

Article

Greenhouse Evaluation of the Agronomic Potential of Urban Wastewater-Based Fertilizers: Sewage Sludge and Struvite for Lettuce Production in Sandy Soil

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Abstract

Environmental impacts of urban wastewater treatment plants (WWTPs) can be reduced by recovering nutrients and organic matter (OM) from their streams for agricultural use, decreasing dependence on conventional fertilizers. This study evaluated dehydrated sewage sludge (SS) as an organic amendment and the partial replacement of mineral P fertilizers in lettuce cultivation. Struvite, a byproduct of WWTPs, was also investigated as a sustainable P source. A 43-day greenhouse pot experiment assessed SS (12 t/ha) and struvite (at two P rates: 30 and 60 kg P₂O₅/ha), both alone and combined. SS significantly increased soil OM ($p < 0.001$), though long-term applications would be required to enhance this effect. The highest struvite rate (60 kg P₂O₅/ha) yielded the greatest extractable soil-P levels (150 ± 8.1 mg P₂O₅/kg), while its combination with SS further increased extractable P (>250 mg P₂O₅/kg), indicating a stable soil P pool. The highest plant dry biomass (8.9 ± 1.1 g, $p < 0.05$) also occurred under the highest struvite dosage. Complementary effects between SS and struvite were observed in foliar K, Ca, Mg, and S contents, although no significant interaction between both was found for P content. Adequate foliar P levels (0.40–0.52%) were achieved only in treatments containing SS, indicating its essential role in improving plant P nutrition.

Keywords: struvite; sewage sludge; nutrients; wastewater; waste-based fertilizers



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1. Introduction

The Mediterranean regions of Europe have been identified as vulnerable to soil degradation, with the highest erosion rates and lowest soil organic carbon (SOC) levels among the European Union (EU) countries [1]. According to 2015 data, over 75% of the land in these regions exhibits very low ($\leq 1\%$) or low ($\leq 2\%$) topsoil organic carbon (OC) content. These low levels result from a combination of factors, including region-specific climate conditions, the impact of global warming, soil erosion, and intensive agriculture practices [1,2]. In contrast, high levels of phosphorus (P) can be found in soil because of the excessive use of mineral and organic P fertilizers [3]. These levels can also often result from the application of manure and sludge as part of organic fertilization strategies, which are

typically guided by crop nitrogen (N) requirements. As a result, more P than necessary is frequently applied, accumulating excess P in the soil [4]. Excessive levels of available P in the soil can lead to environmental problems, such as the risk of eutrophication in surface water and contamination of groundwater. On the other hand, although P in manure and sludge is often in organic forms and not immediately available, reducing the immediate risk of water contamination, it can still accumulate over time in the soil, contributing to long-term P buildup [5,6].

Therefore, it is essential to adopt sustainable agricultural practices that can reverse this trend by increasing soil organic matter (OM) levels while preventing further P accumulation. To achieve this, careful and balanced use of mineral fertilizers is essential, along with a reduction in reliance on these inputs whenever possible. Although mineral fertilizers have played a key role in enhancing agricultural productivity, they have also significantly contributed to greenhouse gas (GHG) emissions in the agri-food sector. According to 2019 data, the production and use of these fertilizers generated approximately 400 Mt of carbon dioxide equivalent, accounting for about 0.8% of global GHG emissions [7]. The European fertilizer industry has set ambitious goals, including a 70% reduction in GHG emissions by 2040 and achieving climate neutrality by 2050, in alignment with the European Green Deal (EGD). Also, under the EGD, the Farm-to-Fork strategy addresses the challenges of building a sustainable food system, with a key objective of reducing nutrient losses by at least 50%, with the expectation of cutting mineral fertilizer use by at least 20%. The use of waste-derived fertilizers and soil amendments provides an environmentally sustainable alternative to the consumption of virgin raw materials, enhancing soil fertility and boosting crop productivity while mitigating the associated environmental issues. By evaluating strategies that enhance the use of waste-derived fertilizers, it is possible to contribute to reducing dependency on conventional mineral fertilizers and minimizing nutrient losses, aligning with the goals of circularity and environmental sustainability outlined in these initiatives. Indeed, the implementation of the new EU Fertilizing Products Regulation (EU) 2019/1009 created opportunities to develop innovative bio-based solutions, promoting the circular economy and fostering more sustainable agricultural practices that can be included in the fertilizer market.

In this context, applying urban sewage sludge (SS) from urban wastewater treatment plants (WWTPs) as a soil conditioner for organic amendment in agricultural soils offers a promising strategy. Applying SS to agricultural soils can offer several agronomic and environmental benefits, as it is rich in OM, P, N, and micronutrients, which enhance soil fertility, stimulate microbial activity, and improve water-holding capacity and soil structure stability [8,9]. Although several countries have banned or restricted the direct land application of this residue [10], its controlled application can help to improve the typically low OC content in soils of Mediterranean regions, where incineration is also not the primary disposal method. Moreover, agricultural soil application of SS must be preceded by proper treatment to grant hygienization and stabilization and to mitigate risks to soil health and the environment. Despite other authors having reported a decline in contaminant concentrations in societal waste [11], application rates must be carefully managed to avoid introducing potentially harmful substances without promoting indiscriminate use of untreated SS. Indeed, according to the European Commission, the levels of potentially toxic metals in SS intended for agricultural use have decreased over time and are often ten times lower than the thresholds set by the Sewage Sludge Directive [12]. In line with this, results from a long-term field experiment from the CRUCIAL project (University of Copenhagen) demonstrated that the application of organic wastes, including SS, corresponding to 100–200 years of legal application, can be both safe and beneficial. These amendments improved soil fertility and structure, did not increase plant uptake

of potentially toxic metals [13], and had no adverse effects on microbial diversity or antibiotic resistance persistence [14], while the quality of soil organic matter (OM) was also maintained or enhanced. However, nowadays, the sustainable management of SS should also consider the development and identification of methods to identify and quantify the presence of other emerging pollutants and microcontaminants [15], which was not considered in this work. Nevertheless, caution is needed when working with this type of waste. Overall, these findings highlight not only the capacity of the soil ecosystem to process modern waste inputs but also its often-underestimated resilience [11].

On the other hand, dependence on conventional mineral fertilizers should be gradually replaced by waste-derived fertilizers, whenever possible. In this context, struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), a byproduct with variability in its composition that can precipitate during wastewater treatment processes, offers a valuable pathway for P recovery and its return to agricultural soils. Struvite has special interest due to its P, N, and Mg content (12.6, 5.7, and 9.9%, respectively [16]) and slow-release behavior that helps to reduce nutrient leaching and supports overall soil health. Several strategies exist to promote the controlled recovery of this mineral within the treatment system. However, in many WWTPs employing secondary treatment and anaerobic digestion, struvite precipitation is frequently observed. This unintended crystallization can cause operational issues in return lines, pumps, and valves, increasing maintenance and pumping costs. In line with this, due to its uncontrolled precipitation, it is important to consider its purity. Due to the presence of high concentrations of other elements (e.g., Ca^{2+} , Na^+ , K^+ , Cl^- , and CO_3^{2-}) in the urban wastewater matrix, there is the possibility of co-precipitation of other minerals, such as hydroxyapatite, brushite, and amorphous compounds along with struvite. Nevertheless, considering that struvite has significant market value and can be included in the European fertilizer market under Regulation (EU) 2019/1009 [16–20], this study intended to evaluate the agronomical potential of this type of struvite, formed as a byproduct of urban wastewater treatment processes (uncontrolled), rather than struvite produced through controlled precipitation processes.

In this context, this study evaluates the combined use of sanitized SS as a soil organic amendment and struvite (a wastewater-derived byproduct) as a sustainable P source for lettuce cultivation, addressing the limited evidence on their comparative performance in sandy soils and contributing to reduced dependence on non-renewable fertilizer sources.

2. Materials and Methods

2.1. Origin and Characterization of Sewage Sludge, Struvite, and Soil

Dewatered SS was sourced from a Portuguese urban WWTP with a treatment capacity of approximately 40,000 m³/day. The system includes activated sludge for biological treatment, followed by anaerobic digestion and mechanical dewatering via centrifugation. The SS was oven-dried at 100 °C for 48 h to ensure hygienization and minimize microbiological risks. No *Salmonella* spp. was detected (according to the ISO 6579:2002 [21] procedure), and *E. coli* levels were below 1.0×10^1 CFU/g (following ISO 16649-2:2001 [22]). However, it is important to note that drying at this temperature may result in partial nitrogen losses as ammonia. Therefore, the use of dried sludge requires balancing the need to preserve nutrients with the need to ensure microbiological safety. Struvite, the waste-based fertilizer, was recovered at the same WWTP as a solid rock-like byproduct formed between anaerobic digestion and dewatering stages. The material was ground and sieved (1–2 mm) to obtain a more uniform particle-size material, suitable for soil application. The general physico-chemical characterization of the materials used in the soil experiment is summarized in Table 1. The heavy metal concentrations in the sewage sludge were all below the legal limits established by Council Directive 86/278/EEC (12 June), thereby allowing its use for agri-

cultural applications. Furthermore, considering the applied dosage of 12 t_{db}/ha of sewage sludge, there is a small risk of heavy metal accumulation in soil. The total metal load, per hectare and per year, remains well below the threshold values specified in the European Directive (Table 1), which are designed to ensure soil protection and prevent long-term contamination. Considering the heavy metal concentrations detected in the struvite, where only zinc was above detection limits of the method, no risk of metal accumulation in the soil is expected.

Table 1. Main physicochemical characteristics of the materials used in soil fertilization and amendments.

	Struvite		Sewage Sludge		
pH	9.38	6.43			
EC (mS/cm)	0.11 ± 0.05	7.23 ± 0.59			
OM (%)	39.1 ± 0.25	70.1 ± 1.23			
TP (mg P/g _{db})	83.8 ± 4.94	15.1 ± 0.85			Heavy metal per
N (mg TKN/g _{db})	28.3 ± 1.20	58.2 ± 0.22			hectare load after 12 t/ha SS
Mg (mg/g _{db})	27.1 ± 2.36	3.85 ± 0.06			application
Ca (mg/g _{db})	24.7 ± 3.21	16.4 ± 0.63			
Heavy metals (mg/kg _{db})	Value	Value	Legal limits according to Council Directive 86/278/EEC *	(kg/ha) **	Legal limit (kg/ha/year) *
Pb	n.d.	28.1 ± 0.25	750–1200	0.34	15
Cr	n.d.	36.8 ± 1.52	-	0.45	-
Zn	12.0 ± 1.35	970 ± 56.1	2500–4000	11.8	30
Cd	n.d.	1.2 ± 0.05	20–40	0.01	0.15
Cu	n.d.	340 ± 32.1	1000–1750	4.15	12
Ni	n.d.	20.9 ± 1.63	300–400	0.25	3

EC—electrical conductivity (EC and pH measured in a 1:10 *w/v* solid–liquid extract with distilled water); OM—organic matter (EPA Method 1684); TP—total phosphorus (EPA Method 365.3); TKN—total N by the Kjeldahl method; n.d.—not detected; Mg, Ca, Pb, Cr, Zn, Cd, Cu, and Ni determined by flame atomic absorption, after aqua regia digestion; db—dry basis; * directive for the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture; ** calculated based on the mean value of heavy metals' concentration.

A sandy soil was collected in Alcochete (Portugal), within a Haplic Arenosols area, according to the IUSS Working Group WRB [23] at a depth of 20 cm. The soil exhibits a coarse texture (916 g/kg sand, 38 g/kg silt, and 56 g/kg clay), is slightly acidic (pH ~6.01), has low salinity (EC of 74.15 µS/cm; 1:2 soil–water extract), and has low organic matter content (6.7 g/kg). Regarding nutrient availability, the soil contains high amounts of extractable P (58.9 mg P/kg, equivalent to 135 mg P₂O₅/kg; Egner–Rhiem method), indicating a high level of fertility. This condition minimizes the risk of P deficiency and allows the experiment to focus on the relative efficiency and release dynamics of the alternative P sources studied. In contrast, K availability is low (27.5 mg K/kg, equivalent to 33 mg K₂O/kg; Egner–Rhiem method), and its content is characteristic of soils with a low fertility level, under the Portuguese Standards used to classify soil fertility [24].

2.2. Experimental Setup

The experiment was conducted for 43 days (from 23 May to 5 July 2024) in a greenhouse without temperature and humidity control. During the experimental period, the median minimum and maximum environmental temperatures ranged from 13–15 °C to 21–24 °C in May, 15–17 °C to 24–27 °C in June, and 18–19 °C to 27–29 °C in July. Mean relative humidity was approximately 69%, 74%, and 66% in May, June, and July, respectively. The pots used were of polypropylene and cylindrical, and the soil sample was 2.90 ± 0.05 kg per pot. Pots were randomly distributed in a wheeled stand, which allowed them to be moved outside and exposed to direct sunlight.

A single application of dehydrated and ground SS was used (S: 13.6 g/pot; 4.7 g/kg of soil; dry matter basis) to evaluate its function as an organic soil amendment. This treatment was compared with the soil without amendment or waste-based fertilizer application (C: control) to assess its effectiveness in increasing soil OM content and contributing to plant-available P.

To assess how struvite, recovered as an operational byproduct from the WWTP, can replace, totally or partially, mineral P fertilizers, two P dosages were applied based on the recommendation for lettuce growth in a soil with high extractable P levels [24]: (i) a lower application rate of 30 kg P₂O₅/ha (13.1 kg P/ha), equivalent to 11.6 mg P₂O₅/kg soil (5.1 mg P/kg soil); and (ii) a higher dosage, doubling the previous value—60 kg P₂O₅/ha (26.2 kg P/ha), equivalent to 23.1 mg P₂O₅/kg soil (10.1 mg P/kg soil). These values were obtained considering the soil existing in a hectare, at a depth of 20 cm and a bulk density of 1.3 g/cm³. Based on these application rates, approximately 60 mg of struvite per kg of soil (0.175 g/pot) was applied for the lowest P dose (E_{1/2}), while the higher dose (E), involved a doubled amount (0.35 g/pot).

For comparison purposes, commercial single superphosphate (18% P₂O₅, 10% CaO, 27% SO₃) was used, providing the same dosages of P, hereafter defined as M_{1/2} for the lowest dosage, about 64 mg superphosphate/kg soil (0.186 g/pot), and M for the highest dosage (0.372 g/pot), doubling the value. Table 2 summarizes the different treatments tested in the pot experiment.

Table 2. Different doses of sludge and of fertilizers used in the pot experiment setup. Both fertilizer doses, superphosphate and struvite, provided the same P to the plant.

Treatment Designation	Sludge (t/ha)	Mineral P Fertilizer (kg P ₂ O ₅ /ha)	Struvite (kg P ₂ O ₅ /ha)
C	0	0	0
CS	12	0	0
M _{1/2}	0	30	0
M _{1/2} S	12	30	0
E _{1/2}	0	0	30
E _{1/2} S	12	0	30
M	0	60	0
MS	12	60	0
E	0	0	60
ES	12	0	60

Treatments were replicated four times, with pots randomly arranged and moved weekly to ensure uniform light exposure throughout the experiment. The soil was kept at approximately 70% of its maximum water-holding capacity (soil water-holding capacity of 19% (m/m), to saturate the soil, measured in the <2 mm disturbed samples). Each pot was planted with one lettuce seedling plant (*Lactuca sativa* L. var. Aphyllion), transplanted 28 days after seeding (two-leaf development stage) in an organic growing media. A basal fertilization with 0.5 g N/plant was added using a nitro-magnesium mineral fertilizer rich in N (27% total N—13.5% nitric N and 13.5% ammoniacal N, 3.5% CaO, 3.5% MgO), and, after 15 days, a top-dressing fertilization provided 0.5 g N/plant using a different mineral fertilizer (15% total N—7.5% nitric N and 7.5% ammoniacal N, 25% CaO). The total N supplied per pot was equivalent across all treatments, ensuring that the observed differences in plant growth were due to experimental inputs rather than differences in N availability. Pots were irrigated periodically, approximately three times a week, with the water content controlled by weighing, to reach the initial 70% maximum water retention capacity. All pots had an individual dish to prevent irrigation water loss, which was made

with deionized water, meeting the requirements of a Type III water ($EC \leq 5.0 \mu\text{S}/\text{cm}$ (25°C), pH between 5.0 and 7.0, ISO 3696:1987 [25]).

2.3. Soil Physicochemical Characterization

At the end of the experiment, soil from each pot was collected, air-dried at room temperature, and sieved through a 2 mm sieve. Subsequently, various parameters were measured to evaluate the treatment's effect.

Soil pH and electrical conductivity (EC) were measured in 1:2.5 (w/v) and 1:2 (w/v) extracts prepared with deionized water, respectively. Total organic carbon (C_{org} , %) was determined after sample combustion at 1200°C , and the CO_2 content was quantified by infrared detection using an elemental analyzer (multi-EA 4000, Analytikjena, Jena, Germany). Organic matter content (OM, %) was estimated based on an assumed average C_{org} content of 58%, using the following formula: $OM = C_{\text{org}} \cdot 1.724$. Mineral N (NH_4^+ and NO_3^-) concentration was determined after extraction with 2 M KCl (1:5 w/v) during 60 min of agitation and centrifugation at 4000 rpm. Then, the supernatant was analyzed by a segmented flow analyzer (San Plus System, Skalar, HQ - Skalar Analytical B.V., Breda, The Netherlands). Extractable P and K were assessed via the Egner–Riehm method, using a 1:20 (w/v) extractant of 0.1 M ammonium lactate in 0.4 M acetic acid [26], shaken for 120 min and centrifuged at 4000 rpm. Although the Egner–Riehm extraction method is commonly used to estimate extractable P and K (especially in Portugal, but also in other European countries), its accuracy can be influenced by soil pH and texture, giving different performances in different soil types [27]. Despite its limitations, the interpretation of the results is made with standard fertilization tables, well adapted to Portuguese soils, and adopted by advisors and farmers for decades in the country [24]. Micronutrients were quantified using the Lakanen and Erviö extraction procedure, with a 1:10 (w/v) solution of 0.5 M ammonium acetate, 0.5 M acetic acid, and 0.02 M EDTA (pH 4.65), agitated for 60 min and centrifuged at 4000 rpm. Non-acid exchangeable cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) were extracted with 1 M ammonium acetate (1:15 w/v), mechanically shaken (J.P. Selecta Rotabit, Fisher Scientific S.L., Madrid, Spain), and centrifuged at 4000 rpm for 15 min. Acid exchangeable cations were measured as hydrogen ions (H^+), extracted using 1 M KCl (1:10 w/v), shaken for 30 min, centrifuged at 4000 rpm, and titrated with 0.05 M NaOH with phenolphthalein indicator.

Quality assurance and quality control procedures were used routinely: all samples were analyzed in triplicate, control standards were measured between each 10 samples, and blanks were measured in parallel to check for possible contamination. Detection and quantification limits were also analytically determined, using standardized procedures.

2.4. Plant Analysis

At the end of the experiment (43 days after transplanting), three non-destructive quantitative parameters were used to evaluate plant physiological status: (i) the chlorophyll relative index was measured using a sensor CL-01 Chlorophyll Meter (Hansatech, Instruments Ltd., Norfolk, UK), (ii) the normalized difference vegetation index (NDVI), using a PlantPEN NDVI 300, and (iii) the photochemical reflectance index (PRI), using a PlantPen PRI 200 (both from PSI (Photon Systems Instruments), Drásov, Czech Republic). Four measurements were made in the first fully developed leaf of each plant, and the mean values were used.

Each lettuce plant was harvested by cutting the whole plant 0.5–1.0 cm above the soil surface, discarding damaged leaves in contact with the soil. The whole plant was weighed to record fresh biomass, then washed with deionized water to remove soil-attached particles, and oven-dried at 60°C for 48 h. Dried biomass was also registered before grinding

the material using a knife mill (Fritsch pulverisette 15, Fritsch GmbH, Idar-Oberstein, Germany). Total N concentration was quantified using the DUMAS combustion method, which determines total nitrogen by burning the sample at high temperature in an oxygen-rich environment and measuring the nitrogen gas (N_2) that is released, using a nitrogen analyzer (VELP Scientific NDA 702 DUMAS Nitrogen Analyzer-TCD detector, Fisher Scientific S.L., Madrid, Spain), with a methodology adapted from the European Standard EN 13654 [28]. Total concentrations of P, Mg, K, Ca, Na, S, Fe, Cu, Zn, Mn, and B were determined after wet digestion with aqua regia (7.5 mL HCl (conc.) + 2.5 mL HNO_3 (conc.) per 0.5 g of sample), using a methodology adapted from the European Standard EN 13650 [29]. Elements were measured in the acid extract by inductively coupled plasma optical emission spectrometry (ICP-OES) using an iCAP 7000 Series ICP Spectrometer (Thermo Fisher Scientific, S.L., Madrid, Spain). Total concentrations of the elements were expressed as mg/kg dry weight at 105 °C. Reagents used were “trace metal grade”, “Suprapur®” (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) or equivalent, and the water was ultrapure (≥ 18.2 M Ω ·cm resistivity, Type I). All samples were analyzed in triplicate, control standards were measured between each 10 samples, and blanks were measured in parallel to check for possible contamination. Detection and quantification limits were analytically determined using standardized procedures and reported when necessary.

Quality assurance and quality control procedures were used routinely: all samples were analyzed in triplicate, control standards were measured between each 10 samples, and blanks were measured in parallel to check for possible contamination. Detection and quantification limits were also analytically determined, using standardized procedures.

2.5. Statistical Analysis

All data were checked for homogeneity of variance (Bartlett’s Test) and normality (Kolmogorov–Smirnov test). No data transformations were necessary. A formal power analysis was not performed due to the exploratory nature of this study and the limited sample size ($n = 4$). Statistical effects of the treatments on the parameters were evaluated using Factorial ANOVA, finding interactions between treatments (two-way ANOVA, sludge application $\times$ fertilizer treatment (0, $M_{1/2}$, $E_{1/2}$, M, E)), using three levels of significance: $p < 0.05$, 0.01, and 0.001. If significant differences were observed, pairwise comparisons of the means were carried out using the post hoc Tukey HSD test ($p < 0.05$). All statistical analyses were performed with STATISTICA 7.0 (Software™ Inc., Tulsa, OK, USA, 2004).

3. Results and Discussion

3.1. Effects of Sewage Sludge and Fertilizer Application on Soil Properties

Table 3 summarizes the effect of different treatments on key soil properties, including pH, EC, OM, and mineral N content (comprising both ammonium and nitrate extractable fractions). Soil pH remained acidic, ranging from 5.00 ± 0.05 to 5.39 ± 0.40 , with no significant differences among treatments ($p > 0.05$). ANOVA results confirmed that neither the sludge nor the fertilizer has a statistically significant effect on soil pH and that no interactions were found between both variation factors (S $\times$ F). Since soil pH affects nutrient availability, as acidic soils can hinder nutrient uptake due to the immobilization of certain elements, such as P, or enhance the mobilization and toxicity of others, such as manganese (Mn) and aluminum (Al) [24], none of the treatments had an influence on soil reactivity. The doses of struvite used in this study, which have an alkaline pH above 9, did not change the soil pH. This suggests that the amounts applied were too low to affect the soil’s buffering capacity. Because struvite releases nutrients slowly, any increase in pH may take longer to appear and might not be noticeable in a short-term experiment. It is also possible that processes in the lettuce root zone help to keep the soil pH stable [30].

Table 3. Effect of sewage sludge and fertilizer application on soil pH, electrical conductivity, organic matter, and mineral N content ($N_{\min} = NH_4^+ - N + NO_3^- - N$) (mean $\pm$ standard deviation, $n = 4$).

Treatment	pH	EC ($\mu S/cm$)	OM (g/kg)	$NH_4^+ - N$ (mg/kg)	$NO_3^- - N$ (mg/kg)	$N_{\min}$ (mg/kg)
C	5.14 $\pm$ 0.12	542 $\pm$ 63 ab	6.7 $\pm$ 0.2 b	49 $\pm$ 13	79 $\pm$ 6	127 $\pm$ 19
CS	5.19 $\pm$ 0.14	620 $\pm$ 96 ab	7.5 $\pm$ 0.8 ab	37 $\pm$ 18	69 $\pm$ 10	105 $\pm$ 27
$M_{1/2}$	5.20 $\pm$ 0.32	470 $\pm$ 53 ab	6.8 $\pm$ 0.4 b	31 $\pm$ 9	61 $\pm$ 6	92 $\pm$ 14
$M_{1/2}S$	5.00 $\pm$ 0.05	625 $\pm$ 51 ab	8.4 $\pm$ 0.3 a	37 $\pm$ 13	67 $\pm$ 3	104 $\pm$ 14
$E_{1/2}$	5.39 $\pm$ 0.40	443 $\pm$ 112 b	6.6 $\pm$ 0.2 b	29 $\pm$ 16	55 $\pm$ 11	83 $\pm$ 26
$E_{1/2}S$	5.15 $\pm$ 0.06	681 $\pm$ 129 a	8.3 $\pm$ 0.4 a	55 $\pm$ 20	69 $\pm$ 14	124 $\pm$ 32
M	5.20 $\pm$ 0.10	512 $\pm$ 102 ab	6.5 $\pm$ 0.2 b	38 $\pm$ 15	66 $\pm$ 12	103 $\pm$ 27
MS	5.10 $\pm$ 0.04	586 $\pm$ 79 ab	7.8 $\pm$ 0.7 ab	45 $\pm$ 8	61 $\pm$ 11	105 $\pm$ 16
E	5.08 $\pm$ 0.20	513 $\pm$ 122 ab	6.9 $\pm$ 0.7 b	41 $\pm$ 21	66 $\pm$ 16	107 $\pm$ 36
ES	5.22 $\pm$ 0.15	533 $\pm$ 71 ab	8.6 $\pm$ 1.0 a	47 $\pm$ 20	56 $\pm$ 8	103 $\pm$ 28
Two-way ANOVA (F values and significance)						
Sludge (S)	1.26 n.s.	15.14 ***	63.05 ***	1.69 n.s.	0.06 n.s.	0.52 n.s.
Fertilizer (F)	0.87 n.s.	0.43 n.s.	2.18 n.s.	0.51 n.s.	1.85 n.s.	0.57 n.s.
S $\times$ F	1.43 n.s.	1.72 n.s.	1.03 n.s.	1.42 n.s.	2.21 n.s.	1.71 n.s.

C: control soil; $M_{1/2}$ and M: mineral P fertilizer, applied at 50% and 100% the reference P-dose, respectively; $E_{1/2}$ and E: struvite, applied at 50% and 100% the reference P-dose, respectively; CS, $M_{1/2}S$, MS, $E_{1/2}S$, ES: the same treatments with sewage sludge application; EC: electrical conductivity; OM: organic matter; results for the same parameter marked with the same letter are not statistically different (Tukey test $p > 0.05$); *** significant at $p < 0.001$; n.s.: not significant.

On the contrary, SS application significantly affected soil EC, with a p -value < 0.001 . The only significant difference for EC was observed in the soil treated with $E_{1/2}S$, reaching $681 \pm 129 \mu S/cm$ values, significantly higher than the EC in the same treatment but without SS. Despite the increase in soil salinity in some of the treatments, the soil remained with very low salinity (410–800 $\mu S/cm$), according to the Portuguese soil salinity classification tables [24], affecting only highly sensitive crops. Previous studies have also shown that SS application increases EC in soil, primarily due to the addition of soluble salts and nutrients dissolved into the soil solution. Additionally, OM mineralization releases more ions, which also contributes to the observed increase in EC [31,32]. According to the literature, most crops are highly susceptible to salinity, with threshold limits around 2000 $\mu S/cm$. In contrast, lettuce (*Lactuca sativa*) is relatively more sensitive to salinity, with reported tolerance thresholds between approximately 1100 and 2000 $\mu S/cm$ [33]. A study conducted with iceberg lettuce plants indicated an optimum soil-salinity level of about 1840 $\mu S/cm$ for fresh plant yield, above which yield begins to decline [34]. Based on the soil EC values observed in the present study, the salinity levels are below the critical threshold and thus likely do not adversely affect productivity.

Regarding the main objective of applying SS to the soil, the OM content in the amended soil was significantly increased by this byproduct ($p < 0.001$). Despite the high OM content of struvite, a consequence of the presence of biosolids from the urban wastewater treatment process in the unpurified byproduct (Table 1), the dosage of this waste-based fertilizer was not sufficient to produce a significant effect on soil OM content. Despite this, based on the results in Table 3 and according to the Portuguese fertility tables [24], the soil is still classified as poor in OM (≤ 10 g/kg for fine- or medium-textured soils). This was a one-time basal application, which may help in the maintenance of soil OM levels and compensate for losses due to mineralization over time, particularly under intensive cropping systems. However, to significantly increase OM content in the soil to higher and more sustainable levels, it is essential to adopt a long-term soil management strategy that promotes OM accumulation. This includes applying SS at moderate doses but at repeated intervals,

thereby enhancing the gradual buildup of OM and improving overall soil health [35]. Assuming repeated applications of SS at regular intervals (e.g., every two years), a long-term projection (30 years) for soil OM (topsoil, 0–20 cm) could be as follows: (1) between 0 and 10 years, a relatively rapid increase of +0.2–0.3% organic carbon compared to the control, reflecting the labile carbon fraction; (2) between 11 and 20 years, a moderate increase of +0.1–0.2% organic carbon compared to the control, as some of the added carbon stabilizes and mineralization begins to balance the input; and (3) between 21 and 30 years, progressively small increments as the system approaches the equilibrium due to cumulative increments over baseline. These values align with empirical data showing 14–37% OM increases after 20 years under sludge doses [36]. Larger gains may occur under favorable conditions with higher application rates, but in most moderate-dose scenarios it is expected that the accumulation is gradual.

Concerning mineral N levels, no statistically significant differences were observed among the treatments ($p > 0.05$). Plants primarily absorb and assimilate N from the soil in inorganic forms, mainly nitrate (NO_3^-) and ammonium (NH_4^+) ions, as well as potentially available amino acids, which tend to be more abundant in soils amended with organic materials, like SS. In this study, the contribution of NH_4^+ to mineral N was lower than the contribution from NO_3^- , for all cases (Table 3). Despite the predominance of NH_4^+ in acidic soils [37], several factors may explain its lower concentrations when compared to NO_3^- , namely (i) rapid nitrification, (ii) plant uptake preferences, or (iii) microbial immobilization processes, which reduce NH_4^+ availability shortly after soil application [38]. However, as evidenced by plant foliar analysis, which will be discussed later, there was no deficiency of N for the plants.

Figure 1 displays the influence of each treatment on the extractability of macro- and micronutrients in the soil at the end of the experiment. Regarding the results for P extractability from soil, the already high baseline P levels make it challenging to clearly demonstrate the effectiveness of struvite. However, the statistical analysis indicates that the extractable P in the treatment with the highest struvite dose (E) was significantly different from the control ($p < 0.05$), with or without even SS application. Despite the presence of a crop during the experimental period, the higher levels of extractable P in fertilized soils with the highest dose of struvite (E) suggest that struvite continued to release P throughout the experiments, consistent with its slow-release nature. However, due to the limitations of the Egner–Riehm extraction method stated before, it is important to carefully interpret these results due to the possible low accuracy of this method in estimating extractable P in these soil conditions.

Additionally, the factors “sludge” and “fertilizer” had a significant effect on P extractability from soil (Table 4; $p < 0.001$), without an interaction between both factors. Indeed, although SS was not primarily applied as a nutrient source but rather as an organic soil amendment, the levels of extractable P exceeded 100 mg P/kg soil (equivalent to more than 250 mg P_2O_5 /kg). These values are considered high and may indicate that the soil has substantial P reserves. However, it is important to emphasize that the P extracted using the Egner–Riehm method, which was used, does not directly reflect the fraction that is immediately available to plants. A part of this “analytically solubilized” P may exist in forms that are available over time, due to chemical interactions with soil, such as P compounds that are fixed with Ca, Fe, or Al through the slow mineralization of organic P present in the SS. Therefore, although the high extractable P levels suggest a significant contribution from SS, actual plant uptake will depend on several factors, including soil chemistry, microbial activity, and climatic conditions that influence P solubility and mobility [27,39]. Nonetheless, this residual P pool in soils amended with SS and the waste-based fertilizer can be

advantageous as a long-term, slow-release source of P, potentially reducing the need for frequent fertilizer applications in subsequent cropping cycles and leaching phenomena.

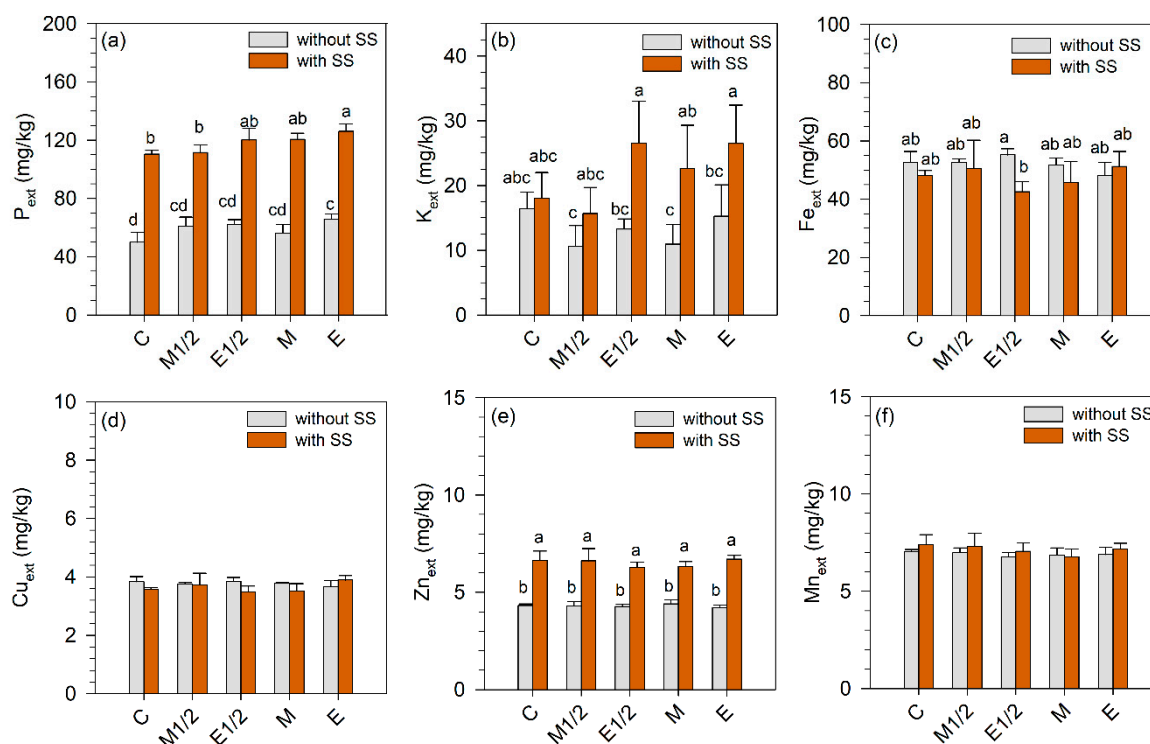


Figure 1. Effect of sludge and fertilizer application on extractable soil macronutrients: (a) P and (b) K (Egner–Rhiem procedure); and extractable micronutrient content: (c) Fe; (d) Cu; (e) Zn; and (f) Mn (Lakanen procedure) at the end of the experiment (mean $\pm$ standard deviation, $n = 4$). Bars marked with different letters represent statistically different results (Tukey test $p < 0.05$). C: control soil; $M_{1/2}$ and M: mineral P fertilizer, applied at 50% and 100% of the reference P-dose, respectively; $E_{1/2}$ and E: struvite, applied at 50% and 100% of the reference P-dose, respectively.

Table 4. Summary of the factorial ANOVA analysis for the effects of the factors “sludge” and “fertilizer”, as well as their interactions, on extractable soil macronutrients: (a) P and (b) K (Egner–Rhiem procedure); and extractable micronutrient content: (c) Fe; (d) Cu; (e) Zn; and (f) Mn (Lakanen procedure), at the end of the experiment ($n = 4$); *, **, *** Significant at $p < 0.05$, 0.01, and 0.001, respectively; n.s.: not significant.

Origin of the variation	F Values and Significance					
	P_{ext}	K_{ext}	Fe_{ext}	Cu_{ext}	Zn_{ext}	Mn_{ext}
Main factors						
Sludge (S)	1204.04 ***	35.82 ***	8.27 **	4.60 *	515.89 ***	3.61 n.s.
Fertilizer (F)	9.48 ***	3.58 *	0.48 n.s.	0.56 n.s.	0.61 n.s.	1.46 n.s.
Interactions						
$S \times F$	1.86 n.s.	2.42 n.s.	2.88 *	2.85 *	1.25 n.s.	0.50 n.s.

Regarding the extractable K after the experiments, it is not possible to verify a consistent path, compared to what happened in the results for P extractability. Indeed, in some cases, the results with and without sludge are statistically equal ($p > 0.05$), for example, in the control soils. The statistical analysis summary presented in Table 4 indicates that the F values for the “sludge” factor are considerably higher for P extractability than for K extractability, despite both showing significant levels at $p < 0.001$. However, in both cases, the interaction effect ($F \times S$) is not statistically significant ($p > 0.05$), which means

that the effect of the waste-based fertilizer (struvite) and the mineral fertilizer is consistent regardless of whether sludge is applied and vice versa, and the combination of both factors does not produce a synergistic or antagonistic effect; the main effects can be separately interpreted. It is important to highlight that the soil used has low levels of extractable K. Potassium is a vital nutrient involved in the production of sugars, starches, and other carbohydrates, as well as in protein synthesis and cell division. A deficiency in K can reduce photosynthetic efficiency, disrupt osmotic balance, and compromise tolerance to abiotic stresses such as drought or salinity. Furthermore, insufficient K can weaken the plant's defense mechanisms, increasing susceptibility to certain pathogens [40]. This underscores the importance of monitoring K status and considering supplemental K treatments in future studies to ensure adequate nutrient supply and optimal plant health.

The same is not true in the case of Fe and Cu extractability from the soil after the experiments, where the interaction effect ($F \times S$) is statistically significant ($p < 0.05$). In both cases, the way that struvite and the mineral fertilizer supplied these micronutrients to the soil was similar and did not significantly differ from the levels observed in the unfertilized control soil. This indicates that neither the struvite nor the mineral fertilizer had a notable impact on Fe and Cu availability in the soil. The extractable levels of these micronutrients remained comparable, regardless of the P fertilization source, suggesting that these external inputs did not enhance their availability after the treatment. On the contrary, the SS application had a statistically significant difference in these micronutrients' availability, particularly for Zn. The extractable Zn levels in soil amended with SS were statistically higher than those in soils treated only with struvite and mineral fertilizer and without fertilization. This suggests that SS-amended soil may provide additional Zn reserves, which are essential for chloroplast function and can positively impact the chlorophyll index in the plants.

Table 5 summarizes the influence of each treatment on soil non-acid exchangeable cations and on cation exchange capacity (CEC). Nutrient availability in soil is strongly influenced by the exchange of cations and anions on the surface of clay minerals, OM, inorganic materials, and plant roots EC [32]. These adsorbed ions can be swapped with others in the soil solution in a reversible way, and CEC refers to the soil's ability to hold and exchange positively charged ions (in this case, K^+ , Ca^{2+} , Mg^{2+} , Na^+ , and H^+), helping to store and supply nutrients to plants [41]. Although CEC significantly increased with SS application to soil ($p < 0.001$), its values remained low (<5 cmol(+)/kg). This suggests a limited capacity to retain cations, which indicates that the nutrients added via SS or struvite are more prone to leaching, reducing nutrient use efficiency and long-term availability of the P applied. This highlights the risk of losses to groundwater or surface runoff, increasing sustainability and environmental concern. Management strategies such as repeated applications, combining with organic amendments that improve CEC, or monitoring nutrient leaching could help optimize P use and minimize environmental impacts.

Table 5. Effect of sludge and fertilizer application on soil exchangeable cations (mean $\pm$ standard deviation, $n = 4$).

Treat.	Non-Acid Exchangeable Cations (cmol(+)/kg)				Exchangeable Acidity (cmol(+)/kg)	CEC (cmol(+)/kg)
	K^+	Ca^{2+}	Mg^{2+}	Na^+	H^+	
C	0.042 ± 0.007 abc	1.70 ± 0.06 c	0.50 ± 0.03 c	0.036 ± 0.006 b	0.113 ± 0.010 d	2.40 ± 0.06 b
CS	0.046 ± 0.010 abc	2.29 ± 0.17 a	0.61 ± 0.05 abc	0.067 ± 0.019 a	0.153 ± 0.027 abcd	3.17 ± 0.21 a
$M_{1/2}$	0.027 ± 0.008 c	1.81 ± 0.14 bc	0.51 ± 0.06 bc	0.027 ± 0.004 b	0.131 ± 0.006 cd	2.50 ± 0.19 b

Table 5. Cont.

Treat.	Non-Acid Exchangeable Cations (cmol(+)/kg)				Exchangeable Acidity (cmol(+)/kg)	CEC (cmol(+)/kg)
	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	H ⁺	
M _{1/2} S	0.040 ± 0.010 abc	2.57 ± 0.29 a	0.64 ± 0.06 ab	0.069 ± 0.005 a	0.152 ± 0.016 abcd	3.47 ± 0.28 a
E _{1/2}	0.034 ± 0.004 bc	1.70 ± 0.10 c	0.48 ± 0.05 c	0.024 ± 0.006 b	0.136 ± 0.034 bcd	2.38 ± 0.13 b
E _{1/2} S	0.068 ± 0.016 a	2.28 ± 0.12 a	0.69 ± 0.07 a	0.082 ± 0.021 a	0.191 ± 0.021 a	3.30 ± 0.21 a
M	0.028 ± 0.008 c	1.77 ± 0.15 bc	0.49 ± 0.05 c	0.027 ± 0.005 b	0.116 ± 0.022 d	2.43 ± 0.21 b
MS	0.058 ± 0.017 ab	2.37 ± 0.30 a	0.59 ± 0.08 abc	0.073 ± 0.011 a	0.162 ± 0.002 abc	3.26 ± 0.20 a
E	0.039 ± 0.012 bc	1.75 ± 0.10 c	0.59 ± 0.05 abc	0.027 ± 0.005 b	0.141 ± 0.015 bcd	2.54 ± 0.16 b
ES	0.068 ± 0.015 a	2.16 ± 0.09 ab	0.67 ± 0.06 a	0.068 ± 0.006 a	0.180 ± 0.016 ab	3.15 ± 0.15 a
Two-way ANOVA (F values and significance)						
S	35.82 ***	119.75 ***	50.69 ***	170.80 ***	44.07 ***	190.18 ***
F	3.58 *	2.38 n.s.	2.89 *	0.41 n.s.	4.08 **	1.27 n.s.
S × F	2.42 n.s.	1.04 n.s.	1.46 n.s.	1.71 n.s.	0.81 n.s.	1.18 n.s.

Treat.: treatment; S: sludge; F: fertilizer; C: control soil; M_{1/2} and M: mineral P fertilizer, applied at 50% and 100% the reference P-dose, respectively; E_{1/2} and E: struvite, applied at 50% and 100% the reference P-dose, respectively; CS, M_{1/2}S, MS, E_{1/2}S, ES: the same treatments with sewage sludge application; CEC: cation exchange capacity; Results for the same parameter marked with the same letter are not statistically different (Tukey test $p > 0.05$); *, **, *** significant at $p < 0.05$, 0.01, and 0.001, respectively; n.s.: not significant.

3.2. Effect of Sludge and Fertilizer Application on Lettuce Growth and Physiological Parameters

Figure 2 illustrates the effect of the different treatments on plant growth and physiological status, including dry mass, chlorophyll relative index, NDVI (normalized difference vegetation index), and PRI (photochemical reflectance index).

The highest dry biomass production was observed in the soil amended with the highest dosage of struvite without sludge (treatment E), reaching about 8.9 ± 1.1 g dry weight. This result was statistically higher than that of the control treatment without sludge (C) but was not significantly different from the biomass yields observed in soils receiving lower struvite dosage (E_{1/2} and E_{1/2}S) or mineral fertilizer (M_{1/2}, M_{1/2}S, M, and MS). The treatments with lower struvite dosage and mineral fertilizers are statistically equal in terms of biomass production. This suggests that, while increasing struvite applications can enhance biomass production, lower doses and conventional fertilization products may offer comparable outcomes under the tested conditions and soil. A literature study indicates that soil treated with 100 and 200 mg P₂O₅/ha of struvite produced greater biomass of *Festuca arundinacea* compared to superphosphate (mineral control) during the second cutting phase of the plant. However, the overall finding suggests that even a lower dosage of 50 mg P₂O₅/ha can support good plant development and maintain adequate nutritional status [42].

According to the data in Table 6, when comparing the individual effects of each factor (fertilizer or sludge) on dry biomass production, it can be concluded that the factors did not independently show a statistically significant effect on this parameter. However, the interaction between these two factors was significant, indicating that the response of plant growth to one factor depends on the presence or absence of the other. This interaction suggests that plant biomass and physiological responses are interdependent when both fertilizers and sludge are present, possibly due to a complementary nutrient supply of both, as discussed earlier. Therefore, integrated fertilization strategies that combine organic and mineral inputs may be more effective than relying solely on either type of input. Indeed, a previous literature study evaluated the use of struvite, SS, and their combination for barley production, with application rates calculated based on recommended P levels for the crop (84 mg P₂O₅/ha). The highest fresh weights of barley were observed in soils amended with the combined residues, while soils treated with either struvite or SS alone also showed statistically higher biomass production compared to the control [43].

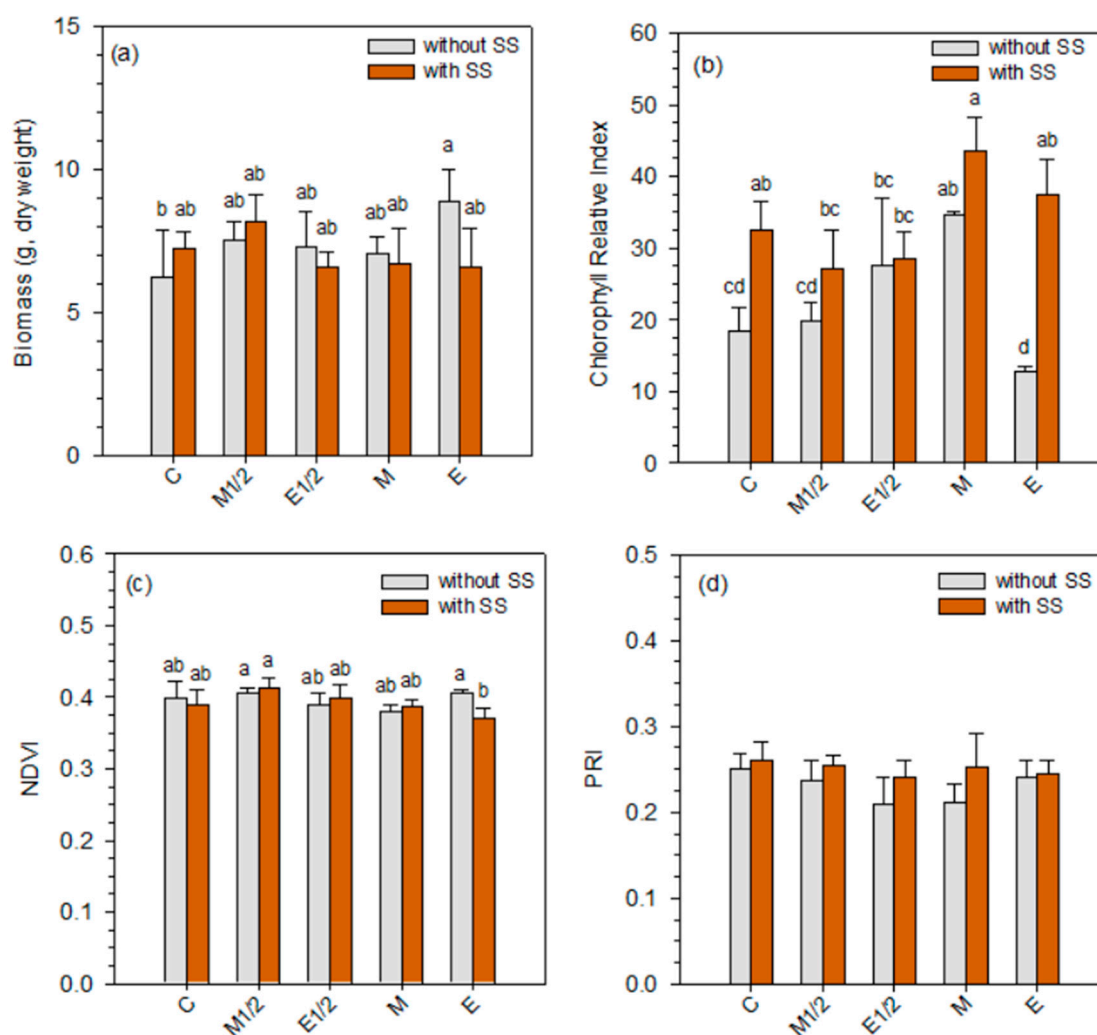


Figure 2. Effect of sludge and fertilizer application on (a) plant dry biomass, (b) chlorophyll relative index, (c) NDVI (normalized difference vegetation index), and (d) PRI at the end of the experiment (mean $\pm$ standard deviation, $n = 4$). Results for the same parameter marked with the same letter are not statistically different (Tukey test $p > 0.05$). C: control soil; M_{1/2} and M: mineral P fertilizer, applied at 50% and 100% the reference P-dose, respectively; E_{1/2} and E: struvite, applied at 50% and 100% the reference P-dose, respectively; CS, M_{1/2}S, MS, E_{1/2}S, and ES: the same treatments with sewage sludge application.

Table 6. Summary of the factorial ANOVA analysis for the effects of the factors “sludge” and “fertilizer”, as well as their interactions, on the dry biomass and physiological parameters (chlorophyll relative index, NDVI, and PRI) of lettuce at the end of the experiment ($n = 4$); *, *** significant at $p < 0.05$, and 0.001, respectively; n.s.: not significant.

F Values and Significance				
Origin of the Variation	Dry Biomass	Chlorophyll Relative Index	NDVI	PRI
Main factors				
Sludge (S)	1.32 n.s.	59.49 ***	1.11 n.s.	7.89 *
Fertilizer (F)	2.34 n.s.	14.92 ***	3.34 *	2.09 n.s.
Interactions				
S $\times$ F	3.48 *	7.42 ***	3.31 *	0.88 n.s.

Although it was expected that higher biomass production would correlate with higher chlorophyll relative index, this was not observed in the present case for the treatment with struvite. As shown in Table 6, both sludge and fertilizer factors, independently, influenced the chlorophyll index ($p < 0.001$). However, there is also a very strong interaction between both factors, and the apparent beneficial effect of sludge application on chlorophyll index is, in fact, influenced by the type and dose of fertilizer, restraining those conclusions. Chlorophyll levels are jointly controlled by climate and soil factors, such as nutritional soil state, namely N content, temperature, and water supply [44]. The observed reduction in chlorophyll relative index in lettuce grown with the highest struvite dosage (E) does not appear to be related to an imbalance in N content. However, rapid biomass accumulation can outpace N uptake, probably leading to a decrease in chlorophyll concentration per unit of leaf area, which is commonly called the “N dilution effect” [45]. On the other hand, this reduction in chlorophyll index, under these treatment conditions, may also point to other factors, such as irregularities in water supply or light and temperature exposure, which could have adversely affected chlorophyll synthesis in these specific plants. Indeed, the NDVI index is also a tool to predict the status of the N in the crops [46], and no such expressive results were verified in this case for treatment E .

PRI is a normalized reflectance index that indicates how plants manage excess light energy during photosynthesis. This index helps to detect early signs of plant stress and tends to decrease, sometimes becoming negative or near zero, under nutrient deficiency or other environmental stress [47]. In this study, PRI values across all treatments remained positive, around 0.2, suggesting that the plants were not experiencing significant stress. These values indicate that the plants were healthy and actively photosynthesizing. Furthermore, the consistently high values observed for both NDVI and PRI reinforce that vegetation across all treatments was healthy, with efficient photosynthesis and no notable signs of stress.

3.3. Effects of Sludge and Fertilizer Application on Lettuce Nutritional Status

Table 7 presents the macro- and micronutrient contents in lettuce leaves to assess the nutritional plant's status following the application of different treatments. According to the Portuguese fertilization tables [24], it is expected that a mature lettuce leaf contains macronutrients in the following concentrations: P—0.4–0.8%; N—3.5–5.0%; K—5.5–9.0%; Ca—1.5–3.5%; Mg—0.15–0.8%; and S—0.18–0.29%.

Considering the P content in the leaves, the minimum threshold for adequate P nutrition in leaves was only achieved in the treatment involving SS amendment. Indeed, the statistical analysis confirms that SS application has a highly significant effect on leaf P concentration ($p < 0.001$), whereas the fertilizer factor and the interaction between sludge and fertilizer were not statistically significant. This aligns with the results of the available P in the soil, previously discussed.

Treatments such as $M_{1/2}S$, $E_{1/2}S$, and ES showed significantly higher P leaf content compared to their non-sludge-amended counterparts. It is important to highlight that only the lettuce grown in soils with SS showed P content in the leaves between 0.4 and 0.52%, indicating that SS effectively enhances P availability and uptake. Furthermore, the percentages of P exported by the plant—calculated based on the amount of P applied through fertilization, excluding previous soil contributions—were more than 50% higher in sludge-amended soils compared to non-sludge treatments. When a reduced dose of mineral fertilizer was combined with SS, the percentage of P exported by the plant was statistically equivalent to that observed with the highest mineral fertilizer dose, also combined with SS. This suggests that the application of SS can substantially reduce the need for mineral P fertilization while maintaining optimal plant growth and P uptake. A two-year field experiment with rice (summer) and wheat (winter) was conducted using struvite-amended

soils at different P application rates (70 or 35 mg P₂O₅/ha for rice and 96 or 48 mg P₂O₅/ha for wheat). The results demonstrated that struvite substitution significantly enhanced plant P uptake, raising P use efficiency from less than 10% in conventional P fertilizers to around 23% with the highest struvite application rate in both crops [48].

Table 7. Effect of each treatment on plant's macro- and micronutrients (mean $\pm$ standard deviation, n = 4).

Macronutrients (g/kg _{db})						
Treatment	P	N	K	Ca	Mg	S
C	3.16 $\pm$ 0.28 b	70.3 $\pm$ 8.9	24.8 $\pm$ 5.0	13.6 $\pm$ 2.6 b	5.5 $\pm$ 0.5 b	3.7 $\pm$ 0.7 bc
CS	4.03 $\pm$ 0.71 ab	67.3 $\pm$ 3.4	18.7 $\pm$ 3.9	13.8 $\pm$ 3.4 b	5.4 $\pm$ 1.0 b	4.0 $\pm$ 0.9 bc
M _{1/2}	3.53 $\pm$ 0.52 b	63.6 $\pm$ 4.7	24.0 $\pm$ 4.1	13.8 $\pm$ 1.6 b	5.7 $\pm$ 0.4 b	3.8 $\pm$ 0.6 bc
M _{1/2} S	5.23 $\pm$ 0.68 a	65.8 $\pm$ 3.5	23.0 $\pm$ 2.8	16.9 $\pm$ 1.5 ab	6.6 $\pm$ 0.5 ab	4.8 $\pm$ 0.4 abc
E _{1/2}	3.40 $\pm$ 0.47 b	65.1 $\pm$ 6.9	20.2 $\pm$ 4.9	12.5 $\pm$ 1.3 b	5.6 $\pm$ 0.4 b	3.5 $\pm$ 0.5 bc
E _{1/2} S	4.98 $\pm$ 0.46 a	72.1 $\pm$ 5.3	24.0 $\pm$ 1.3	18.0 $\pm$ 2.4 ab	6.7 $\pm$ 0.9 ab	5.2 $\pm$ 0.5 ab
M	3.92 $\pm$ 0.07 ab	68.2 $\pm$ 4.9	24.3 $\pm$ 0.6	15.3 $\pm$ 1.1 ab	6.1 $\pm$ 0.2 b	4.2 $\pm$ 0.3 abc
MS	4.56 $\pm$ 0.92 ab	73.1 $\pm$ 3.2	21.7 $\pm$ 5.1	15.6 $\pm$ 2.9 ab	6.0 $\pm$ 0.8 b	4.9 $\pm$ 0.7 abc
E	3.47 $\pm$ 0.75 b	67.9 $\pm$ 9.3	16.5 $\pm$ 6.4	13.4 $\pm$ 3.4 b	5.7 $\pm$ 1.1 b	3.3 $\pm$ 1.2 c
ES	5.10 $\pm$ 0.44 a	70.3 $\pm$ 8.9	23.1 $\pm$ 3.1	20.5 $\pm$ 2.2 a	7.9 $\pm$ 0.9 a	5.8 $\pm$ 0.9 a
Two-way ANOVA (F values and significance)						
Sludge (S)	49.06 ***	1.235 n.s.	0.012 n.s.	18.23 ***	12.73 **	30.88 ***
Fertilizer (F)	2.29 n.s.	1.218 n.s.	1.000 n.s.	1.89 n.s.	3.51 *	1.25 n.s.
S $\times$ F	1.44 n.s.	1.073 n.s.	3.110 *	3.34 *	3.60 *	3.17 *
Micronutrients (mg/kg _{db})						
Treatment	Fe	Cu	Zn	Mn	B	Mo
C	265 $\pm$ 86	12.7 $\pm$ 1.6	217 $\pm$ 34	264 $\pm$ 39	44.3 $\pm$ 4.8 ab	0.34 $\pm$ 0.22
CS	193 $\pm$ 63	11.8 $\pm$ 2.0	202 $\pm$ 43	240 $\pm$ 57	38.3 $\pm$ 8.5 ab	<0.25
M _{1/2}	258 $\pm$ 96	13.5 $\pm$ 2.2	231 $\pm$ 43	289 $\pm$ 35	47.0 $\pm$ 5.1 ab	<0.25
M _{1/2} S	201 $\pm$ 21	14.3 $\pm$ 1.1	251 $\pm$ 45	321 $\pm$ 19	50.6 $\pm$ 3.6 a	<0.25
E _{1/2}	202 $\pm$ 47	12.0 $\pm$ 1.1	207 $\pm$ 37	274 $\pm$ 32	40.7 $\pm$ 4.5 ab	<0.25
E _{1/2} S	284 $\pm$ 78	14.4 $\pm$ 1.0	222 $\pm$ 44	289 $\pm$ 50	47.2 $\pm$ 6.2 ab	<0.25
M	289 $\pm$ 89	14.5 $\pm$ 1.8	233 $\pm$ 6	300 $\pm$ 7	45.9 $\pm$ 2.4 ab	0.35 $\pm$ 0.13
MS	143 $\pm$ 28	13.4 $\pm$ 2.2	209 $\pm$ 53	297 $\pm$ 34	47.0 $\pm$ 0.9 ab	0.29 $\pm$ 0.09
E	148 $\pm$ 49	8.9 $\pm$ 2.0	139 $\pm$ 55	183 $\pm$ 59	32.9 $\pm$ 8.0 b	<0.25
ES	242 $\pm$ 66	12.7 $\pm$ 1.1	156 $\pm$ 28	256 $\pm$ 41	44.9 $\pm$ 4.1 ab	<0.25
Two-way ANOVA (F values and significance)						
Sludge (S)	0.626 n.s.	0.847 n.s.	0.104 n.s.	0.380 n.s.	1.537 n.s.	n.a.
Fertilizer (F)	0.085 n.s.	1.986 n.s.	4.429 **	3.527 *	2.771 *	n.a.
S $\times$ F	1.678 n.s.	1.600 n.s.	0.655 n.s.	1.375 n.s.	2.517 n.s.	n.a.

C: control soil; M_{1/2} and M: mineral P fertilizer, applied at 50% and 100% the reference P-dose, respectively; E_{1/2} and E: struvite, applied at 50% and 100% the reference P-dose, respectively; CS, M_{1/2}S, MS, E_{1/2}S, and ES: the same treatments with sewage sludge application; db: dry basis; results for the same parameter marked with the same letter are not statistically different (Tukey test $p > 0.05$); *, **, *** significant at $p < 0.05$, 0.01, and 0.001, respectively; n.s.: not significant; n.a.: not applicable.

As expected, N content in the leaves was adequate, as N fertilization was applied to prevent any negative effect related to N deficiency. This ensured that the evaluation of struvite and SS focused specifically on their role in P and OM supply, since the objective was to assess their potential as substitutes for mineral P fertilizers.

Concerning the macronutrient content of Ca, Mg, and S in the leaves, significant increases were observed in plants grown in the sludge-amended soils. Indeed, the ES treatment (struvite combined with SS) produced plants with the highest concentration of these macronutrients in the leaves. This suggests a potential synergistic effect between

struvite and SS, enhancing the uptake of K, Ca, Mg, and S by plants ($p < 0.05$). A literature study evaluating struvite and SS applied individually and in combination in a barley crop showed that all treatments, whether applied separately or together, resulted in statistically similar plant P content and uptake, both significantly higher than the control. However, Mg concentration in the plants was higher in the struvite-amended soil [43].

Regarding micronutrient content, the values of Fe, Cu, and Mo did not show a consistent trend, and statistical analysis indicated that no significant treatment effect was achieved with fertilization or SS amendment. Despite the Tukey test indicating no statistical significance between all treatments ($p > 0.05$), the two-way ANOVA analysis showed that fertilizer had a significant effect on Zn and Mn content, indicating that both mineral fertilizer and struvite may influence nutrient availability. This discrepancy can be related to the fact that ANOVA can detect a general difference across groups even if no single pair is different enough to pass Tukey's stricter threshold; the overall effect might be statistically significant but not strong enough for individual pairwise differences to stand out.

4. Conclusions

This study highlights the agronomic potential of struvite and SS from WWTPs as sustainable alternatives to conventional P fertilizers, enhancing soil fertility and plant nutritional status without affecting soil chemical properties.

Despite the alkaline nature of struvite (precipitated under non-controlled conditions), the soil pH remained similar across the treatments, which was also not affected by SS application. In contrast, SS application led to an increase in soil EC and OM, although OM levels remained below the desired increase, suggesting that long-term repeated application would be necessary to enhance soil organic carbon.

Struvite application significantly increased extractable P in the soil, particularly at higher doses, reflecting its slow-release behavior, whereas SS contributed to the soil P pool, supporting long-term nutrient availability. Extractable K, Fe, Cu, and Zn varied across treatments, with SS significantly contributing to the increased Zn availability.

Regarding plant growth parameters, the highest dry biomass was observed in soil amended with struvite at the highest dose. However, lower struvite doses and mineral fertilizer treatments produced comparable biomass yields under the tested conditions, suggesting that moderate application of this waste-derived material may be sufficient to maintain productivity. The combined application of SS and struvite improved plant physiological status, as evidenced by positive chlorophyll indices, NDVI, and PRI values, indicating healthy plants across treatments, without nutritional deficiencies. Leaf nutrient analysis confirmed adequate N levels across all treatments, whereas SS application enhanced P, Ca, Mg, S, and Zn concentrations in plant tissues.

A complementary effect between SS and struvite was observed for several soil and plant parameters in this study. Based on these results, specifically on macronutrient content, it can be concluded that struvite combined with SS (treatments $E_{1/2}S$ and ES) provided the most balanced and nutrient-rich profiles, supporting healthy plant growth.

Overall, integrating struvite and SS into fertilization programs offers a sustainable strategy for recycling waste-derived nutrients and reducing dependency on non-renewable mineral P. As future research, soils with low P availability should be considered to better evaluate the agronomic efficiency of P sources under limiting conditions. Additionally, an environmental assessment to evaluate the leaching potential is recommended to clarify the environmental sustainability of these fertilization strategies.

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