Agronomic potential of excreta-based biofertilizers made from treated human feces and urine for crop production

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RESUMO
O uso de excretas humanas na agricultura é motivado pela escassez de água, degradação dos recursos hídricos e maior demanda por alimentos. Este trabalho investigou o potencial agronômico de biofertilizantes produzidos de fezes e urina humanas tratados para a produção de alimentos. Nesse contexto, uma pesquisa de campo com 18 lisímetros (6 tratamentos e 3 blocos) foi conduzida com a aplicação de quatro biofertilizantes para cultivo de Lactuca sativa var. Valentina. Os tratamentos empregados foram controle negativo (T1), fertilizante químico (T2), fezes tratadas com ureia (T3), fezes compostadas com resíduos orgânicos (T4), urina estocada (T5) e estruvita (T6). O desenvolvimento das plantas foi avaliado semanalmente medindo a altura e o diâmetro de planta. Como resultado, os biofertilizantes promoveram um crescimento maior do que o controle negativo (T1) e inferior ao controle positivo (T2), com exceção da urina estocada, que não apresentou diferença significativa do T1. O tratamento com estruvita se destacou, com altura de planta 98% superior ao controle negativo e número de folhas sem diferença significativa do fertilizante químico. Dessa forma, excretas humanas tratadas apresentaram potencial para fertilizar o solo e permitir a absorção de nutrientes pelas plantas. Apesar da concentração inicial de nutrientes no solo ser muito baixa de acordo com o guia de fertilização da região sul do Brasil, as plantas apresentaram desenvolvimento satisfatório e melhor do que o solo sem fertilização. Como a disponibilização de nutrientes é mais lenta em fertilizantes orgânicos comparado aos fertilizantes químicos, ciclos de cultivo sequenciais provavelmente melhorarão o desenvolvimento das plantas.

Palavras-chave: saneamento ecológico; excretas humanas; fezes; urina; reciclo de nutrientes.

ABSTRACT
The use of human excreta in agriculture is driven by water scarcity, degradation of water resources, and increased demand for food. This work investigated the agronomic potential of biofertilizers made from treated human feces and urine for crop production. In this regard, field research with 18 lysimeters (six treatments and three blocks) was conducted by applying four biofertilizers in the soil to grow Lactuca sativa var. Valentina. Treatments employed were negative control (T1), chemical fertilizer (T2), urea-treated feces (T3), composted feces with organic waste (T4), stored urine (T5), and struvite (T6). Plant development was assessed weekly by measuring the plant height and diameter. As main results, the use of the biofertilizers presented a higher growth than the negative control (T1) and lower than chemical fertilizer (T2), except for the stored urine treatment, which did not exhibit a significant difference from T1. Struvite treatment stood out, showing a height 98% higher than the negative control and final leaf numbers with no significant statistical difference from the chemical fertilizer. Therefore, treated human excreta presented a potential to fertilize the soil and plant uptake. Even though the initial nutrient concentration in the soil was very low, according to the fertilizing guide from southern Brazil, the plants could still grow and present a better development than the soil with no fertilizer. As nutrient availability in organic fertilization is slower than in chemical fertilization, sequential cultivation cycles should improve plant development.

Keywords: ecological sanitation; human excreta; feces; urine; nutrient recycling.
1. INTRODUCTION
The world population is expected to reach the 9.7 billion figure by 2050 (UNITED NATIONS, 2022), which requires a significant effort to overcome the challenges of an ever-growing population. While 26% of the world population suffers from moderate or severe food insecurity (FAO et al., 2019), and also 26% do not have access to safely managed drinking water services (WHO and UNICEF, 2021), there is a concern regarding resources for the future. For example, the demand for food is expected to rise by 60% by 2050 (FAO, 2012), whilst the primary source of phosphorus, an essential fertilizer for food production, is phosphate rock, which is non-renewable and is becoming scarce (CORDELL et al., 2011). Besides, the high prices of chemical fertilizers promote a challenge in providing nutrients for crop production, especially in rural regions with low income (KEMACHEEV AKUL et al., 2011; PETTERSSON and WIKSTRÖM, 2016).

Instead, human excreta are a sustainable source of nutrients for plant development, as they contain the main macronutrients for plant growth: nitrogen, phosphorus, and potassium, as well as carbon, water, and some micronutrients (HARDER et al., 2019). Most of the nutrients excreted per year are in human urine, i.e., 80 – 90% of N, 50 – 80% of P, and 80 – 90% of K (VINNERÅS et al., 2006). Nevertheless, it is mainly composed of water (SENECAL and VINNERÅS, 2006). Despite the availability of nutrients and organic matter, they also have less water (VINNERÅS et al., 2006). Feces, however, need extra caution during manipulation and treatment and should always be considered to contain pathogens (SCHÖNNING et al., 2007). Treatments such as storage can remove pathogens, depending on the temperature and storage duration (VINNERÅS et al., 2008), but do not diminish the volume. Another urine treatment, struvite precipitation, recovers P efficiently and produces a white powder that is easier to carry (LIU et al., 2016).

Regarding feces, even though they contain fewer nutrients than urine, they also have less water (VINNERÅS et al., 2006). Feces, however, need extra caution during manipulation and treatment and should always be considered to contain pathogens (SCHÖNNING et al., 2007). Treatments usually depend on the elevation of the pH, dehydration, and composting. Urea addition promotes the pH elevation and sanitization of feces, producing a brown powder (MAGRI et al., 2013). Composting feces with organic waste elevates the temperature during a time interval to promote sanitization (NIWAGABA et al., 2009).

Studies employing sanitation subproducts for plant growth, e.g., treated human feces and urine, sewage sludge, and pit latrine, demonstrated satisfactory results and, occasionally, presented higher productivity than chemical fertilizers (CHRISPIM et al., 2017; EVERAERT et al., 2017; KUTU et al., 2011; TRIASTUTI et al., 2016). Despite the availability of nutrients and organic matter, the use of human excreta in agriculture is limited by the presence of pathogens and pharmaceuticals. Moreover, it is not easy to compare the productivity of different biofertilizers because studies usually evaluate them separately, so each uses a different soil type, climate, and environmental conditions.

This study aimed to evaluate the productivity of a short-term crop, Lactuca sativa, grown in soil amended with four excreta-based biofertilizers, urea-treated feces, composted feces with organic waste, stored urine, and struvite.

2. MATERIALS AND METHODS

2.1. Soil and biofertilizers properties

Soil type is silt loam with 47.6% sand and 52.4% silt (LEMOS and SANTOS, 1996). Soil nutrient content was 0.2% organic matter, 2.4 mg-P·dm⁻³, 8.8 mg-K·dm⁻³, and 0.0 mg-NH₄·dm⁻³. As soil pH was 4.7, liming was carried out to correct its pH to 7.0 90 days prior to planting in 9.1 tons of dolomitic limestone ha⁻¹.

The urea-treated feces received a static treatment, using a mixture of ash, ground oyster shells, and urea added after each defecation (MAGRI et al., 2013). The treatment of the urea-treated feces lasted 3 months. The composted feces were treated in static windrows using the UFSC composting method (INÁCIO and MILLER, 2009). Waste was added in a volume ratio of 1:3 of feces and kitchen organic waste twice a week for 6 weeks. After, the maturation phase lasted 4 weeks. Human urine was collected from volunteers and stored in plastic containers for 6 months at a temperature between 15 and 20 °C. For struvite production, a 20 L reactor was made in PVC. The reaction was performed using stored urine, with pH around 9.0, and magnesium chloride hexahydrate (MgCl₂ • H₂O), as a source of magnesium, added at a molar ratio of 3.3 Mg:P. The agitation period was 15 minutes at 130 rpm, and struvite was left to precipitate for 60 minutes. The precipitate was dried at 50 °C to constant weight. The nutrient content of each biofertilizer was assessed before cultivation (Table 1).

2.2. Experimental setup

For lettuce cultivation in a controlled soil condition, lysimeters composed of polyethylene tanks (1 m³ volume, 1.51 m diameter, and 0.76 m in height) were built (Figure 1). PVC pipes were connected to each lysimeter for the drainage system leading to 50 L storage tanks. A 10 cm layer of gravel (19 – 25 mm) was placed at the bottom to favor drainage, and geotextile fabrics were placed between the gravel and a 60 cm layer of soil.

The study followed a completely randomized design (CRD) with 6 treatments and 3 replications, being T1 — negative control with no fertilizer; T2 — positive control with urea, triple superphosphate (TSP), and muriate of potash (MOP); T3 — urea-treated feces; T4 — composted feces; T5 — stored urine; and T6 — struvite.

Table 1 – Biofertilizers properties and the amount added in kilograms.

<table>
<thead>
<tr>
<th>Biofertilizer</th>
<th>% N</th>
<th>% P₂O₅</th>
<th>% K₂O</th>
<th>Amount applied (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea-treated feces</td>
<td>0.5</td>
<td>2.2</td>
<td>21</td>
<td>288</td>
</tr>
<tr>
<td>Composted feces</td>
<td>3.7</td>
<td>5.3</td>
<td>40</td>
<td>141</td>
</tr>
<tr>
<td>Stored urine</td>
<td>0.7</td>
<td>0.1</td>
<td>0.2</td>
<td>474</td>
</tr>
<tr>
<td>Struvite</td>
<td>5.2</td>
<td>62.5</td>
<td>64</td>
<td>017</td>
</tr>
</tbody>
</table>

Figure 1 – Lysimeters distribution on the ground and lettuce on the 34th day of the experiment.
Solid fertilizers, *i.e.*, urea, TSP, MOP, urea-treated feces, composted feces, and struvite were mixed into the soil before the transplantation of the seedlings. The urine was applied after transplantation on days 0, 18, and 27 of the experiment, corresponding to 30, 30, and 40% of the mass, respectively. The quantity was calculated using the limiting nutrient according to the fertilizing guide for the region (COMISSÃO DE QUÍMICA E FERTILIDADE DO SOLO, 2016) (Table 1). For the commercial fertilizers, 70.43 g of urea, 96.00 g of TSP, and 72.00 g of MOP were applied per lysimeter.

In each lysimeter, 16 seedlings of *Lactuca sativa* var. Valentina were transplanted to evaluate plant development for 36 days. Each week, 4 plants were randomly selected to measure: plant height using a measuring tape and leaf number. Plant measurement and soil data were treated statistically using SPSS software version 27. Outliers were removed (*α* = 0.05), and normal distribution was verified. Variance analysis (ANOVA) was applied using CRD and Tukey’s post hoc test (*α* = 0.05) to obtain the significant differences.

### 3. RESULTS AND DISCUSSION

Weekly plant height and leaf number did not differ statistically for the block factor (*p* > 0.05), only for treatment (*p* < 0.001), which means that the repetitions from each block are from the same population. Therefore, significant differences presented are due to different treatments applied. Weekly plant height and leaf number measured are shown in Figure 2, and the final images from each treatment are shown in Figure 3.

The treatment using chemical fertilizers (T2) exhibited the highest final height, with 20.9 cm plant height, showing a significant difference from the other treatments (Figure 2a). Struvite (T6), urea-treated feces (T3), and composted feces (T4) had final heights of 16.5, 13.6, and 13.9 cm, respectively, with no significant difference between them. The lowest height group was negative control (T1) with 6.1 cm and stored urine (T5) with 5.8 cm. The use of urea-treated feces, composted feces, and struvite (T3, T4, and T6) led to higher plant height than the negative control (T1) but lower than the chemical fertilizer (T2). Nevertheless, the use of biofertilizers have been shown to improve plant development and lead to heights similar to or better than chemical fertilizers.

For Torgbo *et al.* (2018), the use of co-compost from fecal sludge and municipal waste presented higher plant height than chemical fertilizers. Applying struvite supplemented with KCl in maize production led to a similar result in plant height, with no significant difference from chemical fertilizer (LIU *et al.*, 2011). Pradhan *et al.* (2010) applied urine for beetroot cultivation and found no significant difference from chemical fertilizer.

Until the 22nd day of the experiment, there was no significant statistical difference in the plant height between the plants grown with the chemical fertilizers (T2) and the biofertilizers urea-treated feces, composted feces, and struvite (T3, T4, and T6). Yet, measurements taken on days 29th and 35th showed the biofertilizers were significantly lower than the chemical treatment. A previous study observed the most significant changes in the lettuce height happened between the 3rd and the 5th week of cultivation when fertilized with sludge composted with organic waste (TORGBO *et al.*, 2018), which was the period the chemical fertilizer stood out from the other treatments in the present study. The stabilization in plant height in treatments T3, T4, and T6 while T2 continued to increase its size may indicate that biofertilization lacked nutrients for plant development in the last two weeks.

The highest final leaf number was measured for T2, showing 37 leaves, with no significant difference to the struvite treatment (T6), which had 29 leaves (Figure 2b). The biofertilizer that showed better results was struvite, showing a significant statistical difference from urea-treated feces (T3), composted feces (T4), and urine (T5) treatments. The second group of treatments was composed of urea-treated feces and composted feces, with 23 and 24 leaves, respectively. The lowest leaf number treatments were negative control (T1) and stored urine (T5), showing 7 and 6 leaves, respectively.

The application of the biofertilizers made from human feces (urea-treated and composted) significantly enhanced the leaf numbers compared to the negative control, same as observed by Triastuti *et al.* (2016), who applied latrine compost on *Jatropha curcas* production. The application of struvite showed no significant difference in the lettuce leaf numbers compared to chemical fertilizer in the present study, corroborating the findings when cultivating maize (LIU *et al.*, 2011). Regarding urine, the present study obtained the same results as the negative control, while Germer *et al.* (2011) tested...
the application of urine enriched with P and K on sorghum and had a 6% enhancement in leaf number.

Even though three of four biofertilizers employed in this study showed better plant development in terms of plant height and leaf number compared to no fertilizer, they did not perform as well as the chemical fertilizers, except for struvite on leaf number. In that regard, the treatment T2 was the only one that fully met the demand of all main macronutrients (N, P, and K). The biofertilizers were calculated to fully meet only one nutrient demand, i.e., N, P, or K, which was determined to avoid nutrient buildup in the soil.

Most studies complemented biofertilizers with chemical fertilizers to overcome the lack of one or more nutrients for plant development because the nutrients in organic fertilizers are not proportionally balanced to the plant's needs. The outcomes are similar or better results to employing solely chemical fertilizers (GERMER et al., 2011; GIRIJA et al., 2019; LIU et al., 2011). Therefore, the lettuce cultivated in the present study could probably have shown a growth comparable to the T2 if the other nutrient deficits were met. Nevertheless, this study aimed to evaluate plant development without adding chemical fertilizers in soil with low nutrients concentration.

Another important consideration is that chemical fertilizers have the nutrients readily available for plant uptake, while organic fertilizers have a large parcel of nutrients organically bound to food/waste, which become available slowly through mineralization (MNKENI and AUSTIN, 2009; MOYA et al., 2019; NABEL et al., 2014). This is the case for the use of human feces to grow cabbage, where only higher doses of the biofertilizer led to a significant increase in yield (MNKENI and AUSTIN, 2009). Also, soil tests at the end of another experiment showed high C content, indicating that some portion of N could be organically bound (NABEL et al., 2014). However, N and P present in urine are already available for plant uptake (KIRCHMANN and PETTERSSON, 1995; VINNERÅS, 2002). Therefore, applying a mixture of feces and urine led to different N uptake by spinach, with the highest N in the treatment using the highest proportion of urine to feces (KUTU et al., 2011). Hence, probably sequential cultivation employing organic fertilizers made from human feces or applying some time before planting the seedlings could enhance nutrient mineralization and improve nutrient availability.

Regarding stored urine, the results showed the lowest plant height and leaf number of all biofertilizers applied. Even though nutrients are already

Figure 3 - Picture from the 34th day of the experiment. Treatments: (a) no fertilizer, (b) chemical fertilizer, (c) urea-treated feces, (d) composted feces, (e) stored urine, and (f) struvite.
available, N is in form of ammonia, which is highly volatile (HARDER et al., 2019; SENECAL and VINNERÅS, 2017; UDERT et al., 2006). In this regard, Botto et al. (2018) applied a volume 25% higher to compensate for ammonia volatilization. However, in the present study, the urine mass used was the exact amount needed by the plant based on the N content. Also, soil proprieties such as pH influence biogeochemical processes, impacting nutrient availability and leaching (NEINA, 2019). Therefore, the low result, comparable to the no-fertilizer treatment, possibly indicates an N loss or a problem in nutrient availability for plant uptake.

During the weekly measurement of plant height and leaf number, the control and stored urine treatments showed the lowest values, significantly different from the other applied treatments. These differences are probably due to the lack of nutrients available for crop development. For the treatment with stored urine, this lack may have been due to N losses by volatilization and leaching since the fertilization was calculated to fully meet the demand for N. Urea-treated feces, co-composted feces, and struvite treatments showed the same results for both variables, showing better results than the negative control. For the number of leaves, struvite was the only biofertilizer that presented an effect comparable to the chemical fertilizer, while for height, no biofertilizer was equivalent to the chemical fertilizer. This behavior can be explained by the imbalance of N, P, and K nutrients in biofertilizers, compared to chemical fertilizers, which supplied 100% of the demand for these nutrients recommended by Comissão de Química e Fertilidade do Solo (2016).

4. CONCLUSIONS

Biofertilizers improved the lettuce plant’s development compared to no-fertilizer, especially struvite. Even though the soil was considered poor in nutrients at the beginning of the experiment, the biofertilizers urea-treated feces, composted feces, and struvite were able to promote better plant development than the negative control. Therefore, excreta-based biofertilizers can provide nutrients for plant growth even in soils with low nutrient content. However, the stored urine did not exhibit the same result, as there was no significant difference from the negative control. Hence, we recommend applying a higher quantity or better application to compensate for or avoid nitrogen volatilization losses. Sectioning the application in more than three episodes could also help improve the plants’ nutrient availability.

We recommend investigating the combination of biofertilizers to fully supply the demand of N, P, and K and to better compare to chemical fertilizers. Finally, biofertilizers present the potential to complement chemical fertilizers and, therefore, diminish the use of non-renewable minerals and promoting the recovery of resources.

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