



Impacts of wastewater sludge amendments in restoring nitrogen cycle in *p*-nitrophenol contaminated soil

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Abstract

The possible impacts on nitrogen-cycle in a *p*-nitrophenol (PNP) polluted soil and the effectiveness of wastewater sludge amendments in restoring nitrification potential and urease activity were evaluated by an incubation study. The results indicated that PNP at 250 mg/kg soil inhibited urease activity, nitrification potential, arginine ammonification rate and heterotrophic bacteria counts to some extents. After exposure to PNP, the nitrification potential of the tested soil was dramatically reduced to zero over a period of 30 days. Based on the findings, nitrification potential was postulated as a simple biochemical indicator for PNP pollution in soils. Nitrogen-cycling processes in soils responded positively to the applications of wastewater sludges. A sludge application rate of 200 tons/ha was sufficient for successful biostimulation of these nitrogen processes. The microbial activities in sludge-amended, heavy PNP-polluted soils seemed to recover after 30–45 days, indicating the effectiveness of sludge as a useful soil amendment.

Key words: *p*-nitrophenol; wastewater sludge; soil pollution; nitrogen-cycle; biostimulation

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Introduction

Organic carbon is an essential nutrient for most soil microorganisms, but some organic substances and their derivatives are not utilizable by microflora. Xenobiotic substances are becoming to be problematic for soil because they tend to have a long decomposition period or are not decomposed in the soils. Knowledge of the fate of xenobiotics in terrestrial systems is an important aspect of soil environmental quality. Xenobiotic substances have also been observed to affect soil quality and fertility (Wyszowska et al., 2002; Pampulha et al., 2007; Gianfreda and Rao, 2008). Pollutants interact with soil microorganisms upon their entry to the soil ecosystem. After the normal metabolic activities and functions of soil microorganisms are hindered by pollution, microbial nutrient processes and enzyme activities as well as soil fertility and production will be affected or even collapse.

Among the xenobiotic chemicals, nitrophenols are examples of hazardous compounds that are known to be toxic for many living organisms either directly or indirectly through some of their catabolic metabolites (Gottschalk and Knackmuss, 1993). 4-Nitrophenol (*para*-nitrophenol or *p*-nitrophenol, hereafter abbreviated as PNP) is an important member of the nitrophenol family. It has been used in a number of industries, such as manufacturing of dyes, pesticides, and pharmaceuticals (Spain, 1995).

Nitrophenols are released by these industries into soil as fugitive emissions during manufacturing and production. Widely used insecticides, such as fenitrothion, have caused accumulation of nitrophenols in soil (Hong et al., 2007). In addition, parathion, a pesticide frequently used in the past decades is degraded by microbial hydrolases, a process that releases PNP into the soil (Mulbry and Karns, 1989). The spreading of wastewaters and sewage sludge containing PNP on agricultural lands may also accelerate the accumulation of PNP in terrestrial ecosystems.

Of all the major plant nutrients, nitrogen is often the most important determinant of plant growth and crop yield. The quantity and forms of nitrogen in soils are constantly changing due to biochemical processes (mineralization, nitrification, urea hydrolysis, ammonia volatilization, etc.). Several studies indicated that organic and inorganic pollutants have been shown to adversely affect these processes and reduce soil fertility (Walecka-Hutchison and Walworth, 2007; Contreras-Ramos et al., 2009; Topac et al., 2009).

Understanding the possible impacts on nitrogen processes in agricultural soils polluted by PNP is necessary for efforts to sustain soil fertility to be successful. A great deal of information has been collected on the degradation pathways of nitrophenolic compounds and isolation of phenol-mineralizing bacteria from wastewaters, soils, sediments and sludges (Cho et al., 1998; Qureshi and Purohit,

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2002; Labana et al., 2005; Liu et al., 2007). Nevertheless, detailed information regarding the tolerance of biochemical cycles and indigenous soil bacteria in agricultural soils to nitrophenolic substances remains undocumented. Furthermore, the biostimulating effects of wastewater sludge on restoring nitrogen cycle in soils contaminated by PNP require clarification.

In order to investigate the ecological impact of *p*-nitrophenol pollution on terrestrial ecosystems, a simulation experiment was designed to evaluate the responses of nitrogen processes in soil. The results of the incubation study will aid in defining an economically desirable biostimulation process in agricultural soils polluted with nitrophenols.

1 Materials and methods

1.1 Materials

Surface (0–20 cm) soil samples were collected from an alluvial agricultural field in Bursa, Turkey (Latitude, 40°15'55.18"N; Longitude, 28°47'07.55"E). Properties of soil samples used in this study were as follows: clay 25.4%, silt 18.5%, sand 56.1%, texture sandy clay loam, organic C 1.48%, total N 955 mg/kg, NH₄⁺-N 18.2 mg/kg, NO₃⁻-N 14.4 mg/kg, maximum field water holding capacity 34%, pH in water (1:2.5) 7.68 and EC₍₂₅₎ in water (1:2.5) 415 μS/cm. Soil samples were air dried at room temperature, ground and passed through a 4 mm sieve for incubation study. The first sludge sample (Sludge 1) was sampled from a treatment plant of a canned food company, which treats wastewater with a flow rate of 5500 m³/day in Bursa, Turkey. The second sample (Sludge 2) was obtained from the municipal wastewater treatment plant, which treats wastewater with a flow rate of 64,000 m³/day in Bursa, Turkey. Primary and thickened biological sludges were dewatered by belt press and the fresh sludge cakes were air dried in sand beds in both treatment plants. Sludge samples from drying beds were further dried (> 90%) in laboratory conditions before soil amendments. Characteristics of these sludge samples are presented in Table 1. PNP, with purity ≥ 98.0%, was obtained from the Merck company (Darmstadt, Germany).

1.2 Experimental procedure

Air-dried soil (100 g) was placed into plastic receptacles and different amounts of PNP were added with distilled water to bring the soil to 60% of its maximum field water holding capacity. PNP concentrations in the contaminated soil were 50 and 250 mg/kg dry soil. PNP doses were selected on the basis of earlier studies (Albert et al., 2000; Labana et al., 2005). The air-dried soil without PNP was used in control treatment. Samples were then incubated under controlled conditions in the dark at 28°C for 15 days. Thereafter, two sets of soil samples were analyzed to estimate the effect of PNP on soil nitrogen processes. After the artificial pollution process, two different wastewater sludges with doses of 50 and 200 tons/ha were added to remaining soil pots to evaluate biostimu-

Table 1 Chemical characteristics of the sludge samples*

Parameter	Sludge 1	Sludge 2
pH (1:5, solid:water)	6.75	6.4
EC (1:5, solid:water) (mS/cm)	3.3	3.75
Organic C (%)	38.25	28.44
Total-N (%)	5	5
Total hydrolysable organic-N (%)	4.1	3.3
Ammonium-N (mg/kg)	255	105
Nitrate-N (mg/kg)	150	45
Total P (mg/kg)	6500	8800
Available PO ₄ ³⁻ -P (mg/kg)	250	410
EDTA extractable heavy metals		
Zn (mg/kg)	24.5	196
Cu (mg/kg)	14.2	58.2
Ni (mg/kg)	<5.00	5.88
Cr (mg/kg)	3.8	6.1

* Based on dry weights, Sludges 1 and 2 came from wastewater treatment plants of a canned food company and a municipal wastewater treatment facility in Bursa, respectively.

lation effects. Selected sludge doses were based on our preliminary incubation study (data not shown), evaluating the enhancements in some enzyme activities of sludge-amended agricultural soils. The control treatment without any amendment was also included.

Using a completely randomized design, each treatment was performed in duplicate totaling 96 soil pots at the start of the incubation. Soil in each pot was analyzed with two replicates giving a total of four replicates ($n = 4$) for each parameter to perform analysis of variance. Water loss by evaporation was compensated daily during the pollution and biostimulation processes using distilled water to maintain soil water content. After the biostimulation period of 15, 30 and 45 days, two sets of soil pots were removed and the amounts of ammonium nitrogen, nitrate nitrogen, urease activity, arginine ammonification rate, nitrification potential and heterotrophic bacteria were determined.

1.3 Laboratory analysis

In order to analyze the variation trend of PNP concentrations after the pollution and biostimulation periods, soil samples were mixed with 0.5 mol/L NaOH and swirled for a few seconds. Thereafter, the soil suspension was filtered through a Whatman No. 2V folded filter paper. The yellow color intensity of the filtrate was measured with a spectrophotometer at 410 nm (Jenway 6105 UV/Vis., England). The PNP content of the filtrate was calculated by reference to a calibration graph (Tabatabai, 1982). Recovery of PNP from the soil was (93.2 ± 2.5)% and all PNP concentration data were calibrated by this rate of recovery. The PNP concentrations were given as PNP depletion percentages.

For NO₃⁻-N and NH₄⁺-N concentrations determination, samples were extracted by a 2 mol/L KCl. The concentrations in extracts were analyzed by steam distillation with MgO and Devarda alloy (Keeney and Nelson, 1982).

The urease activity of the soil was determined as described by Tabatabai (1982). Five grams dry soil was treated with 0.2 mL toluene, 9 mL THAM buffer solution (pH 9) and 1 mL of 0.2 mol/L urea solution at 37°C for 2 hr. Following incubation, enzyme activity was stopped by the

addition of 35 mL KCl-Ag₂SO₄ solution. NH₄⁺-N in the soil suspension was determined by vapor distillation. The results were obtained as mg NH₄⁺-N/L and then converted to µg NH₄⁺-N/(g·hr).

The arginine ammonification rate was determined by treating 2 g soil with 0.5 mL of arginine solution (2 g/L) at 30°C for 3 hr followed by extraction with 20 mL of 2 mol/L KCl (Alef and Kleiner, 1986). Ammonium concentrations in the extracts were determined using the indophenol blue method (Keeney and Nelson, 1982). The arginine ammonification rate was calculated as the difference between the arginine-treated and untreated sample values. Arginine ammonification activity was expressed as µg NH₄⁺-N/(g·hr).

Nitrification potentials were determined as described by Hart et al. (1994) using soil slurries with ammonium sulfate as the substrate. Samples were incubated on an orbital shaker at 180 r/min at 25°C for 24 hr. Nitrate from the centrifuged supernatant at 0, 4 and 24 hr was measured using the salicylic acid method as described by Cataldo (1975). Rates of NO₃⁻ formation were calculated using linear regression analysis and nitrification potential was expressed as µg NO₃⁻-N/(kg·hr).

Heterotrophic plate counts in extracts of soil samples (10 g in 100 mL phosphate buffer, prepared according to standard methods) were determined by direct plating onto plate count agar (tryptone 5.0 g/L, yeast extract 2.5 g/L, glucose 1.0 g/L, agar 9.0 g/L) (APHA, 1998). Plates were incubated at 28°C for 72 hr prior to counting colonies. Three replicates per dilution were plated and counted. Results were obtained as CFU/100 mL and converted into CFU/g dry soil.

1.4 Statistical analysis

After the artificial pollution period, Tukey's HSD test was performed for the comparison of the mean values of the control and PNP treated samples. In order to determine whether PNP concentration, sludge application rate and incubation time resulted in changes in soil properties during the biostimulation period, the data were subjected to three-way factorial ANOVA. The effects of sludge application rates and incubation time on soil properties were further tested with two-way factorial ANOVA for each PNP concentration. When significant effects were indicated, comparison of the means was made using Tukey's test. All statistical calculations were performed using Statistica 6.0 software (StatSoft, USA).

2 Results and discussion

2.1 Effect of PNP on soil nitrogen processes

Incubation of soils contaminated with a low dose of PNP (50 mg/kg) at 28°C for 15 days resulted in a PNP depletion of (28 ± 8)% (spectrophotometric measurement at 410 nm). Disappearance of yellow color of PNP may indicate the PNP degradation by soil microorganisms or loss due to adsorption to the soil solids and evaporation to the air. It is documented that phenolic compounds in soil are mainly

transformed by oxidative processes and catalyzed by phenolases and peroxidases produced by natural microflora (Sjoglad and Bollag, 1981; Dec et al., 2003). However, the calculated PNP depletion percentages did not indicate the definite fate of PNP in soil media. This measured loss of PNP may merely signal the beginning of the mineralization process. In view of this, it may be concluded that the degradation of PNP (50 mg/kg) appeared to begin within a rather short period of time (15 days). Likewise, Scow et al. (1986) reported that the mineralization of low concentrations of PNP proceeds with little or no initial acclimation period. On the other hand, PNP depletion percentage (spectrophotometric measurement at 410 nm) was determined as low as (5 ± 3)% for soils contaminated with high level of PNP (250 mg/kg), indicating that a significant portion of the nitrophenolic compound did not undergo any change over the 15-day period. A longer acclimation period was likely required for the PNP dose of 250 mg/kg to produce identifiable results. Accordingly, several studies indicated that the rate of PNP biodegradation was dependent on initial PNP concentration (Scow et al., 1986; Labana et al., 2005).

The response of several soil parameters to PNP contamination was also evaluated at 15 days after exposure. As indicated in Table 2, the NH₄⁺-N concentration in control and in both PNP contaminated soils were found to be similar. Unaffected ammonium concentrations showed that ammonifying bacteria appeared to be less sensitive to PNP contamination in short term. PNP dose of 50 mg/kg did not affect the NO₃⁻-N concentration in comparison to control soil. Inversely, lower NO₃⁻-N concentrations were determined for higher dose of PNP. The effect of nitrite ions originated from PNP degradation on nitrogen turnover processes in the studied soil was assumed as insignificant, since the stoichiometrical amount of NO₂⁻-N in PNP is less than 1.5 mg/kg and the extent of PNP mineralisation seemed to be low or negligible during this period. Levels of urease activity exhibited a decreasing trend (90.78 to 46.59 µg NH₄⁺-N/(mg·hr)) with increasing concentration of PNP. Arginine ammonification rates in control and low-level PNP polluted soils were found to be similar, whereas a slight decrease was observed for PNP doses of 250 mg/kg.

Table 2 indicates that PNP dose of 50 mg/kg resulted in a slight (approx. 9%) reduction in nitrification potential

Table 2 Responses of soil nitrogen parameters after 15-day exposure to PNP

Parameter	Control	50 mg/kg PNP	250 mg/kg PNP
NH ₄ ⁺ -N (mg/kg)	14.35 a*	15.47 a	14.67 a
NO ₃ ⁻ -N (mg/kg)	23.28 a	22.19 a	16.78 b
Urease activity (µg NH ₄ ⁺ -N/(g·hr))	90.78 a	81.59 b	46.59 c
Arginine ammon. rate (µg NH ₄ ⁺ -N/(g·hr))	2.372 a	2.380 a	2.225 b
Nitrification potential (µg NO ₃ ⁻ -N/(kg·hr))	365.7 a	332.0 b	0 c
Heterotrophic bacteria counts (logCFU/g)	5.47 a	5.51 a	5.01 b

*The same letters indicate that the values are not significantly different at 95% level.

of the studied soil. However, higher PNP dose of 250 mg/kg significantly inhibited nitrification activity. Similarly, previous studies have shown that some activities of soil microorganisms such as $^{14}\text{CO}_2$ uptake, nitrate reductase, nitrogenase activity, have been inhibited by nitrophenols and other phenolic compounds (Megharaj et al., 1991, 1992; Okolo et al., 2007). Furthermore, at sufficiently high concentrations, phenols have been shown to be toxic for species capable of using it as a growth substrate (Santos et al., 2001).

As indicated in Table 2, a negative response of heterotrophic bacteria to low levels of PNP was not recorded. The heterotrophic bacteria levels in the control and low-dose (50 mg/kg) PNP treatments were 5.47 and 5.51 logCFU/g, respectively. Exposure to higher levels of PNP (250 mg/kg) resulted in a slight but significant reduction (approx. 0.5 logCFU/g) in bacterial numbers ($p < 0.05$).

The overall evaluation of Table 2 indicated that urease activity levels and nitrification potential values indicated a quick response to low levels of PNP pollution. Heavier pollution decreased ($p < 0.05$) all the studied parameters except for $\text{NH}_4^+\text{-N}$, but the most dramatic response was from nitrification potential for the soil.

2.2 Biostimulating effects of wastewater sludge

Results of the factorial ANOVA test revealed that $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, urease activity and nitrification potential in both Sludge 1 and Sludge 2 amended soils were significantly dependent on pollutant (PNP) dose, sludge application dose and incubation time. The PNP dose was not found to affect arginine ammonification rates and heterotrophic bacteria counts, whereas the effects of sludge application dose and incubation time were found to be highly significant (Table 3).

Incubation of soils with low dose of PNP (50 mg/kg) for further 15 days (first 15 days of biostimulation period) resulted in a PNP depletion of $(86 \pm 6)\%$, $(85 \pm 8)\%$ and $(88 \pm 7)\%$ for control, Sludge 1 amended and Sludge 2 amended soils, respectively (spectrophotometric measurement at 410 nm). After this period no significant depletion occurred in soil pots. The disappearance of yellow color

in control soils with high dose of PNP (250 mg/kg) was calculated as $(30 \pm 6)\%$, $(35 \pm 4)\%$, $(38 \pm 9)\%$ for the biostimulation period of 15, 30 and 45 days, respectively. PNP depletion percentages apparently indicated that both sludge amendments enhanced the degradation of PNP. The approximate PNP depletion percentages in Sludge 1 and Sludge 2 amended soils were found as $(62 \pm 6)\%$ and $(54 \pm 3)\%$, respectively. The results also indicated that the degradation occurred mainly in the first 15 days of the biostimulation period.

The effects of wastewater sludge amendment on $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ content of soils contaminated with PNP are shown in Fig. 1. Figure 1a1 and a2 indicates that Sludge 1 and Sludge 2 amendments either did not increase or only slightly increased the $\text{NH}_4^+\text{-N}$ levels of the soils without PNP. However, more apparent increases were observed in PNP polluted soils. Low $\text{NH}_4^+\text{-N}$ content in sludge amended control soil probably indicated that wastewater sludge amendment quickened the processing of nitrification that occurred following ammonification (Tasatar, 1997; Topac et al., 2007). Soils polluted with PNP (50 and 250 mg/kg) produced greater amounts of $\text{NH}_4^+\text{-N}$, especially at day 15 and 30 of the incubation period. According to PNP depletion percentages calculated for this period, the amount of released $\text{NO}_2^-\text{-N}$ was expected as ca. 4–15 mg/kg. Ammonia formation by the reduction of this PNP-originated nitrite unlikely occurred under the prevailing incubation conditions (well-aerated small soil pots). Therefore, the greater amount of $\text{NH}_4^+\text{-N}$ in PNP polluted soils may be attributed to the apparent inhibition effect of PNP on nitrification process (Table 2). The decreased levels of $\text{NH}_4^+\text{-N}$ determined at day 45 of incubation indicated the probable enhancement of nitrification activity. A proper mechanism explaining this enhancement may be the tolerance and adaptation of soil microorganisms. According to previous studies, this is a possible explanation because a greater amount of soil bacteria can tolerate or even degrade xenobiotic compounds by induction of appropriate genes expression (Top and Springael, 2003).

The $\text{NO}_3^-\text{-N}$ levels in Sludge 1 and Sludge 2 amended

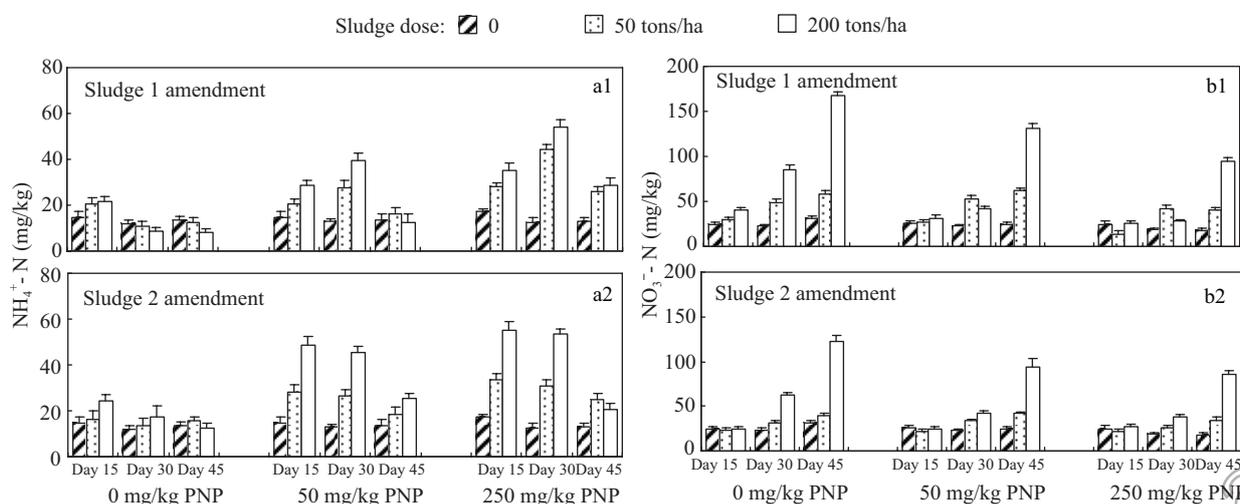


Fig. 1 Effect of wastewater sludge amendment on $\text{NH}_4^+\text{-N}$ content (a) and $\text{NO}_3^-\text{-N}$ content (b) of soils contaminated with PNP.

Table 3 *F*-values and significance of the major effects of treatments and their interactions based on factorial ANOVA

Measured parameter		<i>F</i> -value						
		PNP dose	Time	Sludge dose	PNP dose × Time	PNP dose × Sludge dose	Time × Sludge dose	PNP dose × Time × Sludge dose
NH ₄ ⁺ -N	Sludge 1	366.1**	130.5**	270.4**	58.50**	91.70**	48.00**	18.30**
	Sludge 2	266.3*	155.6**	526.3**	21.70**	78.70**	68.30**	8.500**
NO ₃ ⁻ -N	Sludge 1	345.0*	1281*	1593*	37.00*	126.0*	691.0*	22.00*
	Sludge 2	56.60*	528.0**	710.3**	16.50**	19.60**	296.8**	6.200**
Urease activity	Sludge 1	1975*	205.0*	2004*	97.00*	128.0*	249.0*	59.00*
	Sludge 2	1299**	108.0**	552.0**	5.000*	62.00**	34.00**	10.00**
Arginine ammonification rate	Sludge 1	0.180 ^{ns}	8.900**	138.2**	0.700 ^{ns}	1.500 ^{ns}	3.800*	1.400 ^{ns}
	Sludge 2	0.650 ^{ns}	19.27**	46.73**	1.960 ^{ns}	0.900 ^{ns}	7.300**	0.790 ^{ns}
Nitrification potential	Sludge 1	2105**	163.0**	1187**	147.0**	368.0**	15.00**	21.00**
	Sludge 2	1945**	75.00**	649.0**	117.0**	172.00**	6.000**	9.000**
Heterotrophic plate counts	Sludge 1	0.520 ^{ns}	44.10**	182.1**	1.100 ^{ns}	0.500 ^{ns}	36.00**	0.700 ^{ns}
	Sludge 2	1.130 ^{ns}	30.05**	91.99**	0.060 ^{ns}	1.190 ^{ns}	25.11**	0.300 ^{ns}

ns: no significance, * $p < 0.01$, ** $p < 0.001$.

control soils (0 mg PNP/kg) indicated a gradual increase throughout the incubation period of 45 days (Fig. 1b1 and b2). The NO₃⁻-N concentrations determined at the end of incubation varied in the range of 60–170 mg/kg dry soil and 40–125 mg/kg dry soil for Sludge 1 and Sludge 2 amended soils, respectively. Occurrences of NH₄⁺-N in low levels and NO₃⁻-N in high levels in Sludge 1 and Sludge 2 amended control soils (Fig. 1) clearly indicated that nitrification took place under prevailing experimental conditions. However, only slight or no apparent increase in NO₃⁻-N levels was observed during the first 30 days of incubation in Sludge 1 and Sludge 2 amended soils contaminated with PNP. Thereafter, a significant increase ($p < 0.05$) occurred at day 45 of incubation for both Sludge 1 and Sludge 2 amendments, indicating the probable enhancement of PNP tolerance of nitrification activity. The effect of nitrite ions originated from PNP degradation on nitrate concentrations was not clearly observed during the first 30 days of biostimulation period. Probably, the applied analytical method showed rather low precision to quantify this level of variation.

The effect of wastewater sludge amendment on urease activity level of soils contaminated with PNP is shown in Fig. 2a1 and a2. The trends of variation in urease activity levels were similar in sludge amended control soil and soil contaminated with low dose (50 mg/kg) of

PNP. In the Sludge 1 amendment, the sludge application dose of 50 tons/ha resulted in a slight but significant ($p < 0.05$) increase in urease activity levels with the maximum value of 100 μg NH₄⁺-N/(g·hr). Greater sludge application doses (200 tons/ha) enhanced urease activity of the same soils more markedly. The maximum urease activity level was determined to be 180 μg NH₄⁺-N/(g·hr) for the sludge application dose of 200 tons/ha. Sludge 2 amendments produced a less pronounced effect in urease activity levels in comparison to Sludge 1 amendments. Urease activity levels of the studied soil increased from 66 μg NH₄⁺-N/(g·hr) (control level) to maximum level of 80 and 117 μg NH₄⁺-N/(g·hr) with the addition of low and high doses of Sludge 2, respectively. The enhanced urease activity levels in sludge-amended soils indicated that high levels of substrate in the studied sludge samples activated the synthesis of enzyme by microbial biomass. Previous studies suggested that the development of enzyme activity was directly correlated with organic matter and nutrient content of wastewater sludge (Sastre et al., 1996). Similarly, a number of experiments (Topac et al., 2007, 2008) have also demonstrated that the urolytic activity was stimulated by the addition of wastewater sludge to soil.

With heavier PNP (250 mg/kg) pollution of the studied soil, the responses of soil urolytic activity to addition of sludge (both Sludges 1 and 2) were different. In this

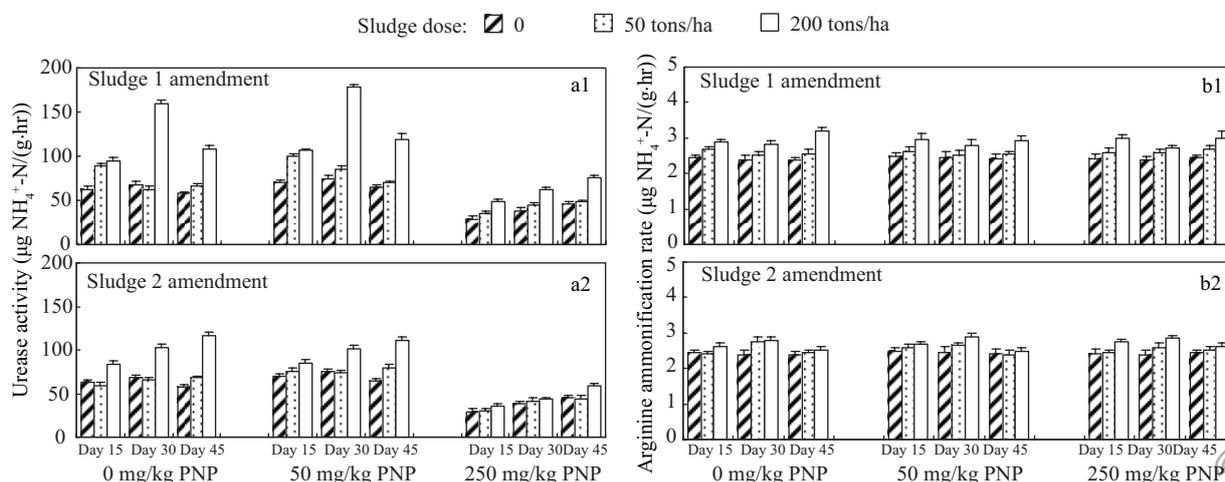


Fig. 2 Effect of wastewater sludge amendment on urease activity (a) and arginine ammonification rate (b) levels of soils contaminated with PNP.

experiment, either slight or no enhancement was observed in sludge-amended soils. The maximum urease activity level of $75 \mu\text{g NH}_4^+\text{-N}/(\text{g}\cdot\text{hr})$ was observed. Thus, the beneficial effect of sludge amendment appeared to be masked by high level of PNP. It can be concluded that urease activity of the studied soil was significantly sensitive to the existence of PNP (250 mg/kg). This result is in line with the previous studies indicating that variations in urease activity of soils can be evaluated as a bioindicator of anthropogenic stress caused by various pollutants (Margesin et al., 2000; Shen et al., 2005; Topac et al., 2009).

The variation of arginine ammonification rate (AAR) in sludge-amended soils containing 0, 50 and 250 mg/kg PNP is shown in Fig. 2b1 and b2. These results indicate that no significant difference was observed between control and PNP polluted soils. AAR levels in soils without sludge amendments were $2.37\text{--}2.49 \mu\text{g NH}_4^+\text{-N}/(\text{g}\cdot\text{hr})$. No significant variation in AAR values was recorded during the overall incubation period. However, sludge amendments significantly increased the AAR values of control and PNP polluted soils. In general, increasing AAR values were observed with increasing doses of sludge in all incubation periods. The AAR values in Sludge 1 amended soils ranged between $2.51\text{--}3.21 \mu\text{g NH}_4^+\text{-N}/(\text{g}\cdot\text{hr})$. The improvement of AAR in Sludge 2 amended soils was lower with the values of $2.38\text{--}2.88 \mu\text{g NH}_4^+\text{-N}/(\text{g}\cdot\text{hr})$. The increase in organic C content of sludge-amended soils resulted in an increase in AAR values (Rietz and Haynes, 2003). As a result, the AAR data indicated that inhibition of nitrogen mineralisation did not occur in PNP-polluted soils.

The effect of wastewater sludge amendment on nitrification potential (NP) of soils contaminated with PNP is shown in Fig. 3a1 and a2. Nitrification potential rates can provide estimated rates of nitrification that could be encountered in a soil and can suggest possible relationships between the properties of a soil and prospects for the flow of N in the soil (Schmidt and Belser, 1982). Because of the high specificity of the implicated bacteria (mainly *Nitrosomonas* spp. for the first step and *Nitrobacter* spp. for the second step), nitrification is a sensitive indicator of soil pollution (Deni and Penninckx, 1999; Smolders et al., 2001; Cela and Sumner, 2002). As indicated in Fig. 3a1

and a2, the nitrification potentials of control soil and soil contaminated with a low dose (50 mg/kg) of PNP were similar. No significant inhibition effect was observed for PNP doses of 50 mg/kg . The responses of control soil and soil contaminated with low dose of PNP to sludge amendments were also found to be similar. Sludge 1 and Sludge 2 amendments with doses of 50 tons/ha did not affect the NP values of soils. However, sludge doses of 200 tons/ha significantly enhanced nitrification potentials with the maximum values of 431 and $411 \mu\text{g NO}_3^-\text{-N}/(\text{kg}\cdot\text{hr})$ for Sludge 1 and Sludge 2, respectively. The determined NP values in contaminated soils with high doses of PNP were quite different. Nitrification in heavily contaminated soils was still entirely inhibited at day 15 of incubation, but thereafter a significant enhancement occurred (0 to $190 \mu\text{g NO}_3^-\text{-N}/(\text{kg}\cdot\text{hr})$). Sludge 1 amendments with doses of 50 and 200 tons/ha significantly increased the NP levels with the values of $150\text{--}250$ and $300\text{--}380 \mu\text{g NO}_3^-\text{-N}/(\text{kg}\cdot\text{hr})$, respectively. For Sludge 2, the counterpart values were considerably lower ($105\text{--}109$ and $255\text{--}345 \mu\text{g NO}_3^-\text{-N}/(\text{kg}\cdot\text{hr})$).

It can be concluded from NP data that 50 tons/ha sludge amendment was not sufficient to increase NP values to control levels. However, a more marked enhancement occurred with higher doses (200 tons/ha) of sludge. In these cases, NP values equaled or even exceeded control values, indicating the effectiveness of both Sludge 1 and Sludge 2 amendments. This study was conducted in a laboratory setting that optimized conditions for organic nitrogen mineralisation and nitrification. Thus, the actual activity enhancement by sludge amendments may be lower under natural field conditions. On the other hand, the products of nitrification, nitrite and nitrate, are known to inhibit nitrification (Azam et al., 1995). As nitrate and nitrite ions leach readily from the soil, nitrification in field conditions is expected to be more active than the measured nitrification from this laboratory experiment.

The variation of heterotrophic plate counts (HPC) in soils contaminated with PNP is shown in Fig. 3b1 and b2. No significant difference was observed between HPC levels of control soil and PNP contaminated soils. Bacterial numbers in those soils ranged from 5.31 to 5.67

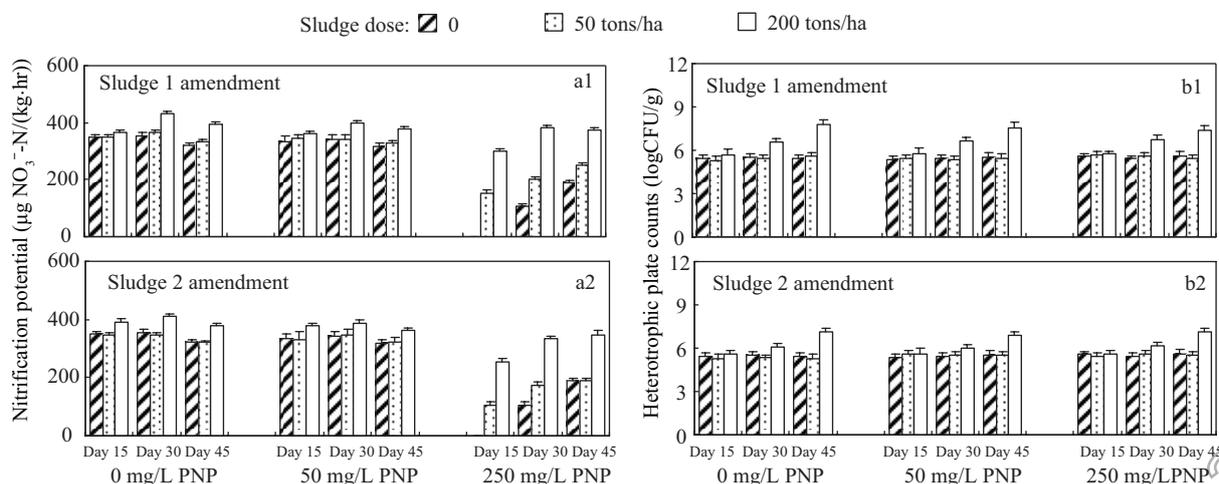


Fig. 3 The effect of wastewater sludge amendment on nitrification potential (a) and heterotrophic plate counts (b) of soils contaminated with PNP.

logCFU/g. As shown in Fig. 3b1 and b2, Sludge 1 and Sludge 2 amendments with doses of 50 tons/ha indicated no effect on HPC. However, sludge amendments with doses of 200 tons/ha significantly increased the colonies of heterotrophic bacteria ($p < 0.05$). Maximum HPC of 7.34 and 7.10 logCFU/g were observed at the end of the incubation period (day 45) for Sludge 1 and Sludge 2 amendments, respectively. This apparent increase in heterotrophic bacteria colonies may contribute to the enhancement of nitrification potential of the studied soil. For instance, a diverse group of heterotrophic bacteria exists that is capable of heterotrophic nitrite oxidation; within this group, each species exhibits differing degrees of mixotrophy and diauxy on nitrite and simple organic compounds (Abeliovich, 2006).

Consequently, the study demonstrated that both sludge samples had beneficial effects on PNP contaminated soils with respect to nitrogen-related processes. It should be noted that nutrient and heavy metal contents as well as degree of maturity may affect the effectiveness of wastewater sludge as a biostimulating agent. Therefore, future studies with a range of sludges from different sources are required to assess the full extent of the biostimulation effect of wastewater sludges.

3 Conclusions

Based on the data obtained in this article, it can be concluded that soil pollution with *p*-nitrophenol impaired even inhibited the nitrogen cycle in soil, and this could result in a deterioration of natural soil structure and nitrogen-induced problems in agricultural production. The levels of urease activity and the nitrification potential values showed a slight but quick response to low level of PNP pollution. Heavier pollution by PNP decreased nitrate N, urease activity, arginine ammonification rate, nitrification potential, and heterotrophic bacteria counts more markedly, the reduction was most dramatic especially with the nitrification potential. After exposure to PNP, nitrification potential of soil was zero over a period of 30 days. Given the high sensitivity of microbial processes to PNP and the probable degradation products, measuring nitrification potential can be accepted as a simple, straight-forward biochemical indicator test for the extent and impact of soil PNP contamination.

Nitrogen cycling in soils was enhanced by the applications of two sludges of different origins. The magnitude of enhancement was generally higher for the wastewater sludge containing a higher amount of hydrolysable organic nitrogen and a lower amount of EDTA-extractable heavy metals. An application dose of 200 tons/ha was sufficient in aiding the recovery of the PNP-inhibited nitrification potential within 30–45 days, but the activity of urease was still significantly lower with this rate of amendment application and after the elapsed time. It may be conjectured that using wastewater sludges in land farming practice appeared to be an efficient bioremediation technique for soils contaminated with PNP. The effectiveness of sludge application under field conditions may be more variable

due to the difficulty in controlling forces of nature such as temperature and rainfall. More studies are needed to monitor the contaminated soil to determine the required applications of sludges.

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