



Research paper

Development of new remediation technologies for contaminated soils based on the application of zero-valent iron nanoparticles and bioremediation with compost[☆]



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ARTICLE INFO

Article history:

Received 29 July 2016

Revised 27 March 2017

Accepted 27 March 2017

Available online 22 May 2017

Keywords:

Bioremediation

Soils

nZVI

Hydrocarbons

Metals

ABSTRACT

This study aimed to develop new techniques for the remediation of contaminated soils based on the application of zero-valent iron nanoparticles (nZVI) and bioremediation with compost from organic wastes and a mixed technique of both. An assessment of the effectiveness of remediation in two soils contaminated with hydrocarbons and heavy metals was carried out, with the aim of looking for positive synergies by combining the two techniques, and demonstrating their viability on an industrial scale. The application of nZVI for *in situ* immobilization of As and Cr in two different soils (Soil I from a contaminated industrial site and Soil II, contaminated artificially) showed a decrease in the concentration of As in Soil I and Soil II, as well as a decrease in Cr concentration for Soil I and Soil II in the leachate of both soils. The addition of compost and nanoparticles under uncontrolled environmental conditions in biopiles was able to produce a decrease in the concentration of aliphatic hydrocarbons of up to 60% in the two soils. Especially, degradation and transformation of longer chains occurred. A significant reduction of ecotoxicity was observed throughout the process in the biopile of soil II, not reaching the LC50 even with 100% of the sample after the treatment, in both earthworm and seeds growth tests.

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1. Introduction

The ceasing of industrial activity has left behind a large amount of contaminated sites on the periphery of urban areas (brown-fields) that are characterized by soils with high concentrations of organic and inorganic compounds (mixed pollution). Mixed pollution generates problems to determine the pollution nature and, consequently, makes the selection of the technologies that must be applied for the removal of contaminants difficult. Each polluted site has its own characteristics related to the typology of pollutants, the concentration levels of these, hydrogeological characteristics of the environment, biogeochemical soil properties, etc.

The soil natural heterogeneity adds to this complexity. Soil properties such as texture, degree of structure development, water retention capacity and/or chemical properties can be determinants for the diffusion of contaminants, and more importantly, for their degree of attachment to the soil mineral and organic matrix. This can be positive when it results in reduced diffusion and high biogeo-chemical stabilization of pollutants in soils, but at the same time limiting when the goal is removing soil contamination. For instance, clayey soils rich in high-reactive silicates and/or containing pH-modifiers such as carbonates can retain easily degradable organic pollutants in primary and secondary organo-mineral complexes, hindering their biological decomposition within the soil matrix.

In this framework, contaminants in soils and sediments can be found in six different ways [1]: as particulate contaminants, as liquid films, adsorbed, absorbed, dissolved in the interstitial pore water, or as solid phases in the pores. For each case, the behavior of the contaminant is different so its hazardousness must be

[☆] Peer review under responsibility of Tomsk Polytechnic University.

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evaluated on the basis of its mobility and availability, which is determined by different chemical and mineralogical procedures. In the case of heavy metal pollution in soils, which poses a worldwide challenge, their mobility can be evaluated extracting the water-soluble fraction of the metal. This fraction measurement represents an approximation to the amount of metals that plants can absorb from the soil under normal conditions [2]. The availability of a metal depends, as that of organic pollutants, on its chemical speciation and on a number of soil parameters such as pH, organic matter concentration, clay mineral carbonates, etc. For instance, the availability of any exchangeable cation is conditioned by the associated minerals that form the exchange complex. Thus, As, in its oxidized form, especially at acid-neutral pH, is chemically adsorbed to oxides of iron and aluminum, aluminosilicates and, to a lesser extent, sheet silicates. In sodium soils, arsenic is more mobile and is released from the solid phase to soil dissolution, such as arsenate anion. In reducing environments, As is in the form of arsenite anion, which is still absorbed by the clay fraction with more force than the anion arsenate. This results in As (III) being manifestly more toxic than As (V), since it forms very stable complexes with the SH groups of the enzymes. The oxidative capacity of manganese oxides is sufficient to oxidize the arsenite to arsenate. As far as Cr is concerned, it is found in the natural environment, especially in tri- and pentavalent forms. The trivalent form gives rise to very stable complexes with organic and inorganic ligands containing oxygen or nitrogen atoms. In the soil, this metal can be found to several depths. In general, normal soil conditions favor the Cr (III) form, which remains quite immobile, being retained on the surface of oxides and silicates, where it forms stable chemical bonds even at acid pH. At pH above 5, $\text{Cr}(\text{OH})_3$ is precipitated as an hydroxide. In higher pH media, Cr (III) can be oxidized to chromate anion, CrO_4^{2-} , which is the toxic form of Cr. The presence of Mn oxides favors this oxidation. The Cr (VI) species is more mobile than Cr (III), especially in the presence of organic matter. The organic matter acts as reducing agent, and, in addition, it complexed, which favors the retention of the Cr (III) produced in the reaction [3].

Organic pollutants are also widespread in brownfields and other polluted sites in industrial and peripheral areas worldwide. They occur as byproducts and residues or spills of the chemical, metallurgical and other industrial activities, and include a wide range of molecules containing C and at least one C–H group. Many of these are the result of liberation of human-made organic components to the environment. On average, 260 Tg of organic compounds are manufactured annually only in the US. Oil byproducts are the most significant source of hydrocarbons into the environment, usually associated with the use of oil-derived fuels [4].

One class of organic pollutants which has gained great attention in environmental studies is polycyclic aromatic hydrocarbon (PAH). PAHs have high resonance energies as a consequence of their dense clouds of pi electrons surrounding the aromatic rings, which make them persistent compounds in the environment and recalcitrant to degradation [5]. This molecular structure is characterized by their hydrophobicity so that they display decreasing solubility in water with increasing molecular mass. The environmental fate of PAHs depends largely on the environment to which they are exposed. The phase or state of a PAH is determined by its vapor pressure and room temperature. Experiments have shown that, at 25 °C, four- and five-ring PAHs are distributed between the solid phase and steam, while PAHs with six and more rings are almost exclusively in the solid phase [6]. PAHs with high molecular weights (from four benzene rings) are more likely to be absorbed to the soil organic matter, and therefore their availability is reduced, but this also makes them less susceptible to remediation [7]. The fate and transport of PAHs is led by physical, chemical and biological processes that are influenced by the nature of the

subsurface environment. Hence, several techniques have been used, with varying results, to achieve acceptable degradation of these recalcitrant compounds (e.g., chemical degradation, biodegradation, phytodegradation and combined degradation methods).

Soil bioremediation is based on the biological degradation of organic pollutants, ideally until their final transformation to CO_2 , inorganic compounds and/or other organic compounds with reduced toxicity. Microorganisms able to degrade hydrocarbons in soils are bacteria, fungi and algae, bacteria being those with the highest and fastest degradation rates [8,9]. Average contents of these microorganisms in polluted soils are usually higher than in non-polluted soils [10]. The success of bioremediation techniques depends on the environmental conditions, number and type of organisms, and the type and structure of the pollutants [8]. Bioremediation techniques are usually based on the stimulation of the natural ability of soil microorganisms to degrade organic pollutants by favoring their activity. This is generally done by granting adequate physical–chemical conditions (nutrients and water availability, aerobic conditions, right pH and red-ox characteristics) [11] and/or by adding exogenous microbial populations especially competent for biodegradation of the targeted pollutants. The addition of organic amendments such as compost is a frequent strategy in this sense and has been used in many sites and contexts [12–14]. In addition, the pre-incubation of small quantities of the polluted soil in contact with this amendment favors the development of the native microbial populations able to degrade pollutants present in the soil. This is known as *assisted* bioremediation including biostimulation.

Currently the most widely used techniques for soil remediation are based on the application of different physical and chemical treatments. Lack of knowledge to properly assess new approaches and technologies tend to please the technologies that have been already proven successful in previous cases, and therefore the classical approach “dig and dump” is still hegemonic. At the same time, there is an inertia to extract the contaminated soil and carry out external treatments, which requires the processing of large amounts of contaminated soil resulting in high costs and making the control of the treatments difficult.

The synergistic use of nZVI and bioremediation is a new strategy for restoring contaminated sites by the *onsite* application of two combined advanced technologies that can contribute to the implementation of recovery alternatives that reduce the volume of waste generated in decontamination processes and therefore reduce the industrial impact on the environment.

Iron nanoparticles perform like strong reducing agents. Their action mechanism involves oxidation–reduction reactions (redox), so that in contact with the medium they are oxidized rapidly and donate their electrons to pollutants, thus reducing them. The pollutants become more stable, less mobile and/or less toxic products. Among the advantages associated with the use of nZVI for soil decontamination, there is the prospect of a notable reduction in the ratio of kg of product per volume of soil to be treated, thanks to the large surface area provided by nanomaterials regarding macroscopic materials. The granular zero valent iron has been used for years with success, especially in permeable reactive barriers (PRBs) for the treatment of chlorinated hydrocarbons (ethanes and ethenes), metals and metalloids (arsenic, chromium and uranium), nitroaromatics, and treatment of perchlorates with limited results [15].

There is a dearth of studies about the decontamination potential of nZVI in soils [16]. The treatment of the metals by nZVI is produced via immobilization [17]. This strategy prevents its transport through the layers of soil, rivers and groundwater. So far, the studies about the immobilization of metals by nZVI in soils have been conducted in “in vitro” conditions. Some of the most important trials have demonstrated the immobilization of Pb and Zn in

nZVI-treated soils through the analysis of the leachate [18], as well as some promising results have been obtained for reducing the mobility of arsenic [19], antimony [20] and some other metals and metalloids in contaminated soils.

Uncertainty about the long-term fate, transformation and ecotoxicity of nZVI in environmental systems is revealed as an important point. It is necessary to predict the fate and physical, chemical and biological consequences of the use of nZVI when used in contaminated sites. Nanoparticles raise fear for their ability to penetrate into the food chain (bioaccumulation), and also by the possibility of facilitating the spread of other non-target pollutants present in the soil [21]. Therefore, a better understanding of the behavior of nanomaterials in sediments and soils, and its degree of toxicity is necessary.

The use of compost in the process of biostimulation in assisted bioremediation strategies is frequent. It grants a source of nutrients and organic matter to stimulate microbial growth in contaminated soils with low natural fertility. When the compost used with this aim is made from waste such as sewage sludge or other organic wastes, it has the advantage of meeting two objectives simultaneously: reducing soil contamination and giving a valuable use to these wastes. The combination of chemical (iron nanoparticles) and biological (bioremediation) technologies is intended to optimize the cost-efficiency of soil remediation from three points of view mainly: it can economize the process, reduce the time for decontamination, and ensure the sustainability of process. This requires a chemical intervention dimensioned so that a rapid reduction in the concentration of target pollutants occurs and thus, a more effective bioremediation and a reduction in time of the global treatment. Simultaneously, bioremediation can provide an early use of the site as it can be compatible with the development of certain activities on the site. Similarly, monitoring the chemical activity process ensures sustainability, as it reduces the use of chemicals that can sometimes be aggressive for the soil and affect its normal functions.

In short, this approach aims to provide solutions for a more efficient treatment and, therefore, more competitive, not only economically but in recovery times, potential side effects, and the sustainability of the application.

The objective of this work was to test the efficiency of a combined technique of soil remediation, using an approach that couples classical bioremediation with organic amendments and the use of zero-valent iron nanoparticles. The work was conducted in two different soils (one artificially polluted with heavy metals and hydrocarbons and one from a brownfield containing a complex mix of organic and inorganic pollutants).

2. Materials and methods

2.1. Chemical reagents, standards and materials

2.1.1. Soils and compost

Two different soils were used for the assays of decontamination by the combined technique: Soil I came from a brownfield in Barakaldo (North of Spain), and it was selected because of its high concentration of hydrocarbons. Soil II, a natural soil from Azkoitia (Spain) without known contamination, was also sampled. Soil II was artificially contaminated, following a preset design and calculations, adapted to the objectives of the assays. The process of controlled contamination was performed by adding diesel, potassium dichromate and sodium arsenate to Soil II. To calculate the target concentrations, the dry weight of the screened soil was considered (487.6 kg of dry soil). These concentrations were established based on the VIE B reference values of the regional Basque Law 4/2015 for the prevention and correction of soil contamination, seeking to overcome these values.

Both soils were initially screened by a metal net 1×1 cm in size, obtaining the following amounts of soil available for the biopiles: Soil I = 212,8 kg (ww); Soil II = 688,1 kg (ww).

Due to its high availability, MSW (municipal solid waste) compost was obtained from Garbiker Bizkaiko Konpostegia plant (Bizkaia, Spain). This compost was obtained from the municipal organic fraction, collected separately. It had a relatively high salt content and a lower C/N ratio than recommended for bioremediation. The chemical characteristics of both soils (concentrations of organic C, total N, available P and K), and the compost, as well as their physical characteristics (water retention capacity and bulk density) were determined following the standard procedures for soils [22] and are shown in Table 1.

Soil I had a basic pH, a high concentration of soluble salts (typical of a marshy soil) and a suitable C/N ratio for bioremediation (recommended C/N ratio for bioremediation is 10) [23–25]. Soil II also had a basic pH, but a low concentration of soluble salts and a lower C/N ratio than recommended (Table 1).

Soil texture, a known factor determining the soil hydric properties, was also measured for Soil I and Soil II. Soil I had a high sand content (74.5%) and low clay content (5.14%), resulting in a loamy sand texture, according to USDA soil texture classification. On the other hand, the artificially contaminated soil (Soil II) had a high content of silt (58%), resulting on a silt loam texture.

The chemical analysis of the metals in soil was performed by ICP-MS (spectrometer with inductively coupled plasma with mass detector Agilent 7700) and ICP-OES (spectrometer with inductively coupled plasma with an optical detector). Samples were homogenized and after that they were mineralized with aqua regia prior to analysis. Digestion of the soil samples was performed in a CEM microwave under the EPA standard 3051_8, 0.5 g of sample was weighed and digested with 9 mL of HNO_3 plus 3 mL of HCl. The resulting extract was filtered and flushed to 50 mL. The leaching of the soil samples extracted from the piles was carried out following the instructions of the standard UNE-EN 12457-4 which provides a standard method for leaching granular waste and sludge. This is a two-stage batch test with a solid-liquid ratio of 10 l/kg for materials with a particle size of less than 10 mm. The leaching of the samples was carried out with a roller stirrer at 10 rpm for 24 h and the filtering of the samples was performed with 0.45 μm membrane filters.

Also aliphatic and aromatic fractions of petroleum hydrocarbons, and TPH or total petroleum hydrocarbons were analyzed by gas chromatography with FID and MS detection, as well as 16 PAHs or polycyclic aromatic hydrocarbons were analyzed by gas chromatography with MS or MS/MS detection.

The preliminary analytical results showed a high uncertainty because of the heterogeneity in the composition of the soil. To avoid this fact, a sampling procedure was designed and applied to ensure better uniformity, based on the standard UNE-EN 932-1. Thus, for the analysis, three samples of each soil were collected, made up of 15 aleatory points. Each sample was around 8 kg of mixture (near 0.5 kg per point), thoroughly mixed and divided into 4 quarters to take the necessary amount for the chemical analysis.

2.1.2. Commercial zero-valent iron nanoparticles

NANOFE 25S commercial nanoparticles were manufactured and supplied by NANOIRON (Rajhrad, Czech Republic). This kind of nanoparticles offers a better balance between reactivity, stability and dispersion.

The product was supplied as an aqueous dispersion, containing by weight: 77% of water, 14–18% zero-valent iron nanoparticles, 2–6% iron oxides, 0–1% carbon and 3% surfactant (PAA based stabilizer and coating).

Table 1
pH, electrical conductivity, nutrient content, moisture, water content at field capacity and wilting point (w.p.) of the soils and the compost.

| Sample | pH | EC (1:5) (mS/cm) | K (mg/kg) (dw) | P (mg/kg) (dw) | N (%) | C (%) | C/N | Moisture (%) | Field capacity (g/g) | w. p. (g/g) |
|-------------|------|------------------|----------------|----------------|-------|-------|------|--------------|----------------------|-------------|
| Soil I | 8.74 | 1.920 | 197.3 | 6.22 | 0.38 | 4.18 | 11.0 | 15.5 | 0.25 | 0.11 |
| Soil II | 7.5 | 0.113 | 46.8 | 4.65 | 0.17 | 1.29 | 7.62 | 22.1 | 0.33 | 0.17 |
| Compost MSW | 8.2 | 4.23 | 14,904 | 318 | 2.5 | 20.3 | 8.12 | 22.1 | 0.91 | 0.63 |

2.1.3. Site conditioning

The soil remediation assays were conducted at Iragaz facilities in Azkoitia (Spain). Before performing the assay a safety basin was prepared to protect the assay from any eventuality. The floor of the basin was sealed with a HDPE sheet to prevent the infiltration of leachates into the subsoil and throughout the wall of the basin. Tents were also set up to protect biopiles from precipitation.

2.2. Preincubation, biopile formation and monitoring

The trials were set up in a sequence with three steps based on previous experiences.

First, the two soils were conditioned as described above, and mixed with zero-valent iron nanoparticles. The nanoparticles used were previously characterized, showing a high degree of dispersion and low sphericity, and a large crystalline iron content and magnetite was observed in X-ray diffractograms (see TEM micrographs and x-ray diffractograms in [26]). The mixture of Soil I and Soil II with nanoparticles was performed by adding 1% of slurry on the weight of wet soil. The mixture was allowed to react for 72 h, and another 1% slurry was subsequently added.

The process in each addition of nanoparticles was monitored. Samples were taken stack according to the UNE-EN 932-1 Standard (Testing to determine the general properties of the aggregates, Part 1: Sampling Methods), and extracted samples were leached according to UNE-EN 12457-4 and later analyzed for the determination of total concentration of As, Cr and Fe in each stage of the process.

Second, a subsample of the two soils was pre-incubated with compost. This was done on the basis of the results of previous experiments in order to allow a better development of microorganism populations native to both soils able to degrade hydrocarbons. The presence of this type of microorganisms is common in these soils. Pre-incubation implies inoculating a small sample of soil to the compost and adjusting temperature, water contents and nutrients to optimal conditions for their development. The availability of C from compost implies that these microorganisms can grow in non-limiting conditions. The development of these populations could allow a better degradation of soil hydrocarbons promoting a more effective bioremediation in the final biopiles. The mixing rate of compost and soil for the incubation was 80–20 on a dry mass basis, for the total amount of compost used in the biopiles (see below). The mixing was done mechanically to ensure a homogeneous distribution of soil in the compost. Water was added to ensure water availability at 70% of the field capacity of the mix. The total contents of C, N, available K and P were controlled, and remained in non-limiting ranges for microbial growth. The pre-incubation was conducted for two weeks. Temperature was controlled daily to ensure adequate conditions (20–25 °C), and the piles were aerated twice a week by mechanical means.

Finally, using the pre-incubated compost, biopiles for bioremediation were set. The final soil–compost proportion was 52.9%–47.1% (dry mass basis) for Soil I and 54.3%–45.7% for Soil II. Both soils had been previously mixed with nanoparticles as described above. Water was added to ensure water retained content between 70% and 100% of the field capacity in each mix. The content of organic C, total N and available P was monitored and verified to remain in a proportion of 100:10:1 during all the bioremediation procedures.

The biopiles were set in open-air cells to better simulate natural conditions. They were protected from rainfall to avoid excess moisture that could hinder microbial activity, and the experiment was run for 66 days. Temperature was measured daily at several points of each biopile and remained within a range of 20–35 °C. Biopiles were aerated by mechanical mixing to avoid the existence of anaerobic conditions. Water was added when needed to ensure adequate moisture conditions (70–100% of field capacity). The decontamination process was monitored by sampling the two biopiles in triplicate using the protocol described above, and analyzing the biopiles at day 0, and at the end of the experiment (day 66). In each sampling, aliphatic and aromatic fractions of petroleum hydrocarbons, TPH and 16 PAHs were analyzed. Those analyses of contaminants were done as described (see Section 2.1.1).

A triplicate analysis of the concentration of hydrocarbons and PAHs in compost was also performed before mixing with soils. An average of 104.3 mg/kg of TPH (>C10–C40 Fraction) and 2.25 mg/kg of the sum of 16 PAHs were obtained.

When the concentration of a contaminant (in this case, a hydrocarbon) falls to non-toxic limits according to the legislation only by the action of the microorganisms present in the soil, the process of decontamination is described as *natural attenuation*. Soil I was left in optimal conditions for natural attenuation for 16 months before the start of the experiment reported in this work. This implies that any possible natural reduction of the concentration of pollutants in this soil was finished before the biopiles were set up. The results obtained demonstrated that natural attenuation did not result in any significant reduction of the concentration of contaminants (see Table S1).

2.3. Toxicity

Ecotoxicity tests of the biopiles (by “*Eisenia foetida*” earthworm mortality test according to OECD 207 [27] and “*Linum usitatissimum* sp.” plant seeds growth test according to OECD 208 [28]) were performed in the initial contaminated soils and in the beginning and the end of the biopiles in accordance with the legal recommendations of the Spanish Royal Decree 9/2005.

These tests were performed in order to verify that the average lethal or effective concentration (LC50) of the soil samples was within the values established in Royal Decree 9/2005, as well as to evaluate the evolution of toxicity throughout the decontamination treatments. According to Spanish law, the soil is contaminated when the average lethal or effective concentration LC50 for soil organisms is less than 10 g of contaminated soil/kg of control substrate. For that purpose, according to the regulations described in the OECD 207 and OECD 208 standards, selected worms and seeds were exposed to samples of contaminated soil at different concentrations on an artificial substrate (control) to finally obtain LC50 or concentration of contaminated soil from which the mortality is higher than 50% of the individuals (case of earthworm tests) or the concentration of contaminated soil from which the percentage of non-germinated seeds is higher than 50% (case of seeds growth tests). In the case of earthworm toxicity tests, the assays were performed with adult individuals of the species “*Eisenia foetida*”, disease-free, cultivated in the laboratory and kept in refrigerated stove at 20 °C with light exposure (600 lux) for 14 days.

Table 2
Initial average concentration of hydrocarbons, heavy metals and other pollutants (mg/kg) of the soils used in the study. Underlined values indicate values above the limits of Industrial VIE B (Basque Law 4/2015).

| Chemical parameter | Soil I | Soil II | Chemical parameter | Soil I | Soil II |
|------------------------|-------------|------------|-----------------------------|--------------|--------------|
| As | 47.3 | <u>244</u> | Naphthalene | <u>159</u> | 1.5 |
| Cd | 4.5 | <0.40 | Phenanthrene | 701 | 2.7 |
| Cr | 31.4 | 52.7 | Pyrene | <u>731</u> | 2.0 |
| Cr (VI) | 0.35 | 1.11 | Sum of 16 PAH | 6737 | 12.1 |
| Cu | 315 | 23.3 | TPH >C10–C40 fraction | <u>16533</u> | <u>11033</u> |
| Pb | <u>1162</u> | 35.8 | >C10–C40 Aliphatic fraction | 962 | 8197 |
| Hg | 15.7 | <0.20 | >C10–C12 Aliphatic fraction | 42 | 709 |
| Mo | 10.6 | 0.5 | >C12–C16 Aliphatic fraction | 73.1 | 2840 |
| Ni | 53.4 | 32.1 | >C16–C21 Aliphatic fraction | 129 | 3160 |
| Zn | 2710 | 101 | >C16–C35 Aliphatic fraction | 710 | 4640 |
| Acenaphthene | <u>124</u> | 0.5 | >C21–C35 Aliphatic fraction | 581 | 1480 |
| Acenaphthylene | 0.5 | 0.1 | >C35–C40 Aliphatic fraction | 138 | 4.5 |
| Anthracene | 225 | 0.4 | >C6–C8 Aliphatic fraction | <5.0 | <5.0 |
| Benz(a)anthracene | <u>754</u> | 0.4 | >C8–C10 Aliphatic fraction | <5.0 | 73.4 |
| Benzo(a)pyrene | <u>556</u> | 0.4 | >C10–C12 Aromatic fraction | 142 | 126 |
| Benzo(b)fluoranthene | <u>772</u> | 0.7 | >C10–C40 Aromatic fraction | 15567 | 2840 |
| Benzo(g,h,i)perylene | 211 | 0.3 | >C12–C16 Aromatic fraction | 545 | 874 |
| Benzo(k)fluoranthene | <u>291</u> | 0.2 | >C16–C21 Aromatic fraction | 3937 | 1137 |
| Chrysene | <u>676</u> | 0.4 | >C21–C35 Aromatic fraction | 9967 | 697 |
| Dibenz(a,h.)anthracene | <u>83.1</u> | 0.1 | >C35–C40 Aromatic fraction | 994 | 5.8 |
| Fluoranthene | <u>1043</u> | 0.7 | >C7–C8 Aromatic fraction | 0.3 | 0.2 |
| Fluorene | <u>130</u> | 1.3 | >C8–C10 Aromatic fraction | <0.80 | 11.2 |
| Indeno(1,2,3.cd)pyrene | <u>283</u> | 0.3 | C6–C7 Aromatic fraction | 0.2 | <0.120 |

After the 14 day period, the substrate of each test was evaluated to observe the worms and determine the number of survivors. The weight of the worms was also considered in order to assess the loss of biomass. In the case of emergence and seeds growth, guaranteed seeds of "*Linum usitatissimum* sp." were used and the tests were carried out at different concentrations of contaminated soil in a germination and growth chamber, at 23 ± 2 °C, and a relative humidity of $70 \pm 20\%$ for 14 days. After this period the percentage of non-germinated seeds and the length of radicle and hypocotyl were evaluated in each test.

2.4. Statistical analysis

For the bioremediation assays, data are presented as \pm standard error of the mean for the results of the different parameters studied. Comparison between biopiles was carried out by using the univariate linear model (ANOVA). Significant differences were based on a probability level of $P < 0.05$ unless otherwise indicated. All statistical analyses were performed using the SPSS 18.0 software (SPSS Inc., 2009). The data obtained from the assays with nanoparticles are presented as \pm standard deviation of the mean values obtained from the analyses of three replicates of each analyte.

3. Results and discussion

3.1. Characterization of soil contamination

Both soils were characterized at the beginning of the experiment, with two aims: First, assessing the presence of contaminants in Soil I (from a brownfield), after a period of natural attenuation. Second, determining the most significant contaminants in Soil I in order to design the procedure for artificially contaminating Soil II. The results of these analyses are shown in Table 2.

In the case of Soil II, the objective was to get an experimental soil with a different contamination than Soil I, so a natural soil was artificially contaminated. As Soil I had a high content of PAH, a predominantly aliphatic hydrocarbon was selected as target contaminant for Soil II, to be addressed by bioremediation. Commercial diesel was added in the proportions needed to attain a concentration of hydrocarbons similar to that present in Soil I. To fur-

ther test the ability of nZVI to immobilizing metalloids such as As [29–32] and to reduce metals such as Cr (VI) to Cr (III) [33], these two toxic species were selected as well.

Soil I and Soil II after being artificially contaminated were analyzed in triplicate to obtain an accurate characterization. The two soils had high concentrations of total hydrocarbons; Soil I also had very high concentrations of PAHs and Pb. Soil II had a high content of As; however, the design values were not obtained for Cr (VI) and TPH (C10–C40). This is attributed to the reduction of Cr (VI) to Cr (III) by reaction with the organic matter in soil and possibly with the hydrocarbons of the diesel added.

3.2. Monitoring of the nanoparticle treatment for metal adsorption

The adsorption of Cr and As was monitored at different stages in order to determine the effectiveness of the immobilization of these metals during the time of treatment with nZVI. The results obtained from the concentration measures of both metals in soil in the different phases (at the first stage with the addition of 1% of nZVI in slurry format and after 72 hours with the addition of other 1% of nZVI) are summarized in Table S2.

Initially both soils had a high iron content, which consequently experienced an increase in its concentration by adding nZVI. The concentration of Cr and As remained unchanged because the determined concentrations were total concentrations of the metal present in soil. The metal adsorption by nZVI does not change the amount of metal present in soil, but nevertheless, it reduces its availability and its diffusion capacity.

As shown in Fig. 1, bar charts showed an elevated dispersion in the results for chemical concentrations of Cr in Soil I and As in Soil II, probably due to the different homogeneity of the mixture of contaminants with Soil I (from contaminated industrial site) and Soil II (artificially contaminated). The diffusion of contaminants through the soil depends largely on the own hydrogeochemical characteristics of each soil [3].

The metals' ability to spread depends on whether the metal is water-soluble. Therefore, in order to evaluate the effectiveness of the treatment with nanoparticles in immobilizing As and Cr, several replicates of each soil leached were analyzed in the different phases of nZVI addition. The leaching of contaminants present in soil is controlled by different parameters and external factors,

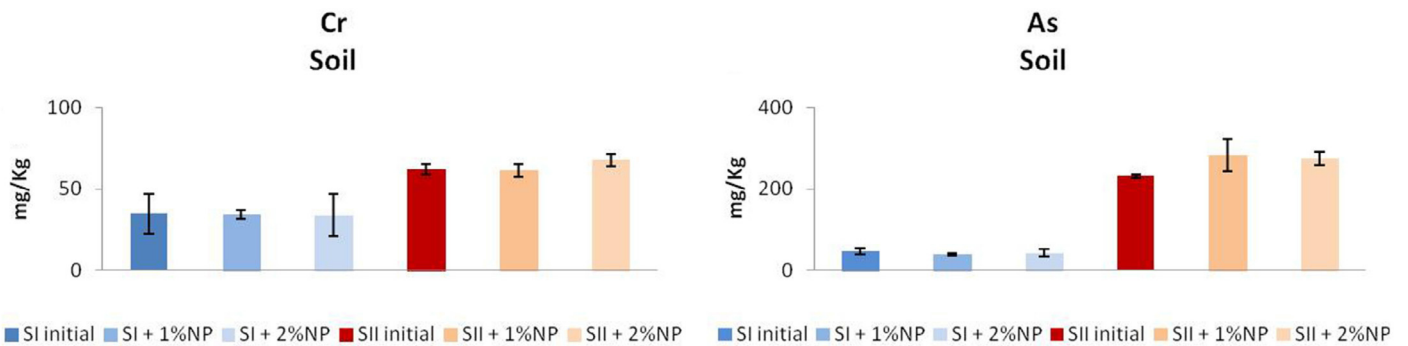


Fig. 1. Evolution of Cr and As concentrations in soil I and soil II in different phases of the treatment with iron nanoparticles. Bars indicate the standard deviations of the means of three replicates of each sample.

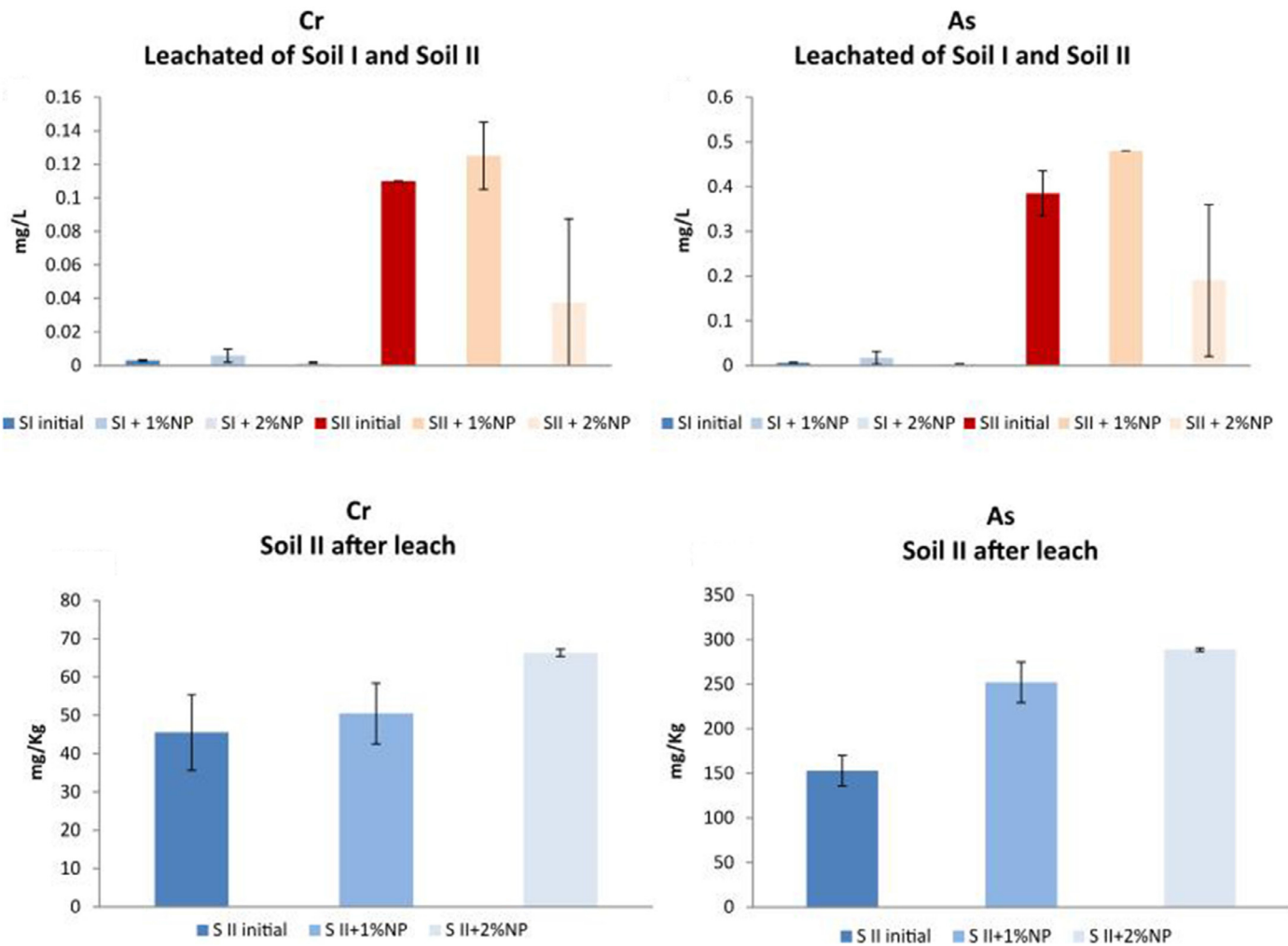


Fig. 2. Bar graph of the mean values of Cr and As concentrations in leachates obtained from three replicates of each sample of Soil I and two replicates of each sample of Soil II and concentration of Cr and As determined for two replicates of Soil II after leaching in the different phases of the treatment. Bars indicate standard deviation of the mean of several replicates of each sample.

including the chemical nature of the soil (pH, reduction properties, content degradable organic material), the nature of leachant, and the contact time of leachant with the soil. Therefore, pH, conductivity and temperature of leachant and leachate were monitored at each stage of the process (Table S3).

The average values of concentration of Cr and As in the leachates of each soil in the different stages of the treatment are shown in Table S4.

For Cr and As in Soil I and Soil II was observed a trend toward the reduction of the concentration of both analytes in the leachate after adding 2% of nZVI. As can be seen (Fig. 2) the reduction of the concentration in the leachate in the case of Soil II produced an increased of the presence of the two metals in the same soil remaining after being leached. The concentrations obtained for each metal in soil and leachate are shown in Tables 3 and 4, as well as the percentage of metal leached. The calculation method of the

Table 3
Cr percentage obtained in the leachate (Leach) of the different phases of the treatment in relation to the Cr concentration determined in soil samples (mean \pm standard deviation of the concentration values of soil and leachates).

| Sample | mg _{Cr} /kg Soil I | mg _{Cr} /L Leach I | %Cr Leach I | Sample | mg _{Cr} /kg Soil II | mg _{Cr} /L Leach II | %Cr Leach II |
|----------|-----------------------------|-----------------------------|-------------|-----------|------------------------------|------------------------------|--------------|
| SI inic. | 34.8 \pm 12.2 | 0.0030 \pm 0.0004 | 0.009 | SII inic. | 61.9 \pm 3.3 | 0.1100 \pm 0.000 | 0.177 |
| SI 1% NP | 34.2 \pm 2.9 | 0.0059 \pm 0.0040 | 0.017 | SII 1% NP | 61.2 \pm 3.9 | 0.1250 \pm 0.020 | 0.204 |
| SI 2% NP | 33.7 \pm 12.9 | 0.0018 \pm 0.0003 | 0.005 | SII 2% NP | 67.8 \pm 3.8 | 0.0375 \pm 0.050 | 0.055 |

Table 4
As percentage obtained in the leachate (Leach) of the different phases of the treatment in relation to the As concentration determined in soil samples (mean \pm standard deviation of the concentration values of soil and leachates).

| Sample | mg _{As} /kg Soil I | mg _{As} /L Leach I | %As Leach I | Sample | mg _{As} /kg Soil II | mg _{As} /L Leach II | %As Leach II |
|----------|-----------------------------|-----------------------------|-------------|-----------|------------------------------|------------------------------|--------------|
| SI inic. | 46.1 \pm 8.0 | 0.0065 \pm 0.0005 | 0.014 | SII inic. | 231.2 \pm 3.7 | 0.3850 \pm 0.05 | 0.166 |
| SI 1% NP | 39.0 \pm 2.1 | 0.0170 \pm 0.0143 | 0.044 | SII 1% NP | 281.7 \pm 39.5 | 0.4800 \pm 0.00 | 0.170 |
| SI 2% NP | 43.2 \pm 9.7 | 0.0034 \pm 0.0002 | 0.008 | SII 2% NP | 274.7 \pm 16.9 | 0.1900 \pm 0.17 | 0.069 |

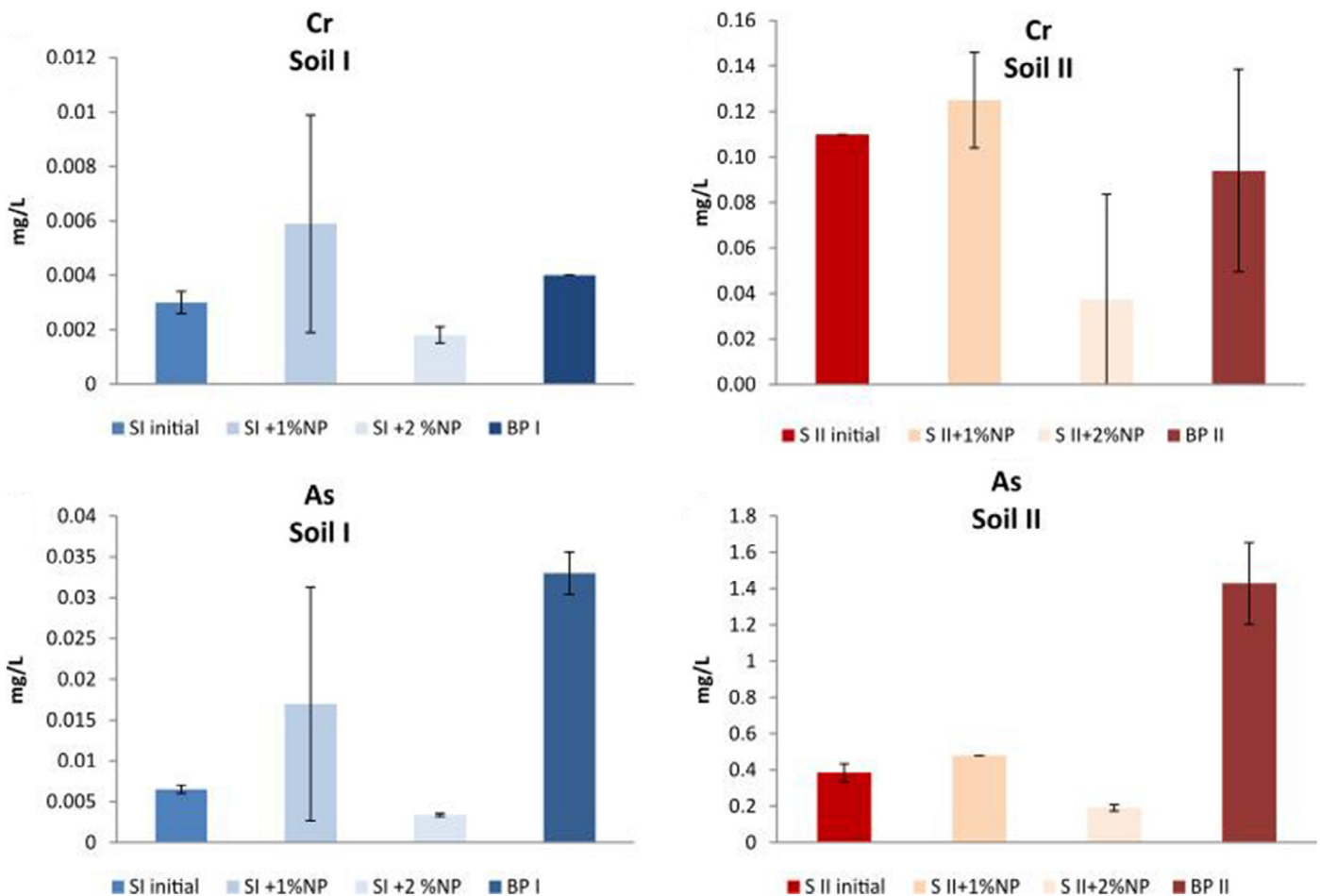


Fig. 3. Representation of the mean values of concentrations of Cr and As in the leachate for Soil I and Soil II through the initial phase of the treatment (addition of 1% of nanoparticles), second phase (addition of 2% of nanoparticles) and third phase (addition of compost (BP final)). Bars indicate standard deviation of the three replicates of each sample (except for the first two phases of Soil II that are two replicates for each sample). The standard deviation of the three replicates of concentration of Cr and As of the biopiles can be seen in Table S6.

percentage of leached analyte is contained in the Supplementary material.

The percentage of each metal in the leachate with respect to that contained in no-leached soil was evaluated. A reduction of the percentage of As and Cr in the leachates could be observed in the second phase of the treatment for Soil I and a similar trend was observed for the Soil II samples. On the contrary, in the first stage of application of nanoparticles, an increase on concentration values of both metals occurred. This fact suggests that initially, nZVI

shows a preference to react with other components of the soil that produce changes that probably involve the increase of the availability of metals in this stage.

To assess the synergistic action of the combination of technologies studied (nZVI and bioremediation with compost), Cr and As concentration in leachate of Soil I and Soil II after being mixed with compost was measured (BP I and BP II respectively).

As can be seen in Fig. 3, an increase in As concentration occurred in Biopile II compared to the concentration obtained for Soil

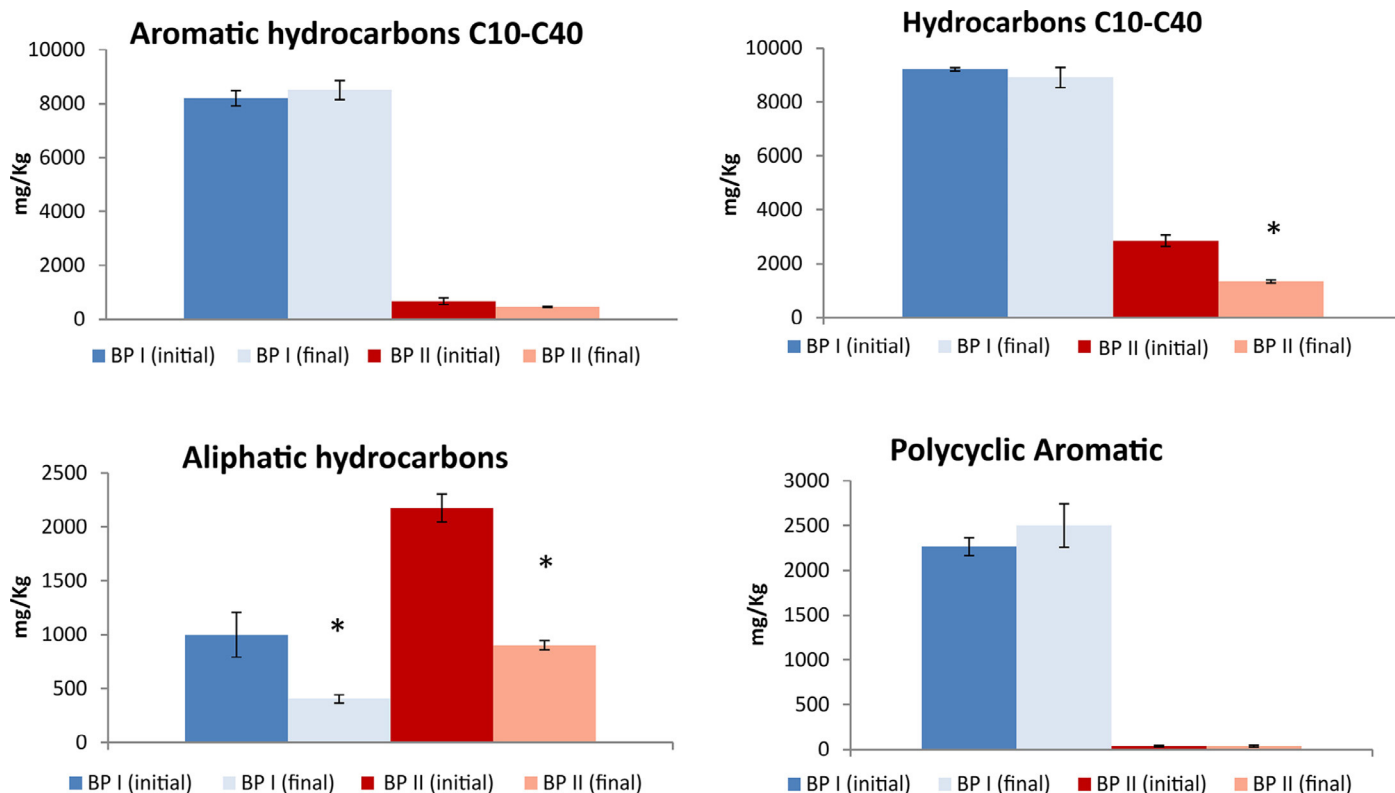


Fig. 4. Evolution of hydrocarbon concentrations (mg/kg) in the biopiles. BP I: Biopile I; BP II: Biopile II. Bars indicate standard error. *Significant differences, n = 3; P < 0.05.

II before mixing with compost. This is probably due to the possibility of interactions of the contaminants with compost that may change the behavior of the metals. Moreover, the immobilization of metals by nZVI may be influenced by other aspects that must be evaluated, as the stability of the immobilization. This fact could not be tested in the framework of this work but it needs to be assessed to improve the decontamination treatment.

3.3. Evolution of hydrocarbons during bioremediation

The effectiveness of decontamination for hydrocarbons was tested by measuring the concentration of total hydrocarbons and their fractions at the start and the end of the bioremediation process, once the biopiles formed. To avoid misinterpretation or biasing of the data because of the dilution effect in biopiles, these concentrations were corrected using the compost-to-soil ratios described above. Fig. 4 represents the evolution of total, aliphatic, aromatic and PAHs in the two biopiles.

As it can be observed, a significant reduction of –53% on average was observed for total hydrocarbons only in BP II. No differences were however observed in BP I for this parameter. However, when the different families of hydrocarbons were studied separately, aliphatic chains showed significant decreases in both biopiles (Table 5). In particular, aliphatic chains in the range C16–C40 displayed reductions close to 50% in BP I and BP II, and shorter chain concentrations were reduced up to 80% in BP II. These results suggest that the combined use of iron nanoparticles and compost bioremediation can be effective in significantly reducing contamination from aliphatic hydrocarbons, especially those with longer chains. The lack of increment of the shorter chains indicates that this reduction was a true decomposition into non-hydrocarbon compounds. The type and fate of these compounds cannot be tested in the framework of this work, but it can be assumed from the type of process that they should include by-products of micro-

Table 5

Percentages of reduction in % over the original soil concentration with observed significant differences (n = 3; P < 0.05)

| | Biopile I | Biopile II |
|--------------------------------|-----------|------------|
| Total hydrocarbons C10–C40 | – | –53% |
| Aliphatic hydrocarbons C10–C12 | – | –80% |
| Aliphatic hydrocarbons C10–C40 | –60% | –59% |
| Aliphatic hydrocarbons C12–C16 | – | –67% |
| Aliphatic hydrocarbons C16–C21 | – | –55% |
| Aliphatic hydrocarbons C16–C35 | –55% | –52% |
| Aliphatic hydrocarbons C21–C35 | –46% | –45% |
| Aliphatic hydrocarbons C35–C40 | –56% | – |
| Aliphatic hydrocarbons C35–C40 | – | –42% |
| Acenaphthylene | – | –58% |

bial degradation, recalcitrant organic compounds associated to the mineral matrix of the soil, and/or CO₂, if their metabolic degradation was complete. The toxicity of these compounds needs to be tested to ensure an effective decontamination (see Section 3.3).

However, aromatic and PAHs remained almost unchanged for both biopiles, except for acenaphthylene in BP II, which showed a reduction of 58% in its concentration (Fig. 5 and Table 5). The probability that the initial and final samples of aromatic hydrocarbons were equal was 54% in BP I, 16% in the BP II and for PAHs it was 43% in BP I and 94% in BP II.

The unsuccessful decontamination in relation to PAHs can be explained from different reasons. PAHs, which are highly widespread in many industrial areas and pose a major concern from the environmental and public health perspectives [4,34], are highly hydrophobic and biochemically recalcitrant. Both characteristics determine their behavior in the environment [35], as microorganisms can only degrade the fraction present in the soil solution [36]. According to Bamforth and Singleton [37] the most significant factors affecting PAH biodegradation are temperature

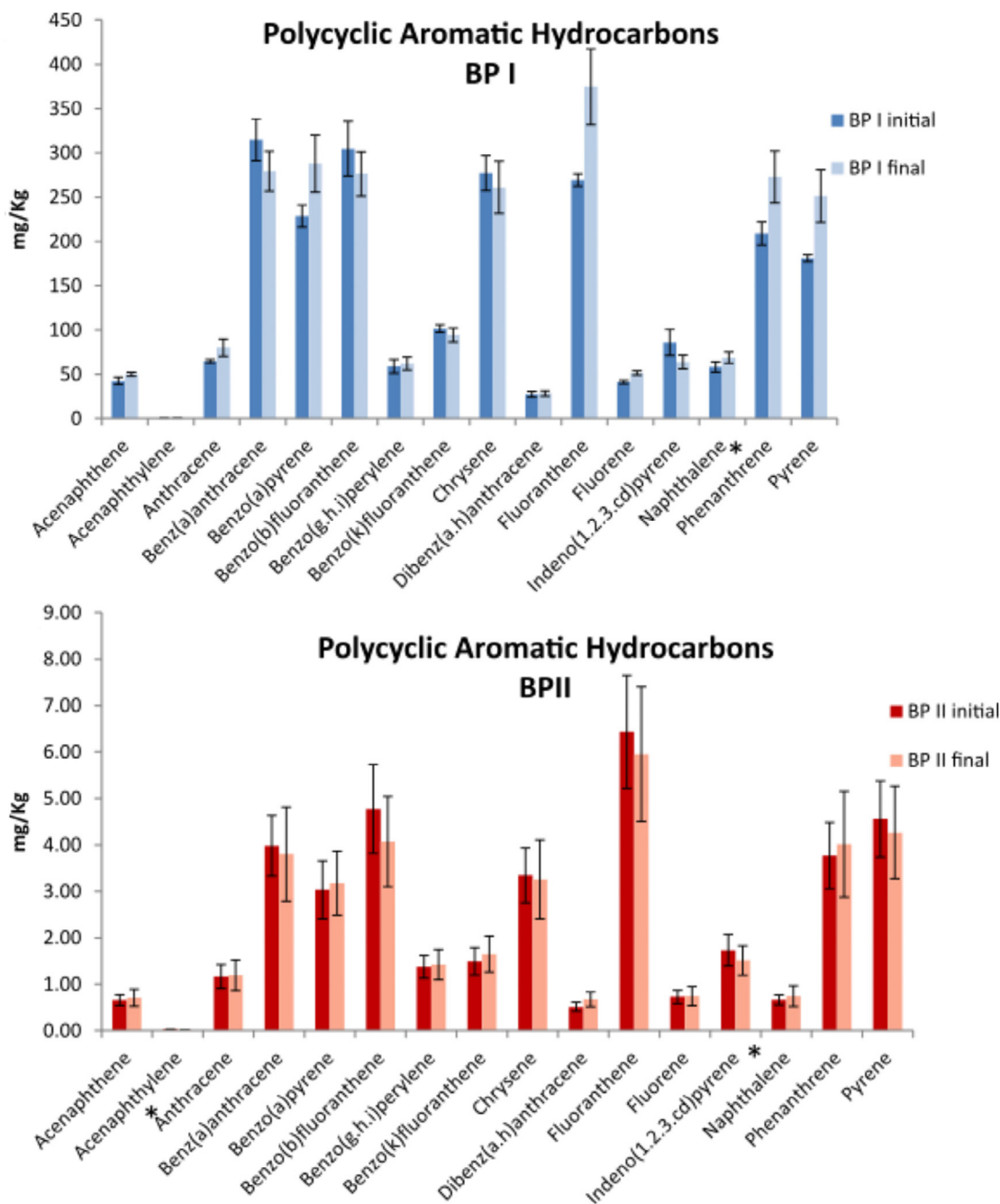


Fig. 5. Evolution of polycyclic aromatic hydrocarbon (PAH) concentrations (mg/kg) in the biopiles. BPI: Biopile I; BPII: Biopile II. Bars indicate standard error. *Significant differences, $n = 3$; $P < 0.05$.

(20–45 °C), soil pH (6–8), oxygen concentration (aerobic conditions required), nutrient availability (C:N:P ratios between 100:15:3 and 120:10:1), moisture conditions (70–100% field capacity), and the own bioavailability of PAHs for microbial activity. Considering that these parameters were kept within non-limiting ranges during the process of bioremediation, and that biodegradation processes of other compounds actually occurred within the biopiles (Table 5), it is likely that the adsorption of PAHs to mineral surfaces, or their occlusion within the organo-mineral matrix of the soil played a role in hindering their biological degradation. Considering the hazardous nature of these compounds, improved bioremediation techniques need to take into account this limitation.

3.4. Toxicity evaluation

According to Spanish law (Royal Decree 9/2005), a contaminated soil is defined when the average lethal or effective concentration LC50 for soil organisms is less than 10 g of contaminated soil/kg of control substrate. Thus, the evolution of ecotoxicity was evaluated by determining LC50 values on the initial contaminated soils (raw contaminated soils) and on the beginning and the end of the treatment in biopiles, i.e. at the beginning of the application of nanoparticles and compost ($t = 0$) and at the end of the treatment ($t = 66$ days). It was observed that initially the two contaminated soils had a moderate toxic nature, since they were below the legal

Table 6

Values of LC50 in the samples of the initial contaminated soils and in the beginning (t=0) and the end (t=66 days) of the treatment in biopiles (BP).

| Sample | BP I (g/kg) | | BP II (g/kg) | |
|----------------------|-------------|--------------|-------------------------------------|-------------------------------------|
| | Earthworm | Seeds growth | Earthworm | Seeds growth |
| Soil | 510 | 596.5 | 460.4 | 877.2 |
| BP initial (t=0) | 319.7 | 874.1 | 618.0 | 936.3 |
| BP final (t=66 days) | 591.2 | 909.0 | Not reached with 100% concentration | Not reached with 100% concentration |

LC 50: in the plant seeds growth test, it is the concentration of the test substance at which the change in emergence is 50% of that of the control. In the earthworm mortality test it is the median lethal concentration, i.e. that concentration of the test substance which kills 50% of the test animals within the test period.

limit of 10 g/kg. However by adding ZVI nanoparticles and compost for the soil treatment in biopiles, samples behaved differently.

Results in Biopile I showed a decreasing toxicity of the mixture for terrestrial plants, mainly reduced at the beginning of the treatment, due to the dilution effect with the compost. During the treatment, toxicity reduction was mild, although it remained at very low values. This low reduction of toxicity could be due to the residual toxicity caused by certain recalcitrant contaminants, whose toxicity was maintained over time. However, an increase in toxicity was observed by adding compost in the test with earthworms, despite the dilution effect of pollutants should have reduced this toxicity. Only a comprehensive assessment of the biochemical processes could provide more accurate information; however, it is possible that this increase in toxicity was caused by these reasons:

- The mixture of the contaminated soil, compost and nZVI drove to an increase of microbiological and biochemical processes, resulting in intermediate compounds of greater toxicity, which led to an increased toxicity in the mix.
- As occurred with the As in Biopile II, the addition of compost could cause an increase in the bioavailability of Pb so that it could enter the metabolic pathways of living organisms more easily than in the initial contaminated soil. Bioavailability is a transcendental in the definition of toxicity of a specific pollutant factor.

During the treatment a further ecotoxicity reduction was observed in the earthworms, reaching final values close to those of the initial soil (Table 6).

In the case of Biopile II, a significant reduction of ecotoxicity was observed throughout the process, not reaching the LC50 even with 100% of the sample after the treatment, in both earthworm and seeds growth tests. This reduction could be possibly related to the decrease in the concentration of aliphatic hydrocarbons.

4. Conclusions

This study shows that the application of nZVI for immobilization of As and Cr in two different soils – Soil I (from a contaminated industrial site) and Soil II (contaminated artificially) – can provide an effective method to reduce the concentration of these analytes in the leachate in successive applications of nZVI, showing a tendency to decrease Cr and As specially in leachate of Soil II. The results obtained are not conclusive because the dispersion of the concentrations of both analytes does not allow an objective analysis of the data.

As observed, the synergistic action of the nanoparticles and compost had as an effect a strong increase of the concentration of As in the leachate, mainly in Biopile II, well above the concentration of this metal found in the leachate of the untreated soil (S initial). The obtained results suggest that the stability of the treatment and the interactions of metals with the components of the two different soils and compost must be evaluated in the future.

On the other hand, the addition of MSW compost and nanoparticles under uncontrolled environmental conditions was able to produce a decrease in the concentration of aliphatic hydrocarbons in the two biopiles up to 60%. Especially degradation and transformation of longer chains occurred. However a significant decrease in the concentrations of aromatic hydrocarbons and PAHs was not achieved in the two assays.

Similarly, a significant reduction of ecotoxicity was observed throughout the process in the biopile II, not reaching the LC50 even with 100% of the sample after the treatment at the end of the experiment, in both earthworm and seeds growth tests. Results in biopile I showed a decreasing toxicity of the biopile for terrestrial plants, mainly reduced at the beginning of the treatment. However, an increase in toxicity was observed by adding compost in the test with earthworms.

In conclusion we believe that the proposed methodology can find a valuable application in the utilization of nZVI and compost as a new strategy for restoring contaminated sites.

Acknowledgments

The authors thank the University of the Basque Country (UPV/EHU) for the technical and human support provided by the Analytical Research Service (SCAB) from SGIKER of the UPV/EHU. The authors gratefully acknowledge Irzagaz partners for trusting and supporting this study. Also, the authors thank administrations that collaborate to carry out this project: CDTi, Gaitek, Spri and Burtzeña Empresa Parkea. The collaboration of C. González in the analytical determinations of soils and composts is also acknowledged.

Appendix. Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.reffit.2017.03.008.

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