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## Soybean nutritional status and seed physiological quality with swine wastewater

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### Key words:

mineral fertilization  
swine waste  
water reuse

### ABSTRACT

Swine wastewater application is a practice that can become viable in agriculture, for minimizing fertilization costs and improving soil conditions. Therefore, it is essential to establish criteria that define appropriate application doses from the agronomic and environmental perspectives. The objective of this study was to evaluate the effects of swine wastewater doses associated with mineral fertilization on soybean nutritional status and physiological quality of seed. The experiment was conducted in the agricultural year of 2010/2011, using twenty-four drainage lysimeters in randomized block design in 4 x 2 factorial scheme, with four swine wastewater doses (0, 100, 200 and 300 m<sup>3</sup> ha<sup>-1</sup>) applied before sowing, associated with presence and absence of mineral fertilization, in three replicates. Leaves at the flowering stage were collected for determinations of N, P, K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup>, Cu<sup>+2</sup>, Zn<sup>+2</sup>, Mn and Fe. Symptoms of toxicity and nutritional deficiency were observed in the crop. Furthermore, higher doses of swine wastewater caused lower physiological quality in soybean seeds.

### Palavras-chave:

fertilização mineral  
dejeito suíno  
reúso de água

## Estado nutricional e qualidade fisiológica de sementes de soja com água residuária da suinocultura

### RESUMO

A disposição de água residuária da suinocultura é uma prática que se pode tornar viável à agricultura, por minimizar custos com fertilização e melhorar as condições do solo. Para tanto, é fundamental o estabelecimento de critérios que definam doses de aplicação adequadas do ponto de vista agrônomo e ambiental. O objetivo do presente trabalho foi avaliar o efeito de doses de água residuária de suinocultura associada à adubação mineral no estado nutricional da planta e na qualidade fisiológica da semente da soja. O experimento, conduzido no ano agrícola 2010/2011, foi realizado em 24 lisímetros de drenagem, em delineamento em blocos casualizados em esquema fatorial 4 x 2, sendo quatro doses de água residuária da suinocultura, 0, 100, 200 e 300 m<sup>3</sup> ha<sup>-1</sup> aplicadas antes da semeadura, combinadas com a presença e ausência de adubação mineral em três repetições. Folhas em estágio de florescimento foram coletadas para a determinação de N, P, K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup>, Cu<sup>+2</sup>, Zn<sup>+2</sup>, Mn e Fe. Sintomas de toxicidade e deficiências nutricionais na cultura foram detectados. Além disto, o aumento da dose de água residuária da suinocultura propiciou menor qualidade fisiológica nas sementes de soja.



## INTRODUCTION

Residues from swine (Caovilla et al., 2010; Sampaio et al., 2010) and cattle (Ayuke et al., 2011) farming, for being rich in nutrients, can be used as biofertilizers in agriculture. In liquid form, these residues are more efficient when disposed on soil surface, supplying plants with water and nutrients (Caovilla et al., 2010; Sampaio et al., 2010). On the other hand, these residues in liquid form require more-detailed studies on their destination in the soil, because they can cause diffuse pollution, which is difficult to detect and control.

Studies show that the main negative effects caused by the use of liquid residues in agriculture are related to water contamination by nitrogen and phosphorus (Smanhotto et al., 2010) and accumulation of heavy metals on soil surface (Bosco et al., 2008b; Lucas et al., 2013). The positive effects are related to the increase in organic matter and nutrients in the soil (Assmann et al., 2007; Bosco et al., 2008a).

Additionally, there is a concern about the indiscriminate use of antimicrobial agents, which can lead to the genetic resistance of soil microorganisms when present in the wastes (Munir et al., 2011; Liu et al., 2013). Thus, in order to search for positive and negative effects of the use of effluents in agriculture, research studies have focused on soil (Sampaio et al., 2010), leachate (Prior et al., 2009; Maggi et al., 2011), runoff (Bosco et al., 2008b; Doblinski et al., 2010; Wang et al., 2013) and on agricultural crops (Kessler et al., 2013a; 2013b). In addition to these topics, some studies also concentrate on the soil biota (Tessaro et al., 2013; Brooks et al., 2014).

Researches on the use of wastewater in the initial development stage of crops are still incipient; however, the study of Pelissari et al. (2009) allows observing a great potential in the development of viable techniques on this line. These authors observed that the time for the production of Eucalyptus seedlings in plant nurseries decreased by 67% when the imported mineral fertilizer was substituted by swine

wastewater. As to the nutritional status of soybean plants, in the study of Kessler et al. (2013a) there was no difference in the contents of  $\text{Cu}^{+2}$ ,  $\text{Fe}^{+2}$ ,  $\text{Mn}^{+2}$ ,  $\text{Ca}^{+2}$  and P; on the other hand, the contents of  $\text{K}^+$ ,  $\text{Mg}^{+2}$ , pH, N, B and  $\text{Zn}^{+2}$  were changed by the management with swine wastewater and mineral fertilization.

The great potential of use of wastewater in agricultural areas refers to grasses and forests, due to the high nutrient cycling power, especially of nitrogen. On the other hand, leguminous plants, such as soybean, are always associated with the production of grasses on a farm, due to crop rotation, as in the Western region of Paraná, Brazil, where agricultural cycles composed of soybean-corn-black oat with intensified applications of swine wastewater are common. In the global context in swine-producing regions, where grain production is also concentrated, the effects of the application of organic biofertilizers (Carvalho et al., 2001), tannery sludge (Costa et al., 2001), biosolids (Currie et al., 2003) and sewage sludge (Vieira et al., 2005) on soybean plants started to be studied.

Based on the above and aiming to contribute to the state of the art on the subject, this study aimed to evaluate the effects of 4 years of swine wastewater application on the nutritional status of soybean and on the physiological quality of its seeds.

## MATERIAL AND METHODS

The experiment was carried out in the agricultural year of 2010/2011. The experimental area is located at the geographical coordinates of  $24^{\circ} 54' 02''$  S and  $53^{\circ} 32' 00''$  W, in Cascavel-PR, Brazil, at a mean altitude of 680 m. The climate is very humid, mesothermal subtropical, with mean annual rainfall of 1800 mm, mean temperature of  $20^{\circ}\text{C}$  and mean relative air humidity of 75%. The means of rainfall and temperature during the experiment are shown in Figure 1. The soil in the area is classified as distroferic Red Latosol.

The experimental area consisted of 24 plots, each one with area of  $1.60\text{ m}^2$ . This area has a history of experiments that have

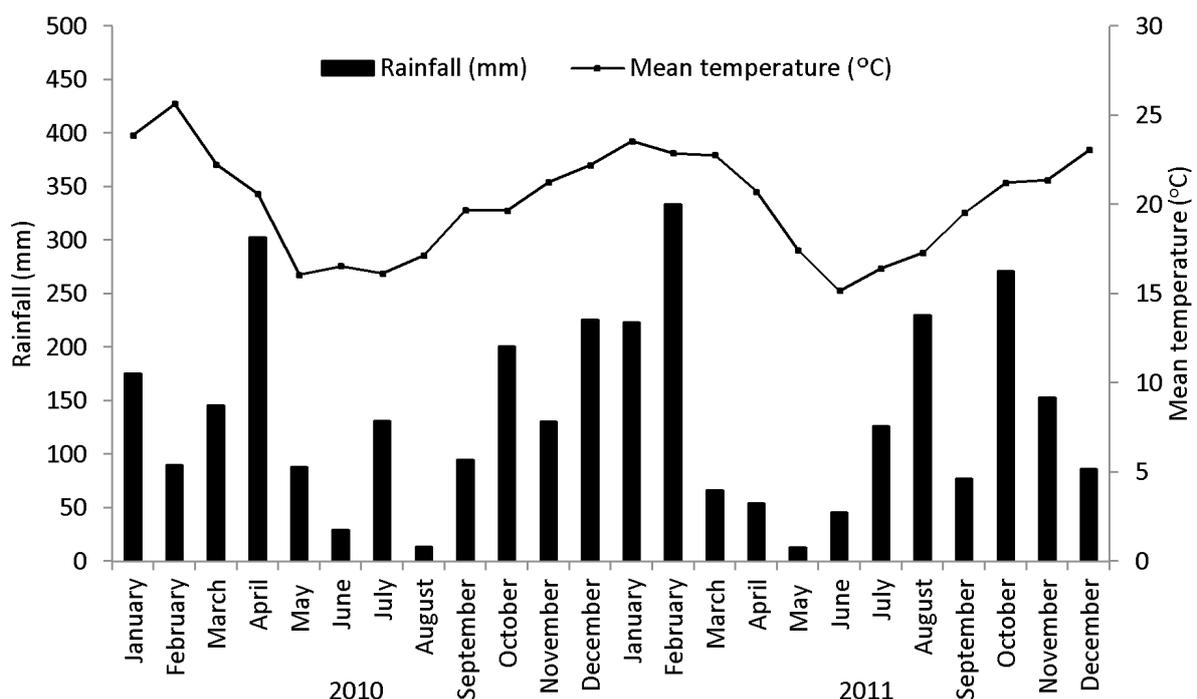


Figure 1. Monthly total rainfall and mean temperature recorded during the experiment

virtually repeated the same agricultural management, only changing the crops. The regional pattern of crop rotation 'corn-black oat-soybean' has been used since the first experiment in 2006, with cultivation of corn (*Zea mays* L.) (1<sup>o</sup>) and soybean (*Glycine max* (L.) Merrill) (2<sup>o</sup>); in 2007, black oat (*Avena strigosa* Schreb.) (3<sup>o</sup>) and soybean (4<sup>o</sup>); in 2008, black oat (5<sup>o</sup>) and baby corn (6<sup>o</sup>); in 2009, corn (7<sup>o</sup>), black oat (8<sup>o</sup>) and soybean (9<sup>o</sup>); and in 2010, corn (10<sup>o</sup>) and black oat (11<sup>o</sup>). This study focused on soybean cultivation (12<sup>o</sup> experiment).

The treatments aimed to study the absence (A) and presence (P) of mineral fertilization (MF) combined with 4 doses of swine wastewater (SW): 0, 100, 200 and 300 m<sup>3</sup> ha<sup>-1</sup>, totaling 8 treatments: (T1) 0 SW-A, environmental control; (T2) 0 SW-P, agronomic control; (T3) 100 SW-A; (T4) 100 SW-P; (T5) 200 SW-A; (T6) 200 SW-P; (T7) 300 SW-A; (T8) 300 SW-P. The presence of MF represented conventional chemical fertilization recommended for the crop.

The swine wastewater was collected in the outlet of the biodigester of a piglet-producing unit and its chemical characteristics are shown in Table 1. Before sowing each crop, SW was applied in the experimental units according to the treatments. The soybean cultivar CD 208 (COODETEC), which has a semi-early cycle of 131 days, was planted using a density of 20 seeds per linear meter and spacing of 45 cm between rows. At sowing, 250 kg ha<sup>-1</sup> of NPK formulation (0:20:20) were applied.

Following the methodology described by EMBRAPA (1999), leaves with petioles were collected for diagnosis, corresponding to the 3<sup>o</sup> and 4<sup>o</sup> trifoliolate leaves from the main stem, in the initial flowering period. These leaves were washed, dried and prepared for the determination of nitrogen (N), phosphorus (P), potassium (K<sup>+</sup>), calcium (Ca<sup>+2</sup>), magnesium (Mg<sup>+2</sup>), copper (Cu<sup>+2</sup>), zinc (Zn<sup>+2</sup>), iron (Fe<sup>+2</sup>) and manganese (Mn), according to the methodology of Tedesco et al. (1995). From the obtained contents, the nutritional interpretation suggested by EMBRAPA (1999) was performed, according Table 2.

Table 1. Physico-chemical characterization of the swine wastewater

Parameters	Results
pH	7.62
EC ( $\mu\text{S cm}^{-1}$ )	1816.00
TC (mg L <sup>-1</sup> )	1331.00
TOC (mg L <sup>-1</sup> )	684.80
TIC (mg L <sup>-1</sup> )	645.90
TN (mg L <sup>-1</sup> )	481.70
NO <sub>3</sub> (mg L <sup>-1</sup> )	103.75
P (mg L <sup>-1</sup> )	22.06
K <sup>+</sup> (mg L <sup>-1</sup> )	8.95
Mg <sup>+2</sup> (mg L <sup>-1</sup> )	67.70
Ca <sup>+2</sup> (mg L <sup>-1</sup> )	52.87
Na <sup>+</sup> (mg L <sup>-1</sup> )	36.70
Cu <sup>+2</sup> (mg L <sup>-1</sup> )	1.86
Zn <sup>+2</sup> (mg L <sup>-1</sup> )	10.22
TS (mg L <sup>-1</sup> )	3498.33
FTS (mg L <sup>-1</sup> )	1310.75
VTS (mg L <sup>-1</sup> )	2187.50
Turbidity (TU)	606.00

EC - Electrical conductivity; TC - Total carbon; TN - Total nitrogen; TOC - Total organic carbon; TIC - Total inorganic carbon; P - Phosphorus; K<sup>+</sup> - Potassium; Na<sup>+</sup> - Sodium; Ca<sup>+2</sup> - Calcium; Mg<sup>+2</sup> - Magnesium; Cu<sup>+2</sup> - Copper; Zn<sup>+2</sup> - Zinc; TS - Total solids; FTS - Fixed total solids; VTS - Volatile total solids

Table 2. Levels of interpretation of nutrients in soybean leaves, according to EMBRAPA (1999)

Nutrients	Very low	Low	Sufficient	High	Very high
N (g kg <sup>-1</sup> )	<32.5	32.5 to 45.0	45.0 to 55.0	55.0 to 70.0	>70.0
P (g kg <sup>-1</sup> )	<1.6	1.6 to 2.5	2.5 to 5.0	5.0 to 8.0	>8.0
K (g kg <sup>-1</sup> )	<12.5	12.5 to 17.0	17.0 to 25.0	25.0 to 27.5	>27.5
Ca <sup>+2</sup> (g kg <sup>-1</sup> )	<2.0	2.0 to 3.5	3.5 to 20.0	20.0 to 30.0	>30.0
Mg <sup>+2</sup> (g kg <sup>-1</sup> )	<1.0	1.0 to 2.5	2.5 to 10.0	10.0 to 15.0	>15.0
Mn (mg kg <sup>-1</sup> )	<15	15 to 20	20 to 100	100 to 250	>250
Fe (mg kg <sup>-1</sup> )	<30	30 to 50	50 to 350	350 to 500	>500
Cu <sup>+2</sup> (mg kg <sup>-1</sup> )		<6	6 to 14	>14	
Zn (mg kg <sup>-1</sup> )	<11	11 to 20	20 to 50	50 to 75	>75

The germination test was performed according to the Rules for Seed Analysis (Brasil, 2009). The seeds were germinated on germitest paper, moistened with distilled water; immediately after, the seeds were placed in a BOD device at 25 °C for seven days and the results were expressed in percentages of germinated, abnormal and dead seeds.

The experimental design was a 4 x 2 factorial scheme with four levels of SW and two of MF, with three replicates. Before the analysis of variance, a descriptive data analysis and an error normality test were performed. For the parameters that did not show normal distribution of errors, data transformation was performed. Tukey test at 0.05 probability level was used for the comparison of means. Regression analysis was performed for the variables germinated, abnormal and dead seeds, as a function of the application of SW doses. The statistical program Minitab 17 was used for the analysis.

## RESULTS AND DISCUSSION

There were no significant differences in the leaf contents of N, P, Mn and Cu<sup>+2</sup> between the treatments (Table 3). Swine wastewater (SW) and mineral fertilization (MF) promoted significant differences in the contents of K<sup>+</sup>, Ca<sup>+2</sup> and Mg<sup>+2</sup>. The content of K<sup>+</sup> increased proportionally to the applied SW doses, which can be explained by the fact that this element is relatively mobile in the water-soil-plant system, contributing and facilitating its transfer to plants (Bertol et al., 2010).

The behavior observed for Ca<sup>+2</sup> and Mg<sup>+2</sup> was contrary to that for K<sup>+</sup>, i.e., its contents decreased with the increase in SW, indicating that these elements were retained in the soil matrix. The data of SW composition (Table 1) show that contents of Ca<sup>+2</sup> and Mg<sup>+2</sup> are higher as compared to K<sup>+</sup>. Since the CEC in Latosols is naturally high (in this experiment: 110-130 mmol<sub>c</sub> dm<sup>-3</sup>), it leads to higher fixation of bivalent elements such as Ca<sup>+2</sup> and Mg<sup>+2</sup>, while K<sup>+</sup> remains in the soil solution, available for absorption by plants.

In the diagnosed contents, the lowest P values were observed in the treatments with doses of 0 and 300 m<sup>3</sup> ha<sup>-1</sup>. It is pertinent to point out that, for Zn<sup>+2</sup>, the highest content was obtained in the treatments fertigated with the dose of 300 m<sup>3</sup> ha<sup>-1</sup>. According to Malavolta et al. (1997), the excess of Zn<sup>+2</sup> reduces the absorption of P. Thus, Zn<sup>+2</sup> possibly induced the antagonistic action on P, decreasing its availability, which was already low due to the small amount applied via SW. This nutritional deficit led to a metabolic dysfunction in the seed, which did not have energy to break down reserve-substances

Table 3. Analysis of variance (p-value) and means comparison test for the nutrients in soybean plants

	N (g kg <sup>-1</sup> )	P (g kg <sup>-1</sup> )	Mn (mg kg <sup>-1</sup> )	Cu <sup>+2</sup> (g kg <sup>-1</sup> )	Zn <sup>+2</sup> (mg kg <sup>-1</sup> )	Mg <sup>+2</sup>	Ca <sup>+2</sup> (g kg <sup>-1</sup> )	K <sup>+</sup>	Fe (mg kg <sup>-1</sup> )
0**	48.94	2.18	36.13	5.81	43.86	5.96a	11.70 a	15.58 a	0 A***
100	46.62	2.62	28.87	5.08	51.01	4.82ab	8.15ab	17.21 ab	0 P
200	45.58	2.83	30.05	4.16	51.94	4.14b	5.94 bc	18.59 bc	100 A
300	49.88	2.43	31.36	4.86	64.65	3.99b	5.27 b	20.57 c	100 P
A	46.60	2.57	27.33	4.41	59.74 A	5.34A	9.24 A	17.01 B	200 A
P	48.91	2.50	35.87	5.54	45.99 B	4.12B	6.29 B	19.70 A	200 P
-	-	-	-	-	-	-	-	-	300 A
-	-	-	-	-	-	-	-	-	300 P
MF	0.45 <sup>ns</sup>	0.55 <sup>ns</sup>	0.07 <sup>ns</sup>	0.35 <sup>ns</sup>	0.02*	0.00*	0.01*	0.00*	MF
SW	0.72 <sup>ns</sup>	0.17 <sup>ns</sup>	0.67 <sup>ns</sup>	0.80 <sup>ns</sup>	0.10 <sup>ns</sup>	0.00*	0.00*	0.00*	SW
SW*MF	0.93 <sup>ns</sup>	0.91 <sup>ns</sup>	0.50 <sup>ns</sup>	0.60 <sup>ns</sup>	0.32 <sup>ns</sup>	0.53 <sup>ns</sup>	0.36 <sup>ns</sup>	0.41 <sup>ns</sup>	SW*MF
CV (%)	15.31	19.91	34.31	58.09	12.91	28.38	12.91	09.62	CV (%)

\*\*\* Means followed by equal lowercase letters in the column do not differ by Tukey test at 0.05 probability level for the follow-up analysis of SW inside MF and equal uppercase letters in the column do not differ by Tukey test at 0.05 probability level for the follow-up analysis of MF inside SW; \*\* Means followed by same lowercase letters in the column do not differ by Tukey test at 0.05 probability level for SW and means followed by same uppercase letters in the column do not differ by Tukey test at 0.05 probability level for MF; A - Absence of MF; P - Presence of MF; \*Significant at 0.05; CV - Coefficient of variation; NS - Not significant

and nourish the embryonic axis, hampering the germination process. Other studies also cite the importance of appropriate P contents for germination, seed emergence and adequate soybean yields (Guerra et al., 2006). On the other hand, high contents of Ca<sup>+2</sup> and Mg<sup>+2</sup> can induce the formation of their phosphates (Novais et al., 2007), contributing to the immobilization of P.

For the macronutrients P and K<sup>+</sup>, not all the treatments supplied plants adequately (Table 2). 33% of P values and 16.66% of K values are below the contents considered as adequate. Plants in the treatment T1 and T7 showed P contents below the adequate range. This was expected, since P is an element of little mobility in the soil, which is the reason for its accumulation in the superficial layer, restricting its availability to plants (Scherer, 2007). It is known that P is a nutrient required in large amounts by plants and it is responsible for storage and permutation of genetic information, with function of energy transfer as a carrier and activator of amino acids (Malavolta et al., 1997). For K<sup>+</sup>, this deficiency was observed in the treatment of environmental control, with values around 14 g kg<sup>-1</sup>.

Part of Zn<sup>+2</sup> values were above the ones established by EMBRAPA (1999). In all treatments, the level of Cu<sup>+2</sup> was considered as low, possibly because it is strongly adsorbed to soil organic and inorganic colloids. In the process of active absorption, there is a competition between Cu<sup>+2</sup> and Zn<sup>+2</sup> for the sites of the carrier (Malavolta et al., 1997), which explains its low content in the analyzed plant tissue; however, despite being low, it did not affect nodulation, which was observed at the field. The values of Zn<sup>+2</sup> varied from sufficient to high and, although the increase in its availability does not constitute an immediate problem, it can lead to plant nutritional imbalances in the long term.

The other elements are within the range considered as sufficient for the soybean crop.

The regression curves of germination percentage as a function of the SW doses for the seeds obtained in the treatments with MF + SW (T4, T6 and T8) and only with SW (T3, T5 and T7) showed determination coefficients (R<sup>2</sup>) of 0.953 and 0.999, respectively (Figure 2). The treatments that combined MF with SW showed an increasing quadratic response, while the germination of seeds treated with SW

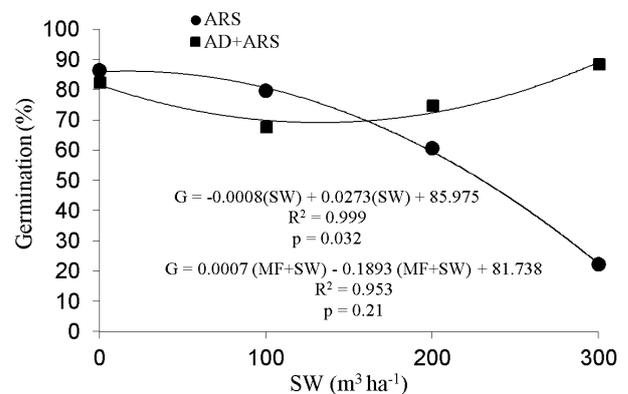


Figure 2. Germination of soybean seeds as a function of the application of swine wastewater (SW) and mineral fertilization + swine wastewater (MF + SW)

showed a decreasing quadratic response. The highest SW doses in the absence of MF changed the physiological potential of the seeds and reduced germination percentage, a sign that the isolated use of this animal residue does not supply plant nutritional requirements in a balanced way, which can lead to negative effects in the production process.

In the comparison between the percentages of germinated, abnormal and dead seeds (Figure 3A), seed germination was equal to 86% for the dose of 100 m<sup>3</sup> ha<sup>-1</sup>. As the dose increased to 200 m<sup>3</sup> ha<sup>-1</sup>, the germination percentage decreased by 10%. In the treatment with dose of 300 m<sup>3</sup> ha<sup>-1</sup>, the germination remained at 22%.

The presence of MF was expressive for the treatments in combination with SW (T4, T6 and T8), since it promoted adequate germination percentage (Figure 3B). The presence of readily available macronutrients in the MF, combined with the increment of micronutrients present in the SW, led to optimal mineral conditions for the formation of grains.

The results indicate that the treatment with SW + MF dose of 300 m<sup>3</sup> ha<sup>-1</sup> promoted balanced fertilization during plant vegetative stage, which allowed the accumulation of reserves that were later translocated to the seeds during the formation of the embryo and storage organs (Carvalho & Nakagawa, 2000). In general, fertilization recommendations are correlated only with crop yield and not with physiological quality of seed (Maeda et al., 1986).

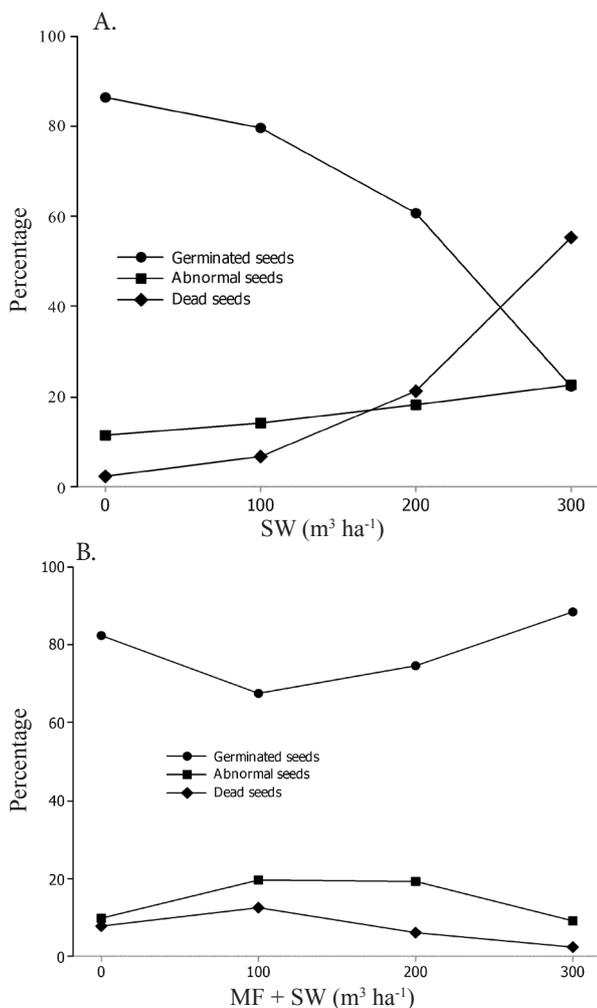


Figure 3. Physiological quality (Germinated, Abnormal and Dead) of soybean seeds produced in the presence of swine wastewater (SW) (A) and mineral fertilization + swine wastewater (MF + SW) (B)

The use of SW as the only nutritional source is not recommended for the fertilization of soils for soybean cultivation intended for seed production.

## CONCLUSIONS

1. As to soybean nutritional status, the association of swine wastewater and mineral fertilization is adequate, except with respect to Cu.

2. The application of swine wastewater is not recommended as a total substitute of mineral fertilizers, especially for crop intended for seed production.

3. The association of two nutritional sources, mineral fertilizer and swine wastewater, from 200 m³ ha⁻¹ on, promotes the production of seeds with adequate physiological quality.

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