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Comparative Evaluation of Chitosan-Based Biopolymer and Conventional Phosphorus Extractants on Phosphorus Availability, Soil pH Dynamics, and Maize Agronomic Performance in Amended Soils

Aghorunse Adeoba Courage[1], *Bankole Ganiyu Olawale[1], Adelana Festus Olugbenga[1], Buzugbe Racheal Ayo[2], Adewuyi Sheriff[2], and Azeez Jamiu Oladipupo[1]

[1]Department of Soil Science and Land Management, Federal University of Agriculture Abeokuta, Ogun state, Nigeria

[2]Department of Chemistry, Federal University of Agriculture Abeokuta, Ogun state, Nigeria

***Corresponding Author:** Tel: +234 703 249 8290; bankolego@funaab.edu.ng

Abstract

Phosphorus (P) availability in tropical soils is commonly restricted by strong fixation processes, mainly through adsorption onto iron and aluminum oxides and precipitation with calcium compounds, which significantly reduce its mobility and plant utilization. This study evaluated a chitosan-based biosorbent (nZvFe-CLCB) as a chemical extractant for assessing soil phosphorus availability, alongside conventional extractants (Bray-1 and Olsen), and examined their relationship with soil pH dynamics and maize performance under different P amendments. Soil samples from six locations in southwestern Nigeria were experimented under screenhouse conditions in a completely randomized design, with three treatments: single superphosphate (SSP), poultry manure (PM), and a control. Soil chemical properties, extractable P, electrical conductivity (EC), pH, maize dry matter weight (DMW), and P uptake were monitored over eight weeks after planting (WAP). Results showed Olsen extractant showed 11 to 15% higher P recovery than Bray-1 and nZvFe-CLCB in amended soils. Bray-1 extractable P was <5% higher than nZvFe-CLCB in native soils. Soil amended with SSP increased maize DMW by 22 to 79% and P uptake by 24 to 118% compared to the control while PM increased DMW by 23 to 102% and P uptake by 25 to 91%. The application of SSP led to soil acidification (pH 5.3) while soil EC increased over time, attaining values of 480–870 µS cm^{-1} (SSP) and 750–960 µS cm^{-1} (PM) at 8 WAP. The nZvFe-CLCB extractant significantly correlated with Bray-1 (r = 0.731*) but not with Olsen and showed a stronger predictive capacity for both DMW (0.633 vs 0.538) and P uptake (0.686* vs 0.629) compared to Olsen. Soil pH showed a significant and positive relationship with all extractants, following the order Bray-1 (0.851**) > nZvFe-CLCB (0.732*) > Olsen (0.688*). Bray-1 had the strongest association with maize DMW (r^2 = 0.777*) and P uptake (r^2 = 0.820**), while soil pH had a strong influence on P availability across methods up to r^2 = 0.851**. The finding concluded that nZvFe-CLCB extractant as an alternative extractant for Bray 1 in predicting plant-available P in tropical soils.

Keywords: Chitosan-based extractant (nZvFe-CLCB); Maize productivity; Phosphorus availability; Soil pH dynamics; Tropical soils

1.0 Introduction

Phosphorus (P) is a limiting macronutrient in terrestrial agroecosystems owing to its low solubility, rapid fixation, and high affinity for binding with minerals in soils (Mishra *et al.,* 2017; Solangi *et al.,* 2023). However, phosphates and chitosan as well as negatively charged soil surfaces form sparingly soluble PO_4^{3-} minerals in acidic conditions and insoluble calcium phosphate minerals in basic conditions, leading to very poor PO_4^{3-} utilization efficiencies of less than 20 percent (Amarasinghe *et al.,* 2022; Ogwu *et al.,* 2025). These transformations are regulated by ligand-exchange reactions and mineral dissolution and formation equilibria, all of which control PO_4^{3-} species distribution and migration within the soil system (Ge *et al.,* 2025).

Soil phosphorus availability is operationally defined through chemical extraction procedures that quantify labile P fractions associated with specific soil pools (Laan *et al.,* 2024). Conventional extractants like Bray I (acid fluoride extraction), Olsen (bicarbonate extraction), and Mehlich-3 (multi-nutrient acidic chelation system) operate on different chemical mechanisms involving proton-promoted dissolution, competitive anion exchange, and metal complexation reactions (Bray and Kurtz, 1945; Olsen *et al.,* 1954; Mehlich, 1984). These extractants give empirical values for the availability of phosphorus but do not adequately account for dynamic processes such as sorption–desorption hysteresis, where phosphorus released during desorption is lower than that previously retained during adsorption due to partial irreversibility of binding on soil mineral surfaces. They also fail to capture shifts in phosphorus speciation driven by pH-dependent transformations (Ge *et al.,* 2025). Consequently, discrepancies between extractable P and actual plant uptake remain a persistent limitation in soil fertility diagnostics.

Phosphorus chemistry in soil is controlled by pH-dependent surface charge development and proton activity, which regulate both PO_4^{3-} ion speciation ($H_2PO_4^-$, HPO_4^{2-}) and the reactivity of soil colloidal surfaces. The distribution between these phosphate species is strongly pH-dependent, with $H_2PO_4^-$ dominating under acidic conditions and HPO_4^{2-} becoming more prevalent as pH increases, thereby influencing adsorption–desorption behaviour on mineral surfaces (Gustafsson *et al.,* 2012; Mahmood *et al.,* 2025). The dominance of positive charges under acidic conditions facilitates PO_4^{3-} adsorption on Fe/Al hydroxides through inner-sphere bidentate or monodentate ligand exchange, while calcium phosphate precipitation prevails under alkaline conditions because of the lower solubility of Ca-P minerals (Penn and Camberato, 2019; Yalin *et al.,* 2025). Soil pH acts as the main factor controlling the buffering capacity, kinetics of phosphorus desorption, and diffusion gradients within the rhizosphere (Custos *et al.,* 2020).

Chitosan is a linear cationic polysaccharide that consists of β-(1→4)-linked D-glucosamine units, obtained through the partial deacetylation of chitin (Desai *et al.,* 2023), and characterised by a high density of ionisable amino functional groups (Das *et al.,* 2024). The protonation of these amino groups under acidic to near-neutral conditions imparts a pH-dependent positive charge to the polymer (Butnariu, 2023), which facilitates electrostatic attraction with anionic species such as phosphate (Wujcicki and Kluczka,2023). In addition, chitosan can participate in ligand exchange and surface complexation reactions with soil mineral phases, enhancing its affinity for soil colloids and influencing phosphorus sorption–desorption equilibria (Laan *et al.,* 2024). This interaction modifies PO_4^{3-} sorption–desorption equilibria through competitive displacement of adsorbed PO_4^{3-}, hydrogen bonding, and coordination with metal centers on oxide surfaces. Additionally, chitosan influences soil chemical microenvironments by altering proton exchange reactions, modifying cation exchange capacity, and affecting enzymatic and microbial phosphorus mineralization pathways (El Hadrami *et al.,* 2010; Wang *et al.,* 2025).

Maize (*Zea mays* L.) is highly responsive to P fertilization due to its relatively high P demand during early vegetative and reproductive growth stages. Phosphorus deficiency in maize results in reduced root elongation, impaired energy metabolism, delayed maturity, and significant yield penalties, particularly in highly weathered tropical soils with strong P-fixation capacity (Gurmu, 2023; Phiri *et al.,* 2024). However, plant uptake efficiency is strongly governed by the synchrony between soil P release and root absorption capacity, which is mediated by soil pH gradients, root exudation of organic acids, and rhizosphere-induced desorption processes.

Current soil P extraction methodologies, particularly Bray-1 and Olsen, are based on operationally defined chemical extractions that quantify discrete labile P fractions. Whilst these methods remain widely adopted in soil fertility evaluation, they are fundamentally equilibrium-based procedures that provide static estimates and do not adequately capture the kinetic and mechanistic processes governing phosphorus mobilisation in the soil–plant system. In highly weathered tropical soils, where P availability is predominantly regulated by adsorption onto Fe and Al (hydr)oxides, inner-sphere surface complexation, and pH-dependent sorption–desorption equilibria, such extractants often exhibit weak or inconsistent correlation with plant-available P and crop uptake. In contrast, chitosan-based materials possess protonable amino functional groups ($–NH_2/–NH_3^+$) capable of participating in electrostatic attraction, ligand exchange reactions, and surface complexation with phosphate species and reactive soil colloids. Despite these well-defined chemical interactions, their application has largely been restricted to sorption, immobilisation, and remediation studies, with limited exploration as analytical extractants for quantifying plant-available phosphorus under varying soil amendment regimes. Coventional P extractants serve only as a diagnostic tool, while new biopolymers like chitosan can be both diagnostic tools as well as reactive chemicals that correlate with plant uptake. The comparison of these two methods in fertilized soils offers a possibility to establish a connection between analytical techniques for soil testing and nutrient uptake. Therefore, this paper aims to compare the effectiveness of chitosan and traditional P extractants in influencing P bioavailability, and maize performance under P fertilization.

2.0 Materials and Methods

2.1 Description of the Experimental Locations and Phases of the Experiment

Chitosan synthesis was carried out in the TETFund-supported Laboratory of the Department of Chemistry, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. Incubation and laboratory analyses were undertaken at the Soils of Forest Island in Africa (SOFIIA) Laboratory. The pot experiment was conducted under screenhouse conditions at the Centre for Agricultural Development and Sustainable Environment (CEADESE), FUNAAB, Ogun State, Nigeria (7°13′N, 3°26′E). The study area falls within the humid tropical rainforest agro-ecological zone of southwestern Nigeria, characterised by a mean annual temperature of about 26–28 °C, annual rainfall of approximately 1,200–1,500 mm, and relative humidity of about 65–80%, and natural photoperiod conditions at the Centre for Agricultural Development and Sustainable Environment

(CEADESE), FUNAAB, Ogun State, Nigeria.

2.2 Chitosan Synthesis

2.2.1 Preparation of chitosan solution

Chitosan flakes with a degree of deacetylation of ≥ 85% and a molecular weight of approximately 100–300 kDa were obtained from an analytical chemical store. A total of 2.0 g of chitosan was dissolved in 100 mL of 5% (v/v) glacial acetic acid solution. The mixture was stirred continuously and heated at 50 °C for 1 h until complete dissolution was achieved. Entrapped air bubbles were removed by allowing the solution to stand at room temperature (Hosseini *et al.,* 2008). The resulting chitosan solution served as the precursor for the preparation of D-glucosamine-based biosorbent materials.

2.2.2 Preparation of chitosan-based biosorbents (nZvFe-CLCB) (Whan *et al.,* 2005)

Chitosan-Epichlorohydrin cross-linked beads was prepared by the suspension of the prepared chitosan beads into a 125 ml solution of 0.10 M ECH containing 0.067 M NaOH to obtain pH 10 for the ECH solution at a suspension ratio of 1:1. The reaction mixture was maintained at 45 ± 2 °C for 2 h under continuous stirring at 200 rpm using a magnetic stirrer to obtain uniform cross-linking.. After 2 hours, the crosslinked chitosan bead was filtered, washed with hot, followed by cold distilled water to remove any excess ECH solution. The chitosan ECH-beads were air-dried at ambient temperature. Prior to cross-linking, chitosan solution was first prepared by dissolving 2 g of chitosan flakes in acetic acid solution under continuous stirring. Thereafter, 30 mL of epichlorohydrin (ECH) was added to the chitosan solution with continuous stirring until a clear and homogeneous mixture was obtained. The resulting solution was then dropped into sodium hydroxide solution to induce bead formation through gelation.

To prepare the nanosized iron-modified beads (nZvFe-CLCB), the dried chitosan–epichlorohydrin cross-linked beads were immersed in an aqueous solution of ferric chloride ($FeCl_3 \cdot 6H_2O$). The suspension was placed on a magnetic stirrer and stirred continuously at 30 °C for 45 min to ensure adequate iron impregnation onto the bead surface. Thereafter, the beads were removed, rinsed to eliminate excess iron solution, and air-dried at room temperature. The dried nZvFe-CLCB biosorbent was then stored in airtight containers and kept for subsequent phosphorus extraction experiments

2.2.3 Extraction of available phosphorus using nZvFe-CLCB biosorbent

Based on the information obtained from the characterization phase, 1 g of air-dried soil was quantitatively transferred into 120 mL extraction bottles containing 30 mL of 0.01 M $CaCl_2$ as the basal electrolyte. Thereafter, 2 g of chitosan-based biosorbent (nZvFe-CLCB) was added to each bottle, and the suspension was shaken for 45 min at 25 °C to allow phosphorus adsorption onto the biosorbent. After equilibration, the biosorbent was separated from the suspension by filtration through Whatman No. 42 filter paper. The retained biosorbent was then transferred into fresh 30 mL portions of 0.01 M $CaCl_2$ and subjected to desorption under identical conditions (25 °C, 45 min shaking). The final suspension was again filtered using Whatman No. 42 filter paper. Phosphorus concentration in the clear filtrate (1 mL aliquot) was determined colorimetrically using the molybdenum blue method (Murphy and Riley, 1962).

2.3 Conventional Extractants

Available phosphorus was also determined using Bray-1 extractant (0.025 N HCl + 0.03 N NH_4F) and Olsen extractant (0.5 M $NaHCO_3$ adjusted to pH 8.5), following standard procedures (Bray and Kurtz, 1945; Olsen *et al.,* 1954).

2.4 Soil Samples Collection and Preparation

Soil samples were collected from six (6) cultivated agricultural fields (Imeko, Alabata, Owode, Ewekoro, Igbeji and Ijale-orile) based on land use history and parent material (Table 1). The samples were collected at a depth of 0-20 cm. Composite samples were made by bulking several samples collected within a unit. The collected soil samples were air-dried, pulverized and sieved with 2 mm mesh size and analyzed for their physical and chemical properties.

2.5 Soil initial analyses

Particle size distribution of the soil samples was determined using the hydrometer method (Bouyoucos, 1951). Soil pH and electrical conductivity (EC) were measured concurrently in a 1:2 soil-to-water suspension using a glass electrode pH meter and an EC meter respectively, following standard procedures (McLean, 1982; Jackson, 1973). Organic matter content was determined by the Walkley and Black wet oxidation method (Walkley and Black, 1934). Total nitrogen was analysed using the modified micro-Kjeldahl digestion technique (Jackson, 1964). Exchangeable bases (Ca, Mg, K and Na) were extracted with 1.0 M ammonium acetate (NH_4OAc) buffered at pH 7.0. Calcium and magnesium concentrations in the extracts were determined using atomic absorption spectrophotometry (AAS), while potassium and sodium were quantified using a flame photometer. Exchangeable acidity (Al^{3+} + H^+) was determined separately, and effective cation exchange capacity (ECEC) was computed as the sum of exchangeable bases and exchangeable acidity. Dithionite–citrate–bicarbonate (DCB) extraction was used for free iron and aluminium oxides, given their role in phosphorus sorption in highly weathered tropical soils, and the concentrations were determined using AAS. Micronutrients were extracted using 0.1 M hydrochloric acid and quantified using AAS. Available phosphorus was extracted using Bray-1 (Bray and Kurtz, 1945) and determined colourimetrically using the molybdenum blue method (Murphy and Riley, 1962) at 880 nm wavelength using a UV–Vis spectrophotometer.

2.6 Treatments Collection and Preparation

Poultry manure was collected from College of Animal Science and Livestock Production farm (FUNAAB), where birds are managed under a semi-intensive production system. Single super phosphate (SSP) was sourced from an agrochemical store in Abeokuta, Nigeria. The manure was air-dried, sieved using 0.5 mm sieve and subjected to nutrient analyses following standard analytical procedures for organic fertilisers (AOAC, 2016; IITA, 1979).

2.6.1 Treatments

The treatments consisted of; control (no amendments), poultry manure (90 kg P ha^{-1}), and Single superphosphate (90 kg P ha^{-1}).

2.6.2 Screenhouse studies

The experiments were laid in a Completely Randomized Design with three treatments, six soils (Alabata, Imeko, Owode, Ewekoro, Igbeji, and Ijale-orile) samples in three replicate. Ten (10) kg of the collected soils were weighed into a 25 liter of pot and poultry manure was applied. Maize seeds (Oba super 6) were sown at two seeds per pot and later thinned to one plant per pot at 2 weeks of poultry manure incorporation. The manure was applied 2 weeks before planting by mixing thoroughly with the soil while SSP was applied after 1 week after planting. Soil moisture was maintained by adjusting it to the pot moisture capacity with distilled water. Soil samples were collected at week 2, 4, 6, and 8 weeks after planting (WAP) and analyzed for available P using nZvFe-CLCB, Bray-1 and Olsen. The experiment was terminated at 8 WAP, maize was harvested, air dried and kept in paper bags; the dry matter weight (DMW) was determined by measuring the loss of water brought about by oven drying at a temperature of 65°C until a constant weight was obtained. The dried plant material was milled and thereafter the P uptake in the plant was determined using the formula:

$$P \text{ uptake } (\text{kg ha}^{-1}) = \left(\frac{\%\, P\ Concentration\ in\ plant \times dry\ matter\ weighr\ (kg\ ha^{-1}}{100}\right)$$

(IITA, 1979; AOAC, 2016)

2.7 Statistical Analysis

Data collected was subjected to Analysis of Variance (ANOVA) using the GenStat (9th edition). Treatment means were separated using Duncan's Multiple Range Test (DMRT) at 5% probability level. Pearson's correlation analysis was performed to determine the relationships among soil pH, extractable phosphorus from the different extractants, and maize dry matter weight (DMW). The correlation coefficients (r) were computed using the GenStat statistical package.

3.0 Results and Discussion

3.1. Physical and Chemical Properties of the Soils and Poultry Manure Used

The physical and chemical properties of the soil used in the study are shown in Table 2. The soils texture ranged from sand to loamy sand and the pH of the soils ranged from moderately acid (sand) to neutral. The soil salinity value was lowest and highest in Owode and sandy Alabata, respectively. The total organic carbon (C) and nitrogen (N) of the soils were low; however, the available phosphorus (P) content in the soils was moderate but lower in Ewekoro.

The dominance of sandy to loamy sand textures across the study locations indicates inherently low nutrient retention capacity, weak aggregation, and reduced surface reactivity. Such soils are typically characterized by limited sorption sites and high susceptibility to nutrient leaching, particularly for mobile nutrients like phosphorus in its soluble forms (Shen *et al.,* 2011). The moderately acidic to near-neutral pH range observed suggests a transitional chemical environment where both Fe/Al-bound P and Ca-associated P reactions may occur simultaneously. According to Penn and Camberato (2019), this pH range is critical because slight shifts can significantly alter PO_4^{3-} speciation and sorption equilibria. The low organic matter reduces microbial activity and limits the formation of organic ligands that could otherwise enhance phosphorus desorption and availability (Laan *et al.,* 2024). The comparatively lower available P in Ewekoro, despite moderate levels overall, may be attributed to higher clay content and associated adsorption processes, which tend to reduce P lability through fixation mechanisms (Gustafsson *et al.,* 2012).

The variation in ECEC across the soils reflects differences in clay mineralogy and organic matter content, with higher values indicating greater capacity for cation retention but also increased potential for PO_4^{3-} adsorption onto reactive surfaces (Mahmood *et al.,* 2025). The consistently high base saturation (>96%)

suggests dominance of basic cations and minimal exchangeable acidity, indicating that soil acidity is not primarily driven by Al toxicity but rather by proton activity in the soil solution. This condition may moderate extreme P fixation but does not eliminate sorption constraints. The distribution of micronutrients, particularly the dominance of Mn and Fe, further supports the presence of active oxide phases, which play a central role in phosphorus retention and transformation in tropical soils. These oxides can strongly bind PO_4^{3-} ions, thereby reducing immediate bioavailability despite moderate extractable P levels (Gustafsson *et al.,* 2012).

The chemical characteristics of poultry manure used for the experiment are shown in Table 3. The pH of the manure was moderately acidic. The manure's total phosphorus (P) content was lower than total nitrogen (N) and recorded low C:N ratio. Also, the concentration of calcium was higher than magnesium (Mg), potassium (K), and sodium (Na). The micronutrients contents of the manure were in the order of magnitude; Zn > Fe > Mn > Cu.

The moderately acidic nature of the poultry manure (PM) suggests its potential to influence soil pH upon application, particularly in buffering alkaline microsites and enhancing nutrient solubility. The low C:N ratio indicates rapid mineralization and nutrient release, which is advantageous for synchronizing nutrient supply with crop demand. However, the relatively lower P content compared to nitrogen implies that P release from the manure may be gradual and mediated through microbial processes rather than immediate dissolution. This aligns with findings by Mamun *et al*. (2022), who reported that organic amendments enhance phosphorus availability through mineralization and organic ligand interactions rather than direct solubilization. The relatively high Ca content in the manure may also influence P dynamics by promoting Ca–P interactions under near-neutral conditions, potentially affecting long-term P solubility.

3.2. Effect of Phosphorus Fertilization on the Agronomic Parameters of Maize at Harvest under Screenhouse Study

The treatment effect was not significant ($P \leq 0.05$) on the number of maize leaves in all locations, except in Imeko soil, though the un-amended plot had the lowest number of leaves in all soils (Table 4). The lack of significant treatment response in leaf numbers suggests that maize leaf initiation was not strongly limited by P supply under most of the studied soils, indicating that early vegetative growth was partially sustained by native soil labile P pools and short-term mineralization processes under controlled moisture conditions. This agrees with Shen *et al*. (2011), who reported that maize may initially rely on internal P remobilization and small labile soil P fractions before external P demand becomes critical. However, the consistently lower leaf number in the control reflects physiological constraints associated with reduced ATP synthesis and impaired nucleic acid metabolism under P deficiency, which limits cell division and early vegetative development (Mansab *et al.,* 2025).

The application of SSP and PM recorded 28 and 37%, 18 and 14%, 35 and 4%, 51 and 9%, 35 and 15%, 15 and 27% higher leaf area than the control in soils of Ewekoro, Alabata, Igbeji, Ijale-orile, Imeko and Owode, respectively. These increases reflect enhanced canopy expansion and improved assimilatory surface area driven by improved P availability, which is critical for energy transfer (ATP/ADP cycling), photosynthate partitioning, and biomass accumulation. The stronger response to SSP is consistent with its high solubility and immediate PO_4^{3-} release, which enhances early root uptake and shoot development, as also reported by Penn and Camberato (2019) in their review of soil P chemistry and plant availability. In contrast, poultry manure (PM) likely released P gradually through microbial mineralization of organic P compounds, leading to delayed synchronization between nutrient release and maize demand (Kacprzak *et al.,* 2023).

Generally, the unamended plot (control) recorded significantly lower maize plant leaf area compared to pots amended with SSP in all locations, except Ewekoro and Owode. The weaker response in these soils may be associated with relatively higher buffering capacity (ECEC and base saturation effects), which can moderate nutrient stress through adsorption–desorption equilibrium processes that maintain minimal P availability even in the absence of fertilizer inputs (Mahmood *et al.,* 2025).

Pot amended with PM was observed to record significantly lower leaf area of maize plant compared to pots treated with SSP in Igbeji, Ijale-orile, and Imeko soils. However, no significant difference was observed between the control and PM amended in soils of Igbeji and Ijale-orile and Imeko. This indicates limited short-term P bioavailability from poultry manure, likely due to immobilization of organic phosphorus compounds, slower enzymatic hydrolysis (phosphatase-mediated mineralization), and temporary microbial P immobilization, which delay plant-accessible PO_4^{3-} release (Laan *et al.,* 2024).

The application of PM had no significant effect on the plant height of maize relative to the control in all locations except Ewekoro; however, soils treated with SSP recorded significantly higher plant height of maize than the control except in soils of Ewekoro and Ijale-orile. This shows the strong dependence of maize stem elongation on readily available P, which supports energy metabolism, root expansion, and meristematic activity required for plant growth (Shen *et al.,* 2011; Gurmu, 2023).

Soil amended with PM recorded the widest stem girth of maize and was observed to be significantly wider than control except in soil of Alabata. This suggests that PM improved biomass partitioning toward structural development, likely through enhanced soil organic matter content, improved aggregation, and gradual nutrient release that supports sustained cambial activity and stem thickening, a response commonly associated with organic amendments (El Hadrami *et al.,* 2010).

3.3. Effect of phosphorus fertilization on soil pH and electrical conductivity under screenhouse study

A consistent decrease was observed in the pH values of all treatments, with sampling time, however the change in pH was slightly lower in unamended soils (Table 5). This temporal decline in soil pH suggests progressive acidification processes driven by rhizosphere activity, nitrification of ammonium-based N fractions, and root exudation of organic acids during maize growth. According to Penn and Camberato (2019), P fertilization can indirectly influence soil pH dynamics through fertilizer dissolution reactions and associated proton release, particularly in acidic to neutral soils where buffering capacity is limited. The lower pH in the control indicates reduced biological and chemical perturbation, consistent with lower nutrient cycling intensity in unamended soil.

The application of SSP and PM had a significant impact on the soil pH when compared with the control, such that soil treated with SSP recorded significant lower pH values than the control from 2 weeks after planting (WAP) in soils of Ewekoro, Igbeji, and Imeko, and from 4 WAP in soils of Alabata, Ijale-orile, and Owode while soils treated with PM recorded significant lower pH values than the control from 4 WAP in soils of Igbeji, and Imeko, and at 8 WAP in all soils except Ijale-orile. The stronger acidifying effect of SSP in early stages can be attributed to its rapid dissolution and release of monocalcium phosphate, which enhances proton activity in the soil solution and promotes localized acidification zones around fertilizer granules. This aligns with Gustafsson *et al*. (2012), who reported that soluble phosphate fertilizers can induce short-term pH depression due to ion exchange reactions and Ca^{2+} displacement. In contrast, PM exerted a delayed pH effect due to gradual decomposition, microbial processing, and release of organic acids during mineralization, resulting in a slower but more sustained modification of soil pH.

Similarly, soils amended with PM recorded significantly higher pH values than SSP treated soils at 8 WAP in all locations except Imeko. This suggests that although poultry manure initially contributes to acidification through organic matter decomposition, it subsequently buffers soil acidity due to the release of basic cations such as Ca^{2+} and Mg^{2+}, as well as the formation of humic substances that enhance soil buffering capacity. El Hadrami *et al*. (2010) noted that organic amendments often moderate extreme pH fluctuations through complexation and cation exchange processes, thereby stabilizing soil reaction over time.

Soil salinity was observed to increase with increase in sampling time in amended soils, however, SSP and PM amended soils recorded significantly higher soil electrical conductivity (EC) than the control (Table 6). The observed increase in EC reflects enhanced ionic strength of the soil solution due to dissolution of fertilizer salts and release of exchangeable cations during decomposition processes. This is a common response in fertilized systems where nutrient additions increase soluble ion concentration and overall osmotic potential of the soil solution (Shen *et al.,* 2011).

The application of SSP had a more pronounced and significant effect on soil EC at the initial week (2 WAP) than soils treated with PM, such that the EC in soils treated with SSP was significantly higher than PM amended soils at 2 WAP in all locations except Imeko. This is attributable to the high solubility of SSP, which rapidly releases Ca^{2+} and $H_2PO_4^-$ ions into the soil solution, thereby sharply increasing EC in the early growth stage. In contrast, poultry manure releases ions more gradually through decomposition-mediated mineralization processes.

Soil amended with PM recorded significantly higher EC than soils treated with SSP at 6 and 8 WAP in all locations except Igbeji and Owode and was significantly higher than all locations at 8 WAP. This late-stage increase indicates cumulative mineralization of organic residues, leading to sustained release of soluble ions over time, which enhances EC beyond that of inorganic fertilizer treatments in the longer term (Laan *et al.,* 2024).

3.4 Effect of extractants on available phosphorus extraction under screenhouse study

The trends across WAP in unamended soils under screenhouse study (Table 7) showed Olsen extracted P content was higher in Ewekoro compared to other locations and was observed to extract significantly higher available P than nZvFe-CLCB across all locations, however, no significant difference was observed between Olsen and Bray-1 extracted P in unamended soils. This pattern indicates that under low external P inputs, soil P exists predominantly in weakly labile pools where bicarbonate-based extraction (Olsen) and acid-based extraction (Bray-1) access a similar reactive fraction. The slightly lower response of nZvFe-CLCB suggests limited desorption efficiency, possibly due to stronger selectivity for specific inorganic P pools and reduced disruption of Fe/Al-bound P complexes under neutral to slightly acidic tropical conditions (Hedley *et al.,* 1982; Sanchez, 2019). Available P content extracted by Bray-1 was observed to be statistically similar to nZvFe-CLCB though higher.

In soils treated with SSP (Table 8), the Olsen extractant was observed to extract significantly higher available P content than Bray-1 and nZvFe-CLCB in all locations, recording 11 and 14% higher available P extracted than Bray-1 and nZvFe-CLCB, respectively. This superiority of Olsen extraction under SSP fertilization reflects a shift in soil P speciation toward more readily desorbable orthophosphate ions and Ca-associated phosphate fractions. The bicarbonate medium likely enhanced displacement of PO_4^{3-} from soil colloidal surfaces, increasing measured labile P pools relative to acid-based extractants. However, no significant difference was observed on the concentration of extractable P between Bray-1 and nZvFe-CLCB.

Table 9 shows that the increase pattern of extractable P by the extractants in soils treated with poultry manure was similar to SSP amended soils (Table 9) though lower in concentrations. The lower absolute P values under PM reflect slower mineralization rates and partial immobilization of organic P within microbial biomass and complex organic matrices, which delay conversion into extractable inorganic forms. The Olsen extractant recorded significantly higher available P values than other nZvFe-CLCB in all locations except Bray-1 in Imeko soils. Similarly, available P contents extracted by Bray-1 were observed not to be significantly different from nZvFe-CLCB all through the sampling periods. In soil treated with PM, the Olsen extracted 11 and 15% higher available P than Bray-1 and nZvFe-CLCB extractants, respectively.

Generally, P availability varied considerably with location, however, the study showed that SSP and PM amended soil increased available P by 250 and 206%, more than the control, respectively, while the average available P values in PM amended soil was 13% lower than SSP. The nZvFe-CLCB extractant gave the lowest P values and was observed to be lower than Olsen and Bray 1 by 13 and 6%, respectively. The Olsen extractant gave the highest P values across all amendments and locations, though, in unamended soil, the three extractants performed similarly, with differences of < 15% observed among extractants.

3.5 Effect of phosphorus fertilizer source on the dry matter and phosphorus uptake of maize

Phosphorus fertilizer sources exerted a significant influence on maize dry matter weight (DMW) and P uptake across the six studied locations, showing variations in nutrient release patterns and soil–fertilizer interactions (Table 10). Across all locations, both SSP and PM significantly enhanced maize growth and P acquisition relative to the unamended control, confirming the fundamental role of P in root development, and biomass accumulation in maize (Marschner, 2012; Brady and Weil, 2017).

Single superphosphate application increased maize DMW by 22 to 79% and P uptake by 24 to 118% compared with the control indicated a high agronomic and P uptake efficiency. This strong response is consistent with the high solubility and immediate plant availability of inorganic phosphate fertilizers, which rapidly supply orthophosphate ions for root uptake. Similar findings have been reported by Fageria *et al*. (2010), who noted that mineral P sources typically produce faster crop responses due to rapid dissolution and diffusion in the soil solution. The higher P uptake observed under SSP also suggests improved P mobility in the rhizosphere, particularly in soils with moderate acidity where P fixation by Ca or Fe compounds may be less restrictive.

In contrast, PM increased maize DMW by 23 to 102% and P uptake by 25 to 91% compared with the control. The relatively higher improvement in biomass production under PM in several locations indicates that organic amendments contributed not only P but also additional nutrients such as N, K, and micronutrients, which collectively enhance vegetative growth and photosynthetic capacity. The gradual mineralization of organic P forms in manure likely ensured sustained nutrient release over time, improving biomass accumulation and enhancing physiological efficiency. This aligns with the findings of Adekiya *et al*. (2020), who reported that organic amendments improve soil biological activity and nutrient retention, thereby enhancing crop dry matter production.

3.6 Relationship between phosphorus extractants, soil pH and maize yield parameter

The interrelationship among P extractants, soil reaction (pH), and maize yield parameters reflect the complex geochemical controls governing P availability in tropical soils. The correlation matrix (Table 11) showed that Bray-1 extractable P had a significant positive relationship with both Olsen (r^2 = 0.785*) and nZvFe-CLCB (r^2 = 0.731*) extractants, indicating a shared ability to access overlapping pools of labile and moderately labile phosphorus fractions. However, the absence of a significant correlation between Olsen and nZvFe-CLCB suggests that these two extractants target distinctly different P pools, showing differences in extraction chemistry—bicarbonate-based desorption versus Fe-oxide disruption mechanisms. This divergence underscores the heterogeneity of soil P forms and supports the view that no single extractant fully captures the dynamic nature of plant-available P (Hedley *et al.,* 1982; Shen *et al.,* 2011).

Bray-1 extractable P exhibited the strongest overall association with maize dry matter yield (DMW) and P uptake, suggesting that acid-based extraction more closely reflects plant-accessible P under the studied soil conditions. This may be attributed to the moderately acidic to neutral pH range of the soils, where Bray-1 effectively solubilizes Fe-bound and exchangeable P fractions that are readily available for root uptake. This aligns with findings by Fageria *et al*. (2010), who reported that Bray-1 performs optimally in acid to slightly acidic tropical soils where Fe and Al-associated P dominate the available pool. The nZvFe-CLCB showed a stronger predictive capacity for both DMW (0.633 vs 0.538) and P uptake (0.686* vs 0.629) compared to Olsen, indicating that its extraction mechanism may better align with biologically relevant P pools influencing biomass production. This suggests that nZvFe-CLCB may be more sensitive to intermediate P fractions that contribute to sustained plant growth rather than immediate uptake alone.

Soil pH showed a significant and positive relationship with all extractants, following the order Bray-1 (0.851**) > nZvFe-CLCB (0.732*) > Olsen (0.688*). This indicates that increasing pH enhanced P extractability across all methods, likely due to reduced phosphate sorption onto Fe and Al oxides and increased desorption into soil solution. Hinsinger (2001) similarly reported that soil pH is a master variable controlling P solubility through its regulation of surface charge and adsorption–desorption equilibria.

4.0 Conclusion

Phosphorus availability in the studied tropical soils was significantly influenced by amendment type, increasing in the order SSP > PM > control, with SSP and PM enhancing extractable P by 250% and 206%, corresponding to approximately 3.5-fold and 3.06-fold increases in available P, respectively. These increases translated into improved maize performance, as dry matter yield increased by 22–79% (SSP) and 23–102% (PM), while P uptake improved by 24–118% and 25–91%, respectively. Soil pH declined under fertilization, with SSP inducing early acidification and PM showing a delayed buffering effect, whereas electrical conductivity increased progressively across treatments.

Consistent with the study objective, extractable P varied among methods in the order Olsen > Bray-1 > nZvFe-CLCB, with Olsen extracting 11–15% more P than Bray-1 and 13–15% more than nZvFe-CLCB. Significant relationships were observed among the extractants (r = 0.731–0.785), with Bray-1 and Olsen showing the closest association. However, the relationship between extractable phosphorus and maize response varied across the methods. Bray-1 showed the strongest association with dry matter yield (r = 0.777) and phosphorus uptake (r = 0.820), followed by nZvFe-CLCB and Olsen. Soil pH exerted a strong influence on phosphorus availability across all extractants. Although the Olsen method extracted higher quantities of phosphorus, it showed weaker correlations with maize performance parameters compared with Bray-1 and nZvFe-CLCB., Bray-1 more accurately reflected plant-available P under the prevailing soil conditions while also nZvFe-CLCB proved to be a functional and efficient extractant for tropical soils by consistently extracting available P and showing a good relationships with maize DMW and P uptake.

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Dedication

This paper is dedicated to the memory of Aghorunse Adeoba Courage, the lead author whose vision, dedication, and tireless work guided this study. We honor his memory and mourn his loss, which came after the completion of the experiment. This article stands as a testament to his belief, and his commitment to research and scientific rigor. His contributions (intellectual, practical, and humane) are woven into these pages and into the results that follow.

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Table 1: Information on location and land use history of the collected soil samples

Locations	Parent material	Longitude	Latitude	Altitude (m)	Land history at collection
Alabata	Base Complex	N7^{0}23'01.814	E3^{0}45'47.255	175	Maize farm with *Chromoleana odorata*
Ewekoro	Sedimentary soils	N6^{0}56'01.814	E3^{0}15'47.255	155	Cassava farm with *Chromoleana odorata*
Igbeji	Basement Complex	N6^{0}58'86.035	E3^{0}55'08.472	160	Natural grassland with trees and *Axonopus fissifolius* as the dominant specie
Ijale-orile	Basement Complex	N7^{0}11'48.386	E3^{0}11'93.612	137	Mixture of crops, fallow land with weeds
Imeko	Sedimentary soils	N7^{0}45'02.527	E3^{0}84'52.595	107	Fallow land with forest
Owode	Sedimentary soils	N6^{0}53'48.386	E3^{0}26'93.612	53	Fallow land with weeds and shrubs

Table2: Some physical and chemical properties of the soils used

Properties	Alabata	Ewekoro	Igbeji	Ijale-orile	Imeko	Owode
Sand (g/kg)	786	710	792	910	886	890
Silt (g/kg)	94	136	68	42	60	36
Clay (g/kg)	120	154	140	48	54	74
Textural class	Loamy sand	Loamy sand	Loamy sand	Sand	Sand	Sand
pH (water)	5.50	6.30	6.80	6.50	6.20	5.80
EC (dS m-1)	0.16	0.15	0.08	0.13	0.05	0.02
Ca ($cmol^{+}{}_{c}/kg$)	3.07	4.47	3.2	2.63	2.58	3.84
Mg ($cmol^{+}/kg$)	1.46	2.44	1.8	1.09	1.12	1.37
K ($cmol^{+}/kg$)	0.22	0.23	0.22	0.18	0.19	0.23
Na ($cmol^{+}/kg$)	0.36	0.23	0.26	0.13	0.17	0.51
Al+H ($cmol^{+}/kg$)	0.11	0.1	0.09	0.15	0.13	0.13
ECEC ($cmol^{+}/kg$)	5.22	7.47	5.57	4.18	4.19	6.08
Base Sat. (%)	97.89	98.66	98.38	96.41	96.89	97.86
Total N (g/kg)	0.14	0.08	0.12	0.09	0.11	0.04
TOC(g/kg)	0.67	0.94	1.37	1.08	0.82	0.79

Available P	22.47	14.43	19.71	18.61	14.31	20.48
Mn (mg/kg)	71.06	70.83	71.7	30.08	70.94	56.9
Fe (mg/kg)	43.97	34.5	36.66	25.38	33.68	30.49
Cu (mg/kg)	5.29	3.68	1.36	5.02	4.11	2.79
Zn (mg/kg)	2.84	2.78	1.39	2.36	1.43	3.75

TOC- Total organic carbon

Table3: Chemical characterization of the poultry manure used

Parameters	Poultry manure
Total N (g/kg)	0.36
Total P (g/kg)	0.28
Total S (g/kg)	0.15
Calcium (g/kg)	0.23
Magnesium (g/kg)	0.14
Potassium (g/kg)	0.12
Sodium (g/kg)	0.15
Org. Carbon (g/kg)	17.02
Copper (mg/kg)	43.950
Iron (mg/kg)	182.80
Manganese (mg/kg)	126.10
Zinc (mg/kg)	199.00
C:N	4.711

Table 4: Effect of phosphorus fertilization on the agronomic parameters of maize at harvest (8 weeks) under screenhouse study

Treatments	NL	LA (cm^2)	PH (cm)	SG (cm)	Treatments	NL	LA (cm^2)	PH (cm)	SG (cm)
		Alabata					Ijale-orile		
Control	10a	658.0b	156.80b	3.0b	Control	9a	442.0b	157.5a	3.2b
SSP	10a	779.0a	181.9a	3.3a	SSP	10a	669.0a	162.5a	3.3b
PM	10a	756.0ab	158.6b	3.2ab	PM	10a	482.0b	169.2a	3.8a
		Ewekoro					Imeko		
Control	10a	604.0b	135.8b	3.3b	Control	10ab	718.0c	163.6b	3.2b
SSP	10a	770.0ab	148.4ab	3.1b	SSP	13a	9660a	186.3a	3.4ab
PM	11a	828.0a	167.6a	3.8a	PM	12b	823.0b	176.2ab	3.9a
		Igbeji					Owode		
Control	9a	511.0b	157.4b	2.1b	Control	10a	584.0b	148.70b	2.8b
SSP	10a	691.0a	193.0a	2.4a	SSP	10a	670.0ab	175.4a	2.9b

PM	10a	533b.0	166.1ab	2.6a	PM	11a	739.0a	152.2b	3.3a

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.SSP- single superphosphate; PM- poultry manure; NL- number of leaves; LA- leaf area; PH- plant height; SG- stem girth

Table 5: Effect of phosphorus fertilization on the pH of soils of different locations under screenhouse study

Treatments	2 WAP	4 WAP	6 WAP	8 WAP	Treatments	2 WAP	4 WAP	6 WAP	8 WAP
	Alabata					Ijale-orile			
Control	7.1a	7.2a	6.6a	6.8a	Control	7.1a	6.8a	7.1a	7.1a
SSP	6.9a	6.3a	6.1b	5.8c	SSP	6.9a	6.4b	6.1b	6.2b
PM	7.1a	6.7ab	6.4ab	6.2b	PM	6.8a	6.6a	6.7a	6.7a
	Ewekoro					Imeko			
Control	7.9a	7.3a	7.5a	7.4a	Control	6.7a	6.7a	7.4a	7.2a
SSP	7.5ab	6.3b	6.2b	6.5b	SSP	5.8b	6.0b	6.2b	6.4b
PM	7.3b	6.9ab	6.4b	6.7b	PM	6.0b	6.2b	6.4b	6.3b
	Igbeji					Owode			
Control	6.8a	6.9a	6.5a	6.6a	Control	6.9a	6.3a	6.9a	7.3a
SSP	6.3b	5.7b	5.3b	5.3c	SSP	6.5a	5.6b	5.8b	5.3c
PM	6.2b	5.9b	5.4b	5.9b	PM	6.7a	6.1a	6.4ab	6.5b

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.

SSP- single superphosphate; PM- poultry manure; WAP-weeks after planting

Table 6: Effect of phosphorus fertilization on electrical conductivity ($\mu S\ cm^{-1}$) of soils of different locations under screenhouse study

Treatments	2 WAP	4 WAP	6 WAP	8 WAP	Treatments	2 WAP	4 WAP	6 WAP	8 WAP
	Alabata					Ijale-orile			
Control	300c	170c	290c	310c	Control	360b	270b	380c	340c
SSP	700a	680a	620b	480b	SSP	500a	430a	540b	590b
PM	510b	650a	880a	870a	PM	310b	390a	610a	750a
	Ewekoro					Imeko			
Control	260b	260b	340c	300c	Control	480c	320c	390c	290c
SSP	430a	520a	660b	550b	SSP	510b	550b	470b	410b
PM	350b	580a	790a	750a	PM	700a	760a	840a	850a
	Igbeji					Owode			

Control	360c	310c	350c	280c	Control	150b	110b	280b	340c
	600a	720a	940a	540b	SSP	540a	750a	850a	590b
PM	480b	660b	810b	780a	PM	470a	780a	960a	800a

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.
SSP- single superphosphate; PM- poultry manure; WAP-weeks after planting

Table 7: Effect of extractants on available phosphorus (mg kg^{-1}) extraction in unamended soil under screenhouse study

	Locations					
Extractants	Imeko	Alabata	Owode	Ewekoro	Igbeji	Ijale-orile
2 WAP						
Bray-1	9.78ab	10.43ab	9.12ab	7.51b	11.07ab	11.04ab
Olsen	10.64a	11.33a	9.92a	13.16a	12.03a	11.99a
nZvFe-CLCB	9.04b	9.63b	8.45b	6.53b	10.62b	10.19b
4 WAP						
Bray1	11.65ab	13.52ab	11.27ab	6.33b	12.96ab	9.45ab
Olsen	12.75a	14.69a	12.26a	15.88a	14.09a	10.27a
nZvFe-CLCB	10.77b	12.49b	10.42b	6.52b	12.27b	8.73b
6 WAP						
Bray-1	16.06ab	9.77ab	9.90ab	6.14b	14.29ab	8.60ab
Olsen	17.46a	10.62a	10.77a	17.68a	15.55a	9.35a
nZvFe-CLCB	14.84b	9.03ab	9.15b	6.34b	13.42b	7.96b
8 WAP						
Bray-1	14.22ab	11.48ab	9.63ab	5.85b	11.97b	7.99a
Olsen	15.46a	12.48a	10.47a	17.36a	13.01a	8.69a
nZvFe-CLCB	13.84b	10.61b	8.90b	6.08b	11.41b	7.95a

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.
WAP-weeks after planting; nZvFe-CLCB: nanosized Zerovalent Iron-Crosslinked Chitosan Beads

Table 8: Effect of extractants on available phosphorus (mg kg^{-1}) extraction in soils amended with SSP under screenhouse study

	Locations					
Extractants	Imeko	Alabata	Owode	Ewekoro	Igbeji	Ijale-orile
2 WAP						
Bray 1	32.83ab	32.57b	27.04b	30.45b	34.46b	34.98b
Olsen	35.67a	37.58a	32.65a	38.22a	40.75a	39.11a
nZvFe-CLCB	30.32b	31.94b	27.75b	28.89b	34.61b	33.25b
4 WAP						
Bray 1	40.72b	43.19b	38.00ab	35.44b	42.84b	35.51b
Olsen	44.27a	52.35a	41.3a	45.66a	48.74a	42.94a
nZvFe-CLCB	34.63b	40.52b	35.10b	34.28b	43.99b	36.50b
6 WAP						
Bray 1	41.83b	44.15b	40.75b	34.14b	38.18b	34.36b
Olsen	49.81a	47.99a	45.39a	46.24a	43.67a	42.79a

nZvFe-CLCB	41.34b	40.79c	38.58b	32.31b	39.12b	32.37b
8 WAP						
Bray 1	40.14b	40.95b	40.79b	28.14b	38.1b	36.07b
Olsen	45.80a	44.51a	47.60a	39.72a	43.45a	41.38a
nZvFe-CLCB	38.93b	37.84c	40.46b	29.76b	37.23b	35.83b

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.
WAP-weeks after planting; nZvFe-CLCB: nanosized Zerovalent Iron-Crosslinked Chitosan Beads

Table 9: Effect of extractants on available phosphorus (mg kg^{-1}) extraction in soils amended with poultry manure under screenhouse study

	Locations					
Extractants	Imeko	Alabata	Owode	Ewekoro	Igbeji	Ijale-orile
2 WAP						
Bray 1	30.66ab	30.70b	29.68b	22.78b	31.06b	32.55b
Olsen	33.32a	34.46a	33.26a	31.77a	35.93a	38.34a
nZvFe-CLCB	28.32b	29.29b	27.42b	21.05b	30.54b	33.75b
4 WAP						
Bray 1	34.72b	32.11b	31.85b	24.92b	34.37b	34.56b
Olsen	39.15a	38.16a	38.97a	29.77a	41.71a	41.91a
nZvFe-CLCB	33.27b	32.44b	33.12b	23.95b	35.45b	35.62b
6 WAP						
Bray 1	38.22ab	37.84ab	36.24ab	23.25b	36.75b	33.59b
Olsen	41.54a	41.13a	39.39a	28.36a	42.44a	38.69a
nZvFe-CLCB	35.31b	34.96b	33.48b	22.41b	34.56b	32.89b
8 WAP						
Bray 1	38.93ab	34.25b	30.94b	23.04b	35.24b	27.61b
Olsen	42.31a	41.56a	37.98a	29.13a	42.66a	33.27a
nZvFe-CLCB	35.96b	35.34b	31.28b	22.21b	34.26b	28.28b

Means with the same letter(s) within the same column are not significantly different at $P \leq 0.05$.
WAP-weeks after planting; nZvFe-CLCB: nanosized Zerovalent Iron-Crosslinked Chitosan Beads

Table 10: Effect of phosphorus fertilizer source on the dry matter and phosphorus uptake of maize

Treatments	DMW (g)	P uptake (g plant)
	Alabata	
Control	105.61c	13.72c
Single superphosphate	174.63a	29.86a
Poultry manure	128.67b	20.32b
	Ewekoro	
Control	75.62c	8.31b
Single superphosphate	122.34b	17.37a
Poultry manure	152.66a	15.87a

	Igbeji	
Control	88.68b	16.84b
Single superphosphate	129.67a	20.87ab
Poultry manure	139.99a	21.13a
	Ijale-orile	
Control	76.66b	12.26b
Single superphosphate	137.31a	24.77a
Poultry manure	132.33a	22.18a
	Imeko	
Control	118.65b	17.79b
Single superphosphate	171.69a	33.65a
Poultry manure	173.33a	29.81a
	Owode	
Control	96.60c	12.56b
Single superphosphate	117.66b	19.53a
Poultry manure	134.01a	21.30a

Table 11: Relationship between phosphorus extractants, soil pH and maize yield parameters

	Bray 1	Olsen	nZvFe-CLCB	DMW	P uptake
Olsen	0.785*				
nZvFe-CLCB	0.731*	0.477			
DMW	0.777*	0.538	0.633		
P uptake	0.820**	0.629	0.686*	0.933***	
Soil pH	0.851**	0.688*	0.732*	0.549	0.687*

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

nZvFe-CLCB: nanosized Zerovalent Iron-Crosslinked Chitosan Beads; DMW- dry matter weight