

Sustainable Fertilization with Iron-Enriched Montmorillonite and Sanitary Sludge Enhances Germination and Growth of *Zea mays* L.

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Abstract

1) Background: Sustainable fertilization strategies are increasingly necessary to reduce the dependence on synthetic inputs, recycle waste resources, and strengthen agricultural resilience in the context of climate change; **2) Methods:** Germination and early development of *Zea mays* (corn) under different treatments: (i) untreated soil (Control); (ii) soil amended with sludge from the Cardeal Wastewater Treatment Plant (SC); (iii) soil amended with sludge modified with Fe-montmorillonite (TechPhos, ST); and (iv) soil amended with a commercial phosphorus salt (PS) were studied, and characterization of soils was performed by XRF, XRD, and FTIR; **3) Results:** physicochemical enhancements corresponded with superior plant performance: ST yielded the highest germination index (171.7), increased shoot and root biomass, greater leaf development, and elevated chlorophyll content compared to both Control and SC treatments. Overall, ST matched or outperformed the commercial fertilizer (PS), highlighting its promise as an effective phosphorus resource; **4) Conclusion:** integration of nanostructured modified montmorillonite with wastewater-derived sludge can enhance the early development of corn while also reducing reliance on conventional fertilizers. Beyond its agronomic advantages, this approach demonstrates how waste valorization supports circular economy principles and enhances soil fertility, underscoring its relevance for future strategies targeting climate change mitigation, food security, and sustainable resource management. Sustainable fertilization strategies are required to reduce dependence on synthetic inputs, enhance waste recycling, and improve agricultural resilience under climate change. This study evaluates the effects of wastewater-derived sludge, particularly when modified with Fe-montmorillonite, on phosphorus availability and early development of *Zea mays*. Methods: Germination and early growth of *Zea mays* were assessed under four treatments: (i) untreated soil (Control); (ii) soil amended with sludge from the Cardeal Wastewater Treatment Plant (SC); (iii) soil amended with Fe-montmorillonite-modified sludge (TechPhos, ST); and (iv) soil amended with a commercial phosphorus salt (PS). Soil characterization was conducted using XRF, XRD, and FTIR. Plant responses were evaluated through laboratory (5 days) and pot (22 days) experiments. Results: ST showed the highest performance, with a germination index of 171.7 and improved biomass, leaf development, and chlorophyll content compared to Control and SC. ST also performed similarly to or better than the commercial fertilizer (PS), indicating high phosphorus efficiency. Conclusions: The

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integration of nanostructured modified montmorillonite with wastewater-derived sludge represents a promising alternative phosphorus source for early maize development. Its application supports waste valorization and circular economy approaches while contributing to improved soil fertility and more sustainable nutrient management under climate change scenarios.

Keywords: Urban wastewater; Bio-based fertilizer; Corn; Germination assays; Outdoor experiments; Nanotechnology; Resource efficiency; Circular economy

1. Introduction

Corn (*Zea mays* L.) is one of the most relevant crops globally in production and yield per hectare, with 1,210 million tons and 5,878 kg ha⁻¹, respectively (FAO, 2023) [1], and has diversified uses as food, feed, and raw material for industries. In recent years, attempts have been made to improve corn yields to face increasing food demands. These efforts include advances in genetics [2-5], crop management [6], precision agriculture and technology [7-9], diversifying agronomic practices, and developing transgenic and hybrid corn varieties with higher yield potential and climate stress tolerance [10,11], all aimed at increasing productivity and ensuring food security.

Phosphorus (P) is an essential nutrient for plant growth, playing a central role in energy transfer, photosynthesis, and cellular development [12]. In major crops such as *Zea mays* L., phosphorus availability is a key limiting factor for early-stage development, directly influencing root establishment, biomass accumulation, and overall productivity [13]. However, despite its agronomic importance, phosphorus management faces a critical global challenge, as phosphate rock (the primary source of P fertilizers) is a finite and non-renewable resource [14-15], raising increasing concerns about its depletion and uneven geopolitical distribution [16-18].

Phosphorus (P) plays a crucial role in corn development, significantly impacting yield and nutrient uptake. Its application is essential throughout growth stages, enhancing productivity [12]. Recent research has demonstrated that P fertilization has a greater effect on corn yield than potassium, and global P has prompted the adoption of sustainable practices like regenerative agriculture [13]. However, the P that exists on planet earth is a finite resource [14]. Phosphate rock remains classified as a critical raw material [15], not only because it is a finite resource, with evolving consumption rates and demands but also due to the political dependency between countries. Due to population growth, the agricultural demand for crop nutrients will increase, but current P use remains beyond the planetary boundaries for sustainability [16]. In many regions, soils are deficient in plant available P, leading to poor crop performance and food insecurity [17], and forage corn also depends heavily on P availability [18].

A common strategy to address phosphorus deficiency in crops involves the application of phosphorus salts—such as monobasic potassium phosphate (KH₂PO₄)—as fertilizers [19, 20]. However, this practice contributes to eutrophication in both marine and freshwater ecosystems [21]. In recent years, it has become increasingly clear that agricultural systems must adopt innovative and sustainable approaches that enhance productivity while reducing environmental impacts. Sustainable fertilization strategies are increasingly necessary to reduce dependence on synthetic inputs, recycle waste materials, and strengthen agricultural resilience amid climate change. These issues have intensified the

need for sustainable strategies to improve phosphorus use efficiency and promote its recovery and reuse within a circular economy framework.

In this context, wastewater treatment plants (WWTPs) represent an important secondary source of phosphorus, as significant amounts of this nutrient are accumulated in sewage sludge. The agricultural reuse of sludge has been widely investigated due to its high organic matter content and nutrient availability, particularly nitrogen and phosphorus [22]. The management of urban wastewater presents a major environmental challenge, with global generation exceeding 45 million tons annually [23]. This raises critical concerns regarding its sustainable disposal and potential reuse, particularly in developing countries where infrastructure and resources are often limited [24]. In this context, phosphorus recovery from urban wastewater offers a sustainable alternative to synthetic inputs, delivering both environmental and economic benefits [25, 26]. This approach supports the production of biobased fertilizers that can be safely and effectively applied to soils, enhancing the sustainable resource use in agricultural systems [27]. However, the direct application of sludge is still limited by factors such as variability in composition, potential contamination, and, importantly, the low bioavailability of phosphorus depending on its chemical speciation.

~~Urban wastewater, which contains high levels of P, represents a valuable and underutilized resource for agricultural systems, while reducing the requirements to continually add P input [22]. The management of urban wastewater presents a major environmental challenge, with global generation exceeding 45 million tons annually [23]. This raises critical concerns regarding its sustainable disposal and potential reuse, particularly in developing countries where infrastructure and resources are often limited [24]. In this context, phosphorus recovery from urban wastewater offers a sustainable alternative to synthetic inputs, delivering both environmental and economic benefits [25, 26]. This approach supports the production of biobased fertilizers that can be safely and effectively applied to soils, enhancing the sustainable resource use in agricultural systems [27].~~

The transition toward more sustainable practices—such as nutrient recycling from wastewater—is being encouraged by governmental policies aimed at reducing both the use and environmental accumulation of mineral phosphate fertilizers. Sewage sludge from wastewater treatment plants (WWTPs) has been applied in agriculture as a fertilizer in several EU countries, including Spain; however, the extent and drivers of this practice—such as its association with low soil organic matter—should be supported by appropriate references to avoid overgeneralization [28]. Nevertheless, the development of efficient, safe, and contaminant-free fertilizers derived from biowaste continues to pose significant challenges [29]. Several approaches have been proposed to recover and reuse phosphorus from wastewater, including chemical precipitation (e.g., struvite formation), adsorption processes, and thermochemical treatments [30]. While these strategies have shown promising results, they often present limitations related to operational costs, scalability, or limited control over nutrient release in soil. Adsorption-based systems have gained increasing attention due to their potential to capture phosphorus efficiently and enable its subsequent reuse. Among the materials investigated for phosphorus adsorption, clay minerals—especially montmorillonite—stand out due to their high surface area, cation exchange capacity, and structural versatility. Furthermore, the modification of these materials with iron species has been shown to significantly enhance their affinity for phosphate ions, due to the strong interactions between iron phases and phosphorus. These properties make Fe-modified clays promising candidates for integrating phosphorus recovery and reuse.

Sewage sludge from wastewater treatment plants (WWTPs) has been widely used for years in agriculture as fertilizer in EU countries, especially where soils generally have low organic matter content [28]. The transition toward more sustainable practices—such as nutrient recycling from wastewater—is being encouraged by governmental policies aimed at reducing both the use and environmental accumulation of mineral phosphate fertilizers. Nevertheless, the development of efficient, safe, and contaminant free fertilizers derived from biowaste continues to pose significant challenges [29].

In the São Paulo State, Brazil, the Cardeal Urban Wastewater Treatment Plant (UWWTP) operates using an Upflow Anaerobic Sludge Blanket (UASB) system. The treatment process includes preliminary steps such as screening and grit removal, followed by a biological reactor, sedimentation, and a disinfection system using sodium hypochlorite. The plant treats an average inflow of 5.0 L/s. In the tertiary treatment stage, polyaluminum chloride (PAC) is added as a coagulant to remove suspended solids, turbidity, and other contaminants. The resulting sludge is directed to drying beds. This sludge has potential for use as a bio-based fertilizer, including in formulations where PAC is replaced by Fe-modified montmorillonite (TechPhos, TP). In the UWWTP, TechPhos, like PAC, is fully incorporated into the sludge produced at the end of the process, with the clay contributing to coagulation.

Despite these advances, an important gap remains in the literature. Most studies focus either on phosphorus removal from aqueous systems or on the agronomic use of recovered products, but rarely address both aspects in an integrated manner. In particular, the incorporation of functional adsorbent materials into sewage sludge and their subsequent performance as fertilizers under plant growth conditions remain poorly understood. This limitation is especially relevant for crops such as *Zea mays* L., which exhibit high phosphorus demand during early developmental stages and are highly sensitive to nutrient availability [31-33]. Therefore, strategies that not only recover phosphorus but also enhance its availability and use efficiency in soil are of great interest. Corn is a valuable indicator for assessing the effectiveness of alternative P sources given its agronomic importance and sensitivity to P availability, making it well-suited for evaluating the influence of recycled fertilization on seed germination and early development. Furthermore, evaluating these responses under both laboratory-controlled conditions and outdoor environments provide insights into the potential field applicability of such sustainable fertilization strategies. Although the recovery of phosphorus from UWWTPs through various adsorption techniques has been previously reported [30], the development and application of novel fertilizer products that integrate nanotechnology with sludge reuse—and their effects on key crops—remain underexplored. Due to its global significance in human and animal nutrition, *Zea mays* L. was chosen as the model species in this study.

Corn has a high phosphorus requirement, especially during the early stages of growth when root and shoot development are particularly sensitive to phosphorus availability [31], because phosphorus influences metabolic processes such as energy transfer, cell division, and photosynthesis, which are essential for rapid seedling establishment and subsequent biomass production [32,33]. Corn is a valuable indicator for assessing the effectiveness of alternative P sources given its agronomic importance and sensitivity to P availability, making it well suited for evaluating the influence of recycled fertilization on seed germination and early development. Furthermore, evaluating these responses under both laboratory controlled conditions and outdoor environments provide insights into the potential field applicability of such sustainable fertilization strategies.

In this context, the present study proposes the use of sewage sludge modified with Fe-enriched montmorillonite (TechPhos) as a multifunctional material that integrates

phosphorus recovery during wastewater treatment with its subsequent application as a soil amendment. Unlike conventional approaches that treat these processes separately, this strategy aims to combine nutrient capture and controlled release within a single material platform. Therefore, this research addresses these knowledge gaps by assessing the effects of (i) untreated soil (Control); (ii) soil amended with sludge of the Cardeal UWWTP (SC); (iii) soil amended with sludge modified with Fe-montmorillonite (ST); and (iv) soil amended with a commercial phosphorous salt (PS), on the germination and early development of *Z. mays*. To evaluate the potential of these recycled fertilizers, two experiments were carried out. The first experiment tested the corn germination and root elongation toxicity test, and was developed under laboratory controlled conditions during 5 days (Experiment 1). The second experiment consisted of studying the first developmental stages of the corn plants in 2L pots, exposed to open air conditions during 22 days (Experiment 2). We hypothesize that the incorporation of Fe-modified montmorillonite into sewage sludge improves phosphorus use efficiency by regulating its retention and gradual release in soil, thereby enhancing seed germination and early plant development compared to untreated sludge and conventional soluble fertilizers. To test this hypothesis, four treatments were evaluated: untreated soil (Control), sewage sludge (SC), sludge modified with Fe-montmorillonite (ST), and a commercial phosphorus fertilizer (PS). The effects of these treatments on the germination and early growth of *Zea mays* L. were assessed under both controlled laboratory conditions and outdoor experiments, combining agronomic analysis with physicochemical characterization to better understand the role of functional materials in sustainable phosphorus management. We hypothesized that SC and ST favor the germination and development of *Z. mays*, by supplying essential nutrients (mainly nitrogen and phosphorus) to seeds and roots, whereas the soil control and PS have limited benefits. In addition, we expected that the number of leaves, and chlorophylls content to be significant predictors of the plant health and development, while anthocyanin content to explain plants stress.

2. Materials and Methods

2.1. Collection of samples

The soil was collected at Estrada Velha do Mar, Jardim Vista Linda, in the city of Ribeirão Pires (23°46'13" S, 46°27'52" W). Following the initial soil analysis, it was determined that liming was necessary to ensure soil parameters met the minimum standards for cultivation. Consequently, liming was carried out. For liming, 229 g of dolomitic limestone was applied per 40 L basket of soil. The limestone used was from the brand Dimy®, with CaO and MgO contents of 24.0% and 17.1%, respectively, and a Neutralization Relative Power (PNR) of 70%.

2.2. Physicochemical characterization

Prior to the experiments, the chemical composition and the concentration (mg kg⁻¹) of each component in the sludge were analyzed before (SC) and after (ST) the addition of TechPhos. Total aluminum, iron, phosphorus, and potassium were determined following EPA Method 6010D (SW-846) [34]. Total nitrogen, Kjeldahl nitrogen, nitrate-nitrogen, and nitrite-nitrogen were analyzed according to the Standard Methods for the Examination of Water and Wastewater (SMEWW) [35]. In addition, the amount (%) of Fixed Solids, Total Solids, and Volatile Solids was determined by SMEWW-2540 solids methods [36]. Subsequently, the parameters of the untreated soil before and after liming, were analyzed

according to procedures described by Van Raij [37]: pH, Organic matter (g L^{-1}), Resin-P (mg L^{-1}), K (mmol L^{-1}), Ca (mmol L^{-1}), Mg (mmol L^{-1}), H^+Al (mmol L^{-1}), Al (mmol L^{-1}), BS (mmol L^{-1}), CEC (mmol L^{-1}), V%, and m%. The Base Sum (BS) was calculated as the sum of exchangeable base cations (Ca^{2+} , Mg^{2+} , and K^+), expressed in mmolc L^{-1} . The Cation Exchange Capacity (CEC) was obtained as the sum of BS and exchangeable acidity ($\text{H}^+ + \text{Al}^{3+}$). Base saturation (V%) and aluminum saturation (m%) were calculated using standard procedures.

$$BS = [\text{Ca}^{2+}] + [\text{Mg}^{2+}] + [\text{K}^+] \quad (\text{Eq. 1})$$

$$CEC = BS + ([\text{H}^+] + [\text{Al}^{3+}]) \quad (\text{Eq. 2})$$

$$V(\%) = 100 \frac{BS}{CEC} \quad (\text{Eq. 3})$$

$$m(\%) = 100 \frac{[\text{Al}^{3+}]}{CEC} \quad (\text{Eq. 4})$$

The Base Sum (BS) was calculated using Equation 1, where the molar concentration of each cation (in brackets) is divided by its valence, and the result is expressed in millimoles of charge per liter (mmolc L^{-1}). The Cation Exchange Capacity (CEC) was determined using Equation 2 and is likewise expressed in mmolc L^{-1} . Base Saturation (V), expressed as a percentage, was obtained using Equation 3, while Aluminum Saturation in the effective CEC (m), also in percentage, was calculated using Equation 4.

$$BS = \frac{[\text{Ca}^{2+}]}{2} + \frac{[\text{Mg}^{2+}]}{2} + \frac{[\text{K}^+]}{1} \quad (\text{Eq. 1})$$

$$CEC = SB + [\text{Na}^+] + (\text{H} + \text{Al}) \quad (\text{Eq. 2})$$

$$V(\%) = 100 \frac{BS}{CEC} \quad (\text{Eq. 3})$$

$$m(\%) = 100 \frac{[\text{Al}^{3+}]}{CEC} \quad (\text{Eq. 4})$$

2.2.1. Elemental Analysis and X-Ray Fluorescence (XRF)

Soil Control, SC, and ST samples were dried at 40°C in a drying oven until no mass change was observed prior to analysis. Elemental analyses of the solids were performed using a Thermo Scientific Flash EA 1112 Elemental Analyzer. The carbon, hydrogen and nitrogen contents were determined by combusting 2–3 mg of the sample, previously dried at 60°C for 12 h and sealed in tin capsules. The quantification of these elements was carried out by gas chromatography of the combustion gases (CO_2 , N_2 and H_2O). All analyses were performed in triplicate, and the results are reported as mean values with standard deviations. The X-Ray Fluorescence (XRF) analysis of the Soil, SC, and ST samples was performed using a Supermini 200 wavelength-dispersive X-ray fluorescence (WDXRF) spectrometer (Rigaku). The solid samples were first dried at 105°C for 12 h and then finely ground to a particle size smaller than $75\ \mu\text{m}$. The resulting powder was homogenized and pressed into pellets using a hydraulic press with a force of approximately 15 tons. The measurement program was set to target major elements of

interest. Each element was measured for a specific counting time (typically between 20 and 100 seconds), under 50 kV Pd tube voltage, and current, collimator, and analyzing crystal parameters adjusted to suit the respective wavelengths. Results were expressed in weight percent.

2.2.2. X-Ray Diffraction (XRD)

Powder X-ray diffraction (XRD) analysis was performed on the Soil, SC and ST samples using a FOCUS D8 diffractometer (Bruker). Data were collected over a 2θ range of 5° to 90° , with a step size of 0.015° . The basal spacing of the clay was calculated using Bragg's equation (Equation 5), where λ represents the wavelength of the Cu $K\alpha$ radiation ($1.54 \text{ \AA}$), d is the interlayer spacing in $\text{\AA}$, θ is the diffraction angle ($^\circ$), and n is an integer ($n = 1$).

$$n \lambda = 2 d \sin(\theta) \text{ (Eq. 5)}$$

2.2.3. Fourier Transform Infrared (FTIR)

Infrared spectra of the Soil, SC and ST samples were acquired using a PerkinElmer Spectrum Two Spectrometer (Massachusetts, USA). The analysis was conducted in Attenuated Total Reflectance (ATR) mode, with a resolution of 4 cm^{-1} and 32 scans collected per sample. Before spectral acquisition, all samples underwent a 24-hour drying period at 100°C .

2.3. Experiment 1

2.3.1. Plant materials and germination test

Experiment 1 was performed under laboratory constant conditions at the São Bernardo do Campo campus of the Universidade Federal do ABC, São Paulo, Brazil. *Zea mays* seeds were acquired from ISLA Company and correspond to the ITAPUÁ 700-hybrid variety, free of transgenics traits, under Renasem N° RS 00567/2019, in accordance with current Brazilian regulations, and treated with 0.25% Vitavax Thiram 200 SC.

Prior to initiating the tests, all glass Petri dishes (100 mm) were sterilized, and corn seeds were surface sterilized by immersion in 2.5% sodium hypochlorite for 5 min to eliminate surface fungi and bacteria, followed by several rinses with distilled water. The germination and radicle and hypocotyl/mesocotyl elongation toxicity test were conducted according to the methodology described in a previous study [26], and following the seed analysis rules guidelines published by the Ministério da Agricultura, Pecuária e Abastecimento – Brazil (2009) [38].

The tests were conducted using 60 *Z. mays* seeds per each triplicated treatment (Control, SC, ST, and PS), totaling 240 seeds, distributed across 12 Petri dishes per replicate of each treatment. Due to the large size of the seeds, only five were randomly placed per dish, ensuring adequate spacing for proper development. Each dish received 4 mL of the respective test solution (Control, SC, ST, or PS) onto a Whatman filter paper. The dishes were then covered with aluminum foil to minimize evaporation and incubated in the dark at $24 \pm 0.5^\circ\text{C}$ and $53.5 \pm 1.2\%$ relative humidity in a controlled chamber. Measurements were recorded every 24 h over 120 h.

Four stock suspensions were prepared in 250 mL glass beakers using distilled water as the suspension medium for the following treatments: (i) 2.5 g Ribeirão Pires soil (Control); (ii) 2.5 g sludge from Cardeal UWWTP without Techphos (SC); (iii) 2.5 g sludge from Cardeal UWWTP with Techphos in a 1:10 mass ratio (w/w) (ST); (iv) 0.2262 g of potassium phosphate monobasic (KH_2PO_4), a compound commonly used as fertilizer (PS).

2.3.2. Germination-related traits

The potential beneficial or detrimental effects of the treatments (SC, ST, and PS) compared to the Control on the germination and growth of *Z. mays* seeds were assessed by evaluating the following endpoints: Germination percentage (GP%; Equation 6); Germination speed index (GSI; Equation 7); Mean germination time (MGT; Equation 8); Germination index (GI; Equation 9); radicle elongation (cm), and ~~hypocotyl~~ **mesocotyl** elongation (cm), both determined by image analysis using the Fiji Software [39]. **Briefly, at the end of the assay, an image was taken to each replicate of radicles and mesocotyls of each treatment and control. Each image was calibrated with a scale reference, by counting pixels along a traced line and converting them into mm. Finally, the segmented line tool was used to measure the slightly curved structures of roots and mesocotyls, and the average lengths were expressed as media and standard deviation (Figure 4 a,b).**

$$GP(\%) = \frac{\text{Number of seed germinated}}{\text{Number of total seeds in plate}} \times 100 \text{ (Eq. 6)}$$

$$GSI = \sum_{i=1}^k \frac{N_i}{T_i} \text{ (Eq. 7)}$$

where, N_i is the number of seeds germinated at time interval I , T_i is the time (days) corresponding to observation i , and k is the total number of observations [40].

$$MGT = \frac{\sum N_i \times T_i}{N} \text{ (Eq. 8)}$$

where, N_i is the number of seeds germinated for each day, T_i is the number of days after sowing, and N is the total number of seeds germinated at the termination of the experiment [41].

$$GI = \frac{[(X_{gs}/X_{gc}) \times 100] \times [(X_{rt}/X_{rc}) \times 100]}{100} \text{ (Eq. 9)}$$

where, X_{gs} is the arithmetic mean of the number of germinated seeds in the treated sample; X_{gc} is the arithmetic mean of the number of germinated seeds in the control; X_{rt} is the arithmetic mean of the root length of the treated sample, and X_{rc} is the arithmetic mean of the root length of the control [42].

The phytotoxicity level of the treated samples with soil Control, SC, ST, and PS, was assessed based on the Germination index and classified based on the criteria described in Shafique et al. [42].

2.4. Experiment 2

Experiment 2 was conducted at the São Bernardo do Campo experimental station (Universidade Federal do ABC; 23° 41' 38" S, 46° 33' 54" O), which has an average annual precipitation of 2133 mm and a mean temperature of 19.2 °C (Climate data. Org.) [43]. The average precipitation during the development of the outdoor experiment was 255 mm, and the temperature **ranged** from a minimum of 18–20°C to a maximum of 30–33°C. Seeds of *Z. mays* were obtained from Isla (Brazil), as in Experiment 1. To eliminate surface fungi and bacteria, seeds were sterilized using 5% sodium hypochlorite solution. Germination was **initiated** in Petri dishes within a greenhouse for the first five days (Supplementary material). Then, germinated seeds were transplanted to 2 L planters (one seed per planter), at approximately 3 cm depth. At this depth, the radicle remains in the upper soil layer where added phosphorus is accessible. Under field conditions, vertical stratification of P can affect availability, but in our controlled system, the depth was shallow and uniform.

Then, the outdoor experiments began, lasting a total of 22 days. A total of 15 replicates for each treatment and control were performed ($n = 60$), accounting for potential variability in open-air environments. Each planter was prepared with a bottom layer of 20 units of expanded clay balls to enhance nutrient uptake, plant growth, aeration and drainage [44]. The remaining volume (2 L) was filled with the Soil (Control), or with soil amended with one of the following treatments: SC, ST, or PS.

Before use, the soil used in all the experiments was carefully sieved to remove large clumps and amended with 250 g limestone ($\text{CaO} + \text{MgO}$) per 30 kg of soil to adjust pH to neutral. For the SC treatment, 20 g of dried sludge from Cardeal UWWTP was mixed with 2 kg of soil per planter. For the ST treatment, 20 g of dried sludge enriched with TechPhos from the same UWWTP was used, also at a dose of 10 g/kg each. For the PS treatment, 1.51 g of monobasic phosphate salt (KH_2PO_4) was added to each 2 kg soil/planter.

To avoid drought stress, 200 mL of tap water was regularly added to each planter as needed following the recommendations of Bangarwa and Dubey [45], plant emergence and early development were monitored and registered daily. The following parameters were recorded to assess plant performance under each treatment (Control, SC, ST, and PS): 1) time emergence of 1st leaf (days); 2) number of leaves; 3) number of nodes; and 4) anthocyanin presence. At the end of the experiment, additional measurements were taken, after carefully separating shoots and roots, including fresh and dry shoot biomass (g), fresh and dry root biomass (g), chlorophyll *a* and chlorophyll *b* content. Details of the experimental setup are provided in the supplementary material (Figure S1).

2.5. Statistical analysis

All statistical analyses were performed using InfoStat version 2020 software. Normality (Shapiro-Wilk) and homoscedasticity (Levene's test) were verified. For parametric data, one-way Analysis of Variance (ANOVA) was employed, followed by Tukey HSD multiple comparison test. When assumptions were not met, the non-parametric Kruskal-Wallis test was used. Association between categorical variables was evaluated using Pearson's Chi-square test. Additionally, Principal Component Analysis (PCA) was conducted to explore multivariate relationships between variables and treatments. Data visualizations were generated using OriginPro version 9 software.

3. Results

3.1. Physical and chemical characteristics of the experimental soils

Table 1 presents the chemical characterization of sludge from the Cardeal Urban Wastewater Treatment Plant (UWWTP) (SC) and after the addition of montmorillonite enriched with Fe (Techphos, ST). The comparison between SC and ST reveals how the addition of Techphos alters the sludge composition.

Table 1. Chemical composition of the original sludge (SC) and after the addition of sludge modified with Fe-montmorillonite (ST).

Component (mg kg^{-1})	SC	ST
Total Aluminum	259.3 ± 52.0	226.4 ± 45.3
Total Nitrogen	200.2 ± 30.0	367.3 ± 55.1
Total Iron	41.1 ± 8.2	54.7 ± 10.9
Total Phosphorus	94.5 ± 23.6	97.7 ± 24.4
Total Potassium	< 50	61.9 ± 15.5
Kjeldahl Nitrogen	185.0 ± 27.8	355.7 ± 53.4
Nitrogen as nitrate	15.2 ± 4.6	11.6 ± 3.5

<u>Nitrogen as nitrite</u> <u>Component (%)</u>	<u>< 10</u> <u>SC</u>	<u>< 10</u> <u>ST</u>
<u>Fixed Solids</u>	<u>46.7 ± 4.7</u>	<u>42.0 ± 4.2</u>
<u>Total Solids</u>	<u>0.5 ± 0.08</u>	<u>1.6 ± 0.24</u>
<u>Volatile Solids</u>	<u>53.3 ± 5.3</u>	<u>58.0 ± 5.8</u>

Total nitrogen increased from 200.2 ± 30.0 mg kg⁻¹ in SC to 367.3 ± 55.1 mg kg⁻¹ in ST, indicating a substantial enrichment that exceeds the associated uncertainty. A similar trend was observed for Kjeldahl nitrogen, reinforcing the contribution of TechPhos to nitrogen retention in the sludge matrix. Total iron also increased from 41.1 ± 8.2 to 54.7 ± 10.9 mg kg⁻¹, consistent with the incorporation of Fe-bearing phases.

In contrast, total phosphorus showed only a marginal increase (94.5 ± 23.6 to 97.7 ± 24.4 mg kg⁻¹), which falls within the estimated uncertainty range. This indicates that the improved agronomic performance observed for ST is unlikely to be associated with changes in total phosphorus content, but rather with differences in phosphorus speciation, particularly the increased proportion of Fe-associated phosphorus.

Potassium, which was below detection limits in SC, was detected in ST (61.94 ± 15.5 mg kg⁻¹), suggesting an additional nutritional contribution. Variations in nitrate concentrations (15.2 ± 4.6 to 11.6 ± 3.5 mg kg⁻¹) remain within uncertainty limits and therefore should not be overinterpreted.

Regarding solids content, fixed and volatile fractions showed moderate variability ($\approx 10\%$), indicating that structural changes in the sludge matrix are consistent but not dominant. Overall, when uncertainties are considered, the most robust compositional changes induced by TechPhos are associated with nitrogen and iron enrichment, rather than total phosphorus concentration.

Although the sources do not establish a direct connection between the use of the characterized sludge and soil liming, the analysis of sludge composition is fundamental for evaluating its potential as a soil conditioner or fertilizer, particularly given its nutrient content. The post-liming Ribeirão Pires soil was used as the Control, and also as the supporting material of the amendments studied in this work (sludge from Cardeal WWTP, TechPhos, and a commercial phosphorus salt). The results of the characterization of the soil Control before and after liming are exhibited in Table 2. In this table, Resin-P denotes Resin-extractable phosphorus, H⁺Al represents total acidity, SB refers to the sum of bases, CEC is the cation exchange capacity, V indicates base saturation, and M signifies aluminum saturation.

Table 2. Ribeirão Pires soil parameters before and after liming. Resin-P (mg L⁻¹): Resin-extractable phosphorus. H⁺Al: total acidity. SB: sum of bases. CEC: cation exchange capacity. V: base saturation. M: aluminum saturation.

<u>Parameter</u>	<u>Initial</u>	<u>Post-liming</u>
<u>pH</u>	<u>4.1 ± 0.12</u>	<u>6.8 ± 0.20</u>
<u>Organic matter (g L⁻¹)</u>	<u>21.0 ± 3.2</u>	<u>23.0 ± 3.5</u>
<u>Resin-P (mg L⁻¹)</u>	<u>< 2</u>	<u>7.0 ± 2.8</u>
<u>K (mmol L⁻¹)</u>	<u>0.7 ± 0.18</u>	<u>0.8 ± 0.20</u>
<u>Ca (mmol L⁻¹)</u>	<u>3.0 ± 0.06</u>	<u>51.0 ± 10.2</u>
<u>Mg (mmol L⁻¹)</u>	<u>1.0 ± 0.2</u>	<u>34.0 ± 6.8</u>
<u>H⁺Al (mmol L⁻¹)</u>	<u>61.0 ± 9.2</u>	<u>15.0 ± 2.3</u>
<u>Al (mmol L⁻¹)</u>	<u>19.4 ± 3.9</u>	<u>< 1.0</u>
<u>SB (mmol L⁻¹)</u>	<u>4.7 ± 0.66</u>	<u>85.8 ± 12.3</u>

CEC (mmol L ⁻¹)	66.0 ± 9.2	101 ± 12.5
V%	7.1 ± 1.4	85.0 ± 16.1
M%	80.0 ± 8.0	<1.0

The liming process resulted in a clear and significant increase in soil pH (from 4.1 ± 0.12 to 6.8 ± 0.20), confirming effective neutralization. Exchangeable calcium and magnesium increased substantially (Ca: 3.0 ± 0.6 to 51.0 ± 10.2 mmol L⁻¹; Mg: 1.0 ± 0.2 to 34.0 ± 6.8 mmol L⁻¹), far exceeding their associated uncertainties, indicating a strong and reliable effect of liming on base cation availability.

Consequently, the sum of bases (SB) increased from 4.7 ± 0.66 to 85.8 ± 12.3 mmol L⁻¹, and the cation exchange capacity (CEC) from 66 ± 9.2 to 101 ± 12.5 mmol L⁻¹. Despite the propagation of uncertainties, these increases remain clearly significant, demonstrating improved soil fertility.

Base saturation (V%) increased from $7.1 \pm 1.4\%$ to $85.0 \pm 16.1\%$. Although the magnitude of this increase is substantial, the relatively high uncertainty associated with V% ($\approx 19\%$ relative error) indicates that this parameter is highly sensitive to propagated variability. Therefore, while the improvement in base saturation is unequivocal, its precise value should be interpreted with caution.

Resin-extractable phosphorus increased from <2 to 7.0 ± 2.8 mg L⁻¹; however, the high uncertainty associated with phosphorus ($\sim 40\%$) suggests considerable variability, limiting the strength of conclusions regarding P availability changes.

Finally, the reduction in total acidity (H⁺Al: 61.0 ± 9.2 to 15.0 ± 2.3 mmol L⁻¹) and aluminum saturation (M%: $80 \pm 8\%$ to $\sim 0\%$) confirms the effective mitigation of aluminum toxicity [47-49]. These changes remain robust even when uncertainty is considered and are consistent with improved conditions for root development and nutrient uptake.

Table 1. Chemical composition of the original sludge (SC) and after the addition of sludge modified with Fe montmorillonite (ST).

Component (mg kg ⁻¹)	SC	ST
Total Aluminum	259.28	226.42
Total Nitrogen	200.2	367.3
Total Iron	41.1	54.7
Total Phosphorus	94.5	97.7
Total Potassium	<50	61.94
Kjeldahl Nitrogen	185.0	355.7
Nitrogen as nitrate	15.2	11.6
Nitrogen as nitrite	<10	<10
Component (%)	SC	ST
Fixed Solids	46.7	42.0
Total Solids	0.5	1.6
Volatile Solids	53.3	58.0

The total aluminum concentration in the sludge decreased from 259.3 mg kg⁻¹ in SC to 226.42 mg kg⁻¹ in ST, indicating a lower overall aluminum content in the system when Techphos is used. Conversely, the total iron concentration in the ST sludge increased from 41.1 mg kg⁻¹ (in SC) to 54.7 mg kg⁻¹. Notably, the addition of Techphos resulted in a significant increase in total nitrogen, rising from 200.2 mg kg⁻¹ in SC to 367.3 mg kg⁻¹ in ST. Consistently, Kjeldahl Nitrogen, which represents organic and ammoniacal nitrogen, also

increased significantly, from 185.0 mg kg⁻¹ in SC to 355.7 mg kg⁻¹ in ST. This suggests that Techphos can enrich the sludge with relevant nitrogen forms.

While phosphorus is a key component in the broader discussion of soil improvement, the concentration of total phosphorus in the sludge showed only a small increase with Techphos addition, from 94.5 mg kg⁻¹ in SC to 97.7 mg kg⁻¹ in ST. Since the TechPhos dosage was calculated to achieve phosphorus removal equivalent to that of PAC, no significant variation in total phosphorus concentration was observed between the SC and ST samples. However, it is important to consider that the proportion of phosphorus coordinated with iron must be greater in ST, whereas in SC, phosphorus is predominantly coordinated with aluminum. Phosphorus binds to Fe/Al surfaces via ligand exchange, forming inner sphere complexes, or as precipitated FePO₄/AlPO₄, depending on conditions. The proportion of phosphorus coordinated with iron should be higher in ST than in SC, since the only metal present in the PAC used for SC treatment is aluminum, whereas TechPhos in ST contains iron. It is important to note that both PAC and TechPhos treatments are effective in removing residual phosphorus from treated domestic wastewater, consistent with the well established ability of aluminum and iron species to facilitate phosphate adsorption.

The analysis of fixed, volatile, and total solids indicates alterations in the stability and organic content of the sludge. Total potassium, which was below the detection limit (<50 mg kg⁻¹) in SC sludge, was detected at 61.94 mg kg⁻¹ in ST sludge. This suggests that ST may be contributing potassium, another essential nutrient. Nitrogen as nitrate decreased slightly from 15.2 mg kg⁻¹ in SC to 11.6 mg kg⁻¹ in ST. Nitrogen as nitrite remained below the detection limit (< 10 mg/kg) in both sludges. These results provide a basis for understanding the chemical composition of SC and ST sludges, especially how the addition of Techphos influences the concentration of nutrients (such as N, P, and K), as well as other elements and the distribution of solids. The increase in nitrogen content may be attributed to the higher agglomeration and adsorption capacity of fine particles present in the effluent after secondary treatment. The enhanced coagulation potential of the clay in TechPhos facilitates a more effective removal of residual organic nitrogen, which is associated with incompletely degraded organic compounds.

Although the sources do not establish a direct connection between the use of the characterized sludge and soil liming, the analysis of sludge composition is fundamental for evaluating its potential as a soil conditioner or fertilizer, particularly given its nutrient content. The post liming Ribeirão Pires soil was used to the Control, and also as the supporting material of the amendments studied in this work (sludge from Cardeal WWTP, Techphos, and a commercial phosphorus salt). The results of the characterization of the soil Control before and after liming are exhibited in Table 2. In this table, Resin P denotes Resin-extractable phosphorus, H⁺Al represents total acidity, SB refers to the sum of bases, CEC is the cation exchange capacity, V indicates base saturation, and M signifies aluminum saturation.

Table 2. The Ribeirão Pires soil parameters before and after liming. Resin P (mg L⁻¹): Resin-extractable phosphorus. H⁺Al: total acidity. SB: sum of bases. CEC: cation exchange capacity. V: base saturation. M: aluminum saturation.

Parameter	Initial	Post-liming
pH	4.1	6.8
Organic matter (g L ⁻¹)	21.0	23.0
Resin P (mg L ⁻¹)	<2	7.0
K (mmol L ⁻¹)	0.7	0.8

Ca (mmol L ⁻¹)	3.0	51.0
Mg (mmol L ⁻¹)	1.0	34.0
H ⁺ Al (mmol L ⁻¹)	61.0	15.0
Al (mmol L ⁻¹)	19.4	<1.0
SB (mmol L ⁻¹)	4.7	85.8
CEC (mmol L ⁻¹)	66.0	101
V%	7.0	85.0
M%	80.0	0

The results of the soil Control characterization before and after liming (Table 2), clearly demonstrate the effectiveness of the liming process. The primary objective of liming, which is soil neutralization, was demonstrably achieved. The soil pH significantly increased from 4.1 to 6.8. This pH level is considered highly suitable for most crops. There was a notable increase in Resin P concentration after liming, from less than 2 mg L⁻¹ to 7 mg L⁻¹.

For liming, a base saturation (V) target of 60% was established; however, the results surpassed this goal, reaching 85% for the limed soil and 90% for the limed soil supplemented with ST. These levels are suitable for plant growth [46], as a base saturation above 50% is generally sufficient to ensure pH neutrality and the availability of essential nutrients like calcium and magnesium. Successful neutralization was also confirmed by the drastic reduction in total acidity (H⁺Al), which fell from 61 mmol L⁻¹ to 15 mmol L⁻¹. Consequently, aluminum saturation (M%) was reduced from 80% to 0%, and aluminum concentration (Al³⁺) decreased from 19.4 mmol L⁻¹ to less than 1.0 mmol L⁻¹. The elimination of aluminum toxicity is fundamental for root development and nutrient absorption by plants [47–49].

3.2. Elemental Analysis and X-Ray Fluorescence (XRF)

The Elemental Analysis (C, H, N), and X-ray fluorescence (XRF) analysis were conducted to characterize the Control, SC and ST samples, with the resulting elemental compositions presented in Table 3.

Table 3. Bulk oxide composition of the elements expressed in weight percent (% wt) of the Control, SC and ST samples, as determined by Elemental Analysis (C, H, N), and XRF. (n=3).

Element	Control	SC	ST
C	1.79 ± 0.02	22.62 ± 0.87	20.46 ± 0.65
H	-	4.25 ± 0.07	3.91 ± 0.04
N	0.14 ± 0.01	2.97 ± 0.06	3.29 ± 0.05
MgO	0.94 ± 0.02	1.07 ± 0.06	1.44 ± 0.18
Al ₂ O ₃	37.79 ± 0.29	23.58 ± 0.58	23.23 ± 0.14
SiO ₂	39.58 ± 0.10	23.39 ± 0.48	24.24 ± 0.21
P ₂ O ₅	0.28 ± 0.05	6.77 ± 0.17	8.28 ± 0.27
SO ₃	0.16 ± 0.02	4.24 ± 0.07	4.34 ± 0.02
K ₂ O	3.76 ± 0.05	0.47 ± 0.02	0.75 ± 0.08
CaO	0.11 ± 0.01	4.99 ± 0.16	4.29 ± 0.20
TiO ₂	1.82 ± 0.03	0.91 ± 0.04	0.63 ± 0.01
Fe ₂ O ₃	13.63 ± 0.06	4.39 ± 0.09	4.78 ± 0.03
Cl	-	0.09 ± 0.01	0.16 ± 0.02
ZnO	-	0.18 ± 0.02	0.15 ± 0.01
MnO	-	0.06 ± 0.01	0.04 ± 0.01

The elemental analyses of the soil control, the original sludge from the Cardeal Wastewater Treatment Plant (SC), and the sludge modified with Fe-montmorillonite (ST) reveal clear compositional distinctions that can explain the observed differences in corn performance (Table 3). The control soil is dominated by aluminosilicate minerals— SiO_2 ($\approx 39.6\%$) and Al_2O_3 ($\approx 37.8\%$)—typical of kaolinitic soils with low fertility and limited nutrient availability ($\text{P}_2\text{O}_5 \approx 0.28\%$, $\text{N} \approx 0.14\%$). In contrast, both SC and ST show markedly higher levels of organic matter ($\text{C} \approx 20\text{--}23\%$), nitrogen ($\approx 3\%$), and phosphorus ($\text{P}_2\text{O}_5 \approx 6.8\text{--}8.3\%$), confirming the nutrient enrichment conferred by the sludge amendments.

This trend aligns with reported sludge compositions in the literature: Wu et al. [50] found 18.46% SiO_2 , 6.14% Al_2O_3 , and 2.39% Fe_2O_3 in conventional sludges, while Azarhomayun et al. [51] reported lower oxide contents (10.1% SiO_2 , 3.7% Al_2O_3 , and 2.12% Fe_2O_3). The higher oxide levels observed here likely reflect the specific treatment process at the Cardeal UWWTP and the presence of mineral coagulants. The slightly reduced carbon content in ST relative to SC is consistent with the greater inorganic contribution of TechPhos. At the same time, the increase in P_2O_5 confirms the higher phosphorus-retention capacity of the Fe-enriched system compared with the polyaluminum chloride (PAC)-based sludge.

3.3. Fourier Transform Infrared (FTIR)

The FTIR spectra for Soil, SC and ST samples are presented in Figure 1.

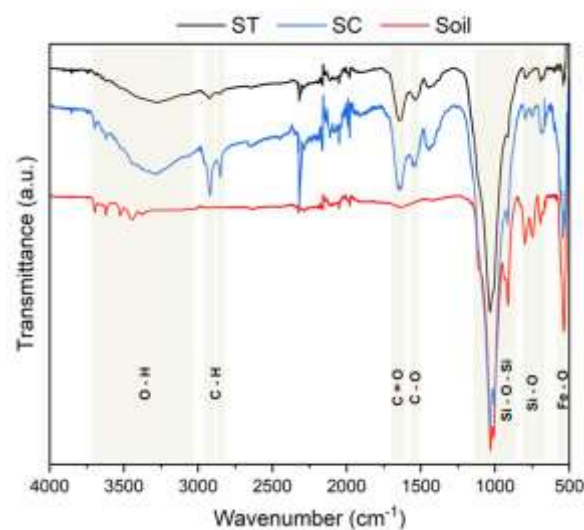


Figure 1. Spectra for Soil (red line), SC (blue line) and ST (black line) FTIR. The highlighted regions correspond to the assignment of bonds.

The signals observed at ~ 3626 and ~ 916 cm^{-1} (in addition to those at $\sim 3600\text{--}3700$, 1640 , $1000\text{--}1100$, ~ 690 , and ~ 550 cm^{-1}) are consistent with the presence of kaolinite/kaolin. This assignment is plausible, as the XRD diffractogram (Figure 2) shows reflections characteristic of kaolinite mainly in the sludge samples. The soil spectrum exhibits an intense band at 540 cm^{-1} , attributed to the Fe–O stretching vibration typical of hematite. In contrast, the possible presence of Fe–O–P bonds resulting from the interaction between iron and phosphate in the sludge samples cannot be clearly confirmed by FTIR. Although both the phosphate anion and its interaction with iron should produce characteristic signals, the main phosphate vibrations (P–O bending, $400\text{--}600$ cm^{-1} ; asymmetric stretching, $1000\text{--}1200$ cm^{-1} ; symmetric stretching, $900\text{--}950$ cm^{-1}) are overlapped by the

intense Si–O absorptions, while the Fe–O–P deformation band coincides with the hematite signal. All samples display Si–O–Si stretching vibrations ($\sim 1030\text{ cm}^{-1}$) and Fe–O stretching in iron oxides ($\sim 470\text{--}570\text{ cm}^{-1}$). However, in sample ST, no significant increase in intensity was observed in the $600\text{--}500\text{ cm}^{-1}$ region compared to SC and Soil, suggesting the absence of clearly detectable crystalline iron phosphate. Nevertheless, a slight broadening and shift of the Si–O–Si band toward lower wavenumbers ($1050\text{--}1000\text{ cm}^{-1}$) in ST may indicate overlapping between Si–O and P–O vibrations, suggesting the presence of adsorbed phosphate or amorphous Fe–O–P interactions. Such poorly crystalline Fe-phosphate species are known to exhibit weak or indistinct infrared signatures, as reported by Arai and Sparks [52]. Therefore, the FTIR results are not conclusive regarding the presence or absence of Fe-phosphate interactions but may suggest subtle evidence of phosphate association with iron- or aluminosilicate-rich sites.

3.4. X-Ray Diffraction (XRD)

Figure 2 shows the diffractograms of the Soil, PAC-treated sludge (SC) and the sludge modified with Fe-Montmorillonite (ST).

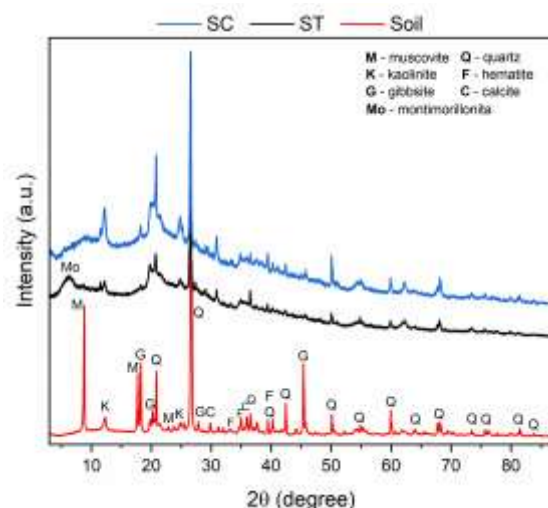


Figure 2. Diffractograms for SC (in blue lines) and ST (in black lines).

The main constituents identified in both diffractograms were quartz (SiO_2) minerals, evidenced by the greater number and intensity of diffraction peaks throughout the analyzed range. Some specific peaks can be associated with different mineral phases present or formed within the material. The peaks at approximately 12° and 25° (2θ) correspond to kaolinite [$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$], appearing with greater intensity in the SC sample, consistent with the addition of PAC. The peak observed near 18° (2θ) in the Soil and SC samples is attributed to gibbsite [$\text{Al}(\text{OH})_3$], while an intense reflection at 26° (2θ) is characteristic of quartz.

The ST sample also shows a signal at approximately 5.8° (2θ), attributed to montmorillonite, which is the predominant mineral phase in its composition. This reflection corresponds to a basal spacing of about $15.1\text{ \AA}$, typical of smectites where calcium acts as the interlayer cation. Other low-intensity peaks distributed across the diffractogram can mainly be assigned to secondary reflections of quartz and kaolinite.

The relative increase in silicon content observed in the ST sample compared to SC may be attributed to the incorporation of aluminosilicate structures originating from TechPhos. In contrast, aluminum levels showed no significant variation between SC and

ST. This can be explained by two opposing effects: in SC, aluminum increases due to the presence of polyaluminum chloride (PAC), whereas in ST, the clay fraction of TechPhos contributes additional aluminosilicate material. As a result, these opposing influences balanced each other, leading to no appreciable change in the overall aluminum content between the samples.

When the data from Tables 1 and 3 are considered together, the compositional trends are coherent. Both analyses indicate Fe enrichment and improved nutrient balance in ST relative to SC, although minor variations in aluminum levels arise from the different analytical bases used—wet-chemical methods ($\text{mg}\cdot\text{kg}^{-1}$) quantify reactive fractions, whereas XRF (% wt) captures total elemental oxides. Thus, the results complement each other and collectively demonstrate that TechPhos incorporation increases the content of N-, Fe-, Si-, and P-bearing phases while maintaining the mineral integrity of the material.

The diffractograms for SC and ST show that no clear evidence of iron phosphate formation was detected, particularly in the ST sample. However, previous studies have shown that the structures formed through the interaction between iron and phosphate are often amorphous, which could explain the baseline displacement observed in the $15\text{--}30^\circ$ (2θ) region. Moreover, the peaks attributed to hematite (Fe_2O_3) are less intense in ST compared to SC and Soil, suggesting that part of the iron originally present as oxide may have transformed, possibly forming Fe-phosphate complexes or bonds. If the resulting phase is poorly crystalline or amorphous, as reported by Bahgat et al. [53], in which $\text{FePO}_4\cdot 2\text{H}_2\text{O}$ precipitates exhibited an amorphous nature, it would not generate detectable diffraction peaks, thereby explaining the absence of an XRD signal even in the presence of iron bound to phosphorus.

3.5. Experiment 1

3.5.1. Germination parameters

The germination dynamics of *Z. mays* seeds were monitored over 120 h under four treatments: Control, SC, ST, and PS. The final number of germinated seeds per treatment was 54 in the Control, 49 in SC, 51 in ST, and 44 in PS, corresponding to average values of 4.5 ± 0.2 , 4.1 ± 0.6 , 4.2 ± 0.2 , and 3.7 ± 0.8 germinated seeds per Petri dish, respectively. Figure 3a illustrates the cumulative germination over time. At 24 h, the number of germinated seeds in the ST treatment was 40.0% higher than in the Control, while PS showed a 20.0% increase. By 48 h, ST still maintained a slight advantage with 5.9% more germinated seeds than the Control. However, no significant differences were found between treatments at any time point. By the end of the experiment, the highest GP% was recorded in the Control ($90.0 \pm 5\%$), followed by ST ($81.7 \pm 12.6\%$), SC ($85 \pm 0.0\%$), and PS ($73.3 \pm 15.3\%$).

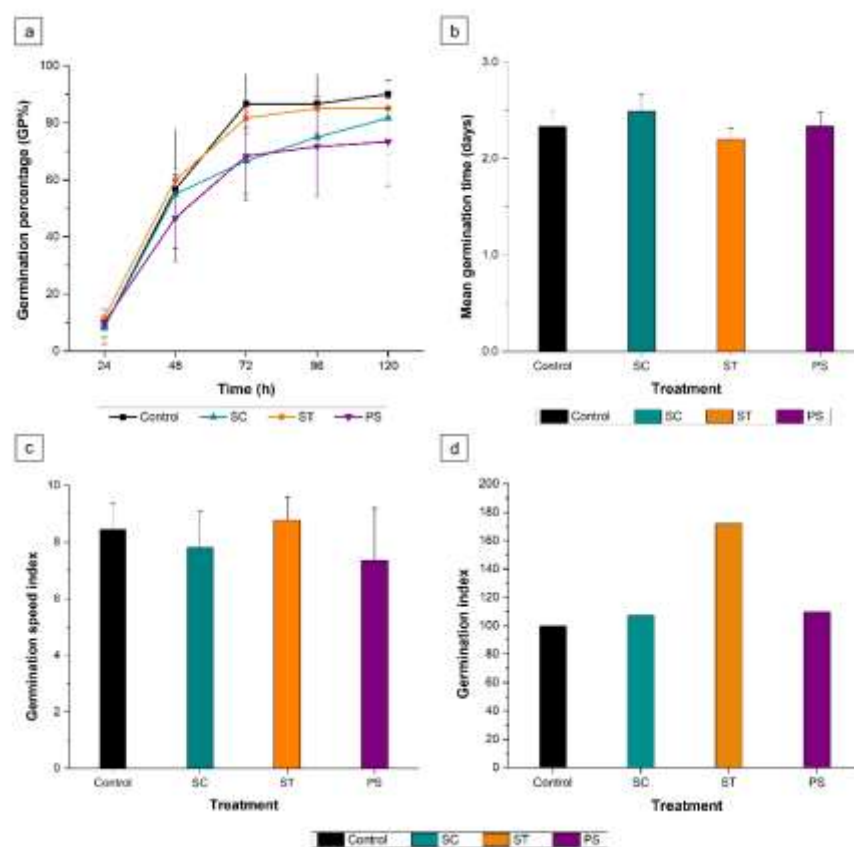


Figure 3. (a) Germination percentage, (b) mean germination time, and (c) germination velocity index of *Zea mays* seeds exposed to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Fe-montmorillonite (TechPhos, ST), and a commercial phosphorus salt (PS). Measurements were taken at 24, 48, 72, and 96 h. (d) Germination index at 120 h under the same treatments. Data are presented as mean $\pm$ standard error ($n = 12$). Means sharing a common letter are not significantly different ($p > 0.01$).

The mean germination time (MGT) increased progressively in all treatments throughout the experiment. Significant differences in MGT were observed mainly at 72 h. At this time point, the SC treatment showed the lowest MGT (1.7 ± 0.1 days), suggesting the fastest germination and was statistically different from the Control (achieving a maximum of 2.7 ± 0.2 days; $p = 0.006$). Although the differences remained at 96 h, at 120 h the gap was reduced, with all treatments reaching a maximum and very similar MGT: SC (2.5 ± 0.2 days), PS (2.3 ± 0.1 days), Control (2.3 ± 0.2 days), and ST (2.2 ± 0.1 days), indicating that the effect of the treatments is focused on the initial speed of germination and not on the final germination.

To further characterize seed performance, integrative parameters were calculated at the 120-h mark (Figures 3b, 3c). The Mean Germination Time (MGT) showed consistent values across treatments, with ST presenting the lowest numerical value (2.20 ± 0.12 days), followed by the Control and PS (both at 2.33 days), and SC (2.26 ± 0.48 days).

Similarly, the Germination velocity (GV), which indicates the speed of the process, confirmed the high vigor of the seeds, particularly in the ST treatment, which reached a peak value of 8.78 ± 0.81 , while PS showed the lowest velocity (7.34 ± 1.85) (Figure 3c).

The germination speed index (GSI) showed a progressive increase throughout the trial in all treatments, reaching maximum values between 72 and 96 h, at which point germination stabilized. At 120 h, the Control and ST treatments presented the highest GSI

values (8.4 ± 0.9 and 8.8 ± 0.8 , respectively), followed by SC (7.8 ± 1.3) and PS (7.3 ± 1.8), with the greatest variability. Although no statistically significant differences were found between the Control and the treatments, the germination rate was higher in the ST treatment.

The germination index (GI), which integrates both the speed and the final percentage of germination, further supported the positive influence of SC and ST on seed performance. At the end of the assay, GI values were 106.9 for SC, 171.7 for ST, and 106.5 for PS (Figure 3d).

The Germination Index (GI), which integrates both speed and final percentage, further supported the positive influence of the treatments (Figure 3d). Germination Index values were 106.9 for SC, 171.7 for ST, and 106.5 for PS. According to the classification by Shafique et al. (2021), none of the treatments exhibited phytotoxicity. These results, especially for the ST treatment, underscore the potential of using sludge enriched with TechPhos® as a sustainable alternative that maintains seed vigor and enhances early development in *Z. mays*.

3.5.2. Radicle and hypocotyl-mesocotyl elongation

The average elongation of *Z. mays* radicles was 57.9 ± 13.3 mm in the Control, 68.3 ± 18.6 mm in SC, 105.3 ± 19.9 mm in ST, and 77.9 ± 15.8 mm in PS (Figure 4a). Compared to the Control, radicle length increased by 17.9% in SC, 81.8% in ST, and 34.4% in PS. All treatments showed statistically significant differences (ANOVA, $p < 0.001$). The Tukey post hoc test revealed that ST differed significantly from all other treatments (Control, SC, and PS; $p < 0.001$), and PS also differed significantly from the Control ($p < 0.001$). These results indicate that all three treatments provided more favorable conditions than Control for radicle growth.

The average elongation of *Z. mays* hypocotyl-mesocotyls was 23.8 ± 7.1 mm in the Control, 34.2 ± 8.2 mm in SC, 40.4 ± 8.0 mm in ST, and 25.0 ± 6.7 mm in PS (Figure 4b). Relative to the Control, hypocotyl-mesocotyl length increased by 43.6% in SC, 69.6% in ST, and 7.7% in PS, all treatments differed significantly (ANOVA, $p < 0.001$). According to the Tukey post hoc test, ST was significantly different from Control, PS ($p < 0.001$), and SC ($p < 0.004$). Additionally, SC differed significantly from both the Control and PS ($p < 0.001$). These findings suggest that SC and especially ST enhanced hypocotyl-mesocotyl development compared to the Control.

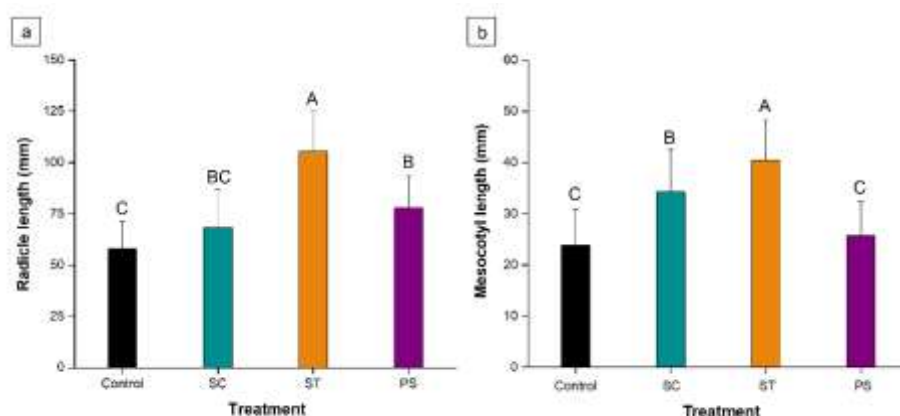


Figure 4. Mean radicle length (a) and hypocotyl-mesocotyl length (b) (cm) of germinating *Zea mays* seedlings after 22 days of exposure to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Fe-montmorillonite (TechPhos, ST), and a commercial phosphorus salt

(PS). Data are presented as mean $\pm$ standard deviation ($n = 12$). Means sharing a common letter are not significantly different ($p > 0.01$).

3.6. Experiment 2

3.6.1. Germination time and emergence time of first leaf

The impact of treatments (Control, SC, ST, PS) on key variables of early phenological development was evaluated: germination day and first leaf emergence day (Figure 5). Results for germination day showed highly significant statistical differences between treatments ($H = 44.26$, $p < 0.0001$). Post-hoc analysis revealed that ST and SC treatments showed significantly longer germination times, with means of 4.0 days for both, forming a distinct subgroup ('A'). PS and Control treatments exhibited significantly shorter germination times, with means of 3.0 days for both, clustering in the same statistical subgroup ('B'). (Figure 5a).

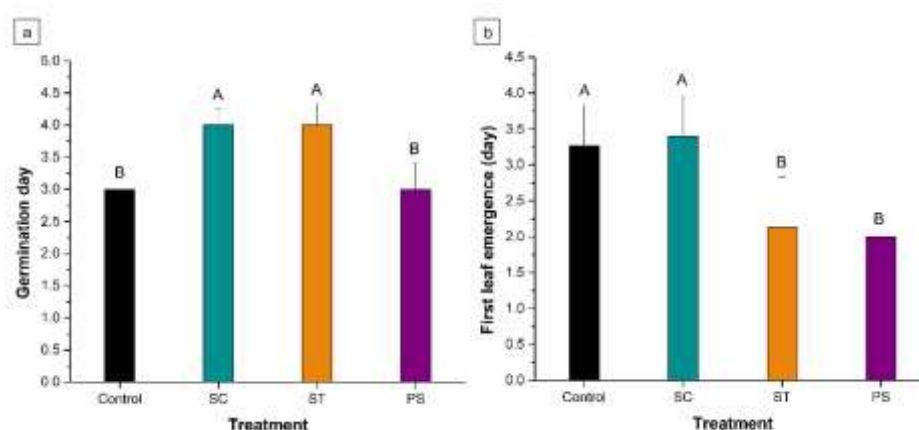


Figure 5. (a) Germination day, and (b) day of first leaf emergence in *Zea mays* seedlings exposed to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Ferromorillonite (TechPhos, ST), and a commercial phosphorus salt (PS). Data are presented as mean $\pm$ standard deviation ($n = 15$). Means sharing a common letter are not significantly different ($p > 0.01$).

For first leaf emergence day, highly significant differences between treatments were detected ($H = 30.09$, $p < 0.0001$). PS and ST treatments recorded the fastest emergence day, with means of 2.00 ± 0.00 and 2.13 ± 0.52 days respectively, clustering in statistical subgroup 'A'. SC and Control treatments showed significantly delayed emergence, with means of 3.40 ± 0.50 and 3.27 ± 0.59 days, forming subgroup 'B' (Figure 5b).

While Control and PS treatments achieved faster germination, PS and ST were effective in accelerating subsequent emergence and initial development of the first leaf. SC and ST treatments, despite slower germination, differed in first leaf emergence, with ST being significantly faster than SC.

3.6.2. Shoot and root growth

Shoot and root biomass growth was evaluated for the different treatments. For shoot dry biomass, ANOVA revealed highly significant differences between treatments ($F = 45.64$, $p < 0.0001$). PS treatment displayed the highest value (1.31 ± 0.25 g), being significantly superior to SC (0.84 ± 0.31 g) and Control (0.33 ± 0.10 g). The ST treatment (1.12 ± 0.20 g) did not differ significantly from PS or SC, but was significantly superior to Control (Figure 6a). Control presented the lowest shoot dry mass, being significantly

different from all other treatments (Figure 6a), where PS displays the highest value highest bar, followed by ST, SC, and Control. Regarding shoot fresh biomass, the Kruskal-Wallis test confirmed highly significant differences between treatments ($H = 32.27$, $p < 0.0001$). Post-hoc analysis showed that ST (7.06 ± 1.53 g) and PS (6.72 ± 1.18 g) treatments induced significantly greater shoot fresh mass, with no significant differences between them. These two treatments were significantly superior to SC (4.8 ± 1.72 g) and than the Control (0.87 ± 0.27 g), although they were statistically comparable to the SC treatment (4.8 ± 1.72 g), which did not differ significantly from each other.

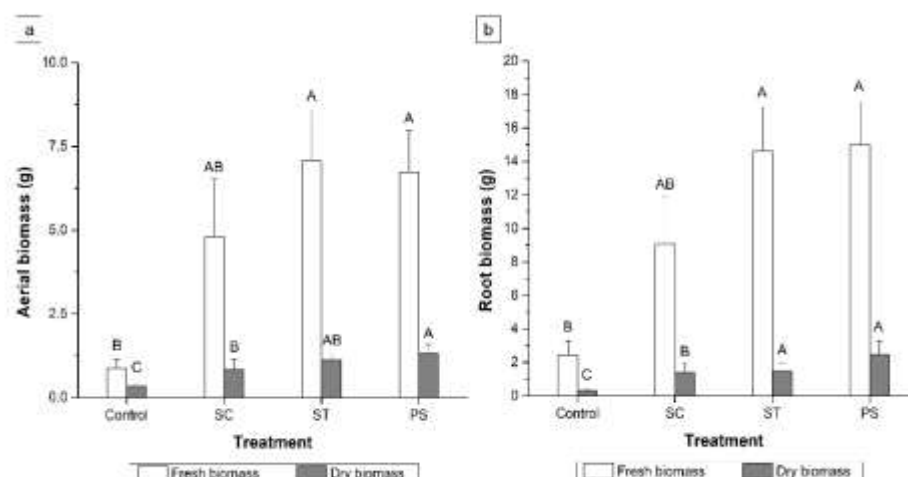


Figure 6. (a) Aerial biomass (g) and (b) Root biomass (g) of *Zea mays* plants after 22 days of exposure to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Ferromontmorillonite (TechPhos, ST), and a commercial phosphorus salt (PS). Data are presented as mean $\pm$ standard deviation ($n = 15$). Means sharing a common letter are not significantly different ($p > 0.01$).

For root fresh biomass, Kruskal-Wallis results also indicated highly significant differences ($H = 32.96$, $p < 0.0001$). The PS (14.99 ± 2.57 g) and ST (14.63 ± 2.61 g) showed the highest root fresh biomass, being significantly greater than the Control (2.45 ± 0.84 g), while the SC treatment (9.06 ± 2.81 g) remained statistically similar to both the Control and the top-performing treatments (Figure 6b), with no significant differences between them. SC treatment (9.06 ± 2.81 g) was significantly superior to Control (2.45 ± 0.84 g), but significantly inferior to PS and ST. Control presented the lowest root fresh mass. The Figure 6b illustrates that root fresh biomass follows the pattern (PS, ST > SC > Control). Finally, for root dry biomass, significant differences were observed ($H = 28.50$, $p < 0.0001$). The PS treatment (2.48 ± 0.81 g), ST (1.49 ± 0.43 g), and SC (1.39 ± 0.53 g) treatments were all significantly superior to Control (0.33 ± 0.11 g), with no significant differences detected among the three. These results demonstrate that PS and ST treatments are the most consistent and effective promoters of growth and biomass accumulation in both shoot and root tissues.

3.6.3. Growth dynamics

Growth dynamics, evaluated through leaf number over time, showed distinct developmental patterns between treatments (Figure 7a). Throughout the observation period (22 days), PS and ST treatments exhibited consistently higher leaf production rates, reaching greater accumulated leaf numbers. SC treatment presented intermediate

dynamics, while Control showed the smallest increase in leaf number throughout the study.

Statistical evaluation of leaf number revealed highly significant differences between treatments ($H = 65.74$, $p < 0.0001$). Post-hoc analysis of mean ranks (Figure 7b) enabled treatment grouping: Control (4.04 ± 1.56 leaves) and SC (4.25 ± 1.80 leaves) formed a statistically indistinguishable group ('A'), characterized by lower leaf number. ST (4.86 ± 1.75 leaves) and PS (5.04 ± 1.51 leaves) constituted a second group ('B'), showing significantly higher leaf number with no significant differences between them.

3.6.4. Physiological state and pigment content

Physiological state evaluation focused on chlorophyll content and anthocyanin presence. For Chlorophyll-*a* content, analysis of variance (ANOVA) indicated significant differences between treatments ($F = 3.64$, $p = 0.0142$). Post-hoc Tukey test showed that Control (11.13 ± 3.40 g/cm²), SC (11.80 ± 2.72 g/cm²), and ST (11.14 ± 1.86 g/cm²) treatments did not differ significantly among themselves, presenting the highest levels of this pigment. PS treatment (8.95 ± 2.20 g/cm²) exhibited significantly lower Chlorophyll-*a* content ($p < 0.05$) compared to the other three treatments (Figure 7a).

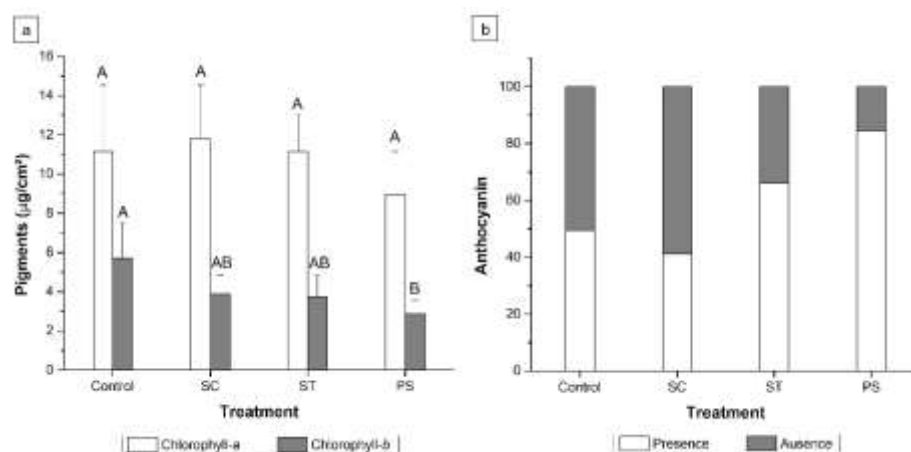


Figure 7. (a) Chlorophyll-*a* and Chlorophyll-*b* content and (b) proportion of plants showing anthocyanin pigmentation in *Zea mays* after 22 days of exposure to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Fe-montmorillonite (TechPhos, ST), and a commercial phosphorus salt (PS). Data are presented as mean $\pm$ standard deviation ($n = 15$). Means sharing a common letter are not significantly different ($p > 0.01$).

For Chlorophyll-*b*, the non-parametric Kruskal-Wallis test detected highly significant differences between groups ($H = 20.40$, $p = 0.0001$). Post-hoc rank analysis revealed a clear distinction: Control (5.69 ± 1.79 g/cm²) presented the highest Chlorophyll-*b* content, being significantly different ($p < 0.05$) from all other treatments. ST (3.76 ± 1.06 g/cm²) and SC (3.87 ± 0.93 g/cm²) treatments showed intermediate levels, with no significant differences between them. PS treatment (2.87 ± 0.69 g/cm²) recorded the lowest Chlorophyll-*b* content, differing significantly ($p < 0.05$) from all other groups. Figure 7a visualizes these differences, with a progressive decrease in Chlorophyll-*b* content from Control through ST/SC to PS. Regarding anthocyanin presence, no statistically significant association between treatments and presence/absence of these pigments were found (Chi-square = 12.00, $p = 0.21$) (Figure 7b).

3.7. Principal Component Analysis

Principal Component Analysis (PCA) was conducted to synthesize and integrate the data across treatments, with the goal of revealing the underlying structure of phenotypic variability in plants exposed to different experimental conditions (Figure 8). Correlation analyses were performed between the variables evaluated (Table S1. Supplementary material).

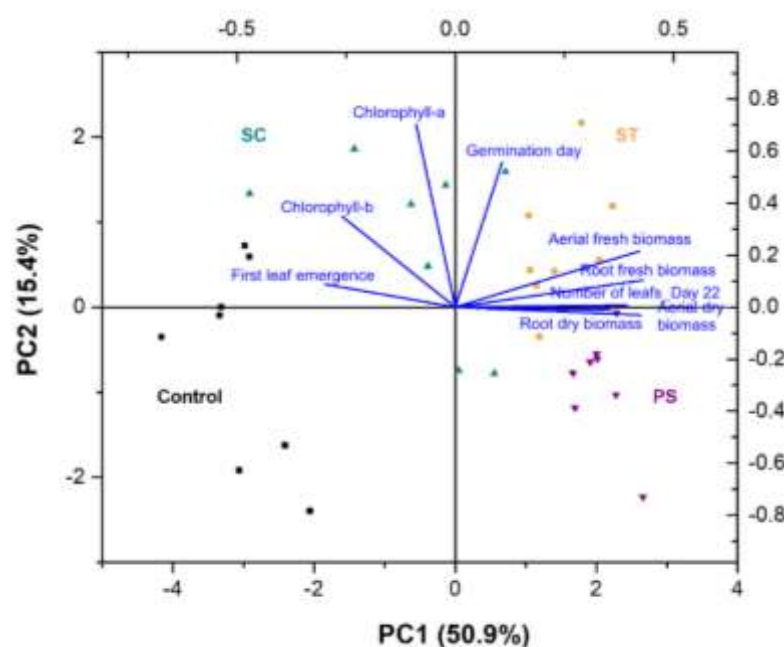


Figure 8. Principal component analysis (PCA) based on morphological and physiological variables measured in *Zea mays* plants exposure to untreated soil (Control), sludge from the Cardeal UWWTP (SC), sludge modified with Fe-montmorillonite (TechPhos, ST), and a commercial phosphorus salt (PS). Treatments are represented in the score plot, with separation along principal components indicating multivariate differences in plant response.

This analysis revealed that three principal components possess eigenvalues greater than unity, explaining a combined proportion of 77.5% of the total variance observed across the nine evaluated variables. The first principal component (PC1), which accounts for 50.9% of the variance exhibited high positive loadings with root fresh biomass, aerial dry and fresh biomass, number of leaves at day 22, and root dry biomass, along with a negative loading with first leaf emergence day. This configuration indicates that PC1 represents early establishment and the plant's capacity to generate biomass and vegetative structures, although the first leave emergence is not necessarily correlated with biomass. In corn and other plants, the timing of first leaf emergence does not directly predict biomass accumulation, as plant growth is influenced by multiple factors such as nutrient uptake, stress conditions, and developmental overlap between vegetative and reproductive stages, specially under the climate change scenario [70]

The second principal component (PC2), accounting for 15.4% of the variance, is characterized by high positive loadings of chlorophyll-*a*, the germination day, and chlorophyll-*b*. The third principal component (PC3), explaining 11.2% of the variance, appears to capture residual variations related to initial development timing and fresh biomass (see Table S2, Supplementary Material). While PC3 contributes to the total explanation of variability, the biplot visualization (Figure 8) focuses on PC1 and PC2 as they effectively discriminate the main treatment effects, while exhibiting less prominent

loadings, appears to capture residual variations related to initial development timing and fresh biomass, contributing to the total explanation of variability, although its interpretation requires further investigation.

Visualization of observation scores in the biplot (Figure 8) enabled clear discrimination of plant response strategies under the Control, SC, ST, and PS treatments. Plants in the Control group consistently clustered in the lower-left quadrant of the biplot, indicating the lowest growth vigor and biomass accumulation. In contrast, ST and PS treatments were located on the right side of the biplot (high PC1 scores), demonstrating superior efficacy in promoting growth. However, these treatments differed along the PC2 axis: ST concentrated in the upper-right quadrant, indicating that its growth vigor is associated with high chlorophyll content. The PS treatment was positioned in the lower-right quadrant, suggesting that its growth efficacy is associated with lower chlorophyll levels and faster germination. The SC treatment showed a more dispersed and centralized distribution, indicating a more variable or moderate effect across both dimensions.

Among the evaluated treatments, ST consistently promoted superior performance in terms of seed germination, biomass accumulation, and foliar development. While none of the treatments exhibited phytotoxic effects, both PS and ST significantly enhanced shoot and root growth, with ST showing comparable efficacy to PS and superior performance relative to SC and Control. Notably, ST also resulted in higher chlorophyll content, indicating a more sustained physiological benefit over time. Despite slower initial germination, ST led to faster first leaf emergence than SC and was statistically similar to PS in terms of shoot biomass. In contrast, SC alone exhibited intermediate results, enhancing growth compared to Control, but underperforming when compared to ST and PS. Root biomass followed a similar trend, with ST and PS inducing the highest values, SC showing moderate improvements, and Control consistently exhibiting the lowest performance across all parameters. Growth dynamics further reinforced these findings, with ST and PS treatments associated with greater leaf number over time. However, PS treatment presented significantly lower chlorophyll-*a* and chlorophyll-*b* content, suggesting that while it accelerated early growth stages, its long-term contribution to plant development may be limited. PCA analysis corroborated these results, which revealed distinct physiological response strategies. Plants under ST treatment were associated with high chlorophyll content and sustained vigor, whereas PS treatment clustered with fast germination but lower pigment content. SC treatment only showed moderate or variable effects.

Importantly, the comparable growth responses between SC and ST suggest that the nutrients inherently present in the SC sludge may be sufficient to support early corn development. However, the enhanced performance of ST indicates that the incorporation of TechPhos provides additional nutritional or structural benefits beyond phosphorus alone. These results reveal distinct treatment strategies in influencing plant development, providing a foundation for future research on underlying physiological mechanisms and optimization of cultivation protocols. The ability of PCA to visualize these interactions in a low-dimensional space demonstrates the utility of this technique for interpreting complex data.

4. Discussion

4.1. Physicochemical characterization

4.2. Experiment 1

Considering the germination parameters in Experiment 1, a GI value of 171.7 indicates that the ST treatment was 71.7% more effective than the Control. This outcome reflects the composite nature of the GI metric, which integrates both seed germination and root elongation. While the number of germinated seeds per plate showed no significant difference between ST (4.2 ± 0.2) and Control (4.5 ± 0.2), **radicle was 81% longer in ST than in the control** the radicle length in the ST treatment was 81.8% longer than that observed in the Control. Therefore, the elevated GI value is primarily attributed to enhanced radicle elongation in the ST treatment, rather than increased germination rates. The radicle elongation indicate that all three treatments provided more favorable conditions than Control for radicle growth, and the average elongation of *Z. mays* **hypocotyls mesocotyls** show that ST was significantly different from Control, PS, and SC. These findings suggest that SC and **especially** ST enhanced **hypocotyl mesocotyl** development compared to the Control.

According to the classification proposed by Shafique et al. [42], none of the treatments exhibited phytotoxicity. On the contrary, SC, PS and especially ST enhanced seed germination and root development in *Z. mays*. These observations provide valuable insights into the effects of sludge-based treatments on *Z. mays* germination and early development. The superior performance of the ST treatment underscores the potential of using sludge enriched with TechPhos as a sustainable alternative to conventional phosphorus fertilization.

4.3. Experiment 2

The results on shoot and root growth demonstrate that PS and ST treatments were the most consistent and effective promoters of growth and biomass accumulation in both shoot and root tissues, and the growth dynamics evaluated through leaf number over time, confirms that ST and PS promote superior foliar development, resulting in greater leaf quantity in plants under these treatments compared to Control and SC, and the pigment analysis showed a progressive decrease in Chlorophyll-*b* content from Control through ST/SC to PS. Regarding anthocyanin content, results suggests that under the conditions of this study, treatments did not differentially influence anthocyanin occurrence.

Overall, considering the physicochemical properties of the soil treatments—Control, SC, ST, and PS—along with the morphophysiological responses of corn plants in both experiments, several consistent patterns emerge. Among the key indicators of soil fertility, base saturation—which reflects the proportion of cation exchange sites occupied by essential nutrients such as calcium, magnesium, potassium, and sodium—is particularly important.

Studies suggest that base saturation levels ranging from 45% to 80% are generally adequate for optimal plant growth, depending on the crop species. In this context, liming proved beneficial for reaching suitable base saturation levels: 85% in the limed soil and 90% in the limed soil supplemented with ST, both of which fall within the range considered favorable for plant development [54].

Liming significantly improved key soil acidity parameters, including a fourfold reduction in potential acidity (H^+Al), pH neutralization to 6.8, an 80-fold decrease in aluminum saturation (M%), and a 19-fold reduction in exchangeable aluminum (Al^{3+}). The mitigation of aluminum toxicity is particularly critical, promoting root growth and enhancing nutrient uptake [55]. These chemical improvements also led to increased

nutrient availability: calcium concentrations rose by 17-fold and 34-fold following liming and ST application, respectively. Likewise, the sum of bases (SB) increased 18-fold, and extractable phosphorus reached 87.0 mg/L. Collectively, these changes suggest that none of the treatments (Control, SC, ST, or PS) posed toxicity risks to corn plants' development.

Comparison of the SC and ST treatments by FTIR analysis revealed characteristic signals of both organic and inorganic components. Notably, the ST amendment exhibited a higher phosphorus content, as confirmed by XRF analysis. Specifically, ST contained significantly greater concentrations of SiO_2 and P_2O_5 than SC, indicating an enhanced phosphorus-retention capacity in the TechPhos-enriched sludge compared to the polyaluminum chloride (PAC)-based SC treatment. The presence of silicon is particularly relevant, as it has been shown to mitigate oxidative stress in various plant species by increasing relative water content and activating antioxidant defense mechanisms [56]. In the context of the stress actually exerted by climate change, the application of silicon nanoparticles (Si NPs) has been reported to alleviate drought-induced stress in corn seedlings. Potassium-silica nanoparticles can enhance growth, yield, and drought tolerance in canola cultivars by harnessing the synergistic effects of potassium and silicon. Similarly, the use of Fe_2O_3 nanoparticles has shown promise in increasing biomass, yield, essential oil content, and phytochemical properties, while potassium promotes cell division and the synthesis of carbohydrates and proteins, ultimately contributing to improved grain yield [57-60].

Our findings show that in addition to increased phosphorus content, the ST amendment also contained higher concentrations of total nitrogen (1.8-fold), potassium (1.24-fold), Kjeldahl nitrogen (1.9-fold), and phosphorus, primarily in association with iron (1.04-fold). The enhanced nutrient availability likely underpinned the superior growth performance of corn in ST-treated soils, suggesting potential benefits for agricultural productivity. In contrast, the reduced development of corn plants observed in the untreated soil (Control) may be attributed to its lower nutrient content. Although corn plants also showed satisfactory development under the PS amendment, it is widely recognized that the frequent application of synthetic fertilizers at high rates, while effective for maximizing yields, can lead to environmental contamination—particularly of aquatic ecosystems.

Under sludge-based fertilization, especially in ST treatment, corn plants demonstrated improved performance compared to those grown in soil alone or with SC and PS treatments. This enhancement is likely due to better nutrient availability, improved root development, and increased plant resilience facilitated by the biofertilizer properties of the amendments.

These results are in line with those of Al-Suhaibani et al. [22], who emphasized the environmental and economic advantages of using treated wastewater in agriculture, including reduced dependence on freshwater resources. Treated wastewater is a cost-effective source of essential macronutrients (N, P, and K) and trace elements (Ca, Mg, B, Fe, Mn, and Zn), offering a sustainable alternative to synthetic fertilizers. Supporting this, Chojnacka et al. [61] demonstrated that the reuse of treated municipal wastewater could fully meet crop phosphorus and potassium demands based on its nutrient composition. Furthermore, a recent study by Ammeri et al. [62] highlights the potential of treated wastewater reuse as a strategy to mitigate global water scarcity. In addition, our previous study demonstrated that TechPhos is not toxic to lettuce (*Lactuca sativa*), soy (*Glycine max*), and rice (*Oryza sativa*) at 0.4 g L^{-1} , and enhanced germination and root growth of *L. sativa* and *G. max* seeds [63]. However, long-term studies are still needed to assess the

environmental fate of TechPhos, particularly its effects on soil health and microbial communities. Addressing these knowledge gaps will be essential for ensuring the safe and effective integration of phosphorus-supplying nanomaterials (PSNs) into sustainable agricultural practices.

The viability of P availability from sewage sludge depends strongly on the chemical form in which P is retained, particularly its association with metals such as aluminum (Al) or iron (Fe). In the SC sample, where phosphorus is predominantly bound to aluminum (P–Al), it tends to form highly stable complexes such as aluminum phosphate (AlPO_4). These compounds are sparingly soluble, especially under acidic conditions, which are common in many tropical and subtropical soils. As a result, the bioavailability of P from Al-bound forms is typically low. Although liming and neutralization of the soil can slightly increase P solubility, the release remains limited. Greenhouse studies have shown that even after multiple growing cycles, phosphorus recovery from alum-precipitated sludge remains modest, indicating a slow and inefficient nutrient release profile [64]. In contrast, sludge in which phosphorus is primarily associated with iron (P–Fe) presents a more favorable scenario for phosphorus resources shortage and crop development. Iron phosphates or phosphorus adsorbed to iron oxyhydroxides are more sensitive to changes in soil redox conditions [65]. Under anaerobic or reducing environments, Fe^{3+} can be biologically reduced to Fe^{2+} , leading to the dissolution of iron-bound phosphorus and its release into the soil solution [66]. This mechanism significantly enhances the availability of phosphorus to plants. Experimental data from greenhouse trials indicate that liming Fe-based sludges can further increase P uptake by plants—often more effectively than Al-based sludges under the same conditions [67]. Previous study has been developed to take advantage of the phosphorus recovery potential. For example, it was proposed to use of ash from commercially pure CaO additive in dry sludge calcination. Although a high rate of conversion of the non-apatite inorganic phosphorus fraction into apatite phosphorus was obtained in the tests [68], this approach is costly because sludge incineration needs up to 950 °C to obtain apatite phosphorus. Our findings indicate that the biogeochemical behavior of Fe allows for greater flexibility and responsiveness in phosphorus release, particularly in soils with variable redox or pH conditions. Therefore, from an agronomic and environmental standpoint, sewage sludge resource in which phosphorus is primarily associated with iron is a more suitable and effective candidate for use as a biofertilizer compared to sludge dominated by aluminum-bound phosphorus.

The characterization data (Sections 3.1–3.4) and agronomic results (Section 3.6) are largely consistent, demonstrating that the sludge modified with Fe-montmorillonite (TechPhos, ST) improves nutrient content, reduces aluminum toxicity, and enhances corn growth compared with untreated soil (Control) and the polyaluminum-based sludge (SC). However, some analytical inconsistencies should be acknowledged. The total Fe content increased from SC to ST according to wet-chemical analysis, while XRF data expressed as Fe_2O_3 (% wt) showed an apparent decrease, likely reflecting differences in analytical basis—soluble versus total oxide fractions. Similarly, the reported increase in phosphorus after TechPhos addition was relatively small (94.5 to 97.7 mg kg^{-1}), suggesting that the improved plant response results more from changes in Fe–P coordination and nutrient bioavailability than from bulk P concentration. Moreover, part of the improvements in soil chemistry (notably Ca and Mg increases) derive from liming rather than from the amendments themselves. Despite these limitations, the compositional trends, supported by agronomic performance, clearly indicate that the integration of Fe-rich nanostructured clays into wastewater-derived sludge promotes a more favorable chemical environment for early plant development. Moreover, long-term field experiments in tropical Brazil

demonstrated that sewage sludge can enhance soil microbial activity, enzymatic functions, and nutrient content, improving soil fertility and productivity in maize and soybean crops [69].

This study demonstrates the potential of sludge-based treatments to enhance the germination and early development of *Zea mays* L. Overall, these findings highlight the feasibility of repurposing urban wastewater sludge—especially when combined with functionalized clay materials such as TP—as a sustainable alternative to conventional nitrogen, potassium, and phosphorus fertilization in nutrient budgeting. This approach not only reduces dependence on synthetic fertilizers but also promotes circular economy practices by valorizing underutilized resources like wastewaters, with clear environmental, agronomic, and economic advantages for food production.

However, environmental risks accompany these benefits. Biosolids often contain heavy metals such as Zn, Ni, Pb, and Cr, and organic pollutants that may accumulate with continuous application [71]. In this work, we detected Zn and Mn in low concentrations. The use of sewage sludge as a biofertilizer demands strict control of contaminants—particularly heavy metals—alongside continuous agronomic and environmental monitoring to minimize risks to human health, soil quality, and water resources. Based on four years of monitoring urban wastewater treatment plants in Argentina, authors consistently observed that when systems receive exclusively domestic sewage, without industrial inputs, ecotoxicologically relevant heavy metals (Cd, Cr, Pb, Hg) are typically absent or below detection limits in influent streams. For the effluent evaluated in this study, all elements detectable by XRF were reported in Table 3, reflecting a composition consistent with other domestic effluents. While this supports the assumption that metal contamination is not significant under the studied conditions, it is critical to acknowledge that this conclusion depends on strict source control and may not hold under changes in wastewater composition or infrastructure conditions. Regulatory frameworks define safe use parameters. In Brazil, CONAMA Resolution 498/2020 and MAPA Normative Instruction 61/2020 establish metal and pathogen limits, aligning with U.S. (EPA 40 CFR Part 503) and EU (Directive 86/278/EEC; Regulation 2019/1009) standards [72–74]. These frameworks promote biosolid valorization under controlled and monitored conditions. In controlled conditions, sewage sludge can be transformed from an environmental liability into a renewable agricultural resource when adequately treated and applied.

It should be emphasized that our experiment was short-term and conducted at the pot-scale, and therefore not directly scalable. Pot experiments with *Brassica napus* and *Zea mays*, play a key role in assessing the potential of plants before their use in field conditions [75]. While this represents a promising approach, further validation is required. The experimental framework outlined in this work may serve as a basis for pilot-scale trials, with the potential to scale up for industrial applications.

5. Conclusions

This study conducted laboratory and pot-scale experiments and demonstrated that phosphorus recovery from sewage sludge can support the sustainable development of *Zea mays* by conserving finite phosphorus reserves. In this approach, the adsorbent is retained within the final biosolids rather than separated, thereby enabling their direct application as a biofertilizer. Such integration reduces the operational demands of wastewater treatment plants (WWTPs) while simultaneously transforming waste into a value-added resource.

Furthermore, the findings highlight the potential of Fe-montmorillonite-modified sludge to advance circular economy practices and enhance soil fertility at larger scales.

This strategy is proposed as a future pathway to mitigate climate change impacts and promote sustainable resource utilization. Importantly, it aligns with the United Nations Sustainable Development Goal 2 (SDG 2), which emphasizes food security and sustainable agriculture.

Supplementary Materials: The following supporting information can be downloaded at: [**Author Contributions:** Conceptualization, W.A.C., A.L.U., and A.M.G.; methodology, A.L.U., A.M.G., N.R., and L.F.L.M.; software, N.R., and L.F.L.M.; validation, W.A.C., A.L.U., and A.M.G.; investigation, W.A.C., A.L.U., and A.M.G.; resources, W.A.C., L.R., and A.M.G.; data curation, N.R., and L.F. L.M.; writing—original draft preparation, A.M.G., and W.A.C.; writing—review and editing, W.A.C.; A.M.G., N.R., and L.R.; supervision, W.A.C., and A.M.G.; project administration, W.A.C., and L.R.; funding acquisition, W.A.C., L.R., and A.M.G.; All authors have read and agreed to the published version of the manuscript.](https://www.mdpi.com/article/doi/s1, Figure S1: Experiment 2; Table S1. Correlations and statistical significance among the analyzed variables.</p>
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Conflicts of Interest: The authors declare no conflicts of interest.

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