Photodegradation of polycyclic aromatic hydrocarbons in soils under a climate change base scenario

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ABSTRACT

The photodegradation of polycyclic aromatic hydrocarbons (PAHs) in two typical Mediterranean soils, either coarse- or fine-textured, was here investigated. Soil samples, spiked with the 16 USEPA priority PAHs, were incubated in a climate chamber at stable conditions of temperature (20°C) and light (9.6 W m^{-2}) for 28 days, simulating a climate change base scenario. PAH concentrations in soils were analyzed throughout the experiment, and correlated with data obtained by means of Microtox® ecotoxicity test. Photodegradation was found to be dependent on exposure time, molecular weight of each hydrocarbon, and soil texture. Fine-textured soil was able to enhance sorption, being PAHs more photodegraded than in coarse-textured soil. According to the EC_{50} values reported by Microtox®, a higher detoxification was observed in fine-textured soil, being correlated with the outcomes of the analytical study. Significant photodegradation rates were detected for a number of PAHs, namely phenanthrene, anthracene, benzo(*a*)pyrene, and indeno(*123-cd*)pyrene. Benzo(*a*)pyrene, commonly used as an indicator for PAH pollution, was completely removed after 7 days of light exposure. In addition to the PAH chemical analysis and the ecotoxicity tests, a hydrogen isotope analysis of benzo(*a*)pyrene was also carried out. The degradation of this specific compound was associated to a high enrichment in ^2H, obtaining a maximum δ^2H isotopic shift of +232‰. This strong isotopic effect observed in benzo(*a*)pyrene suggests that compound-specific isotope analysis (CSIA) may be a powerful tool to monitor *in situ* degradation of PAHs. Moreover, hydrogen isotopes of benzo(*a*)pyrene evidenced a degradation process of unknown origin occurring in the darkness.

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Photodegradation
Soil
Ecotoxicity
Hydrogen isotopes

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of semi-volatile organic compounds composed of two or more fused aromatic rings. Although these chemicals are mostly released to air, soil is considered as one of the major sinks of atmospheric PAHs (Nadal et al., 2011; Wang et al., 2014), being deposited via dry and wet processes (Nadal et al., 2004). PAH fate in the environment includes volatilization, adsorption on soil particles, leaching, microbial degradation, chemical oxidation, and photo-oxidation (Hartiash and Kaushik, 2009). Photodegradation is an important transformation pathway for most PAHs in the environment (Zhang et al., 2006), having been largely studied in water (Bertilsson and Widenfalk, 2002; de Bruyn et al., 2012; Fasnacht and Blough, 2003; García-Martínez et al., 2005; Jacobs et al., 2008; Jing et al., 2014; Luo et al., 2014; Rivas et al., 2000; Shemer and Linden, 2007; Singh et al., 2013; Xia et al., 2009). In contrast, the knowledge regarding the photodegradation process of PAHs in soils is rather limited (Balmer et al., 2000; Frank et al., 2002; Gong et al., 2001; Xiaozhen et al., 2005). It has been reported that soil depth has an important role in the photodegradation of these chemicals, enhancing the resistance of PAHs to be photodegraded. In addition, temperature, soil particle size and humic acids also have a significant influence on photodegradation in soils under UV-B radiation (Zhang et al., 2010). Photodegradation of PAHs in soils has been shown to be not only limited by the light penetration capacity in soils, but also by its wavelength (Cavoski et al., 2007; Xiaozhen et al., 2005). Consequently, photodegradation depends on a number of variables, such as soil type, thickness of the soil layer, as well as light absorption spectrum of each compound (Zhang et al., 2010). This degradation process may play a key role on the fate of PAHs in areas such as the Mediterranean region, with high sunlight presence during the whole year. In turn, some PAH metabolites, which can even be more toxic than their parental compounds, may be generated during the degradation process. Overall, although PAH levels might be reduced in soils exposed to sunlight, toxicity may be increased (Huang et al., 1995; Mallakin et al., 1999; McConkey et al., 1997).

Compound-specific isotope analysis (CSIA) is a very valuable tool, which can be used to monitor *in situ* degradation processes of chemical pollutants, and as a source identification technique (Elsayed et al., 2014; Imfeld et al., 2014). CSIA is capable of discriminating degradation from other attenuation processes naturally occurring in the environment, that do not generate destruction of pollutants, such as dispersion,

volatilization or sorption. CSIA is based on the isotopic effect produced during a degradation process, which is known as isotopic fractionation (Meckenstock et al., 2004). This effect is based on the enrichment of heavy isotopes in the reacting compound, which is linked to the different strength of the bonds that contain heavy and light isotopes. Since nondestructive natural attenuation processes frequently do not entail significant isotope fractionation, a significant enrichment of the heavy isotope of organic pollutants confirms that a degradation process is occurring. Unfortunately, research on the hydrogen isotopic fractionation of PAHs during degradation is very scarce. To the best of our knowledge, the only precedent is the study of Bergmann et al. (2011), who reported a high hydrogen isotopic shift of naphthalene in two different microbial cultures.

The present investigation aimed at assessing the photodegradation of 16 US EPA priority PAHs in two types of Mediterranean soils. Laboratory experiments were conducted in a climate chamber to simulate the current Mediterranean environmental conditions, keeping temperature and sunlight stable. Temporal changes of PAH concentrations and ecotoxicity levels were investigated, and jointly evaluated. Moreover, hydrogen isotopic analysis of benzo(*a*)pyrene, considered one of the most toxic PAHs and probably carcinogenic to humans (Aina et al., 2006), was complementarily performed to verify the findings.

2. Materials and methods

2.1. Soil characteristics

Two common Mediterranean soils were selected to perform the photodegradation experiments. Physicochemical properties of both soils are given in Table 1. Soil samples were collected from the A horizon of remote areas of Catalonia (NE of Spain). The Arenosol soil, with granitic origin, is an acidic and coarse-textured soil that can be classified as Haplic Arenosol, according to the (FAO-UNESCO, 1998). It is commonly used for ecotoxicity tests in terrestrial environments. In turn, Regosol soil is an alkaline calcareous fine-textured soil formed of sedimentary materials, being classified as Calcaric Regosol (FAO-UNESCO, 1998). Both soils are characterized by owing a low organic matter content (Table 1). In order to quantify titanium, iron, aluminum and manganese oxides, ammonium oxalate was used as extractant, according to the method described by Drees and Ulery (2008).

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130 *2.2. Experimental design*

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132 Photodegradation experiments were carried out in a Binder KBWF 240 climate
133 chamber (Binder GmbH, Tuttlingen, Germany) with constant lighting, temperature and
134 humidity. Temperature and daylight were set at 20 ºC and 9.6 W/m^2, respectively, as
135 current environmental conditions in the Mediterranean area. Because photodegradation
136 reactions occur mainly in the surface, soil was air-dried. Consequently, to avoid the
137 presence of water and any potentially associated biodegradation process, humidity was
138 kept constant at 40%. Ten grams of air-dried soil were deployed in uncovered glass Petri
139 dishes forming a thick layer of 1 mm of soil. A stock solution containing 16 US EPA
140 priority PAHs at 2000 µg mL^{-1} in dichloromethane:benzene was provided by Supelco®
141 (99.0% purity, Bellefonte, PA, USA). Each sample was 10-times spiked with 25 µL of
142 this stock solution diluted with an hexane/dichloromethane (1:1) mixture (Scharlau
143 Chemie S.A., Barcelona, Spain; hexane: 96% purity, dichloromethane: 99.5% of purity)
144 to an individual PAH concentration of 100 µg mL^{-1}, leading to a Σ16 PAHs concentration
145 of 40 µg g^{-1} in soil. Afterwards, samples were incubated inside the climate chamber. In
146 order to differentiate concentration decreases due to slow sorption and/or volatilization
147 processes from photodegradation, a number of dark control samples covered with
148 aluminum foil were exposed to the same environmental conditions. Duplicates of
149 irradiated samples and dark controls of each soil were removed from the climate chamber
150 after 1, 2, 3, 4, 5, 6, 7, 14, and 28 days. To verify the lack of any biotic reactions, before
151 the experiment was initiated, soils were incubated at the same conditions in manometric
152 respirometers (Oxitop®, WTW). Negligible oxygen consumption was observed during the
153 incubation period, discarding biotic processes. Ten grams of soil without any spiking of
154 PAHs were used as blank soil samples.

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156 *2.3. PAH analysis*

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158 Prior to analysis, PAHs were extracted from soil samples by using 30 mL of a mixture of
159 hexane/dichloromethane (1:1) (Scharlau Chemie S.A., Barcelona) in a Milestone Start E
160 Microwave Extraction System (Milestone s.r.l., Sorisole, Italy), according to the US EPA
161 method 3546. Subsequently, samples were filtered, concentrated to 1 mL and further
162 evaporated with a gentle stream of purified N_2. Since any interference with target analytes

was found, cleanup was discarded in order to achieve suitable recoveries for low molecular weight PAHs. Regarding the quality control, d_{10}-fluorene (98.3% purity, Supelco®) was used as surrogate, while d_8-naphthalene (99.8% purity, Supelco®) and d_{12}-benzo(*a*)pyrene (98.5% purity, Supelco®) were used as internal standards. Dried samples were dissolved with a solution of internal standards at 50 μg mL^{-1} concentration in hexane/dichloromethane (1:1) mixture (Scharlau Chemie S.A., 99.5% of purity). Blank soil samples were also extracted following the same procedure in order to assure that collected soils were PAH-free. All samples were analyzed by means of gas chromatography-mass spectrometry (GC-MS) in accordance to the US EPA method 8270. A Hewlett-Packard G1099A/MSD5973 equipment with an HP-5MS 5% Phenyl Methyl Siloxane column (20 m x 0.25 mm x 0.25 μm) was used to quantify the content of the 16 PAHs under study in soil. The final experimental conditions were: 1 μL injection at 310 ºC in split-splitless, and pulsed splitless mode at 35 psi (for 0.05 min). Transfer line temperature was set at 280 ºC. Ultra-pure (99.9999%) helium was used as carrier gas, at a total flow rate of 1.4 mL min^{-1}. The GC oven temperature started at 80 ºC, being consecutively increased at 15 ºC min^{-1} until 180 ºC, at 8 ºC min^{-1} until 250 ºC, and at 3 ºC min^{-1} up to 300 ºC. At the end of each ramp, temperature was held for 1 min. Finally, an increase of 20 ºC min^{-1} was executed until reaching 320 ºC, holding this temperature for 6 min. The detector was set to quantify the analytes covering specific masses ranging from 40 to 600 atomic mass units. The mass spectrometer and source temperatures were 150 ºC and 230 ºC, respectively. Samples were quantified using a six-point calibration curve (5, 10, 25, 50, 60, 80 μg mL^{-1}). Sample preparation for the PAH analyses was performed at the "Laboratory of Environmental Engineering" of THE Universitat Rovira i Virgili (URV), while concentrations were determined at the "Servei de Recursos Científics i Tècnics" of the same institution (SRCiT-URV).

2.4. Statistical analysis

Results were statistically evaluated using XLSTAT Statistical Software for Excel. Repeated measures of the ANOVA were used to state significant differences between irradiated and non-irradiated samples through the time. A regression analysis was also executed to study the relationship between concentrations and time. Probability levels were considered as statistically significant at $p<0.05$.

197 *2.5. Ecotoxicological tests*

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199 Soils were extracted by using an ultrasonic bath mixture (1:1) of n-hexane 95% (UV-
200 IR-HPLC) PAI-ACS (Panreac, Castellar del Vallès, Barcelona, Spain) and acetone (Reag.
201 Ph. Eur) PA-ACS-ISO (Panreac), following the US EPA method 3550C. Blank soil
202 samples were simultaneously extracted following the same procedure. Afterwards, soil
203 extracts were filtered, and the solvent was completely dried with a rotatory evaporator,
204 being finally reconstituted with 2 mL of dimethyl sulfoxide (UV-IR-HPLC-GPC) to a
205 concentration of 2-4% in Microtox diluent (2% NaCl of aqueous solution). Ecotoxicity
206 values were quantified by means of the Microtox® 500 Analyser (SDI, USA), following
207 the ISO 11348-1:2007 norm. The bioluminescent bacteria *V. fischeri* was used to measure
208 the inhibition of light emission when organisms were exposed to soil extract samples.
209 EC_{50} values were estimated as the sample concentration causing 50% of light inhibition
210 on the test organisms (Roig et al., 2013). Sample preparation and Microtox® test were
211 performed at the "Laboratory of Environmental Engineering" of the URV.

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213 *2.6. Hydrogen isotope analysis of benzo(a)pyrene*

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215 For hydrogen isotope analysis, soil samples were also extracted with the same
216 Milestone Start E Microwave Extraction System (Milestone s.r.l., Sorisole, Italy),
217 according to the US EPA method 3546. The extract was treated by following the same
218 procedure used to analyze PAH levels. Once the extract was completely dried, it was
219 dissolved in 62.5 μL of dichloromethane (99.5%, Scharlau Chemie S.A.) free of any
220 deuterated PAHs that could interfere in δ^2H analysis. The hydrogen isotope composition
221 of benzo(*a*)pyrene was analyzed using a gas chromatography-pyrolysis-isotope ratio
222 mass spectrometry system (GC-Pyr-IRMS) consisting of a Trace GC Ultra equiped with
223 a split/splitless injector, coupled to a Delta V Advantage IRMS (Thermo Scientific
224 GmbH, Bremen, Germany), through a combustion interface.

225 The GC/Pyr/IRMS system was equipped with an Agilent Technologies DB-1 column
226 (30 m × 0.25 mm, 1.0 μm film thickness; Santa Clara, CA, USA). The oven temperature
227 program was kept at 50 °C for 1 min, heated again until 160 °C at a rate of 25 °C min^{-1},
228 then up to 320 °C at a rate of 3 °C min^{-1}, being finally held at 320 °C for 20 min. The
229 injector was set to splitless mode at a temperature of 280 °C. Helium was used as a carrier
230 gas with a gas flow rate of 1.0 mL min^{-1}.

Hydrogen isotope ratios are reported relative to an international standard (Vienna Standard Mean Ocean Water, VSMOW), using the delta notation:

$$\delta^2H\ (‰) = (R/(R_{std}-1)) \times 1000$$

where R and R_{std} are the isotope ratios (H^2/H^1) of the sample and the standard, respectively. All the measurements were run in duplicate, and the standard deviations of the δ^2H values obtained were below ±10‰. The analytical system was daily verified using PAH control standards with known hydrogen isotope ratios, which were previously determined using a Carlo-Erba 1108 (Carlo-Erba, Milano, Italy) elemental analyzer (EA) coupled in continuous flow to a Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The samples were prepared for the isotopic analyses in the "Mineralogia Aplicada i Geoquímica de Fluids" Research Group laboratory and analyzed at the "Centres Científics i Tecnològics" of the Universitat de Barcelona (CCiT-UB).

3. Results and discussion

3.1. Photodegradation of PAHs in soils

The trends in the levels of naphthalene, phenanthrene, pyrene, benzo(*a*)pyrene, and benzo(g,h,i)perylene in Arenosol and Regosol soils, are depicted in Fig. 1. Those compounds were selected as representatives of 2-, 3-, 4-, 5- and 6-ringed PAHs, respectively. The results for other PAHs are shown in Supporting Information (SI, Fig. S1). A different behavior for the 16 PAHs in coarse- and fine-textured soils over the time was observed, leading to different photodegradation rates, which were calculated considering the difference between irradiated samples and dark controls (Table 2). Statistical significances for the different exponential and linear rates are shown in Table 3. Three main processes might be related to the concentration decreases: volatilization (Wang et al., 2015), sorption (Liu et al., 2007; Zhang et al., 2014), and photodegradation (EL-Saeid et al., 2015). However, the contribution of each process was different according to the physicochemical properties of each compound (SI, Table S1), as well as to the texture of each soil. In general terms, higher photodegradation rates were noted in some compounds for fine-textured soil, when comparing irradiated samples and dark controls. As expected, the lowest PAH recoveries were found for the most volatile compounds (naphthalene, acenaphthylene and acenaphthene). PAH recoveries 1 h after

soil contamination were found to be higher in fine-textured Regosol soil (14%-127%) than in heterogeneous coarse-textured Arenosol soil (7%-92%). The latter has lower organic matter content, as well as a lower amount of fine fraction, causing a weaker retention of PAHs.

Volatilization was probably the most significant process for 2- and 3-ringed PAHs. No differences of naphthalene concentrations were found between irradiated and dark control samples, indicating that naphthalene was not photodegraded in any soil, either Arenosol or Regosol. Because of its high vapor pressure, the decreasing content of naphthalene (85%) observed during the first hour after being spiked (SI, Table S1) could be related to volatilization processes (Liu et al., 2011). Similar results were also obtained for acenaphthylene, being 80% decreased in the same period of time elapsed. Regarding acenaphthene, similar and constant concentrations were noted for irradiated and dark control samples over the time, with slight decreasing rates in Arenosol and Regosol soils (1.5% and 2%, respectively). Consequently, a significant decrease of the concentration of this compound was not observed ($p>0.05$), indicating that photodegradation did not occur.

Fluorene, phenanthrene, anthracene and fluoranthene showed higher decreasing concentration rates in Regosol than in Arenosol soil. In Regosol soil, phenantrene and anthracene exhibited photodegradation rates of 33% and 40%, respectively, after 28 days of light exposure, being the differences between controls and irradiated samples statistically significant after 2 days.

In Arenosol soil, pyrene, benzo(*a*)anthracene, chrysene and benzo(*b*+*k*)fluoranthene kept their concentrations constant in both irradiated samples and dark controls, suggesting lack of photodegradation. In contrast, decreasing concentrations of the same 4-ringed PAHs were found in fine-textured Regosol soil. Decreasing rates were up to 30% for these compounds at the end of the experiment. In turn, pyrene concentration was only slightly decreased (17%) over the experiment. This ratio is 10% lower than that reported by (Zhang et al., 2010) for pyrene in soil samples irradiated using UV lamps with a wavelength of 254 nm. However, no significant differences were noted in the current study between irradiated and control samples ($p>0.05$).

Benzo(*a*)pyrene was sorbed to soil during the first day according to the fast concentration decrease, in both soils and both irradiated and non-irradiated samples. A decreased rate of 23% was estimated in coarse-textured Arenosol soil, which is in agreement with the findings of Zhang et al. (2006). In turn, higher rates were observed in fine-textured Regosol soil, showing a complete removal of benzo(*a*)pyrene after 7 days

of incubation. Zhang et al. (2008) reported that titanium dioxide (TiO_2) under UV light, accelerates the photodegradation process of phenanthrene, pyrene and benzo(*a*)pyrene on surface soil, being benzo(*a*)pyrene the most quickly degraded. Although the TiO_2 content in fine-textured Regosol soil is lower than that in Arenosol soil, the higher content of other photocatalysts (e.g., Fe_2O_3, Al_2O_3, MnO_2, or TiO_2) in the fine-textured soil, as well as the higher fine fraction due to its clay content, might be responsible of this complete degradation (Gupta and Gupta, 2015; Zhang et al., 2006; Zhao et al., 2004).

As a consequence of the high constant concentrations in irradiated samples and dark controls, it can be confirmed that dibenzo(*a,h*)anthracene tended to be less adsorbed than other PAHs in both soils, since concentrations in dark controls were constant over the experiment. This compound seemed to suffer a slight photodegradation in the coarse-textured soil, while the degradation rate was substantially higher in fine-textured soil (<5% and 28%, respectively) over the experiment. After 28 days of exposure, only 12% of the indeno(*123-cd*)pyrene was photodegraded in Arenosol soil, while up to 69% was removed in fine-textured Regosol soil. Benzo(*ghi*)perylene was adsorbed more quickly in the coarsed soil, finding a slightly higher decrease of its concentration in dark controls over the experiment when comparing to dibenzo(*a,h*)anthracene. The photodegradation of benzo(*ghi*)perylene started in the 14th day of exposure, being the photodegradation rate <5% after 28 days. Contrastingly, this PAH was less adsorbed in Regosol soil, where a photodegradation of up to 25 % was noted at the end of the experiment.

Phenantrene, pyrene, benzo(*a*)pyrene and benzo(*ghi*)perylene showed a concentration decrease in dark conditions in Arenosol soil, indicating that unknown degradation processes, other than photodegradation, could be also occurring in the dark conditions for this type of soil. In contrast, in the Regosol soil, these same compounds showed constant concentrations in dark conditions, being therefore different from the levels observed under light conditions.

Focusing on the differences between soil textures, our findings agree with those previously reported by Xiaozhen et al. (2005). These authors found that the photolysis rate of antrazine and the photolytic depth increased gradually from sand through silt to clay. Therefore, photochemical reactions may be accelerated when soil particles are smaller. This is likely related to the increase of the surface area per mass, hence showing a greater catalytic capability. By contrast, Zhang et al. (2010) found that the increase of soil particle size allows a higher scattering and permeation of light, therefore speeding up any photodegradation process. It must be stated that the current experiment was

performed with the top soil layer (1 mm of depth), since the objective was to analyze the PAH photodegradation in soils due to atmospheric deposition. Consequently, in the present study the role of light penetration is discarded. Several studies have also highlighted the active function of Fe_2O_3, MnO and TiO_2 to boost photodegradation processes. Zhao et al. (2004) found that the addition of α-Fe_2O_3 or TiO_2 enhanced the photocatalytic degradation of gamma-hexachlorocyclohexane (γ-HCH) in the soil surface. Similarly, Zhang et al. (2006) stated that the content of Fe_2O_3 and other semiconductor oxides, such as TiO_2 and MnO_2, in soils improved the photodegradation of benzo(*a*)pyrene. Notwithstanding, the presence of oxides available in fine-textured Regosol soil, as well as its clay content, might have some influence on the high photodegradation rates, even in the PAHs of high molecular weight. Nadal et al. (2006) reported that high molecular weight PAHs could not be photodegraded in an organic solvent after one week of UV-B exposure. In contrast, Guieysse et al. (2004) confirmed found out that UV-photolysis acts preferentially on large PAHs. In any case, the complexity of soils could give place to an enhancement and acceleration of photodegradation reactions.

3.2. Effect of photodegradation over ecotoxicity of PAHs

Microtox® has been established as a fast, useful and sensitive method to assess the toxicity of soils spiked with PAHs (Khan et al., 2012). According to Salizzato et al. (1997), 5 min.-EC_{50} values were found to be suitable for these organic compounds. In the present study, blank samples in Arenosol and Regosol soils, showed ecotoxicity values of 113 and 182 mg of soil mL^{-1} Microtox® diluent, respectively. These results are 10-times higher than toxicity results found in spiked soil samples before any irradiation, showing values of 12.9 and 15.6 mg of soil mL^{-1} Microtox® diluent, in Arenosol and Regosol soil samples, respectively.

The EC_{50} values of irradiated samples and dark controls in Arenosol and fine-textured Regosol soils are depicted in Fig. 2. The coefficient of determination (R^2) of EC_{50} vs. Σ16 PAH concentrations over the time was 0.75 and 0.78 in coarse- and fine-textured soils, respectively. Both irradiated samples and dark controls tended to increase their EC_{50} over time. This slow detoxification would be mainly consequence of the volatilization, sorption and/or photodegradation of PAHs. In Arenosol samples, EC_{50} of irradiated and control samples showed a very similar trend. Therefore, no ecotoxicity differences were

found, independently on the exposure to light, being in full agreement with the findings from the chemical analysis of PAHs. In contrast, EC_{50} in irradiated and dark control samples of fine-textured soils showed a different pattern (Fig. 2). Excluding data regarding one day after incubation, the EC_{50} curve of irradiated samples was more pronounced than that of dark controls, indicating a lower toxicity. Taking into account that irradiated and dark control samples were exposed to the same conditions, excepting light exposure, it is clear that light enhances the detoxification of fine-textured Regosol soil. Similarly to Arenosol soil findings, the current toxicity results also agree with the high photodegradation rates observed in the analytical experiment. Those 3-, 4-, 5- and 6-ringed PAHs, which were highly photodegraded, could be the responsible of the toxicity decrease. In the period of time elapsed between day 1 and before 2 and 3 days after incubation time in Regosol and Arenosol soils, respectively, the toxicity of irradiated samples was higher than in dark controls. This could be linked to the potential formation of metabolites, such as some oxygenated PAHs, even more toxic than the parental compounds (Bandowe et al., 2014; Knecht et al., 2013; Lundstedt et al., 2007). Anyhow, this finding deserves further investigation, which should confirm the relationship between the generation of by-products and the ecotoxicological status of soil.

The results of the Microtox® ecotoxicity test demonstrated that the light, together with other mechanisms such as sorption, volatilization, and abiotic degradation, enhances PAH detoxification on surface soil. This process is especially remarkable in fine-textured soils, which contain materials capable to act as photocatalysts. Anyway, although photodegradation and detoxification occurred, spiked soil samples did not achieve toxicity levels of blank samples 28 days after light exposure. Consequently, a longer exposure time, or an increase of light intensity, would be required to completely remove PAHs from soils. However, the outcomes of both chemical and ecotoxicological analyses indicate that photodegradation is an important process of PAH removal in soil.

3.3. Hydrogen isotope effects on benzo(a)pyrene

The hydrogen isotopic composition of benzo(*a*)pyrene in irradiated samples and dark controls is shown in Fig. 3. In Arenosol soil, benzo(*a*)pyrene of irradiated samples experienced a change in its hydrogen isotopic composition (after only 5 days of experiment) from -39‰ to +193‰. In agreement with data on PAH levels, this high isotopic shift clearly confirms degradation of benzo(*a*)pyrene under the selected climate

conditions. Due to their too low concentrations, δ^2H could not be obtained from the subsequent samples. Unexpectedly, a hydrogen isotopic change of benzo(*a*)pyrene was also observed in dark control samples. Therefore, unknown degradation processes could be also occurring in absence of light (Fig. 3). Under darkness conditions, benzo(*a*)pyrene in Arenosol suffered a progressive enrichment in δ^2H. Despite being slower than in irradiated samples, it increased from -39‰ to +181‰ after 28 days of incubation in the dark. It is suggested that there could be some abiotic degradation of benzo(*a*)pyrene, which would be the result of its reaction with organic and/or mineral phases of the soil.

The hydrogen isotope results of benzo(*a*)pyrene in Regosol soil samples were similar to those corresponding to Arenosol soil. Benzo(*a*)pyrene hydrogen isotopic composition changed from -39‰ to +68‰ after only 3 days of experiment (Fig. 3). Five days after starting, a decrease in the δ^2H value was observed, most likely as a result of reversible sorption processes, which might have released benzo(*a*)pyrene molecules with a lower degradation degree, and consequently, with a lower δ^2H. However, this isotopic shift also confirms that benzo(*a*)pyrene in Regosol soil is degraded under the same climate conditions. Dark controls also showed a slow degradation, with an isotopic change from -39‰ to +35‰ after 28 days of experiment. Similarly to Arenosol soil samples, dark controls of Regosol soil showed a slower isotopic enrichment in ^2H with respect to irradiated samples, confirming that the same process of PAH loss might be occurring. The evolution of the hydrogen isotopic composition of the dark controls shows fluctuations over the time that could be linked to sorption/desorption effects. Consequently, the hydrogen isotope analysis also seems to confirm that sorption processes of PAHs in soil were present, which is in agreement with the data from the chemical analysis of PAHs. Notwithstanding, since the relationship between the lack of hydrogen isotopic fractionation of PAHs with sorption processes in soil has not been described in the scientific literature, this hypothesis cannot be confirmed yet. Our results corroborate that benzo(*a*)pyrene is not only photodegraded, but also that this degradation is associated to a significant isotopic change. Moreover, they highlight the great potential of CSIA to be used as a powerful tool to monitor *in situ* PAH degradation. Furthermore, the abiotic degradation of benzo(*a*)pyrene without light intervention was proved to be a potentially relevant pathway of PAH loss in soil. However, further studies are still necessary to confirm the mechanisms of PAH degradation in dark conditions.

4. Conclusions

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436 The photodegradation of PAHs in soils is highly dependent on the exposure time, the
437 molecular weight of each hydrocarbon, and the soil texture. Low molecular weight PAHs
438 are more influenced by volatilization and sorption, while medium and high molecular
439 weight PAHs are able to undergo different photodegradation ratios. Soil properties
440 (texture and metal oxides) were found to influence on volatilization, sorption and
441 photodegradation of PAHs. Photodegradation in soils is a mechanism that mostly occurs
442 in soil surface, being able to partially detoxify soil. Moreover, this process can be
443 enhanced by solid phase soil composition, especially in soils with a finer fraction, as well
444 as by the presence of semiconductor minerals, such as metal oxides. The evolution of 16
445 PAH concentrations over the time agrees well with Microtox® results, with a faster
446 detoxification in fine-textured Regosol soil. However, after 28 days of incubation, soil
447 samples were not completely detoxified. It is important to note that photodegradation is
448 not the only process of PAH loss in soils. Other mechanisms, such as biodegradation and
449 sorption, may also have important roles on the PAH behavior in soils. Moreover, in a
450 climate change context, where an increase of solar radiation is expected,
451 photodegradation could become a very important process in PAH dynamics in soils.
452 Finally, the complementary analyses of hydrogen isotopes of benzo(*a*)pyrene
453 confirmed, at a molecular level, that this compound is degraded not only under light
454 conditions, but also in the darkness. Furthermore, the strong isotopic effect observed in
455 benzo(*a*)pyrene makes the CSIA a potentially suitable technique to give evidence of PAH
456 degradation. Since its degradation involves a high hydrogen isotopic variation, CSIA is
457 also a powerful tool to quantify *in situ* the degradation efficiency.
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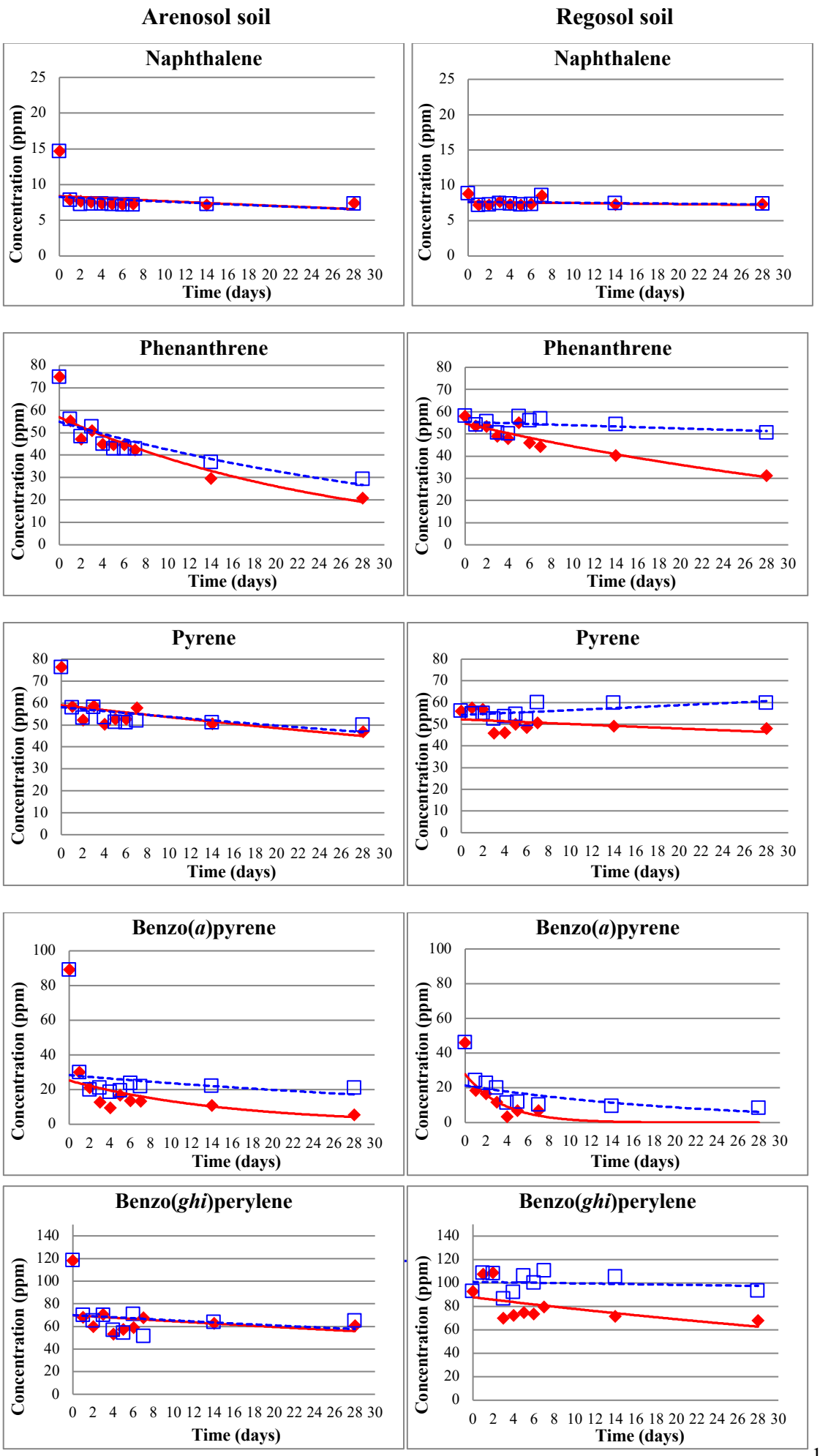
Arenosol soil
Regosol soil
Naphthalene
Phenanthrene
Pyrene
Benzo(a)pyrene
Benzo(ghi)perylene
Concentration (ppm)
Time (days)
Irradiated samples
Dark controls

Fig. 1. Concentration of various PAHs in irradiated and dark control soil samples.

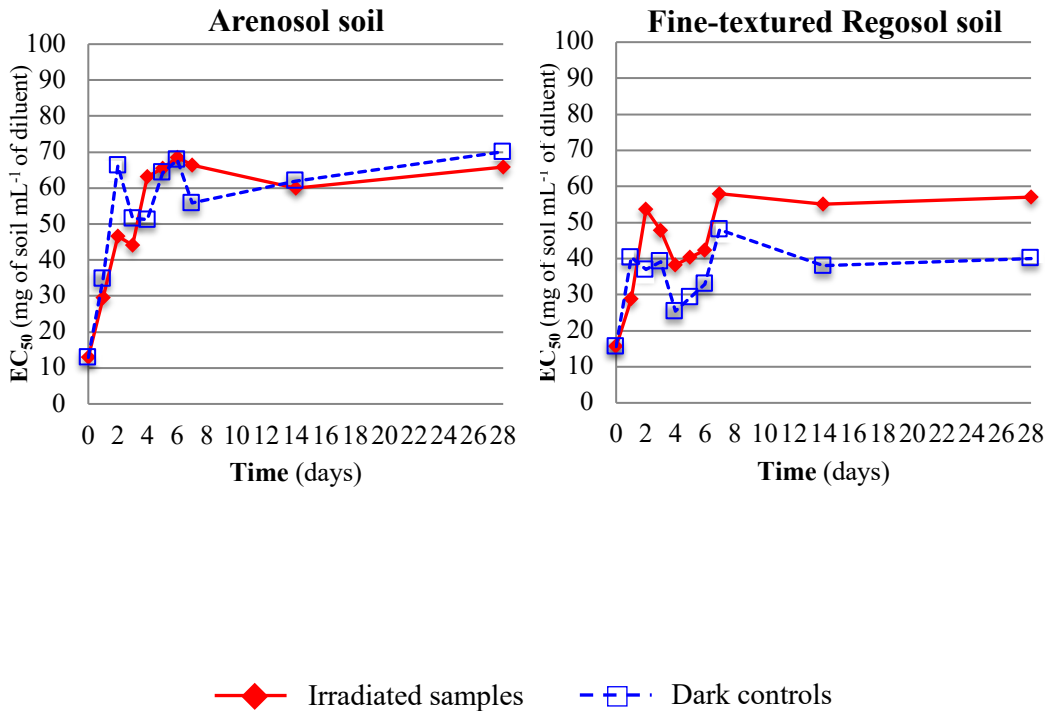

Fig. 2. Ecotoxicity of Arenosol and Regosol soil samples spiked with 16 PAHs.

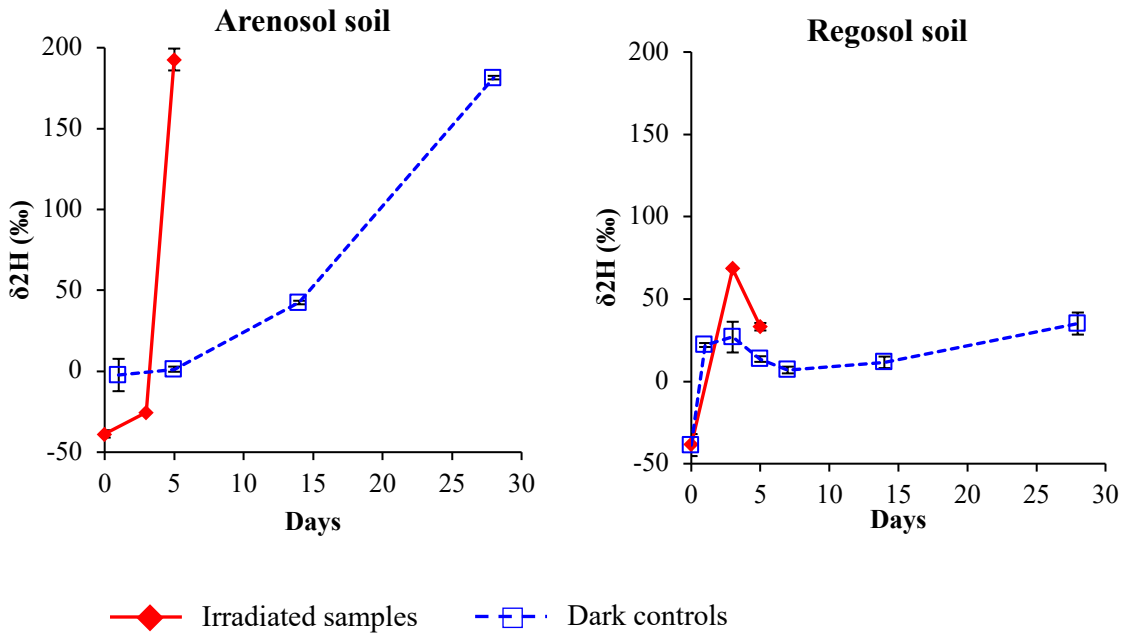

Fig. 3. Hydrogen isotopic composition of benzo(*a*)pyrene in Arenosol and Regosol soils in irradiated and dark control experiments.

Table 1

Physico-chemical properties of the selected Mediterranean soils.

	Arenosol soil	**Regosol soil**
pH	5.8	8.0
Electrical conductivity at 25 ºC (dS m^{-1})[a]	0.06	0.13
Organic C (%)[b]	0.71	1.70
Total Kjeldahl N (%)	0.07	0.18
C/N	10.1	9.44
$CaCO_3$ (%)	0.10	23.20
Texture: sand/silt/clay (%)[c]	74.1/14.0/11.9	43.4/22.3/34.3
Cation exchange capacity (meq 100 g^{-1})[d]	12.60	18.23
Exchangeable calcium (mg CaO kg^{-1})[d]	4.80	12.55
TiO_2 (mg kg^{-1})	429	41.3
MnO_2 (mg kg^{-1})	573	648
Al_2O_3 (mg kg^{-1})	3008	6070
Fe_2O_3 (mg kg^{-1})	6686	13492

Analytical methods: [a]Aqueous extracts 1:2.5; [b]Oxidizable C by Walkley-Black method; [c]Robinson Pipette method; [d]1 N ammonium acetate extracts.

Table 2

Statistical significance (p) of the regression associated to the photodegradation of PAHs.

Compound	**Arenosol soil**		**Regosol soil**	
	regression	**p**	**regression**	**p**
Naphthalene	exponential	0.277	exponential	0.955
Acenaphthylene	exponential	0.571	exponential	**0.023**
Acenaphthene	exponential	**0.005**	exponential	**<0.0001**
Fluorene	exponential	**0.006**	exponential	**<0.0001**
Phenanthrene	exponential	**<0.0001**	exponential	**<0.0001**
Anthracene	exponential	**<0.0001**	exponential	**<0.0001**
Fluoranthene	exponential	0.548	exponential	0.152
Pyrene	exponential	**0.06**	linear	0.134
Benzo(*a*)anthracene + chrysene	exponential	0.137	linear	0.095
Benzo(*b*+*k*)fluoranthene	exponential	**0.039**	linear	0.454
Benzo(*a*)pyrene	exponential	**0.017**	exponential	0.114
Benzo(*ghi*)perylene	exponential	0.409	exponential	0.120
Dibenzo(*a,h*)anthracene	exponential	0.058	exponential	0.131
Indeno(*123-cd*)pyrene	linear	0.289	exponential	**0.004**

In bold, statistically significant regression.

Table 3

Photodegradation rates (%) of the 16 PAHs under study in Arenosol and Regosol soils.

	Arenosol soil	**Regosol soil**
Naphthalene	0	0
Acenaphthylene	0	0
Acenaphthene	1.5	2
Fluorene	3	9.5
Phenanthrene	11.2	33.2
Anthracene	19.7	39.8
Fluoranthene	0	12.5
Pyrene	0	17.1
Benzo(*a*)anthracene + chrysene	0	30
Benzo(*b*+*k*)fluoranthene	0	30
Benzo(*a*)pyrene	23	4.9*
Benzo(*ghi*)perylene	3.6	24.6
Dibenzo(*a,h*)anthracene	2	28.3
Indeno(*123-cd*)pyrene	11.7	68.9

*completely degraded after 7 days of light exposure.