

Application of the Humic Substances and Ammonia in Order to Minimize Losses on Nitrogen Fertilization

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Abstract

Nitrogen (N) is an element absorbed in larger quantities by plants due to the vast number of chemical and biological reactions. Despite the high nutritional need of this element by the plants, it is not a constituent of any terrestrial rock. To avoid high production costs of N, there is an increasing interest in the use of organic fertilizers in Brazil, including the use of humic substances (HS). In this work we evaluated the chelating properties of HS, when used together with a source of N. The nitrogen source used in this experiment was the ammonia. We checked the optimum conditions for interaction between the HS and ammonia such as: the ratio of reaction of each compound; minimum incorporation time between these compounds and the amount of ammonia that can be adsorbed by HS (income). The best ratio of ammonia/HS was 1/50 (v/v). Higher amounts of ammonia added to 50 mL of HS caused a greater loss of N. We also concluded that the application of ammonia/HS in the soil can be made immediately after their mixing, without the need of higher contact time between these substances in order to have the adsorption. No loss of ammonia was detected after 72 hours of its application together with HS. Finally, by using a factorial design, the best working area was found by settling the time of incorporation and volatilization in 24 h, and the volumes of HS and NH₃ in 100 and 2 mL, respectively.

Keywords

Organic Fertilizer, Adsorption, Volatilization, Experimental Planning

1. Introduction

Nitrogen (N) is an element absorbed in larger quantities by plants due to the

multitude of chemical and biological reactions which are involved; among them, one can mention protein synthesis [1]. Despite the high nutritional need of such element by the plants, this is not a constituent of any terrestrial rock, and it is present only in the atmosphere (78%). This fact adds to nitrogen an elevated production cost due to the high energy consumption for its industrial setting, known as Haber-Bosch process. This cost, consequently is passed on to farmers [2]. For this reason, interest in the use of organic fertilizers in Brazil has increased significantly in recent years, among them, the use of humic substances (HS).

HS are formed when substances that are considered non-humic (waxes, carbohydrates, proteins and nucleic acids), are degraded by microbial action [3]. Generally, these formed compounds are rich in carbon, hydrogen and oxygen; they can be bound to functional groups, such as OCH_3 (methoxyl), OH (alcohols) and COOH (carboxyl). These humic compounds may be different because they depend on the nature of the units that constitute them [4] [5].

This organic material contains about 90% of the organic carbon reserves [6] [7], and can be divided into three parts: humin, fulvic acids, that are capable of complexing Fe, Cu, Ca and Mg and humic acids, formed by polymers, aliphatic and aromatic compounds, and may still form humates when combined with metals [8]. Another feature of the HS is its influence on several soil properties such as: stability of aggregates, infiltration and water retention, among others. In addition to improving the agriculture production [9], the HS decrease the costs of fertilizers and defensives, and the concern about environmental pollution is grown.

From these facts reported in the literature, this study aimed to evaluate the chelating properties of HS, when used together with a source of N_2 . In this work we sought to evaluate the interaction process between nitrogen and HS looking to find the optimum conditions for interaction between the HS and ammonia such as: concentration of each compound in order to avoid waste at the time of application; minimum incorporation time between these compounds and the amount of ammonia that can be desorbed by HS (income).

2. Materials and Methods

2.1. Working Solutions and Reagents

Hydrochloric acid (Sigma-Aldrich, St. Louis, MO, EUA), phenolphthalein (99% purity) and ethanol A.R. (Merk Darmstadt, Germany). Hydrochloric acid working solutions (0.1 ; 0.5 and $0.1 \text{ mol}\cdot\text{L}^{-1}$) were prepared from a $10 \text{ mol}\cdot\text{L}^{-1}$ stock solution, and phenolphthalein was solubilized in 95% ethanol.

Commercial grade Eurochem ammonia with 25% nitrogen (Deutschland, Germany) was used. The HS (humic extract of the peat) was provided by Agrolatino Company located in Matão (city of state of São Paulo, Brazil).

2.2. Procedures

To evaluate the best ratio of interaction between ammonia and HS, the minimal

incorporation time and the yield between these two compounds, the following standard procedure was adopted: HS and ammonia (solution A) were added in an Erlenmeyer and, in other similar container, 350 mL of water and three drops of phenolphthalein (solution B). This system was connected through a small glass tube with built-in tap between the flasks. At the end of each conical flask, a stopper was placed so that the ammonia would not be lost to the environment and could be quantified. This system can be seen in **Figure 1**.

To complete the study and better understand the system used, a series of experiments were made via factorial design to verify which factors are most influenced by the fixation of ammonia in HS.

3. Results and Discussion

In order to evaluate the best conditions of application of the product together with HS, the experiments were carried out using ammonia and humic substances and titrating the result of this reaction with HCl $0.1 \text{ mol}\cdot\text{L}^{-1}$

The first experiment assessed the amount of ammonia which may be added to 50 mL of HS without causing saturation of the nitrogen in the fertilizer, giving as consequence the loss of the product to the environment. The amount of ammonia was ranged from 1 to 6 mL (from 1 to 1 mL). The values of ammonia added to HS were calculated based on the concentration applied in the different cultures ($200 \text{ mL}\cdot\text{m}^2$) and the 50 mL of HS are equivalent the amount of organic material applied per hectare ($200 \text{ m}^3\cdot\text{ha}^{-1}$). This system was left static for 24 hours, because some organic materials need to stay in rest to have a best incorporation. The resulting of the reaction was titrated after 24 hours of the tap opening among the erlenmeyers.

Observing **Figure 2**, it can be concluded that in this situation the best ratio of ammonia/HS is 1/50 because, it can be observed that the loss of ammonia was

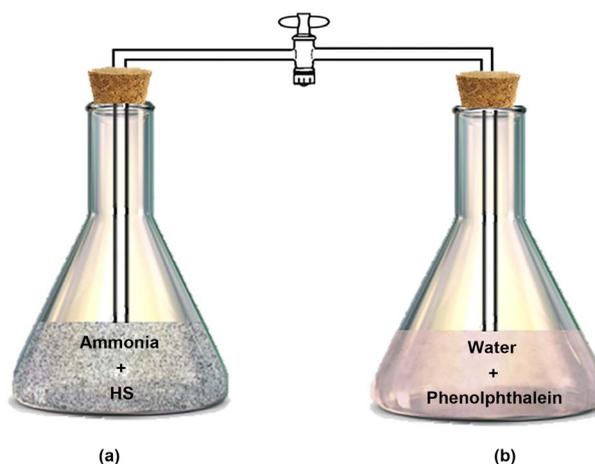


Figure 1. Scheme of the quantification of ammonia unfixated by HS (a) transferred across the tube with tap to other erlenmeyer with water and phenolphthalein (b). Both the erlenmeyers were sealed with stopper to avoid losing the environment.

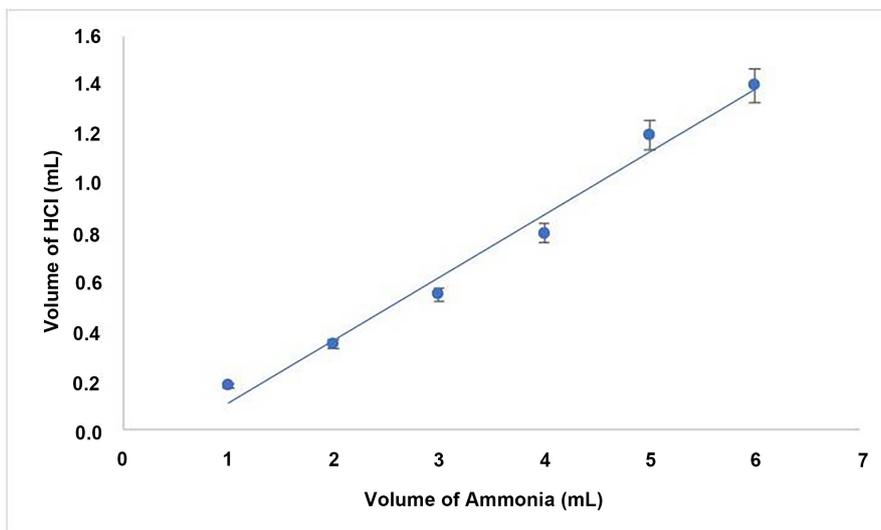


Figure 2. Titrated HCl volume in solution B (water and ammonia) when the amount of ammonia added to HS was varied for the purpose of calculating the displaced ammonia concentration of system A.

proportional when more of this source of the nitrogen was added to the organic material.

This may have been due to the absorption of ammonia and the humic substance being strongly affected by the presence of polar functional groups, such as carbonyls, carboxyls and esters, which increase ϵ in relation to the absorption of unsubstituted rings [10]. Probably the ratio 1/50 mL was the best found due to this filling of ammonia absorption sites.

Studies have shown that urea-applied SH reduce NH_3 volatilization because of their high capacity to exchange cations (CEC), acidity and high buffering capacity [11] [12]. According to [13] [14], the carboxylic and phenolic groups are the most abundant ionizable sites in HS and determine the acidic character, the CEC and the buffer capacity of the acidity. According to the literature, [11] [15] found that the application of combined urea HS extracted from soil reduced NH_3 volatilization by 30%. In addition, the mode of application and the dose of urea are also determinant in the volatilization potential [16] [17].

Losses of nitrogenized fertilizers to the environment, in recent years, proved to be a growing concern related to environmental pollution [18]. These losses, from the inadequate management and its dependence on soil and weather conditions during application, in particular the water resources, for nitrate, and the atmosphere, for nitrous oxide [18] [19], has stimulated the search for management systems that increase the efficiency of this form of fertilization with greater use of nitrogen, which is involved in a multitude of chemical and biological reactions of plant metabolism. Thus, it provides increased productivity and profitability for producers, considering the cost/benefit and sustainability of the agroecosystem [20] [21].

To confirm these results, the amount of HS in the system varied from 10 to

120 mL (10 to 10 mL) using 1 mL of urea as established in the previous experiment. The results can be seen in **Figure 3**, where it was confirmed that ammonia loss is minimized when 1 mL of ammonia is used for 50 mL of the organic material. There are no reports in the literature of the average amount of urea/HS ratio that must be mixed to minimize volatilization losses, as this will depend on the nature of HS being used, as cited by Stvenson, who reports that the nature of the organic material defines the amount of ammonia that it will chelate in its structure and not the amount with which both are applied, since in the organic structures there are reactions of molecular condensation that favor the incorporation of nitrogen compounds in the structures [22] [23] [24].

Another parameter evaluated was the time required for the HS, in contact with ammonia, to be incorporated into the sites of this organic material with the purpose of reducing volatilization losses. Although there are several studies confirming the application of nitrogen along with HS, there are no studies showing its N time to this organic material. Thus, 50 mL of HS were placed in contact with 1 mL of ammonia and, in another flask, water and phenolphthalein. This system remained closed for 24, 48 and 72 hours. Then, the valves were opened and the water/phenolphthalein solution from all systems was titrated 24 hours after their opening of the system.

The volume of the titration of the three time periods were, on average, 0.83 mL, as shown in **Table 1**.

Thus, it can be observed that it is not necessary to leave the humic material and ammonia in contact for a specified time so that they are adsorbed, as the HCl titrated values were within 5% of experimental error. This fact shows that in the field, the application of ammonia/HS can be made immediately after mixing, without need of rest to occur adsorption between the substances.

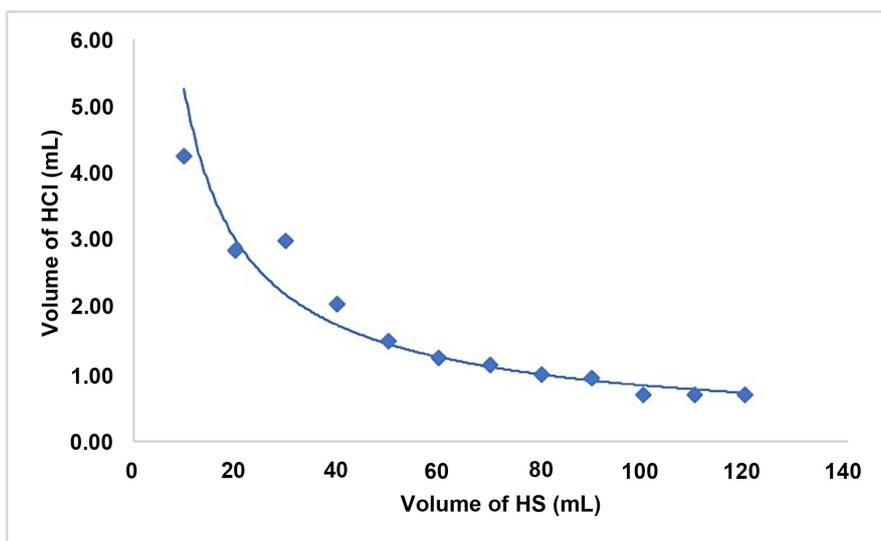


Figure 3. Titrated HCl volume (mL) in solution B (water and ammonia) when the amount of HS added to ammonia was varied in order to calculate the ammonia concentration displaced from system A. The curve was fitted as a power function.

Table 1. Volume of HCl used in the titration at different times of incorporation of HS, in contact with ammonia.

Time of incorporation (h)	Titrated volume (mL)
24	0.80
48	0.85
72	0.83
Mean	0.83

In addition to assessing the time of incorporation of these two important materials for agriculture, it could also be observed the time of ammonia dissipation to the environment after its application in combination with the organic material. For this, the system described previously was kept closed for 24 hours and titrated after 24, 48, 72, 96 and 120 hours of opening the valves, thus obtaining, after each titration, the following volumes, which can be seen in **Table 2**.

Observing **Table 2**, it is possible to conclude that there is a loss of ammonia until 72 hours after the valve is opened, after this time the system comes into equilibrium. This may also occur in the environment where an amount of nitrogen fertilizer, applied in conjunction with the HS, is lost to the environment but, after a certain time, the system stabilizes.

The N losses in soil may occur due to the presence of several enzymes such as L-asparaginase, L-glutaminase, amidase and urease. Urease is the main catalyst enzyme for urea hydrolysis to CO₂ and NH₃ and is widely distributed in nature. The rate of hydrolysis of urea to ammonia is influenced by the type of organic material applied as well as its amount added. Furthermore, the intensity of the effect of organic matter on urease activity will depend on its availability as an energy source for microorganisms and also on the ease of microbial decomposition of organic matter [25].

According to the literature, one way to increase the efficiency of nitrogen fertilizer use may be the use of fertilizer associated with humic substances where they observed a synergistic effect on the association of humic acids from coal with urea compared to the application of conventional urea only [26]. Nevertheless, there are few studies about this mechanism by which humic acids help to increase the efficiency of nitrogen fertilizer use.

A similar study showed that the application of urea together with HS reduced its volatilization, and the period with the highest losses in these conditions was between 37 and 41 hours, similar to the one reported in this study [27] [28].

Another fact that attracted the attention of many researchers is the HS's ability to incorporate certain fertilizers, including nitrogen fertilizers, and to make controlled release of this material according to the need of plants. Thus, the capacity of incorporation of ammonia by HS was evaluated, calculating its yield from ammonia titration (without addition of HS) under the same conditions of previous experiments. The titration consumed 1.5×10^{-3} moles of HCl. On the other

Table 2. Time of ammonia volatilization into the environment when applied in conjunction with the HS.

Time of volatilization (hours)	Titrated volume (mL)
24	0.8
48	1.4
72	1.6
96	1.6
120	1.6

hand, the number of moles of HCl consumed in the titration of a sample of aquatic ammonia together with HS under the same conditions was 1.6×10^{-4} moles. From these results, it can be seen that 90% of ammonia is adsorbed to HS and only 10% are lost by the environment.

To better understand what are the variables that influence the most in ammonia setting by HS, it was decided to perform a 2^4 full factorial design, with duplicate, generated by 10.0 Statistica software, in which four factors were studied at two different levels. The factors studied were: amounts of HS (20 and 100 mL) and NH_3 (2 and 4 mL); volatilization time (24 and 48 h) and incorporation time (24 and 48h). The design generated 32 experiments that were performed randomly to avoid interference of systematic errors. **Table 3** shows the factors encoded with signal “+1” to the top level, and signal “-1” to the lower level.

The full factorial design is used to identify which variables are more important to the studied response; observe their interactions; predict the system response in a study on a condition that has not been realized in practice; to know, in advance, how many experiments should be performed and generate less chemical and mathematical waste and save time [29]. The coding is important, since it gives equal importance to all variables, which are therefore written in a single scale, -1 and +1, regardless of its original value. It is necessary that the experiments are carried out randomly, so that systematic errors are minimized [30].

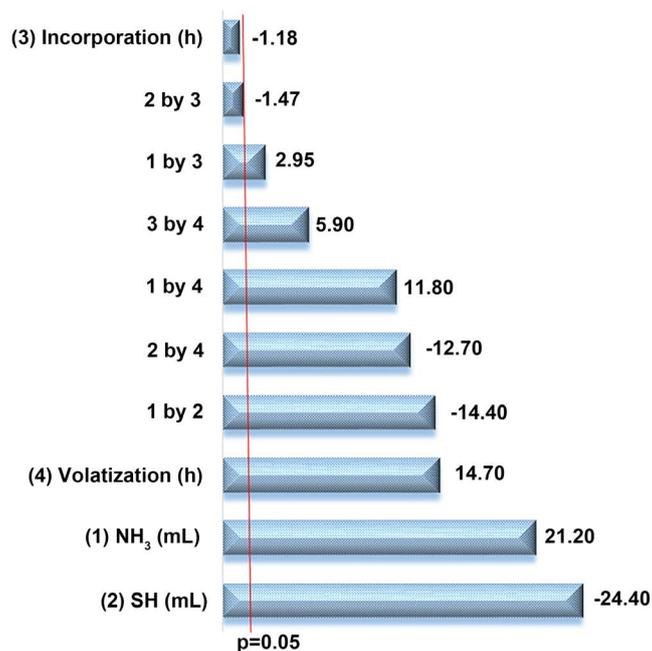
With the factorial design, one can estimate the effect of the variables studied, and to conclude which of them were statistically significant (95% confidence) and thus define the best fixing region of ammonia in HS, within the space of variables studied.

The generated Pareto plot confirmed that the incorporation time at the levels tested was not a significant variable, showing that the compounds can be applied immediately after mixing in the field. The most significant parameters shown in the Pareto graph were the amount of HS and ammonia that consequently encompasses the volatilization time. These conditions should be taken into account because the quantities applied will minimize the losses of this form of fertilization by volatilization (**Figure 4**).

Figure 3 shows the response surfaces obtained by 2^4 factorial design with replicates, fixing the incorporation in the lower level (24 h), as this is not a significant

Table 3. First 2⁴ factorial design, with replicates, and the encoded factors to determine the best region of ammonia fixation in HS.

Factors	Levels	
	(-1)	(+1)
HS (mL)	20	100
NH ₃ (mL)	2	4
Incorporation (h)	24	48
Volatilization (h)	24	48

**Figure 4.** Pareto chart of standardized of the parameters used in fertilization.

variable and because 24 h is better to the process, as it is a shorter incorporation time.

In response surface of **Figure 5(a)** it can be seen that the greater the amount of SH (20 mL) and the lower the amount of NH₃ (2 mL), there is a decrease in the volume of HCl titrated. In the response surface of **Figure 5(b)** it is possible to observe that, when using the largest amount of SH (40 mL) and lower time of volatilization (24 h), there is a lower consumption of HCl for titration.

Therefore, **Figure 5** shows the response surface obtained with the independent variables; volatilization and volume of NH₃, leading to a better working area, utilizing a shorter time of volatilization (24 h) and less amount of NH₃ (2 mL).

Thus, in these conditions, it can be concluded that the best working area is settling the time of incorporation and volatilization in 24 h, and the volumes of HS and NH₃ in 100 and 2 mL, respectively. It is noteworthy that this planning

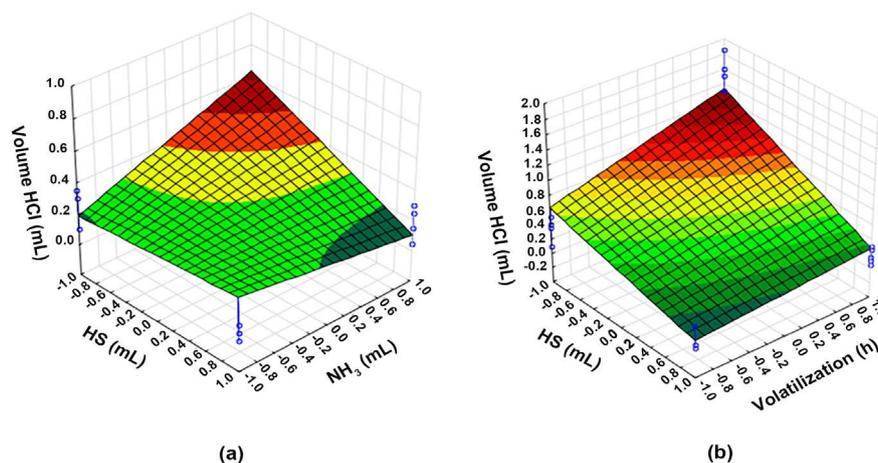


Figure 5. Response surfaces obtained by factorial planning 2^4 for the purposes of the application of HS to humic acid.

was done to identify what are the significant variables for this procedure, and that additional details only point to a great area of work and cannot define the optimal working condition.

4. Conclusions

From these results it can be seen that it is necessary to previously evaluate the amount of SH and ammonia to be used in the field, and that the use of HS with conventional fertilizers may be an alternative, both with the aim of minimizing the environmental pollution, as to avoid the excesses, as the larger unused amount is lost to the environment, representing economic losses and unnecessary costs to the producer, also raising the value of the product within the entire production chain.

Thus, the search for management systems that increase fertilizer efficiency with increased benefit from nitrogen, increased productivity and profitability to the producer has become a major challenge in the current scenario. The currently available literature on this subject is still scarce for comparisons between methods that can be correlated to this study.

This research showed that the factorial design is a good tool, assisting in the study of the variables for fixing the ammonia in HS, leading to better working conditions with less time and lower cost of reagents.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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