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DOCTORAL THESIS

THE USE OF BY-PRODUCTS AS SOIL AMENDMENTS IN THE AGRICULTURAL/FORESTRY SECTOR

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ABSTRACT

Sewage sludge is a solid or semi-solid residue generated during wastewater treatment, rich in macro- and micronutrients, and potentially useful as an agricultural or forestry input. In addition to providing nutrients, it can improve soil physical characteristics, such as aeration and water retention capacity. However, its application should be done with caution, considering the possible presence of potentially toxic elements that can harm plant development. To deepen our understanding of the effects of sewage sludge application on seedling production, this thesis was structured in two phases. The first consisted of a systematic literature review followed by a meta-analysis, based on the PRISMA 2021 protocol, analyzing recent articles with quantitative data on the effects of sewage sludge on soils and plants. The second phase involved two pot experiments conducted for 120 days under greenhouse conditions, with different tree species used in urban landscaping, grown in substrates with varying proportions of domestic sewage sludge. The experiments were conducted to evaluate the effects of sewage sludge on soil chemical parameters, plant growth through non-destructive analyses (monthly stem height and diameter), and nutrient concentrations accumulated in plants through final chemical analyses of tissues separated between shoots and roots. In both experiments, statistical analyses included ANOVA and Tukey's test at a 5% significance level, as well as multivariate analyses (PCA and PFA) to identify the factors that most contributed to the variations between treatments.

The first experiment was conducted in Sassari, Sardinia, Italy, using *Nerium oleander* as the species. The treatments consisted of T0 (100% commercial substrate), T1 (25% centrifuged sewage sludge (SS) + 50% urban soil (US) + 25% perlite), T2 (50% SS + 25% US + 25% perlite) and T3 (75% SS + 25% perlite), each with six replicates (pots). The control treatment (T0) showed the best initial performance in height, stem diameter and aerial biomass, followed by T1, T2 and

T3. In the treatments with ≥50% sludge (T2 and T3), leaf chlorosis was observed in the seedlings during the first 60 days, possibly associated with the high initial electrical conductivity (T3 = 25.9 mS/cm) representing substrate salinity, which may have limited nutrient uptake. Despite this, from the third monthly assessment onwards, a gradual recovery in growth was observed in T2 and T3, possibly due to salt leaching and progressive nutrient release. Total and aboveground biomass were significantly higher in T0, while nutrient concentrations showed distinct patterns, with macronutrients exhibiting greater translocation to the shoots, while micronutrients accumulated primarily in the roots, with the exception of Mn, Zn, and B, which were more abundant in the aboveground tissues. Heavy metal levels in soil and plants remained below the limits established by European Directive 86/278/EEC and Italian Legislative Decree No. 99/1992.
The second experiment was conducted in Botucatu (São Paulo, Brazil), with the native species *Schinus terebinthifolius* (Aroeira-pimenteira). The treatments consisted of T0 (commercial control substrate), T1 (4% dewatered and stabilized sewage sludge + 71% urban soil (red latosol) + 25% vermiculite), T2 (8% SS + 67% urban soil + 25% vermiculite) and T3 (12% SS + 63% urban soil + 25% vermiculite), all with six replicates. With monthly monitoring of pH and electrical conductivity (EC) of the leachate, a sharp decrease in salinity was observed over time, with T3 decreasing from 13.1 mS/cm to 5.08 mS/cm. The control treatment (T0) presented higher values of pH, organic matter, potassium, magnesium, sum of bases (SB), base saturation (V%), and boron. In contrast, nutrients such as available phosphorus, calcium, aluminum, sulfur, copper, iron, zinc, and manganese increased proportionally to the sludge dose applied. Regarding seedling development, T0 showed greater growth in the first 30 days, but the sludge treatments outperformed the control at the end of the experiment. There were no significant differences between the sludge treatments in total biomass and chlorophyll content, but all were superior to the control. In the chemical analysis of the plants, N, Ca, and Mn were more abundant in the shoots, while Mg, B, Cu, Fe, and Zn were concentrated in the roots. Phosphorus, potassium, and sulfur were evenly distributed between roots and shoots. Heavy metal levels in the soil remained below the limits established by CONAMA resolution 498/2020. Based on the two experiments, it is concluded that the application of sewage sludge, when monitored for salinity and composition, is viable and safe for use as a substrate in seedling production. Percentages between 12% and 25% proved to be more balanced, combining nutritional benefits without compromising initial plant growth. None of the treatments exceeded the legal limits for heavy metals or presented severe symptoms of toxicity at the end of the experiment. For future studies, we recommend the use of controls with local soil in addition to the commercial substrate, different irrigation depths, and more extensive monitoring of the elements present in the leachate throughout the cycle.

Keywords: Sewage sludge, biosolids, urban afforestation, heavy metals, plant development.

RESUMO

O lodo de esgoto é um resíduo sólido ou semissólido gerado no tratamento de águas residuais, rico em macro e micronutrientes e com potencial para ser utilizado como insumo agrícola ou florestal. Além de fornecer nutrientes, pode melhorar características físicas do solo, como a aeração e a capacidade de retenção de água. No entanto, sua aplicação deve ser feita com cautela, considerando a possível presença de elementos com potencial tóxico que podem prejudicar o desenvolvimento vegetal. Com o objetivo de aprofundar o conhecimento sobre os efeitos da aplicação de lodo de esgoto na produção de mudas, esta tese foi estruturada em duas etapas. A primeira consistiu em uma revisão sistemática da literatura seguida de meta-análise, baseada no protocolo PRISMA 2021, analisando artigos recentes com dados quantitativos sobre os efeitos do lodo de esgoto em solos e plantas. A segunda etapa envolveu dois experimentos em vasos conduzidos por 120 dias em condições de estufa, com diferentes espécies arbóreas utilizadas em arborização urbana, cultivadas sob substratos com proporções variadas de lodo de esgoto doméstico. Os experimentos foram instalados com o objetivo de avaliar os efeitos do lodo de esgoto nos parâmetros químicos do solo, no crescimento vegetal por meio de análises não destrutivas (altura e diâmetro do caule mensais) e nas concentrações de nutrientes acumulados nas plantas, por meio de análises químicas finais em tecidos separados entre parte aérea e raízes. Em ambos experimentos as análises estatísticas incluíram ANOVA e teste de Tukey a 5% de significância, além de análises multivariadas (PCA e PFA) para identificação dos fatores que mais contribuíram para as variações entre os tratamentos.

O primeiro experimento foi realizado em Sassari, Sardenha (Itália), utilizando *Nerium oleander* como espécie. Os tratamentos consistiram em T0 (100% substrato comercial), T1 (25% lodo de esgoto centrifugado (SS) + 50% solo urbano (US) + 25% perlita), T2 (50% SS + 25% US + 25% perlita) e T3 (75% SS + 25% perlita), cada um com seis repetições (vasos). O tratamento controle (T0) apresentou o melhor desempenho inicial em altura, diâmetro do caule e biomassa aérea, seguido por T1, T2 e T3. Nos tratamentos com ≥50% de lodo (T2 e T3), foi observada clorose foliar nas mudas durante os primeiros 60 dias, possivelmente associada à elevada condutividade elétrica inicial (T3 = 25,9 mS/cm) representando salinidade do substrato, o que pode ter limitado a absorção de nutrientes. Apesar disso, a partir da terceira avaliação mensal, observou-se recuperação gradativa no crescimento de T2 e T3, possivelmente devido à lixiviação de sais e liberação progressiva de

nutrientes. A biomassa total e aérea foi significativamente maior no T0, enquanto as concentrações de nutrientes mostraram padrões distintos, onde os macronutrientes apresentaram maior translocação para a parte aérea, enquanto os micronutrientes se acumularam majoritariamente nas raízes, com exceção de Mn, Zn e B, que foram mais abundantes nos tecidos aéreos. Os teores de metais pesados no solo e nas plantas permaneceram abaixo dos limites estabelecidos pela Diretiva Europeia 86/278/CEE e pelo Decreto Legislativo Italiano n.º 99/1992.
O segundo experimento foi conduzido em Botucatu (São Paulo, Brasil), com a espécie nativa *Schinus terebinthifolius* (aroeira-pimenteira). Os tratamentos consistiram em T0 (substrato comercial controle), T1 (4% lodo de esgoto desidratado e estabilizado + 71% solo urbano (latossolo vermelho) + 25% vermiculita), T2 (8% SS + 67% solo urbano + 25% vermiculita) e T3 (12% SS + 63% solo urbano + 25% vermiculita), todos com seis repetições. Com o monitoramento mensal do pH e da condutividade elétrica (EC) dos lixiviados, observou-se queda acentuada da salinidade ao longo do tempo, com o T3 passando de 13,1 mS/cm para 5,08 mS/cm. O tratamento controle (T0) apresentou maiores valores de pH, matéria orgânica, potássio, magnésio, soma de bases (SB), saturação por bases (V%) e boro. Em contrapartida, nutrientes como fósforo disponível, cálcio, alumínio, enxofre, cobre, ferro, zinco e manganês aumentaram proporcionalmente à dose de lodo aplicada. Em relação ao desenvolvimento das mudas, o T0 apresentou maior crescimento nos primeiros 30 dias, mas os tratamentos com lodo superaram o controle ao final do experimento. Não houve diferenças significativas entre os tratamentos com lodo quanto à biomassa total e teor de clorofila, mas todos foram superiores ao controle. Na análise química das plantas, N, Ca e Mn foram mais abundantes na parte aérea, enquanto Mg, B, Cu, Fe e Zn se concentraram nas raízes. Fósforo, potássio e enxofre apresentaram distribuição equilibrada entre raízes e parte aérea. Os teores de metais pesados no solo permaneceram abaixo dos limites estabelecidos pela resolução CONAMA 498/2020.
Com base nos dois experimentos, conclui-se que a aplicação de lodo de esgoto, quando monitorada quanto à salinidade e composição, é viável e segura para uso como substrato na produção de mudas. Percentuais entre 12% e 25% mostraram-se mais equilibrados, combinando benefícios nutricionais sem comprometer o crescimento inicial das plantas. Nenhum dos tratamentos ultrapassou os limites legais de metais pesados nem apresentou sintomas graves de toxicidade ao final do experimento. Para futuros estudos, recomenda-se o uso de controles com solo local além do substrato comercial, diferentes lâminas de irrigação e monitoramento mais amplo dos elementos presentes nos lixiviados ao longo do ciclo.

Palavras-chave: Lodo de esgoto, biossólido, arborização urbana, metais pesados, desenvolvimento vegetal.

With gratitude, dedicated to my beloved wife, my family, and my supervisors.

GENERAL INTRODUCTION OF THE THESIS

Rapid urban growth and the consequent increase in waste generation have highlighted the need for sustainable solutions for managing and reusing these materials. Furthermore, with the growing scarcity of natural resources, the traditional linear production model has become unsustainable, highlighting the urgent need to adopt more sustainable practices (Hegab et al. 2023). The logic of exploitation followed by disposal is progressively being replaced by approaches that value efficiency and the circular economy, in which waste ceases to be a problem and becomes a valuable input for new production chains (Sariatli 2017).

Among the waste generated in urban centers, sewage sludge stands out for both its volume and its potential uses. Sewage sludge, a solid or semi-solid fraction resulting from effluent treatment, presents a strategic alternative, especially after proper treatment. This waste can be used as a source of energy, fertilizer, soil amendment, or even as a means of recovering essential nutrients, such as phosphorus (Turrión et al. 2018). Its chemical composition naturally rich in macro and micronutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc and copper, makes it attractive for agriculture and other vegetative practices (Dhanker et al. 2021; Rusănescu et al. 2022).

In addition to providing nutrients to plants, sewage sludge can improve soil physical properties, such as water retention capacity, aeration, and increased organic carbon content, creating a more favorable environment for plant growth (Feng et al. 2024; Marin e Rusănescu 2023). These benefits are particularly relevant in regions with degraded soils or in urban areas with poor soil quality. Studies also indicate that sludge can act synergistically with chemical fertilizers, increasing nutritional efficiency and agricultural and forestry productivity (Abreu-Junior et al. 2017).

Although the application of this material often results in improvements in soil fertility, positive effects are not always guaranteed (Markowicz et al. 2021). Therefore, the use of this waste presents challenges, as when poorly managed or applied in inadequate concentrations, sludge can have negative impacts on both the environment and agricultural production. Potentially phytotoxic compounds, heavy metals, pathogens, and persistent organic substances can contaminate soil and water bodies, even compromising human health (Goldan et al. 2022; Çirka et al. 2021; Kicińska et al. 2019). Studies indicate that high doses can inhibit seed germination, harm the initial development of seedlings, and, in more severe cases, cause the death of some plant species (Wu et al. 2017; Filipi et al. 2023). It is also important to emphasize that it is essential to consider the interaction between the sludge and the plant species used, as some plants have a greater capacity for absorption or restricted translocation of heavy metals (Chu et al. 2018).

Despite its recognized potential, the use of this material remains uneven across regions and restricted to certain applications. In Brazil, for example, experience with the use of sewage sludge is still limited. The state of Rio de Janeiro alone generates approximately 133,000 tons per year, most of which is still destined for landfills, reflecting the underutilization of this resource (Abreu et al. 2017). Brazilian legislation is regulated by CONAMA Resolution No. 375/2006, most recently updated (RESOLUÇÃO Nº 498 2020), which establishes criteria for the agricultural use of sludge, including stabilization requirements, limits for heavy metals, and the absence of pathogenic organisms. The National Solid Waste Policy, (LEI Nº 12.305 2010), also reinforces the need for the agricultural use of waste such as sludge, prioritizing its environmentally appropriate disposal.

Within the European Union, directives constitute regulatory instruments that establish objectives and principles common to the member states of the economic group, with each country being responsible for transposing these guidelines into its national legal system through specific legislation. In this context, (Direttiva 86/278/CEE, 1986) defines measures to promote the safe agricultural use of sewage sludge, ensuring the protection of soil, vegetation, animals, and human health. In accordance with this European determination, Italy, through (Decreto Legislativo nº 99/1992, 1992), incorporated the aforementioned directive into its legal system, regulating the agricultural use of sewage sludge. This standard establishes limits for heavy metals and application criteria, fully complying with the provisions of the European Directive.

In the Sardinia region, specifically, the agricultural use of sludge is already a well-established practice. Approximately 68% of the sewage sludge generated in the region is reused, predominantly in forage production (Pinna et al. 2022). The area of application has remained stable over the past ten years, at approximately 2,300 hectares. However, regional regulations, according to the Regional Guidelines for the Management and Authorization of the Use of Sewage Sludge in Agriculture (Sardegna 1999), are strictly limited to agricultural use, not including specific regulations for urban or landscape applications.

Despite the widespread recognition of the agronomic value of sewage sludge, its use in landscaping, ecological restoration, and urban reforestation remains significantly underexplored. These regulatory gaps hinder the advancement of urban planning solutions. Although ornamental species are widely recognized for providing relevant ecosystem services, there is a scarcity of studies on their behavior in sludge-containing substrates. Given this gap between potential and practical application, this thesis seeks to contribute to filling this knowledge gap.

Based on the above, the thesis was structured into four chapters, which complement each other, integrating theoretical, experimental, and comparative approaches to the use of sewage sludge in agricultural and landscaping systems. The first chapter presents a systematic literature review, followed by a meta-analysis of quantitative data, bringing together scientific articles that investigated the effects of sewage sludge application on soil organic matter and nutrient levels. The objective was to compile and interpret the results of various studies published over a decade to identify patterns, gaps, challenges, and opportunities related to the use of sewage sludge. This synthesis allowed us to understand the current state of scientific knowledge on the topic and identify aspects still underexplored in the literature. Thus, Chapter 1 constituted the theoretical and methodological foundation that underpinned the case studies developed in Chapters 2 and 3, guiding the definition of the experimental objectives and variables analyzed.

Chapters 2 and 3 consist of two experimental case studies, developed independently but using similar methodologies. The second chapter addresses the experiment conducted in Sassari, Sardinia (Italy), using the oleander species (Nerium oleander), widely used in landscaping projects and urban green spaces throughout the Mediterranean region. Chapter 3 presents the study conducted in Botucatu, São Paulo state (Brazil), using the pepper tree species (Schinus terebinthifolius), native to the Atlantic Forest and widely used in reforestation and urban greening projects.

Chapter 4 is comprehensive and comparative, dedicated to the combined analysis of the results obtained in both experiments, seeking a conclusion from the work performed. This chapter discusses the similarities and differences observed between the two experiments, aiming to identify response patterns and assess the consistency of the effects of using sewage sludge.

In light of the above, the general objective of this thesis was to evaluate the feasibility of using domestic sewage sludge on species widely used in urban forestry in different geographic contexts, considering both established scientific aspects and practical application under real-world cultivation conditions.

More specifically, the study sought to: (i) evaluate the effects of different concentrations of domestic sewage sludge on the morphological and physiological development of plant species under controlled conditions; (ii) determine the levels of macro and micronutrients in the aerial and root parts of the plants, as well as the levels of heavy metal accumulation; and (iii) compare the results obtained in two distinct geographic contexts (Italy and Brazil) to identify response patterns and verify the consistency of the observed effects.

The results are expected to provide technical and scientific support for the safe and efficient use of sewage sludge in urban systems, contributing to the improvement of sustainable waste management practices. Furthermore, the findings of this research can serve as a basis for improving environmental regulations and developing public policies aimed at the reuse of organic waste in sustainable urban planning.

CONTENTS

1 CHAPTER 1 – Sustainable Impact of Sewage Sludge Application on Soil Organic Matter and Nutrients: A Meta-Analysis

*Note: This chapter corresponds to the article entitled "A Systematic Review and Meta-Analysis of the Sustainable Impact of Sewage Sludge Application on Soil Organic Matter and Nutrient Content", published in the journal "Sustainability" in 2024, under DOI "https://doi.org/10.3390/su16229865", and is reproduced here with the authors' permission.

1.1 Introduction

When discussing sustainable development, it is essential to mention the United Nations 2030 Agenda, launched in 2015 with the goal of establishing Sustainable Development Goals (SDGs) and clear targets to be achieved by 2030. According to ONU data, more than 4.2 billion people still lack access to clean water and basic sanitation services (ONU, [s.d.]). SDG 6 (clean water and sanitation) supports the development of strategies and the implementation of water and wastewater treatment systems. With this global proposal to improve water and wastewater treatment systems, there is a noticeable trend toward a significant increase in sludge production in the coming years.

The production of solid waste and sewage sludge is experiencing exponential growth, driven by the increasing global population and the pursuit of sustainable development. Research into the reuse of sludge is ongoing, as this so-called "waste" can be transformed into a resource, given that it contains numerous organic compounds, as well as macro- and micronutrients beneficial to soils and plants (Ferraz et al. 2016). However, in order to determine the appropriate application rates and to consider this material as a raw input, studies are required to ensure its safety. This is critical because many types of sludge contain high concentrations of heavy metals and pathogenic residues, which may lead to bioaccumulation in various parts of plants and soils (Guoqing et al. 2019). Numerous studies have been conducted to improve the disposal of sewage sludge, including research focused on the development of forest and ornamental species (Xue et al. 2015), (Abreu-Junior et al. 2017), (Abreu-Junior et al. 2019), (Goulart et al. 2019), and (Guerrini et al. 2021), studies aiming to increase food productivity (Marin e Rusănescu 2023), (Ceccanti et al. 2022), (Oliveira et al. 2023) and (Prates et al. 2022) and investigations related to soil remediation (Hechmi et al. 2020), (Zoghlami et al. 2016), (Picariello et al. 2020), (Hechmi et al. 2021) and (Dhanker et al. 2021).

The use of the PRISMA protocol (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) plays a crucial role in conducting rigorous and transparent literature reviews (Page et al. 2021). This well-established protocol provides a set of methodological guidelines that support researchers in planning, conducting, and reporting systematic reviews in a consistent and comprehensive manner. By adopting PRISMA, reviewers ensure that their research is carried out in a structured way, minimizing bias and maximizing the quality of the findings. Although widely applied in the healthcare field (Spano et al. 2020), the protocol can be adapted for use in various areas of study, as it offers robust methodological guidance. Tailoring the protocol to the specific needs and standards of each field is essential to ensure the quality of systematic reviews and meta-analyses.

Therefore, the main objective of this study was to perform a systematic analysis of the studies available on research platforms, following the PRISMA protocol. Based on this analysis, we were able to direct our efforts toward the development of a thesis with greater originality and authenticity.

1.2 Materials and Methods

1.2.1 PRISMA protocol

In this study, we adopted the PRISMA protocol (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) to conduct the systematic review and meta-analysis, drawing on its concise and insightful methodological guidelines.

The foundations for improving the reporting of systematic reviews date back to the 1990s, when a group of renowned researchers began efforts in this direction. Before the development of PRISMA in 2009, there was the QUOROM Statement

(Quality of Reporting of Meta-analyses), which focused primarily on systematic reviews of controlled clinical trials.

However, PRISMA marked a significant advancement by broadening its scope to include systematic reviews of various study types, not limited to clinical trials. Today, PRISMA is widely recognized and adopted globally as an essential guideline for conducting and reporting systematic reviews and meta-analyses. Its ongoing maintenance and regular updates reflect the research community's active commitment to upholding the highest methodological standards. The official PRISMA website provides access to the latest versions, extensions, and supporting resources, further consolidating its pivotal role in advancing evidence-based research. The most recent version of the PRISMA protocol (2020 version) was used to select relevant publications that would be included in this work. This protocol outlines a detailed 27-step process to ensure transparency, consistency, and quality in the systematic reviews and meta-analyses (PRISMA Statement 2020).

In summary, the adoption of the PRISMA protocol in this work reflects the need to enhance the quality and transparency of systematic reviews and meta-analyses, thereby contributing to high-quality scientific research.

1.2.2 Database

The literature review focused on identifying studies that compared the use of sewage sludge with control treatments, evaluating the effects on soil chemical attributes. The application contexts included the rehabilitation of degraded areas, its use as fertilizer in agricultural practices, and the recovery of soils and landscapes. The parameters selected for analysis were those with consistent and recurring quantitative data, including organic matter (OM), nitrogen (N), phosphorus (P), copper (Cu), and zinc (Zn), as they had a significant and comparable presence among the studies. Although other elements such as potassium (K) and potentially toxic metals, such as cadmium (Cd) and lead (Pb), are also of interest, the scarcity of data made their inclusion in this meta-analysis unfeasible.

The literature search stage was completed in August 2023, with access made through the credentials of the University of Sassari (UNISS), using the Scopus and Web of Science databases. These two platforms were chosen after comparative assessments demonstrated their greater scope in relation to other databases, which had a high rate of duplication among records. The keywords used in the search were (“sewage sludge” OR “byproduct”) AND (“urban soil” OR “landscape” OR “soil remediation” OR “forestry”).

Initially, duplicate records were eliminated. Then, a set of criteria was established for the inclusion of studies: (a) written in English; (b) accepted and published articles; (c) inserted in areas of knowledge aligned with biological sciences applied to sustainability and soil science. For the Scopus database, the filters used were “environmental sciences”, “agricultural sciences” and “biological sciences”, while on the Web of Science platform, they were “environmental sciences”, “environmental engineering”, “soil sciences” and “ecology”. It was not possible to precisely quantify the number of studies by thematic area, since many of them were classified in more than one area in the search platform filters; (d) publication between 2013 and August 2023; (e) studies whose abstract made it clear that domestic sewage sludge was used in soils; (f) studies that presented quantitative data. Qualitative studies, book chapters, case reports, investigations that used materials other than sewage sludge, which could mask the result strictly by the addition of sewage sludge, and studies with missing information were eliminated, even after attempts to contact the respective authors.

After this initial screening, eligible articles were independently evaluated by at least two researchers from this study. The selection was conducted in successive stages, based on language, type of document, thematic area, year of publication, abstract, and articles with the presence of quantitative data. The methodological structure strictly followed the PRISMA protocol guidelines: identification, screening, eligibility assessment and inclusion of studies.

1.2.3 Data extraction

The PRISMA diagram shows the detailed article screening process (Fig. 1). After selecting the articles, we included 37 studies in our analysis.

Data extraction was performed manually by reading the selected articles in full by two different reviewers. The data were extracted and organized into spreadsheets, where all important data were described individually for each study (Ahn e Kang 2018).

Due to the wide variety of sewage sludge concentrations used in the analyzed studies, which also used different units of measurement, in some cases the concentrations were expressed in mg/ha and in others in percentage, three main classifications were adopted: low concentration, with applications of up to 30% or 25 mg/ha of sewage sludge; medium concentration, with applications between 30% and 70% or between 25 mg/ha and 50 mg/ha; and high concentration, with applications above 70% or above 50 mg/ha (dos Santos Sousa et al. 2022).

The values of organic matter, nitrogen and phosphorus were expressed as percentages, while those of copper and zinc were presented in mg/kg. In studies that did not provide soil density, a density of 1 g/cm^3 was assumed to convert the values into percentages. When the studies did not present the standard error, the value corresponding to 10% of the measured value was adopted (Luo et al. 2006). For studies in which the data were not shared, but that allowed the extraction of graphs, the measurements were performed using the ImageJ software (Softonic 2024).

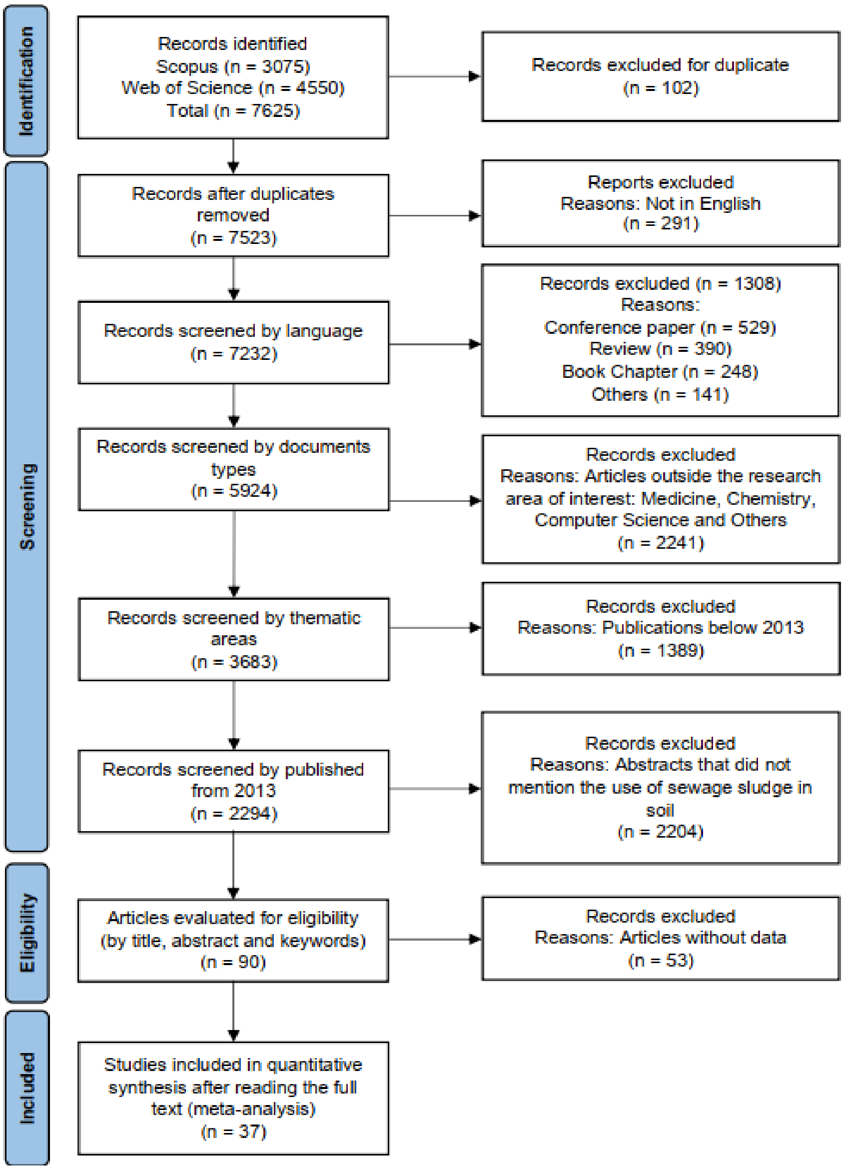


Fig. 1. Flowchart of the PRISMA approach illustrating the steps involved in bibliographic searches and the inclusion and exclusion criteria.

Not all studies included in the meta-analysis presented data on the five parameters analyzed and some studies offered more than one comparison, either with different controls at the same sewage sludge dosage or, in some cases, the same control compared with different types of sewage sludge, resulting in a discrepancy between the number of articles analyzed and the total number of analyses performed, as can be seen in Table 1.

Variable outcome	Number of studies	Number of comparisons	Outcome		
			SS rate	Environment	Continent
OM	24	57	Low (25) Medium (16) High (16)	Field (45) Controlled (12)	Africa (17) America (18) Asia (19) Europe (1) Oceania (2)
N	19	51	Low (20) Medium (16) High (15)	Field (42) Controlled (9)	Africa (22) America (13) Asia (11) Europe (3) Oceania (2)
P	25	61	Low (25) Medium (20) High (16)	Field (52) Controlled (9)	Africa (25) America (20) Asia (7) Europe (4) Oceania (5)
Cu	28	98	Low (39) Medium (31) High (28)	Field (56) Controlled (42)	Africa (19) America (21) Asia (44) Europe (10) Oceania (4)
Zn	24	88	Low (35) Medium (28) High (25)	Field (50) Controlled (38)	Africa (21) America (20) Asia (36) Europe (7) Oceania (4)

Table 1. Characteristics of articles included in the meta-analysis. Variable outcome: parameter analyzed; Number of studies: number of studies analyzed for the respective parameter; Number of comparisons: total number of comparisons within the selected studies; SS rate: sewage sludge application rate; Environment: field or controlled (greenhouse); Continent: continent where the study was conducted.

1.2.4 Statistical analysis

The variables analyzed in the meta-analysis were: soil organic matter content, total nitrogen, total phosphorus, copper, and zinc. The selected publications did not include all of these variables simultaneously, which is why random-effects models were applied for each variable and for each comparison between the control and the three analyzed sewage sludge application rates (low, medium, and high).

To obtain the effect size of the treatments compared to the control for each comparison, the response ratio, i.e., the transformed logarithmic ratio of the means, was calculated, since the variables are always quantitative and continuous (Lajeunesse 2011), as was the sample variance. The random-effects models applied were multilevel (Fernández-Castilla et al. 2020).

The choice to use random effects was made because the studies exhibit great heterogeneity due to the collection of data from studies with different objectives and methodologies, thus presenting different comparisons between studies. The use of the multilevel model was necessary to allow for the inclusion of moderators, adjusting for influences both between comparisons within the same study and between studies (Fernández-Castilla et al. 2020).

Three moderators were applied to the multilevel random effects models: a) sewage sludge application concentration, b) continent where the studies were conducted, and c) conditions under which the studies were conducted. The first moderator covers different sewage sludge application concentrations, which can be low, medium, or high; the second moderator covers studies conducted in the Americas, Europe, Africa, Asia, or Oceania; and the third includes experiments conducted in field conditions or in a controlled environment, such as a greenhouse or laboratory. Heterogeneity was calculated using the QE test (Viechtbauer 2010).

To calculate effect sizes, random effects models, and forest plots, the “metafor” package in the R Studio statistical software was used (Posit 2024).

1.3 Results and Discussion

1.3.1 Distribution Analysis

1.3.1.1 Publications by Year (2013–2023)

Analyzing the period studied, we noted a growing number of studies. It is noteworthy that approximately 60% of the studies found were published from 2019 onwards, demonstrating a growing number of investigations related to the topic over the last five years. The year with the highest number of publications was 2021, with six studies, accounting for approximately 16% of the total publications. 2014 recorded no publications, while 2013 recorded only two, representing approximately 5% of the total (Fig. 2).

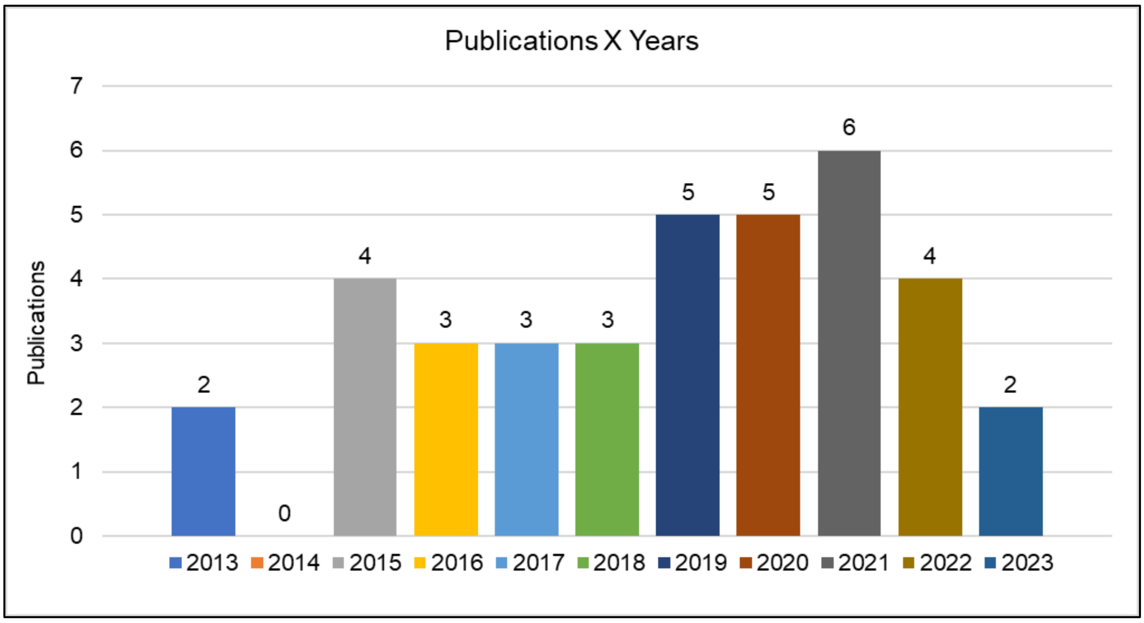


Fig. 2. Number of articles published each year over the last 10 years.

1.3.1.2 Analysis by origin

The Americas was the continent contributing the largest number of studies after the systemic analysis, with 11 studies, followed by Asia, with 9 studies, Africa, Europe and Oceania, with 8, 6 and 3 studies respectively (Fig. 3).

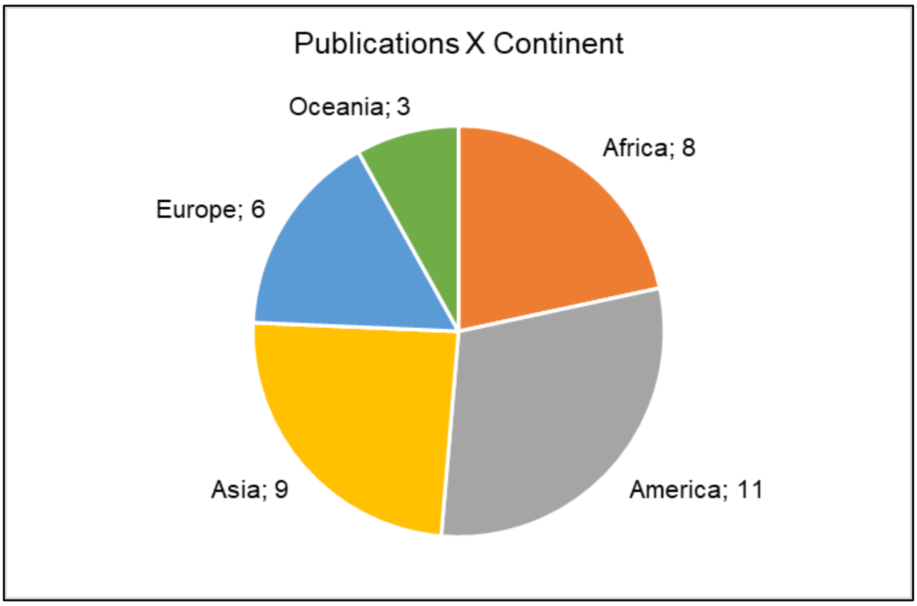


Fig. 3. Number of articles found by continent.

Figure 4 shows the distribution of published articles on a global scale. America and Asia account for 29.7% and 24.3% of studies, respectively, followed by Africa with 21.6%, and Europe and Oceania with 16.2% and 8.1%, respectively.

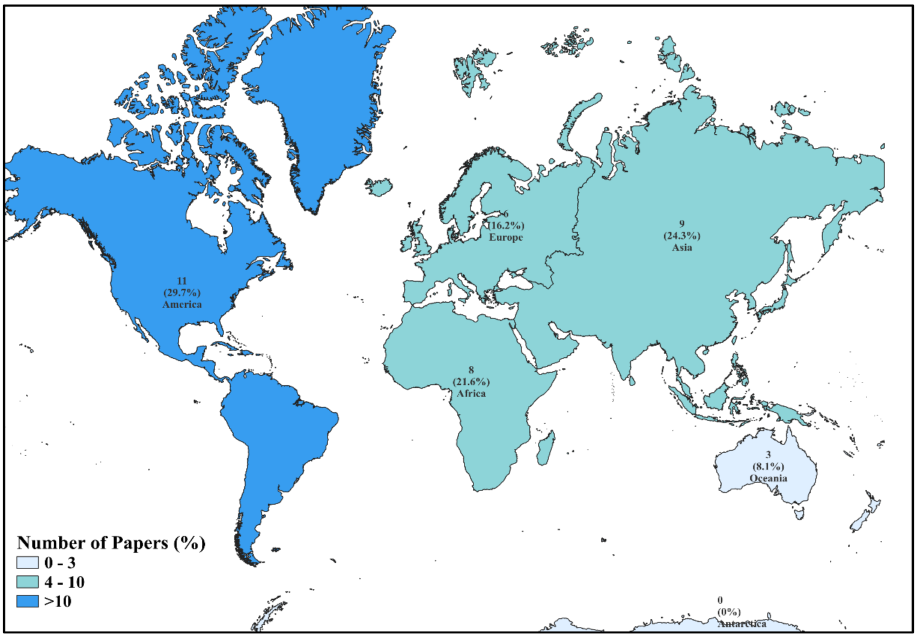


Fig. 4. Distribution of articles found by continent on a global scale.

Brazil stood out as the country with the highest number of studies, with a total of 11, equivalent to approximately 29.7% of the total studies. Tunisia came in second with six studies, followed by China with five studies. The studies included in this research using sewage sludge over the past 10 years were found in 14 countries (Fig. 5). Several countries, however, submitted fewer studies, with only one copy each, including India, Saudi Arabia, South Korea, Turkey, Italy, Lithuania, Poland, and Romania.

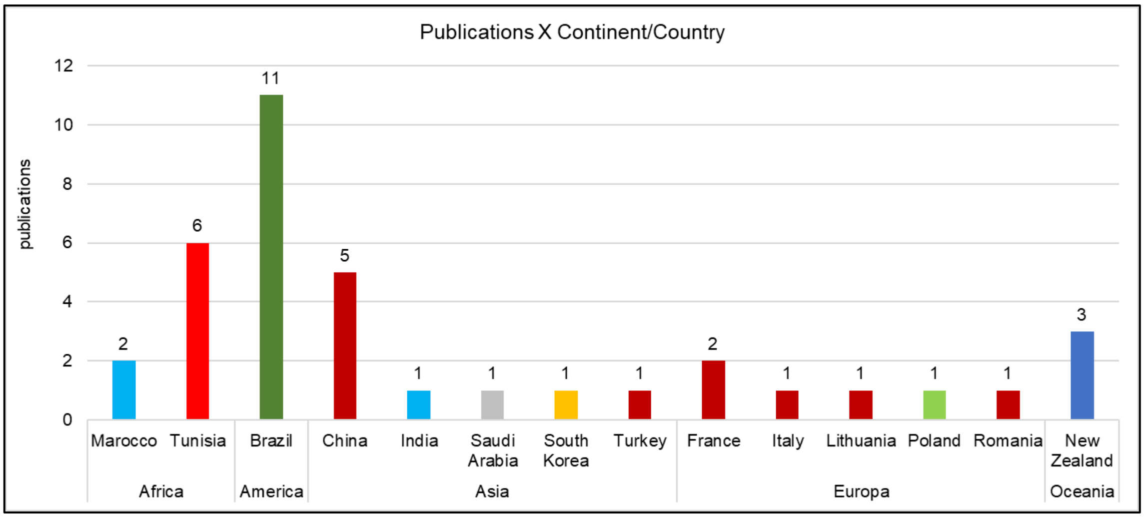


Fig. 5. Number of articles found by country.

Looking at it overall, we note that the number of countries that have submitted studies on sewage sludge in the last 10 years and that were included in the study is low, given the scope of the topic and the fact that it is an issue that affects all national populations (Fig. 6). In addition to the few countries submitting studies on the topic, it is noteworthy that many studies are concentrated in just a few regions. The three countries with the highest number of studies combined account for approximately 60% of the total.

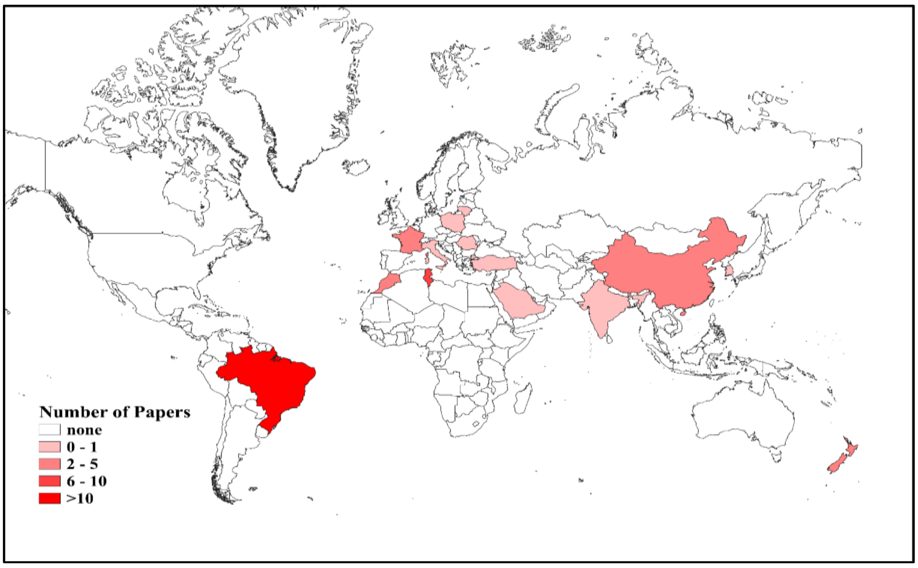


Fig. 6. Distribution of selected articles across countries on a global scale.

1.3.1.3 Analysis by vegetative typology

Of the 37 articles resulting from the PRISMA screening, 13, or approximately 35%, were studies on agri-food crops. After agri-food species, we have forestry species, which accounted for 12 studies, corresponding to 32.4% of the total. Of the 6 plant species found, 67.6% were concentrated in these two categories (Fig. 7).

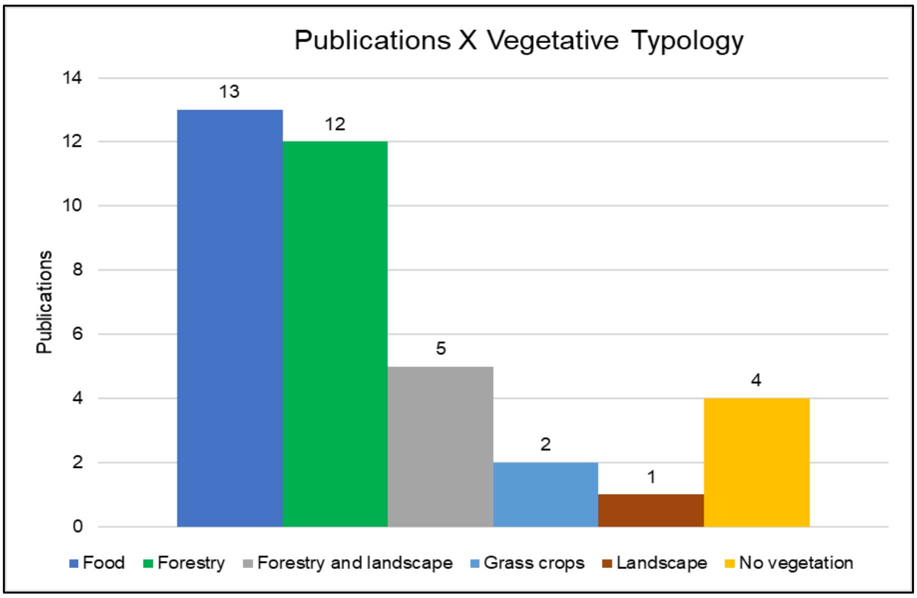


Fig. 7. Number of publications according to the classification of the investigated vegetation.

1.3.2 Statistical analysis

To perform the statistical analyses, a multilevel random-effects model was applied, due to the heterogeneity of the projects and also because comparisons exist not only between projects but within them. Given these variations, it was possible to apply three moderators, three different sewage sludge application concentrations, five continents where the work was carried out, and finally two experimental fields, conducted both in the field and in a controlled environment.

Table 2 shows the results of the Residual Heterogeneity Test (EQ) and the Moderator Test (MQ) found for the five parameters analyzed in the study.

	Test of Moderators (QM)	Test for Residual Heterogeneity (QE)
SO	QM(df = 7) = 439.7723, p-val < .0001	QE(df = 49) = 2520.0525, p-val < .0001
N	QM(df = 7) = 634.7836, p-val < .0001	QE(df = 43) = 1059.2760, p-val < .0001
P	QM(df = 7) = 14.2133, p-val = 0.0475	QE(df = 53) = 3752.1448, p-val < .0001
Cu	QM(df = 7) = 3791.4413, p-val < .0001	QE(df = 80) = 7287.3183, p-val < .0001
Zn	QM(df = 7) = 14047.1569, p-val < .0001	QE(df = 90) = 9568.2894, p-val < .0001

Table 2. Moderator Test (MQ) and Residual Heterogeneity Test (EQ) for the 5 parameters analyzed. SO: Organic Matter; N: Nitrogen; P: Phosphorus; Cu: Copper; Zn: Zinc.

Phosphorus had the lowest QM value, meaning that moderators did not have as significant an impact on the variation in results compared to the other parameters analyzed. According to the results, in the ascending sequence of impacts followed by phosphorus, the concentration of organic matter, nitrogen, copper, and finally zinc had a value approximately 1,000 times greater than that of phosphorus.

The p-value was highly significant for all parameters analyzed, but phosphorus was the only one with a p-value = 0.0475, indicating a confidence level of approximately 95% for the moderators' influence. For the other parameters, the p-value was less than 0.0001, indicating a confidence level greater than 99.9%, confirming that moderators have a stronger and more consistent impact on the results.

Although the moderator test showed that the evaluated factors explained some of the variation between comparisons, the results of the residual heterogeneity test indicate that a significant amount of unexplained heterogeneity still exists. This can be evidenced by the high QE values for all parameters, regardless of the degrees of freedom analyzed. Zinc, which had the highest QM value and the highest QE value (9568.2894), along with the highest number of degrees of freedom (df = 90), was the parameter that demonstrated the greatest unexplained residual variation, suggesting that the moderators included in the model were not sufficient to fully explain the heterogeneity present between comparisons. In all cases, the p-values were less than 0.001, indicating that residual heterogeneity is significant for all parameters.

Overall, from what is shown in Figure 8, it is clear that the application of sewage sludge, compared to the no-application control, favored greater effects on soil organic matter, nitrogen, copper, and zinc levels, especially in the medium and high application classes. For the organic matter variable, the low application class already achieved a significant effect (i.e., its confidence interval does not exceed 1) of 1.51 (i.e., 50% more than the control), while the application rate of the highest class achieved a medium application rate effect of 2.2. Nitrogen content was significantly higher than the control only in the medium (1.45) and high (1.84) residue application rate classes. Phosphorus content, however, did not respond to the application of sewage sludge, with wide confidence intervals, indicating high heterogeneity not captured by the model.

The effects of sewage sludge application on zinc and copper contents followed very similar trends, with increasing and significant effects in the medium and high application classes, with the latter showing mean effects reaching 2.2 (zinc) and 2.9 (copper).

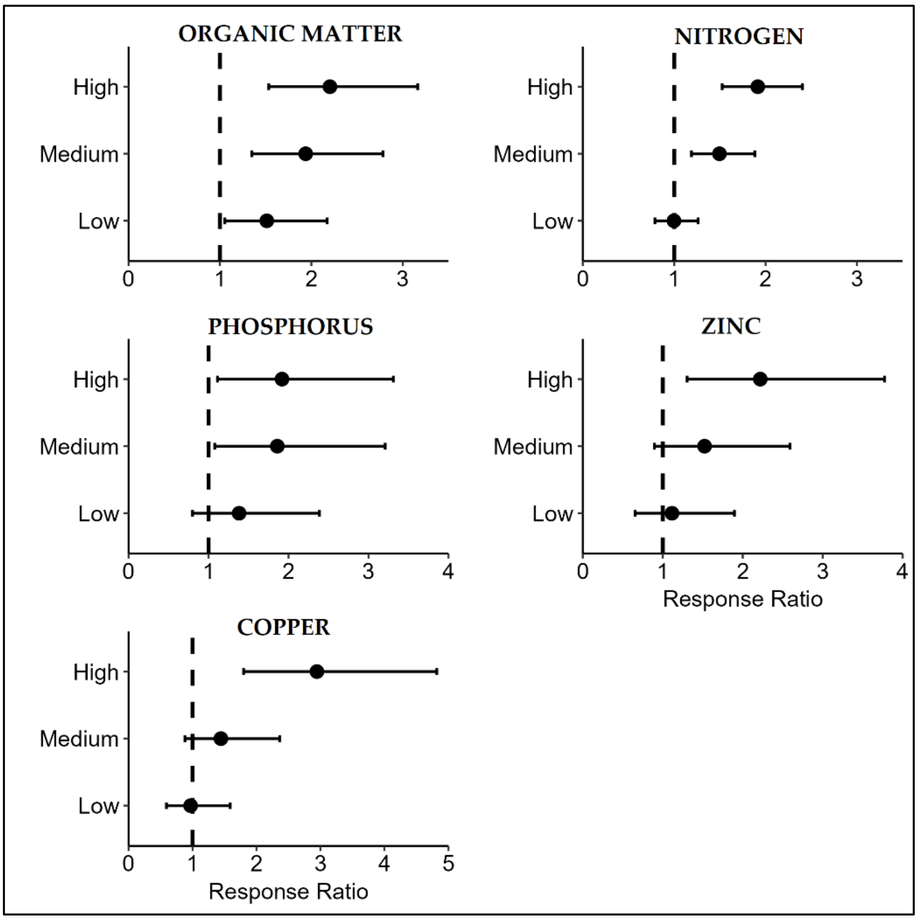


Fig. 8. Meta Regression applied with moderators of different sewage sludge concentration applications.

Using continents as moderators, a different pattern was found for each variable studied. Organic matter and phosphorus, for example, did not have different mean effects across continents, although sludge application had a significant effect on organic matter content in all. Phosphorus levels, in turn, were not affected by sewage sludge application, although studies conducted in the Americas showed a mean effect of 6.3 (with a non-intercepting confidence interval of 1) (Fig. 9).

Mean effects were also higher in the Americas for copper and zinc contents, with estimated values of 8.9, almost 9 times higher than the mean of the control without sewage sludge application. In the other continents, the confidence intervals intersect with 1, making it impossible to conclude that there was a significant difference in application.

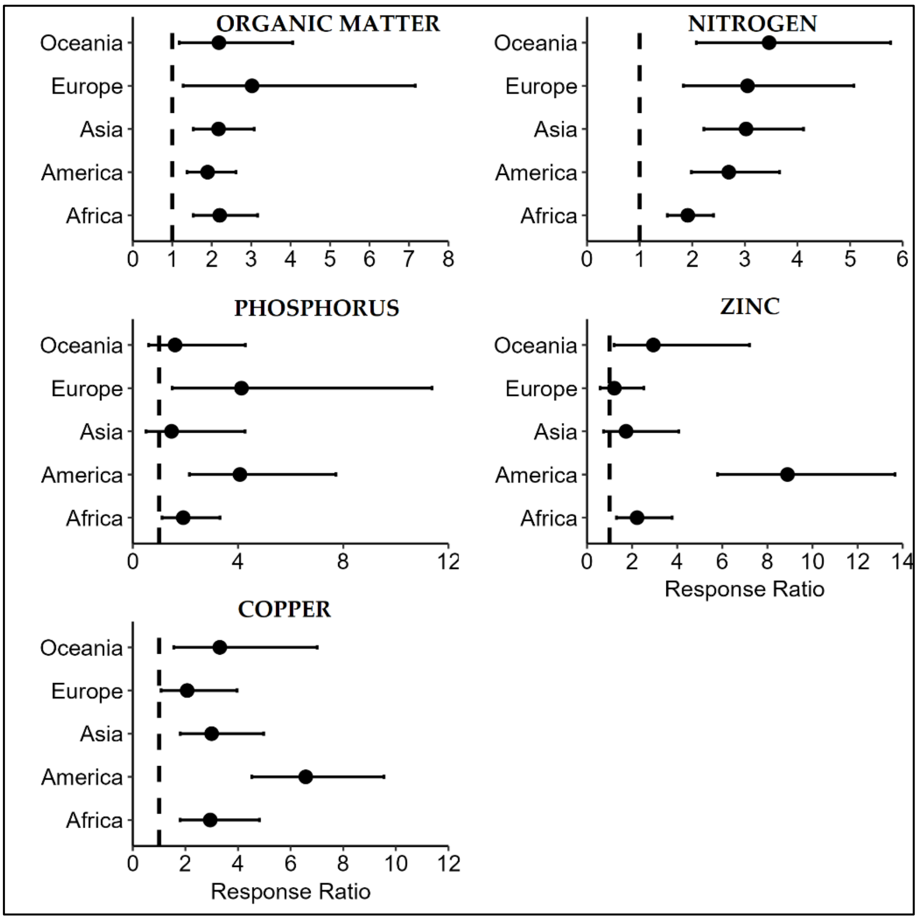


Fig. 9. Meta Regression applied with continent moderators.

Depending on the application site, the use of sewage sludge was positive for the levels of all variables, with the sole exception of phosphorus, where the confidence intervals intersect 1 in both the greenhouse and field experiments. The difference between greenhouse and field studies was found only for nitrogen content, where the application of sludge produced a greater effect when carried out in the greenhouse (average effect of 2.7 versus 1.84 in the field). Although for the other variables, the greenhouse effect always produced a higher average effect than the field studies, their confidence intervals are very wide and show a large overlap (Fig. 10).

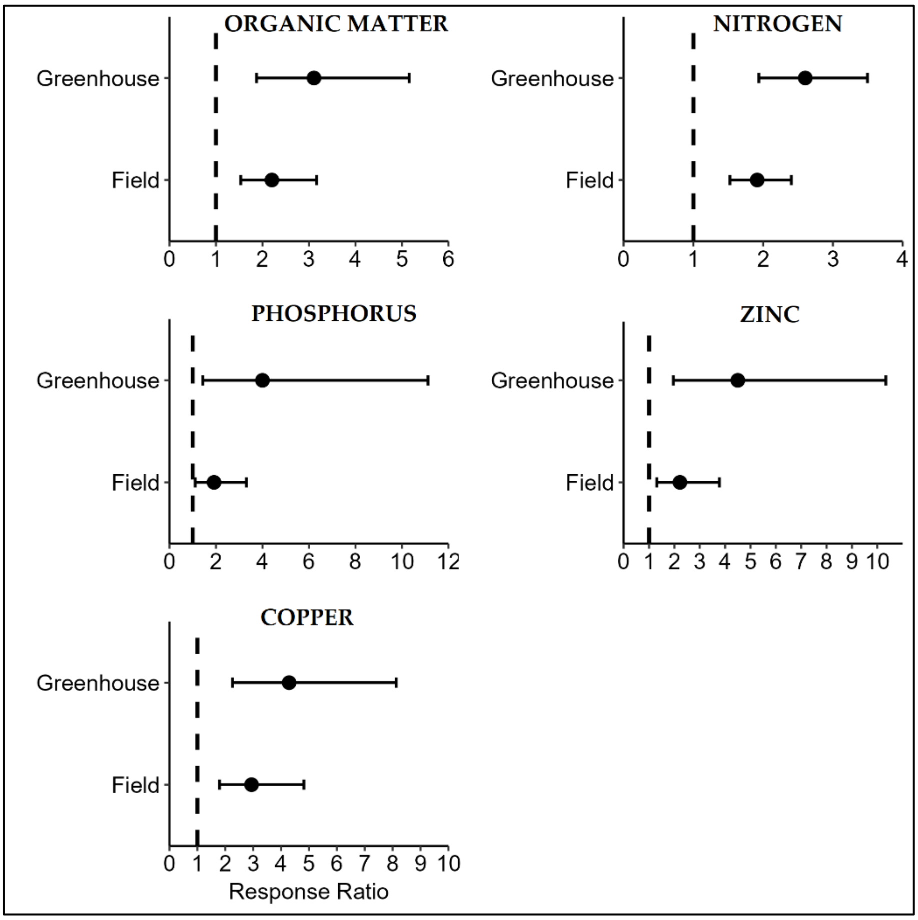


Fig. 10. Meta Regression applied with moderators of conditions in which the studies were conducted.

1.4 Conclusion

It has been observed that over the last 10 years, taking into account the methodological applications applied, there has been an increase in the number of studies found. 75.7% of the selected studies are concentrated primarily in the Americas, Asia, and Africa. The countries that contributed the largest number of scientific studies were Brazil, followed by Tunisia, which together totaled 17 studies, equal to approximately 46%.

The selected moderators proved effective in explaining part of the variation observed between studies, with highly significant QM values for all parameters analyzed. However, the residual heterogeneity (QE) test showed that, even after including these moderators, significant differences between studies remained.

It can be concluded that the application of sewage sludge produced significant increases in soil organic matter, nitrogen, copper, and zinc levels, especially at medium and high doses. Even at low doses, organic matter showed a significant effect, with a 50% increase compared to the control. On the other hand, phosphorus did not respond consistently to sludge application.

The results demonstrate that sewage sludge application has a positive impact on soil organic matter, nitrogen, copper, and zinc levels, with more pronounced effects at medium and high doses. Organic matter increased significantly across all continents, while phosphorus did not show a consistent response, except in studies conducted in the Americas, where a significant increase was observed. Furthermore, sludge use substantially increased copper and zinc levels, especially in the Americas. Regarding application sites, the effects were strongest in greenhouses. These results suggest that sewage sludge can be effective in improving soil fertility, with impacts varying depending on geographic location and application environment.

2 CHAPTER 2 – Effects of Different Concentrations of Sewage Sludge on the Development of Oleander (*Nerium oleander*)

2.1 Materials and methods

2.1.1 Experimental Position

The experiment was conducted at the Sassari Wastewater Treatment Plant (WTP), located in the same municipality, in the Sardinia region, Italy (latitude 40°42'9.97" N; longitude 8°31'30.51" E) (Fig. 11). The plant is managed by Acciona Agua SA, which acted as an institutional partner in the development of the experiment. The region's climate is classified as Mediterranean, characterized by hot, dry summers and mild, wet winters. The average annual temperature is approximately 15°C, with average highs of up to 30°C in the summer months and lows of around 7°C in the winter, in line with the average climate patterns observed in northern Sardinia (ARPA Sardegna 2025). Average annual rainfall in the urban area of Sassari is around 588 mm, reaching up to 648 mm in higher altitudes. Annual rainfall varies between 500 mm and 800 mm, with the lowest levels recorded in coastal areas and the highest inland areas. Total annual sunshine exceeds 2,500 hours, reflecting high levels of solar radiation characteristic of the region (ARPA Sardegna 2025).

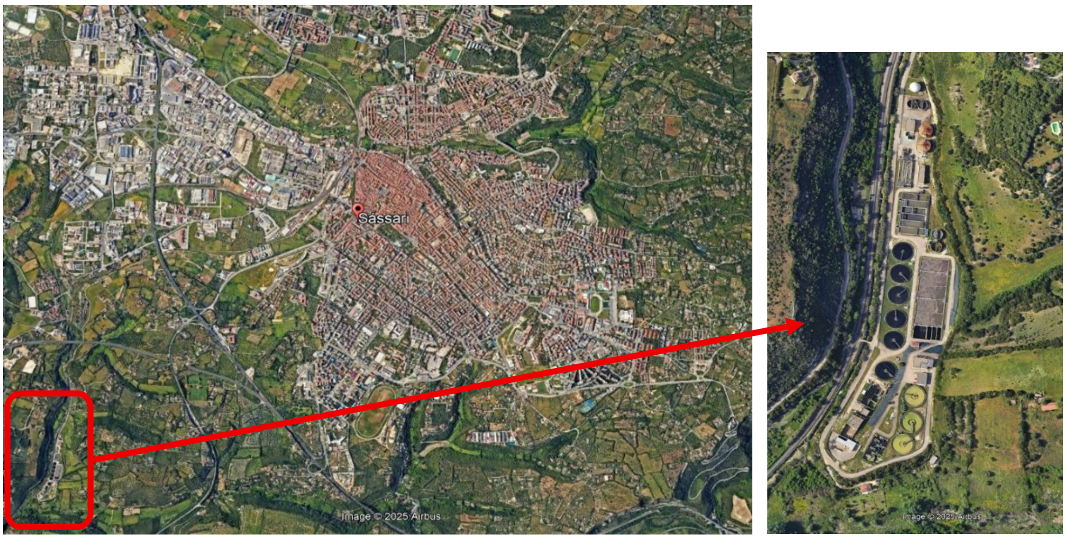


Fig. 11. Wastewater Treatment Plant (WTP) where the experiment was carried out in the municipality of Sassari.

During the experimental period, between June and October, weather conditions are typical of the region's hot, dry season. Average monthly temperatures during this period range between 21°C and 32°C, with July and August being the hottest months. Precipitation is scarce between June and August, averaging less than 20 mm/month, gradually increasing in October to approximately 80 mm. Insolation is high, averaging 10 to 12 hours of sunlight per day during the summer months. These climatic conditions characterize a dry environment with high temperatures and intense sunlight (ARPA Sardegna 2025).

2.1.2 Specie and culture media

The species used in the experiment was the oleander (Nerium oleander), native to the Mediterranean region and widely used in urban reforestation throughout Italy, including on the island of Sardinia. Its popularity in landscapes is due to its hardiness and versatility, which allow the plant to tolerate adverse conditions common in urban environments, such as compacted soil and limited water availability. These characteristics make the oleander an ideal species for a variety of landscape contexts, requiring little maintenance and offering high aesthetic value. The oleander seedlings used in the experiment were donated by the La Mandra of La Giua forest nursery, part of the regional territorial service of Sassari, an autonomous region of Sardinia. (Fig. 12 and 13).


Fig. 12 and 13. Oleander plants used in the experiment.

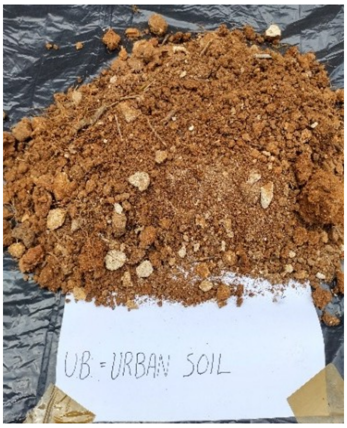
Four materials were selected to formulate the treatments used in the experiment: commercial substrate, urban soil, sewage sludge, and expanded perlite. The commercial substrate used consisted of a peat-based product widely used in the production of forest seedlings in nurseries in the region. Its selection as the control treatment was based on the fact that it is the same input routinely used by the La Mandra of La Giua forest nursery, which supplied the seedlings used in the experiment. Therefore, the use of this substrate served as a comparative reference for the other treatments tested (Fig. 14).

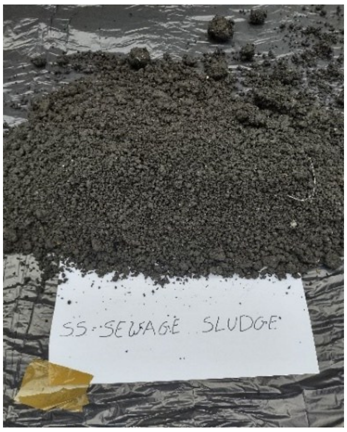
Fig. 14. Commercial substrate used in the experiment.

The urban soil used in the treatments was collected in the municipality of Sassari (Sardinia, Italy), at coordinates 40° 42′ 59″ N and 8° 35′ 02″ E. The collection area corresponds to an urban sector in the early stages of road construction, characterized by soil recently exposed by earthworks. Collection was performed manually using a metal shovel. Initially, the surface layer of approximately 2 cm, corresponding to the organic fraction and surface debris, was removed, and then the soil was collected at a depth of 0 to 20 cm (Fig. 15).

The sewage sludge used was provided by the Funtana Veglina Wastewater Treatment Plant, also located in Sassari, and came primarily from domestic effluent treated through a secondary process. After anaerobic digestion, the sludge is transferred to a holding tank (100% moisture) and then subjected to a mechanical centrifuge. At this stage, a cationic polyelectrolyte is added, which promotes particle flocculation, increasing floc size and facilitating solid-liquid separation during centrifugation. The resulting material has a moisture content between 70% and 90%. After treatment, the sludge is released for use in the field, provided it meets the standards established by the "*Direttive Regionali per la Gestione e L'autorizzazione All'utilizzo dei Fanghi di Depurazione in Agricoltura, regolata dal D.Lgs nº. 99 del 1992 che attua la Direttiva 86/278/CEE*" on environmental protection. The sludge was collected immediately after passing through the mechanical centrifuge and subsequently mixed with the other products. (Fig. 16).

Finally, expanded perlite, an inorganic material of glassy origin, was used to increase aeration, drainage, and water retention in the substrate mixtures (Fig. 17), ensuring better physical conditions for the root environment.





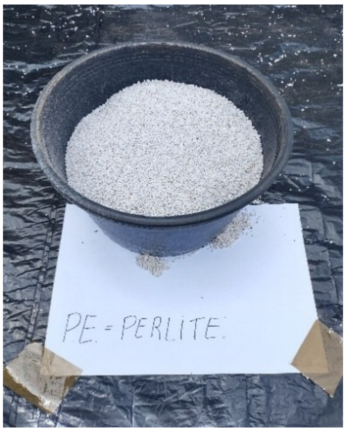


Fig. 15, 16 and 17. Urban soil used in the experiment (left); sewage sludge used in the experiment (center); perlite (right).

2.1.3 Description of the experiment

The experiment began on May 13, 2024, with the initial collection, organization, and preparation of the materials that would comprise the treatments. Field work continued until October 20, 2024, the date that marked the end of the experimental cycle, with the final measurements and collection of destructive samples. On May 20, the mixtures (Fig. 18 and 19) were prepared, formulated according to the proportions previously established for each treatment.

After the initial homogenization of the components, the mixtures underwent a 30-day stabilization period, during which they remained spread over a plastic tarp installed on an asphalted area to avoid any risk of soil contamination. During this period, the mixtures were manually stirred once a day (Fig. 20) to promote aeration and uniformity. Stabilization progress was monitored by daily weighing using a precision digital scale and a container of known volume, allowing for monitoring of mass variation over time (Fig. 21 and 22). The criterion adopted for concluding the stabilization period was achieving a constant weight.

The pre-stabilization procedure is essential in experiments involving organic waste, as it helps standardize the physical conditions of the substrates and reduces the uncontrolled release of potentially phytotoxic soluble (Elgarahy et al. 2024).

Fig. 18 and 19. Beginning of the material mixing process (left); mixtures immediately after initial homogenizations (right).

Fig. 20. Daily manual stirring of the mixtures during the stabilization period.

Fig. 21 and 22. Collecting the mixture and (left); weighing the mixture (right).

The mixtures were formulated with different volumetric proportions of dry materials, using a clear plastic bucket with a known volume of 12 liters to standardize measurements. The treatments consisted of the following proportions of centrifuged sewage sludge: 0% (T0), 25% (T1), 50% (T2), and 75% (T3). The complete formulations for each treatment were:

- T0: 100% commercial substrate, used by the local seedling nursery;
- T1: 25% centrifuged sewage sludge, 50% urban soil, and 25% perlite;
- T2: 50% sewage sludge, 25% urban soil, and 25% perlite;
- T3: 75% sewage sludge and 25% perlite.

Each treatment had 6 (six) replicates, totaling 24 pots used throughout the experiment, as represented in the experimental design (Fig. 23).

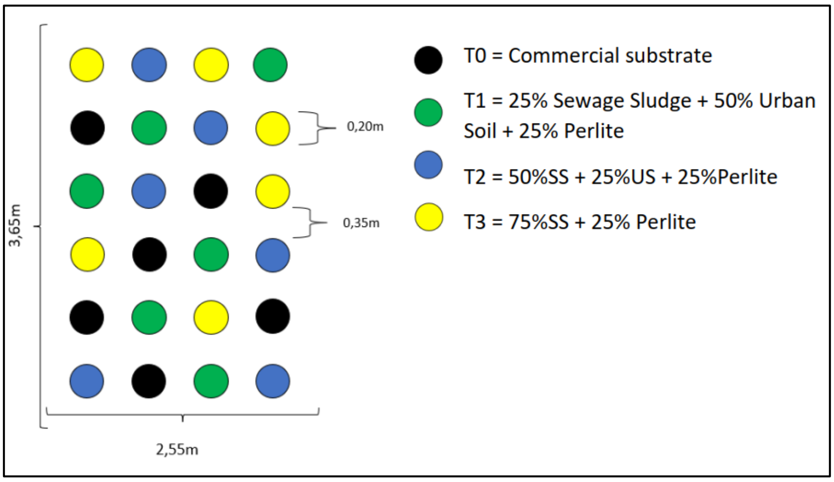


Fig 23. Experimental design.

Before transplanting, the seedlings were carefully removed from the 1-liter plastic bags in which they were stored, and approximately 1 cm of the lower end of the root system was removed (Fig. 24). This is a commonly adopted procedure to prevent root curling and promote proper development after planting.

During the pre-transplanting phase, a base fertilization was applied with 1.5g of NPK 10-10-10 granular chemical fertilizer per liter of mix, corresponding to 19g per pot, considering a total volume of 9.5 liters (Fig. 25). The dosage used was previously validated with two commercial nurseries in the region to ensure its suitability for local production conditions. To ensure the homogenization of the fertilizer with the substrate, the weighed quantity of fertilizer was placed in a raffia bag together with the 9.5 liters of mixture intended for each pot (Fig. 26) and then shaken manually for approximately one minute (Fig. 27), promoting uniform distribution of the fertilizer.

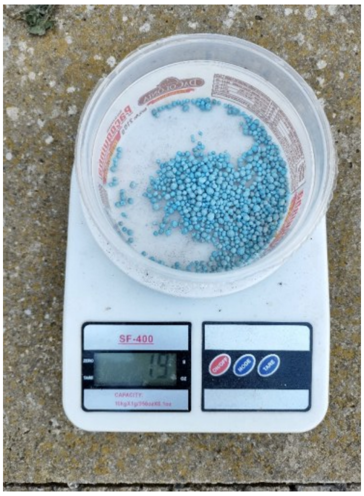


Fig. 24 and 25. Removal of 1 cm of the base of the root ball (left); weighing of the granulated chemical fertilizer NPK 10-10-10 (right).

Fig. 26 and 27. Application of fertilizer and substrate in the raffia bag (left); manual homogenization of the mixture (right).

To promote drainage and minimize substrate loss during irrigation, a layer of approximately 500 grams of expanded clay (equivalent to about 1 liter) was added to the bottom of each pot before planting, acting as a physical retention barrier (Fig. 28). After the mixtures had stabilized, and still before transplanting, representative samples of each treatment, weighing approximately 0.5 kg, were collected (Fig. 29). The samples were packaged in zip-lock plastic bags, properly labeled with the corresponding nomenclature for each treatment (Fig. 30). Subsequently, the materials were sent for laboratory analysis, with some being sent to the Laboratory of the University of Sassari (UNISS) at the Nuoro site, and some to AGRIS – Agency for Agricultural Research, located in Cagliari, Sardinia.



Fig. 28, 29 and 30. Addition of expanded clay to the bottom of the pots (left); weighing of substrate samples by treatment (center); identification and packaging of samples for sending to laboratories (right).

The seedlings were transplanted into colored plastic pots with a volume of 9.5 liters on June 20, 2024. Considering that the available seedlings presented morphological variations in height and number of stems, an internal standardization criterion was chosen for each replicate within the treatments, in order to maintain experimental consistency. In replicates 1, 2, and 3, seedlings with a single stem and similar height were used. Repeat 4 consisted of seedlings with two stems, while repeats 5 and 6 received seedlings with three stems, also of similar height (Fig. 31). The distribution of seedlings between treatments was performed randomly, in order to ensure statistical neutrality in the allocation of morphological variations. The choice to include seedlings with different numbers of stems reflects the landscape versatility of the species, commonly used in different contexts, from urban reforestation, in which the use of individuals with a single stem predominates, to central flowerbeds and ornamental areas, where cultivation with multiple stems is preferred for its denser and more expansive aesthetic effect.

Fig. 31. Seedlings organized according to the number of stems per replicate and accompanied by the identification plates corresponding to the treatments.

The pots were placed in a greenhouse measuring 3 × 4 meters (total area of 12 m²), installed on the premises of the company Acciona Agua. The site was previously leveled, and the structure was mounted on a waterproof tarp to avoid leaching and prevent soil contamination (Fig. 32, 33, 34 and 35).

Fig. 32, 33, 34 and 35. Leveling the ground; installation of the white waterproof tarpaulin; assembly of the greenhouse structure; and greenhouse finished and ready for the start of the experiment.

The internal arrangement of the pots followed a four-column, six-row arrangement, as illustrated in figure 36, allowing for clear identification of the units and ensuring uniform spacing between replicates. Irrigation was provided via a double-outlet drip irrigation system, fixed to the pots with stakes, ensuring adequate water distribution across all treatments. The system was automated, operating with a timer programmed to activate irrigation for 1 minute during the night. This timing was chosen to avoid supplying water heated by sunlight throughout the day, as the experiment was conducted in the middle of summer. This measure aimed to minimize the risk of heat stress in the plants, as accumulated water in the piping could reach high temperatures, impairing seedling development.

After approximately 30 days of the experiment, it was observed that the automated irrigation frequency was promoting excessive moisture in the substrate. Therefore, the drip system was deactivated, and irrigation began to be performed manually, based on the water needs visually observed in each plant. This verification was done by tactile inspection, evaluating the moisture level in the substrate and the presence of residues adhering to the fingers after touching, a procedure that allowed greater control and precision in water replacement.

Fig. 36. Arrangement of replicates within the greenhouse with irrigation installed.

To minimize potential influences associated with the position of the pots within the greenhouse, the seedlings were repositioned every 15 days throughout the experiment (Fig. 37). The new arrangements were always defined randomly using the online platform sorteador.com.br, ensuring neutrality in the spatial distribution of the replicates.

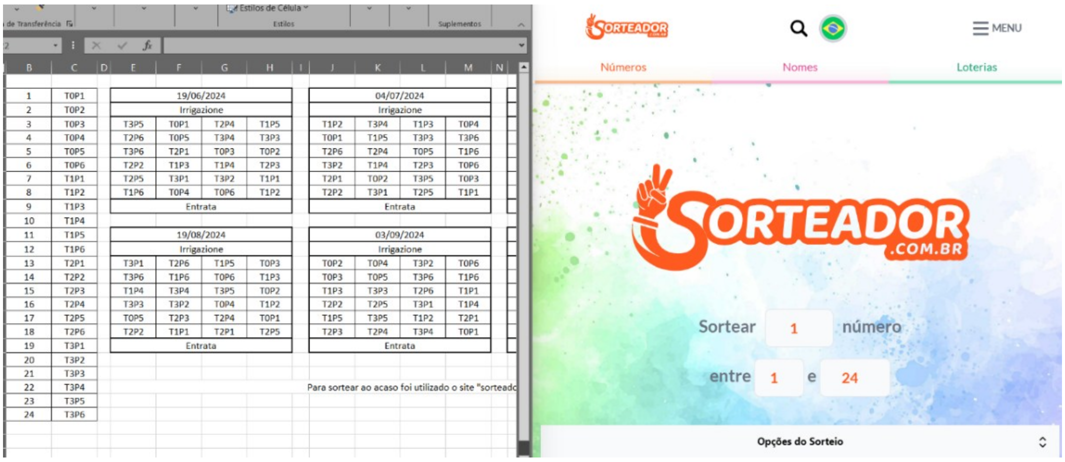


Fig. 37. Site used to perform causality in the allocation of repetitions.

2.1.4 Analysis performed

Soil analyses were conducted at two distinct points throughout the experiment. The first stage occurred after the mixture stabilization period, before transplanting the seedlings, and the second was conducted at the end of the experiment, immediately after the destructive analyses of the plants, where individual samples were collected from each replicate.

In the initial stage, samples of pure urban soil, pure sewage sludge, commercial substrate (T0), and the mixtures corresponding to treatments T1, T2, and T3 were collected, totaling six samples. Approximately 500 grams of each material were separated, packaged in appropriate containers, and sent for analysis. The samples were divided in two, with one part sent to the Laboratory of the University of Sassari (UNISS), on the Nuoro campus, and the other to AGRIS (Agency for Agricultural Research), located in Cagliari, Sardinia. All analyses followed the protocols established in the Italian Ministerial Decree of September 13, 1999, approving the Official Methods of Chemical Analysis of Soil.

In addition to chemical determinations, all samples underwent physical characterization, including analysis of soil texture and particle size. For this purpose, the wet sieving and sedimentation methodology (pipette method) was adopted, which consists of separating the textural fractions by washing with sieves to remove larger particles (sand), followed by controlled sedimentation in suspension and collection by pipetting of the silt and clay fractions, based on their specific settling times. The contents of organic carbon (C-org), organic matter (SOM), inorganic carbon (C-inorg), and consequently total carbon (c-total) were determined using a high-precision elemental analyzer.

Soil chemical fertility variables, such as exchangeable calcium, exchangeable magnesium, exchangeable sodium, and exchangeable potassium, as well as Ca/Mg and Mg/K ratios, the degree of base saturation, the sum of exchangeable bases (SB), and the percentage of exchangeable sodium (ESP), were quantified through extraction with barium chloride and triethanolamine. The cation exchange capacity (CEC) was also determined using the same extractant solution.

The quantification of heavy metals, cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn), was performed by digesting the samples in aqua regia (mixture of nitric acid (HNO_3) and hydrochloric acid (HCl)), according to a widely recognized methodology for the total extraction of metallic elements from solid matrices. The procedure consists of dissolving the metals in a hot nitrochloride solution. Before digestion, the samples are pretreated with hydrogen peroxide (H_2O_2) to facilitate the decomposition of organic matter and increase metal extraction efficiency. Digestion is performed in a closed system using a microwave or a reflux hotplate. The concentrations of the extracted metals were determined by atomic absorption spectrophotometry (AAS), using flame atomization (FAAS) for the elements with higher concentrations, and electrothermal atomization (GAAS) for the elements present in lower or trace amounts.

The available fraction of heavy metals was determined based on protocol TI018 rev. 03 of 2024, entitled "*Indice di disponibilità dei metalli pequenti (Cd, Cu, Fe, Mn, Ni, Pb, Zn) del suolo*", developed by AGRIS – Agency for Agricultural

Research and Economic Analysis, of the Autonomous Region of Sardinia. This method aims to estimate the fraction potentially absorbable by plants. Metal concentrations are read by flame atomic absorption spectrophotometry (FAAS) or inductively coupled plasma optical emission spectrometry (ICP-OES), depending on the analytical sensitivity required for each element.

Final soil analyses were conducted individually for each experimental replicate. During the destructive analysis of the plants, the remaining substrate in each pot was carefully homogenized during root system removal. A sample of approximately 500 grams of soil was then collected per experimental unit and packaged in properly labeled zip-lock plastic bags.

The samples were sent to the responsible laboratory, following the same procedures adopted for the initial analyses. This included both the sample preparation steps in the laboratory and the subsequent application of the previously described methodologies to evaluate the chemical properties of the replicates.

Plant analyses were conducted at two distinct points throughout the experiment: monthly non-destructive measurements to monitor development and growth, and final destructive analyses to characterize the nutritional status of the vegetative parts, both aerial and root.

The monthly assessments included measuring the height and stem diameter of the seedlings. Height was measured using a 10-meter metal tape measure with millimeter markings, considering the distance between the base of the stem and the apical bud of the plant (Fig. 38). Stem diameter was measured using a Caliper 0–150 mm digital caliper, with accurate readings in millimeters (Fig. 39). The first measurement was taken on June 20, 2024, the date of transplantation, and subsequent measurements were taken every 30 days, always on the 20th of the following month, totaling five measurements over the 120-day duration of the experiment, ending with the last measurement on October 20, 2024.

For seedlings with multiple stems, individual measurements were taken on each stem, and the average of the values obtained was then used to represent both the height and diameter of each replicate.

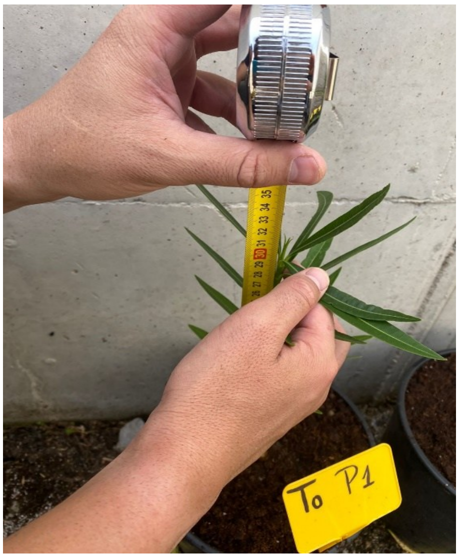


Fig. 38 and 39. Height measurement; neck diameter measurement.

After the final seedling development assessment, carried out on October 20, 2024, the destructive analysis of the plants began. The procedure began with the careful removal of the seedlings from the pots, followed by thorough washing of the roots under running water to remove all adhering soil residue, a crucial step to avoid interfering with the nutritional analysis results (Fig. 40). After cleaning, the shoots and roots of each replicate were separated. The shoots were manually fragmented using pruning shears, placed in properly labeled kraft paper bags, and sent to the laboratory for chemical analysis. Similarly, the roots were placed in the same bags, with corresponding identification, and also sent for analysis (Fig. 41).

Fig. 40. Plants with root systems properly washed to eliminate soil residues.

Fig. 41. Samples of aerial parts (AP) and roots (PR) properly separated, identified and packaged in kraft paper bags for sending to the laboratory.

The preparation of the plant samples for analysis, including oven drying, grinding, and homogenization, was carried out entirely by the technical team of the responsible laboratory. The chemical analyses conducted included the quantification of total nitrogen, macroelements (calcium, magnesium, phosphorus, potassium and sulfur), micronutrients (zinc, copper, manganese, iron, sodium and boron), as well as heavy metals (copper, boron, chromium, nickel, cadmium and cobalt), allowing a detailed assessment of the nutritional status of the plants at the end of the experiment.

2.1.5 Statistical analysis

Univariate and multivariate statistics will be performed using R (R Core Team, 2023) and RStudio (RStudio Team, 2023). Statistical analyses will begin with analysis of variance (ANOVA) to identify significant differences between treatments (T0, T1, T2, and T3) regarding soil chemical parameters, seedling vegetative development (monthly measurements of height and stem diameter), and nutritional content of shoots and roots. ANOVA will be considered applicable when the data meet the assumptions of normality of residuals and homogeneity of variances. To compare treatment means, the Tukey-HSD (Honestly Significant Difference) test will be applied, with a significance level of $p < 0.05$, to identify which treatments differ statistically. Multivariate analyses were conducted using Principal Component Analysis (PCA), which was used to reduce data dimensionality and facilitate visualization. This enabled the identification of patterns and clustering among variables and treatments, without assuming causal relationships. To apply PCA, the data were organized into three main groups: (A) soil physical-chemical parameters; (B) final plant morphological variables (height, diameter, aerial biomass, and root biomass); (C) plant chemical and nutritional parameters, analyzed by repetition, with C1 representing the chemical analyses of the aerial part and C2 representing the chemical analyses of the roots. PCA was performed for the following group combinations: A+B, A+C1, A+C2, B+C1, B+C2, and C1+C2, aiming to reduce the dimensionality of databases with a large number of variables and identify possible structural patterns among the datasets. Additionally, a Principal Factor Analysis (PFA) was conducted, encompassing all available information, to identify latent (unobservable) factors that explain the correlations between the measured variables. These multivariate analyses contributed to a more integrated and comprehensive understanding of treatment behavior across multiple dimensions.

2.2 Results

2.2.1 Soil

- Moisture and density of treatments

The initial moisture content results for the pure materials showed contrasting behaviors, with sewage sludge (SS) having the highest initial moisture content (69.7%), while urban soil (US) had the lowest (12.6%). The commercial substrate (T0) also had a high initial moisture content (64.1%), a value close to that of the sludge (Fig. 42).

As for the final results, after 120 days of cultivation, a general trend of increasing moisture content was observed in treatments T1, T2, and T3 compared to their respective initial conditions. Treatment T3 (75% sludge) had the highest final moisture content among the treatments (57.2%), followed by T0 (53.2%), T2 (41.36%), and T1 (26.42%). This trend suggests that the increased proportion of sewage sludge in the mixtures is positively correlated with the water retention capacity of the treatments, possibly due to the high amount of organic matter in the sludge. The high moisture content observed in the control treatment (T0) also reinforces this aspect, considering its composition is predominantly peat-based.

The initial density values of the materials varied among themselves. Urban soil (US) had the highest initial density (1.005 g/cm³), while sewage sludge (SS) and commercial substrate (T0) had the lowest values, at 0.150 g/cm³ and 0.127 g/cm³, respectively (Fig. 42). This difference is due to the higher concentration of mineral particles in urban soil, in contrast to the high presence of organic matter in the other components.

Regarding the mixtures, a tendency for density values to decrease was observed after 120 days of cultivation, although this variation was subtle. Treatment T0, in turn, had the lowest final density (0.166 g/cm³), followed by T3 (0.279 g/cm³), T2 (0.445 g/cm³), and T1 (0.636 g/cm³). The results indicate that the increased proportion of sewage sludge in the mixtures is associated with a lower final density, possibly due to the decomposition of organic matter and the greater porosity of the substrate.

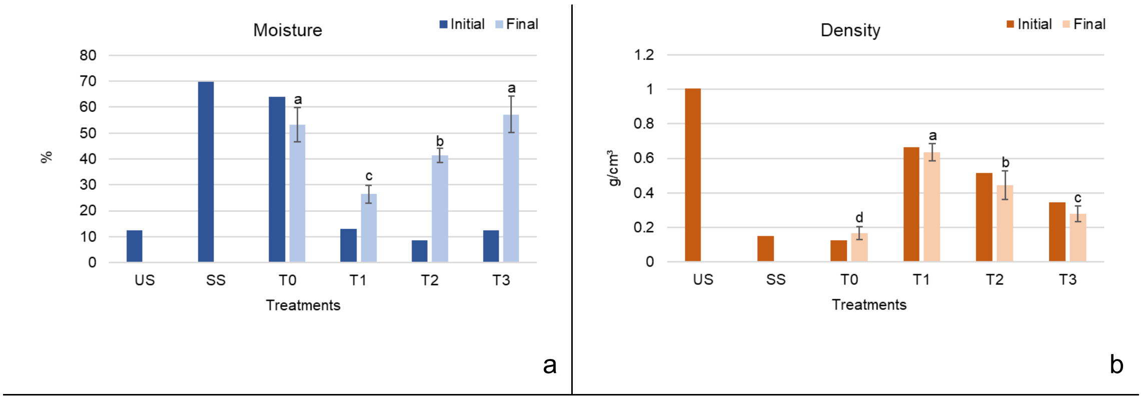


Fig. 42. (a) Moisture content and (b) density of the materials used of the treatments in the experiment. US: pure urban soil; SS: pure sewage sludge; T0: commercial substrate; T1: 25% sludge; T2: 50% sludge; T3: 75% sludge. For the final measurements, different letters indicate significant differences between treatments according to Tukey’s HSD test at a 5% significance level.

- Macronutrients and physical and chemical parameters

In table 3, the initial analysis of the pure materials shows differences between the urban soil (US) and the sewage sludge (SS). When compared to each other, the sludge presented the highest contents of total nitrogen (36.7g/kg), total phosphorus (7911mg/kg), available phosphorus (P-Olsen, 189mg/kg), organic carbon (205g/kg) and organic matter (353g/kg), values higher than those observed in the urban soil, which presented, respectively, 1.1g/kg, 200mg/kg, 4mg/kg, 20g/kg and 34g/kg. These results confirm the high fertility and organic richness of the sludge. Among the treatments, the control treatment (T0 - commercial substrate) presented the highest values of organic carbon (437g/kg), organic matter (753g/kg) and C.S.C (77.8meq./100g). Regarding total nitrogen, total phosphorus, and available phosphorus, the levels were higher than those of urban soil, but lower than those of pure sludge, at 2.8g/kg, 1000mg/kg, and 68mg/kg, respectively.

The mixtures that created treatments T1, T2, and T3 showed gradual values according to the proportion of incorporated sludge. For organic carbon and organic matter, the values exceeded those of urban soil, but did not reach the levels observed in the control treatment. In contrast, the levels of nitrogen, total phosphorus, and available phosphorus increased significantly, and even the treatment with the lowest sludge addition (T1) showed higher values than T0.

The pH of the pure materials showed slightly alkaline behavior, with values of 7.15 in the sludge and 7.39 in the urban soil. The treatments containing these materials also presented alkaline pH, with the exception of T0, which was more acidic (pH 5.69), probably due to its peat-based composition. The electrical conductivity (EC) followed a trend similar to that of available phosphorus. The pure sludge presented 4.68 mS/cm (1:2), the urban soil 0.426 mS/cm, and the control treatment 2.53 mS/cm. Treatments T1, T2, and T3, however, presented higher EC than the sludge itself, suggesting that the stabilization period promoted the release of previously retained soluble salts. This effect is intensified by the fact that the sludge was used in natura, only centrifuged to compose the mixtures. Finally, the Cation Exchange Capacity (CEC) was directly proportional to the sludge proportion in the treatments. The sludge registered a C.S.C of 65.4 meq/100g, reflecting its high cation retention capacity, attributed to the high organic matter content. Treatments T1, T2, and T3 showed progressive increases, reaching 72.3 meq/100g in T3, a value similar to that of T0 (77.8 meq/100g), which was rich in peat.

Parameter	SS	US	T0	T1	T2	T3
pH	7,2	7,4	5,7	7,2	7,9	7,8
Total Nitrogen (g/Kg)	36,7	1,1	2,8	8,6	19,9	30,2
P available Olsen (mg/Kg)	189,0	4,0	68,0	130,0	515,0	710,0
Total P (mg/Kg)	7911,0	200,0	1000,0	2350,0	4650,0	5495,0
Ec (mS/cm 1:2)	4,7	0,4	2,53	6,2	9,6	25,9
Organic Carbon (g/Kg)	205,0	20,0	437,0	58,0	131,0	221,0
Organic Substances (g/Kg)	353,0	34,0	753,0	100,0	226,0	381,0
C.S.C (mequiv./100g)	65,4	24,0	77,8	32,1	54,4	72,3

Table 3. Initial values of physicochemical parameters of the materials used (SS, US, T0, T1, T2 and T3), including pH, nutrient contents, electrical conductivity (EC) and cation exchange capacity (CSC).

Table 4 presents the means and standard deviations of the macronutrients and physical-chemical parameters evaluated in the different treatments. Figure 43 shows the comparison between the initial and final values of the experiment, as well as the statistically significant differences observed in the final analyses between the treatments. Overall, it can be seen that the incorporation of sewage sludge significantly altered the soil's chemical composition, with specific variations for each parameter analyzed.

Parameter	T0	T1	T2	T3
	------------------------------------ Final		---------------------------------------	
pH	5,53 ± 0,12	6,48 ± 0,08	5,96 ± 0,15	5,37 ± 0,31
Total Nitrogen (g/Kg)	4,33 ± 0,34	3,53 ± 0,43	6,5 ± 0,86	19,45 ± 7,83
P available (mg/Kg)	162,33 ± 28,84	124,17 ± 23,38	175,8 ± 13,44	221,25 ± 24,65
Total P (mg/Kg)	1023,7 ± 117,73	1108,5 ± 159,6	2982 ± 444,65	6917,25 ± 2140,8
Ec (mS/cm 1:2)	5,79 ± 0,55	3,48 ± 0,75	2,44 ± 0,53	4,18 ± 1,17
Organic Carbon (g/Kg)	289,5 ± 45,87	43,83 ± 2,93	66 ± 7,84	113,25 ± 24,06

Organic Substances (g/Kg)	499,17 ± 78,93	75,67 ± 4,72	114,2 ± 13,10	195,25 ± 41,32
C.S.C (mequiv./100g)	77,87 ± 14,50	28,22 ± 1,29	37,24 ± 2,41	39,53 ± 5,71

Table 4. Means and standard deviations of macronutrients and physical and chemical parameters from final soil samples for treatments T0, T1, T2 and T3.

Regarding pH (a), treatment T0 showed the smallest variation between the initial and final analyses. However, a gradual reduction in pH was observed with increasing sludge dose, with T1 significantly higher than the others in the final measurements.

Total nitrogen (b) showed a significant increase with increasing sludge doses, with treatment T3 standing out, which recorded the highest final content and a statistically significant difference compared to the others, approximately five times higher than the values observed in the other treatments. Treatments T0, T1, and T2 maintained lower and similar levels, with no significant differences.

Similar behavior was observed for total phosphorus and available phosphorus (Olsen method) (c and d). In both cases, phosphorus levels increased as the sludge proportions applied increased. Total phosphorus showed progressive growth with increasing sludge dose, with T3 having the highest final level (6917.25 mg/kg) and a statistically significant difference compared to the others. T2 presented intermediate values, while T0 and T1 remained with less significant levels. For available phosphorus, T3 again stood out with the highest final value (221.25 mg/kg), followed by T2 and T0, while T1 presented the lowest value (124.17 mg/kg), differing significantly from the others. The initial electrical conductivity (EC) increased sharply as the sludge proportions increased, with T3 presenting the highest value (25.9 mS/cm), approximately ten times higher than T0 (2.53 mS/cm). However, in the final analyses, T0 presented the highest EC value (5.79 mS/cm), significantly higher than the other treatments, suggesting possible salt loss due to leaching in treatments T1, T2, and T3.

The parameters organic carbon, organic matter, and cation exchange capacity (CEC) showed similar behavior. Treatments T1, T2, and T3, in the initial samples, showed increases in these attributes as a function of the sludge dose applied, although always with lower values than those observed in T0. In the final analyses, T0 remained the treatment with the highest values, registering 289.5

g/kg of organic carbon, 499.17 g/kg of organic matter, and 77.87 meq/100g of CEC. In comparison, T1 presented 43.83 g/kg, 75.67 g/kg and 28.22 meq/100g; T2, 66.00 g/kg, 114.20 g/kg and 37.24 meq/100g; and T3, 113.25 g/kg, 195.25 g/kg and 39.53 meq/100g, respectively. In all these parameters, T0 was significantly superior to the other treatments.

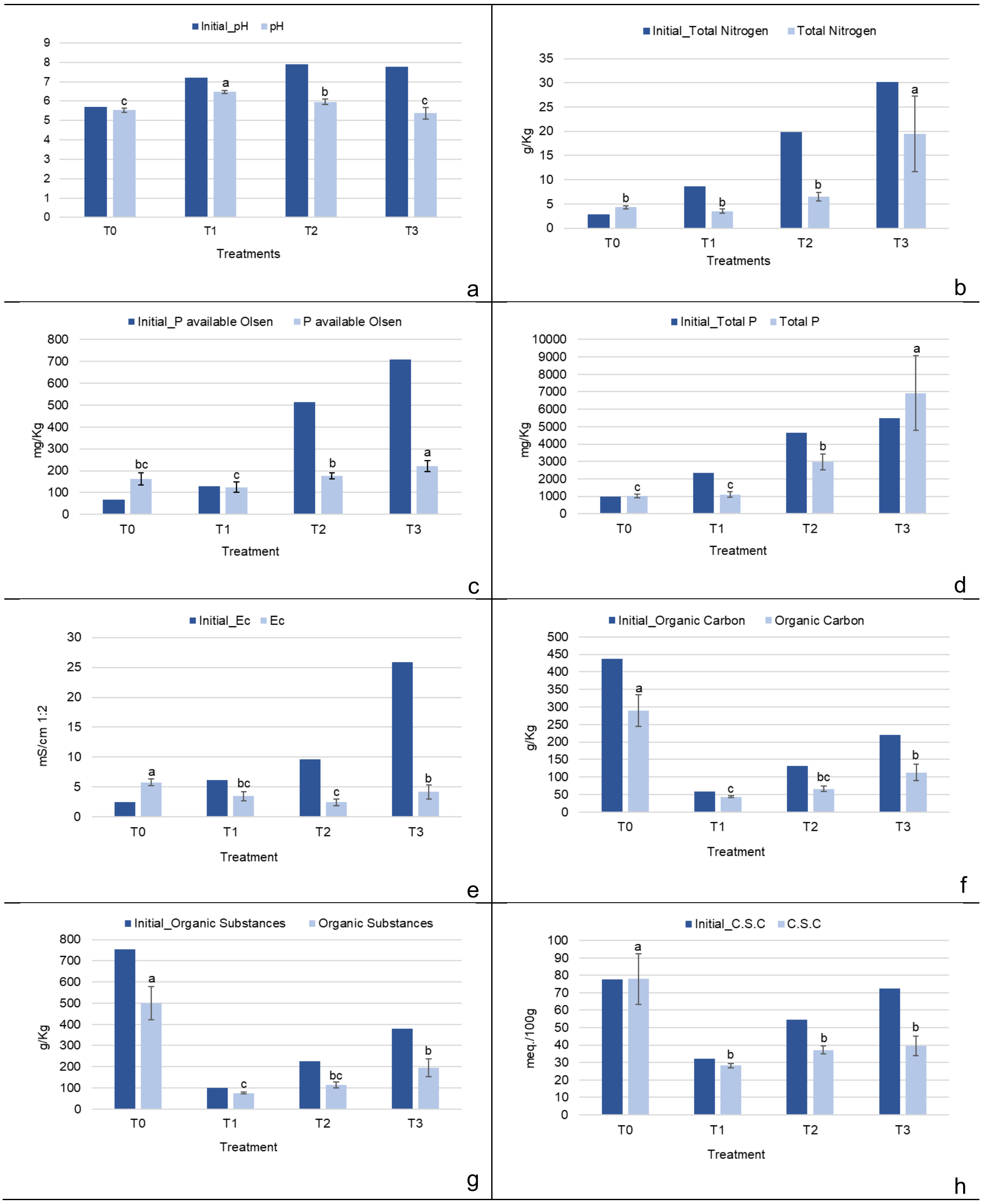


Fig. 43. Comparison between the initial and final values of the soil physical-chemical parameters by treatment (T0, T1, T2 and T3), including: pH; total nitrogen (g/kg), P available Olsen (mg/kg), total phosphorus (mg/kg), electrical conductivity (mS/cm, 1:2), organic carbon (g/kg), organic substance (g/kg) and C.S.C (meq/100g). For final measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level.

- Micronutrients and Heavy Metals

Overall, table 5 shows that the Cu, Zn, and Pb increased proportionally to the increase in sewage sludge doses in the treatments, demonstrating the direct influence of the waste on the concentration of these elements. On the other hand, the levels of Mn, Co, Cr, and Ni showed less linear variations, suggesting that their concentrations result from an interaction between the soil and the sludge input. Cd, in turn, remained at very low levels in all treatments.

Parameter	SS	US	T0	T1	T2	T3
Cu (mg/Kg)	400,0	7,3	29,5	58,7	156,0	313,0
Zn (mg/Kg)	1187,0	39,5	29,9	209,0	614,0	1179,0
Mn (mg/Kg)	181,0	422,0	77,9	424,0	341,0	184,0
Co (mg/Kg)	3,7	7,3	1,2	7,4	5,4	3,8
Pb (mg/Kg)	95,0	14,9	5,5	27,0	46,3	75,6
Cr (mg/Kg)	36,0	32,6	6,3	36,0	35,1	30,6
Ni (mg/Kg)	23,7	18,3	4,0	21,8	21,8	21,8
Cd (mg/Kg)	1,6	0,2	0,2	0,4	1,0	1,9

Table 5. Micronutrients and Heavy Metals in the initial samples.

Copper (Cu) levels increased significantly throughout the experiment, in line with the increased proportions of sewage sludge applied. In the initial analyses, the (SS) contained 400 mg/kg, which explains the progressive enrichment of the treatments according to the amount incorporated. In the final values, which can be seen in table 6, T3 presented the highest copper concentration (239.25 mg/kg), differing significantly from the other treatments according to the Tukey test ($p < 0.05$), as shown in figure 45 (a). Treatments T0, T1, and T2 exhibited lower values (26.2 mg/kg, 49.7 mg/kg, and 78.2 mg/kg, respectively), with no significant differences among them, indicating that only the higher sludge doses were capable of promoting significant accumulation of the element.

Like copper, zinc (Zn) also showed a pronounced increase as a function of the sludge doses applied. In the initial analyses, the sludge presented 1187 mg/kg of Zn, a value much higher than that of the urban soil (39.5 mg/kg) and the commercial substrate (29.9 mg/kg). In the final results, T3 again stood out with the highest content (862.25 mg/kg), differing significantly from the other treatments. Treatments T2 (299.4 mg/kg) and T1 (186.7 mg/kg) showed intermediate increases, while T0 (33.3 mg/kg) maintained the lowest value.

In contrast, the manganese (Mn) and cobalt (Co) contents showed similar behavior, but opposite to that observed for Cu and Zn, not following the increase in sludge doses. For Manganese, in the initial analyses, the urban soil and the treatment with the lowest amount of sludge (T1) already presented naturally high values, between 422 and 424 mg/kg, respectively, suggesting that the element originates predominantly from the soil, and not from the sludge. In the final results, T1 maintained the highest content (425.5 mg/kg), differing significantly from the others. Treatments T2 (251.8 mg/kg) and T3 (237.5 mg/kg) presented intermediate and similar values, while T0 (94.6 mg/kg) remained with the lowest content. For cobalt (Co), the urban soil (7.3 mg/kg) and T1 (7.4 mg/kg) initially already presented relatively high values, reinforcing that the element is not directly associated with the sludge, but rather with the mineral composition of the soil used. In the final results, T1 maintained the highest content (6.75 mg/kg), differing significantly from the others, while T2 (4.42 mg/kg) and T3 (3.65 mg/kg) presented intermediate and similar values. T0 (2.26 mg/kg) continued to have the lowest content.

Lead (Pb) levels, in turn, showed behavior closer to that of copper and zinc, with an increase proportional to the sludge doses applied. In the initial analyses, the sludge (95 mg/kg) and T3 (75.6 mg/kg) already presented higher values. In the final results, T3 maintained the highest content (53.2 mg/kg), differing significantly from the others. Treatments T1 (24.9 mg/kg) and T2 (26.5 mg/kg) presented intermediate and similar values, while T0 (5.7 mg/kg) remained with the lowest value.

Chromium (Cr) levels remained stable among the sludge treatments, with no clear upward trend with the applied dose. Initially, both the sludge (36 mg/kg) and treatments T1, T2, and T3 already had similar values, between 30 and 36 mg/kg, while T0 had a lower level (6.3 mg/kg). In the final results, the sludge treatments (T1: 30.8 mg/kg; T2: 26.9 mg/kg; T3: 37.3 mg/kg) remained statistically similar, differing only from T0 (8.7 mg/kg), which maintained the lowest level. A similar pattern was observed for nickel (Ni), which showed moderate variations among treatments, with a slight increase at the higher sludge doses. In the initial analyses, the sludge (23.7 mg/kg) and treatments T1, T2, and T3 already had similar values, while T0 maintained the lowest level (4 mg/kg). In the final results, T3 stood out with the highest value (24.6 mg/kg), although there was no significant difference compared to T1 (19.1 mg/kg) and T2 (16.3 mg/kg). T0 (9.35 mg/kg) remained the treatment with the lowest content, differing from the others.

Finally, cadmium (Cd) behaved similarly to copper, zinc, and lead. In the initial analyses, sludge (1.6 mg/kg) and T3 (1.9 mg/kg) exhibited the highest values, followed by T2 (1.0 mg/kg) and T1 (0.4 mg/kg), while T0 (0.2 mg/kg) maintained the lowest content. In the final results, this trend was maintained, with T3 presenting the highest content (1.35 mg/kg), differing significantly from the others, while T2 (0.58 mg/kg) and T1 (0.48 mg/kg) were statistically similar to each other, and T0 (0.23 mg/kg) remained with the lowest value.

Parameter	T0	T1	T2	T3
	------------------------------------ Final	---------------------------------------		
Cu (mg/Kg)	26,2 ± 2,57	49,73 ± 2,85	78,24 ± 5,44	239,25 ± 77,95
Zn (mg/Kg)	33,2 ± 2,86	186,67 ± 8,36	299,4 ± 15,01	862,25 ± 280,59
Mn (mg/Kg)	94,55 ± 10,76	425,5 ± 37,60	251,8 ± 19,28	237,5 ± 41,39
Co (mg/Kg)	2,26 ± 0,54	6,75 ± 0,39	4,42 ± 0,37	3,65 ± 0,88
Pb (mg/Kg)	5,69 ± 0,61	24,95 ± 0,71	26,54 ± 1,28	53,2 ± 14,19
Cr (mg/Kg)	8,72 ± 1,41	30,82 ± 2,07	26,88 ± 3,80	37,28 ± 20,47
Ni (mg/Kg)	9,35 ± 5,38	19,12 ± 1,20	16,26 ± 2,19	24,63 ± 13,98
Cd (mg/Kg)	0,23 ± 0,05	0,48 ± 0,04	0,58 ± 0,04	1,35 ± 0,44

Table 6. Means and standard deviations of macronutrients and physical and chemical parameters of initial soil samples for treatments T0, T1, T2 and T3

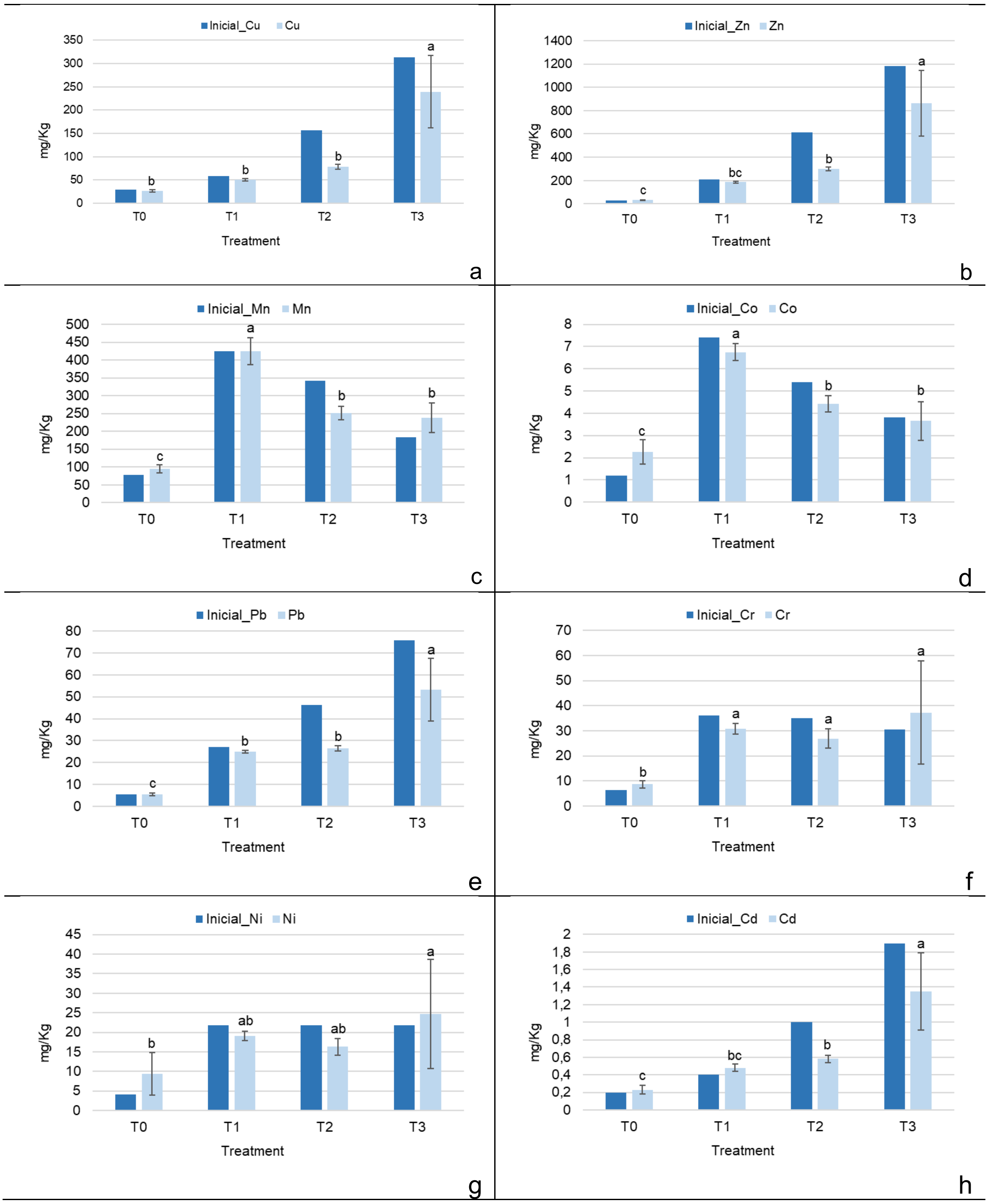


Fig. 45. Comparison between the initial and final total contents of the micronutrients copper (Cu), zinc (Zn), and manganese (Mn), and the heavy metals cobalt (Co), lead (Pb), chromium (Cr), nickel (Ni), and cadmium (Cd), extracted by aqua regia, across treatments T0, T1, T2, and T3. For final measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level. All values are expressed in mg/kg.

2.2.2 Vegetative Development

For both height and stem diameter, it was observed that the treatments with higher proportions of sewage sludge (T2 and T3) showed slower initial development, possibly associated with a period of adaptation or physiological stress resulting from the chemical and physical characteristics of the mixtures. Nevertheless, over the course of the experiment, these plants demonstrated gradual recovery.

Table 7 presents the cumulative increases in height and stem diameter of the seedlings throughout the experiment. Height growth varied between treatments, initially showing a general downward trend as the proportion of sewage sludge in the mixtures increased. Treatment T0 presented the highest absolute values in all measurements, reaching 41.7 cm at the end of the experiment. However, the difference between the first and last increments was relatively small (from 35.6 cm to 41.7 cm), indicating that seedling growth in this treatment was concentrated primarily in the early stages of the experiment. The treatments that received sludge showed lower initial values but more significant proportional increases over time. T1 increased from 18.95 cm in July to 36 cm in October, representing a gain of approximately 90% between the last increase and the first. T2 and T3, which recorded the smallest initial increases (4.33 cm and 2.27 cm, respectively), reached 24.7 cm and 15.58 cm by the end of the experiment, with relative increases of approximately 471% and 586%, respectively, in contrast to the 17% growth observed in T0. Overall, the increase in height followed the T0 > T1 > T2 > T3 trend in absolute terms.

The increase in collar diameter showed a similar pattern. T0 recorded the highest final value (5.46 mm), followed by T1 (4.91 mm), T2 (4.31 mm), and T3 (2.28 mm). Although T0 maintained an advantage in absolute values, the difference between the first and last increments was smaller (from 3.42 mm to 5.46 mm) when compared to the other treatments. In relative terms, the gains were 59.6% for T0, 180.6% for T1, 543% for T2, and 484.6% for T3, confirming that, despite the smaller final dimensions, the sludge treatments showed higher recoveries in the intermediate and final phases of the experiment.

Height increment	T0	T1	T2	T3
20.07.24	35,6 ± 8,47	18,95 ± 5,67	4,33 ± 3,71	2,27 ± 2,19
20.08.24	39,44 ± 11,03	31,52 ± 5,77	13,91 ± 8,16	5,54 ± 3,78
20.09.24	40,63 ± 11,43	33,44 ± 7,71	21,81 ± 10,55	11,93 ± 5,42
20.10.24	41,7 ± 11,43	36 ± 8,72	24,7 ± 10,83	15,58 ± 4,89
Diameter increment	T0	T1	T2	T3
20.07.24	3,42 ± 0,65	1,75 ± 0,64	0,67 ± 0,48	0,39 ± 0,50
20.08.24	4,14 ± 1,39	3,25 ± 0,93	1,81 ± 1,14	0,91 ± 0,36
20.09.24	4,51 ± 1,30	4,1 ± 1,27	2,4 ± 1,61	1,41 ± 0,41
20.10.24	5,46 ± 1,76	4,91 ± 1,69	4,31 ± 3,10	2,28 ± 0,53

Table 7. Means and standard deviations values of the monthly increments in height (cm) and collar diameter (mm) of the seedlings over 120 days of experiment, by treatment (T0, T1, T2 and T3). Measurements performed at monthly intervals.

Figures 46 (a) and 46 (c) show the increments in seedling height and collar diameter over the five measurements, highlighting both the variations between periods within each treatment (capital letters) and the differences between treatments at each assessment date (lowercase letters).
Regarding height, it can be seen that the T0 treatment showed no significant differences among the four monthly increments. In treatments T1, T2, and T3, the first increment measurement differed significantly from the three subsequent measurements, which, in turn, did not differ from each other.
When comparing the treatments in each assessment period, it can be seen that, on the first date (July 20, 2024), the increment in T0 was significantly greater than that in T1, which, in turn, exceeded those in T2 and T3, which did not differ from each other. In the second increment, T0 and T1 showed no significant difference, but both differed from T2 and T3. In the third and fourth measurements, the pattern of significance was similar, with T0 not differing from T1, T1 not differing from T2, and T2 not differing from T3.
Regarding neck diameter, T0 also showed no significant differences between the evaluation periods. Treatments T1 and T2 showed differences only between the first measurement and the three subsequent measurements. T3, in turn, showed no significant differences between consecutive measurements (fourth and third, third and second, and second and first).

In the comparison between treatments, during the first measurement, T0 differed significantly from T1, which also differed from T2 and T3, which remained statistically similar. In the second and third measurements, the pattern was similar, with T0 not differing from T1, T1 not differing from T2, and T2 not differing from T3. In the fourth measurement of diameter increase, no significant differences were observed between treatments.

Figure 46 (b) presents the absolute mean seedling heights across the five measurements. Initially, seedlings in all treatments exhibited a similar height pattern; however, in the second measurement, treatment T0 demonstrated significantly superior initial development compared to the others. T1 (25% SS) also outperformed treatments T2 and T3 (50% and 75% SS). From the third measurement onward, both the control and T1 treatments showed a tendency toward stabilization, with a reduction in height growth, while treatments with higher sludge proportions (T2 and T3) began to exhibit more pronounced growth, maintaining this trend until the end of the experiment.

Figure 46 (d) shows the average seedling collar diameters. Similar to height growth, all treatments showed continuous diameter increases throughout the experimental period. Treatment T0 maintained the highest average values across all evaluations, followed by T1, T2, and T3, which maintained this same sequence over time.

In the initial measurements, it was observed that the treatments with a higher sludge proportion (T2 and T3) showed slower initial growth, possibly associated with a seedling adaptation phase to the substrate conditions. From the third measurement onward, these treatments began to show more significant increases, indicating a gradual recovery in growth. Even so, T0 and T1 maintained an advantage in absolute diameter values until the end of the experiment, reflecting better overall seedling development performance.

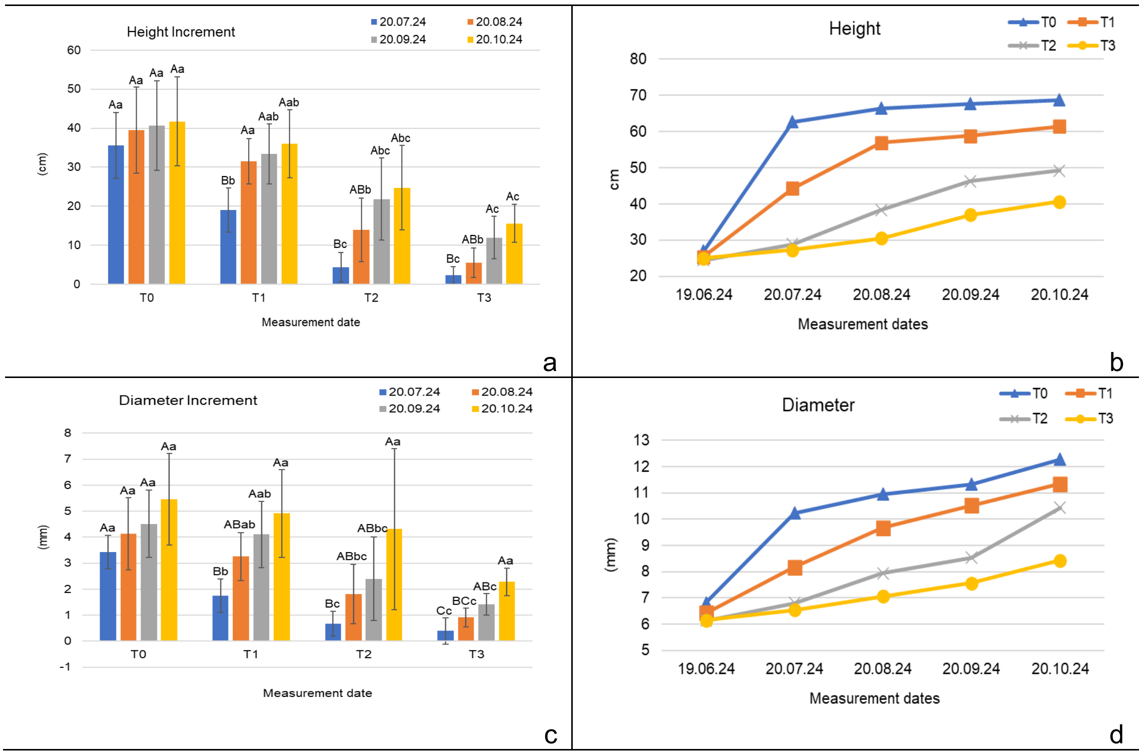


Fig. 46. (a) Average cumulative height increment over monthly measurements; (b) average absolute height per month; (c) average cumulative diameter increment over monthly measurements; (d) average absolute diameter per month. Different capital letters indicated significant differences ($p < 0.05$) between increments within each treatment, while different lowercase letters indicated significant differences ($p < 0.05$) between treatments within the same increment measurement. Comparisons between treatments T0, T1, T2, and T3.

Table 8 presents the means, standard deviations, minimum, maximum and median values of aerial, root and total biomass of seedlings in the different treatments.

Treatment	Parameter	Mean	Standard Deviation	Min	Max	Median
T0		54,12	11,402	35,59	69,89	55,33
T1	Dry Mass - Aerial Part	38,21	6,478	31,65	47,97	36,41
T2		29,83	5,18	23,29	36,84	28,7
T3		22,07	11,5	7,53	33,13	23,8
T0		31,26	4,385	24,82	35,84	31,18
T1	Dry Mass - Root Part	29,09	11,88	21,84	52,89	25,41
T2		18,49	3,248	13,96	23,1	18,45
T3		22,56	8,853	11,65	30,65	23,97
T0	Total Dry Mass	85,38	15,542	60,41	105,64	86,51

T1	67,3	10,156	56,68	84,54	66,07
T2	48,32	7,255	41,3	55,89	47,15
T3	44,63	15,394	26,67	63,78	44,03

Table 8. Mean values, standard deviations, minimum, maximum and median values of seedling dry mass by treatment (T0, T1, T2 and T3), at the end of the experiment. The data are organized by aerial part, root part and total dry mass, expressed in grams (g).

Figure 47 illustrates the significant differences between treatments according to Tukey's test ($p < 0.05$) for the mean values of shoot, root, and total dry mass at the end of the experiment.

Shoot dry mass decreased proportionally with increasing sewage sludge doses. Treatment T0 obtained the highest mean value (54.12 g), differing significantly from the others. T1 (38.21 g) showed intermediate performance, with no significant difference compared to T2 (29.83 g), which, in turn, also did not differ from T3 (22.07 g).

For root dry mass, no statistically significant differences were observed between treatments, although T0 maintained the highest mean (31.26 g), followed by T1, T3, and T2, with values of 29.09 g, 22.56 g, and 18.49 g, respectively. This trend indicates that root growth was less influenced by sludge proportions than shoot development.

Total dry mass, resulting from the sum of shoot and root biomass, followed the same pattern as that of the shoot, with a gradual reduction as sludge doses increased. T0 had the highest average value (85.38 g), differing significantly from treatments T2 (48.32 g) and T3 (44.63 g). T1 (67.3 g) remained in the middle, not statistically different from the others.

Overall, the results indicate that total biomass accumulation decreased with increasing sewage sludge proportions, with this reduction being more significant in the shoot, while the root system showed greater stability between treatments.

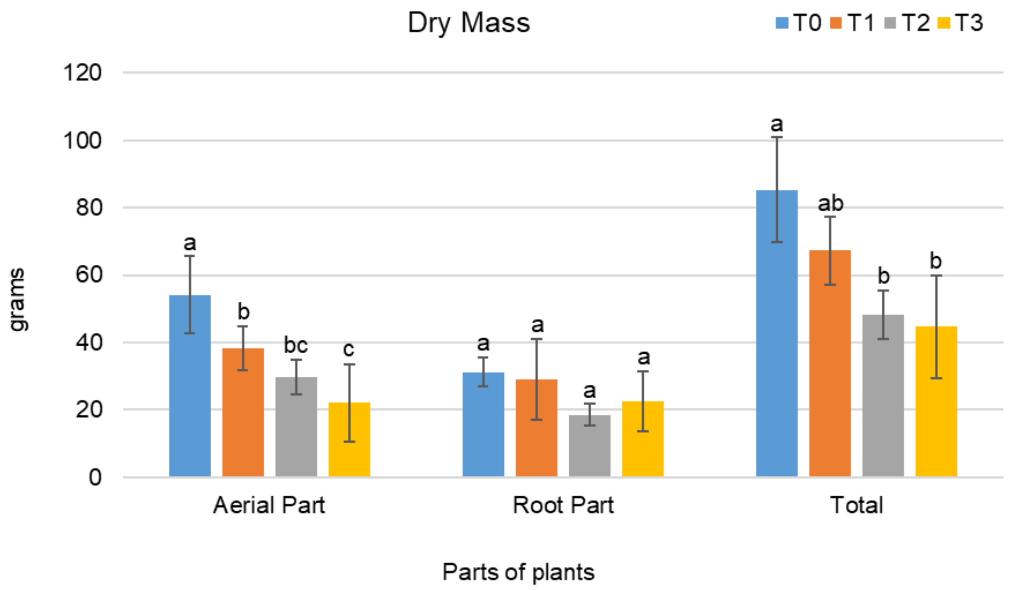


Fig. 47. Comparison of the average dry mass (g) of seedlings by treatment (T0, T1, T2 and T3), separated by aerial part, root part and total. For final measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level.

2.2.3 Vegetative Chemical Analysis

For the analysis of plant nutritional data, replicate 4 of treatment T1 was excluded due to anomalous values, thus characterizing it as an outlier. After contacting the laboratory responsible for the chemical analysis, the possibility of sample contamination was raised, which reinforced the need to exclude it from the calculations of means and standard deviations. This exclusion was applied to both shoot and root data.

The results of the chemical analyses of plant tissues were presented in a consolidated format, but with a distinction between shoot and root parts within the tables and each graph. For better organization and comprehension, the nutritional data were divided into two subsections: the first containing the levels of carbon, essential macronutrients (nitrogen, phosphorus, and potassium), and exchange cations (calcium, magnesium, and sulfur), and the second containing the levels of micronutrients and heavy metals detected in the samples. It is worth noting that, in the chemical analyses of the shoots and roots, the elements lead (Pb), cadmium (Cd), and cobalt (Co) were not considered in the comparisons, as the values reported by the laboratory were below the detection limits (e.g., <1.5; <2.5), making statistical calculations impossible. A similar situation occurred with chromium (Cr) and nickel (Ni) in the shoots of the T0 treatment, which limited the comparisons of these two elements to the T1, T2, and T3 treatments. For the root part, however, a complete comparison was possible between all treatments.

- Carbon (C), essential macronutrients (N, P, K), and exchange cations (Ca, Mg and S)

Table 9 presents the mean values and standard deviations of the carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) contents determined in the aerial and root parts of the seedlings in the different treatments.

Aerial Parameter	T0	T1	T2	T3
C (%)	40,07 ± 0,97	38,84 ± 0,69	40,78 ± 0,86	40,58 ± 1,35
N (%)	1,62 ± 0,22	2,02 ± 0,11	2,16 ± 0,26	2,2 ± 0,43
P (%)	0,31 ± 0,03	0,35 ± 0,09	0,45 ± 0,12	0,47 ± 0,09
K (%)	1,89 ± 0,16	1,56 ± 0,07	2,03 ± 0,17	1,69 ± 0,19
Ca (%)	1,84 ± 0,26	2,36 ± 0,18	2,01 ± 0,20	1,64 ± 0,25
Mg (%)	0,26 ± 0,03	0,28 ± 0,02	0,32 ± 0,04	0,3 ± 0,04
S (%)	0,38 ± 0,03	0,3 ± 0,04	0,39 ± 0,11	0,35 ± 0,07
Root Parameter	**T0**	**T1**	**T2**	**T3**
C (%)	39,92 ± 0,52	39,2 ± 0,40	39,84 ± 0,87	40,98 ± 2,07
N (%)	0,81 ± 0,09	1,05 ± 0,11	0,94 ± 0,18	0,92 ± 0,25
P (%)	0,2 ± 0,03	0,19 ± 0,01	0,19 ± 0,05	0,17 ± 0,09
K (%)	1,38 ± 0,19	1,53 ± 0,14	1,34 ± 0,29	0,96 ± 0,38
Ca (%)	0,91 ± 0,06	1,35 ± 0,11	0,96 ± 0,11	0,88 ± 0,09
Mg (%)	0,2 ± 0,03	0,23 ± 0,02	0,21 ± 0,03	0,2 ± 0,06
S (%)	0,23 ± 0,05	0,15 ± 0,02	0,11 ± 0,02	0,11 ± 0,04

Table 9. Means and standard deviations values of carbon (C), essential macronutrients (N, P, K), and exchange cations (Ca, Mg and S) in the aerial and root parts of the plants, by treatment (T0, T1, T2 and T3). Expressed in %.

Figure 48 (a–g) show the mean concentrations of the analyzed chemical elements and the significant differences between treatments, according to Tukey's test ($p < 0.05$), highlighting the variations in nutrient absorption and distribution between the shoots and roots of the seedlings.

In general, element concentrations were higher in the shoots, indicating greater nutrient accumulation in photosynthetic tissues compared to the roots.

Carbon (C) contents showed a similar pattern among the plant parts, ranging from 38.84% to 40.78% in the shoots and from 39.2% to 40.98% in the roots. The differences between treatments were small and without a clear trend. Only T1 showed a significantly lower value in the shoots compared to the others, while there was no statistical difference between treatments in the roots. Nitrogen (N) showed distinct behavior, with substantially higher concentrations in the shoots, ranging from 1.62% (T0) to 2.20% (T3), while in the roots, values remained close to 1%. A trend toward increasing N concentrations was observed with increasing sludge doses, with treatments T1, T2, and T3 being statistically similar to each other and significantly higher than T0. No significant differences were observed among the roots in the treatments.

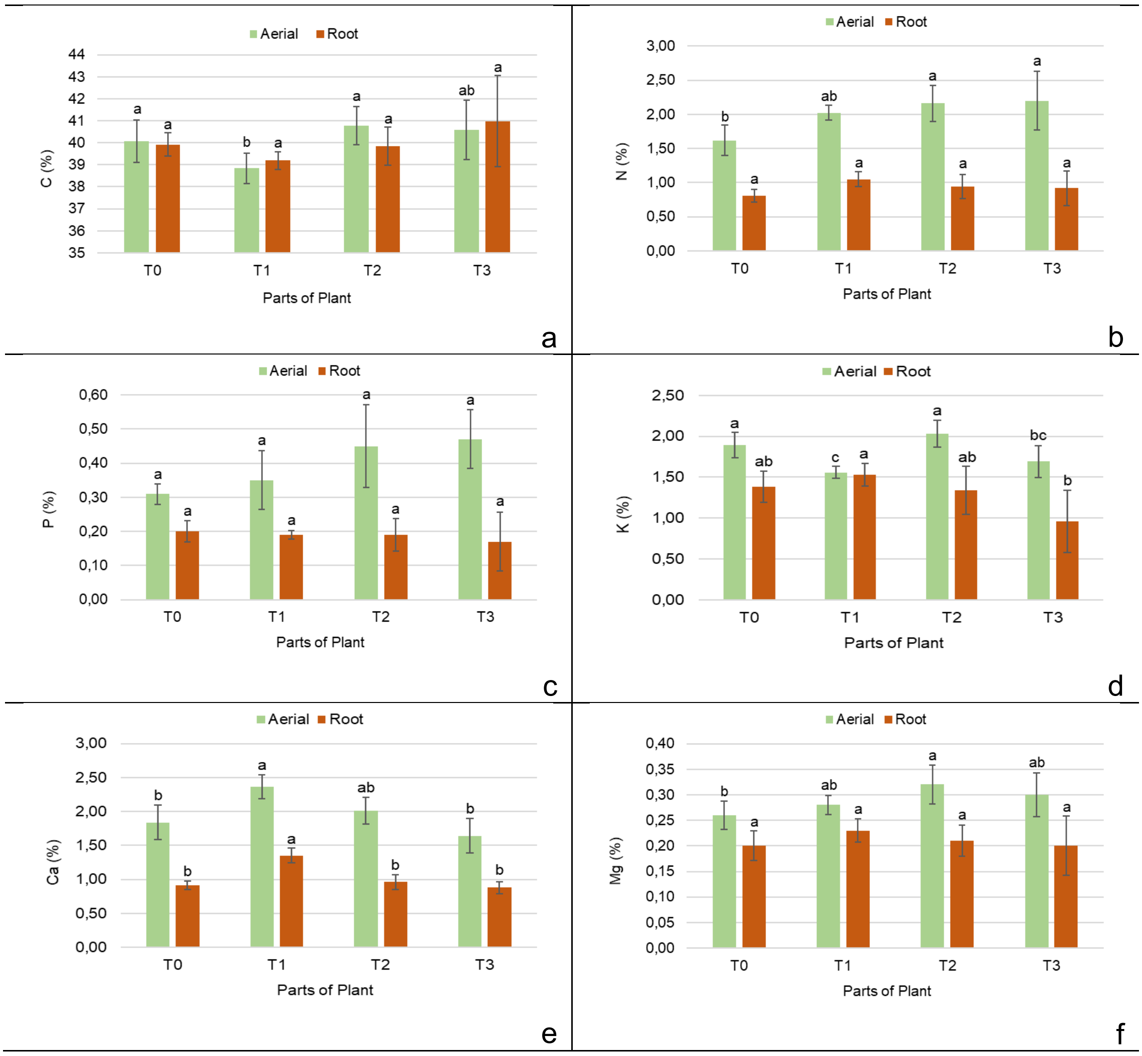
Phosphorus (P) concentrations ranged from 0.31% to 0.47% in the shoots and from 0.17% to 0.20% in the roots. Although the highest values in the shoots were observed in treatments T2 and T3, the differences were not statistically significant. Similarly, concentrations in the roots remained stable, with no distinction between treatments.

In the shoots, potassium (K) concentrations did not show a clear trend. Treatments T0 and T2 did not differ significantly from each other, but were distinct from T3, which showed no difference compared to T1. In the roots, treatments T0, T1, and T2 differed significantly from T3.

Calcium (Ca) showed a similar behavior to potassium, with higher concentrations in the shoots (1.64% to 2.36%) than in the roots (0.88% to 1.35%). Treatment T1 stood out with the highest mean value in the shoots, differing significantly from treatments T0 and T3, but with no difference compared to T2. In the roots, the differences were slight, although T1 had a significantly higher value than the other treatments.

Magnesium (Mg) levels ranged from 0.26% to 0.32% in the shoots and from 0.20% to 0.23% in the roots. In the shoots, the treatments with sludge addition (T1, T2, and T3) did not differ from each other, but showed significantly different values than T0. In the roots, concentrations remained homogeneous, with no significant differences between treatments.

Finally, sulfur (S) concentrations, like those of other nutrients, were higher in the shoots (0.35% to 0.39%) compared to the roots (0.11% to 0.23%). There were no significant differences between the treatments in the shoots; however, in the roots, T0 had significantly higher levels than the treatments with sludge addition (T1, T2, and T3).



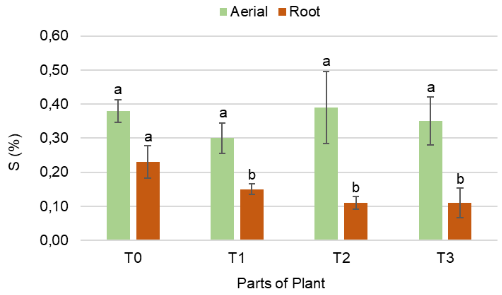


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Fig. 48. Chemical analysis of the percentages of carbon (C) (a), and macronutrients nitrogen (N) (b), phosphorus (P) (c) and potassium (K) (d), as well as the exchange cations calcium (Ca) (e), magnesium (Mg) (f) and sulfur (S) (g), in the roots of plants subjected to treatments T0, T1, T2 and T3. Different letters indicate significant difference between treatments by Tukey's HSD test ($p < 0.05$), applied separately to data from the aerial part and the root part. The results are expressed as a percentage (%).

- Micronutrients (Cu, Zn, Mn, Fe, Na and B) and heavy metals (Cr and Ni).

Table 10 presents the mean values and standard deviations of the concentrations of micronutrients (Cu, Zn, Mn, Fe, Na, and B) and heavy metals (Cr and Ni) determined in the aerial and root parts of seedlings subjected to the different treatments. The results demonstrate the accumulation pattern of these elements as a function of sewage sludge doses, as well as their distribution among the plant parts.

In general, a distinct behavior was observed regarding macronutrients and base cations: while these were predominantly concentrated in the aerial parts, micronutrients presented higher levels in the roots, with the exception of Zn, Mn, and B, which accumulated in greater quantities in the aerial parts. The treatments with sludge addition (T1, T2, and T3) presented higher values for most elements, both in the aerial parts and in the roots, when compared to the control (T0). In the shoots, copper (Cu) showed a progressive increase with increasing sludge doses, rising from 2.62 mg/kg (T0) to 6.50 mg/kg (T1), 11.98 mg/kg (T2), and 9.75 mg/kg (T3). Manganese (Mn) also showed a significant increase, ranging from 92.83 mg/kg in the control to 295.6 mg/kg, 595 mg/kg, and 500.75 mg/kg in subsequent treatments, being one of the elements with the highest concentration in the shoots.

Zinc (Zn), iron (Fe), chromium (Cr), and nickel (Ni) levels followed a similar trend, with consistent increases across treatments. The average Zn concentrations were 26.12, 51.08, 77.36, and 86.13 mg/kg, while Fe was 110.12, 159.4, 203.8, and 223.25 mg/kg for T0, T1, T2, and T3, respectively. Cr and Ni were not detected in the control treatment, but were present in the sludge mixtures, ranging from 8.45 to 15.91 mg/kg and from 4.43 to 7.82 mg/kg, respectively.

Sodium (Na) varied without a defined trend, ranging from 1251.6 mg/kg (T2) to 1668.25 mg/kg (T3), while boron (B) maintained similar values between treatments, between 42.6 mg/kg (T2) and 56.4 mg/kg (T0). In the roots, concentrations were generally higher than those observed in the shoots, confirming the preferential accumulation of micronutrients in this part of the plant. Cu varied from 6.06 mg/kg (T0) to 16.52 mg/kg (T1), maintaining similar values in treatments with higher sludge proportions (12.33 mg/kg and 12.79 mg/kg). Zn showed a similar behavior, with levels of 22.07 mg/kg (T0) and increasing to 56.1 mg/kg (T1), remaining stable at 54.96 mg/kg (T2) and 51.18 mg/kg (T3).

Mn showed continuous growth with sludge doses, increasing from 51.03 mg/kg (T0) to 171.15 mg/kg (T3). Fe stood out as one of the most abundant elements, ranging from 960.5 mg/kg (T0) to 1921.2 mg/kg (T1), 1141.4 mg/kg (T2), and 1327.75 mg/kg (T3).

Na levels remained high in all samples, ranging from 1764.4 mg/kg (T2) to 2525.4 mg/kg (T1), with no dose-response pattern. B showed small variations, ranging from 27.83 mg/kg (T3) to 36.68 mg/kg (T1).

Regarding metals, Cr was detected in all treatments, ranging from 95.51 mg/kg (T2) to 174.09 mg/kg (T1), while Ni levels ranged from 46.82 mg/kg (T2) to 82.75 mg/kg (T1), both with higher levels in the roots.

Overall, the results indicate a more pronounced accumulation of Fe, Mn, Zn, Cr, and Ni in the roots, and higher levels of Zn, Mn, and B in the shoots.

Aerial Parameter	T0	T1	T2	T3
Cu (mg/Kg)	2,62 ± 0,45	6,5 ± 0,99	11,98 ± 2,71	9,75 ± 2,56
Zn (mg/Kg)	26,12 ± 3,45	51,08 ± 5,14	77,36 ± 16,73	86,13 ± 5,75

Mn (mg/Kg)	92,83 ± 23,87	295,6 ± 49,58	595 ± 78,26	500,75 ± 50,17
Fe (mg/Kg)	110,12 ± 28,59	159,4 ± 22,43	203,8 ± 38,89	223,25 ± 49,82
Na (mg/Kg)	1377,83 ± 146,54	1540,2 ± 280,99	1251,6 ± 172,94	1668,25 ± 615,31
B (mg/Kg)	56,4 ± 13,51	47,5 ± 7,34	42,62 ± 3,77	51,63 ± 9,94
Cr (mg/Kg)	-	8,45 ± 2,20	12,96 ± 7,18	15,91 ± 6,80
Ni (mg/Kg)	-	4,43 ± 1,45	6,42 ± 3,09	7,82 ± 3,42
Root Parameter	T0	T1	T2	T3
Cu (mg/Kg)	6,06 ± 1,76	16,52 ± 2,33	12,33 ± 2,33	12,79 ± 6,25
Zn (mg/Kg)	22,07 ± 3,09	56,1 ± 6,80	54,96 ± 19,49	51,18 ± 23,31
Mn (mg/Kg)	51,03 ± 11,14	126 ± 15,38	169,6 ± 29,64	171,15 ± 89,95
Fe (mg/Kg)	960,5 ± 364,09	1921,2 ± 420,41	1141,4 ± 455,21	1327,75 ± 710,59
Na (mg/Kg)	2147,83 ± 382,14	2525,4 ± 554,66	1764,4 ± 232,67	2000,75 ± 719,83
B (mg/Kg)	31,3 ± 4,46	36,68 ± 5,96	28,1 ± 3,37	27,83 ± 5,84
Cr (mg/Kg)	108,89 ± 59,53	174,09 ± 51,29	95,51 ± 48,65	128,52 ± 39,03
Ni (mg/Kg)	51,6 ± 28,13	82,75 ± 23,49	46,82 ± 22,84	63,19 ± 19,02

Table 10. Means and standard deviations values of micronutrient and heavy metals contents (expressed in mg/Kg) in the aerial and root parts of the plants, by treatment (T0, T1, T2 and T3).

Figure 49 (a–h) show the significance comparisons between treatments for micronutrient and heavy metal concentrations, considering both the shoots and roots of the seedlings. In addition to the statistical differences between treatments, it is possible to observe in which plant compartments the greatest accumulation of each element occurred.

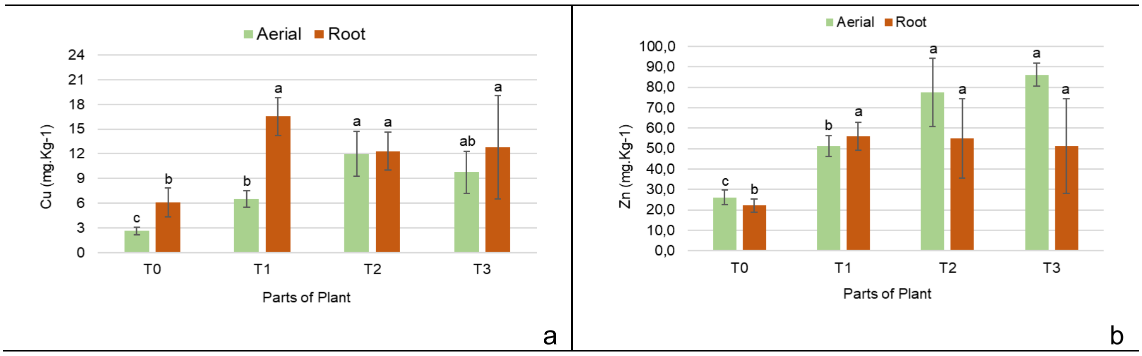
Copper (Cu) did not show a defined pattern across plant parts. In the shoots, treatments T2 and T3 exhibited the highest concentrations, differing significantly from T1, which in turn was higher than T0. In the roots, the treatments with sludge addition (T1, T2, and T3) did not differ from each other but presented significantly higher values than the control.

Zinc (Zn) showed a more consistent behavior, with concentrations predominating in the shoots. Only treatment T1 showed a higher concentration in the roots, while T0, T2, and T3 maintained accumulation in the shoots. Among the treatments, in the aerial part, T2 and T3 did not differ significantly from each other and presented the highest averages, followed by T1, which exceeded T0. In the roots, the sludge treatments did not differ from each other, but all presented significantly higher concentrations than the control.

Manganese (Mn) showed a similar pattern to Zn, with accumulation predominating in the aerial parts. Treatments T2 and T3 recorded the highest averages, differing from T1 and T0, which exhibited the lowest levels. In the roots, the values were lower and without significant differences between the treatments that received sludge, differing only from the control.

Iron (Fe) showed the opposite behavior, concentrating in the roots. Concentrations were significantly higher in the sludge treatments (T1, T2, and T3) compared to T0, while the differences between the residue treatments were not significant. In the aerial part, the same pattern was observed, with low levels and no statistical difference between the treatments that received sludge, but higher than the control. Neither sodium (Na) nor boron (B) showed significant differences between treatments in any part of the plant. Na was more concentrated in the roots, regardless of treatment, while B was higher in the aerial parts.

Finally, chromium (Cr) and nickel (Ni) exhibited the same accumulation pattern, with much higher concentrations in the roots than in the aerial parts. In both cases, no significant differences were observed between treatments.



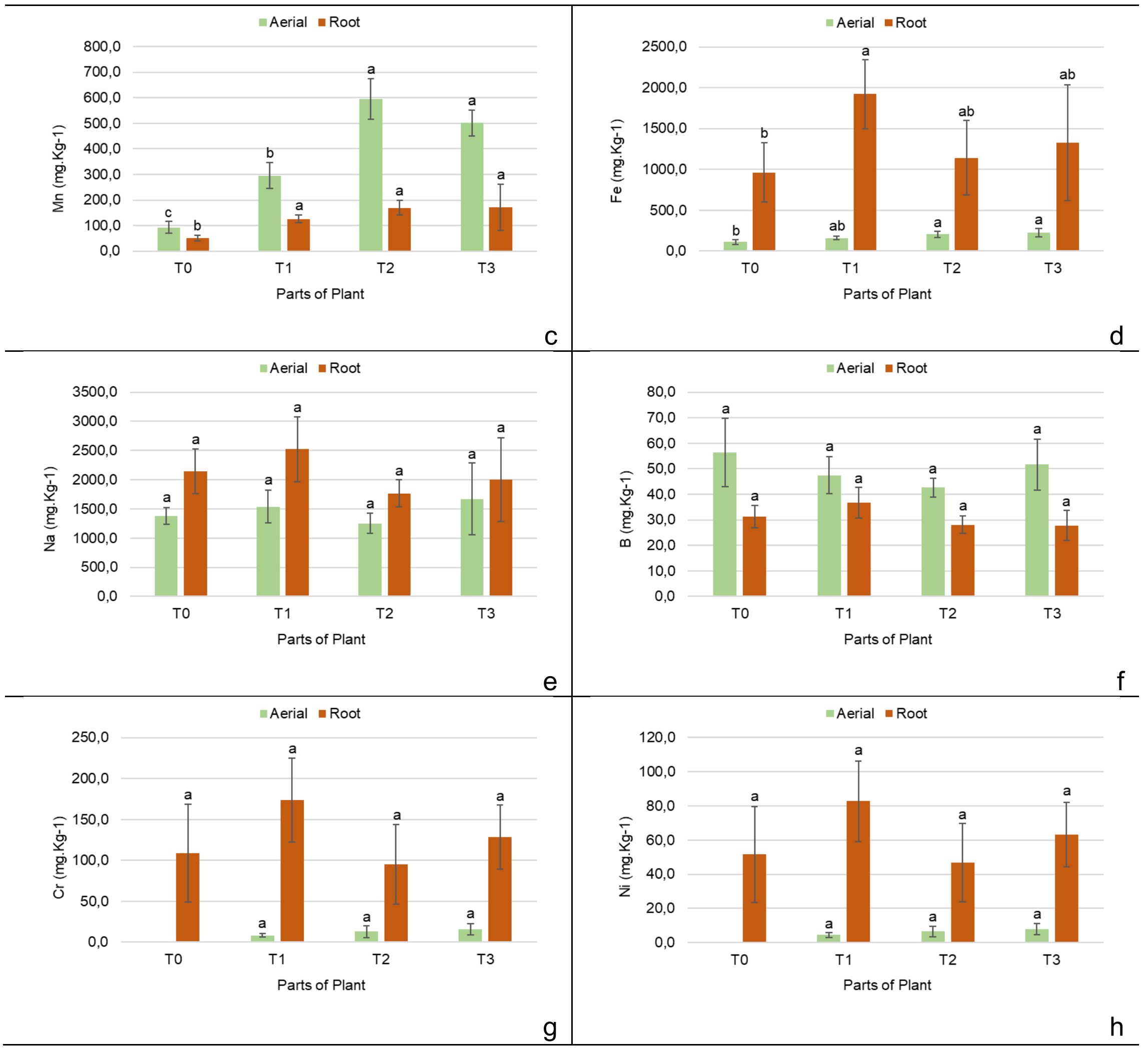


Fig. 49. Comparison of the levels of Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Sodium (Na), Boron (B), chromium (Cr) and nickel (Ni) levels in the aerial and root parts of plants in treatments T0, T1, T2, and T3. Different letters indicate significant difference between treatments by Tukey's HSD test ($p < 0.05$), applied separately to data from the aerial part and the root part. Results are expressed in mg/kg.

2.2.4 Multivariate Statistics

We applied Principal Component Analysis (PCA) to summarize the variability of the data and facilitate visualization, highlighting patterns and groupings between variables and treatments, without inferring causality. The input data were organized into three sets: (A) physical-chemical parameters of the soil at the end of the experiment; (B) final morphological variables of the plant (height, diameter, aboveground biomass and root biomass); and (C) chemical and nutritional parameters of the plant, evaluated by replication, with C1 being the aboveground part and C2 the roots. PCA was applied to the combinations A+B, A+C1, A+C2, B+C1, B+C2 and C1+C2, with the aim of reducing the dimensionality of matrices with many variables and revealing possible structural patterns between the sets. PCA analysis reveals the distribution between treatments along two main axes, highlighting the chemical and structural relationships induced by the progressive application of sewage sludge in urban substrates for the production of Nerium oleander. These results exemplify the multifaceted effects of sludge on essential properties, nutrients, and potentially toxic elements (PTEs), as well as the vegetative response of plants, offering important practical and applicable information for the management of alternative substrates in urban environments. In figure 50, you can see the principal component analysis (PCA), applied to groups A (soil physicochemical parameters) and B (final morphological variables of the plants), revealed striking distinctions between the treatments tested. The first two components together explained 74.3% of the total variance of the data, with 43.8% attributed to Dimension 1 (PCA1) and 30.5% to Dimension 2 (PCA2). In PCA1, the variables with the greatest contribution were shoot biomass, cation exchange capacity (CSC), and organic carbon, while in PCA2, pH, available phosphorus (P_av), and manganese (Mn) stood out. The biplot graph showed a clear separation between the treatments, with T0 (peat-based control) clearly isolated from the others, showing an association with organic carbon (OC), CSC, and plant growth variables, a result consistent with the composition of the commercial substrate. Treatment T3, with 75% sewage sludge, clustered near the vectors of phosphorus, copper, and zinc, suggesting a greater accumulation of these nutrients. Treatment T2 formed an intermediate group, reflecting a certain balance between morphological and chemical attributes. Treatment T1 showed a stronger relationship with high pH and manganese concentration, which is consistent with its higher proportion of calcareous urban soil, which presented a high Mn content. The directions and lengths of the vectors revealed significant positive correlations between OC, CSC, and plant growth parameters, while pH and Mn showed negative correlations with these variables. Furthermore, high concentrations of micronutrients, as well as high available phosphorus, also demonstrated negative correlations with plant development.

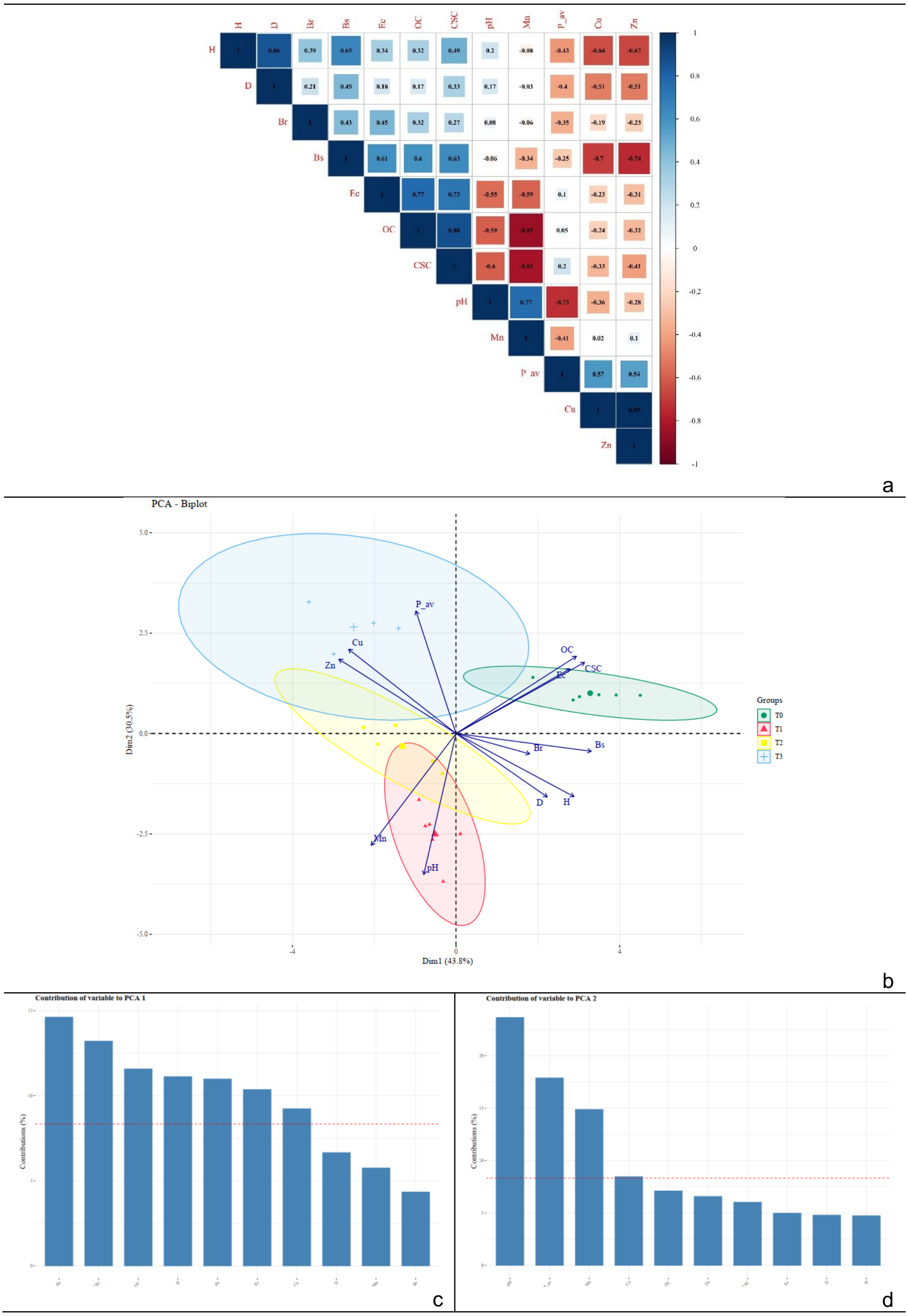


Fig. 50. PCA between soil physicochemical parameters and plant growth parameters (A + B). (a) Pearson correlation matrix; (b) PCA Biplot; (c) PCA1 contribution; (d) PCA2 contribution.

In figure 51, you can see the principal component analysis (PCA), applied to groups A (physicochemical parameters of the soil) and C1 (chemical analyses of the aerial part of the plants), revealed well-defined patterns among the treatments. The first two components together explained 65.2% of the total variance of the data, with 41.7% attributed to Dimension 1 (PCA1) and 23.5% to Dimension 2 (PCA2). In PCA1, the main contributions were highlighted by the levels of zinc, copper, and manganese accumulated in the aerial part, while in PCA2, the most influential variables were pH, available phosphorus, and manganese. The biplot graph showed a clear separation between the groups, with treatment T0 located on the left, associated with the vectors of organic carbon (OC), cation exchange capacity (CSC), and electrical conductivity (Ec), which reflects the more stable and homogeneous composition of the commercial peat-based substrate. On the other hand, treatment T3 was strongly associated with copper, zinc, and available phosphorus vectors, suggesting a greater accumulation of these elements in both the soil and the aerial parts of the plants. Group T1 showed a relationship with high pH and manganese levels, as well as greater calcium accumulation in the aerial parts, consistent with its higher proportion of calcareous urban soil, while T2 formed an intermediate group, distributed between the two extremes. The directions and intensities of the vectors indicated positive correlations between the nutrients accumulated in the aerial parts and the levels of available phosphorus, copper, and zinc in the soil, while pH and manganese showed negative correlations with these variables, as well as with fertility indicators such as OC and CSC.

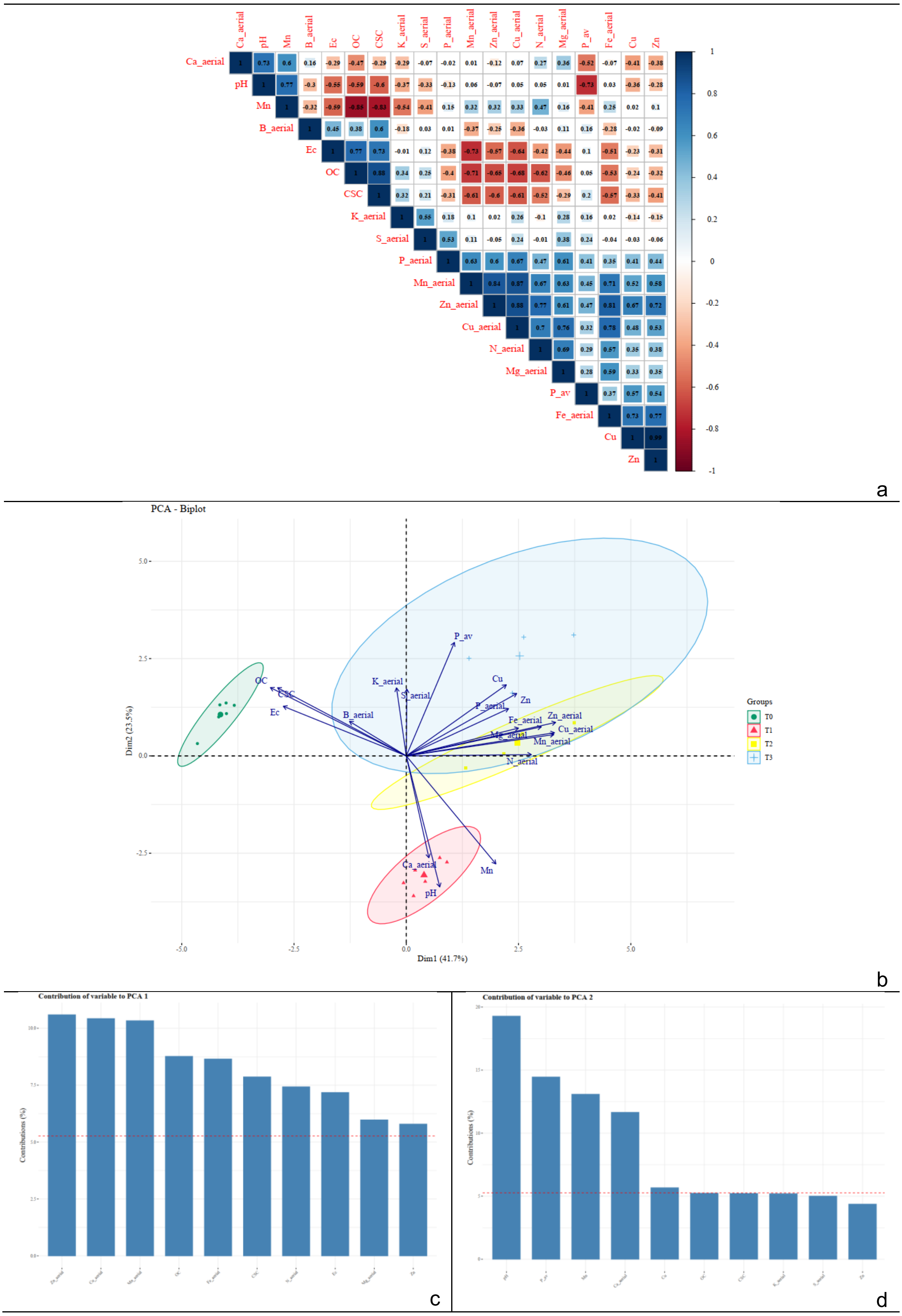


Fig. 51. PCA between soil physicochemical parameters and chemical parameters of the aerial part of plants (A + C1). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2.

In figure 52, you can see the principal component analysis (PCA), conducted using groups A (physicochemical parameters of the soil) and C2 (chemical analyses of the root system of the plants), allowed the identification of patterns, especially between the control treatment (T0) and the treatments with sewage sludge application, regardless of the percentage applied. The first two components together explained 70.7% of the total variance of the data, with 43.3% attributed to Dimension 1 (PCA1) and 27.4% to Dimension 2 (PCA2). In PCA1, the variables that contributed most were the concentrations of Cu and N in the root, in addition to Mn in the soil. In PCA2, the levels of S in the root and Zn and Ca in the soil stood out. The biplot graph revealed a clear separation between T0, located in the upper left quadrant, strongly associated with the vectors of OC, CSC, Ec and sulfur in the root, and the other treatments. Regarding the clustering of treatments with sludge application, a progressive differentiation was observed between them, with T1 showing the greatest distinction, followed by T2 and T3. Groups T1, T2, and T3 formed partially overlapping areas, with T1 being more associated with pH, while T2 and T3 were related to high and varied concentrations of metallic macro and micronutrients, both in the soil (Cu, Zn, and Mn) and in the roots (N, Ca, Mg, Mn, Zn, and Cu). The directions and lengths of the vectors indicated neutral to positive correlations between the nutrients accumulated in the soil and roots, while S, OC, and CSC showed negative correlations with these variables, reinforcing the role of substrate type in the nutritional dynamics observed in the plants.

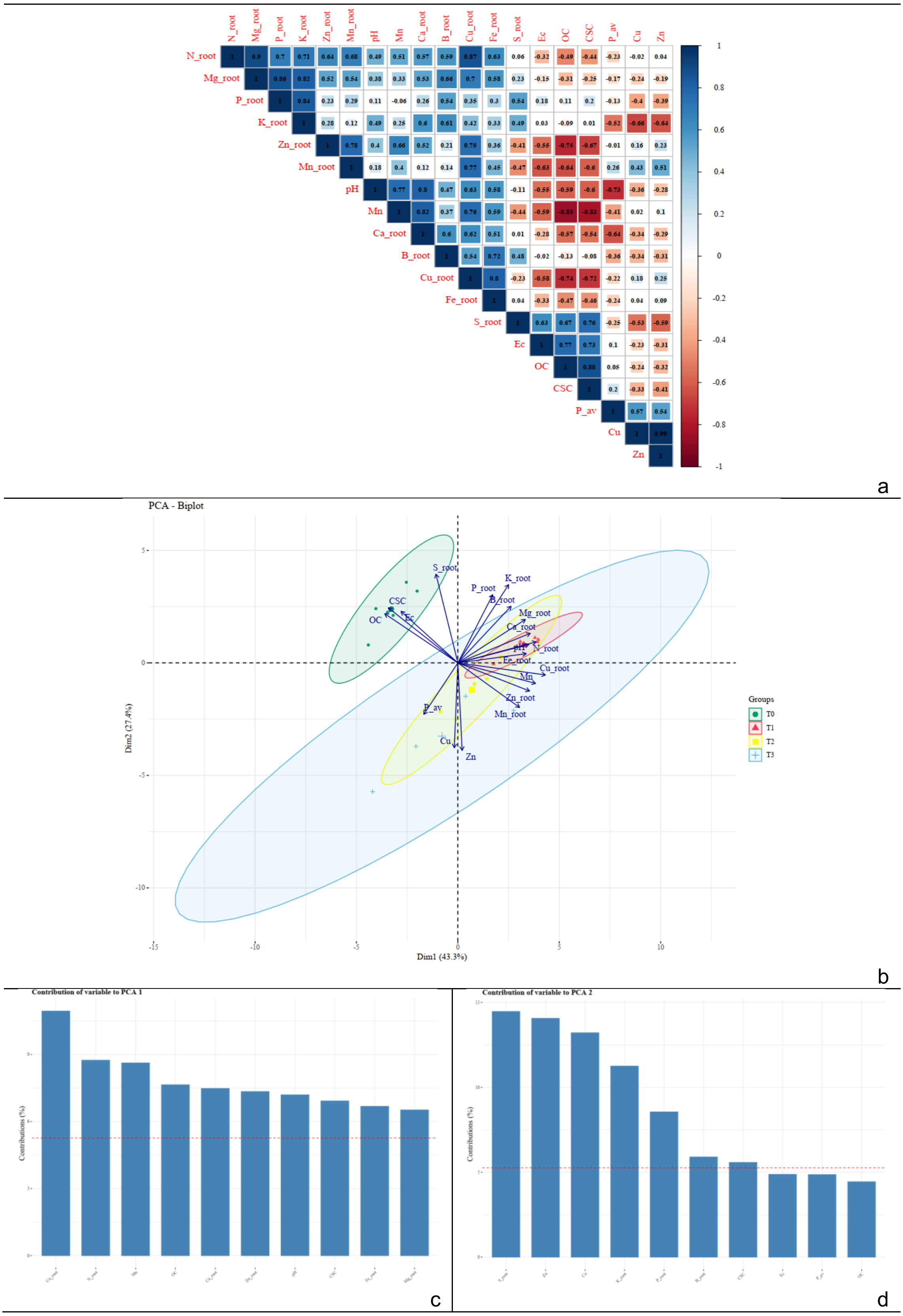


Fig. 52. PCA between soil physicochemical parameters and chemical parameters of the root part of plants (A + C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2.

In figure 53, you can see the principal component analysis (PCA), performed based on groups B (final morphological variables of the plant) and C1 (chemical analyses of the aerial part), revealed important patterns for understanding the relationship between mineral nutrition and plant development. The first two components together explained 60.2% of the total variance of the data, with 43.9% attributed to Dimension 1 (PCA1) and 16.3% to Dimension 2 (PCA2). In PCA1, the main contributors were the Cu, Mn, and Zn contents in the aerial part, as well as the aerial biomass (Bs), while in PCA2 the stem diameter (D) and the Ca and Mg contents in the aerial part stood out. The biplot graph demonstrated a clear separation between treatments, with T0 located on the left of the graph, strongly associated with morphological variables such as aerial and root biomass, height and stem diameter, as well as the boron content in the aerial part. Treatment T1 appeared in a centralized position, more directly associated with calcium accumulation, but still maintaining some proximity to morphological parameters. In contrast, treatments T2 and T3 did not show significant differences between them, mainly associated with higher levels of metallic micronutrients in the aerial part, such as Cu, Zn, Fe, and Mn, in addition to N and P. The directions and intensities of the vectors indicated positive correlations between the accumulation of these nutrients in the aerial part and the treatments with a higher proportion of sewage sludge, while the morphological parameters showed a stronger correlation with the control treatment and with the lowest percentage of sludge (25%). These results indicate that, although treatments T2 and T3 promoted greater absorption and translocation of metallic nutrients, this did not result in clear improvements in plant growth and development when compared to treatment T1 and, especially, to the control.

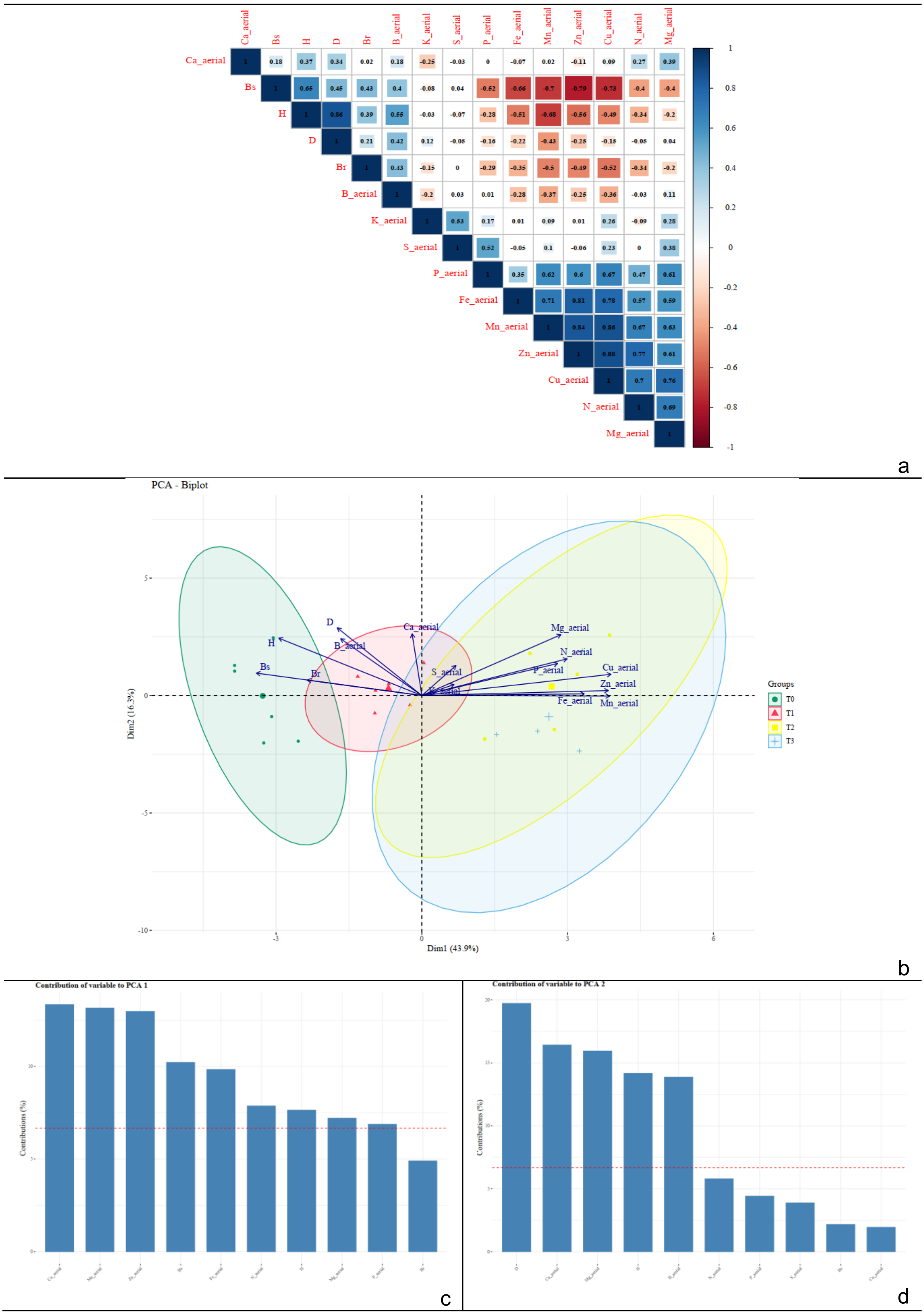


Fig. 53. PCA between plant growth parameters and chemical parameters of the aerial part of plants (B+C1). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

In figure 54, you can see the principal component analysis (PCA), performed based on groups B and C2, revealed distinct distribution patterns among the treatments, allowing us to relate plant development to nutrient accumulation in the roots. The first two components together explained 72.6% of the total data variance, with 42.6% attributed to Dimension 1 (PCA1) and 30% to Dimension 2 (PCA2). In PCA1, the most prominent variables were the Mg, K, and N contents in the root, while in PCA2, the most influential variables were S and Mn in the root, followed by shoot biomass. The biplot graph indicated a clear separation between the groups, with treatment T0 strongly associated with morphological variables such as shoot and root biomass (Bs and Br), height (H), stem diameter (D), and boron content in the roots. Treatment T1 formed a centralized group, showing intermediate behavior, associated with moderate nutrient contents and balanced morphological performance. Treatment T2 showed less clustering, being more related to the accumulation of K, Ca, and Mg in the roots. Treatment T3 was the least clustered, with a more dispersed distribution, associated with high levels of metallic micronutrients such as Cu, Zn, and Mn in the roots, but with an inverse correlation to the morphological parameters. The directions and intensities of the vectors indicated positive correlations between the accumulation of macro and micronutrients in the root and the treatments with higher application of sewage sludge, while the morphological variables showed a stronger correlation with the control treatment (T0), suggesting that the excess of certain nutrients did not result in better plant growth performance.

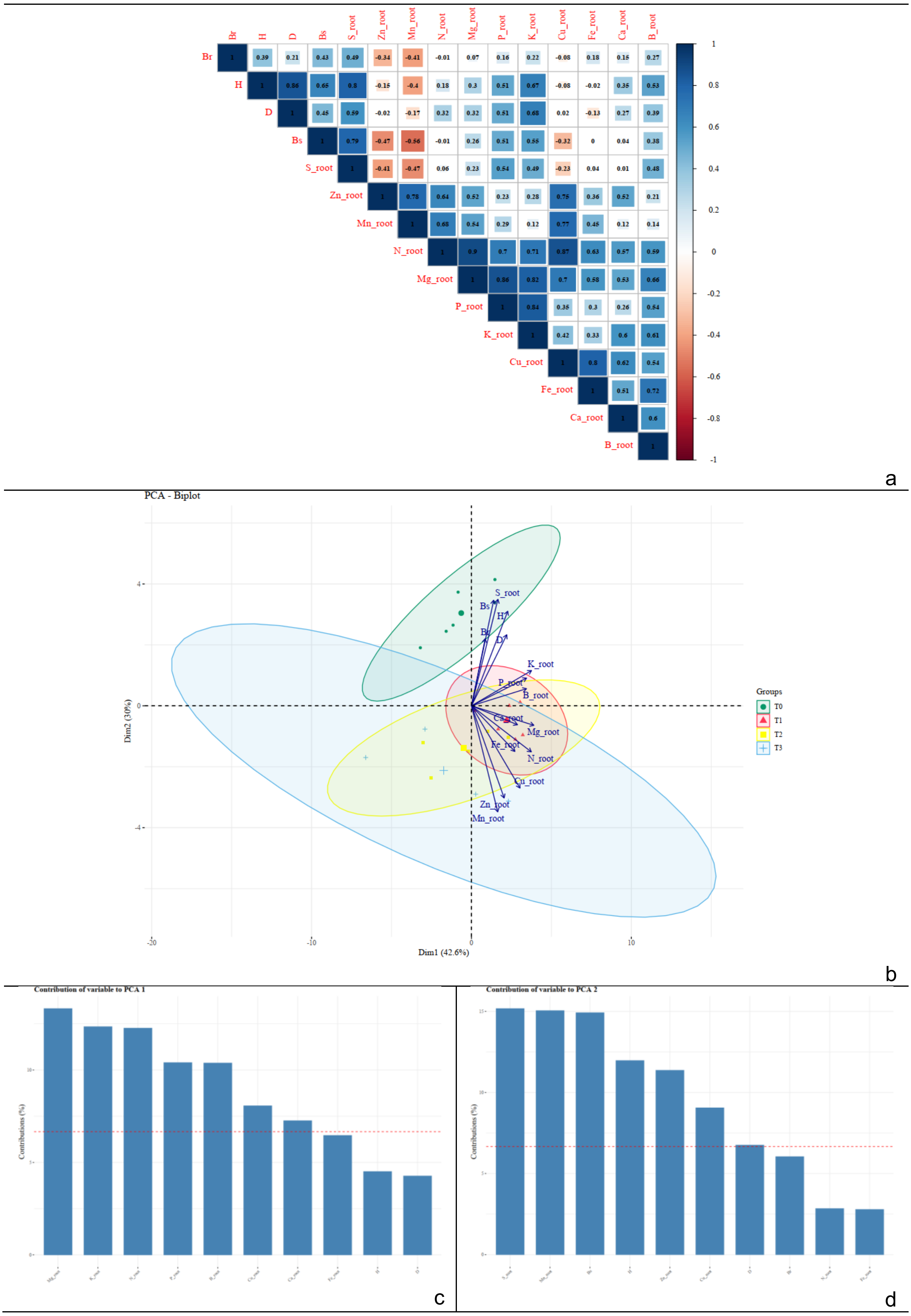


Fig. 54. PCA between plant growth parameters and chemical parameters of the root part of plants (B+C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

In figure 55, you can see the principal component analysis (PCA), performed between groups C1 and C2, allowed the identification of relationships between nutrient accumulation in different parts of the plant. The first two components together explained 63.6% of the total variance, with 34.2% attributed to Dimension 1 (PCA1) and 29.4% to Dimension 2 (PCA2). In PCA1, the levels of Zn, Cu, and Mn in the aerial part stood out, while in PCA2, the variables with the greatest contribution were B, Mg, Fe, and Cu in the root. The biplot graph showed a separation between treatments, mainly with T0 (control) clearly distinct from the treatments with sewage sludge application. T0 was strongly associated with sulfur accumulated in the root, a factor that differentiated it from the others. Treatment T1 formed a more compact and centralized group, related to moderate levels of macro and micronutrients such as B, K, P, and Mg in the roots, in addition to Ca in the aerial part. Treatments T2 and T3 showed similar behavior, with no significant differences between them, exhibiting similar nutritional patterns in terms of nutrient absorption and redistribution in the plant. T2 showed slight dispersion and indicated a balanced absorption dynamic, with significant nutrient accumulation in the roots and translocation to the aerial parts. T3, in turn, was the most dispersed and least clustered group among the treatments, strongly associated with the accumulation of metallic micronutrients such as Cu, Zn, Mn, and Fe, both in the root and in the aerial parts, revealing, like T2, a significant absorption of these elements from the soil and their subsequent translocation to the aerial tissues. The directions and lengths of the vectors indicated positive correlations between the accumulation of micronutrients in different parts of the plant and the treatments with higher sludge application, while sulfur in the roots, associated with T0, showed an inverse correlation. These results suggest that the intensive application of sewage sludge favors the accumulation of certain nutrients, especially metals, in plants.

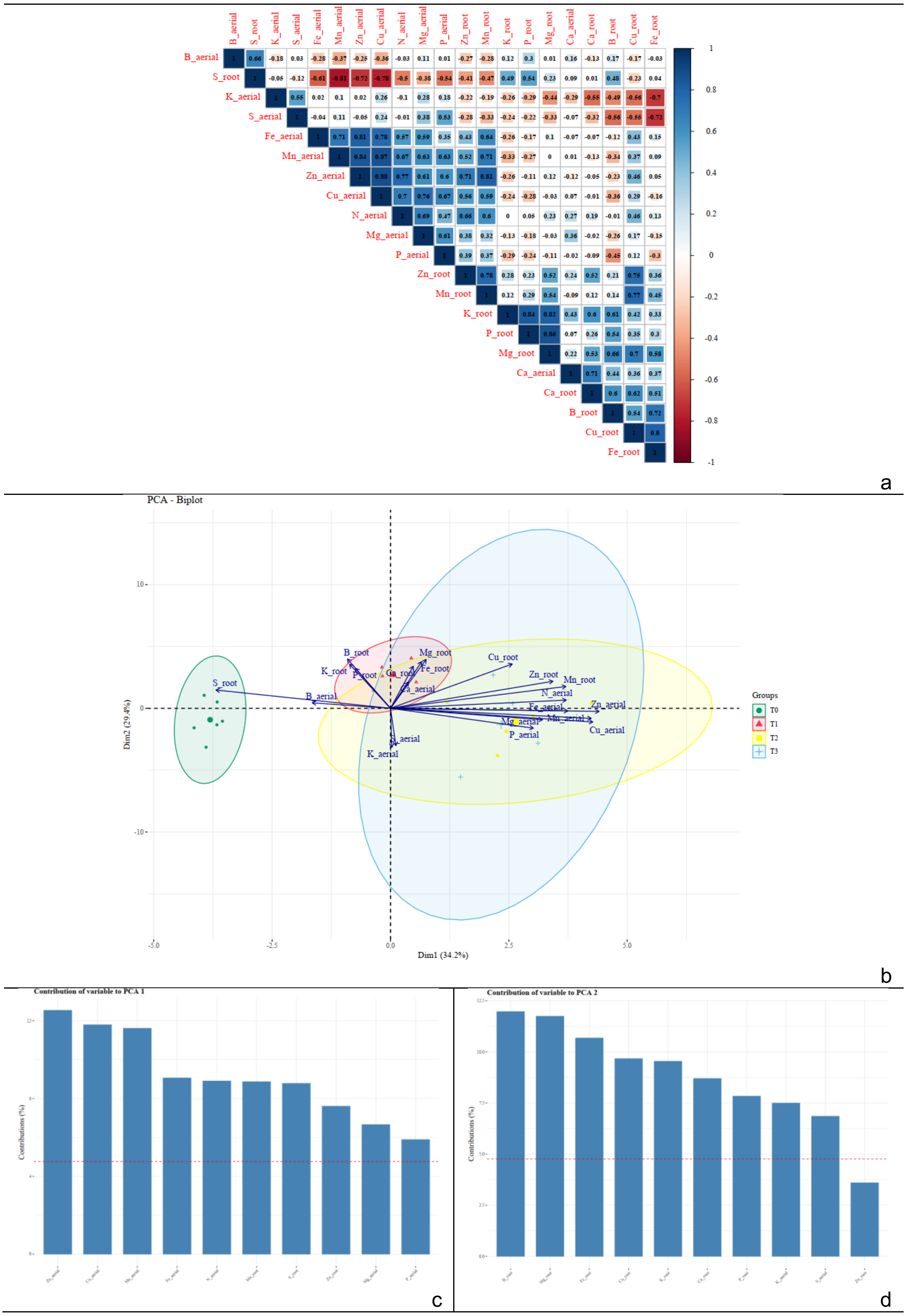


Fig. 55. PCA between chemical parameters of the aerial part of plants and chemical parameters of the root part of plants (C1+C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

The PFA extracts a total of 8 factors, all with eigenvalues greater than 1. Overall, the eight extracted factors account for a total variance of 92% (Table 11).

The first factor (F1) shows a strong positive correlation between a series of nutrients and microelements, especially P_tot (0.975), N (0.970), Zn (0.918), Cu (0.900), Fe (0.914), Pb (0.876) and Cd (0.924). These indicators unmistakably reflect the mineral richness and accumulation of both nutrients and potentially phytotoxic elements resulting from the massive addition of sewage sludge, a phenomenon well-documented in the agronomic literature (Yakamercan et al. 2021; Filipi et al. 2023). However, there is also a strong negative correlation between these factors and vegetative growth parameters: plant height (H: −0.594), diameter (D: −0.442), aboveground biomass (Bs: −0.749), and a weaker one for root biomass (Br: −0.356). This pattern suggests that even with a substrate rich in macro- and micronutrients, there is a contraction of plant performance: growth and biomass are depressed when potentially toxic elements and nutrients exceed optimal uptake thresholds (Singh e Agrawal 2008), likely due to ionic toxicity effects, osmotic imbalances, or metabolic disruptions in plant tissues. Based on these observations, this factor can be termed a “nutrient-metal cluster with inhibitory effect on biomass,” as it reflects mineral richness and the potential phytotoxicity that may compromise plant growth if PTE levels become too high.

The second factor (F2) is dominated by a multicorrelation among variables such as pH (0.749), Mn (0.905), Co (0.862), OM (−0.908), OC (−0.907), CEC (−0.845), EC (−0.780), as well as root (Br: −0.611) and aboveground biomass (Bs: −0.498). The negative correlation in OM, OC, CEC, and EC indicates that substrate portions richer in peat and lower in salinity yield greater root mass, a more balanced pH, and lower concentrations of mobile metals. The increase in Mn and Co on the positive side highlights the role of these micronutrients in root physiology and their close dependence on substrate matrix stability, as confirmed by (Vítková et al. 2024), who stressed the crucial function of organic content in activating/controlling metal ion bioavailability. For these reasons, this factor can be named “organic stability-cation exchange and root health,” emphasizing the

centrality of commercial organic components for maintaining ionic balance and promoting efficient root morphology even with variations in pH and micronutrients.
The third factor (F3) features the strongest positive correlations for Crroot (0.968) and Niroot (0.964), as well as Feroot (0.825), Broot (0.692), and Naroot (0.621). This suggests the efficiency (or risk) of heavy metal accumulation in roots, Cr and Ni in particular, a phenomenon that the literature associates with oxidative stress and potential impacts on plant health (Singh e Agrawal 2008). This factor can be defined as "metal accumulation and risk in roots," indicating the roots' ability to sequester heavy metals under conditions of strong substrate input.
The fourth factor (F4) reveals the strongest correlations for the root absorption capacities of Mg (0.913), P (0.893), N (0.866), and K (0.761), with a strong negative correlation for Croot (−0.739). This means that during intense uptake of essential nutrients, root carbon accumulation decreases, likely due to higher cellular turnover or the use of structural reserves (Silva et al. 2022). This factor may therefore be named "root uptake efficiency for NPK and Mg," indicating a dynamic nutritional state where the root prioritizes immediate absorption over accumulating structural organic reserves.
The other four factors have very low importance in terms of variance (<5), representing minor elements with weaker correlations, as they are already extensively explained by the first four factors.
Ultimately, interpretation of the PFA clearly reveals a multilayered framework in which: i) sludge-enriched substrate provides greater nutritional potential but, if not carefully managed regarding concentrations, can harm growth by exceeding phytotoxicity limits; ii) organic stability from peat supports a healthy, vigorous root environment; iii) there is a risk of heavy metal accumulation in root tissues; and iv) the root's nutritional response to N, P, K, Mg is sensitive to the organic/mineral status. Ultimately, extremely balanced and rational peat–sludge management strategies are required to guarantee productivity and phytosanitary safety, as also confirmed by recent international best practices (Abreu-Junior et al. 2017; Guerrini et al. 2017; Filipi et al. 2023).

Parameters	F1	F2	F3	F4	F5	F6	F7	F8

H	-0,594	-0,205	0,034	0,304	0,022	0,322	-0,255	0,519
D	-0,442	-0,089	-0,162	0,404	0,274	0,357	-0,280	0,439
Bs	-0,749	-0,498	0,203	0,228	-0,074	0,044	0,050	0,050
Br	-0,356	-0,611	0,196	0,045	-0,152	0,308	0,395	0,180
pH	-0,284	0,749	0,268	0,162	-0,094	0,346	-0,128	-0,047
Ec	-0,245	-0,780	-0,052	-0,043	-0,229	0,036	0,065	0,204
P_av	0,535	-0,317	-0,077	-0,106	0,246	-0,612	0,130	0,086
P_tot	0,975	-0,037	-0,048	-0,068	0,024	-0,154	0,094	-0,033
N	0,970	-0,145	-0,007	-0,133	-0,055	-0,017	0,064	0,002
OC	-0,246	-0,907	-0,104	-0,100	-0,167	0,117	-0,119	-0,007
OM	-0,247	-0,908	-0,104	-0,100	-0,166	0,116	-0,120	-0,009
CSC	-0,334	-0,845	-0,049	-0,105	0,002	-0,095	-0,230	0,262
Cu	0,900	-0,000	-0,042	-0,110	0,090	-0,008	0,387	0,048
Zn	0,918	0,086	-0,032	-0,085	0,087	-0,017	0,351	0,025
Mn	-0,005	0,905	0,242	0,108	-0,075	0,097	0,215	0,058
Co	-0,137	0,862	0,353	0,048	0,078	0,085	0,236	0,040
Pb	0,876	0,318	0,016	-0,097	0,080	0,018	0,333	-0,001
Cr	0,356	0,523	-0,002	-0,085	0,211	-0,045	0,700	0,058
Ni	0,284	0,307	0,058	-0,153	0,262	-0,028	0,808	0,036
Cd	0,924	0,116	-0,020	-0,141	0,019	0,046	0,311	-0,015
N_aerial	0,198	0,470	-0,033	0,179	0,550	-0,349	0,362	0,068
C_aerial	0,487	-0,334	-0,186	-0,110	0,174	0,037	-0,120	-0,663
Ca_aerial	-0,520	0,559	0,286	-0,058	0,321	0,212	0,090	0,322
Mg_aerial	0,152	0,328	-0,237	-0,062	0,792	-0,104	0,238	0,183
K_aerial	-0,085	-0,230	-0,498	-0,308	0,450	-0,061	-0,449	-0,275
P_aerial	0,296	0,361	-0,563	-0,054	0,151	-0,357	0,239	0,294
S_aerial	-0,185	-0,157	-0,667	-0,272	0,228	-0,122	0,145	0,010
Zn_aerial	0,673	0,461	-0,194	0,170	0,365	-0,283	0,100	0,000
Cu_aerial	0,457	0,564	-0,385	-0,025	0,518	-0,135	0,046	-0,071
Mn_aerial	0,502	0,537	-0,126	-0,051	0,375	-0,433	0,103	-0,193
Fe_aerial	0,731	0,303	-0,068	0,147	0,490	0,122	0,127	-0,150
Na_aerial	0,475	-0,003	0,156	-0,361	-0,041	0,610	0,127	0,082
B_aerial	-0,146	-0,465	0,163	0,059	0,159	-0,034	0,181	0,774
Cr_aerial	0,734	0,394	-0,038	0,218	0,412	0,140	0,072	-0,154
Ni_aerial	0,740	0,421	0,007	0,151	0,384	0,143	0,059	-0,198
N_root	0,087	0,370	0,213	0,866	0,091	0,077	0,028	0,081
P_root	-0,278	-0,172	0,122	0,893	0,003	-0,112	-0,111	0,125

K_root	-0,568	0,142	0,087	0,761	0,022	0,130	-0,150	0,097
C_root	0,397	-0,166	-0,284	-0,739	0,219	0,055	0,059	0,057
Ca_root	-0,360	0,690	0,229	0,308	-0,151	0,301	0,097	0,190
Mg_root	-0,123	0,186	0,251	0,913	0,038	-0,069	0,010	-0,002
S_root	-0,480	-0,613	0,286	0,259	-0,050	0,186	-0,217	0,382
Cu_root	0,274	0,629	0,384	0,576	0,016	0,027	0,081	0,056
Zn_root	0,222	0,702	-0,000	0,436	0,123	-0,282	-0,044	0,110
Mn_root	0,560	0,453	0,090	0,545	0,161	-0,333	-0,056	-0,097
Fe_root	0,127	0,331	0,825	0,354	-0,120	0,037	0,101	-0,036
Na_root	-0,181	-0,030	0,621	0,583	-0,157	0,111	0,232	-0,039
B_root	-0,230	0,082	0,692	0,475	0,040	0,229	-0,115	0,151
Cr_root	0,021	0,149	0,968	0,055	0,010	-0,076	0,060	0,094
Ni_root	0,050	0,160	0,964	0,055	0,023	-0,090	0,064	0,113
Proportional variance (%)	40	22	10	7	5	3	3	2
Cumulative variance (%)	40	62	72	79	84	87	90	92
Eigenvalues	22.720	12.586	5.929	3.889	3.034	1.992	1.495	1.266

Table 11. Exploratory Factor Analysis (PFA) performed with soil nutritional variables, plant morphological parameters, and chemical data of the aerial and root parts at the end of the experiment.

2.3 Discussion

2.3.1 Soil

The positive correlation between sludge proportion and soil moisture aligns with findings in previous research, which consistently link elevated organic matter (characteristic of SS) to enhanced water retention and aggregate stability (Singh e Agrawal 2008; Eid et al. 2021). The commercial substrate (T0), predominantly peat-based, also demonstrated substantial moisture retention, corroborating prior research on peat substrates, which favors water availability (Raviv et al. 2002). The observed differences are likely attributable to the distinct physical structure and organic composition of the peat and sludge materials, with sludge's colloidal properties and organic matrix facilitating greater pore space and higher capillarity (Giannakis et al. 2014). These results provide further evidence of the role of organic amendments in improving substrate moisture content, which is particularly relevant for seedling establishment and nutrient cycling in urban and restored soils. The increased moisture retention observed in higher sludge treatments may therefore contribute positively to plant growth and resilience under variable moisture regimes, as noted in comparable studies (Singh e Agrawal 2008; Eid et al. 2021).

Statistical analysis indicates that all substrates containing sludge maintained significantly lower densities than urban soil ($p < 0.05$), supporting the view that sludge application, primarily due to its substantial organic matter content, enhances soil porosity and reduces compaction. These findings align with the literature, which widely documents that organic amendments decrease bulk density and improve soil structure (Wong et al. 1999; Castaldi et al. 2005). The minimal density found in the commercial substrate is consistent with its high-peat composition, historically linked to substantial porosity and very low weight (Raviv et al. 2002). Sludge's reduction of soil density has broad implications for root growth and soil aeration, corroborating previous research showing that sludge-enriched soils foster favorable conditions for seedling root penetration and overall plant establishment (Ferreras et al. 2006). These density modifications through organic amendments serve as an effective strategy for urban and restoration soils, echoing results from comparable experiments and supporting their application in both agronomic and ecological restoration contexts (Wong et al. 1999; Castaldi et al. 2005).

The obtained results for pH show a distinct pattern consistent with the well-documented buffering effect of urban soils and sewage sludge, attributed to their mineral constituents and organic matter content (Singh e Agrawal 2008; Abdul Khaliq et al. 2017). In contrast, peat-based commercial substrates are widely recognized for promoting acidification due to their high humic substances and lack of buffering minerals (Raviv et al. 2002). Significant differences in final pH between treatments support the suggestion that increasing the proportion of sludge results in only a mild decrease in pH, as the inherent alkalinity of sludge and urban soil counteracts the acidifying effects of the substrate over time. This outcome aligns with previous studies, where sludge application typically sustains a near-neutral pH and counterbalances organic-acid-mediated decreases (Wong et al. 1999; Abdul Khaliq et al. 2017). Maintaining neutral to slightly alkaline pH through sludge incorporation can be strategically valuable for improving nutrient availability, reducing phytotoxic risks from heavy metals, and sustaining microbial activity in restoration and urban planting contexts (Singh e Agrawal 2008; Giannakis et al. 2014).

Nitrogen input from sludge is likely the main factor responsible for the increases observed in treatments with higher residue proportions. This N supply capacity was confirmed by (Sevilla-Perea et al. 2014). Mineralization of organic matter during the experimental period may have contributed to the gradual release of available N forms, which may have been partially absorbed by plants and partially retained in the substrate. This is consistent with reviews and experimental studies that describe sludge/biosolids as a relevant N source, with rates influenced by the degree of residue stabilization and its organic composition (Singh e Agrawal 2008; Rigby et al. 2016; Adegbidi e Briggs 2003; Sevilla-Perea et al. 2014). Similar results were reported by (Antolín et al. 2005), who observed increased soil fertility and yield in barley fertilized with sludge under Mediterranean conditions, and by recent studies showing greater N availability and better crop performance in systems amended with biosolids (Paramashivam et al. 2017; Silva-Leal et al. 2021). These results suggest that, although total nitrogen increased with increased sludge, the efficiency of mineralization and plant uptake may vary depending on the balance between organic and inorganic fractions and the degree of sludge stabilization. This is also confirmed by evidence that mineralizable N decreases with more intense stabilization processes (Sevilla-Perea et al. 2014). Moderate sludge proportions tend to balance input and risk, promoting improved nitrogen fertility without excess, in line with meta-analyses and field studies with biosolids (Silva-Leal et al. 2021; Paramashivam et al. 2017).

The results obtained for available phosphorus and total phosphorus were similar. The increase in available P observed in treatments T2 and T3 is possibly related to the mineralization of organic matter and the release of soluble P during sludge decomposition, a phenomenon widely documented in systems receiving biosolids (Singh e Agrawal 2008). The gradual release of phosphorus may also be associated with the oxidation of organic compounds and the transformation of less soluble forms into more bioavailable fractions, especially under pH conditions near neutrality (Bai et al. 2013; Rigby et al. 2016). Furthermore, the initially slightly alkaline pH of the sludge treatments may have favored the partial precipitation of calcium phosphates, which explains the difference between total P and available P observed in T3, indicating a retention of part of the P in less mobile forms (Cordell et al. 2009; Alvarenga et al. 2015). Similar results were reported by (Antolín et al. 2005), who observed a significant increase in available and total P in Mediterranean soils after sewage sludge application, associated with increased plant productivity and improved fertility. In agreement, (Sevilla-Perea et al. 2014) found that P mineralization in soils with biosolids follows a sharp initial release and subsequent stabilization, depending on the C/P ratio and organic matter content. The electrical conductivity (EC) of the substrates increased proportionally to the addition of sewage sludge, reaching high values in treatment T3 (25.9 mS/cm). This increase is related to the high concentration of soluble salts present in the sludge (Shan et al. 2021). Similar results were reported by (Roca-Pérez et al. 2009), who observed EC values above 15 mS/cm in mixtures with fresh sludge, influenced by the degree of maturation and the origin of the material. After 120 days, there was a significant reduction in EC, ranging from 2.4 to 5.8 mS/cm. This decrease can be explained by the leaching of soluble salts caused by irrigation, as also demonstrated by (Yang et al. 2018), who observed that after 8 weeks of sludge application, soil EC began to decline sharply. (Song e Lee 2010) also reported that growing ornamental species in sludge substrates promotes a gradual decrease in salinity due to plant nutrient uptake. The stabilization observed at the end of the experiment indicates that the substrates reached an adequate saline balance. According to (Schafer e Lerner 2022), values between 1.8 and 4 mS/cm are considered acceptable for most tolerant ornamental species.

The control treatment (T0), composed of a peat-based substrate, presented high levels of organic carbon and organic substances, a result of the properties of this

material. Peat is naturally rich in stable humic and fulvic compounds, which have a low decomposition rate and high carbon retention capacity (Méndez et al. 2017; Szajdak et al. 2020). According to (Pipes e Yavitt 2022), peat decomposition is extremely slow due to the presence of recalcitrant humic substances and the scarcity of easily degradable compounds. In the sewage sludge treatments, the levels of organic carbon and organic substances varied according to the proportion of residue applied. During cultivation, microbial mineralization and natural decomposition of organic matter likely occurred, forming more stable mineral and humic compounds, as described by (Ma et al. 2019). Even with this reduction, the treatments with higher sludge proportions (T2 and T3) maintained higher carbon and organic matter contents than T1. This shows that increasing the sludge dose increased the substrate's capacity to retain organic compounds. Similar results were reported by (Albiach et al. 2001; Pedra et al. 2007), who found an increase in total carbon in soil-substrate mixtures with higher sludge proportions. Consistently, (Paganini et al. 2024) also observed an increase in organic matter in applications with high sludge concentrations.
The control treatment presented the highest CSC values, resulting from the high concentration of humic and fulvic compounds naturally present in peat-based materials. These compounds contain negatively charged functional groups responsible for cation retention and high ion exchange capacity (Méndez et al. 2017). According to (Pipes e Yavitt 2022), peat maintains a stable chemical structure and resistance to oxidation, which contributes to the persistence of its active cation exchange sites even after prolonged cultivation periods. In sewage sludge treatments, CSC values were lower than in the control, but increased with the proportion of sludge applied. This indicates that the sludge contributed to cation retention due to the presence of negatively charged organic and mineral compounds and adsorption potential (Giannakis et al. 2014). According to (Eid et al. 2021; Abdul Khaliq et al. 2017), increased sludge application improves substrate fertility by increasing organic matter content, resulting in higher CSC and greater nutrient availability. The slight reduction in CSC observed at the end of the experiment may be associated with the decomposition of organic matter and the release of ions during mineralization, temporarily reducing the active

functional groups available for exchange (Singh e Agrawal 2008; Rigby et al. 2016).

The increase in copper content observed with increasing sewage sludge proportions is directly related to the chemical composition of the biosolids, which typically contains Cu from domestic and industrial effluents (Guoqing et al. 2019; Ma et al. 2019). According to the data obtained, this behavior can be explained by the high affinity of copper with organic compounds and minerals in the substrate, forming stable complexes with organic matter and metal oxides (Ferraz et al. 2016). This interaction reduces its mobility in the medium and hinders its leaching, which explains the progressive accumulation observed in treatments with a higher sludge proportion. These results are consistent with what was observed by (Wierzbowska et al. 2018), who reported similar behavior in soils fertilized with organic residues, in which Cu remained predominantly adsorbed to humic compounds. However, some of the metal can be gradually released during the decomposition of organic matter, maintaining sufficient bioavailable concentrations for plant metabolism (Ceccanti et al. 2022). This controlled release ensures the supply of the micronutrient to plants without causing toxic effects, which is in agreement with (Baioui et al. 2017), who emphasize that Cu is essential for photosynthesis and the synthesis of redox enzymes, but its excess can generate oxidative stress.

Zinc showed a similar behavior to copper, with an increasing trend with increasing sludge doses, confirming that biosolids are a significant source of this micronutrient (Paganini et al. 2024). The observed increase is consistent with the Zn content typically found in composted urban waste and its strong affinity for organic compounds, as also reported by (Ma et al. 2019; Guoqing et al. 2019). According to (Eid et al. 2021), the addition of sewage sludge tends to improve substrate fertility and the availability of essential micronutrients, such as zinc, which favors enzymatic metabolism and chlorophyll synthesis in plants. However, according to (Wu et al. 2017; Ceccanti et al. 2022) , high concentrations of Zn can alter microbial activity and interfere with the absorption of other nutrients, especially when there is an imbalance between bioavailable forms and those bound to organic matter. The presence However, the presence of humic compounds and reactive minerals acts as a stabilizing factor, reducing leaching and potential toxicity. This is also confirmed by (Giannakis et al. 2014; Ferraz et al. 2016), who emphasize that Zn tends to complex with organic matter and adsorb to mineral surfaces, remaining in stable forms with low mobility.

Manganese behaved differently from other micronutrients, which can be explained by the initial input from the urban soil used in the substrate formulation. The Mediterranean limestone soil added to the mixtures naturally presented high Mn levels (422 mg/kg), which explains the high concentrations in the treatments containing a higher proportion of this soil. These results are consistent with the observations of (Manta et al. 2002), who reported Mn concentrations between 142 and 1259 mg/kg in Mediterranean urban soils in Sicily, and of (Roca-Perez et al. 2010), who also identified high manganese levels in eastern Spain, attributed to accumulation in areas with intense urban and industrial activity. Mn bioavailability is strongly controlled by redox conditions and organic matter content, factors that regulate its solubility and the balance between oxidized and reduced forms (Ma et al. 2019; Guoqing et al. 2019). According to (Baioui et al. 2017), manganese exhibits highly dynamic behavior, varying between oxidation states depending on the chemical characteristics of the soil. This effect was also reported by (Wierzbowska et al. 2018), who observed a reduction in the soluble fraction of Mn in soils rich in organic carbon. On the other hand, the gradual mineralization of organic matter can release small amounts of the metal, maintaining its availability over time without causing toxic effects, as described by (Wu et al. 2017; Ceccanti et al. 2022).

Cobalt's behavior was similar. to that observed for manganese, with a significant increase in treatments containing urban soil, mainly due to its high initial content in this material. The Mediterranean limestone soil used in the mixtures presented naturally high Co concentrations, higher than those of the sludge itself, which explains the higher values in treatments containing a higher proportion of soil. This pattern is consistent with that observed by (Manta et al. 2002; Roca-Perez et al. 2010), who reported high levels of cobalt and other heavy metals in Mediterranean urban soils, attributed to limestone geology and the accumulation of anthropogenic waste. The final results obtained suggest that, in substrates containing sludge, cobalt tends to be adsorbed and complexed by organic matter and iron and manganese oxides, reducing its soluble fraction and, consequently, its leaching potential. This behavior is reported by (Malinowska e Jankowski 2020) and also by (Zhai et al. 2003), who found that cobalt was predominantly bound to Fe and Mn oxides in soil, representing 23–35%, while 4–20% was bound to organic matter. Similarly, (Liu et al. 2018) found that Co accumulates predominantly in sparingly soluble forms when associated with humic compounds, contributing to its immobilization and reducing potential toxicity. On the other hand, a small fraction of the element may remain available to plants. According to (Baioui et al. 2017; Wu et al. 2017), Co is absorbed in reduced amounts, performing important physiological functions, such as biological nitrogen fixation and the activation of antioxidant enzymes. This controlled availability results from the balance between the immobilization promoted by organic matter and the gradual release resulting from mineralization, a process also reported by (Liu et al. 2018; Wu et al. 2017).

Lead (Pb) levels showed a similar behavior to copper and zinc, increasing proportionally to sewage sludge application, a pattern also described by (Wu et al. 2017). However, in the final analyses, a reduction in Pb levels was observed in the sludge treatments, a result consistent with (Bogusz e Oleszczuk 2020), who attributed this decrease to leaching or plant uptake. This dynamic can be explained by the metal's chemical form, as (Buthnoo e Sungthong 2025) report that Pb in sludge occurs predominantly in residual forms with low bioavailability. This finding is reinforced by (Wong et al. 2001), who found that approximately 85% of the Pb present in sludge is in the residual fraction, which helps explain its low mobility in the soil observed in this experiment.

Chromium (Cr) levels in the sludge treatments remained stable, with no tendency to increase proportionally to the applied dose, a behavior already evident in the initial analyses. The lack of significant variation between treatments is consistent with (Navarro-Pedreño et al. 2003), who observed that, in calcareous soils, Cr applied via sludge has low mobility in the profile, despite an increase in its concentration in the soil solution and leachate. This retention can be explained by the strong association of Cr with less bioavailable fractions, as shown by

(Alvarenga et al. 2007), who identified Cr primarily bound to the Fe-Mn oxide fraction (58.6%) and organic matter (33%), which reduces its mobility and limits its uptake by plants.

Nickel (Ni) levels showed the same behavior as Cr. There was a moderate increase with increasing sludge doses, reflecting the greater contribution of the metal via residue. Values remained relatively constant in treatments with sewage sludge application, suggesting soil retention. This trend is consistent with (Navarro-Pedreño et al. 2003), who observed that Ni applied with sludge is retained in the surface horizons of calcareous soils, without presenting significant mobility in the profile. Ni adsorption in mineral and organic soil fractions is documented, as demonstrated by (Mamindy-Pajany et al. 2013), who reported strong immobilization of the metal in urban soils amended with sludge, especially at neutral to slightly alkaline pH. Furthermore, (Mlaiki et al. 2025) highlighted that the adsorption and weak transport of Ni in soils with sludge are associated with the mineralization of organic matter and the soil's cation exchange capacity.

Cadmium (Cd) levels gradually increased with increasing sludge proportions, a behavior similar to that observed for lead (Pb). This trend reflects the greater input of the metal via biosolids and its high sensitivity to the chemical characteristics of the substrate, especially pH and organic matter, as demonstrated by (Bogusz e Oleszczuk 2020; Penido et al. 2019). Despite the higher initial Cd content in the sludge treatments, a slight reduction was observed between the initial and final values. This behavior can be explained by the highly mobile nature of Cd in sludge-treated systems, since the metal has low affinity for stable organic complexes and tends to remain in exchangeable forms, as reported by (Rossi e Beni 2018). The observed retention is also in line with the results of (Bergkvist et al. 2003), who, after 41 years of sludge application, found that 92% of the Cd remained retained in the surface soil layer, with negligible losses due to leaching.

It is worth highlighting that, regardless of the concentrations applied, the initial and final values of all potentially toxic metals remained below the limits established by (Direttiva 86/278/CEE, 1986) and by Italian Legislative (Decreto Legislativo nº 99/1992, 1992), not representing a risk of soil contamination.

2.3.2 Vegetative Development

The treatments with higher sludge proportions (T2 and T3) showed slower initial development, while the control treatment (T0), composed of a commercial peat-based substrate, showed the highest absolute values since the first evaluations. This behavior may be associated with the inadequate physicochemical properties of fresh sludge, which compromise the initial growth of forest seedlings (Alonso et al. 2024), in addition to the physiological stress caused by factors such as high salinity and accumulation of heavy metals in mixtures with high sludge content, which impair nutrient uptake and can cause necrosis, delaying plant development, as demonstrated by (Rani et al. 2017) in *Nerium oleander* seedlings. This result is also consistent with the findings of (Žaltauskaitė et al. 2017), who reported inhibition of shoot growth and dry biomass production, both aboveground and root, in *Salix viminalis* under high sewage sludge concentrations, and with the results obtained by (Lima Filho et al. 2021), who observed similar behavior in *Ceiba speciosa* seedlings, reinforcing that high sludge doses can significantly impair early development.

This pattern is further corroborated by the results of (Kominko et al. 2022), who highlighted that the application of sewage sludge in high proportions can cause phytotoxic effects, especially during early development, due to the slow mineralization of organic matter and the uncontrolled release of potentially toxic compounds. However, after the third measurement, an improvement in growth parameters was observed in treatments with higher sludge doses, suggesting that, after the initial adaptation phase, the sludge gradually began to act as a source of nutrients, especially nitrogen and phosphorus, favoring growth in more advanced stages, as noted by (Fuentes et al. 2007). According to (Ma et al. 2019), this recovery may also be associated with the presence of organic matter and beneficial microorganisms, which over time contribute to improving the substrate structure, increasing microbial activity and water retention capacity, optimizing the availability of essential nutrients.

Similar results were reported by (da Silva Santos et al. 2021), who observed that the use of sludge in moderate proportions increased the physiological quality of

Alibertia edulis seedlings, promoting greater biomass accumulation and better physiological performance in the intermediate growth phases. The superiority of the control treatment (T0), in turn, is related to the ideal properties of the commercial substrate used, such as high porosity, low electrical conductivity, and nutritional balance, which favored the good development of the seedlings from the beginning, as described by (Giannakis et al. 2014). This behavior is also consistent with the findings of (Ozdemir et al. 2017), who highlighted the importance of adequate physical properties of the substrate, such as good aeration and structural stability, in the growth of ornamental seedlings.

The intermediate performance observed in treatment T1 (25% sludge) indicates that moderate sludge proportions can achieve a balance between adequate nutrient supply and the absence of toxic effects. This result was also observed by (Manca et al. 2020) in *Eucalyptus urograndis* seedlings grown with substrates based on sludge and sugarcane bagasse, and by (Rani et al. 2017) in experiments with *Nerium oleander* using tannery sludge. Similar data were reported by (Nobrega et al. 2025), who indicated that proportions between 25% and 35% of urban organic waste, such as sewage sludge, are effective in promoting the physiological quality of tropical forest seedlings. These findings reinforce the existence of a critical limit for safe sludge application, above which the harm to development outweighs its potential nutritional benefits. Another aspect to consider is the accumulation of potentially toxic compounds, such as ammonium and heavy metals, especially zinc and copper, in substrates with high sludge content, which can interfere with plant metabolism, as demonstrated by (Bourioug et al. 2015) in studies with larch seedlings. Mohamed et al. (2018) also warned that the continuous use of sludge can alter soil nutrient availability and alter pH, negatively impacting growth, especially in the early stages.

Although Nerium oleander has potential as a phytoremediation species (Ibrahim e Afandi 2020; Asmaa et al. 2023; Rani et al. 2017) confirmed that sewage sludge content greater than 20% can be detrimental to the species' development, leading to a reduction in aboveground and root biomass, and consequently, total biomass. This limitation may be associated with the accumulation of heavy metals, which increase the osmotic pressure of the soil solution, hindering the absorption of minerals and water by the roots, as explained by (Kominko et al. 2022).

2.3.3 Vegetative Chemical Analysis

The concentration of carbon (C) in both the aerial parts and roots of oleander seedlings showed variations between treatments, however, without significant differences between plant parts, indicating a balanced distribution of the element in the tissues. According to the data obtained, the C content was statistically lower in the aerial part of T1 compared to the other treatments, which may be related to the lower addition of sewage sludge in this treatment, reflecting a lower supply of organic matter available for plant incorporation. Sewage sludge is recognized for its high organic carbon load, the application of which favors the stabilization of organic matter, improves soil structure, and stimulates beneficial microbial activity, as pointed out by (Kauser et al. 2025). As recorded by (O'Connor et al. 2005), the application of biosolids as nutrient sources or soil amendments for plant growth remains the best option for recycling organic carbon (C) and nutrients into biosolids. The trend of higher C values in treatments T2 and T3, even without showing a significant difference, can also be attributed to the greater availability of organic compounds promoted by the increased sludge fraction, which is consistent with what was observed by (Lu et al. 2012), who reported greater incorporation of C in plant tissues grown in substrates with biosolids. However, the absence of significant differences between roots and shoots within the same treatment suggests that the plant allocated carbon in a balanced way, probably in response to a growing environment without relevant water or nutritional stress, as proposed by (Sardans et al. 2012) in studies with C allocation under different soil conditions. Contrary to what was reported by (López-Piñeiro et al. 2008), who observed greater C accumulation in the shoots in soils with biosolids, the results here indicate that the presence of urban soil (UB), with low organic matter, may have limited this process, especially in treatments with a lower proportion of sludge. Future investigations could evaluate the quality of the incorporated organic matter (humic and labile fractions), as well as the microbial activity in the substrate, to better clarify the dynamics of carbon assimilation and allocation by plants.

The high content of organic and ammoniacal nitrogen in treated sewage sludge, promoting plant nutrition, was highlighted by (Fytili e Zabaniotou 2008). Although the nitrogen content in the aerial part did not show significant differences between the sewage sludge treatments, the total accumulation of the nutrient was greater in the substrates containing biosolids, when compared to the control, as also observed by (Alonso et al. 2018) in Ceiba speciosa crops when testing percentages of 25 to 100% sludge. Similarly, (Zabotto et al. 2020) concluded that, regardless of the proportion used, the use of sewage sludge resulted in foliar nitrogen concentrations in Eucalyptus urograndis more than double those observed in the control treatment. The greater translocation of N to the aerial parts can be explained by the high demand for this nutrient in metabolic processes related to leaf and stem growth, especially in plants in the vegetative development phase, as reported by (Tegeder e Masclaux-Daubresse 2018). The lower concentration of N in the roots, regardless of the treatment, may be associated with the high mobility of nitrogen, being rapidly directed to the aerial parts after absorption (Zhang et al. 2022). Furthermore, the supply of nitrogen via sludge may have promoted more intense microbial activity, accelerating the mineralization of organic N and the availability of assimilable forms by the roots. Contrary to what was reported by (Bustamante et al. 2008), who observed an accumulation of N in the root zone in poorly decomposed substrates, here the homogeneity of the mixture and the cultivation time (120 days) may have favored the full mineralization and absorption of the nutrient. Thus, the superiority of the sludge treatments compared to the control confirms the agronomic potential of this residue for nitrogen supply, especially when applied in balanced doses. Future studies could evaluate the dynamics of nitrogen release over time, correlating it with the active microbiota and the type of sludge stabilization used.

The translocation of phosphorus (P) from the roots to the aerial parts of plants, even without statistically significant differences between treatments, showed an increasing trend as the proportion of sewage sludge in the mixtures increased. This pattern can be explained by the greater availability of P in the sludge-enriched substrate. The efficiency in translocation can be attributed to the greater metabolic demand for phosphorus in the leaves, since this element participates in cellular and biochemical processes and is a component of essential molecules such as nucleic acids, ATP, and membrane lipids, as highlighted by (Torres et al. 2025). These results are also consistent with the findings of (Karak e Bhattacharyya 2011), who observed greater absorption and mobility of phosphorus in substrates with the addition of biosolids. Similar results were reported by (Zabotto et al. 2020), who observed that phosphorus concentrations in Eucalyptus urograndis leaves were at least four times higher than in the control in all sewage sludge treatments, with no significant differences between the doses tested. This same pattern was observed by (Alonso et al. 2018), who found higher phosphorus levels in the aerial parts of Ceiba speciosa under sewage sludge application of 25% to 100%, regardless of the dose applied. However, it is important to consider that, in some cases, as reported by (Warman e Termeer 2005), organic amendments need to be mineralized to release sufficient available P, a process that is often slow in recently amended soils. This could partly explain the lack of statistical significance between the sludge treatments, even though there was a clear trend of increasing levels. Complementarily, (Pedrero et al. 2010) reported similar behavior in other ornamental species treated with biosolids, reinforcing that the trend observed in this study aligns with what has been verified in the literature. Although the values obtained here did not differ statistically, the increasing trend suggests that the increase in phosphorus content in the substrate positively influences the mobility of the nutrient.

The concentration of K in biosolids is usually naturally low, since it is a highly soluble element, frequently diluted and lost in wastewater during the treatment process, as highlighted by (Kirchmann et al. 2017). This characteristic is reflected in the data from the present study, which did not demonstrate a consistent pattern between the aerial and root parts in the different treatments. Similarly, (Zabotto et al. 2020) observed that, even with the application of different doses of sludge, there was no significant difference compared to the control, with K deficiency identified in the leaves of Eucalyptus urograndis, with visual symptoms such as marginal chlorosis after 60 days of planting. The absence of clear variations in K concentrations can be explained by the already known limitation of this nutrient in biosolids, as mentioned by (Rusan e Athamneh 2004), reinforcing that, even with the addition of sludge, the supply of K may be insufficient to alter the nutritional status of the plants. This limitation is also attributed to the fact that K tends to remain in the liquid phase during wastewater treatment, resulting in low retention in centrifuged sludge, as reported by (Garcia et al. 2010). Despite this, the data showed greater K accumulation in the aerial part, especially in the control and T2 treatments, a behavior consistent with its high mobility via the phloem and physiological importance in osmotic regulation and enzymatic activation. On the other hand, a reduction in K concentration in the roots was observed in treatment T3, possibly associated with increased substrate salinity, as indicated by high electrical conductivity (EC) values, which may compromise K absorption by competing with ions such as Na^+, Mg^{2+}, and NH_4^+, as demonstrated by (Gómez-Muñoz et al. 2022). This imbalance in the K/Na ratio can affect root accumulation and cause physiological disturbances, as also verified by (Pereira et al. 2024) in chrysanthemums fertilized with biosolids. Still, the efficient translocation of absorbed K to the aerial parts even under saline stress, as observed by (Ferreira et al. 2023), suggests a possible adaptive response of plants to maintain essential metabolic functions. Thus, moderate proportions of sludge, as in T2, seem to offer a better balance between K availability and salinity control, corroborating the findings of (Shan et al. 2021) in rice grown in saline soils with biosolids.

According to the results obtained, the higher the dose of sludge used, the lower the concentration of Ca, both in the roots and in the aerial parts, which may be related to changes in the availability or absorption of the nutrient. Calcium is essential for plants, participating in several structural functions in cell walls and membranes, as highlighted by (Esperschuetz et al. 2017). Its absorption occurs predominantly at the tips of actively growing roots, depending on a continuous flow via the xylem, as pointed out by (White 2001). High concentrations of sludge can modify the chemical composition of the rhizosphere or impair root growth, reducing Ca absorption (Esperschuetz et al. 2017). Despite this, a higher concentration of Ca was observed in the aerial part in all treatments, indicating efficient translocation of the nutrient, a behavior consistent with its low mobility in the phloem and the need for constant supply by the roots (White 2001). The prominence of treatment T1 may be associated with the higher proportion of urban soil containing Mediterranean limestone, a natural source of calcium carbonate, which increases the availability of the nutrient, as reported by (Tejada et al. 2009) in alkaline soils with organic residues. In treatments with higher sludge content, the greater organic matter and cation exchange capacity may have reduced the immediate availability of Ca due to competition with cations such as Mg^{2+} and K^{+} or complexation with organic compounds, as described by (Regasa et al. 2025). Contrary to what was observed by (Ros et al. 2003), who reported Ca accumulation in roots under compacted soils, the results of this study suggest that the physical structure of the mixtures did not present compaction, given the upward transport of the nutrient. Future studies should consider the influence of moisture and physical structure of the substrate on Ca mobility, especially in systems with a high load of organic residues.

The distribution of magnesium (Mg) among the plant parts revealed a higher concentration in the aerial part in all treatments, with a significant difference between treatments T0 and T2, suggesting a slight positive influence of the mixtures with sewage sludge on the translocation of this nutrient. The application of sludge resulted in higher foliar levels than the control, although treatments T1, T2, and T3 did not differ from each other, a pattern also observed by (Zabotto et al. 2020) when applying increasing doses of sludge to Eucalyptus urograndis. Similar results were reported by (Alonso et al. 2018) with Ceiba speciosa, reinforcing that, even without a statistical difference between sludge doses, there is a tendency for foliar accumulation of Mg when biosolids are incorporated. This behavior may be related to the mobile nature of Mg and its importance as a component of chlorophyll, which demands preferential allocation in photosynthetic tissues, as discussed by (Warman e Termeer 2005). The greater availability of exchangeable Mg in substrates with sludge may be linked to the presence of organic compounds that promote complexation and solubilization of nutrients, as demonstrated by (Ferreira et al. 2023) in amaranths fertilized with biosolids. Despite this positive trend in the aerial part, the low overall concentration of Mg in both compartments may be linked to the low Mg content in the biosolids themselves, as indicated by (Rusan e Athamneh 2004). The absence of a significant difference in the roots between treatments suggests that, although the supply was sufficient to maintain basal requirements, there was no accumulated excess in this compartment. Still, factors such as antagonism with K^+ or Ca^{2+} may have limited absorption, as reported by (Gómez-Muñoz et al. 2022). Additionally, the increased salinity in treatments with more sludge may have reduced root absorption of Mg through ionic competition, as discussed by (Pereira et al. 2024). Overall, the results reinforce the role of sludge as a complementary source of Mg, but suggest that adjustments to the ionic balance of the substrates are necessary to optimize its absorption.

The preferential translocation of sulfur (S) to the aerial part, observed consistently in all treatments, reflects its high mobility and importance in the biosynthesis of sulfur-containing amino acids such as cysteine and methionine. According to (Churka Blum et al. 2013), S is rapidly redistributed from the roots to the leaves when the supply is adequate and growth is active, as occurred in all formulations tested. In the analysis of the aerial part, there were no significant differences between treatments, a result similar to that found by (Esperschuetz et al. 2017) in three woody species fertilized with sewage sludge. On the other hand, (Zabotto et al. 2020) found a doubling of the S concentration in the leaves of Eucalyptus urograndis with the application of biosolids, regardless of the dose, suggesting that different species and substrates may respond differently. The greater retention of S in the roots of the control (T0) was the only statistically significant difference, possibly due to the high cation exchange capacity and acidity of the peat, which limits ion mobility, as pointed out by (Lehmann et al. 2003). The sludge itself may contain soluble forms of S, accelerating its mobilization. Contrary to what was observed by (Wilhelm Scherer 2009), who reported S accumulation in compacted soils, the data here indicate that the substrate allowed good transpiration flow, essential for sulfur mobility.

According to (Shukla et al. 2011; Kumar e Chopra 2014)**,** heavy metal levels in plants are directly related to the concentrations of these elements in the environment, and applications of sewage sludge, especially in high doses, significantly increase the absorption of these metals, as highlighted by (Shomar et al. 2013). This behavior was confirmed by (Chu et al. 2018), who observed an increase in Cu and Zn in the roots and shoots of five urban ornamental species fertilized with biosolids. In the present study, a higher concentration of Cu was found in plants treated with sludge, although without a consistent pattern between roots and shoots, a pattern also observed by (Chu et al. 2018). Copper, transported mainly in its reduced form, plays an essential role in several metabolic processes, including photosynthesis, respiration, carbohydrate metabolism, and chlorophyll biosynthesis, as discussed by (Mir et al. 2021). In studies with *Loropetalum chindense*, (Dai et al. 2006) observed greater accumulation of Cu in the roots when cultivated in soils treated with sludge, and attributed this behavior to the mobility of Cu influenced by soluble organic compounds, whose stability can be reduced at acidic pH, increasing its bioavailability. In the present experiment, the absence of preferential accumulation in a specific part of the plant may be associated with variations in pH and organic composition of the substrates, influencing the behavior of Cu. Furthermore, according to (Mir et al. 2021), both copper deficiency and excess can cause serious physiological dysfunctions in plants. Despite the increased concentration in the sludge treatments, the values remained within the range considered adequate for most species, between 5 and 20 mg/kg of dry matter, as established by (Farid et al. 2021), indicating the absence of toxicity symptoms and the viability of sludge use.

The increase in Zn concentrations in both roots and shoots is directly related to the increased doses of sewage sludge applied, as observed by (Chu et al. 2018). This pattern of accumulation in the shoots was also recorded by (Zabotto et al. 2020) when evaluating different sludge proportions in Eucalyptus urograndis cultivation. In a 12-week study, (Dai et al. 2006) found that Osmanthus fragrans and Cinnamomum camphora accumulated more Zn in the stems than in the leaves, while in Buxus radicans and Viburnum macrocephalum the levels were similar between roots, stems, and leaves, although generally low in the roots when sludge was applied. Similarly, (Chu et al. 2018) when studying woody species, found in A. macrorrhiza a high translocation factor of Zn from the roots to the aerial parts, a pattern also identified in this study, especially in treatments with concentrations equal to or greater than 50% sewage sludge. Although zinc plays important roles in the roots, it is a micronutrient predominantly used in the aerial parts, participating in processes such as cell division and protein synthesis (Jain et al. 2010), in addition to acting in the expansion and functionality of floral tissues, such as pistil, anthers and pollen, as reported by (Hafeez et al. 2013). These factors explain the tendency for preferential translocation of Zn from the root to the aerial part. Although the application of higher doses of sludge promoted a slight reduction in biomass and plant development, no visual symptoms of Zn phytotoxicity were observed. This is in agreement with (Kaur e Garg 2021), who point out several adverse effects associated with excess Zn in tissues, but also reinforced by (Asare et al. 2025), according to which toxicity symptoms generally manifest only when foliar concentrations exceed 100 to 700 mg/kg of dry mass, a range above the values observed in this study.

The concentration of manganese (Mn) increased significantly in the aerial parts of plants in treatments with a higher proportion of sewage sludge (T2 and T3), indicating an efficient translocation of this micronutrient in response to its greater availability in the substrate. This trend is consistent with the findings of (Singh e Agrawal 2007), who observed significant accumulation of Mn in plant tissues grown in soils enriched with biosolids. In addition to the sludge used, which had concentrations approximately twice as high as those of the control treatment, the high concentration of Mn observed in our experiment may be strongly associated with the composition of the urban soil used in the mixtures, which contained about six times more Mn than the pure commercial substrate. This characteristic can occur in Mediterranean soils, as demonstrated by (Manta et al. 2002) in Italy and (Roca-Perez et al. 2010) in Spain. The low Mn content recorded in the control treatment (T0), both in roots and aerial parts, is probably related to the low initial content of this element in the peat-based substrate, as also reported by (Bourioug et al. 2015), who associated the low Mn content in Larix decidua with the low concentration of the element in the sludge used. Similar to the results obtained in this work, (Pascual et al. 2004) observed that the application of sewage sludge resulted in higher Mn concentrations in barley roots, regardless of water availability. Likewise, (Zabotto et al. 2020) reported that the use of sewage sludge significantly increased foliar Mn content in Eucalyptus urograndis under greenhouse cultivation conditions. Unlike the behavior observed for metals such as chromium (Cr) and nickel (Ni), manganese exhibits greater mobility in the xylem, which justifies its preferential accumulation in the aerial parts. This is related to its essential role in physiological processes, especially in photosynthesis, where it acts in the oxidation of water in the oxygen evolution center of photosystem II, as described by (Gong et al. 2011). The greater translocation of Mn from roots to photosynthetic tissues is therefore associated with its primary biological function in leaves.

Iron (Fe) concentration was significantly higher in the roots in all treatments, especially in T1, suggesting low translocation of the element to the aerial part, a typical behavior of this low-mobility micronutrient, as described by (Morrissey e Guerinot 2009). Treatment T1, which combined Mediterranean limestone soil and sewage sludge, increased the substrate pH, reducing Fe solubility and favoring its adsorption by the roots, limiting foliar transport. The lower presence of Fe in the aerial part in the control (T0) can be attributed to the low initial supply of the nutrient, restricting both absorption and redistribution. Furthermore, the Fe present in sludge is frequently bound to poorly soluble forms, such as oxides and hydroxides, hindering its systemic translocation (Singh e Agrawal 2007). The tendency for root accumulation in treatments with biosolids confirms the root's barrier role to iron, especially in environments with a pH close to neutrality. According to (Mousavi et al. 2017), the continuous application of biosolids can increase the concentration of Fe in the soil, but the nutrient tends to accumulate in the rhizosphere due to the presence of organic matter and humic acids, which keep it in less mobile forms. Competition with cations such as Mn, Zn, and Cu, present in high concentrations in the sludge, can also reduce Fe mobility, as reported by (Warman e Termeer 2005). Similarly, (Manca et al. 2020) observed Fe retention in the roots of Eucalyptus cultivated with sludge and bagasse, associated with the presence of organic compounds. Root retention may also be a protective mechanism against foliar toxicity, as proposed by (Esperschuetz et al. 2017). Although the differences in the aerial part were not very significant, there was a tendency for greater accumulation in the treatments with sludge, indicating possible partial translocation favored by the greater availability of the nutrient, which may be advantageous in cases of deficiency.

The distribution of sodium (Na) in plants did not show statistically significant differences between treatments, regardless of the proportion of sewage sludge. However, there was a limitation in the translocation of Na to photosynthetic tissues as a way to avoid toxicity, as also reported by (Kronzucker e Britto 2011). This retention is linked to the non-essential nature of Na for most plants, which passively absorb it and accumulate it in the roots when present in excess. The sewage sludge used presented high values of electrical conductivity, which justifies the presence of Na, since urban waste frequently contains salts from domestic and industrial activities (Singh e Agrawal 2008). Sodium mobility depends on the physicochemical characteristics of the soil and the cultivated species, and it commonly competes with cations such as K^+, Ca^{2+}, and Mg^{2+}, which can affect nutritional balance, as demonstrated by (Shan et al. 2021). The similarity between treatments indicates that even substrates with lower sludge content already presented sufficient Na concentrations to activate physiological mechanisms of root retention. Furthermore, the maintenance of low foliar levels suggests the action of efficient homeostatic mechanisms to prevent photosynthetic damage, as observed by (Ferreira et al. 2023) in amaranths fertilized with biosolids. Therefore, the general trend indicates that oleander balanced sodium absorption and exclusion in the different treatments, reinforcing the need to monitor substrate salinity when using urban waste as a source of organic matter.

Boron (B) did not show statistically significant differences between treatments in any part of the plant, a behavior also reported by (Zabotto et al. 2020) in Eucalyptus urograndis fertilized with different doses of sewage sludge. Even so, a tendency towards greater accumulation of B in the aerial parts of the seedlings was observed, consistent with the physiology of the micronutrient, whose mobility depends on the species and water conditions. In woody plants with a high transpiration rate, transport occurs mainly via the xylem and accumulates in young tissues, as described by (Brown et al. 2002). This pattern is favored by constant moisture and adequate supply, which promote continuous sap flow. The balanced distribution between roots and aerial parts may have been influenced by the pH close to neutrality, ideal for B absorption, as pointed out by (Shorrocks 1997). The higher concentration in the aerial parts, even without statistical difference, also indicates possible translocation, in the form of complexes with polyols, as reported by (Shireen et al. 2018). The absence of significant variations between treatments may be associated with the complexation of B with organic compounds present in the sludge, reducing its immediate bioavailability, a phenomenon observed by (Simón et al. 2013) in substrates rich in organic matter. Furthermore, the dynamics of B adsorption and desorption, regulated by pH, clay type, and organic matter, may have attenuated the effects of the different doses, as suggested by (Xin e Huang 2017), who highlight that the efficiency of B translocation depends more on plant physiology than on soil concentration. Thus, the data suggest that oleander prioritized the allocation of B to metabolically active tissues, justifying its greater presence in the aerial part.

The predominance of chromium (Cr) bioaccumulation in the roots, observed in all treatments, reflects the typical behavior of this element. The absence of significant differences between treatments regarding root concentration reinforces this pattern, characteristic of plant defense mechanisms that limit the toxic effects of the metal, as discussed by (Shanker et al. 2005). This behavior was also verified by (Dai et al. 2006) in a 12-week study with four herbaceous species cultivated in soils with sewage sludge, where *Dendranthema morifolium* presented the highest Cr levels in the roots, followed by *Begonia semperflorens* and *Loropetalum chinense*, with a decreasing distribution root > stem > leaf. In the present study, the detection of Cr in the aerial part only in the treatments with sludge (T1, T2, and T3), although without statistical significance, suggests the beginning of systemic translocation of the metal. This mobility may be associated with the presence of more bioavailable forms such as Cr^{6+}, which, although less common in organic and anaerobic environments, can occur in partially oxidized or poorly stabilized biosolids, as pointed out by (Dixit et al. 2015). The greater microbial activity induced by sludge may also favor redox changes, promoting temporary mobilization of Cr. This result is partially consistent with (Choppala et al. 2012), who observed greater Cr mobility in soils with fresh organic matter, although most of the metal still accumulated in the roots. The absence of Cr in the aerial part of the control treatment (T0) reinforces the role of sludge as a source of the element, as demonstrated by (Smith 2009) in studies with urban waste. Despite the absence of phytotoxicity symptoms, the presence of Cr in leaves justifies monitoring for the risk of bioaccumulation.

The distribution of nickel (Ni) in plants followed the same trend observed for chromium, with significant bioconcentration in the roots and low translocation to the aerial part in all treatments. This is consistent with the typical behavior of Ni in non-hyperaccumulator plants, which restrict its mobility in the phloem due to the risk of cellular toxicity, as reported by (Gajewska e Skłodowska 2008). This result is confirmed by (Dai et al. 2006), who observed Ni accumulation 26 times greater in the roots of *Dendranthema morifolium* than in the leaves, suggesting root retention as a defense mechanism. Such retention can be explained by the formation of complexes with cell wall pectins and by the precipitation of the metal in vacuoles, preventing its arrival at metabolically active tissues. According to (McGrath et al. 2001), the bioavailability of nickel in soils depends on factors such as pH, organic matter, and CEC, which control its adsorption and desorption. The organic matter in substrates with sludge can temporarily complex Ni, but also facilitate its release and absorption under constant moisture. The absence of significant differences between treatments reinforces the hypothesis that the oleander root system acts as an effective barrier against excessive Ni transport, a behavior consistent with the findings of (Yusuf et al. 2011), who also observed root retention of the metal in contaminated soils. Unlike what was observed by (Baker et al. 2000) in hyperaccumulator species such as Alyssum, oleander demonstrated exclusionary behavior. Future studies should investigate the role of specific transporters to elucidate the physiological mechanisms of Ni absorption and retention in this species.

2.4 Conclusions

Based on the results obtained, it is concluded that the application of sewage sludge positively influences several soil and substrate properties, promoting improvements in physical, chemical, and nutritional parameters. Greater moisture retention, lower density, and maintenance of a slightly acidic pH were observed in treatments with higher proportions of sludge, favoring nutrient availability and root development. There was an increase in organic matter, organic carbon, and cation exchange capacity (CEC) levels, although lower than the values of the commercial substrate, due to its peat-rich composition. Elements such as copper and zinc accumulated in a controlled manner, complexed with organic compounds, while heavy metals such as lead, nickel, cadmium, chromium, and cobalt remained below legal limits (Legislative Decree No. 99/1992), without representing a risk of contamination.

Regarding the growth of *Nerium oleander*, the control treatment (T0) showed the best performance from the beginning, reflecting the ideal properties of peat. In contrast, treatments with higher sludge proportions (T2 and T3) showed impaired initial development, with necrosis and death of some seedlings, probably due to saline stress and the presence of metals. From the third month onwards, gradual recovery was observed, highlighting the late nutritional effect of the sludge. Treatment T1 (25% sludge) showed intermediate performance, with stable growth and absence of toxic effects, indicating a good balance between nutrient supply and safety. Even so, T2 and T3 remained statistically inferior to T0 and T1 for variables such as height, diameter, and aboveground biomass, showing that high sludge proportions compromise initial development.

Nutrient concentrations in the aerial and root parts revealed a physiological pattern consistent with the species. N, P, Mg, and Zn were more significant in the aerial part, while Fe, Ni, Cr, and some Cu and Na were retained in the roots as a physiological barrier. Treatment T1 stood out for its higher availability of Ca, originating from the urban limestone soil, while T0 showed greater sulfur accumulation in the roots. Potassium exhibited unstable behavior, while sodium was efficiently retained in the roots, preventing toxicity. The absence of visual symptoms and the control of metal concentrations reinforce the viability of using sludge, provided it is monitored and dosed appropriately.

Principal component analysis (PCA) reinforced that the commercial substrate (T0) was more associated with plant growth, while treatments with higher proportions of sludge concentrated metallic micronutrients (Cu, Zn, Mn, and Fe) in roots and aerial parts, without resulting in better seedling performance. Treatment T1 showed the best balance between nutrient accumulation and plant development, consolidating itself as the best alternative among the sludge treatments.

Thus, the data obtained indicate that, for calcareous soils and under pot cultivation conditions in a protected environment, the ideal proportion of sewage sludge as a substrate component is approximately 25%, at least in short-term experiments, ensuring agronomic benefits without compromising plant development.

Finally, a limitation of the study is the absence of tests under different irrigation depths, considering scenarios of water deficit and excess water. It would also be interesting to evaluate long-term responses or under field conditions. In future studies, monthly monitoring of leachates is recommended to track nutrient losses and possible substrate leaching, paying special attention to electrical conductivity (EC), which can affect nutrient availability and plants performance.

3 Effects of Different Concentrations of Sewage Sludge on the Development of Aroeira-pimenteira (*Schinus terebinthifolia*)

3.1 Materials and Methods

3.1.1 Experimental Position

The experiment was carried out at the Soils Department of the São Paulo State University (UNESP), Botucatu campus, São Paulo state, Brazil (latitude 22°50′49″S; longitude 48°26′4″W) (Fig. 56). The region has a humid subtropical climate and is traditionally classified as Cwa according to the Köppen system, based on historical series from 1966 onwards. This classification is characterized by hot and humid summers and winters with significant reduction in precipitation. However, more recent studies, based on data from the period 1991 to 2020, indicate a possible reclassification to Aw, since the average temperature of the coldest month exceeds 18°C (Franco et al. 2023).

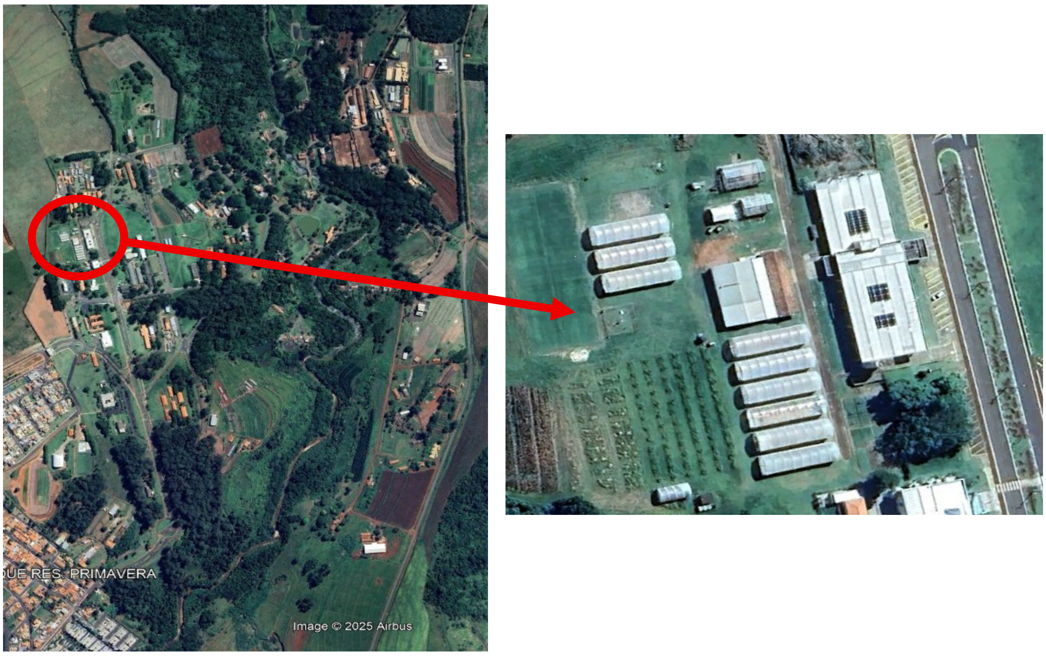


Fig. 56. Location of the Department of Soils at the São Paulo State University (UNESP).

The average annual temperature is approximately 21°C, with minimums around 17°C and maximums close to 27°C throughout the year. The average annual precipitation is around 1,500 mm and the average relative humidity is around 70% (Franco et al. 2023).

During the experimental period, between January and May, the weather conditions are typical of the transition between late summer and autumn. During this period, average temperatures range from 19°C to 24°C, with the hottest days concentrated between January and March, a period in which the highest volume of precipitation is also observed, which tends to decrease progressively until May. Solar radiation is relatively high, especially in the months of January and February.

3.1.2 Specie and culture media

The species used in the experiment was the Aroeira-pimenteira (*Schinus terebinthifolia*), a species native to the Brazilian Atlantic Forest, with a wide natural distribution from Rio Grande do Sul to the Northeast, including the state of São Paulo. The species can also be found naturally in Argentina, Paraguay and Uruguay. Recognized for its hardiness, rapid growth and ability to adapt to different types of soil and water regimes, prolonged flowering and persistent fruiting, the aroeira has been widely used in urban afforestation projects, revegetation of degraded areas and landscaping in public and private spaces. Its aromatic leaves, attractive fruits and resistance to periods of drought and poor soils make it a functional and aesthetically valued species in urban and rural compositions (Carvalho 2003). In São Paulo, its use has been increasing mainly due to its tolerance to urban environments and low maintenance costs, and it is recommended by environmental agencies for planting on sidewalks, central flowerbeds and areas of ecological restoration. The pepper tree seedlings used in this experiment were provided by the nursery of the School of Agricultural Sciences (FCA) of UNESP, Botucatu campus, a unit focused primarily on the production of species for research and university extension purposes. The seedlings were standardized, with uniformity in height, neck diameter and age, ensuring greater control over the initial conditions of the experiment (Fig. 57).

Fig. 57. Aroeira-pimenteira seedlings selected at the FCA nursery taking into account homogeneity

Four materials were used to compose the treatments proposed in this experiment: commercial substrate, urban soil, dehydrated and stabilized sewage sludge, and vermiculite.

The commercial substrate used was a light, stable, homogeneous material free of contaminants, which is used in the production of seedlings and in the cultivation of plants in pots. Its formulation is composed mainly of peat, expanded vermiculite, and carbonized rice husk, components that provide good physical and chemical properties, such as water retention, aeration, and low apparent density. The substrate was purchased from agricultural establishments in Botucatu and belongs to the internal class LXXXVI of the Carolina Soil brand (Fig. 58). It is widely used by regional production nurseries.

Fig. 58. Commercial substrate used as control.

The urban soil used in the experiment was provided by the Soil Department of the São Paulo State University (UNESP). The material came from an area located near the entrance to the UNESP campus in Botucatu (coordinates: 22°51'17"S, 48°26'13"W) (Fig. 59). The field collection was carried out with the aid of an excavator, which initially removed the surface layer approximately 10 cm deep, corresponding to the organic horizon. Then, the mineral soil was collected and used for the experimental tests.

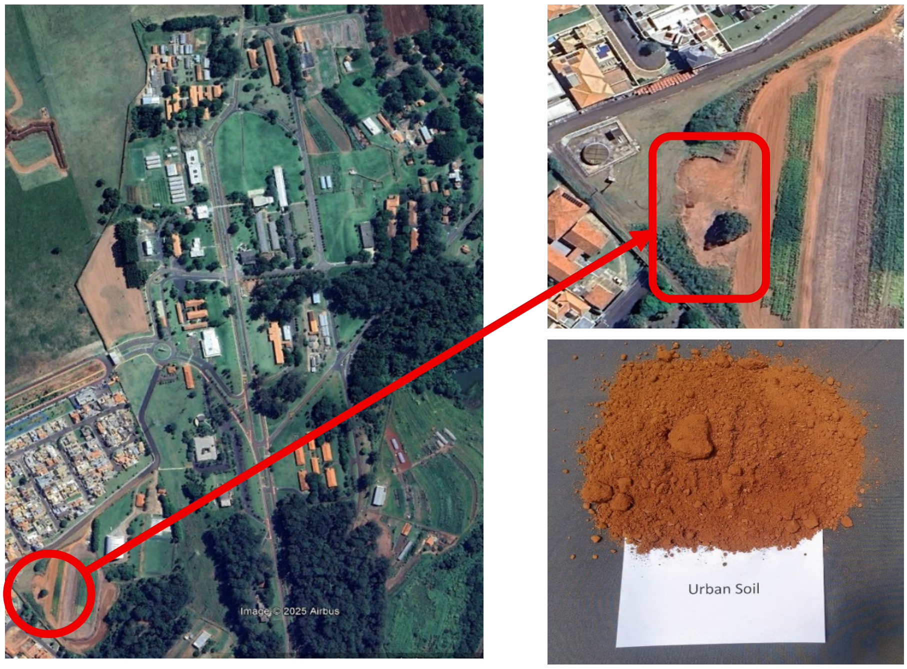


Fig. 59. Urban soil collection site within the UNESP campus and collected material.

The sewage sludge used in this experiment came from the Lageado Sewage Treatment Plant (STP), located in the municipality of Botucatu (SP) and operated by the Basic Sanitation Company of the State of São Paulo (SABESP). The effluents received by this unit are predominantly of domestic origin and undergo a treatment system consisting of preliminary, primary and secondary stages, at the end of which biological sludge is obtained. This solid fraction is sent to a drying and stabilization process conducted in a greenhouse installed on the premises of the STP itself. The structure consists of two parallel channels, approximately 80 meters long and 5 meters wide each (Fig. 60). In this process, the sludge remains for approximately 70 days, being mechanically stirred daily. Continuous stirring allows the gradual conduction of the material along the greenhouse, promoting its homogenization, efficient aeration and thermal elevation due to the activation of aerobic fermentation. This process causes the temperature to reach approximately 70°C, an essential condition for stabilizing the organic matter and microbiologically sanitizing the sludge. These conditions favor biological and thermal treatment, resulting in the degradation of unstable organic matter and the inactivation of pathogenic microorganisms, ensuring the sanitary stability of the compost. The final product complies with national Resolution CONAMA No. 375/2006, and is suitable for application in agricultural soils with environmental safety (Fig. 61, 62 and 63).

Fig. 60. Fresh sewage sludge deposited at the entrance of the two parallel channels.



Fig. 61, 62 and 63. Daily turning of sewage sludge for stabilization purposes; end of the channel with stabilized material; stabilized and sieved sludge ready for use in treatments.

Medium-weight expanded vermiculite was also used (Fig. 64), which was also supplied by the FCA seedling nursery at UNESP, with the aim of improving water retention and increasing aeration of the treatments, favoring the oxygenation of the root systems.

Fig. 64. Vermiculite used in treatment mixtures.

3.1.3 Description of the experiment

The experiment began in January 2025, with the acquisition of materials and initial preparation for the treatment setup. Before use, urban soil and sewage sludge were sieved through a 5 mm mesh to standardize the materials and remove larger fragments. Because the sewage sludge was already stabilized at the SABESP Sewage Treatment Plant (STP), it did not require additional stabilization steps after mixing.

The mixtures were formulated with different volumetric proportions of dry materials. A clear plastic bucket with a known volume of 10 liters was used to standardize the measurements. The treatments consisted of the following sewage sludge proportions: 0% (T0), 4% (T1), 8% (T2), and 12% (T3). The complete formulations were:

- T0: 100% commercial substrate (Carolina Soil), widely used by nurseries in the region and by the FCA/UNESP forest nursery;
- T1: 4% stabilized and screened sewage sludge, 71% dry and screened urban soil, and 25% medium-sized vermiculite;
- T2: 8% sewage sludge, 67% urban soil, and 25% vermiculite;
- T3: 12% sewage sludge, 63% urban soil, and 25% vermiculite.

Each treatment had 7 (seven) replicates, totaling 28 pots (Fig. 65). However, only six replicates per treatment (24 pots in total) were used in the analyses. The seventh replicate was included as a reserve to replace any losses during the experiment.

Fig. 65. 4 treatments, 7 (seven) repetitions, totaling 28 pots

The materials were mixed using a 100-liter concrete mixer (Fig. 66). After adding the components for each treatment, the mixer was run continuously for 3 minutes to ensure complete homogenization. Once this step was completed, samples were collected for initial nutritional characterization analyses (Fig. 67). From each treatment, three subsamples of approximately 500 grams were placed in plastic bags and subsequently combined to form a composite sample per treatment (Fig. 68).

Fig. 66, 67 and 68. Concrete mixer used for homogenization of treatments; collection of sewage sludge, urban soil and the 4 treatments after homogenization (upper right corner); samples prepared for sending to the Soils Department (lower right corner).

These samples were sent to the Soil Department Laboratory at FCA/UNESP for chemical analysis. Pure samples of urban soil and sewage sludge were also sent, and the soil was also subjected to physical analysis (texture and grain size).

Before planting the seedlings, a base fertilization was applied with NPK 10-10-10 chemical fertilizer, in granulated form, at a dosage of 2 grams per liter of substrate. After weighing the fertilizer, the mixtures were placed in a concrete mixer along with the fertilizers, where they remained for an additional 2 minutes to ensure uniform distribution of the NPK throughout the substrate volume. This dosage was previously verified with local nurseries and is in accordance with commercial practices.

The aroeira-pimenteira seedlings used were in 150 cm³ polyethylene tubes (Fig. 69). Transplanting into 9.5-liter black polyethylene pots took place on January 22, 2025. To promote drainage and prevent substrate loss during irrigation, the pots received a fine-mesh screen at the bottom with a layer of approximately 500 g of gravel (Fig. 70).

Fig. 69 and 70. Seedlings stored in 150cm³ tubes; pots prepared with screen and coarse gravel before transplanting the seedlings.

The seedlings were uniform in height, diameter, and age, as well as in good phytosanitary condition. The plants were randomly assigned to treatments.
After transplanting, the pots were placed in a greenhouse on the premises of the Soil Department of FCA/UNESP, Botucatu campus. The greenhouse is enclosed, with controlled access, and features a waterproof floor and a transparent plastic covering, ensuring growing conditions protected from the elements (Fig. 71).

Fig. 71. Greenhouse used to develop the experiment.

To minimize the effects of micro environmental variations within the greenhouse, the pots were repositioned every 30 days. The new positioning was determined randomly using a platform (sorteador.com.br), ensuring a random distribution of replicates throughout the experiment.

3.1.4 Analysis performed

Soil analyses were performed at two distinct time points: initially, before the experiment was implemented, and later, at the end of the experimental period, immediately after the destructive analyses of the plants.

For the initial analyses, representative samples of urban soil, dewatered and stabilized sewage sludge used in the experiment, and mixtures corresponding to the four treatments (T0, T1, T2, and T3) were collected. Three samples, each weighing approximately 500 grams, were then homogenized to form a composite sample for each material and treatment. The samples were sent to the FCA/UNESP Soil Laboratory in Botucatu, where they were oven-dried at 105°C until they reached constant weight. After drying, the materials were sieved with 2 mm sieves (BERTEL brand), standardizing the samples for analysis.

In addition to chemical analyses, only the urban soil sample was subjected to physical characterization, with assessment of texture and grain size. Grain size analysis was performed by fractionation using the pipette method, which allows for the distinction between fine sand (0–0.25 mm) and coarse sand (0.25–2.0 mm) fractions, providing a detailed description of the soil's textural distribution.

Initial chemical analyses followed the protocols described by (Raij, B. van et al. 2001), reference adopted for evaluating the fertility of tropical soils. The pH in a $CaCl_2$ solution (0.01 $molL^{-1}$), organic matter (OM), resin-extracted phosphorus (P), exchangeable aluminum (Al^{3+}), potential acidity (H^+Al), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sum of bases (SB), cation exchange capacity (CEC), base saturation (V%), in addition to sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined.

The pH was determined in a calcium chloride suspension ($CaCl_2$ 0.01 $molL^{-1}$), using a properly calibrated pH meter. Potential acidity (H^+Al) was estimated by the SMP buffer method, while exchangeable aluminum (Al^{3+}) was extracted with potassium chloride and quantified by titrimetric.

Organic matter was determined by oxidation via sodium dichromate, with colorimetric readings performed on a visible light spectrophotometer (VIS). The phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) contents were extracted using ion exchange resin. Phosphorus was quantified with ammonium molybdate reagent by VIS spectrophotometry, while the K, Ca, and Mg cations were determined by atomic absorption spectrophotometry with the addition of lanthanum oxide as a matrix modifier, using an Agilent 200 Series AA equipment (Fig. 72).

The available sulfur content ($S\text{-}SO_4^{2-}$) was obtained by extraction with monocalcium phosphate, with turbidimetric reading based on the formation of barium sulfate, also performed on a VIS spectrophotometer. The micronutrients iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) were extracted with DTPA solution and quantified by atomic absorption spectrophotometry. Finally, boron (B) was determined after extraction in hot water, with microwave heating, and quantified by colorimetry using the azomethine-H reagent, with readings performed on a Femto VIS spectrophotometer, model 600plus (Fig. 73).


Fig. 72 and 73. Agilent 200 Series AA equipment; Femto VIS spectrophotometer, model 600plus

Final soil analyses were performed individually and repeatedly. During the destructive plant analysis stage, when the roots were removed from the pots, the remaining substrate was homogenized, and approximately 500 grams of soil was collected per pot and packaged in kraft paper bags. (Fig. 74 and 75). These samples were sent to the laboratory, where they were also dried in an oven with forced air circulation at 105°C until weight stabilization (Fig. 76 and 77) and subsequently sieved through a 2 mm sieve (BERTEL brand) (Fig. 78 and 79), maintaining the same preparation protocol used in the initial analyses.

Fig. 74 and 75. Collection of approximately 500 grams of soil per repetition.

Fig. 76 and 77. Samples inside the forced-air drying oven and then on the bench for further sieving.


Fig. 78 and 79. Sieving of dry samples and samples ready to be sent for chemical analysis.

Before submitting the final samples for chemical analysis, dry soil density was determined. To do this, a soil collection pipe with a known volume of 10 cm^3 (Fig. 80), was used, which was filled with dry soil and then weighed on a precision scale, taking three measurements per sample (Fig. 81). The average of these three weighing was used as a representative value. It should be noted that the use of tubes provides accurate soil density estimates, with a margin of error generally less than 5%. Bulk density was then calculated based on the formula:

$$\text{Dry soil density (g/cm}^3\text{)} = \frac{\text{Dry soil mass (g)}}{\text{Cylinder volume (cm}^3\text{)}}$$

Fig. 80 and 81. 10cm³ Labimport Soil Collection pipe and weighing dry soil.

All methodological procedures adopted for the preparation, extraction, and analysis of the final soil samples followed the same protocols applied to the initial samples. This included the drying, sieving, and homogenization steps, as well as the methods used to determine the soil's chemical properties.

Plant analyses were performed at two distinct stages: monthly non-destructive assessments to assess plant development and final destructive analyses to assess nutritional status.

Monthly evaluations consisted of measuring the height, stem diameter, pH, and electrical conductivity (Ec) of each replicate. Height was measured using a 10-meter tape measure with millimeter markings, measuring the distance from the plant stem to the apical bud (Fig. 82), while stem diameter was obtained using a digital caliper, model Starrett 797, precision in millimeters (Fig. 83).

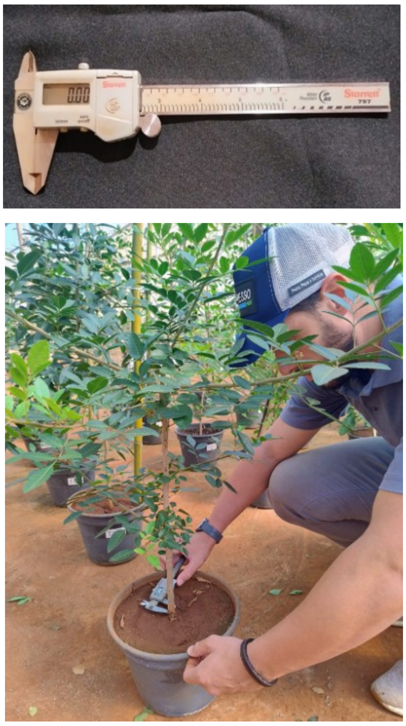


Fig. 82 and 83. Monthly height measurements (left); neck diameter measurement (right).

For pH and EC determinations, the leachate collection technique was used. A plastic bag was placed around the base of each pot, followed by slow, controlled irrigation, allowing drainage and collection of excess solution. (Fig. 84). The collected solution was taken to the laboratory, where readings were taken with specific pH and electrical conductivity meters, both from the Lucadema brand. (Fig. 85 and 86).

Fig. 84. Leachate collection for pH and electrical conductivity (EC) measurement

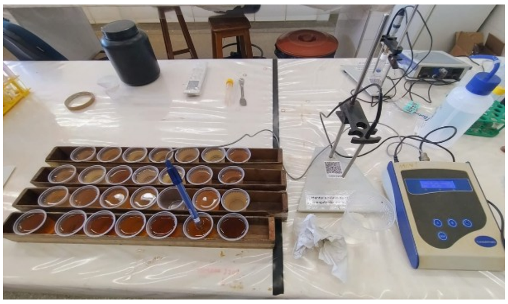
 

Fig. 85 and 86. pH reading (left); electrical conductivity measurement (right).

On the morning of May 22, 2025, the seedlings' green color index was measured to indirectly and non-destructively estimate leaf chlorophyll content, an indicator strongly associated with plant nutritional status, particularly nitrogen. The assessment was performed using a FALKER ClorofiLOG device, model CFL1030, which provides readings expressed in ICF (Leaf Chlorophyll Index) units (Fig. 87). For each replicate, two mature leaves were selected, one on each side of the plant, avoiding very young or senescent leaves, which could mask the results (Fig. 88). The average of the two readings was used as a representative value for the replicate, in order to minimize possible instrumental errors or specific physiological variations between the leaves evaluated.

Fig. 87 and 88. ClorofiLOG FALKER, model CFL 1030 and leaf chlorophyll index measurement.

Destructive plant analyses were performed on May 22 and 23, 2025. At this time, the plants were segmented into shoots and roots using pruning shears. The shoots were immediately weighed on a digital scale to obtain the wet weight, then placed in kraft paper bags (Fig. 89, 90 and 91) and sent to a forced air circulation oven maintained at 65°C. The samples remained for four days until they reached a constant weight, after which the dry weight of the shoots was determined.

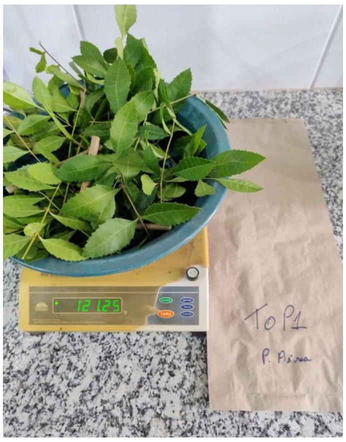




Fig. 89, 90 and 91. Segmentation of the aerial part (left); weighing of the moist aerial parts (center); packaging in kraft paper before drying in an oven (right).

The roots were carefully removed from the pots immediately after the aerial parts were cut (Fig. 92). Then, adhering soil was removed and the roots were washed in running water until all particulates were removed (Fig. 93). After initial cleaning, the roots were rinsed with deionized water (Fig. 94), a procedure adopted to eliminate soluble mineral residues and avoid contamination that could interfere with subsequent analyses. The roots were then dried with paper towels, weighed to obtain the root wet weight (Fig. 95), and subsequently stored in the same oven used for the aerial parts, also remaining for four days at 65°C, until reaching a constant weight (root dry weight) (Fig. 96).

Fig. 92, 93 and 94. Roots removed from the pot (left); roots after initial wash (center); roots rinsed with deionized water (right).

Fig. 95 and 96. Roots weighed to obtain root wet weight (left); oven with samples of shoot and root parts in the drying process (right).

After sample weight stabilization, the dry weights of the shoots and roots were determined (Fig. 97). The plant samples were then prepared for nutritional analysis, with shoots and roots processed separately in each replicate.

The dried roots were ground in a knife grinder located in the Soil Department of FCA/UNESP, equipment developed by Indústria Aparelhos Científicos e Técnicos

Ltda. (CITEC) (Fig. 98). After grinding, representative samples with approximately 100 grams of material per repetition were formed, properly packaged in paper bags and labeled for shipment to the laboratory. (Fig. 99).

Fig. 97, 98 and 99. Weighing of the dry root (left); grinder used for initial grinding of the roots (center); samples prepared for sending to the laboratory for nutritional analysis (right).

The aerial samples, because they had larger stem diameters, were first ground through a higher-powered Marconi grinder available at the Bioprocess Department of Fazenda Lageado, UNESP, Botucatu (Fig. 100, 101, 102 and 103). The material was then ground again using the same blade equipment used for the roots, ensuring greater uniformity among the samples.

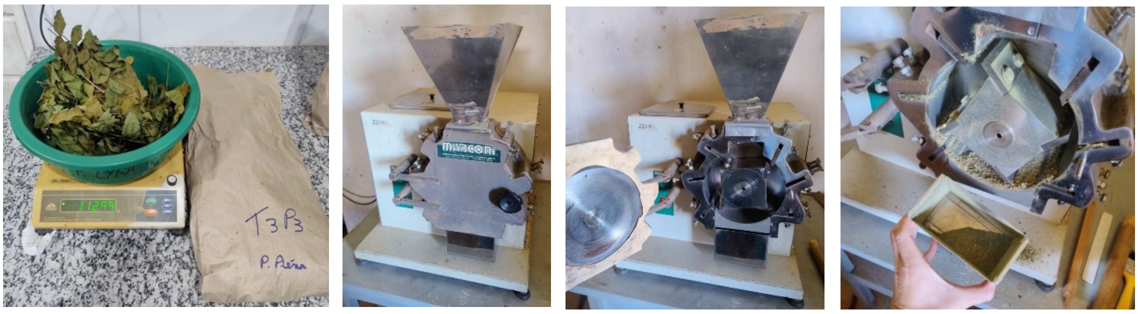


Fig. 100, 101, 102 and 103. Weighing of the dry aerial part (left); grinder used to grind the aerial part (other photos).

Between crushing one sample and another, the equipment was cleaned, including opening the machines, removing the screen, brushing with a brush to eliminate larger residues, and finally, using compressed air or a vacuum cleaner, in order to avoid cross-contamination between samples (Fig. 104 and 105).

Fig. 104 and 105. Cleaning the shredder with a brush (left) and with a vacuum cleaner (right).

The properly homogenized samples were then stored individually in identified paper bags and sent to the Laboratory of the Soil Department of FCA/UNESP for analysis of nutritional contents (Fig. 106).

Fig. 106. Homogenized samples stored individually and sent for analysis.

Nutritional analyses of plant tissues, both aerial and root, were performed at the Soil Department of FCA/UNESP, based on the methodologies described by (Malavolta, E. et al. 1997). Different digestion and reading procedures were used to quantify mineral elements.

The samples were initially subjected to nitro-perchloric digestion, a method that allows the simultaneous determination of several macro and micronutrients, including phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), sodium (Na), and aluminum (Al). Phosphorus was quantified colorimetrically using metavanadate reagent, with readings performed on a Femto 600plus visible light spectrophotometer (VIS). Sulfur was determined using the turbidimetric method, based on the formation of barium sulfate, also with readings on a VIS spectrophotometer. The other elements (K, Ca, Mg, Cu, Fe, Mn, Zn, Na, and Al) were analyzed by atomic absorption spectrophotometry using an Agilent 200 Series AA equipment.

Total nitrogen (N) was determined using the sulfuric digestion method, followed by the semi-micro Kjeldahl technique, widely used for nitrogen analysis in plant tissues. This procedure involved digesting organic matter in concentrated sulfuric acid, followed by quantification of N in the ammonium form.

Boron (B) analysis was performed by dry digestion, with the sample incinerated in a muffle furnace for subsequent solubilization and colorimetric reading using the azomethine-H reagent in a VIS spectrophotometer.

3.1.5 Statistical analysis

Univariate and multivariate statistics will be performed using R (R Core Team, 2023) and RStudio (RStudio Team, 2023). Statistical analyses will begin with analysis of variance (ANOVA) to identify significant differences between treatments (T0, T1, T2, and T3) regarding soil chemical parameters, seedling vegetative development (monthly measurements of height and stem diameter), and nutritional content of shoots and roots. ANOVA will be considered applicable when the data meet the assumptions of normality of residuals and homogeneity of variances. To compare treatment means, the Tukey-HSD (Honestly Significant Difference) test will be applied, with a significance level of $p < 0.05$, to identify which treatments differ statistically. Multivariate analyses were conducted using Principal Component Analysis (PCA), which was used to reduce data dimensionality and facilitate visualization. This enabled the identification of patterns and clustering among variables and treatments, without assuming causal relationships. To apply PCA, the data were organized into three main groups: (A) soil physical-chemical parameters; (B) final plant morphological variables (height, diameter, aerial biomass, and root biomass); (C) plant chemical and nutritional parameters, analyzed by repetition, with C1 representing the chemical analyses of the aerial part and C2 representing the chemical analyses of the roots. PCA was performed for the following group combinations: A+B, A+C1, A+C2, B+C1, B+C2, and C1+C2, aiming to reduce the dimensionality of databases with a large number of variables and identify possible structural patterns among the datasets. Additionally, a Principal Factor Analysis (PFA) was conducted, encompassing all available information, to identify latent (unobservable) factors that explain the correlations between the measured variables. These multivariate analyses contributed to a more integrated and comprehensive understanding of treatment behavior across multiple dimensions.

3.2 Results

3.2.1 Soil

- Soil dry mass density, pH and Ec

The initial dry soil density values varied among the materials evaluated. Table 12 shows that pure urban soil presented the highest value (1.108 g/cm³), while sewage sludge, being a predominantly organic material, exhibited a lower density (0.610 g/cm³). Among the treatments, the control (T0), corresponding to the commercial substrate, presented the lowest initial density (0.192 g/cm³), while the treatments with sludge addition (T1, T2, and T3) showed similar values of 0.938 g/cm³, 0.936 g/cm³, and 0.899 g/cm³, respectively. This higher density observed in the sludge treatments is due to the higher proportion of urban soil in the mixtures.

Treatment	Parameter	Mean	Standard Deviation	Min	Max	Median
Pure soil		1,108	0,02	1,085	1,124	1,114
Pure Sludge		0,610	0,022	0,592	0,635	0,603
T0_Initial		0,192	0,007	0,186	0,2	0,189
T1_Initial		0,938	0,041	0,892	0,97	0,952
T2_Initial	Soil dry	0,936	0,019	0,916	0,955	0,938
T3_Initial	mass density	0,899	0,056	0,842	0,955	0,899
T0		0,165	0,009	0,147	0,172	0,168
T1		0,937	0,043	0,899	1,009	0,922
T2		0,897	0,028	0,865	0,948	0,895
T3		0,902	0,03	0,863	0,934	0,907

Table 12. Mean, standard deviation, minimum, maximum and median of dry soil density (g/cm^3) for pure materials (soil and sewage sludge), for treatments after initial mixing (T0, T1, T2 and T3 – Initial) and after cultivation for 120 days (T0, T1, T2 and T3).

Figure 107 shows that the pure urban soil did not differ significantly from the T1, T2, and T3 treatments, which also presented statistically similar results. In contrast, these treatments differed from the pure sewage sludge and the commercial substrate (T0), which, in turn, did not present significant differences. At the end of the experiment, the dry soil densities remained stable, with no significant variations between the sludge mixture treatments (T1, T2, and T3), remaining statistically similar. A significant difference was observed only in relation to the control treatment (T0), which continued to present a lower density compared to the others.

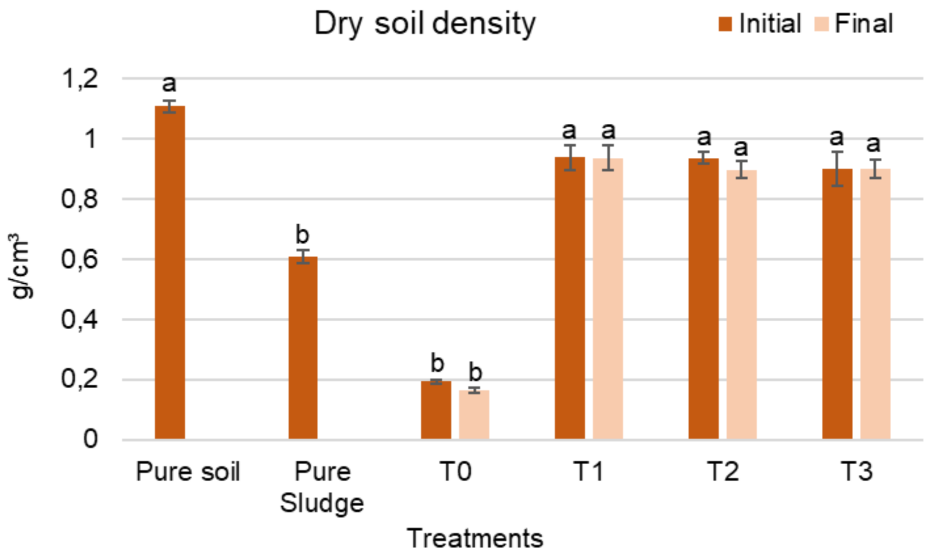


Fig. 107. Dry soil density (g/cm³) of pure materials (soil and sewage sludge), commercial substrate (T0) and experimental treatments (T1, T2 and T3), evaluated before (initial) and after (final) the 120-day cultivation period. For final measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level.

Table 13 shows that the pH and electrical conductivity (Ec) values showed similar trends throughout the experiment, although the Ec variation was more pronounced. Regarding pH, treatment T0 maintained values close to neutrality (≈7.0) throughout the experimental period, while treatments T1, T2, and T3 presented slightly more acidic values, reflecting the greater proportion of urban soil in the mixtures, characterized by natural acidity. After four months of monitoring, a gradual reduction in pH was observed in all treatments, with the decrease being more pronounced in those that received higher proportions of sludge. T0 varied from 7.04 to 6.84, T1 from 6.51 to 6.39, T2 from 6.40 to 5.48, and T3, which presented the greatest acidification, went from 6.39 to 5.05, indicating a trend of progressive acidification associated with the decomposition of organic matter.

For electrical conductivity (Ec), the initial results revealed an increase proportional to the sludge doses applied. In the first measurement (30 days), T0 presented 0.34 mS/cm, while T1, T2 and T3 reached, respectively, 5.62 mS/cm, 8.88 mS/cm and 13.1 mS/cm, corresponding to an increase of approximately 3750% in T3 compared to the control. This initial increase reflects the higher concentration of soluble salts from the sewage sludge. However, during subsequent measurements, a significant reduction in Ec was observed in all treatments, especially from the third month onwards, when the values dropped to 0.17 mS/cm (T0), 3.35 mS/cm (T1), 3.14 mS/cm (T2) and 4.65 mS/cm (T3). This reduction continued until the last measurement (May), with values between 0.16 mS/cm (T0) and 5.08 mS/cm (T3), indicating stabilization of electrical conductivity at levels suitable for plant development.

pH	T0	T1	T2	T3
06.02.25	7,04 ± 0,11	6,51 ± 0,20	6,4 ± 0,19	6,39 ± 0,06
06.03.25	6,77 ± 0,11	5,94 ± 0,27	5,69 ± 0,19	5,67 ± 0,14
07.04.25	7,27 ± 0,16	6,34 ± 0,26	5,69 ± 0,19	5,35 ± 0,34
06.05.25	6,84 ± 0,10	6,39 ± 0,14	5,48 ± 0,50	5,05 ± 0,43
Ec (mS/cm)	T0	T1	T2	T3
06.02.25	0,34 ± 0,05	5,62 ± 2,79	8,88 ± 3,65	13,1 ± 4,08
06.03.25	0,26 ± 0,10	6,48 ± 2,04	7,14 ± 2,07	11,78 ± 3,87
07.04.25	0,17 ± 0,04	3,35 ± 0,80	3,14 ± 1,66	4,65 ± 1,45
06.05.25	0,16 ± 0,06	3,04 ± 1,25	3,2 ± 2,10	5,08 ± 2,64

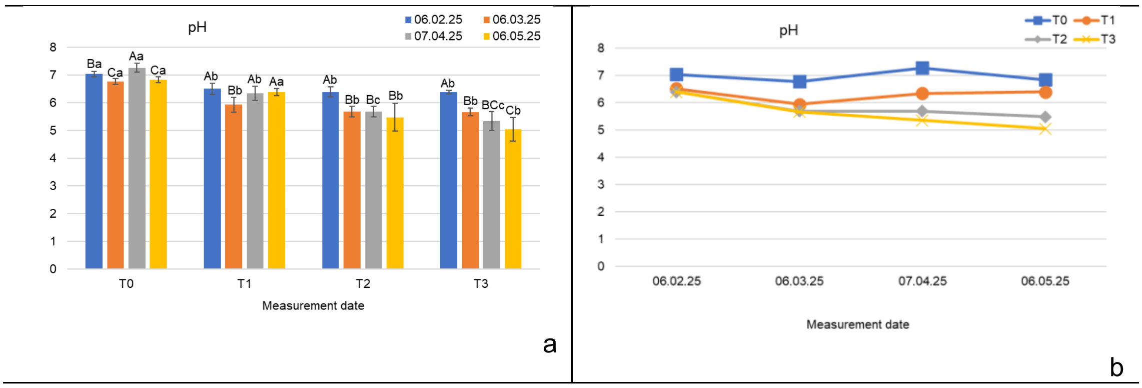
Table 13. Means and standard deviations values of monthly pH and electrical conductivity (Ec) measurements of leached material from replicates of treatments T0, T1, T2 and T3.

Figure 108 (a) highlights significant differences both between the measurement periods within each treatment and between the treatments themselves for pH. Considering the comparisons between the different measurement times (capital letters), it can be seen that all treatments showed significant variations over time. This trend was more pronounced in the treatments with a higher proportion of sewage sludge (T2 and T3), where the first measurement differed significantly from the three subsequent ones, indicating progressive acidification throughout the experiment.

In the comparison between treatments, taking into account the evaluation period (lowercase letters), T0 maintained significantly higher pH values in almost all measurements, except for the fourth, when it did not differ statistically from T1. This trend can also be observed in the line graph in figure 108 (b), which illustrates a slight reduction in pH across all treatments, most evident in those receiving higher proportions of sewage sludge.

Figure 108 (c) presents the statistical differences for electrical conductivity (Ec). Capital letters indicate the comparison between measurements within each treatment, while lowercase letters represent the comparison between treatments within the same analysis period. Overall, no significant differences were observed between the first and second measurements in any of the treatments. However, from the third evaluation onward, T0 and T3 began to differ significantly, while T1 showed a difference only in the last measurement, and T2 remained stable, with no significant variations over time.

When comparing treatments within each period, the results show that, in the first two measurements, T3 presented significantly higher EC values than the others. Treatments T1 and T2 exhibited intermediate values, with no significant difference between them, but still higher than the control (T0). In the third measurement, a marked reduction in electrical conductivity was observed in the sludge treatments, such that T1, T2, and T3 did not differ from each other, maintaining a difference only in relation to T0, which presented the lowest value. In the last measurement, none of the treatments presented statistically significant differences, indicating stabilization of salt concentrations between the mixtures. This trend is illustrated in figure 108 (d), which shows the gradual reduction of Ec over time, especially in treatments that contained sewage sludge in their compositions.



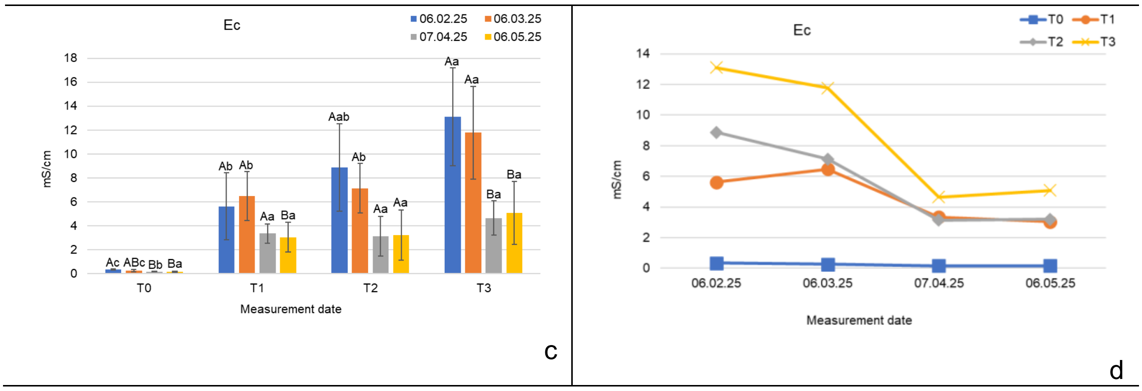


Fig. 108. pH (a, b) and electrical conductivity (Ec) values in mS/cm (c, d) of the leached material in treatments T0, T1, T2 and T3 over the different measurement dates. Different capital letters indicated significant differences ($p < 0.05$) between measurements within each treatment, while different lowercase letters indicated significant differences ($p < 0.05$) between treatments within the same measurement.

- Chemical and physical parameters of soil

Table 14 shows the initial physical and chemical parameters of the materials used in the mixtures, as well as the mixtures themselves before the experiment began. The urban soil used had a sandy loam texture (medium texture), composed of 61.2% sand, 31.9% clay, and 6.9% silt, characteristics that provide good drainage and aeration, in addition to a moderate water and nutrient retention capacity. The acidic pH (4.0) and low base saturation (V% = 13.09%) indicate initial conditions of high acidity and low fertility, with low levels of calcium (4.51 mmol/dm³), magnesium (2.82 mmol/dm³), and potassium (0.78 mmol/dm³). Organic matter (20.41 g/dm³) and phosphorus (14.56 mg/dm³) also showed low values. The sewage sludge had a pH of 5.9 and high organic matter content (109.05 g/dm³), as well as high nutrient concentrations, particularly calcium (290.3 mmol/dm³), magnesium (37.53 mmol/dm³), phosphorus (375.49 mg/dm³), and sulfur (8836.78 g/dm³). These values resulted in a base sum (SB = 330.42 mmol/dm³) and cation exchange capacity (CEC = 363.27 mmol/dm³) significantly higher than those of urban soil, with a base saturation of 90.96% and no exchangeable aluminum (Al^{3+} = 0).

In experimental treatments T1, T2, and T3, a gradual variation in chemical properties was observed depending on the incorporated sludge content. T0 (commercial substrate) presented pH 6.0, CTC of 109.95 mmol/dm³, SB of 96.39

mmol/dm³ and high phosphorus content (226.13 mg/dm³), with no presence of exchangeable aluminum. In treatments T1, T2 and T3, which received increasing amounts of sludge, a progressive increase in calcium, magnesium and phosphorus contents was observed, accompanied by an increase in CTC and base saturation, which varied from 68.4% for T1 to 79.23% for T3. Organic matter increased proportionally to sludge doses, rising from 18.29 g/dm³ (T1) to 26.01 g/dm³ (T3), and sulfur (S) increased from 466.78 g/dm³ (T1) to 1,695.21 g/dm³ (T3), demonstrating an increase in the levels of these elements with the increase in sludge proportion.

Regarding micronutrients, marked differences were observed between urban soil and sewage sludge. The soil presented low levels of B (0.31 g/dm³), Cu (2.32 g/dm³), Fe (35.52 g/dm³), Mn (4.61 g/dm³), and Zn (0.32 g/dm³), while the sludge exhibited higher values, with B = 2.78 g/dm³, Cu = 10.6 g/dm³, Fe = 530.94 g/dm³, Mn = 16.72 g/dm³, and Zn = 129.03 g/dm³.

In the experimental treatments, a gradual increase in the levels of all micronutrients was observed as the sludge proportions increased. Iron (Fe) varied from 7.56 g/dm³ at T0 to 108 g/dm³ at T3, and zinc (Zn) from 2.88 g/dm³ to 18.19 g/dm³, representing the largest increases. Manganese (Mn) and copper (Cu) also increased consistently, going from 2.84 g/dm³ and 1.22 g/dm³ at T0, to 11.19 g/dm³ and 3.62 g/dm³ (T3), respectively. Boron (B) showed a more discreet increase, varying from 0.40 g/dm³ (T1) to 0.49 g/dm³ (T3).

Parameter	Pure Soil	Pure Sludge	T0	T1	T2	T3
			------------------- Initial			---------------------
Sand (%)	61,2	-	-	-	-	-
Clay (%)	31,9	-	-	-	-	-
Silt (%)	6,9	-	-	-	-	-
Soil Texture	Medium	-	-	-	-	-
pH (CaCl2)	4	5,9	6	5	5	5,3
M.O. (g/dm3)	20,41	109,05	65,26	18,29	20,56	26,01
P resina (mg/dm3)	14,56	375,49	226,13	31	174,08	211,64
Al3+ (mmolc/dm3)	10,72	0	0	0	0	0
H+Al (mmolc/dm3)	53,88	32,85	13,56	35,36	35,73	29,88

K (mmolc/dm3)	0,78	2,59	10,56	1,74	1,66	1,62
Ca (mmolc/dm3)	4,51	290,3	14,83	22,22	44,22	52,01
Mg (mmolc/dm3)	2,82	37,53	71	52,59	53,9	60,37
SB (mmolc/dm3)	8,11	330,42	96,39	76,55	99,78	114
CTC (mmolc/dm3)	61,99	363,27	109,95	111,91	135,51	143,87
V% (%)	13,09	90,96	87,66	68,4	73,63	79,23
S (g/dm3)	23,72	8836,78	161,59	466,78	1036,71	1695,21
B (g/dm3)	0,31	2,78	1,33	0,4	0,39	0,49
Cu (g/dm3)	2,32	10,6	1,22	2,27	2,9	3,62
Fe (g/dm3)	35,52	530,94	7,56	49,52	77,59	108
Mn (g/dm3)	4,61	16,72	2,84	6,67	8,77	11,19
Zn (g/dm3)	0,32	129,03	2,88	5,93	11,25	18,19

Table 14. Initial chemical and physical characterization of the materials used in the experiment: pure urban soil, pure sewage sludge, and mixtures formed in treatments T0, T1, T2, and T3 before the start of the experiment. Data include physical attributes, macronutrients, and micronutrients.

Following the initial characterization of the materials and experimental mixtures, which provided an understanding of the starting conditions of the substrates, final chemical analyses were performed at the end of the experiment (Table 15). The objective was to evaluate the fertility parameters and nutrient availability in each treatment. The comparison between the initial and final phases allowed for the identification of the main trends of variation in the soil chemical attributes. Table 18 presents the mean values obtained for each treatment, along with their respective standard deviations.

Parameter	T0	T1	T2	T3
	------------------------------------ Final	--		
pH (CaCl2)	5,55 ± 0,15	4,52 ± 0,15	4,25 ± 0,05	4,27 ± 0,05
C (%)	24,79 ± 1,57	1,54 ± 0,13	1,99 ± 0,34	2,32 ± 0,22
M.O. (g/dm3)	73,58 ± 4,23	35,82 ± 17,49	36,34 ± 2,62	36,53 ± 5,35
P resina (mg/dm3)	42,78 ± 5,74	62,12 ± 35,93	166,59 ± 60,20	195,36 ± 93,02
Al3+ (mmolc/dm3)	0,00	4,21 ± 1,58	7,44 ± 1,68	8,73 ± 1,09
H+Al (mmolc/dm3)	17,15 ± 2,18	46,98 ± 4,17	66,77 ± 9,41	69,79 ± 8,29
K (mmolc/dm3)	4,63 ± 0,76	1,26 ± 0,40	1,17 ± 0,32	1 ± 0,40
Ca (mmolc/dm3)	37,93 ± 7,01	28,62 ± 8,62	39,7 ± 7,97	51,12 ± 17,49
Mg (mmolc/dm3)	99,13 ± 11,68	54,7 ± 15,69	52,86 ± 11,71	39,5 ± 12,08

SB (mmolc/dm3)	141,7 ± 16,40	84,58 ± 21,94	93,73 ± 18,24	91,62 ± 28,07
CTC (mmolc/dm3)	158,85 ± 16,70	131,56 ± 24,78	160,5 ± 25,21	161,4 ± 32,12
V% (%)	89,13 ± 1,54	63,53 ± 5,08	58,17 ± 4,09	55,66 ± 7,53
S (g/dm3)	6,69 ± 2,81	185,07 ± 160,05	411,45 ± 197,66	541,61 ± 213,21
B (g/dm3)	0,38 ± 0,05	0,24 ± 0,04	0,29 ± 0,02	0,36 ± 0,04
Cu (g/dm3)	2,37 ± 0,09	2,95 ± 0,38	3,95 ± 0,50	4,48 ± 0,26
Fe (g/dm3)	17,58 ± 2,79	74,93 ± 10,87	120,78 ± 8,37	148,13 ± 9,36
Mn (g/dm3)	5,95 ± 0,98	7,49 ± 1,71	9,05 ± 1,32	8,71 ± 1,69
Zn (g/dm3)	3,92 ± 0,36	6,2 ± 4,00	14,92 ± 2,97	20,6 ± 3,45

Table 15. Physicochemical parameters of treatments T0, T1, T2 and T3 at the end of the 120-day experimental period

Figure 109 shows a comparison between the initial and final values of the chemical parameters, as well as the significant differences between the treatments at the end of the experiment.

There was a general reduction in pH values in all treatments compared to the initial conditions. T0 had the highest final pH (5.55), while T1, T2, and T3 recorded more acidic values of 4.52, 4.25, and 4.27, respectively (Table 18). T0 was significantly higher than T1, which, in turn, differed from treatments T2 and T3.

T0 also had the highest final values of carbon (24.79%) and organic matter (73.58 g/dm^3), differing significantly from the others. Treatments T1, T2, and T3 maintained similar values, ranging from 1.54 to 2.32% C and 35.82 to 36.53 g/dm^3 of M.O., with no significant differences, regardless of the sludge proportion applied.

For available phosphorus (P - resin), final levels ranged from 42.78 mg/dm^3 in T0 to 195.36 mg/dm^3 in T3, demonstrating an increase with increasing sludge doses. Treatments T2 and T3 presented significantly higher values than T0 and T1, which maintained the lowest levels and did not differ from each other.

Exchangeable aluminum (Al^{3+}) values, absent in the initial analyses, were detected only in the treatments that received sludge, ranging from 4.21 $mmolc/dm^3$ (T1) to 8.73 $mmolc/dm^3$ (T3). T2 and T3 presented significantly higher values than T1, while T0 maintained no detectable aluminum presence. This behavior was also reflected in potential acidity (H+Al), which decreased from 17.15 $mmolc/dm^3$ (T0) to 69.79 $mmolc/dm^3$ (T3). Treatments T2 and T3 did not differ from each other, but both presented higher values than T1, while T0 had the lowest value.

Potassium (K^+) levels decreased compared to initial values, with T0 maintaining the highest final level (4.63 mmolc/dm³) and differing significantly from the others, while T1, T2, and T3 presented similar values.

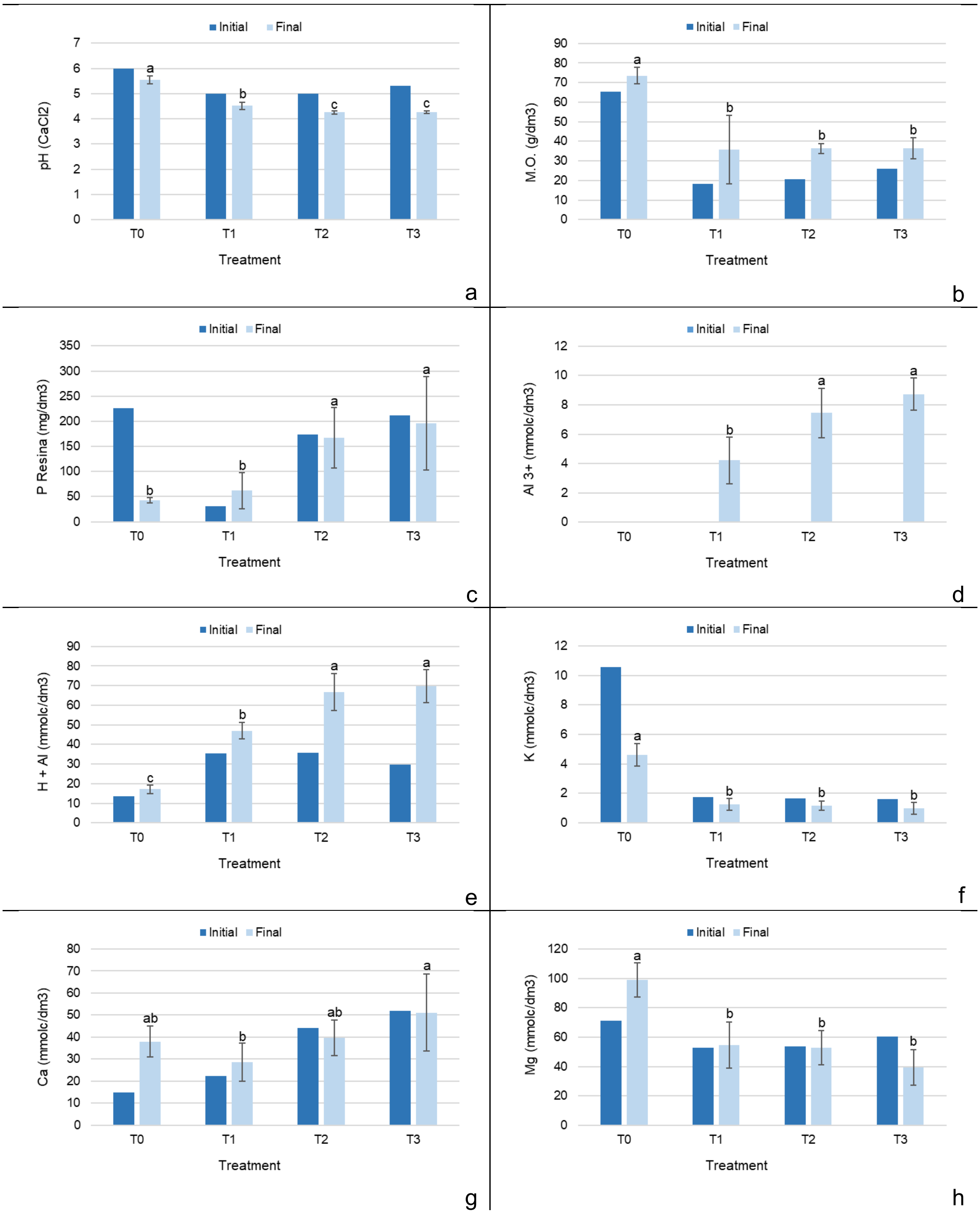
Calcium (Ca^+) and magnesium (Mg^+) levels behaved differently. Calcium increased proportionally to the sludge doses, reaching 51.12 mmolc/dm³ in T3, not differing from the T0 and T2 treatments, but being significantly higher than T1. Magnesium maintained higher levels in T0 (99.13 mmolc/dm³), differing significantly from the other treatments, while T1, T2, and T3 (54.7, 52.86, and 39.5 mmolc/dm³, respectively) were statistically similar.

The sum of bases (SB) followed the same trend as magnesium, with T0 presenting the highest value (141.7 mmolc/dm³), significantly higher than the sludge treatments, T1, T2, and T3, which did not differ from each other. The cation exchange capacity (CEC) showed a slight increase, but without significant differences among the treatments, remaining between 131.56 and 161.4 mmolc/dm³. Base saturation (V%) gradually decreased with increasing sludge doses, ranging from 89.13% (T0) to 55.66% (T3). T0 differed significantly from the others, while T1, T2, and T3 remained similar.

Sulfur (S) contents increased proportionally to sludge doses, ranging from 6.69 g/dm³ (T0) to 541.61 g/dm³ (T3), with significant accumulation in the final mixtures. Treatments T2 and T3 were significantly higher than T0 and T1, while T1 still had a higher value than T0.

Boron (B), although initially higher in T0, remained stable at the end of the experiment. Treatments T3 and T0 differed significantly from T1 and T2.

Among the metallic micronutrients, a consistent increase in the concentrations of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) was observed, accompanying the increase in sludge proportions. Copper varied from 2.37 g/dm³ (T0) to 4.48 g/dm³ (T3), and iron, from 17.58 g/dm³ (T0) to 148.13 g/dm³ (T3), both presenting significant differences among all treatments, with T3 superior to the others. Manganese increased from 5.95 g/dm³ (T0) to 8.71 g/dm³ (T3), differing significantly only from the control, while T1, T2 and T3 remained statistically similar. Zinc showed similar behavior to Cu and Fe, with a pronounced increase in mixtures with higher sludge content, ranging from 3.92 g/dm³ (T0) to 20.6 g/dm³ (T3), with significant differences between treatments, being T3 > T2 > T0, T1.



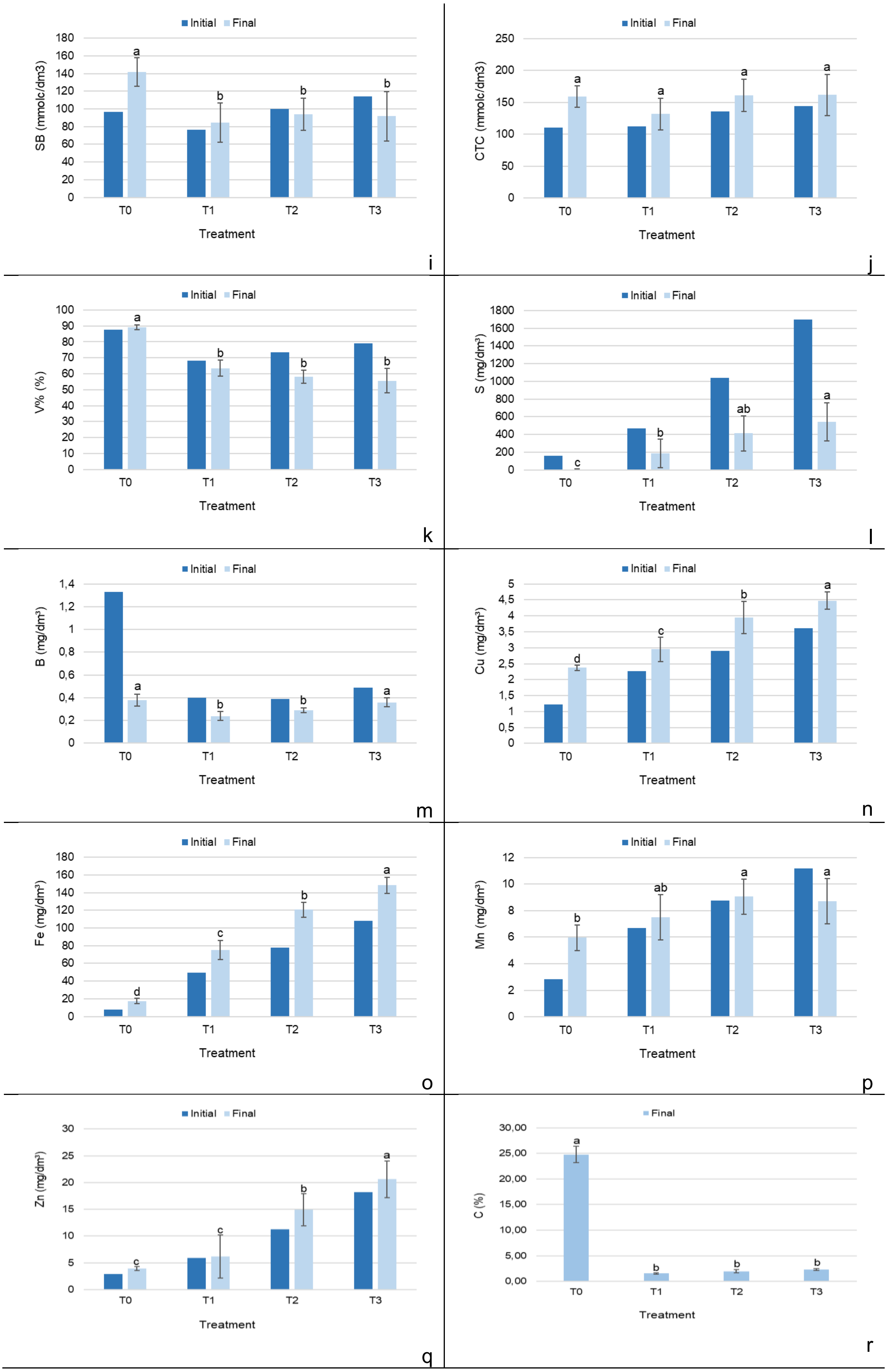

Initial
Final
SB (mmolc/dm3)
Treatment
T0
T1
T2
T3
a
b
b
b
i
CTC (mmolc/dm3)
a
a
a
a
j
V% (%)
a
b
b
b
k
S (mg/dm³)
c
b
ab
a
l
B (mg/dm³)
a
b
b
a
m
Cu (mg/dm³)
d
c
b
a
n
Fe (mg/dm³)
d
c
b
a
o
Mn (mg/dm³)
b
ab
a
a
p
Zn (mg/dm³)
c
c
b
a
q
C (%)
a
b
b
b
r

Fig. 109. Comparison between the initial and final values of soil physicochemical attributes for treatments T0, T1, T2, and T3. The parameters evaluated include (a) pH; (b) organic matter (OM); (c) available phosphorus (P resin); (d) exchangeable acidity (Al^{3+}); (e) potential acidity (H+Al); (f) potassium (K); (g) calcium (Ca); (h) magnesium (Mg); (i) sum of bases (SB); (j) cation exchange capacity (CEC); (k) base saturation (V%); (l) sulfur (S); (m) boron (B); (n) copper (Cu); (o) iron (Fe); (p) manganese (Mn); (q) zinc (Zn); and (r) Carbon. For final measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level.

3.2.2 Vegetative Development

Table 16 presents the cumulative increases in seedling height and collar diameter throughout the experiment. It can be seen that the treatments with higher proportions of sewage sludge (T2 and T3) showed slower initial development, possibly due to a period of adaptation to the chemical and physical conditions of the mixtures. However, throughout the evaluations, these treatments demonstrated progressive recovery and continuous growth, surpassing T0 in the second height increase measurement and in the last diameter increase measurement.

Height growth varied among treatments. In the first evaluation (February), the values were lower in the sludge treatments, especially T2 (7.57 cm) and T3 (13.2 cm), while T0 and T1 recorded 26.57 cm and 15.5 cm, respectively. Following evaluations, a more pronounced increase was observed in plants grown with sludge, particularly in T1, which reached 85.17 cm at the end of the experiment, representing the highest value among the treatments. Treatments T2 (84.27 cm) and T3 (80.22 cm) showed similar final values, demonstrating consistent growth similar to that of T1. T0, although maintaining good initial performance, reached 70.45 cm at the end of the period, being surpassed by the treatments with sludge. Thus, it can be observed that, after a slower start, the treatments with sludge showed better cumulative height performance.

The increase in stem diameter showed a similar pattern. At the beginning of the experiment, differences between treatments ranged from 0.03 mm (T2) to 1.17 mm (T0). As measurements progressed, the treatments with sludge demonstrated better relative performance, especially T1, which reached 7.1 mm at the end of the period—the highest value observed. Treatments T2 (6.66 mm) and T3 (6.34 mm) maintained results close to those of the control (6.62 mm), indicating that the use of sewage sludge did not compromise the growth in stem thickness and, in some cases, provided a superior increase.

Height increment	T0	T1	T2	T3
22.02.25	26,57 ± 6,23	15,5 ± 6,54	7,57 ± 4,77	13,2 ± 4,27
22.03.25	35,78 ± 11,46	44,13 ± 13,72	31,07 ± 9,46	40,6 ± 9,31
22.04.25	49,2 ± 9,91	68,78 ± 18,21	57,42 ± 12,28	60,33 ± 15,39
22.05.25	70,45 ± 15,56	85,17 ± 23,38	84,27 ± 14,06	80,22 ± 21,08
Diameter increment	T0	T1	T2	T3
22.02.25	1,17 ± 0,41	0,47 ± 0,53	0,03 ± 0,03	0,2 ± 0,14
22.03.25	2,2 ± 0,49	2,24 ± 0,69	1,41 ± 0,57	1,21 ± 0,48
22.04.25	4,32 ± 0,77	4,54 ± 1,1	4,11 ± 0,67	3,73 ± 1,00
22.05.25	6,62 ± 1,07	7,1 ± 1,35	6,66 ± 1,03	6,34 ± 1,18

Table 16. Means and standard deviations values of the cumulative increments in height (cm) and collar diameter (mm) of the seedlings throughout the experiment by treatment (T0, T1, T2 and T3). The increments were calculated in relation to the initial measurement taken on the first day of the experiment.

Figures 110 (a–d) show the increments and absolute growth in plant height and stem diameter over the five monthly measurements of the experiment. Figures 110 (a) and (c) show the average monthly increments, while figures 110 (b) and (d) illustrate the absolute growth in height and diameter using line graphs.

In Figures 110(a) and (c), different capital letters indicate significant differences ($p < 0.05$) between the measurement periods within each treatment, while different lowercase letters indicate significant differences ($p < 0.05$) between the treatments on the same measurement date.

In figure 110 (a), it can be seen that the height increment in treatment T0 was significantly higher only in the last measurement, differing from the second and third, which, in turn, were different from the first. Treatment T1 showed a similar trend, with the last increment not differing from the third measurement, but being higher than the second and first. T2 showed significant differences between all evaluated periods, demonstrating continuous and consistent growth over time. T3, on the other hand, showed a higher final increase, differing from the second and third measurements, which did not differ from each other but were distinct from the first.

When comparing treatments within each period, it is noted that in the first measurement (February 22, 2025), T0 showed a significantly higher increase than the sludge treatments (T1, T2, and T3). From the second measurement onward, however, there were no significant differences between treatments, although the mean values of T1, T2, and T3 exceeded T0 in subsequent measurements.

Figure 110 (c) shows a similar behavior for the increase in neck diameter. In all treatments, the increases increased progressively over time, with significant differences between periods, except between the first and second measurements of T3.

When comparing treatments within each period, T0 showed a greater increase in the first measurement, differing significantly from the sludge treatments. In the second measurement, the increments of T1, T2, and T3 were similar, although T3 showed a difference compared to the others. In the last two measurements, no significant differences were observed between treatments, indicating a tendency toward uniform growth.

The line graph in figure 110 (b), representing absolute height, shows that T0 showed faster initial growth, but from the third measurement onward, it was surpassed by the treatments with sludge addition (T1, T2, and T3), which maintained more continuous growth until the end of the experiment. In figure 110 (d), regarding absolute diameter, T0 showed a slight advantage in the initial measurements, but this difference disappeared in subsequent evaluations, resulting in similar final values between the treatments.

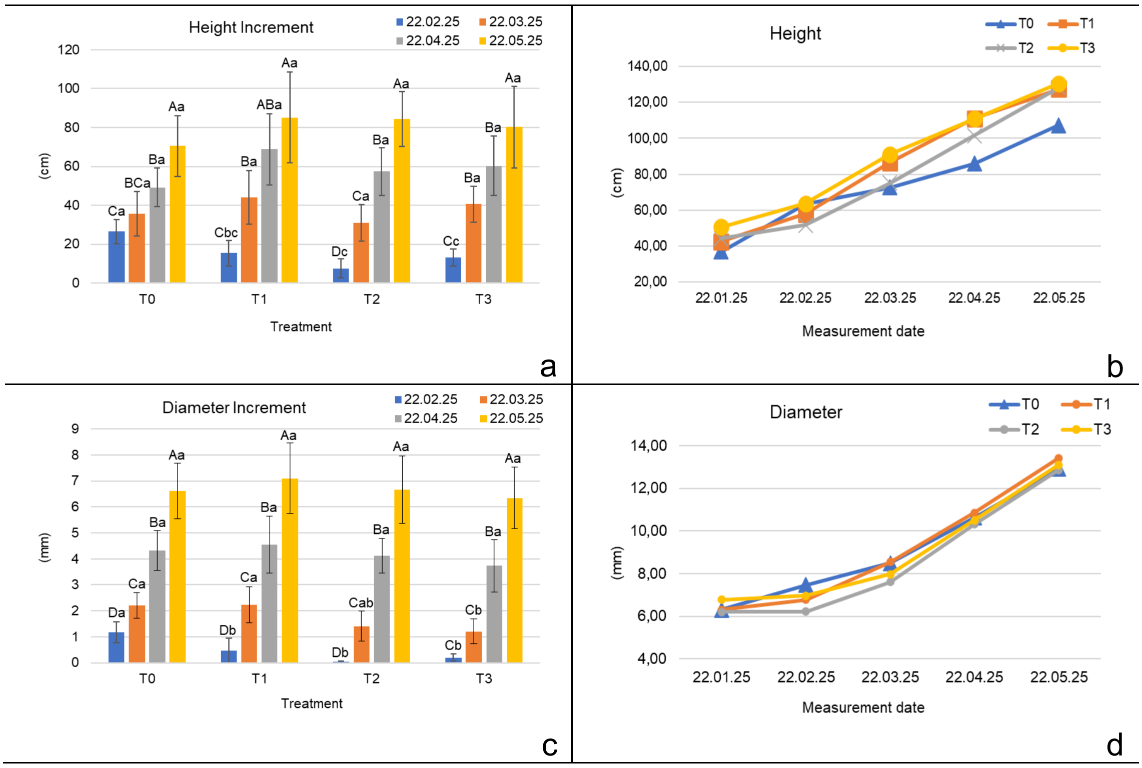


Fig. 110. (a) Average cumulative height increment over monthly measurements; (b) average absolute height per month; (c) average cumulative diameter increment over monthly measurements; (d) average absolute diameter per month. Different capital letters indicated significant differences ($p < 0.05$) between increments within each treatment, while different lowercase letters indicated significant differences ($p < 0.05$) between treatments within the same increment measurement. Comparisons between treatments T0, T1, T2, and T3.

Indirect quantification of leaf chlorophyll content was performed using the Falker ClorofiLOG 1030 equipment, which estimates the Leaf Chlorophyll Index (LCI) non-destructively. The instrument's operating principle is based on measuring light transmittance at different wavelengths, specifically in the red and near-infrared regions, which are absorbed at different intensities by the chlorophyll molecules present in the leaves. From these readings, the device separately calculates the relative indices of chlorophyll a and chlorophyll b, as well as the total chlorophyll index (a + b), indirectly reflecting the photosynthetic pigment content. This methodology allows for a rapid, accurate, and non-destructive assessment of the physiological state of plants, also constituting an indirect method for estimating nitrogen concentration in plant tissues.

Table 17 shows that the mean values of the Leaf Chlorophyll Index (LCI) varied among the treatments evaluated. For chlorophyll a, a significant increase was observed in treatments T1 (40.72), T2 (40.23) and T3 (39.05) in relation to the control treatment T0 (32.29). Chlorophyll b showed a similar behavior, with higher values in T1 (28.34), T2 (26.68) and T3 (25.87), compared to the lowest value observed in the control (12.45). When analyzing total chlorophyll (a + b), the highest indices were recorded in treatments T1 (69.06), T2 (66.90) and T3 (64.92), in contrast to T0 (44.74), indicating an increase in the relative content of photosynthetic pigments in response to treatments with sewage sludge application.

Treatment	Parameter (ICF)	Mean	Standard Deviation	Min	Max	Median
T0	Average chlorophyll A	32,29	2,330	28,85	35	32,48
T1		40,72	0,534	40,2	41,7	40,68
T2		40,23	0,887	38,95	41,5	40,25
T3		39,05	1,666	36,5	40,7	39,65
T0	Average chlorophyll B	12,45	2,091	9,8	15,95	12,63
T1		28,34	1,835	25,75	30,85	28,7
T2		26,68	2,397	24,85	30,5	25,43
T3		25,87	4,339	19,1	29,2	28,05
T0	Average total chlorophyll	44,74	4,242	38,65	50,4	45,1
T1		69,06	2,122	66,35	71,6	69,23
T2		66,9	3,182	63,95	72	65,55
T3		64,92	5,990	55,6	69,75	67,7

Table 17. Means, standard deviations, minimum, maximum and median values of the chlorophyll A, B and total indices (ICF) obtained in the seedlings of treatments T0, T1, T2 and T3.

Figure 111 shows the statistical analysis performed using Tukey's test ($p < 0.05$), which showed a significant difference between the control treatment (T0) and the other treatments (T1, T2, and T3) for the total chlorophyll index, the latter being grouped under the same letter ("a"), indicating no significant difference between them. For chlorophyll a and b, a similar behavior was observed, with T0 differing statistically from the other treatments, which presented higher and statistically equivalent values. These results demonstrate that the treatments promoted a significant increase in leaf chlorophyll content, suggesting an improvement in the photosynthetic efficiency of the plants.

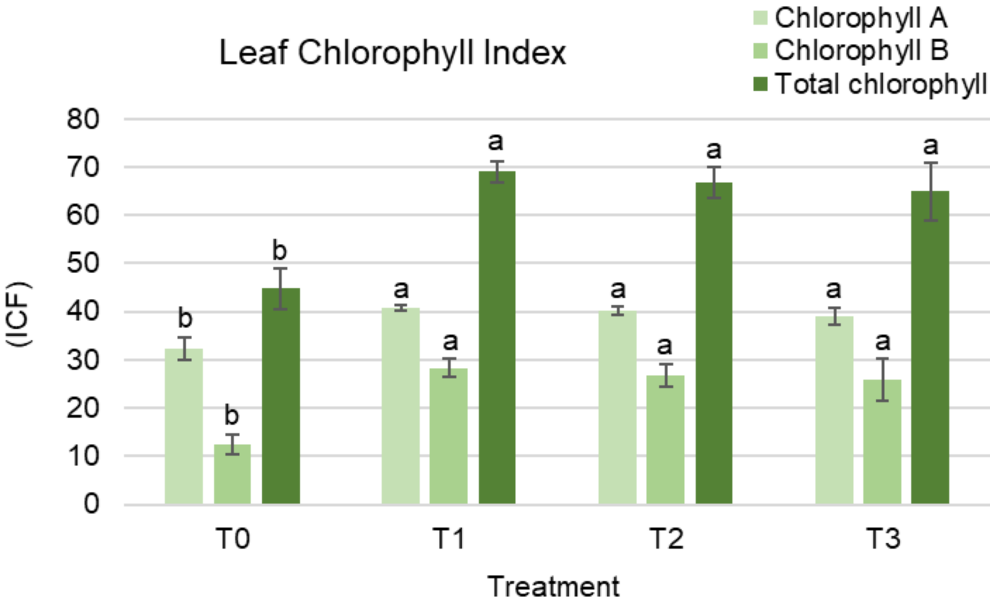


Fig. 111. Chlorophyll A, chlorophyll B and total chlorophyll (ICF) indices in the leaves of seedlings from treatments T0, T1, T2 and T3. For the measurements, different letters mean significant difference between treatments by Tukey's HSD test at a 5% significance level.

Regarding dry biomass, table 18 shows an increase in the average values of the aerial part in the treatments that received percentages of sewage sludge in their formulations, in relation to the control treatment. Treatment T2 presented the highest average value (100.81 g), followed by T1 (100.62 g) and T3 (99.54 g), while the control treatment T0 presented the lowest value (57.18 g). For the dry biomass of the root part, the values presented less variation between the treatments, with T3 (25.71 g) being the highest, followed by T1 (22.68 g) and T0 (20.29 g), while T2 (16.75 g) presented the lowest value. Considering the total dry biomass, which corresponds to the sum of the aerial and root parts, the highest values were observed in treatments T3 (125.25 g), T1 (123.29 g) and T2 (117.56 g), in contrast to the lowest value recorded in T0 (77.47 g), which shows the increase in the production of total dry mass of the plants when subjected to the application of sewage sludge.

Treatment	Parameter	Mean	Standard Deviation	Min	Max	Median
T0		57,18	18,567	33,65	84,86	53,95

T1	Dry Mass of	100,62	12,537	76,52	112,37	104,45
T2	Shoot Part	100,81	17,638	73,87	121,53	98,26
T3	(g)	99,54	22,32	76,55	134,86	96,89
T0		20,29	7,083	11,92	29,89	18,23
T1	Dry Mass of	22,68	7,606	12,95	31,43	21,37
T2	Root Part (g)	16,75	5,58	10,34	26,68	16,29
T3		25,71	7,818	18,82	35,53	22,63
T0		77,47	24,994	45,57	112,67	71,36
T1	Total Dry	123,29	17,278	89,47	137,17	130,16
T2	Mass (g)	117,56	20,044	84,21	137,15	118,14
T3		125,25	22,69	95,98	153,68	121,68

Table 18. Mean, standard deviation, minimum, maximum and median values of shoot dry mass, root dry mass and total dry mass of plants in treatments T0, T1, T2 and T3

As shown in Figure 112, the statistical analysis revealed that the treatments with sewage sludge addition (T1, T2, and T3) presented significant differences compared to the control treatment (T0) for shoot dry biomass and total dry biomass. For root dry biomass, no statistically significant differences were observed among any of the treatments. These results suggest that the application of sewage sludge contributed to the increase in plant dry biomass, especially in the shoot.

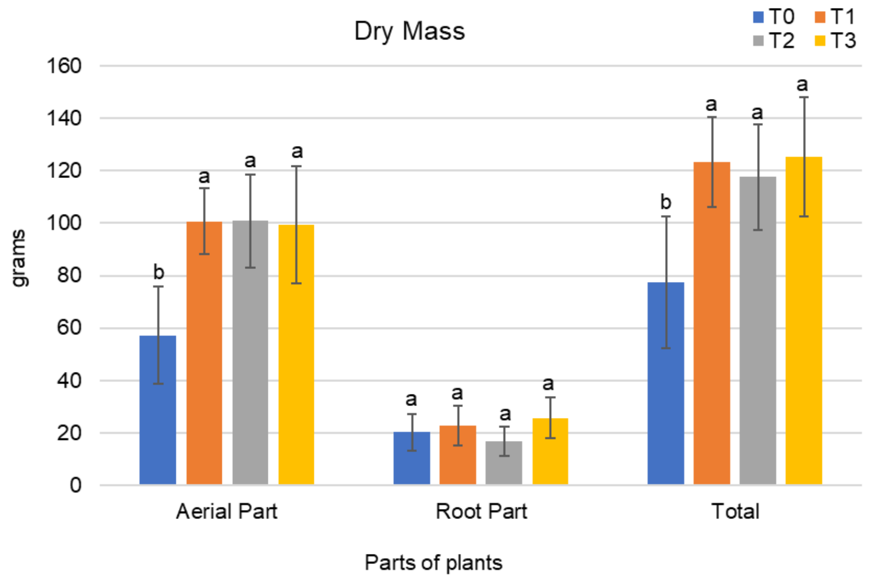


Fig. 112. Comparison of the dry mass (grams) of seedlings by treatment (T0, T1, T2 and T3), separated by aerial part, root part and total in treatments T0, T1, T2, and T3. For the

measures, different letters mean significant difference between treatments by Tukey's HSD test at a significance level of 5%.

3.2.3 Vegetative Chemical Analysis

As shown in table 19, the macronutrient content in the aerial and root parts of the plants varied between treatments. In general, it was observed that nitrogen (N) showed an increasing behavior in response to treatments with sewage sludge application, both in the aerial and root parts, reaching maximum values in T2 (22.61 g kg^{-1}) and T3 (20.72 g kg^{-1}) in the aerial part, and in T3 (15.87 g kg^{-1}) in the root, compared to the lowest values of the control treatment (8.19 g kg^{-1} and 6.93 g kg^{-1}, respectively). Sulfur (S) showed a similar trend to nitrogen, with a marked increase in treatments T1, T2, and T3, with T1 (3.46 g kg^{-1}), T2 (3.78 g kg^{-1}), and T3 (3.39 g kg^{-1}) standing out in the shoot and T3 (4.22 g kg^{-1}) in the root, evidencing a positive correlation between the increase in N and S.
In contrast, phosphorus (P) showed a distinct behavior, with a reduction in mean values in treatments T1, T2, and T3 compared to the control in the shoot (2.31 g kg^{-1} in T0 versus 1.56 to 1.93 g kg^{-1} in the others). However, no major differences were observed in the root, with the four treatments ranging from 1.67 to 1.94 g kg^{-1}. Potassium (K) also showed a decreasing trend in response to increasing sewage sludge application, with the highest levels recorded in the control (16 g kg^{-1} in the shoot and 17.55 g kg^{-1} in the root), while the lowest values were observed in T2 (12.07 g kg^{-1}) and T3 (13.27 g kg^{-1}) in the shoot, and T3 (11.2 g kg^{-1}) in the root. Calcium (Ca) and magnesium (Mg) showed a more balanced behavior among the plant parts, with a slight increase in the treatments compared to the control. In the shoot, the highest calcium levels were observed in T2 (5.85 g kg^{-1}), followed by T3 (4.97 g kg^{-1}), while in the root the values ranged from 2.77 to 3.42 g kg^{-1} without significant variations. For magnesium, the root part showed higher levels in T1 (4.32 g kg^{-1}) and T2 (4.97 g kg^{-1}), while in the aerial part the values remained stable between 2.0 and 2.42 g kg^{-1}.

Aerial Parameter	T0	T1	T2	T3

N (g/Kg)	8,19 ± 1,30	18,91 ± 2,35	22,61 ± 1,66	20,72 ± 3,82
P (g/Kg)	2,31 ± 0,66	1,64 ± 0,22	1,93 ± 0,38	1,56 ± 0,48
K (g/Kg)	16 ± 2,30	13,77 ± 1,29	12,07 ± 2,58	13,27 ± 2,71
Ca (g/Kg)	3,55 ± 0,60	4,63 ± 0,30	5,85 ± 0,52	4,97 ± 0,90
Mg (g/Kg)	2,27 ± 0,34	2,42 ± 0,17	2,27 ± 0,21	2 ± 0,25
S (g/Kg)	1,36 ± 0,25	3,46 ± 0,50	3,78 ± 0,56	3,39 ± 1,32
Root Parameter	T0	T1	T2	T3
N (g/Kg)	6,93 ± 0,56	13,49 ± 1,99	15,04 ± 1,48	15,87 ± 1,80
P (g/Kg)	1,87 ± 0,29	1,67 ± 0,27	1,94 ± 0,31	1,84 ± 0,48
K (g/Kg)	17,55 ± 1,30	14,7 ± 1,98	11,68 ± 2,28	11,2 ± 2,41
Ca (g/Kg)	3,42 ± 0,21	2,77 ± 0,37	2,85 ± 0,18	2,77 ± 0,33
Mg (g/Kg)	2,7 ± 0,36	4,32 ± 0,67	4,97 ± 0,95	3 ± 0,01
S (g/Kg)	1,51 ± 0,19	3,63 ± 0,82	2,93 ± 0,35	4,22 ± 1,35

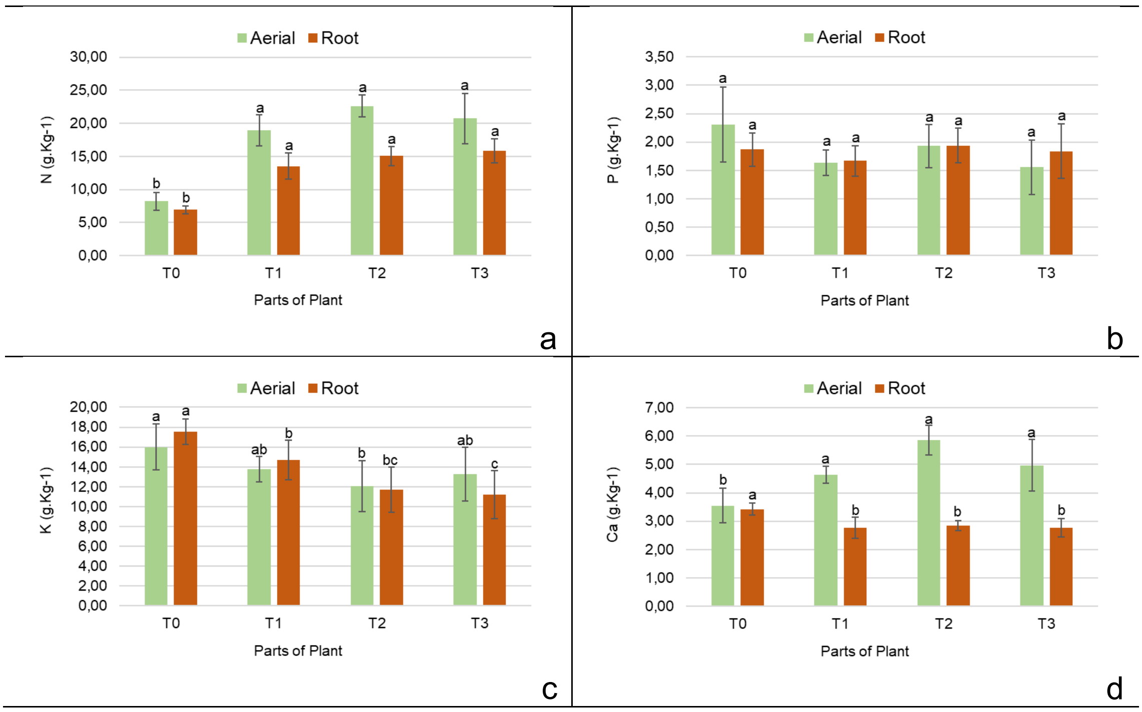
Table 19. Mean and standard deviation values of macronutrient contents (N, P, K, Ca, Mg and S) in the aerial and root parts of seedlings subjected to treatments T0, T1, T2 and T3.

Fig. 113 (a–f) shows the significant differences between treatments, according to the Tukey test ($p < 0.05$), applied separately to the shoot and root data.
The nitrogen (N) results (Fig. 113 a) indicate significant differences between treatments, both in the shoot and root parts. Treatment T0 presented the lowest mean values, differing statistically from treatments T1, T2, and T3, which exhibited higher and statistically similar levels. This behavior demonstrates that the application of sewage sludge promoted a significant increase in nitrogen absorption and accumulation in plants, which corroborates the results previously observed indirectly through the leaf chlorophyll index. A similar trend was observed for sulfur (Fig. 113 f), in which treatment T0 presented the lowest mean values, differing from treatments T1, T2, and T3, which maintained higher and statistically equivalent levels. Regarding phosphorus (P) (Fig. 113 b), no statistically significant differences were observed between treatments, either in the shoots or in the roots. Values fluctuated slightly between the conditions evaluated, with no clear upward or downward trend. For potassium (K) (Fig. 113 c), significant differences were observed in both parts of the plants. In the shoots, treatment T0 presented the highest average value, differing from T2, which had the lowest content, while T1 and T3 exhibited intermediate values, with no

statistical distinction from the others. In the roots, a similar pattern was observed, with T0 presenting the highest K content and T3 the lowest, while T1 and T2 remained at intermediate levels. Overall, the results indicate a reduction in potassium content in plants subjected to sewage sludge application.

The behavior of calcium (Ca) (Fig. 113 d) differed between plant parts. In the shoots, the treatments with sludge addition showed higher levels, without significantly differing from each other, but higher than the T0 control, which exhibited the lowest mean value. In the roots, an inverse relationship was observed, with the T0 treatment presenting the highest calcium content, significantly different from the others, regardless of the sludge dose applied. This pattern suggests that the application of sewage sludge stimulated the translocation of calcium from the roots to the shoots.

On the other hand, magnesium (Mg) levels (Fig. 113 e) showed significant differences only in the roots. Treatments T1 and T2 showed the highest mean values, differing statistically from T0 and T3, which exhibited the lowest levels. In the shoots, however, no significant differences were observed, with values remaining similar among all treatments.



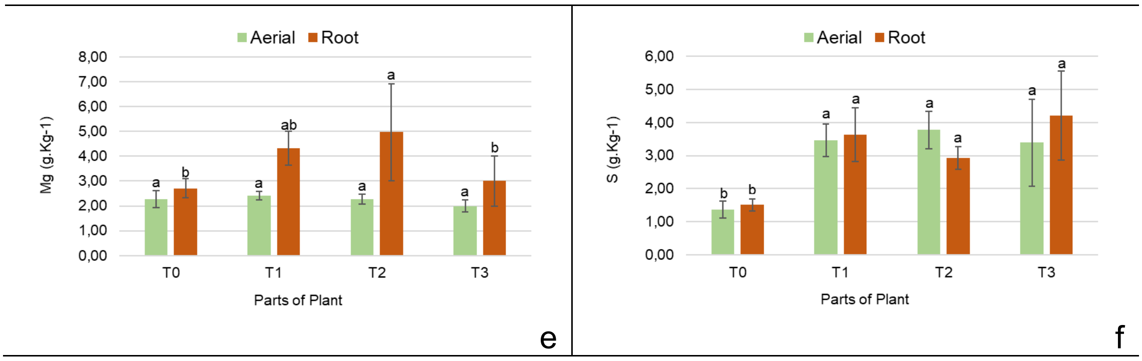


Fig. 113. Macronutrient contents in the aerial and root parts of seedlings in different treatments (T0, T1, T2 and T3). (a) Nitrogen (N); (b) Phosphorus (P); (c) Potassium (K); (d) Calcium (Ca); (e) Magnesium (Mg); (f) Sulfur (S). Different letters indicate significant difference between treatments by Tukey's HSD test ($p < 0.05$), applied separately to data from the aerial part and the root part. Results expressed in g/kg.

Table 20 shows that micronutrient levels in the shoots and roots of plants varied between treatments, reflecting different responses to sewage sludge application. Boron (B) levels were relatively stable in the shoots, ranging from 9.09 to 12.66 mg kg^{-1}, with no clear upward or downward trend. However, in the roots, levels progressively increased, from 9.68 mg kg^{-1} at T0 to 19 mg kg^{-1} at T3, suggesting greater accumulation of the element in the roots of plants subjected to treatments with higher percentages of sludge application.

Copper (Cu) also showed a consistent increase in response to treatments. In the shoots, levels increased moderately, ranging from 3.67 mg kg^{-1} at T0 to 9.5 mg kg^{-1} at T2. In the roots, the increase was more significant and continuous, with values of 18.5 mg kg^{-1} (T0), 26.83 mg kg^{-1} (T1), 39.33 mg kg^{-1} (T2), and 46.33 mg kg^{-1} (T3), demonstrating greater copper retention in root tissues due to sludge application.

Regarding iron (Fe), a marked accumulation was observed in the roots, directly associated with sewage sludge application. Levels increased from 569.67 mg kg^{-1} (T0) to values above 5000 mg kg^{-1} in the T2 and T3 treatments, representing an increase more than eightfold compared to the control. In the shoots, values remained stable between treatments, ranging from 87.67 to 100.67 mg kg^{-1}, indicating restricted translocation of the element to the upper tissues and predominant accumulation in the root zone.

The behavior of manganese (Mn) was similar to that observed for iron, showing a strong upward trend in response to the percentage of sewage sludge application. In the shoots, levels increased progressively, from 82.33 mg kg^{-1} (T0) to 261.67 mg kg^{-1} (T3), passing through 188.5 mg kg^{-1} (T1) and 253.17 mg kg^{-1} (T2). In the roots, there was also a significant increase, from 33.83 mg kg^{-1} (T0) to 107.83 mg kg^{-1} (T3). This consistent increase indicates that sewage sludge contributed significantly to increased manganese availability and uptake by plants, and was also the micronutrient that exhibited the greatest translocation from root to shoot.

Finally, zinc (Zn) showed similar results to copper, showing a progressive increase with treatment, both in shoot and root growth. In shoot growth, levels increased from 13.33 mg kg^{-1} (T0) to 81.83 mg kg^{-1} (T3), then 31.17 mg kg^{-1} (T1) and 56.33 mg kg^{-1} (T2). In the roots, the increase was even more pronounced, ranging from 26.67 mg kg^{-1} (T0) to 189.46 mg kg^{-1} (T3), with intermediate values of 86 mg kg^{-1} (T1) and 141.83 mg kg^{-1} (T2). As observed for copper and iron, zinc demonstrated preferential accumulation in the roots, but also with positive effects on the shoot.

Aerial Parameter	T0	T1	T2	T3
B (mg/Kg)	12,66 ± 1,74	9,09 ± 1,44	11,79 ± 1,39	11,79 ± 3,45
Cu (mg/Kg)	3,67 ± 1,03	8,5 ± 1,98	9,5 ± 2,59	8 ± 3,23
Fe (mg/Kg)	100 ± 35,32	97,5 ± 16,38	100,67 ± 11,69	87,67 ± 9,93
Mn (mg/Kg)	82,33 ± 12,74	188,5 ± 15,76	253,17 ± 64,10	261,67 ± 53,26
Zn (mg/Kg)	13,33 ± 11,61	31,17 ± 5,91	56,33 ± 9,37	81,83 ± 16,89
Root Parameter	T0	T1	T2	T3
B (mg/Kg)	9,68 ± 1,15	15,01 ± 1,45	16,06 ± 3,05	19 ± 3,28
Cu (mg/Kg)	18,5 ± 3,62	26,83 ± 7,14	39,33 ± 8,78	46,33 ± 9,61
Fe (mg/Kg)	569,67 ± 90,40	3813,67 ± 1048,90	5227,17 ± 2893,20	5203,83 ± 1742,85
Mn (mg/Kg)	33,83 ± 7,52	95 ± 26,86	90,33 ± 19,44	107,83 ± 22,99
Zn (mg/Kg)	26,67 ± 6,74	86 ± 32,04	141,83 ± 10,61	189 ± 46,85

Table 20. Mean and standard deviations values of micronutrient contents (B Cu, Fe, Mn and Zn) in the aerial and root parts of seedlings subjected to treatments T0, T1, T2 and T3.

Fig. 114 (a – e) shows the mean levels of the micronutrients boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) in the shoots and roots of plants subjected to the different treatments. Differences between treatments were assessed using Tukey's test ($p < 0.05$), applied separately to the data for each plant part.

Regarding boron (B) (Fig. 114 a), significant differences were observed only in the roots of the plants. Treatment T0 had the lowest mean value, differing statistically from T2 and T3, which exhibited the highest levels, while T1 maintained an intermediate value, with no significant difference compared to the others. In the shoots, the values were statistically similar between treatments, with no significant differences.

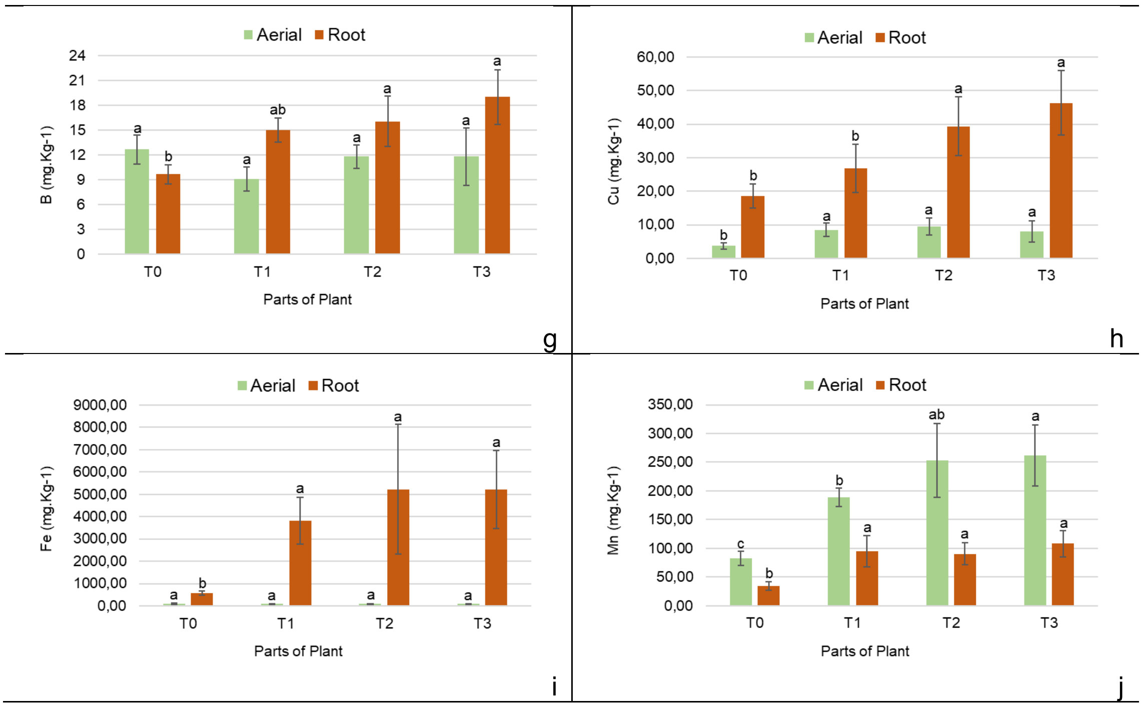
Similarly, copper (Cu) also showed significant differences, especially in the roots (Fig. 114 b). Cu levels increased progressively as the percentage of sewage sludge applied increased, with T0 and T1 presenting the lowest average values, statistically differing from T2 and T3, which presented the highest levels. In the shoots, the values were lower, but there was still a significant difference, with T0 maintaining the lowest value, while T1, T2, and T3 presented higher and statistically equivalent levels.

In the case of iron (Fe), significant accumulation of the element was observed in the roots, with marked statistical differences between the treatments. T0 presented significantly lower values than those observed in T1, T2, and T3, which exhibited much higher and statistically similar levels, as shown in fig. 114 c. In the shoots, no significant differences were observed, maintaining low values compared to those recorded in the roots.

The opposite behavior was observed for manganese (Mn) (Fig. 114 d), which presented significant differences, mainly in the shoots of the plants. Treatments T2 and T3 presented the highest averages, with no significant differences between them, while T1 showed intermediate values, differing statistically from both T2 and T3, as well as from T0, which presented the lowest average values. In the roots, the levels were lower than in the shoots, but there was still a significant difference between T0 and the other treatments, with T1, T2, and T3 exhibiting higher and statistically similar values.

Finally, the results for zinc (Zn) (Fig. 114 e) reveal a progressive increase in levels in both parts of the plant, with significant differences between treatments. In the shoots, treatment T3 presented the highest average value, differing statistically from T2, which in turn was higher than T0 and T1, which did not differ from each other. In the root parts, all treatments presented significant differences, following the order T3 > T2 > T1 > T0.

Overall, the results obtained for micronutrients indicate that the application of sewage sludge significantly increased the absorption and accumulation of essential elements, predominantly in the roots. Among the nutrients evaluated, copper (Cu) and iron (Fe) stood out for their most significant accumulation in root tissues, while manganese (Mn) showed a distinct pattern, with higher levels in the shoots. Zinc (Zn) showed a progressive increase in both parts of the plant, although with greater retention in the roots, and boron (B) showed discreet variations and relatively stable behavior between treatments. Thus, it can be inferred that manganese showed greater translocation from the roots to the shoots, while copper, iron, and zinc remained more concentrated in the roots, and boron showed little variation between different parts of the plants.



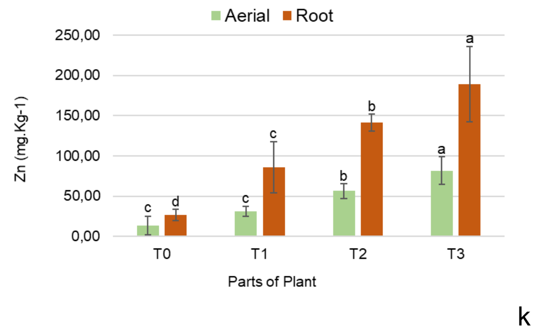


Fig. 114. Micronutrient contents in the aerial and root parts of seedlings subjected to different treatments (T0, T1, T2 and T3). (g) Boron (B); (h) Copper (Cu); (i) Iron (Fe); (j) Manganese (Mn) and (k) Zinc (Zn). Different letters indicate significant difference between treatments by Tukey's HSD test ($p < 0.05$), applied separately to data from the aerial part and the root part. The results are expressed in mg/kg.

3.2.4 Multivariate Statistics

We applied Principal Component Analysis (PCA) to summarize the variability of the data and facilitate visualization, highlighting patterns and groupings between variables and treatments, without inferring causality. The input data were organized into three sets: (A) physical-chemical parameters of the soil at the end of the experiment; (B) final morphological variables of the plant (height, diameter, aboveground biomass and root biomass); and (C) chemical and nutritional parameters of the plant, evaluated by replication, with C1 being the aboveground part and C2 the roots. PCA was applied to the combinations A+B, A+C1, A+C2, B+C1, B+C2 and C1+C2, with the aim of reducing the dimensionality of matrices with many variables and revealing possible structural patterns between the sets. In figure 115, you can see that the PCA analysis reveals the distribution between treatments along two main axes, highlighting the chemical and structural relationships induced by the progressive application of sewage sludge in urban substrates for the production of Nerium oleander. These results exemplify the multifaceted effects of sludge on essential properties, nutrients, and potentially toxic elements (PTEs), as well as the vegetative response of plants, offering important practical and applicable information for the management of alternative substrates in urban environments.

Principal component analysis (PCA), performed with groups A (soil physicochemical parameters) and B (final morphological variables of the plants), revealed relevant structural patterns among the treatments, highlighting the clear separation of the control treatment (T0) in relation to the treatments with sewage sludge application. Dimension 1 (PCA1) explained 45.3% of the data variance, while Dimension 2 (PCA2) accounted for 18.7%, totaling 64% of the explained variance. In PCA1, the largest contributors were pH, Zn, Cu, and organic carbon (OC). In PCA2, cation exchange capacity (CSC) and stem diameter (D) stood out, followed by root biomass. The biplot graph revealed good clustering of treatments, where T0 was positioned on the left, strongly associated with pH and OC, while T2 and T3 correlated with Cu, Zn, available phosphorus (P_av), and shoot dry biomass (Bs). Treatment T1 occupied a more central position, reflecting a balance between the different parameters analyzed. It is also observed that, as the proportions of sludge applied increased (4%, 8%, and 12% for T1, T2, and T3, respectively), the treatments approached the vectors associated with nutritional levels. The directions of the vectors indicate positive correlations between micronutrient levels and shoot biomass, suggesting that the increasing use of sludge contributed to plant development, evidence also confirmed in the field. In contrast, the CSC vector showed a negative correlation with the other growth parameters.

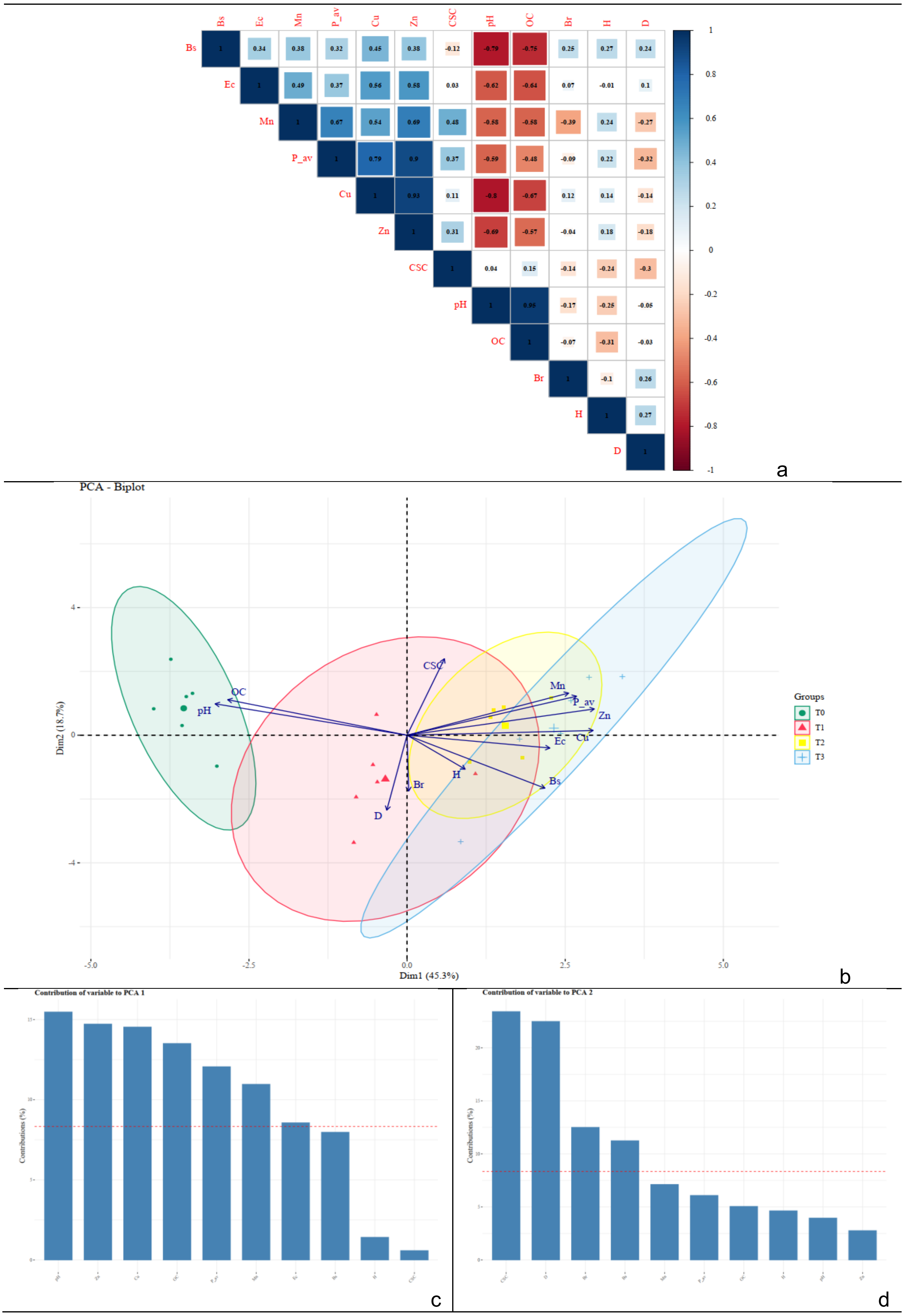


Fig. 115. PCA between soil physicochemical parameters and plant growth parameters (A + B). (a) Pearson correlation matrix; (b) PCA Biplot; (c) PCA1 contribution; (d) PCA2 contribution.

In figure 116, you can see the principal component analysis (PCA), conducted between groups A (soil physicochemical parameters) and C1 (nutrients accumulated in the aerial part), again showed a clear separation between the control treatment (T0) and the treatments with sewage sludge application. Dimension 1 (PCA1) explained 49.7% of the data variance, while Dimension 2 (PCA2) contributed 12.5%, totaling 62.2% of the explained variance. In PCA1, the variables with the greatest contribution were soil pH and N and Mg in the aerial part, in addition to soil organic carbon (OC). In PCA2, the highlights were the levels of K, P, and Mg accumulated in the aerial part. The biplot demonstrated that T0 remained isolated, strongly related to the pH and OC vectors, reflecting the characteristics of the commercial substrate used, peat-based, with a higher pH and higher organic matter content. On the other hand, treatments T1, T2, and T3, composed of high percentages of acidic latosol, were associated with vectors related to nutrients accumulated in both the soil and the aerial part of the plant. Treatments T0, T1, and T2 showed well-defined clustering, while T3 exhibited greater dispersion, indicating variability in plant responses to increased sludge content. Specifically, T1 showed a strong association with K, P, Mg, B, and Fe levels in the aerial part. The directions of the vectors show positive correlations between micronutrient levels and nutritional parameters of the aerial part, reinforcing the beneficial effect of sewage sludge on nutrient absorption and availability by plants.

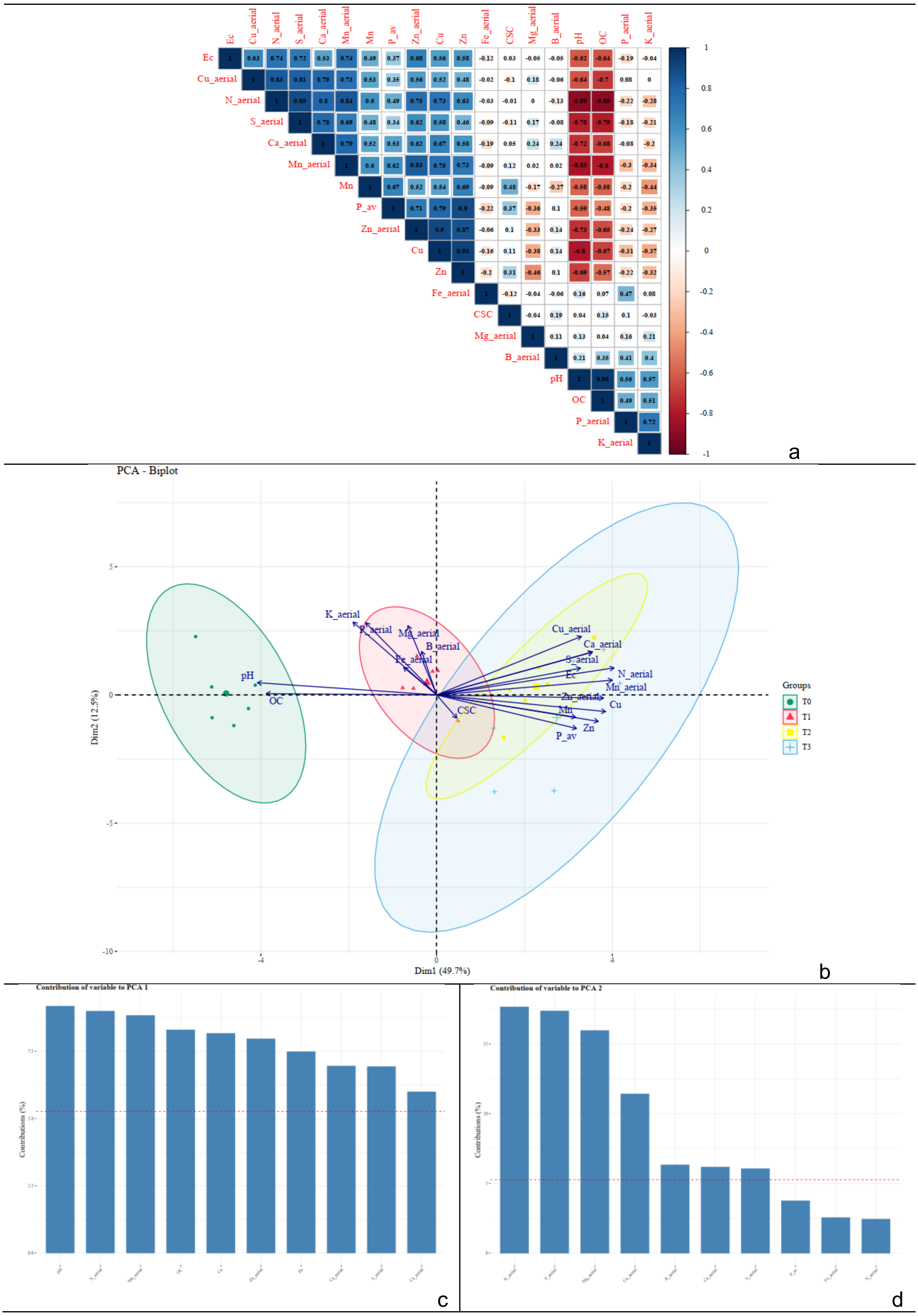


Fig. 116. PCA between soil physicochemical parameters and chemical parameters of the aerial part of plants (A + C1). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2.

In figure 117, you can see the principal component analysis (PCA), performed between groups A (soil physicochemical parameters) and C2 (nutrients accumulated in the root system), showed a structure similar to previous analyses, highlighting a clear separation between the control treatment (T0) and the treatments with sewage sludge application. Dimension 1 (PCA1) explained 55.6% of the variance, while Dimension 2 (PCA2) accounted for 11.2%, totaling 66.8% of the explained variance. In PCA1, soil pH, as well as the levels of N and Zn accumulated in the roots, stood out as the main contributors. In PCA2, P and Ca in the root were the main contributors, followed by available phosphorus in the soil. The biplot demonstrated that T0 remained strongly associated with the pH and organic carbon (OC) vectors, typical characteristics of the peat-based substrate. In contrast, treatments T1, T2, and T3 were progressively distributed from left to right, following the increase in the proportions of sludge applied. These treatments clustered close to the vectors associated with nutrients accumulated in the soil and roots, such as Cu, Zn, N, S, B, and Mn. Treatment T3, with the highest proportion of sludge, showed greater dispersion, indicating a more pronounced variability in plant responses. The directions of the vectors indicate positive correlations between the use of sludge and the increase in nutrient concentrations in both the soil and roots, while pH and OC showed a negative correlation with these nutrients, with the exception of the cations K and Ca.

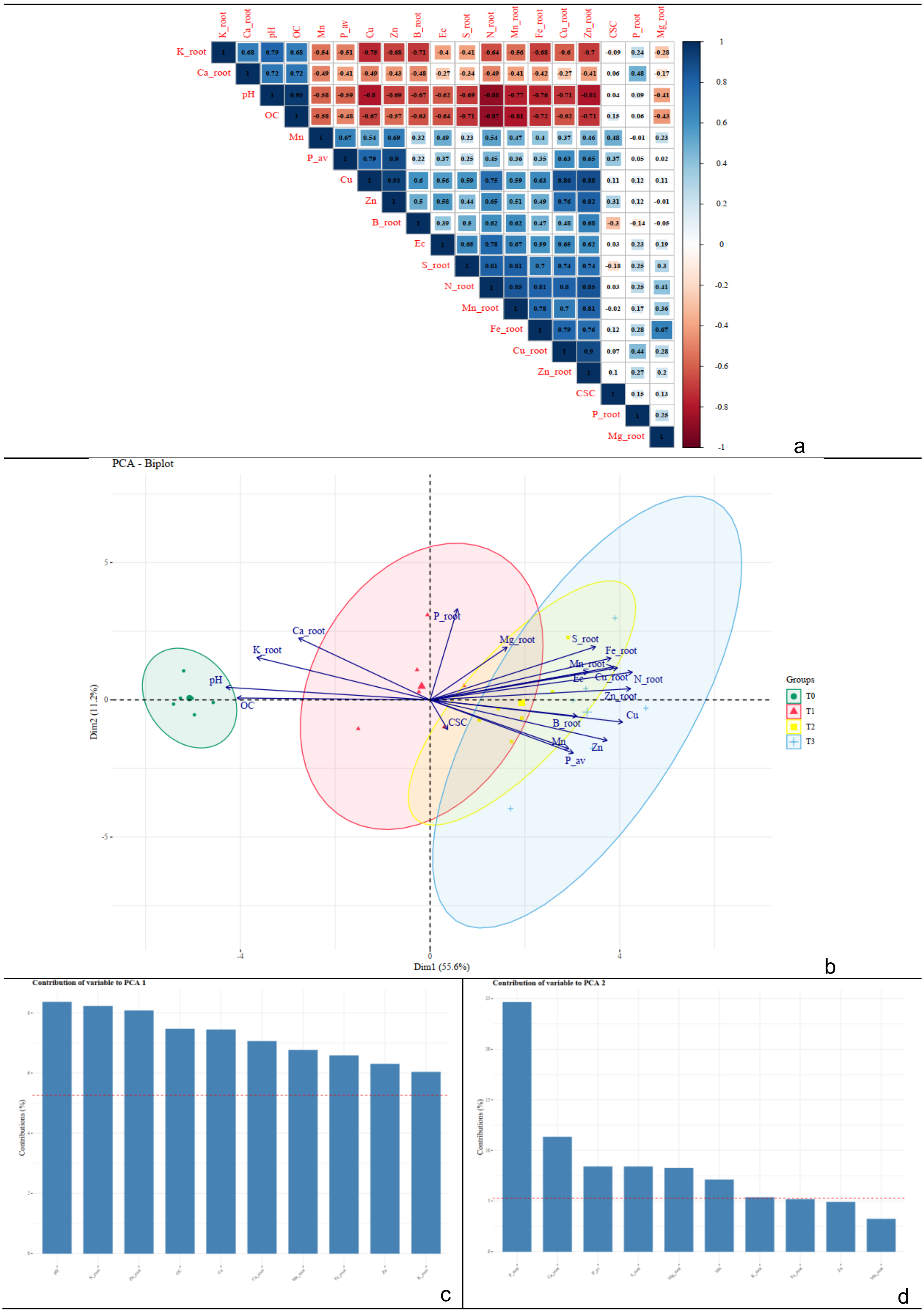


Fig. 117. PCA between soil physicochemical parameters and chemical parameters of the root part of plants (A + C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2.

In figure 118, you can see the principal component analysis (PCA), performed between groups B (final morphological variables of the plant) and C1 (nutrients accumulated in the aerial part), showed good separation between treatments, highlighting a well-defined clustering. Dimension 1 (PCA1) explained 36.2% of the data variance, while Dimension 2 (PCA2) represented 18.4%, totaling 54.6% of the explained variance. In PCA1, the main contributors were the levels of N, Mg, S, and Ca in the aerial part. In PCA2, phosphorus (P) and potassium (K) stood out, followed by the dry biomass of the aerial part (Bs). The biplot indicated that treatment T0 was more associated with the vectors of P, K, B, and Fe in the aerial part, but in the opposite direction to the vectors related to plant growth, indicating less seedling development. Treatment T1 showed a consistent and balanced grouping, situated between the nutrient and growth vectors, such as height (H), diameter (D), and root biomass (Br). Treatments T2 and T3 were located to the right of the graph, with T2 showing greater compaction of points. Both showed a strong association with the levels of Mn, N, Ca, Zn, S, and Cu in the aerial part, as well as a positive correlation with morphological parameters. The directions and intensities of the vectors show a positive correlation between the accumulation of macro and micronutrients and plant development, in contrast to the vectors of P, K, B, and Fe, which showed a negative relationship with growth. These results reinforce the positive effect of increasing sewage sludge application on increasing nutrition and stimulating seedling growth, especially in treatments with higher SS levels.

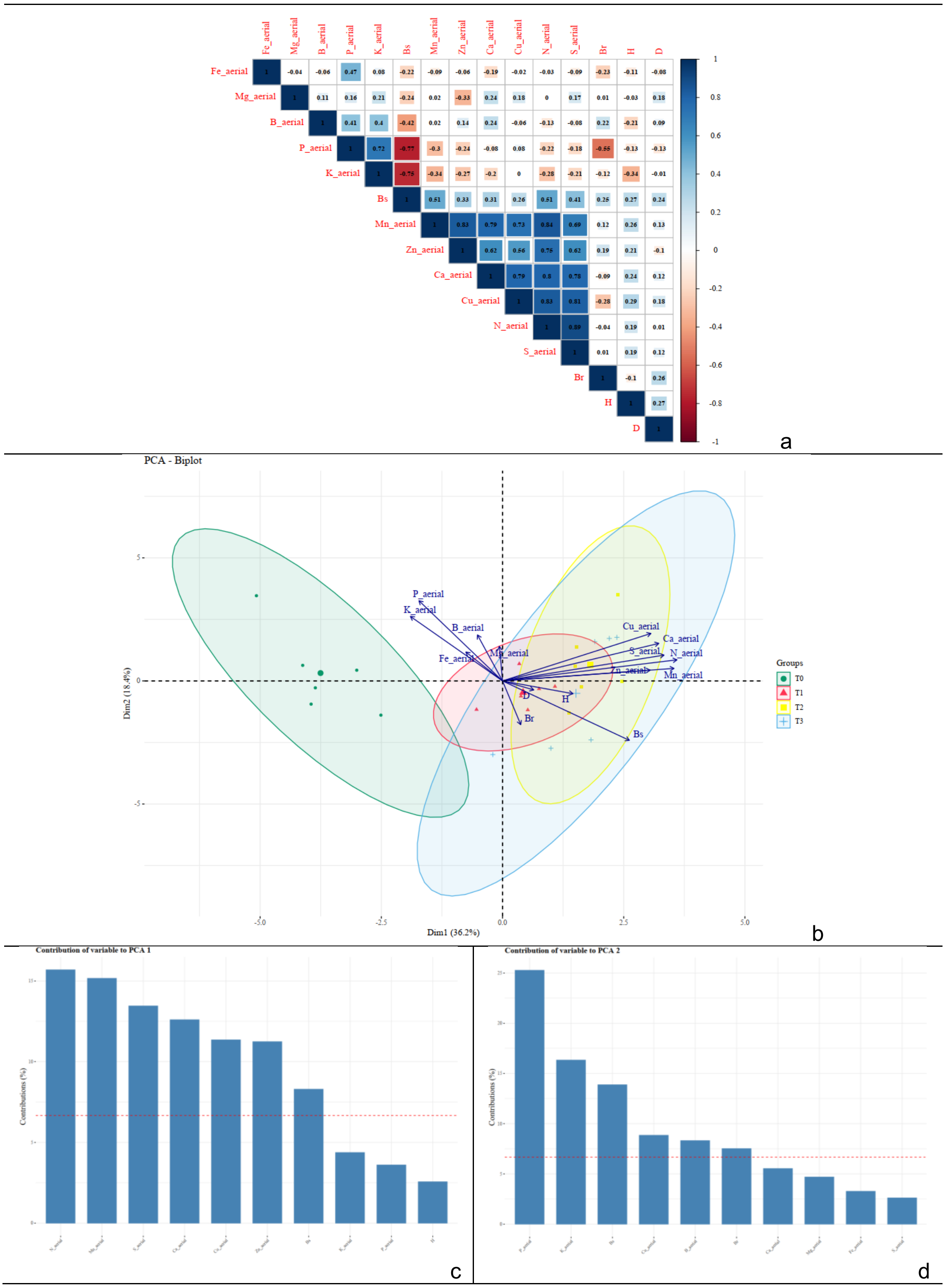


Fig. 118. PCA between plant growth parameters and chemical parameters of the aerial part of plants (B+C1). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

In this principal component analysis (PCA) (Fig. 119), the final morphological variables of the plant (B) and the nutrients accumulated in the roots (C2) were jointly evaluated. Dimension 1 (PCA1) explained 46.1% of the data variance, while Dimension 2 (PCA2) accounted for 15.1%, totaling 61.2% of the explained variance. In PCA1, the largest contributors were the N, Zn, and Mn contents in the roots. In PCA2, P in the root stood out, followed by Ca in the same structure and, in third place, the dry biomass of the aerial part (Bs). The arrangement of treatments in the biplot showed that T1, T2, and T3 presented quite similar behaviors, with partially overlapping groupings, while treatment T0 stood out for being positioned in the opposite direction to the plant growth variables and most of the nutrients accumulated in the roots. Treatment T0 showed a positive association only with potassium (K) and calcium (Ca) cations in the roots, being the only nutritional vectors aligned with the control. In contrast, T1, T2, and T3 showed a strong positive correlation with aboveground and root biomass, as well as high concentrations of Cu, Zn, Mn, Fe, N, and S in the roots. The directions and intensities of the vectors confirm these relationships, indicating that the increasing application of sewage sludge promoted the accumulation of nutrients in the underground part of the plants, directly favoring their development. The compaction of the points in the T0 group also reveals less variability between the repetitions of this treatment.

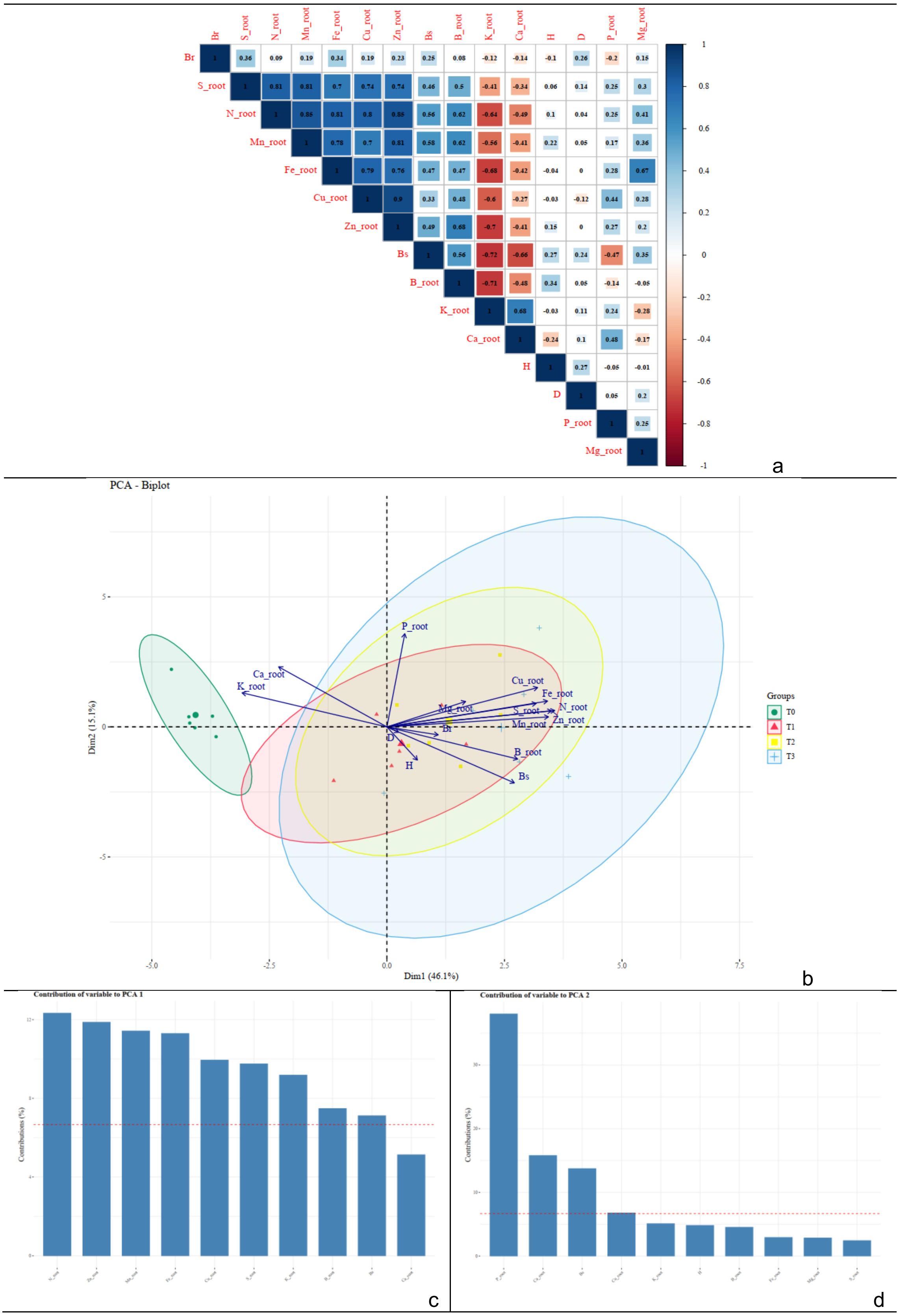


Fig. 119. PCA between plant growth parameters and chemical parameters of the root part of plants (B+C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

The final principal component analysis (PCA) (Fig. 120), performed with groups C1 (nutrients accumulated in the aerial part) and C2 (nutrients accumulated in the roots), revealed patterns consistent with those observed in previous analyses. Dimension 1 (PCA1) explained 48.6% of the data variance, while Dimension 2 (PCA2) contributed 16.8%, totaling 65.4% of the explained variance. In PCA1, the main contributors were the N and Zn contents in the roots, as well as N and Mn in the aerial part. In PCA2, the P contents stood out mainly, both in the root and in the aerial part, followed by K accumulated in the aerial part. The biplot indicated that treatment T0 was again positioned in opposition to most of the nutritional vectors, reflecting a low correlation with the nutrients accumulated in plant tissues. However, this treatment still showed a positive association with K cations, both in the root and shoot, as well as Ca in the roots and P in the shoot, which were the only nutrients aligned with their vector. In contrast, the treatments with sludge application (T1, T2, and T3) showed a gradual shift to the right in the biplot, following the increase in the sludge doses applied. These treatments showed a strong association with the main nutritional vectors, especially N, Mn, Zn, Cu, and S, accumulated in both the roots and shoot, highlighting the direct influence of sludge addition on the absorption and accumulation of these elements. The directions of the vectors reinforce the trend of a positive correlation between the increase in sludge doses and the nutritional enrichment of the plants, confirming a pattern consistent with the results obtained in the other PCA analyses.

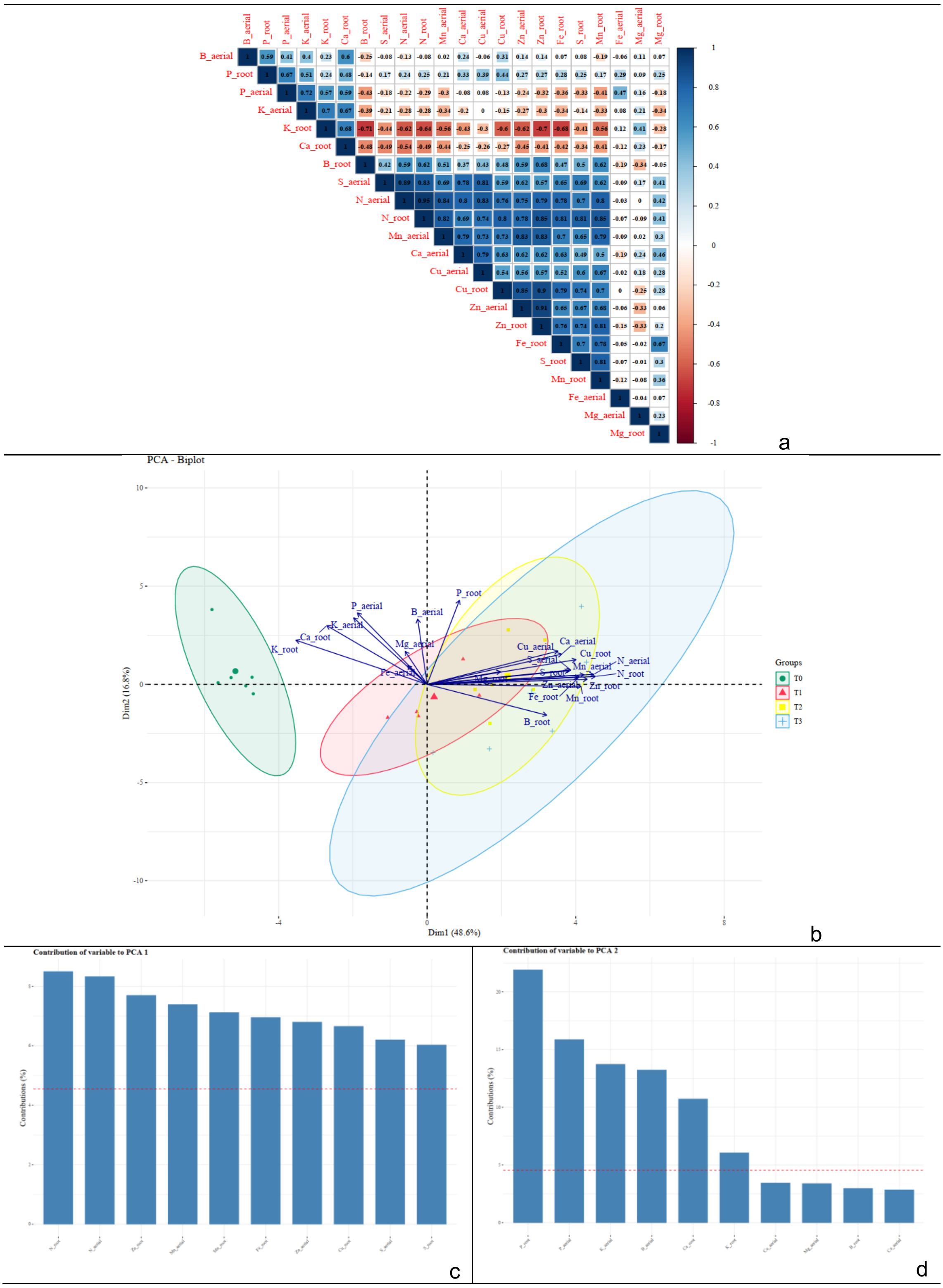


Fig. 120. PCA between chemical parameters of the aerial part of plants and chemical parameters of the root part of plants (C1+C2). (a) Pearson correlation matrix; (b) PCA Biplot; (c) Contribution of PCA1; (d) Contribution of PCA2

In this case, as well, the PFA extracts a total of eight factors (eigenvalues> 1), which together account for as much as 90% of the total variance (Table 21). The first factor of the Botucatu PFA is characterized by a strong positive association of elements such as Al^{3+} in the soil (0.846), H+Al (0.755), available phosphorus (P_res; 0.693), Cu (0.943), Fe (0.892), Zn (0.874), and Cu_aerial, Zn_aerial, Zn_root, Curoot with very high values (often >0.85). Positive correlations are also observed with N_aerial (r = 0.639), N_root (r = 0.687), Mn_root (r = 0.552), and B_root (r = 0.673). This "richness" of metals and nutrients is paralleled by a strong negative correlation with soil pH (−0.811), organic matter (−0.517), K (−0.588), Kroot (−0.738), V (−0.751), Mg (−0.770), and other fertility parameters. This pattern suggests that, in treatments with more acidic substrates and lower organic matter (where the sludge content is high), there is a substantial increase in the availability of mineral elements, micronutrients, and heavy metals, all of which are mobilized by acidity and accelerated mineralization. Nevertheless, this scenario severely penalizes the base of chemical fertility (pH, organic matter, exchange bases), in accordance with the findings of (Yakamercan et al. 2021; Filipi et al. 2023), which indicate that acidification triggers the massive release of elements from the sludge and soil, with potential risks of ionic toxicity and physiological imbalances in plants. Therefore, this factor can be termed "induced acidity and soil PTE/nutrient richness," which is fundamental in explaining how, in acidic and weakly buffered environments, sludge causes a synchronous mobilization of elements which, beyond a certain threshold, become phytotoxic and may negatively impact plant growth.

The second factor displays a positive correlation among P_aerial (0.839), K_aerial (0.794), P_root (0.861), Kroot (0.542), Ca_root (0.704), and organic matter in the soil (0.464), indicating a pronounced capacity for potassium and phosphorus accumulation in both aerial and root plant parts, where organic matter content is higher. Conversely, these parameters are negatively correlated with Mn (−0.234), shoot dry mass (−0.747), and total dry mass (−0.735); this suggests that treatments and conditions favoring uptake in these pools may also reduce biomass productivity if excessively unbalanced. This factor may thus be designated as "compartmental K/P uptake and organic stability," as it describes a nutritional strategy adopted by the plant, enhanced by potassium and phosphorus, and supported by substrates richer in organic fraction.

The third factor reveals a strong positive correlation among Ca (0.870), soil cation exchange capacity (CTC; 0.940), soil base saturation (SB; 0.667), and S (0.612), with a negative correlation to K (−0.646). This suggests that in soils with high CEC and base saturation, conditions favor the slow release of essential nutrients, which is ideal for maintaining long-term agronomic fertility stability (Silva et al. 2022). Thus, this factor may be named "importance of CEC in modulating nutrient release," crucial for maintaining plant health and productivity over time.

The fourth factor is strongly (positively) correlated with Mg_root (0.797) and Ca_aerial (0.438), showing fewer and weaker correlations with other elements. Root uptake of magnesium, facilitated by rapid sludge mineralization and high mobility in tropical soils, is often accompanied by calcium absorption in the aerial part and osmotic changes reflected in reserve dynamics and growth (Singh e Agrawal 2008). Therefore, this factor can be described as "radical Mg uptake and mobility efficiency."

Overall, the Botucatu PFA highlights, as expected in tropical systems using high SS concentrations, the importance of balancing mineral availability, the status of acidic and organic substrates, and cation-exchange capacity. The primary risk evidenced is the depletion of organic buffers and acid induction, which, while facilitating rapid inorganic nutrition (especially metals and macronutrients), imposes significant phytotoxicity and physiological response constraints (Yakamercan et al. 2021; Filipi et al. 2023). Thus, with careful management and monitoring, nutritional uptake can be maximized, but the system must be balanced to avoid both mass deficit and risks from excessive metals.

Parameters	F1	F2	F3	F4	F5	F6	F7	F8
H	0,091	-0,168	-0,129	-0,063	-0,139	-0,001	0,192	0,853
D	-0,143	0,040	-0,294	0,131	0,480	0,164	0,176	0,533
Bs	0,377	-0,747	-0,056	0,118	0,193	0,045	0,312	0,165
Br	0,125	-0,232	-0,109	0,122	0,822	0,127	-0,094	-0,111
pH	-0,811	0,040	-0,204	0,077	-0,058	-0,061	-0,372	0,025
Ec	0,502	0,095	0,103	-0,224	0,173	0,120	0,679	-0,116

pH	-0,692	0,411	0,028	-0,177	-0,008	-0,059	-0,544	-0,037
M.O.	-0,517	0,464	0,084	-0,130	0,200	0,088	-0,543	0,064
P_res	0,693	-0,105	0,477	-0,004	-0,237	0,102	0,086	0,187
Al3+	0,846	-0,313	0,016	0,058	0,045	-0,026	0,313	0,061
H+Al	0,755	-0,281	0,212	0,161	0,002	0,015	0,481	-0,006
K	-0,588	0,410	0,067	-0,041	0,021	0,132	-0,646	-0,043
Ca	0,357	0,015	0,870	-0,065	0,006	-0,058	-0,124	-0,053
Mg	-0,770	0,304	0,343	0,029	-0,054	0,085	-0,350	-0,086
SB	-0,528	0,285	0,667	-0,005	-0,042	0,054	-0,383	-0,097
CTC	0,025	0,092	0,940	0,128	-0,046	0,074	-0,038	-0,117
V%	-0,751	0,324	0,250	-0,097	-0,052	-0,007	-0,472	-0,068
S	0,569	-0,238	0,612	-0,016	-0,074	0,081	0,388	0,006
B	0,178	0,485	0,292	-0,223	0,042	0,245	-0,608	-0,047
Cu	0,943	-0,097	0,105	0,053	-0,067	0,081	0,165	0,020
Fe	0,892	-0,217	0,107	0,041	-0,009	0,057	0,355	-0,004
Mn	0,391	-0,234	0,535	-0,004	-0,427	0,015	0,468	0,103
Zn	0,874	-0,059	0,359	-0,111	-0,137	0,095	0,142	0,126
N_aerial	0,639	-0,060	-0,043	0,142	-0,099	-0,015	0,722	-0,032
P_aerial	-0,209	0,839	0,019	-0,019	-0,341	-0,257	-0,119	0,023
K_aerial	-0,294	0,794	-0,100	-0,285	0,059	0,069	-0,029	-0,220
Ca_aerial	0,561	0,135	-0,028	0,438	-0,227	0,316	0,525	0,150
Mg_aerial	-0,495	0,224	-0,128	0,365	-0,042	0,358	0,385	-0,089
S_aerial	0,452	-0,007	-0,126	0,152	-0,081	0,145	0,758	-0,024
B_aerial	0,240	0,681	0,067	0,389	0,251	0,239	-0,348	0,021
Cu_aerial	0,425	0,221	-0,141	0,054	-0,237	0,100	0,754	0,149
Fe_aerial	-0,090	0,202	-0,109	0,092	-0,139	-0,866	0,015	-0,038
Mn_aerial	0,667	-0,041	0,136	0,096	0,077	0,097	0,598	0,171
Zn_aerial	0,890	0,040	0,126	-0,089	0,075	-0,023	0,301	0,073
N_root	0,687	-0,068	-0,005	0,078	0,097	-0,022	0,665	-0,095
P_root	0,213	0,861	0,061	0,221	0,067	-0,235	0,184	0,058
K_root	-0,738	0,542	-0,072	-0,158	0,060	0,028	-0,099	0,136
Ca_root	-0,366	0,704	-0,031	0,030	0,138	0,252	-0,338	-0,001
Mg_root	0,018	-0,117	0,098	0,797	0,173	-0,148	0,434	-0,036
S_root	0,566	0,013	-0,186	0,019	0,397	-0,007	0,551	-0,120
B_root	0,673	-0,334	-0,356	-0,185	-0,039	0,058	0,178	0,061
Cu_root	0,876	0,162	0,052	0,151	0,159	-0,077	0,275	-0,134
Fe_root	0,601	-0,103	0,070	0,421	0,260	-0,076	0,481	-0,233

Mn_root	0,552	-0,156	-0,008	-0,014	0,257	-0,061	0,639	-0,008
Zn_root	0,897	-0,027	0,076	0,026	0,197	-0,007	0,303	0,040
Proportional variance (%)	51	11	9	6	5	3	3	2
Cumulative variance (%)	51	62	71	77	82	85	88	90
Eigenvalues	24.881	5.576	4.337	3.016	2.318	1.640	1.243	1.030

Table 21. Exploratory Factor Analysis (PFA) performed with soil nutritional variables, plant morphological parameters, and chemical data of the aerial and root parts at the end of the experiment

3.3 Discussion

3.3.1 Soil

The observed values for dry soil density throughout the experiment directly reflected the physical nature and composition of the materials used in the different treatments. Pure urban soil showed the highest initial density, consistent with its sandy-loam texture and high mineral particle content, a typical result of soils with a predominance of sand and clay and a low silt fraction, as described by (Kampf 2005; Raviv et al. 1998), who relate high densities to the presence of dense solid particles. In contrast, pure sewage sludge and the commercial control substrate exhibited the lowest density values, which is explained by their high proportions of organic matter and high porosity. Substrate T0, for example, is composed of peat, expanded vermiculite, and carbonized rice husk, all lightweight and highly porous materials, falling within the ideal density range for commercial substrates (0.1 to 0.3 g/cm³), as indicated by (Kampf 2005). Studies such as those by (Nappi e Barberis 1993) reinforce that organic waste, including sewage sludge, has low apparent density due to the lower specific mass of its components. Treatments T1, T2, and T3 showed high and similar initial densities (0.899 to 0.938 g/cm³), demonstrating that urban soil was the dominant component in defining the density of the mixtures. This behavior reinforces the idea that the final density of the substrate is strongly influenced by the proportion of mineral material, even with the inclusion of lighter components, as also pointed out by (Caravaca et al. 2002). Statistical analysis showed that the densities of the treatments with SS did not differ significantly from pure urban soil, but differed from T0 and SS, which shows that the high proportion of soil canceled out the expected density reduction with the addition of sludge. This result is consistent with the findings of (Ferraz et al. 2016), who reported similar behavior in mixtures with high soil content and reduced proportions of organic matter. At the end of the cycle, the densities of the sludge treatments remained practically unchanged, with no statistically significant differences, suggesting structural stability provided by the mineral predominance, capable of resisting compaction and decomposition of organic matter. This stability pattern is confirmed by (Feng et al. 2024; Marin e Rusănescu 2023), who demonstrated that substrates rich in mineral components maintain their physical characteristics over time, unlike organic ones, which are more susceptible to structural collapse. T0, in turn, remained the substrate with the lowest final density, an expected behavior given its composition, and desirable in container cultivation systems as it favors root growth and water management, aspects highlighted by (Kampf 2005) as fundamental in seedling production.

The initial pH differences observed between the control treatment (T0) and the sludge treatments (T1, T2, T3) directly reflect the chemical characteristics of the materials used in the formulations. The pure commercial substrate (T0), composed of high-quality materials such as peat, vermiculite, and carbonized rice husk, showed a pH close to neutrality throughout the experiment, starting at 7.04, which characterizes a slightly basic medium. According to (Barbosa et al. 2017), this behavior can be explained by the low intrinsic acidity of its organic components, which have a high buffering capacity and a lower tendency to release hydrogen ions in solution. In contrast, treatments T1, T2, and T3, containing higher proportions of urban soil and sewage sludge, exhibited slightly more acidic initial values (between 5.3 and 5.0), reflecting the natural acidity of urban soil, whose pH, in its pure form, was only 4.0. Throughout the monitoring, all treatments showed a tendency toward acidification, more pronounced in the formulations with higher doses of sludge. The final pH of T3, for example, decreased from 5.3 to 4.27, this being the largest drop among the treatments.

This progressive acidification is a result of the microbial decomposition of the organic matter in the sludge, a process that releases organic and inorganic acids into the soil, as described by (Ferreras et al. 2006). Although some sludges, when alkalized, can act by raising the soil pH, the sludge used in this experiment had a moderately acidic initial pH (5.9), which did not favor this corrective effect.

This drop in pH also favored the mobilization of exchangeable aluminum (Al^{3+}), one of the main indicators of acidity in tropical soils. While initial analyses of the mixtures did not detect Al^{3+}, at the end of the experiment its presence was significant in the sludge treatments, with values ranging from 4.21 to 8.73 mmolc/dm³, mainly in T3. This mobilization indicates that the pH reached critical levels, below 5.5, a range in which Al^{3+} becomes soluble and can exert toxic effects on plants. (Corrêa et al. 2007) explain that, below this threshold, previously insoluble Al^{3+} is released into solution, increasing the active acidity of the soil. The literature also points out that, at pH below 5, this ion tends to precipitate as $Al(OH)_3$, a behavior described by (McBRIDE et al. 1997) and confirmed by (Filius et al. 1998).

The more pronounced acidification in the sludge treatments, especially in T2 and T3, is therefore directly related to the higher organic load introduced by the increasing doses of SS. Although this process is partly expected and even desirable in excessively alkaline soils, in contexts like this, with naturally acidic soils and low buffering capacity, it can induce adverse effects such as the solubilization of Al^{3+} and the reduction of the availability of basic nutrients. Even so, the organic matter present in the sludge contributed positively to the increase in cation exchange capacity (CEC), resulting from the oxidation and transformation of humic compounds, as evidenced by (Maio et al. 2011; Mondal et al. 2015), who highlight the role of organic matter in promoting chemical and microbiological improvements in soils.

The results of electrical conductivity (Ec) indicate a classic dynamic of systems enriched with organic waste. A significant increase in the initial phase, followed by a progressive reduction and stabilization of soluble salt levels in the substrate. This trajectory was particularly evident in the treatments that received sewage sludge, whose application showed a direct influence on the initial increase in Ec.

Treatment T3, which received the highest dose of sludge (12%), presented the highest Ec value in the first month, reaching 13.1 mS/cm, while the control T0, composed only of commercial substrate, registered an initial value of 0.34 mS/cm. As verified by (Guerrini e Trigueiro 2004), the occurrence of salinity and the presence of elements that may be toxic to plants, as well as high density or low porosity, can restrict or even prevent the use of biosolids in the substrate for seedling production. The initial salinity of the mixtures is directly related to the amount of sludge applied, with sewage sludge being a material with a high concentration of soluble and conductive salts. The chemical behavior of this material is well supported by the literature. (Shan et al. 2021) demonstrated that sewage sludge significantly contributes to increasing the soil EC, attributing this effect to its high intrinsic salinity. (Roca-Pérez et al. 2009) highlight that, although sludge composts bring agronomic benefits, increased salinity is one of the main limiting factors for their use. Similarly, (Cai et al. 2010) compared sludge composts with conventional agricultural soils and observed an initial EC of 5.01 dS/m for the compost, compared to only 0.20 dS/m in the control soil, associating this increase with the presence of soluble ions.

However, as time progressed, especially from the third month onwards, there was a significant reduction in EC in all treatments, particularly in T2 and T3. In treatment T3, EC fell from 13.1 mS/cm to 5.08 mS/cm at the end of the experimental cycle. This reduction can be largely attributed to the leaching process, favored by regular irrigation and the physical structure of the containers used (pots). Leaching is a physical process of removing soluble ions from the substrate by the passage of water, resulting in the loss of salts through drainage. (Savvas e Gruda 2018) suggest that, in cultivation systems with organic substrates, controlled leaching is the most efficient strategy to reduce high EC levels, provided it is accompanied by careful irrigation management. The observation by (Schafer et al. 2015) that continuous monitoring of EC is essential to avoid chemical imbalances reinforces the importance of the frequent control carried out during the experiment, as evidenced by the monthly measurements. In addition to leaching, the decomposition of organic matter present in the sludge also contributed to the modulation of Ec over time. The progressive acidification observed in treatments with higher sludge doses is directly related to the mineralization of organic matter, the release of organic acids, and the alteration of ionic dynamics in the soil. (Shan et al. 2021) note that, although lower pH increases solubility (which theoretically could increase Ec), the application of sewage sludge (SS) increases the N and P content and reduces the pH, so that the increase in nutrient concentration (from decomposed OM) can "compensate" for the adverse effects of high salinity. Thus, the continuous transformation of organic compounds tends to promote a reorganization of the ionic balance, which, added to leaching, reduces the total concentration of free salts in solution. Thus, the reduction in Ec is not solely a result of the physical removal of ions, but also of a chemical reconfiguration in the system as the waste products stabilize and biological processes settle in (Schafer et al. 2015).

The organic matter (OM) results showed that treatment T0, composed exclusively of commercial peat-based substrate, maintained the highest values at the end of the experiment, reaching 73.58 g/dm³. This result is directly attributed to the highly organic nature of the material, also reflected in the low apparent density (0.165 g/cm³) and high buffering capacity of the substrate. Peat is known for its high carbon content and resistance to rapid decomposition, which justifies the preservation of OM over time, in line with observations by (Kampf 2005; Roca-Pérez et al. 2009). In contrast, treatments T1, T2, and T3, which included increasing proportions of sewage sludge (4%, 8%, and 12%, respectively) combined with a high proportion of urban mineral soil (63% to 71%), presented final OM values... significantly lower, ranging between 35.82 and 36.53 g/dm³. The high proportion of mineral soil caused a dilution effect on the organic content, while the active mineralization of the sludge organic matter, especially the labile fraction, reduced the expected accumulation even with the increased dose. The absence of a significant difference between the three sludge treatments can be explained by the small variation in dosage and the rapid decomposition of the organic matter, as pointed out by (Hechmi et al. 2020; Zoghlami et al. 2016).

Treatment T0, composed solely of commercial peat-based substrate, showed the highest values for total carbon and organic matter. This result is attributed to the high stability and carbon content of the organic materials used, known for their high water retention capacity and low density (Wu et al. 2017; Ferraz et al. 2016; De Lucia e Cristiano 2015). This behavior is consistent with peat's potential to maintain organic matter and improve soil physical properties, as reported by (Alvarenga et al. 2007). The observed stability is partly due to the predominance of humified carbon fractions, which are less susceptible to microbial degradation, as discussed by (Schmidt et al. 2011). In the sludge treatments, the lower C and OM contents can be explained by the dilution effect, caused by the high proportion of urban soil with low organic content, and by the rapid decomposition of the labile carbon fraction of the sludge. Despite the high OM content in the sludge (109.05 g/dm³), its participation in the mixtures was insufficient to significantly increase the final values, as also observed by (Ferraz et al. 2016). The rapid decomposition of labile carbon, common in organic residues, results in losses due to mineralization in the first weeks, as shown by (Bergkvist et al. 2003) and confirmed by (Fernández et al. 2007; Lloret et al. 2016), who related the chemical instability of the sludge to the biological activity of the soil. The acidification of the substrates with sludge, especially in T3, reinforces this mineralizing dynamic. The recorded pH drop (from 6.39 to 5.05) is associated with the production of organic acids and the release of protons during nitrogen mineralization, a process widely documented by (Pereira et al. 2020; Shan et al. 2021).

The final levels of available phosphorus (P resin) showed significant variation between treatments, demonstrating an increase proportional to the increase in sewage sludge (SS) doses, rising from 42.78 mg/dm³ in the control (T0) to 195.36 mg/dm³ in T3. This trend is widely confirmed in the literature, as sewage sludge is recognized as an important source of nutrients, especially phosphorus, contributing to the recovery of degraded soils (Alvarenga et al. 2007; Fytili e Zabaniotou 2008). In the present study, the SS presented an initial available phosphorus content of 375.49 mg/dm³, a value significantly higher than that of urban soil (14.56 mg/dm³), a result consistent with that observed by (Ferraz et al. 2016), who highlighted the potential of SS to increase P levels in the soil. The increase in resin P in treatments with higher SS content also indicates the presence of bioaccessible phosphorus fractions, that is, forms that are not immediately soluble, but which become available with the mineralization of organic matter. (Frišták et al. 2018) highlight that even pyrolyzed sludge contains phosphorus fractions that become accessible over time. It is observed that T1 presented the lowest resin P content at the end of the experiment along with the control treatment, with no statistical difference between them, which indicates that there is a minimum threshold of SS addition necessary to significantly improve phosphorus availability. Treatments T2 and T3, on the other hand, presented significantly higher values, demonstrating that from 8% sludge onwards, there is saturation of the system and maintenance of high levels of residual phosphorus even after cultivation. This behavior is consistent with the findings of (Arrobas et al. 2024), who observed a sustained increase in phosphorus with increasing doses of sludge.

This residual effect is further confirmed by the results of (Alleoni et al. 2012), who, in a long-term experiment with SS application in Latosol cultivated with Eucalyptus grandis, reported maintenance of available phosphorus levels for up to 36 months.

The results demonstrated that exchangeable aluminum (Al^{3+}) was detected only in the initial composition of the pure soil and in the final analysis of the treatments (T1, T2, and T3), which is directly related to the initial composition of the urban soil and the acidification of the substrates over time. The presence of this ion, absent in the initial analysis, was observed at the end of the experiment with increasing values as the sludge dose increased, reaching 8.73 mmolc/dm³ in T3. The mobilization of Al^{3+} occurred mainly due to the reduction of pH below 5.5, a critical threshold for aluminum solubilization in tropical soils (Roca-Pérez et al. 2009). The control T0, by maintaining a final pH of 5.55, did not present exchangeable aluminum, which reinforces the relationship between acidification and the availability of this element. The mineralization of organic matter present in the sludge, associated with sulfur oxidation (which increased proportionally to the sludge dose applied), promoted the release of H^+ ions and organic acids, intensifying the acidity of the medium, as described by (Roig et al. 2004; Roca-Pérez et al. 2009). Furthermore, the potential acidity (H+Al) increased significantly in the sludge treatments, reflecting the higher load of exchangeable aluminum in systems T2 and T3, indicating that the higher doses of organic residue accelerated the acidification processes. This effect was potentiated by the low buffering capacity of the Brazilian urban soil used in the mixtures, a highly weathered soil with physicochemical characteristics typical of Oxisols, which favor the mobilization of aluminum and iron in acidic environments, as pointed out by (Firmano e Alleoni 2021). The presence of Al^{3+} at high levels represents a physiological risk to plants, as its toxicity can cause root damage, inhibiting growth and nutrient absorption, as discussed by (Koyama et al. 2001).

The basic cations calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), along with derived parameters such as the sum of bases (SB) and base saturation (V%), showed distinct behaviors among the treatments, strongly influenced by the high nutrient load provided by the sewage sludge and subsequent chemical changes, such as acidification and ionic competition. Calcium was the cation that stood out the most, showing a significant increase in the sludge treatments, especially in T3, a direct reflection of the high concentration of Ca in the pure sludge (290.3 mmolc dm^{-3}). This increase is related to the common practice of alkaline stabilization of sludge with lime in Brazil, as described by (Bittencourt et al. 2014). This technique, by forming $Ca(OH)_2$, raises the soil pH and makes calcium available quickly and efficiently, making alkalized sludge an effective source for acidity correction and nutrient supply, as pointed out by (Ferraz et al. 2016). Studies such as that of (Barbosa et al. 2017) reinforce this effectiveness, demonstrating that sludge treated with lime surpasses limestone in raising pH and making Ca^{2+} available. However, high calcium levels can promote ionic antagonisms, interfering with potassium and magnesium absorption, and increasing their leaching to deeper layers. (Ferraz et al. 2016) reported that this unbalanced Ca:Mg and Ca:K ratio may be associated with reduced productivity.

The potential acidity ($H^{+} + Al^{3+}$) increased, and V% decreased with increasing sludge doses, indicating that, despite the initial supply of bases, the mineralization of organic matter can generate acidification, compromising the corrective effectiveness of the sludge in the medium term. In contrast, potassium (K^{+}) levels showed a tendency to decrease throughout the experiment in the sludge treatments, with T0 being the only one to maintain a high level at the end.

This decrease is attributed to the low concentration of potassium in the sludge, since this nutrient is the most subject to losses in the liquid fraction during treatment, as highlighted by (Barbosa et al. 2017; Singh e Agrawal 2008). Competition between cations also contributes to the displacement of K^+, favored by the abundance of Ca^{2+} and Mg^{2+}, as discussed by (Farhat et al. 2016), who observed an increase in potassium leaching with the use of biosolids. Soil alkalization, although beneficial in certain aspects, can intensify the mobility of K^+ and Mg^{2+} in tropical soils with low CEC, as pointed out by (Farhat et al. 2016). Thus, the literature recognizes that sludge has a limited effect on K^+ levels, and its deficiency must be supplied by complementary sources, as suggested by (Bettiol e Ghini 2011; Carmo et al. 2016). Magnesium, in turn, showed the opposite behavior to calcium, with T0 maintaining the highest levels at the end and the sludge treatments showing decreasing values with increasing dose. This difference may be associated with the greater cation retention capacity of the commercial substrate (rich in peat), and competition with Ca^{2+} in the sludge treatments, which may have favored Mg^{2+} leaching. Although (Barbosa et al. 2017) reported an increase in Mg^{2+} with sludge, the results of this study differ, possibly due to the substrate composition and intensity of ionic competition.

As for SB, it followed the trend of magnesium, being higher at T0, while V% showed a decline proportional to the increase in sludge doses, going from 89.13% at T0 to 55.66% at T3. Despite the initial contribution of basic cations by the sludge, the organic decomposition process generates acids that reduce the pH and favor the solubilization of Al^{3+}, which increases the potential acidity and reduces the proportion of bases in the CEC, as explained by (Fontes e Alleoni 2006). The pH at T3, for example, dropped from 5.3 to 4.27, evidencing this effect. This behavior differs from that expected in alkaline amendments, where pH and V% tend to increase. (Meriño-Gergichevich et al. 2010) highlight that, in acidic soils, the release of Al^{3+} can generate phytotoxicity, requiring additional correction with alkaline sources. Thus, although sewage sludge initially contributes nutrients, subsequent microbial acidification seems to dominate soil chemistry, reducing base saturation and highlighting the complexity of its use as a soil conditioner.

Sulfur (S), an essential macronutrient for plant development, showed high availability in the substrates after the application of sewage sludge, highlighting its role as an important nutritional source. This increase is directly related to the naturally rich composition of sludge in sulfur compounds, as pointed out by (Mondal et al. 2015; Maio et al. 2011), who emphasize the potential of biosolids to supply sulfur in relevant quantities to the soil. The presence of sulfur in the sludge triggers microbial oxidation reactions that convert sulfur compounds into sulfuric acid, releasing H^+ ions and contributing substantially to soil acidification. This effect was particularly evident in treatments with higher doses of sludge, reflecting the intense mineralization of organic matter containing sulfur. The literature corroborates this process, with studies such as those by (Boaro et al. 2014; Kampf 2005) demonstrating that the oxidation of elemental S in substrates is an efficient mechanism for reducing pH, directly influencing soil chemistry and the balance of nutrients available to plants.

Regarding Boron (B), treatment T0, composed exclusively of commercial peat-based substrate, showed the highest initial concentration (1.33 g/dm³) and maintained a high final value (0.38 g/dm³) even after 120 days of cultivation. This result is directly related to the high proportion of organic matter (65.26 g/dm³) present in this substrate, which favors the retention of micronutrients such as Boron through complexation and adsorption mechanisms. Studies such as those by (Roca-Perez et al. 2010) confirm the essential role of organic matter in reducing losses due to leaching, especially in environments with frequent irrigation. In treatments T1 and T2, the lowest final concentrations of boron (0.24 and 0.29 g/dm³, respectively) can be attributed to the dilution of the organic substrate by the introduction of urban soil, a material with a low boron content (0.31 g/dm³) and reduced nutrient retention capacity. The amount of sludge incorporated in these treatments was not sufficient to compensate for this reduction, and as (Ferraz et al. 2016) point out, soils with low organic matter lose more micronutrients through leaching. This trend is reinforced by (Dechen e Nachtigall 2006), who highlight the high solubility of boron and its mobility in irrigated systems. Treatment T3, which received 12% SS, showed the second highest final boron value (0.36 g/dm³), statistically similar to T0 and higher than

T1 and T2, indicating that the higher dose of sludge was sufficient to increase the organic matter content and, therefore, improve micronutrient retention. Higher doses of sludge increase organic matter and favor the availability of trace elements in the soil. The composition of the sludge itself, which contained 2.78 g/dm^3 of boron, also contributed to this result, as discussed by (Dechen e Nachtigall 2006). Although sludge is widely recognized as a source of micronutrients, including boron (Fytili e Zabaniotou 2008), there are reports of variations in its composition, as observed by (Warman e Termeer 2005), who identified low levels of the element in sludge-derived compounds.

Regarding metallic micronutrients, a significant increase in copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) levels was observed in the treatments that received sewage sludge, with levels increasing proportionally to the applied dose. This behavior is consistent with the results of (Singh e Agrawal 2008), who point to sludge as an important source of these elements. Additionally, (Janaszek e Kowalik 2023) highlighted that, although sludge contains high concentrations of heavy metals, many of these metals are partially immobilized by the organic matrix of the residue, which reduces their mobility in the soil. In the present study, the soil acidity observed in treatments T2 and T3 ($pH < 5.0$) possibly contributed to the increased solubility of the metals, a phenomenon described by (Morera et al. 2001), who report greater mobility of metals in more acidic environments. Nevertheless, the absence of phytotoxicity symptoms in the plants indicates that micronutrient levels remained within agronomically safe limits, even in the face of chemical changes in the soil (RESOLUÇÃO Nº 498 2020).

The significant increase in copper (Cu) concentrations in sewage sludge treatments is directly related to the high load of this element in urban biosolids, mainly originating from industrial effluents and the corrosion of metal pipelines, as highlighted by (Alloway 2012). Cu has a high affinity for organic matter, forming stable complexes with humic fractions of the soil, which favors its retention without necessarily compromising its availability to plants (McBride 1995). (Smith 2009) adds that copper, when associated with organic matter, tends to persist in partially available forms. Furthermore, (Janaszek e Kowalik 2023) reinforce that the application of sludge promotes the immobilization of heavy metals through organic complexation, reducing their mobility even in acidified environments, which may explain the absence of toxicity symptoms in the present study, even with the increase in Cu concentrations in the soil.

The significant increase in iron concentrations reflects the substantial contribution of this element via sewage sludge, whose origin is mainly associated with the use of ferric salts, such as ferric chloride, in chemical flocculation at treatment plants, in addition to the presence of metallic particles from pipeline corrosion (Smith 2009; Fytili e Zabaniotou 2008). Iron is widely recognized as one of the most abundant elements in biosolids, being mostly retained in the solid fraction due to its low solubility at neutral to alkaline pH (Alloway 2012). However, in systems rich in organic matter, such as treatments with a higher proportion of sludge, Fe can form soluble complexes with humic and fulvic acids, which increases its availability in the soil (McBride 1995). This mechanism helps explain the high levels observed in T3. According to (Smith 2009), iron from sludge tends to stabilize in forms that are not very mobile, but still accessible to plants, especially in organic soils. (Fytili e Zabaniotou 2008) also highlight that the mobility and availability of iron depend strongly on the redox environment and organic content, being favored by soils with high biological activity, a condition promoted by the application of stabilized organic waste, such as sewage sludge.

The increase in manganese (Mn) concentrations in the treatments was more moderate compared to the other micronutrients, with statistical significance only between the control and the other treatments. This subtle response can be explained by the highly redox-sensitive behavior of Mn, which in well-aerated environments tends to oxidize, forming insoluble oxides and reducing its bioavailability. Mn bioavailability is strongly controlled by redox conditions and organic matter content, which regulate the balance between its oxidized and reduced forms (Ma et al. 2015; Guoqing et al. 2019). (Baioui et al. 2017) highlight that Mn exhibits dynamic behavior, alternating between oxidation states according to the chemical characteristics of the soil. The gradual mineralization of matter can release small amounts of the metal over time, maintaining its availability without reaching toxic levels, as reported by (Wu et al. 2017; Ceccanti et al. 2022). Finally, (McBride 1995) highlights that Mn tends to accumulate less stably in soils with good drainage and aeration.

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3.3.2 Vegetative Development

Analysis of the morphological parameters of height and stem diameter of Schinus terebinthifolia seedlings reveals patterns consistent with the physiological dynamics of the plants in response to the application of sewage sludge in different proportions. Initially, the treatments with higher sludge content (T2 and T3) showed slower growth, both in height and diameter, which may be attributed to a stress effect or the need for initial adaptation of the seedlings to the chemical characteristics of the substrate (Alonso et al. 2018)**.** Furthermore, as can be seen in the experiment results, initially the treatments with higher proportions of sewage sludge presented high Ec, a reality that interferes with the development of the seedlings. (Shan et al. 2021) demonstrated that sewage sludge contributes significantly to increasing the soil Ec, attributing this effect to its high intrinsic salinity, as did (Roca-Pérez et al. 2009) highlighted that, although sludge compounds bring agronomic benefits, increased salinity is one of the main limiting factors for their use. Substrates rich in organic matter and nutrients, such as those containing biosolids, may present slow or irregular nutrient release, in addition to possible osmotic or toxicity effects due to high concentrations of heavy metals or soluble salts (Bettiol e Ghini 2011; Alonso et al. 2018). Despite this initial limitation, the data show a clear recovery over time. Subsequent measurements indicate consistent cumulative growth, with treatments T1, T2, and T3 surpassing the control (T0) in both height and diameter in the final phase of the experiment. All values were within the recommended range for native species of the Atlantic Forest (5–10 mm), according to (Tsakaldimi et al. 2013), which indicates that even treatments with a higher proportion of sludge produced seedlings that were morphologically suitable for planting in the field. This positive final performance is associated with the ability of sewage sludge to supply essential macronutrients, especially nitrogen (N) and phosphorus (P), which favor cell expansion and the development of active growth tissues. (Guerrini e Trigueiro 2004; Faria et al. 2013; Trigueiro e Guerrini 2014) have already demonstrated that the use of biosolids significantly improves soil fertility and contributes to the growth of forest species. In addition, the increase in height and diameter may also be related to the improvement in the physical structure of the substrate, promoted by the presence of organic matter and the greater capacity for water and nutrient retention, as suggested by (Scheer et al. 2012; Cabreira et al. 2017).

Another relevant aspect is the fact that stem diameter is a more robust indicator of seedling quality than height alone, as it reflects the plant's robustness and resistance to post-planting stresses, such as water deficit and wind (Grossnickle 2012; Davide 2020). In this sense, treatments with sewage sludge proved effective in promoting diameter growth compatible with the requirements for field planting, without compromising the structural integrity of the seedlings.

Regarding dry biomass, the analysis of the parameters revealed clear and consistent effects of biosolids application on dry matter accumulation, especially in the aerial part of the plants. The dry mass of the aerial part and the total dry mass were significantly higher in the treatments with sewage sludge (T1, T2, and T3) compared to the control (T0), showing that the use of biosolids favored the growth and productivity of the seedlings. On the other hand, the root dry mass did not show statistically significant differences, revealing a distinct behavior in the allocation of biomass among the plant compartments. The significant increase in aerial dry mass in the sludge treatments is directly related to the greater availability of essential nutrients, especially nitrogen and phosphorus, present in sewage sludge. These nutrients are fundamental for the development of photosynthetic tissues and supporting structures of the aerial part. As demonstrated by (Guerrini e Trigueiro 2004; Faria et al. 2013; Scheer et al. 2012), the addition of biosolids improves substrate fertility, promotes nutrient absorption, and stimulates aboveground biomass formation. (Chu et al. 2018) also highlight that aboveground biomass growth tends to be more pronounced in plants grown in substrates with sludge compounds, both in forest and ornamental species, which supports the findings of the present study.

In addition to the nutritional contribution, the conditioning effect of biosolids on the physical and chemical properties of the substrate may also have favored the formation of aboveground biomass. The presence of organic matter in the sludge improves soil structure, water retention capacity, aeration, and microbial activity, all factors that contribute to the growth of photosynthetic tissues (Ribeiro et al. 2009).

On the other hand, the absence of a significant difference in root biomass between treatments suggests a possible redistribution of the plant's energy investment, prioritizing shoot growth in environments with high nutrient availability. As discussed by (Zabotto et al. 2020), plants grown in poor substrates tend to develop more extensive root systems as a strategy to search for scarce nutrients. In fertile environments, such as in the sludge treatments, the investment in root growth is reduced, since nutrient absorption can be achieved with a less dense root system.

It is also important to consider that, although sewage sludge promoted gains in shoot biomass, very high concentrations of heavy metals or salts in the substrate can restrict root growth due to toxicity or osmotic effects, as suggested by (Júnio Ramos et al. 2009). In the present experiment, this may explain why the root dry mass in the more concentrated treatments (T2 and T3) did not proportionally increase the dry mass of the aerial part, resulting in a higher aerial/root biomass ratio.

The significant increase in the Leaf Chlorophyll Index (LCI), including chlorophyll A and B pigments, observed in the sewage sludge treatments (T1, T2 and T3), compared to the control (T0), shows a positive physiological response of the plants to the application of biosolids. This result is justified in the literature, being mainly attributed to the high availability of nitrogen in biosolids, the improvement of substrate fertility and the consequent stimulation of photosynthesis (Katircioglu 2021).

The ICF is a parameter used as a non-destructive indicator of chlorophyll content in leaves, which, in turn, is closely linked to the nutritional status of plants, especially nitrogen availability. The chlorophyll molecule contains nitrogen atoms in its structure, and this nutrient is also essential for the biosynthesis of the Rubisco enzyme, responsible for carbon fixation in photosynthesis (Mascarello et al. 2016; Duru 2002). Thus, when there is a greater supply of nitrogen, as in substrates enriched with sewage sludge, there is stimulation of the synthesis of photosynthetic pigments, resulting in leaves with a more intense green color and greater photosynthetic capacity (Madeira et al. 2009).

Several studies confirm the role of sewage sludge as an efficient source of N and promoter of increased ICF. (Zabotto et al. 2020), for example, observed that Eucalyptus urograndis seedlings grown with different doses of biosolids showed significantly higher chlorophyll values than those in the control treatment, with evident intense green coloration in the leaves. This increase was attributed to the higher content of organic matter and nitrogen in the substrates, which also correlated with the Green Color Index (GCI), another physiological indicator related to chlorophyll content.

In addition, (Trigueiro e Guerrini 2014) highlight that the application of biosolids improves not only the nitrogen content in the soil, but also its absorption by plants. This factor is directly related to the increase in the ICF, since N is a key element in the constitution of chlorophylls. (Usman et al. 2012) reinforce that the use of sewage sludge as fertilizer provides increases in the content of nutrients such as

N, P and K, whose effects are reflected in physiological parameters such as photosynthesis and plant vigor.

In a broader context, the increase in ICF is also linked to improvements in the photochemical efficiency of plants. The presence of more abundant and healthy pigments favors the functioning of the photosynthetic reaction centers, which can result in higher CO_2 assimilation rates and stomatal conductance, as demonstrated by (Zabotto et al. 2020). These functional improvements explain why biosolids treatments, in addition to increasing ICF, also promoted greater biomass accumulation and vegetative growth.

3.3.3 Vegetative Chemical Analysis

Nitrogen was the nutrient with the greatest increase in plant tissues after sewage sludge application. The increasing behavior of N with increasing doses was observed in both aerial parts and roots. The sludge treatments (T1, T2, and T3) stood out statistically in relation to the control, highlighting the potential of biosolids as an efficient source of nitrogen. This increase is justified by the composition of the sludge, recognized as an organic fertilizer rich in nutrients and organic matter (Paganini et al. 2024). Its application increases the levels of macro and micronutrients and improves soil fertility (Alonso et al. 2018; LIMA et al. 2011; Contin et al. 2012; Bai et al. 2017). (Chu et al. 2018; Zabotto et al. 2020) highlight that urban biosolids enrich degraded soils, and (Guedes et al. 2006) observed increased productivity with greater nutrient availability. The mineralization of N, largely present in organic form in the sludge, transforms it into inorganic forms (NH_4^+ and NO_3^-), which are absorbed by plants (Hernández et al. 2002). This process is efficient in the first year after application, ensuring a continuous supply throughout the plant cycle. As a result, there is greater absorption and accumulation of N, as demonstrated by (Zabotto et al. 2020), who reported significantly higher levels in the leaves. This accumulation favors vegetative growth and biomass, according to (Scheer et al. 2012).

Phosphorus showed distinct behavior among the plant compartments, with a slight tendency for reduction in average values in the aerial part in the treatments with sewage sludge. In the root system, the values remained more stable, and, in general, no statistically significant differences were observed between treatments, both in the aerial and root parts. This result, although at first glance counterintuitive, is consistent with studies that demonstrate that sewage sludge, despite being a rich source of total phosphorus, does not always provide a direct increase in foliar P levels due to biochemical, edaphic, and physiological limitations (Warman e Termeer 2005). One of the most recurring explanations is the effect of nutritional dilution. The greater biomass production stimulated by the high N and S levels in the SS treatments may have diluted the P concentration in the tissues. This has already been observed by (Cadot et al. 2018), who reported a decrease in P in wheat and barley grains with increased application of nitrogen fertilizers. (Bhattacharya et al. 2010) also indicated that increased plant biomass, even with different types of organic residues, can reduce relative phosphorus concentrations. Therefore, although sewage sludge favors growth, this structural gain may not be reflected in higher foliar P contents due to its dilution in plant tissues. Furthermore, according to (Fontes e Alleoni 2006), the dynamics of phosphorus in the soil are complex, especially in highly weathered tropical environments. Even when the experimental data indicate an increase in available P in substrates T2 and T3, effective absorption by plants may be limited by fixation processes, in which P binds to iron and aluminum oxides, reducing its availability (Fontes e Alleoni 2006). (Warman e Termeer 2005; Krogstad et al. 2005) reinforce that the mineralization of organic P in biosolids is slow, and only a small fraction is available in the short term. (Pantano et al. 2016; Guimarães et al. 2018) also observe that, in many cases, the availability of nitrogen, which is more easily assimilated, can outweigh the absorption of P during growth. In long-term experiments, (Selles et al. 1995) demonstrated that the concentration of available P in the soil does not always follow the balance of applied and exported P, indicating that accumulation in the soil does not necessarily translate into greater plant absorption. Another relevant factor is the influence of soil pH on phosphorus availability. (Manca et al. 2020) demonstrated an inverse correlation between pH and P content in the aerial parts of eucalyptus seedlings.

Potassium showed the opposite behavior to nitrogen and sulfur, with a decreasing trend in plant tissues as the application of sewage sludge increased. This decrease is explained by the low K content in biosolids, its high solubility, and the loss mechanisms during sewage treatment, which cause the element to remain in the liquid fraction and not be retained in the sludge (Andreoli et al. 2014). In addition to the low concentration, sewage sludge is considered nutritionally unbalanced in relation to K, which limits its efficiency as a source of this nutrient (Ferraz et al. 2016; Chen et al. 2022). (Manca et al. 2020) also demonstrated very low K_2O levels in substrates based on sewage sludge and sugarcane bagasse (0.07–0.11%), in contrast to the commercial substrate (0.20%). Reduced K absorption in plants can be aggravated by cationic antagonisms, especially with calcium, whose concentration is high in lime-treated sludge, potentially inducing K deficiency. This excess of Ca also favors K leaching to deeper layers, reducing its availability (Ferraz et al. 2016). (Chen et al. 2022) also observed that exchangeable K in the soil decreases over time in areas treated with sludge. Even with the greater plant growth in treatments with SS, the lower concentration of K in the tissues can be attributed to nutrient dilution, a phenomenon in which the increase in biomass proportionally reduces the concentration of nutrients (Bhattacharya et al. 2010; Bourioug et al. 2015). In Eucalyptus grandis, the control showed foliar K concentrations up to 40% higher than those observed with mineral fertilizers or SS (Ferraz et al. 2016).

Calcium showed a more balanced behavior in the treatments with sewage sludge, with a tendency to increase compared to the control. In the aerial part, the treatments registered significantly higher concentrations than the control, while in the root there were no significant variations. This increase in the aerial part is directly related to the high Ca content present in sewage sludge, especially those conditioned with lime, or that receive industrial waste rich in calcium compounds, such as calcium sulfate (Ferraz et al. 2016). Literature shows that the application of sludge tends to increase macronutrient levels in the soil, and in some cases, the Ca content in the substrate increased from 0.3% in commercial substrates to up to 14.3% in those treated with sludge (Guerrini e Trigueiro 2004; Ribeiro et al. 2009; Faria et al. 2013; Ferraz et al. 2016) also observed that the use of lime-stabilized sludge increased the pH and Ca levels in the soil by up to 250 times in the 0–5 cm layer. The higher concentration of Ca in the aerial part, but not in the roots, can be explained by the translocation dynamics of this nutrient, which is passive and occurs predominantly through the xylem. As Ca is immobile in the phloem, it tends to accumulate in older tissues and in the aerial part, especially under conditions of greater availability and absorption (White e Broadley 2003). This trend was observed by (Alonso et al. 2018; Manca et al. 2020), whose studies revealed that Ca is frequently among the most concentrated macronutrients in the aerial part, with levels above 34 g kg^{-1} in some treatments. Furthermore, foliar Ca concentration has been positively associated with the presence of boron and potassium, and negatively influenced by soil pH, reinforcing the importance of the balance between these elements for healthy plant growth (Kabata 2010). (Wolf e McGrath 2011) add that the application of sewage sludge can increase the cation exchange capacity (CEC) of the soil, allowing greater retention of Ca^{2+} and its consequent absorption. However, the increase in Ca in the plant can also result in antagonistic effects on the absorption of other cations, mainly potassium and, to a lesser extent, magnesium. (Ferraz et al. 2016) report that the application of sludge enriched with Ca led to a significant increase in Ca:K and Ca:Mg ratios in leaves, which may limit the adequate absorption of these nutrients. Studies by (Manca et al. 2020) also evidenced complex cationic interactions, revealing that the behavior of Mg in the aerial part was positively influenced by pH and Ca, and negatively correlated with K ($r = -0.79$, $p < 0.05$), reinforcing the competition between these nutrients in the absorption process.

The behavior of magnesium showed significant variations, especially at the highest dose (T3), where Mg levels in the roots were lower than those observed in T1 and T2. This result can be attributed to complex interactions with other cations, such as calcium and sodium, which increase with the application of sewage sludge. (Manca et al. 2020) demonstrated that Mg in the aerial part was positively correlated with pH, but negatively with potassium, revealing cationic competition that affects nutrient absorption and redistribution. This scenario is aggravated by the increase in pH, common in soils treated with lime-stabilized sludge, which can promote leaching of Mg and K to subsurface layers (Ferraz et al. 2016). Although the Mg levels in the sludge were initially higher, the final content in the substrate decreased in T1, T2, and T3 compared to T0, suggesting depletion or high absorption. It is important to highlight that the accumulation of Mg in roots may reflect a peak absorption or a root retention strategy, as observed by (Manca et al. 2020) in experiments with sewage sludge and sugarcane bagasse. (Zabotto et al. 2020) also reported that foliar Mg levels in Eucalyptus urograndis were not significantly altered by sludge treatments, and (Ferraz et al. 2016) demonstrated that, in some cases, the highest foliar concentration was obtained with mineral fertilization. This suggests that the Mg supplied by sewage sludge may not be easily assimilated or translocated at higher doses. Furthermore, the low concentration of Mg in plant tissues, even with increased biomass growth, may be a result of nutrient dilution, as pointed out by (Bhattacharya et al. 2010). (Wolf e McGrath 2011) observe that the use of sludge increases the cation exchange capacity (CEC) of the soil, which should favor the retention of cations such as Ca^{2+}, Mg^{2+}, and K^{+}. However, the high presence of Ca at the higher doses may have promoted competition, reducing the availability of Mg for absorption.

Sulfur showed a positive response to the application of sewage sludge, with a significant increase in the levels in the aerial part and roots. This increase is directly linked to the release of S during the decomposition of the organic matter in the sludge, which is a significant source of this nutrient (Zabotto et al. 2020). Studies with Eucalyptus grandis show that SS increases sulfur levels in the soil, especially in the surface layers, with movement of sulfate (SO_4^{2-}) to deeper layers (Guerrini e Trigueiro 2004; Ribeiro et al. 2009). (Zabotto et al. 2020) also recorded higher foliar S levels than adult trees treated with SS, reinforcing its value as a nutritional source. (Chu et al. 2018) observed simultaneous accumulation of S, N, P, and K in plants treated with biosolids, demonstrating the combined effect of these nutrients. The correlation between N and S levels is explained by the integrated biochemical function of these elements, both essential components of amino acids such as methionine and cysteine, in addition to participating in the synthesis of proteins and enzymes (Zabotto et al. 2020; Manca et al. 2020). This relationship is strengthened by the presence of organic matter, which acts as the main reservoir of these macronutrients. Multivariate data from Principal Component Analysis (PFA) demonstrate a strong association between OM, N_t, and S_t in the substrates (Zabotto et al. 2020; Manca et al. 2020). In Eucalyptus grandis, foliar concentrations of S were up to 70% higher in treatments with SS compared to the control, as observed throughout the growth cycle (Zabotto et al. 2020). (Chu et al. 2018) confirm that increased SS application promotes greater absorption of these nutrients, directly contributing to vegetative growth. The use of sewage sludge is effective not only as a source of S but also as a promoter of nutritional synergies between macronutrients essential for plant development.

In contrast to macronutrients, micronutrients tended to show higher levels in the roots, reflecting a possible protection strategy or reduced translocation, although Mn also accumulated significantly in the shoots.

Boron analysis showed discrete variations in the shoots and a progressive and significant increase in the roots in response to sewage sludge application. This pattern is supported by several studies that highlight B as an essential micronutrient for plant growth, playing a fundamental role in cell wall formation, especially through its binding to pectin molecules (O'Neill et al. 2004). Sewage sludge is recognized as a relevant source of B, and its application can increase the availability of the nutrient in the soil. (Manca et al. 2020) demonstrated that substrates enriched with biosolids presented high levels of B, which were positively correlated with plant morphological parameters, such as height and stem diameter. The application of SS in tropical soils, especially for species such as eucalyptus, has proven efficient in correcting deficiencies of this micronutrient (Manca et al. 2020). Retention in the roots can be explained by cationic interactions that affect its absorption. B in the root showed a negative correlation with potassium and a positive correlation with calcium and sodium, indicating competition and a direct influence of substrate chemistry on the nutrient dynamics (Manca et al. 2020). Additionally, the balance between B and Ca is considered crucial for normal plant growth, acting in fundamental metabolic processes (Kabata 2010). B deficiency, on the other hand, compromises stomatal function and K absorption, aggravating water stress situations (Wimmer e Eichert 2013).

Copper showed a progressive accumulation behavior in response to increased doses of sewage sludge (SS), both in the aerial part and in the roots, with treatments T2 and T3 standing out, registering the highest concentrations, especially in the roots. In the aerial part, all sludge treatments showed significantly higher values than the control, indicating that the increase in the SS dose promoted a direct increase in Cu levels in plant tissues. This result can be justified by the composition of the sludge used, being a significant source of this micronutrient/metalloid. Literature indicates that the use of sewage sludge-based compounds increases the levels of heavy metals in growing substrates, including copper and zinc, as evidenced by (Chu et al. 2018; Ferraz et al. 2016). The progressive increase in Cu concentration in roots and shoots is a recurring pattern when using biosolids, as also reported by (Chu et al. 2018), who observed an increase in Cu levels in the soil, control, and at high doses.

Another relevant aspect is the preferential retention of Cu in the roots, a typical behavior of heavy metals in non-hyperaccumulator plants, where the low translocation of copper to the shoots is related to its affinity with soil and organic matter components, and to the action of roots as retention barriers (Rather et al. 2020). (Chu et al. 2018) confirmed that the translocation factor (TF) of Cu is generally less than 1.0, which demonstrates a physiological limitation to the mobility of this element to the leaves and stems. Organic matter, in turn, plays a fundamental role in this process, acting as a sink for Cu and other metals (Fe, Zn, Mn), controlling their variability in the substrate, as demonstrated by (Manca et al. 2020), who also pointed out that the roots showed lower Cu levels in treatments with less available phosphorus, and proportional increases when the P content was higher, suggesting an interdependence between the absorption of these two elements.

Iron showed a marked accumulation pattern in the roots of plants treated with sewage sludge. This accumulation was statistically significant in the roots, but there was no significant variation in the aerial part, where the levels remained low and stable. This pattern of root accumulation and low translocation to aerial tissues is supported by the literature, which highlights sewage sludge as a primary source of iron, as well as the role of organic matter and physiological mechanisms of the plant in the root retention of heavy metals (Ferraz et al. 2016). The presence of organic matter in the substrate, in turn, also contributes to this accumulation, acting as a sink for metals and promoting Fe retention in the soil, as demonstrated by (Manca et al. 2020). Regarding distribution in the plant body, several studies indicate that iron, like copper, tends to accumulate in the roots due to physiological mechanisms that restrict translocation. These heavy metals are preferentially retained in the roots as a way to protect the aerial part from potential toxicity, a behavior opposite to that of macronutrients such as N or P (Manca et al. 2020). The low translocation of Fe is therefore related to its complexation with organic compounds in the soil and the ability of roots to immobilize heavy metals, preventing them from reaching toxic levels in the leaves, as demonstrated by (Júnio Ramos et al. 2009). Despite being an essential micronutrient, high iron concentration in the roots may be associated with negative effects on root growth, as observed by (Zabotto et al. 2020; Júnio Ramos et al. 2009). At high doses, excess Fe in the root system can compromise plant development. This restriction in iron mobility, accompanied by its accumulation in the roots, is strongly linked to the composition of the substrate with sewage sludge and the increase in organic matter and pH, which together influence the availability, retention, and distribution of metals in the soil-plant system (Manca et al. 2020).

Manganese showed a progressive increase in concentrations in the aerial part. Among the micronutrients evaluated, Mn was the one that showed the greatest translocation from the root to the aerial part, evidencing a pattern of behavior opposite to that observed for Fe, Cu, and Zn, which accumulated mainly in the roots. These results are compatible with those demonstrated by (Chu et al. 2018), which highlights the mobility of Mn in the plant, its dynamic behavior in the soil as a function of pH and organic matter, in addition to its interaction with other nutrients. Mobility is favored by the high organic matter content of biosolids, which acts as a regulator of the availability of heavy metals in the soil, including Mn. Multivariate analyses performed by (Manca et al. 2020) demonstrated that Mn is associated with the same factor as N, S, and OM, reinforcing the joint influence of these components on absorption dynamics. This Mn translocation pattern is confirmed in the study by (Chu et al. 2018), where Mn was classified as one of the micronutrients with the greatest mobility capacity compared to other heavy metals. Furthermore, soil chemistry and pH played a crucial role in Mn absorption. According to (Kabata 2010), Mn is highly sensitive to soil acidity and redox conditions, but its solubility can be modulated by complexation with organic compounds. (Manca et al. 2020) observed a positive correlation between Mn, phosphorus, and sodium in the substrate, which may be linked to the solubility of Mn phosphates and the mutual influence of these elements on root absorption processes. In a study conducted by (Bourioug et al. 2015) with Larix decidua, plants grown in soil amended with sewage sludge showed a substantial increase in Mn concentrations in both needles and lateral roots, corroborating the tendency for aerial accumulation and effective absorption of Mn in environments with SS.

Analysis of zinc behavior demonstrated a progressive and significant accumulation in plant tissues, especially in the roots, with an increase proportional to the doses of sewage sludge, following the order T3 > T2 > T1 > T0. This pattern is consistent with the literature, which highlights Zn as an element present in high concentrations in biosolids and with low mobility within the plant (Júnio Ramos et al. 2009; Zabotto et al. 2020). The sewage sludge used had 129.03 g/dm³ of Zn, justifying the observed increase. (Chu et al. 2018) also confirm that the application of sludge increases the levels of Cu and Zn in the substrates, a pattern also observed by (Ferraz et al. 2016) in studies with eucalyptus.

The greater retention of Zn in the roots is explained as a plant defense mechanism, which restricts the translocation of heavy metals to the aerial parts. (Manca et al. 2020) highlight the role of organic matter as the main "sink" for these metals, promoting their immobilization in the soil and rhizosphere. (Júnio Ramos et al. 2009) also observed inhibition of root growth in Eucalyptus urophylla with increasing Zn, indicating possible toxicity at high concentrations. Physiologically, Zn acts synergistically with nitrogen, being an enzymatic cofactor in several metabolic processes, which justifies the positive correlation between Zn in the substrate and N in the shoots (Kabata-Pendias e Mukherjee 2007). Cations such as Ca and Na also favor its absorption (Kabata 2010). Despite the significant increase in Zn, (Dechen e Nachtigall 2006) report an absence of visible symptoms of toxicity, such as chlorosis or necrosis. (Chu et al. 2018) confirm that, even with high levels of Zn, the ecological risk was considered low.

3.4 Conclusions

The results of this study demonstrate that the application of sewage sludge significantly altered the physical and chemical attributes of the soil, with effects directly proportional to the applied dose. Treatments with sludge showed higher density and lower levels of organic matter and total carbon compared to the control (T0), reflecting the influence of the mineral soil. The gradual reduction in pH favored the solubilization of exchangeable aluminum, especially in T3, while electrical conductivity increased initially and subsequently decreased due to leaching. Higher doses increased CEC and available phosphorus, confirming the fertilizing potential of sludge. However, reductions in K^+ and Mg^{2+} indicate ionic competition and losses due to leaching. Micronutrients such as Cu, Zn, and Fe increased with sludge doses but remained within safe agronomic limits. Seedling performance was also positively influenced, particularly treatments T2 and T3, which surpassed T0 in height, stem diameter, biomass, and chlorophyll index. Nitrogen showed the greatest accumulation in the tissues, followed by sulfur, reinforcing sludge as an efficient source of nutrients. Micronutrients were mostly retained in the roots, except for manganese, which accumulated in the aerial part. Principal component analyses confirmed the positive and dose-dependent influence of sludge on soil fertility, nutrition, and plant growth. The T0 treatment appeared distant from the nutritional and growth vectors, while the sludge treatments formed clusters associated with better performance. Taken together, the data confirm that the controlled use of sewage sludge improves nutritional dynamics and plant development, consolidating its potential as a sustainable input in the production of forest seedlings.

It was concluded that the highest dose of sewage sludge tested (12%) was quite satisfactory, promoting significant gains in terms of nutritional value and seedling development, without causing toxicity symptoms. However, it is important to previously assess the electrical conductivity (EC) of the sludge, since high salinity levels can compromise the absorption of water and nutrients by plants, harming their growth and, in more severe cases, leading to seedling death. This initial characterization step is essential to ensure the safe and efficient use of the residue.

The short evaluation period represents a limitation of this study. Future research should include long-term experiments, as well as investigating the cumulative effects on the soil, the environmental risks of continuous use of the residue, and the impact of different irrigation depths, simulating conditions of water deficit and excess.

4 Conclusion and Final Remarks

This thesis aimed to evaluate the use of sewage sludge as a substrate component in the seedling growth process in a nursery, considering different edaphoclimatic contexts and application proportions. The research was structured in three chapters, the first being a systematic review with meta-analysis, followed by two case studies applied under different experimental conditions. The systematic review and meta-analysis were fundamental to establishing the theoretical and methodological bases that supported the subsequent experiments. From the analysis of quantitative studies available in the literature, it was possible to identify consistent trends of increased organic matter and nutrients such as nitrogen and phosphorus after sludge application, in addition to pointing out important gaps regarding the effects of salinity, metal accumulation, and interactions with different soil types. These data served as guidelines for defining the doses and parameters analyzed in the case studies, contributing to a more targeted experimental design.

Case study 1, conducted in Italy, used Nerium oleander as an indicator species and mixtures with 25%, 50%, and 75% of sewage sludge balanced with urban calcareous soil. The results showed that the sludge promoted improvements in moisture retention, organic matter, CEC, and nutrients such as nitrogen and phosphorus, although the control (commercial peat-based substrate) maintained the best growth performance. High doses of sludge (50% and 75%) resulted in initial stress in the seedlings, possibly due to high salinity and the presence of metals, with only partial recovery over time. The treatment with 25% sludge stood out as the most balanced proportion, providing nutritional gains without compromising seedling development, and is therefore recommended as a safe limit for calcareous soils in short-term evaluations.

Case study 2, in turn, was conducted in Brazil with the native species Brazilian peppertree (Schinus terebinthifolius), in an acidic red latosol, using increasing doses of dehydrated sewage sludge (4%, 8%, and 12%). Unlike the first study, all sludge proportions promoted significant gains in seedling growth, especially the 12% treatment, which showed the best results in height, biomass, and nutritional content. The positive behavior was attributed to the greater initial fertility of the sludge and its ability to supply essential nutrients such as N, P, and S, even in the long term, without the need for chemical fertilization. The retention of metallic micronutrients in the roots, with the exception of Mn, also contributed to the safety of using the residue. In addition, the monthly monitoring of the leachates allowed the identification of the dynamics of electrical conductivity over time, an important methodological differential that contributed to a more complete understanding of the system.

The comparison between the two case studies shows that the ideal sludge dose is strongly conditioned by the type of soil and the composition of the mixture used. While in the calcareous soil of case study 1 the safe dose was 25%, in the latosol of case study 2 the application of up to 12% proved to be efficient and safe. This indicates that the agronomic response to sludge does not depend exclusively on the characteristics of the residue, but rather on the interaction between the sludge, the soil and the other components of the substrate. Acidic and weathered soils, such as latosols, can benefit more from the input of organic matter and nutrients promoted by the sludge, while calcareous soils require greater caution, especially regarding salinity and the release of metals.

Therefore, the integrated analysis of the two studies suggests that the ideal application range for sewage sludge is between 12% and 25%, always considering the physical-chemical characteristics of the soil, the degree of sludge stabilization and the objectives of the crop. Furthermore, the importance of periodic monitoring of leachates is highlighted, especially regarding electrical conductivity and nutrient loss, as a way to anticipate salinization risks and assess fertilization efficiency.

Finally, the main limitations of this thesis involve the short evaluation period in both case studies and the absence of experiments under different water regimes. Thus, it is recommended that future research explore the long-term behavior of plants and soil under field conditions and test different irrigation depths, simulating both water deficit and excess. Such approaches will allow for a better understanding of the cumulative effects of sewage sludge application and will contribute to the development of safer and more efficient protocols for its sustainable agricultural use.

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Starting a doctoral journey, especially when you're already in the job market and need to move to another country, is not simple. It's a path full of uncertainties, challenges, and situations that take us out of our comfort zone. The difficulties are many, including adapting to a new language, dealing with bureaucracy, understanding a new culture, and, in parallel, the technical challenges: structuring an experimental project, conducting extensive literature reviews, implementing experiments, conducting field data collection, working in the laboratory, analyzing data, discussing interpretations, writing articles, and finally, writing the thesis.

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