

Municipal Sludge–Derived Biochar Enriched with *Pseudomonas* sp. Isolate RA-21 Enhances Soil Properties and *Capsicum annuum* Growth

(Bioarang Terbitan Enapcemar Perbandaran Diperkaya dengan *Pseudomonas* sp. Pencilan RA-21 Meningkatkan Sifat Tanah dan Pertumbuhan *Capsicum annuum*)

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ABSTRACT

The growing world population has increased the demand for food to meet global needs, which contributed to the growth and vibrancy of the agricultural sector. There is an urgent need for soil treatment to restore healthy soil conditions due to extensive and unregulated agricultural activities that have degraded soil fertility, resulting in reduced crop yields. Sludge from a municipal wastewater treatment plant is potentially used in biochar production. Thus, the combination of biochar with plant growth-promoting bacteria offers an ecologically friendly alternative for improving soil health and agricultural output. ICP-MS and Brunauer–Emmett–Teller (BET) analysis were used to determine the quality of the developed biochar. An indigenous *Pseudomonas* sp. isolate, RA21, was mixed with biochar and used as a soil conditioner to promote chili plant growth. Plant height, leaf size, and stem diameter were measured to compare treated and untreated plants. ICP-MS analysis indicates that zinc and iron were the dominant metals present in all samples. BET analysis shows that approximately 70% of the biochar pore volume is composed of mesopores. The Fe and Zn nutrients in biochar serve as nutrient sources for plant uptake, while the high proportion of mesopores provides sites for bacterial colonization and enhances adsorption capacity. The application of a soil conditioner resulted in greater plant growth variables such as height, leaf size, and stem diameter when compared to the control group, and was equivalent to commercial fertiliser. Using soil conditioner resulted in more leaf area (651 cm²) in chilli plants compared to those treated with commercial fertiliser (629 cm²). Biochar, in particular, serves as a transporter for bacteria, allowing for optimal growth and activity while also providing nutrients that promote healthy plant development. The combination of sludge-derived biochar with the indigenous *Pseudomonas* sp. strain RA21 has shown encouraging results as a soil conditioner by increasing nutrient availability, allowing for the value-added use of municipal sludge within a circular economy framework.

Keywords: Biochar; *Pseudomonas* sp.; sludge-derived biochar; soil conditioner

ABSTRAK

Populasi dunia yang semakin meningkat telah meningkatkan permintaan terhadap makanan untuk memenuhi keperluan global, yang mana menyumbang kepada pertumbuhan dan merencanakan sektor pertanian. Terdapat keperluan mendesak untuk merawat tanah bagi memulihkan semula keadaan tanah yang sihat akibat daripada aktiviti pertanian yang meluas dan tidak terkawal yang telah merosotkan kesuburan tanah, sekali gus mengurangkan hasil tanaman. Sisa pepejal dari loji rawatan air sisa bandar berpotensi digunakan dalam penghasilan bioarang. Oleh itu, gabungan bioarang dengan bakteria penggalak pertumbuhan tumbuhan menawarkan alternatif yang mesra alam untuk meningkatkan kesihatan tanah dan hasil pertanian. Analisis ICP-MS dan Brunauer–Emmet–Teller (BET) digunakan untuk menentukan kualiti bioarang yang dihasilkan. Bakteria *Pseudomonas* sp. pencilan RA21 dicampurkan dengan bioarang dan digunakan sebagai perapi tanah untuk merangsang pertumbuhan tanaman cili. Ketinggian pokok, saiz daun dan diameter batang diukur untuk membandingkan tanaman yang dirawat dan tidak dirawat. Analisis ICP-MS menunjukkan zink dan ferum merupakan logam berat utama yang terdapat dalam semua sampel. Analisis BET menunjukkan kira-kira 70% liang bioarang terdiri daripada liang mikro. Nutrien Fe dan Zn dalam bioarang berfungsi sebagai sumber nutrien untuk penyerapan tumbuhan, manakala peratusan mesoliang yang tinggi menyediakan tapak bagi pengkolonian bakteria serta meningkatkan kapasiti penyerapan. Penggunaan perapi tanah menghasilkan keluasan daun yang lebih besar (651 cm²) pada tanaman cili berbanding tanaman yang dirawat dengan baja komersial (629 cm²). Bioarang khususnya bertindak sebagai pengangkut bagi bakteria,

mbolehkan pertumbuhan dan aktiviti yang optimum di samping membekalkan nutrien yang menyokong perkembangan tumbuhan yang sihat. Gabungan bioarang terhasil daripada enap cemar dengan strain *Pseudomonas* sp. pencilan RA21 menunjukkan hasil yang memberangsangkan sebagai bahan pembaik tanah melalui peningkatan ketersediaan nutrien, sekali gus membolehkan penggunaan nilai tambah enap cemar perbandaran dalam kerangka ekonomi kitaran.

Kata kunci: Bioarang; bioarang berasaskan enap cemar; pembaik tanah; *Pseudomonas* sp.

INTRODUCTION

Land, particularly soil, is a vital component of human survival and ecological function. It forms the physical basis for plant development and is the major source of food for humans (Silver et al. 2021). Soil supports a varied population of creatures and serves as a primary repository for carbon and other nutrients necessary for life. Its retention of water capability promotes healthy plant development while improving soil stability and minimizing erosion, which can harm human activities. Furthermore, soil plays an important role in controlling atmospheric composition by influencing the dynamics of greenhouse gases. Given that land resources account for over 95% of global food production and raw material supply, soil productivity must be maintained (Pozza & Damien 2020). However, the growing dependence on intense and unsustainable fertilizer techniques to increase crop yields puts soil health and prolonged agricultural viability at risk.

Accordingly, the use of inorganic chemical fertilizers is rising alongside the growing demand for the global food supply. The use of chemical fertilizers on a large scale has harmed ecosystems and the environment. This issue has also led to soil degradation in terms of nutrient content, soil acidity, water leaching and nutrients, as well as the loss of other organic matter in the soil. In addition, this uncontrolled land use has also led to a declining trend in microbial diversity. For instance, biofertilizers have a substantially greater microbial population (about 3.5×10^7 CFU/mL) than chemical fertilizers (2.6×10^5 CFU/mL), indicating that chemical fertilizer use diminishes microbial richness and diversity (Adam et al. 2025). Soil microorganisms contribute to supplying organic matter and nitrogen, which plants utilize for growth. The high acid content in chemical fertilizers will change the pH of the soil and make it less suitable for microbes to reproduce (Naz et al. 2022a). The natural environment can provide a suitable habitat for these microbes to produce a high-quality substrate for plants and increase soil aeration and water content in the soil (Tauro et al. 2021).

The massive use of chemical fertilizers as a source of nutrients for plants has worsened the situation by contributing to soil acidity, releasing greenhouse gases, polluting water resources, and affecting aquatic life (Surbala Devi & Borah 2023; Zhang et al. 2022a). According to Zhang et al. (2022b), applying fertilizers on a large scale, particularly at nitrogen levels exceeded 350 mg kg^{-1} caused soil acidification and disrupted microbial activity. In addition, the utilization of chemical and organic fertilizers cannot conserve soil nutrients. This phenomenon

occurs when fertilizer nutrients are unable to permeate the soil efficiently due to poor soil conditions, which impede water and nutrient absorption. Soils that have inadequate cation exchange capacity (CEC) and pH have a decreased ability to hold onto and exchange nutrients (Howe et al. 2024). As a result, the fertilizers are not effective on plants because the nutrients from the fertilizers are only found in the topsoil and cannot be absorbed by the roots (Chakraborty et al. 2022; Nurhafizhoh et al. 2022; Priya, Sarkar & Maji 2024).

Therefore, the application of soil conditioners to improve soil structure is one of the solutions to the soil fertility problem. A soil conditioner is a chemical or material applied to the soil to improve its qualities, such as water absorption and structural stability, which are vital for plant development and fertility. At the same time, these soil conditioners improve soil deficiencies in terms of its structure and nutrient content (Mukherjee 2013). Several types of soil conditioners are being used to improve soil conditions, including organic and inorganic conditioners. Organic conditioners include compost, manure, biochar and sewage sludge, while inorganic conditioners include gypsum, lime, sulfur, and zeolite (Shinde, Sarkar & Naik 2019).

In this study, sewage sludge was chosen as a soil conditioner because it contains higher quantities of nitrogen and phosphorus than other soil conditioners, such as municipal solid waste (El-Naggar & El-Ghamry 2001). In 200 kg of sludge, nitrogen content is estimated to be 6 kg of total weight, 8 kg of phosphorus, and 10 kg of various dissolved salts (Iticescu et al. 2018). The sewage sludge that underwent the high-temperature combustion process produced biochar that had a high total surface area, the ability to absorb organic compounds, and a large number of fine cavities of $20 \mu\text{m}$ in size, facilitating the adhesion of bacteria (Mian, Ao & Deng 2023). This is important for the attachment of plant growth-promoting microorganisms, as well as increasing the ability to hold water in them before being introduced into the infertile soil as a treatment approach.

Pseudomonas aeruginosa was chosen as a plant growth-promoting bacterium and associated with biochar due to its capacity to improve plant development, stimulate microbial diversity and activity, and minimize heavy metal pollution (Zhao, Zhao & Wang 2025). The chili plant, *Capsicum annum*, was planted into the treated soil, fertilizer, and control soil mediums. Several parameters, such as plant height, leaf area, and stem diameter, were measured and recorded as one of the

indicators of the effectiveness of the treated soil. At the end of the experiment, growth parameters of chili plants in treated soil outperformed the control plants, indicating the success of the soil treatment process using biochar and microorganisms.

MATERIALS AND METHODS

MATERIALS

Municipal sludge was obtained from Indah Water Konsortium Berhad, Malaysia (IWK) sewage wastewater treatment plant (STP). All chemicals were purchased from Fisher Scientific (M) Sdn. Bhd.

BACTERIAL SAMPLE COLLECTION, ISOLATION AND IDENTIFICATION

The sample was taken from sediment near Sungai Hulu Langat in Selangor (2.931881, 101.775708). Five-centimeter soil depth was taken using a spatula and transferred into a sampling bottle. The soil sample was kept in the chiller at 4 °C prior to bacterial isolation. The 100 µL of liquid (10 g soil combined with 0.85% sodium chloride) was spread over Nutrient Agar and incubated for 5 days at 37 °C. The chosen pure colony was further investigated for possible plant growth-promoting microorganisms. The bacterial DNA was collected using the Bio Basic Kit (EZ-10 Spin Column Genomic DNA Minipreps Kit, Bacteria), which was then utilized for PCR. In this study, the universal primers used were 27F (5'AGAGTTTGATCATGCTCAG 3') and 1429R (5'GGTACCTTGTTACGACTT 3') for forward and reverse primers, respectively (Vanhee et al. 2024). The obtained forward and reverse nucleotide sequences were aligned and analyzed via NCBI BLAST to reaffirm the bacterial species identification.

BIOCHAR PRODUCTION

The production of biochar was conducted according to Agrafioti et al. (2013) with slight modifications. Sludge from three Indah Water Konsortium (IWK) wastewater treatment plants (WWTP) was pre-dried separately using the oven (Memmert) at 100 °C for several days until a constant mass was reached. The dried sludge was crushed with a mortar and pestle before it was sieved through a 4 mm sieve. Sieved sludge was burned in a furnace at 600 °C for 4 h with a temperature increment of 17 °C/min. The produced biochar was weighed and kept in an airtight container for future use.

HEAVY METAL ANALYSIS

Heavy metals analysis for each biochar was performed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyzer. Metals from biochar samples were extracted following the method described by Zhao (2019) with slight modifications. Ten milligrams of biochar sample

were dissolved in aqua regia solution containing 20 mL concentrated HCl, 4 mL HNO₃, 1 mL hydrofluoric acid, and 1 mL hydrogen peroxide. The solution was heated on a hot plate for 15 min and then cooled to room temperature before being filtered to separate the insoluble biochar. The filtrate was transferred into a 100 mL volumetric flask and made up with distilled water until it reached the mark. The prepared samples were sent for ICP-MS analysis according to the standard setup (Zhao 2019).

POROSITY AND SURFACE AREA ANALYSIS

The surface area and porosity of the biochar were assessed using the Brunauer-Emmett-Teller (BET) method for mesopore analysis. The samples were dry materials in powder or fine granular form, with a minimum mass of 1 g. Degassing was carried out at a temperature below the point of melting, up to 350 °C. Biochar samples were taken and sent to the analysis laboratory to determine the surface area and porosity of biochar. The total surface area is determined from the gradients and intersections of the BET graph using the BET equation and the cross-sectional area of the nitrogen molecule (Walton & Snurr 2007).

pH VALUE MEASUREMENT

Soil pH measurement was conducted using the method outlined by Braus and Thea (2021) with slight modifications. The soil sample was mixed with 0.01 M KCl at a ratio of 1:1. The tube was vortexed until well-mixed and let rest for 40 min. The tube was then centrifuged at 8000 rpm for 60 s. The aqueous phase was taken out and measured for pH using a pH meter (Metrohm) (Braus & Thea 2021).

SOIL CONDITIONER FORMULATION

The *Pseudomonas* sp. isolate RA21 with ~1.0 optical density was cultured in nutrient broth for 24 h at 25 °C under shaking conditions. The bacterial culture was incorporated into biochar in a 1:1 (v/v) ratio. Soil conditioners that are made from biochar were moistened and incubated aerobically in open trays for 30 days at a temperature of 25 °C to promote microbial colonization and nutrient stabilization between the soil, the biochar, and the activity of microorganisms. The produced conditioner was mixed with soil in a 1:1 (w/w) ratio in a polybag for the cultivation of chili plants, *C. annuum*, comprising a final concentration of 50% growth medium (topsoil + coconut husk) and 50% soil conditioner.

THE APPLICATION OF BIOCHAR TO CHILLI PLANTS

Capsicum annuum seeds were sown for a few weeks in a greenhouse. After the seedlings reached the 4-leaf stage and had the same height, they were transferred into polybags with a dimension of 14 cm high and 18 cm in diameter with water drainage. Chili plants were transplanted into three different soil mediums, namely control (soil only),

fertilizer soil (commercial fertilizer AB), and treated soil (soil conditioner). Each treatment had three replicates, with 2 kg of soil per polybag. Those plants were watered once per day and placed in an open area for direct sunlight.

GROWTH MEASUREMENT OF *Capsicum annuum* L.

Three growth parameters were evaluated: plant height (measured with a ruler from base to highest point), leaf area (length \times width), and stem diameter (measured with a digital caliper). Plants grown in untreated, fertilized, and conditioner-treated soil under the same environmental conditions as previously maintained were compared. Each parameter was measured at the end of week 4 propagation, whereas each treatment was measured every week after transplanting in three biological replicates (three plants per treatment). Measurement data were recorded and calculated for statistical analysis.

STATISTICAL ANALYSIS

All statistical analysis was conducted using Minitab 16. Post hoc Tukey's HSD with two-way ANOVA was used to rank the data and further analyze the significant differences between the obtained results. All analyses were performed at $\alpha = 0.05$. All sampling and analysis were conducted in biological triplicates and data were presented as mean \pm standard deviation.

RESULTS AND DISCUSSION

HEAVY METAL CONTENT IN SLUDGE

Heavy metals analysis is important before any sludge sample can be used as a soil conditioner. ICP-MS was used

to determine the existence of harmful metal elements in the sludge sample. Three sites of IWK STP were chosen and labelled as Sample A, Sample B and Sample C. Figure 1 represents ICP-MS data in the form of concentration in parts per billion (PPB) against the type of metals present in the sludge samples. From the graph (Figure 1), nine dominant metals were detected in all three samples. Zinc (Zn), Manganese (Mn), and iron (Fe) were the most prevalent metals in all three samples, causing environmental heavy metal toxicity and ecosystem instabilities (Das et al. 2023).

Zinc is the most abundant metal present in the IWK STP sludge, with the highest concentration of less than 900 ppb for Sample B. A similar finding was reported in 2021, where sludge from a municipal WWTP in Poland showed zinc as a dominant metal presence (Wojciula, Boruszko & Pajewska 2021). The same pattern was reported in several places, such as in Spain and China, where each municipal sludge showed zinc as the dominant metal presence. However, the concentration is different for each place (Sánchez et al. 2017; Yang, Huang & Lai 2017). High zinc concentration in municipal wastewater sludge is common, as this metal can come from various sources, such as kitchen households (brass metal) and galvanized pipelines along the WWTP (Shamuyarira & Gumbo 2014). Zinc's allowable limits in the environment vary depending on the location. The US EPA stated the allowable limit for zinc in aquatic environments safe for humans is to be less than 26000 ppb (Li et al. 2019), and the Contaminated Land Management and Control Guidelines (CLMCG), Malaysia, specified Zn limits of 2.30×10^3 mg/kg in domestic soil and 3.50×10^4 mg/kg in commercial soil (Abdul Rashid, Wan Yaacob & Umor 2023).

Manganese was detected in all three samples, with Sample C having the highest manganese concentration,

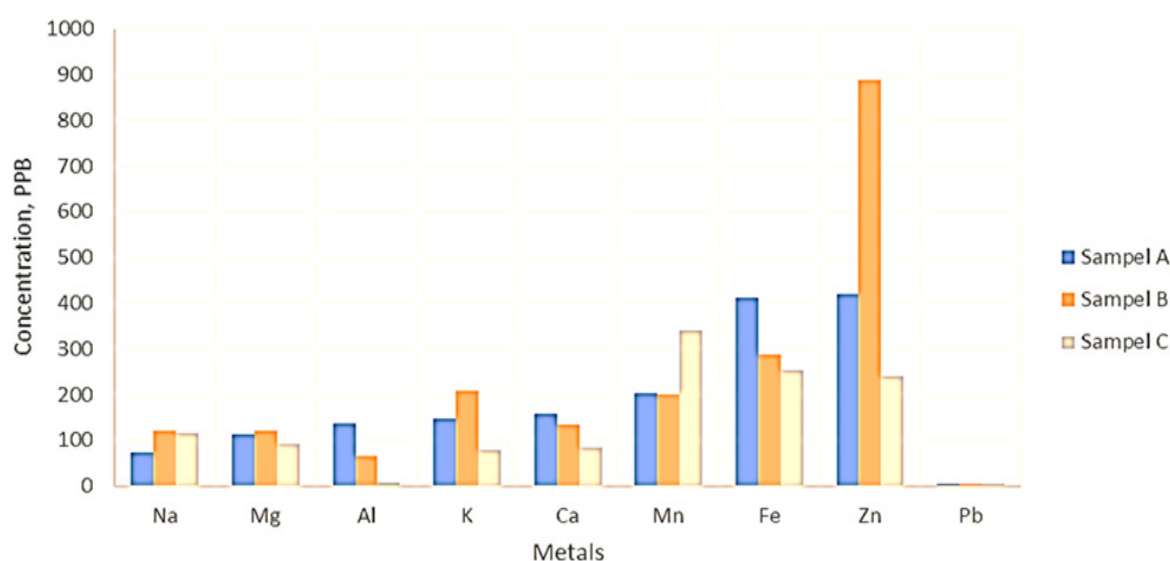


FIGURE 1. Concentration of heavy metals in sludge labeled as Sample A, Sample B and Sample C using an ICP-MS analyzer

approximately 350 ppb, with other samples showing below 200 ppb. The report on the physicochemical properties of municipal sludge in Saudi Arabia showed that the manganese element was present in the sludge waste, where most of the sites reported the manganese concentrations were below 2.0×10^5 ppb (Al-Malack et al. 2008). Compared to previous studies, the manganese concentration in IWK sludge is significantly lower than in other countries. The allowable limit in Malaysia for manganese in a raw water sample is 200 ppb, while the WHO stated the allowable limit for manganese is 400 ppb (Abagale et al. 2013; Hassimi et al. 2011). Malaysia's CLMCG allows for manganese levels of 1.80×10^5 ppb and 2.60×10^6 ppb in residential and industrial soils, respectively (Abdul Rashid, Wan Yaacob & Umor 2023). Based on this fact, the presence of zinc and manganese in IWK STP sludge is within acceptable limits and can be further used as a soil conditioner.

BIOCHAR POROSITY PROFILE

Biochar's surface profile and its porosity were determined using BET analysis. Based on Table 1, it is estimated that the pore diameter is approximately 3.6725 nm based on the value given by the average pore adsorption diameter. The size indicated that the biochar produced in this experiment consists of mesopores with a pore density of $0.1122 \text{ cm}^3/\text{g}$. On the other hand, the percentage of mesopores in biochar can be obtained by dividing the mesopore area by the t-plot and BET surface area. From the calculation, it can be estimated that the coverage percentage of mesopores is approximately 70% of biochar. The BET surface area was $122.20 \text{ m}^2/\text{g}$, which is substantially higher than the average range of $23\text{--}46 \text{ m}^2/\text{g}$ (Abu Sari, Ishak & Abu Bakar 2014).

The isotherm plot is presented in Figure 2 as the amount adsorbed against relative pressure. Based on the isotherm plot, it is indicated that the biochar surface consists of several pore types, including micropores and mesopores. At relatively high pressure, the slope shows increased uptake of adsorbate as pores become filled; the inflection point typically occurs near the completion of the first monolayer. This plot pattern is similar to that described by Fu et al. (2021), which represents a combination of micropores and mesopores.

Figure 3 represents the pore diameter distribution graph. The peak in Figure 3 indicates the pore size diameter of the biochar sample. The peak is between

3.6 and 4.1 nm; thus, it is estimated that the pore size of biochar is approximately 3.8 nm. The International Union of Pure and Applied Chemistry (IUPAC) defines biochar pores as macropores ($>50 \text{ nm}$), mesopores (2-50 nm), and micropores ($<2 \text{ nm}$) (Gray et al. 2014). In the present study, the average pore size of the biochar was 3.8 nm, indicating a primarily mesopore structure.

Biochar's multi-pore characteristic is essential for its function. The presence of micropore and mesopore in biochar contributes to its adsorption and desorption abilities (Gan et al. 2021). It is reported that the presence of micropores is vital in increasing surface area that promotes liquid-solid adsorption. Macropores, on the other hand, are essential for hydrology, root attachment, movement, and aeration (Freddo, Chao & Brian 2012). Micropore presence also proved to improve microorganisms' growth and activity on their surface through the adsorption of dissolved organic matter (Hameed et al. 2017; Oni, Olubukola & Obembe 2019). Additionally, biochar promotes microorganism propagation by alleviating nutrient leaching (Yaashikaa et al. 2020). In summary, the presence of multiple pores on biochar is essential in improving soil condition through several parameters, such as hydrology and aeration. Additionally, the presence of multiple pores promotes microorganism growth by providing an attachment site and nutrient availability for microorganisms to undergo their activity.

SOIL pH PROFILE

The soil pH profile is important during the plantation process. Soil pH significantly influences plant growth by affecting nutrient availability and interactions with other elements in the soil. Figure 4 represents the soil pH profile of untreated soil (control) and treated soil using chemical fertilizer and soil conditioner. Each pH profile represents the pH value after 4 weeks of soil treatment, except for the control soil that represents the pH value at day 0 until week 4. The initial pH for each treatment was slightly acidic, with a pH value of 6.59. For the control soil, the pH value for the soil remains unchanged after 4 weeks. A similar pattern was also shown by soil in the presence of commercial fertilizer. However, a slight increment in pH value was observed for soil treated with soil conditioner. After 4 weeks of treatment, the soil in the presence of soil conditioner showed a pH value of 6.9, a 4.5% increment.

TABLE 1. BET data analysis for biochar surface porosity characteristics for sludge samples

Parameter	Value
BET surface area	122.1989 m^2/g
Mesopore area t-plot	85.4392 m^2/g
Pore density	0.112193 cm^3/g
Average pore adsorption diameter	3.6725 nm

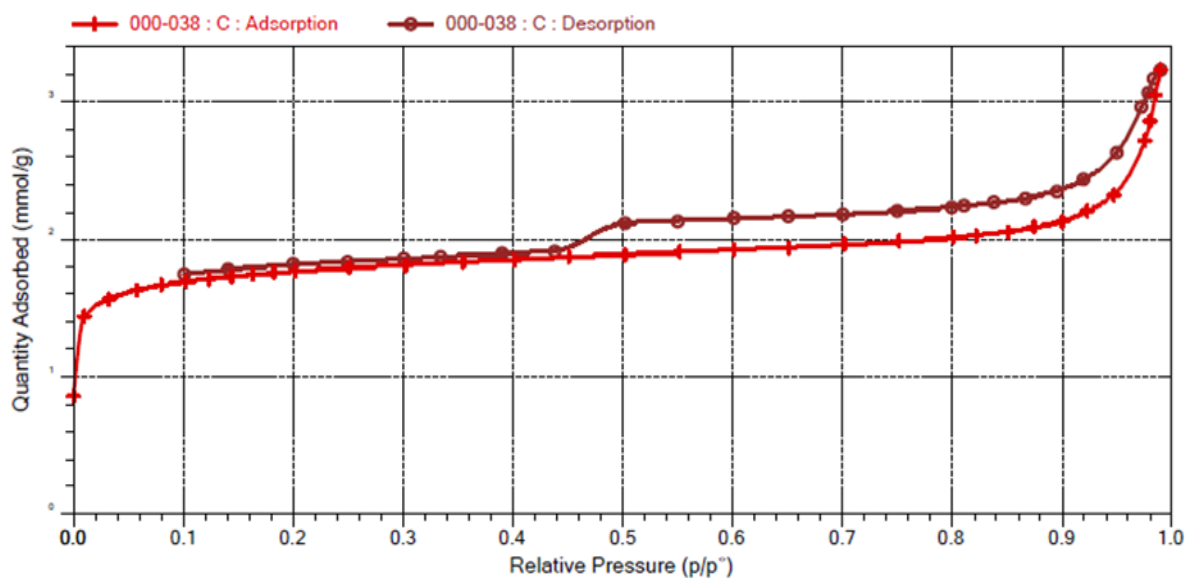


FIGURE 2. Nitrogen adsorption and desorption isotherm of biochar sample. The inset shows the amplified adsorption isotherm at a low p/p° range between 0 and 0.35

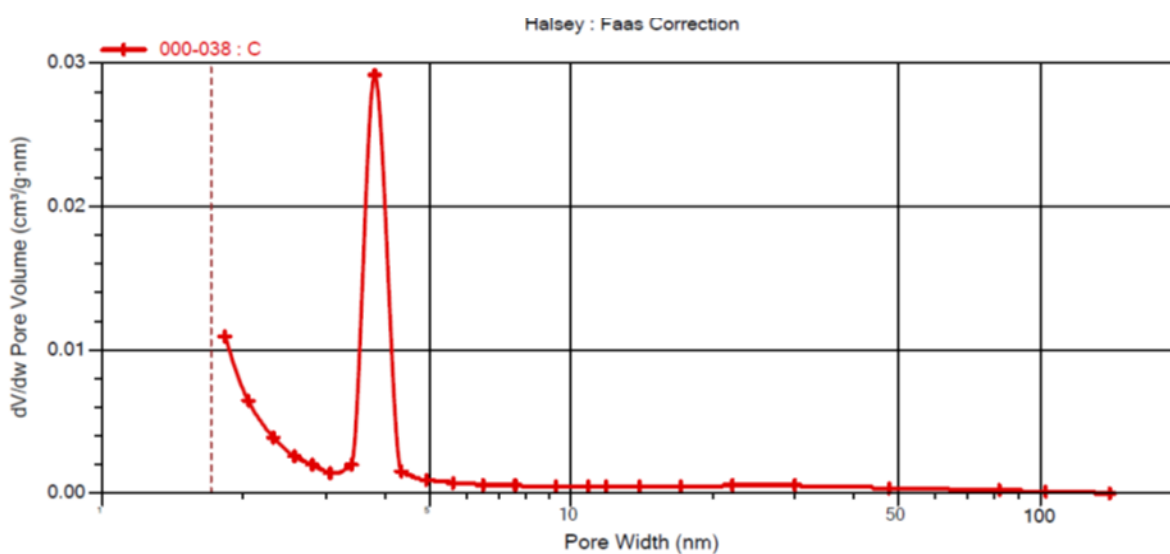
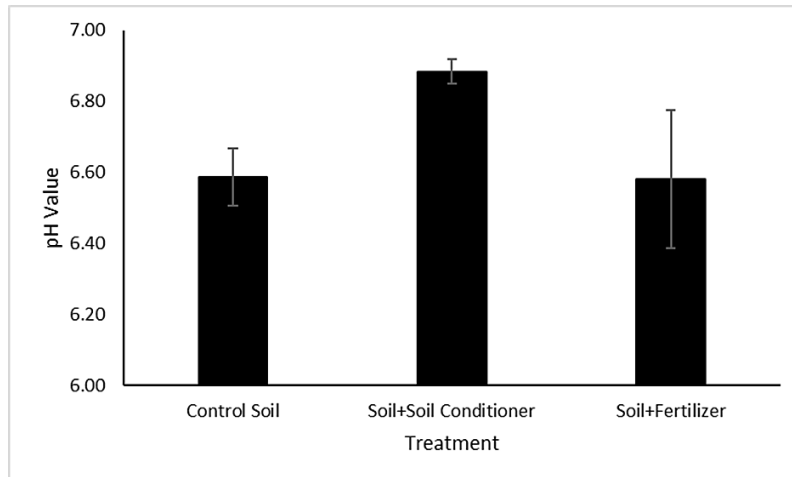


FIGURE 3. Distribution graph of biochar pore diameter

in a longer time. People exposed to lead were reported to have numerous illnesses, even when exposed for a short time at low concentrations. In pregnant women, exposure to lead may cause miscarriage and affect fetal neuron development (Naz et al. 2022b). Similar to lead, the accumulation of cadmium had impacts on human health. Local farmers consumed rice irrigated with cadmium- and zinc-rich mining waste and were diagnosed with bone softening and kidney failure (Robertsa 2014).

In comparison with plants treated with soil conditioner, plants with soil conditioner showed a similar height to *C. annuum* planted with commercial chemical fertilizer. This indirectly shows that soil conditioners in the presence

of *P. aeruginosa* are involved in the regulation of nitrogen sources in soil. As mentioned before, the existence of nitrogen-fixing microorganisms helps in metabolizing N_2 from the environment into the nitrogen source required by the plant to grow (Sedlacek, Giguere & Pjevac 2020). Some of the plant growth-promoting microbes (PGPMs) used in formulating soil conditioners are *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Streptomyces* (Lopes, Dias-Filho & Gurgel 2021). PGPM commonly grows at the root part and its surrounding soil. It helps in fixing N_2 from the environment and facilitates nitrogen uptake by plants. PGPM presence not only helps in nitrogen-fixing, but it also acts as a phytostimulator



The error bar represents the standard deviation of three experimental replicates

FIGURE 4. Soil pH profile of untreated (control) and treated soils

For soil treated with commercial fertilizer (Figure 4), the pH profile remains unchanged. In a common situation, the presence of commercial fertilizer shows a tendency to reduce the soil pH value. In other words, fertilizer is prone to acidifying soil conditions (Nur Amalina et al. 2020). There is a report claiming that the presence of chemical fertilizer promotes a pH average increment of 0.07 annually (Wang et al. 2019). However, in this research, no significant change in soil pH value was observed. This is most probably due to the ability of chili plants to absorb ammonium ions (NH_4^+) during the ammonium conversion to nitrate process. By absorbing NH_4^+ , proton ion (H^+) dissociation during this process is controlled, thus, controlling the soil acidification process (Ferrón-Carrillo, Tatiana & Miguel 2021).

The result obtained from soil conditioner treatment corroborates the previous claim that the presence of biochar soil conditioner increases acidic soil pH value (Geng et al. 2022; Shetty & Prakash 2020). This is also supported by Chen et al. (2022), who found that rice straw-derived biochar enhanced soil acidity, resulting in a pH of around 5.0. This might occur due to better hydration systems and the presence of effective microorganisms in regulating pH levels in the soil. This analysis suggests that soil conditioner helps maintain soil pH and creates conditions suitable for agricultural activities. Specifically, soil acidity has a substantial impact on biochar performance because it increases the availability of key minerals like potassium, stimulates the metabolic activity of bacteria and biochar, and increases cation exchange capacity (Zhang et al. 2022a), encouraging healthy chili plant development.

EFFECTS OF TREATMENT ON PLANT GROWTH PARAMETERS

PLANT HEIGHT

Plant height is a reliable indicator of soil fertility and nutrient availability; thus, it was used to evaluate the effects of soil

treatments. If the soil is scarce in nutrients, it no longer functions as a supporting medium and nutrient provider to the plant. Both plants grown on soil fortified with soil conditioner and fertilizer showed significant height (~57%) when compared to the control soil. A comparable study utilizing *Pseudomonas* in combination with biochar has demonstrated greater plant development, as measured by higher root and shoot biomass. Furthermore, this combination treatment was able to remove toxic substances in soil, plant roots, and shoots. (Zhao, Zhao & Wang 2025).

Commercial fertilizer is known for its variation in nitrogen, phosphorus, and potassium (N-P-K) ratio content. Nitrogen is an essential element required by the plant to undergo metabolism and grow. Although nitrogen is present in the environment in approximately 78%, it exists in the presence of gas form (N_2), which is a disadvantage to the plant since it cannot be taken directly from the environment. Plants require help from soil microorganisms, particularly nitrogen-fixing bacteria, to convert N_2 gas into NH_4^+ (Sedlacek, Giguere & Pjevac 2020). However, the presence of effective microorganisms that are able to fix and convert nitrogen varies in soil. According to Li et al. (2017), the majority of the *Pseudomonas* genus are capable of nitrogen fixation; however, more evaluations, such as the acetylene reduction assay (ARA), are required in future research to evaluate if *Pseudomonas* sp. isolate RA21 can carry out nitrogen fixation.

Nitrogen in commercial fertilizers helps in providing sufficient nutrients for plants to grow (Xu et al. 2020). However, prolonged use of commercial fertilizers has shown a negative impact on the environment, such as eutrophication and production of toxic gases, namely methane, carbon dioxide, and ammonia (Chandini, Ravendra & Om 2019). Moreover, most commercial chemical fertilizers contain heavy metals, including arsenic, lead, and cadmium (Savci 2012). The presence of these metals in chemical fertilizers may contribute to soil and water contamination, which might risk human health

that regulates plant hormones such as auxin, gibberellin, cytokinin, and ethylene (Bhat, Rehana & Shazia 2019; Khan et al. 2020).

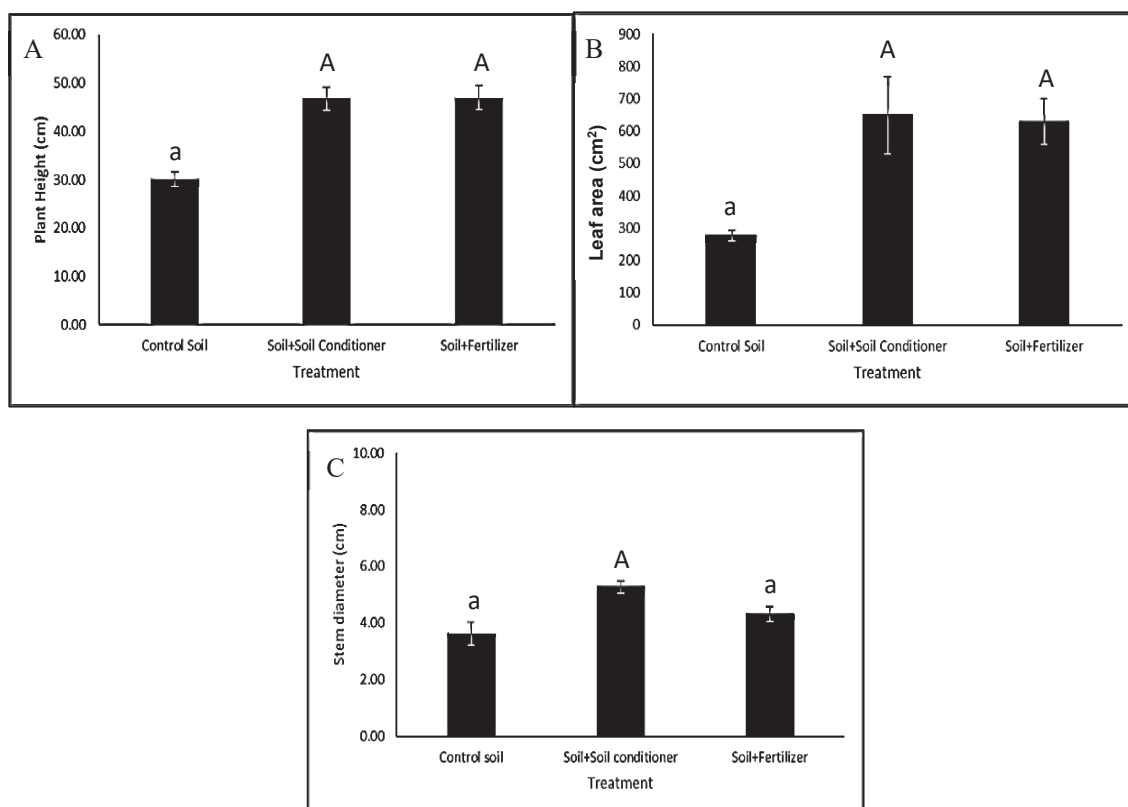
The genus *Pseudomonas* is a bacterium grouped under the gamma proteobacteria subclass. It is abundant in the rhizosphere region and known for its outstanding performance as a plant growth-promoting bacterium in regulating phytohormone secretion, acting as a biocontrol, solubilizing nutrient, and inducing systemic resistance against disease (Ghadamgahi et al. 2022; Sah, Krishnani & Singh 2021; Singh et al. 2022). It is reported that the presence of *Pseudomonas* in the soil promotes shoot height up to 1.28-fold (Jimtha John et al. 2017). Based on the data provided, the integration of *Pseudomonas* sp. isolate RA21 in biochar as a soil conditioner has the potential to be used in agricultural applications. The presence of *Pseudomonas* sp. isolate RA21 has been found to improve plant growth in ways equivalent to conventional chemical fertilizers.

LEAF SIZE

The chili leaf size was determined based on the area calculation (length \times width). In Figure 5(b), the soil treated

with a soil conditioner and fertilizer produced larger leaf sizes compared to the control. Leaf size for treatment with soil conditioner is approximately 651 cm², while leaf size for commercial fertilizer is approximately 629 cm². The leaf size in plants treated with soil conditioner was slightly larger than in those treated with commercial fertilizer.

The presence of the *Pseudomonas* sp. isolate RA21 in the soil significantly boosts the leaf size (Figure 5(b)). Although the mechanism of *Pseudomonas* sp. isolate RA21 is not evaluated in this experiment, several previous reports elaborate on the possible mechanism in detail. The slightly larger leaf size observed in soil conditioner treatment may be linked to improved iron availability via siderophore production. Siderophores are low-molecular-weight, water-soluble organic ligands that function as iron-binding compounds, facilitating iron transport and absorption in plants (Sah, Krishnani & Singh 2021). Under aerobic conditions, Fe (II) is oxidized to the less plant-available Fe (III); however, siderophores chelate Fe (III), enabling plant uptake and enhancing biocontrol by limiting iron availability to competing microorganisms (Romera et al. 2019; Sah, Singh & Singh 2017). Iron is an important metal in regulating biological metabolism, photosynthesis



The error bar represents the standard deviation of three replicates. Different letters indicate that the difference is significant ($p < 0.05$; Tukey HSD)

FIGURE 5. Growth parameter of *Capsicum annum* L. under treatments. (A) Plant height, (B) leaf area, and (C) stem diameter over week 4 of treatments. The control setup represents soil without any additional substance. Soil + soil conditioner represents soil fortified with lab-made soil conditioner containing *Pseudomonas* sp. isolate RA-21 mixed with biochar from municipal sludge. Soil + fertilizer represents soil fortified with commercial fertilizer

and chlorophyll synthesis. Its role as a central component in the electron chain and a co-factor in enzymes helps in balance metabolism in living organisms, including plants (Schmidt, Thomine & Buckhout 2020). Bacterial siderophore synthesis and iron uptake are critical for plant growth; hence, future research will include siderophore quantification, chlorophyll content measurement, and iron uptake evaluations to confirm the proposed process.

STEM DIAMETER

Figure 5(c) represents the *C. annuum* stem diameter for control and treated soil. The graph shows that the soil added with soil conditioner has the widest stem diameter at 5.29 cm, followed by soil with commercial fertilizer at 4.33 cm and control soil at 3.63 cm. The *Pseudomonas* sp. isolate RA21 treatment increased stem diameter by 45.7% compared to the control and by 22.1% compared to fertilizer. Based on this data, it can be said that the presence of *Pseudomonas* sp. isolate RA21 had a significant effect on *C. annuum* stem growth, as it was significantly higher ($p < 0.05$) compared to fertilizer and control.

Pseudomonas sp., was found to improve plant biomass, including stem diameter size (Waghunde & Sabalpara 2021). The increase in plant biomass associated with PGPM is often attributed to the regulation of phytohormones, siderophores, and enzymes that enhance nutrient uptake from the environment. The presence of *P. aeruginosa* can induce phytohormones that affect ACC deaminase enzymes, which lowers ethylene synthesis in plants, resulting in enhanced growth, including stem diameter (Sah, Krishnani & Singh 2021). Other than inducing phytohormones, the presence of *Pseudomonas* promotes the secretion of siderophores and helps in environmental phosphate solubilization (Nordstedt et al. 2020). This ability helps in plant nutrient uptake, especially for N and P elements from the environment, to increase plant biomass even in extreme conditions (Mehmood et al. 2023). The enhanced phosphate solubilization and nitrogen uptake may directly promote xylem/phloem development, resulting in thicker stems. The effect of RA21 on stem thickening was consistent throughout the growth period (Figure 5(c)), suggesting sustained microbial activity. Thus, increased stem diameter may contribute to higher structural stability and improve fruit-bearing capacity in *Capsicum* crops.

Plant biomass is regulated by various mechanisms and metabolic processes. These metabolisms are mainly affected by the reaction of phytohormones, siderophores, and enzymes induced in the presence of *Pseudomonas* bacteria in the ecosystem. Hence, additional studies such as phytohormone assays and nutritional analysis in stem tissue should be conducted to verify the mechanism in plant stems.

CONCLUSION

In conclusion, sludge from municipal wastewater treatment is possibly used as biochar for soil treatment since there are no harmful heavy metals present in it. The absence of potentially hazardous heavy metals is encouraging; yet, large-scale applications require regulatory compliance and continuous monitoring. The combination of biochar and the indigenous *Pseudomonas* sp. isolate RA21 has proven effective as a soil conditioner. This was supported by an improvement in chili plant growth rate, leaf size, and stem diameter profile. The results serve as a baseline for further analysis before applying this soil conditioner in real-world environments. This finding is sufficient to conclude that biochar from municipal waste sludge incorporated with *Pseudomonas* sp. isolate RA21 demonstrates promising effects as a soil conditioner. This technique (biochar + bacteria) promotes waste valorisation (recycling municipal sewage), agricultural sustainability, and lower chemical fertilizer usage. However, the study was limited to pot trials and a single crop, and longer-term/multi-crop studies are needed.

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