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Short communication

Chloride ion as nitrification inhibitor and its biocidal potential in soils

Michele Xavier Vieira Megda [a,*], Eduardo Mariano [a], José Marcos Leite [a], Marcio Mahmoud Megda [a], Paulo Cesar Ocheuze Trivelin [b]

[a] ESALQ/USP, Av. Pádua Dias 11, CP 9, Piracicaba, CEP 13418-900 São Paulo, Brazil
[b] CENA/USP, Av. Centenário 303, CP 96, Piracicaba, CEP 13400-970 São Paulo, Brazil

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ABSTRACT

The accumulation of chlorine ion in soils and plants due to the continued use of potassium chloride triggers a series of physiological disorders in plants and microorganisms deserving special attention. This study aimed to evaluate the inhibition potential of chloride ions added to soils as ammonium chloride (AC) and/or KCl in the reaction of soil nitrification. The experiment was conducted under laboratory conditions. The soil was incubated for 21 days at 70% of maximum water retention capacity at 25 °C. The addition of AC, combined with KCl, decreased nitrate concentration in the soil. The increase of Cl content in the soil with the use of AC resulted in an inhibition of soil nitrification. The agricultural potential of using AC as N source was shown in many crops, although, caution regarding excess is recommended, which results in a build-up of salt in the soil and causes injury to the root system and microorganisms.
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Plant growth and soil microorganisms are largely affected by salt concentration, especially in arid and semi-arid regions, causing serious damages to agriculture. Although the chemical and physical properties of soils and their effects on plant growth are largely investigated (Wickramasinghe et al., 1985; Rietz and Haynes, 2003; Ghollarata and Raiesi, 2007; Wang et al., 2008), only recently have studies been conducted on the microbial activity in this environment (Rousk et al., 2011; Asghar et al., 2012; Yan and Marschner, 2012, 2013).

Several studies under laboratory and field conditions showed that chloride ions, even at low concentrations, have the potential to inhibit soil nitrification, becoming a potential biocide (Darrah et al., 1987; Yuan et al., 2007; Souri, 2010; Chowdhury et al., 2011). Chloride ions and their soil derivatives have a strong oxidant action and are, therefore, a potent biocide (Wong et al., 1988; Chen and Wong, 2004) with the capacity to greatly reduce microbial population.

Ammonium chloride poses as an alternative N source for the culture containing 25% of N and 65% of Cl. Studies showed good efficiency of AC in rice, cotton and sugarcane production (Tisdale et al., 1985; Ashraf et al., 2005; Vieira-Megda et al., 2012). However, the use of high doses of AC may lead to a reduction of crop yield and microbial activity, mainly combined with other chlorinated fertilizers such as KCl (Yahya, 1998; Vieira et al., 2010). This study was conducted to evaluate the chloride ion potential related to the application of KCl and AC as nitrification inhibitors.

The soil used in this study was classified as Typic Hapludox (Soil Survey Staff, 2010) and the following chemical soil characteristics were assessed in the 0–20-cm layer: pH 4.5 in 0.01 mol L^{-1} $CaCl_2$ (Van Raij et al., 2001); 23.8 g kg^{-1} organic carbon and 1.7 g kg^{-1} total N, both assessed using a mass spectrometer coupled to an automatic analyzer of C and N (Barrie and Prosser, 1996); 130 $mmol_c$ kg^{-1} cation exchange capacity (CEC) (Van Raij et al., 2001).

The following treatments (with 4 replicates) were used: AC (100 mg kg^{-1} N and 260 mg kg^{-1} Cl); AC (100 mg kg^{-1} N and 260 mg kg^{-1} Cl) + KCl 100 (100 mg kg^{-1} Cl); and AC (100 mg kg^{-1} N and 260 mg kg^{-1} Cl) + KCl 200 (200 mg kg^{-1} Cl). The use of AC doses (50, 100 and 200 mg kg^{-1} N, equivalent to 130, 260 and 520 mg kg^{-1} Cl, respectively) was also assessed. A control treatment (without the application of N or Cl) was included.

The soil samples were pre-incubated for a 20-d period under dark conditions to restore microbial activity. At the end of the pre-incubation period, the Cl sources were solubilized in deionized water, in an amount sufficient to increase soil moisture to 70% of the maximum capacity of water retention (MCWR), and, afterward, they were homogeneously incorporated into the soil. Subsequently,

* Corresponding author. Present address: Centro de Energia Nuclear na Agricultura – CENA/USP, Av. Centenário 303, CP 96, Piracicaba, CEP 13400-970 São Paulo, Brazil. Tel.: +55 19 34294738.

E-mail addresses: marcio_agr@yahoo.com.br, micheleagronomia@yahoo.com.br (M.X. Vieira Megda), emariano@cena.usp.br (E. Mariano), josemarcosleite@yahoo.com.br (J.M. Leite), pcotrive@cena.usp.br (P.C. Ocheuze Trivelin).

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soil subsamples were placed into 250-ml plastic pots and the units of experimental soil were incubated for a period of 21 days (d) in a Biochemical Oxygen Demand (BOD)-type chamber and maintained at 25 ± 1 °C. Inorganic form of N–NO_3^- and the electric conductivity of the soil solution was estimated after one, seven, 14 and 21 d of incubation using the method proposed by Van Raij et al. (2001). The soil microbial biomass C (SMB-C), on the other hand, was assessed after one and 21 d of incubation using the fumigation-extraction method proposed by Vance et al. (1987).

The N–NO_3^- concentration in the AC treatment was higher than in the treatments with KCl at seven, 14 and 21 d of incubation, and the increased KCl dose led to a decrease in N–NO_3^- concentration in the soil (Fig. 1a). These results support the hypothesis that high chloride contents in the soil lead to a reduction of microbial nitrification (Golden et al., 1980). High salt contents in the soil result in the excessive production of oxygen-reactive species (Halliwell and Gutteridge, 1985; Thompson et al., 1987). These species may have a cytotoxic effect causing oxidative damage to lipids (Wise and Naylor, 1987), proteins and nucleic acids (Imlay and Linn, 1988).

Concentrations of N–NO_3^- in the soil after 1 d of incubation were 15, 12 and 11 mg kg^{-1} for doses of 130, 260 and 520 mg kg^{-1} Cl-AC, respectively (Fig. 1b). After seven and 14 d of incubation with a dose of 130-mg kg^{-1} Cl, N–NO_3^- contents in the soil were twice as high as the values observed in the soil with 520 mg kg^{-1} Cl. Souri (2010) assessed the N–NO_3^- concentration in the soil following the application of KCl doses and found similar results.

The N–NO_3^- concentrations were approximately 79, 82 and 64 mg kg^{-1} for 130-, 260- and 520-mg kg^{-1} doses of Cl-AC, respectively, after 21 d of incubation (Fig. 1b). A comparative analysis showed that N–NO_3^- contents in the soil at the end of the incubation period in treatments AC + KCl 200 containing 460 mg kg^{-1} Cl^- (Figure 1a) and 520 mg kg^{-1} Cl-AC (Fig. 1b) were 58 and 64 mg kg^{-1}, respectively.

The addition of AC combined with KCl promoted a decrease in SMB-C after 1 d of incubation (Fig. 2a). However, AC + KCl treatment showed a higher SMB-C after 21 d of incubation than the AC application alone. The results show that the biocide effect of soil chloride on SMB exhibits a short-term effect following the application of salts, and this effect is minimized over time, which is known as resilience time.

The highest SMB-C concentration was observed in 520-mg kg^{-1} dose of Cl-AC 1 d after application (Fig. 2b). This result may be explained by the increased microbial activity stimulation caused by the addition of high doses of N to the soil (200 mg kg^{-1}) in the highest AC dose used (520 mg kg^{-1} of Cl). According to Jenkinson et al. (1985), for providing appropriate conditions for

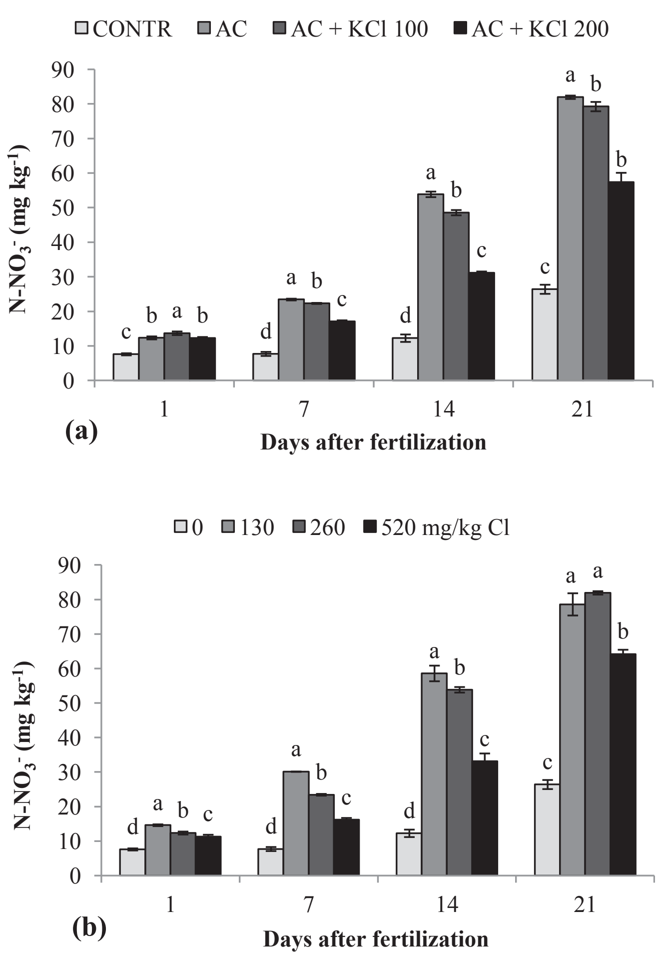

Fig. 1. Soil concentrations of nitrate-N as a function of time related to the application of nitrogen (N) and chloride (Cl). **(a)** AC: ammonium chloride (100 mg kg^{-1} N and 260 mg kg^{-1} Cl); **(b)** chloride doses (0, 130, 260, 520 mg kg^{-1} Cl). The number next to the KCl source refers to the dose of Cl in mg kg^{-1}. Identical letters for identical incubation times do not differ according to Tukey's test ($p < 0.01$). The vertical bar refers to the standard deviation.

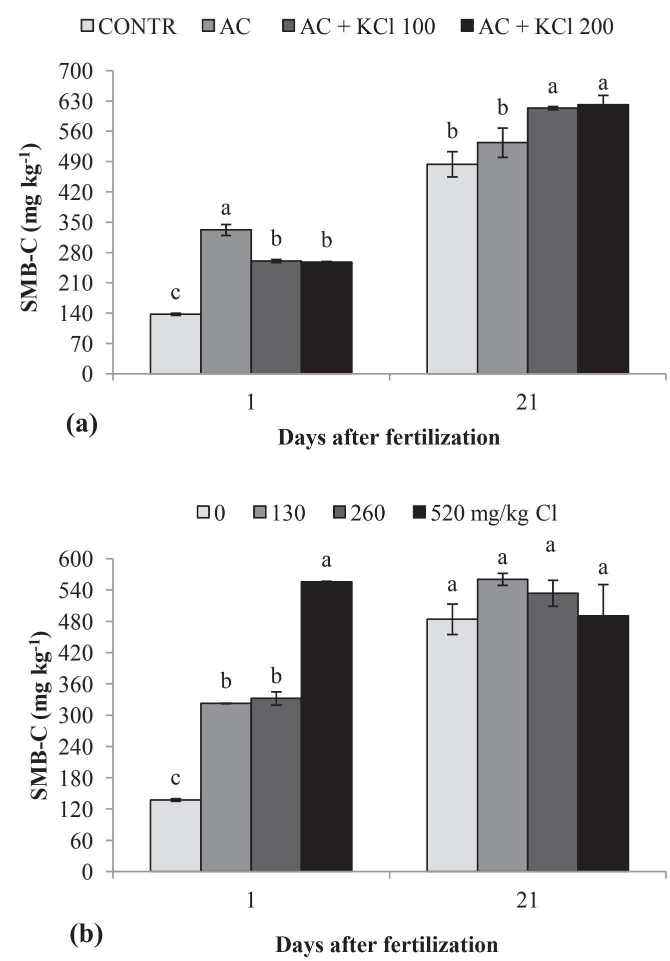

Fig. 2. Soil microbial biomass carbon concentrations (SMB-C) as a function of time related to the application of nitrogen (N) and chloride (Cl). **(a)** AC: ammonium chloride (100 mg kg^{-1} N and 260 mg kg^{-1} Cl); **(b)** chloride doses (0, 130, 260, 520 mg kg^{-1} Cl). The number next to the KCl source refers to the dose of Cl in mg kg^{-1}. Identical letters within the same time period indicate no difference according to Tukey's test ($p < 0.01$). The vertical bar refers to the standard deviation.

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