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Research paper

# Toxicity standards of biosolid leachate

## *Padrões de toxicidade do lixiviado de biossólido*

Rafael Lucas Alexandre[1]; Caroline Menezes Manzatto[1]; Emilly Giacobbo[1]; Robert Willian Florentino[1]; Kelly Geronazzo Martins[2]

[1]Master's student in Sanitary and Environmental Engineering, State University of the Center-West, Irati, Paraná, Brazil.
Orcid: 0009-0008-2540-3224. E-mail: rafael.lucas.alexandre@gmail.com;
Orcid: 0009-0008-4669-2758. E-mail: carolmmanzatto@gmail.com;
Orcid: 0009-0000-3166-9530. E-mail: emillygiacobbo26@gmail.com;
Orcid:0009-0004-3261-8785. E-mail: robertwilliam1517@gmail.com.
[2]Professor of the Environmental Engineering Undergraduate Course, State University of the Center-West, Irati, Paraná, Brazil. Orcid: 0000-0002-0447-4444. E-mail: kellygm77@gmail.com.

**ABSTRACT**: Sewage treatment in Brazilian cities generates biosolids that can be useful in the agricultural field, although they pose contamination risks to the environment and to human health. Ecotoxicity tests are essential to ensure the safety of such a use, since they assess biosolids' toxicity in the soil and in water. The aim of the current study is to assess biosolids leachate phytotoxicity by using lettuce seeds. In order to do so, Petri dishes were used to incubate the seeds, which received the biosolids leachate and remained under controlled temperature conditions, in the dark, for 168 hours. Overall, the herein assessed parameters have evidenced that the biosolids leachate diluted up to 60% had positive effect on (i.e., it stimulated) seedling growth and germination. However, phytotoxic effect was observed when it was applied without any dilution, since it reduced seed germination and growth parameters. Therefore, the lower the leachate concentration, the higher the *Lactuca sativa* root growth, a fact that highlights the phytotoxic effect of this leachate.

**Keywords**: Phytotoxicity; *Lactuca sativa*; sewage sludge.

***RESUMO:*** *O tratamento de esgoto nas cidades brasileiras gera biossólidos que podem ser úteis na agricultura, mas contêm riscos de contaminação ambiental e à saúde. Testes de ecotoxicidade são cruciais para garantir a segurança desse uso, avaliando a toxicidade do biossólido no solo e na água. Diante do exposto, o presente estudo teve por objetivo avaliar a fitotoxicidade de lixiviado de biossólido, utilizando sementes de alface. Para isso, foram utilizadas placas de Petri para a incubação das sementes, que receberam o lixiviado do biossólido e permaneceram em temperatura controlada e ausência de luz durante 168 horas. De forma geral, os parâmetros avaliados, demostraram que o lixiviado de biossólido quando diluído até 60% apresenta efeito positivo, ou seja, estimula o crescimento das plântulas bem como a germinação, entretanto, quando aplicado sem qualquer diluição, é observado efeito fitotóxico, reduzindo a germinação das sementes e os parâmetros de crescimento. Nesse contexto, conclui-se que quanto menor a concentração do lixiviado, maior o crescimento das raízes da Lactuca sativa, o que demostra efeito fitotóxico do lixiviado.*

***Palavras-chave****: Fitotoxicidade; Lactuca sativa; lodo de esgoto.*

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## INTRODUCTION

Urban centers have received increasing numbers of new inhabitants throughout history and the need of implementing waste treatment systems has also increased together with the development generated by these populations. According to the Panorama of Basic Sanitation in Brazil, the country has approximately 362,400 kilometers of sewage collection networks, and they assist 55% of the country's population, which comprises approximately 214 million inhabitants. Therefore, Brazil collects sanitary sewage from approximately 117 million people (Brasil, 2021).

The collected sewage is sent to sewage treatment plants (STPs) where it undergoes several treatment levels, namely: preliminary level, which accounts for removing coarse solids; primary level, which removes suspended solids and BOD from the sewage; and secondary level, which accounts for the soluble BOD removal process. Some WWTPs also apply tertiary treatment to remove nutrients, pathogenic organisms, non-biodegradable compounds, heavy metals, as well as remaining suspended solids and inorganic materials (Sperling, 2018).

Biosolids are the waste resulting from sewage treatment. Improperly handled biosolids can lead to major environmental damage. On the other hand, this by-product can be incorporated to agricultural soil due to the large amount of nutrients found in its composition. In addition to help improving soil chemical, physical and biological aspects, this incorporation can generate savings in soil fertilization for farmers (Polleti *et al*., 2017). Anjos and Mattizzo (2000) applied biosolids leachate to the soil and observed that constant applications of it can increase the nitric nitrogen content in the leachate of soil treated with biosolids.

However, sludge also carries toxic elements and pathogenic organisms. The literature review by Alonso (2018) presented concepts concerning the production of biosolids deriving from sewage sludge, as well as its featuring, chemical and microbiological conditions, its association with organic micropollutants and its likely applicability for forest seedlings' growing purposes. The aforementioned author addressed the issue associated with the presence of heavy metals, pathogens and bacteria, as well as with organic compounds, such as medicines, cleaning products and pesticides in biosolids, since these elements can turn the use of biosolids into a public and environmental health issue due to their contamination factor.

Groundwater contamination depends on several factors capable of changing qualitative water features, such as atmospheric deposition, dissolution and/or hydrolysis processes in the aquifer, as well as its mixing with sewage or saline water. Groundwater contamination is associated with domestic and industrial discharges, with slurry deriving from landfills and with incorrect organic waste application in the soil. These practices contaminate water due to the release and mobilization of pathogenic organisms, anions, mainly the nitrogenous ones, and metals found in the soil (Freitas; Brilhante; Almeida, 2001). In addition, nitrate leaching, which is one of the biggest issues associated with biosolids leachate, has two adverse health effects, namely: (1) methemoglobinemia development, which mainly affects children by causing the 'blue baby' syndrome; and (2) potential formation of carcinogenic nitrosamines and nitrosamides (Feng; Wang; Feng, 2005; Bouchard *et al*., 1992).

Conama Resolution n. 498/2020 sets the criteria and procedures for biosolids' production and application in the soil. Article 7 provides biosolids, from their generation to their application, which must be in harmony with the environment based on quality

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monitoring (Brasil, 2020). Therefore, it is essential assessing biosolids' features to dispose it in a way not to harm the environment and human health. Some biosolids' disposal options comprise landfills, using them in agricultural areas, their incineration and recovery of degraded areas (Bettiol; Camargo, 2006).

Thus, it is paramount carrying out ecotoxicity tests to check this waste's toxicity level in the soil, since, in combination to physicochemical analyses, these tests can contribute to soil and water conservation processes (Michelan *et al*., 2021; Polleti *et al*., 2017).

Seeds can be used in toxicological tests applied to biosolids given their low cost and easy analysis. This assessment can be based on germination index data, relative growth index and radicle elongation index (Mendes *et al*., 2020). Lettuce (*Lactuca sativa*) provides one of the seeds mostly used in toxicity tests, since it is easy to assess and its results come out in a short period-of-time. Root and stem growth, as well as number of germinated seeds, were assessed after seed germination and pollutant application. Overall, the lower the root growth, the stronger the pollutant's toxic effect (Mendes *et al*., 2020; Barbosa *et al.*, 2022).

The current study was conducted on bench scale to assess the phytotoxicity of biosolids leachate deriving from a waste treatment plant, based on using lettuce seeds as indicators to analyze plant germination and growth rates.

## MATERIALS AND METHODS

The study was carried out in the Chemistry and Ecotoxicology Laboratories of the Environmental Engineering Department, at Midwestern State University, Irati Campus/Paraná State. The experiment used 21 sterilized Petri dishes covered with filter paper, waste and seeds. The herein used biosolids were provided by Rio das Antas WWTP, in Irati County – PR, where the sludge (which mainly derived from domestic sewage) was subjected to liming process in 2022 (based on using 35% virgin lime) and to stabilization process for three months. Biosolids' physical-chemical and microbiological features were in compliance with CONAMA Resolution n. 498 (Brasil, 2020).

Approximately 100g of treated sludge was inserted in a glass funnel suspended by universal support and, then, 200 ml of distilled water was gradually added to the biosolids sample to get the leachate, which was placed in a beaker.

The produced leachate was collected and diluted in deionized water at the following concentrations: 60%, 70%, 80%, 90% and 100% (raw leachate).

### Phytotoxicity test

Zinc sulfate solutions were used as positive control (+) to assess seeds' sensitivity to a known toxic compound, whereas distilled water was used as negative control (-) to check the batch's health.

Experiments conducted with control samples were carried out by following the same pattern adopted in tests conducted with test solutions.

*Lactuca sativa* (commonly known as white lettuce) seeds deriving from organic crops were herein used. The experiment was carried out in triplicate, based on using 10 seeds per Petri dish (210 seeds, in total).

Petri dishes were previously cleaned and dried, and covered with qualitative filter paper (9 cm, in diameter), which was moistened with leachate dilutions. All 10 seeds were evenly distributed on each plate based on using tweezers. The Petri dishes were wrapped in parafilm to help mitigating leachate evaporation.

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After the experiments were prepared, the plates were kept in incubator at constant temperature of 22 °C ± 2°C, for 168 hours, in the dark, for growth analysis purposes.

Seeds germinated in each plate were counted after 7-day incubation and the root and stem length of each seedling was measured. This stage was carried out in laboratory environment based on using tweezers and millimeter ruler.

The germination count and the length of the lettuce seedlings' stems and radicles were used as basis for calculations carried out through the following formulas:

**Relative Growth Index "Equation** (1)

$$RGI = \frac{MSG}{MG_{NC}} \quad (1)$$

Wherein:
RGI = Relative Growth Index;
MSG = Mean Sample Growth, in centimeters;
MGnc = Mean Growth of the Negative Control, in centimeters.

**Radicle elongation index "Equation (2)"**

$$REI = \frac{MREs}{MREnc} \quad (2)$$

Wherein:
REI = Root Elongation Index;
MREs = Mean Radicle Elongation in the Sample, in centimeters;
MREnc = Mean Radicle Elongation in the Negative Control, in centimeters.

REI values were classified into three categories, based on the observed toxic effects:

- Radicle elongation inhibition (I): $0 < REI > 0.8$;
- No significant effect (NES): $0.8 \leq REI \geq 1.2$;
- Radicle elongation boosting (E): $REI > 1.2$.

**Germination index "Equation (3)"**

$$GI = \left(\frac{RGI_S * GS}{GS_{nc}}\right) * 100 \quad (3)$$

Wherein:
GI = Germination Index;
RGIs = Relative Growth Index in the sample;
GS = Number of Germinated Seeds;
GSnc = Number of Germinated Seeds in the Negative Control.

**Data analysis**

The experiment has followed a completely randomized design, with three repetitions per treatment. Data were subjected to one-factor analysis of variance (dilution and controls) and differences between means were checked through Tukey test, at 5% significance level.

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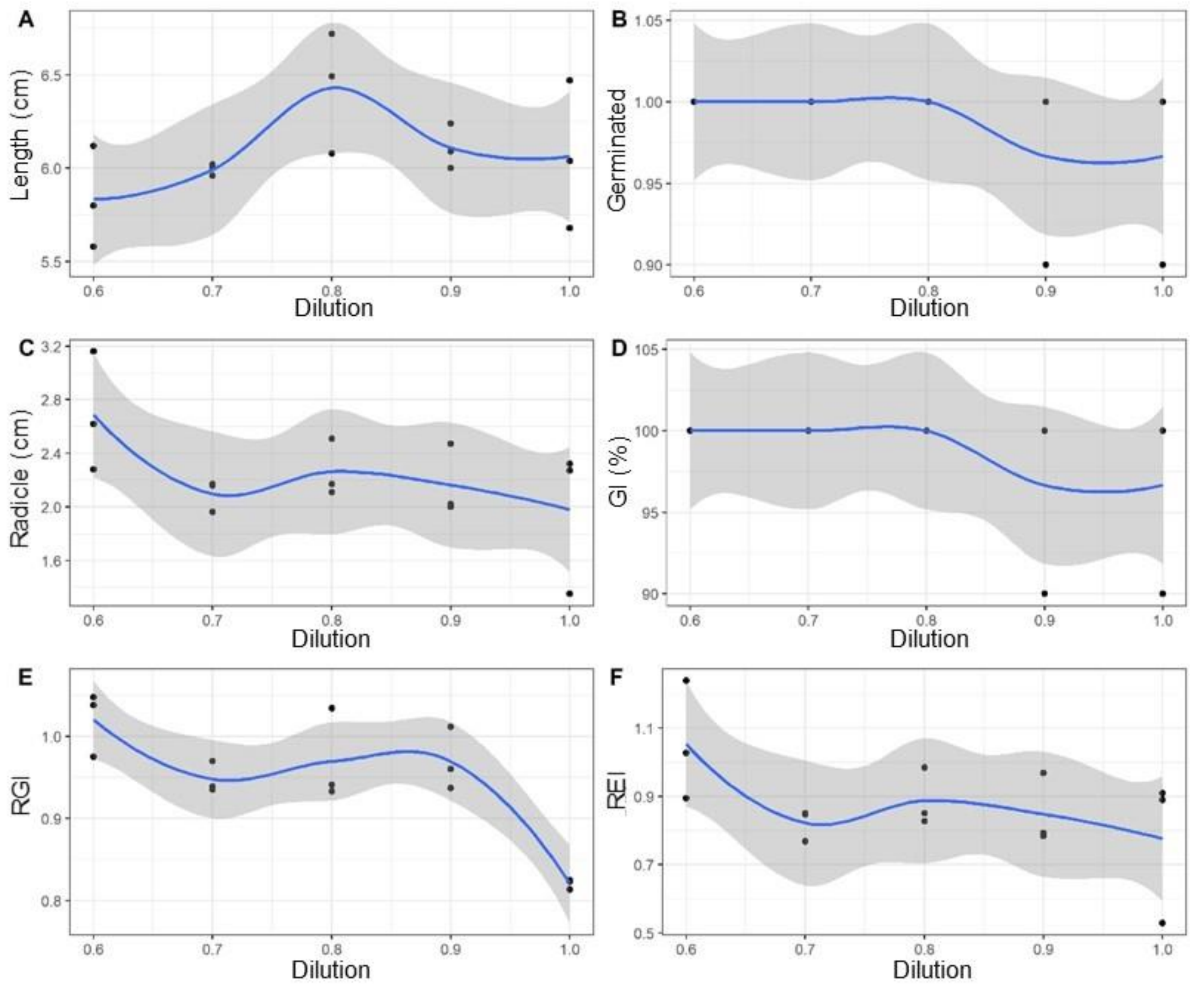
Homogeneity and Gaussianity assumptions were checked through Bartlett and Shapiro-Wilks tests, respectively, at 95% confidence level.

## RESULTS AND DISCUSSIONS

At least 90% of seeds have germinated ion all plates, regardless of whether they were covered with diluted or non-diluted biosolids leachate. This finding has evidenced the leachate's low phytotoxicity level. Steffler *et al*. (2018) also found similar results when they used biosolids deriving from the same WWTP in their research. According to the aforementioned authors, biosolids' doses up to 25 $t.ha^{-1}$ did not significantly increase nitrate concentrations in the percolate. On the other hand, doses higher than 80 $t.ha^{-1}$ should not be applied because they would lead to harmful nitrate concentrations in groundwater. In other words, similar to the current findings, the aforementioned authors observed that high biosolids doses had negative effects.

**Figure 1** - Total length, germination, radicle growth, GI, RGI and RAI values



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The other investigated variables have evidenced the significant effect of the adopted dilutions, with emphasis on the raw leachate, which resulted in lower radicle growth, total plant length, root elongation and growth index values. The herein adopted dilutions had positive influence on lettuce germination.

Based on **Figure 1**, biosolids leachate doses up to 80% have boosted total lettuce seedlings' length, but values recorded for this parameter have gradually decreased at higher doses of it. Seed germination remained constant at leachate doses up to 80% - all seeds have germinated. At least one seed has failed to germinate at leachate doses of 90% to 100%, and it has evidenced that high doses of it have toxic effect on lettuce seeds.

The leachate dose at 60% had the most stimulating effect on the relative growth index, whereas the other doses of it had negative effect on this parameter, as shown in **Table 1**.

**Table 1 -** Average values for relative growth index (RGI), radicle elongation index (REI) and germination index (GI) according to each concentration of biosolid leachate

| Concentration of sludge leachate Sewage Treatment Station (%) | RGI (cm) | REI (cm) |
|---|---|---|
| 60 | 1.02 | 1.05 |
| 70 | 0.95 | 0.82 |
| 80 | 0.97 | 0.89 |
| 90 | 0.97 | 0.85 |
| 100 | 0.92 | 0.78 |
| Control (-) | 1.00 | 1.00 |
| Control (+) | 0.80 | 0.73 |

Based on **Table 2**, leachate doses of 60%, 70%, 80%, 90% and the Negative Control did not have significant effect on seed root elongation index, since their values ranges from 0.8 to 1.2. On the other hand, both the 100% leachate dose and the Positive Control have inhibited root elongation, whose value was lower than 0.8.

Yet, based on **Table 2**, variables presenting the same letter did not show statistically significant difference from each other, whereas variables presenting different letters have shown statistically significant difference from each other.

**Table 2 -** Comparison of statistical equality between dilutions.

| Dilution | Length (cm) | Germination (%) | RL (cm) | SE (cm) | REI | GI (%) | RGI |
|---|---|---|---|---|---|---|---|
| control + | 4.19 b | 0.97 a | 1.86 a | 0.73 a | 0.73 a | 96.67 a | 0.80 b |
| control - | 5.25 a | 1.00 a | 2.55 a | 1.00 a | 1.00 a | 100 a | 1.00 a |
| 100% | 4.31 b | 1.00 a | 1.98 a | 0.78 a | 0.78 a | 100 a | 0.82 b |
| 90% | 5.09 a | 1.00 a | 2.16 a | 0.85 a | 0.85 a | 100 a | 0.97 a |
| 80% | 5.09 a | 1.00 a | 2.26 a | 0.89 a | 0.89 a | 100 a | 0.97 a |
| 70% | 4.98 a | 1.00 a | 2.10 a | 0.82 a | 0.82 a | 100 a | 0.95 a |
| 60% | 5.36 a | 1.00 a | 2.69 a | 1.05 a | 1.05 a | 100 a | 1.02 a |

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Therefore, one can conclude that the negative control and the 90%, 80%, 70% and 60% dilutions did not statistically differ from each other, and that they were significantly different from the positive control and from the raw leachate, which were statistically equal to each other.

Polleti *et al*. (2017) conducted a similar experiment with lettuce seeds and observed significantly different root growth among plants subjected to different treatment types the seeds were subjected to.

## CONCLUSIONS

Although there were no statistically significant differences in the herein assessed parameters between dilutions, it was clear that lettuce seedlings' growth and leachate concentration presented inversely proportional correlation, i.e., the lower the leachate concentration, the higher the growth of *Lactuca sativa* roots – this finding highlights the phytotoxic effect of the investigated leachate.

Overall, the 60% dilution was the least harmful to lettuce seeds, although there were no statistically significant differences in parameters, such as length and ICR, among the 60%, 70%, 80% and 90% dilutions. This finding means that these dilutions had no toxic effect on lettuce seedlings' growth.

On the other hand, both the positive control and the 100% dilution had toxic effect on this parameter, whereas none of the dilutions or the control has shown statistically significant differences in the other assessed parameters.

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