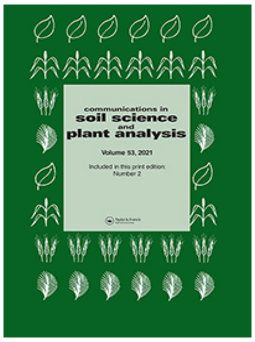

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Tannery Sludge Applied in High Doses in Elephant Grass as an Alternative Fertilization

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ABSTRACT

Tannery sludge has great potential for use in agriculture, however it is a potentially toxic waste, and it is necessary to establish the ideal dose for each crop. The objective of this study was to evaluate the influence of fertilization with liquid tannery sludge on growth, chlorophyll *a* fluorescence and gas exchange of *Pennisetum purpureum* in two production cycles. A 2 × 6 + 1 split plot scheme was used, where the first factor was composed of two productive cycles, the second factor different fertilizations doses: 0, 30, 70, 110, 150, 190 t ha^{-1} of liquid tannery sludge and an additional treatment with nitrogen, phosphorus and potassium control. Plant height, number of leaves and tillers, canopy and stem diameter, leaf area, dry matter, chlorophyll index (SPAD), chlorophyll *a* fluorescence and gas exchange were evaluated. The highest production of total dry matter was observed in the second production cycle. The range between 72–100 t ha^{-1} of liquid tannery sludge showed the greatest gains in growth and production of elephant grass. At doses above 150 t ha^{-1} of liquid tannery sludge, the photochemical apparatus begins to show possible damage to the photosystem II (PSII) and reductions in gas exchange.

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Gas exchange; *Pennisetum purpureum* Schum; quantum efficiency; sustainability; toxicity

Introduction

The tanning industries occupy an important market niche in many developing countries such as Brazil, India, Bangladesh, among others (Goswami et al. 2018; Silva et al. 2010; Vig et al. 2011). In Brazil, this sector has 244 tanneries that are responsible for generating around 30 thousand direct jobs and turn over 2 billion dollars every year (CICB 2020).

At the same time that these industries occupy a prominent place in the national economy, they are also highly polluting, because during the tanning process, leather is treated with various chemical products (Gasemloo et al. 2019; Martines et al. 2010), such as sodium hydroxide, ammonium hydroxide, sodium sulfide, ammonium sulfate, sulfuric acid, formic acid and chromium salts (Martines et al. 2010). Thus, at the end of the tanning process, a potentially toxic waste is generated, which can contaminate the soil and water if it is improperly disposed of.

However, tannery sludge has a high load of organic matter and mineral nutrients, which are essential for plant development, so that its use in the agricultural environment has been explored by several authors around the world (Allué et al. 2013; Berilli et al. 2020; Rani, Kumar, and Arya 2017).

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The use of this residue in agriculture has been characterized as a viable alternative to solve the problem of the final deposition of the residue in the environment, and, at the same time, to contribute to the increase of soil fertility and to reduce production costs (Berilli et al. 2019; Nunes et al. 2018).

Plant development in soils fertilized with tannery residues will depend on some factors, notably the tolerance in which the species under study has to potentially toxic elements such as chromium (Cr) and sodium (Na). Cr, for example, when in excess can contribute to the generation of free radicals, lipid peroxidation of leaves and roots and the activation or inhibition of the antioxidant defense system (Gill et al. 2015). Na can increase soil salinity, cause stomatal closure and impair CO_2 assimilation (Cocozza et al. 2019). Thus, it is essential to know which plant species best adapt to these conditions.

Elephant grass (*Pennisetum purpureum*) is a perennial grass, quite common in tropical regions. It is rapidly growing due to its enormous efficiency in converting solar energy into biomass, in parts due to its C4 metabolism, capable of inhibiting photorespiration (Zhu, Long, and Ort 2008). With an average productivity of around 45 t ha^{-1} $year^{-1}$ of dry matter, it is a great option to be grown in small areas, with the purpose of maintaining a stock of fodder for animals during the driest seasons of the year, when the quantity and quality of pasture are reduced (Olajuyin et al. 2018).

Despite the fact that *P. purpureum* forage is widely cultivated in Brazil, little is known about the use of residues with fertilizing potential, as is the case with tannery sludge in the productive potential and physiology of this plant. Therefore, the objective of this study was to evaluate the influence of fertilization with liquid tannery sludge on growth, chlorophyll *a* fluorescence and gas exchange of *P. purpureum* in two production cycles.

Material and methods

Experiment location

The experiment was carried out at the Federal Institute of Education, Science and Technology of Espírito Santo – Campus Itapina, located in the municipality of Colatina, geographic coordinates of 19° 32′ 22” south latitude; 40° 37′ 50” west longitude and altitude of 71 meters, Northwest region of the state of Espírito Santo, Brazil (Figure 1). The region’s climate is Tropical Aw, according to the Köppen climate classification, with a well-defined rainy season between October and January and an average climatological precipitation of 1029.9 mm (Sales et al. 2018).

Experimental design and treatments

A scheme of plots subdivided in time 2 × 6 + 1 was used, considering the first factor (plots) two productive cycles of elephant grass (first and second production cycle) and the second factor (sub-plots) different fertilization doses and was also used an additional treatment. The treatments were arranged in a randomized block design, with four replications. The fertilizations used were composed of six doses of tannery sludge in liquid form (0, 30, 70, 110, 150, 190 t ha^{-1} of liquid tannery sludge) and an additional treatment, receiving fertilization with NPK (nitrogen, phosphorus and potassium), according to the recommendation of fertilization given by Ribeiro, Guimarães, and Alvares (1999). Fertilization occurred only at the beginning of the experiment, and the chemical characteristics of the tannery sludge are shown in Table 1.

Plant material

Elephant grass seedlings (*Pennisetum purpureum* Schum) were used from stem sections of the middle part of parent plants. The irrigation was done by a sprinkler system using a Mini Cannon Plona KS 1500 sprinkler, every two days, for a period of one hour a day.

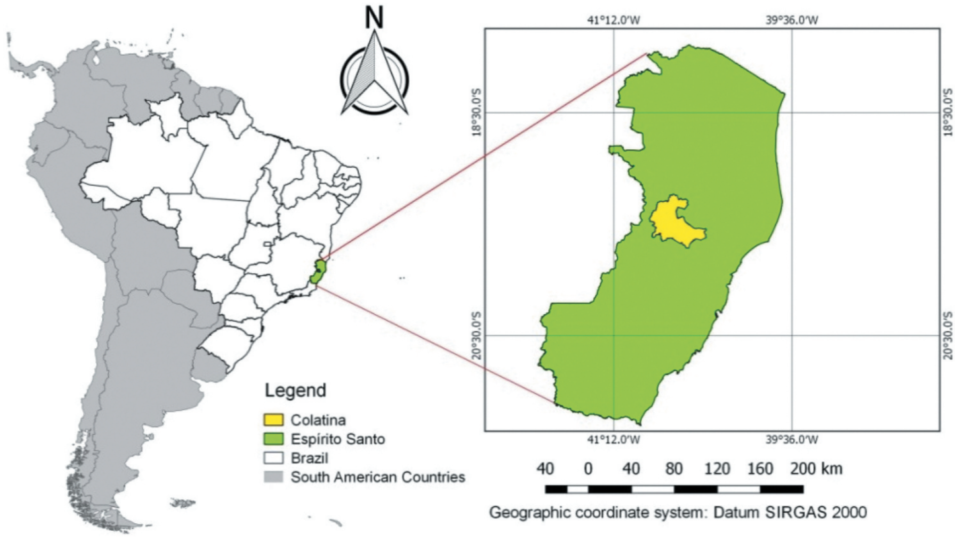

Figure 1. Geographic location of Colatina, located in the state of Espírito Santo, Brazil.

Table 1. Characteristics of bovine tannery sludge used in treatments.

pH	N	P	K	Ca	Mg	C	EC	Fe	Cu	Zn	Mn
		– – – – – – –% – – – – – – –					dS m^{-1}	– – – – -mg dm^{-3} – – – –			
12.3	3.7	0.09	0.08	2.7	0.1	0.9	17.3	57.0	1.0	0.9	0.9

pH: Hydrogen Potencial; N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; C: Organic Carbon; EC: Electric Conductivity; Fe: Iron; Cu: Copper; Zn: Zinc; Mn: Manganese.

Table 2. Chemical characteristics of the substrate used.

pH	P	K	Ca	Mg	Al	Na	C	MO	Fe	Cu	Zn	Mn	S	B
	-mg dm^{-3}-			– – cmol$_c$ dm^{-3} – –			– g dm^{-3} –			– – – – – mg dm^{-3} – – – – -				
5.0	5.1	48.0	0.8	1.3	0.0	0.03	4.7	8.1	7.0	0.6	0.8	7.9	112.0	0.4

pH: Hydrogen Potencial; N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; Al: Aluminium; Na: Sodium; C: Organic Carbon; MO: Organic Matter; Fe: Iron; Cu: Copper; Zn: Zinc; Mn: Manganese; S: Sulfur; B: Boron.

Each section of the stem had a knot, and were planted 5 cm deep in plastic bags for seedlings (11x20 cm), which were kept in a nursery until transplanting. About 20 days after planting the stems, the seedlings were transplanted in 18-liter pots, when they reached approximately 30 cm in height and 6 fully expanded leaves. The pots were filled with substrate composed of soil classified as dystrophic red Latosol (Table 2) sieved through a 15 mm sieve. The planting spacing was 0.3 m between plants and 1 m between rows.

Trial period and evaluated characteristics

The experiment was implemented in early October 2013 and the first cut of elephant grass occurred 120 days after planting. The second cut occurred 120 days after regrowth, ending the experiment in June 2014. The meteorological data of the period in which the study was carried out were obtained from the meteorological database of Xavier, King, and Scanlon (2016), using the data set arranged in a regular grid of 0.25° × 0.25°, covering the entire Brazilian territory (Figure 2).

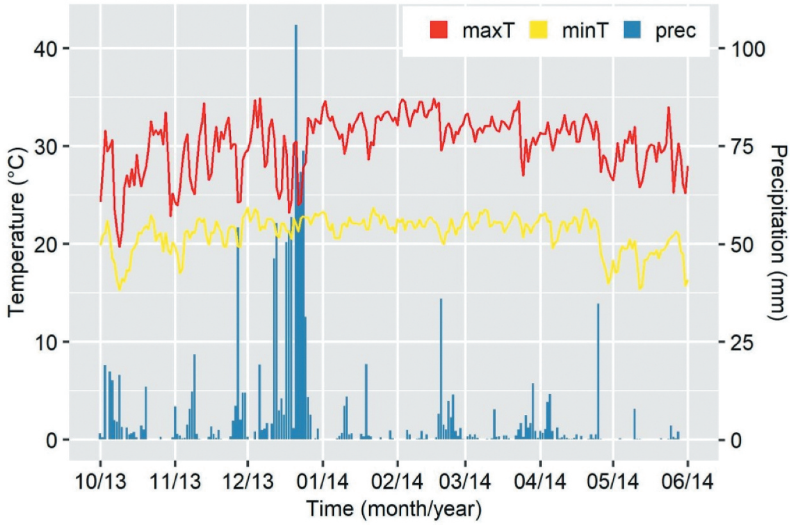

Figure 2. Maximum, minimum temperatures and rainfall observed during the experimental period, for the municipality of Colatina, state of Espírito Santo, Brazil.

The following characteristics were evaluated in the first and second production cycle: number of leaves, plant height (PH) (cm), canopy diameter (CD) (m), stem diameter (SD) (mm), number of tillers emitted (NT) and leaf area (LA) (m^2). Leaf dry matter (LDM) (g), stem dry matter (SDM) (g) and total dry matter (TDM) (g) were also determined. For this, the material was dried in a forced circulation oven at 65°C (± 2°C) for 72 hours, and then weighed on an analytical balance. The green intensity of the leaves was evaluated using a portable chlorophyll meter (SPAD-502, Minolta, Japan). Five points of each sheet were read, according to the method of Swiader and Moore (2002).

In the second production cycle, in addition to the characteristics already mentioned, photochemical efficiency was also evaluated, using three leaves per plant, in the middle part of the leaf, between 8:00–10:00 am by means of a non-Pocket-PEA fluorimeter modulated (Hansatech Instruments Ltd, King's Lynn, Norfolk, United Kingdom), using a saturation pulse of 3.500 µmol m^{-2} s^{-1} of actinic light. The parameters of initial fluorescence (Fo), maximum fluorescence (Fm), which correspond to the complete reduction of primary receptors of photosystem II, variable flowering (Fv) and the maximum photochemical efficiency of photosystem II (Fv/Fm), were evaluated. Gas exchange analyzes were also performed in the second production cycle, being evaluated the net photosynthetic rate (P_N) (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mol CO_2 m^{-2} s^{-1}), transpiration rate (E) (µmol H_2O m^{-2} s^{-1}) and leaf temperature (°C). For this purpose, punctual measurements were taken on days without cloudiness between 8 and 10 hours, using as standard intermediate leaves of the crown fully expanded and without visual anomalies. Photosynthetically active radiation was standardized in artificial light saturating 1000 µmol photons m-2 s-1 and CO2 at a chamber concentration of 420 ppm, using an infrared gas analyzer (model LI-6400, Li-COR, Lincoln, Nebraska, USA).

Before the analysis of variance, the assumptions of normality of errors and homogeneity of variances were verified by the Shapiro-Wilk and Bartlet tests, respectively. The data obtained were subjected to analysis of variance by the F test and when significant, were performed the Tukey test at 5% probability ($p < .05$) for the production cycle.

Later, regressions were developed with the tannery sludge doses. In choosing the equations, the significance of the models, the biological meaning and the coefficient of determination were considered. The entire statistical procedure was performed by the open source program R (R Core Team 2019).

Results

Analysis of variance, production cycles and fertilizations

In this study, no significant interaction was observed between the factors studied (production cycle x fertilization) for all characteristics evaluated in elephant grass (Table 3). In the absence of significant interaction, the isolated effects of each factor on growth characteristics were analyzed. The coefficient of variation remained within the standards for elephant grass, as in most cases the value was less than 20% (Clemente and Muniz 2002).

The production cycles did not promote significant changes in the number of leaves, canopy diameter, plant height, stem diameter, leaf chlorophyll index and number of tillers. Despite this, other characteristics such as leaf area, leaf dry matter, stem dry matter and total dry matter were significantly higher during the second production cycle (Table 4).

The different doses of tannery sludge were adjusted in quadratic regressions, for all evaluated characteristics (Figures 3 and 4). In Figure 3 it is observed that the inflection point of the regression curve was higher than conventional fertilization for the characteristic plant height (Figure 3b), stem diameter (Figure 3c) and number of tillers (Figure 3d). For the number of leaves (Figure 3a), the tannery sludge dose that maximized the gain was 77 t ha^{-1}, showing a gain of 271 leaves (Figure 3a). This value was lower than that obtained by the control treatment (NPK), which had a value of 305 leaves.

For the characteristics plant height (Figure 3b), stem diameter (Figure 3c) and tillering number (Figure 3d), the tannery sludge doses that maximized the gains were 84, 100 and 82 t ha^{-1}, respectively. The maximum height obtained for elephant grass plants was 2.41 m (84 t ha^{-1}), a value very close to

Table 3. Analysis of the variance of the number of leaves (NL), canopy diameter (CD), plant height (PH), stem diameter (SD), Number of tillers (NT), leaf area (LA), leaf dry matter (LDM), stem dry matter (SDM), total dry matter (TDM), leaf chlorophyll index (SPAD) of elephant grass in two production cycles, fertilized with liquid tannery sludge.

		Medium square				
SV	DF	NL	CD (m)	PH (m)	SD (mm)	NT
Production Cycles (C)	1	0003.60^{ns}	000.03^{ns}	000.01^{ns}	004.75^{ns}	020.15^{ns}
Fertilization (F)	5	6720.80**	000.16**	000.25*	009.13**	126.05**
C × F	5	0853.00^{ns}	000.03^{ns}	000.02^{ns}	001.40^{ns}	009.74^{ns}
CV_{plot} (%)		0016.30	007.08	010.98	008.65	015.48
$CV_{subplot}$ (%)		0017.26	009.06	011.70	009.46	014.84
Overall average		0249.02	001.49	002.27	012.22	019.89
SV	DF	LA (m^2)	LDM (g)	SDM (g)	TDM (g)	SPAD
Production Cycles (C)	1	3709*	563996*	964382**	1380205*	0 57.05^{ns}
Fertilization (F)	5	9310**	11957**	232655**	369007**	0 26.47^{ns}
C × F	5	1258^{ns}	1288^{ns}	36057^{ns}	55884^{ns}	0 19.61^{ns}
CV_{plot} (%)		0009.84	023.50	020.11	023.21	008.30
$CV_{subplot}$ (%)		0016.49	017.85	018.67	017.57	008.86
Overall average		0001.81	257.36	684.38	935.29	036.95

* Significant at 5% probability ($p < 0.05$); ** Significant at 1% probability ($p < 0.01$); ns: not significant. SV: Source of variation. DF: degrees of freedom. CV: coefficient of variation.

Table 4. Average values of number of leaves (NL), canopy diameter (CD), plant height (PH), stem diameter (SD), Number of tillers (NT), leaf area (LA), leaf dry matter (LDM), stem dry matter (SDM), total dry matter (TDM), leaf chlorophyll index (SPAD) of elephant grass during the first and second production cycle.

Production Cycle	NL	CD (m)	PH (m)	SD (mm)	NT
1° Cycle	248.74 a	1.51 a	2.27 a	8.65 a	20.53 a
2° Cycle	249.28 a	1.46 a	2.25 a	9.46 a	19.23 a
-	LA	LDM	SDM	TDM	SPAD
1° Cycle	1.72 b	223.08 b	542.63 b	765.72 b	38.00 a
2° Cycle	1.90 a	291.64 a	826.12 a	1104.85 a	35.82 a

Means followed by the same letter in the column do not differ at the 5% probability level ($p < 0.05$) by the Tukey test.

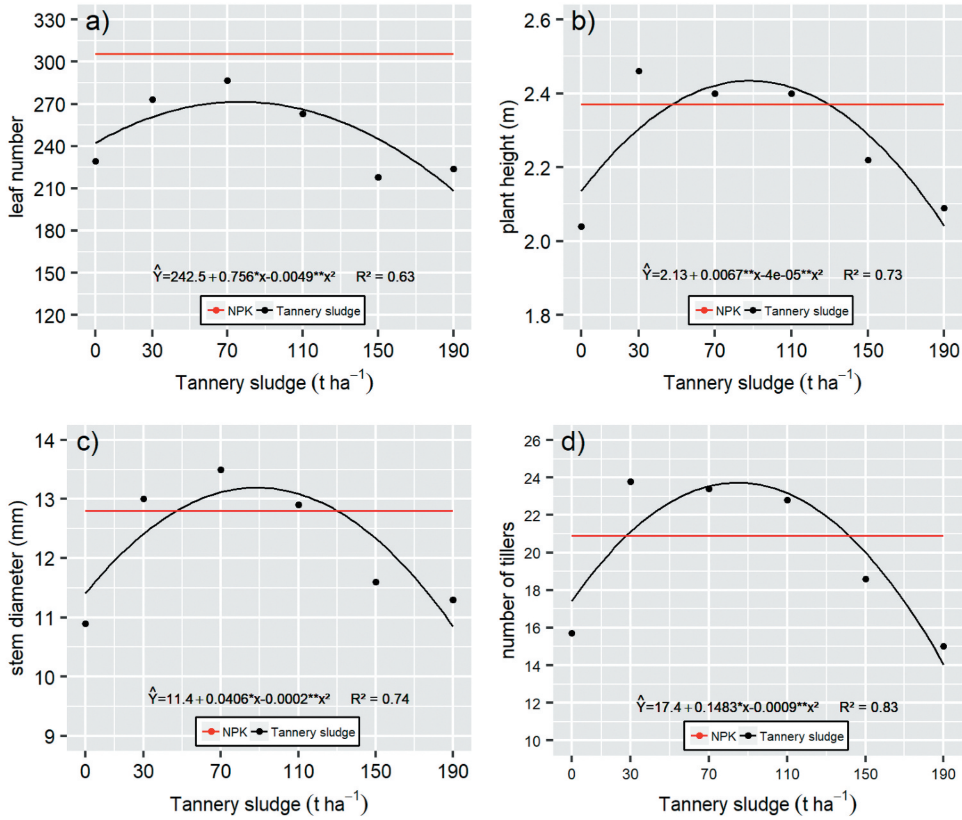

Figure 3. Number of leaves (a), plant height (b), stem diameter (c) and number of tillers (d) of elephant grass subjected to different fertilizations of liquid tannery sludge and an additional treatment with NPK. * significant at 5% and ** significant at 1%.

that obtained by treatment with NPK, which showed a value of 2.37 m. The same response pattern was observed for stem diameter, in which it had a maximum value of 13.3 mm (dose of 100 t ha^{-1}), whereas plants fertilized with NPK had 12.8 mm, that is, a difference of approximately 4%.

When evaluating the canopy diameter (Figure 4a), it is observed that the proportion that maximized the gain was 72 t ha^{-1} of liquid tannery sludge, showing a gain of 1.6 m. The value was equal to that obtained by the treatment with NPK, which presented 1.6 m. For the characteristics dry leaf, stem dry matter and total dry matter (Figure 4), the treatment with NPK showed values higher than the maximum inflection point obtained by the tannery sludge doses.

Fertilization with tannery sludge that maximized the leaf dry matter, stem dry matter and total dry matter characteristics were 76, 83 and 84 t ha^{-1}, respectively (Figure 4). The best adjustments are observed in the stem dry matter (Figure 4c) and total dry matter (Figure 4d), with determination coefficient values of 0.95 and 0.93, respectively.

The maximum leaf area (Figure 4e) estimated, at 2.12 m^2, was obtained with the application of 86 t ha^{-1} of tannery sludge. This value was lower than that obtained by the NPK treatment, which presented 2.35 m^2 of leaf area, that is, approximately 11% more in photosynthetic area.

Physiological analyzes during the second production cycle

The initial fluorescence showed few variations between treatments, ranging from 5000 to 5500 (Figure 5a). The maximum fluorescence (Figure 5b) and variable fluorescence (Figure 5c) obtained a similar response pattern, with 0 to 30 t ha^{-1}, showing a considerable increase in the indexes, followed by a progressive drop with increasing doses of liquid tannery sludge, notably at a dose of 190 t ha^{-1}.

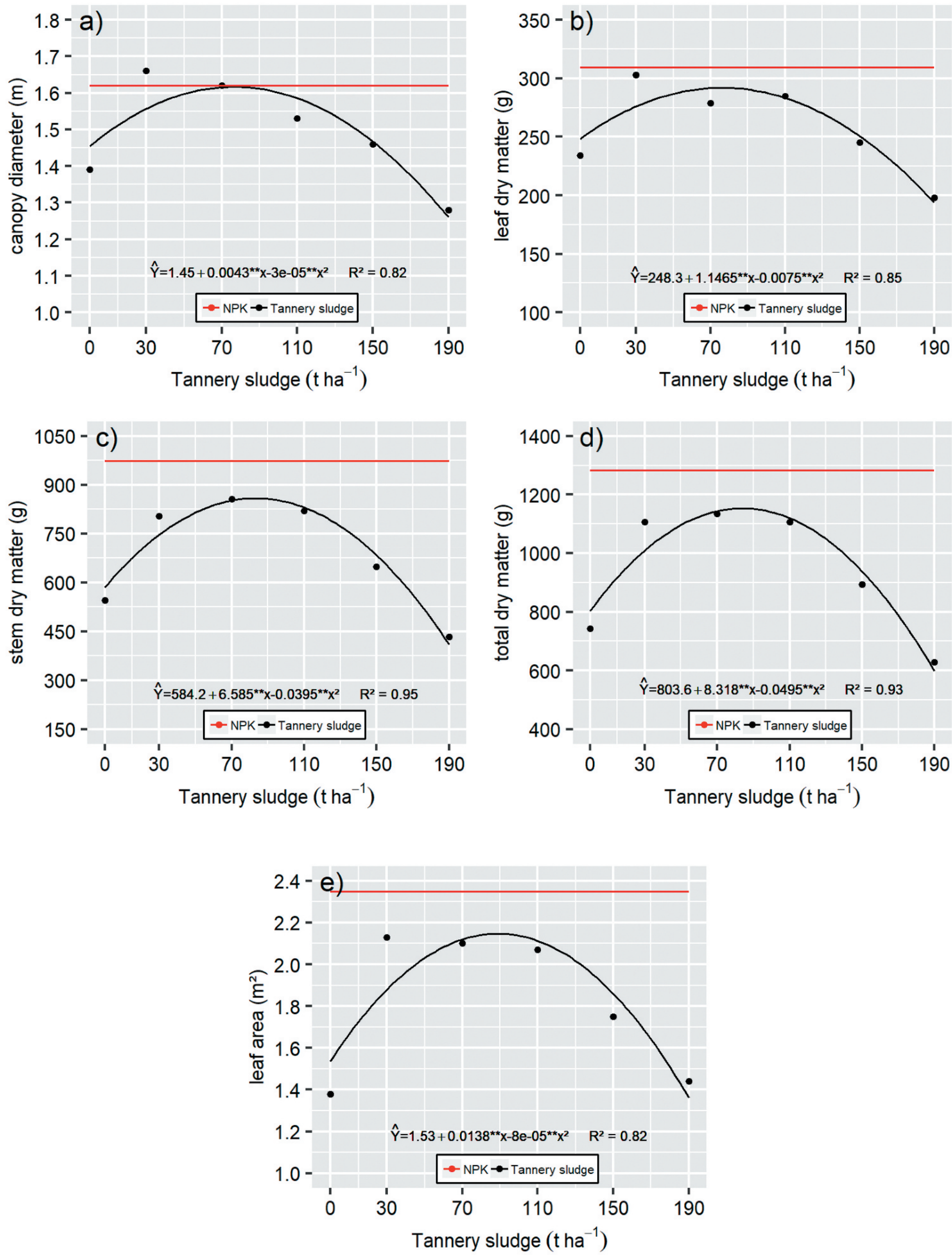

Figure 4. Canopy diameter (a), leaf dry matter (b), stem dry matter (c), total dry matter (d) and leaf area (e) of elephant grass subjected to different fertilizations of liquid tannery sludge and a treatment additional with NPK. * significant at 5% and ** significant at 1%.

The applications of 30 and 70 t ha^{-1} of liquid tannery sludge showed numerical values higher than fertilization with NPK for both characteristics, maximum fluorescence (Figure 5b) and variable fluorescence (Figure 5c). The maximum quantum yield (Fv/Fm) during the second production cycle ranged from 0.63 to 0.78 (Figure 5d). The highest values of Fv/Fm were observed in plants that received applications of 30 and 70 t ha^{-1} of liquid tannery sludge and fertilization with NPK, with values of 0.78, 0.76 and 0.75, respectively.

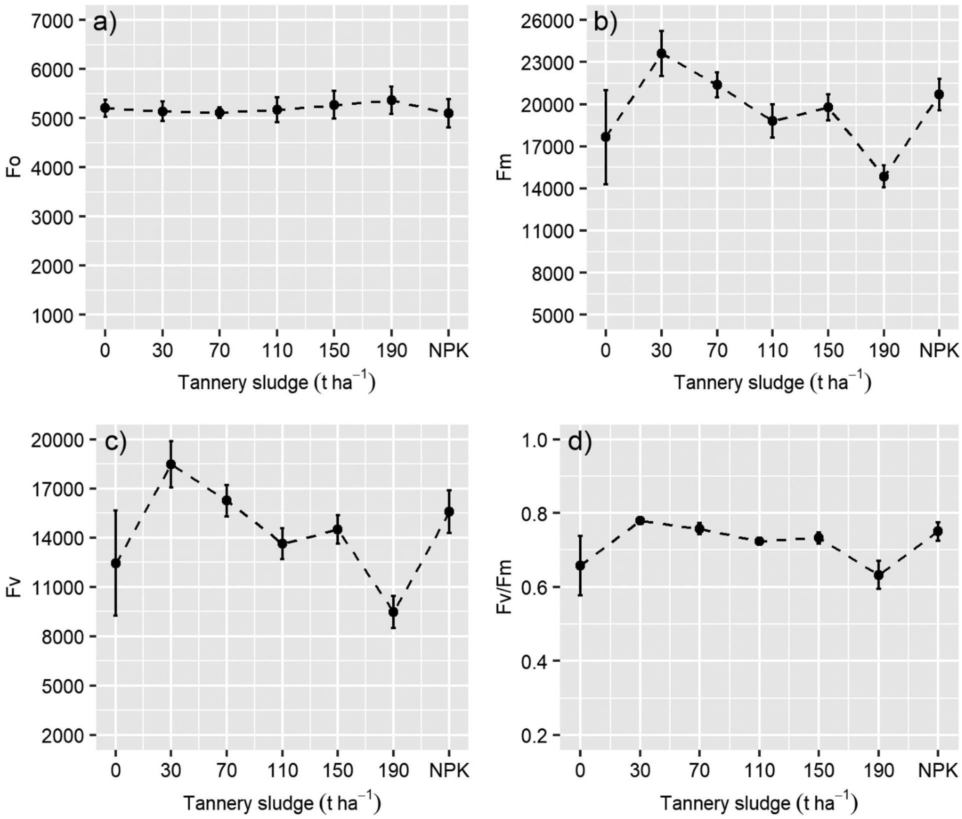

Figure 5. Initial fluorescence (a), maximum fluorescence (b), variable fluorescence (c) and maximum quantum production (d) of elephant grass subjected to different fertilizations of liquid tannery sludge and NPK fertilization during the second production cycle. Bars with standard deviation from the mean.

The tannery sludge dose that promoted the highest numerical value for liquid photosynthesis was 30 t ha^{-1}, obtaining results similar to NPK fertilization, with values of 34.9 and 35.8 μmol CO_2 m^{-2} s^{-1}, respectively (Figure 6a). The photosynthesis values seem to be reflective of the stomatal effects, since gs and E present the same behavior, that is, higher value obtained with 30 t ha^{-1} of tannery sludge (Figure 6b,c).

When analyzing the transpiratory rates (Figure 6c), it is observed that fertilization with NPK and with 30, 70 and 110 t ha^{-1} of liquid tannery sludge were very close, with values of 6.9, 7.0, 6.3 e 6.1 mol H_2O m^{-2} s^{-1}, respectivamente. Regarding the leaf temperature (Figure 6d), there was an opposite effect, with an increase in temperature as the doses of tannery sludge increased, with the lowest temperatures observed in conventional NPK fertilization (30.7°C) followed by fertilization with 30 (31.2°C), 0 (31.4°C), 70 (31.4°C), 110 (31.5°C), 150 (31.7°C) e 190 (31.9°C) t ha^{-1} of tannery sludge.

Discussion

Productive cycles and fertilizations

The larger leaf area obtained in the second production cycle was not accompanied by a higher leaf emission, indicating that there was a significant increase only in the expansion of the leaves, allowing a greater production of dry matter (Table 4). Possibly the largest leaf area increased the photosynthetic capacity of plants, which, in turn, provided greater carbon fixation, which are responsible for their maintenance and growth (Sales et al. 2017), justifying the greater gain in leaf dry matter, stem dry matter and total dry matter (Table 4).

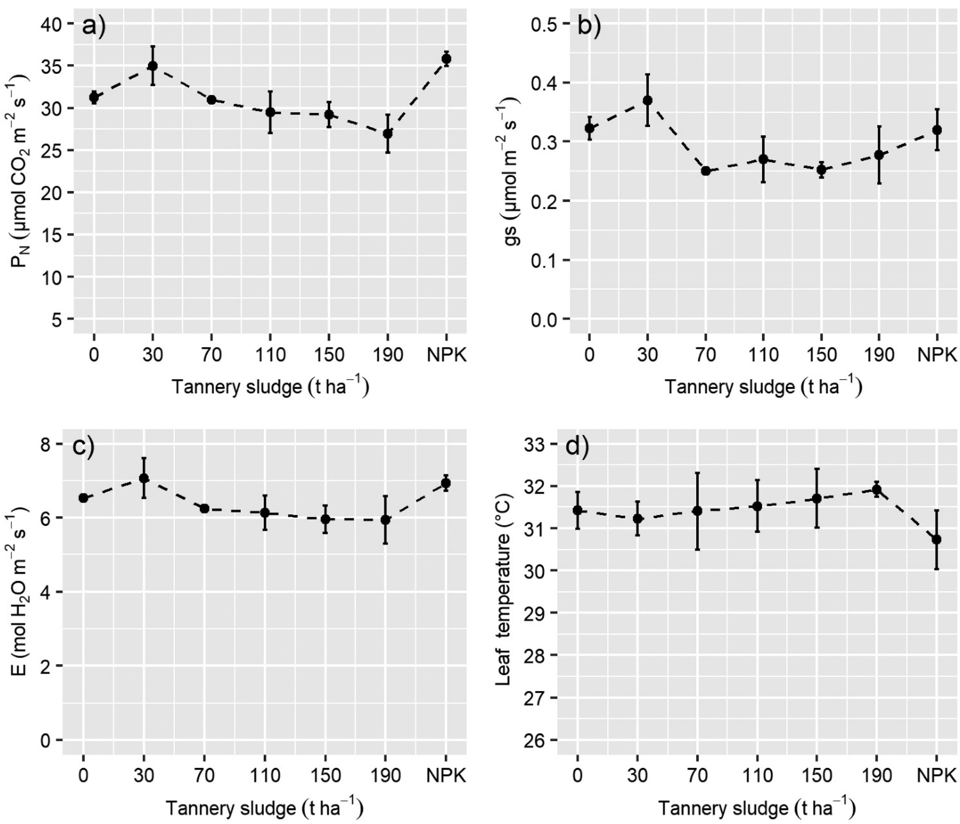

Figure 6. Liquid photosynthesis (a), stomatal conductance (b), transpiration (c) and leaf temperature (d) of elephant grass subjected to different fertilizations of liquid tannery sludge and NPK fertilization during the second production cycle. Bars with standard deviation from the mean.

Therefore, it was observed that the second cycle of elephant grass was the most productive. In contrast to these results, the authors Santos et al. (2014) found no difference in three genotypes of elephant grass fertilized with nitrogen, during the first and second production cut.

When assessing the different fertilizations applied to elephant grass, a similar response pattern is evident among the characteristics evaluated (Figures 3 and 4). The quadratic adjustments found in the tannery sludge doses (Figures 3 and 4) reveal that there was an initial gain in the characteristics evaluated with the increase in the tannery sludge dose and, subsequently, a decrease occurs. This same response pattern was also observed in *Capsicum annuum* L. and *Capsicum chinense* Jacq. seedlings when grown in substrate with tannery sludge (Almeida et al. 2017; Berilli et al. 2019).

Tannery sludge is responsible for providing essential nutrients to plants, in addition, it can also raise pH and increase Cr and Na levels (Table 1). Therefore, the increase in the dose of tannery sludge possibly increased the levels of these elements. Thus, it is of fundamental importance to find a balance between nutrition and toxicity due to the harmful effects of high pH, Cr and Na. The addition of tannery sludge in low amounts can lead to conditions of nutritional insufficiency, and additions in high doses can cause toxicity to the plants.

Despite the quadratic equations between the different evaluated characteristics having presented different ideal proportions (Figures 3 and 4), a range that corresponds to the best gains for the evaluated characteristics is perceived. This ideal range showed a variation of 72–100 t ha^{-1} of liquid tannery sludge. The application of tannery sludge outside this range promoted some harmful effect, either due to lack of nutrients or due to toxicity.

The high doses possibly cause an increase in salinity, an excess of Cr and an increase in soil pH, impairing the normal growth of plants. The phytotoxicity induced by CR is mediated mainly by reactive oxygen species (ROSs), which cause cellular and extracellular damage to plants (Wakeel et al. 2018). Among the changes caused by the high concentration of Cr, we can mention necrosis, programmed cell death, interruption of the cell cycle and suppression of cell division that reduce growth (Wakeel, Xu, and Gan 2020).

In turn, the salinity caused by the tannery residue can lead to soil sodicity, causing dispersion of the clay, which reduces the growth of crops (Upadhyay and Singh 2015). The saline effect can also inhibit plant growth, creating osmotic stress and causing an imbalance in nutrient absorption.

High pH is another factor that can hinder the development of plants, as it reduces the absorption of nutrients. The activity of microorganisms, as well as the solubility and availability of nutrients are some of the most important processes that depend on pH (Gentili et al. 2018). Therefore, fertilization with tannery sludge has a high potential, due to the supply of several essential nutrients and organic matter, however, precautions must be taken regarding the dose to be applied, seeking the balance between plant nutrition and toxicity by harmful elements.

In general, the maximum inflection point of the regressions obtained with the applications of tannery sludge was close to the control treatment (NPK), demonstrating to be efficient in the supply of nutrients to the plants. Tannery sludge is composed of organic materials of animal origin mixed with inorganic salts. Some of the components present in this residue are essential nutrients for plant development, such as N, P, K, Ca and Mg, in addition to organic carbon (Banerjee et al. 2018). These essential nutrients were responsible for equating fertilization in the range of 72–100 t ha^{-1} of liquid tannery sludge to the control treatment with NPK (Figures 3 and 4).

Physiological analyzes during the second production cycle

There was an increase in maximum fluorescence (Figure 5b) and variable fluorescence (Figure 5c) with the application of 30 t ha^{-1}, followed by a small variation with the increase in the doses of liquid tannery sludge. The decline in the values of Fm and Fv/Fm were acute at the dose of 190 t ha^{-1}, which reflects a reduction in the PSII's ability to reduce the primary acceptor QA (Calatayud and Barreno 2001). The drop in Fm values with the dose of 190 t ha^{-1} of tannery sludge can be interpreted as a slowly reversible damage to the light collecting complex in the PSII (photoinhibition). One of the factors that may have contributed to this drop in the values of Fm and Fv may be related to the salinity present in the tannery residue (Table 1) at the dose of 190 t ha^{-1} of tannery sludge. The PSII is relatively sensitive to salt stress (Silva et al. 2019).

Therefore, non-fertilization and the application of doses greater than 150 t ha^{-1} of liquid tannery sludge had the potential to cause stress to elephant grass, and this is strongly linked to the elements with potential for toxicity present in the waste. The decline in Fv/Fm can be caused by an increase in initial fluorescence (Fo) or a decline in Fm (Dias and Marenco 2006). As seen in Figure 5d, there was a decline in Fm, often associated with the protective interconversion of violaxanthin to zeaxanthin, in two independent reactions (xanthophyll cycle), which leads to the dissipation of non-radioactive energy (Demmig-Adams and Adams 1992).

The elements Cr and Na present in the tannery sludge in high quantity (Table 1) are probably the main responsible for this reduction in Fv/Fm. The Cr element decreased the efficiency of the photochemical processes of the PSII, causing a reduction in the primary charge separation rate or disconnecting the light-collecting systems from the PSII (Mathur, Kalaji, and Jajoo 2016). This impairs the transfer of electrons between PSII and PSI (Khalida et al. 2012). On the other hand, salt stress can reduce the maximum quantum yield of the primary photochemical step, whose function is essential for the electron transport chain in the chloroplast and mitochondria (Ogaya et al. 2011).

The photosynthesis, stomatal conductance and transpiration values (Figure 6) were 33, 28 and 17% respectively higher with NPK fertilization, when compared to the treatment with 190 t ha^{-1} of liquid tannery sludge, during the second production cycle. The lower stomatal conductance (Figure 6b) reduces the CO_2 concentration in the sub-stomatal chambers, which contributed to the lower photosynthesis (Figure 6a). According to Andrade et al. (2019), stomatal conductance has a positive correlation with transpiratory rates.

Fertilization above 150 t ha^{-1} of liquid tannery sludge possibly raised soil saline levels, negatively affecting gas exchange due to the partial closure of stomata, which leads to a decline in photosynthetic rates. Saline stress is generally associated with water stress, since high concentrations of Na in the soil restrict the absorption of water by plants (Płażek et al. 2013).

In relation to the increase in leaf temperature (Figure 6d) arising from the increase in fertilizations with tannery sludge, we can also relate this response to the saline effects from the tannery residue. Soil salinity contributes to stomatal closure. The temperature of the leaves increases as transpiration decreases, due to less energy dissipation in the form of latent heat, contributing to the warming of the leaf surface. Transpiration is the largest and most efficient method of heat dissipation present in the plant kingdom (Pokorny et al. 2010; Urban et al. 2017), which makes transpiration inverse to leaf temperature.

The effects observed on the physiological characteristics show that the elephant grass suffered reductions in the efficiency of its photosynthetic apparatus at the dose of 190 t ha^{-1} of liquid tannery sludge, while the other fertilizations with tannery sludge promoted stomatal changes and, consequently, reflecting on photosynthesis. This reduction shows the sensitivity that this species has to the elements Cr and Na, being necessary caution in the dosage of application of this residue as a form of fertilization.

Conclusion

The results obtained in our study suggest that fertilization with liquid tannery sludge is a viable and sustainable alternative to fertilization. The range for using tannery sludge as a form of fertilization is between 72–100 t ha^{-1} of liquid tannery sludge for *P. purpureum*. Doses of liquid tannery sludge above 150 t ha^{-1} promote reduction in the quantum efficiency of photosystem II, gas exchange and plant growth, causing toxicity.

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Disclosure statement

I, Rodrigo Amaro de Salles, author responsible for the submission of the manuscript entitled “Tannery sludge applied in high doses in elephant grass as an alternative fertilization” and all the co-authors presented here, declare that WE DO NOT HAVE, CONFLICT OF INTERESTS.

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