY
CYCLODEXTRINS AND RHAMNOLIPIDS. SIDE EFFECTS ON REMOVAL
AND AVAILABILITY OF POTENTIALLY TOXIC ELEMENTS.**

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Abstract

This study evaluated the effect of several cyclodextrins (CDs) and a rhamnolipid (RL) on the removal of polycyclic aromatic hydrocarbons (PAHs) from a co-contaminated soil which had received historically creosote and inorganic wood preservatives for almost 100 years, and the effect of such extractions on the potentially toxic elements (PTEs). The influence on such processes of an electrolyte (0.01 M $\text{Ca}(\text{NO}_3)_2$) was also studied. Up to 15.4% of the $\Sigma 16$ PAHs were extracted using RL in the absence of the electrolyte as washing solution, but decreases until reaching 0.60% in the presence of Ca^{2+} due to RL precipitation and partial inactivation. Only up to 2% of the $\Sigma 16$ PAHs was extracted with CDs (4-ring PAHs in higher concentrations), but the electrolyte had no effect on extraction. In relation to PTEs, CDs proved to be inefficient for their extraction, and even RL in the presence of the background electrolyte. But in the absence of electrolyte PTEs extraction by RL increased. Apart from that, the availability of Ni, Cr, and As, those more associated to Fe and Al soil surfaces, increased after extraction with RLs in the presence of Ca^{2+} (about 100% for Cr and Ni and 200% for As). Under these conditions Fe and Al availability increased two- and ten-fold, respectively, indicating that Fe-Al soil surfaces were altered. Therefore, the ionic strength and the cations present in the soil solution of soils have to be considered when RLs are used as extractants for remediation purposes.

Key words: Soil washing, PAHs, toxic elements, cyclodextrin, rhamnolipid

1. Introduction

Creosote has been used as a wood preserving agent for many years. It is a very complex mixture of organic compounds produced from the high temperature carbonization of bituminous coal, and contains approximately 85% PAHs, 10% phenolic compounds, and 5% nitrogen-, sulfur- or oxygen-containing heterocycles (USEPA, 1992). Also some Potentially Toxic Elements (PTEs) such as Cr, Cu or As, are used as wood preserving agents; the most frequent are chromated copper arsenate (CCA), ammoniacal copper arsenate (ACA), and ammoniacal copper-zinc arsenate (ACZA) (Pollard et al., 1994). Wood used to be first treated with inorganic preservatives and then with creosote. The waste sludge produced by these treatments has generally been landfilled, and older wood preserver sites contain contaminated soil which leads to secondary contamination of groundwater. The existence of such a complex and problematic matrix of organic and inorganic contaminants with high differences in their physical-chemical properties makes quite difficult the remediation of these contaminated sites.

Soil washing is a treatment process to remediate both organic and inorganic pollutants from contaminated soils. It uses solutions of extractants (organic compounds, acids, tensioactives, etc.) to remove pollutants from soils, being more appropriate for soils that contain at least 50% sand and gravel (Morillo and Villaverde, 2017). Surfactant enhanced washing has been widely employed to remediate soils, but synthetic surfactants may cause environmental problems due to their toxicity and low biodegradability (Ying, 2006), remaining a portion of the applied chemicals in the soil, and/or changing soil physicochemical characteristics. To avoid such problems, new extractants are being used in soil washing, such as biosurfactants or CDs. Washing of contaminated soils with eco-friendly extractants results in lower soil toxicity and can facilitate their subsequent bioremediation.

Biosurfactants are applied to soil remediation due to their benign features like low critical micelle concentration (CMC) values and better biocompatibility (Mao et al., 2015). Biosurfactants are surface-active compounds mainly generated by bacteria, fungi and yeast. Those commonly used for soil remediation include glycolipid (e.g., rhamnolipids, fructose lipids, sophorolipids) and lipopeptide (e.g., surfactin, polymyxin) compounds. In addition to the positive impact on solubilization and desorption of soil pollutants, biosurfactants can also stimulate microbes to decompose the contaminants (Lai et al., 2009; Bezza and Chirwa, 2017), but there is controversy about this. Rhamnolipids are the most extensively studied and used biosurfactant in remediation of soils (Yan et al., 2011; Zeng et al., 2018). There are studies dealing with the washing of organic contaminants from soils using RLs (Lai et al., 2009), but a few studies have been done on washing of PAHs from soils, and most of them on artificially contaminated soils (Bordas et al., 2005; An et al., 2011; Zhang, 2015). pH is an important parameter for the rhamnolipid-enhanced solubilisation of PAHs, but its resulting impact is inconsistent in the literature. Whereas Shin et al. (2008) observed the highest solubility at pH 5 and decreased at pH 7, Li et al., (2015) obtained the highest solubilisation at pH 11. The viability of biosurfactants for metals removal has been also demonstrated (Mulligan et al., 1999; Juwarkar et al., 2007; Díaz et al., 2015). In the case that the removing of both organic and inorganic contaminants from soils is required, mixtures of extractants are usually employed (Reddy et al., 2011; Tian et al., 2017). In spite of the good results obtained in studies in the laboratory on RL-enhanced soil remediation, they have not yet been significantly employed at field scale due mainly to the cost for commercial RL supply. However, it is expected that a large-scale industrial production in microbial surfactants will emerge in the next few years because

consumers increasingly demand for environmentally friendly products (Henkel et al., 2017).

Cyclodextrins (CDs) are alternative agents to enhance the apparent solubility of hydrophobic compounds and their removal from soil (Flaherty et al., 2013; Mousset et al., 2014). CDs have a low-polarity cavity where organic compounds of the appropriate shape and size can form inclusion complexes (Morillo et al., 2012), increasing their apparent solubility and availability for biodegradation (Villaverde et al., 2012; Sánchez-Trujillo et al., 2014; Morillo et al., 2014). CDs have several advantages such as their non-toxicity to microorganisms, high biodegradability and negligible sorption to soils, and relative insensitivity to pH and ionic strength effects. CDs have demonstrated to be effective extractants also for PTEs. Laboratory studies have shown the ability of some derivatives of CDs to complex metals (Skold et al., 2009; Prochowicz et al., 2016), but it has been only observed as a collateral effect when using CDs to remove organic contaminants from soils. As far as we know, there are no published studies dealing with the extraction of only PTEs from soils using CDs.

The majority of washing studies on contaminated soils are carried out to extract only organic or inorganic contaminants, but without describing the effect of the selected extractant on the rest of other contaminants present in the soil. There are few studies using only one extractant (biosurfactant or CDs) to remove simultaneously PTEs and hydrophobic contaminants, especially in the case of biosurfactants. Wang and Brusseau (1995), Chatain et al. (2004), Skold et al. (2008) or Ye et al. (2014) used carboxymethyl- β -CD for soil washing of metals and various organic contaminants; Yang et al. (2010) and Sun et al. (2013) observed the simultaneous removal of PAHs and metals using two amino acids derived from β -CD or methyl- β -CD, respectively; and Wang et al. (2010) studied the extraction of phenanthrene and lead from soil using

glycine- β -CD. On the contrary, in relation to biosurfactants, as far as we know, there are no studies using rhamnolipids for the same purpose. Only Song et al. (2008) evaluated the performance of another biosurfactant (saponin) for removing phenanthrene and cadmium from co-contaminated soils.

The effect of any remediation treatment used for hydrophobic contaminants removal from co-contaminated soils will have effects on extraction and availability of PTEs present in the soil, and this fact have to be considered in order to avoid a secondary contamination of the surrounding environment or an increased toxicity in the treated soil which can affect to a subsequent bioremediation process. This is the main novelty that brings this study, and therefore, its specific objective was to evaluate the performance of several cyclodextrins and the rhamnolipid JBR 425 (RL) on the removal of the 16 priority PAHs defined by the US-EPA from an aged creosote-contaminated soil, and the possibility of mobilization of PTEs (Cd, Cr, Cu, Pb, Zn, Ni, As) present also as co-contaminants.

The wood treatment plant where the co-contaminated soil was taken was in use from 1906 till 1998 for impregnation of railway sleepers to preserve and waterproof these wooden structures, and, therefore, the contaminants are highly resistant to their mobilization because of such aged contamination.

Cations in the soil solution are determinant for the ionic strength of soils, but can significantly affect the self-assembly behavior of some surfactants and their solubilization capacity (Helvacı et al., 2004; Chen et al., 2016). This study evaluates also the effect of Ca^{2+} cations (as 0.01 M $\text{Ca}(\text{NO}_3)_2$, solution which is often used to simulate ionic strength of soil solution) on the solubilization and extraction of PTEs and PAHs from the selected soil when using CDs or RLs.

2. Materials and Methods

2.1. Collection and preparation of contaminated soil samples

The soil was collected from a former wood treatment plant (38° 01' 39.1'' N 4° 03' 58.6'' W Jaen, Spain), and obtained at a depth of 0-20 cm. The soil was air-dried at room temperature, crushed, passed through a 2 mm sieve and stored at -20°C. The main properties of the polluted soil are shown in Table 1. The soil has a sandy loam texture.

2.2. Chemicals and reagents

Sixteen priority USEPA PAHs were purchased from Accustandard (New Haven, CT, USA). Deuterated surrogate PAHs (naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂) from Sigma-Aldrich (St.Louis, MO, USA). Anthracene-d₁₀ (Dr.Ehrenstorfer, Augsburg, Germany) was used as internal standard. β -CD, HP- β -CD, HP- γ -CD, and RAMEB (randomly methylated- β -CD, purity 97%) were supplied by Cyclolab (Budapest, Hungary). Rhamnolipid biosurfactant JBR 425 (RL) was supplied by Jeneil Biosurfactant Co., (TX, USA). It is an aqueous solution of a mixture of two forms of rhamnolipid (molar ratio of 0.97/1) at 25 wt% in water, with a CMC value around 30 mg/l at the lowest surface tension of 28 mN/m (Mulligan and Roshtkhari, 2016). Solvents were from Merck (Darmstadt, Germany) and Solid Phase Extraction (SPE) alumina cartridges (5 g, 20 mL) were from Waters Corporation (Milford, MA, USA).

2.3. Exhaustive extraction of PAHs and PTEs

Exhaustive extractions were carried out to quantify the amount of PAHs and PTEs in the creosote-polluted soil. The exhaustive extraction of PAHs from soil was performed

in triplicate. 0.2 g of soil were spiked with 1 μg of the surrogates standard and placed in a glass centrifugation tube with 20 mL hexane/dichloromethane (1:1). This tube was vortexed (1 min) and placed in an ultrasonic bath (10 min). This process was repeated three times without changing the solvent. The sample was centrifuged and the supernatant placed in vials to be evaporated (Turbo Vap LV, MA, USA) to 1 mL at 20°C under a nitrogen stream prior to clean up with SPE cartridges with 5 g alumina. They were conditioned with 40 mL hexane:dichloromethane (1:1) and 2 mL of the extract were loaded onto the column and eluted with 40 mL hexane:dichloromethane (1:1). This extract was evaporated to 1.5 mL at 20°C under a nitrogen stream and dosed in a chromatographic vial. 1 μg of anthracene- d_{10} was added as internal standard. Extraction of pseudo-total soil PTEs contents was carried out by microwave-assisted (Ethos 900, Milestone, Italy) aqua-regia digestion.

2.4. Non-exhaustive extraction of PAHs and PTEs from soil using an electrolyte solution and aqueous β -CD, HP- β -CD, RAMEB or rhamnolipid solutions

Three sequential batch extractions were performed using the electrolyte and aqueous solutions of the CDs and RL. 0.3 g of soil were placed in glass tubes containing 10 mL of 0.01 M β -CD, HP- β -CD, RAMEB or RL solutions with and without 0.01 M $\text{Ca}(\text{NO}_3)_2$ as background electrolyte, used to mimic the characteristics and ionic strength of soil-water solutions. Extractions with only distilled water or 0.01 M $\text{Ca}(\text{NO}_3)_2$ were also performed for comparison. Extraction experiments were carried out in triplicate. The glass tubes were shaken on a horizontal shaker for 20 h at a constant temperature of 20 °C. After centrifugation the supernatant was removed and the extracted PAHs and PTEs analyzed. Another 10 mL of a fresh water, electrolyte, CDs or RL solutions were

added. The extractions were carried out three times in total, and the extracts were analyzed individually.

For PAHs analysis, a 3 mL aliquot of the supernatant was liquid-liquid extracted with 3 mL of hexane. 1 mL of the organic phase was transferred to a glass vial and anthracene- d_{10} added before analysis to a final concentration of $1 \mu\text{g mL}^{-1}$.

For determination of PTEs, 10 mL of supernatant were treated with 1 mL of H_2O_2 and 1 mL of concentrated HNO_3 , and maintained at reflux (1h) to obtain a clear supernatant where PTEs content was determined. The remaining soil was washed three times with deionized water to completely eliminate the extractants residues, and was air dried. Then, available PTEs content in the soil after extractions was determined by 0.05 M EDTA extraction at pH 7.00 (Ure et al., 1993).

2.5 Instrumental analysis

Analysis of PAHs was performed by gas chromatography coupled to mass spectrometry (GC-MS) using an Agilent GC 6890 coupled with a quadrupole mass spectrometer (Agilent 5973N, CA, USA). Electron ionization mode was used at 70 eV. A HP-5 MS column coated with 5%-phenyl-methylpolysiloxane of 30 m x 0.25 mm I.D and 0.25 μm film thickness (J&W Scientific, CA, USA) was used with the following temperature programme: 60°C (1 min) to 175°C at 6 °C/min (4 min) to 235°C at 3°C/min (5 min) and raised to 315°C at 8°C/min. 2 μL of sample was injected in splitless mode and the valve was closed for 1.5 min. Helium was used as a carrier gas at 1.9 mL/min. Temperatures for the injector, transfer line and ion source were 280, 270 and 230°C, respectively. Time scheduled selected ion monitoring (SIM) was used with four retention time windows. Three ions were monitored for each compound to improve the sensitivity and selectivity. The software used was MSD ChemStation 5.0.0.0 (Agilent, CA, USA). The

quantification procedure with deuterated surrogate standard is explained in a previous study (Sánchez-Trujillo et al., 2013). The Quality Control/ Quality Assurance description is given in Supplementary Data (Document 1).

PTEs were determined by ICP-MS (IRIS Advantage spectrometer Thermo Jarrel Ash Corporation, MA, USA). Quality control was carried out with the reference materials (BCR-700 for EDTA and BCR-141R for aqua-regia extractable metals, from JRC-IRMM, European Commission). All measurements were done in triplicate. Deviations of $\pm 10\%$ were considered satisfactory.

Statistical analysis was performed using statistical package SPSS Version 23.0. Standard deviation is shown in parenthesis, and analysis of variance (Oneway ANOVA) was selected for comparison between treatments at 95 % confidence level ($P < 0.05$).

3. Results and Discussion

3.1 PAHs and PTEs content in the contaminated soil

The concentration of each PAH and their sum (Σ PAH) in the soil studied are shown in Table 2. According to the range of values of the classification of Maliszewska-Kordybach (Maliszewska-Kordybach, 1996) (< 200 , 200–600, 600–1000 and $> 1000 \mu\text{g kg}^{-1}$ for ‘not contaminated’, ‘weakly contaminated’, ‘contaminated’ and ‘heavily contaminated’ samples, respectively), a PAH content of 414.1 mg kg^{-1} corresponds to a heavily contaminated soil. The Spanish Government has proposed a “risk assessment value” of 50 mg kg^{-1} for total petroleum hydrocarbons in soils (Ministerio de la Presidencia, 2005), and this polluted soil exceeds this limit by 8.3-fold only with its content of 16 PAHs.

The chemical composition of creosote varies according to the production process and, in addition, there are several factors playing an important role in the accumulation or dissipation of PAHs in soil (humidity, mean temperature, organic matter content and aging of the soil) (Morillo et al., 2008), being frequent to have a wide variety in PAH soil content in the different wood preserving facilities. The more abundant constituents in creosote are 2- and 3-ring PAHs, amounting to more than 60%, and the less abundant are 5- and 6-ring PAHs, with percentages lower than 5% (Mueller et al., 1996; Bestari et al., 1998). PAHs in creosote complement each other with respect to wood preservation. The lighter molecular weight PAHs are generally more toxic to organisms, and those with heavier molecular weight help “retain” the more toxic lighter components within the wood by minimizing leaching or volatilization. However, medium and high molecular weight PAHs are accumulated in aged contaminated soils because low molecular weight PAHs decreased over time due to their higher volatility, water solubility and bioavailability to be degraded by soil microorganisms.

In the creosote-polluted soil under study, benzo(b)fluoranthene (5-rings) and fluoranthene, pyrene and crysene (4-ring PAHs) presented the higher percentages (18.6, 13.0, 11.1, and 10.9%, respectively), amounting to 53.6% with respect to the $\Sigma 16$ PAHs (Table 2). Moreover, the sum of 4-, 5-, and 6-ring PAHs amounts to 93.9%. The reason for the extremely low content in 2- and 3-ring PAHs in this soil in comparison to other creosote contaminated soils (Hansen et al., 2004; Juhasz et al., 2005; Realp et al., 2008; García-Delgado et al., 2015) is probably related to the region of Spain where it is situated, characterized by a very warm climate in the summer and mild in the winter, with a mean annual temperature of 17.9 °C and very dry conditions (mean annual rainfall about 490 mm), factors that favored their loss through volatilization.

In relation to PTEs, table 1 shows the pseudo-total content of PTEs present in this contaminated soil. Concentrations exceed the values established as normal in soils and those which are considered toxic for plants (Table S2) (Cabrera et al., 1999). It is remarkable the high concentration of Pb (1127 mg kg^{-1}) which exceeds the normal value in soil by 32.2-fold, and that of As, Cu and Zn, exceeding by 15.6-, 9.6- and 4.5-fold, respectively, their normal values. The variations in the levels of As, Cu and Cr measured in soils near inorganic arsenical treated wood is quite broad, ranging from 15 to 3200 mg kg^{-1} for As, from 20 to 2400 mg kg^{-1} for Cr, and from 10 to 9800 mg kg^{-1} for Cu (Guemiza et al., 2017).

In this wood treatment plant it is known that Tanalith (a mixture of sodium fluoride, sodium dichromate, arsenate and dinitrophenol) started to be used from 1960's (personal communication), and it is the origin of As and Cr contamination. Probably ACZA (ammoniacal copper-zinc-arsenate) was also used as wood preservative and would explain the high concentrations of Cu and Zn. But, as far as we know, a wood preservative containing Pb does not exist. However, it is known that creosote could be applied either at full strength or diluted with petroleum oil or coal tar, and the presence of Pb could come from it (Sudell, 1992).

3.2 PAHs extraction with cyclodextrins and rhamnolipids

The ability of RL and several CDs to remove individual PAHs from the polluted soil is observed in Figures 1 and 2. PAHs extraction using distilled water was quite similar to that obtained with $0.01 \text{ M Ca(NO}_3)_2$ solution and only this later is presented in both figures. It is noteworthy the much higher extraction percentages of PAHs obtained with RLs in the absence of $0.01 \text{ M Ca(NO}_3)_2$. The extraction reached up to 27% in the case of

acenaphthene or 20% for anthracene and pyrene (Figure 1), giving as result up to 15.4% of the total PAHs extracted (Table 3). The removal of PAHs by RLs may be attributed to a decreased interfacial tension which decreases the repulsive forces between hydrophobic compounds adsorbed on soils and the aqueous phase, allowing the formation of co-aggregates or micelles to incorporate such compounds, which are responsible for their solubilization (Zeng et al., 2018).

However, in the presence of 0.01 M $\text{Ca}(\text{NO}_3)_2$ the extraction of PAHs from this creosote-polluted soil decreased drastically. Solutions with different cations can significantly affect the self-assembly behavior of RL. Electrolytes directly affect the carboxylate groups of the rhamnolipids and Helvacı et al. (2004) found that the addition of NaCl could cause a decrease in CMC and surface tension of RL solution. Chen et al. (2016) observed changes in self-assembly of RL with different cations, and the residual RL concentration in water decreased significantly with 0.01 M Ca^{2+} and Mg^{2+} due to the precipitation of RL, decreasing the solubilization of triclosan from sediments.

The percentages of PAHs extracted by RL in other studies are quite different because they depend on the concentration of RL used and the type of soil. Most of the studies have been carried out on not aged soils artificially contaminated, and percentages up to 60-70% can be reached (Bordas et al., 2005; Zhang, 2015), but also lower recoveries are observed (10-17%, Shin et al., 2006; 7-9%, An et al., 2011). Such marked disparity in the results obtained could be in part due to the different ionic strength of the soil solution in each study, which is often not considered.

Table 4 shows the percentage of extraction enhancement obtained for each PAH when using solutions of the different extractants with respect to the extraction obtained with only distilled water (ratio of the percentage extracted with CDs or RLs solutions to that obtained with water, which was the same as that using $\text{Ca}(\text{NO}_3)_2$ solution). It should be

noted the extremely high enhancement factor of extraction obtained with RLs in the absence of Ca^{2+} , increasing up to more than 117- and 120-fold higher in the cases of fluoranthene and pyrene, between 11.4- and 24.6-fold higher for other 8 PAHs, and higher than 7.92-fold for the rest of PAHs. That is, even 5- and 6-ring PAHs, extremely difficult to be extracted from soils with aqueous solutions due to their high hydrophobicity, are effectively released with RL solutions in absence of the electrolyte.

The high PAHs extraction obtained with RL contrast with those observed with the different CDs (Figure 2), with only a maximum extraction of 2% in the case of HP- β -CD (Table 3). With CDs as extractants, a decrease in percentage of PAHs extracted is observed as the number of rings increased. As observed in Table 4, in the case of using β -CD, 8 of the 14 PAHs determined were extracted in lower amounts than with $\text{Ca}(\text{NO}_3)_2$ solution. On the contrary, HP- β -CD, RAMEB and HP- γ -CD extracted higher amounts than $\text{Ca}(\text{NO}_3)_2$ solution, but not for 6-ring PAHs when the extraction was carried out with the different CDs (Table 4). The reasons for the low extraction of PAHs in this particular soil by using CDs is the low content of 2-, 3-ring PAHs, which are practically absent (6.14% of the total PAHs content), in contrast to the majority of creosote-polluted soils, as previously mentioned. The molecular sizes of these LMW PAHs were the most favorable for the formation of inclusion complexes with β -CD derivatives due to their stereoselective diffusion to the nonpolar cavity (Morillo et al., 2012). However, 4-ring PAHs were those extracted in higher concentration by the different extractants, that can be due to their higher contents in relation with 2-, 3-ring PAHs (Table 2) (Wu et al., 2010). In a previous paper (Sánchez-Trujillo et al., 2013), the same procedure using different CDs was used to extract PAHs from another industrial soil where 2- and 3-ring PAHs amounted to 73.7%. Whereas in that polluted soil up to 26.6%, 26.4%, 9.02% and 6.94% of 3-ring PAHs could be extracted by

HPBCD, RAMEB, BCD and HP γ CD, respectively, in this creosote-polluted soil only 5.07%, 5.07%, 2.82% and 3.79% were extracted, respectively.

The presence of PTEs in this co-contaminated soil may have exerted also some effects to decrease PAHs extraction. The bonding of phenanthrene adsorption was strengthened in the presence of adsorbed Pb²⁺ more than with Cd²⁺ (Zhang et al., 2011). Liang et al. (2016) also reported that in the presence of Cu, Pb and Cr the sorption of naphthalene, phenanthrene and pyrene to soils were enhanced by 21.1-107%. The extent of increment was positively associated with the hydrophobicity of the PAHs and the electronegativity and radius of the metal cations. The sorption of metals increased the zeta potentials of soil surfaces, becoming neutralized and more hydrophobic, and increasing the affinity of PAH for soil surfaces. The cation- π bonding between the adsorbed metal cations and the aromatic rings of PAHs has a significant role to their improved sorption to soil. Therefore, one of the factors which can contribute to the low extraction of PAHs from this contaminated soil is the presence of high concentrations of PTEs.

The presence of a mixture of PAHs in this co-contaminated soil may have also exerted some effects on individual PAHs extraction. However, the effect of PAH concentration or property on desorption was not always consistent in different studies. Doick et al. (2006) observed in single and co-contaminant spiked and aged soils with phenanthrene and/or pyrene that the quantity of phenanthrene recovered was significantly greater in co-contaminated than in single phenanthrene spiked soil, and the quantity of pyrene extracted was lower when phenanthrene was present. Also Madrid et al. (Madrid et al., 2016) observed that the co-contamination of soil with fluorene and pyrene did not affect fluorene dissipation evolution, but significantly inhibited pyrene dissipation, in comparison to their respective single systems.

3.3 PTEs removal and available PTEs content in the polluted soil before and after extraction treatments

3.3.1 PTEs removal

Table 5 shows the percentage of total PTEs extracted from the polluted soil after three consecutive extractions with various extractants. Metal ions are able to form complexes with certain CD derivatives, such as methyl- β -CD, carboxymethyl- β -CD, or glycine- β -CD, demonstrated their significant effect on inorganic pollutant removal from soil (Norkus, 2009). However, β -CD, HP- β -CD or RAMEB proved to be inefficient for extraction of PTEs from contaminated soils (Khodadoust et al., 2005; Ehsan et al., 2007). In the present study the percentages of PTEs extracted with the different CDs were in general similar to those extracted by $\text{Ca}(\text{NO}_3)_2$ solution. Therefore, the use of β -CD, HP- β -CD or RAMEB to extract PAHs (particularly those with high percentages of low molecular weight PAHs) could be an advantage in those soils where the release of PTEs is not convenient to prevent an increase of the soil toxicity.

The extraction of PTEs with RLs prepared in $\text{Ca}(\text{NO}_3)_2$ solution as background electrolyte was also not statistically different from that obtained with only 0.01 M $\text{Ca}(\text{NO}_3)_2$, except in the case of Cu and Ni (Table 5). This low extraction of PTEs reached with RLs contrasts with the results of a number of studies which demonstrated previously their viability for the removal of metals from soils (Sarubo et al., 2015; Chen et al., 2017). Slizovskiy et al. (2011) observed that the rhamnolipid JBR 425 was effective in decreasing Zn, Cu, Pb, and Cd in soil by 39, 56, 68, and 43%, respectively, compared with less than 6% removal by water alone. Juwarkar et al. (2008) used a di-rhamnolipid biosurfactant to remove Cr, Pb, Cd, Cu and Ni from soil. They reported

that the biosurfactant selectively removed the PTEs in the order: $\text{Cd} = \text{Cr} > \text{Pb} = \text{Cu} > \text{Ni}$. However, when PTEs were extracted with RL solutions prepared only in water, up to 21.3% of pseudo-total Cd content could be extracted, about 13-14% Pb, Zn, or Ni, about 7% Cu or As, and 5.43% Cr, with the sequence: $\text{Cd} > \text{Ni} \geq \text{Zn} \geq \text{Pb} > \text{Cu} \geq \text{As} > \text{Cr}$, quite similar to that obtained by Juwarkar et al. (2008), except in the case of Cr. Probably the higher percentage of Pb extracted in relation to Cu in our case was related to its much higher concentration (1127 and 289 mg kg^{-1} for Pb and Cu, respectively).

When RLs are prepared with 0.01 M $\text{Ca}(\text{NO}_3)_2$ used as background electrolyte PTEs extraction was very low due to their precipitation, as mentioned in section 3.2. For this reason, the total amount of Ca^{2+} determined in the supernatants after finishing three consecutive extractions with RL was 41219 mg kg^{-1} , much lower than Ca^{2+} in the supernatants after extraction with CDs or $\text{Ca}(\text{NO}_3)_2$ solutions ($> 65000 \text{ mg kg}^{-1}$, data not shown). Torrens et al. (1998) also observed that increasing concentrations of K^+ in solution increased RL sorption, and decreased the removal of soil-bound cadmium. Therefore, RL sorption/precipitation in saline soils has to be considered, as well as its use as a feasible remediation option.

Arsenic was the only studied PTE which presents different chemical characteristics from those of the rest of elements, but, nevertheless Wang and Mulligan (2009, 2014) showed its extraction from soils using RLs. As far as we know, these were the only studies demonstrating the capacity of RLs to extract As until now.

In the present paper, the extraction of As by $\text{Ca}(\text{NO}_3)_2$ solution or by RLs in the presence of the background electrolyte was not detected. The behavior of arsenic in soil is analogous to that of phosphate because of their chemical similarity. Like phosphate, As is fixed to Al and Fe-containing soil particles, and thus is relatively immobile.

Furthermore, Fe, Al and Ca influence this fixation forming insoluble precipitates with arsenate (Arai, 2010). However, when the treatment with RLs was performed in the absence of Ca^{2+} from the background electrolyte, up to 7% As was extracted, showing that RLs can be used to remediate soils containing As but only in those cases where the ionic strength of the soil solution was low.

3.3.2 PTEs availability

The resulting treated soil obtained after using different extractants should be studied in relation to not only the total concentration of PTEs in the system, which may not be the most appropriate measure, but also the availability of the PTEs which remain in the treated soil. PTE availability determines both the risk of a possible metal leaching through the soil profile, and the physiological and toxic effects of available PTEs on biodegradation of the organic pollutants.

Table 6 shows the available soil PTE content initially present in the polluted soil, and after the extraction treatment with different extractants in 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution as background electrolyte. The available metals were also determined after their extraction with the same extractants but in the absence of the background electrolyte, but only data relative to RL are reported in Table 6 because with the CDs the results were similar to those obtained in the presence of the electrolyte. The availability of the various metals follows different behaviour depending on both the extractant and the metals.

The availability of Zn decreased after extraction with CDs, and also with RL in presence of $\text{Ca}(\text{NO}_3)_2$. Zn is known to be more mobile in soil than many other metals, and probably a great part of its available content has been mobilized previously during

the soil washing procedure. Also Cd presents similar properties, but the decreased availability observed in Table 6 was not statistically significant.

The effect of the different extractants on Cu and Pb availability was also not significant. Both elements are the least mobile of all the PTEs studied here. They form very strong complexes with soil organic matter, with a very high content in this soil (8.11%), and other soil components, such as iron and manganese oxides or clay minerals (Hough, 2010), and therefore their availability have not changed with the extractants.

Availability increased about 100% in the case of Cr and Ni and 200% for As when the soil had been previously extracted with RL prepared in 0.01 M $\text{Ca}(\text{NO}_3)_2$ solution as background electrolyte. On the contrary, when the previous extraction was carried out in the absence of the electrolyte, the availability of these elements was similar to that presented initially in the soil. The remaining concentrations of RLs adsorbed and/or precipitated on the soil surface due to the presence of the electrolyte seem to exert a powerful effect on the subsequent increased availability of these PTEs.

Cr exists generally as occluded lattice forms and associated with Fe and Al oxides (Chirenge et al., 2003). Ni is associated mainly with Fe and Mn oxides. Also As is retained mainly on the sorption sites of variable charge minerals (Fe, Mn, and Al oxides, allophanic materials, and imogolite) and edges of phyllosilicates, and carbonates (Pigna et al., 2015), but has a strong affinity for the surfaces of minerals containing Fe. According to Ochoa-Loza et al. (2007), RLs adsorb also on surface sites of oxides and clay minerals, and the presence of RLs inhibits the formation of Fe and Al hydroxide precipitates (Wang and Mulligan, 2009), which will reduce the adsorption of As, Ni and Cr, enhancing their removal. This possibility is supported by the higher amounts of available Fe and Al observed when the soil had been treated with RLs in 0.01 M

$\text{Ca}(\text{NO}_3)_2$ solution as background electrolyte (Table S3), which increased two-fold and ten-fold, respectively, the values obtained when the soil had been extracted with RLs without electrolyte or CDs (prepared both, in water or in 0.01 M $\text{Ca}(\text{NO}_3)_2$ solutions).

All these factors must be taken into account when different extractants are employed for remediation of co-contaminated soils, because they can increase the bioavailability of organic contaminants, but also that of PTEs present (Ren et al., 2018), with the consequent possibility of inhibiting pollutant biodegradation and/or increase PTEs leaching. At this time, however, few data exist concerning the impact of washing solutions or other soil treatment processes on metal bioavailability, particularly with field-aged samples.

Conclusions

The conclusions that arise from this study indicate that β -CD derivatives are suitable to be used in washing processes of contaminated soils containing high percentages of 2-, 3-, 4-ring PAHs, even with high contents in PTEs, because these will not be removed. The presence of inorganic cations in the washing solutions has no effects on PAH extraction.

On the contrary, extractions using RLs is advantageous even for the removal of PAHs with higher molecular weights, extremely difficult to be extracted due to their high hydrophobicity. However, the ionic strength and the cations present in the soil solution have to be considered to avoid RLs inactivation due to their precipitation, as well as to minimize the risks of increasing PTEs extraction and availability.

Results obtained from this investigation indicated that previous information about the content in organic and inorganic contaminants, and their mutual effects when using

selected extractants is needed for the election of effective and safe remediation agents for the co-contaminated soils.

Assessment of ecotoxicological effects would be particularly important because some extractants can release PTEs that, otherwise, would not be available due to their strong binding to soil particles. Consequently, PTEs uptake by receptor organisms may be enhanced. Research about assessment of the use of different ionic strength and cations as well as ecotoxicological effects derived from CDs and RLs application to co-contaminated soils is currently being performed.

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Figure legends

Figure 1. Percentage of individual PAHs extracted from the polluted soil using aqueous solutions of $\text{Ca}(\text{NO}_3)_2$ and RL in presence and absence of $\text{Ca}(\text{NO}_3)_2$.

Figure 2. Percentage of individual PAHs extracted from the polluted soil using aqueous solutions of $\text{Ca}(\text{NO}_3)_2$ and several CDs.

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Table 1. Physico-chemical characteristics and potentially toxic elements (PTEs) concentrations (mg kg^{-1}) of the polluted soil. Standard deviation in parenthesis.

OM (%)	8.11 (0.30)	<u>Pseudo total content of PTEs (aqua regia)</u>	
CaCO ₃ (%)	23.2 (3.1)	Cr	41.3 (5.9)
pH	8.14 (0.16)	Cu	289 (55)
Clay (%)	10.4 (1.3)	Ni	31.1 (4.6)
Silt (%)	14.8 (2.8)	Pb	1127 (123)
Fine sand (%)	8.10 (1.17)	Zn	402 (32)
Coarse sand (%)	66.7 (3.0)	Cd	1.30 (0.10)
CEC (cmol kg^{-1})	7.51 (0.64)	As	93.6 (4.6)

Table 2. Content of PAHs in the creosote contaminated soil (mg kg^{-1}) and percentage of individual PAHs related to $\Sigma 16$ PAHs. Standard deviation in parenthesis.

PAHs	mg kg^{-1}		%	
Naphtalene	1.72	(0.15)	0.41	(0.04)
Acenaphthylene	5.20	(0.70)	1.26	(0.17)
Acenaphthene	0.78	(0.22)	0.19	(0.05)
Fluorene	0.57	(0.12)	0.14	(0.03)
Phenanthrene	7.04	(2.04)	1.70	(0.50)
Anthracene	10.1	(0.36)	2.44	(0.09)
Fluoranthene	53.9	(4.70)	13.0	(1.13)
Pyrene	46.1	(2.50)	11.1	(0.60)
Benzo[a]anthracene	24.5	(1.71)	5.92	(0.41)
Chrysene	45.1	(5.78)	10.9	(1.40)
Benzo[b]fluoranthene	77.0	(15.0)	18.6	(3.67)
Benzo[k]fluoranthene	18.9	(5.07)	4.56	(1.23)
Benzo[a]pyrene	32.3	(7.35)	7.80	(1.78)
Dibenzo[a,h]anthracene	21.2	(2.66)	5.12	(0.64)
Indeno[1,2,3-cd]pyrene	36.7	(7.19)	8.86	(1.74)
Benzo[ghi]perylene	33.0	(5.42)	7.97	(1.31)
Σ 2,3-rings	25.4	(3.59)	6.14	(0.88)
Σ 4-rings	169.6	(14.7)	40.95	(3.54)
Σ 5-rings	149.4	(30.0)	36.08	(7.32)
Σ 6-rings	69.7	(12.6)	16.83	(3.05)
$\Sigma 16$ PAHs	414.1	(60.9)	100	(14.8)

Table 3. Concentration ($\mu\text{g/g}$) and percentages of PAHs extracted (separated by number of rings) using different extractants. Extractions using only distilled water or 0.01 M $\text{Ca}(\text{NO}_3)_2$ solutions were similar, as well as extractions using CDs in water or in $\text{Ca}(\text{NO}_3)_2$ solutions as background electrolyte. Standard deviation in parenthesis.

PAHs	2-rings		3-rings		4-rings		5-rings		6-rings		$\Sigma 16$ PAHs	
	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%
H₂O/Ca(NO₃)₂	0.07 (0.0 1)	4.34 (0.5 8)	0.36 (0.1 0)	1.52 (0.4 3)	0.71 (0.1 8)	0.42 (0.1 1)	1.40 (0.5 9)	0.94 (0.4 2)	0.92 (0.5 8)	1.32 (0.7 9)	3.46 (1.0 7)	0.84 (0.3 5)
β-CD	0.07 (0.0 2)	4.35 (1.2 0)	0.67 (0.0 9)	2.82 (0.3 8)	0.97 (0.2 6)	0.57 (0.1 5)	0.97 (0.2 2)	0.65 (0.1 4)	0.57 (0.0 8)	0.82 (0.1 2)	3.25 (0.6 6)	0.79 (0.1 6)
HP-β-CD	0.10 (0.0 1)	5.84 (0.6 1)	1.20 (0.2 6)	5.07 (1.1 1)	4.22 (1.1 1)	2.49 (0.6 5)	2.28 (0.5 0)	1.52 (0.3 4)	0.49 (0.1 6)	0.70 (0.2 3)	8.29 (2.0 4)	2.00 (0.4 9)
RAMEB	0.10 (0.0 0)	5.66 (0.1 4)	1.20 (0.0 9)	5.07 (0.3 8)	2.06 (0.1 3)	1.22 (0.0 8)	1.82 (0.3 4)	1.22 (0.2 2)	0.67 (0.1 6)	0.97 (0.2 3)	5.86 (0.7 2)	1.41 (0.1 7)
HP-γ-CD	0.09 (0.0 2)	5.17 (1.0 1)	0.90 (0.0 8)	3.79 (0.3 3)	1.25 (0.1 8)	0.74 (0.1 0)	2.13 (0.9 7)	1.43 (0.6 5)	0.63 (0.1 2)	0.90 (0.1 8)	5.00 (1.3 7)	1.21 (0.3 3)
RL	0.04 (0.0 1)	2.25 (0.4 2)	0.53 (0.0 9)	2.25 (0.2 0)	1.11 (0.1 6)	0.65 (0.0 9)	0.29 (0.0 6)	0.20 (0.0 4)	0.53 (0.1 2)	0.77 (0.1 4)	2.50 (0.4 4)	0.60 (0.1 1)
RL (H₂O)*	0.10 (0.0 1)	5.81 (0.8 2)	3.16 (0.2 4)	13.4 (1.0 2)	30.8 (4.7 5)	18.2 (2.8 0)	16.8 (2.6 9)	11.3 (1.8 0)	11.9 (2.1 8)	17.1 (3.1 3)	62.9 (9.8 7)	15.4 (2.3 9)

* not prepared in 0.01 M $\text{Ca}(\text{NO}_3)_2$.

Table 4. Extraction enhancement effect of different extractants on PAHs with respect to the extraction obtained with only distilled water (or 0.01 M $\text{Ca}(\text{NO}_3)_2$ aqueous solution, which was the same).

	β -CD	HP- β -CD	RAMEB	HP- γ -CD	RL	RL (H_2O)*
Naphtalene	1.00	1.35	1.30	1.19	0.52	1.34
Acenaphtylene	1.51	3.38	3.29	2.24	0.41	11.7
Acenaphtene**	nd	nd	nd	nd	nd	nd
Fluorene**	nd	nd	nd	nd	nd	nd
Phenanthrene	1.88	2.42	1.51	1.44	0.11	18.6
Anthracene	1.77	3.26	3.58	2.09	1.91	7.92
Fluoranthene	0.85	8.88	1.36	1.56	2.53	117
Pyrene	0.64	7.45	2.76	2.66	4.79	121
Benzo (a) anthracene	1.56	4.10	3.04	1.49	0.22	18.0
Chrysene	1.54	6.15	3.21	1.78	1.49	24.6
Benzo (b) fluoranthene	0.68	1.74	1.32	1.10	0.28	15.2
Benzo (k) fluoranthene	0.46	1.30	1.30	0.70	0.16	11.4
Benzo(a)pyrene	0.92	1.56	0.61	2.83	0.15	8.86
Dibenzo (a,h) anthracene	0.51	1.94	2.98	0.90	0.19	10.6
Indeno(1,2,3-cd)pyrene	0.58	0.44	0.85	0.87	0.84	12.2
Benzo (g,h,i) perylene	0.67	0.65	0.59	0.45	0.26	13.9

*not prepared in 0.01 M $\text{Ca}(\text{NO}_3)_2$.

**nd: not determined. These PAHs were not extracted with distilled water or $\text{Ca}(\text{NO}_3)_2$ aqueous solution

Table 5. Percentages of PTEs extracted from the polluted soil using solutions of different extractants. Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

Extractant	As	Cd	Cr	Cu	Ni	Pb	Zn
Ca(NO₃)₂	n.d.	7.71 a	2.16 a	n.d.	3.23 a	0.32 a	2.71 a
BCD	n.d.	4.28 a	1.32 a	n.d.	2.60 a	0.40 a	3.13 a
HPBCD	n.d.	7.81 a	2.87 ab	n.d.	3.71 a	0.53 a	2.64 a
RAMEB	n.d.	3.36 a	1.01 a	n.d.	2.46 a	0.21 a	2.06 a
RL	n.d.	9.72 a	1.37 a	0.62 a	6.40 a	0.50 a	3.95 a
RL(H₂O)*	7.02	21.3 b	5.43 b	7.46 b	14.4 b	12.9 b	13.2 b

*not prepared in 0.01 M Ca(NO₃)₂.

Table 6. Available PTEs content (mg kg^{-1} ; EDTA) initially present in soil and after extractions with different extractants. Values in the same row followed by the same letter are not significantly different ($P < 0.05$)

	Initial	BCD	HPBCD	RAMEB	RL	RL(H₂O)*
Cr	0.24 a	0.20 a	0.27 a	0.22 a	0.52 b	0.15 a
Cu	35.4 a	36.8 a	37.6 a	35.3 a	40.8 a	43.2 a
Ni	0.29 ab	0.19 a	0.25 a	0.26 a	0.57 c	0.34 b
Pb	266 a	301 a	291 a	274 a	283 a	220 a
Zn	38.3 b	29.0 a	29.8 a	25.9 a	31.0 a	35.9 b
Cd	0.32 a	0.28 a	0.28 a	0.25 a	0.26 a	0.24 a
As	1.03 a	2.06 ab	2.00 ab	2.18 ab	3.08 b	1.03 a

Highlights

- PAHs extraction on metal availability is studied in creosote-contaminated soil
- Rhamnolipids (RLs) and cyclodextrins (CDs) used as eco-friendly extractants
- PTEs not extracted with the selected CDs. Increased PTEs extraction and availability with RL.
- Higher Σ PAHs extraction with rhamnolipids (RLs) than with cyclodextrins (CDs)
- PAHs extracted with RL falls greatly in Ca^{2+} solutions due to RL precipitation

Figure 1

Figure 2