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Nitrogen recycling through fertilization of Bermuda grass using human urine diluted in water

Reciclagem de nitrogênio pela adubação de grama Bermuda usando urina humana diluída em água

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ABSTRACT: The purpose of this study was to analyze the nutrient accumulation and development of Bermuda grass in the establishment phase using diluted human urine as source of nutrients. The experiment was conducted in greenhouse with a completely randomized design using six treatments which were consisted of five urine doses (5, 10, 15, 20 and 25 mL of urine per liter of water) and control (soil without fertilization), with four replicates. There were significant differences between treatments for dry matter in the plants. Higher concentrations of urine led to higher production of dry matter until the 20 mL of urine per liter of water treatment. A maximum accumulated dry matter of 528g was found for an application of 18 mL/L. This value was obtained from a mathematical regression based on experimental data. The highest concentrations of nitrogen and phosphorus in leaves after 120 days of planting were observed at the 10 mL/L treatment. The use of diluted human urine promoted good development and an adequate accumulation of nutrients in plants tissue allowing the diversion of man made nutrients from unwanted environmental reservoirs.

KEY WORDS: *Cynodon dactylon*, nutrient cycling, diluted human urine, ecological sanitation.

RESUMO: O objetivo deste estudo foi analisar o acúmulo de nutrientes e desenvolvimento da grama Bermuda na fase de estabelecimento, utilizando urina humana diluída como fonte de nutrientes. O experimento foi conduzido em casa de vegetação em delineamento inteiramente casualizado, com seis tratamentos, que consistiram de cinco doses de urina (5, 10, 15, 20 e 25 mL de urina por litro de água) e controle (solo sem adubação), com quatro repetições. Houve diferenças significativas entre os tratamentos para matéria seca das plantas. Concentrações mais elevadas de urina levaram a uma maior produção de matéria seca, até a dose de 20 mL/L. A máxima matéria seca acumulada da poda, (528.0 g) foi encontrada para uma aplicação de 18mL/L. Este valor foi obtido a partir de regressão matemática com base em dados experimentais. As maiores concentrações de nitrogênio e fósforo nas folhas, 120 dias após o plantio, foram observadas no tratamento de 10.0 mL/L. O uso da urina humana diluída promoveu bom desenvolvimento e acumulo adequado de nutrientes no tecido das plantas, permitindo o desvio dos nutrientes produzidos pelo homem dos reservatórios ambientais indesejados.

PALAVRAS-CHAVE: *Cynodon dactylon*, ciclagem de nutrientes, urina humana diluída, saneamento ecológico.

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Introducion

The application of chemical fertilizers has become essential to provide nutrients in optimal amounts for plant development (FRANCO e NETO, 2007). It is well known the close relationship that exists between rates of fertilizer use and productivity (ISHERWOOD, 1998). Among the various agricultural inputs water and fertilizers are the main contributors to agricultural production.

Nitrogen (N) is the most required nutrient in the majority of crops (MALAVOLTA, 2006) directly reflecting on the world's consumption of nitrogenous fertilizers. Improper handling of it causes groundwater contamination, contributes to the eutrophication of aquatic environments and compromises the water quality of springs (BARTON e COLMER, 2006). It also affects food security and causes harm to public health due to contamination by nitrate.

To ensure productivity standards required by agricultural systems, atmospheric nitrogen is transformed into reactive N in the forms of ammonium nitrate, urea, calcium nitrate, ammonium bicarbonate and several varieties of nitrogenous fertilizers (ERISMAN, 2007). Recent studies show that this transformation is carried out at such a high rate that natural processes of denitrification can not regulate the nitrogen flow on earth, causing its accumulation in environmental reservoirs (ROCKSTRÖM et al., 2009) leading the presence of nitrates in aquifers and water courses eutrophication.

According to Rockstrom et al. (2009) the maximum conversion of reactive N to inert N for all human activities is about 35 million tons of N per year (Mt N/year). At a global level at least 200 Mt N/year are currently converted by anthropogenic activities, with 120 Mt N/year to produce industrial ammonia, 50 to 70 Mt N/year through biological nitrogen fixation in agriculture, and 30 to 40 Mt N/year to produce energy by fuels combustion (SUTTON et al., 2011).

In this scenario, it is necessary to mitigate changes in the global biogeochemical N cycle caused by the conversion of atmospheric inert N to reactive N. Thus, the management model of anthropogenic N flows in agricultural systems should be based on cyclic flows, following the logic of natural systems where there are no losses with the use of all available resources.

In contrast to the current model of agriculture the application of techniques for water reuse and nutrients cycling, used in ecological sanitation systems (Ecosan), promotes a new way of dealing with what is, nowadays, considered waste. Ecosan is based on the systematic reuse and recycling of nutrients and water in an hygienically safe and closed circuit. Ecosan systems enable the recovery of nutrients, for example from human urine for the benefit of agriculture (WERNER et al., 2003).

Among the resources available for agriculture human excreta are a potential alternative to enhance sustainability and conservation of natural resources, favoring nutrients recycling. Due to the high concentration of nutrients in urine the interest in its separation, at the source, from other sewage streams has increased in recent years (JONSSON et al., 2004). The low presence of human pathogenic organisms in urine reinforces this tendency.

This use of excreta is widely practiced in many parts of the world. Chinese people have used human composts and animal waste for thousands of years, and Japan introduced this mechanism in agriculture in the twelfth century. In Europe, until recently, it was common among farmers to recycle human waste and animal manure (ESREY, 1998).

Studies have shown the benefits of human urine in crop production (GANROT, 2005; KARAK e BHATTACHARYYA, 2011). However, despite numerous studies in other countries (KARAK e BHATTACHARYYA, 2011), in Brazil there are few studies related to its use as agricultural fertilizer (BOTTO, 2013). Besides the high N concentration in urine it also possesses other necessary elements for plant growth, such as phosphorus and potassium (JONSSON et al., 2004).

The fertilization of grass with human urine includes further advantages. As it is not an edible culture it may find less cultural restrictions from the public, although a safe application of this product can be guaranteed by the low presence of pathogen sand proper handling (JONSSON et al., 2004). Additionally transportation logistics are facilitated as grass is a urban and a rural culture.

This study investigates the effects of application of different concentrations of human urine in water on the growth of Bermuda grass at its establishment phase. Nutrient accumulation in plant tissue was also evaluated.

Material and Methods

Research site - The experiment was conducted in a greenhouse at experimental area of the Soil and Water Engineering Group at the Federal University of Reconcavo of Bahia (NEAS/ UFRB), located in Cruz das Almas, Bahia, coordinates 22º 42' S and 47º 38' W and 220 m altitude. The climate is classified as humid to sub-humid with 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 used in the experiment was an Oxisol with low fertility. It was collected at a depth of 0-20 cm in the university campus. The soil analysis before planting was performed on the Soil Laboratory at the School of Agriculture Luis de Queiroz (ESALQ / USP) according to the methods described by Raij (2001). The following chemical composition was obtained: pH ($CaCl_2$): 6.2; Organic matter: 13 g/dm^3; P (resin): < 2 mg/dm^3; S (SO_4^{-2}): < 3 mg/dm^3; Na: 1.9 mmolc/dm^3; K: 18.4 mmolc/dm^3; Ca: 10 mmolc/dm^3; Mg: <1 mmolc/dm^3; Al: 28 mmolc/dm^3; H + Al: 30 mmolc/dm^3 and base saturation (BS): 58.0 mmolc/dm^3. The granulometric composition of the soil was 800, 13 and 187 g/kg of sand, silt and clay, respectively.

Experimental design - The experiment was conducted in a completely randomized design consisting in 24 experimental plots, each one in a polyethylene container, divided in six treatments. Each treatment was composed of 4 plots to allow replication. Five of them were treated with urine solutions consisting in 5, 10, 15, 20 and 25 mL of urine per liter of water, respectively. The sixth plot (control) was left without fertilization.

Treatments with urine received additional doses of phosphorous and potassium, so that all treatments were fertilized with the same dose of these nutrients. To allow a better understanding of the effect of nitrogen from the urine all the nitrogen provided to the plants came from this source. To ensure that all treatments contained the same concentration of phosphorus and potassium, five samples of human urine where analyzed prior to assembly of the experiment to determine their average chemical composition (Table 1), 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 through historical data and 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.

A commercial fertilizer containing simple superphosphate and potassium chloride was applied based on the recommendation of Godoy and Bôas (2003). Each treatment was fixed to represent an equivalent supply of 150 kg/ha of P_2O_5 and 150 kg/ha of K_2O. The potassium fertilizer was applied at planting (50%) and the second fraction 60 days later. All phosphorus was applied at planting.

Seeds of Bermuda grass (*Cynodon dactylon)* were used following the manufacturer's recommendations for an equivalent dose of 25 kg/ha. The planting was carried out in polyethylene containers with a capacity of 100L

Table 1. Characteristics of human urine and water used in irrigation.

Determinations	Unit	Water	Urine
pH		8.40	8.70
Electrical conductivity	dS m^{-1}	0.78	24.35
N-total	mg L^{-1}	nd	6937.50
P total	mg L^{-1}	nd	923.33
K_2O	mg L^{-1}	7.03	1483.75
Ca^{+2}	mg L^{-1}	5.21	65.00
Mg^{+2}	mg L^{-1}	8.50	10.00
S	mg L^{-1}	nd	1655.00
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}	106.90	2937.50
B	mg L^{-1}	nd	0.50
Cl^-	mg L^{-1}	0	4093.75

nd – not determined

and 0.41 m^2 with drains and filled with soil over a 3 cm thick layer of gravel and a geotextile blanket.

Collection and application of human urine - The human urine used in the experiment was directly collected in a black plastic reservoir with a capacity of 20L installed in the male's toilets of the student's residence at UFRB campus. The students voluntarily contributed by urinating directly into the reservoir. The urine was collected for up to 3 days and was used up to 4 days after collection.

The solutions of urine in water were prepared at the time of irrigation using the values of class A evaporation pan, installed inside the greenhouse, applying 100 % of the evaporated blade. The urine was added to water accordingly to the concentrations defined for each treatment. The plots were irrigated every two days.

The chemical characterization of urine used throughout the experiment was done by collecting samples for all irrigations, which were stored under refrigeration. A composite sample was obtained monthly considering the weighted average of single samples, based on the volume of water applied on each irrigation.

Grass management and evaluation - The grass was cut to approximate 2 cm height. Pruning was performed when at least one of the treatments had 10 cm height of with a frequency of 7-14 days. After cutting the material was placed in paper bags and taken to an oven with forced air circulation at 65°C for 72 hours to determine its dry mass. All collected prunings were used for calculating the dry matter accumulation of the pruning during the establishment phase of grass. Pruning collected at 30, 60, 90 and 120 days after planting (DAP) were used for analysis of the leaves nutrients. Analyses to determine the levels of nutrients were performed in the laboratory of mineral nutrition of plants at the College of Agriculture Luis de Queiroz (ESALQ/USP), by the method described by Malavolta et al. (1997).

For calculating the dry matter of shoots and roots, after the end of the experiment, plants were removed from the recipients, divided into shoots and roots, and brought to a drying oven with forced air circulation at 65°C, until constant weight.

Statistical analysis - All variables were subjected to analysis of variance followed by regression analysis. The variance was calculated by taking the average of the four replicates for each treatment. The estimation of the optimal dose of urine, which provides greater accumulated dry mass of pruning, dried root weight and total dry mass were determined by the first derivative of the respective regression equations obtained by equating the first derivative of the equation to zero. For both analyses SISVAR System software (Analysis of Variance, version 5.3) was used. Results were analyzed by F-test at a 5% significance level.

Results and Discussion

Plant growth - From the results fron the Statistical analysis it can be stated that the production of dry matter accumulation (DMA) in the cutting grass collected from the Bermuda grass growth stage was influenced positively by urine applied doses (Table 2).

In Figure 1 it can be observed that the control treatment, which did not receive urine, had the lowest accumulated dry mass of pruning, 160.9 g/plot. Increasing doses of urine to the dose of 20 mL/L provided increasing increments in the production of dry matter accumulation due to the growth. However, the dose of 25 mL/L urine showed an accumulated dry mass of 485 g/plot less than the mass of 519 g/plot observed at the dose of 20 mL/L. Regression showed that the 528 g maximum dry matter accumulated pruning along the establishment phase of grass would be obtained with a solution of 18mL of urine per liter of water.

Table 2. Summary of the analysis of variance, coefficient of variation (CV%) and estimation of parameters of dry matter accumulated (DMA), root dry weight (RDW), total dry mass (TDM) and concentration in leaves of NPK.

Source of variation	DF	MS DMA	RDW	TDM	N	P	K
Treatment	5	75082.95**	507.30*	1442.52**	184.88**	4.93^{NS}	51.54**
Residue	18						
General Average		414.37	65.91	93.17	34.86	5.02	22.85
CV (%)		9.75	17.12	14.03	7.53	12.67	12.28

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

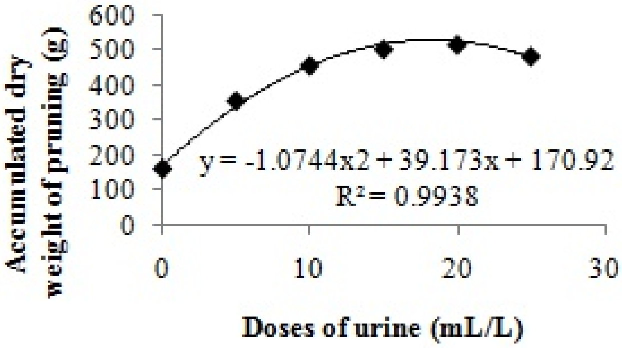

Figure 1. Accumulated dry weight of pruning, of bermuda grass, depending on the dose of urine mL/L.

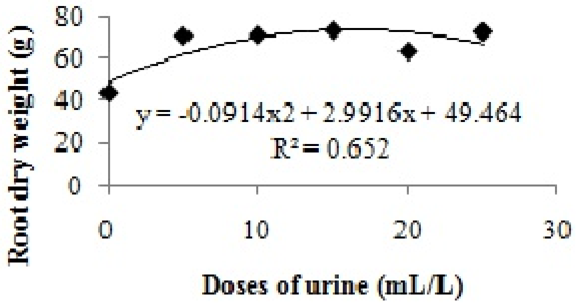

Figure 2. Accumulated dry weight of root dry mass of bermuda grass, depending on the dose of urine mL/L.

The observed increase of dry mass from pruning with increasing doses of urine concentration was expected since Bowman et al. (2002) argue that Nitrogen is the nutrient that the grass needs in a greater quantity and according to Wiecko (2006) the supply of nitrogen accelerates the growth of grass leaves.

Similarly to the results observed in this experiment Snyder e Cisar (2000), using increasing doses of N in Bermuda grass, found an increase in dry matter of cuttings for one year. Backes et al. (2010a) found that application of increasing doses of sewage sludge in emerald grass provided a mass increase of cuttings due to the high concentration of nitrogen present in the sludge used. The results observed with human urine were similar to those found with the application of sewage sludge reported by Backes et al (2010a).

The lower growth of the control treatment without fertilization can be associated with nitrogen deficiency and according to Taiz e Zeiger (2013) the deficiency of nitrogen can inhibit plant growth causing them to have severe deficiency symptoms within a few months without fertilization. Urine can, therefore, be considered a viable source of nitrogen for Bermuda grass. However, it was also observed a decrease in DMA in the treatment which received the 25 mL/L dose which, according to Wiecko (2006) can occur when lawns receive excessive amounts of N indicating that above 20 mL/L doses may affect the successful development of Bermuda grass.

For the root dry weight (RDW) variable a significant difference between treatments (Table 2), similar to the behavior observed in the DMA form, was observed. The treatment, which did not receive human urine, showed a lower root mass. As can be seen in Figure 2, there was an increase in RDW with increasing doses of urine. According to the regression analysis, a maximum root production of 74 g, would be obtained at a 16mL/L dose representing an increase of 67%, compared to the control treatment.

With a 25 mL/L dose there was a greater dry weight of the roots compared to 20 mL/L dose. This fact may be explained by considering that, at this doses plants growth may have been directed to the roots instead of their leaves which according to Souza e Fernandes(2006) occurs in adverse conditions where the plant reduces shoot growth due to the mobilization of reserves, occurring elongation of the root system.

The excess nitrogen in the 25 mL/L dose may have configured an adverse condition to the growth of grass resulting in an increased root system. However Wiecko (2006) cites that appropriate doses of nitrogen fertilizer promote a suitable rooting and a healthy root system however, excessive doses promote great shoot growth in expense of the root system. In our experiment an excess of nitrogen benefited root growth in expense of leaves growth. This point needs further analyses which are out of the scope of this work.

For the total dry mass (TDM) a significant difference between treatments (Table 2) was found. A maximum TDM of 107g would be obtained at a dose of 17 ml/L urine in water (Figure 3) with 93% increase when compared to the control treatment.

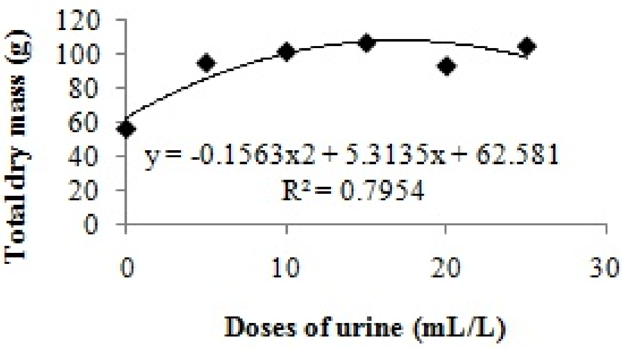

Figure 3. Accumulated dry weight of total dry mass of bermuda grass, depending on the dose of urine mL/L.

Similar to the results obtained in this experiment studies by other authors have also shown that the use of human urine promotes the growth of plants, as tests carried out by Heinonen-Tanski et al. (2005) evaluating the use of urine as a source of nutrients in a cucumber crop and obtained similar or slightly better results in terms of yield, when compared to treatment with commercial fertilizer.

NPK accumulation in leaves - Although in all treatments P and K were complemented to the five diluted solution of urine concentrations of N, P and K in the leaves presented significant differences at 120 day after planting (DAP) time, which means in the end of the experiment (see Figure 4). However, there was no significant linear or quadratic effect for the accumulation of N and P (Figure 4a and 4b). The variation of K showed a significant linear behavior (Figure 4c). The highest concentration of nitrogen at 120 DAP was found in plants that received the 10 mL/L dose, presenting a 17% higher value when compared to the control treatment. Similar behavior was observed for the P levels, where as for K there was a decrease in the leaves concentration with the increasing of urine concentration in water solution.

According to Wiecko (2006) the ideal concentration of N in dry weight for Bermuda grass is between 30 and 50 g/kg then in this experiment all the treatments provided accumulations within the referenced range. Similar results were obtained by Backes et al. (2010b).These authors obtained concentrations of 28 g/kg using sewage sludge to fertilize Emerald grass. The concentrations of P and K for all treatments are also on the range recommended in the literature which according to Wiecko (2006) is 1.5 - 5 g/kg for P and 1 - 40 g/kg for K. The greater root growth associated to changes in its architecture provides increased absorption of P. In addition, the processes that conserve the absorbed P involve: reduction in growth rate, increased biomass production per unit of P absorbed, remobilization of internal P, changes in the metabolism of C that by passes the steps that require P and use of alternative airways (VANCE et al., 2003). This information justifies the results, as the control treatment with lower growth showed similar concentrations of P, as compared to other treatments.

According to Malavolta (2006) potassium is the second most important mineral nutrient required by plants in quantitative terms. Potassium is vital for photosynthesis and in situations of disability it causes a reduction in this process and an increase in respiration resulting in decreased accumulation of carbohydrates (NOVAIS et al., 2007). The highest vegetative growth observed with increasing doses of urine suggests the need of higher rates of potassium given the great amount required by the plant. As K fertilization was the same for all treatments and the increase in urine doses provided greater growth, due to the higher supply of N,

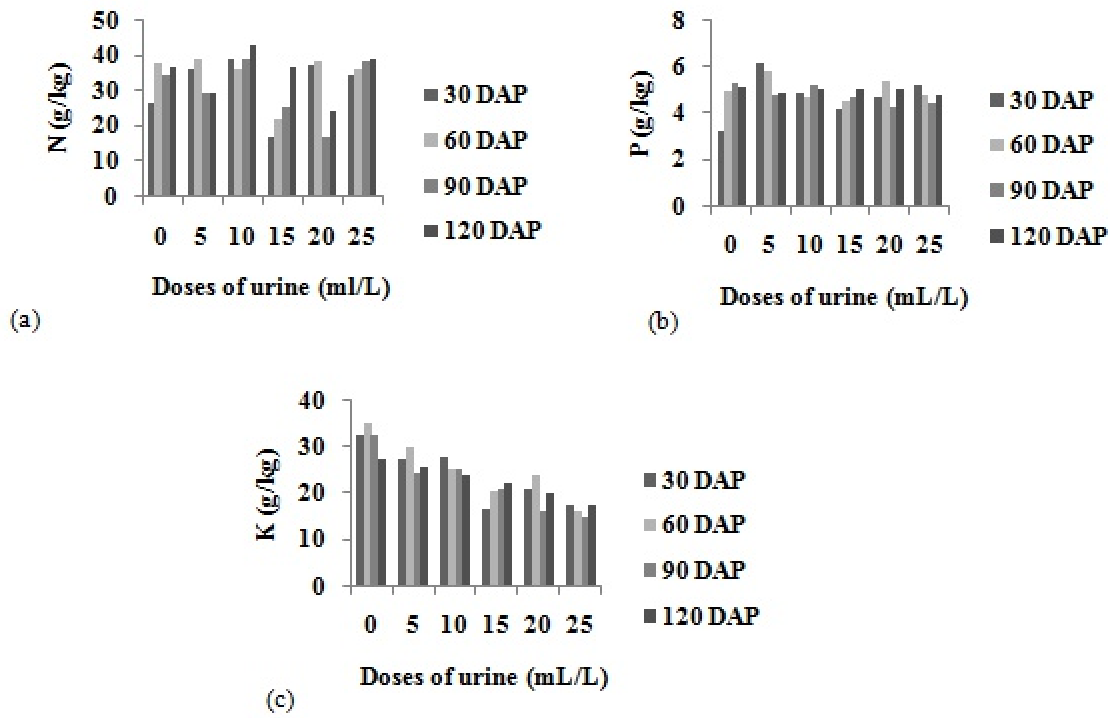

Figure 4. Nitrogen, phosphorus and potassium concentration in leaves, depending on the application doses of urine along the establishment phase of grass.

there was decrease in concentration of K in leaves.

The information about nutrients concentration in the leaves of grasses has great importance regarding to fertilization. According to Kopp e Guillard (2002) after pruning the nutrients present in the leaves are still availiable when if left in the field, contributing to the cycling of nutrients and optimizing the use of nitrogen. In addition, the use of cuttings can reduce nitrogen fertilizer rates in 20-30% due to N recycling through the ground (PESSARAKLI, 2008).

To evaluate the amount of nutrients absorbed by flap emerald grass Backes et al. (2010a) observed that the higher the dose of sewage sludge applied the lower the percentage of nutrients extracted by the grass from the soil. Tidåkeret al. (2007) reported that human urine can promote better recovery of nutrients by the plant compared to sewage sludge.

Conclusions

New ways to close the loop of nitrogen in the ecosphere are needed to face the increasing concentration of reactive forms of this nutrient. An important route that has to be redirected is the discharge of nitrogen from human wastes in the natural environment. By recycling nitrogen from wastes less of this element will be necessary to be extracted from the atmosphere. Urine is, by far, where human wastes more concentrates nitrogen and, at the same time, presents a very low presence of pathogens when compared to other wastes. For these reason urine is investigated as a fertilizer. However, there is a need to define optimized means to manage human urine for this purposes. This work investigates the use of human urine, segregated before mixing it to wastewater, as a fertilizer for Bermuda grass, a culture that is developed both in urban and rural areas.

It is shown that Bermuda grass shows great development when irrigated with solutions of urine in water. In the investigated range of dilutions from zero (control) to 25 mL of urine per liter of water, best results for accumulated dry matter from pruning along the establishment phase were found for the fertilizing solution of 18.2 mL of urine per liter of water. The amount found was three times the obtained in the control plot where no fertilizer was used. With respect to maximum total dry mass the best urine solution found was 17 mL/L which allowed a total dry mass, 120 days after planting, 93% higher than the control case. For the accumulation of N and P in leaves, 120 days after planting, best result was observed in a 10 mL/L dose .

It can be concluded that the use of human urine promoted good development of shoots and roots, as well as accumulation of nutrients in the plant tissue, which can be recycled thus reducing the use of synthetic nitrogen fertilizer.

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