

## Effect of Nitrate in Irrigation Water on Iron Reduction and Phosphate Release in Anoxic Paddy Soil Condition

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Since  $\text{NO}_3^-$  is a more favorable electron acceptor than Fe, high  $\text{NO}_3^-$  loads function as a redox buffer limiting the reduction of Fe and following release of  $\text{PO}_4^{3-}$  in flooded paddy soil. The effect  $\text{NO}_3^-$  loaded through irrigation water on Fe reduction and  $\text{PO}_4^{3-}$  release in paddy soil was investigated. Pot experiment was conducted where irrigation water containing 5 or 10 mg N L<sup>-1</sup> of  $\text{NO}_3^-$  was continuously applied at 1 cm day<sup>-1</sup>, and changes of  $\text{NO}_3^-$ ,  $\text{Fe}^{2+}$  and  $\text{PO}_4^{3-}$  concentrations in soil solution at 5 and 10 cm depths beneath the soil surface were monitored as a function of time. Irrigation of rice paddy with water containing 5 mg N L<sup>-1</sup> of  $\text{NO}_3^-$  led to reduced release of  $\text{Fe}^{2+}$  and prevented solubilization of P at 5 cm depth beneath the soil surface. And application of irrigation water containing 10 mg N L<sup>-1</sup> of  $\text{NO}_3^-$  could further suppress Fe reduction and solubilization of P through 10 cm depth soil layer beneath the surface. These results suggest that the introduction of high level  $\text{NO}_3^-$  with irrigation water in rice paddy can strongly limit Fe reduction and P solubilization in root zone soil layer in addition to the excessive supply of N to rice plants.

**Key words:** Nitrate, Iron, Phosphate, Paddy soil, Oxidation-reduction, Irrigation water

### Introduction

Oxygen is only sparingly soluble in water and diffuses much more slowly through water than through air (Schlesinger, 1997). What little oxygen that is present in flooded rice paddy soils in the form of dissolved  $\text{O}_2$  is quickly consumed through metabolic processes. Although energy yields are much greater with  $\text{O}_2$  than with any other terminal electron acceptor, under anoxic conditions anaerobic and facultative microbes can use alternative electron acceptors such as  $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{4+}$ ,  $\text{SO}_4^{2-}$ , and  $\text{CO}_2$  to produce energy and build biomass (McBride, 1994). Therefore, microbial transformations of elements play a large role in biogeochemical cycling of nutrients in anaerobic soils.

In general, submergence enhances available  $\text{PO}_4^{3-}$  levels in flooded anoxic rice paddy soils, which is attributable to reduction of hydrous ferric compounds, particularly Fe-oxides (Sanyal and De Datta, 1991; Ponnamperna, 1972). Liberation of sorbed and/or co-precipitated P increases solution or extractable  $\text{PO}_4^{3-}$  in

anoxic flooded soils. However, it has been found that the presence of  $\text{NO}_3^-$  in water acts as an effective redox buffer and substantially delaying the release of  $\text{PO}_4^{3-}$  in various anoxic flooded ecosystems including rice paddies (Anderson, 1982; Lucassen et al., 2004; Sallade and Sims, 1997; Young and Ross, 2001; Surridge et al., 2007). The inhibition of  $\text{PO}_4^{3-}$  release is mostly due to the suppression of  $\text{Fe}^{3+}$  reduction under the presence of  $\text{NO}_3^-$ , a more preferential electron acceptor. Also the enzymatic re-oxidation of  $\text{Fe}^{2+}$  during  $\text{NO}_3^-$  reduction results in the formation of oxidized Fe phases and re-adsorption of dissolved  $\text{PO}_4^{3-}$  (Matocha and Coyne, 2007; Straub et al., 2004; Weber et al., 2006). Enhanced  $\text{SO}_4^{2-}$  reduction and iron-sulfide mineral (e.g. FeS,  $\text{FeS}_2$ ) formation, caused by inputs of high  $\text{SO}_4^{2-}$  water, is responsible for increased pore water  $\text{PO}_4^{3-}$  accumulation in aquatic ecosystems (Smolders and Roelofs, 1993; Murray, 1995; Roden and Edmonds, 1997). However, high concentration of  $\text{NO}_3^-$  in the water can suppress  $\text{SO}_4^{2-}$  reduction and accompanied  $\text{PO}_4^{3-}$  accumulation (Lucassen, et al., 2004). Oxidation-reduction reactions involving  $\text{NO}_3^-$ , Fe, and  $\text{SO}_4^{2-}$ , which control the solubility of P in anoxic flooded soil environments, can be summarized as shown in Fig. 1.

Therefore, in flooded soil environments, where  $\text{NO}_3^-$  is continuously supplied and removed by reduction process

Received : January 28, 2010 Accepted : February 16, 2010

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(denitrification), P solubilization can be considerably inhibited with suppression of Fe reduction and re-oxidization of reduced Fe. It has been found that high  $\text{NO}_3^-$  concentrations in river water and groundwater greatly inhibit eutrophication of fresh water wetlands by limiting  $\text{PO}_4^{3-}$  availability (Anderson, 1982; Lucassen et al., 2004; Surridge et al., 2007). In paddy soil condition, Fe reduction was significantly inhibited with application of  $\text{NO}_3^-$  ( $40 \text{ mg N kg}^{-1}$ ) and concentration of  $\text{PO}_4^{3-}$  in soil solution was significantly lower compared with the  $\text{PO}_4^{3-}$  concentration found in the soil of no  $\text{NO}_3^-$  application (Chung, 2009). These results indicate that  $\text{NO}_3^-$  introduced in the anoxic layer of rice paddy soil with fertilization and irrigation can inhibit reduction of Fe and accompanied P solubilization.

During the early stage of rice growth, where relatively large amount of N is applied as a basal fertilization, denitrification in the anoxic root zone soil layer can lead to reduced release of  $\text{Fe}^{2+}$  and  $\text{PO}_4^{3-}$ . And solubilization of P in the anoxic root zone soil layer would be continuously limited during the growth period by the input of irrigation water with high  $\text{NO}_3^-$  concentrations. Under these circumstances the availability of P and Fe in the soil could be limited and deficiency of these nutrients in rice plants could be caused in some extreme cases. It has been found that  $\text{PO}_4^{3-}$  release from the lake sediment usually does not take place with the concentration of  $\text{NO}_3^-$  higher than 5

$\text{mg/L}^{-1}$  in overlying water (Anderson, 1982). Rice paddies are mostly under flooded condition during the growth period and continuously irrigated. Most of the irrigation waters are supplied from rivers, dams and reservoirs, and in many parts of our country the concentration of  $\text{NO}_3^-$  in those water resources frequently exceeds even  $5 \text{ mg L}^{-1}$  (Chung et al., 1997; Chung et al., 1998; Ministry of Environment, 2009).

In this study the effect of surface applied  $\text{NO}_3^-$  with irrigation water on the release of  $\text{PO}_4^{3-}$  in root zone soil layer was investigated using a rice paddy model system.

## Materials and Methods

**Soil** A Yuga silt loam (fine silty, mixed, mesic family of Fluvaquentic Endoaquepts) was collected from the surface layers (0-20 cm) of a rice paddy in Daegu University Experimental Farm (Table 1). The soil was air-dried and crushed to pass 5 mm sieve. Reagent grade  $\text{KH}_2\text{PO}_4$  solution was sprayed on the soil and mixed thoroughly to adjust available P level to  $58 \text{ mg P kg}^{-1}$ , and the treated soil was kept for 30 days at  $20^\circ\text{C}$  for stabilization of the added P before experiment.

**Experimental system construction** The flooded rice paddy model consisted of a 10-L plastic cylinder with

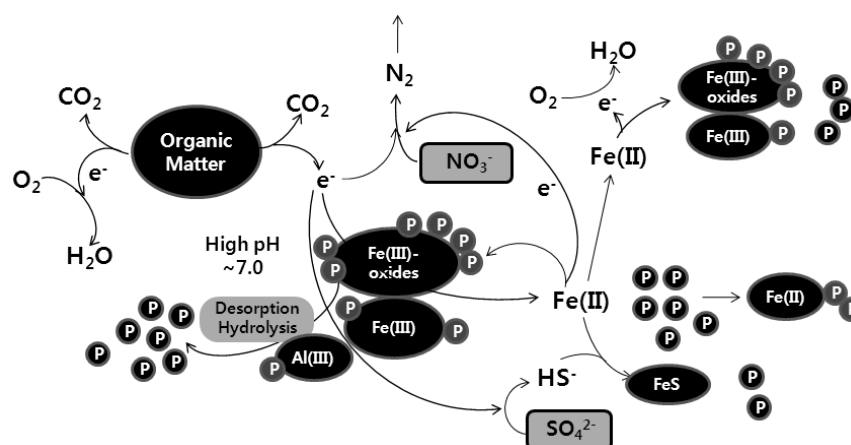


Fig. 1. Oxidation-reduction reactions which control the release of phosphate in anoxic flooded paddy soils.

Table 1. Some physical and chemical characteristics of the Yuga silt loam soil.

pH (1:5 $\text{H}_2\text{O}$ )	Organic matter $\text{g kg}^{-1}$	Available P $\text{mg kg}^{-1}$	Total P $\text{mg kg}^{-1}$	Oxide Fe $\text{g kg}^{-1}$	Particle separate		
					Sand	Silt	Clay
6.5	26.0	17.3	1304	13.5	22.2	54.0	23.8

a sealed bottom, 24 cm long with a diameter of 23 cm (Fig. 2). The prepared soil was packed uniformly to a height of 15 cm from bottom of the cylinder. Two soil moisture samplers (Eijkelkamp, Giesbeek, The Netherlands) were placed at each depth of 5 and 10 cm beneath the soil surface for soil solution sample collection. A platinum electrode for redox potential measurement was placed at a depth of 10 cm beneath the soil surface. Two drain outlets were installed on the bottom of the cylinder. In this experiment, three experimental systems were constructed and used for the irrigation treatments of three different  $\text{NO}_3^-$  concentrations.

**Experimental procedure** The prepared experimental systems were flooded with 0.02 M  $\text{CaCl}_2$  solution supplied from bottom of the soil column through drain outlets and 5 cm flooding depth above the soil surface was maintained with the drain outlets closed until the redox potential measured at 10 cm soil depth lowered below -200 mV. Irrigation waters containing three levels of  $\text{NO}_3^-$  (0, 5 and 10 mg N  $\text{L}^{-1}$ ) were used in the experiment. Irrigation waters were prepared by dissolving a reagent grade  $\text{KNO}_3$  in distilled water containing 0.02 M  $\text{CaCl}_2$ . After the redox potential at 10 cm soil depth was lowered below -200 mV, irrigation water containing  $\text{NO}_3^-$  was added to maintain 5 cm flooding depth after removing the pre-flooded water (0.02 M  $\text{CaCl}_2$  solution) above the soil surface. The drain outlets were opened and the percolation rate was maintained at 1 cm  $\text{day}^{-1}$  during the experiment. Floodwater depth of 5 cm was maintained throughout the

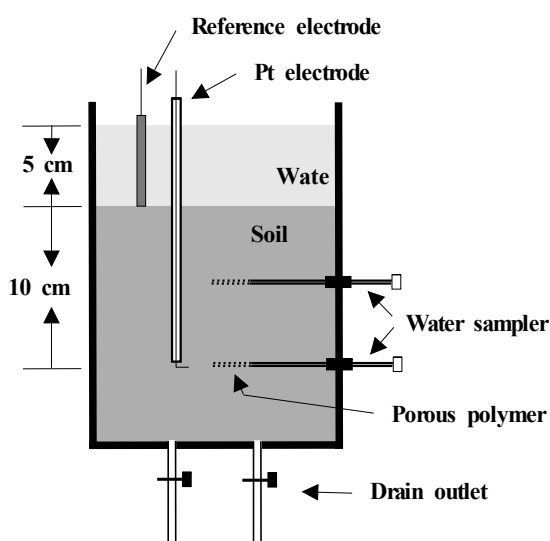


Fig. 2. Schematic diagram of the experimental paddy soil system.

experiment by daily addition of irrigation water. The experiment was conducted at  $25 \pm 2^\circ\text{C}$ .

Daily soil water sampling and monitoring of the effect of  $\text{NO}_3^-$  on Fe reduction and P solubilization were initiated 5 days after flooding with  $\text{NO}_3^-$  containing irrigation water. Daily changes of redox potential at 10 cm depth soil were also monitored. Two soil solution samples of 15 mL were collected at each depth of 5 and 10 cm beneath the soil surface using a syringe connected to the soil moisture sampler.

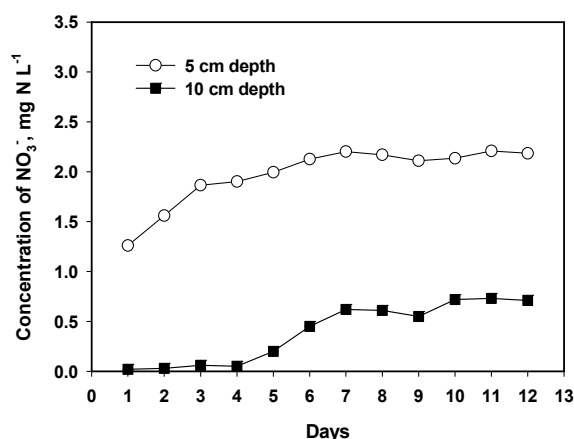
**Analytical methods** Soil sample was air-dried and passed through a 2-mm sieve. Soil pH was determined using a pH meter in deionized water with a 1:5 soil/solution ratio. Organic matter was determined by using Walkley and Black procedure (Nelson and Sommers, 1982). Total and available P contents were measured using perchloric acid digestion and Bray No. 1 procedures (RDA, 1988). Content of free iron oxides was measured using Na-dithionite-extraction method (Olson and Ellis, 1982). Soil particle size distribution was determined by micro-pipette method (Miller and Miller, 1987).

Soil solution samples were passed through 0.45  $\mu\text{m}$  membrane filter before analysis. Flow injection autoanalyzer (FIAstar-5000 system, FOSS Tecator, Höganäs, Sweden) was used in the analysis of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ , and  $\text{Fe}^{2+}$  was determined using 1,10-phenanthroline method (Stucki and Anderson, 1981). Soil redox potential was determined using Eh meter (Fujihara Factory, Tokyo, Japan) and the reference electrode ( $\text{Ag}/\text{AgCl}/\text{Sat. KCl}$ ) potential 206 mV was adjusted.

## Results and Discussion

In the treatment of irrigation water without  $\text{NO}_3^-$ ,  $\text{NO}_3^-$  was not detected in soil solutions sampled at 5 and 10 cm depths during the experiment. Any  $\text{NO}_3^-$  presented in the soil could be completely removed by denitrification during the pre-flooding period. In the treatment of irrigation water containing 5 mg N  $\text{L}^{-1}$  of  $\text{NO}_3^-$ , trace amount of  $\text{NO}_3^-$  was detected at 5 cm depth but at 10 cm depth  $\text{NO}_3^-$  was not detected during the experiment. In the treatment of irrigation water containing 10 mg N  $\text{L}^{-1}$  of  $\text{NO}_3^-$ , as shown in Fig. 3, concentration of  $\text{NO}_3^-$  in the solution was rapidly increased from 1.2 mg N  $\text{L}^{-1}$  at the initial measurement and 5 days after the initial

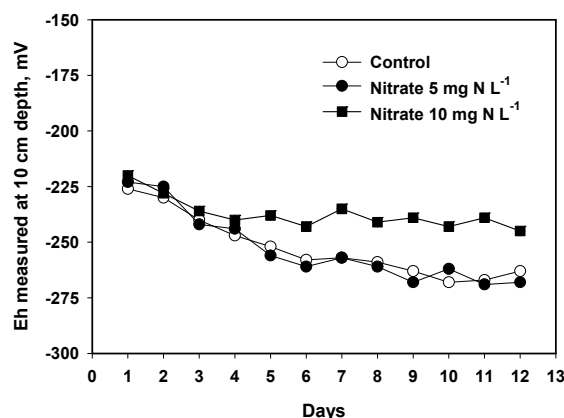
measurement more than  $2.0 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  was found at 5 cm depth. At 10 cm depth, concentration of  $\text{NO}_3^-$  in the soil solution was below  $0.2 \text{ mg N L}^{-1}$  in the early period of measurement and 6 days after the initial measurement  $0.4 \sim 0.7 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  was found. In all of the treatments,  $\text{NO}_3^-$  was not found in the soil solution collected from the drain outlets. Since there was not any loss of  $\text{NO}_3^-$  via leaching and plant absorption in the experimental systems, the  $\text{NO}_3^-$  removal from irrigation water during percolation could be due to the denitrification process where  $\text{NO}_3^-$  preferentially used as an electron acceptor under anoxic soil condition (Sposito, 1989; Matocha and Coyne, 2007). Kasuya (1999) also found that most of the  $\text{NO}_3^-$  in percolating water was removed in the shallow plow layer (0~3 cm) by denitrification in rice paddies where groundwaters containing  $5 \sim 27 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  were irrigated.



**Fig. 3.** Changes of soil solution concentrations of  $\text{NO}_3^-$  at 5 and 10 cm depth beneath the soil surface in the application of irrigation water containing  $10 \text{ mg N L}^{-1}$   $\text{NO}_3^-$ . The shown data are average values from duplicate determinations.

Changes of redox potentials at 10 cm depth beneath the soil surface 5 days after applications of irrigation water containing  $\text{NO}_3^-$  are shown in Fig. 4. During the experiment, the redox potential was continuously lowered from about  $-225 \text{ mV}$  in all treatments. Redox potential changes between the treatments of no  $\text{NO}_3^-$  application and  $5 \text{ mg N L}^{-1}$   $\text{NO}_3^-$  application were not different. But, in the soil of  $10 \text{ mg N L}^{-1}$   $\text{NO}_3^-$  application, redox potential at 10 cm depth was lowered much slowly and remained higher than those found in the other treatments. Nitrate supplied through irrigation water of  $5 \text{ mg N L}^{-1}$  at percolating rate of  $1 \text{ cm day}^{-1}$  can be completely removed

by denitrification within the surface 5 cm depth soil layer. Therefore, under this condition irrigation water of  $5 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  can affect the redox processes within the top 5 cm depth soil but any effects on the redox processes, including Fe reduction and P solubilization, in the soil layer below 5 cm depth cannot be expected. However, application of irrigation water containing  $10 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  at  $1 \text{ cm day}^{-1}$  percolation rate is expected to affect the redox processes up to the 10 cm depth of soil, since  $\text{NO}_3^-$  in the irrigation water can reach to 10 cm depth soil layer beneath the soil surface. However, the depth of soil layer in which redox processes are influenced by  $\text{NO}_3^-$  introduced through irrigation water would be various depending on  $\text{NO}_3^-$  concentration and percolating rate of irrigation water, and various soil environmental conditions controlling denitrification activities.



**Fig. 4.** Changes of redox potentials measured at 10 cm depth beneath the soil surface in the applications of irrigation water containing  $\text{NO}_3^-$ .

Effects of  $\text{NO}_3^-$  introduced through irrigation water on the reduction of Fe in anoxic soil layer are shown in Fig. 5. In the treatment of irrigation water containing no  $\text{NO}_3^-$ , concentrations of  $\text{Fe}^{2+}$  in soil solution sampled at 5 and 10 cm depths was continuously increased from the initial concentrations of  $2.3\text{-}2.7 \text{ mg L}^{-1}$ , and it was higher at 10 cm depth compared to the concentration found at 5 cm depth. This result indicates that reducing condition in the soil is continuously developed during the experiment and the reducing condition is stronger at deeper soil layer. In the treatment of irrigation water containing  $5 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$ , concentration of  $\text{Fe}^{2+}$  in soil solution collected at 5 cm depth beneath the soil surface was relatively lower than the concentration found in the irrigation treatment without  $\text{NO}_3^-$ , and the concentration of  $\text{Fe}^{2+}$  was not

changed during the experiment. In the treatment of irrigation water containing  $10 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$ , concentration of  $\text{Fe}^{2+}$  in soil solution collected at 5 cm depth beneath the soil surface was much lower than those found in the other treatments, and the concentration of  $\text{Fe}^{2+}$  was slowly decreased during the experiment. These results indicate that Fe reduction can be more strongly suppressed in the soil layer where more  $\text{NO}_3^-$  is introduced and more denitrification occurs. At 10 cm depth beneath the soil surface, concentration of  $\text{Fe}^{2+}$  in soil solution continuously increased during the experiment from the initial concentrations of  $2.7 \text{ mg L}^{-1}$  in all treatments. Concentrations of  $\text{Fe}^{2+}$  in soil solution between the treatments of no  $\text{NO}_3^-$  application and  $5 \text{ mg N L}^{-1}$   $\text{NO}_3^-$  application were not different, but in the treatment of  $10 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  application concentration of  $\text{Fe}^{2+}$  in soil solution at 10 cm depth was considerably lower than those found in the other two treatments. Comparing the concentrations of  $\text{Fe}^{2+}$  in soil solutions between 5 cm and 10 cm depth, the concentrations were higher in the soil

solutions collected at 10 cm depth. This result could be due to the stronger reducing condition developed in the deeper soil layer and the greater suppression of Fe reduction by denitrification in the shallow soil layer.

Effects of  $\text{NO}_3^-$  introduced through irrigation water on the release of  $\text{PO}_4^{3-}$  in anoxic soil layer are shown in Fig. 6, and the concentrations of  $\text{PO}_4^{3-}$  were slowly and continuously increased during the experiment. At 5 cm depth beneath the soil surface, concentrations of  $\text{PO}_4^{3-}$  in the treatment of irrigation water containing  $5 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$  were relatively lower than the concentrations found in the treatment of no  $\text{NO}_3^-$  in irrigation water. And in the treatment of irrigation water containing  $10 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$ , the concentrations of  $\text{PO}_4^{3-}$  were further lower, and during the last 1 week period of experiment the concentration difference was about  $0.1 \text{ mg P L}^{-1}$  comparing to the treatment of no  $\text{NO}_3^-$  irrigation water. At 10 cm depth beneath the soil surface, the concentrations of  $\text{PO}_4^{3-}$  in soil solution found in the treatments of no  $\text{NO}_3^-$  application and  $5 \text{ mg N L}^{-1}$   $\text{NO}_3^-$  application were not

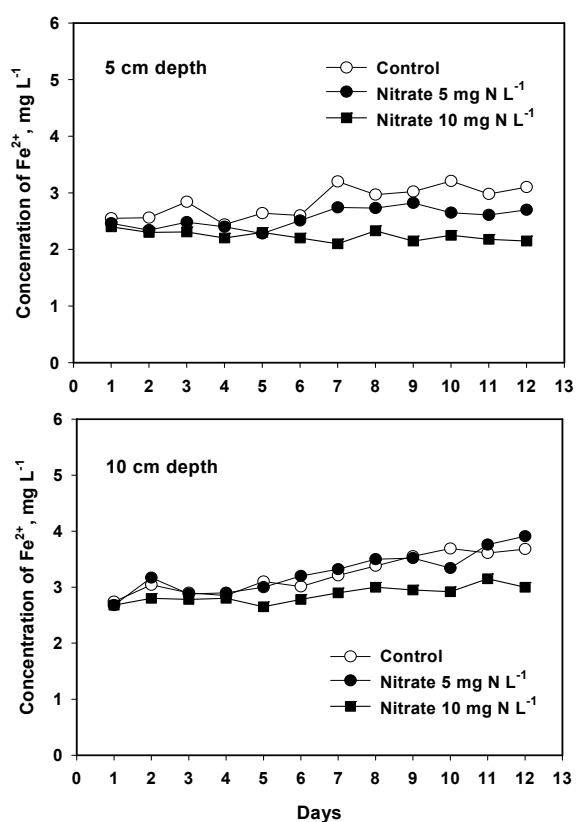


Fig. 5. Changes of soil solution concentrations of  $\text{Fe}^{2+}$  at 5 and 10 cm depth beneath the soil surface in the applications of irrigation water containing  $\text{NO}_3^-$ . The shown data are average values from duplicate determinations.

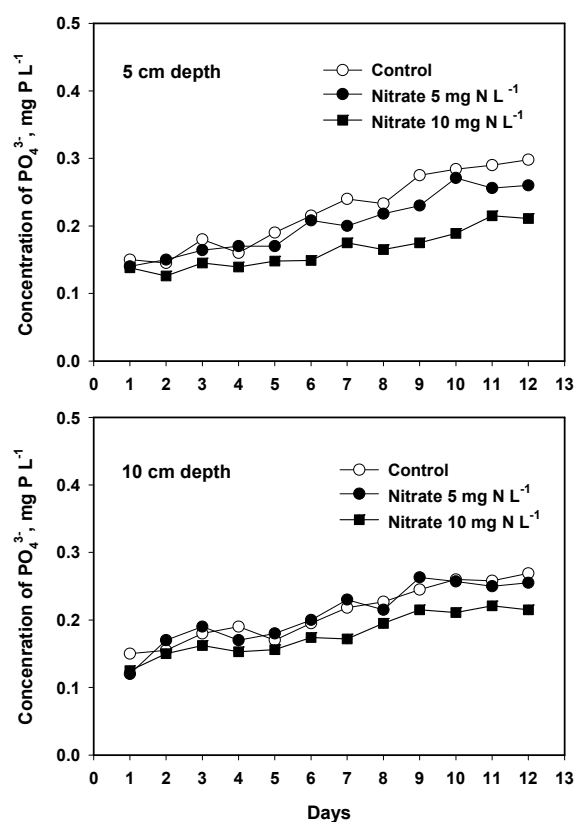


Fig. 6. Changes of soil solution concentrations of  $\text{PO}_4^{3-}$  at 5 and 10 cm depth beneath the soil surface in the applications of irrigation water containing  $\text{NO}_3^-$ . The shown data are average values from duplicate determinations.

different. But, in the treatment of  $10 \text{ mg N L}^{-1}$  of  $\text{NO}_3^-$ , concentrations of  $\text{PO}_4^{3-}$  in soil solution were considerably lower than those found in the other two treatments. These results coincided with the impaired Fe reduction caused by  $\text{NO}_3^-$  introduced through irrigation water (Fig. 5).

The processes involved in the inhibition of soil Fe reduction and  $\text{PO}_4^{3-}$  solubilization by  $\text{NO}_3^-$  are complicated as presented in Fig. 1, and not completely understood yet. However, a continuous flow of  $\text{NO}_3^-$ -rich irrigation water through the soil layer can lead to a higher redox potential in the soil than the same water without  $\text{NO}_3^-$ . And addition of  $\text{NO}_3^-$ , an energetically more favorable electron acceptor than Fe, to soil under anoxic conditions prevents a release of  $\text{PO}_4^{3-}$  by limiting Fe reduction from Fe- $\text{PO}_4^{3-}$  complexes and by anoxic oxidation of  $\text{Fe}^{2+}$  (Straub et al., 2004; Matocha and Coyne, 2007). If  $\text{NO}_3^-$  concentrations are high,  $\text{SO}_4^{2-}$  reduction and Fe immobilization with  $\text{FeS}_x$  formation are also impaired and mobilization of  $\text{PO}_4^{3-}$  from iron-phosphate complexes is prevented (Lucassen et al., 2004). Although it cannot be explained in detail, inhibition of Fe reduction by  $\text{NO}_3^-$  would be one of the main mechanisms which control  $\text{PO}_4^{3-}$  mobilization from iron-phosphate complexes in flooded anoxic soil conditions including rice paddy. When  $\text{NO}_3^-$  concentration exceeded about  $0.5 \text{ mg N L}^{-1}$  in the water of shallow and very productive lakes, usually no  $\text{PO}_4^{3-}$  release from the sediment took place (Anderson, 1982).

Though a considerable reducing condition is developed in flooded anoxic rice paddy soils, if  $\text{NO}_3^-$  is continuously added through irrigation water and fertilizations during the growth period, the solubilization of  $\text{PO}_4^{3-}$  in root zone soil generally found in rice paddy fields after flooding would be limited. This phenomenon could be a mechanism which can reduce  $\text{PO}_4^{3-}$  loss through leaching and other ways to the water systems around wet land systems including rice paddies (Smolders and Roelofs, 1993; Lucassen et al., 2004; Surridge et al., 2007). However, considering the rice roots are distributed within 10 cm depth beneath the soil surface, availability of P for rice plants would be greatly limited by addition of  $\text{NO}_3^-$  in paddy fields. Irrigation with water containing N more than  $5 \text{ mg L}^{-1}$  can cause various damages on rice plants with excess N uptake (Hidaka, 1993). Therefore, introduction of excessive amount of  $\text{NO}_3^-$  in rice paddy through irrigation in addition to fertilization should be restricted in order to secure the availability of N and P during the

growth period.

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## 관개용수 중의 질산 이온이 농토양의 철 환원과 인 용출에 미치는 영향

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담수상태의 토양이나 습지생태계에서 NO<sub>3</sub><sup>-</sup>는 환원상태의 발달을 지연시키는 완충역할을 할 수 있다. 농토양에서 관개용수를 통하여 공급되는 NO<sub>3</sub><sup>-</sup>가 Fe의 환원과 그에 따른 P의 가용화에 미치는 영향을 조사하였다. 관개용수중의 NO<sub>3</sub><sup>-</sup> 함량이 5 mg N L<sup>-1</sup> 수준일 경우 5 cm 깊이 토양에 도달하기 전에 대부분 탈질작용에 의해 제거되었으며, 5 cm 깊이 토양에서 일어나는 Fe의 환원과 P의 용출이 저해되었고 10 cm 깊이 토양의 환원현상에는 영향을 미치지 못하였다. NO<sub>3</sub><sup>-</sup> 함량이 10 mg N L<sup>-1</sup> 수준인 관개용수를 공급하였을 경우에는 10 cm 깊이 토양층까지 NO<sub>3</sub><sup>-</sup>가 잔류 유입되었으며, Fe의 환원과 P의 용출을 현저히 억제하는 것으로 나타났다. 이상의 결과를 보면 관개용수를 통하여 NO<sub>3</sub><sup>-</sup>를 포함한 질소가 과도하게 농토양으로 유입되면 질소과다현상을 유발할 뿐만 아니라 P의 가용화를 억제함으로써 인 결핍을 초래할 수도 있을 것이다.

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