Geoderma 317 (2018) 8–14

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Persulfate treatments of phenanthrene-contaminated soil: Effect of the application parameters

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ARTICLE INFO

Handling Editor: Yvan Capowiez

Keywords:
Advanced oxidation processes
Polycyclic aromatic hydrocarbons
Environmental remediation
Pretreatment

ABSTRACT

The aim of this work was to study the effects of the parameters involved in persulfate (PS) application, namely, the cation counterion, mode of persulfate addition and soil-moisture content, to optimize the use of low doses of PS coupled with bioremediation technologies. Soil microcosms were contaminated with 110 ± 20 mg of phenanthrene per kilogram of dry soil. The efficiency of each treatment in phenanthrene elimination was evaluated. Additionally, the impact on cultivable autochthonous heterotrophic bacteria populations was examined.

The addition of sodium persulfate in successive doses (14.9 g/kg_{DS}) showed the greatest efficiency in phenanthrene elimination (36%) after 7 days of treatment, but inhibited further bioremediation. Sodium persulfate was more efficient than ammonium. Increased moisture enhanced the bioremediation but inhibited the oxidative treatment. The persulfate-ion decomposition rate decreased at high moisture levels, possibly through diluting activators within the soil.

The results suggest that, for oxidative treatments of contaminated soil, the application of low doses of sodium PS at low soil-moisture levels is the most effective option for an elimination of phenanthrene that is compatible with bioremediation.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are composed of two or more aromatic rings that are fused together in a linear, angular or cluster arrangement when a pair of carbon atoms is shared between them (Dhote et al., 2010). PAHs are mainly formed during natural and anthropogenic combustion processes of fuels (Samburova et al., 2016). There are sixteen PAHs that are considered as priority pollutants by the United States Environmental Protection Agency (US EPA) and the European Community, due to their toxic, mutagenic, and carcinogenic properties(Schneider et al., 2002; Tobiszewski and Namieśnik, 2012; Usman et al., 2016) and their recalcitrant nature.

A strategy frequently used for the treatment of soils polluted with PAHs is bioremediation through the degradative action of microorganisms because of the low cost and the low impact on the site to be treated. Although accelerated bioremediation processes have been applied (Ward et al., 2003), most of the soil bioremediation processes are relatively slow processes, and frequently it either takes a long time or desired end points may not be achieved due to the lack of suboptimal environmental conditions for selection and growth promotion of the biological system employed (Singh et al., 2009).

Chemical-oxidation technology is a potent option for soil restoration that can effectively eliminate an extensive range of contaminants, including PAHs. In addition, chemical oxidation offers the potential of a complementary remediation strategy that could deliver effective and efficient results while avoiding the principal drawbacks associated with bioremediation alone (Sutton et al., 2011).

In ISCO, inorganic oxidants are injected into the subsurface to degrade organic contaminants into carbon dioxide or other less toxic products (Siegrist et al., 2011). Two common oxidants applied in ISCO are hydrogen peroxide and persulfate, both of which oxidize organic contaminants by generating highly reactive radical species, such as hydroxyl and sulfate radicals (Deng et al., 2014). Hydrogen peroxide decomposes very quickly and can produce substantial heat, which not only induces potential safety problems but also causes the escape of volatile organic compounds. As a result, persulfate has been increasingly used in recent years for the remediation of organic contaminated sites, because persulfate tends to have much longer lifetime in the subsurface and it is safer to handle and more applicable to subsurface than hydrogen peroxide (Tsitonaki et al., 2010).

Persulfate (PS) has several advantages such as high aqueous solubility, high stability at room temperature and relatively low cost

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https://doi.org/10.1016/j.geoderma.2017.12.016
Received 6 July 2017; Received in revised form 6 December 2017; Accepted 12 December 2017
Available online 30 December 2017

(Peluffo et al., 2016). It reacts slowly with many organic compounds (Furman et al., 2010), but can be chemically (Tsitonaki et al., 2010), photochemically (Lin and Lee, 2015; Rosso et al., 1999) or thermally (Mora et al., 2009) activated to generate the more strongly oxidizing sulfate radicals, $SO_4^{\cdot -}$ with a redox potential E° ($SO_4^{\cdot -}$ / SO_4^{2-}) of 2.6 V (Wardman, 1989). These radicals are responsible for the effectiveness of the PS ion in oxidative treatments of soils (Osgerby, 2006). Moreover, since iron is a natural constituent of soil, its addition is not necessary for PS activation (Osgerby, 2006).

The possibility of combining PS application with biologic remediation is an attractive strategy. Although the operational conditions in oxidative treatments will have a significant influence on the results (Lemaire et al., 2013b), the impact of that approach in combination with bioremediation processes requires a thorough understanding of the impact of each step on soil geochemistry, biota, and contaminant dynamics (Sutton et al., 2011).

Discrepant results were obtained depending on the dosage of oxidant—the main parameter studied in the oxidative treatments of soil (Ranc et al., 2016). The general tendency in this approach was to apply high quantities of PS, but they produced a wide range of results. In some instances, the effectiveness of the treatment can increase with the dosage of oxidant (Huang et al., 2005). However, other authors reported that PAH degradation ratios with PS were up to 80% and not directly correlated with the oxidant dose (Lemaire et al., 2013a; Mora et al., 2014).

Nevertheless, the dosage of oxidant poses other problems when it is intended to be used in conjunction with bioremediation. Because the addition of PS causes a decrease in the pH and an increase in the salt content, at a high dose the consequent environmental perturbation would limit the efficiency of bioremediation (Lemaire et al., 2013a). An alternative strategy—the one proposed here—is the addition of PS in a series of low doses to minimize the impact on the autochthonous microbial community.

Another key factor in the application of PS is the nature of the salt employed. Most of the researchers use the persulfate sodium salt as the oxidant because it has higher solubility than the potassium counterion. Ammonium persulfate, however, is quite soluble (58.2 g/100 ml H_2O for the NH_4^+ *versus* 55.6 g/100 ml H_2O for the Na^+ salt at 20 °C) (Osgerby, 2006). It was reported that the addition of nutrients to the soil, in the form of nitrogen, phosphorous and carbon compounds, allows the native microbial population to develop and augment. Thus, such addition is translated into an increase of microorganisms capable of metabolizing the pollutant, therefore enhancing the biodegradation rate (Calvo et al., 2009). Then, the use of ammonium persulfate could increase the amount of available nitrogen in the soil, thus potentially improving the growth of bacterial populations. This possibility was thus investigated in the present work.

Soil hydrocarbon biodegradation may be limited by the water available for microbial growth and metabolism. A decrease in moisture content results in a decrease in microbial activity, while rewetting causes a large and rapid increase in activity (Ayotamuno et al., 2006). Generally, optimum activity occurs when the soil moisture is 50%–80% of the field capacity (Calvo et al., 2009). However, when oxidant is added, most of the treatments tested in the laboratory and carried out in batch reactors have been designed under the assumption that the best option is the application of the oxidant in an aqueous solution at a soil:solution ratio varying between 1:1 (Andreottola et al., 2010) and 1:10 (Usman et al., 2012); even though the rational use of water could be considered as a factor to reduce costs and the environmental impact of the treatment.

Hence, attention should be given to the control of the moisture level in the soil to combine the chemical treatment with the biological one. Accordingly, the present experiments assayed the controlled addition of water up to 44.5% and 65% of the field capacity (24.9% and 36.5% MC, respectively). Moreover, working with this moisture content is more representative of the unsaturated zone of soils (Ranc et al., 2016).

The aim of this work was to study the effects of the parameters involved in PS application (*e.g.*, the cation counterion, mode of PS addition, and soil-moisture content) on PAH degradation and its impact on the cultivable autochthonous heterotrophic bacteria populations in order to optimize an ISCO pre-treatment that could be coupled to bioremediation processes. Moreover, due to the moisture content range studied, the conclusions obtained here could be applied to the non-saturated soil zone that has been much less studied in oxidation treatments.

2. Materials and methods

2.1. Soil characteristics

The uncontaminated soil from an area near the city of La Plata, Argentina (34° 50′ S, 58°1 0′ W) that was selected for the study and analyzed in the Laboratory of Soil Science at the University of La Plata had the following physicochemical properties: a clay loam texture, a pH of 6.6, 4.67% organic carbon (Walkley-Black method), 3.89 mg/kg total nitrogen, 4.0 mg/kg of available phosphorus (Bray Kurtz No. 1 method), an electrical conductivity of 3.3 ± 0.3 dS/m (measured on the saturated-paste extract), and 110 ± 1 ppm of Fe (extracted with EDTA and following the EPA method 7950).

2.2. Soil treatment systems

Soil microcosms consisting of 150 g of sieved soil (2-mm mesh) were placed in a glass container of 250 g capacity. They were contaminated with 110 ± 20 mg of phenanthrene per kilogram of dry soil (kg_{DS}). Phenanthrene was delivered in an acetone solution and mixed manually into the soil with a spatula, as reported (Festa et al., 2016).

The treatments applied are shortly described in Table 1. Persulfate salts were dissolved on the minimum amount of water to deliver into soil, maintaining the desired soil-moisture content. In five treatments, the microcosms were maintained at 24.9% (w/w) soil-moisture content (MC). To each of 2 microcosms sodium PS was added in a single application at 8.6 and 43.0 g/kg_{DS} (NaPS1 and NaPS2, respectively), while in another one the oxidant was introduced in successive additions of 5 g/kg_{DS} (NaPSsucc) each up to a total concentration of 19.3 g/kg_{DS} after 28 days (14.9 g/kg_{DS} after 7 days). In addition, two treatments were prepared with ammonium PS, at 38.4 g/kg_{DS} added either all at once (NH4PS2) or in successive increments of 5 g/kg_{DS} (NH4PSsucc) up to a final concentration of 57.7 g/kg_{DS} after 28 days (15.1 g/kg_{DS} after 7 days). As mentioned above, we tested the utilization of NH_4^+, which would also have the ability to stimulate the intrinsic degradation capacities of autochthonous microbiota (Silva-Castro et al., 2016). It should be mentioned that the molar concentrations of PS in sodium and ammonium salts is affected by their molecular weights (238 and 228, respectively), *e.g.* 5 g of sodium PS corresponds to 0.021 mol while 5 g of ammonium PS corresponds to 0.022 mol.

In two treatments, NaPS3 and NaPS4, sodium PS was applied at

Table 1
Experimental conditions of the persulfate (PS) treatments tested.

Treatment	Application	PS added (g/kg_{DS})	doses	Moisture content (% w/w)
Bio1	none	0	–	24.9
NaPS1	single	8.6	1.8	24.9
NaPS2	single	41.7	8.6	24.9
NaPSsucc	successive	14.9 (at 7 days)	3.1	24.9
NH4PS2	single	38.4	8.3	24.9
NH4PSsucc	successive	15.1 (at 7 days)	3.2	24.9
Bio2	none	0	–	36.5
NaPS3	single	11.1	2.3	36.5
NaPS4	single	55.3	11.4	36.5

11.1 and 55.3 g/kg_{DS}, respectively, keeping the microcosms at a 36.5% MC.

Control microcosms testing bioremediation alone at the two soil-moisture contents (*i.e.*, 24.9 and 36.5% MC) were contaminated with phenanthrene but without oxidant addition. The soil-moisture content was determined along the treatment in order to maintain it at 24.5% and 36.5% MC.

All microcosms were carried out in triplicate trays and incubated under controlled conditions to achieve a simultaneous biologic process with respect to temperature and oxygen availability (closed but not hermetic flasks).

2.3. Physical and chemical analysis

For pH determination, 5 g of soil sample was mixed with 5 ml of ultrapure water in a 15 ml polycarbonate tube, stirred for 5 min, and then left to stand for about an hour (EPA method 9045D) before measuring the pH of the aqueous phase with a glass electrode (Phoenix Electrode Company).

To determine the phenanthrene concentration in the different treatments, 1 g of soil sample was mixed with 10 ml of ethyl acetate in a 15 ml polycarbonate tube. Phenanthrene was extracted by ultrasound in a bath (Testlab Ultrasonic TB04TA, 40 kHz, 160 W) for 60 min (Luque-García and Luque de Castro, 2003; Song et al., 2007). The resulting mixture was then centrifuged at 3000 rpm for 10 min (Presvac model DCS-16 RV) and the solution was filtered (Magna Nylon, Osmonics Inc., pore size 0.45 μm). Of the resulting filtrate, 10 μl was injected into a Hewlett–Packard Model 1050 (Ti series) high-performance liquid chromatograph with multiwavelength detection containing a C18 Restek Pinnacle II column (particle size 5 μm, 2.1 mm i.d., 250 mm). The elution with a mixture of 80/20 (*v*/v) methanol/H_3PO_4 (15 mM) was performed at 0.5 ml/min and constant flux.

2.4. Microbiological analysis

In order to determine total cultivable heterotrophic bacteria, 10 g (wet weight) of soil sample was suspended in 90 ml of 0.85% (v/v) NaCl, agitated for 30 min on a rotary shaker (at 250 rpm), and then decanted for 5–10 min. The total cultivable heterotrophic bacteria were assayed in duplicate after 1/10 serial dilutions. These suspensions were spread on R2A medium plates (Reasoner and Geldreich, 1985) and the resulting colonies were counted after 28 days of incubation at 24 ± 2 °C.

2.5. Spectrophotometric determination of PS concentrations

To study the effect of the moisture content of the system on the rate of PS decomposition, a set of six experiments was performed (*cf.* the experimental conditions in Table 2). Systems A and B were prepared with ammonium PS at 20 g/kg_{DS} and with sodium PS at 20 g/kg_{DS}, respectively, and at a moisture content of 20%. Systems C through G were prepared with sodium PS at 20 g/kg_{DS} and the increasing moisture contents listed in Table 2. All experiments were carried out in triplicate trays. The systems were incubated at 25 °C for 14 days, and the PS concentration was determined periodically. Systems D through G were constantly shaken.

Table 2
Experimental conditions for the determination of persulfate (PS) decomposition.

System	Application	Moisture content (% w/w)
A	ammonium PS	20 ± 1
B	sodium PS	20 ± 1
C	sodium PS	33 ± 1
D[a]	sodium PS	50 ± 1
E[a]	sodium PS	67 ± 1
F[a]	sodium PS	83 ± 1
G[a]	sodium PS	91 ± 1

[a] Incubation with constant shaking.

The PS concentration was determined by a spectrophotometric method. For systems A through C, samples of 4.5 g of soil were mixed with 4.5 ml of H_2O, and the aqueous phase was filtered (Magna Nylon, Osmonics Inc., pore size 0.45 μm). For systems D through G, the measurement was performed on the supernatant. In both procedures, an aliquot of 0.1 ml of the aqueous phase was mixed with 9.4 ml of water and 0.5 ml $NaHCO_3$/KI (0.05 g $NaHCO_3$, 1.00 g KI dissolved in 10.0 ml of ultrapure water). Absorbance at 350 nm was measured in a quartz cuvette (Shimadzu UV-1800 spectrophotometer). The detection limit was 0.05 g/kg_{DS} (Liang et al., 2008a).

2.6. Statistics

All experiments in this study were performed by triplicates. The mean and standard deviations of triplicate independent experiments were calculated. The mean values were compared by parametric two way ANOVA test. All statistical analyses were performed using Microsoft Excel 2010.

3. Results and discussion

3.1. Effects of the parameters involved in PS application

Fig. 1 shows the percent elimination of phenanthrene after 7 days of PS action at 24.9% MC. In NaPS2 microcosm the elimination of phenanthrene was significantly higher ($p < 0.05$) (33 ± 6%) than in NH4PS2 and Bio1 microcosms (phenanthrene degradation of about 20%). Even though microcosms NaPS2 and NH4PS2 received similar additions of oxidant (Table 1), the former proved more efficient, thus demonstrating that the presence of NH_4^+ as the persulfate counterion did not enhance the degradation of phenanthrene, but rather had an inhibitory effect on phenanthrene elimination. The same result was found in the treatments with successive additions of PS, NaPSsucc and NH4PSsucc (Fig. 1). Although in all treatments, the PS anion generated highly reactive sulfate radicals (Osgerby, 2006), the difference between the results in NaPS and NH4PS microcosms could be attributed to the reaction of those radicals with NH_4^+, with the rate constant of that process reported to be $3 \cdot 10^5\ M^{-1}\ s^{-1}$, while sulfate radicals do not react with Na^+ at all (Neta et al., 1988). The effect of NH_4^+ was relevant because their presence, close to the production of the radicals, allows the reaction without involving diffusion processes. Thus, NH_4^+ competed with phenanthrene for reaction with sulfate radicals thus

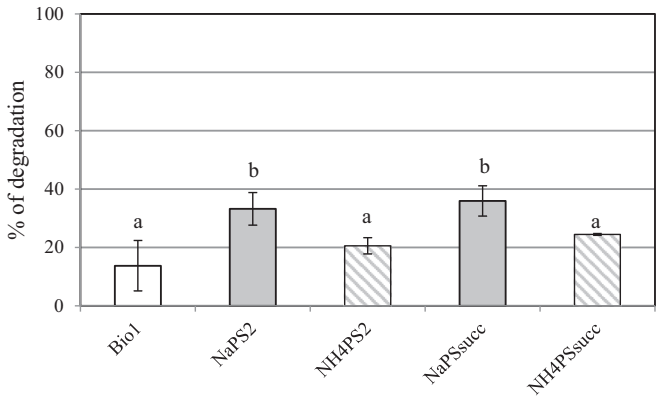

Fig. 1. Percent elimination of phenanthrene after 7 days for persulfate treatments at 24.9% soil-moisture content: Bio1, NaPS2, NH4PS2, NaPSsucc and NH4PSsucc. The results are the average of 3 independent experiments. The bars represent standard deviations. Values followed by the same letter are not significantly different at the 5% level (by the two-way ANOVA, Tukey test) compared with Bio1.

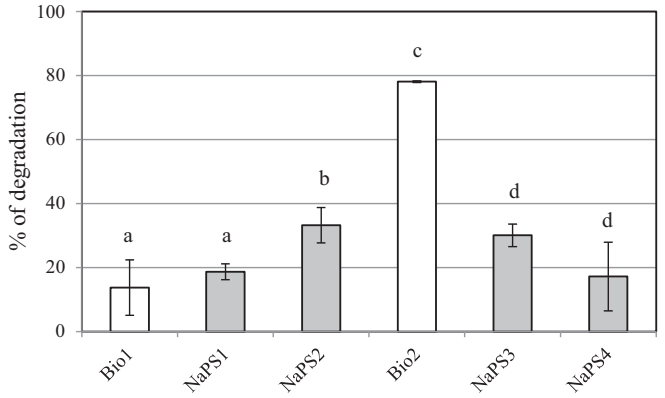

Fig. 2. Percent elimination of phenanthrene after 7 days for persulfate treatments at 24.9 and 36.5% soil-moisture content: Bio1, NaPS1, NaPS2, Bio2, NaPS3, NaPS4. The results are the average of 3 independent experiments. The bars represent standard deviations. Values followed by the same letter are not significantly different at the 5% level (by the two-way ANOVA, Tukey test) compared with Bio1 and Bio2.

reducing the effectiveness of the PS treatment.

NaPSsucc proved to be the most efficient treatment of the soil at 7 days (Fig. 1) because it had a similar percentage of degradation of phenanthrene to that of NaPS2. Here we have to emphasize that NaPSsucc exhibited the same degradation of phenanthrene using about a third of the amount required by NaPS2. This result highlights the lack of correlation between the dosage of oxidant and the efficiency on pollutant degradation, due to the nature of free radical reactions (Lemaire et al., 2013a; Mora et al., 2014). Sulfate radicals -main responsible on these oxidative treatments- are able to quickly react with a wide range of compounds, including soil organic matter (Bosio et al., 2008; David Gara et al., 2008). The soil used on this study has a quite high content of organic matter (4.67% organic carbon) and then an important outflow of sulfate radical by secondary reactions is expected.

As shown in Fig. 2, significant differences ($p < 0.05$) in the degradation of phenanthrene were found between Bio1 (13 ± 4%) and Bio2 (78.1 ± 0.2%)—with those microcosms being at 24.9 and 36.5% MC, respectively (Fig. 2). Holman and Tsang (1995) determined that a water content of 50%–70% field capacity (that is, 28%–39% MC for the soil used here) was optimum for the biodegradation of aromatic hydrocarbons at a maximum rate. This observation is in agreement with the results of these experiments, where Bio2 (at 36.5% MC) exhibited a higher biodegradation percentage of phenanthrene than Bio1 (24.9% MC), both in the absence of chemical oxidants.

Nevertheless, the treatments NaPS3 and NaPS4 (addition of sodium PS at the highest moisture content) were less efficient in the degradation of phenanthrene than Bio2 while NaPS1 and NaPS2 (similar additions of sodium PS but at lower moisture content) were better than Bio1. Moreover, the four experimental interventions resulted in different concentrations of remaining PS (Table 3). After 2 and 7 days of treatment, the PS concentration measured in the microcosms at MC of 36.5% (NaPS3 and NaPS4) was significantly higher than in the equivalent environments at MC of 24.9% (NaPS1 and NaPS2; Table 3). This difference would indicate that in the microcosms at MC of 36.5%, the PS activation was diminished by the presence of higher amounts of water. However, the treatments with higher amount of PS (NaPS2 and NaPS4) did not present significant differences between them (by anova analysis), possibly do to the inhibition of biological processes. On the other hand, NaPS3 was more efficient than NaPS1, showing that for lower oxidant amount, the biological process seems to be improved by the increment on MC.

A recent review (Ranc et al., 2016) analyzed fifty-three papers published since 2000 on oxidation of PAH-contaminated soils with permanganate, hydrogen peroxide, persulfate and ozone. They found out that oxidation is mostly performed by batch experiments and column experiments are fewer. No comments about unsaturated treatments -like ours- were done. They consider than the main key soil parameters to be considered for oxidation studies in case of artificial contaminations are soil organic matter content and initial and final concentrations of each targeted PAH. For artificially spiked soils, they informed values for PAH degradation from 25% to 90% depending on the amount of PS used (always higher than 5 doses) and the experimental conditions. The levels of phenanthrene degradation reached in our work seem to be low, but they are consistent with the moderate amount of oxidant and the high organic matter content of the soil.

Table 3
Initial PS concentrations and after 2 and 7 days of treatment.

Treatment	PS added (g/Kg_{DS})	PS (g/Kg_{DS}) after 2 days	PS (g/Kg_{DS}) after 7 days
NaPS1	8.61	0.14	< 0.12
NaPS2	41.7	1.13	< 0.70
NaPS3	11.1	3.43	0.59
NaPS4	55.3	12.8	4.0

3.2. Activation of the persulfate anion

The persulfate interaction with soil compounds can affect treatment efficiency by either improving activation or by diminishing its persistence (Oliveira et al., 2016). Oliveira and coworkers studied the interaction between persulfate and three different soils, in a series of batch tests. They found persulfate half-life between 5 and 80 days, depending on experimental conditions and soil characteristics (Oliveira et al., 2016).

We performed a systematic study of the rate of decomposition of the PS anion as a function of the percentage of water in the soil. The decrease in PS concentration with time for the systems A, B, E, and F listed in Table 2 is plotted in the inset of Fig. 3: the decomposition of ammonium PS (System A) was faster than that of sodium PS (System B) at the same soil-moisture content (20%), while the decrease in the sodium salt was progressively slower at the higher soil-moisture levels of 67% (System E) and 83% (System F).

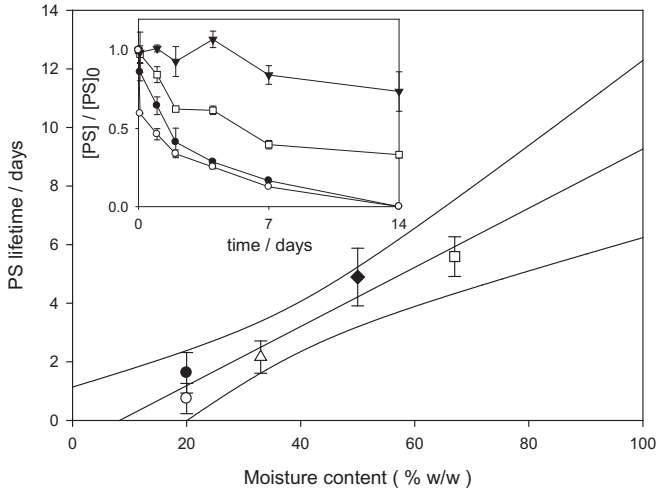

Fig. 3. Half-life of the persulfate anion as a function of the percent moisture content of the soil. In the figure, the half-life of persulfate decomposition on the *ordinate* is plotted as a function of the percent soil-moisture content on the *abscissa*. The solid least-squares-regression line is shown among the data points along with the dashed confidence-interval curves. *Inset:* the decay curve for persulfate decomposition on the *ordinate* is plotted as a function of time in days on the *abscissa*. Key to symbols: ammonium persulfate at 20% soil-moisture content (○) and with sodium persulfate at 20% (●), 33% (△), 50% (◆), 67% (□), and 83% (▼) soil-moisture content. The results are the average of three independent experiments, and the bars represent the standard deviations.

The half-life of PS for each experiment was determined by interpolation: from each PS decay curve (*cf.* the inset to Fig. 3), at the point of 50% decomposition of PS a perpendicular was dropped to determine the intersection with the *abscissa* corresponding to the half time.

The half-life for sodium PS decomposition increased with the moisture content (Fig. 3). The linear regression fitted to the data points gave an intercept of - 0.9 ± 0.8 and a slope of 0.10 ± 0.02 with $r^2 = 0.97$. Systems F and G were not included in the calculation because the PS decomposition after 14 days remained < 50% of the initial values.

Assuming that all the PS added to the system remained in the aqueous phase, we prepared a series of aqueous solutions (with neat water, without organic matter) with the initial concentrations of PS corresponding to those of systems A through G (data not shown). Only 5% of the PS became decomposed in 14 days, regardless of the initial PS concentration. In our experiments with soil, when the percent MC increases, the behavior of the systems tends to be similar to that observed for those aqueous solutions.

Therefore, the differences between the systems depicted in Fig. 2 could be explained by the presence of activators of PS in the soil. Depending on the addition of water (*i.e.*, the percent MC), the concentration of activators in the aqueous phase could decrease, thus blunting their effect.

A variety of metals -such as iron, copper, silver, manganese, cerium, and cobalt- could function as activators of PS decomposition (Liang et al., 2004). The most common activator for PS treatments is iron (Fe^{2+}, Fe^{3+}), which is always present in the soil (in these soils, at 110 ± 1 ppm upon extraction with EDTA). To explore this possibility, the concentration of Fe was determined directly in the aqueous phases of systems D through G without extraction. Accordingly, the aqueous phase was filtered off and injected directly into the spectrophotometer. For all the samples, the amount of Fe was constant, at a concentration of 0.2 ± 0.1 ppm. This value indicates that the presence of Fe in the aqueous phase was not responsible for the different results observed in our experiments.

The mineral-mediated decomposition of PS and the generation of oxidants were studied with four oxides of iron and manganese, two clay minerals, and natural soil (Ahmad et al., 2010).The results of this research demonstrated that synthetic iron and manganese oxides can activate PS to generate oxidants. The efficiency of iron and manganese oxides in the natural soil, however, has a strong dependence on the amount of those oxides present (Ahmad et al., 2010).

Although we were not able to identify the activator of PS decomposition, the behavior observed was consistent with the publication of Ferreira and coworkers. They reported that natural activation of PS is dependent on several factors, such as the type and quantity of minerals present, degree of crystallinity, release of soluble metals into the aqueous phase, and the phenolic moieties of the soil organic matter (Ferreira et al., 2017). In our experiments the amounts of soil and PS were maintained, with different volumes of water being added, thus causing an overall dilution of the system, including the aforementioned oxides.

3.3. Impact on bacterial population by PS application

The residual amount of PS on soil was lower than 8% of the initial addition after a week of treatment (see Table 3). Therefore, later degradation could not be the direct oxidation of the contaminant but a biological one.

A decrease in the initial soil pH was observed in all the microcosms after the addition of PS (Table 4), as reported by others researchers (Huang et al., 2005; Mora et al., 2014; Tsitonaki et al., 2008). Nevertheless, the soil buffering capacity allowed only moderate variations in the pH values in most of the microcosms, with these dropping less than might have been expected (Liang et al., 2008b). The amount of PS added to microcosms NaPS2, NaPS4, and NH4PS2, however, surpassed this capacity, resulting in extremely acid pH values throughout the entire experiment. A low pH can constitute a significant environmental perturbation that would limit the survival and activity of the soil microorganisms and as a consequence, the intended biodegradation (Mora et al., 2014). The application of PS in successive small additions (NaPSsucc and NH4PSsucc) did not diminish the acidification of the systems.

Table 4
Initial pH and pH, number of cultivable heterotrophic bacteria and percent elimination of phenanthrene after 28 days of incubation.

Treatment	Initial	After 28 days		
	pH	pH	Log of CFU/g_{DS}	% elimination of phenanthrene
Bio1	7.0 ± 0.1[a]	6.0 ± 0.1	8.4 ± 0.6	78 ± 4
NaPS1	4.6 ± 0.1[b]	5.2 ± 0.1	9.2 ± 0.7	84 ± 2
NaPS2	2.7 ± 0.1[b]	2.9 ± 0.1	5.6 ± 0.1	34 ± 5
NaPSsucc	4.6 ± 0.1[c]	3.2 ± 0.1	5.1 ± 0.2	36 ± 9
NH4PS2	2.6 ± 0.1[b]	2.9 ± 0.1	4.8 ± 0.2	16 ± 7
NH4PSsucc	4.4 ± 0.1[c]	2.6 ± 0.1	5.1 ± 0.1	38 ± 7
Bio2	6.9 ± 0.1[a]	5.8 ± 0.1	8.3 ± 0.2	94 ± 6
NaPS3	4.4 ± 0.1[b]	4.7 ± 0.1	7.6 ± 0.2	94 ± 1
NaPS4	3.5 ± 0.1[b]	2.6 ± 0.1	5.4 ± 0.2	55 ± 12

[a] Without PS.
[b] One day after PS addition.
[c] One day after the first PS addition.

The number of cultivable heterotrophic bacteria was determined in all microcosms after 28 days of treatment. The microcosms with the lowest PS concentration, NaPS1 and NaPS3, supported levels of cultivable heterotrophic bacteria comparable to those of the respective bioremediation controls; whereas the bacterial survival in the other microcosms was more than two orders of magnitude lower (Table 4). These results would suggest that the soil acidity of these latter microcosms might be sufficient to limit biodegradation. Thus, the final values of pH and of cultivable heterotrophic bacteria are the main parameters to determine the impact of PS applications on the biota.

The percent elimination of phenanthrene after 28 days of incubation correlated with the number of cultivable heterotrophic bacteria, as shown in Table 4.

At the end of the treatment, phenanthrene degradation was similar for both microcosms without oxidant, Bio1 and Bio2, although they showed significant differences after a week (see Fig. 2). In treatments NaPS1 and NaPS3 (with a lower amount of PS), the elimination of phenanthrene was not statistically different from that of Bio1 and Bio2 respectively, according to the slight modification in pH values and in the number of cultivable heterotrophic bacteria.

Moreover, the addition of NH_4^+ did not improve the number of cultivable heterotrophic bacteria at the end of incubation. This observation indicates that the enhancement of bioremediation by NH_4^+ addition was not relevant under these experimental conditions.

The use of PS at a moderate dose was efficient for the elimination of phenanthrene at short time but inhibited further bioremediation. Martinez-Pascual and coworkers reported that the microbial populations were initially negatively affected by the use of oxidants, but a population rebound was observed at a late stage (Martínez-Pascual et al., 2015). Then, subsequent steps involving natural attenuation could be possible at longer time. Silva-Castro and coworkers informed that the addition of fertilizer, as post-treatment after oxidation, produced a rapid increase in heterotrophic microbiota (Silva-Castro et al., 2013). The impact on soil properties by PS could be overcome by various bioremediation strategies such as stimulation by organic or inorganic amendments or composting (Ranc et al., 2016). Thus, further biological treatments could be applied to overcome the PS effect on soil properties.

4. Conclusions

Our results suggest that, for oxidative treatments of contaminated soil, the application of low doses of sodium PS in the presence of the low soil-moisture levels is the most effective option for an elimination of phenanthrene that is compatible with bioremediation.

Moreover, the conditioning of the contaminated soil by incubation, airing, and by slightly increasing the soil-moisture content seems to be the best strategy because it has good results at short and long terms, with the minimum cost. This one emphasized the importance of carefully selection of the adequate treatment conditions based on the particularities of the polluted site: the addition of some water to reach the "Bio2" conditions could be more convenient than the addition of a more expensive reagent as PS, if polluted soil is treated or pollution is in the unsaturated zone.

Acknowledgements

This work was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 2013–2015, 112-20120100112CO) and Proyecto de Investigación Orientado CONICET-Fundación YPF. Mora V. C. and Rosso J. A. are research members of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina), and Morelli I. S. is research member of CIC-PBA (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina). Peluffo M. is doctoral fellow of CONICET.

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