

## **SOIL REDOX AND pH EFFECTS ON NITRATE REDUCTION UNDER FLOODED CONDITIONS**

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### **ABSTRACT**

In an incubation experiment, both biological and chemical reduction of nitrate were studied under flooded conditions at different redox (0, 200 and 400 mV) and pH (5, 7 and 9) values in the presence and absence of HgCl<sub>2</sub> as a sterilant.

Nitrate reduction followed a linear pattern with the time of incubation at all combined pH – Eh treatments. At a given pH value, NO<sub>3</sub><sup>-</sup> reduction increased by 2 to 3 folds due to lowering Eh from 400 to 0 mV. The highest reduction rate was found at pH 9 combined with Eh 0 mV. At 48 hours of incubation, for instance, and pH 9 the reduction was 73% at 0 mV but didn't exceed 24% at Eh 400 mV in the absence of HgCl<sub>2</sub>. The corresponding values in the presence of HgCl<sub>2</sub> were 80% and 42%, respectively. Catalytic action of HgCl<sub>2</sub> under alkaline conditions was responsible for such increase by enhancing the chemical reduction. Under acid conditions, at 0 and 400 mV without HgCl<sub>2</sub> the values were 64% and 30%, but were 25% and 14% with HgCl<sub>2</sub>. Such decreased values were attributed to the poisoning action of soluble mercury under acid conditions to soil biota. It also indicated the importance of chemical reduction under acid and well aerated conditions which are different than those suitable for nitrate reducing microorganisms.

**Key Words:** Chemical Nitrate Reduction, Biological Nitrate Reduction, Soil Redox Potential

### **INTRODUCTION**

For both economic and hygienic-environmental reasons, nitrogen loss out of the soil profile should be avoided. Terman (1979) concluded that less than half of nitrogen fertilizer applied in the world actually enters the crop. However, it is still extremely difficult to elucidate to what extent NO<sub>3</sub><sup>-</sup> reduction is the result of biological or chemical reactions. In recent years, loss of nitrogen due to chemical process has gained wide research interest (El-Sebaay, 1991; El-Sebaay and Khaled 1991; Hojberg *et al* 1996; Abbasi and Adams, 1999; El-Sebaay, 2000). Loss due to denitrification has received a lot of attention although it represent a small portion of the total NO<sub>3</sub><sup>-</sup> loss. Under field conditions, Hojberg *et al* (1996) concluded that denitrification never made up more than 10% of the total NO<sub>3</sub><sup>-</sup> reduction. While, Abbasi and Adams (1999) declared that, on average, the total denitrification ranged between 20 to 30% of the applied nitrogen. On the other hand, nitrogen loss due to microbial assimilation is known to be minimal. In this concern, Chen *et al* (1972) observed only 7% and 3% conversion of added <sup>15</sup>NO<sub>3</sub><sup>-</sup>-N to <sup>15</sup>N-labelled organic-N and NH<sub>4</sub><sup>+</sup>-N, respectively. Also, Stanford *et al* (1975) and Buresh and Patrick (1978) concluded that NH<sub>4</sub><sup>+</sup> rather than NO<sub>3</sub><sup>-</sup> was the primary form of N-assimilation. Of the many environmental factors governing NO<sub>3</sub><sup>-</sup> reduction, the aeration status is very important (Lindsay, 1979; Firestone, 1982 and El-Sebaay, 2000). Redox potential (Eh) in combination

with soil reaction (pH) reflect this aeration status. It is worth to mention that  $\text{NO}_3^-$  can be reduced not only under anaerobic conditions but also in an aerobic environment where anaerobic microsites exist (Bremner and Blackmer, 1978 and Nelson, 1982). However, under flooded conditions due to limited supply of  $\text{O}_2$ , most of applied  $\text{NO}_3^-$  was reduced within few days (George et al 1993; Peoples et al, 1995; Abbasi and Adams, 1999). The reason why numerous studies on  $\text{NO}_3^-$  reduction often give conflicting results is because redox conditions are generally neither measured nor controlled. On the other side, Atta and Van Cleemput (1981); Tiedje *et al* (1982) and Peoples et al (1995) stated that Eh values below 200 mV induced  $\text{NO}_3^-$  reduction. Theoretical calculations made by El-Sebaay (2000) confirmed that a small decrease in Eh or pH can cause marked increase in the chemical reduction of nitrate.

Because it is still difficult to elucidate whether biological or chemical  $\text{NO}_3^-$  reduction is more important, an attempt was made in this study to investigate  $\text{NO}_3^-$  reduction in a clay soil through an incubation experiment at controlled Eh and pH values under sterilized and unsterilized flooded conditions.

## **MATERIALS AND METHODS**

The study was carried out to investigate  $\text{NO}_3^-$  reduction at controlled conditions of pH (5, 7 and 9) and Eh (0, +200 and +400 mV) in a fine Vertic Torrifluvents soil sample from Shalakan Farm (Agric. Res. St., Ain Shams Univ.). It is a clay soil with 7.75 pH, 3.82%  $\text{CaCO}_3$ , 1.23% O.M. and 12.1 ppm  $\text{NO}_3^-$ -N. Soil suspension was obtained by adding 300 ml distilled water to 150 g soil in an incubation round flask of 500 ml capacity and equipped to allow measuring of pH and Eh in the suspension as described by El-Sebaay (1986). In order to increase the biological activity 0.2% ground wheat straw was added to the suspension. Before the start of incubation at different pH and Eh values, the soil suspension was preincubated under laboratory conditions (25 to 31°C) for two weeks to obtain reducing conditions. The attained value was -25 mV. Afterwards, the control of pH was done manually according to Patrick et al (1973) by adding 2N NaOH or 2N HCl solution. The pH value just before starting of incubation was 7.32. The Eh value was stepwise increased to the desired value by injecting oxygen (air) to the suspension using a syringe inserted through a rubber septum. After attaining the desired pH ( $\pm 0.03$  unit) and Eh ( $\pm 5$  mV), 100 ppm  $\text{NO}_3^-$ -N (on soil weight basis) was added as  $\text{KNO}_3$  solution by a syringe. The suspension was continuously stirred using a magnetic stirrer and a stirring bar. Concentration of  $\text{NO}_3^-$ -N in the suspension was determined every 12 hours for 4 days. At each time, 5 ml clear suspension was succeeded by a syringe and analysed for the remained  $\text{NO}_3^-$ -N by the modified kjeldahl method using Devarda's alloy as mentioned by Baruah and Barthakur (1997). Redox potential was measured in a similar way followed by El-Sebaay (1986) using a portable Voltmeter.

In order to distinguish between the chemical and biological nitrate reduction, the same experiment was repeated but the suspension was treated with 0.3% HgCl<sub>2</sub> (on soil weight basis) as a sterilant before NO<sub>3</sub><sup>-</sup>-N addition.

## RESULTS AND DISCUSSION

Nitrate concentration at different combinations of pH and Eh values is illustrated in Figure (1). It is clear that, at all pH-Eh combinations nitrate reduction as a function of incubation time followed a linear pattern. Moreover, its reduction was markedly higher under reduced than under oxidized conditions. To clarify this, the % reduction at 48 hours, as an example, was calculated and given at the left part of Table (1). It shows that at pH 9 and 0 mV 73% of NO<sub>3</sub><sup>-</sup>-N has disappeared, while the corresponding value at 400 mV was only 24%. This means that, by lowering the Eh from 400 to 0 mV the amount of nitrate reduced increased by about three folds. This finding can be attributed to the high depletion of O<sub>2</sub> concentration under reduced conditions (0 mV) and to the possible formation and temporary accumulation of nitrite (NO<sub>2</sub><sup>-</sup>) under oxidized conditions, which poison the nitrate reducing biota as was mentioned by Van Cleemput et al (1976); Burns et al (1996) and Stevens et al (1998). Moreover, under reduced flooded conditions, the possible existence of native electron donors such as Fe<sup>2+</sup>, Mn<sup>2+</sup> and S<sup>2-</sup> is expected and can donate electrons to reduce nitrate through chemical reactions (Nelson and Bremner, 1970; Bulla et al 1970; Ghoshal and Larsson, 1977). Moreover, comparing the slope of the linear pattern (Figure, 1) declared that under oxidized conditions (e.g. 400 mV) the rate of NO<sub>3</sub><sup>-</sup> disappearance was faster under acid than under alkaline conditions.

**Table 1. Percentage nitrate reduction at 48 hours and at different pH and Eh combinations**

PH	Eh	Without HgCl <sub>2</sub>		With HgCl <sub>2</sub>	
		0 mV	400 mV	0 mV	400 mV
9		73	24	80	42
5		64	30	25	14

This can be explained by the known reverse relationship between pH and Eh, beside the predominance of chemical NO<sub>3</sub><sup>-</sup> reduction under acidic and oxidized conditions over the biological one which is enhanced by alkaline and reduced conditions as was found by El-Sebaay and Khaled (1991), Hojberg et al (1996) and El-Sebaay (2000). On the other hand, under comparable reduced conditions (e.g. 0 mV) nitrate was completely reduced at pH 9 within 84 hours (Figure 1), while about 18% of NO<sub>3</sub><sup>-</sup> was still existing at pH 5 within that time. It seems that nitrate reduction works better under low pH value when combined with high Eh than with low Eh values. Similar conclusion was drawn by Lindsay et al. (1981) and El-Sebaay (2000) who

**Figure (1):Nitrate concentration at different ph and Eh values in the unsterilized soil sample**

**Figure (2):Nitrate concentration at different ph and Eh values in the sterilized soil sample**

confirmed that acid oxidized conditions favour chemical reduction of nitrate under oxidized conditions. However, even at 400 mV and pH 9 there was still some nitrate reduction. This means that  $\text{NO}_3^-$  can lose some of its stability under well aerated (oxidized) and alkaline conditions. These findings disagree with that obtained by Patrick (1960) who concluded that nitrate became stable under flooded conditions at pH 5 and Eh of 344 mV.

Data presented in Figure (2) show the effect of soil sterilization by mercuric chloride on nitrate reduction at different pH-Eh combinations. Similar to unsterilized conditions,  $\text{NO}_3^-$  reduction at a given pH value proceeded faster under reduced than oxidized sterilized conditions. However, under oxidized alkaline and even neutral conditions the rate of reduction was markedly higher in the sterilized soil sample. This can easily be recognized when comparing the corresponding slope line of similar treatments under both sterilized and unsterilized conditions in Figures (1 and 2). Also, the % reduction (Table 1) at pH 9 and Eh 400 mV was 24 and 42% without and with  $\text{HgCl}_2$ , respectively. It means that under these conditions, the chemical reduction is of greater importance than the biological one. This can be explained by the action of  $\text{HgCl}_2$  in catalyzing the chemical reduction under alkaline and oxidized conditions. In contrast, opposite behaviour was found under acid oxidized conditions. Since, at 48 hours of incubation (Table 1) least % reduction occurred (14%) under sterilized acid conditions. Twice this value (30%) was obtained under corresponding unsterilized conditions. This means that under acid conditions the presence of  $\text{O}_2$  (Eh 400 mV) seemed to be more effective in lowering  $\text{NO}_3^-$  reduction rate than the addition of  $\text{HgCl}_2$ . However, the presence of Hg in a soluble form under this acid condition might cause a poisoning effect on the soil biota and consequently suppressed the biological  $\text{NO}_3^-$  reduction. Therefore, the 30% reduction was mostly due to chemical and not biological reduction. It is worth to mention that although an appreciable amount of the sterilant (not stop nitrate reduction completely under conditions suitable for biological reduction (e.g. pH 7 and Eh 0 mV). Adsorption and complexing processes with soil constituents might have suppressed its activity.

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## **تأثير جهد الأكسدة والاختزال ورقم حموضة التربة على اختزال النترات تحت ظروف الغمر**

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نظراً لاهمية اختزال النترات واثار ذلك على النواحي الاقتصادية والبيئية بجانب صعوبة تحديد ما اذا كان اختزالها اساساً بيولوجياً ام يشاركه الفعل الكيميائى بالتربة . لذا اجريت تجربة معملياً لتتبع اختزال النترات المضافة لعينة تربة طينية القوام (100 جزء فى المليون) تحت ظروف مختلفة ومتحكم فيها من جهد الاكسدة والاختزال (Eh) تراوحت بين صفر ، 400 ملليفولت ومن رقم الحموضة (pH) تراوحت من 5 الى 9 باعتبارهما المسئولان عن ظروف التهوية بالتربة . كما اجريت تجربة مماثلة اضيف فيها كلوريد زئبق سام للكائنات الحية بالتربة للتمييز بين الاختزال البيولوجى والكيميائى للنترات .

اوضحت النتائج ان اختزال النترات تحت ظروف الغمر يتبع علاقة خطية مع الزمن تحت المعاملات المختلفة من pH ، Eh ، وانه عند اى قيمة معينة من الـ pH فان اختزال النترات يزيد بخفض الـ Eh حيث زاد معدل الاختزال بمقدار 2-3 مرات تحت الظروف سيئة التهوية (صفر ملليفولت) عنها تحت جيدة التهوية (400 ملليفولت) . وكانت اعلى معدلات اختزال على الاطلاق عند قيم الـ pH المرتفعة المصاحبة لقيم الـ Eh المنخفضة . فعلى سبيل المثال عند 48 ساعة من التحضين تحت pH 9 وفى غياب  $HgCl_2$  اختزل نحو 73% من النترات عندما كان Eh صفر ملليفولت بينما لم يزد عن 24% عند 400 Eh ملليفولت . وكانت القيم المقابلة نتيجة الاختزال الكيميائى (فى وجود  $HgCl_2$ ) هى 80% ، 42% على الترتيب . اما تحت الظروف الحامضية سيئة التهوية فى غياب وجود  $HgCl_2$  فكانت نسب الاختزال 64% ، 25% على الترتيب . بينما تحت الظروف جيدة التهوية فكانت 30% ، 14% بنفس الترتيب السابق . وهذا يؤكد على حدوث الاختزال الكيميائى بنسبة محسوسة بجانب الاختزال البيولوجى للنترات بالتربة.

وتوصى الدراسة بعدم الاكتفاء بتتبع الاختزال البيولوجى للنترات بالتربة باعتباره ضار بالبيئة ولكن باخذ الاختزال الكيميائى فى الاعتبار خاصة تحت الظروف الغير مناسبة للاختزال البيولوجى مثل الظروف الحامضية وجيدة التهوية .



