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Novel Investigation on Ammonium Thiosulphate (ATS) as an Inhibitor of Soil Urease and Nitrification

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Abstract

Among the numerous products so far promoted as effective urease and/or nitrification inhibitors, it is possible to detect a renewed interest in environmentally friendly tools, such as ammonium thiosulphate (ATS, $(NH_4)_2S_2O_3$) which is currently used as fertilizer for N and S nutrition. Among contradictory results accounted in the current literature, there is reported that ammonium thiosulphate (ATS) exerts inhibitory activity at large but unlikely agronomic rates of 2500 - 5000 mg·kg^{-1} soil. We carried out a novel experiment aimed to: a) verify the inhibitory action of ATS, even when applied in soil at low rates (25 and 100 mg·kg^{-1} soil as S-ATS), towards urease activity and nitrification in urea treated and not treated soils; b) investigate on the influence of ATS on the soil microbial biomass as it is generally assumed that soil microorganisms are the main agents of urea hydrolysis. For these purposes we selected an arable sandy soil and a grassland sandy-loam soil which are treated with urea or/and ATS. Results obtained from this novel investigation showed that a) ATS significantly decreased urease activity in both soil types and that the exerted inhibitory effect was moderate and short-term; b) ATS retarded the hydrolysis of urea and lowered nitrate production in the urea treated soils; c) the available fraction of iron and manganese in the used soils might be involved in the mechanism of inhibition; d) ATS did not affect the size of soil microbial biomass pool; e) the efficiency of ATS as urease and nitrification inhibitor was more evident in the sandy soil. Because of its properties, ATS may be applicable on many crops without being harmful on the soil microbial pool.

Keywords

Ammonium Thiosulphate, Inhibitor, Urease, Nitrification, Soils

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1. Introduction

To cope with an increasing global population, rapid depletion of natural resources, increasing environmental pressures and climate changes, there is a worldwide necessity to radically change the approach to consumption and production and to concomitantly optimize the efficiency of use of natural and anthropogenic resources [1]. Modern agriculture nowadays feeds 6 billion people, and the global cereal production has doubled in the past 40 years, mainly because of the increased yields resulting from greater fertilizer input, irrigation and pesticides, new crops varieties, and other technologies implemented during the "Green Revolution" [2].

There is a general consensus that, under the current trends, the global population could increase by 40% within 2050 and the global grain demand would roughly double [3]. Doubling and sustaining grain production without compromising the three pillars of sustainability (*i.e.* environmental integrity, public health and economic benefits) is currently a major challenge and it will be even a greater challenge in the future [4] [5]. Agricultural practices influence not only grain yields and food production in general, but also the state of the global environment. Intensive agriculture depends, to a great degree, on the use of synthetic fertilizers and industrially produced N is the main source of N input in the crop production system worldwide.

However, nitrogen is not only an essential plant nutrient that guarantees high-yield production, it is also a pollutant. Large external inputs of N fertilizer coupled with decreasing N use efficiency contribute to severe environmental pollution, including the degradation of downstream water quality, development of photochemical smog, and rise of global concentrations of gaseous N-oxides, which are known to be powerful greenhouse gases [6] [7].

It is well known that only 30% - 50% of applied N is taken up by crops. The unaccounted percentage represents N losses due to volatilization or leaching processes [8] [9]. N losses can therefore be deleterious for offsite ecosystems, especially considering that in the past half century the global use of N fertilizer increased about tenfold and is expected to further increase by 2050 unless there is a substantial increase in fertilizer efficiency.

Most of the N fertilizers applied to soils for agriculture production are in the form of N-NH_4^+ (ammonium-sulphate, ammonium-nitrate) or NH_4^+ compounds. Urea in particular is the most widely used N source in agriculture worldwide [10] [11], but its efficiency is markedly reduced by nitrous/ammonia and nitrate losses that are significantly affected by weather conditions and vary between soil types [12]-[16].

In most arable soils urea is rapidly converted to ammonia by urease (a metalloenzyme able to catalyse the hydrolysis of urea to ammonia and carbamate). This leads to volatilization of ammonia and emissions of nitrous oxides. In addition, ammonium in soil is rapidly oxidized via chemical and biological pathways, the latter leading first to the production of nitrite (NO_2^-) and then to nitrate (NO_3^-). Nitrate is soluble, negatively charged, mobile in soil and therefore subjected to leaching. For these reasons, controlling the processes of nitrification and/or urease hydrolysis is a potential tool to restrict N leaching and ammonia/nitrous volatilization from soils.

A concomitant achievement of high agricultural yields, food security and environmental sustainability is possible, but this would require considerable changes in nutrient management [17]; firstly, by avoiding the overuse of N fertilizers, and secondly by achieving a higher efficiency of N fertilization which would be the key to optimizing the trade-off among yield, profit, and environmental protection.

With increasing public concern regarding health issues, environmental protection and natural resource sustainability, there is a shift toward the development of environmentally friendly agricultural practices that aim to maximize N fertilizer efficiency. Accordingly, the goal of the precision fertilization is to get the right rate of fertilizer to match the actual N needs of crops and supply at the right time of the growing stage, thereby minimizing nutrient losses and raising profitability. Improving the efficiency of urea-based fertilizers through new technologies and management strategies would therefore be of tangible interest. One of the most promising approaches is fertilization with urea in combination with urease/nitrification inhibitors. Commercial products address either on urease activity or nitrification. However, the strong and long-lasting inhibitory effect exhibited by most of them might be detrimental for crops with short growing periods (especially maize ~130 dd), which require high N uptake over a very short period of time.

Among the numerous products so far promoted as urease or nitrification inhibitors, there is nowadays a renewed interest concerning the use of environmentally friendly tools, such as ammonium thiosulphate $((NH_4)_2S_2O_3)$ (ATS) which is currently used as fertilizer for N and S nutrition. The ability of the fertilizer ammonium thiosulphate in conjunction with urea to retard the hydrolysis of urea by soil urease and nitrification was reported by several experts [18]-[24]. However, in their reports the authors evidenced inhibitory effects only when ATS was

used at rates of 2500 mg·kg^{-1} soil. By contrast, McCarty *et al.* [25] found that ATS showed little, if any, potential value for retarding hydrolysis of urea fertilizer in soil, even at rates higher than 5000 mg·kg^{-1} soil. To contribute to the knowledge we carried out a novel experiment aimed to verify the inhibitory effect of ATS applied to soil at lower rates (25 and 100 mg·kg^{-1} soil as S-ATS), towards urease and nitrification in original and in urea treated soils, and hypothesize the mechanisms involved. To these purposes we selected an arable sandy soil and a grassland sandy-loam soil which were amended with urea or/and ATS.

2. Materials and Methods

2.1. Soil

Composite samples of two soils from NE Italy were collected at 2 - 10 cm depth. We sampled a grassland sandy-loam soil (A) (Gorizia 45˚56'6,7"N, 13˚37'95"E, a grassland Mollic Hapludalf soil, USDA 1999) and an arable sandy soil (B) (San Martino 46˚1'0"N, 12˚52'0"E, a Fluventic Eutrudept arable soil, USDA 1999). Each fresh soil sample was sieved to 2 mm to remove rocks and root materials, thoroughly hand-mixed, and divided into two subsamples. One subsample was stored at 4˚C until incubation began. The other subsample was air-dried to measure soil parameters, according to SSSA standards [26].

Selected chemical characteristics of the used soils are reported in **Table 1**.

2.2. Soil Treatments

Soil samples were adjusted to 35% of their respective water holding capacity (WHC) and pre-conditioned in airtight plastic containers at 20˚C for 7 days to allow microbial activity to regain its equilibria after the initial disturbance [27].

Soil samples were then treated with ATS, and/or urea solutions in order to add 25 ppm of S (corresponding to 32.5 kg S-ATS ha^{-1}), 100 ppm of S (corresponding to 130 kg S-ATS ha^{-1}) and 150 kg N ha^{-1} to soil as urea or urea plus ATS (**Table 2**). The ATS and urea working solutions were prepared by diluting urea and the commercial product Secofit® TS (ATS) in water. The concentration of the applied solution was calculated to bring the soil to 40% WHC after addition. The control (T0) received only distilled water. The treated soils were then placed in airtight plastic boxes. Two jars containing respectively distilled water and 1 M NaOH were put into each box to prevent changes in water content and to trap the evolved CO_2. The boxes were then placed in an incubator at 20˚C for 15 days. The soil moisture level was controlled twice a week and adjusted when necessary. To avoid the onset of anaerobic conditions, the boxes were opened once a day and the soil samples thoroughly mixed. The applied doses were calculated on the basis of the soil density (1.3 t·m^{-3}).

Table 1. Selected parameters for soil characterization.

Soil	Sand	Silt	Clay	pH	CSC	C_{ORG}	N_{TOT}	C_{MIC}	Available Fe	Available Mn
	(%)	(%)	(%)		cmol$^+$ kg^{-1}	g·kg^{-1}	g·kg^{-1}	µg·g^{-1}	mg·kg^{-1}	mg·kg^{-1}
Sandy loam (A)	37	48	15	7.8	8.2	25.4 ± 1.6	2.8 ± 0.26	720 ± 54	21 ± 1.8	23 ± 1.7
Sandy (B)	68	28	3	8.3	3.5	6.5 ± 0.4	0.9 ± 0.08	155 ± 13	8 ± 0.7	12 ± 1.0

Table 2. Scheme of the soil treatments.

	Soil treatment
T0	Control soil
T1	Urea 150 kg N ha^{-1}
T2	ATS as 32.5 kg S-ATS ha^{-1} (*i.e.* 25 ppm S-ATS)
T3	ATS as 130 kg S-ATS ha^{-1} (*i.e.* 100 ppm S-ATS)
T4	150 kg N ha^{-1} as ATS (32.5 kg S ha^{-1}) + urea (134 kg N ha^{-1})
T5	150 kg N ha^{-1} as ATS (130 kg S-ATS ha^{-1} + urea (85 kg N ha^{-1})

The scheme of the soil treatments is shown in **Table 2**.

2.3. N-NH_4^+ Determination

The amount of ammonium was determined by extracting NH_4^+ from soil with 0.5 M K_2SO_4 (1:5 w/v). The ammonium produced during the incubation period was then determined colorimetrically by a modified indophenol-blue reaction, based on the reaction of Na salicylate with NH_3 and Na-dichlorisocyanurate. The intensity of the developed blue colour was measured at 690 nm [28].

2.4. N-NO_3^- Determination

N-NO_3^- was extracted from soil with K_2SO_4 (10 g sample and 50 ml of 0.5 M K_2SO_4) and the NO_3^- content in the extracts was recorded at 220 nm. The true concentration of N-NO_3^- was obtained by subtracting the spectral interference at 275 nm due to the presence of organic matter from the results obtained at 220 nm [29].

2.5. Soil Microbial Biomass Carbon (MBC)

Soil microbial biomass carbon (MBC), which represents the quantity of C in the cells of living microorganisms in the soil, was determined according with the fumigation-extraction method [30]. Moist soil portions, each corresponding to 5 g of oven dried soil, were fumigated with ethanol-free chloroform for 24 h, then extracted with 20 ml 0.5 M K_2SO_4 for 30 min. A set of non-fumigated soil samples was similarly extracted at the time fumigation commenced. The K_2SO_4 extractable organic carbon (OC) was determined by an OC analyser (Shimadzu TOC-VCSN). MBC was calculated from the equation MBC = K × EC, where EC = [(OC extracted by K_2SO_4 from fumigated samples) (OC extracted by K_2SO_4 from non-fumigated samples)] and K (conversion factor) = 2.22.

2.6. Determination of Available Iron and Manganese

Assessment of available Fe and Mn was conducted according to the DTPA/TEA method developed by Lindsay and Norwell [31]. Briefly, 20 ml of DTPA/TEA were added to 10 g soil and the mixture was shaken for 2 h. After centrifugation the supernatant was collected, filtered, stored at 20˚C and subsequently analyzed by ICP-AES (Perkin Elmer, Optima 3000).

3. Results and Discussion

3.1. Influence of ATS on Urease Activity in Untreated or in Urea Treated Soils

There is general agreement that the soil urease strongly prevails in the hydrolytic process of urea and that the production of NH_4^+ can be a reliable expression for determining the enzymatic activity. As shown in **Figure 1(a)** and **Figure 1(b)**, ATS significantly impaired urease activity in both soil types, but the exerted inhibitory effect was short-lived.

The maximum enzymatic inhibition occurred within the first three days when urease reached its lowest level in both the soils, and recovered afterwards with a different course. In comparison with the respective control, after application of 25 ppm S-ATS urease activity decreased by 23% in the sandy-loam soil (A). However, the enzymatic activity recovered to the initial values within few days. In the sandy soil (B), application of the same dose of ATP resulted in a decrease in urease activity by 53%. Conversely to soil A, the enzymatic activity did not recover afterwards, remaining at a lower level than that observed in the respective control soil. At the higher ATS dose (100 ppm S-ATS) the inhibition reached 43% in soil A and 82% in soil B, and persisted in both the soils until the end of the experiment. Our results therefore indicate that ATS had a more marked inhibitory effect in the sandy soil (B) which is characterized by a lower content of organic matter and higher pH.

Concerning the treatments with urea, since samples were supplied with equal initial amounts of total N (*i.e.* 150 kg N ha^{-1}) albeit N originated in different proportions from urea and ATS, the amount of extracted NH_4^+ in amended soils resulted from hydrolysis of urea per se or urea plus ammonium from ATS. Therefore, in order to ascertain the net influence of ATS on the hydrolytic process of urea we normalized the data by subtracting NH_4^+ produced from ATS to the corresponding soil samples treated with urea. As evidenced by **Figure 2(a)** and **Figure 2(b)**, the ammonium produced was ATS-dose and soil type dependent.
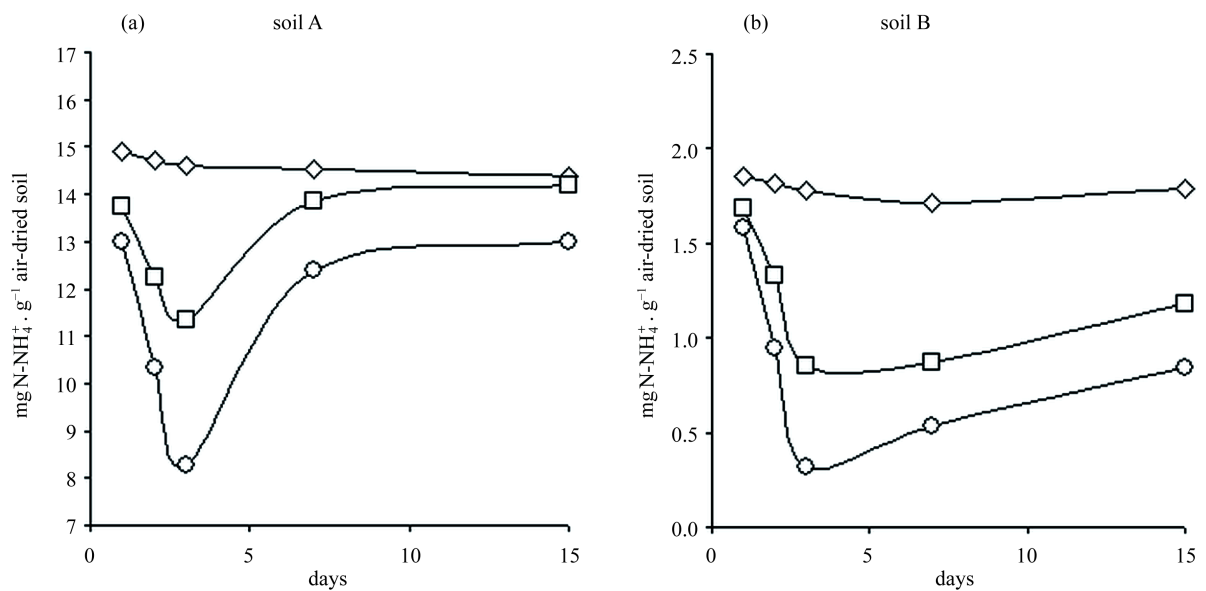
Figure 1. Dynamic of urease activity in sandy-loam soil (a) and in sandy soil (b). amended with 25 ppm (□) or 100 ppm (○) S-ATS, and no treated soil (◊).
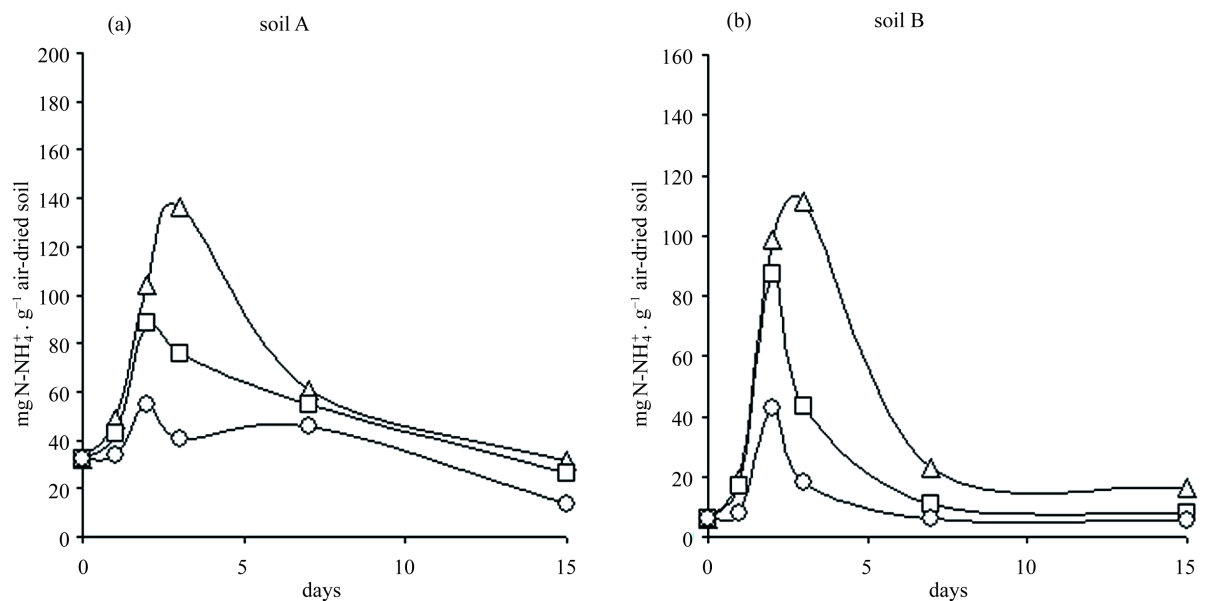

Figure 2. Dynamic of N-NH$_4^+$ concentration in samples of sandy-loam soil (a) and in sandy soil (b). Soils were treated with urea (Δ), urea + 25 ppm S-ATS (□), and urea + 100 ppm S-ATS (○).

Application of urea alone significantly affected the ammonium production in both types of soil already after the first day, achieving the maximum level within 3 days of incubation, followed by a subsequent decline with different courses in soils A or B. In the sandy soil (B) characterised by a lower amount of organic carbon and a higher pH, after reaching the maximum, NH$_4^+$ concentration sharply decreased to background values at the end of the first week. On the other hand, in the well textured sandy-loam soil (A) ammonium levels remained constant over time.

Comparing the levels of NH$_4^+$ in the treated soils, we observed that in sandy-loam soil (A) application of 25 ppm or 100 ppm S-ATS reduced the production of ammonium, and therefore urease activity, by 37% and 68%,

respectively. Even more evident was the inhibition of urease activity in the sandy soil (B) where the depression of NH_4^+ production reached 70% at the lower S-ATS rate and 88% at the higher.
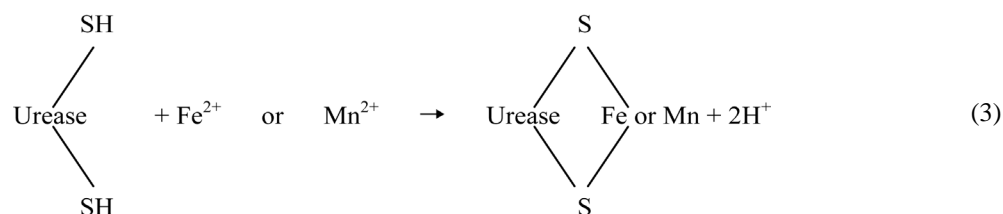
The influence of soil properties on urease activity has been documented previously. For example, MacGarity and Myers [32] observed that urease was highly correlated with organic carbon, and Zantua *et al*. [33] reported evidence for the persistence of urease in soils high in organic matter. Concerning results obtained in our experiment, we suppose that the persistence of urease activity in ATS-treated soils could be a consequence of enzyme-protecting mechanisms exerted by soil components The adsorption of enzymes by minerals, humus and organo-mineral colloids is a common phenomenon in soil environment [34]. In particular, the functional groups occurring on the surfaces of soil inorganic and organic colloids show great affinity with extracellular urease, and formation of urease-colloidal complexes results in urease being stabilized and protected against hydrolytic process [35]-[38]. What is more, humic acids behave as supramolecules which are able to polymerize and aggregate, and form supramolecular ensembles with proteins, such as the enzymes [39] and hence retain the enzyme activity [40]-[45].

Moreover, specific chemisms which could have promoted the formation of water-soluble Shiff bases through reaction of ammonia produced from urea-hydrolysis with aldehydic/carbonyl functional groups of dissolved organic compounds can be co-responsible for the prolonged persistence of in the well-textured soil A. The formation of Shiff bases could have facilitated the persistence of ammonium into the soil solution thus reducing the risk of ammonia losses, and consequently increased potential efficiency of the N-fertilizer.

Even though the specific mechanism through which ATS affects urease has still not been clearly assessed, the involvement of indirect pathways have been hypothesized. Thiosulphate is unstable when added to soils and is oxidized (via biological and chemical pathways), among other intermediate compounds, to the stable tetrathionate which is thought to be the actual urease inhibitor, by reacting with –SH groups in jack bean urease to give a S-sulpho derivative [46], as:

$$RSH + S_4O_6^{2-} \rightarrow RS-S_2O_3H + S_2O_3^{2-} \tag{1}$$

$$RS-S_2O_3H + O_2 \rightarrow RS-SO_3H + SO_2 \tag{2}$$
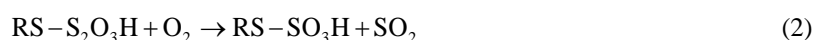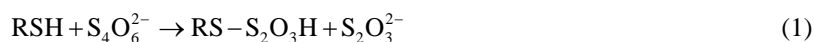
There is general agreement that the formation of tetrathionate is largely abiotic, presumably mediated by transition metals, including Fe and Mn oxides as electron acceptors. Furthermore, Goos [18] and Sullivan and Havlin [24] assumed that reduced iron and manganese may also inhibit urease activity by altering the conformation of the sulphydryl groups in the catalytic center of the enzyme, according to the following simplified reactions:

$$
\begin{array}{c}
\text{SH} \\
/ \\
\text{Urease} \qquad + \text{Fe}^{2+} \quad \text{or} \quad \text{Mn}^{2+} \quad \rightarrow \quad \text{Urease} \quad \text{Fe or Mn} + 2\text{H}^+ \\
\backslash \\
\text{SH}
\end{array}
\tag{3}
$$

Being both a reducing and acidifying agent, thiosulphate has been recently proposed also as a tool for optimizing phytoremediation by enhancing the availability of inorganic contaminants in soil [47].

Since it was reasonable to suppose that even available fraction of Fe and Mn in examined soils would be improved by ATS addition we carried out a preliminary experiment to verify whether ATS would affect the mobility of Fe and Mn in soils during the incubation period, and to find a possible correlation between soil urease activity and the concentration of the available fraction of Fe or Mn.

Compared to the respective control soil, we observed that the available fraction of iron and manganese in both tested soils sharply increased after the application of ATS, reaching the highest amounts (+11.7% and +17% for Fe and Mn, respectively for soil B) within three days and decreased afterwards.

Due to the likely similar dynamics of urease activity (as N- NH_4^+ production), we checked whether urease activity and available Fe and Mn were correlated, regardless of soil type and treatment in order to generalize a relation between the two parameters. By plotting the proportion of soil urease activity (Tn/T0) against available Fe and Mn (**Figure 3**) we observed a linear and significative correlation ($y = -4.7951x + 583.65$, $R^2 = 0.9031$) thereby providing evidence that the soluble fractions of iron and manganese indeed affect soil urease activity.
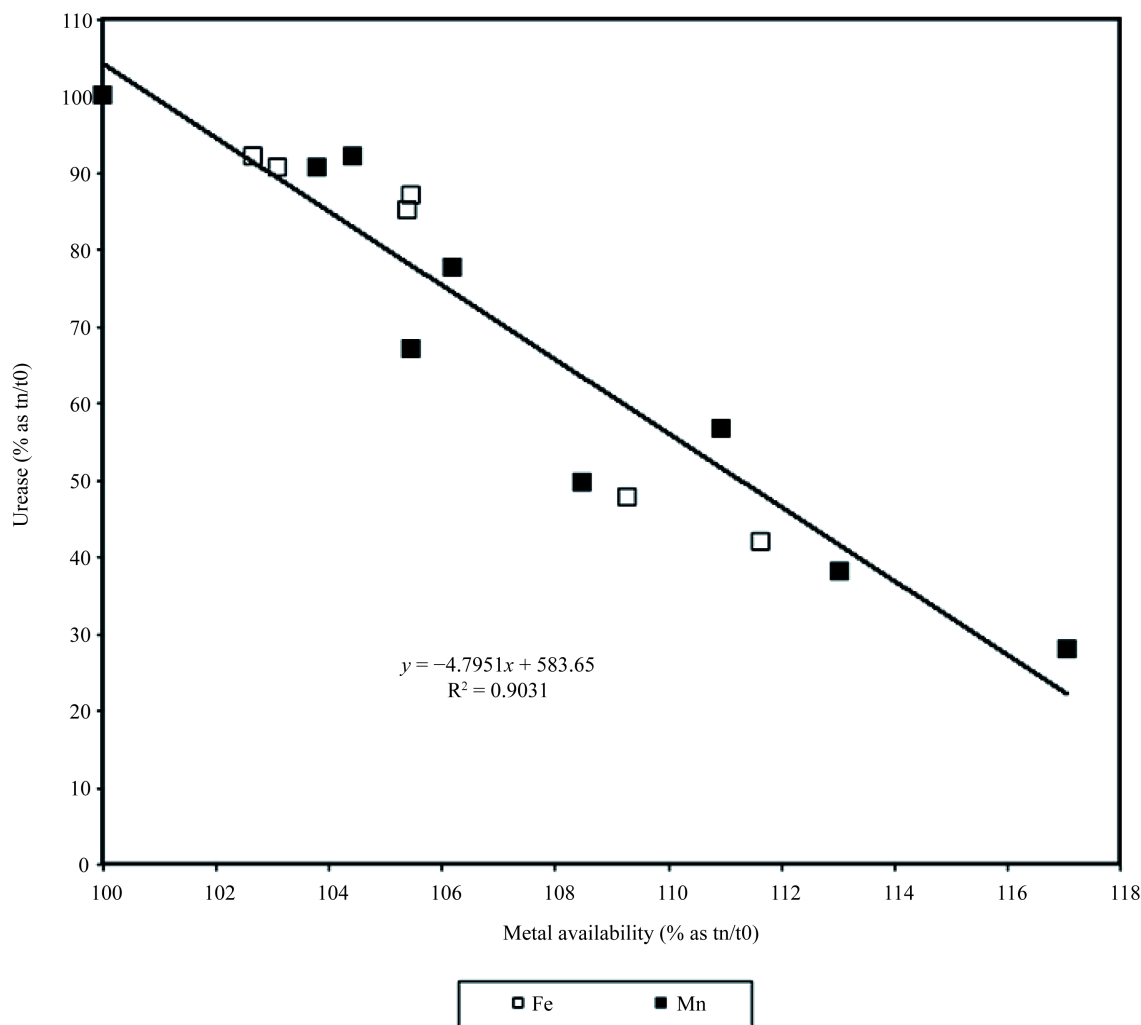
Figure 3. Relationship between soil urease and available Fe (□) and Mn (■).

3.2. Influence of ATS on Nitrification in Untreated or in Urea Treated Soils

Even though ATS did not significantly affect the amount of nitrate extracted from both of the soils, we observed that ATS exerted an evident and significant inhibitory effect on nitrification in urea treated soils. **Figure 4(a)** and **Figure 4(b)** show the dynamics of the normalized amounts of nitrate (expressed as N- NO_3^-) in soils treated with urea (T1) or urea plus ATS (*i.e.* T4, T5).

Generally, when no ATS was applied (T1) nitrate rapidly increased throughout the two weeks of incubation, whereas the presence of thiosulphate delayed the initial nitrification rate and significantly reduced NO_3^- formation. Evidence for the ability of ATS to delay nitrate formation has been reported in previous studies. Bremner [48] reported that the addition of ATS significantly retarded ammonium oxidation only at application greater than 500 ppm. Our results, however, showed evidence that ATS delayed NO_3^- formation even when applied at considerably lower rates (25 ppm S-ATS).

Similarly to the results obtained for soil urease, the inhibition of nitrate production was more evident in the sandy-loamy soil (B), confirming the influence of soil properties on enzymatic processes, and nitrification in particular [49].

Several abiotic and biotic processes are considered to be involved in inhibition of nitrification by ATS. Janzen and Bettany [50] stated that the inhibitory effect of thiosulphste on nitrification may be largely attributable to a direct toxic effect of thiosulphate or its oxidation products (tetrathionate, carbon disulphide) on *Nitrobacter sp.* and other NO_2^- oxidizing microorganisms.
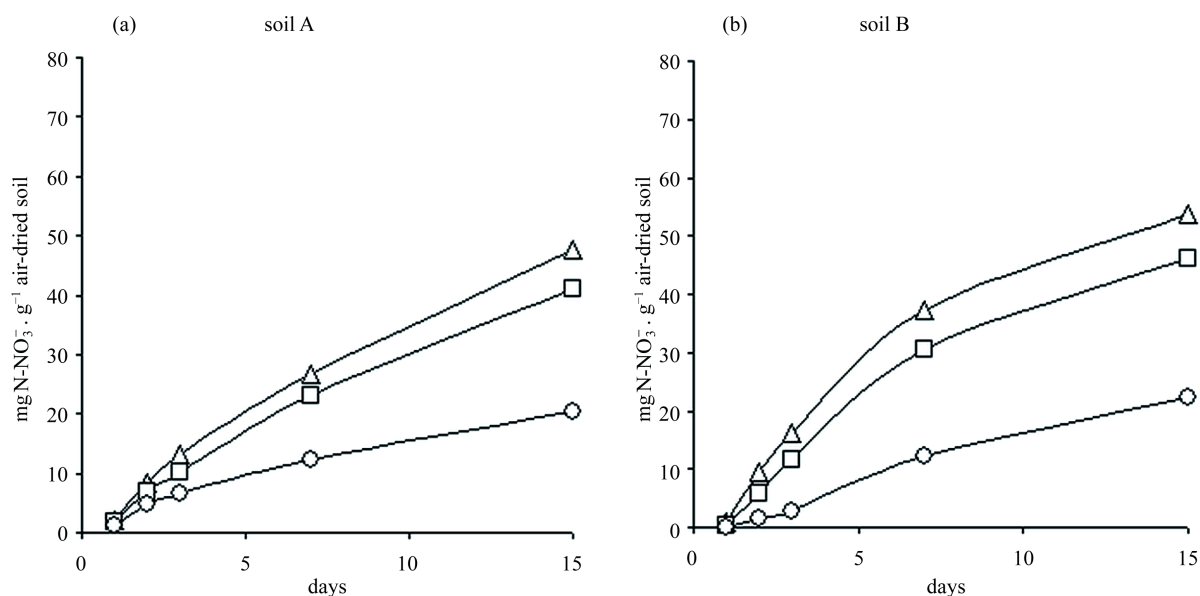
Figure 4. Changes in Nitrate $\left(\text{N-NO}_3^-\right)$ concentration in samples of sandy-loam soil (a) and in sandy soil (b). Soils were treated with urea (△), urea + 25 ppm S-ATS (□), and urea + 100 ppm S-ATS (○).

In our experiment, we noticed that the rate of NH_4^+ disappearance did not correspond to the rate of NO_3^- formation, suggesting that ATS led to the stabilization of N- NH_4^+, and thus delaying the production of nitrate [51] [52]. In addition, our results are in accordance with those reported by Al Kanani *et al*. [53] who attributed the non-concomitant NH_4^+ to NO_3^- conversion to the accumulation of nitrite, adversely affecting the amount and the activity of soil microorganisms, thus retarding nitrification

3.3. Influence of ATS on the Size of the Soil Microbial Biomass in Untreated or in Urea Treated Soils

It is generally assumed that soil microorganisms are the main agents of urea hydrolysis [54], and as we found that ATS inhibit soil urease activity, especially in sandy soil B, changes in the size of soil microbial biomass would be expected. The maintenance of soil fertility depends also on the size and activity of the soil microbial biomass, a small fraction of soil organic matter (1% - 4%). In addition, microbes, having both mass and activity and being in intimate contact with the soil microenvironment are, in many ways, ideal monitors of soil quality or perturbation [55] [56]. The evaluation of the size of soil microbial biomass, expressed as soil microbial biomass carbon (MBC), gives useful information about the deviation of the soil environment from its equilibrium. In our experiment, we found that the original size of soil microbial biomass did not change significantly in none of the ATS-amended soils. In effect, the amount of MBC ranged from 150 to 160 $\mu\text{g}\cdot\text{g}^{-1}$ soil in sandy soil (B) and from 710 to 735 $\mu\text{g}\cdot\text{g}^{-1}$ soil in sandy-loam soil (A), thus revealing that all the ATS-treatments had no effect on the total amount of original microbial pool.

4. Conclusion

The use of moderate inhibitors able to enhance the N availability in soils to meet the N demand of crops during a specific plants' physiological stage is consistent with the aims of precision fertilization. Results obtained in our experiment provide evidence that ATS alone or combined with urea exerted moderate inhibitory effects, both on soil urease activity and nitrification. In addition, we found that the inhibition was more evident when ATS applied to a sandy soil than to a sandy-loam soil, even at a low rate (25 ppm S-ATS). These characteristics are valuable in the context of optimizing the efficiency of N fertilization and also reducing nitrate leaching and ammonia volatilization in the early stages of crop growth when root growth and the plants' N uptake ability after the application of urea are limited. In addition, we observed that the microbial biomass size did not change sig-

nificantly throughout the experiment neither of the investigated soils, revealing that ATS application had no negative effect on the native microbial pool, thus preserving the biotic component of soils. Our results also provide evidence that ATS application can be considered as an environmentally friendly tool among agronomic practices as it can be used both as liquid fertilizer for N and S plant nutrition and as a weak inhibitor of urease and nitrification. For all these characteristics we assume that farmers may use this product for many purposes and in different environmental conditions and in different farming systems. Because of its properties ATS could be applicable on many crops such as maize, wheat, rye, rape, etc., and in general on crops of high N demands and with a short growing period without being harmful on the soil microbial pool.

Acknowledgements

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