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# Use of stabilization pond sludge in cultivation of roses

Jorge L. da Paixão Filho1, Giuliano Gabrielli2, Bruno Coraucci Filho3 & Adriano L. Tonetti4

- $^1\,FEC/UNICAMP, Campinas, SP.\ E-mail: jorgepaixao@gmail.com\ (Autor\ correspondente)$
- <sup>2</sup> FEC/UNICAMP, Campinas, SP. E-mail: giulianogabrielli01@gmail.com
- <sup>3</sup> FEC/UNICAMP, Campinas, SP. E-mail: bruno@fe.unicamp.br
- <sup>4</sup> FEC/UNICAMP, Campinas, SP. E-mail: adriano@fec.unicamp.br

## Key words:

nitrogen roots leaf area agriculture facultative ponds

#### ABSTRACT

One option for the increasing production of sewage sludge (SS) is its agricultural use in crops with high nutritional demand, such as the rose. Therefore, the aim of this work was to study the application of SS from primary stabilization pond in roses of the variety 'Carola'. For the experiment, the roses were planted in 20 L recipients using increasing doses of sludge (T2 - 12 Mg ha $^{-1}$ , T3 - 24 Mg ha $^{-1}$  and T4 - 36 Mg ha $^{-1}$ ), mineral fertilizer (T5) and control treatment (T1, without N) in a randomized block design. The evaluated parameters were: roots dry mass, aerial dry mass, total biomass, leaflets area and nitrogen concentration in the leaf. The SS application resulted in better development of the rose, increasing the roots and the aerial biomasses, the leaflets area, and nitrogen content in the leaves compared to the control (T1). There was no statistical difference between the use of mineral fertilizer (T5), and the SS under the highest dose (T4). With these benefits, the use in agriculture of sewage sludge derived from stabilization ponds proved to be an adequate option for its disposal.

#### Palavras-chave:

nitrogênio raízes área foliar agricultura lagoa facultativa

## Uso de lodo de lagoa de estabilização no cultivo de roseira

### RESUMO

Uma opção para a crescente geração de lodo de esgoto (LE) é seu uso na agricultura em cultura com alta demanda de nutrientes, como a roseira. Portanto, o objetivo deste trabalho foi estudar a aplicação do lodo de lagoa facultativa primária em roseiras da variedade 'Carola'. Para a realização do experimento as roseiras foram plantadas em baldes de 20 L utilizando-se doses crescentes de lodo (T2 - 12 Mg ha¹, T3 - 24 Mg ha¹ e T4 - 36 Mg ha¹), adubo mineral (T5) e testemunha absoluta (T1) com delineamento em blocos casualizados. Os parâmetros avaliados foram: massa seca das raízes, massa seca da parte aérea, biomassa total, área dos folíolos e concentração de nitrogênio no tecido foliar. A aplicação de LE resultou em melhor desenvolvimento da roseira aumentando a biomassa das raízes, da parte aérea, área dos folíolos e o teor de nitrogênio nas folhas quando comparado com o tratamento controle (T1). Também foi constatado que não houve diferença estatística entre o uso de adubo mineral (T5) e LE na maior dose (T4). Com esses benefícios o uso de lodo de lagoa facultativa em roseira mostrou ser uma opção adequada para o destino deste resíduo.

#### Introduction

The volume of treated sewage in Brazil has grown significantly in recent years, creating a demand for the proper disposal of the sludge produced, which should be environmentally friendly and economically feasible.

An alternative for the disposal of sewage sludge (SS) is its use in agriculture, since it contains nitrogen, phosphorus, and organic matter, which can improve agricultural production (Tsutiya, 2001). In the crop of sunflowers the application of SS improved the production of grain and yield of oil (Lobo & Grassi Filho, 2007).

The use of SS improves the physical properties of the soil, such as increased macro porosity and decreased micro porosity (Maria et al., 2010), and can improve root penetration and

absorption of nutrients, as shown in SS application in peach palm, that showed an increase in root biomass with the increase of the applied dose (Vega et al., 2005). The knowledge of the root system is an important parameter to avoid contamination of groundwater by ions such as nitrates (NO<sub>3</sub><sup>-</sup>) which is easily leached into the soil profile.

To evaluate the nitrogen absorption, a diagnosis is made on a specific plant organ, such as a leaf. The SS application increased the foliar nitrogen concentration in the eucalyptus (*Eucalyptus grandis*), when compared to the mineral fertilizer (Guedes & Poggiani, 2003). Other authors also concluded that its application can increase the absorption of elements, which are normally present in lower concentrations in the sludge, such as the phosphorus (Chiba et al., 2008).

The floriculture is a promising market with high income per planting area; however, Brazil lacks research on new flower varieties, and the efficient use of farm inputs (Barbieri & Stumpf, 2005). To meet the high demand, farmers are using high rates of fertilizer and water (Tamimi et al., 1999), which may cause environmental damage and a waste of resources.

In this context, further studies are needed on the use of SS by the Brazilian rose producers. Hence, the purpose of this study was to evaluate the use of SS from primary stabilization ponds on the rose formation.

### MATERIAL AND METHODS

The experiment was performed in October 2010 at the University of Campinas (UNICAMP), in the experimental area of the Department of Sanitation and Environment, School of Civil Engineering, Architecture, and Planning, in Campinas-SP. According to CEPAGRI (Center of Meteorological and Climate Research Applied to the Agriculture) the mean annual air temperature at the site is of 22.6 °C, with average rainfall of 1424.5 mm year 1 and relative humidity between 47 and 73%. The climate, according to the Koppen classification, is Cwa altitude tropical with rainy summers, and dry winters.

The soil used in the experiment was taken from the university campus and analysed in the Soil Laboratory at the IAC (Agronomic Institute of Campinas), presenting a clayey texture, field capacity of  $0.28~g~g^{-1}$ , and wilting point of  $0.24~g~g^{-1}$ . Table 1 shows the fertility results. Based on these data, dolomitic limestone was applied to raise the base saturation of soil to 80%, and castor bean cake to increase the organic matter, according to Malavolta et al. (2002).

For the experiment 20 L recipients were used, distributed in area fenced with black agricultural cloth, and were subjected to natural environmental conditions. To improve drainage, eight holes were drilled in the bottom of each recipient, and a 5 cm layer of gravel was added to it, followed by the soil.

The SS used in the experiment originated from the primary stabilization pond belonging to the wastewater treatment plant of the city of Coronel Macedo, SP operated by the SABESP, earlier described by França et al. (2011) (Table 2). This sludge was obtained from the pond's first dredging, occurring 18 years after the beginning of its operation, and it was subsequently dewatered for 2 years in BAG. This sludge is in conformity with the rules for agricultural use, established by the National Council for the Environment (CONAMA, 2006) Resolution No. 375.

A mineralization fraction (MF) was established for the calculation of the available nitrogen in the SS, since neither the literature nor Resolution No. 375 provided a mineralization fraction for sewage sludge produced by stabilizing ponds. Due

**Table 2.** Characterization of the sludge from the city of Coronel Macedo, SP

Moisture (% v/v) 70.9 Copper (mg kg <sup>-1</sup> ) pH 8.1 Chromium (mg kg <sup>-1</sup> ) Volatile Solids (% v/v) 5.9 Sulfur (g kg <sup>-1</sup> )	84.4 26.4
	26.4
Volotile Calida (0/ v/v) E.O. Cultur (a kg-1)	
Volatile Solids (% v/v) 5.9 Sulfur (g kg <sup>-1</sup> )	10.3
Total Solids (% v/v) 29.1 Iron (mg kg <sup>-1</sup> )	19407
Organic Carbon (g kg <sup>-1</sup> ) 131 Phosphorus (g kg <sup>-1</sup> )	3.7
Ammonium nitrogen (mg kg <sup>-1</sup> ) 955 Magnesium (g kg <sup>-1</sup> )	0.86
Nitrate-nitrite nitrogen (mg kg <sup>-1</sup> ) 39.5 Manganese (mg kg <sup>-1</sup> )	180
Total Kjeldahl Nitrogen (g kg <sup>-1</sup> ) 14.2 Mercury (mg kg <sup>-1</sup> )	<1.0
Aluminium (mg kg <sup>-1</sup> ) 12175 Molybdenum (mg kg <sup>-1</sup> )	8.0
Arsenic (mg kg <sup>-1</sup> ) 3.3 Nickel (mg kg <sup>-1</sup> )	6.1
Barium (mg kg <sup>-1</sup> ) 260 Selenium (mg kg <sup>-1</sup> )	<1.0
Boron (mg kg $^{-1}$ ) <1.0 Zinc (mg kg $^{-1}$ )	316
Cadmium (mg kg <sup>-1</sup> ) 0.6 Potassium (mg kg <sup>-1</sup> )	216
Calcium (g kg <sup>-1</sup> ) 6.1 Sodium (mg kg <sup>-1</sup> )	585
Lead (mg kg <sup>-1</sup> ) 37.5	

to the long period that the sludge remained in the pond, a 20% MF was established.

The Eq. (1) was used to calculate the available N in sewage sludge as recommended by Resolution No. 375.

$$N_{\text{available}} = \left(\frac{MF}{100}\right) X \left(N_{\text{kj}} - N_{\text{NH}_3}\right) + N_{\text{NH}_3} + \left(N_{\text{NO}_3} + N_{\text{NO}_2}\right) \left(1\right)$$

where:

 $N_{available}$  - Available Nitrogen, mg kg $^{-1}$ 

Nkj - Kjeldahl Nitrogen, mg kg-1

 $N_{\mathrm{NH3}}$  - Ammonium Nitrogen, mg kg $^{\text{-1}}$ 

 $N_{_{\rm NO3}}$  - Nitrogen as nitrate, mg  $kg^{_{\rm -1}}$ 

 $N_{_{\rm NO2}}$  - Nitrogen as nitrite, mg  $kg^{\mbox{\tiny -1}}$ 

MF - Mineralization Fraction

To calculate the available nitrogen in the SS a new analysis of the content of nitrogen was performed, and the values obtained are shown in Table 3.

During the growing phase, the rose has the following needs: 100 kg ha<sup>-1</sup> of nitrogen, 280 kg ha<sup>-1</sup> of phosphorus and 300 kg ha<sup>-1</sup> of potassium (Raij et al., 1997). With the crop's nitrogen requirements and the available nitrogen in the sewage sludge it was possible to establish the recommended dose for cultivation according to Resolution No. 375. Based on this information, the

**Table 3.** Nitrogen values for the sewage sludge after two years of dewatering in BAG

Results*
64.4
19.6
736
35.7

<sup>\*</sup> On dry weight basis

Table 1. Chemical composition of the soil

SOM	рΗ	Р	K	Ca	Mg	Al	H+AI	SB	CEC	V	S	В	Cu	Fe	Mn	Zn
g dm <sup>-3</sup>	hii	mg dm <sup>-3</sup>			n	ımol₀ dn	n <sup>-3</sup>			%			mg (	dm <sup>-3</sup>		
16	5.1	2	< 0.1	30	4	-	20	33.9	54.1	63	-	0.1	3.1	11	3.6	0.8

SOM - Soil organic matter

following treatments were applied: T1 - control (without N); T2 - 12 Mg ha $^{-1}$  of sludge (dry basis - DB) (50% of the recommended dose); T3 - 24 Mg ha $^{-1}$  sludge (DB - recommended dose); T4 - 36 Mg ha $^{-1}$  sludge (DB - 150% of the recommended dose), and T5 - mineral fertilization (100 kg N ha $^{-1}$ ).

Ammonium sulfate was used as nitrogen source in the treatment with mineral fertilizer (T5), and in all treatments, (T1, T2, T3, T4 and T5), simple superphosphate and potassium chloride as the phosphorus and potassium sources were applied. The experimental design was in a randomized block with six replications of five treatments, and two plants per replications, totaling 60 plants.

The transplantation of the forty-day old rose seedlings to the soil occurred in two occasions: the first one on 23/08/2010 and the second one on 07/10/2010. The experiment began in 16/02/11 (Day 0) with the application of the SS and mineral fertilizer. The experiment was done in blocks because of the age differences between plants, so each block had plants of the same age. They were irrigated with 4 mm day 1 by drip irrigation system.

For this research, the variety of rose chosen was resistant to diseases and pests. A second relevant factor was consumer preference. So, the variety 'Carola' with red petals was the chosen one.

The sample collection method for the analysis of the nitrogen contents in the leaves was described by Sonneveld & Voogt (2009). The analyses were performed at 67 and 241 days after application of SS. The leaves were rinsed with distilled water, and placed in paper bags, dried until reached the constant weight in a 65 °C oven, followed by grinding in a Wiley mill. The digestion was performed with sulfuric acid and hydrogen peroxide ( $\rm H_2SO_4 + \rm H_2O_2)$  and the nitrogen determination using the Kjeldahl method.

The area of the leaflets was determined using the non-destructible method proposed by Rouphael et al. (2010), which uses the leaflets length (L) and width (W). The analysis was performed in five leaflets located in the central region of all plants. The area of the leaflets was evaluated in three different moments. The first analysis was performed after the plants were initially fertilized (84 days after application of SS) and the subsequent analyses were performed to check the residual effect of the sludge application (228 and 285 days after application of SS).

For the determination of the dry mass of roots (RDM) and the aerial parts (ADM), stem was cut at ground level; thus, separating the aerial parts (leaves, branches and stem) from the radicular system. A jet of running water was applied for the soil removal from roots. At the laboratory, the material was again washed with distilled water, stored in paper bags, and placed in a 65 °C oven until reaching the constant weight. The total biomass (RDM + ADM) was also analysed. The analysis of biomass was made at the end of the experiment (302 days after application of SS). The experiment was kept for a long period to evaluate the mineralization of the residual organic nitrogen.

The analysis of variance (ANOVA) (p < 0.05) was performed according to a complete block design, considering time as

subplot. Tukey test ( $\alpha$  < 0.05) was used for comparison of means. The program used for statistical analysis was BioEstat version 5.0.

#### RESULTS AND DISCUSSION

Table 4 shows a significant increase in RDM values with the addition of the SS to the rose. The only treatment which did not show statistical differences when compared with T1 was T2. Similar root development was observed in the field experiment with the peach palm (*Bactris gasipaes*), conducted by Vega et al. (2005). The authors concluded that the increase in radicular biomass was proportional to the dose of SS applied, and that with the highest doses (400 kg N ha<sup>-1</sup>) occurred a deepening of the radicular system and an increase in fine roots, which are responsible for absorption of most nutrients and water from the soil.

The lowest RDM was observed in the control treatment (T1). A significant increase in RDM was noted in the treatment which received the highest dose of sludge (T4). Similar results were reported by Pedroza et al. (2003) when studying the application of limed sewage sludge in cotton (*Gossypium hirsutum*) and by Singh & Agrawal (2010) evaluating the application in rice (*Oryza sativa*). They also observed that by increasing the application rate, the radicular biomass of both crops increased.

Comparing the treatments that received the recommended dose of nitrogen (100 kg ha<sup>-1</sup>), which were T3 (sludge) and T5 (mineral fertilizers), a higher rate of RDM was observed in the treatment receiving the mineral fertilizer (T5). This fact can be attributed to the non-mineralization of the nitrogen in T3, or to an overestimation of the mineralization fraction values (MF); Thus, determining the mineralization fraction for each type of sludge with potential use in agriculture is necessary for a more precise recommended dose.

There is a concern for groundwater contamination by nitrate leaching with the soil application of sewage sludge (Boeira, 2009). However, since its application stimulates root growth in rose, only a small amount of nitrate is likely to leach, due to greater volume of soil being explored by the radicular system.

The aerial dry mass (ADM) of rose was influenced by the applied dose of sludge, with higher growth when the nitrogen

**Table 4.** Root dry mass (RDM), aerial dry mass (ADM) and total biomass of rose obtained under different treatments#

Treatments	RDM	ADM	Total Biomass
		g plant <sup>-1</sup>	
T1	13.40 a	16.10 a	29.50 a
T2	18.20 a	28.10 ab	48.30 ab
T3	32.50 b	35.80 b	68.30 b
T4	41.20 bc	51.73 c	92.93 c
T5	49.00 c	55.00 c	104.00 c

<sup>\*</sup>T1 - control (without N); T2 - 12 Mg ha<sup>-1</sup> of sludge (dry basis - DB) (50% of the recommended dose); T3 - 24 Mg ha<sup>-1</sup> sludge (DB - recommended dose); T4 - 36 Mg ha<sup>-1</sup> sludge (DB - 150% of the recommended dose), and T5 - mineral fertilization (100 kg N ha<sup>-1</sup>)

<sup>\*</sup>Same letters in the column show no statistical difference by the Tukey test (p < 0.05)

dose was 50% above the recommended (T4) (Table 4), and in the treatment with mineral fertilizer (T5), both treatments showed no significant differences. These results are similar to RDM, showing again that SS dose of T4 was more adequate. A similar result with the increased SS dosage was also observed by Boen & Haraldsen (2011), with ryegrass (*Lolium perenne*).

According to RDM and ADM, the highest accumulation of the total biomass (RDM + ADM) was observed in the treatment with mineral fertilizer (T5) followed by the treatment (T4). However, there was no statistical difference between them (Table 4). Lobo & Grassi Filho (2007) using SS in sunflowers also observed increase of total biomass (stalk+leaves), showing that its use is beneficial for the growth of roots and shoots.

An increase in the area of the leaflets with the application of the SS was observed during all the periods studied (Table 5) which is consistent with results on other species, rice (*Oryza sativa*), cotton (*Gossypium hirsutum*) and tobosagrass (*Hilaria mutica*), receiving SS (Mata-González et al., 2002; Bezerra et al., 2005; Singh & Agrawal, 2010).

In the first analysis the largest area was observed in T4. Greater rose leaf area influences the photosynthesis and the photo-assimilates production; thus, resulting in greater biomass, and the likelihood of higher productivity (Casarini, 2004). The relationship between photosynthesis and plant growth with application of SS was studied by Antolín et al. (2010) in alfalfa crop (*Medicago sativa*), and the results showed an increase in photosynthesis when the SS was applied, as well as a greater plant growth.

In the second and third analysis (Table 5) the roses were at the beginning of the production phase and a new application of fertilizer or sludge was needed. However, this application was not performed, since the mineralization process of the organic matter in the sludge was still in progress.

When comparing the area of the leaflets between dates, the areas of the leaflets were reduced in the treatment 4, in the second and third analysis, and in the treatment 2 in the second analysis. The reduction in leaf area may be indicative of deficiency of N and P (Casarini & Folegatti, 2006) or may have been a transport of these nutrients to other plant parts such as the roots (Cabrera et al., 1995).

Table 6 shows the nitrogen concentration in the rose leaves at the beginning and at the end of the experiment. The first

**Table 5.** Mean area of five rose leaflets at different times under different treatments\*

Treatments#	Analysis I 11/5/2011	Analysis II 2/10/2011	Analysis III 28/11/2011
		cm²	
T1	8.90 aA*	8.80 aA	10.30 aA
T2	12.10 bB	10.20 aA	11.50 aB
T3	12.80 bA	11.40 bA	11.60 aA
T4	14.66 cB	11.90 bA	11.95 bA
T5	12.00 bA	11.20 bA	11.97 bA

 $<sup>^{\#}</sup>$ T1 - control (without N); T2 - 12 Mg ha<sup>-1</sup> of sludge (dry basis - DB) (50% of the recommended dose); T3 - 24 Mg ha<sup>-1</sup> sludge (DB - recommended dose); T4 - 36 Mg ha<sup>-1</sup> sludge (DB - 150% of the recommended dose), and T5 - mineral fertilization (100 kg N ha<sup>-1</sup>)

**Table 6.** Analysis of the nitrogen content in the leaves of the 'Carola' rose under different treatments<sup>#</sup>

Treatment	Analysis I 24/4/2011	Analysis II 14/11/2011
	g l	kg <sup>-1</sup>
T1	14.84 aA*	14.96 aA
T2	16.92 abA	16.08 aA
T3	19.65 bcA	16.40 aA
T4	21.43 cA	13.30 aB
T5	18.45 abcA	15.30 aA

 $<sup>^{*}</sup>$ T1 - control (without N); T2 - 12 Mg ha<sup>-1</sup> of sludge (dry basis - DB) (50% of the recommended dose); T3 - 24 Mg ha<sup>-1</sup> sludge (DB - recommended dose); T4 - 36 Mg ha<sup>-1</sup> sludge (DB - 150% of the recommended dose), and T5 - mineral fertilization (100 kg N ha<sup>-1</sup>)

evaluation shows an increase in the concentration of this nutrient in the foliar tissue with the increased rate of sludge. Simonete et al. (2003) encountered comparable results in corn. Similarly, Casarini (2004) observed an increase in the concentration of foliar nitrogen by applying increased dose using fertigation in the rose variety "Versilha".

The T4 presented the highest foliar nitrogen content (21.43 g N kg $^{-1}$ ), which showed a mean concentration 44% higher than the T1, and 16% higher than the T5 (mineral fertilizer). Likewise, Guedes & Poggiani (2003) in a field experiment, assessed the macro and micro nutrient levels in *Eucalyptus grandis* leaves, and found that the highest nitrogen concentrations in plants were the ones treated with sewage sludge. So, the concentration increase of nitrogen in leaf tissue of rose evidences that sewage sludge can replace the application of mineral fertilizer, reducing the cost of production and minimizing the natural resources consumption.

In the analysis between dates, the foliar nitrogen concentration showed no statistical difference for the treatments, except treatment T4 that showed content of nitrogen lower than in first analysis. Ahmad et al. (2012) also found a larger concentration of nitrogen in rose in the beginning of the studied period, with a reduction in the second and the third analysis.

The concentration levels of foliar nitrogen in the roses from the first and second sampling were below the recommended for the crop, 3.0% or 30 g kg<sup>-1</sup>, according to Cabrera (2000). Nevertheless, the N content below the optimum in the leaf may not mean a nutrient deficiency, since, according to the age of the leaf and the growth stage of the flower stems, there is a decrease in leaf N concentration due to the redistribution of this element in the plant to supply the demand of the new developing tissues (Cabrera et al., 1995). A second important factor on the concentration of nutrients in the leaves is that roses in soilless systems and greenhouses have higher concentrations of nutrients in the leaves than roses planted in the field of the same species (Ahmad et al., 2012).

#### Conclusions

1. Application of sludge from primary stabilization ponds improved the biomass of the roots and aerial parts, leaf area and foliar nitrogen content.

<sup>\*</sup>Different lowercase letters within column indicate significant differences between treatments for p<0.05; same capital letters in line show no statistic difference for the time trials (p<0.05)

<sup>\*</sup> Different lowercase letters within column indicate significant differences between treatments for p < 0.05; same capital letters in line show no statistic difference for the time trials (p < 0.05)

- 2. The optimum dose of sewage sludge from stabilization pond applied to rose was 36 Mg ha<sup>-1</sup> of dry sludge.
- 3. The sewage sludge from primary stabilization pond can be used as nitrogen fertilizer by the rose producers in Brazil.

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