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The Effect of Interaction of Microbially Immobilized Biochar and Salinity Stress on Soil Chemical Properties (pH, EC, C-organic, and CEC) in Red Spinach (*Amaranthus tricolor* L.) Cultivation

Nofia Wahyu Puspita¹, Rossyda Priyadarshini^{1,*}, Fitri Wijayanti¹

Abstract

Soil salinity reduces nutrient availability and inhibits plant growth. While red spinach (*Amaranthus tricolor* L.) exhibits tolerance to suboptimal conditions, its growth declines significantly under high salinity stress. This study evaluated the efficacy of microbially immobilized biochar in ameliorating the chemical properties of saline-stressed soil and enhancing red spinach growth. The experiment employed a Completely Randomized Design (CRD) with two factors: biochar dose (0, 15, 30, and 45 t ha⁻¹) and NaCl salinity levels (0, 4, 6, and 8 mS cm⁻¹), with three replications totaling 48 experimental units. Soil parameters measured included pH, electrical conductivity (EC), organic carbon (C), and cation exchange capacity (CEC) at 0 and 28 days after planting (DAP), while fresh plant weight was recorded at 28 DAP. Data were analyzed using ANOVA followed by a 5% Least Significant Difference (LSD) test. Results indicated that biochar application significantly influenced soil pH and CEC. The 30 t ha⁻¹ treatment yielded the highest pH (7.31) at 28 DAP, whereas the 45 t ha⁻¹ treatment achieved the maximum CEC (61.84 cmol kg⁻¹). NaCl stress significantly increased soil EC from 0.93 to 8.69 mS cm⁻¹ at the highest dose, while organic carbon remained unaffected by either treatment. Although the 15 t ha⁻¹ dose was optimal for pH improvement and the 45 t ha⁻¹ dose for CEC enhancement, the 45 t ha⁻¹ treatment overall proved most effective in improving the chemical properties of saline soil and supporting red spinach growth, maintaining stable pH, EC, CEC, and organic carbon values through 28 DAP.

Keywords: Functional Microbes, Organic Soil Conditioner, Salinity Stress, Salt-Tolerant Plants, Soil Chemical Fertility

1. Introduction

Fertile agricultural land continues to decline each year. One factor contributing to the reduction in fertile land is increased soil salinity. Soil salinity is the accumulation of high levels of soluble salts (NaCl, Na₂CO₃, Na₂SO₄) in the soil, which affects plant growth and development (Rahayu et al., 2019). Soil is said to be saline generally has an electrical conductivity value in saturated soil conditions (*electric conductivity of saturation extract*, E_c) equal to or greater than 4.0 dS.m⁻¹, and an exchangeable sodium percentage (*ESP*) >15% and a soil pH value of around 8.5 (Hendri and Saidi, 2020). The main problems faced by red spinach plants in saline soils are osmotic stress and ion

toxicity. These conditions make it difficult for plants to absorb water even when there is sufficient water in the soil, and affect the ability of plant roots to absorb nutrients.

Sodium chloride (NaCl) is the main salt found in saline soil. In saline soil, NaCl levels range from 2-6% (Karolinoerita and Annisa, 2020). The high dissolved-salt content in saline soil, particularly Na⁺, reduces the exchangeable Ca, Mg, and K, thereby reducing the availability of nutrients for plants. Furthermore, plant growth is inhibited by the osmotic and toxic effects of excessive salt ions (Hendri and Saidi, 2020). Therefore, efforts are needed to increase nutrient availability in saline soil. Efforts to increase nutrient availability in saline soil

*Correspondence: rossyda_p@upnjatim.ac.id

1) Universitas Pembangunan Nasional Veteran Jawa Timur - Jl. Rungkut Madya, Gn. Anyar, Kec. Gn. Anyar, Surabaya, Jawa Timur, Indonesia

include improving soil chemical properties, as these are among the soil properties that play a role in determining soil fertility (Sihotang, 2021).

Improving the chemical properties of soil on salinity-affected land can be achieved by adding soil amendments, such as biochar. Biochar can increase ion and cation exchange capacity, soil nutrient availability, and nutrient adsorption, and reduce nutrient loss due to leaching (Liang et al., 2021). However, biochar is biologically *inert* due to its carbon-rich, highly stable structure. To optimize biochar's function, microbial immobilization within biochar is an important strategy that simultaneously enhances the soil's biological and chemical benefits. Microbes immobilized in biochar can aid in the restoration of contaminated soil, including saline soil used for cultivating red spinach (*Amaranthus tricolor*).

Red spinach has the advantage of being an easy-to-cultivate, nutrient-rich, and tolerant plant to less-than-optimal environmental conditions (2020). However, at certain salinity levels, spinach growth decreases significantly. This is consistent with Xu and Mou's (2016) statement that salt stress reduces spinach germination, root elongation, seedling growth, chlorophyll content, and photosynthesis, and increases membrane permeability. Based on research by (2021), the addition of biochar at a dose of 45 g/kg decreased soil pH, slightly increased soil EC, and significantly increased total phosphorus and total nitrogen content with increasing biochar application rates. Total N and total P increased by 34.5% and 317.4%, respectively, in soil with the highest biochar application rate. However, the application of biochar did not significantly increase spinach growth indicators (plant height, number of leaves, fresh weight and dry weight, and nitrogen concentration) under saline conditions.

Based on a review of various previous studies, it can be concluded that the application of single biochar and separate microbial inoculation has shown positive results in the amelioration of saline soils. However, research combining these two approaches through immobilization technology, especially for red amaranth cultivation, is still very limited. Therefore, this study combines biochar with microbial immobilization technology to produce microbially immobilized biochar, an integrated, innovative ameliorant material. This study uses biochar as a microbial *carrier* that not only protects against extreme environmental conditions but also provides an ideal habitat for the development and activity of rhizosphere microorganisms. Based on this novelty, this study aims to examine the effect of the interaction between microbially immobilized biochar and various levels of salinity stress (NaCl) on the temporal dynamics of changes in soil chemical properties, including pH, EC, organic carbon, and CEC, and analyze its implications for the growth and productivity of red amaranth (*Amaranthus tricolor* L.) in saline land conditions.

2. Material and Methods

2.1. Time and Place

The research was conducted from December 2024 to April 2025. This research was conducted in the Greenhouse, Land Resources Laboratory, and Plant Health Laboratory, located at the Faculty of Agriculture, National Development University "Veteran", East Java. The research location is located at coordinates 7°20'1.97" South Latitude and 112°47'28.21" East Longitude, with an altitude of 6 m above sea level. The average temperature ranges from 25°C to 33°C, and the average humidity is 90%.

2.2. Tools and materials

The tools used are divided into field tools and laboratory analysis tools. The tools needed for media preparation include a 2 mm sieve, a research label, a polybag, a ruler, a shovel, a hoe, a trowel, and scales.

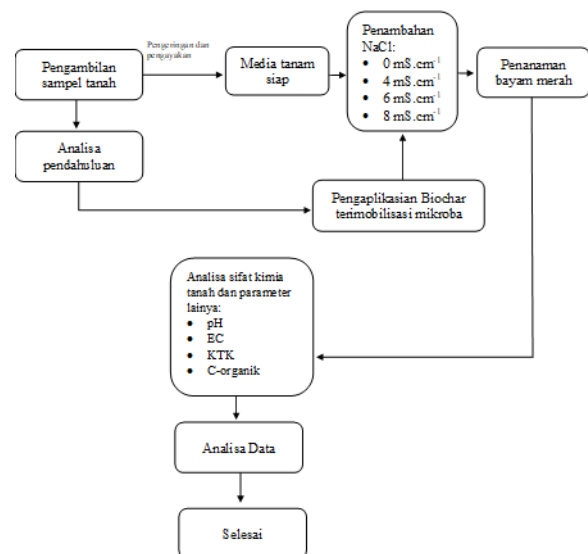


Figure 1. Research Implementation Flowchart

The tools used in the Land Resources Laboratory and Plant Health Laboratory include 0.5 mm and 2 mm sieves, analytical balance, film bottle, glass beaker, test tube, reciprocating shaker, pH meter, 100 ml volumetric flask, film bottle, pipette and pump, vortex, glass funnel, plastic funnel, filter paper, digestion tube, 100 ml Erlenmeyer, test tube, burette and static, Leibig cooler and spectrophotometer, autoclave, petri dish, test tube, micropipette and tip, analytical balance, triangle, glass beaker, Erlenmeyer, handcounter, and Bunsen. The materials used in this study are soil, NaCl, and microbially immobilized biochar. The materials used for laboratory analysis were soil samples passing through 0.5 and 2 mm sieves, ammonium acetate (NH₄OAc), 1 M pH 7.0, 50% alcohol, 10% NaCl, quartz sand, concentrated sulfuric acid (H₂SO₄) 95-97%, potassium dichromate (K₂Cr₂O₇) 1 N, tartrate buffer solution, phenol, 5% NaOCl, boiling stones, liquid

paraffin, 1 % boric acid, Conway indicator, 40 % NaOH, and Devarda alloy powder.

2.3. Research Methods

The research was conducted using an experimental method arranged in a factorial Completely Randomized Design (CRD) consisting of two factors. The first factor is the dose of microbially immobilized biochar consisting of 4 levels, namely 0 ton.ha⁻¹, 15 ton.ha⁻¹, 30 ton.ha⁻¹, and 45 ton.ha⁻¹. The second factor is salinity stress (NaCl), with 4 levels: 0 mS.cm⁻¹, 4 mS.cm⁻¹, 6 mS.cm⁻¹, 8 mS.cm⁻¹. The combination of treatments consisted of 16 treatments, each repeated 3 times, for a total of 48 sample units. The observation parameters consisted of two: soil sample analysis, including pH, EC, CEC, and C-organic analysis, and C-organic analysis. As well as the growth parameters of red spinach plants, namely the wet weight of red spinach.

2.4. Data Analysis

The research data were analyzed statistically using analysis of variance (ANOVA) within a completely randomized factorial design to determine whether each

treatment had an effect. If a significant effect of the treatment was found, further testing was conducted using the Honestly Significant Difference Test (HSD) at the error level 5%. Data analysis, including analysis of variance (ANOVA) and the Honestly Significant Difference (HSD) test at a 5% error rate, was conducted in Microsoft Excel 2019.

3. Results and Discussion

Soil with high salt content can degrade its physical, chemical, and biological properties. This condition impacts soil fertility and agricultural productivity. This study focuses on the effects of microbially immobilized biochar and various salinity stresses on soil chemical properties to improve soil quality and agricultural productivity. Soil media observations were carried out at 0 DAP and 28 DAP with observation parameters including pH, C-organic, CEC, and C-organic. The results of the study on changes in soil chemical properties at 0 DAP are shown in Table 1, and those at 28 DAP are shown in Table 2. Observations of the fresh weight of red spinach plants were conducted at the end of the observation (28 DAP) and are shown in Table 3.

Table 1 Soil Chemical Properties (pH, EC, C-organic, and CEC) after Treatment at 0 HST

Treatment	Observation to-			
	pH	EC	KTK	C-organic
Biochar				
B0	6.63 ± 0.14	4.57 ± 4.05	45.09 ± 3.14	0.54 ± 0.47
B1	6.90 ± 0.69	5.21 ± 5.25	49.73 ± 6.36	1.00 ± 0.87
B2	6.80 ± 0.22	4.80 ± 4.73	47.23 ± 10.15	0.64 ± 0.47
B3	6.84 ± 0.58	5.67 ± 5.11	61.84 ± 10.61	1.13 ± 0.20
BNJ 5%	tn.	tn.	tn.	tn.
NaCl				
N0	7.12 ± 0.62 ^b	0.75 ± 0.33 ^a	61.95 ± 12.12	0.87 ± 0.97
N1	6.76 ± 0.18 ^{ab}	4.92 ± 1.05 ^b	50.62 ± 8.19	0.97 ± 0.08
N2	6.68 ± 0.26 ^{ab}	6.72 ± 1.13 ^c	46.48 ± 2.17	0.73 ± 0.65
N3	6.60 ± 0.16 ^a	7.88 ± 2.06 ^c	44.84 ± 4.74	0.74 ± 0.69
BNJ 5%	0.45 [*]	1.59 ^{**}	tn.	tn.

Information: Numbers followed by the same letter in the same column indicate no significant difference in the 5% BNJ test.

Table 2. Soil Chemical Properties Values (pH, EC, C-organic, and CEC) after Treatment at 28 HST.

Treatment	Observation to-			
	pH	EC	KTK	C-organic
Biochar				
B0	6.77 ± 0.32 ^a	6.14 ± 6.06	44.33 ± 5.55 ^a	1.20 ± 0.48
B1	7.31 ± 1.05 ^b	5.97 ± 5.89	44.56 ± 7.16 ^{ab}	1.88 ± 0.81
B2	7.09 ± 0.32 ^{ab}	5.39 ± 4.84	44.79 ± 6.14 ^{ab}	1.50 ± 0.66
B3	7.18 ± 0.68 ^{ab}	5.28 ± 4.83	50.98 ± 2.07 ^b	1.83 ± 0.88
BNJ 5%	0.42 ^{**}	tn.	3.85 [*]	tn.
NaCl				
N0	7.63 ± 0.85 ^b	0.93 ± 0.39 ^a	45.53 ± 7.02	1.75 ± 1.31
N1	7.07 ± 0.22 ^a	5.24 ± 0.61 ^b	46.58 ± 7.95	1.80 ± 0.17
N2	6.86 ± 0.35 ^a	7.92 ± 1.95 ^c	48.76 ± 4.42	1.62 ± 0.78
N3	6.78 ± 0.17 ^a	8.69 ± 1.54 ^c	43.80 ± 6.80	1.24 ± 0.26
BNJ 5%	0.45 [*]	1.40 ^{**}	tn.	tn.

Information: numbers followed by the same letter in the same column indicate no significant difference in the 5% BNJ test.

3.1. Soil pH

Soil pH parameters at 7 HST (Days After Treatment) showed significant differences in the single-factor NaCl treatment (Table 1). Soil pH values based on the effect of administration of NaCl range from 6.60 to 7.12, which indicates a neutral criterion. Treatment N0 gave the highest result, namely 7.12, which was significantly different with N3 treatment with a pH value of 6.60 (Table 1). Based on the soil pH data in Table 1, the NaCl treatment decreased soil pH from 8.60 (initial soil sample) to 7.12. This is in accordance with the statement (Kome et al., 2018) that NaCl salinity can affect soil pH through a cation exchange mechanism, where Na⁺ ions replace base cations such as Ca²⁺ and Mg²⁺ in the soil sorption complex. This process can cause a decrease in soil pH because the base cations are leached and replaced by H⁺ ions.

The soil pH parameter values at 28 HST observations showed significant differences in the single factor of NaCl treatment and in the microbially immobilized biochar, followed by the 5% BNJ test (Table 2). Treatment B1 showed the highest value of 7.31, which was significantly

higher than the B0 control (6.77). This indicates that providing biochar at level B1 significantly increased the pH compared to the control (without biochar). The increase in pH caused by the provision of biochar during the decomposition of organic matter can release base cations (Ca²⁺, Mg²⁺ and K⁺), thereby increasing the concentration of OH⁻ ions (Atmojo, 2013; Arifin et al., 2022). This is in line with the research of (2024): there was an increase in soil pH, especially at a high dose of 100 g/polybag biochar (B2), and each additional dose of rice husk biochar further increased soil pH. Biochar has functional groups that can absorb acid cations such as Al³⁺ and H⁺ ions in the soil, thereby reducing the concentration of H⁺ in the solution.

In the NaCl factor, the results of the 5% BNJ test showed a different pattern, with treatment N0 (without NaCl) giving the highest value, namely 7.63, denoted by letter b, which was significantly different from all NaCl treatments. Treatments N1 (7.07), N2 (6.86), and N3 (6.78), which were all noted with the letter a, showed that the three treatments were not significantly different from each other.

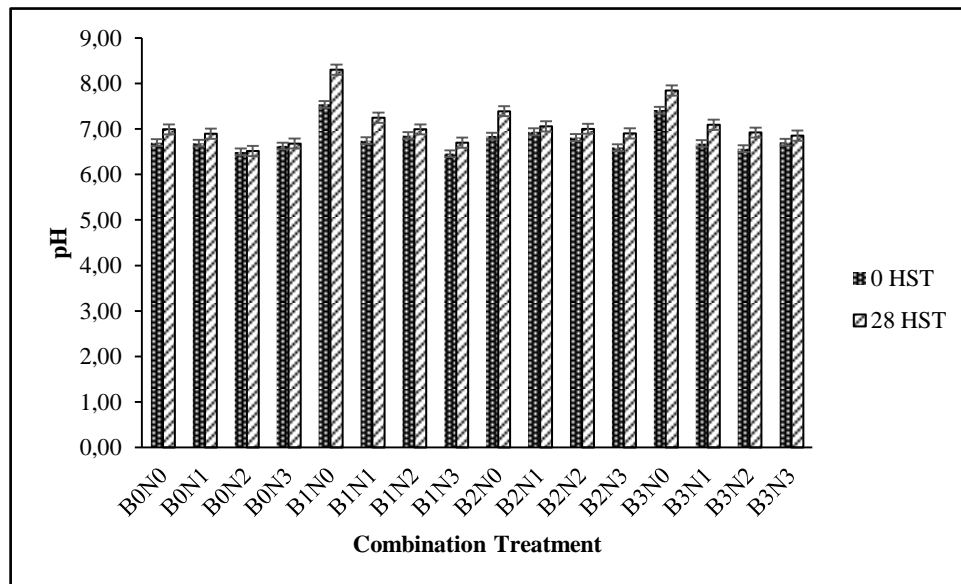


Figure 21 Histogram of Soil pH Value after Treatment. Information: B0 (Control), B1 (Biochar 82.5 grams per polybag), B2 (Biochar 165 grams per polybag); B3 (Biochar 247.5 grams per polybag), N0 (Control), N1 (NaCl 24.2 grams per polybag), N2 (NaCl 36.3 grams per polybag); N3 (NaCl 48.4 grams per polybag).

Figure 2 shows a consistent pattern: biochar treatment tends to increase soil pH, whereas NaCl addition lowers pH, especially at higher concentrations. The pattern of increasing soil pH is clearly visible in treatments containing biochar, especially in the sample codes B1N1, B2N1, and B3N1, which show higher pH values than the control (B0). This increase occurs because biochar has alkaline properties with high cation base content, such as K⁺, Na⁺, Ca²⁺, and Mg²⁺, which can increase soil pH. A decrease in soil pH was observed in treatments containing NaCl, especially at higher concentration levels such as N2 and N3. Based on Figure 2, it can be concluded that

applying biochar at the optimal dose (B1) without NaCl (N1) is the best strategy to maintain healthy soil pH and support optimal plant growth.

3.2. EC land

Soil electrical conductivity is the ability of the soil to conduct an electric current. Soil salinity levels are grouped based on the electrical conductivity (DHL) value, namely non-saline soil (<1.0 dS/m), very low (<1.1 - 2 dS/m), moderate (<2.1 - 4 dS/m), rather high (4-8 dS/m), and high (8.8-16 dS/m) (Mutmainah et al., 2022). High EC values will cause plants to experience stress due to high salt

concentrations. Soil EC parameters at 7 DAP and 28 DAP showed significant differences in the single-factor NaCl treatment. Soil EC values based on the effect of NaCl administration at 0 and 28 DAP ranged from 0.75 mS/cm to 8.69 mS/cm, indicating a high level of salinity (Table 1 and Table 2). The fact that a very significant difference was already seen at 0 HST indicates that Na^+ and Cl^- ions from NaCl have a direct effect on the soil system affecting immobilization. The significant difference in soil EC values has implications for the activity of the inoculated *Pseudomonas fluorescens*. *Soil microorganisms, including Pseudomonas* sp. bacteria can survive at salinity levels of 4.69 dS m^{-1} (3000 ppm) and 7.81 dS m^{-1} (5000 ppm). Above a concentration of 7.81 dS m^{-1} (5000 ppm), the number of surviving.

Soil EC parameters at 0 DAP and 28 DAP showed no significant differences in the single-factor treatment of microbially immobilized biochar, but biochar application could reduce soil EC. This indicates that the ability of microbially immobilized biochar to suppress the negative effects of salinity is not optimal in the early phase of plant

growth. This condition may be caused by the short interaction time among biochar, microbes, and soil, as well as by high osmotic pressure resulting from NaCl accumulation in the growing medium, which inhibits root water and nutrient absorption (Murtaza et al., 2024). Treatments B2 and B3 had the lowest EC content compared to the other treatments (table 1 and table 2). Based on this, treatments B2 and B3 are the best treatments in reducing soil EC. This is in line with the research of Chen et al., (2024) where the application of 1%, 2.5%, and 5% biochar is effective in suppressing the EC of saline-alkali soil, and based on response modeling, the optimal dose of biochar to reduce soil EC is in the range of $\pm 3.16\%$. Treatment B0 (control) produced the highest EC value of 6.28 mS/cm among other treatments. In the initial stage of application, biochar is still in a fresh state, with a pore structure that has not fully integrated with the soil matrix and has not formed a conducive habitat for microbial colonization. Therefore, at this stage the influence of biochar has not been able to significantly reduce the negative impact of NaCl.

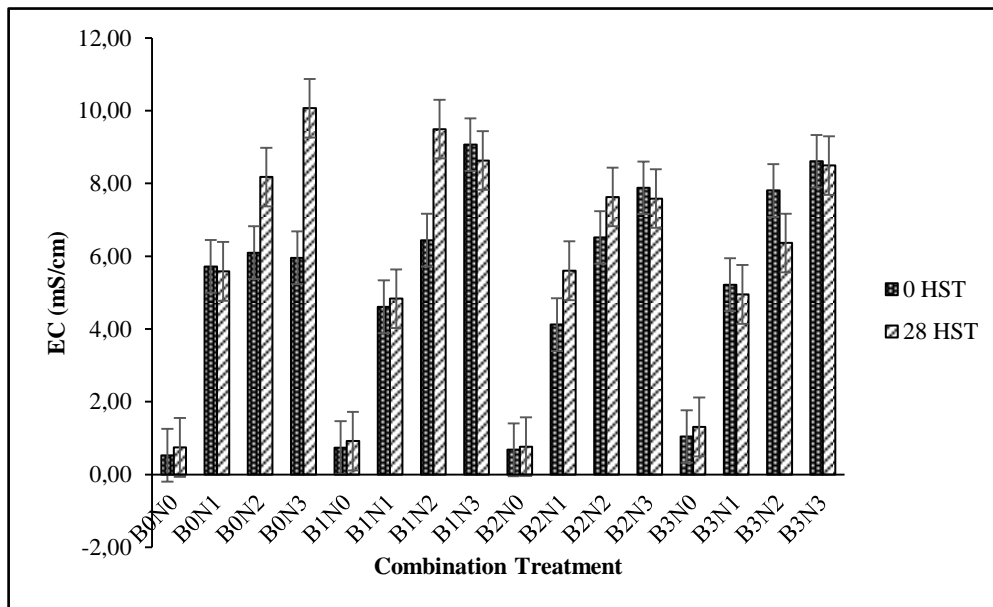


Figure 3. Histogram of Soil ECT Values after Treatment. Information: B0 (Control), B1 (Biochar 82.5 grams per polybag), B2 (Biochar 165 grams per polybag); B3 (Biochar 247.5 grams per polybag), N0 (Control), N1 (NaCl 24.2 grams per polybag), N2 (NaCl 36.3 grams per polybag); N3 (NaCl 48.4 grams per polybag).

Based on Figure 3, the soil EC analysis showed an increase in soil electrical conductivity (EC) across various NaCl salinity treatments (N0, N1, N2, N3) during the observation period from 0 HST to 42 HST (Days After Planting). The high EC indicates that the soil solution is at its maximum concentration, indicating high salinity stress. EC values above 4 mS/cm are generally considered high salinity levels and can inhibit the growth of most crops.

3.3. C-organic

Soil organic C parameters at 0 DAP and 28 DAP

showed no significant differences in the single-factor treatment of microbially immobilized biochar. Based on Table 1, soil organic C parameters at the 0 DAP observation, the application of *Pseudomonas fluorescens*-immobilized biochar treatment increased soil organic C from 0.99% (initial soil sample) to 1.13% as the biochar dosage increased. Nuryani and Handayani (2003); Yosephine et al. (2020) stated that organic matter added to the soil after decomposition can increase soil carbon content, as well as the organic acids derived from the weathering of organic matter. Based on Table 2, at the 28

DAP observation, the B3 treatment showed a higher organic C content than the 0 DAP treatment. This indicates that high-dose biochar is beginning to show its slow-release effect, as the carbon stored in its pore structure is gradually released.

The NaCl factor showed no significant effect on the C-Organic value. The higher the NaCl concentration, the lower the soil C-organic content. The decrease in value is evident in the N2 and N3 treatments (Table 2), indicating that at higher NaCl concentrations, the toxic effects of salt begin to dominate and inhibit bacterial activity. Salinity

conditions reduce the number of bacterial cells, thereby affecting nodule formation (Mutmainah et al., 2022). With decreased microbial activity, the mineralization of organic matter slows, thereby reducing the release of organic C.

This is in line with Qu et al., (2018) research on the addition of NaCl at concentrations of 3, 6, 9, and 12 g/L significantly inhibited the decomposition process of soil organic carbon (SOC) and emissions of CO₂-C and CH₄-C gases during 28 days of incubation compared to the control treatment without salt, indicating that high salinity can suppress organic C mineralization in tidal soil.

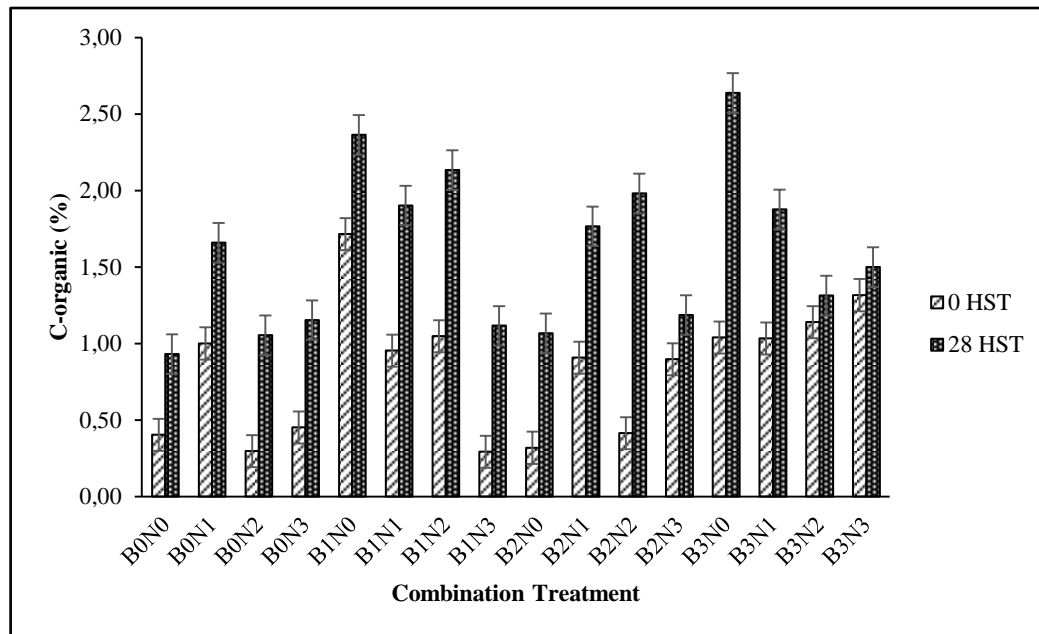


Figure 4. Histogram of Soil Organic C Value after Treatment. Information: B0 (Control), B1 (Biochar 82.5 grams per polybag), B2 (Biochar 165 grams per polybag); B3 (Biochar 247.5 grams per polybag), N0 (Control), N1 (NaCl 24.2 grams per polybag), N2 (NaCl 36.3 grams per polybag); N3 (NaCl 48.4 grams per polybag).

Based on Figure 4, biochar and microbes can increase the organic C content of saline soil. The initial soil value before treatment was 0.99% and is considered low. Based on Figure 4, the best treatment is the B3N1 combination, which shows consistently high C-organic content, especially at 28 HST. B3N1 shows that high doses of biochar with mild osmotic stress of NaCl are effective in maintaining high C-organic content due to the direct contribution of carbon from the biochar itself. This is in line with the research of Wang et al. (2025). Application of biochar to saline-alkali soil at doses of 10, 20, and 30 t ha⁻¹ significantly increased the soil organic carbon (SOC) content and the C/N ratio of the soil compared to the control without biochar, indicating the role of biochar in improving C-organic storage in soils degraded by salinity. A high NaCl concentration (N3) consistently inhibited at 28 HST, as evidenced by lower C-organic values in the B0N3, B1N3, B2N3, and B3N3 combinations.

3.4. KTK

Soil cation exchange capacity (CEC) is also a chemical property that plays a significant role in maintaining and improving soil fertility. Cation exchange capacity (CEC) is the ability of soil colloids to adsorb and exchange cations. CEC can be influenced by soil texture and organic matter content. The value of CEC depends on soil texture, clay mineral type, and the amount of organic matter present.

and organic matter content. The higher the clay content or finer the texture, the higher the CEC. Similarly, the higher the organic matter content, the higher the CEC.

The soil CEC parameters at 0 DAP and 28 DAP showed significant differences in the single-factor treatment with microbially immobilized biochar. In contrast, the NaCl treatment did not show a significant effect at all observation intervals. At 28 DAP, the B3 treatment (50.98) was significantly different from B0 (44.33) (Table 2). This was because the biochar began to decompose stably and increased soil moisture and absorbed excess Na⁺ ions, helping reduce salinity stress and increase plant water use efficiency (Acharya et al., 2024).

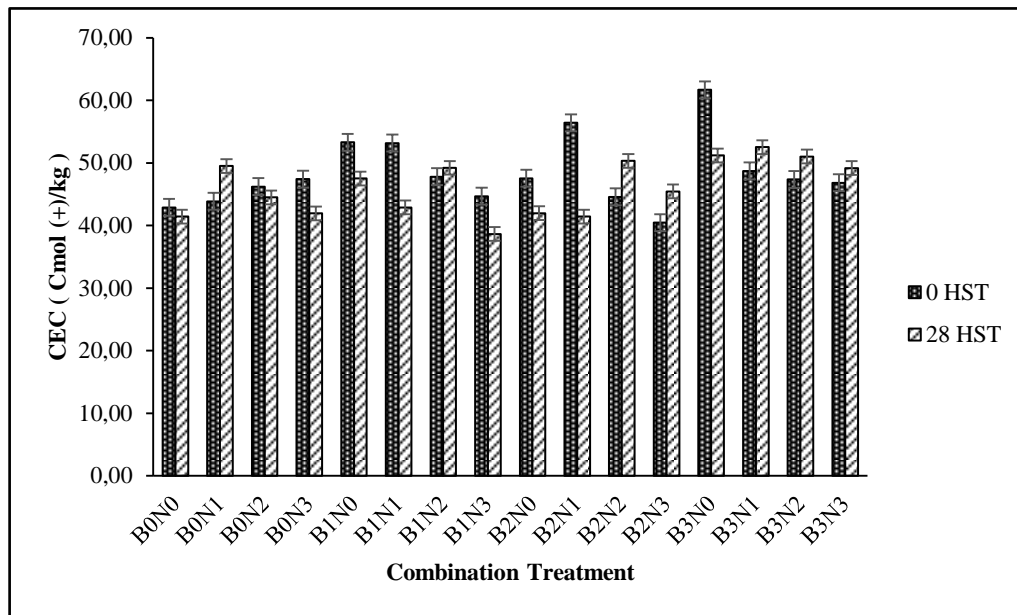


Figure 5. Histogram of Soil CEC Values after Treatment. Information: B0 (Control), B1 (Biochar 82.5 grams per polybag), B2 (Biochar 165 grams per polybag); B3 (Biochar 247.5 grams per polybag), N0 (Control), N1 (NaCl 24.2 grams per polybag), N2 (NaCl 36.3 grams per polybag); N3 (NaCl 48.4 grams per polybag).

Based on Figure 5, at 28 HST, the B3N0 and B3N1 treatments had the highest CEC content among the treatment combinations. In low NaCl (N1) conditions, Na⁺ ions were present in increased amounts, but had not yet reached toxic levels that could damage the soil colloidal structure. In addition, this was because the *Pseudomonas fluorescens* bacteria immobilized on biochar were halophilic, allowing them to still grow at a salinity of 4 mS/cm (N1) (Arisandi et al., 2017). The B3N1 treatment was the best at maintaining CEC availability in the soil. This is in line with research (Abdou et al., 2024). Application of biochar at a dose of 20 tons ha⁻¹ on saline soil significantly increased the cation exchange capacity (CEC) of the soil compared to without biochar, accompanied by an increase in organic matter content in saline land.

High-dose biochar administration (B3) provides a much larger number of negatively charged groups, surface area, and porosity than low and medium doses, thereby significantly increasing the soil's cation exchange capacity (CEC).

3.5. Wet Weight of Red Spinach Plants

Plant wet weight is an important parameter for assessing vegetative growth, especially in environments subject to salinity stress. Plant wet weight is an indicator for determining plant biomass because biomass formation is significantly influenced by the plant's ability to absorb water and nutrients from the growing medium. Based on this, the interaction between biochar soil amendments and salinity stress induced by NaCl is thought to play a role in determining the plant's ability to adapt and maintain growth.

The results of the analysis of variance showed that the combination of biochar and NaCl did not significantly affect spinach plant fresh weight. In the treatment without NaCl (N0), plant fresh weight tended to be higher across all biochar treatment levels. The B3N1 treatment yielded the highest amount at 36.40 g, but was not significantly different from the other treatments (Table 4.3). The salinity stress treatment via NaCl application had a significant effect on plant fresh weight. Increasing the NaCl concentration resulted in a significant decrease in fresh weight across all biochar application doses. This is shown in the graph (Figure 6).

Table 3. Wet weight of red spinach plants

Biochar	NaCl			
	N0	N1	N2	N3
B0	40.28 ± 8.61	30.28 ± 2.83	24.48 ± 4.60	39.12 ± 2.03
B1	40.33 ± 10.30	21.12 ± 7.72	32.04 ± 0.00	24.74 ± 7.30
B2	41.90 ± 9.23	22.53 ± 5.31	21.41 ± 10.40	32.04 ± 0.00
B3	41.93 ± 4.36	36.40 ± 0.00	32.04 ± 0.00	32.04 ± 0.00
BNJ 5%	tn.			

Information: Numbers followed by the same letter in the same column indicate no significant difference in the 5% BNJ test.

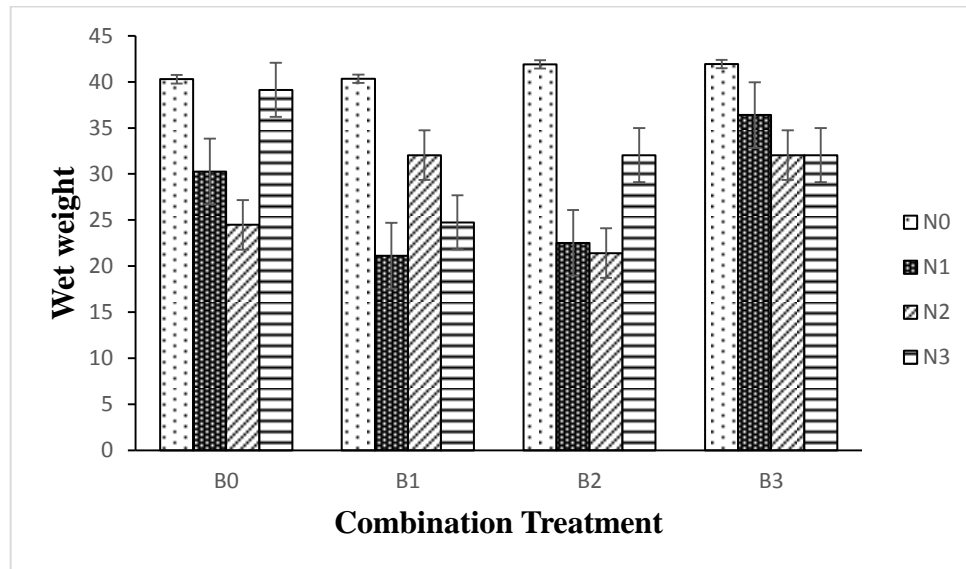


Figure 6. Histogram of Wet Weight Values of Red Spinach After Treatment. Information: B0 (Control), B1 (Biochar 82.5 grams per polybag), B2 (Biochar 165 grams per polybag); B3 (Biochar 247.5 grams per polybag), N0 (Control), N1 (NaCl 24.2 grams per polybag), N2 (NaCl 36.3 grams per polybag); N3 (NaCl 48.4 grams per polybag).

The administration of high concentrations of NaCl, namely the N2 and N3 treatments, resulted in the lowest wet weight of plants compared to other treatments. This is in line with the opinion of (2022) that high salinity levels can inhibit plant growth by suppressing cell division and elongation, reducing protein synthesis, and reducing plant biomass accumulation.

4. Conclusion

The B3N1 treatment (biochar 45 tons/ha & NaCl 4 mS/cm) was the best treatment, consistently producing low and stable EC, high organic C, optimal CEC, and the highest fresh weight of red spinach. High doses of biochar effectively maintain soil chemical quality by increasing nutrient availability without Na^+ competing with essential

cations. For red spinach cultivation on saline land, applying microbially immobilized biochar at 45 tons/ha is recommended for optimal results.

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