TREATMENT OF Pb – NAPHTHALENE CO – CONTAMINATION IN SOILS BY ZERO VALENT IRON NANOPARTICLES (NZVI) AND BIOREMEDIATION

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Abstract: The co-contamination of soils with heavy metals and organic contaminants is a problem that requires a different and innovative approach to remediate these kinds of sites. The different nature of these two contaminant groups makes the problem complicated. Recently, zero-valent iron nanoparticles (nZVI) application has been integrated with bioremediation to be used for the remediation of halogenated organics. However, the use of this process for the remediation of co-contaminated soils has not been investigated. In this study, nZVI application integrated with bioremediation approaches (bioattenuation and biostimulation) was applied to Pb and naphthalene spiked soils in laboratory-scale reactors. Soil Pb fractions and naphthalene concentrations were monitored for 90 days. The nZVI decreased the mobility and bioavailability of Pb significantly by reducing exchangeable and carbonate bound fractions from 66.2 % to 25.8 – 37.2 % range. The nZVI also caused naphthalene degradation in the range of 38.8 – 58.5 %. Significant biodegradation of naphthalene occurred at samples subjected to nZVI and biostimulation. The highest overall naphthalene degradation (76.7 %) was obtained from the nZVI application which was integrated with biostimulation.

Keywords: Nano zero-valent iron, heavy metal, polyaromatic hydrocarbon, soil, Pb, naphthalene

Pb ve Naftalen ile Kirlenmiş Toprakların Sıfır Değerlikli Demir Nanotanecikleri (SDDN) ve Biyolojik Arıtımla İyileştirilmesi

Öz: Ağır metal ve organik kirleticilerin bir arada bulunduğu kirlenmiş sahaların arıtımı farklı ve yenilikçi arıtım yaklaşımlarının uygulanmasını zorunlu kılmaktadır. Bu iki kirletici grubunun birbirinden farklı özellikleri sorunun çözümünü zorlaştırmaktadır. Son dönemde, sıfır değerlikli demir nanotanecikleri (SDDN) ve biyolojik arıtımın beraber kullanıldığı teknolojiler halojenli organik kirleticilerin giderimi üzerine uygulanmıştır. Ancak bu prosesin ağır metal ve organik kirleticilerin bir arada bulunduğu toprakların arıtımında kullanımı incelenmemiştir. Bu çalışmada SDDN uygulaması, biyolojik arıtımın dışarıdan müdahale olmadan (bioattenuation) ve nütrient ilavesi ile gerçekleştirilmesi (biostimulation) stratejileriyle beraber Pb ve naftalen ile kirletilen topraklara laboratuvar ölçekli reaktörlerde uygulanmıştır. Toprak Pb fraksiyonları ve naftalen konsantrasyonu 90 gün boyunca periyodik analizlerle takip edilmiştir. SDDN uygulaması, Pb'nin değişebilir ve karbonatlara bağlı fraksiyonlarının toplamda 66,2 % olan oranını, 25,8 – 37,2 % bandına indirerek, metalin hareketliliğini ve biyoelverişliliğini önemli ölçüde düşürmüştür. SDDN uygulaması ayrıca 38,8 – 58,5 % aralığında naftalen giderimi sağlamıştır. SDDN uygulaması nütrient ilavesi ile gerçekleştirildiğinde (biostimulation) anlamlı düzeyde biyolojik naftalen giderimi gerçekleştiği görülmüştür. En yüksek genel naftalen giderim oranı (76,7 %) SDDN uygulaması ile nütrient ilavesinin birlikte uygulanmasıyla elde edilmiştir.

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1. INTRODUCTION

Soil contamination has become one of the most serious environmental problems nowadays especially due to the increase of industrialization. Co-contaminated sites where heavy metals and organic contaminants coexist are an important component of this problem which urgently needs to be solved worldwide (Dong et al., 2013). Conventional soil remediation methods are often insufficient in the treatment of co-contaminated sites. The fact that the physical and chemical structures of the contaminants are quite different from each other makes the treatment of such sites difficult. Heavy metals are mostly water/acid-soluble, usually mobile in soil and groundwater, and integrated to soil Fe-Mn oxides and organic matter. On the contrary, most of the organic pollutants are hydrophobic, easily integrated with the organic substances in the soil and, difficult to treat (Cang et al., 2013). Another factor that complicates the treatment of co-contaminated sites is that heavy metals inhibit the biodegradation processes of organic pollutants (Al-Saleh and Obuekwe, 2005; Sneath et al., 2013; Sprocati et al., 2012). Nevertheless, it has become a necessity to develop appropriate remediation technologies for sites co-contaminated with heavy metals and organic contaminants (Perez et al., 2010).

One of the innovative remediation technologies available for complicated contamination scenarios is zero-valent iron nanoparticles (nZVI). It is typically Fe^0 particles with diameters less than 100 nm. In aqueous solutions, it reacts with water and oxygen to form an iron (hydro)oxide shell on its outer surface. Therefore, it consists of a core-shell structure (O'Carrol et al., 2013). Due to its high reducing power and adsorption capacity, this nanomaterial can be used on a wide range of contaminants including heavy metals and halogenated hydrocarbons (Pazinski and Krebz, 2020). In recent years, the integration of nZVI application with other physical, chemical or, biological remediation methods attracts attention. For example, bioremediation or phytoremediation was combined with nZVI application for the remediation of halogenated organics (Jiang et al., 2018). Bioremediation can be defined as a process in which organic contaminants are degraded by microorganisms for their energy or nutrient needs. There are different approaches of bioremediation such as bioattenuation and biostimulation. The natural degradation potential of the microbial community present in the media is utilized in the bioattenuation approach. In the biostimulation approach, necessary nutrients are supplied in order to increase the rate and efficiency of biodegradation (Bose et al., 2021).

The main remediation strategy for heavy metal contaminated soils is the immobilization of heavy metals by mechanisms such as reduction, adsorption or, precipitation. Therefore nZVI is a suitable material for the immobilization of heavy metals in the soil environment. On the other hand, immobilization decreases the bioavailability of heavy metals and thus alleviates the toxic effects of heavy metals on microorganisms (Zhao et al., 2021). This is important considering that a wide range of organic contaminants are readily biodegradable and bioremediation methods utilize mostly microorganisms. Thus, the integration of nZVI technology and different bioremediation approaches might be a promising option for the remediation of heavy metal and organic contaminants.

In this lab-scale study, soils co-contaminated with a heavy metal (Pb) and an organic contaminant (naphthalene) were remediated with an integrated approach using nZVI application and bioremediation subsequently. Bioattenuation and biostimulation approaches were combined separately with the nZVI application. Nitrogen and phosphorus were added as the necessary nutrients to improve biodegradation in the biostimulation step. The immobilization of Pb was monitored using soil Pb fractions analyses and the degradation of naphthalene was quantified using gas chromatography.

2. MATERIALS AND METHODS

2.1. Materials

The soil samples were collected from an arable site on the campus of Zonguldak Bülent Ecevit University Çaycuma Vocational School. This sampling site has not been used for agricultural or industrial purposes. The collected soil was air-dried in the laboratory, mixed and homogenized, passed through a 2 mm sieve, and then stored in plastic bags at room temperature to be used in the experiments. The soil consists of 87.5 % sand, 9.2 % silt and, 3.3 % clay. It is a slightly alkaline soil with a pH of 8.1 and has a low organic matter content (0.72 mg g^{-1} dry soil). A commercial nZVI product (NanoferStar, Nanoiron, Czechia) was used in the experiments. The average particle size and specific surface area of the nZVI were 50 nm and > 25 m^2 g^{-1}, respectively, according to the manufacturer.

2.2. Preparation of Reactor Sets

The soil samples were spiked with $(CH_3COO)_2Pb \cdot 3H_2O$ (Merck, Germany) and, naphthalene (Acros Organics, Czechia) solutions to prepare the contaminated soil samples. Firstly, heavy metal-containing solutions providing the desired pollutant concentration (500 mg kg^{-1} Pb) were mixed with the soil samples to ensure homogeneous distribution and incubated for one month. After this process, naphthalene was dissolved in acetone in an amount to provide a pollutant concentration of 200 mg kg^{-1} and mixed homogeneously with the soil. In order to keep the negative effects of acetone on the soil microorganism population to a minimum, the solution was first mixed with one-fifth of the total soil amount, then the soil was aerated and then mixed with the remaining soil. Sterile control samples were autoclaved (Nüve Steamart OT32V) before the spiking procedure to monitor abiotic effects during the experiments. Reactor sets were prepared as two separate groups. The first group was established to monitor the remediation performance of nZVI application and bioattenuation (Table 1). The second group was prepared to monitor the remediation performance of nZVI application and biostimulation (Table 2).

Table 1. Reactors prepared for nZVI+bioattenuation treatment

Reactor name	Variables			
	Pb	Naphthalene	nZVI	Microorganism
Pb_sterile	+	-	-	-
pah_sterile	-	+	-	-
Pb_pah_sterile	+	+	-	-
Pb_pah	+	+	-	+
Pb_pah_nzvi_sterile	+	+	+	-
Pb_pah_nzvi	+	+	+	+

The spiked soil samples were handled according to the experimental plan shown in Table 1 and Table 2. Then, 100 g of soil samples were placed in 250 ml standard beakers which were used as reactors. The nZVI application was carried out at 25 °C at a concentration of 0.01 g g^{-1} dry soil and a reaction time of 72 hours. The reactors were kept in an oven at constant temperature and in the dark until the end of the reaction period. At the end of the reaction period, soil Pb fractions and naphthalene analyzes were carried out. After the nZVI application, the bioremediation phase was started. At this point, nutrient addition was made to the samples in the reactors selected for biostimulation treatment to increase the activity of the microorganism population. $(NH_4)_2SO_4$ was added as a nitrogen source with a C:N ratio of 10:1 and K_2HPO_4 was added as a source of phosphorus with a C:P ratio of 100:1. Then, the reactors were

incubated for 3 months under the same conditions. During the incubation, naphthalene concentrations were analyzed every 15 days. To minimize evaporation losses, especially for naphthalene content, the beakers were closed with three layers of parafilm during the incubation period. The moisture content of the soil samples was kept constant at 50 % of the water holding capacity of the soil. On sampling days, the necessary amount of water was added to the samples with reduced moisture content to keep the soil moisture contents at an equal level. All of the soil samples and reactors were prepared in triplicate and the results of the analyses were given as averages of triplicate samples.

Table 2. Reactors prepared for nZVI+biostimulation treatment

Reactor name	Variables				
	Pb	Naphthalene	nZVI	Microorganism	Nutrient
Pb_pah_sterile_n	+	+	-	-	+
Pb_pah_n	+	+	-	+	+
Pb_pah_nzvi_sterile_n	+	+	+	-	+
Pb_pah_nzvi_n	+	+	+	+	+

2.3. Application of the nZVI

In order to increase the reactivity of the nZVI product and obtain the maximum remediation efficiency, the nZVI was subjected to an activation procedure provided by the manufacturer before soil application. In the first stage of the activation process, the nZVI product which is in powder form was mixed with deionized water with an nZVI ratio of 20%. Mixing was continued for 10 minutes using a kitchen mixer (Vestel Mix&Go Blender, 300 Watt). After the mixing, the suspension was incubated in a closed environment at room temperature for 48 hours. Then, the nZVI suspension was added to the soil samples at a concentration of 0.01 g nZVI g^{-1} dry soil and mixed to ensure homogeneous distribution.

2.4. Analytical Methods

Total Pb in the soil was extracted by following EPA3051A procedure. Briefly, an acid digestion solution (HNO$_3$:HCl 3:1, v/v) was added to one gram of the soil samples. Then, the soils were subjected to an extraction program (175 C$^°$ 10 min, 100 C$^°$ 10 min) in a microwave oven (Speedwave, Berghof, Germany). The Pb concentration of the supernatant was determined using a Flame Atomic Absorption Spectrophotometer (FAAS).

The soil Pb fractions were determined by a modified version of Tessier's sequential extraction procedure (Gasparatos et al., 2015; Yin et al., 2016). In this method, the soil is subjected to a series of extraction solutions and the Pb content of each fraction is analyzed in each extraction step. The extraction solutions and procedures at each extraction step were provided in Table 3. The Pb concentrations in the supernatants obtained from the extraction procedure were determined by FAAS.

Table 3. The sequential extraction steps for soil Pb fractions analysis (Gasparatos et al., 2015; Yin et al., 2016)

Fraction	Solution	Procedure
Exchangeable (F1)	8 ml of 1 M MgCl$_2$	1 h agitation
Carbonate-bound (F2)	8 ml of 1 M CH$_3$COONa (pH=5 with CH$_3$COOH)	5 h agitation
Fe-Mn oxides-bound (F3)	20 ml 0.04 M NH$_2$OH·HCl	İntermittent agitation at 96±3 °C for 6 h
Organic matter-bound (F4)	3 ml 0.02 M HNO$_3$ and 5 ml % 30 H$_2$O$_2$ (v/v) (pH=2 with HNO$_3$)	Agitation at 85±2 °C for 2 h
	3 ml % 30 H$_2$O$_2$ (added on previous solution)	Agitation at 85±2 °C for 3 h
	5 ml 3.2 M CH$_3$COONH$_4$ (added on previous solution)	Agitation for 30 min
Residual (F5)	Calculated by: Total Pb – (F1+F2+F3+F4)	

USEPA 3550C ultrasonic extraction method was used for the extraction of naphthalene content in soil samples. 1 g of soil sample was mixed with 1 g of Na$_2$SO$_4$ (Merck, Germany) and 10 mL of hexane (Merck, Germany) and sonicated in an ultrasonic bath (Daihan WiseClean WUC-D06H) at 25 °C for 10 min. Then, the solvent was separated from the soil by centrifugation (Nuve NF200, Turkey) for 10 minutes at 2000 rpm. The solvent volume was reduced to approximately 1 ml by evaporation with nitrogen gas. The cleanup step was based on the USEPA Method 3610B. Briefly, glass wool was placed on the bottom of the 100 mm x 10 mm chromatography column, 10 g aluminum oxide (0.063 – 0.2 mm, Merck, Germany) was suspended with the solvent to be used and poured into the column. After allowing the alumina to settle in the column, the solvent was drained, and 1 g of Na$_2$SO$_4$ was added to the column. Finally, another 20 mL of solvent was passed through the column to allow the filling materials to settle. After the preparation of the column, the concentrated solvent obtained from the extraction process is introduced to the column and 10 ml of acetone (Sigma Aldrich, Germany) was passed through the column. The solvent passed through the column was evaporated with nitrogen gas to 1 ml and transferred to vials for gas chromatography analysis. Gas chromatography analyzes were performed by the Thermo Scientific Trace GC Ultra Gas Chromatograph instrument. The column used was a TR-5MS (30 m x 0.25 mm ID x 0.25 μm film thickness) and the detector was a Flame Ionization Detector (FID). Calibration with the external standard method was established using naphthalene standard calibration solution (AS-E0053 Naphthalene 1000 μg/ml, AccuStandard, USA). The calibration range was selected as 1 – 100 ppm and 5-point calibration was created.

3. RESULTS AND DISCUSSION

3.1. Immobilization of Pb by the nZVI

The results of the soil Pb fractions analyses were given in Figure 1. The initial total Pb content of the soil before it was used in the experiments, which was measured as 7.9 ppm, consisted of approximately 45% F3 and 55% F5 fractions (data not shown). The mobility and bioavailability of metals in the soil vary according to the distribution of the metal fractions (Tessier et al., 1979). Soil metal fractions can be sorted according to their decreasing mobility and availability as follows: exchangeable (F1) > bound to carbonates (F2) > bound to Fe-Mn oxides (F3) > bound to organic matter (F4) > residue fraction (F5) (Gil-Diaz et al., 2014). In this context, when the results of the Pb fractions analyses were examined, it was seen that the Pb content entering the soil after the Pb spiking was highly distributed to the F2 (64.9 %) and F3 (27.8 %) (Control sample in Fig. 1). While the F1 fraction, the fraction with the highest mobility, had a percentage of 1,3%; the F4 and F5 fractions with the lowest mobility were dispersed at ratios of 0.6% and 5.3%, respectively. Thus, it was inferred that the soil Pb content before the nZVI application consisted of a total of 66.2 % (342.1 ppm Pb) of high mobility F1 and F2 fractions.
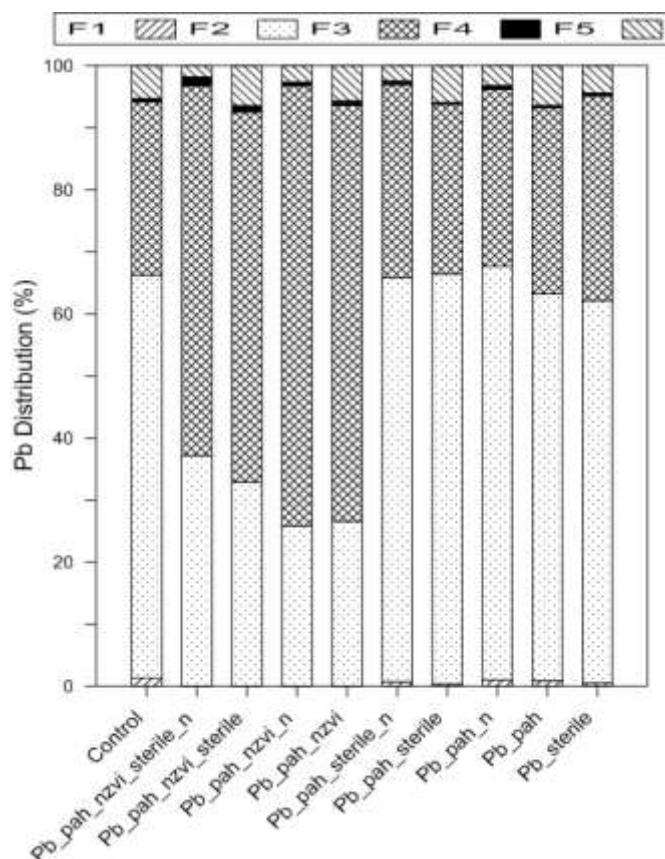

Figure 1:
Distribution of the soil Pb fractions in soil samples(F1:Exchangeable, F2: Carbonate-bound, F3: Fe-Mn oxides-bound, F4:Organic matter bound , F5: Residual)

After the nZVI application, it is seen in Figure 1 that there were drastic changes in Pb fractions in all soil samples which were subjected to the nZVI (Pb_pah_nzvi_sterile_n, Pb_pah_nzvi_sterile, Pb_pah_nzvi_n and, Pb_pah_nzvi). On the other hand, there were no

significant changes in the control reactors without nZVI. The Pb content which was initially found as the F1 fraction in the soil, completely dispersed to the other fractions after the nZVI application. In the F2 fraction, the values decreased from 64.9 % to 37.2%, 32.9%, 25.8% and 26.5%, respectively (Pb_pah_nzvi_sterile_n, Pb__nzvi_sterile, Pb_pah_nzvi_n and Pb_pah_nzvi). In the F3 fraction, the values increased from 27.8% to 59.6%, 59.7%, 71.0% and 67.0%, respectively (Pb_pah_nzvi_sterile_n, Pb_pah_nzvi_sterile, Pb_pah_nzvi_n and Pb_pah_nzvi). There were no significant changes in the F4 fraction. In the F5 fraction, there were minor increases in only two reactors (Pb_pah_nzvi_sterile and Pb_pah_nzvi). These results showed that the exchangeable and carbonate-bound fractions with high mobility decreased and Fe-Mn oxides-bound fraction with lower mobility increased. Considering this situation, it can be said that the mobility and availability of Pb content were reduced by the nZVI application. These results were in parallel with the results reported by Gil-Diaz et al. (2014) who carried out immobilization experiments with nZVI in two different heavy metal-contaminated soils (acidic and calcareous soils). They reported that there was a decrease in the carbonate-bound fraction and an increase in the Fe-Mn oxides-bound fraction, especially for the calcareous soil which showed similar characteristics to the soil used in this study.

3.2. The Effect of nZVI on Naphthalene

In Figure 2, the naphthalene removal results obtained from the analyzes performed immediately after the nZVI application was given. The soil samples were initially contaminated with 200 ppm naphthalene and after the nZVI application, naphthalene concentrations in Pb_pah_nzvi_sterile_n, Pb_pah_nzvi_sterile, Pb_pah_nzvi_n and, Pb_pah_nzvi reactors decreased by 49.5 %, 38.8 %, 58.5% and, 57.6 %, respectively. These results showed that nZVI also contributed to the naphthalene removal in addition to the immobilization of Pb.
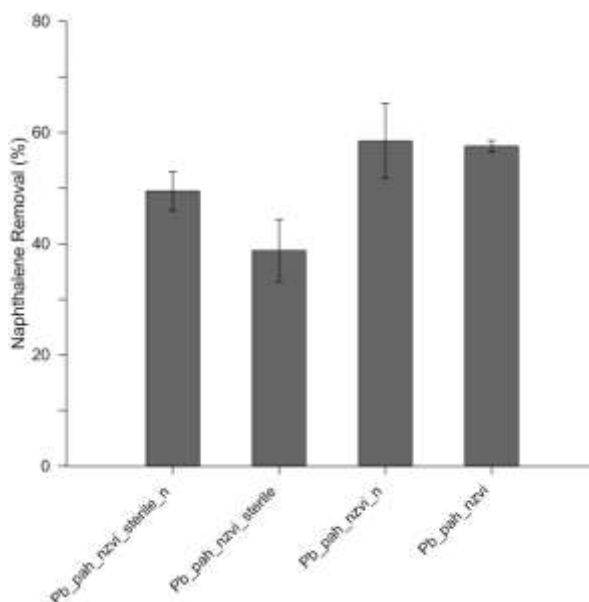

Figure 2:
Naphthalene removal percentages after the nZVI application. Error bars represent the standard deviation of triplicate samples.

Since nZVI is a highly reactive material, it has been tested on many organic pollutants as well as inorganic pollutants. In particular, the process of degradation of halogenated organic

compounds by nZVI utilizing reducing mechanisms has been successfully carried out in many studies and applications (Zhao et al., 2016). There is a limited number of studies in the literature on the effect of nZVI on PAH compounds. Chang et al. (2005) reported that they achieved 70% pyrene ($C_{16}H_{10}$) removal with nZVI in soil. In a similar laboratory-scale study, Chang and Kang (2009) obtained 44% removal efficiency in optimum conditions in a soil which they spiked with pyrene in their batch experiments. The researchers stated that the pollutant removal occurred as a result of the strong redox conditions caused by nZVI. Reductions in the range of 38.8 – 58.5% obtained for naphthalene in this study are consistent with the studies mentioned above. We know from one of our previous studies that O_2 content in the reactor headspace remains above 15% for this reactor configuration (Kahraman et al. 2017). So, the contribution of the anaerobic reductive degradation appears to be minimum. On the other hand, oxidation through Fenton-like reactions may be considered as a suspected degradation mechanism. It is known that H_2O_2 might form in the presence of nZVI and water in aerobic athmosphere. Eventually this leads to production of ·OH radical which is a strong oxidant (Li et al. 2021).

In another study, Oleszczuk and Koltowski (2017) reported that nZVI did not cause the removal of PAH compounds in soil samples exposed to long-term PAH contamination. The researchers, who monitored a wide range of 2,3,4,5 and 6-ringed PAH compounds, reported that this result may be due to the fact that the soil they studied was rich in nutrients and organic matter, reducing the reactivity of nZVI. In this context, although it is a fact that soil content and structure affect the efficiency of the process, the results of this study suggest that the decrease in the naphthalene content shown in Figure 2 is due to the degradation of naphthalene by the effect of nZVI.

3.3. Application of nZVI and Bioattenuation

After the application of nZVI to the reactors according to the experimental program and the analyses related to the effects of nZVI on Pb fractions and naphthalene, the reactors were incubated for 90 days for the bioremediation step. During this period, regular naphthalene analyzes were conducted to monitor the biodegradation performance. In the bioremediation step, two different biological treatment strategies were tested namely bioattenuation and biostimulation. The naphthalene removal percentages of the reactors subjected to the bioattenuation treatment were presented in Figure 3. In order to reveal the extent of biodegradation more clearly, the naphthalene removals determined after the nZVI application were not included in the calculations.

As it was shown in Figure 3, according to the results of the sterile control reactors, there were abiotic losses mainly due to evaporation although the reactors were kept closed. In all three sets of the sterile control reactors, the variation over the experimental period had a similar trend. At the end of the experimental period, the naphthalene removal results of reactors Pb_pah_nzvi_sterile, Pb_pah_sterile and, Pah__sterile were 30.2%, 20.9% and, 26.9%, respectively. The removal percentages of the Pb_pah_nzvi_sterile were not significantly different from other sterile reactors which suggests that the nZVI was effective on naphthalene at the first contact but it did not cause any significant change thereafter.

The trend of the Pb_pah reactor during the experimental period was similar to the sterile control reactors. However, when the Pb_pah and Pb_pah_sterile reactors were compared, it was observed that the removal rates started to decrease after 60 days and there was a significant difference between the removal of the Pb_pah and the Pb_pah_sterile at the end of the experimental period. This can be considered as an indication that the toxic properties of Pb and naphthalene in the soil suppressed the microbial activity to a great extent, but it did not stop the microbial activity completely. In the Pb_pah_nzvi reactor in which nZVI was applied, it was observed that the results differentiated from other samples, especially between 30-60 days. The naphthalene removal rate was lower but then it increased to the levels of other reactors. At the end of the experimental period, it was calculated that there was a 31.7 % removal of

naphthalene in the Pb_pah and 18.2 % in the Pb_pah_nzvi sample. The low levels of naphthalene degradation were due to the toxic effects of the contaminants as well as the negative effects of the nZVI on the microbial population.
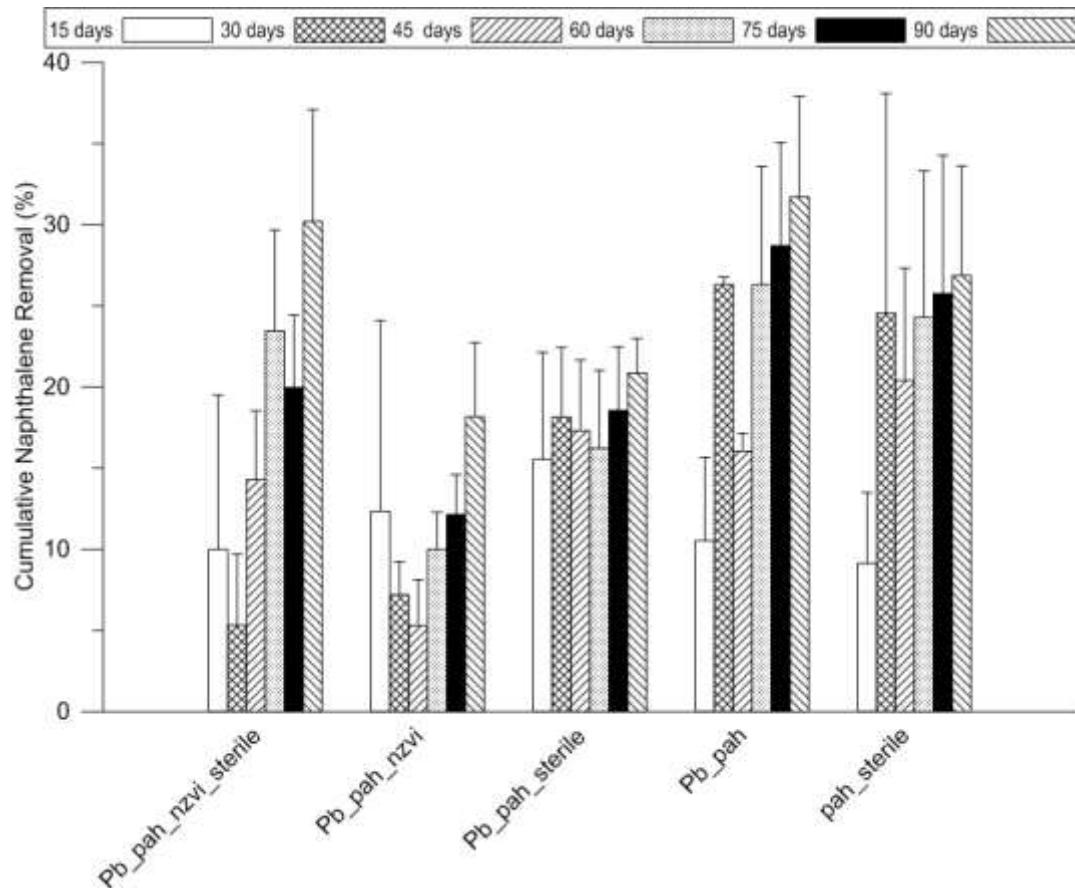

Figure 3:
Cumulative naphthalene removal percentages of bioattenuation reactor set throughout the experimental period. Error bars represent the standard deviation of triplicate samples.

3.4. The Application of nZVI and Biostimulation

The naphthalene removal percentages of the reactors subjected to the biostimulation treatment were presented in Figure 4. N and P were added to the soils after the nZVI application to stimulate microbial activity. In order to reveal the extent of biodegradation more clearly, the naphthalene removals determined after the nZVI application were not included in the calculations.
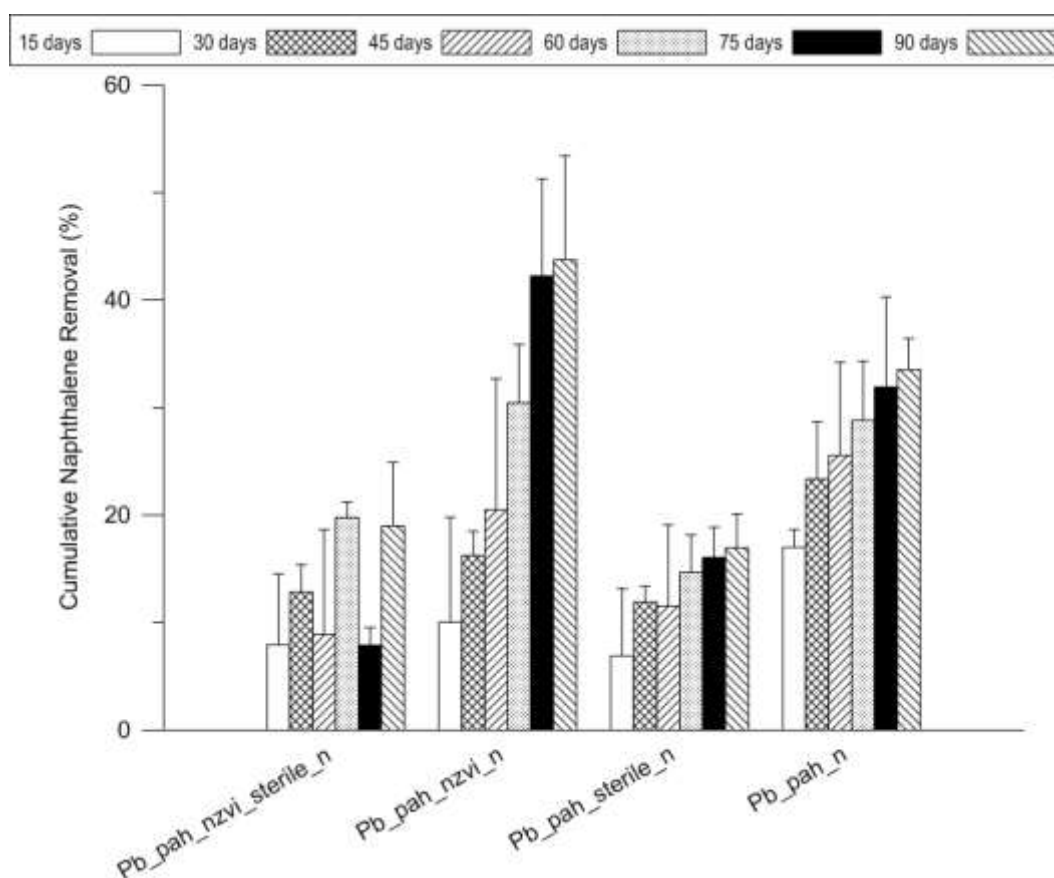
Figure 4:

Cumulative naphthalene removal percentages of biostimulation reactor set throughout the experimental period. Error bars represent the standard deviation of triplicate samples.

As seen in Figure 4, there was no significant difference in the removal percentages of the sterile control reactors (Pb_pah_sterile_n and Pb_pah_nzvi_sterile_n) throughout the experimental period. The naphthalene removal percentages of Pb_pah_sterile_n and Pb_pah_nzvi_sterile_n samples at the end of the experiment were 16.9% and 19.0%, respectively. When the removal rates of the sterile reactors were compared, it can be said that the losses due to evaporation were less for the biostimulation treatment although there were mostly no significant differences.

The removal percentages of the Pb_pah_n reactor deviated from the sterile reactors after 45 days and 33.5 % removal was calculated at the end of the experimental period. It can be said that after a 45-days of acclimation phase, the microorganism population became active. The increase started after 60 days in the Pb_pah reactor (bioattenuation treatment) but the increase in the Pb_pah_n started after 45 days, indicating that the nutrient addition accelerated the biodegradation. As for the Pb_pah_nzvi_n sample, it was seen that similar to the Pb_pah_n reactor, naphthalene removal increased after the 45th day and separated from the results of the sterile control reactors with significant differences and reached 43.8 %. Thus, although the positive effect of the nutrient addition was observed, it should also be noted that the nZVI did not have a slowing effect on microbial activity. Especially after the 60th day, the average values measured in the Pb_pah_nzvi_n reactor were higher than the results of the Pb_pah_n reactor. Although there was no significant difference at the end of the experimental period, it is possible that higher removal levels might have been obtained in the Pb_pah_nzvi_n reactor in a longer

experimental period. Future studies with increased experimental durations are needed to clarify this point.

3.5. Overall Process Performance

The process applied in this study is a process implemented through the integrated application of nZVI and biological soil treatment methods. Considering the heterogeneous structure of the soil and the presence of contaminants with different properties, the applicability of the remediation method needs to be evaluated from different aspects. In this context, the overall remediation efficiency obtained from the process is the most important data that should be considered in the general evaluation. The overall naphthalene removal percentages of the processes applied in the study were presented in Figure 5 (including comparisons with the relevant control reactors). These results include the degradation due to both nZVI and biodegradation as well as the abiotic losses.
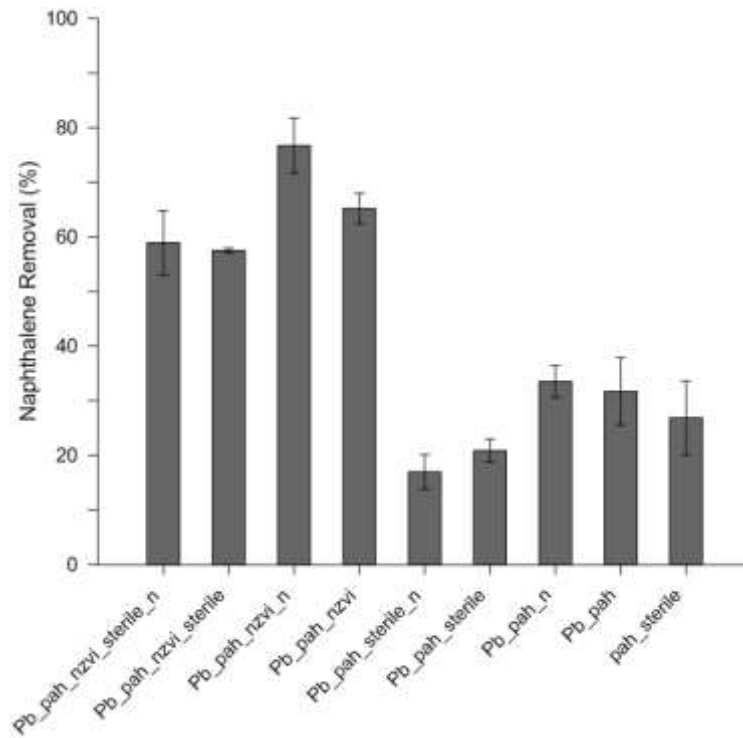

Figure 5:
Overall naphthalene degradation results. Error bars represent the standard deviation of triplicate samples.

As seen in Figure 5, losses caused by abiotic factors were 17.0%, 20.9% and 26.9%, respectively, in Pb_pah_sterile_n, Pb_pah_sterile and Pah_sterile control reactors. The abiotic loss was mainly due to the evaporation and occurred at an average value of 21.6 %. For the Pb_pah and Pb_pah_n reactors in which the nZVI was not applied and the sterilization was not performed, the naphthalene removal percentages of 31.7 % and 33.5% were calculated, respectively. It can be said that these soil samples were in a biologically active state and the biodegradation occurred at a decreased rate.

As emphasized in the previous sections, the direct effect of the nZVI on naphthalene was detected in the sterile control reactors Pb_pah_nzvi_sterile_n and Pb_pah_nzvi_sterile (58.9% and 57.5% respectively). The removal efficiencies of these reactors were higher as compared to the reactors which were not subjected to the nZVI application. The nZVI application and bioattenuation treatment were combined and tested in the Pb_pah_nzvi. The average efficiency of the Pb_pah_nzvi reactor was 65.2 % at the end of the experimental period and this value was significantly higher than the Pb_pah_nzvi_sterile. The nZVI application and biostimulation treatment were combined and tested in the Pb_pah_nzvi_n. A removal efficiency of 76.7 % was obtained in the Pb_pah_nzvi_n which was the highest naphthalene removal efficiency obtained in the study. According to these results, it can be said that the integration of bioremediation approaches with the nZVI application might increase the treatment efficiency of the heavy metal-organic co-contaminated soils, especially when used with a strategy to improve biological activity such as nutrient addition.

4. CONCLUSION

In this study, laboratory-scale experiments were carried out by applying nZVI and bioremediation to natural soil samples contaminated with Pb and naphthalene contaminants. Bioattenuation and biostimulation approaches were used as the bioremediation processes after the nZVI application. Process performance was measured on Pb immobilization and naphthalene removal parameters. The results after the nZVI application showed that highly mobile exchangeable and carbonate-bound fractions were reduced and the Pb content of the Fe-Mn oxides bound fraction with lower mobility increased. Thus, the mobility and availability of Pb content were greatly reduced by the nZVI application. Only nZVI application provided naphthalene removal in the range of 38.8 – 58.5%. When the nZVI was applied together with bioattenuation or biostimulation 65.2% and 76.7% naphthalene removal was obtained, respectively. The results showed that the integrated use of the nZVI and bioremediation processes has the potential to be used in the treatment of soils containing heavy metals and organic contaminants, but the effect of the selected biological treatment strategy on the process efficiency should be considered.

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CONFLICT OF INTEREST

The author (s) acknowledge that there is no known conflict of interest or common interest with any institution / organization or person.

AUTHOR CONTRIBUTION

Bekir Fatih Kahraman determination of conceptual and/or design processes of the study, data collecting, data analysis and interpretation, creating the manuscript, final approval and, full liability of the study. Ahmet Altın determination of conceptual and/or design processes of the study, management of conceptual and design processes of the study, data analysis and interpretation, critical review of intellectual content, final approval and, full liability of the study.

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