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## Effectiveness of biochar and microbes on the dynamics of available phosphorus in NaCl-stressed soil

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## Original article

## ABSTRACT

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**Introduction:** Saline soils are characterized by high concentrations of soluble salts and alkaline pH, which limit nutrient availability, particularly phosphorus (P), and reduce soil productivity. This study aimed to evaluate the effectiveness of biochar and microbial inoculants in improving soil chemical properties and P availability under NaCl-induced salinity stress. **Methods:** The experiment was conducted from December 2024 to March 2025 in a greenhouse using a completely randomized factorial design with two factors: microbial inoculation (control, *Pseudomonas sp.* and *Azotobacter*) and biochar types (control, coconut shell, rice husk, mangrove wood and cassava stem) at a rate of 30 tons/ha. Treatments were replicated three times, resulting in 45 experimental units. Soil samples were incubated for 12 weeks with observations every two weeks and analysed for pH, electrical conductivity (EC), available P, and exchangeable Na. **Results:** The results showed that biochar and microbial inoculants significantly influenced soil pH, EC, and nutrient dynamics. Biochar application reduced EC through ion adsorption and improved soil porosity, while also enhancing P availability via mineral ash contribution and cation exchange processes. Microbial inoculation, particularly *Azotobacter*, increased P availability through the production of organic acids and phosphatase enzymes, and its effect was more pronounced when combined with biochar. The highest available P was consistently observed in the combination of *Azotobacter* or *Pseudomonas sp.* and cassava stem biochar. Furthermore, both amendments reduced exchangeable Na, thereby improving soil structure and nutrient balance. **Conclusion:** In conclusion, the synergistic application of biochar and microbes effectively ameliorates saline soils, enhances phosphorus availability, and represents a sustainable strategy for soil fertility improvement under salinity stress.

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## INTRODUCTION

Marginal lands are characterized by limited soil fertility, poor water availability, and suboptimal environmental conditions that restrict crop growth and productivity. Among them, saline soils represent one of the most challenging types of marginal land. In Indonesia, saline soils cover approximately 0.6 million hectares, posing a serious constraint to agricultural sustainability and food security (Badrudin et al., 2023). Salinity stress affects soil chemical and physical properties, leading to reduced nutrient availability, osmotic imbalance, and ion toxicity that collectively hinder plant growth and yield.

Saline soils are commonly defined by high concentrations of soluble salts, including NaCl, Na<sub>2</sub>CO<sub>3</sub>, and Na<sub>2</sub>SO<sub>4</sub>. They are typically characterized by electrical conductivity (EC) values greater than 4 mS/cm, alkaline pH (> 8.5), and exchangeable sodium percentage (Na-dd) below 15%. These conditions significantly alter nutrient dynamics in the soil. In particular, phosphorus (P) availability is severely constrained. Although calcium (Ca<sup>2+</sup>) is important for soil structural stability, under alkaline conditions it often precipitates with phosphate ions, forming insoluble compounds such as monocalcium phosphate [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>], dicalcium phosphate (CaHPO<sub>4</sub>), and tricalcium phosphate [Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>]. These compounds are poorly available to plants, thereby limiting phosphorus uptake and reducing crop productivity (Xie et al., 2022). Consequently, strategies to manage soil pH, Ca<sup>2+</sup> dynamics, and phosphorus solubility are essential for improving crop performance on saline soils.

Recent studies suggest that biochar applications can play an important role in ameliorating saline soils. Biochar is a carbon-rich material produced from biomass pyrolysis under limited oxygen conditions (Saifullah et al., 2018). Its application has been reported to increase soil organic carbon, enhance cation exchange capacity (CEC), and buffer soil pH toward neutral conditions (Joseph et al., 2021). These changes improve nutrient retention and availability while also reducing the toxic effects of excessive sodium. Additionally, biochar derived from agricultural residues such as rice husks,

cassava stems, coconut shells, and mangrove wood provides porous structures that improve soil aeration and water retention, while also contributing organic acids that can mobilize phosphorus (Dimitriadou et al., 2025).

In addition to biochar, soil microbes particularly phosphate-solubilizing bacteria (PSB)—offer a complementary approach to improving phosphorus availability in saline soils. Microbes such as *Azotobacter* and *Pseudomonas sp.* have been widely reported to solubilize insoluble phosphate compounds through the secretion of organic acids and phosphatase enzymes (Li et al., 2023). Moreover, their adaptability to saline conditions makes them suitable for application in stressed soils; for example, *Azotobacter* can survive in environments with salinity levels up to 8 mS/cm, while *Pseudomonas sp.* has been shown to increase available phosphorus by over 11% in saline (Dey et al., 2021). The porous structure and large surface area of this biochar not only improve the physical properties of the soil but also have the potential to create an ideal microhabitat to protect and support beneficial soil microorganism populations.

The combination of biochar and phosphate-solubilizing microbes has the potential to create a synergistic effect. Biochar improves soil physicochemical properties and provides a stable habitat that supports microbial growth and activity. In turn, microbes enhance nutrient cycling and increase the bioavailability of phosphorus that would otherwise remain bound to calcium or other cations. Despite these promising interactions, studies examining the integrated use of biochar and microbes in saline soils remain limited. Most previous research has focused on their individual effects, leaving a gap in understanding their combined influence on phosphorus dynamics under salinity stress. Addressing this gap is crucial; for advancing our understanding of the synergistic mechanisms between biochar and microbes, while also providing practical insight for developing sustainable soil management strategies that enhance phosphorus availability and crop productivity in saline affected soils.

This study aims to evaluate the combined and individual effect of biochar and microbial inoculation on the dynamics of available phosphorus and other soil quality parameters in NaCl-stressed soils. Through this research, specific attention was given to identifying changes in soil phosphorus availability and related chemical properties following the application of biochar, microbes, and their combination. Furthermore, the study aimed to assess the interaction effect between biochar and microbial inoculation under salinity stress conditions. The findings of this research provide insights for management approaches and contribute to environmentally friendly strategies for improving crop productivity in saline affected areas.

## METHODS

### Location and time of research

The study was conducted from December 2024 to March 2025. Treatments were carried out in a greenhouse, while soil analysis was performed at the Soil Resources Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur. Soil samples were collected from saline-affected land located at coordinates 7°22'7.875" S, 112°46'24.493" E in Sedati District, Sidoarjo Regency. The samples were taken at a depth of 0–20 cm using a hoe from a banana plantation with an initial EC of 0.72 mS/cm. The soil was air-dried, ground, and sieved to 2 mm, then placed into polybags (2 kg oven-dry soil weight each) and saturated to field capacity. Salinity was standardized by applying NaCl solution (13.2 g NaCl in 100 ml distilled water per polybag) to achieve an electrical conductivity (EC) of approximately 6 mS/cm, which was monitored weekly. Subekti et al. (2020) reported that rice fields affected by seawater intrusion in Indramayu, West Java, had moderate and high salinity levels with EC values of 7,23 and 10,51 dS/m, respectively. Moderate salinity still allowed plant growth, while higher levels caused severe yield loss. Based on this, an EC level of 6 mS/cm was chosen in this study to represent moderate saline conditions typical of Indonesian coastal soils. This level provides sufficient stress to affect nutrient and microbial activity without completely inhibiting biological processes, allowing effective evaluation of biochar and microbial treatments.

### Research design

The experiment was arranged in a completely randomized factorial design (CRD) with two factors: (1) microbial inoculation consisting of control, *Pseudomonas sp.*, and *Azotobacter* (25 ml/kg or equivalent to 50 ml/polybag), and (2) biochar application consisting of control, coconut shell, rice husk, mangrove wood, and cassava stem, biochars at a rate of 30 tons/ha. In total, there were 15 treatment combinations with three replications, resulting in 45 experimental units. Biochar was applied at a rate of 30 g/polybag to the soil surface. The microbial inoculants, *Pseudomonas sp.* (cultured in King's B broth) and *Azotobacter* (cultured in Ashby broth), were propagated by incubation for 24 hours prior to application.

The equipment used in this study was divided into two categories: field equipment and laboratory equipment. Field equipment included a hoe, shovel, trowel, sack, camera, GPS, 2 mm sieve, research labels, polybags, and a balance. Laboratory equipment included 0.5 mm and 2 mm sieves, an analytical balance, film bottles, funnels, measuring cylinders, beakers, test tubes, stove, reciprocal shaker, pH meter, 100 ml volumetric flasks, volumetric pipettes, graduated pipettes, 100 ml Erlenmeyer flasks, test tubes, filter paper, funnels, pump, vortex mixer, spectrophotometer, Petri dishes, micropipettes, inoculating loop, Bunsen burner, autoclave, laminar air flow (LAF) cabinet, and stationery.

The materials used in this study included NaCl, biochar, and microbial media (Nutrient Agar, King's B, and Ashby). The materials used for laboratory analysis included soil samples sieved to 0.5 mm and 2 mm, ammonium acetate (NH<sub>4</sub>OAc) 1 M pH 7.0, Olsen's extractant, PO<sub>4</sub> standard solution 1000 ppm, ascorbic acid, concentrated P reagent, and distilled water.

Soil chemical analysis was performed following the latest guidelines, including ASTM D4972 (2019/2024) for soil pH measurement with a soil-water suspension (1:2.5), as well as available phosphorus extraction methods using 0.5 M NaHCO<sub>3</sub> solution (Olsen, Bowman 2023; Nur Kholifah et al., 2025). Exchangeable cations, particularly Na<sup>+</sup>, were determined by the 1 M ammonium acetate (NH<sub>4</sub>OAc) extraction method at pH 7.0 according to modern laboratory guidelines (Sikora et al., 2020; Iowa State University, 2018).

### Data analysis

Soil sampling during incubation was conducted every two weeks for six intervals. The samples were analyzed for chemical parameters (pH, EC, available P, and exchangeable Na). Data were subjected to analysis of variance (ANOVA) at the 5% significance level, followed by the Honest Significant Difference (HSD) test when treatment effects were significant. Correlation and determination analyses were also performed to examine the strength of relationships among variables.

## RESULTS AND DISCUSSION

A preliminary analysis was conducted prior to the experiment to characterize the soil properties before treatment application and to evaluate its initial fertility status.

Table 1. soil characteristics before treatment

No	Parameter	Unit	Result	Criteria (*)
1	pH H <sub>2</sub> O	-	8.60	Alkaline
2	EC	mS/cm	0.71	Low
3	C-Organic	%	0.99	Very Low
4	KTK	Cmol (+)/kg	42.58	High
5	P-available	Ppm	56.26	High
6	P-Potential	mg/100g	151.94	High
7	Ca-exchangeable	Cmol (+)/kg	37.43	Very High
9	Na-exchangeable	Cmol (+)/kg	7.11	Very High
10	Microbial population	CFU/ml	1.2 x 10 <sup>6</sup>	-
11	Texture	-	Sand 15% Dust 85%	Silt

(\*) : Assessment criteria for soil analysis results based on the 2023 soil and fertilizer instruments standards testing center

Preliminary soil analysis (Table 1) showed that the soil had an alkaline reaction with a pH (H<sub>2</sub>O) of 8.60. classified as alkaline according to the Soil and Fertilizer Testing Standard Instrument Center. The high pH was associated with sodium (Na<sup>+</sup>) accumulation, which limited phosphorus (P) availability due to precipitation with Ca<sup>2+</sup> and also inhibited microbial activity. Consequently, organic matter decomposition and nutrient mineralization were not optimal. The initial electrical conductivity (EC) was 0.71 mS/cm. indicating low salinity.

The organic carbon content was 0.99%. categorized as low, likely due to high salt concentrations that suppressed microbial decomposition of organic matter. Low organic matter negatively affected soil structure, water-holding capacity, aeration, and nutrient supply (Hassani et al., 2024). Available P was 56.26 ppm and potential P was 106.91 mg/100 g, both classified as high. However, under alkaline conditions, much of the P was bound to Ca<sup>2+</sup>, making it less available to plants. This indicates that high total P does not necessarily correspond to high plant-available P, as its release depends on microbial activity and phosphatase enzymes, which are inhibited under osmotic stress.

Exchangeable Na<sup>+</sup> was 7.11 cmol(+)/kg and Ca<sup>2+</sup> was 37.43 cmol(+)/kg. both very high. Excess Na<sup>+</sup> caused clay dispersion, degraded soil structure, and reduced porosity. Although the cation exchange capacity (CEC) was high (42.58 cmol(+)/kg), it was dominated by Na<sup>+</sup>, which is detrimental to soil quality. Thus, soil amendments such as organic matter or biochar are required to reduce Na<sup>+</sup> toxicity and improve nutrient balance. Soil texture was dominated by 85% silt and 15% sand, classifying it as silty soil. High Na<sup>+</sup> concentration displaced Ca<sup>2+</sup> and Mg<sup>2+</sup>, leading to clay dispersion into finer particles that clogged soil pores. This condition resulted in poor drainage, compaction when dry, and unstable soil structure, which are typical characteristics of NaCl-affected soils.

### Effect of biochar and microbes on soil pH

The analysis of variance revealed that both individual and combined applications of biochar and microbial amendments had a significant positive effect on soil pH. The detailed results are presented in Figure 1 and Table 2.

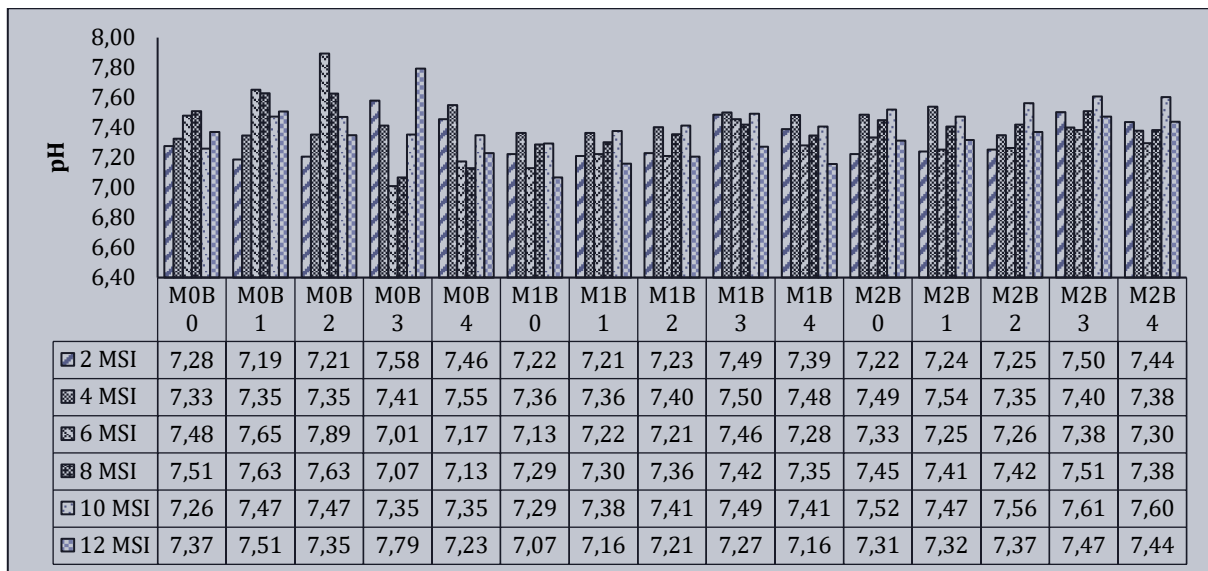


Figure 1. Diagram of Soil pH Values After Treatment

Table 2. Soil pH Values after Treatment Application

	Mikroba	Biochar				
		B0	B1	B2	B3	B4
2 WAI	M0	7.28	7.19	7.21	7.58	7.46
	M1	7.22	7.21	7.23	7.49	7.39
	M2	7.22	7.24	7.25	7.50	7.44
	HSD 5%	ns				
4 WAI	M0	7.33	7.35	7.35	7.41	7.55
	M1	7.36	7.36	7.40	7.50	7.48
	M2	7.49	7.54	7.35	7.40	7.38
	HSD 5%	ns				
6 WAI	M0	7.48 <sup>e</sup>	7.65 <sup>d</sup>	7.89 <sup>cd</sup>	7.01 <sup>bc</sup>	7.17 <sup>abc</sup>
	M1	7.13 <sup>abc</sup>	7.22 <sup>abc</sup>	7.21 <sup>abc</sup>	7.46 <sup>a</sup>	7.28 <sup>ab</sup>
	M2	7.33 <sup>ab</sup>	7.25 <sup>ab</sup>	7.26 <sup>ab</sup>	7.38 <sup>a</sup>	7.30 <sup>ab</sup>
	HSD 5%	0.43**				
8 WAI	M0	7.51 <sup>ab</sup>	7.63 <sup>a</sup>	7.63 <sup>a</sup>	7.07 <sup>b</sup>	7.13 <sup>b</sup>
	M1	7.29 <sup>ab</sup>	7.30 <sup>ab</sup>	7.36 <sup>ab</sup>	7.42 <sup>ab</sup>	7.35 <sup>ab</sup>
	M2	7.45 <sup>ab</sup>	7.41 <sup>ab</sup>	7.42 <sup>ab</sup>	7.51 <sup>ab</sup>	7.38 <sup>ab</sup>
	HSD 5%	0.47**				
10 WAI	M0	7.26	7.47	7.47	7.35	7.35
	M1	7.29	7.38	7.41	7.49	7.41
	M2	7.52	7.47	7.56	7.61	7.60
	HSD 5%	ns				
12 WAI	M0	7.37 <sup>bc</sup>	7.51 <sup>ab</sup>	7.35 <sup>bc</sup>	7.79 <sup>a</sup>	7.23 <sup>bc</sup>
	M1	7.07 <sup>c</sup>	7.16 <sup>bc</sup>	7.21 <sup>bc</sup>	7.27 <sup>bc</sup>	7.16 <sup>bc</sup>
	M2	7.31 <sup>bc</sup>	7.32 <sup>bc</sup>	7.37 <sup>bc</sup>	7.47 <sup>ab</sup>	7.44 <sup>ab</sup>
	HSD 5%	0.37*				

Note : Figures followed by the same letter are not significantly difference according to HSD 5% test.

Based on Table 2 the results of the 5% Honest Significant Difference test on soil pH after treatment show that at 2 and 4 WAI, all results are the same not significantly different (ns), and the pH between the biochar and mikroba

combinations is not yet significantly different. Differences begin to appear at 6 WAI in the combinations and in each amendment, indicating that biochar, microbes, and the combination of both have a very significant effect. At 8 WAI, the combination of the two obtained a HSD value of 0.47 and showed very significant differences. The combination of treatments B3 (mangrove wood biochar) and B4 (cassava stem biochar) tended to have a neutral pH value compared to the control (without biochar). Cassava stem and mangrove wood biochar contain very high levels of organic matter, enabling them to neutralize pH (Priyadarshini et al., 2024).

The initial soil pH was 8.60, which is alkaline. At 2 WAI to 12 WAI, the pH showed lower values than the initial soil pH, but the values were fluctuating. This could be influenced by microbiological processes such as nitrification, which lowers pH, and denitrification, which increases pH through the consumption of  $H^+$  ions (Yan et al., 2015). During nitrification, soil bacteria oxidize  $NH_4^+$  to  $NO_3^-$ , a process that releases  $H^+$  ions into the soil solution and contributes to soil acidification (Bergamasco et al., 2019). These changes were not only influenced by microbial processes such as nitrification, denitrification, and  $CO_2$  production from organic matter decomposition but also by the applied treatments. The combination of cassava stem biochar (B4) and microbial inoculation resulted in a more moderate and stable pH decrease compared to other treatments. Cassava stem biochar, which has alkaline properties and high buffering capacity, can adsorb  $Na^+$  ions and neutralize excessive alkalinity, while providing favorable microsites for microbial activity. Meanwhile, *Pseudomonas sp.* and *Azotobacter* are known to produce organic acids that locally lower soil pH and solubilize phosphate compounds, contributing to enhanced phosphorus availability. Microbial respiration is carried out by breaking down organic matter, which produces  $CO_2$ . The gas produced dissolves in water and forms carbonic acid, which ionizes into  $H^+$  and  $HCO_3^-$  ions, thereby lowering the soil pH (Obia et al., 2015).

### Effect of biochar and microbes on electrical conductivity

The analysis of variance revealed that applications of biochar and microbial amendments had a significant positive effect on electrical conductivity. The detailed results are presented in Figure 2 and Table 3.

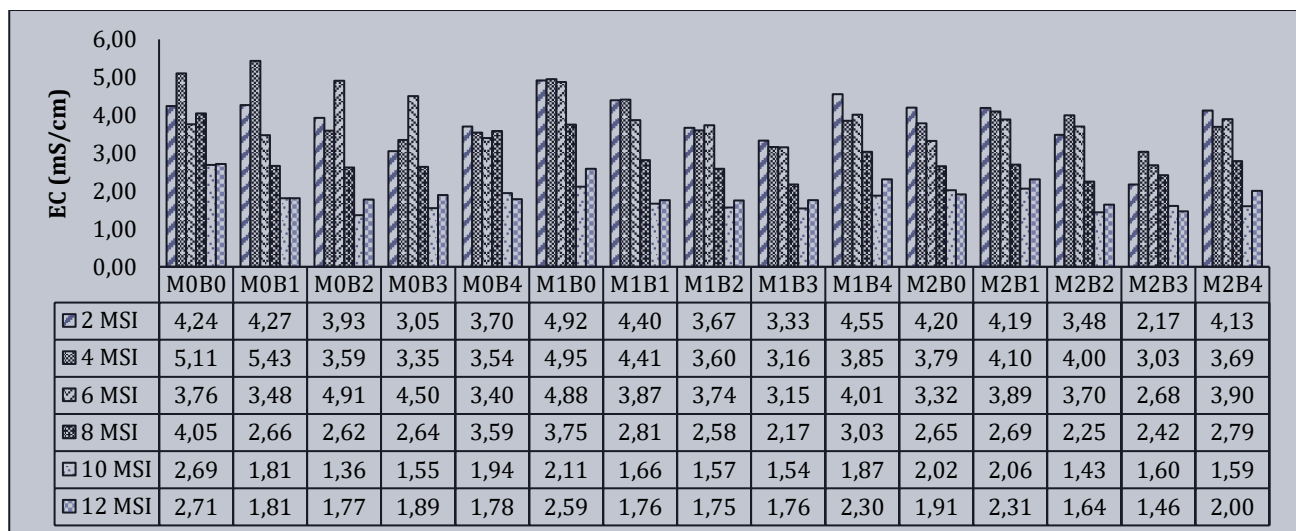


Figure 2. Diagram of electrical conductivity after treatment

Based on Table 3 the HSD test results indicated that each amendment significantly affected soil EC. From 2 to 6 weeks after incubation (WAI), microbial treatments had no significant effect, but by 8 WAI they showed a highly significant difference. EC values declined over time in all treatments, with varying rates from 3.68 to 1.99 mS/cm in the control (M0), 4.05 to 2.03 mS/cm with *Pseudomonas sp.* (M1), and 4.80 to 1.96 mS/cm with *Azotobacter* (M2). *Azotobacter* was more effective in reducing soil electrical conductivity (EC) than *Pseudomonas sp.* and the uninoculated control due to its ability to fix atmospheric nitrogen, produce extracellular polysaccharides (EPS) that bind salt ions ( $Na^+$  and  $Cl^-$ ), and secrete organic acids that can precipitate or immobilize  $Na^+$  ions. This reduction was attributed to microbial immobilization of ions into microbial biomass, where bacteria absorb and store nutrients within their cells, thereby lowering ion concentrations in the soil solution (Xiang et al., 2022).

Biochar application also significantly reduced EC at 2, 8, 10, and 12 WAI. The decrease was due to its high surface area, abundant micro and macropores, and negatively charged functional groups that adsorb cations and anions ( $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $SO_4^{2-}$ ), reducing free ions contributing to EC. Furthermore, biochar improved soil porosity and water infiltration, enhancing leaching of soluble salts below the root zone and lowering salinity. In this polybag experiment downward leaching of salts is limited due to restricted percolation; therefore, the observed decrease in measured salinity is unlikely to result from leaching below the root zone. Instead, the reduction in soil salinity and EC associated with biochar addition is more plausibly explained by mechanisms operating within the soil–biochar matrix, including adsorption of soluble salts onto biochar surfaces, increased cation exchange capacity and consequent displacement/retention of  $Na^+$  by

exchangeable Ca<sup>2+</sup>/Mg<sup>2+</sup>, microbial-mediated immobilization or precipitation of salts, and improved aggregation and water distribution within the substrate. Cassava stem biochar is high porosity, surface functional groups and alkaline character can retain Na<sup>+</sup> and other ions in the solid phase or within biochar pores, thereby lowering the activity of salts in the soil solution and reducing EC in the measured extracts (dos Santos, 2022)

Table 3. Electrical conductivity values after amendment application

Microbial Treatment	Incubation					
	2 WAI	4 WAI	6 WAI	8 WAI	10 WAI	12 WAI
M0	3.68	4.20	4.00	3.11 <sup>a</sup>	3.11	1.99
M1	4.05	3.99	3.39	2.86 <sup>ab</sup>	2.86	2.03
M2	4.8	3.72	3.42	2.56 <sup>b</sup>	2.56	1.86
HSD 5%	ns	ns	ns	0.34 <sup>**</sup>	ns	ns

Biochar Treatment	Inkubasi					
	2 WAI	4 WAI	6 WAI	8 WAI	10 WAI	12 WAI
B0	4.45	4.61 <sup>a</sup>	3.98	3.48 <sup>a</sup>	2.27 <sup>a</sup>	2.40 <sup>a</sup>
B1	4.28	4.64 <sup>a</sup>	3.74	2.72 <sup>bc</sup>	1.84 <sup>ab</sup>	1.95 <sup>ab</sup>
B2	3.69	3.73 <sup>ab</sup>	4.11	2.48 <sup>c</sup>	1.45 <sup>b</sup>	1.72 <sup>b</sup>
B3	4.34	3.17 <sup>b</sup>	3.44	2.4 <sup>c</sup>	1.56 <sup>b</sup>	1.70 <sup>b</sup>
B4	4.12	3.69 <sup>ab</sup>	3.76	3.13 <sup>ab</sup>	1.80 <sup>ab</sup>	2.03 <sup>ab</sup>
HSD 5%	ns	1.18 <sup>**</sup>	ns	0.51 <sup>**</sup>	0.47 <sup>**</sup>	0.57 <sup>**</sup>

Note : Figures followed by the same letter are not significantly difference according to HSD 5% test.

**Effect of biochar and microbes on available P**

The analysis of variance revealed that combined applications of biochar and microbial amendments had a significant positive effect on available P. The detailed results are presented in Figure 3 and Table 4.

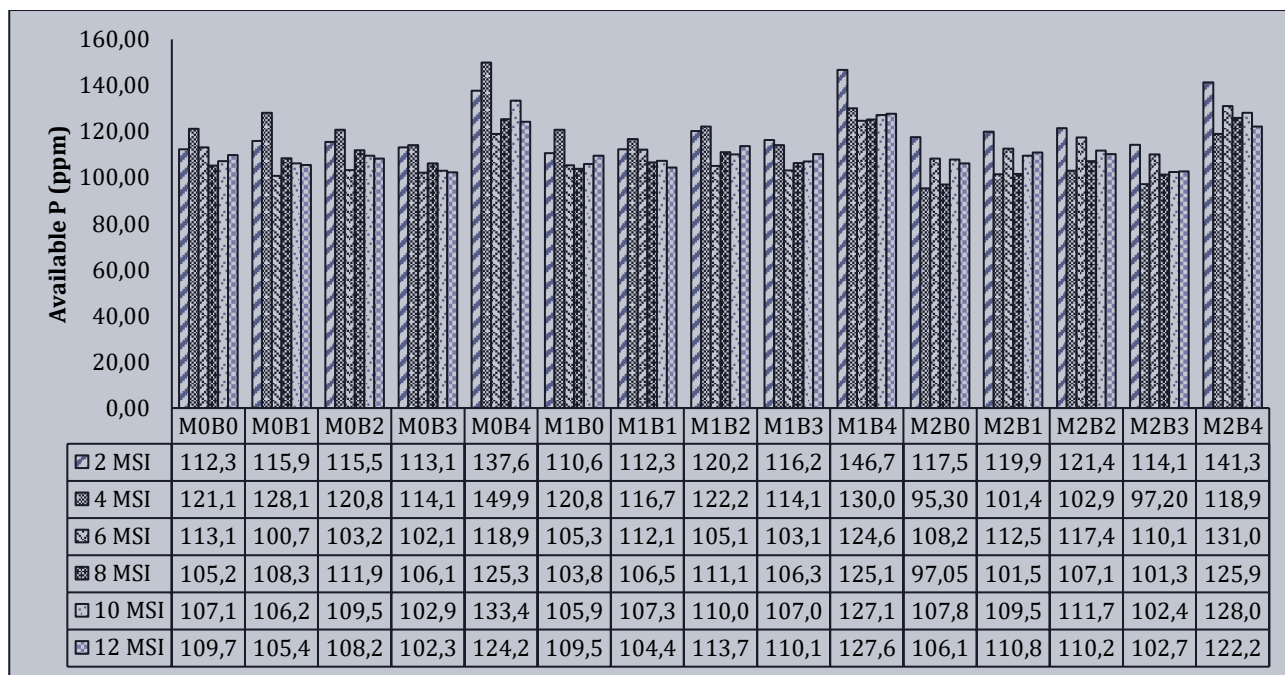


Figure 3. Diagram available P after treatment

Table 4 presents the changes in available phosphorus (P) under different treatments of biochar and microbial inoculation. The combination of *Azotobacter* (M2) with cassava stem biochar (B4) exhibited the highest and most consistent increase in available P, particularly during the early incubation phase (2–4 WAI). This result indicates a strong synergistic interaction between *Azotobacter* and cassava stem biochar in enhancing P availability under saline conditions. The improvement may be attributed to the ability of *Azotobacter* to solubilize phosphate through the production of organic acids and to fix atmospheric nitrogen, while cassava stem biochar contributes by improving cation exchange capacity (CEC), reducing Na<sup>+</sup> activity, and providing favorable microhabitats for microbial proliferation. In contrast, treatments without microbial inoculation (M0) showed only limited increases in available P, suggesting that microbial activity played a pivotal role in mobilizing phosphorus in the biochar-amended saline soils. These findings align with previous studies reporting that biochar improves P availability through direct P contribution from its ash, neutralization of saline soil that reduces Ca-bound P, and modification of soil surface properties affecting phosphate sorption-desorption (Joseph et al., 2021).

Table 4. Available P after treatment application

	Microbial	Biochar				
		B0	B1	B2	B3	B4
2 WAI	M0	112.34 <sup>bc</sup>	115.95 <sup>bc</sup>	115.53 <sup>bc</sup>	113.10 <sup>bc</sup>	137.66 <sup>a</sup>
	M1	110.69 <sup>c</sup>	112.35 <sup>bc</sup>	120.25 <sup>bc</sup>	116.29 <sup>bc</sup>	146.75 <sup>a</sup>
	M2	117.55 <sup>bc</sup>	119.93 <sup>bc</sup>	121.40 <sup>bc</sup>	114.19 <sup>bc</sup>	141.32 <sup>a</sup>
	HSD 5%	9.597*				
4 WAI	M0	121.18	128.17	120.81	114.10	149.97
	M1	120.80	116.72	122.21	114.17	130.06
	M2	95.30	101.40	102.98	97.20	118.98
	HSD 5%	ns				
6 WAI	M0	113.15	100.70	103.26	102.18	118.92
	M1	105.32	112.11	105.10	103.16	124.63
	M2	108.25	112.52	117.48	110.13	131.03
	HSD 5%	ns				
8 WAI	M0	105.22	108.34	111.94	106.16	125.35
	M1	103.87	106.53	111.10	106.34	125.19
	M2	97.05	101.55	107.11	101.35	125.90
	HSD 5%	ns				
10 WAI	M0	107.18	106.21	109.50	102.98	133.45
	M1	105.95	107.31	110.05	107.01	127.15
	M2	107.85	109.54	111.72	102.49	128.07
	HSD 5%	ns				
12 WAI	M0	109.74	105.48	108.28	102.32	124.27
	M1	109.54	104.41	113.70	110.16	127.69
	M2	106.19	110.88	110.20	102.77	122.21
	HSD 5%	ns				

Note : Figures followed by the same letter are not significantly difference according to HSD 5% test.

Biochar, a carbon-rich product of biomass pyrolysis, contributes nutrients including P, as agricultural feedstock biochar contains mineral-rich ash (Nepal et al., 2023). Its porous structure enhances cation exchange capacity (CEC), thereby retaining  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and  $\text{Mg}^{2+}$  and preventing P precipitation with these cations (Bai et al., 2024). In saline soils, biochar can also exchange  $\text{Na}^+$  with  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , reducing clay dispersion and improving soil structure. Biochar pores not only provide physical protection for microbes under saline stress but also supply organic carbon that supports microbial metabolism, while microbes release previously bound P.

Microbes also play a crucial role in enhancing P availability. Phosphate-solubilizing microbes (PSM) release organic acids that dissolve bound phosphate and secrete phosphatase enzymes that mineralize organic P into plant-available forms, thereby facilitating improved phosphorus uptake by plants and contributing to overall soil health and fertility (Pang et al., 2024). Accordingly, *Azotobacter* (M2) consistently provided higher available P compared to no-microbe treatments (M0). The effect was more pronounced when combined with biochar, which not only acts as a microbial habitat but also supplies carbon sources. Previous studies confirmed the synergistic role of biochar–microbe combinations in increasing soil P availability and plant P uptake (Bai et al., 2024). This synergy occurs because biochar provides protective microhabitats under saline stress, supplies organic carbon to sustain microbial metabolism, while microbes mobilize bound P, jointly enhancing P availability (Silva et al., 2023).

#### Effect of biochar and microbes on exchangeable Na

Exchangeable sodium (Na-dd) is the amount of sodium ions present on the surface of soil colloids (clay and organic matter) that can be exchanged with other cations in the soil solution. The Na-dd value indicates how much  $\text{Na}^+$  is available for exchange in the soil. If the Na-dd content is high in the soil, it will replace  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Akhtar et al., 2015). This will result in soil structure damage, closed pores, reduced water infiltration, and disrupted plant growth.

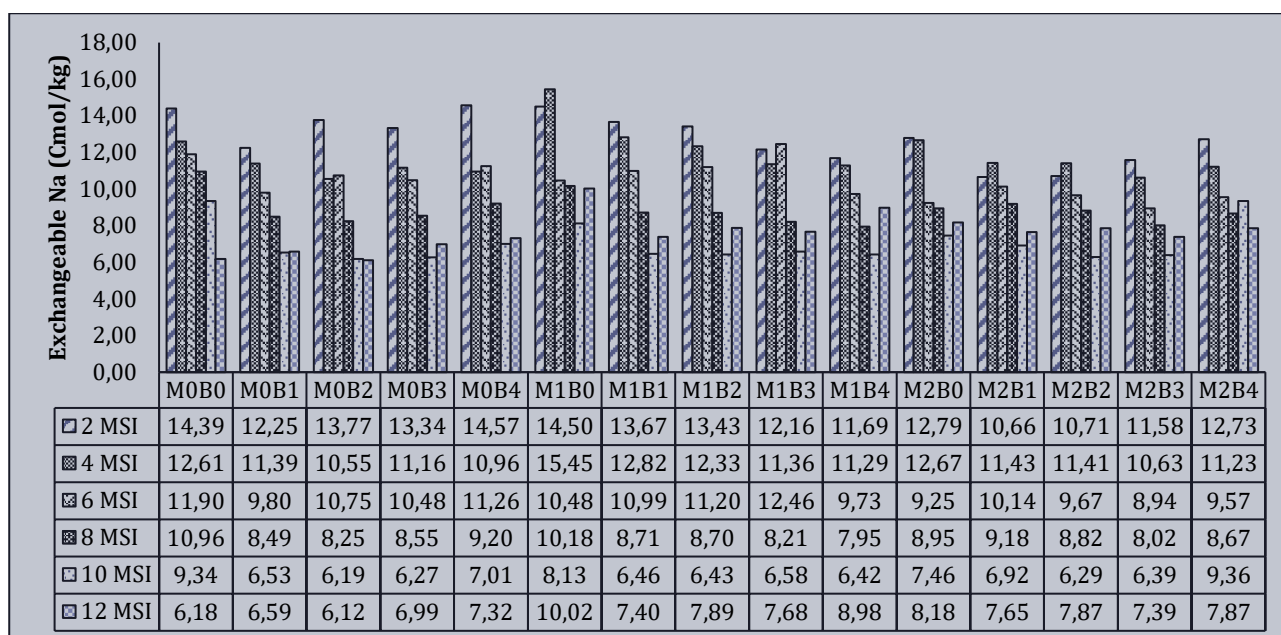


Figure 4. Diagram exchangeable Na values after treatment

Table 5. Exchangeable Na values after amendment application

	Microbial	Biochar				
		B0	B1	B2	B3	B4
2 WAI	M0	14,39 <sup>cd</sup>	12,25 <sup>abcd</sup>	13,77 <sup>cd</sup>	13,34 <sup>abcd</sup>	14,57 <sup>d</sup>
	M1	14,50 <sup>cd</sup>	13,67 <sup>bcd</sup>	13,43 <sup>abcd</sup>	12,16 <sup>abcd</sup>	11,69 <sup>abcd</sup>
	M2	12,79 <sup>abcd</sup>	10,66 <sup>a</sup>	10,71 <sup>ab</sup>	11,58 <sup>abc</sup>	12,73 <sup>abcd</sup>
	HSD 5%	2,98*				
4 WAI	M0	12,61	11,39	10,55	11,16	10,96
	M1	15,45	12,82	12,33	11,36	11,29
	M2	12,67	11,43	11,41	10,63	11,23
	HSD 5%	ns				
6 WAI	M0	11,90	9,80	10,75	10,48	11,26
	M1	10,48	10,99	11,20	12,46	9,73
	M2	9,25	10,14	9,67	8,94	9,57
	HSD 5%	ns				
8 WAI	M0	10,96	8,49	8,25	8,55	9,20
	M1	10,18	8,71	8,70	8,21	7,95
	M2	8,95	9,18	8,82	8,02	8,67
	HSD 5%	ns				
10 WAI	M0	9,34 <sup>c</sup>	6,53 <sup>ab</sup>	6,19 <sup>a</sup>	6,27 <sup>a</sup>	7,01 <sup>ab</sup>
	M1	8,13 <sup>bc</sup>	6,46 <sup>ab</sup>	6,43 <sup>ab</sup>	6,58 <sup>ab</sup>	6,42 <sup>ab</sup>
	M2	7,46 <sup>ab</sup>	6,92 <sup>ab</sup>	6,29 <sup>a</sup>	6,39 <sup>ab</sup>	9,36 <sup>c</sup>
	HSD 5%	1.770**				
12 WAI	M0	6,18	6,59	6,12	6,99	7,32
	M1	10,02	7,40	7,89	7,68	8,98
	M2	8,18	7,65	7,87	7,39	7,87
	HSD 5%	ns				

Note : Figures followed by the same letter are not significantly difference according to HSD 5% test

Based on Table 5, the 5% HSD analysis for exchangeable sodium (Na-dd) showed that the application of microbes and biochar had varying effects depending on the incubation period. Significant effects were observed at the 2th and 10th weeks of incubation, with HSD values of 2.98\* and 1.770\*\*, respectively. During these periods, the combined treatments of microbes and biochar, particularly M1 (*Pseudomonas sp.*) and M2 (*Azotobacter*) with biochar types B2 (rice husk) and B3 (mangrove wood), were more effective in reducing Na-dd levels compared to the control (M0B0). Rice husk and mangrove wood biochars effectively reduced exchangeable sodium (Na-dd) in saline soils due to their high ash and silica contents, alkaline nature, and porous structure. The rice husk biochar contains abundant amorphous silica and base cations ( $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ), which enhance ion exchange and replace  $\text{Na}^+$  on soil colloid surfaces. Meanwhile, mangrove wood biochar has a large surface area and high porosity, allowing efficient adsorption and immobilization of  $\text{Na}^+$  ions (Phuong et al., 2019; Sadegh-Zadeh et al., 2018). Both biochars improve soil cation exchange capacity and aggregation, leading to reduced sodium accumulation and improved soil structure under saline conditions. Microbes applied to the soil were able to increase the activity of soil microorganisms, which play a role in improving soil aggregate structure and increasing cation exchange capacity, so that  $\text{Na}^+$  ions can be exchanged and removed from the soil colloid adsorption complex. Certain microbes, such as salt-solubilizing bacteria and bacteria that produce exopolysaccharides (EPS), are also known to reduce soil salinity by forming biofilms that bind harmful ions such as  $\text{Na}^+$  (Aasfar et al., 2021). In addition, the calcium and magnesium content in biochar can compete with sodium in cation exchange complexes, causing  $\text{Na}^+$  ions to desorb from soil particles and wash them out of the root zone (Tan et al., 2021)

## CONCLUSION

The combined application of biochar and microbial inoculants effectively improved soil chemical properties (pH, EC, and Exchangeable Na) and phosphorus availability under saline conditions. Cassava stem biochar (B4) showed superior performance in enhancing available phosphorus due to its high ash content and mineral composition. However, it was less effective in reducing exchangeable sodium (Na-dd) compared to other biochar types. *Azotobacter* and *Pseudomonas sp.* contributed to phosphorus solubilization and nutrient balance through organic acid production. These results highlight that integrating biochar with beneficial microbes can improve soil fertility and partially mitigate salinity stress in degraded soils.

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