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Effects of Atrazine and Paraquat on Nitrifying Bacteria

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Abstract. The influence of atrazine and paraquat on a mixed culture of nitrifying bacteria was studied in an aqueous system. At a concentration as low as 1 μg/mL paraquat exerted a complete inhibition of ammonium and nitrite oxidation for 40 days. Atrazine (1 μg/mL and 2 μg/mL), however, caused only an inhibition of ammonium oxidation for a short period. After 16–18 days of inhibition the ammonium oxidizing activity was resumed. In the presence of atrazine (1 μg/mL), however, the rate of nitrite oxidation was increased. Within a period of 28 days, in culture media a partial degradation of atrazine occurred.

Herbicides play an important role in modern agriculture. However, they can also enter aquatic ecosystems by direct routes such as application for weed control or by indirect routes such as agricultural land drainage and runoff after intensive irrigation or heavy rainfall. Besides other factors, the magnitude of herbicide losses from soil is mostly related to the intensity of rainfall and proximity to the application date.

Due to increasing interest in non-tillage practices the use of paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) has enormously increased in recent years. Paraquat is also used for fence lines, pipelines and as a desiccant on potato vines, sugarcane, cotton and sunflower plants. All of these uses increase the possibility that more paraquat will enter aquatic ecosystems. Similar to paraquat, atrazine (2-chlor-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a widely used herbicide for a variety of crops. It is also used directly in aquatic systems for the control of aquatic weeds. In aquatic environments, nitrification causes depletion of oxygen and accumulation of nitrate (Hall and Jeffries 1984). Numerous studies on the influence of these two herbicides on nitrification in soil have been reported. Domsch and Paul (1974) reported that in soils these herbicides did not exert any effect on nitrification. However, very little is known about the influence on nitrification in aquatic ecosystems.

The aim of this work was to study the influence of atrazine and paraquat on a mixed culture of nitrifying bacteria in liquid media.

Materials and Methods

A mixed culture of nitrifying bacteria was enriched and isolated from a soil sample as previously described (Gadkari 1984b).

Media

No. 1: The medium for ammonium and nitrite oxidation was composed of $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 20 mg; $MgSO_4.7H_2O$, 100 mg; $FeSO_4.7H_2O$, 10 mg; $CaCl_2.2H_2O$, 10 mg; neutral red, 1 mg. The inorganic salts were dissolved in 1,000 mL HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid)-buffer (0.075 M), and the medium was adjusted with 6N KOH to pH 8.0.

No. 2: The medium for nitrite oxidation was prepared as previously described (Gadkari 1984b). It contained $NaNO_2$, 1.0 g; NaCl, 0.5 g; $MgSO_4.7H_2O$, 50 mg; KH_2PO_4, 150 mg; $(NH_4)_2Mo_7O_{24}.4H_2O$, 50 μg; $FeSO_4.7H_2O$, 150 μg; $CaCO_3$, 7 mg; and distilled water, 1,000 mL. The medium was adjusted with 6N KOH to pH 7.8.

Experimental Procedure

Ammonium and nitrite oxidation: Preculture and experimental cultures were grown in mineral medium No. 1. From precultures, 10 mL was transferred into 300 mL triple-baffled Erlen-

meyer flasks (flask with three indentations), each containing 150 mL of culture medium when added ammonium had been oxidized 70–80%.

Nitrite oxidation: Precultures were grown in medium No. 1 and experimental cultures were cultivated in medium No. 2. From a preculture in which 70–80% of the produced nitrite had been oxidized, 10 mL was transferred into 300 mL triple-baffled Erlenmeyer flasks, each containing 150 mL of culture medium.

All cultures were incubated on a rotary shaker (200 rpm) at 30°C. Every day, 0.5 to 1.0 mL aliquots were removed for chemical analysis.

Chemical Analysis

Ammonium, nitrite and nitrate were determined as described previously (Gadkari 1984b).

Calculations

Theoretically, 1.0 g $(NH_4)_2SO_4$/L gives after total oxidation of ammonium 696.96 mg NO_2^-/L. When this amount of nitrite is completely oxidized, the expected amount of NO_3^- is 939.39 mg/L. One g of added $NaNO_2$ after total oxidation, will maximally result in 898.55 mg NO_3^-/L.

Herbicides

Atrazine was obtained from Serva (Heidelberg, W. Germany) and paraquat was obtained from Riedel-de-Haeen (Hannover, W. Germany). A stock solution of atrazine was prepared by dissolving 15 mg atrazine in 1.0 mL chloroform. The atrazine solution (10 µL or 20 µL) was added to the growth medium (150 mL) to achieve a final herbicide concentration of 1 µg/mL or 2 µg/mL. 10 or 20 µL of chloroform/150 mL growth medium had no inhibitory effect on the nitrifying bacteria. A stock solution of paraquat was prepared by dissolving 150 mg paraquat in 100 mL distilled water. The paraquat solution (0.5 mL or 1.0 mL) was added to the growth medium (150 mL) to achieve a final concentration of 5 µg/mL or 10 µg/mL. Prior to aseptic addition in the growth medium, both herbicides were sterilized by passing through a sterile membrane filter (pore size, 0.22 µM).

Biotest

Culture media treated with herbicides were tested for their herbicidal effects by performing a biotest (Gadkari 1984a) in a petri plate with round filter paper (70 mm Ø). With 3 mL of a culture solution, the filter paper was moistened and on it 10 cress seeds were evenly distributed. From each culture, 3 replicates were prepared. For comparison, untreated culture medium was included. The petri plates were incubated at ambient temperature (18°–22°C). After 4 days of incubation the germination of seeds were recorded. In the control, the seedlings showed a healthy root and shoot development. The leaves were dark green. In the case of herbicides, even after 7 days of incubation there was either no growth or only small deformed roots were developed.

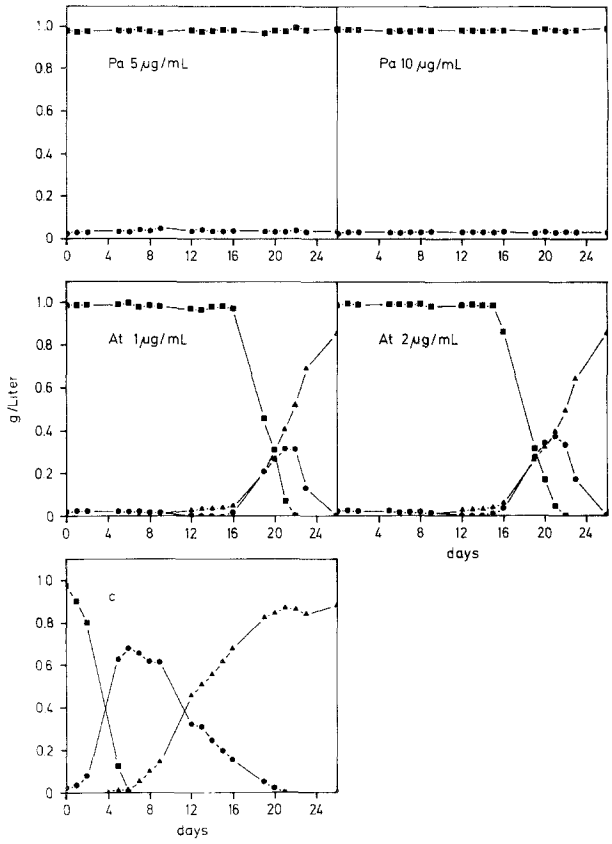

Fig. 1. Ammonium and nitrite oxidation in the presence of atrazine and paraquat. C, control; At, atrazine; Pa, Paraquat. ■——■, $(NH_4)_2SO_4$; ●——●, NO_2^-; ▲——▲, NO_3^-

Results

Control cultures of nitrifying bacteria were able to oxidize ammonium within 6–8 days (Figure 1). Nitrite formed via ammonium oxidation was further oxidized to nitrate over an incubation period of 20–22 days. In the presence of atrazine (1 µg/mL and 2 µg/mL) the ammonium oxidation was inhibited for 16–18 days (Figure 1). Thereafter, the rate of ammonium oxidation in these cultures was similar to that of the control. The total nitrification process was completed within 26–28 days.

To verify whether, after an inhibition period (16–18 days), the resulting rapid ammonium oxidation was due to adaptation of the culture to atrazine or not, a second experiment was performed where the preculture was grown in the presence of 2 µg atrazine/mL. From the preculture after complete

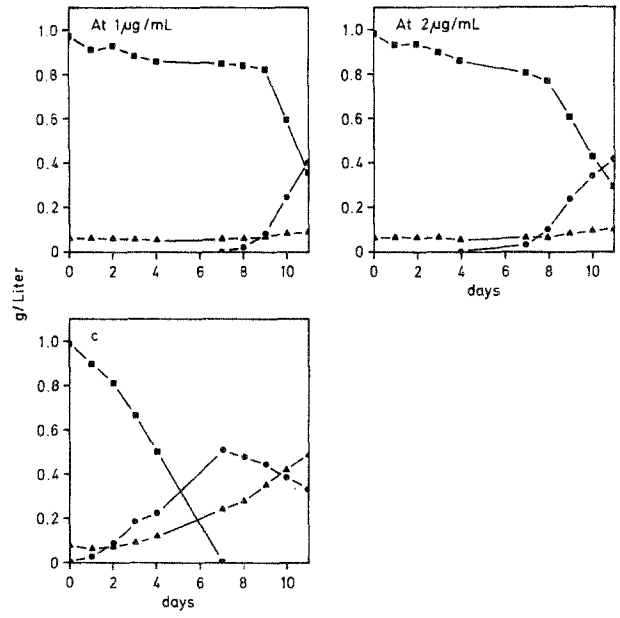

Fig. 2. Ammonium and nitrite oxidation in the presence of atrazine. Preculture was grown in the presence of 2 µg atrazine/mL. C, control; At, atrazine; ■——■, $(NH_4)_2SO_4$; ●——●, NO_2^-; ▲——▲, NO_3^-

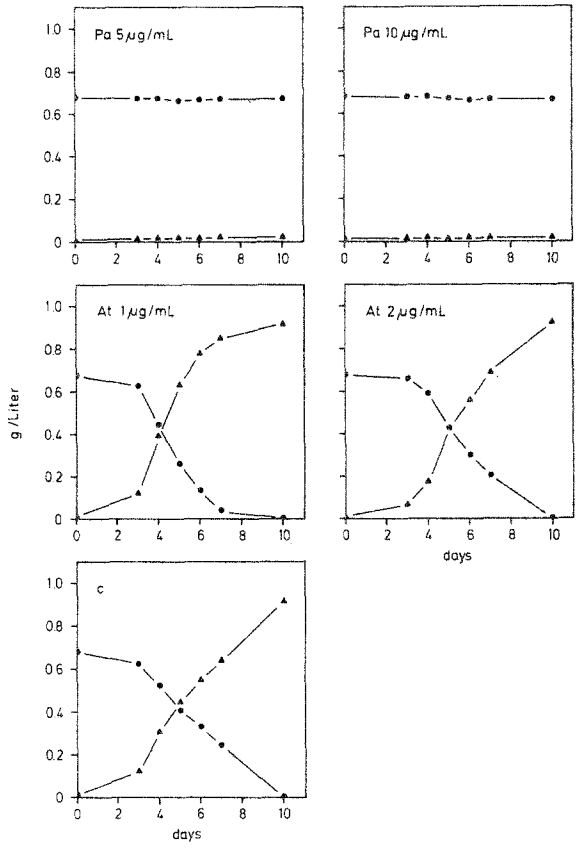

Fig. 3. Nitrite oxidation in the presence of atrazine and paraquat. C, control, At, atrazine; Pa, paraquat. ●——●, NO_2^-; ▲——▲, NO_3^-

oxidation of added ammonium new media containing atrazine (1 µg/mL and 2 µg/mL) and for comparison purpose untreated media were inoculated. The results given in Figure 2 revealed that in the presence of atrazine the ammonium oxidation was inhibited for 6–8 days. A biotest performed at the end of the experiments (28–30 days) showed that the culture media containing atrazine had partially lost their herbicidal activities.

Compared to cultures supplemented with atrazine, cultures in the presence of paraquat were very sensitive. In the presence of 5 µg/mL and 10 µg/mL paraquat, ammonium oxidation was totally inhibited (Figure 1). Even after a prolonged incubation period (40 days) the nitrifying activity was not resumed.

In order to understand the effects of atrazine and paraquat on the second oxidation step (nitrite oxidation), the cultures were inoculated only into medium No. 2. Under these conditions, atrazine did not exert any inhibitory influence on nitrite oxidation (Figure 3). Compared to the control, in the presence of 1 µg/mL atrazine, the rate of nitrite oxidation was slightly increased. In contrast, paraquat (5 µg/mL and 10 µg/mL) caused a complete inhibition of nitrite oxidation (Figure 3) during the total incubation period. Even after a prolonged incubation period (28 days) nitrite oxidation was not detected. The results presented in Figure 4 revealed that in the presence of a very low concentration of paraquat (1 µg/mL), ammonium and nitrite oxidation were inhibited for more than 18 days.

Discussion

In aqueous systems paraquat (0.9 µmol/L) was lethal to *Chlorella fusca* (Weber 1981). Paraquat (5 ppm) was also toxic to freshwater copepods (Naqvi *et al.* 1981). In this study, paraquat (1 µg/mL) caused complete inhibition of nitrification. In soil, even higher concentrations (100 µg/mL) of paraquat did not exert any influence on nitrification. It is known that paraquat is strongly adsorbed by negatively charged clay surfaces and, therefore, is not available to and cannot act on microorganisms (Mathur *et al.* 1976). In aqueous solution, the situation is different. In the absence of sufficient clay particles paraquat may remain in solution and thus be able to exert an inhibitory effect on nitrification. In aquatic systems for weed control, use of para-

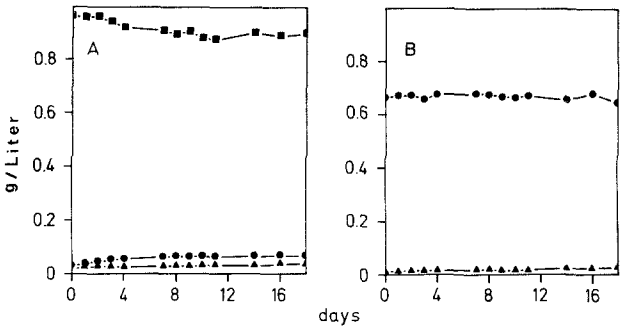

Fig. 4. Influence of 1 μg paraquat/mL on ammonium and nitrite oxidation. A, ammonium oxidation in medium No. 1; B, nitrite oxidation in medium No. 2. ■——■, $(NH_4)_2SO_4$; ●——●, NO_2^-; ▲——▲, NO_3^-

quat may be helpful to reduce the consumption of oxygen and the accumulation of nitrate.

In the presence of oxygen (good aeration), paraquat produces toxic superoxide radicals (Hassan and Fridovich 1978). In the present work, the nitrifying cultures were continuously shaken and thus were provided with sufficient oxygen. It is plausible that the effect of paraquat on nitrifying bacteria was due to superoxide radicals. Yamanaka (1983) reported that 4 μM paraquat inhibited the growth of *Nitrobacter agilis*, a nitrite oxidizer, but did not affect growth of *Nitrosomonas europaea*, which is an ammonium oxidizer. The results obtained in this study, where nitrite oxidation is concerned, are in accordance with the results of Yamanaka (1983), but clearly differ regarding ammonium oxidation. In the present work, ammonium oxidation was inhibited by 1 μg paraquat/mL (3.88 μM). In soil, among ammonium oxidizers, *Nitrosolobus* and *Nitrosospira* species are often dominant (Walker and Wikramasinghe 1979). The mixed culture used in this study most probably contained dominantly other species of ammonium oxidizers than *Nitrosomonas*. Since Yamanaka (1983) used pure culture of *Nitrosomonas europaea*, this may be a reason for the different results.

The observed average concentration of atrazine in various aquatic systems ranges from 15 μg/L to 200 μg/L (Glotfelty *et al.* 1984; Lay *et al.* 1984). Application of 100 μg/L atrazine caused complete disappearance of cyanobacteria (Herman *et al.* 1986). One mg atrazine/kg caused reduction of biomass and changes in species composition of periphyton (Kosinski 1984). At a concentration of 20 μg/L, atrazine reduces the nonpredatory insects in an aquatic system (Dewey 1986). Brockway *et al.* (1984) have stated that atrazine (50 μg/L) has a negative impact on primary producers. They also reported that in the presence of atrazine, nitrate concentration was increased and oxygen concentration was decreased. In this study, when compared to the control, the rate of nitrite oxidation was increased in the presence of 1 μg atrazine/mL (4.6 μM), which is in agreement with the results of Brockway *et al.* (1984).

In the present work, during an incubation period of 28 days, a partial loss of herbicidal activity of atrazine occurred. Apparently, atrazine at concentrations usually found in aquatic systems has no adverse effect on nitrification. On the contrary, it activates the second step of nitrification. However, paraquat (1 μg/mL) caused complete inhibition of nitrification. Thus, application of paraquat to aquatic systems may be helpful to improve the oxygen status of aquatic environments. Additionally, due to inhibition of nitrification, the production of nitrate may be restricted.

Since laboratory studies give only an indication of the effects herbicides may exert in nature, they require additional studies in natural environments.

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