

## DIFFERENTIAL SENSITIVITY OF $\text{NH}_4^+$ AND $\text{NO}_2^-$ OXIDATION TO $\text{CdCl}_2$ AND $\text{AlCl}_3$ IN AN AQUEOUS SYSTEM

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### Abstract

The effects of different concentrations of  $\text{CdCl}_2$  and  $\text{AlCl}_3$  on  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation in an aqueous system were investigated.  $\text{AlCl}_3$  had no effect on  $\text{NH}_4^+$  removal and its oxidation to  $\text{NO}_2^-$ , whereas  $\text{NO}_2^-$  disappearance was greatly enhanced by  $\text{AlCl}_3$ . In contrast,  $\text{CdCl}_2$  delayed removal of  $\text{NH}_4^+$  and its subsequent oxidation to  $\text{NO}_2^-$  and also decreased the rate of  $\text{NO}_2^-$  disappearance. When compared to the controls,  $\text{NO}_3^-$  production from  $\text{NH}_4^+$  oxidation decreased severely by  $\text{CdCl}_2$  whereas slight increase in  $\text{NO}_3^-$  production was observed in the presence of  $\text{AlCl}_3$ . The results presented provide evidence for differential sensitivity of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation to  $\text{AlCl}_3$  and  $\text{CdCl}_2$  in an aquatic environment.

**Keywords:** Sensitivity;  $\text{CdCl}_2$ ;  $\text{AlCl}_3$

### Introduction

Nitrification, the biological oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  is carried out by nitrifying bacteria; *Nitrosomonas* which oxidizes  $\text{NH}_4^+$  to  $\text{NO}_2^-$  and *Nitrobacter* which oxidizes  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Addition of toxic substances to soil and water bodies may exert a significant impact on the performance of nitrifying bacteria and hence on the nitrogen balance in the environment [2,7,14]. Lee *et al.* [7] reported inhibitory effects of  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  on *Nitrosomonas* sp. and *Nitrobacter* sp. in continuous flow stirred tank reactors. Sato *et al.* [11-13] found that the growth inhibition of *Nitrosomonas europaea* in batch system was highly correlated to the amine compounds of copper and nickel. Significant reduction of nitrifying activity was detected when simulated acid rain was added to copper-amended soil [2]. Addition of lead salts influenced the nitrification processes in soil

[6]. The nitrate formation decreased with increase in lead salts concentrations. Singha *et al.* [14] studied the effect of  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$  alone and in combination on the N transformation in a loamy-sand soil. Addition of metals in combination caused a severe reduction in nitrification and ammonification. Comparison of the results of bacteria-plate-counts in the presence of heavy metals showed that all tested heavy metals had more toxic effects on isolated soil microorganisms in agar media than in soil [1].

Removal of ammonia from water bodies is an important factor in the survival of aquatic biota. Uptake of ammonia by phytoplanktons and biological nitrification are among the major mechanisms for the removal of ammonia [3,5]. The presence of  $\text{AlCl}_3$  and  $\text{CdCl}_2$  in agricultural and industrial waste waters is an ever increasing hazard to various biological activities. To our knowledge, there is no report of impacts of  $\text{AlCl}_3$

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or CdCl<sub>2</sub> on nitrification in an aquatic system. Experiments on the effects of heavy metals on nitrification are mainly carried out in different soil types. In the present study, we report the effects of the AlCl<sub>3</sub> and CdCl<sub>2</sub> on the fate of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> in an aqueous system.

### Materials and Methods

Water samples containing different populations of bacteria including *Nitrosomonas* sp. and *Nitrobacter* sp. were collected in 25-L containers from a pond 25 km west of Shiraz, immediately transferred to the laboratory and kept aerated at room temperature. Either AlCl<sub>3</sub> or CdCl<sub>2</sub> was added to 1-L flasks containing 900 ml of water sample to produce the concentration range of 0, 15, 60, 120 and 300 μM AlCl<sub>3</sub> and 0, 5, 25, 50 and 100 μM CdCl<sub>2</sub>. Then, either ammonium sulfate or sodium nitrite was added to each flask to give a final concentration of 2600 μg/L for NH<sub>4</sub><sup>+</sup>-N and 1200 μg/L for NO<sub>2</sub><sup>-</sup>-N at the beginning of experiments. The pH of each flask was adjusted to 7.8 with 0.1 M NaOH and the flasks were placed on orbital shaker (model SGM-300-010U) at 130 rpm and kept at room temperature (~25°C) for the entire length of the experiments. There were three replicates for each experiment.

Samples were taken every other day from each flask and the amount of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were determined by the colorimetric procedure described by Stainton *et al.* [15]. Ammonium concentration was measured at 640 nm and NO<sub>2</sub><sup>-</sup> concentration was determined at 543 nm using Shimatzo double - beam spectrophotometer (model UV-160 A). Nitrate was analyzed after being reduced to NO<sub>2</sub><sup>-</sup> by passing through a cadmium column and measuring NO<sub>2</sub><sup>-</sup> as described.

### Results

#### *Effects of AlCl<sub>3</sub> and CdCl<sub>2</sub> on NH<sub>4</sub><sup>+</sup> Removal*

As shown in Figure 1, the NH<sub>4</sub><sup>+</sup> content of water samples was depleted 11 days after the start of the experiment. The presence of 15, 60, 120 and 300 μM AlCl<sub>3</sub> in the media had no effect on the NH<sub>4</sub><sup>+</sup> depletion. In contrast, NH<sub>4</sub><sup>+</sup> depletion was affected by CdCl<sub>2</sub> (Fig. 2). In the absence of CdCl<sub>2</sub>, NH<sub>4</sub><sup>+</sup> disappeared after 11 days from the start of the experiment. In the presence of 5, 25 and 50 μM CdCl<sub>2</sub>, the removal of NH<sub>4</sub><sup>+</sup> began after a lag period and completed on day 15, 21, and 25, respectively. The lag period was longer with increased CdCl<sub>2</sub> concentrations, but the rate of NH<sub>4</sub><sup>+</sup> removal (slopes of the lines) were nearly identical up to 50 μM

CdCl<sub>2</sub>. At 100 μM CdCl<sub>2</sub>, only 54% decrease in NH<sub>4</sub><sup>+</sup> was observed after 27 days from the start of the experiment. Since the values for three replications in each treatment were close to each other, to avoid crowding, only the means are presented and the bars for standard errors are not included in the figures.

#### *Effects of the AlCl<sub>3</sub> and CdCl<sub>2</sub> on NO<sub>2</sub><sup>-</sup> Removal*

As shown in Figure 3, AlCl<sub>3</sub> stimulated the removal of NO<sub>2</sub><sup>-</sup>. In the absence of AlCl<sub>3</sub>, a 75% decrease in NO<sub>2</sub><sup>-</sup> content of water samples occurred after 21 days from the start of the experiment. The rate of NO<sub>2</sub><sup>-</sup> removal increased with increasing AlCl<sub>3</sub> concentration. At 300 μM AlCl<sub>3</sub>, complete depletion of NO<sub>2</sub><sup>-</sup> occurred on day 17. In contrast, NO<sub>2</sub><sup>-</sup> removal decreased with increasing CdCl<sub>2</sub> concentration. In control water samples, NO<sub>2</sub><sup>-</sup> depletion was completed after 23 days, where as at 100 μM CdCl<sub>2</sub>, NO<sub>2</sub><sup>-</sup> content remained relatively unchanged (Fig. 4).

#### *Effects of AlCl<sub>3</sub> and CdCl<sub>2</sub> on NO<sub>2</sub><sup>-</sup> Production from NH<sub>4</sub><sup>+</sup> and Its Subsequent Disappearance*

In the presence of different concentrations of AlCl<sub>3</sub> the production of NO<sub>2</sub><sup>-</sup> increased up to 11 to 13 days from the start of experiment (Fig. 5). These results and the trend of NH<sub>4</sub><sup>+</sup> removal shown in Figure 1, suggest that NH<sub>4</sub><sup>+</sup> oxidation is the principal source of observed NO<sub>2</sub><sup>-</sup> production. Nitrite produced by NH<sub>4</sub><sup>+</sup> oxidation subsequently disappeared from the media (Fig. 5). The pattern of NO<sub>2</sub><sup>-</sup> disappearance as affected by different concentrations of AlCl<sub>3</sub> is similar to the pattern of NO<sub>2</sub><sup>-</sup> removal shown in Figure 3. This suggests the endogenously produced NO<sub>2</sub><sup>-</sup> and exogenous NO<sub>2</sub><sup>-</sup> are probably removed by the same mechanism.

In the presence of different concentrations of CdCl<sub>2</sub> (Fig. 6), the increase in NO<sub>2</sub><sup>-</sup> closely followed the decrease in NH<sub>4</sub><sup>+</sup> shown in Figure 2. The production of NO<sub>2</sub><sup>-</sup> from NH<sub>4</sub><sup>+</sup> oxidation was delayed by CdCl<sub>2</sub>, but the amounts of NO<sub>2</sub><sup>-</sup> produced in the presence of 5, 25 and 50 μM CdCl<sub>2</sub> were essentially the same as in control (Fig. 6). CdCl<sub>2</sub> slowed the rate of NO<sub>2</sub><sup>-</sup> disappearance which is similar to the pattern of exogenous NO<sub>2</sub><sup>-</sup> removal shown in Figure 4.

#### *Effect of AlCl<sub>3</sub> and CdCl<sub>2</sub> on NO<sub>3</sub><sup>-</sup> Production*

The amount of nitrate as the end product of nitrification was not significantly affected by AlCl<sub>3</sub>. At 0, 15, 60, 120 and 300 μM AlCl<sub>3</sub>, the amounts of NO<sub>3</sub><sup>-</sup>-N produced from NH<sub>4</sub><sup>+</sup> oxidation by day 31 were 1975, 2114, 2114, 2118 and 2114 μg/L, respectively. In

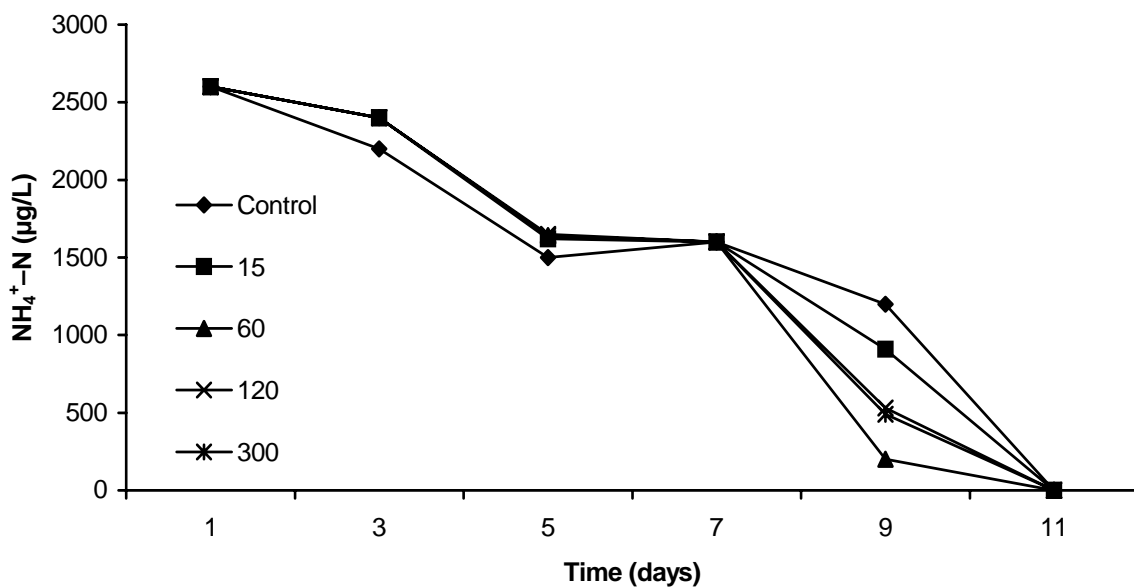


Figure 1.  $\text{NH}_4^+$  removal as affected by different concentrations ( $\mu\text{M}$ ) of  $\text{AlCl}_3$ .

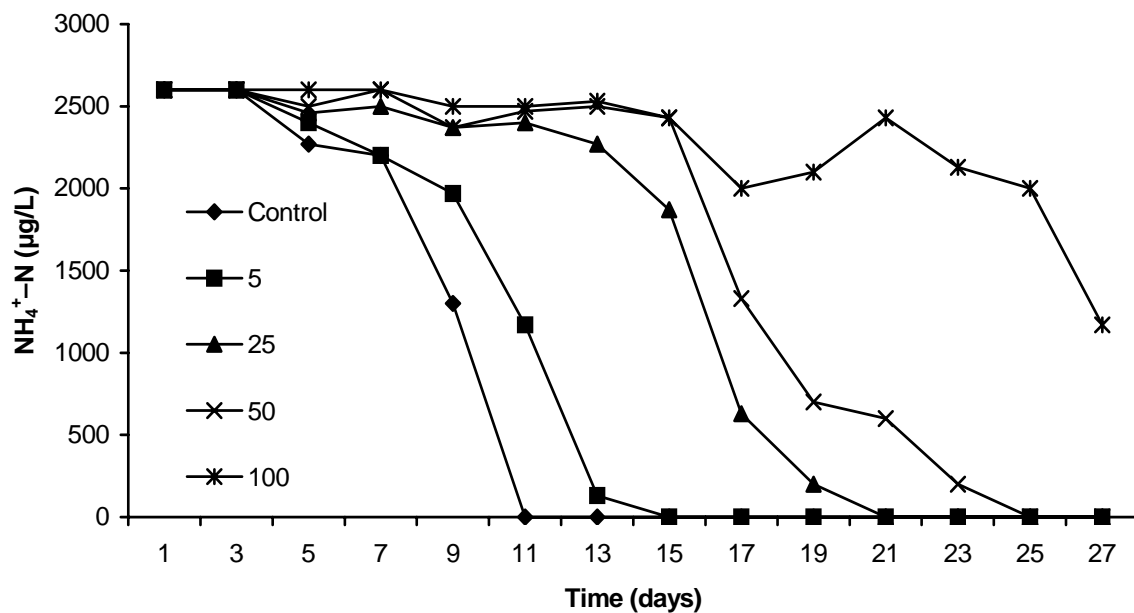


Figure 2.  $\text{NH}_4^+$  removal as affected by different concentrations ( $\mu\text{M}$ ) of  $\text{CdCl}_2$ .

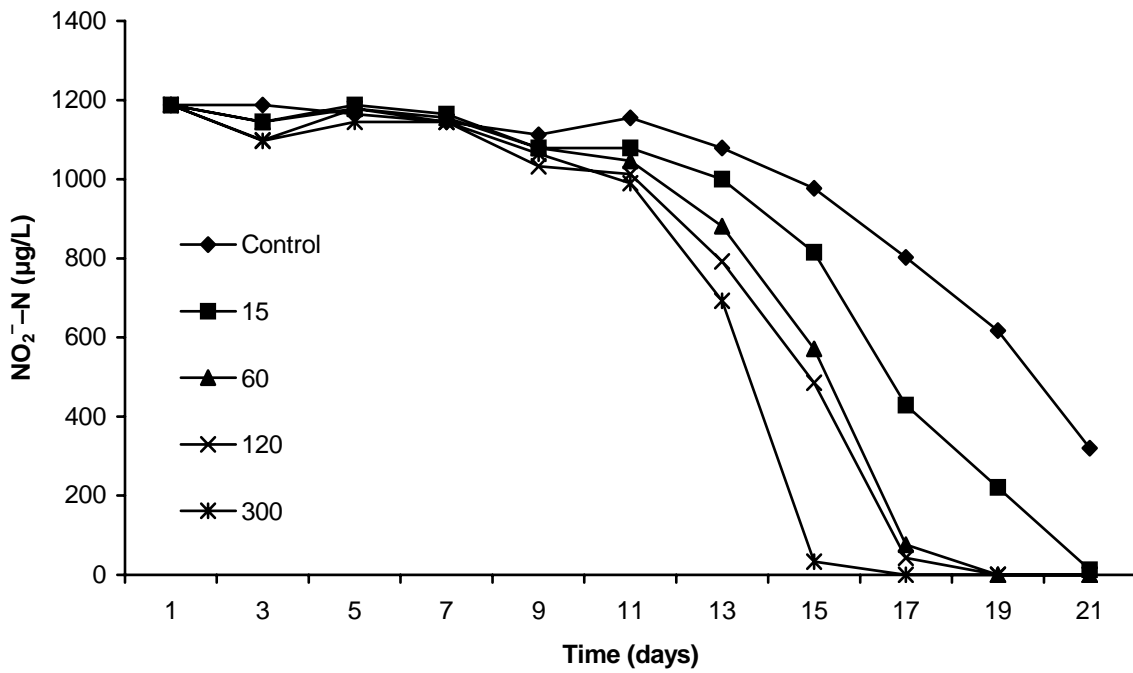


Figure 3. Effects of different concentrations ( $\mu\text{M}$ ) of  $\text{AlCl}_3$  on  $\text{NO}_2^-$  removal.

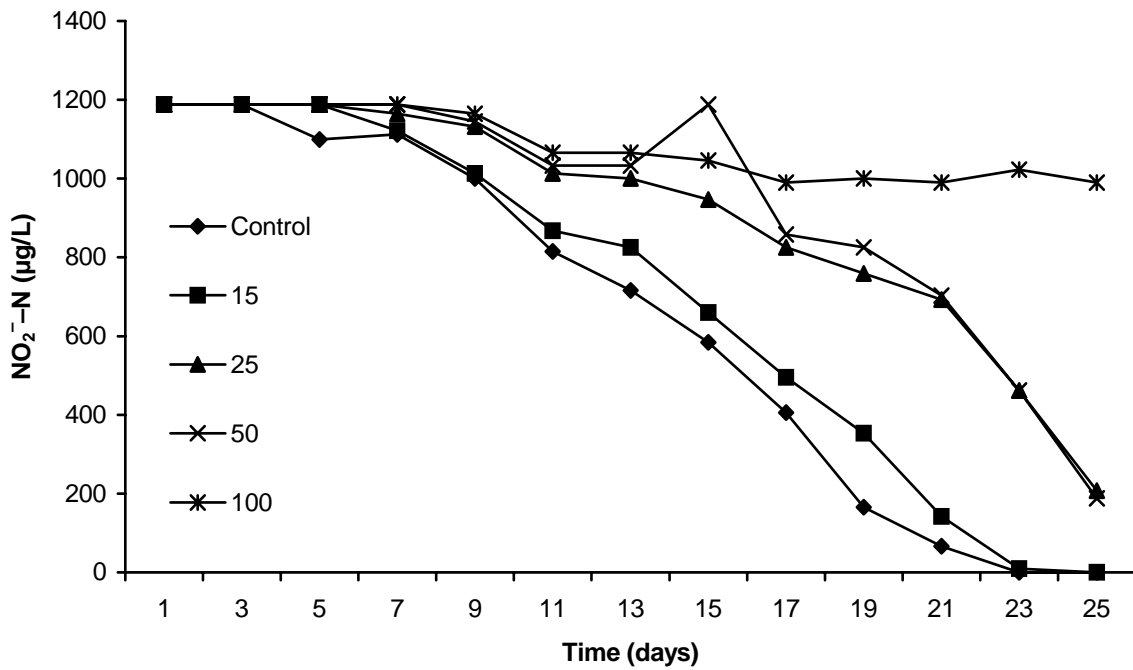
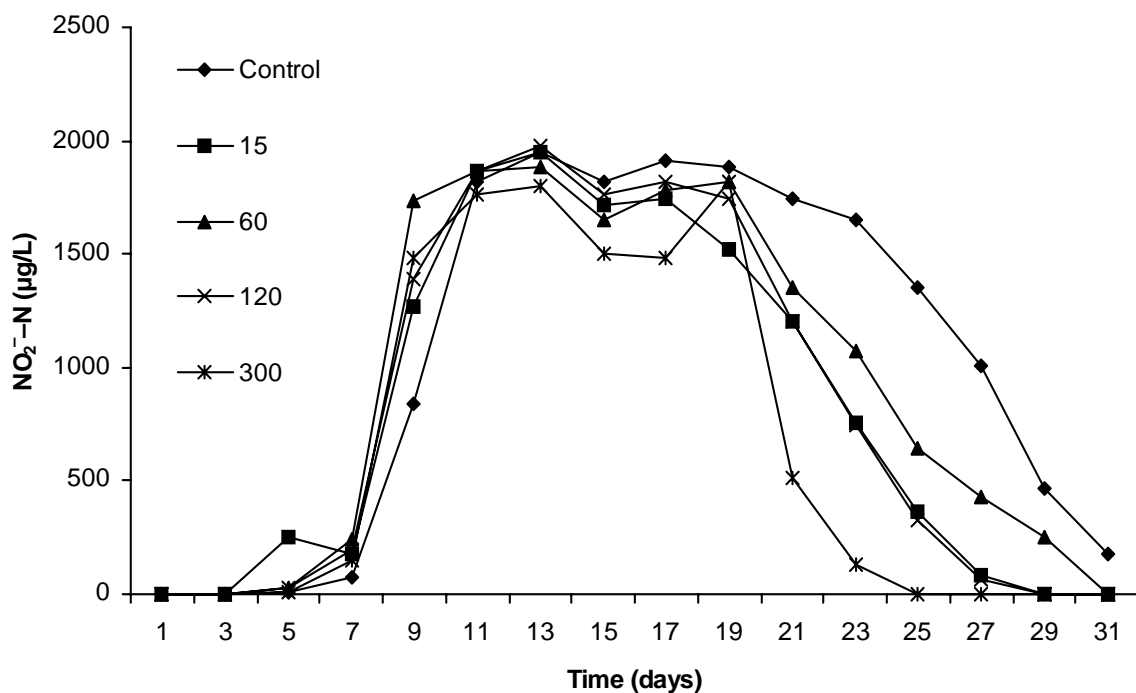
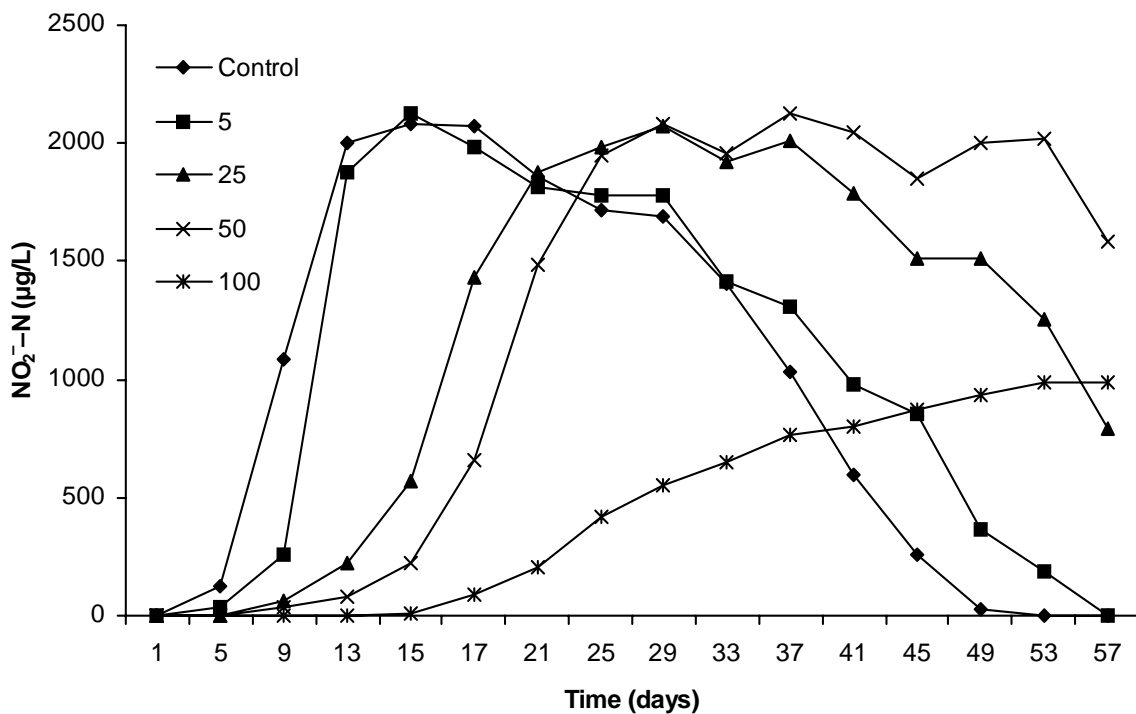


Figure 4. Effects of different concentrations ( $\mu\text{M}$ ) of  $\text{CdCl}_2$  on  $\text{NO}_2^-$  removal.



**Figure 5.** Production of  $\text{NO}_2^-$  from  $\text{NH}_4^+$  and its subsequent disappearance as affected by different concentrations ( $\mu\text{M}$ ) of  $\text{AlCl}_3$ . At the start of experiment, 2600  $\mu\text{g/L}$   $\text{NH}_4^+ \text{-N}$ , as ammonium sulfate, was added to each flask.



**Figure 6.** Production of  $\text{NO}_2^-$  from  $\text{NH}_4^+$  and its subsequent disappearance as affected by different concentrations ( $\mu\text{M}$ ) of  $\text{CdCl}_2$ . At the start of experiment, 2600  $\mu\text{g/L}$   $\text{NH}_4^+ \text{-N}$ , as ammonium sulfate, was added to each flask.

contrast,  $\text{NO}_3^-$  production from  $\text{NH}_4^+$  oxidation was severely reduced in the presence of  $\text{CdCl}_2$ . By day 57,  $\text{NO}_3^-$ -N produced at 0, 5, and 25  $\mu\text{M}$   $\text{CdCl}_2$  were 1975, 1805 and 756  $\mu\text{g/L}$ , respectively. At 50 and 100  $\mu\text{M}$   $\text{CdCl}_2$ ,  $\text{NO}_3^-$  production was completely inhibited. Although these results are in agreement with those shown in Figures 5 and 6, the amounts of  $\text{NO}_3^-$  recovered were not stoichiometrically equal to the amount of  $\text{NH}_4^+$  added at the beginning of the experiments. Nitrate assimilation by microorganisms present in culture solutions or lost by denitrification may account for the differences.

### Discussion

In the present work, biological nitrification might have been responsible for the removal of  $\text{NH}_4^+$  and  $\text{NO}_2^-$ . If so, it is suggested that  $\text{AlCl}_3$  possibly enhanced  $\text{NO}_2^-$  oxidation by increasing the activity of *Nitrobacter* sp. but had no effect on the oxidation of  $\text{NH}_4^+$  by *Nitrosomonas* sp. In contrast,  $\text{CdCl}_2$  possibly reduced the activities of both groups of bacteria. Differential sensitivity of nitrifying bacteria to light,  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  has been reported by several investigators [3,7]. Olson [10] and Ward *et al.* [16] have reported the differential photoinhibition of the two steps of the nitrification process and later Diab and Shilo [3] showed that *Nitrobacter* sp. was more sensitive to light than *Nitrosomonas* sp. which could possibly cause accumulation of nitrite in water bodies especially in fish ponds. Lee *et al.* [7] showed that *Nitrosomonas* sp. was more sensitive to  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  than *Nitrobacter* sp. Inhibition of nitrification by cadmium has been shown in several experiments [3,4,14]. Laboratory addition of 10, 100 and 500 mg  $\text{Cd}^{2+}/\text{kg}$  dry soil as  $\text{CdCl}_2$ , lowered the ability of soil to nitrify 100  $\mu\text{g}$  added  $\text{NH}_4^+$ -N/g dry soil [4]. Nitrification in a loamy-sand soil amended with sewage sludge decreased significantly in the presence of  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$  [14]. Influence of  $\text{Al}^{3+}$  on nitrification has also been studied by several investigators [8,9]. In alfalfa and straw-amended soil, aluminum sulfate retarded nitrification [8]. Mandal and Parsons [9] showed that addition of  $\text{AlCl}_3$  and 0.1 M HCl to peat, inhibited nitrification but had stimulatory effect on ammonification. They suggested the presence of an active acid adapted strain of nitrifiers in the peat.

Further investigations on the effects of  $\text{Al}^{3+}$  and heavy metals on the activity and viability of isolated *Nitrosomonas* and *Nitrobacter* are in progress for better understanding of the nitrogen balance in aquatic environment.

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