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DIFFERENTIAL SENSITIVITY OF NH_4^+ AND NO_2^- OXIDATION TO $CdCl_2$ AND $AlCl_3$ IN AN AQUEOUS SYSTEM

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Abstract

The effects of different concentrations of $CdCl_2$ and $AlCl_3$ on NH_4^+ and NO_2^- oxidation in an aqueous system were investigated. $AlCl_3$ had no effect on NH_4^+ removal and its oxidation to NO_2^-, whereas NO_2^- disappearance was greatly enhanced by $AlCl_3$. In contrast, $CdCl_2$ delayed removal of NH_4^+ and its subsequent oxidation to NO_2^- and also decreased the rate of NO_2^- disappearance. When compared to the controls, NO_3^- production from NH_4^+ oxidation decreased severely by $CdCl_2$ whereas slight increase in NO_3^- production was observed in the presence of $AlCl_3$. The results presented provide evidence for differential sensitivity of NH_4^+ and NO_2^- oxidation to $AlCl_3$ and $CdCl_2$ in an aquatic environment.

Keywords: Sensitivity; $CdCl_2$; $AlCl_3$

Introduction

Nitrification, the biological oxidation of NH_4^+ to NO_3^- is carried out by nitrifying bacteria; *Nitrosomonas* which oxidizes NH_4^+ to NO_2^- and *Nitrobacter* which oxidizes NO_2^- to 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 Cu^{2+} and 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 Zn^{2+}, Cd^{2+} and 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 $AlCl_3$ and $CdCl_2$ 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 $AlCl_3$

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or $CdCl_2$ 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 $AlCl_3$ and $CdCl_2$ on the fate of NH_4^+ and NO_2^- 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 $AlCl_3$ or $CdCl_2$ 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 $AlCl_3$ and 0, 5, 25, 50 and 100 μM $CdCl_2$. Then, either ammonium sulfate or sodium nitrite was added to each flask to give a final concentration of 2600 μg/L for NH_4^+–N and 1200 μg/L for NO_2^-–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_4^+, NO_2^- and NO_3^- were determined by the colorimetric procedure described by Stainton *et al.* [15]. Ammonium concentration was measured at 640 nm and NO_2^- concentration was determined at 543 nm using Shimatzo double - beam spectrophotometer (model UV-160 A). Nitrate was analyzed after being reduced to NO_2^- by passing through a cadmium column and measuring NO_2^- as described.

Results

Effects of $AlCl_3$ and $CdCl_2$ on NH_4^+ Removal

As shown in Figure 1, the NH_4^+ content of water samples was depleted 11 days after the start of the experiment. The presence of 15, 60, 120 and 300 μM $AlCl_3$ in the media had no effect on the NH_4^+ depletion. In contrast, NH_4^+ depletion was affected by $CdCl_2$ (Fig. 2). In the absence of $CdCl_2$, NH_4^+ disappeared after 11 days from the start of the experiment. In the presence of 5, 25 and 50 μM $CdCl_2$, the removal of NH_4^+ began after a lag period and completed on day 15, 21, and 25, respectively. The lag period was longer with increased $CdCl_2$ concentrations, but the rate of NH_4^+ removal (slopes of the lines) were nearly identical up to 50 μM $CdCl_2$. At 100 μM $CdCl_2$, only 54% decrease in NH_4^+ 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 $AlCl_3$ and $CdCl_2$ on NO_2^- Removal

As shown in Figure 3, $AlCl_3$ stimulated the removal of NO_2^-. In the absence of $AlCl_3$, a 75% decrease in NO_2^- content of water samples occurred after 21 days from the start of the experiment. The rate of NO_2^- removal increased with increasing $AlCl_3$ concentration. At 300 μM $AlCl_3$, complete depletion of NO_2^- occurred on day 17. In contrast, NO_2^- removal decreased with increasing $CdCl_2$ concentration. In control water samples, NO_2^- depletion was completed after 23 days, where as at 100 μM $CdCl_2$, NO_2^- content remained relatively unchanged (Fig. 4).

Effects of $AlCl_3$ and $CdCl_2$ on NO_2^- Production from NH_4^+ and Its Subsequent Disappearance

In the presence of different concentrations of $AlCl_3$ the production of NO_2^- increased up to 11 to 13 days from the start of experiment (Fig. 5). These results and the trend of NH_4^+ removal shown in Figure 1, suggest that NH_4^+ oxidation is the principal source of observed NO_2^- production. Nitrite produced by NH_4^+ oxidation subsequently disappeared from the media (Fig. 5). The pattern of NO_2^- disappearance as affected by different concentrations of $AlCl_3$ is similar to the pattern of NO_2^- removal shown in Figure 3. This suggests the endogenously produced NO_2^- and exogenous NO_2^- are probably removed by the same mechanism.

In the presence of different concentrations of $CdCl_2$ (Fig. 6), the increase in NO_2^- closely followed the decrease in NH_4^+ shown in Figure 2. The production of NO_2^- from NH_4^+ oxidation was delayed by $CdCl_2$, but the amounts of NO_2^- produced in the presence of 5, 25 and 50 μM $CdCl_2$ were essentially the same as in control (Fig. 6). $CdCl_2$ slowed the rate of NO_2^- disappearance which is similar to the pattern of exogenous NO_2^- removal shown in Figure 4.

Effect of $AlCl_3$ and $CdCl_2$ on NO_3^- Production

The amount of nitrate as the end product of nitrification was not significantly affected by $AlCl_3$. At 0, 15, 60, 120 and 300 μM $AlCl_3$, the amounts of NO_3^-–N produced from NH_4^+ oxidation by day 31 were 1975, 2114, 2114, 2118 and 2114 μg/L, respectively. In

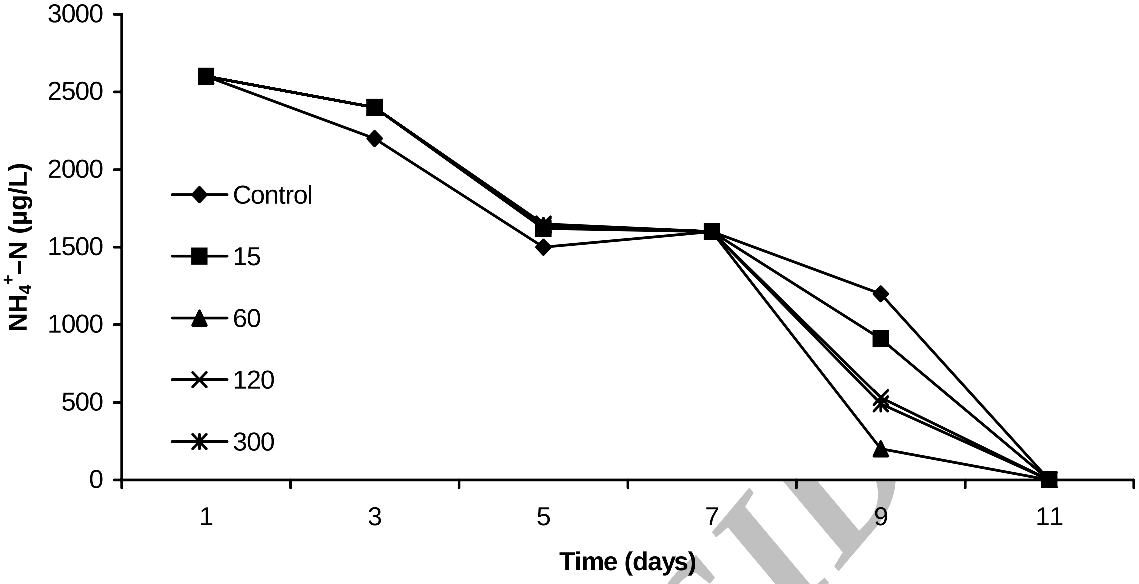

Figure 1. NH_4^+ removal as affected by different concentrations (µM) of $AlCl_3$.

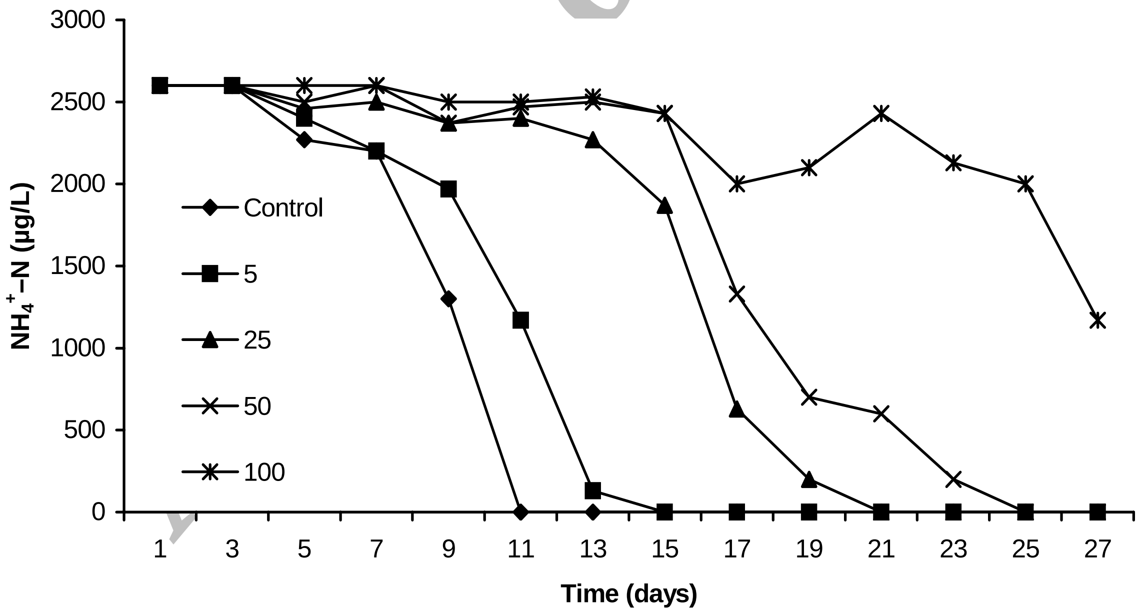

Figure 2. NH_4^+ removal as affected by different concentrations (µM) of $CdCl_2$.

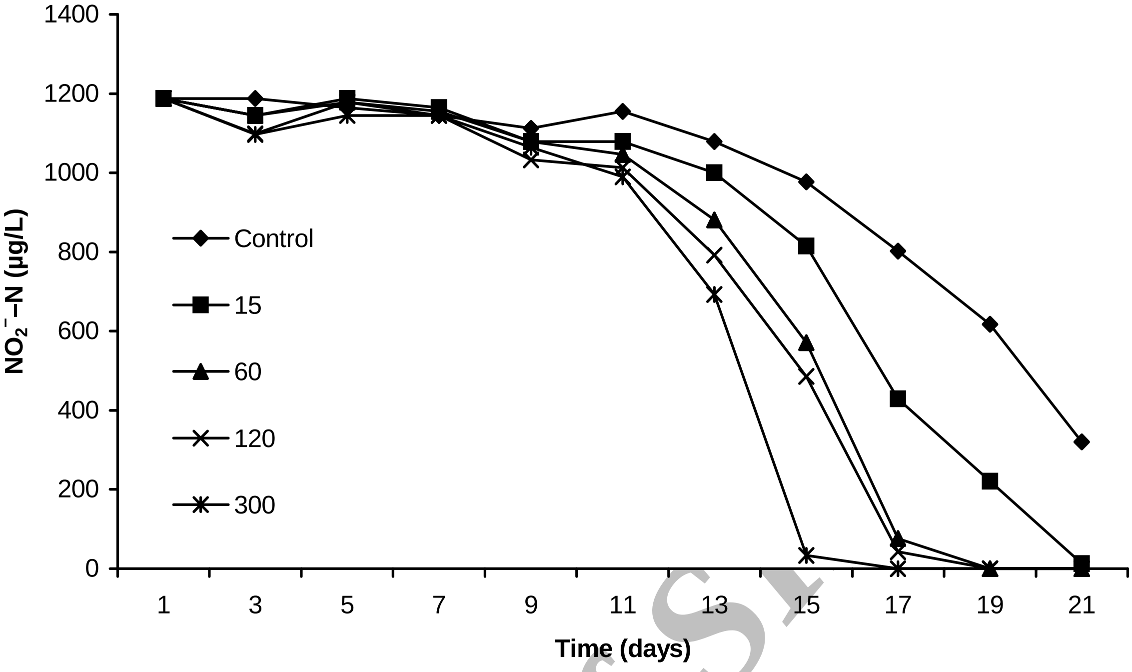

Figure 3. Effects of different concentrations (μM) of $AlCl_3$ on NO_2^- removal.

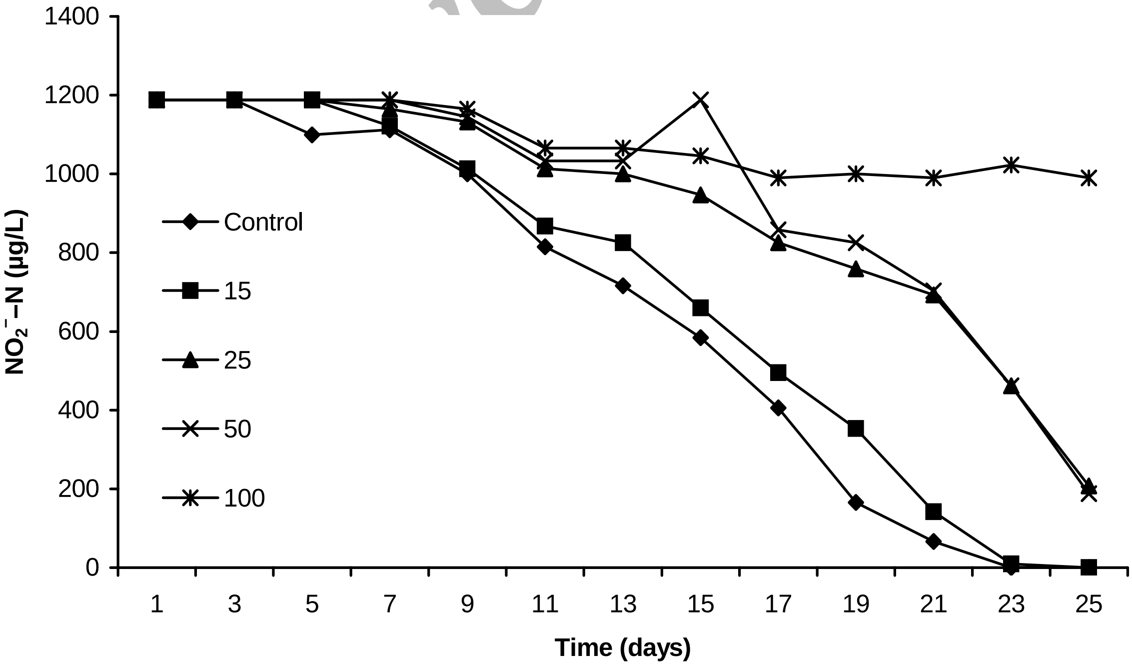

Figure 4. Effects of different concentrations (μM) of $CdCl_2$ on NO_2^- removal.

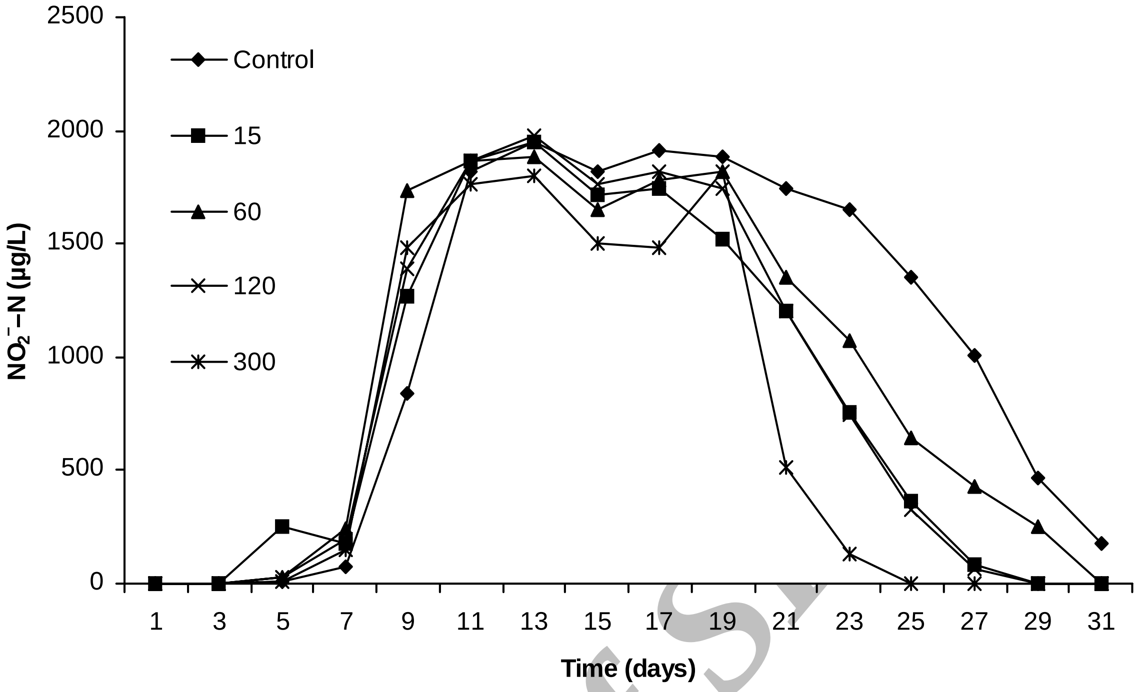

Figure 5. Production of NO_2^- from NH_4^+ and its subsequent disappearance as affected by different concentrations (µM) of $AlCl_3$. At the start of experiment, 2600 µg/L NH_4^+–N, as ammonium sulfate, was added to each flask.

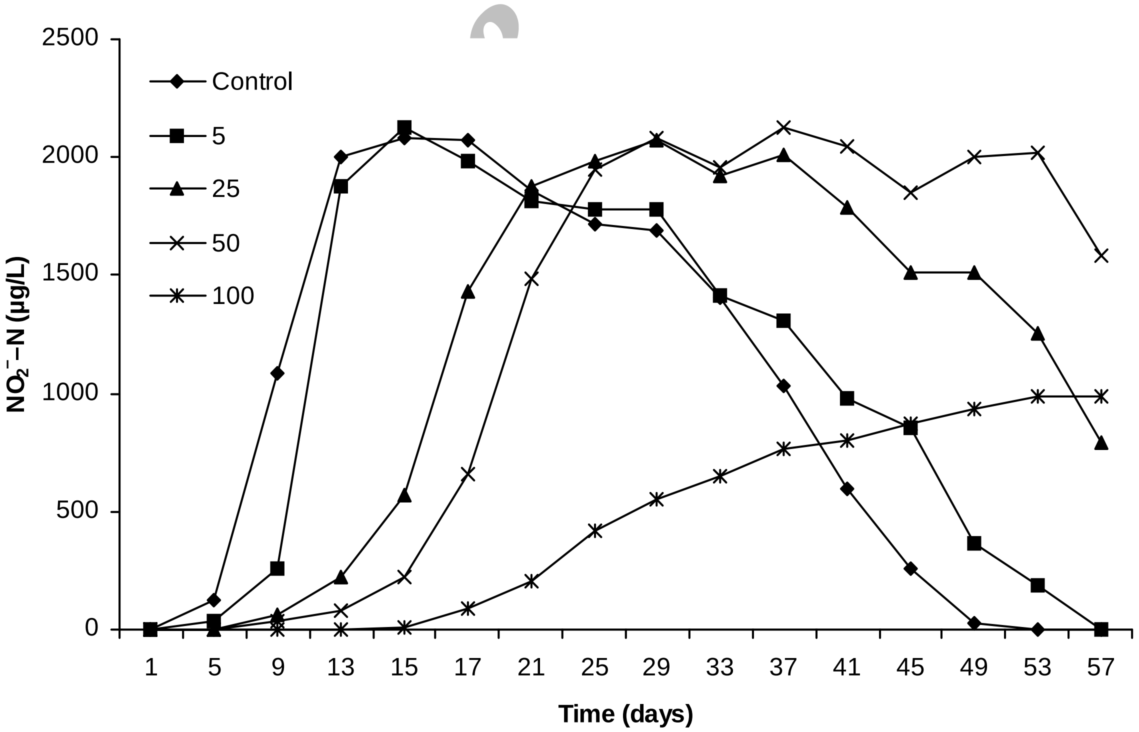

Figure 6. Production of NO_2^- from NH_4^+ and its subsequent disappearance as affected by different concentrations (µM) of $CdCl_2$. At the start of experiment, 2600 µg/L NH_4^+–N, as ammonium sulfate, was added to each flask.

contrast, NO_3^- production from NH_4^+ oxidation was severely reduced in the presence of $CdCl_2$. By day 57, NO_3^-–N produced at 0, 5, and 25 μM $CdCl_2$ were 1975, 1805 and 756 μg/L, respectively. At 50 and 100 μM $CdCl_2$, NO_3^- production was completely inhibited. Although these results are in agreement with those shown in Figures 5 and 6, the amounts of NO_3^- recovered were not stoichiometrically equal to the amount of 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 NH_4^+ and NO_2^-. If so, it is suggested that $AlCl_3$ possibly enhanced NO_2^- oxidation by increasing the activity of *Nitrobacter* sp. but had no effect on the oxidation of NH_4^+ by *Nitrosomonas* sp. In contrast, $CdCl_2$ possibly reduced the activities of both groups of bacteria. Differential sensitivity of nitrifying bacteria to light, Cu^{2+} and 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 Cu^{2+} and 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 Cd^{2+}/kg dry soil as $CdCl_2$, lowered the ability of soil to nitrify 100 μg added NH_4^+–N/g dry soil [4]. Nitrification in a loamy-sand soil amended with sewage sludge decreased significantly in the presence of Zn^{2+}, $Cd^{2+}$and Ni^{2+} [14]. Influence of 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 $AlCl_3$ 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 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.

Acknowledgements

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