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SHORT COMMUNICATION / COMUNICACIÓN BREVE

EFFECT OF VERMISTABILIZED SEWAGE SLUDGE ON THE GERMINATION AND GROWTH OF *Schinus molle* AND *Cedrela odorata*

Efecto del lodo residual vermiestabilizado sobre la germinación y crecimiento de *Schinus molle* y *Cedrela odorata*

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Key words: biosolid, wastewater, humic extract, seeds, forest species.

ABSTRACT

The purpose of this study is to examine the effect of vermicompost derived from biosolids produced from wastewater treatment (named here as "biosolid humus") on the germination and growth of the forest species *Schinus molle* and *Cedrela odorata* under greenhouse conditions. We applied four treatments, consisting of a mixture of biosolid humus and substrate, in proportions of 0 (control), 5, 10, and 20%. There were no significant differences in the species' germination, which is considered to be the production of the first pair of true leaves. The germination percentage value tended to be higher for *S. molle* than *C. odorata* species under the 5 and 10% treatments. This result indicates that biosolid humus favors the germination process. The effect of biosolid humus resulted in more significant growth of *C. odorata* than *S. molle*. Both species showed differential stem length and number of leaves responses to the treatment using 20% biosolid humus, significantly lower for *C. odorata*. However, the opposite was true for germination parameters, resulting in a stimulating effect on *S. mole.* This study indicates that biosolid humus affects the germination and growth of the two species. Before incorporating this fertilization into regular seedling and reforestation processes, further studies are necessary to determine the optimal dosage levels of biosolid humus and prevent potential salinity effects.

Palabras clave: biosólido, aguas residuales, extracto húmico, semillas, especies forestales.

RESUMEN

El propósito de este estudio fue examinar el efecto del vermicompostaje obtenido a partir de biosólidos de una planta de tratamiento de aguas (denominado en este artículo "humus de biosólido") sobre la germinación y crecimiento de las especies forestales *Schinus molle* y *Cedrela odorata* en condiciones de invernadero. Se aplicaron cuatro tratamientos, consistentes en una mezcla de humus de biosólido y sustrato, en proporciones de 0 (control), 5, 10 y 20 %. No hubo diferencias significativas en la germinación de las especies, considerada como la producción del primer par de hojas verdaderas. El valor del porcentaje de germinación fue mayor para *S. molle* que para las especies de *C. odorata* con tratamientos de 5 y 10 %. Este resultado indica que el humus de biosólido favorece el proceso de germinación. El efecto del humus de biosólido resultó más significativo sobre el crecimiento de *C. odorata* que de *S. molle*. Ambas especies mostraron respuestas diferenciales al tratamiento utilizando 20 % de humus de biosólido para variables como longitud del tallo y número de hojas, significativamente menores para *C. odorata.* Sin embargo, ocurrió lo contrario para los parámetros de germinación, resultando en un efecto estimulante sobre *S. molle*. Este estudio indica que el humus de biosólido afecta la germinación y el crecimiento de las dos especies, pero son necesarios más estudios sobre sus niveles de dosificación antes de incorporar este tipo de fertilización en el proceso regular de producción de plántulas y reforestación.

INTRODUCTION

Various methods are employed to minimize environmental impact, ensure sustainability, and promote the reuse, recycling, and recovery of organic waste from industrial and urban development (Collivignarelli et al. 2019). Among these methods, a common approach implemented in wastewater treatment plants (WWTP) combines physical, chemical, and microbiological processes to remove solid and liquid pollutants (Agrawal et al. 2020). However, conventional practices such as incineration or landfill disposal of resulting sewage sludge often contribute to the expansion of contaminated areas (Rorat et al. 2019).

Recently, alternative treatments for WWTP sludge, such as aerobic or anaerobic digestion, alkaline stabilization, thermal drying, acid oxidation/disinfection, and composting, have gained preference (US-EPA 2019). These methods aim to produce biosolids—organic materials rich in macronutrients, such as nitrogen and phosphorus. Biosolids must undergo appropriate stabilization and processing to be suitable for use as fertilizers and soil conditioners to improve low-fertility soils and restore degraded lands (Collivignarelli et al. 2019).

An emerging method for sludge stabilization involves using earthworms, known as vermistabilization. This biological process transforms sludge into high-value organic microbial fertilizer (vermicompost) through the joint action of earthworms and microorganisms. However, it requires the sludge to be dehydrated to facilitate the passage of earthworms (Sinha et al. 2010, Edwards and Arancon 2022).

The utilization of biosolids in agriculture and engineering is becoming increasingly common (Collivignarelli et al. 2019, Eurostat 2019) due to their ability to enhance soil nutrient availability, soil texture, and water retention capacity (Donoso et al., 2016). Notably, incorporating biosolids into soils of deforested areas, which are often depleted of nutrients and organic matter, helps mitigate environmental stress that inhibits plant growth (Xue et al. 2015, Castán et al. 2016).

While this practice also promotes the germination and growth of some forest species, its success depends on factors such as the source and application method of biosolids, as well as the age of the plants (Xue et al. 2015, Pérez-Piqueres et al. 2018). Consequently, biosolid humus benefits forest restoration (Campoe et al. 2014).

The natural forest area in Colombia is dwindling annually due to agricultural expansion, livestock production, and illegal logging (Minagricultura 2021). Strategies for species conservation, promoting reforestation, and conducting tree-planting campaigns have been initiated (Córdoba et al. 2019). Clearly, these endeavors will require a substantial number of plants, and utilizing biosolids reduces the environmental impact and enhances the survival prospects of trees during reforestation efforts.

Therefore, this study aims to assess the impact of biosolid humus produced at the WWTP of the Universidad Militar Nueva Granada (UMNG) on the germination and growth of two forest species, *Cedrela odorata* and *Schinus molle*. The former is a native timber species found in tropical and subtropical America, facing a threatened status (Calixto et al. 2015, UEIA-USFS 2018), while the latter is a woody tree native to the central Andes; it thrives in subtropical and tropical regions of South America and is utilized for medicinal, culinary, and urban reforestation purposes (Ramos-Montaño 2020, UEIA-USFS 2018).

MATERIALS AND METHODS

Study area

The experiment took place at the Nueva Granada campus of UMNG in Cajicá, Colombia (4º 56' 33" N, 74º 00' 45" W), situated at an elevation of 2670 masl.

Production of vermicompost from the WWTP of Nueva Granada campus

Sewage sludge from the WWTP of Nueva Granada campus was collected and air-dried before being incorporated into beds at the vermicomposting facility. *Eisenia foetida* earthworms were utilized at a rate of 3.5 kg per square meter. The beds, measuring 2 × 1 × 0.5 m, were monitored daily to maintain pH levels between 5 and 8, temperatures between 16 and 25 ºC, and humidity between 25 and 60%. The conversion process into vermicompost typically takes approximately four months (Silva-Leal et al. 2016).

After completion, a sample of biosolid humus was collected for content analysis at an analytical chemistry facility. The composition, cationic relations, and heavy metal content of this biosolid humus are detailed in **table I**.

Germination experiments

Seeds of both species came from a commercial provider. The labels indicated a harvesting period of less than a year, a germination percentage of 60%, and seed origin from natural forests. An in vitro germination test verified the actual germination percentage, resulting in lower percentages than indicated on the labels (30% for *S. molle* and 37% for *C. odorata*).

The substrate consisted of a mixture of natural soil (60%), burnt rice husk (20%), and crude rice husk

TABLE I. CHEMICAL SOIL ANALYSIS.

Variable	Unit	Result				
		Biosolid humus	Control	TTO5	TTO10	TTO20
pH (Acidity reaction)	–logH+	5.70	4.80	5.10	4.90	5.00
Electrical Conductivity	dS/m	6.40	1.10	2.00	1.90	2.80
Major elements						
Ammoniacal Nitrogen (NH_4)	%	0.035	0.006	0.015	0.021	0.013
Nitric Nitrogen (NO_3)	%	0.05	0.015	0.021	0.018	0.029
Nitrogen (N)	%	0.91	1.02	0.46	0.36	0.50
Phosphorus (P)	%	0.70	0.12	0.20	0.21	0.25
Potassium (K)	%	0.23	0.09	0.15	0.14	0.12
Calcium (Ca)	%	2.10	0.16	0.33	0.29	0.24
Magnesium (Mg)	%	0.31	0.22	0.17	0.17	0.17
Sodium (Na)	%	0.064	0.070	0.081	0.091	0.081
Sulfur (S)	%	0.62	0.08	0.08	0.12	0.13
Sample Parameters						
Water percent	%	19.8	27.14	24.46	26.22	24.20
Carbon:nitrogen relation	p:p	10.3	11.06	22.54	28.29	18.83
Organic carbon	%	9.4	11.28	10.30	10.23	9.39
Organic material	%	20.4	24.47	22.35	22.19	20.38
Heavy metals						
Cd (8 mg/kg)	mg/kg	2.5	0.4	0.6	0.6	0.7
Pb (300 mg/kg)	mg/kg	32.7	14.0	15.3	16.3	15.4
Cr (1000 mg/kg)	mg/kg	37.7	12.7	14.0	14.8	13.8
Minor elements						
Iron (Fe)	ppm	12 129	12 660.9	15 796.0	10 242.1	9741.8
Manganese (Mn)	ppm	284	157.4	108.0	106.2	96.3
Copper (Cu)	ppm	19.1	4.2	5.8	5.1	7.6
Zinc (Zn)	ppm	457	11.0	33.2	30.4	59.8
Boron (B)	ppm	43.5	7.6	9.5	7.2	11.3

The maximum heavy metal content allowed by Decree 1287-2014 (Minvivienda 2014) are given in parenthesis. Control: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment, TTO20: 20% biosolid humus treatment.

(20%), which was disinfected using the fumigant Basamid before use. Basamid granules (35 g/m^2) were incorporated into the substrate at 10 cm from the surface with a soil moisture content of 30%. The area was covered with plastic to prevent gas emissions. After one month, a soil quality control test was conducted at a biological laboratory to certify the absence of pathogens (Rippa et al. 2023).

Four treatments were established by mixing substrate and biosolid humus. The final proportions of biosolid humus were 0% (control), 5% (TTO5), 10% (TTO10), and 20% (TTO20). A randomized block design with two repetitions for each treatment was employed, resulting in 90 plants per treatment. The mixtures were dispensed into germination trays, with one seed sown directly into each cell. The germination trays were maintained under greenhouse conditions (Gutiérrez-Ginés et al. 2023, Onchoke and Fateru 2024).

Germination, seedling emergence, and other germination parameters were recorded daily for a period of two months. The following equations were used to calculate different germination parameters:

Equation 1 calculates the germination percentage (Kader 2005, ISTA 2023):

$$Germination\ Percentage\ (GP) = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds} \times 100 \quad (1)$$

Equation 2 calculates the velocity of germination (Jones and Sanders 1987):

$$Coefficient\ of\ Velocity\ of\ Germination\ (CVG) = \frac{\sum_{i=1}^{k} Ni}{\sum_{i=1}^{k} NiTi} \times 100 \quad (2)$$

where Ni corresponds to the number of seeds germinated every day and Ti is the number of days from sowing corresponding to

Equation 3 calculates the time spread of germination, which is the difference between the time of the last germination (T_n) and the time for the first germination (T_i) (Kader 2005):

$$Time\ Spread\ of\ Germination\ (TSG) = T_n - T_i \quad (3)$$

The higher the *TSG* value, the more significant the difference in germination speed between the "fast" and "slow" germinating members of a seed lot.

Equation 4 calculates the germination index (Benech et al. 1991):

$$Germination\ Index\ (GI) = (10 \times n1) + (9 \times n2) + \ldots + (1 \times n10) \quad (4)$$

Where $n1$, $n2$ . . . $n10$ are the number of germinated seeds on the first, second, and subsequent days until the 10th day, while 10, 9... and 1 are weights given to the number of germinated seeds on the first, second, and subsequent days.

Growth and development

The four treatments used to evaluate germination were also employed to monitor growth and development using a randomized block design. Stem length and the number of leaves were measured weekly for 13 weeks to observe various developmental stages, from seedling emergence to true leaf production and the first stem bifurcation. The relative chlorophyll content was also determined using a portable chlorophyll meter (SPAD-502) when leaf size permitted.

Statistical analysis

Data were analyzed using the InfoStat statistical package. Analysis of variance (Anova) was performed at the $p \leq 0.05$ confidence level for data meeting the required assumptions. For growth and development data, the Kruskal-Wallis test was employed, and the means of these data were compared using non-parametric Tukey and Dunnett tests ($p \leq 0.05$). The R-project statistical program and box plot diagrams were used to illustrate the relationship between treatments.

RESULTS

Influence of biosolid humus on the germination

Figures 1 and **2**, along with **table II**, present each species' germination percentage, speed, propagation time, and index. *S. mole* experienced a stimulating or neutral effect in some cases, such as the coefficient of velocity of germination (CVG), for most of the parameters evaluated. However, an adverse effect was observed in plants subjected to TTO20. The treatment exhibited a retardant effect on the assessed parameters (**Figs. 1a** and **2**; **Table II**). Conversely, *C. odorata* displayed a different behavior, experiencing a retardant effect on the germination percentage (GP) and the germination index (GI) with all treatments and no apparent effect on the other germination parameters assessed (**Fig. 1b** and **Table II**).

Influence of biosolid humus on growth

Figure 3 illustrates the behavior of the three selected variables used to estimate plant growth: stem length, leaf count, and chlorophyll content. Remarkably, the treatment amended with 20% vermistabilized biosolids demonstrated a general

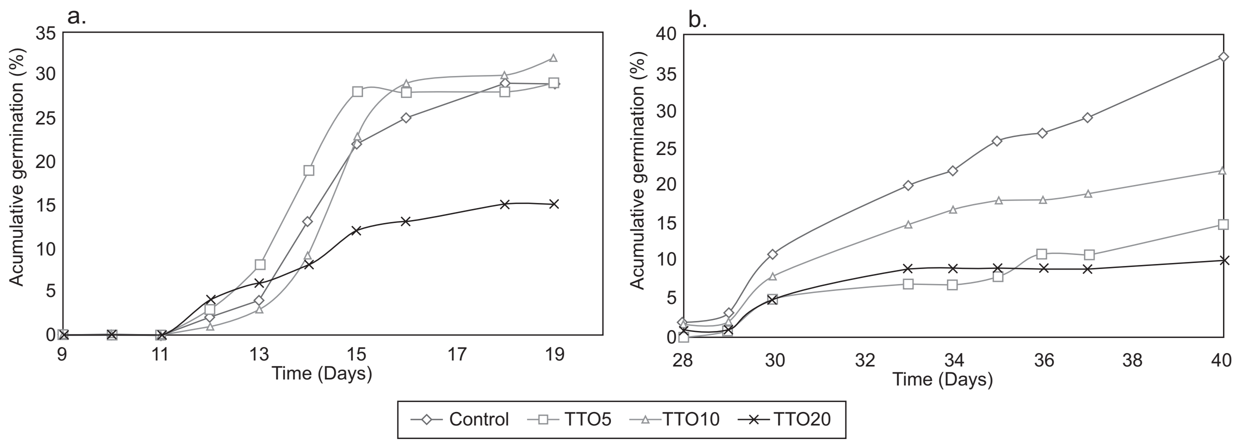

Fig. 1. Cumulative germination curve in substrates amended with three biosolid humus contents (a) *S. molle*, (b) *C. odorata*. Control: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment; TTO20: 20% biosolid humus treatment.

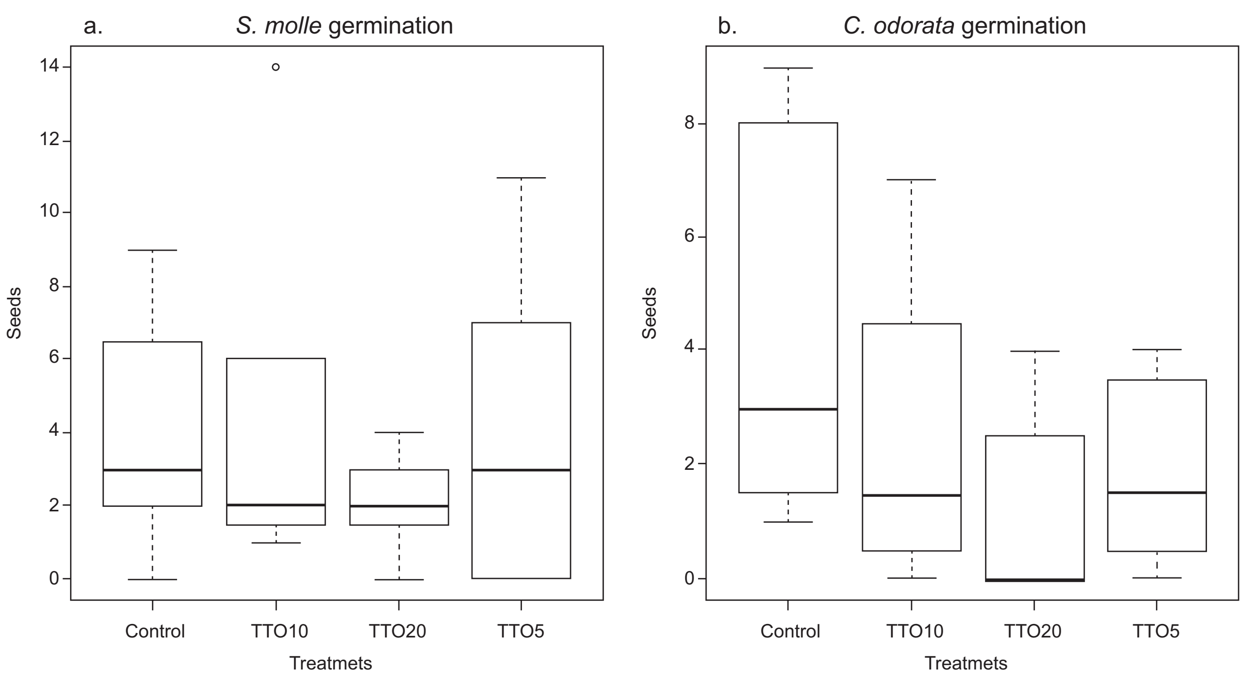

Fig. 2. Distribution of the germinated seeds in substrates amended with three biosolid humus contents: (a) *S. molle* and (b) *C. odorata*. Control: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment, and TTO20: 20% biosolid humus treatment. The error bars indicate 95% confidence interval. The error bars indicate the 95% confidence interval; the lower and upper ends of the box represent the 25th and 75th percentiles, respectively; the line within the box represents the median, and outliers are represented as dots.

TABLE II. GERMINATION PARAMETERS UNDER DIFFERENT PROPORTIONS OF BIOSOLID HUMUS.

Parameters	*S. molle* seed				*C. odorata* seed			
	Control	TTO5	TTO10	TTO20	Control	TTO5	TTO10	TTO20
Germination percentage	32.2 ± 3.9	32.2 ± 4.9	35.6 ± 5.2	16.7 ± 1.6	38.9 ± 3.8	16.7 ± 1.8	22.2 ± 3.0	10.0 ± 2.0
Time spread of germination (day)	6 ± 2.16	7 ± 2.56	7 ± 2.56	6 ± 2.16	11 ± 3.6	11 ± 5.35	10 ± 3.62	10 ± 3.62
Coefficient of velocity of germination	6.73	7.09	6.61	7.01	2.91	2.90	2.98	3.08
Germination index	174.00	199.00	185.00	99.00	246.00	104.00	155.00	78.00

The F-value was evaluated to determine significant differences between treatments.
Control: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment, and TTO20: 20% biosolid humus treatment.

stimulatory effect on all variables for both species. However, the magnitude of this effect varied when considering each variable for each species individually.

For instance, while stem length was more stimulated in *S. molle* than in *C. odorata*, the effect on leaf count was greater for *C. odorata* than for *S. molle*. Additionally, although the impact on chlorophyll content was subtle for both species, it was still detectable.

DISCUSSION

The vermicomposting process plays a vital role in solid organic waste recovery, transforming compost into a valuable source of organic matter for soil enhancement, fertilization, or incorporation into crop substrates (Kiyasudeen et al. 2016, Huang et al. 2024). In vermistabilized biosolids, nutrient availability is enhanced through earthworm activity, which breaks down organic substrates, stimulates microbial activity, and accelerates mineralization rates (Kiyasudeen et al. 2016, Lei et al. 2024).

Consequently, vermicomposting products are anticipated to exhibit elevated nutrient concentrations and heavy metal content (Wang et al. 2024). Biosolids produced over four months using vermiculture at UMNG complied with "category A" criteria as per Decree 1287-2014 (Minvivienda 2014), indicating heavy metal levels below the permissible limits (**Table I**).

Germination curves illustrate the impact of biosolid humus on the germination process for both species (**Fig. 1**). The treatments showed no statistically significant differences in germination for either species. Although the reported germination rates under regular conditions for *S. molle* and *C. odorata* are 50% and 80%, respectively (Chan et al. 2012), our findings indicate an inhibitory effect on germination for both species.

Notably, *S. molle* exhibited its highest germination percentage under the TTO10 treatment (GP = 35.56 ± 5.21), while the lowest was observed at TTO20 (GP = 16.67 ± 1.63). Similarly, for *C. odorata*, the highest germination percentage was recorded under the TTO10 treatment (GP = 22.22 ± 2.97), with the lowest at TTO20 (GP = 10.00 ± 2.01). Both values were lower than the control (GP = 38.89 ± 3.80). Various dosages were included in the treatments based on previous studies, suggesting that low vermicomposting dosages favor germination (Ievinsh 2011, Azizi et al. 2024).

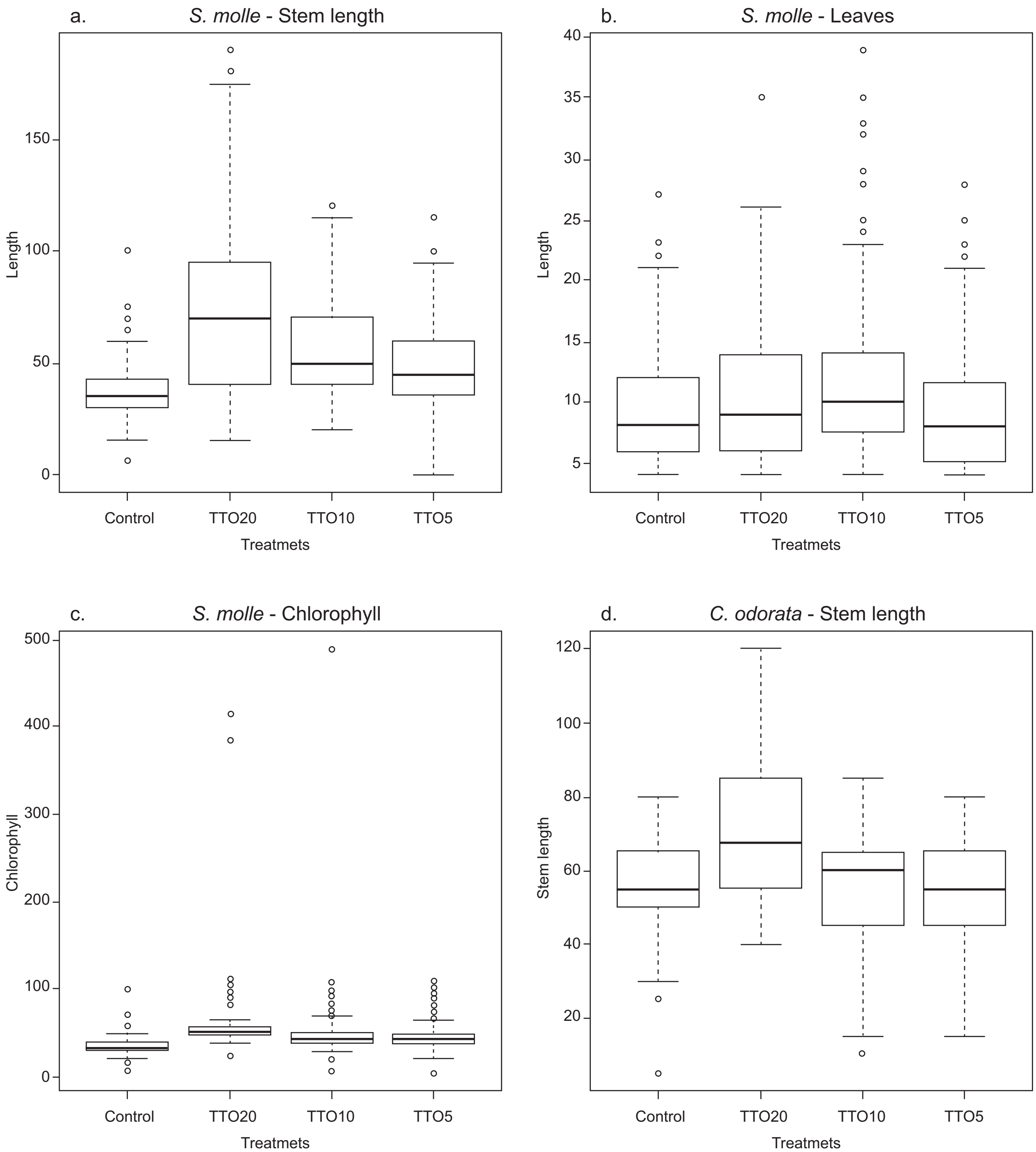

Fig. 3. Behavior of *S. molle* for (a) stem length, (b) number of leaves, (c) chlorophyll content (SPAD Index), and behavior of *C. odorata* for (d) stem length, (e) number of leaves, (f) chlorophyll content (SPAD Index). CONTROL: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment, TTO20: 20% biosolid humus treatment. The error bars indicate the 95% confidence interval; the lower and upper ends of the box represent the 25th and 75th percentiles, respectively; the line within the box represents the median, and outliers are represented as dots.

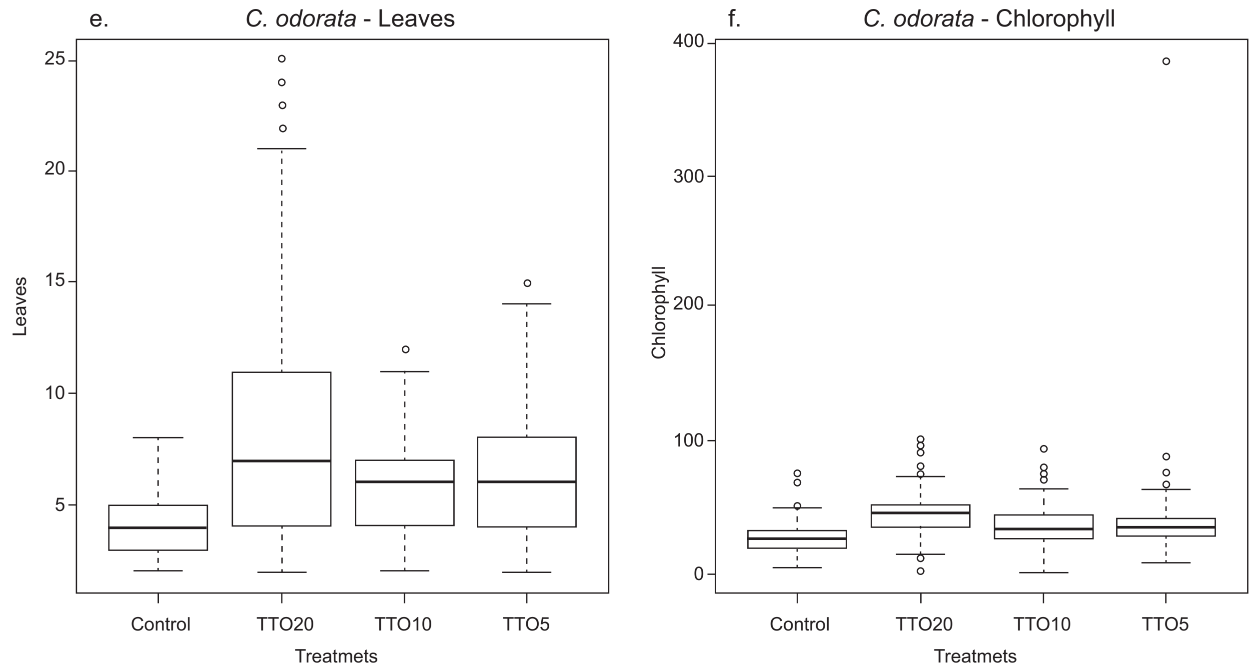

Fig. 3. Behavior of *S. molle* for (a) stem length, (b) number of leaves, (c) chlorophyll content (SPAD Index), and behavior of *C. odorata* for (d) stem length, (e) number of leaves, (f) chlorophyll content (SPAD Index). CONTROL: 0% biosolid humus treatment, TTO5: 5% biosolid humus treatment, TTO10: 10% biosolid humus treatment, TTO20: 20% biosolid humus treatment. The error bars indicate the 95% confidence interval; the lower and upper ends of the box represent the 25th and 75th percentiles, respectively; the line within the box represents the median, and outliers are represented as dots.

Luo et al. (2018) reviewed several characteristics of biosolid humus, including high conductivity, salinity, and low molecular weight of organic acids, and their influence on germination processes. They highlighted compost as a potential inhibitor of radicle emergence due to factors such as the lack of standardized methods for assessing compost toxicity.

Consequently, our results may be associated with the soil's electrical conductivity (EC), which was measured at 6.4 dS/m (**Table I**). This high value likely elevated the EC of the substrate mix, impacting biological processes, as higher proportions of biosolid humus in the substrate mixture correlated with lower germination percentages (**Figs. 1** and **2**).

In **figure 3**, the effects of vermistabilized biosolid on stem length (F-value = 11.35), number of leaves (F-value = 0.802), and chlorophyll content (F-value = 0.891) exhibited significant differences at the TTO20 treatment in both species (Blouin et al. 2019, Rehman et al. 2023).

These responses are likely attributed to earthworm activities, which promote mineralization and enhance nutrient availability, thereby contributing to the nutritional enrichment of biosolid humus in the soil and ultimately affecting growth and photosynthetic activity (Calixto et al. 2015, Shi et al. 2024). However, further studies are warranted to delve into these aspects comprehensively.

CONCLUSIONS

Our results validate the potential of sewage sludge stabilized with *Eisenia foetida* to stimulate the growth of *C. odorata* more effectively than *S. molle*. However, given the significance of these species as urban trees and their crucial role in ecosystem recovery, it is essential to highlight the differential responses observed during germination. Interestingly, we observed a stimulating effect on *S. molle*.

Therefore, while compost can help maintain optimal soil nutrition, its application should be approached cautiously, preferably post-germination for *C. odorata*. In contrast, *S. molle* could benefit from low doses applied before and after germination.

Notably, both species displayed varied responses to the 20% biosolid humus treatment, with *C. odorata* exhibiting significantly lower stem length and leaf number values.

These findings underscore the need for further research to refine dosage strategies and explore subsequent applications, aiming to maximize the use of these species in environmental restoration endeavors and broaden the utilization of compost derived from wastewater treatment plants.

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