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Full Length Research Paper

Bermudagrass fertilization with human urine as a tool to close nutrient cycles: The use of micronutrients

Olivia Silva Nepomuceno Santos[1]*, Marcelo Batista Teixeira[2], Hans Raj Gheyi[2], Luciano Matos Queiroz[3], Vital Pedro da Silva Paz[2], Cassia da Silva Linge[4] and Asher Kiperstok[3]

[1]Federal Institute of Education, Science and Technology of Bahia, Campus Seabra. Brazil.
[2]Center for Soil and Water Engineering, Federal University of Recôncavo of Bahia, Brazil.
[3]Department of Environmental Engineering Polytechnic School, Federal University of Bahia, Brazil.
[4]Department of Agricultural and Environmental Sciences – Production, Faculty of Agricultural Sciences and Food, University of Milan, Italy.

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Segregating human urine from wastewater may significantly contribute to diminish the nitrogen and phosphorous problem, considered to be one of the major planetary limits already exceeded. Application of urine diluted in water in agriculture contributes to both sides of the problem. On one hand, it allows reduction of nutrient discharges into receiving bodies, on the other, it reduces the need for reactive nitrogen and its energy demand. Advantages of application of urine inbermudagrass and macronutrients accumulation are shown in the previous paper. This paper presents the results of the evaluation of accumulation of micronutrients (B, Fe, Mn, Cu and Zn) in soil and in leaves of bermudagrass irrigated with different dilutions of human urine in water. The experiment was conducted in a greenhouse in a completely randomized design with six treatments consistingof six urine doses (0 - control, 5, 10, 15, 20 and 25 ml of urine per liter of water), with four replicates. As for concentrations in the leaves, there was a significant difference between treatments for all nutrients, with the largest accumulations, of B, Fe and Mn, 120 days after planting, observed in the 10 mL L^{-1} human urine dilution in water. There were significant differences among treatments in the soil layer 0-20 cm, for Fe, Mn and pH, concentrations as well as the levels of B and Mn in the soil layer 20-40 cm, and for B, Mn and pH between the layers analysed, in treatments 15, 20 and 25 ml L^{-1}. Dilutions between 10 and 20 ml L^{-1} induced a greater accumulation of micronutrients in the plant tissue. The use of urine diluted in water provided adequate levels of micronutrients in the leaves in most of treatments, and it did not cause metal accumulation in the soil above the recommended levels for the bermudagrass cultivation.

Key words: *Cynodon dactylon,* nutrient recycling, water reuse.

INTRODUCTION

The impoverishment of the soil, which increases the need for the application of fertilizer, is accentuated by

*Corresponding author. E-mail: olivianepomuceno@gmail.com, Tel: 00- 55-71-32839892.
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conventional sanitation systems because the nutrients excreted primarily in urine and the feces are mixed with water and other waste streams and discharged into water bodies. This final discharge causes not only a deficit of nutrients in the soil, but it also presents environmental and sanitary risks.

Human excreta are potential sources of nutrients for plants, but are usually considered as waste and disposed of, causing an increase in demand for energy and chemicals to remove nitrogen and phosphorus in sewage treatment plants (Spångberg et al., 2014). The main source of these nutrients in the domestic sewage is human urine (Tidåker et al., 2007), which could be collected at source and exploited in agriculture. By this mean, other significant energy savings may also be obtained as the the process of transforming inert nitrogen (N_2) from the atmosphere, to reactive nitrogen (Nr) demand about 13 kWh kg^{-1} of Nitrogen.

The use of human excreta in agriculture is a potential alternative in the pursuit of ensuring the sustainability of agricultural and conservation of natural resources as it enables recycling of nutrients. The high concentration of these elements in human excreta and the means to separate them at the source, has increased researchers interest in recent years (Jonsson et al., 2004, Richert et al., 2010).

Studies show the benefits of application of diluted human urine, segregated from wastewater streams, in crop production (Ganrot, 2005; Lienert and Larsen, 2010; Karak and Bhattacharyya, 2011). Besides the large presence of nitrogen, other important nutrients, such as phosphorus, potassium and micronutrients (Jonsson et al., 2004), are also present in urine.

In earlier publication, accumulation of macronutrients from urine used to irrigate bermuda grass (*Cynodon dactylon*) was presented (accepted for publication in 2015). Here, accumulation of micronutrients in plant and soil is investigated.

Research has been conducted in various parts of the world, to assess the fate of nutrients in the soil-plant system, however, there is little data available regarding the effect of the use of segregated excreta on grass and the use of micronutrients (Provin et al., 2008). Many factors influence the dynamics of these elements in the soil, such as pH, organic matter, vegetation and management factors, such as the addition of organic waste (McDowell, 2003).

Grassland species have different nutrient requirements as well as different levels of maintenance requiring different nutritional managements (Wiecko, 2008). Evaluation of the fate of nutrients in the soil, while receiving different compounds, is important because of the potential for leaching and runoff. Application of an alternative source can minimize the requirements for conventional lawn fertilization (Provin et al., 2008).

It would be more beneficial to plants if a small amount of fertilizer was applied every day. However, this procedure is rarely used because it is impractical and costly. The use of fertilizers with the irrigation water becomes more viable and can be applied often enough to maintain adequate and balanced levels of nutrients in the soil (Wiecko, 2008).

Nutrients in urine can be applied with irrigation water. Grass cultivation is an attractive option for diluted urine fertilization because its high nutrient demand and its cultivation in urban centers. Furthermore, possible improvements in plant development can be obtained by fractionating nutrient supply, by applying urine in the irrigation water.

The presence of micronutrients in urine demands investigation about its accumulation in soil and utilization by plants. Micronutrients present in high concentrations in the soil solution can reach levels which may betoxic to plants and microorganisms and may affect functionality, biodiversity and the sustainability of ecosystems.

According to Levy et al. (2011), wastewater use in arid and semiarid soils also involves risk from toxic levels of boron accumulation in soil. On the other hand, plants that can often accumulate nutrients in their tissues, without harming or causing visible phytotoxic effects, could be an alternative for decontamination of soils (Santos, 2005).

The objective of this study was to evaluate the accumulation of micronutrients in the soil and in the leaves of bermudagrass irrigated with different dilutions of human urine.

MATERIALS AND METHODS

Research site

The assay was carried out in a greenhouse in the experimental area of the Soil and Water Engineering Group at the Federal University of Recôncavo of Bahia (NEAS/UFRB), located in Cruz das Almas, Bahia, at latitude 22° 42' S, longitude 47° 38' W and altitude of 220 m. Climate is classified as humid to sub-humid, with a mean annual temperature and relative humidity of 80% and 24ºC, respectively, and average annual rainfall of 1,143 mm (D' Angiolella et al., 1998).

The soil, Oxisolo f low fertility, was collected in 0-20 cm depth from the UFRB campus. According to the results of the analysis, the chemical compositionof the soil is shown in Table 1. The granulometric composition of the soil was 800, 13 and 187 gkg^{-1} of sand, silt and clay respectively, of sandy loam texture.

Experimental design

A completely randomized design (CRD) was used with six treatments which consisted of five different dilutions of human urine in water (T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), and a control (T0 - irrigation without urine and soil without any fertilization). Each plot consisted of a polyethylene container with a capacity of 100 L and 0.41 m^2, with four replications, totaling 24 experimental plots.

Seeds of bermudagrass (*Cynodon dactylon*) were used following the manufacturer's recommendations for an equivalent dose of 25 $kgha^{-1}$. The planting was carried out in plastic containers made of polyethylene with a capacity of 100 L and 0.41 m^2 with drains and

Table 1. Chemical characteristics of the soil used in the experiment.

pH ($CaCl_2$)	M.O.	P (resin)	S-SO_4^{2-}	Na	K	Ca	Mg	Al	H+Al	Base saturation (BS) - %	B	Cu	Fe	Mn	Zn
	g dm^{-3}	mg dm^{-3}		mmol$_c$ dm^{-3}							mg dm^{-3}				
6.2	13	<2	<3	1.9	18.4	10	<1	28	30	58.0	0.22	<0.3	15	11.1	4.7

Table 2. Characteristics of human urine and water used in irrigation.

Property	Unit	Water	Urine
pH		8.40	8.70
Electrical conductivity	dS m^{-1}	0.78	24.35
N-total	mg L^{-1}	nd	6,937.50
P-total	mg L^{-1}	nd	923.33
K_2O	mg L^{-1}	0.18	1,483.75
Ca^{2+}	mg L^{-1}	0.26	65.00
Mg^{2+}	mg L^{-1}	0.70	10.00
S	mg L^{-1}	nd	1,655.00
Fe - total	mg L^{-1}	nd	1.63
Mn - total	mg L^{-1}	nd	1.63
Cu	mg L^{-1}	nd	0.88
Zn	mg L^{-1}	nd	1.13
Na	mg L^{-1}	4.65	2,937.50
B	mg L^{-1}	nd	0.50
Cl^-	mg L^{-1}	0	4,093.75

nd, not determined.

filled with aforementioned soil over a 3 cm thick layer of gravel and a geotextile blanket.

Fertilization, collection and application of human urine

Human urine used in the experiment was collected in the males' toilets of a student residence, and stored in a 20 L black reservoir. The students voluntarily contributed by urinating directly into the reservoir. The urine was collected for up to 3 days and was used within 4 days after collection. Single samples of urine used in each treatment were stored under refrigeration. A composite sample was produced each month using the weighted average of the volumes of water applied in each treatment. Irrigation management was performed using the values of class A evaporation pan, installed inside the greenhouse, applying 100% of the evaporated depth.

Treatments with urine received additional doses of phosphorous and potassium so that all treatments were fertilized with the same dose of these nutrients. In order to gain a better understanding of the effect of nitrogen from the urine, all the nitrogen provided to the plants came from this source. In order to ensure that all treatments contained the same concentration of phosphorus and potassium, five samples of human urine where analysed before assembly of the experiment to determine their mean chemical composition (Table 2), which was taken into account for the calculation of fertilization.

The supply of nutrients throughout the stage of establishment of grass was estimated, based on predicted values of evaporation using historical data, and this was used to manage the amount of irrigation water. Chemical fertilization was performed based on the value obtained by the difference between the amount of nutrients supplied by urine and the recommendation for the crop.

Commercial fertilizers, composed of simple superphosphate and potassium chloride, were applied based on the recommendations of Godoy and Boas (2003). Each treatment was fixed to supply an equivalent of 150 kgha^{-1} of phosphorus and 150 kgha^{-1} of potassium. The potassium fertilizer was applied at planting and 60 days after planting (DAP).

Soil was raised to field capacity before planting, establishing an irrigation interval of two days. Irrigation was performed manually, using a watering can and the distance between the plots was 50 cm.

Grass management and evaluation

Grass was maintained at a height of approximately 2.0 cm. Pruning/cutting was performed when at least one of the treatments achieved a height of 10 cm with a frequency of 7 to 14 days. After trimming, the material was placed in paper bags and taken to an oven with forced air circulation at 65°C for 72 h to reach a constant weight.

This same material was used to determine the concentration of boron, iron, manganese, copper and zinc in leaves, respecting the interval equal to 30 days. Analysis to determine the levels of micronutrientes were performed in the Laboratory of Mineral Nutrition of Plants at the College of Agriculture Luis de Queiroz

Table 3. Summary of analysis of variance, coefficient of variation (CV%) and estimation of parameters B, Fe, Cu, Mn and Zn, 120 days after planting (DAP) in leaves of bermudagrass.

Source of variation	Mean square					
	DF	B	Fe	Cu	Mn	Zn
Treatment	5	26.55**	2,134.34**	22.08**	3,112.21**	354.41**
Residue	18	3.04	168.51	1.43	618.04	24.62
Mean (mg kg^{-1})		7.76	118.31	11.14	187.83	61.68
CV (%)		22.47	10.97	10.74	13.24	8.04

*Significant at p <0.05 by F test; **Significant at p <0.01 by F test; DF, degree of freedom.

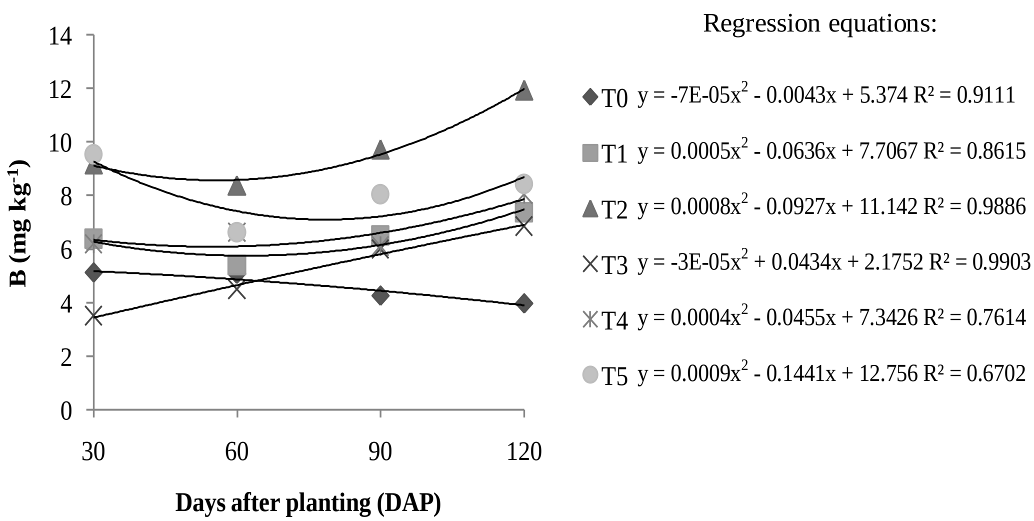

Figure 1. Concentration of B in leaves of bermudagrass (*Cynodon dactylon*) as a function of dose in urine (T0 - 0 ml L^{-1}, T1 - 5 mL L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evaluated to four periods.

(ESALQ/USP), by the method described by Malavolta et al. (1997). At the end of the experiment, soil samples were collected at 0-20 and 20-40 cm to determine the levels of B, Fe, Mn, Cu, Zn and pH. Analysis were performed at the ESALQ/USP laboratory, according to the methods described by Raij (2001).

Statistical analysis

Data collected were subjected to analysis of variance followed by regression analysis. For both analysis, SISVAR System software - Analysis of Variance, version 5.3 (Ferreira, 2010) was used.

RESULTS AND DISCUSSION

Concentration of micronutrients in the leaves

Boron

Micronutrients concentrations in plants' tissue of bermudagrass are presented in Table 3. Results show that nutrient concentrations 120 days after planting, were positively influenced by the urine application.

For concentrations of B in plant tissue at the end of the experiment (Figure 1), the highest values (11.9 $mgkg^{-1}$) were observed in samples that received dose of 10 ml L^{-1} of urine, greater than the 200% observed in the control treatment - T0 - (<4.0 $mgkg^{-1}$). In addition, urine use caused the plants to show adequate levels of B in their tissues, which according to Wiecko (2006) is in the range of 6 to 30 $mgkg^{-1}$, a value which was observed in all treatments except the control.

Assessment of each treatment over time (Table 4) shows that, for boron, a significant effect was observed only in the treatment of 10 ml L^{-1} (T2 treatment). For this concentration of urine in water, the B concentration in the leaves rose from 9.2 to 11.3 $mgkg^{-1}$ in the interval of 30 to 120 days after planting (DAP), a 30% increase.

Boron translocation from roots to shoots may be influenced by the level of supply in the soil. Plants with adequate supply retain higher proportions in roots than plants subject to disabilities and short-term deprivation which can cause a sharp fall in levels in shoots (Dannel

Table 4. Summary of analysis of variancefor the effect of time on the concentration of B, Fe, Cu, Mn and Zn in the leaves, for each treatment.

Source of variation	DF	Treatment	Mean square				
			B	Fe	Cu	Mn	Zn
Period	3	T0	1.34^{NS}	448.34**	3.80 ^{NS}	12,237.18**	29.43 NS
Residue	12		2.48	24.07	1.57	332.23	20.17
Mean (mg kg^{-1})			4.60	115.71	12.65	162.65	63.56
CV (%)			34.19	4.24	9.93	11.21	7.07
Period	3	T1	2.59 ^{NS}	186.93^{NS}	13.72**	3,114.64*	150.76*
Residue	12		4.12	184.97	1.77	731.50	35.78
Mean(mg kg^{-1})			6.40	122.78	12.81	204.21	63.53
CV (%)			31.70	11.08	10.39	13.24	9.42
Period	3	T2	9.25*	497.18^{NS}	4.01**	5,566.64**	95.27NS
Residue	12		1.76	476.57	0.59	599.57	47.17
Mean(mg kg^{-1})			9.80	146.68	11.46	189.65	67.68
CV (%)			13.53	14.88	6.75	12.91	10.15
Period	3	T3	8.95^{NS}	186.30^{NS}	2.20^{NS}	7,814.47**	80.41*
Residue	12		3.10	85.21	0.76	962.06	15.64
Mean(mg kg^{-1})			5.20	98.21	10.37	181.46	44.75
CV (%)			33.87	9.40	8.40	17.09	8.84
Period	3	T4	3.20^{NS}	452.55^{NS}	13.80**	3,781.16NS	394.12**
Residue	12		1.14	255.10	0.42	1,551.72	29.53
Mean(mg kg^{-1})			6.73	111.21	10.78	171.37	49.37
CV (%)			15.92	14.36	6.02	22.99	11.01
Period	3	T5	5.79^{NS}	50.56^{NS}	10.47**	2,411.43*	155.79*
Residue	12		2.75	70.18	0.72	591.17	27.17
Mean(mg kg^{-1})			8.14	114.56	8.09	161.34	61.75
CV (%)			20.36	7.31	10.51	15.07	8.44

*Significance ($p < 0.05$) by F test; **Significance ($p < 0.01$) by F test; NS, not significant; DF, degree of freedom.

et al., 1998; Li et al., 2001; Noguchi et al., 2000). Although it is an essential element for plants, boron is also toxic when present in excess (Takano et al., 2008).

Grasses grown in soils with low concentrations of B may undergo slow growth and may not even complete their life cycle and may also present characteristic deficiency symptoms such as stunted leaves (Samples and Savoy, 2008). Results show that urine used as a source of nutrients led to an increase in boron concentrations in the leaves without causing excesses that could affect plant development.

Iron

Similar to boron, the highest concentrations of Fe in plants tissue, at 120 DAP, were also observed under the dose of 10 ml L^{-1}ofurine in water (T2) with gains of up to 35% compared to the control treatment (T0) (Figure 2).

In the case of this micronutrient, all treatments were within the recommended range which, according to Wiecko (2006), is 50 to 350 mgkg^{-1}. It is worthwhile to mention that highest dose of urine presented contennts of Fe even lower than control.

Concentrations of Fe varied significantly over time (Table 4) just for the control treatment T0, with values of 100 and 120 mgkg^{-1}, at 30 and 120 DAP, respectively (Figure 2).

According to Foth (1990), acidic soils have sufficient Fe concentrations to meet the needs of plants. Analysing the soil's pH, it appears that this justifies the results obtained for the concentrations of Fe in the leaves.

The application of urine did not influence negatively the Fe concentration in the plant tissue. Application of urine, therefore, may represent a good alternative for grasses in the tropics which often suffer from iron deficiencies, especially in alkaline and sodic soils in arid and semiarid regions demanding foliar application of the element. Munshaw et al. (2006) claimed that the application of Fe to bermudagrass provides intense green color in spring and promotes better recovery of the lawn.

Copper

The highest concentrations of copper, at 120 DAP, were

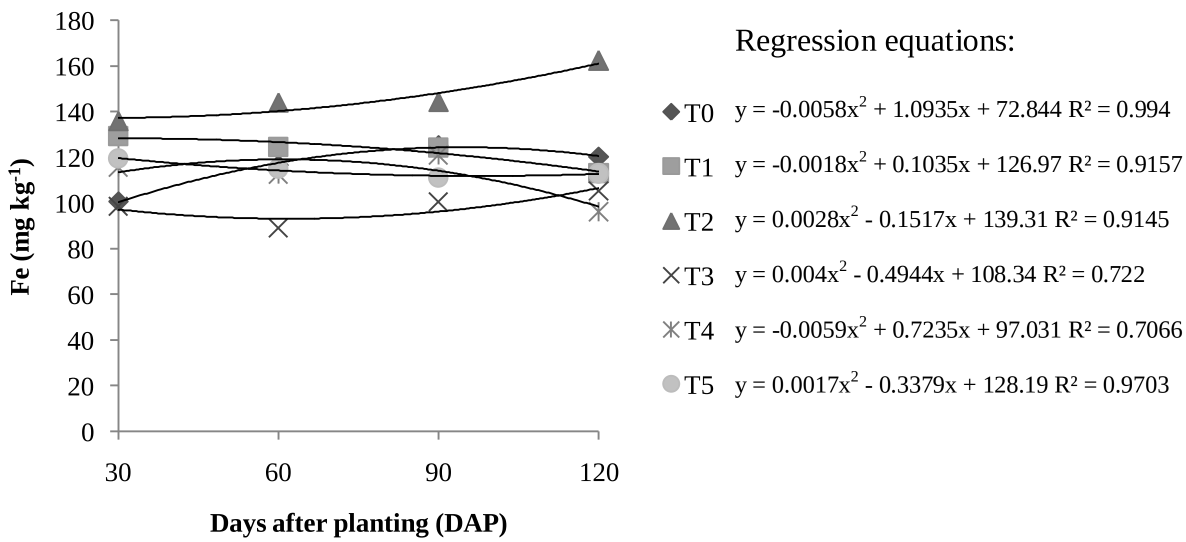

Figure 2. Concentration of Fe in leaves of bermudagrass (*Cynodon dactylon*) as a function of dose in urine (T0 – 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evaluated four periods.

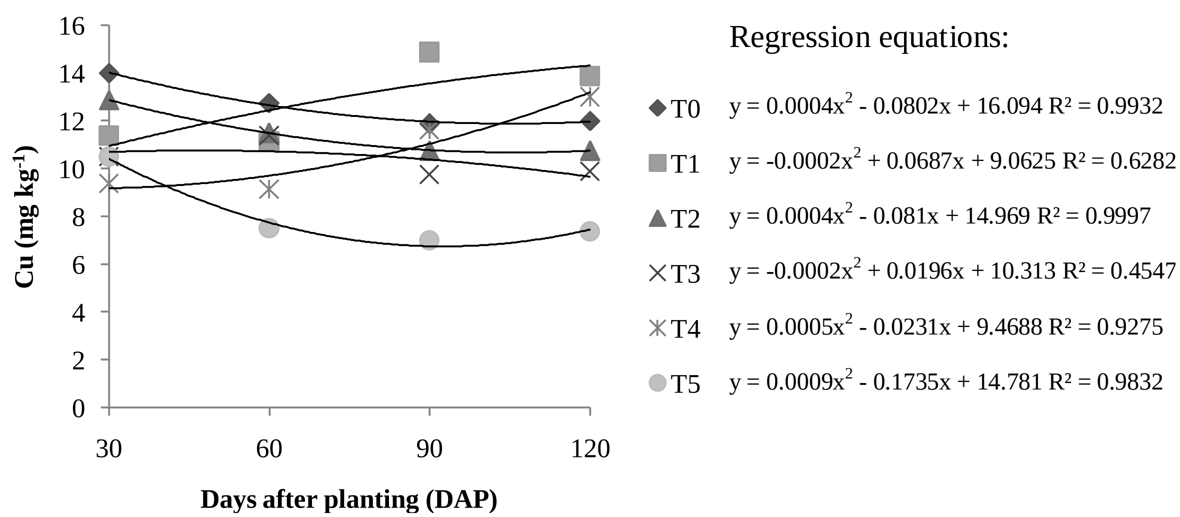

Figure 3. Concentration of Cu in leaves of bermudagrass (*Cynodon dactylon*) as a function of dose in urine (T0 – 0 ml L^{-1}, T1 – 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 – 20 ml L^{-1}, T5 – 25 ml L^{-1}), valued over four periods.

observed in treatments with a dose of 5 ml L^{-1} of urine (Figure 3), while the lowest concentrations were observed in the T5 treatment (20 mL L^{-1}) with 7.4 $mgkg^{-1}$, a value 47% lower than that observed in the treatment T1. However, all the observed values are within the optimal range 5 to 50 $mgkg^{-1}$ (Wiecko, 2006). Accumulation over timeshows that copper had a significant effect in all treatments except contol (Table 4). Like many other micronutrients, symptoms of copper deficiency also appear in younger leaves of grass (Broyer, 1954). Although little research has been carried out on the impacts of copper fertilizers on lawns (Heydari and Balestra, 2008), studies have shown that copper affects the growth of some species of grassland and excessive application may cause root lesion in some grass species (Broyer, 1954). These findings reinforce the potential use of human urine, because of the adequate levels of micronutrients in leaf tissues obtained by its use.

Manganese

The highest concentrations of Mn in plant tissue were observed in T2, at 120 DAP, with 12.4% increases compared to the control treatment (Figure 4). All

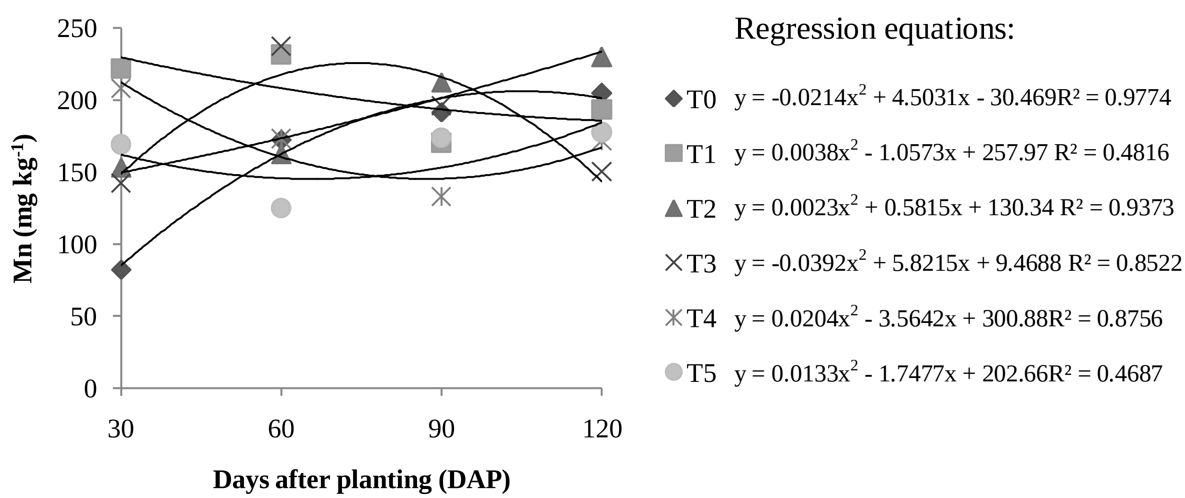

Figure 4. Concentration of Mn in leaves of bermudagrass (*Cynodondactylon*) as a function of dose of urine (T0 - 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 1 5 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), valued into four periods.

treatments also maintained adequate levels of this concentration in leaves - which, according to Wiecko (2006) is between 25 and 300 $mgkg^{-1}$ for grass. For Mn, there was a significant effect over time for all treatments except T4 (Table 4). T2 showed the highest concentration, at 120 DAP, which was 53.2% higher than in T3.

Manganese deficiency is not common in bermudagrass, however, if it occurs, it usually results in chlorosis of young leaves. Low absorption of Mn sometimes occurs with excessive application of nitrogen and potassium. Manganese concentrations can also be affected by pH values (Broyer, 1954; Snyder et al., 2008). In the experiment, high N concentration did not affect the absorption of Mn.

Evaluating the use of human urine in the cultivation of cabbage (*Brassica oleracea*), Pradhan et al. (2007) found that Mn behaved differently with respect to other micronutrients under similar concentrations between plants fertilized with urine and commercial fertilizer.

Zinc

The highest concentrations of Zn were observed in T1 (72 $mgkg^{-1}$). There was a significant variation in Zn concentration overtime (Table 4) in relation to T3 , T4 and T5 doses with values 60.5, 12.5 and 23.4% lower than in T1 (Figure 5). Zn deficiency is uncommon in grasses (Snyder et al., 2008) however, a deficiency can impair plant growth, generating small and stunted leaves (Wiedenhoeft, 2006).

These symptoms were not observed during the course of experiment. Contrary to that observed with the use of urine, in a study with bermudagrass and use of sewage sludge by Lane (1988), increasing Zn doses led to increasing concentrations of Zn in the leaves. The author attributed this fact to a greater growth of plants that received higher doses of nutrients.

Provin (2008) evaluated the influence of the use of sewage sludge on soil nutrient dynamics in growing bermudagrass observing that concentrations of Zn and Fe in leaves increased with increasing doses of these substances.

Concentration of micronutrients in the soil and pH

Boron

Concentrations of boron in the soil did not vary significantly with increasing concentrations of urine in the irrigation water, in layer 1 (0 to 20 cm), but there was significant difference between treatments in the layer 2-20 to 40 cm (Figure 6a and Table 5).

Differences in concentrations were observed between layers in all treatments with urine (Table 6). Apparently, boron did not leach from the first to the second layer of the soil. An appropriate concentration of B in the soil was maintained. For growing lawns, according to Wiecko (2006), B concentration in soil should be between 0.25 and 0.75 $mgdm^{-3}$.

Although this range does not represent toxicity for many plants in heavy soils, higher levels can cause problems, especially for trees and shrubs. Grasses are generally much more tolerant toboron than other plants if they are pruned and the clippings are removed regularly (Harivandi, 1983).

Iron and manganese

No significant effects were observed between treatments for the concentration of Fe in the soil layer 2 (Table 5).

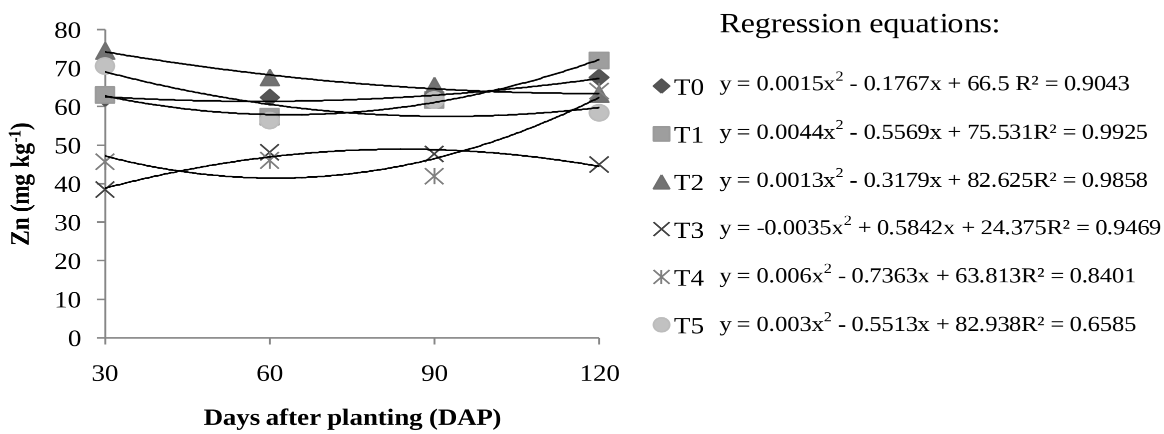

Figure 5. Concentration of Zn in leaves of bermudagrass (*Cynodon dactylon*) as a function of dose of urine (T0 - 0 ml L^{-1}, T1 - 5 ml L^{-1}, T2 - 10 ml L^{-1}, T3 - 15 ml L^{-1}, T4 - 20 ml L^{-1}, T5 - 25 ml L^{-1}), evalated in four periods.

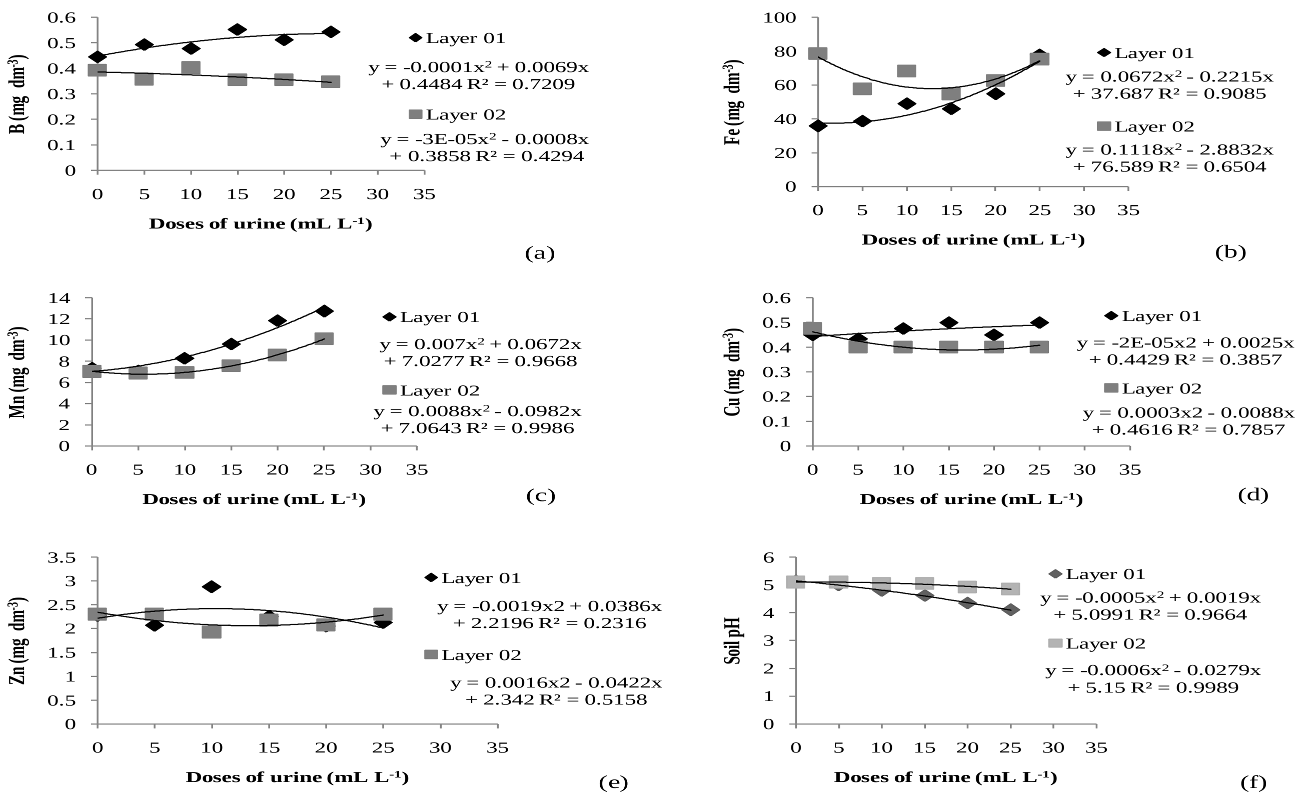

Figure 6. Content of B, Fe, Mn, Cu and Zn and pH in the soil depending on the doses of urine in water, in the different soil layers, 120 DAP.

However, a significant difference was observed between the two layers analyzed, for T0 and T1 treatments, as seen in Figure 6b and Table 6. Fe concentration in the deeper layer was more than twice than in the upper level before urine application (T0). As higher concentrations of urine in the irrigation water were applied, concentrations of this element in layer 1 increased and the differences between layers were reduced. For 25 mg L^{-1} (T5) values were almost the same.

Only T1 and T3 concentrations remained within the range recommended for growing lawns, which is 7.5 to 60 mg dm^{-3} (Wiecko, 2006). For all treatments there was a lower concentration of Fe in the upper layer of the soil,

Table 5. Summary of analysis of variancefor the concentrations of B, Fe, Cu, Mn, Zn and pH in the soil layers 0-20 and 20-40 cm.

Source of variation	DF	Mean square					
		B	Fe	Cu	Mn	Zn	pH
		(Layer 1- 0-20 cm)					
Treat	5	0.00657^{NS}	912.40*	0.00320^{NS}	22.381**	0.38144^{NS}	0.62841**
Residue	18	0.00251	225.82	0.00523	0.96606	0.49717	0.00819
Mean (mg dm^{-3})		0.50375	50.31	0.46791	9.48	2.27	4.67
CV (%)		9.96	29.86	15.46	10.37	31.01	1.94
		(Layer 2 – 20-40 cm)					
Treat	5	0.00212*	362.86^{NS}	0.00375^{NS}	6.61**	0.09541^{NS}	0.04175^{NS}
Residue	18	0.00071	477.83	0.004861	1.49	0.082361	0.04430
Mean (mg dm^{-3})		0.36830	66.16	0.41250	7.95	2.17	5.01
CV (%)		7.27	33.04	16.90	15.55	13.17	4.20

*Significance (p <0.05) by F test; **Significance (p <0.01) by F test; NS, not significant; DF, degree of freedom.

where most of the plant roots are found, causing greater absorption, as observed in the nutrient levels in the leaves.

For Mn, a significant effect of treatments on the two layers was observed for treatments T2, T3, T4 and T5 (Figure 6c and Table 6). No difference was observed between layers for the T1 treatment and control which showed 7 mg dm^{-3} in both layers. For all treatments with urine, there was a greater accumulation of Mn in the upper layers of the soil.

Copper and Zinc

No significant difference between treatments was observed for concentrations of Cu and Zn in soil or between the two layers (Figure 6d and e), except for the concentration of Cu in the treatment T5 (Table 6). Values observed were inside Wiecko's (2006) recommended range for growing lawns, 0.5 - 5 mg dm^{-3} for Cu and 2-5 mg dm^{-3} for Zn. In the case of T2 the identified value of 1.9 mg dm^{-3} at Layer 2, is below the range. However, in Layer 1 where most of the roots develop, all treatments showed adequate levels.

According to Foth (1990), copper tends to be found adsorbed in a fraction strongly complexed with inorganic or organic matter. As a result, copper is rather immobile in soil and concentrations in the solution tend to be very low. As for availability to plants, Zinc and copper exhibit similar behaviors.

Piedade et al. (2009) evaluated the effects of irrigation with wastewater in four species of grass. The values for most micronutrients were found to be superior to those observed at the start of the experimental phase because the application of waste water to the ground causes increases in the levels of micronutrients. As expected, similar results were observed when using human urine diluted in water, since the nutrients present in wastewater are mainly derived from human urine (Tidåker et al., 2007). The use of urine's micronutrients in the soil, 120 DAP, did not lead to levels above those recommended for growing grass for lawns.

pH

The values of soil's pH showeda significant difference between the layers for T3, T4 and T5 (Table 6), with the lowest values (4.1) observed in the 0-20 cm layer in treatment T5. There were significant differences between treatments in layer 2, as can be seen in Figure 6f and Table 5. The use of urine reduced the soil's pH. OnlyT1 presented the same valueas the control case (5.1).

Pradhan et al. (2010) compared theeffect of human urine with that of commercial fertilizers in the cultivation of sugar beet and found no significant differences. For urine treatmentsthese authors observed values of 7.31 and 7.13 at the beginning and end of the experiment.For commercial fertilizers they found a pH 7.17.

The main influence of pH on plant growth is on the availability of micronutrientsas boron, copper, and zinc are leachable and can be deficient in leached, acid soils. On the other hand, they can become insoluble (fixed) and therefore unavailable

Table 6. Summary of the analysis of variance to the concentrations of B, Fe, Cu, Mn, Zn and pH for the 0-20 and 20-40 cm of soil layers, for each treatment.

Source of variation	DF	Treatment	Mean square					
			B	Fe	Cu	Mn	Zn	pH
Layer	1	T0	0.00551^{NS}	3,655.12*	0.00125^{NS}	0.21125^{NS}	0.00125^{NS}	0.00500^{NS}
Residue	6		0.00229	367.29	0.00291	1.37	0.24458	0.00833
Mean (mg dm^{-3})			0.41875	57.12	0.46250	7.18	2.28	5.12
CV (%)			11.44	33.55	11.68	16.30	21.62	1.78
Layer	1	T1	0.03645**	728.28**	0.00211^{NS}	0.07411^{NS}	0.10811^{NS}	0.02^{NS}
Residue	6		0.00109	41.56	0.00111	1.90	0.16444	0.03666
Mean (mg dm^{-3})			0.42500	48.20	0.41625	6.97	2.18	5.05
CV (%)			7.77	13.37	8.01	19.78	18.57	3.79
Layer	1	T2	0.01125**	741.12^{NS}	0.01125^{NS}	3.64**	1.80^{NS}	0.125^{NS}
Residue	6		0.03666	267.79	0.00458	0.84250	1.21	0.02500
Mean (mg dm^{-3})			0.44	58.62	0.43750	7.60	2.4	4.92
CV (%)			6.39	27.91	15.47	12.08	46.01	3.21
Layer	1	T3	0.07801**	153.12^{NS}	2.02^{NS}	8.40**	0.01125^{NS}	0.36120**
Residue	6		0.00106	238.45	0.01	0.92250	0.04291	0.00958
Mean (mg dm^{-3})			0.45375	50.37	0.45000	8.60	2.21	4.83
CV (%)			7.18	30.65	22.22	11.17	9.36	2.02
Layer	1	T4	0.04961**	120.12^{NS}	0.00500^{NS}	21.45**	0.00125^{NS}	0.66120*
Residue	6		0.00266	99.62	0.01166	1.26	0.02625	0.06291
Mean (mg dm^{-3})			0.43375	58.62	0.42500	10.21	2.06	4.63
CV (%)			11.90	17.03	25.41	11.03	7.86	5.41
Layer	1	T5	0.07605**	12.50^{NS}	0.02000**	13.52000**	0.06125^{NS}	1.12500**
Residue	6		0.00179	1,096.25	0.00	1.05	0.04	0.01
Mean (mg dm^{-3})			0.44500	76.50	0.45000	11.42	2.21	4.47
CV (%)			9.51	43.28	0.0	9.01	9.18	2.74

*Significance ($p < 0.05$) by F test; **Significance ($p < 0.01$) by F test; NS - not significant; DF, degree of freedom

for plants in alkaline soils (Foth, 1990). In acid soils, the presence of iron may provoke deficiencies of other elements, such as molybdenum, due to its reaction to form insoluble compounds. However, even at lower pH values, no deficiencies were observed for the micronutrients assessed, with appropriate levels found in leaves.

Conclusions

The results presented in this study allow us to conclude that:

1. Irrigation with diluted human urine positively influences the levels of micronutrients present in plant tissue and soil. Adequate levels of micronutrients were verified in bermudagrass when irrigated with human urine. This represents an additional advantage for nitrogen and phosphorous cycling through human urine use in agriculture;
2. The highest accumulation of B, Fe and Mn in leaves, 120 days after planting, were observed under 10 ml urine per liter of water;

3. Concentrations between 5 and 15 mL L^{-1} showed better results as they promoted good nutrient accumulation in the plant tissue;

4. As the use of urine promotes the recycling of nutrients which reduces the amount of industrial fertilizers needed, it can be a viable alternative for the discharge of excreta in rural and urban areas. It allows improvements in plant development, permitting a better fractionation of nutrient supply. Furthermore, the use of urine does not cause accumulation of micronutrients considered toxic for growing bermudagrass, both in the leaves and in the soil.

Conflict of Interest

The authors verify that there are no competing interests.

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