Kinetic approach to evaluate the effects of 3,3'-diaminobenzidine on N mineralization in soils

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ABSTRACT

It has been demonstrated that azo dyes, the xenobiotics widely use in industries, can pose threats to public health. 3,3'-diaminobenzidine (DAB), as a benzidine analogue, is generated from reduction of azo dyes by intestinal or environmental microorganisms. The compound was applied at doses of 0 and 100 mg N/kg into two soils of contrasting textural properties belonging among Haplic Calsisols (Juzdan soil) and Calcaric Cambisols (Shervedan soil) and the effects on N mineralization kinetics were evaluated. Kinetic behavior of N mineralization in either the control or the DAB-treated soils consistently followed first-order models. In the early 7–10 days of the incubation period, net N mineralization was adversely influenced by DAB. After the early 7–10 days of incubation, the accumulation of inorganic N was greater in DAB-treated soils than those of control soils. This finding indicated that the degradation of DAB was started. Potentially mineralizable N in Haplic Calsisols and Calcaric Cambisols were 1.1 and 1.4 times greater than those of controls, respectively. Similarly, initial potential rates of N mineralization in the DAB-treated soils were 1.3 and 1.1 times greater than those of controls, respectively. The potentially mineralizable N and initial potential rates of N mineralization in both soils were significantly (LSD, $P < 0.05$) increased when soils were treated with DAB. About 95 and 82% of the initial DAB-N were mineralized to inorganic N after 60 days of aerobic incubation, respectively. Overall, DAB can be a potentially toxic xenobiotic for soil N mineralization shortly after application and the rate of its inhibition or stimulation is time-dependent.

Keywords: xenobiotics; N mineralization; soil contamination; diaminobenzidine

Soil is a complex microhabitat regulating plant productivity and the maintenance of biogeochemical cycles by the activity of microorganisms able to degrade organic including xenobiotics (Nannipieri et al. 2003). It was comprehensively shown that azo dyes can pose threats to public health (Pielesz et al. 2002). Azo dyes are synthetic organic colorants used for research purposes and in the textile, hair dying, paper making and food industries (Meyer 1981). One of the analogues of benzidine is 3,3'-diaminobenzidine (DAB). DAB is an aromatic compound with molecular weight of 214.27, which shows its important potential toxicity and carcinogenic properties. This substance, as other benzidine analogues, is generated from the reduction of azo dyes by intestinal or environmental microorganisms (Chung et al. 1992, Chung and Stevens 1993)

Considerable amounts of information currently exist about the effects of DAB on animal cells and human health (Chung et al. 1992, Choudhary 1996). The toxicity of azo dyes for microbial populations and the processes of biotransformation and mineralization of organic compounds in aquatic environments is well documented (Chung and Stevens 1993), but far less is known about the effects of this substance on soil biological activities (Pozo et al. 2003). The negative effects of DAB on the growth and nitrogenase activity of *Azotobacter vinelandii* and *Azotobacter chroococcum* strains were reported previously (Chung et al. 1998, Pozo et al. 2000). Lower nitrogenase activity, ATP content and growth of *Azotobacter* and lower number of nitrifying and denitrifying bacteria (Pozo et al. 2003) were also reported when exposed to 5–50 mg DAB/kg (Pozo et al. 2000). In contrast, soil dehydrogenase, phosphatase and arylsulfatase activities responded positively to DAB (Pozo et al. 2003).

There is a lack of information on the effects of DAB on N availability in soil. Since DAB is an N containing compound, it can be hypothesized that DAB provides soil microbiota with a source of N that is presumably mineralizable to inorganic N. For this purpose, a kinetic approach is required to investigate the fate of DAB-N in soil. Therefore, the objective of this study was to investigate the effects of DAB on kinetic parameters of N mineralization in soils.

MATERIALS AND METHODS

Soil sampling. Two samples (0–15 cm) were collected from soils developed in the arid climate of Central Iran. The Juzdan soil was taken from an agricultural field (31°30'N, 51°55'E) under irrigated alfalfa cultivation. The Shervedan soil was taken from the Isfahan University of Technology, Research Station in Shervedan (32°30'N, 51°36'E). This soil was under irrigated conventional corn (*Zea mays* L.) monoculture. Both soils are calcareous and belong to the arid regions of Central Iran with 120 mm mean annual precipitation. The general properties of the soils are summarized in Table 1.

Triplicates of 100 g of sieved soil were placed in sterile glass vials. DAB was dissolved in acetone and the stock solution was added to the dishes, in order to reach concentration of 100 mg DAB/kg of dry weight soil. After thorough mixing, the treated soils were incubated at 25°C and 50% water holding capacity (WHC) under aerobic conditions for 60 days. Controls without DAB received the same amount of distilled water and acetone. During the 60 days of incubation, inorganic N was monitored by destructive sampling on 12 occasions at 0, 1, 2, 4, 7, 10, 15, 20, 25, 30, 40 and 60 days after incubation. Inorganic N was extracted with 2M KCl and measured by a steam distillation procedure (Keeney and Nelson 1982).

A first order kinetics equation was used to calculate the potentially mineralizable N (N_0) and N mineralization rate constant (k):

$$N_m = N_0 (1 - e^{-kt})$$

Where: N_m is the N mineralized at any specific time (t), and k is the first-order rate constant. The initial potential rate of N mineralization was calculated as $N_0 \times k$ (Burket and Dick 1998).

RESULTS AND DISCUSSION

The amounts of N mineralized in the Juzdan soil are shown for the DAB-treated and the control soils (Figure 1). During the early 7 days of incubation no significant difference was observed between the two treatments; however, the amounts of inorganic N released in the control soil were insignificantly greater than the DAB-treated soil. A similar trend was also observed for the Shervedan soil, so that N mineralized in the DAB-treated and the control soils during the first 10 days of incubation was not significantly different although, the amounts of inorganic N released was slightly greater in the control soil (Figure 2). This implies that DAB did not contribute to N mineralization process during the first 7 (in Juzdan soil) and 10 (in Shervedan soil) days of incubation. Moreover, the rate of N mineralized during the period was slightly greater in the control soil compared to the soils treated with 100 mg DAB/kg (Figures 1 and 2); it indicates that DAB can slightly inhibit N mineralization during the early period. Since N mineralization is basically a microbial process (Tate 2000), it can be hypoth-

Table 1. General properties of the soils studied

Soil properties	Juzdan	Shervedan
Soil type (FAO classification)	haplic calcisols	calcaric cambisols
Texture	sandy clay loam	clay
pH	7.5	8.0
Organic C (g/kg)	17.7	17.4
Electrical conductivity (dS/m)	5.1	1.8
Water saturation percentage (%)	43.8	57.7
Ca-carbonate equivalent (%)	46.5	37.0
Cation exchange capacity ($cmol_c$/kg)	29.8	39.4

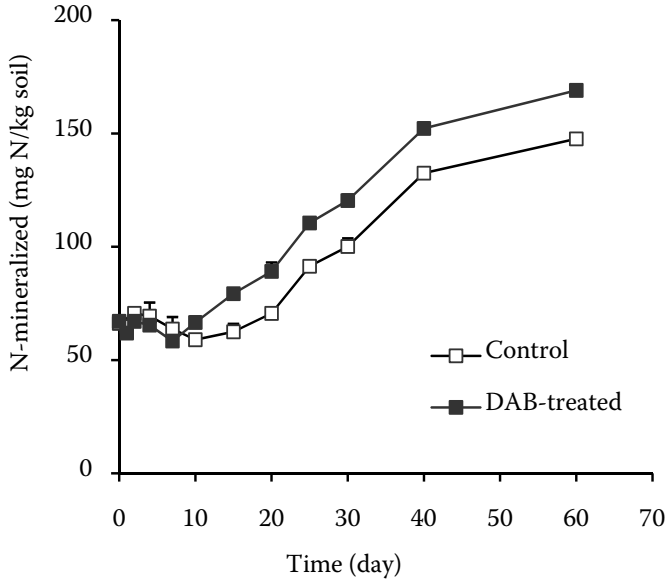

Figure 1. Inorganic nitrogen mineralized in the Juzdan soil during the incubation time

esized that N mineralizing populations of soil are adversely affected.

Following the early period (7–10 days), DAB application resulted in an increase of the amounts of N mineralized in both soils (Figures 1 and 2). The difference between DAB-treated and control soils remained significant during the rest of incubation period in both soils (Figures 1 and 2). It was shown that DAB enhanced the growth of viable soil bacteria for all tested doses (0–50 mg/kg), and heterotrophic counts were significantly higher compared with control after 14 days of treatments (Pozo et al. 2003). The promoting effects of benzidine and benzidine analogues on the growth and biological activity of *Azotobacter* were also reported, indicating that these microorganisms can tolerate high concentrations of benzidine (Pozo et al. 2000). These results are in agreement with our findings and indicate that DAB can stimulate the activity of soil microorganisms which participate in N mineralization processes. The positive effects of DAB on soil dehydrogenase are also reported (Pozo et al. 2003). Soil dehydrogenase activity and soil microbial biomass are associated (Dick et al. 1996); therefore, it can be concluded that DAB enhances soil microbial biomass. To our knowledge, the effect of DAB on the soil enzymes contributing to N mineralization (i.e. amidohydrolases) has not been investigated.

The effects of xenobiotic compounds on soil biological properties are time-dependent (Pozo et al. 2003, Yao et al. 2006). Viera et al. (2007) showed that as time elapsed, the negative effects of sulfentrazone herbicides on soil microbial biomass C decreased. We observed that the pattern of DAB effects on N mineralization varied

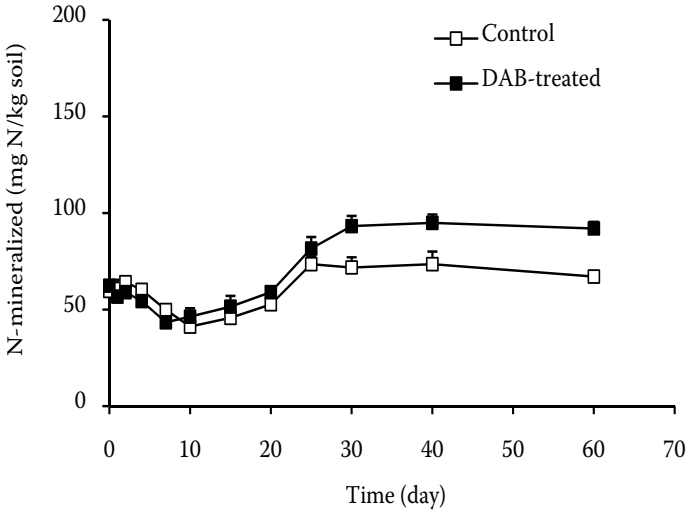

Figure 2. Inorganic nitrogen mineralized in the Shervedan soil during the incubation time

Table 2. Potentially mineralizable N (N_0) and initial potential rates (kN_0) of DAB-treated and control soils

Soil	DAB (mg/kg soil)	N_0	kN_0
Juzdan	0	175.9 (1.60)[b]	5.3 (0.05)[b]
	100	192.5 (2.20)[a]	7.1 (1.05)[a]
Shervedan	0	74.4 (1.70)[b]	5.7 (0.31)[b]
	100	102.1(1.74)[a]	6.1 (0.11)[a]

Values in parenthesis are standard deviation (n = 3). Dissimilar letters in each column for each soil indicate significant difference (LSD, $P < 0.05$)

with time (Figures 1 and 2). While the cumulative concentrations of inorganic N mineralized were statistically similar for control and DAB-treated soil during early 7–10 days, DAB-treated soil supported greater amounts of inorganic N after the early period (Figures 1 and 2). This finding is consistent with that of Pozo et al. (2003) which showed that the maximum difference of bacterial population between control and DAB-treated soil occurred after 14 days of incubation. Apparently, a time lag is required for soil microbiota to start degradation of DAB and consequently to release its N as inorganic N. The mechanism underlying the lag time is not well understood and needs further researches.

All results of N mineralization from DAB-treated soils conformed well to the exponential model $N_m = N_0 (1 - e^{-kt})$ described earlier. The first-order kinetic model was widely used in literature to describe the C (Ajwa and Tabatabai 1994, Martinez and Tabatabai 1997) and N mineralization kinetics (El-Gharous et al. 1990, Nourbakhsh and Alinejadian 2009).

The values of potentially mineralizable N (N_0) and initial potential rates of N mineralization (kN_0) are shown in Table 2. The N_0 values in DAB-treated soils were consistently greater than those of control in both soils. In the Juzdan and Shervedan soils, the average values of N_0 for DAB-treated soils were 1.1 and 1.4 times greater than those of controls, respectively. Similar trend was observed for kN_0. In the Juzdan and Shervedan soils, the average values of kN_0 for DAB-treated soils were 1.3 and 1.1 times greater than those of controls, respectively. In the current study the values of N_0 were calculated according to a 60-day aerobic incubation. These results imply that during the incubation period, DAB was gradually used as a source of N by the soil microbial population. The effects of management practices on N mineralization in soil were extensively investigated (Deng and Tabatabai 2000, Nourbakhsh and Alinejadian 2009). The greater rate of N mineralization in organic amendment-treated soils was attributed to the higher availability of organic N (Zaman et al. 2004). Therefore, the greater levels of aerobic N mineralization indices (N_0 and kN_0) in the DAB-treated soils can be attributed to the higher input of N that entered into the soils as DAB-N. The DAB-induced inorganic N (ΔN_i) was calculated as:

$$\Delta N_i \text{ (mg/kg)} = N_{i\ (DAB)} - N_{i\ (C)}$$

Where: $N_{i\ (DAB)}$ and $N_{i(C)}$ are the inorganic N in the DAB-treated and control soils, respectively. Dividing ΔN_i by the total N that was initially added to the soils as DAB-N (100 mg/kg) would provide the percentage of DAB-N that is mineralized to inorganic N during the incubation period. It was observed that 95 and 82% of the initial DAB-N were mineralized to inorganic N after 60 days of aerobic incubation, respectively (data not shown). Most of DAB-N was obviously converted to inorganic N; however, the fate of the rest of DAB molecule (C skeleton) in soil is still obscure.

Overall, our results clearly declare that in a short period of time (early 7–10 days of the incubation) DAB has a potential inhibitory effect on N mineralization, but in following gradual degradation of DAB in soil, most of DAB-N would be released as inorganic N. The results have supported our hypothesis suggesting that DAB can be used as N-containing substrate by native soil microbiota. Our results were obtained from two calcareous soils that predominantly occur in arid and semi-arid areas. To better understand the dynamics of DAB in soil, a study can be performed on acidic and/or tropical soils. Moreover, recognition of the transient organic molecules that are generated in DAB-contaminated soils is of great importance for further studies.

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