Journal of Hazardous Materials 170 (2009) 1006–1013

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Effect of a sulfonated azo dye and sulfanilic acid on nitrogen transformation processes in soil

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ARTICLE INFO

Article history:
Received 5 November 2008
Received in revised form 2 May 2009
Accepted 17 May 2009
Available online 22 May 2009

Keywords:
Ammonification
Azo dyes
Nitrification
Nitrifying bacteria
Soil pollution

ABSTRACT

Introduction of organic dyes into soil via wastewater and sludge applications has been of increasing concern especially in developing or under-developed countries where appropriate management strategies are scarce. Assessing the response of terrestrial ecosystems to organic dyes and estimating the inhibition concentrations will probably contribute to soil remediation studies in regions affected by the same problem. Hence, an incubation study was conducted in order to investigate the impact of a sulfonated azo dye, Reactive Black 5 (RB5) and sulfanilic acid (SA), a typical representative of aromatic sulfonated amines, on soil nitrogen transformation processes. The results apparently showed that nitrogen related processes in soil can be used as bioindicators of anthropogenic stress caused by organic dyes. It was found that urease activity, arginine ammonification rate, nitrification potential and ammonium oxidising bacteria numbers decreased by 10–20% and 7–28% in the presence of RB5 (>20 mg/kg dry soil) and SA (>8 mg/kg dry soil), respectively. Accordingly, it was concluded that organic dye pollution may restrict the nitrogen-use-efficiency of plants, thus further reducing the productivity of terrestrial ecosystems. Furthermore, the response of soil microbiota to SA suggested that inhibition effects of the organic dye may continue after the possible reduction of the parent dye to associated aromatic amines.

1. Introduction

Azo dyes, which are used in a number of industries including textile, paper printing, food, leather, pharmaceutical and cosmetic, are synthetic compounds and hence xenobiotics [1]. These compounds are characterised by the presence of aromatic moieties linked together with azo groups (—N=N—). Given that useful dyes must possess a high degree of chemical and photolytic stability, the removal of dyes from effluent is problematic. Stability against microbial attack is also a required feature of azo dyes [2]. Consequently, they are less amenable to biodegradation and often pass through activated sludge facilities with little or no reduction in colour [3].

Water-soluble dyes such as the sulfonated azo dyes generally enter the environment through wastewater discharges. The release of azo dyes into the environment is a concern given the coloration of natural waters and inherent toxicity, mutagenicity and carcinogenicity of the dyes and associated biotransformation products. Therefore, considerable attention has focused on evaluating the fate of azo dyes during wastewater treatment and in the natural environment. Land application of sewage sludge and long-term irrigation from natural streams polluted with textile effluent also account for the movement of organic dyes into agricultural soil. Zhou [4] found that the average concentration of total organic dyes in surface soil near printing, dyeing and synthetic dye enterprises in China was up to 12.3–456.2 mg/kg dry weight.

Although several authors have reported on alternating anaerobic and aerobic conditions in azo dye degradation and the toxic effect of azo dyes on aquatic environments [5–8] few studies have concentrated on the effect of azo dyes and dye derivatives on terrestrial ecosystems [4,9,10]. Biodegradation, partition, sorption and immobilisation represent the main processes that may affect the accumulation of azo dyes and degradation products in soil environments. Soil is a biologically balanced system, and any drastic change in its environment can modify microbial populations and soil enzymatic activities involved in various nutrient cycles, which can affect soil fertility.

Thus, the likely persistence and largely unknown effect of azo dyes and azo dye-derived aromatic amines in the soil environment makes these compounds desirable targets for soil quality monitoring and environmental distribution studies. Evaluation of the effect of parent azo dyes in addition to anaerobic degradation products may assist in the prediction of harmful effects on soil fertility caused by the accumulation and possible degradation of the dyes in soil environments. In an effort to assess the impact of a sulfonated azo dye and sulfanilic acid on nitrogen transformation processes in soil

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doi:10.1016/j.jhazmat.2009.05.080

and to supplement the environmental risk assessment for these xenobiotics, soil was treated with varying doses of the sulfonated azo dye Reactive Black 5 and sulfanilic acid, a typical representative of aromatic sulfonated amines. For this purpose an incubation study was carried out for 50 days under controlled conditions and the urease activity, nitrification potential, arginine ammonification rate and nitrifying bacteria counts were determined during the incubation period and nitrogen transformation processes in contaminated soil were evaluated.

2. Materials and methods

2.1. Materials

Soil samples were collected from the top 20 cm of an agricultural field located in Bursa-Balabancık village (Latitude, 40°15′55.1″N; longitude, 28°47′07.55″E). The commercial product Reactive Black 5-RB5 (diazo, 150% [w/w] dye) was obtained from DyStar (Leverkusen, Germany) as a powder. To simulate dyehouse processes, 4 g/l dye solution was hydrolysed in 0.1 M NaOH at 80 °C for 4 h followed by neutralisation with 33% (w/v) hydrochloric acid. Sulfanilic acid (SA) was of analytic grade and obtained from Merck AG, Germany.

2.2. Incubation study

In an effort to evaluate the effect of different RB5 and SA concentrations on soil nitrogen processes, 100 g soil portions were placed into plastic receptacles and different amounts of RB5 and SA were added with distilled water to bring the soil to 60% of its field capacity. Control treatment without RB5 or SA was also included. The RB5 and SA concentrations in the contaminated soil were 5, 10, 20, 40, 80 mg/kg dry soil and 2, 4, 8, 16, 32 mg/kg dry soil, respectively. SA concentrations were calculated from the applied RB5 doses, assuming that 2 mol SA was theoretically obtained from the reduction of 1 mol RB5. Samples were then incubated under controlled conditions in the dark at 28 °C for 1, 10, 20, 30, 40 and 50 days. The experiment was planned with a completely randomised design and each treatment was performed in duplicate to give a total of 132 experimental units at the start of the incubation. Water loss by evaporation was compensated daily using distilled water to maintain soil water content. At each sampling time two sets of soil pots were removed and the urease activity (day 1, 10, 20, 30, 40 and 50), arginine ammonification rate (day 1, 10, 30 and 50), nitrification potential (day 1, 10, 30 and 50) and nitrifying bacteria counts (day 1, 20 and 50) were determined.

2.3. Laboratory analysis

The soil samples were analysed for particle size distribution by the hydrometer method [11]. Electrical conductivity (EC) and pH of soil were measured in sample extracts obtained by shaking the material with distilled water at 1:2.5 (w/v) sample:water ratio using a conductivity meter and pH meter, respectively [12,13]. Field capacity was determined using the method of saturation followed by free draining [14]. Nitrate and ammonium nitrogen concentrations were determined in samples which were extracted using 2 M KCl. The concentrations in extracts were analysed by steam distillation with MgO and Devarda alloy [15]. Total nitrogen content of samples was measured by Kjeldahl digestion method [16]. Easily oxidisable carbon contents were determined by potassium dichromate oxidation followed by spectrophotometric measurement at 590 nm [17].

The urease activity of the soil was determined as described by Tabatabai [18]. Five grams dry soil was treated with 0.2 ml toluene, 9 ml THAM buffer solution (pH 9) and 1 ml 0.2 M urea solution at 37 °C for 2 h. Following incubation, enzyme activity was stopped by the addition of 35 ml KCl (2.5 M)–Ag_2SO_4 (100 ppm) solution and NH^+_4–N in the soil suspension was determined by vapour distillation. The results were obtained as mg NH_4^+–N l^{-1} and then converted to μg NH_4^+–N g^{-1} h^{-1}.

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 h followed by extraction with 20 ml 2 M KCl [19]. Ammonium concentrations in the extracts were determined using the indophenol blue method [20]. The arginine ammonification rate was calculated as the difference between the arginine-treated and untreated sample values. Arginine ammonification activity was expressed as μg NH_4^+–N g^{-1} dw soil h^{-1}.

Nitrification potentials were determined using the shaken slurry method with ammonium sulphate as the substrate [21]. Samples were incubated on an orbital shaker at 180 rpm at 25 °C for 24 h. Nitrate from the centrifuged supernatant at 0, 4 and 24 h was measured using the salicylic acid method as described by Cataldo et al. [22]. Rates of NO_3^- formation were calculated using linear regression analysis and nitrification potential was expressed as μg NO_3^-–N g^{-1} dw soil h^{-1}.

Populations of ammonium oxidisers (AOB) and nitrite oxidisers (NOB) were enumerated using the most probable number dilution technique. Ammonium sulphate was added to the medium of Schmidt and Belser [23] for the determination of ammonium oxidisers and sodium nitrite was added for the determination of nitrite oxidisers. Each dilution was performed in triplicate. Assessment for positive AOB and NOB tubes was performed following five weeks of incubation at 28 °C. The presence of nitrite or nitrate, taken as evidence of NH_4^+ oxidisers, was checked using a drop test with the Griess–Ilosvay reagent [23]. Using the same approach, the number of NO_2^- oxidisers was estimated from the disappearance of NO_2^-, and taken as evidence of NO_2^- oxidisers. The ammonium and nitrite oxidiser populations were calculated using the MPN table of Cochran [24].

2.4. Statistical analysis

In an effort to determine whether RB5 and SA treatment resulted in changes in soil properties over time, values pertaining to the urease activity, arginine ammonification rate, nitrification potential and nitrifying bacteria counts were subjected to two-way analysis of variance (ANOVA). The effect of treatment on soil properties was further tested with one-way ANOVA for each incubation period. When significant effects were indicated by ANOVA, post hoc analysis was performed using Tukey's HSD multiple comparison test. Pearson's product moment correlation coefficients between the examined parameters and pollutant concentrations were also computed. All statistical calculations were performed using STATISTICA 6.0 software.

3. Results and discussion

The data presented in Table 1 depicted that the soil used in the incubation study was sandy clay loam in texture (sand: 56.1%, silt: 18.5% and clay: 25.4%). The soil was slightly alkaline (pH 7.68) in reaction and the electrical conductivity was 415 μS/cm. The field capacity of the experimental soil was 34%. The soil had 955 mg/kg total N and contained relatively low quantities of mineral N (18.2 and 14.4 mg/kg ammonium-N and nitrate-N, respectively). The results indicated that soil organic carbon content was moderate with the value of 1.48%.

The RB5 and the associated aromatic amine, SA were amended to above-mentioned soil samples and the response was monitored throughout an incubation period of 50 days. Considerable variation in all of the examined soil properties were observed for the dif-

Table 1
General properties of soil samples used in the incubation study.

Parameters[a]	Soil
Sand (%)	56.1
Silt (%)	18.5
Clay (%)	25.4
Texture	Sandy clay loam
pH (1:2.5, solid:water)	7.68
EC (1:2.5, solid:water) (μS/cm)	415
Field capacity (%)	34
Total N (mg/kg)	955
Ammonium N (mg/kg)	18.2
Nitrate N (mg/kg)	14.4
Organic C (%)	1.48

[a] Dry weight basis.

ferent RB5 doses in the soil at different sampling times. Results of the two-way ANOVA test revealed that the urease activity, nitrification potential, arginine ammonification rate and number of ammonium and nitrite oxidising bacteria in azo dye-treated soil were significantly dependent on pollutant doses and incubation time. The interaction between RB5 dose and incubation time was also found to be significant ($p < 0.01$). In other words, the effect of pollutant dose was dependent on the incubation period. Similar statistical results were obtained in for SA-treated soil except for the nitrite oxidising bacteria. The main effect of pollutant dose did not affect the number of nitrite oxidisers in SA-treated soil (Table 2).

3.1. Nitrogen mineralisation potential

Investigations of soil urease activity has attracted considerable attention due to the increasing use of urea as a fertiliser to increase soil productivity. Urease plays a key role in the N cycle, transforming urea to ammonium [25]. Like many other soil enzymes, urease can be either intracellular or extracellular, and extracellular urease can be either free or immobilised on mineral or organic soil components [26]. Extracellular urease activity was found to range from 27% to 63% of the total urease activity in soil [27].

Fig. 1 shows the variation in urease activity of RB5- and SA-treated soil throughout the incubation period. The urease activity in soil containing 5, 10 and 20 mg/kg RB5 was equal to or higher than the respective control values throughout the first 30 days of the incubation (Fig. 1a), and thereafter decreased slightly. On the other hand, the urease activity in soil which had been treated with the highest RB5 doses (40 and 80 mg/kg) was significantly lower than that of the respective control soil for all incubation periods ($p < 0.05$). The average inhibition of urease activity in these soils was 15% and 20% for RB5 doses of 40 and 80 mg/kg, respectively.

Soil urease activity exhibited a stronger and faster response to SA treatment (Fig. 1b). The urease activity for all SA-treated soil samples was significantly lower than the respective control soil during an incubation up to 40 days ($p < 0.05$), after which time the activity approximated control values. Average inhibition values showed that SA doses of 2 and 4 mg/kg were responsible for a reduction in urease activity of 7% and 9%, respectively. Higher SA doses (8, 16 and 32 mg/kg) appeared to result in greater inhibition with average values in the range of 18% to 25%.

Urease activity is a frequently used test for determining the influence of various anthropogenic pollutants (heavy metals, pesticides, insecticides, crude oil, etc.) on the microbiological quality of soil [28–30]. Reduced urease activity observed in this study may be referred to as a simple assessment tool for evaluating anthropogenic stress caused by organic dyes. The urease inhibition observed in this study suggested a possible binding of RB5 and SA to extracellular urease in a reversible and/or irreversible manner that results in changes in the protein conformation and activity. The indirect inhibitory effect may be ascribed to RB5/SA-induced changes in size, structure and functionality of the microbial community. The inhibitory effect caused by RB5 and SA treatment may also be partly attributed to immobilisation of free urease on the modified components [31] of the soil investigated. Catalysis by an immobilised enzyme may differ from that of the free form because of chemical or conformational changes in enzyme structure, which occurs

Table 2
Results of two-way ANOVA for the analysis of the main effect of contaminant doses and incubation time.

Sources of variation	Reactive Black 5 (RB5)				Sulfanilic acid (SA)			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Dependent variable: urease activity								
Doses	5	1598.2	59.293	<0.001	5	1800.4	224.73	<0.001
Time	5	422.97	15.692	<0.001	5	314.37	39.239	<0.001
Doses × time	25	175.64	6.5159	<0.001	25	79.869	9.9691	<0.001
Error	72	26.955			72	8.0116		
Dependent variable: nitrification potential								
Doses	3	13395	256.00	<0.001	3	22123	787.15	<0.001
Time	5	4575.2	87.438	<0.001	5	12490	444.42	<0.001
Doses × time	15	368.81	7.0485	<0.001	15	5277.1	187.76	<0.001
Error	48	52.325			48	28.105		
Dependent variable: arginine ammonification potential								
Doses	3	0.4164	65.835	<0.001	3	1.6263	380.41	<0.001
Time	5	1.292	178.53	<0.001	5	1.1981	280.26	<0.001
Doses × time	15	0.0845	13.359	<0.001	15	0.0442	10.350	<0.001
Error	48	0.0063			48	0.0043		
Dependent variable: ammonium oxidising bacteria								
Doses	2	0.4484	8.2104	<0.001	2	0.3379	13.646	<0.001
Time	5	1.4339	26.256	<0.001	5	1.6942	68.422	<0.001
Doses × time	10	0.3079	5.6374	<0.001	10	0.3688	14.893	<0.001
Error	36	0.0546			36	0.0248		
Dependent variable: nitrite oxidising bacteria								
Doses	2	0.4001	14.741	<0.001	2	0.0471	1.4744	n.s.
Time	5	0.2663	9.8111	<0.001	5	1.2771	39.971	<0.001
Doses × time	10	0.1324	4.8812	<0.001	10	0.0401	1.2547	n.s.
Error	36	0.0271			36	0.0320		

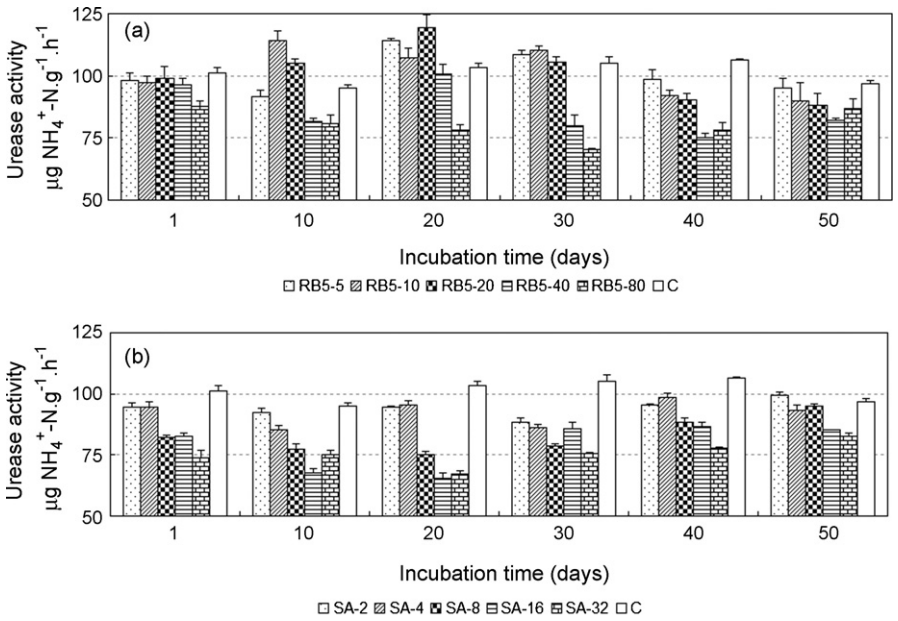

Fig. 1. Variation in urease activity of soil treated with varying doses of Reactive Black 5 (RB5) and sulfanilic acid (SA).

following immobilisation, modification of the microenvironment surrounding the enzyme due to the physical and chemical nature of the immobilising support, and steric hindrance effects on substrate accessibility to the active site [32].

Fig. 2 shows the rate of arginine ammonification in RB5- and SA-treated soil. Arginine is one of the 20 essential amino acids. Micro-organisms catabolise arginine via one or more of four major pathways: (1) the arginine–urease or arginase–urea amidolyase pathway, (2) the arginine transmidinase pathway, (3) the arginine deiminase pathway (4) and the arginine decarboxylase pathway. Except in the arginine transmidinase pathway, ammonium is an end-product [33]. Arginine ammonification reflects the N mineralisation capacity and more than 50 bacterial strains are known to utilise arginine as a C and N source [19,34]. As shown in Fig. 2a, no marked inhibition was observed in RB5-treated soil for the first 10 days of incubation. Furthermore, lower RB5 doses seemed to activate arginine ammonification in comparison to control values. Arginine ammonification rates in soil treated with 20, 40 and 80 mg/kg RB5 were all significantly lower than those in the respective control soil samples on the 30th day of incubation ($p < 0.05$). The percent inhibition decreased toward the end of the incubation. Since many organisms are believed to have the ability to ammonify nitrogenous substances [35], any activity lost in organisms affected by RB5 would presumably be compensated for by the activity of less sensitive species.

SA exposure with doses lower than 16 mg/kg did not inhibit nitrogen mineralisation, as estimated by the arginine ammonification rates (Fig. 2b). However, soil exposed to 16 and 32 mg/kg SA possessed significantly less KCl-extractable ammonium for the overall incubation period ($p < 0.05$). The average inhibition pro-

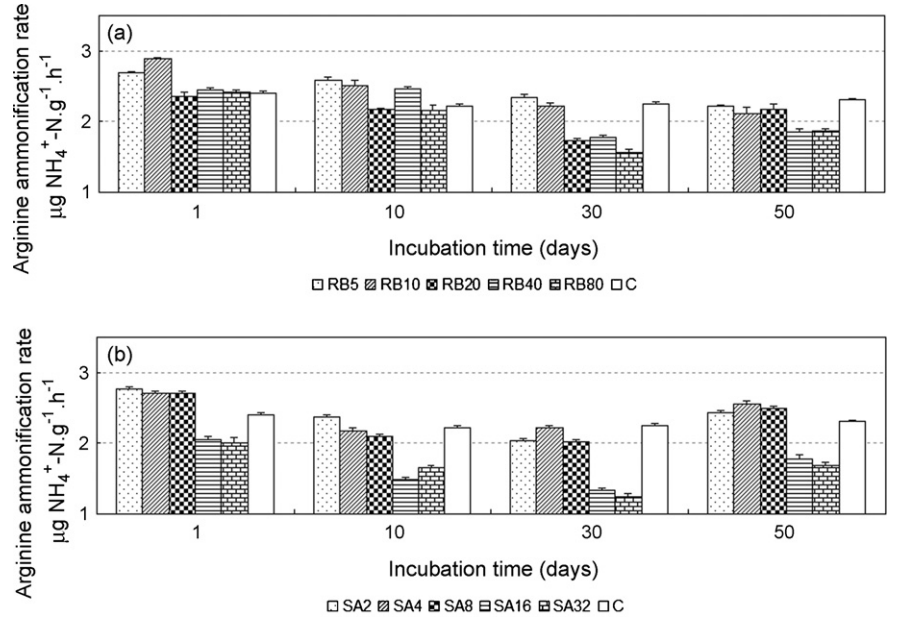

Fig. 2. Variation in arginine ammonification rate of soil treated with varying doses of Reactive Black 5 (RB5) and sulfanilic acid (SA).

duced by the SA doses of 16 and 32 mg/kg was 28% and 29%, respectively. The average percent inhibition established that the inhibitory effect of SA treatment on arginine ammonification, and to a lesser extent urea hydrolysis, in the soil investigated is more noticeable compared with RB5 treatment. According to observed inhibition of nitrogen mineralisation, it may be concluded that restricted urea hydrolysis and arginine ammonification in RB5 and SA contaminated soils may indicate a possible lack of availability of nitrogen. Iron deficiency of crops grown in dye-polluted soils was reported in a previous study [9]; however, nitrogen related data were scarce in published literature.

3.2. Nitrification potential and countable nitrifying bacteria numbers

The effect of RB5 and SA treatment on the nitrification potential of the soil investigated is shown in Fig. 3. The nitrification potential of soil treated with low levels of RB5 (5 and 10 mg/kg) did not significantly differ from control values during the overall incubation period (Fig. 3a). On the other hand, higher RB5 doses (>10 mg/kg) apparently decreased the nitrification potential of soil in comparison to control values ($p < 0.05$). The average percent inhibition of soil treated with 20, 40 and 80 mg/kg RB5 was 17%, 19% and 21%, respectively. Similarly, Li and Bishop [36] identified the azo dye inhibition effects on nitrifying biofilms and stated that the ammonium consumption and nitrate production rates decreased as Acid Orange 7, a sulfonated azo dye (15 and 25 mg/l), exposed to the biofilms.

Fig. 3b shows little difference between the nitrification potential of SA-treated and control soil on the first day of incubation, after which time a sharp decrease was observed for SA doses of 8, 16 and 32 mg/kg. Maximum inhibition of soil nitrification potential (13% to 28%) for the aforementioned treatments was observed on the 30th day of incubation, although this marked inhibitory effect completely disappeared at the end of the incubation period (50 days). As for the increasing activity with the incubation proceeded, a proper mechanism explaining this situation may be the tolerance and adaptation of soil micro-organisms. There can be an increase in population size of soil micro-organisms that tolerate or even degrade xenobiotic compound by induction of appropriate genes [37]. Several studies indicated that SA can be degraded by the adapted soil micro-organisms. For example, aerobic degradation of SA was observed with inoculum sources that were historically polluted with sulfonated aromatic amines [38].

A range of chemolithotrophic and chemoorganotrophic bacteria and fungi are capable of biological nitrification in soil, with the chemolithotrophic ammonia-oxidising bacteria (AOB) probably being the most important group [39]. Chemolithotrophic ammonia-oxidising bacteria play an important role in the conversion of ammonia to nitrite and are responsible for the rate limiting step of nitrification, making these bacteria key to the global nitrogen cycle [40]. Chemolithotrophic AOB obtain energy for growth from the oxidation of ammonia and assimilate carbon as carbon dioxide. Fig. 4 depicts the response of culturable AOB population sizes to RB5 and SA treatment. The estimates of total AOB numbers initially ranged between 4.11 and 4.45 log MPN/g soil in control and RB5-treated soil (Fig. 4a). Bacterial numbers decreased markedly in response to RB5 treatment ($p < 0.05$). The average inhibition of AOB numbers in that period was 5% to 13%, and at the end of the incubation the bacterial numbers tended to approximate control values.

The addition of SA elicited a different pattern in relation to the AOB population sizes (Fig. 4b). No inhibitory effect was observed in bacterial numbers during the first 20 days of incubation, where the average AOB number for that period was 4.41 and 4.36 log MPN/g for control and SA-treated soil, respectively. Subsequently, an apparent decrease was observed in soil treated with higher SA doses (16 and 32 mg/kg) and culturable bacterial numbers decreased to an average value of 3.40 log MPN/g soil.

Results from this study also demonstrated that the nitrification potential of the soil investigated correlated significantly with the population size of AOB ($r = 0.83$ and 0.74 for RB5 and SA treatment, respectively). Culturable AOB numbers and nitrification potential values in soil treated with RB5 (>20 mg/kg soil) and SA (>16 mg/kg soil) support the general view that nitrification and AOB are sensitive to soil pollutants [41]. Oranusi and Ogugbue [42] determined the tolerance of Nitrobacter to Orange II and Direct Blue 71 dyes by examining the total viable count and reported that Nitrobacter had a low tolerance to the azo dyes and that the level of tolerance decreased with time. They concluded that exposure of these nitrifying bacteria to the aforementioned dyes could adversely affect their activity in the biogeochemical cycle of nitrogen. Similarly, Yongjie and Bishop [43] reported that Acid Orange 7 inhibited all

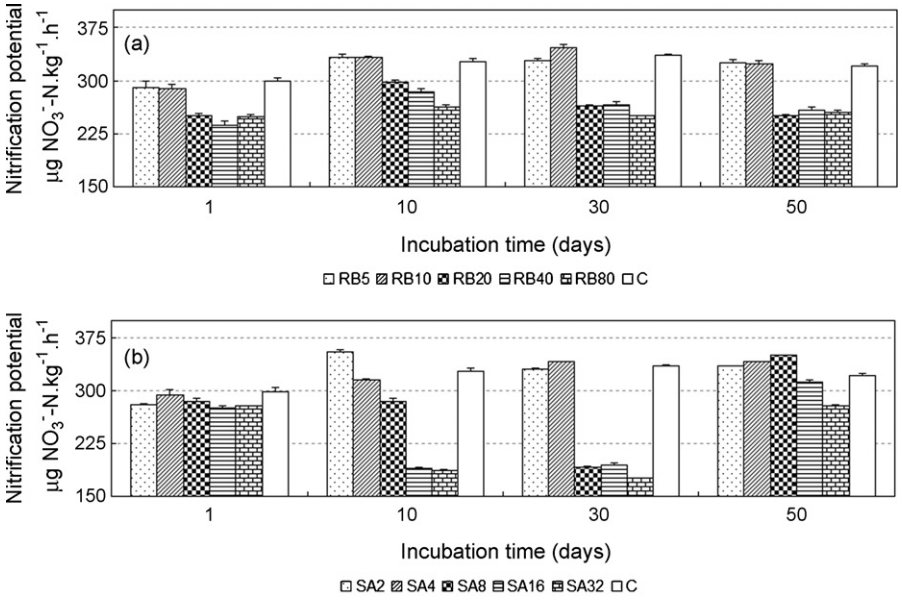

Fig. 3. Variation in nitrification potential of soil treated with varying doses of Reactive Black 5 (RB5) and sulfanilic acid (SA).

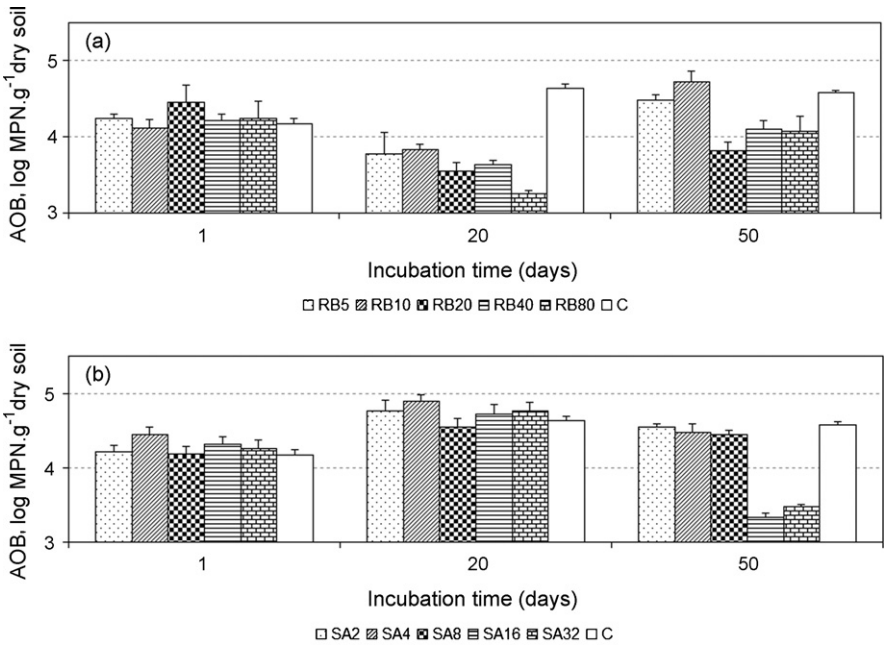

Fig. 4. Variation in ammonium oxidising bacteria of soil treated with varying doses of Reactive Black 5 (RB5) and sulfanilic acid (SA).

stages of the nitrification process and decreased substrate utilisation. Another study indicated that the first nitrification step, conversion of ammonium to nitrite by *Nitrosomanas,* was inhibited by the dye (azo dye acid black 1) bearing wastewater [44].

The effect of RB5 and SA treatment on culturable nitrite-oxidising bacteria (NOB) numbers of the soil investigated is shown in Fig. 5. Nitrite-oxidising bacteria catalyse the second step of aerobic nitrification, being the oxidation of nitrite to nitrate [45]. As shown in Fig. 5a, culturable bacterial numbers were not affected by the addition of varying doses of RB5 during the first 20 days of incubation. Culturable NOB numbers determined at the 50th day of incubation indicated that addition of 40 and 80 mg/kg RB5 resulted in a 16% to 30% decrease, whereas no inhibition was observed at lower doses. On the other hand, SA exposure did not decrease NOB numbers during the overall incubation period. The average population size of NOB was 3.46 and 3.55 log MPN/g soil for control and SA-treated soil, respectively (Fig. 5b). Nitrite oxidisers are generally believed to be lithoautotrophs. However, members of the genus *Nitrobacter* have been observed to grow heterotrophically, with each species exhibiting differing degrees of mixotrophy and diauxy on nitrite and simple organic compounds [46]. The possession of heterotrophic capabilities may help nitrite oxidisers maintain viability when nitrite oxidation is inhibited or when there is insufficient nitrite available, and may account for the observation that soil NOB were less sensitive to SA than AOB.

Pearson's correlation coefficients between pollutant concentrations and the soil parameters investigated are shown in Table 3. The urease activity, arginine ammonification rate, nitrification poten-

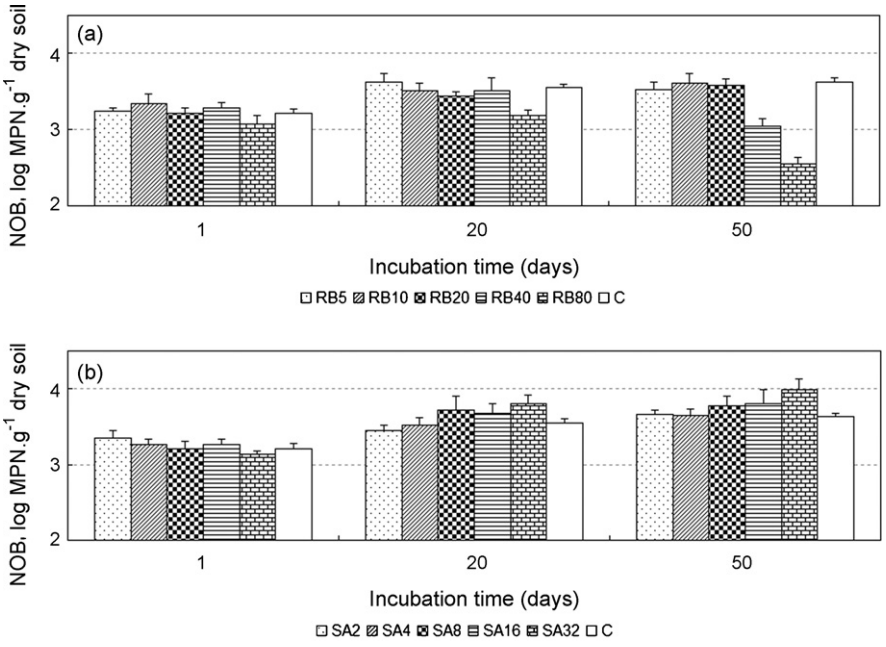

Fig. 5. Variation in nitrite oxidising bacteria of soil treated with varying doses of Reactive Black 5 (RB5) and sulfanilic acid (SA).

Table 3
Pearson's correlation coefficients (r) between the parameters examined and pollutant concentrations in RB5- and SA-treated soils.

	UA	NP	AAR	AOB	NOB
Reactive Black 5	−0.66*	−0.71*	−0.44*	−0.37	−0.63*
Sulfanilic acid	−0.73*	−0.64*	−0.71*	−0.34	0.22

Correlation coefficients marked by (*) are significant at the 0.001 level (two-tailed).

tial and nitrite oxidising bacteria counts in RB5-treated soil were all correlated to pollutant concentrations. These parameters were negatively correlated with the applied doses of RB5. In the case of SA treatment, the urease activity, nitrification potential and arginine ammonification rate correlated negatively with the applied SA doses. The number of ammonium and nitrite oxidisers in soil did not correlate with the SA concentration. It can be concluded from Table 3 that the nitrification potential indicated a stronger negative response ($r = -0.71$) to RB5 treatment, whereas stronger negative responses to increasing amounts of SA were observed for the urease activity and arginine ammonification rate ($r = -0.73$ and $r = -0.71$, respectively).

The aerobic mineralisation of azo compounds has been restricted to a few bacterial strains which utilise certain carboxyl-substituted structures. Adaptation experiments with these cultures to growth with the industrially important sulfonated analogues were unsuccessful [47]. Under the current experimental conditions (small soil pots and aerated incubation) the breakdown of dye molecule through reduction of azo linkage was not expected in RB5 added soils. Hence, the observed inhibition effects in RB5 added soils may be attributed mainly to the parent azo dye. In natural soil environments, organic dyes in well-aerated surface soils may probably be resistant to biodegradation. However, excess water in heavy-textured soils (for example, after heavy rainfall) can promote the formation of anoxic conditions in dye-contaminated soils. The cleavage of azo bond may be possible under such circumstances resulting in the formation of aromatic amines. Therefore, pollution of soils with organic dyes may also refer the aromatic amine pollution. The results of the current study apparently indicated that inhibition effects of RB5 on nitrogen related processes may continue after the possible reduction of parent dye to SA.

4. Conclusion

Monitoring of dye-polluted sites is yet often restricted to the quantification of the contaminant by means of complex chemical analyses. Because of the widespread soil pollution through dye-containing wastewater and sludge applications, simple risk assessment tools are urgently required especially in developing and under-developed countries. In view of the results obtained in the present paper, it can be concluded that nitrogen related processes in soil can be used as bioindicators of anthropogenic stress caused by organic dyes. The assessment of the potential nitrogen mineralisation and nitrification will probably facilitate the monitoring procedures because of their rapid response to dye-induced changes. The results of the study depicted that urease activity, arginine ammonification rate, nitrification potential and ammonium oxidising bacteria numbers decreased by 10–20% and 7–28% in the presence of RB5 (>20 mg/kg dry soil) and SA (>8 mg/kg dry soil), respectively. According to decreased levels of nitrogen mineralisation-nitrification and reduced numbers of nitrifiers observed in this study, it was concluded that organic dye pollution may restrict the nitrogen-use-efficiency of plants, thus further reducing the productivity of terrestrial ecosystems. Moreover, the response of soil microbiota to SA suggested that inhibition effects of organic dye may continue after the possible reduction of parent azo dye to associated aromatic amines. The need for complete mineralisation of organic dyes must be taken into consideration when deciding for the optimal remediation technology.

Acknowledgement

This study was supported by the Research Fund of the Uludağ University, project number 2007-24.

References

[1] A. Stolz, Basic and applied aspects in the microbial degradation of azo dyes, Appl. Microbiol. Biotechnol. 56 (2001) 69–80.
[2] H. Leub, Textile dyeing, in: K. Hunger (Ed.), Industrial Dyes, Chemistry, Properties and Applications, Wiley VCH, Weinheim, 2003, pp. 339–425.
[3] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[4] Q. Zhou, Chemical pollution and transport of organic dyes in water–soil–crop systems of the Chinese Coast, Bull. Environ. Contam. Toxicol. 66 (2001) 784–793.
[5] J.A. Libra, M. Borchert, L. Vigelahn, T. Storm, Two stage biological treatment of a diazo reactive textile dye and the fate of the dye metabolites, Chemosphere 56 (2004) 167–180.
[6] A. Pandey, P. Singh, L. Iyengar, Bacterial decolorization and degradation of azo dyes, Int. Biodeterior. Biodegrad. 59 (2007) 73–84.
[7] R.M. Melgoza, A. Cruz, G. Buitron, Anaerobic/aerobic treatment of colorants present in textile effluents, Water Sci. Technol. 50 (2004) 149–155.
[8] C. Wang, A. Yediler, D. Lienert, Z. Wang, A. Kettrup, Toxicity evaluation of reactive dyestuffs, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria *Vibrio fischeri*, Chemosphere 46 (2002) 339–344.
[9] Q. Zhou, J. Xu, Y. Cheng, Inhibitory effect of reactive X-3b red dye (RRD) on iron uptake by three crops, Plant Soil 261 (2004) 155–162.
[10] C. Pozo, M.V. Martĭnez-Toledo, B. Rodelas, J. González-López, Response of soil microbiota to the addition of 3,3-diaminobenzidine, Appl. Soil Ecol. 23 (2003) 119–126.
[11] G.W. Gee, J.W. Bauder, Particle size analysis, in: A. Klute (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1986, pp. 383–411.
[12] J.D. Rhoades, Soluble salts, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 167–179.
[13] E.O. Mc Lean, Soil pH and lime requirement, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp.199–224.
[14] D.K. Cassel, D.R. Nielsen, Field capacity and available water capacity, in: A. Klute (Ed.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods, ASA and SSSA, Agronomy Monograph No 9, Madison, WI, 1986, pp. 901–926.
[15] D.R. Keeney, D.W Nelson, Nitogen-inorganic forms, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No 9, Madison, WI, 1982, pp. 643–698.
[16] J.M. Bremner, C.S. Mulvaney, Nitrogen-total, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 595–624.
[17] D.W. Nelson, L.E. Sommers, Total Carbon, Organic Carbon and Organic Matter, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 539–579.
[18] M.A. Tabatabai, Soil enzymes, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 903–947.
[19] K. Alef, D. Kleiner, Arginine ammonification, a simple method to estimate microbial activity potentials in soil, Soil Biol. Biochem. 18 (1986) 233–235.
[20] D.R. Keeney, D.W. Nelson, Nitogen-inorganic forms, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 643–693.
[21] S.C. Hart, J.M. Stark, E.A. Davidson, M.K. Firestone, Nitrogen mineralisation, immobilization, and nitrification, in: R.W. Weaver, J.S. Angle, B.S. Bottomley (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties, ASA and SSSA, Book Series No. 5, Madison, WI, 1994, pp. 985–1018.
[22] D.A. Cataldo, M. Haroon, L.E. Schrader, V.L. Young, Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid, Commun. Soil Sci. Plan 6 (1975) 71–80.
[23] E.L. Schmidt, I.W. Besler, Nitrifying bacteria, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, ASA and SSSA, Agronomy Monograph No. 9, Madison, WI, 1982, pp. 1027–1042.
[24] W.G. Cochran, Estimation of bacterial densities by means of the most probable number, Biometrics 6 (1950) 105–116.
[25] L. Gianfreda, F. Sannino, N. Ortega, P. Nannipieri, Activity of free and immobilized urease in soil: effects of pesticides, Soil Biol. Biochem. 26 (1994) 777–784.

[26] P. Nannipieri, P. Sequi, P. Fusi, Humus and enzyme activity, in: A. Piccolo (Ed.), Humic Substances in Terrestrial Ecosystems, Elsevier, Amsterdam, 1996, pp. 293–327.
[27] S. Klose, M.A. Tabatabai, Urease activity of microbial biomass in soils, Soil Biol. Biochem. 31 (1999) 205–211.
[28] R. Margesin, G. Walder, F. Schinner, The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil, Acta Biotechnol. 20 (2000) 313–333.
[29] G. Shen, Y. Lu, Q. Zhou, J. Hong, Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme, Chemosphere 61 (2005) 1175–1182.
[30] D.K. Singh, S. Kumar, Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments, Chemosphere 71 (2008) 412–418.
[31] K.M. Rao, V.T. Rao, G.R. Babu, On interaction of a clayey soil with textile dye waste, Electron. J. Geotechnol. Eng. 13 (2008) 1–18.
[32] P. Nannipieri, L. Gianfreda, Kinetics of enzyme reactions in soil environments, in: P.M. Huang, N. Senesi, J. Buffle (Eds.), Structure and Surface Reactions of Soil Particles, J. Wiley & Sons, New York, 1998, pp. 449–479.
[33] A.T. Abdelal, Arginine catabolism by microorganisms, Annu. Rev. Microbiol. 33 (1979) 139–168.
[34] O. Dilly, Ammonification of amino acids in field, grassland and forest soils, in: H. Insam, A. Rangger (Eds.), Microbial Communities. Functional versus Structural Approaches, Springer, Berlin, 1997, pp. 248–260.
[35] J.N. Ladd, R.B. Jackson, Biochemistry of ammonification, in: F.J. Stevenson (Ed.), Nitrogen in Agricultural Soils, American Society of Agronomy Inc./Crop Science Society of America Inc./Soil Science Society of America Inc., Madison, WI, 1982, pp. 173–228.
[36] J. Li, P.L. Bishop, In situ identification of azo dye inhibition effects on nitrifying biofilms using microelectrodes, Water Sci. Technol. 46 (2002) 207–214.
[37] E.M. Top, D. Springael, The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds, Curr. Opin. Biotechnol. 14 (2003) 262–269.
[38] N.C.G. Tan, A. van Leeuwen, E.M. van Voorthuizen, P. Slenders, F.X. Prenafeta-Boldu, H. Temmink, G. Lettinga, J.A. Field, Fate and biodegredradability of sulfonated aromatic amines, Biodegradation 16 (2005) 527–537.
[39] K. Killham, Nitrification in coniferous forest soils, Plant Soil 128 (1990) 31–44.
[40] J.I. Prosser, T.M. Embley, Cultivation-based and molecular approaches to characterisation of terrestrial and aquatic nitrifiers, Antonie Van Leeuwenhoek 81 (2002) 165–179.
[41] L.E. Sverdrup, F. Ekelund, P.H. Krough, T. Nielsen, K. Johnsen, Soil microbial toxicity of eight polycyclic aromatic compounds: effects on nitrification, the genetic diversity of bacteria, and the total number of protozoan, Environ. Toxicol. Chem. 21 (2002) 1644–1650.
[42] N.A. Oranusi, C.J. Ogugbue, Toxic response of *Nitrobacter* to Orange II and Direct Blue 71, J. Appl. Sci. Environ. Manage. 6 (2002) 87–90.
[43] H. Yongjie, P.L. Bishop, Effect of Acid Orange 7 on nitrification process, J. Environ. Eng. ASCE 120 (1994) 108–121.
[44] R.W. Martin, C.R. Baillod, J.R. Mihelcic, Low-temperature inhibition of the activated sludge process by an industrial discharge containing the azo dye acid black 1, Water Res. 39 (2005) 17–28.
[45] R.M. Maier, I.L. Pepper, C.P. Gerba, Environmental Microbiology, second ed., Academic Press, London, 2008.
[46] E. Bock, H. Koops, H. Harms, Cell biology of nitrifying bacteria, in: J.I. Prosser (Ed.), Nitrification, IRL Press, Washington DC, 1986, pp. 17–38.
[47] T. Zimmermann, F. Gasser, H.G. Kulla, T. Leisinger, Comparison of two bacterial azoreductases acquired during adaptation to growth on azo dyes, Arch. Microbiol. 138 (1984) 37–43.