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Research Article

Potential *Fungal-bacterial Biofilm* for Bioremediation Polluted Soils of Chromium and Its Impact on Maize Growth

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Abstract: Recently, biofilm has emerged as a notable agent in bioremediation. The present study was conducted to determine the potential of microbes described in forming fungal-bacterial biofilm (FBB) as a bioremediation agent for chromium-contaminated soil. Parameters observed include total chromium concentration in soil, *Zea mays* growth, total chromium concentration in tissue, and its effects on microbial populations. The study commenced with biofilm formation assays and pot experiments in a greenhouse using combinations of chemical fertilizer (CF) on *Zea mays*. This research employed a Completely Randomized Design (CRD) with five treatments and four replications: A (100% CF), B (75% CF + 25% FBB), C (50% CF + 50% FBB), D (25% CF + 75% FBB), E (100% FBB). The results indicate that all treatments could reduce total chromium concentration below the threshold limit (2.5 mg kg^{-1}), the lowest chromium concentration found in treatments D and E at 1.25 mg kg^{-1}. FBB alone or in combination with CF did not enhance *Zea mays* growth. Treatment E exhibited plant height, crown dry weight, and root dry weight sequentially 20.31%, 84.10%, and 76.15% lower than treatment A. FBB could increase chromium accumulation in plants, with treatment E having the highest chromium concentration in crown and roots, at 15.47 µg g^{-1} and 15.59 µg g^{-1}. Application of 100% FBB increased soil bacterial population by 44.02% compared to treatment A. In conclusion, the microorganisms identified can form FBB and serve as bioremediation agents by enhancing heavy metal accumulation in plants (phytoextraction).

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1. Introduction

Agricultural land is fundamental to the provision of global food. Agricultural land, particularly soil, must be healthy, safe, and free from harmful substances that can enter plant tissues, as food products must be safe for consumption. However, environmental pollution due to metals has been increasing recently, such as in China, where 20 hectares of cultivated land, comprising 20% of total arable land, have been contaminated by Cr (VI) with concentrations ranging from 30-200 mg kg^{-1} (Xia et al., 2020). In addition, several agricultural lands in Waru, Kebakkramat, and Karanganyar are located in the area

of a textile-to-textile industry factory, which produces waste containing heavy metals such as Cu, Ni, Pb, Cr, Hg, Zn, Ag dan Cd posing a potential threat to irrigation water used for agricultural land due to the accumulation of these heavy metals (Yasinta et al., 2018).

Chromium is known as one of the heavy metal elements commonly found in wastewater. Chromium is widely used in leather tanning, iron casting, textile dyeing, the paint industry, and others (Prabhakaran et al., 2019; Wadood et al., 2021; Bhunia et al., 2022). Exposure to chromium for plants, animals, and humans has various negative impacts. Chromium in plants can disrupt seed germination and development, reduce root elongation, cause abnormal growth, and result in decreased yields (Rahman and Singh, 2019), while in humans, chromium can lead to liver damage, lung cancer, blood cancer, and affect the reproductive system (Renu et al., 2021). According to the Republic of Indonesia Regulation No. 22 (2021) concerning Environmental Protection and Management, the threshold limit value for chromium, especially Cr (VI), is 2.5 mg kg^{-1}.

Various techniques have been employed to remediate chromium. One method that can be utilized is bioremediation, harnessing living organisms as single isolates or microbial consortia, one of which is the microbial biofilm. Microbial biofilms are communities of microorganisms that adhere to one substrate or the other through the production of extracellular polymeric substances (EPS), one example of its metabolites is the production of siderophores, which are commonly found in microorganisms, fungi and plants (Maurya et al., 2022b; Atbaş and Taşkın, 2024). Microbial biofilms can be formed from single species or multi-species, known as fungal-bacterial biofilms (FBB). In extreme environmental conditions, such as locations with high concentrations of heavy metals, biofilms can benefit plants above them by absorbing soluble organic molecules and nutrients through concentration gradients. In contrast, heavy metals are sedimented on the surface of biofilms (Henagamage et al., 2022).

Based on the description above, FBB shows potential as a bioremediation agent for heavy metals, particularly chromium, in agricultural environments. In this study, FBB was formed from bacteria and fungi previously discovered in the West Slope of Mount Lawu in previous research (Rosariastuti et al., 2023), and its bioremediation ability was tested using *Zea mays* plants. *Zea mays* were chosen to tolerate heavy metal-contaminated environments such as As, Pb, Cd, and Cr (Kumar and Saini, 2016; Zeng et al., 2024). This research combined doses of FBB and CF through pot experiments in a greenhouse. The aim was to assess the bioremediation ability of FBB and its effect on the growth of *Zea mays* in chromium-polluted soil. This study is expected to solve agricultural land pollution, mainly due to chromium contamination.

2. Material and Methods

2.1. Experimental site and design

The study was carried out at the Laboratory of Soil Biology and Biotechnology, Laboratory Chemistry and Soil Fertility, Laboratory Physics and Soil Conservation, and pot experiments were carried out in Greenhouse A, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia. This research started from May 2023 to December 2023. The study was divided into two stages. The first stage involved the preparation of FBB, including isolate preparation, compatibility testing, FBB culture preparation, and biofilm formation testing. The second phase consisted of greenhouse experiments using a Completely Randomized Design with five treatment units and four replications, so there were 20 experimental units in total. The treatment combinations comprised Treatment A (100% CF), B (75% CF + 25% FBB), C (50% CF + 50% FBB), D (25% CF + 75% FBB), E (100% FBB).

2.2. Materials

This study utilized Andisol obtained from Ngargoyoso District, Karanganyar, Central Java, Indonesia, as the planting medium. Mandala brand organic fertilizer was used as the initial planting medium for seeding the test plants. Inorganic fertilizers used as treatments included Urea fertilizer produced by PT. Pupuk Indonesia (Persero) Gresik, SP-36 produced by PT. Petrokimia Gresik and KCl branded Mahkota produced by Canpotex Limited. The test plant used was corn P27 with the Pioneer trademark. The pollutant used was chromium in $K_2Cr_2O_7$, sourced from Merck KgaA in Germany.

The selective mediums utilized are Pikovskaya medium, YEMA medium, Jensen medium, PDA medium, NA medium, NB medium, and PDB medium. In this research, other materials used include Phosphate Buffered Saline, *Crystal violet*, 30% H_2O_2, HCl 2N, 4% $Na_4P_2O_7$, concentrated H_2SO_4, 10% NaCl, Zn granules, NaOH, H_3BO_3, HCl 0.1N, concentrated HNO_3, and concentrated $HClO_4$. The equipment used during the research included an Atomic Absorption Spectrophotometer (AAS) with series Thermo Ice 3000, UV-Vis Spectrophotometer, vortex, autoclave, mixer, shaker, etc.

2.3. FBB preparation

Four bacterial strains, namely N1 (unidentified nitrogen-fixing bacteria), N2 (*Beta proteobacteria bacterium*), H1 (*Paraburkholderia terrae*), and Y1 (*Paraburkholderia caribensis*) (Rosariastuti et al., 2023), and five fungal strains consisting of S2B2 (*Aspergillus*), Y2b (*Penicillium*), A5 (*Aspergillus*), Y1b (*Aspergillus aculeatus*), and P1b (*Talaromyces purpureogenus*) were obtained from various land uses on the West Slope of Mount Lawu. These bacteria and fungi are well-tested for the growth of plants, such as the production of IAA, siderophores, phosphate solubilization, nitrogen fixation, and potassium solubilization. All strains were inoculated on their respective selective media: YEMA, Jensen, Pikovskaya, and PDA. Compatibility testing using the modified streak method was conducted to determine whether all strains could grow together (Singh et al., 2019; Robles-Morales et al., 2021).

2.4. Study biofilm and inoculum preparation

Each strain was inoculated into 100 ml of NB culture medium for bacteria and PDB for fungi. Cultures were shaken at 90 rpm for approximately 72 hours at ambient temperature. Subsequently, 1 ml of each culture was taken and inoculated into a new mixed medium, PDB+NB, and then stored in shakers at 90 rpm at ambient temperature until the density of bacterial cells on fungal filaments achieved 10^{10} cells/ml. The bacterial cells on the fungal filaments were then observed under a microscope using Lactophenol cotton blue staining (Henagamage, 2019).

Fungal-bacterial biofilm (FBB) establishments were observed qualitatively by the crystal violet binding assay method. The formed FBB suspension was taken in a volume of 2 ml and then incubated for 24 hours in an incubator at 37 °C without shaking. Media without FBB was used as a control. After 24 hours, the media were decanted, and the tubes were washed twice using PBS. Subsequently, staining was carried out with 2 ml of crystal violet (1%, v/v) for 15 minutes. The tubes were re-washed with 2 ml PBS and dried upside down for 30 minutes. The bound stains were FBB (O'Toole, 2010; Maurya et al., 2022a).

The provided FBB consisted of FBB formed from bacterial and fungal isolates on a mixed PDB+NB medium. Inoculum was prepared when the bacterial cell density on the fungi reached 10^{10} cells/ml (Pandit et al., 2020). The dosage of biofilm provided was 10^7 cells/gram of soil by dissolving the biofilm in distilled water to a volume of 20 ml (Liu et al., 2023).

2.5. Soil and plant preparation

The soil was composited, sieved, and weighed at 5 kg per plastic bag, then sterilized using the wet steam method in a pot for 3 hours per day over 3 days. The sterilized soil was transferred to pots 1 day before planting, with Chemical Fertilizer (CF) added according to the treatment, with doses for urea at 300 kg.ha^{-1}, SP-36 at 100 kg.ha^{-1}, and KCl at 50 kg.ha^{-1}(Sofyan et al., 2019). Corn seeds were initially sown in trays using a soil and manure mixture at a 1:1 until there were 1-2 leaves (Sutami et al., 2021).

2.6. Pot experimental

All pots were set up to a completely randomized design inside the greenhouse and labeled with treatment indicators. Seedlings with 1-2 leaves were transferred into pots, with three seedlings per pot. They are kept for 7 days, after which the best one is to be maintained until it reaches the generative phase, at this point, it is harvested 100 days after sowing (DAS). FBB was applied after the plants were 7 days old from transplanting, simultaneously introducing the pollutant at a concentration of 5 mg kg^{-1} chromium in the form of $K_2Cr_2O_7$. Plant growth, including plant height, was observed weekly, while fresh and dry weights were measured at harvest.

2.7. Plant harvesting and sample collection

The plants were harvested after 100 days in the late sunny afternoon. The plants were measured for height, and fresh weight was recorded from the roots to the crown of the plants. The plants were then oven-dried to determine their dry weight. Soil samples were taken in two stages: fresh soil samples for bacterial and fungal population analysis. Samples for chromium concentration analysis were then air-dried and sieved with 0.5 and 2 mm sizes.

2.8. Soil analysis

The observation parameters tested as initial soil characteristics include pH in H_2O and NaF, Cation Exchange Capacity (CEC), Organic Carbon (C.Organic), Total Chromium, Total Bacteria and Fungi, and soil texture. pH in H_2O was measured using the electrometric method (Faria et al., 2023), pH in NaF with the NaF extraction method (Arifin et al., 2022), CEC with the ammonium saturation method (Mattila and Rajala, 2022), soil Organic Carbon with the Walkley and Black method black (Źróbek-Rozanska, 2016), total chromium with the wet digestion method (Rosariastuti et al., 2018), bacterial and fungal populations with the standard plate count method (Tan et al., 2021), and soil texture with the pipette method (Igaz et al., 2020).

2.9. Determination of chromium concentration in soil and plant tissues

The bioremediation effectiveness of CF and FBB combination against heavy metals was measured by analyzing their availability in root and crown biomass soil samples. Each root and crown of the plant was wind-dried and then dried in an oven under 70 °C for 48 hours until the weight was constant. Soil and plant samples are ground and sieved to 2 mm to standardize particle size. The samples were wet-ashed until the white smoke disappeared and a clear solution/white precipitate remained. The filtrate extract was measured for total chromium concentration using an Atomic Absorption Spectrophotometer (AAS) (Rosariastuti et al., 2018).

2.10. Data analysis

The obtained data is then statistically analyzed using analysis of variance (ANOVA) to determine the effects of CF and FBB treatments. Subsequently, if there are significant effects, the Duncan Multiple Range Test (DMRT) analysis is conducted at a confidence level of 95% to identify differences between treatments.

3. Results

3.1. Compatibility testing, biofilm formation study and microscopy

Compatibility testing was conducted on all isolates used in biofilm formation. The results obtained from this compatibility test showed that all grown isolates did not produce any inhibition zones, indicating that they could be cultured together (Figures 1a and 1b). In the FBB, bacterial strains would live on fungal filaments. These fungal filaments act as surfaces for bacterial colonization. Figure 2 shows various bacteria living on fungal filaments.

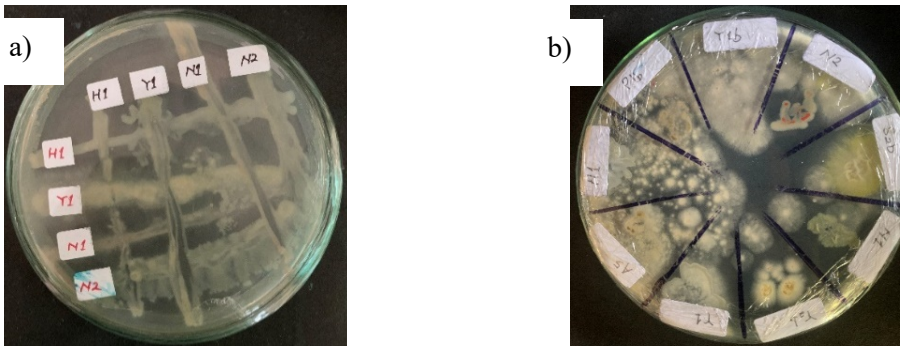

Figure 1. Compatibility Test of Biofilm-forming Fungi and Bacteria a) Compatibility test among bacterial strains, b) Compatibility test between bacteria and fungi. N1: Nitrogen-fixing bacteria (unidentified), N2: *Beta proteobacteria bacterium*, H1: *Paraburkhlorderia terrae*, Y2: *Paraburkholderia caribensis*, S2b2: *Aspergillus*, Y_2b: *Penicillium*, A5: *Aspergillus*, Y1b: *Aspergillus aculeatus*, P1b: *Talaromyces purpureogenus*.

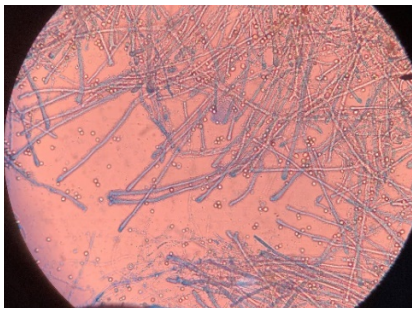

Figure 2. Microscopic image of the biofilm showing bacterial colonization living on fungal filaments.

The observation results indicate the presence of residual strain stains on the FBB culture produced (Figure 3a) compared to the control, which does not contain any strain stains. Furthermore, the residual strain stains become thicker as the biofilm formation progresses. Figure 3b shows that the strain stains on the third day are thicker compared to the first and second days. The residual strain stains on the reaction tubes are indicated as adherent biofilm cells (Ruiz et al., 2021).

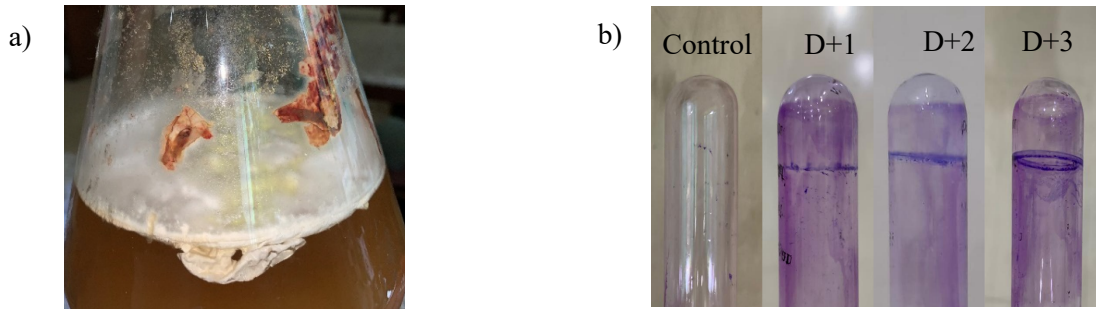

Figure 3. Crystal Violet Biofilm Assay. a) Biofilm development through co-culture inoculation; b) Crystal violet biofilm assay at 1,2 and 3 days.

3.2. Characteristics of initial soil

The initial soil used in this study has been sterilized and has not received any treatment. Characteristics of the Initial soil are presented in Table 1.

Table 1. Characteristics of initial soil

Parameters	Result	Unit	Classification
pH H_2O	6.25	-	Neutral[a]
pH NaF	11.40	-	High[a]
CEC	21.29	cmol(+).kg^{-1}	High[a]
Organic Carbon	6.85	%	High[a]
Total Chromium	2.11	mg kg^{-1}	Below quality standard[b]
Total Bacteria	2.59	Log 10 CFU g^{-1}	
Total Fungi	1.51	Log 10 CFU g^{-1}	
Sand	27		
Silt	70	%	*Silt Loam*
Clay	3		

Note: [a]) Level according to the Indonesian Soil and Fertiliser Instrument Standard Research Institute (2023), [b]) Level according to the Republic of Indonesia Regulation no. 22 concerning Implementation of Environmental Protection and Management (2021). Source: Primary Data (2022).

Initial soil characteristics in the growing medium indicate that before any remediation process, the soil had a pH H_2O of 6.25 and a pH NaF of 11.40. The cation exchange capacity was 21.29 cmol(+) kg^{-1}, with an organic carbon content of 6.85%. The initial total chromium concentration in the soil was 2.11 mg kg^{-1}. The initial soil's bacterial and fungal colony counts were 2.59 Log 10 CFU g^{-1} and 1.51 Log 10 CFU g^{-1}, respectively. Additionally, the soil used falls under silt loam texture.

3.3. Assessment of FBB's ability to reduce total chromium concentration in soil

The research results indicate that all treatments reduced the total chromium concentration in the soil below the threshold value (2.5 mg kg^{-1}), as depicted in Figure 4.

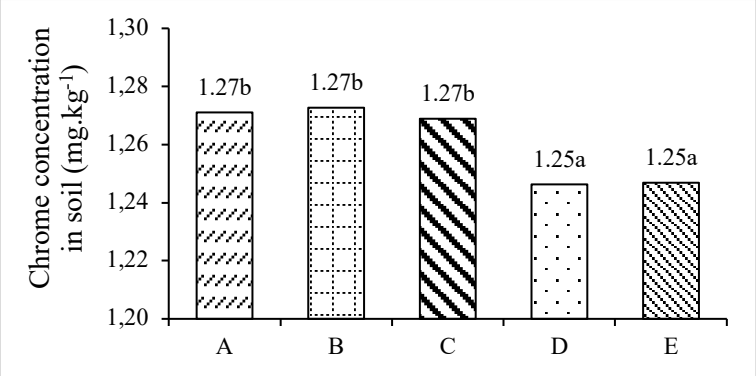

Figure 4. Total chromium concentration in the soil after FBB's ability test.

ANOVA results indicate that the treatments significantly affect the total chromium concentration in soil ($P < 0.05$). Based on the DMRT results, the concentration of total soil chromium in treatments A, B, and C did not have significant differences, with final total chromium concentrations of 1.27 mg kg^{-1} and remediation effectiveness values of 82.12%, 82.09%, and 82.15%, respectively. Treatments D and E showed lower total chromium concentrations in the soil than the other treatments, at 1.25 mg $kg^{-1,}$ with remediation effectiveness values of 82.48% and 82.46%, respectively, and no significant difference between them. Treatment D is the best treatment for reducing total chromium in soil with the highest remediation effectiveness value (Table 2).

Table 2. Effectiveness of bioremediation

Treatment	Initial Level (mg kg^{-1})	Final Level (mg kg^{-1})	Bioremediation Effectiveness (%)	Description
A	7.11	1.27	82.12	Decrease
B	7.11	1.27	82.09	Decrease
C	7.11	1.27	82.15	Decrease
D	7.11	1.25	82.48	Decrease
E	7.11	1.25	82.46	Decrease

Source: Primary Data.

3.4. Assessment of FBB's influence on plant growth

This experiment analyzes the impact of FBB application on the growth of *Zea mays*. Results of the treatment of *Zea mays* height are presented in Figure 5.

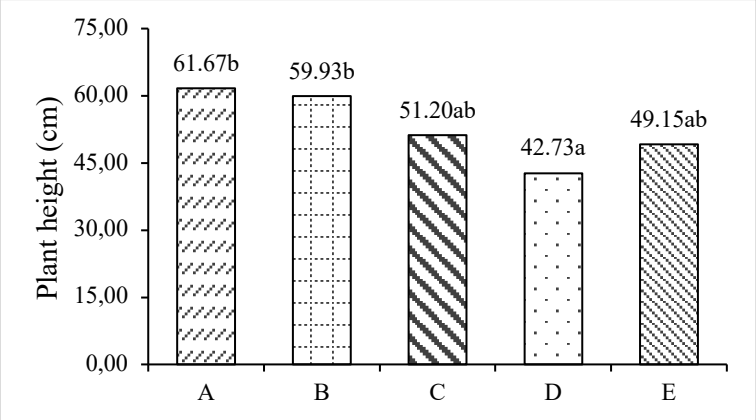

Figure 5. Effect of FBB on the height of *Zea mays* plants.

The ANOVA results indicate that applying FBB with CF combination significantly affects plant height ($P < 0.05$). Based on the DMRT results, treatment A exhibits the highest plant growth with a height of 61.67 cm, and there is no significant difference with treatment B, which has a height of 59.93 cm. However, treatments A and B significantly differ from treatment D, which has the lowest plant height at 42.73 cm (Figure 5). The research results on the dry weight of the crown and root of the plants can be seen in Figure 6.

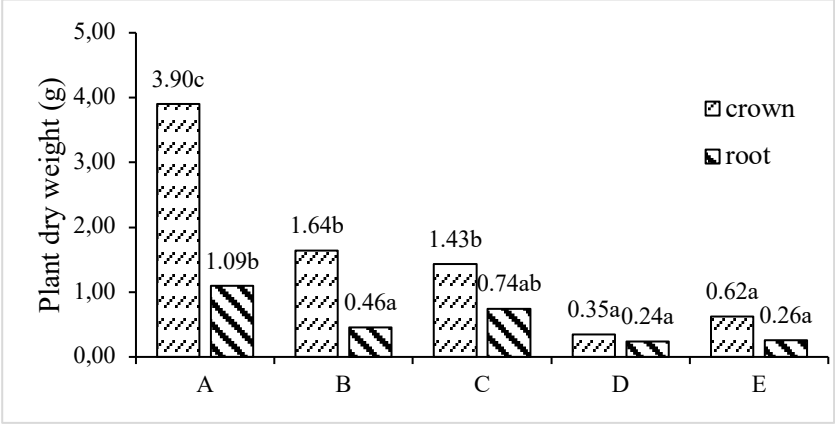

Figure 6. Effect of treatments on the dry weight of *Zea mays* plants crown and roots.

ANOVA results indicate that applying FBB with CF combination significantly affects the crown's dry weight and Zea mays' roots ($P < 0.05$). Based on the DMRT results, the crown dry weight

is heavier than the root dry weight (Figure 6). The highest dry weight in the crown and roots was obtained in plants treated with treatment A, which are 3.90 g and 1.09 g, respectively. The lowest root dry weight in treatment D was 0.24 g. However, there is no significant difference between treatments B and E. Overall, the best plant growth is achieved in treatment A, which is 100% CF, followed by treatment B, with 75% CF and 25% FBB.

3.5. The effect of FBB on total chromium concentration in plant tissue

The results of total chromium concentration in various parts of *Zea mays* plants grown in chromium-contaminated soil are presented in Figure 7.

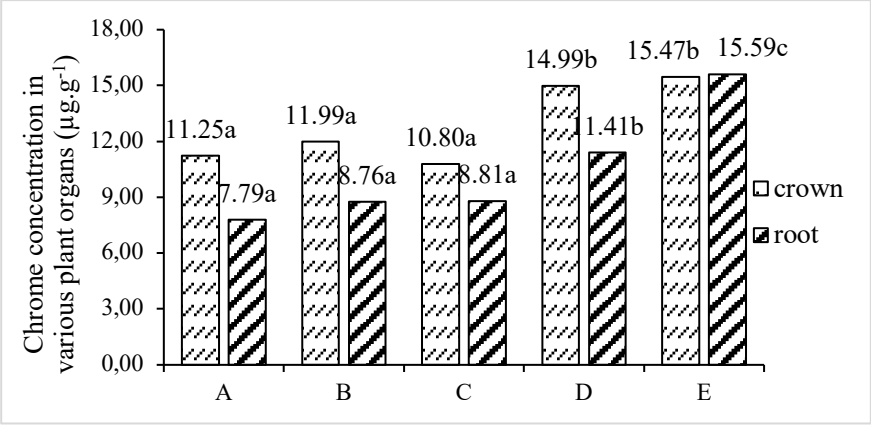

Figure 7. Total chromium concentration in various parts of *Zea mays* plant.

The ANOVA results show that the treatment of FBB with CF combination significantly affects the total chromium concentration in plant tissue ($P < 0.05$). DMRT showed the highest concentration of total chromium in the crown and root of the plant was obtained in the treatment E (100% FBB), which was 15.47 µg g^{-1} and 15.59 µg g^{-1}, respectively, and it differs significantly from all other treatments. The lowest total chromium concentration in the crown was obtained in treatment C, which was 10.80 µg g^{-1}, but there is no significant difference between treatments A and B.

3.6. Rhizosphere microbial populations

Observation of the Population of microbes in the *Zea mays* plants inoculated with FBB under various treatments is depicted in Figure 8.

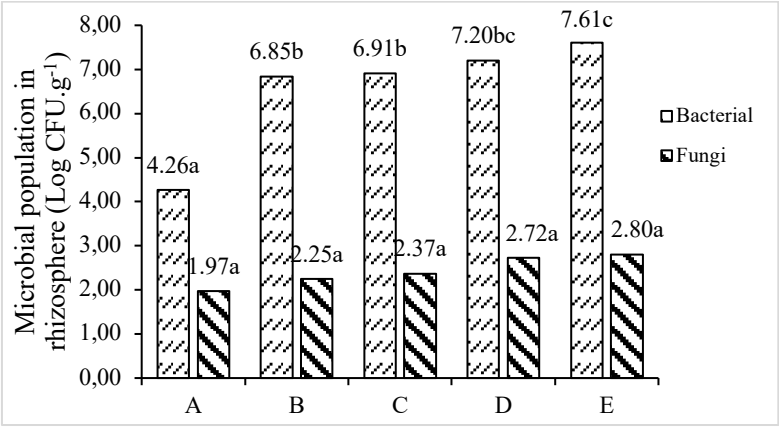

Figure 8. After the pot experiment, the microbial Population in the rhizosphere of *Zea mays* plants in a chromium-contaminated environment.

The ANOVA result indicates that the treatment of FBB with a combination of CF significantly affects the bacterial population ($P < 0.05$) but does not significantly affect the fungal population ($P > 0.05$). DMRT results show that treatment E with 100% FBB has the highest bacterial population density at 7.61 Log CFU g^{-1}, showing significant differences compared to all other treatments, while the lowest bacterial population density is observed in treatment A, without the addition of FBB, at 4.26 Log CFU g^{-1}. However, the combination of FBB and CF treatments did not result in significant differences in fungal population density based on DMRT results. Treatment E, with 100% FBB application, did not significantly differ from treatment A, which did not receive FBB.

4. Discussion

The research results indicate that all tested bacterial and fungal isolates exhibited strong compatibility, characterized by the absence of inhibition zones or clear zones, allowing them to co-culture in the same environment. This is consistent with previous studies where the lack of inhibition zones in compatibility tests indicates that all isolates can coexist in the same environment (Jia et al., 2023). When cultured together, isolates could form biofilms, as evidenced by the increasing thickness of the residual spots in the reaction tubes over time, indicating biofilm development from the FBB that had been cultured. Previous research also identified *Proteobacteria* and *Paraburkhlordie* phyla as bacterial genera with high biofilm formation abilities (Liu et al., 2023; Dong et al., 2024; Ghitti et al., 2024; Zhou et al., 2024).

The initial soil used is Andisol from Ngargoyoso, Central Java, Indonesia. As a planting medium, the soil has a neutral pH before undergoing the bioremediation process, while the high NaF pH indicates that the soil has a high allophane content. According to (Arifin et al., 2022), Andisols are characterized by a high allophane content, as indicated by a NaF pH > 9. The initial soil's cation exchange capacity (CEC) and organic carbon (C.organic) are classified as high characteristics. Soils dominated by amorphous minerals like Andisols will have varying charges, resulting in high CEC, and the high organic carbon content also leads to high CEC (Rizqiyah et al., 2023). The initial soil total chromium concentration was below the threshold value. According to the Republic of Indonesia Regulation No. 22 (2021) concerning the Organization of Environmental Protection and Management, the threshold value for Cr is 2.5 mg kg^{-1}. The number of bacterial and fungal colonies in the initial soil after sterilization is low. According to Sutami et al., (2021), wet steam sterilization can kill up to 83.71% of bacteria. The soil texture falls into the category of silt clay. According to Sigaye et al., (2021), the dominant soil texture in Andisols is silty loam and clay.

The research results demonstrate that the total chromium concentration in the soil decreases with increasing FBB treatment. Furthermore, it was found that the treatment without FBB but with 100% CF reduced the total chromium concentration in the soil to the same level as the treatments with 25% and 50% FBB. This indicates that *Zea mays* plants, without bioremediation agents, can already absorb the chromium heavy metal. Previous studies have also revealed *Zea mays* ability to remediate hexavalent chromium toxicity, where it can accumulate hexavalent chromium in the roots and stems (Adiloğlu and Göker, 2021). Meanwhile, in soil treated with a combination of 25% CF + 75% FBB and 100% FBB, the total chromium concentration was reduced even further than other treatments (below the threshold value of 2.5 mg kg^{-1}). The best treatment, as identified by the test results on the decrease in total soil chromium concentration, is Treatment D, which has the highest remediation effectiveness value of 82.48%. This indicated that FBB acts as a bioremediation agent capable of restoring soil from heavy metal exposure through mechanisms such as deposition in EPS biofilm tissue or increasing solubility for plant uptake. According to previous research, biofilm mode eliminates metals through its negatively-charged functional groups such as phosphate, sulfate, hydroxyl, and carboxyl, which aid in the absorption of positive metal ions such as Cr (VI), Cd (II), and As (V) (Priyadarshanee and Das, 2021; Maity et al., 2023), thereby reducing the distance between root tissues and heavy metals. Subsequently, this heavy metal accumulation is efficiently transported to the upper parts of the plant (Zhao et al., 2023). Other studies have revealed that biofilms composed of rhizobacteria are implicated in the biogeochemical cycle on heavy metals by increasing heavy metal solubility due to the organic acid synthesis such as IAA, phosphate solvents, and redox processes (Sharma and Saraf, 2023).

The combined treatment of FBB with CF affected the biomass of *Zea mays* planted in chromium-polluted soil. The research showed that chromium exposure inhibited the growth of *Zea*

mays, affecting plant height, root dry weight, and crown dry weight. This research also found that the *Zea mays* growth decreased as the dosage of CF decreased. The best treatment obtained from the growth assessment of *Zea mays* was treatment A with 100% CF, as CF could quickly provide the necessary nutrients for the plants. Adding CF at different metal concentration treatments could increase plant dry weight because the plants received sufficient nutrition compared to other treatments (Seddiki et al., 2023). In this study, an increase in the dosage of FBB was accompanied by a decrease in the growth of *Zea mays* because FBB could increase the solubility of total chromium concentration, allowing chromium to be absorbed by the plants and affecting germination, root development, and elongation. The findings of this study suggest that FBB formed from microbes in the western slope of Mount Lawu as a bioremediation agent would be more optimally used in conjunction with chromium-tolerant phytoremediation plants. For example, research by Ferina et al. (2017) stated that using bioremediation agent *Agrobacterium sp I3* and the phytoremediation plant Mendong was more effective in remediating chromium than treatments without the Mendong plant. However, the use of *Zea mays* plants as phytoremediation plants can cause stress due to decreased root penetration, which disrupts the plant's ability to explore the soil for water and nutrients (Bashir et al., 2020), disrupts cell elongation and biosynthesis, and reduces chlorophyll content (Irfan et al., 2021), resulting in decreased fresh weight and dry biomass of the plants (Vishnupradeep et al., 2022).

Our results found that the total chromium concentration in plant tissues increases with higher FBB treatments. Chromium enters plant tissues through several mechanisms, with the most easily absorbed chromium valences being Cr (III) and Cr (VI). Plants absorb chromium through the competition for macro and micronutrient uptake, such as Ca, Fe, Zn, Mg, and Cu, resulting in the deficiency of these essential nutrients (Wakeel and Xu, 2020; Kapoor et al., 2022). Plants absorb Cr (III) passively through cation diffusion in the cell wall and Cr (VI) through active absorption on phosphate and sulfate transporters (Srivastava et al., 2021). The accumulation of chromium in *Zea mays* in this study appears to be higher in the crown compared to the root tissue. This contrasts with the findings of (Peternella et al., 2021), where Cr was more abundant in maize roots than in other parts as a natural defense mechanism of plants. This suggests that the FBB mechanism, as a bioremediation agent, increases the accumulation of heavy metals in plants or phytoextraction. Therefore, FBB may not be suitable for use in *Zea mays* or other food crops. High accumulation of heavy metals in these food crops can be hazardous to humans if they enter the food tissue. The findings of this research suggest that FBB should ideally be used in conjunction with phytoremediation plants such as *Cirsium libanoticum, Matricaria chamomilla, Papaver dubium,* or others, which according to (İnci et al., 2024), are capable of accumulating chromium at levels of 1.84, 1.79, 1.74 $mg.kg^{-1}$, respectively. However, these values are lower than annual food crops. They pose no threat to living organisms.

Chromium metal presence in soil can cause toxicity to soil microbes. However, biofilms contain EPS (extracellular polymeric substances), primarily proteins, polysaccharides, and nucleic acids, which can protect microbial populations from toxic contaminants (Maurya et al., 2022). Our result showed that the provision of FBB can protect rhizobacteria in the soil. This is evidenced by increasing the number of bacteria populations in the soil treated with FBB compared to those without FBB. The best treatment obtained from the test results on rhizosphere microbial populations is the treatment with 100% FBB, which can result in a 44.02% higher bacterial population than the treatment without FBB. In other research, it was found that due to the presence of Cr (VI) contamination, there is an increase in EPS content, such as proteins and carbohydrates, as well as the presence of functional groups that act as protective shields for bacteria (Maurya et al., 2022).

Conclusion

FBB, in combination with CF, can be employed as a bioremediation agent to restore chromium-contaminated soil below the threshold value. The combination of FBB and CF can enhance the accumulation of chromium concentrations in *Zea mays*, indicating that the mechanism of FBB as a bioremediation is by improving heavy metals uptake in plants (phytoextraction) and translocating metals from plant roots to plant crowns. According to research findings, FBB, in combination with CF, is more suitable for application in plants tolerant to heavy metal compounds because when applied to *Zea mays*, it decreases plant growth due to the high concentration of chromium absorbed by the plants. Zea mays is a food crop that is unsuitable for use as a phytoremediation plant. Other results show that using FBB

in combination with CF as a bioremediation agent effectively maintains microbial populations in chromium-contaminated soil. Therefore, there is a need for more in-depth research on the use of FBB in phytoremediation plants to restore more land contaminated by the presence of other heavy metals.

Ethical Statement

Ethical approval from this study was obtained from Indonesian patent (S00201910796).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

The first author is tasked with conducting the research, analyzing the gathered data, composing a draft manuscript of the research findings, and drawing conclusions based on the research results.

The second author serves as the research supervisor, outlining the broader research framework, providing guidance throughout the research process, offering feedback on presenting findings, and refining the manuscript.

The third author is responsible for providing additional input on the research framework and refining the manuscript.

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