Evaluation of PAH removal efficiency in an artificial soil amended with different types of organic wastes

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Abstract The removal of polycyclic aromatic hydrocarbons (PAHs) from a spiked OECD (Organisation for Economic Co-operation and Development) artificial soil was investigated. Laboratory-scale thermally insulated bioremediation reactors were used to implement biostimulation strategy of composting. The selected PAHs included anthracene, chrysene, benzo(k)fluoranthene, and benzo(a)pyrene with an initial concentration of 658 mg of USEPA 16 PAHs kg⁻¹ soil (d/w). The contaminants' removal was improved by amending the contaminated soil with four different types of fresh organic waste. After 140 days of incubation, the removal of three-ring and four-ring PAHs in all reactors was higher than five-ring PAHs. The reactor displaying a mesophilic phase during bioremediation ended with a removal of 89 and 59 % for three-ring and four-ring PAHs, respectively. In contrast the reactor displaying a thermophilic phase ended with 71 and 41 % removal for three-ring and four-ring PAHs, respectively. The highest five-ring PAH removal was obtained for reactors with buffalo manure and sewage sludge amendments (40 and 33 %, respectively), while food and kitchen

waste and fruit and vegetable waste amendments showed less efficiency (26 and 8 %, respectively). Microtox[®] test data indicated lower toxicity in reactor amended with sewage sludge considering that this setup reached the highest PAH removal and DHA (dehydrogenase activity) compared to others.

Keywords Polycyclic aromatic hydrocarbons · Bioremediation · Biostimulation strategy · Composting of organic waste · OECD artificial soil

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment and are present in soils, sediments, groundwater, and the atmosphere [29, 31]. The highest concentrations of these organic pollutants are found in soils located in the areas of industrial activities and nearby urban zones that render the necessity for their removal even more urgent [30, 31].

Generally, the main causes of their presence in soils are accidental forest fires as well as volcanic eruptions as natural phenomena, but most of the contamination originates from anthropogenic sources [20, 29, 37]. PAHs are a global concern both for environment and human health. Moreover, their concentration in contaminated soils from industrial areas can be variable depending on the activity on the site [18].

PAHs are chemical compounds composed of two or more fused aromatic rings containing carbon (C) and hydrogen (H) atoms [31, 37]. Only 16 of them have been recognized as “priority pollutants” listed by U.S. Environmental Protection Agency (US EPA) and the European Commission, and seven of them are classified as probable human carcinogens [20, 37].

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Lower molecular weight (LMW) PAH compounds, containing two or three rings, have shown significant acute toxicity and other adverse effects, while many higher molecular weight (HMW) PAHs, containing 4–7 rings are carcinogenic, mutagenic, or teratogenic to a large number of different living organisms [20, 37, 40]. Their high hydrophobicity makes them easily adsorbed onto the soil organic matter and thus less available for biological uptake, forming persistent micro pollutants in soils [20, 31]. PAHs hydrophobicity increases as the rings' number of PAHs molecule increases [26].

Among several treatment technologies available to remove PAHs from soil, bioremediation is one of the most suitable to deal with these environmental contaminants [15, 37]. Considering that PAHs biodegradation is a complex natural chemico-biological process, it can be influenced by limiting factors such as microorganism nature, water content, aeration, physico-chemical properties of contaminants, their concentration, and bioavailability [27, 34, 37].

Among the various bioremediation technologies, land-farming is the most favorable for remediation of PAHs-contaminated soil due to the need for low capital costs and low technology to be implemented. This technique also allows the handling of large volumes of soil [30]. To overcome possible limitations, it could be successfully applied when combined with composting of organic waste [13, 42, 46]. Moreover, it could be a successful method for treating wastes such as animal manure, sewage sludge, and municipal organic solid waste, if final residues of treatments would not be toxic. Due to high content of readily biodegradable organic matter and water in such wastes, incineration or landfill disposal is not economical [17]. Thus, treatment of PAHs-contaminated soil combined with composting of organic waste could be an interesting option and a sustainable method. It would enable eco-friendly disposal of such waste and enhance the biodegradation rate of PAHs.

Even if the composting approach is found to have a potential for the remediation of PAHs-contaminated soils [3, 7], there are not many studies related to its application with fresh organic waste, and it is still an emerging *ex situ* bioremediation technology [27]. Previous experiments dealing with PAHs-contaminated soil composted with fresh green and bio-waste showed that about 50 % of the PAHs were removed during 60 days of experimental activities [46]. Meanwhile Atagana [7] managed to remove almost 100 % of PAHs concentrations while composting contaminated soils with poultry manure, but for a much longer period of time (i.e. 19 months). Moreover, Guerin [13] showed interesting results by applying cow manure and fresh green tree waste material, where removal of high molecular weight PAHs was at least 50 % during the 7-month-long treatment period.

Due to so many variations, there is a need to optimize the bioremediation process. Hence, the aim of this paper is to implement bioremediation by adding different fresh organic waste to a PAHs-contaminated soil to enrich the soil with organic matter, nutrients, and microorganisms. The focus of this research is to study the effectiveness of four types of fresh organic waste added to a LMW and HMW PAHs-contaminated soil, and to monitor the removal of these compounds according to their properties. In addition, this study evaluates the microbial activity of the monitored processes as well as soil toxicity level due to the possible formation of even more toxic metabolites and intermediates as a result of incomplete PAHs degradation.

Materials and methods

Chemicals

Four PAHs listed by U.S. EPA as priority pollutants were purchased from Sigma-Aldrich: anthracene (Anth) (purity ≥ 99 %), chrysene (Chry) (purity 98 %), benzo(k)fluoranthene (B(k)F) (purity ≥ 99 %), and benzo(a)pyrene (B(a)P) (purity ≥ 96 %).

Soil and organic amendments

The soil used in these experiments was an artificial Organisation for Economic Co-operation and Development (OECD) soil prepared and stored according to OECD Guideline recommendations [28]. It consisted of 10 % sphagnum-peat, 70 % quartz sand (274,739, Sigma-Aldrich), 20 % kaolinite clay (03584 Fluka, Sigma-Aldrich), and 1 % calcium carbonate (C4830, Sigma-Aldrich, purity ≥ 99 %). The dry constituents of the soil were mixed thoroughly 2 weeks before starting the soil spiking procedure and stored at room temperature until use. The mixed dry soil was moistened with MilliQ water 48 h prior spiking to reach a stable soil pH and stored in the fridge.

Buffalo manure (BM) was obtained from a buffalo farm in Caserta area, Campania Region, Italy. Food and kitchen waste (FKW) was prepared using food chopper by mixing the following masses of ingredients expressed in weight percentages: fruit and vegetables 79 %, meat 8 %, dairy products 2 %, bakery 6 %, pasta and rice 5 %. Fruit and vegetable waste (FVW) was prepared by mixing masses of fresh fruits (48 %) and vegetables (52 %), according to their average production in Italy [25]. The mixture was homogenized according to the same way as done for FKW. Activated sewage sludge (SS) was collected from the wastewater treatment plant in Nola, Campania Region, Italy. Prior to its application, the sludge was centrifuged to reduce the initial moisture content (99 %) until 88 %.

Table 1 Characteristics of soil and organic wastes

Parameter/material	Soil	BM	FKW	FVW	SS
Moisture content (% w/w) ^a	6.2 ± 0.1 ^d	83.6 ± 0.2	81.2 ± 2.9	90.8 ± 0.2	88.0 ± 0.1
Volatile solids (% d/w) ^b	6.3 ± 0.1	63.0 ± 0.2	94.2 ± 0.7	93.8 ± 0.4	59.3 ± 0.6
Fixed solids (% d/w)	93.7 ± 0.1	37.0 ± 0.2	5.8 ± 0.7	6.2 ± 0.4	40.7 ± 0.6
Organic matter (OM) (% w/w) ^c	13.0 ± 3.4	12.9 ± 0.4	11.5 ± 1.2	10.5 ± 1.7	9.0 ± 4.8
Bulk density, ρ_b (g cm ⁻³)	0.9 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	1.1 ± 0.0	0.9 ± 0.0

^a w/w: wet weight^b d/w: dry weight^c Conversion factor 1.724 based on TOC content [38]^d Standard error of mean of three replicates

These organic co-substrates were selected to evaluate their effectiveness on the bioremediation processes of PAHs-contaminated soil. The properties of the soil and organic amendments are presented in Table 1.

Soil spiking

OECD soil was spiked at the initial concentration of both anthracene and chrysene of 235 mg kg⁻¹ soil (d/w), while initial concentration of five-ring PAHs was 94 mg kg⁻¹ soil (d/w) for each of them. The soil was air-dried at room temperature for 24 h and sieve at 2-mm prior to homogenization. The soil was then mixed two times for 5–10 s with lab-blender, closed for 5 min, and again mixed in the same way. Afterward, the soil was air-dried for 3 days under a fume hood in darkness conditions until the total evaporation of solvent [10, 35]. Furthermore, the soil was stored at room temperature for 37 days in glass containers in the dark to avoid photolysis [32].

Composting reactors

Experiments were performed at laboratory scale in thermally insulated composting–bioremediation reactors made of Plexiglas[®] [9, 33], with an operational capacity of 1.1 L. Air supply was provided through air pump (Newair, Newa, Tecnoindustriasrl, Italy) and air distribution pipes were installed at the bottom of reactors. Prior to entering reactors, air was humidified with distilled water to decrease drying of soil/organic amendment mixture. The content of the reactor was placed on a Plexiglas[®] plate with small holes (ø 1 mm), to provide uniform air distribution and in the same time to collect the leachates. Air outlet was placed on the top of reactors through air filter made of tubes of glass silk (HTS-AL-10, Distrelec, Italy) where volatile compounds were collected. The outlet tube was packed with 200 mg of resin (Amberlite XAD-2, Sigma-Aldrich, Italy) and 300 mg of coconut activated carbon (Carbo-active granules, Newa, Italy). Air filter tubes were replaced at each sampling interval.

Experimental conditions

Experiments were performed in four reactors (one per organic amendment) at room temperature and lasted 140 days. The spiked soil prepared as described in “Soil spiking” was manually mixed with bulking agent at a ratio of 1:1.5 (v/v) to increase porosity and oxygen diffusion. As bulking agent, corn cobs chopped to 1–4 cm size, $\rho_b = 0.17$ (g cm⁻³) were used. The contaminated soil to organic waste ratio was 5:1 on dry weight basis for all reactors, i.e. for RBM reactor (soil amended with BM), RFW (soil amended with FKW), RFVW (soil amended with FVW), and RSS (soil amended with SS). The quantity of soil used in reactors RBM and RFW was 350 g per each, while in RFVW and RSS was 300 g per each of them. During the preparation of the mixtures, MilliQ water was added to set the moisture content at 60 % as the best for PAH removal through the composting process [3, 36, 46]. The soil moisture level was maintained by spraying MilliQ water on daily basis.

Representative samples were collected in triplicate for each reactor immediately after filling reactors at day 0, and after 14, 28, 56, 84, 112, and 140 days of treatment. The content of approximately 150 g was collected from three different points within the matrix of each composting reactor, and sub-samples were extracted from them and used for carrying out the analyses.

The operating temperature, continuously monitored by a digital thermometer, showed different trends in each reactor according to the microbial activity, since the experiments were conducted at room temperature without any temperature control system. During the 140 days of treatment, temperatures reached thermophilic range in RBM and RFW, while temperatures in RSS and RFVW were lower and limited to mesophilic range. Based on results of Lukić et al. [23], the operating temperature in RBM reached 23, 56, 56, 26, 25, 26, and 24 °C at the day 0, 14, 28, 56, 84, 112, and 140 of experimental activities, respectively. A peak of temperature was reached after 29 days at 56.6 °C followed by the cooling and maturation stages of the process.

Similarly, the thermophilic range of temperature was also reached in RFWK with the temperature of 23, 33, 57, 44, 39, 26, and 30 °C, respectively, at the same days of the experimental activities as mentioned above. Likewise, a peak of temperature was reached after 27 days at 57.6 °C followed by the cooling and maturation stages of the process. Unlike, the temperature trend in RFWV showed only mesophilic range with temperatures of 21, 20, 21, 20, 21, 27, and 27 °C at the day 0, 14, 28, 56, 84, 112, and 140 of experimental activities, respectively. After 116 days, a peak of temperature at 30 °C was reached. Finally, the temperature trend in RSS displayed mesophilic range, i.e. 20, 20, 22, 20, 22, 28, and 28 °C, respectively, at the same sampling days as already mentioned above, with a peak of temperature at 30 °C after 116 days.

Physical and chemical analyses

Moisture content was analyzed by weighing the samples extracted from the reactors before and after drying them for 24 h in an oven at 105 °C [5], while volatile solids and fixed solids were determined according to the Standard Method 2540E [4]. Organic matter content was calculated according to TOC values [Chemical Oxygen Demand (COD) Closed Reflux method] [1] (Table 2). All results are presented as average of triplicates with standard deviation. Since soil was mixed with organic amendments, PAH concentrations were calculated based on the inorganic ash content of the compost mixture, to avoid potential bias due to dilution by organic waste. Bulking agent was physically removed from samples, and was not considered within any analysis.

PAHs analyses

PAHs compounds were extracted using microwave extraction as operating procedure [43]. In extraction vessel, 1 g of sample was mixed with 40 ml of acetone and hexane in the same quantity. An internal standard of 320 µl (chrysene-d12, Supelco, Italy) was added to all samples to monitor extraction efficiency. The extraction vessels were properly closed in extracting frames and placed in a Start D Microwave Digestion System (Milestone). After the completion of extraction process for 35 min and cooling to the room temperature, 1 ml of each sample was stored in glass vial in freezer for the next analyses.

Concentrations of PAHs in the extracts were determined by gas chromatography/mass spectrometry (GC/MS) according to EPA Method 8270D [44]. Analysis was performed on Agilent Technologies 6850 Network GC System coupled with a mass selective detector (Agilent Technologies 5973 Network) and a 30 m Zebron Phase ZB-5MS capillary column (0.25 mm inside diameter, 0.25 µm film thickness). The injection volume of samples was 2.0 µl, and run time was about 37 min for one sample. GC–MS system was calibrated prior to the analysis of samples using five calibration standards (5, 2.5, 1, 0.5, and 0.25 ppm). Standard curves were prepared by injecting a mixture of 14 PAHs (Mix A, Sigma-Aldrich, Italy) diluted with acetone/hexane mixture (1:1). PAHs concentration was calculated by manual integration of the chromatograms.

Determination of microbial dehydrogenase activity

The determination of microbial dehydrogenase activity (DHA) on samples collected in triplicate from each reactor was performed at the end of bioremediation experiments. Dehydrogenase activity was determined by colorimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,3,5-triphenylformazan (TPF) according to the method of Casida [11] and Kizilkaya [19]. Actually, 2.5 ml of reagent water and 30 mg glucose were added to six grams of solid sample. The enzymatic reaction started when 1 ml of 3 % 2,3,5-triphenyltetrazolium chloride solution was added to the suspension. The samples were incubated for 24 h at 37 °C and subsequently extracted with methanol as a solvent. The red methanolic solutions of formazan were measured spectrophotometrically at 485 nm (Perkin Elmer UV/VIS Spectrometer Lambda 10), and results were expressed as µg TPF g⁻¹ dry sample.

Toxicity test

To evaluate the soil toxicity level, samples were collected prior to the initiation of the composting process as well as its termination. The aqueous extract was obtained by mixing 2 g homogenized soil with 20 ml distilled water for 16 h using an orbital shaker set at 200 rpm. After centrifugation at 4000g (IEC Centra GP8R) for 20 min at 4 °C, 15 ml of the aqueous phase was stored in the freezer [6]. Bioassay was performed on a Microtox Model 500

Table 2 Organic matter content in reactors on the beginning of bioremediation

Parameter/reactors	RBM (soil + BM)	RFWK (soil + FKW)	RFWV (soil + FVW)	RSS (soil + SS)
OM concentration (g kg ⁻¹ w/w)	117.2 ± 29.2 ^A ab ^B	141.2 ± 5.3 b	80.4 ± 6.0 a	108.1 ± 4.9 ab

^A Standard error of mean of three replicates at 95 % confidence level

^B Different letters in the row indicate on significantly different values between treatments (ANOVA, $p < 0.05$)

Analyzer (Modern Water). Test was based on measurement of bioluminescence differences in the marine bacterium *Vibrio fischeri* (LUMISTox LCK 487, Hach Lange, France) by exposure to the 1 ml filtered aqueous extract of soil sample [12]. Actually, when soil toxicity is high, bioluminescence in the marine bacterium *Vibrio fischeri* is low. Acute toxicity was measured as a function of decreased luminescence after 5 and 15 min exposure time at 15 °C. High inhibition at time 5 and 15 min, i.e. $I_{(5)}$ and $I_{(15)}$, respectively, (%), indicates high soil toxicity.

Statistics

Data were analyzed by one-way analysis of variance (ANOVA) to test for significant differences of each contaminant removal within every treatment, and total contaminant reduction between treatments. All the experiments were done as triplicates. Statistical analysis was performed using the R software (R i386, 3.1.1 version). The differences between individual means were tested using Tukey multiple comparison test, where significant p values were obtained at the level of $p < 0.05$. On the figures and in the tables, the letters (a, b, c, d) represent the homogenous groups obtained by the comparison of average values.

Results and discussion

PAH removal

Efficiency of different organic amendments in simulated composting treatment

According to the PAH concentrations commonly detected in naturally contaminated soil by industrial activities, the

soil was spiked to reach a concentration of 658 mg total PAH kg^{-1} (d/w) in all experiments. Selected concentration was considered according to the concentrations of each PAH as well as the concentration of total PAHs in naturally contaminated soil [2, 18]. Total PAH removal in each reactor after 140 days of bioremediation treatment is shown in Fig. 1. In all treatments, removal of the LMW PAH, anthracene, was higher than HMW PAHs. Hence, the removal of Anth was much higher than that three pollutants regardless the applied organic amendments. Interestingly, the most favorable results were reached in RSS and RFVW reactors (89 and 85 % for Anth, respectively). In contrast, reactors operated under thermophilic condition display lower anthracene removal, i.e. 71 % in RFWK and 69 % in RBM.

Similarly, the highest chrysene removal occurred in RSS (59 %). The chrysene removal in RFVW was slightly lower than in RSS and equal to 50 %, while in the other two reactors a chrysene removal lower than 50 % was observed.

Benzo(a)pyrene removal was slightly higher in all reactors compared to benzo(k)fluoranthene. Furthermore, considerably better removal has been reached in reactors filled with BM and SS compared to reactors filled with FKW and FVW amendments, but without being statistically significant compared to RFWK. Thus, B(a)P removal was 40 and 33 %, and B(k)F removal was 37 and 33 % in RBM and RSS, respectively. In other two reactors filled with food waste, the decrease of B(a)P and B(k)F concentrations did not exceed 30 %. Moreover, concentrations of B(k)F and B(a)P in RFWK and RFVW on the end of treatment did not show significant differences compared to their initial concentrations (ANOVA, $p < 0.05$).

PAHs with larger number of fused aromatic rings and higher molecular weights showed more resistance to

Fig. 1 PAH removal yield (%) after 140 days of simulated composting treatments. Vertical bars represent the standard deviations of mean of three replicates at 95 % confidence level

degradation due to lower bioavailability [27, 31], resulting in a decrease in total removal that was less than 50 % for both B(a)P and B(k)F, in all treatments. Moreover, even if they have the same molecular weights and the same number of five-rings, B(a)P is characterized by $\log K_{ow} = 6.04$, while B(k)F by $\log K_{ow} = 6.84$. This difference in hydrophobic properties influences their behavior under the same operating conditions and consequently their degradation. Actually, the larger octanol–water partition coefficient is related to the higher potential of bioaccumulation, which is the main responsible for the lower biodegradability of such compounds [18].

Interestingly, treatments with activated SS and FVW amendments were found to be more successful for the removal of three- and four-ring PAHs. Moreover, both RSS and RFVW showed only mesophilic temperature range during the treatment. In the literature a mesophilic phase has been found to be more favorable in specific cases due to the richest microbial diversity able to degrade lower molecular weights organic pollutants with great success, but it was not found to be so efficient in the degradation of recalcitrant PAHs [3, 27]. In agreement with results of this research, Antizar-Ladislao et al. [3] obtained a decrease of LMW PAHs concentration such as naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, and phenanthrene by an average of 89 % at 38 °C which is twice compared to concentration reduced at 55 °C by an average of 45 %. Otherwise, the reduced concentration for four-ring PAHs such as fluoranthene, pyrene, benzo(a)anthracene, and chrysene was not so different and it was by an average of 67 % at 38 °C compared to an average of 69 % at 55 °C.

Treatments with BM and SS amendments have been showed to be more efficient for PAH removal than treatments with food waste amendments. Indeed, the removal of Anth in RBM was slightly lower compared to RFW. Similarly, the removal of both Anth and Chry was lower in RBM compared to RFVW. In contrast, the removal of B(k)F and B(a)P in RBM was pretty higher compared to RFW and RFVW. Since the removal of five-ring PAHs is more difficult to reach compared to PAHs with lower molecular weights, the treatment with BM amendment could be considered as more efficient for PAH removal than treatments with food waste amendments. However, a clear distinction was also found between these two amendments firstly mentioned. Indeed, it was found higher total PAH removal in RSS compared to RBM, but removal efficiency of five-ring PAHs was slightly better in RBM than in RSS. Those interesting results might be achieved due to the influence of high temperature occurring during the thermophilic phase, which contributed to higher removal efficiency of recalcitrant PAHs in RBM treatment. Actually, due to increased diffusion rate and contaminants

solubility, PAHs bioavailability has been enhanced leading to a potential improvement of their biodegradation [14, 34, 45]. Otherwise, higher removal of total PAHs in RSS compared to RBM may be achieved due to very rich microbial diversity typical for activated sewage sludge [21]. As microorganisms are key player in the degradation of organic pollutants, greater extent of contaminants metabolization in RSS would be proportional to higher microbial diversity and density in activated sewage sludge compared to other waste products [14, 17, 27]. Accordingly, in the study of Antizar-Ladislao et al. [3] B(a)P removal was not reached through mineralization during composting at 38 °C in soil amended with green waste, while in this study B(a)P removal reached even 33 % during composting at lower temperature in soil amended with SS. Nonetheless, temperature profile in RSS was in the range of mesophilic stage throughout the treatment with a peak at 30 °C [23]. Further, the equal amount of B(k)F removal, which is also recalcitrant organic pollutant and difficult to be removed from PAH contaminated soil, was reached in RSS, i.e. 33 %. Thus, even if Namkoong et al. [27] pointed out larger removal of contaminants from soil using SS amendment compared to compost, hereby stressing more significant contribution of SS in removing recalcitrant contaminants compared to other organic wastes. Moreover, the nutrient content of an organic waste has to be considered as an important parameter during the treatment of PAH removal. Actually, Lukić et al. [23] have shown that SS contained the lowest C:N ratio and the highest content of soluble fraction and protein compared to BM, FKW, and FVW. Accordingly, the treatment amended with SS was the most successful in PAH removal compared to other three treatments amended with organic wastes which contained a less favorable nutrient content.

Although being the most efficient, SS treatment has shown its benefits by providing the fastest removal for three out of four PAHs indicators, i.e. except for Chry. Indeed, in the early stage of experiment a significant difference between concentration values for Anth at day 0 and day 14 in treatment with SS amendment was found (Fig. 2). It was considered as the most rapid removal, since the first significant differences of concentration values for Anth were observed between day 0 and day 56 in RBM treatment and between day 28 and day 56 in RFVW treatment (ANOVA, $p < 0.05$). Nevertheless, the slowest removal of three-ring PAH indicator was observed in RFW among the values at day 0 and 84. However, the first significant differences between concentration values for Chry at day 0 and day 28 in RFW reactor was found. It was considered as the most rapid removal of four-ring PAH indicator. Even if the most efficient removal of Chry was observed in RSS, the first significant differences between its concentration values were observed a bit later

Fig. 2 Dissipation of each contaminant among the treatments. Vertical bars represent the standard deviations of mean of three replicates at 95 % confidence level, which could be covered with

marker. Different letters indicate on significantly different values of PAH concentrations through incubation time for each contaminant separately (ANOVA, $p < 0.05$)

compared to RFKW, i.e. between values at day 84 and 112 (ANOVA, $p < 0.05$). Heavier molecular weight organic pollutants, i.e. five-ring PAHs such as B(k)F and B(a)P, showed faster removal in RSS than in treatment with BM amendment. Actually, the first significant differences in RSS were observed among the values at day 0 and day 14, and at day 28 and 56, respectively (ANOVA, $p < 0.05$). For comparison, the first significant differences in RBM were observed among the values at day 28 and day 84 for both contaminants (ANOVA, $p < 0.05$).

The delay in other treatments could be due to the lack of appropriate number of microorganisms able to degrade certain organic pollutants. It would take longer time to increase their number, therefore, increasing the removal time. Another reason can be that the microorganisms in other reactors needed to be adapted to the presence of organic pollutants and to activate appropriate enzymes. In contrast, RSS could avoid that phase considering that activated SS could easily contain PAHs in trace or other organic contaminants. Thus, SS could be expected to inoculate contaminated soils with microorganisms already adapted on such kind of pollutants and able to provide appropriate enzymes for their removal [41] compared to food wastes and buffalo manure where it might be not so common.

It is very interesting to compare the obtained results with data published by Straube et al. [42] by performing landfarming technology with biostimulation (addition of bulking agent and dried blood as a slow-release nitrogen

source) and bioaugmentation (addition of *Pseudomonas aeruginosa* strain 64). Even if they managed to achieve a total PAH removal of 86 and 87 %, respectively, after 16 months, the removal was mainly related to three- and four-ring PAHs. Besides, the maximum Anth removal in their study was 72 % after 16 months, while treatment with SS amendment performed in this research work resulted in 89 % removal in less than 5 months. Moreover, treatments performed by Straube et al. [42] did not manage to remove recalcitrant PAHs like B(k)F and B(a)P at all after 16 months, while all treatments with organic wastes described in this research study were able to remove them with different efficiency in about 5 months. Chry had a different pathway, actually landfarming with bioaugmentation showed the highest removal 78 % after 16 months, but in the first 6 months of treatment the removal had not been detected yet [42]. Conversely, RSS resulted in 59 % of removal after only 140 days, and could be expected to reach similar level of removal as what was achieved by Straube et al. [42] for a much shorter time.

Great potential of activated SS to enhance PAH removal from contaminated soils should be also investigated associated with thermophilic phase of composting process. It would ensure greater diffusion rate and enhanced bioavailability of contaminants that is supposed to facilitate their access to microorganisms [21]. Actually, increased bioavailability enhances the biodegradation processes and ensures bioremediation as a feasible method [31].

Volatilization and leaching

Total volatilization loss and leaching were monitored in all reactors, since PAH removal can also be subjected to volatilization and leaching [40]. As expected [31], only Anth was detected in its volatile form but in a very low concentration in all reactors. Indeed, Anth volatilization was below 0.5 % on total removal in all treatments. Furthermore, the highest range of volatilization was observed after 3 weeks of treatment and decreased until the end of process (Fig. 3). Actually, the higher metabolism by microorganisms affects the lower volatilization throughout the treatment. Therefore, higher range of volatilization is more typical in the earlier stages of process when volatile contaminants have not been completely subjected to metabolism by microorganisms.

The leaching of PAHs from the soil was very low, and did not occur in all reactors. Actually, two out of four PAHs, i.e. Chry and B(k)F, were detected in the leachate of RSS, while in RFVW only B(k)F was detected. All of them were present in extremely low quantity, i.e. 0.2 and 2.4 % in relation to the initial concentrations of B(k)F in RFVW and RSS, respectively, and 1.3 % of Chry in RSS leachate. There is no determined relationship among total PAH removal and their leaching. Possibly presence of persistent organic pollutants in leachate is expected, since they are resistant to other removal mechanisms such as microbial degradation and volatilization. Accordingly, B(k)F has been found in leachate of treatments where some contaminants were detected, since it is considered as the most recalcitrant indicator in experiments.

Bioassays

Dehydrogenase activity (DHA) in bio-reactors

Metabolic activity of microorganisms measured as a biological activity indicator for PAH removal was dehydrogenase activity.

Unfortunately, biological activities might be not consistent with contaminants removal, and often do not correspond to residual contaminants concentrations [24]. Nevertheless, monitored biological activities within this research work are related with PAHs concentrations at the end of bioremediation (Fig. 4).

According to the statistical analysis, significant differences of dehydrogenase activities were not observed (ANOVA, $p < 0.05$), but different values of these biological indicators in $\mu\text{g TPF g}^{-1} \text{ d/w}$ between reactors were obtained. Those values are positively correlated ($r = 0.8$) with removal of HMW PAHs, i.e. as values for DHA activity increase, values for total removal of Chry, B(k)F, and B(a)P also increase in certain reactors. Accordingly, the highest activity has been measured in reactor with SS amendment ($2601 \mu\text{g TPF g}^{-1} \text{ d/w}$) that is twice higher than the activity measured in reactor amended with FVW ($1269 \mu\text{g TPF g}^{-1} \text{ d/w}$). Consequently, DHA activity in RFKW was 40 % higher ($1792 \mu\text{g TPF g}^{-1} \text{ d/w}$), while RBM shown almost 70 % higher microbial activity ($2141 \mu\text{g TPF g}^{-1} \text{ d/w}$) than RFVW.

Dehydrogenase enzymes are the catalysts of metabolic process such as biological oxidation of organic compounds and subsequently the detoxification of xenobiotic [8, 11, 24]. Considering that PAHs biodegradation is a complex metabolic process catalyzed by dehydrogenase enzymes, their activity is crucial for the overall success of bioremediation [8, 24]. Further, for a successful utilization of DHA activities as bioindicator for PAH removal, it is useful to make them suitable and correlate the measurements with other bioindicators during bioremediation such as microbial biomass, soil enzymatic activities (urease, protease, β -glucosidase, phosphatase, arylsulphatase), seed germination, earthworm survival, and microbial bioluminescence [16, 24].

Soil toxicity

Microtox[®] test was used to perform an assessment of toxicity level, due to the fact that incomplete degradation

Fig. 3 Anthracene volatilization during the bioremediation treatment

Fig. 4 Dehydrogenase activities in each reactor after 140 days of bioremediation. Vertical bars are standard deviations of mean of three replicates at 95 % confidence level. Different letters indicate on significantly different values between treatments (ANOVA, $p < 0.05$)

Table 3 Decrease/increase of acute toxicity in soil at the end of composting treatment, measured with Microtox test at inhibition time of 5 and 15 min

Reactors	Inhibition at time 5 min (%)		Inhibition at time 15 min (%)		Toxicity evolution (%)	
	0 day	140 days	0 day	140 days	Inhibition at time 5 min ($I_{(5)}$)	Inhibition at time 15 min ($I_{(15)}$)
RBM	19.7 ± 8.3	36.1 ± 7.8	17.3 ± 9.4	38.2 ± 6.3	+98.2 ± 37.9	+200.8 ± 170.4
RFKW	37.6 ± 0.8	11.6 ± 1.8	34.4 ± 2.3	13.1 ± 0.2	−69.5 ± 4.3	−61.7 ± 1.9
RFVW	40.6 ± 1.1	21.2 ± 6.9	34.8 ± 0.2	22.7 ± 8.4	−48.2 ± 15.6	−34.7 ± 24.6
RSS	20.3 ± 0.0	7.1 ± 0.0	20.3 ± 0.0	8.9 ± 1.8	−64.8 ± 0.0	−56.0 ± 8.8

of PAHs could lead to the formation of metabolites such oxy-PAHs that might be more toxic than their parent compounds [34]. Therefore, prior implementing the bioremediation process, the contaminated samples demonstrated certain toxic effects due to the presence of organic pollutants. After composting treatment, the overall acute toxicity was considerably decreased, especially for RFKW treatment (69–62 %) and then for RSS (65–56 %) (Table 3). Decrease of acute toxicity for RFVW was pretty lower compared to RFKW and RSS treatments and amounted 48–35 %. In contrast, for RBM, an increase of acute toxicity was detected.

Results of Microtox[®] test provided information on the bioavailable fraction of organic pollutants and confirmed the effectiveness of simulated composting treatments. Moreover, they demonstrated that toxicity level is not always in correlation with the contaminants concentration. Actually, the highest toxicity decrease was found in RFKW, while the residual PAHs concentrations were among the highest compared to other treatments. Indeed, the toxicity of the PAHs was reduced but not their extractability, and it confirms that the extraction with organic solvents is not correlated with the bioavailability of the pollutants [34]. The largest decrease in bioavailability shown in RFKW could occur due to the stronger sorption of pollutants in considerably higher amount of organic matter present in the beginning of process, than in the other three treatments (Table 2). Considered bioavailability is that one related to *Vibrio fischeri* used for the Microtox[®]

test. This is a good example to show usefulness of bioassays to estimate the bioavailable fraction of organic pollutants [22]. On the other hand, in RBM a significant removal of target contaminants was observed, but the decline toxicity was not detected. Even more, acute toxicity was higher on the end of composting treatment that could be the consequence of oxy-PAHs formation as a result of incomplete degradation [39], as explained above.

Within this set of experiments, microbial activities in reactors would be in accordance with Microtox[®] test data. Indeed, RFVW reactor with the lowest DHA activity showed also the lowest decline toxicity, while RSS reactor with the highest microbial activity, showed considerably higher decrease of toxicity compared to RFVW. In RSS the results indicated more than one-third higher decrease of toxicity after inhibition at time 5 min and even more than 60 % after inhibition at time 15 min. Results of Microtox[®] test for RFKW could not be related to DHA activity due to possible reduction of pollutant's bioavailability as it is explained in previous paragraph, neither for RBM since was detected an increase of toxicity mentioned above.

Conclusions

This research work confirmed the potential success and high efficiency of bioremediation using composting of organic waste as biostimulation strategy to degrade persistent PAHs and to shorten their total removal time.

By considering all the data and results obtained, it can be concluded that activated SS is the most favorable organic amendment to be considered and used in composting treatments. Actually, RSS reactor was found to be the most efficient treatment in overall PAH removal considering that it showed the highest removal of Anth and Chry, and the fastest removal of Anth, B(k)F, and B(a)P compared to other treatments. Even if B(k)F and B(a)P removal was a bit higher in RBM than in RSS, it is not considered as efficient due to the considerable increase of acute toxicity at the end of composting treatment compared to its initial value. Accordingly, this study highlights the importance of monitoring the changes in soil toxicity prior and after bioremediation. Ecotoxicity test should be used when biodegradation products are not monitored in addition to chemical analysis of contaminants. Furthermore, this research work showed that DHA enzymatic activities could be used successfully as bioindicator for PAH removal.

On the other hand, it could be interesting also to study PAH removal at constant temperature displaying a mesophilic phase only with BM and FKW amendments. It could provide information about time needed for microorganisms to adapt their physiological processes in response to present pollutants. Moreover, longer mesophilic phase could facilitate PAH removal due to the richest microbial diversity and possible increased microbial activity. This study confirmed the greatest success of treatment subjected to mesophilic phase. In this regard, there is a need for further studies to clarify all specificities and details to upgrade this approach and make use of all benefits of such complex process in the most efficient way.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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