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Interaction between *Eichornia crassipes* with *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 in Cr^{6+} reduction in liquid media

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Abstract. *Wulandari AD, Meitiniarti VI, Kasmiyati S. 2024. Interaction between* Eichornia crassipes *with* Microbacterium *sp. SpR3 and* Micrococcus luteus *RT-9 in Cr6+ reduction in liquid media. Biodiversitas 25: 957-963.* Water Pollution caused by Cr^{6+} contamination is difficult to remove from the environment. One of the efforts to reduce Cr^{6+} contamination in water is by bioremediation. Bioremediation using the interaction of rhizosphere bacteria with aquatic plants can be employed to decrease the content of Cr^{6+} in water environment. This study aimed to determine the effect of adding *Microbacterium* sp. SpR3, *Micrococcus luteus* RT-9, and a mixture of both bacteria on the ability of *Eichornia crassipes* to reduce the concentration of Cr^{6+} in the growth media and the plant's growth potential. This study was conducted in the Microbiology Laboratory of the Faculty of Biology at Satya Wacana Christian University. *Eichornia crassipes plans of* similar size were grown in 450 mL of Hoaglands solution with Cr^{6+} 5 $mg.L^{-1}$ in a container. The treatment in this study involves the addition of 50 mL (10% of the working volume) of *Microbacterium* sp SpR3 culture, *Micrococcus luteus* RT- 9, and a combination of both Cr^{6+}-reducing bacteria. The reduction rate of Cr^{6+} concentration in the mixture of both types of bacteria reached 88.06%, which was higher compared to the control without the addition of the inoculum (87.08%). The inoculation of both Cr^{6+} reducing bacteria did not hibit the growth rate of the plants. The ability of *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 to reduce Cr^{6+} in liquid media can be employed in the bioremediation of Cr^{6+} contaminated wastewater.

Keywords: Bioremediation, Cr^{6+}, Cr^{6+}-reducing bacteria, wastewater, water hyacinth

INTRODUCTION

Global water pollution is caused by population growth, increasing human activity, increasing industrialization, poor utilization of water resources, and unplanned urbanization (Obinna and Ebere 2019). Unlike pollution caused by organic matter, heavy metal contamination in wastewater cannot be degraded by chemical or biological processes, so it tends to accumulate in the environment. The presence of heavy metal chromium in the form of Cr^{6+} in the biosphere can damage ecosystems and disrupt the survival of biota. Several studies have stated that the wastewater from the leather tanning industry activities contains 1.07-7.80 $mg.L^{-1}$ Cr^{6+} (Agarwal et al. 2013), industrial waste, domestic waste, and agricultural waste contain 0.01 $mg.L^{-1}$ Cr^{6+} (Vardhan et al. 2019). The quality standard for Cr^{6+} content in determined industrial waste is 0.3 $mg.L^{-1}$ (MENLHK 2014).

The active and non-biodegradable nature of Cr^{6+} makes it hard to be removed from the environment and is accumulated in the contaminated biological tissues. Cr^{6+} gets absorbed in the human body through exposure to oral, dermal, and inhalation routes. Cr^{6+} has mutagenic and carcinogenic effects in humans and animals that cause cancer, hypokretosis, mental disorders (Coelho et al. 2015), teratogens, mouth ulcers, indigestion, acute tubular necrosis, vomiting, abdominal pain, kidney failure, and mutagens in humans (Hossan et al. 2020). Furthermore, it is also highly toxic to plants that cause chlorosis and decrease plant biomass production (Agarwal et al. 2013; Augustynowicz et al. 2015). Cr^{6+} contamination in water harms the ecological function of water, which causes changes in the shape and physical condition of water structures such as taste, color, odor, and viscosity. It also decreases the availability of nutrients in water such as reducing dissolved oxygen, decreasing salinity, reducing the availability of organic acids, reducing the availability of sulfur, and lowering pH (Obinna and Ebere 2019).

Considering the danger of Cr^{6+} pollution in living things, efforts are needed to eliminate or reduce Cr^{6+} pollution in aquatic environments. Alternative efforts that can be done are bioremediation. Bioremediation is a technique used to remove contaminants from ecosystems by utilizing biological mechanisms in microbes and plants (Ojuederie and Babalola 2017). Agarwal research (2013) showed that with bioremediation techniques, Cr^{6+} levels in leather tanning waste could be reduced by 92.10% using *Pseudomonas fluorescence* in a 72 hours experiment and by 99.47% using *Eichornia crassipes* in 20 days experiment. *Pistia stratiotes* plants can also reduce Cr^{6+} levels by 80.90% (Tabinda et al. 2018).

Eichornia crassipes can be used as a bioremediator for heavy metals because of their high absorption rate (hyperaccumulator), tolerance of highly polluted environments, and very high biomass production rates (De Laet et al. 2019). Microorganisms can also be used as bioremediators in remediating heavy metal-polluted environments as have been widely practiced. Isolated of

Microbacterium sp SpR3 from tanning wastewater and the rhizosphere of *Acalypha indica* was reported to be able to reduce Cr^{6+} levels in the soil by 16.04 $mg.L^{-1}$ in 7 days (Innation et al. 2021). *Micrococcus luteus* RT-9 bacteria were also reported to have tolerance of Cr^{6+} up to 100 µg mL^{-1} Cr^{6+} (Meitiniarti et al. 2022). Endophytic and rhizosphere bacteria can absorb metal ions into their cell walls, thereby increasing the uptake of Cr^{6+} by plants. The synergistic interaction between plants and bacteria makes the phytoremediation process more efficient. Upadhyay (2017) reported that *Bacillus subtilis* MNU16 can reduce 75% of Cr^{6+} from 50 to 13.23 $mg.L^{-1}$ within 72 h. Bioremediation utilizing non-food plants (phytoremediation) associated with microorganisms can be a reliable source of reducing Cr^{6+} in water. Bacteria possessing catabolic genes survive and proliferate in the close vicinity of the root and in the internal tissues of the host plant without causing pathogenicity. Bacteria which are known to degrade toxic Cr^{6+} has been suggested as an additional chromosome-encode chromate-resistance mechanism. In addition to plasmid encode tolerance, Cr^{6+}can be also converted and reduced to be less toxic by the enzyme chromium reductase. Water hyacinths also accumulate hexavalent chromium in their tissues which can help remediate wastewater (Irawati 2017). Considering the high chromate accumulating capability of *Eichornia crassipes*, plant assisted-bioremediation is an emerging field, in which plants' roots in conjunction with their rhizospheric microorganisms used to remediate Cr^{6+} contamination in wastewater. This study was initiated to evaluate the effect of *Microbacterium* sp SpR3 and *Micrococcus luteus* RT-9 in conjunction with *Eichornia crassipes* on the removal of Cr^{6+} from liquid media.

MATERIALS AND METHODS

Research designed

The research was conducted using a completely randomized design, and was carried out for 21 days. *Eichornia crassipes* in the vegetative phase with root length of 15-20 cm, leaf length and width of 20-22 cm, wet weight of 300 ± 5 g, and number of leaves of 4-5 were acclimated in two stages, namely 1) acclimation with tap water for three days to remove dirt attached to the roots, and 2) acclimation in Hoagland's solution for seven days. Then *Eichornia crassipes* were then grown in 450 mL of Hoaglands solution with Cr^{6+} 5 $mg.L^{-1}$ in the container.

To prepare 1000 mL Hoagland's solution, mix 1 mL of 1 M $NH_4H_2PO_4$, 6 mL of 1 M KNO_3, 4 mL of $Ca(NO_3)_2.4H_2O$ 1 M, and 2 mL of $MgSO_4.7H_2O$ 1 M (Li and Cheng 2014). Two mL of microelements were added, consisting of 0.33 mL H_3BO_4 10 mM, 0.33 mL H_2MoO 0.4 mM, 0.33 mL $CuSO_4$ 0.4 mM, 0.33 mL $MnSO_4$ 1 mM, 0.33 mL $ZnSO_4$ 1.75 mM, 0.33 mL KCl 25 mM, and 1 mL $FeCl_3$ 12.5 mM (Tabinda et al. 2018). Stock Cr 100 $mg.L^{-1}$ solution was prepared by dissolving 282.90 mg $K_2Cr_2O_7$ in 1000 mL of distilled water as an addition to Cr-containing media (Hossan et al. 2020).

The treatments by adding a 50 mL (10% of the working volume) combination of Cr^{6+} reducing bacteria inoculum. *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 isolates, obtained from the Microbiology Laboratory, Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga, were maintained on Luria Berthani (LB) slanted media and rejuvenated every 24 hours. Bacterial isolates from 24-hour-old plate cultures were inoculated into a liquid LB medium containing 25 $mg.L^{-1}$ Cr^{6+}. The composition of 1000 mL of liquid Luria Berthani (LB) media is 10 g of peptone, 5 g of yeast extract, and 5 g of NaCl. Afterward, the media was sterilized using hot steam (autoclave 121°C for 15 minutes) (Irawati et al. 2017). The addition of Cr to the media is for maintaining the ability of bacteria to resist Cr. The bacterial culture was incubated at 30°C with agitation at 125 rpm for 12 hours under aerobic conditions until it reached an OD_{600nm} = 1 (Pramono et al. 2013). Inoculums were prepared by harvesting *Microbacterium* sp SpR3 and *Micrococcus luteus* RT-9 from each liquid media using a centrifuge at 3000 rpm for 5 minutes. The obtained cell pellet is resuspended with physiological saline according to the inoculum volume ratio (Table 1). For the control treatment (R0M0), the inoculum volume was replaced with 50 mL of physiological saline.

Determination of Cr^{6+} content in Hoaglands solution

Ten mL of liquid media samples were taken from each treatment and filtered using filter paper. Five mL of liquid media filtrate with five drops of 0.18M H_2SO_4 was added to acidify the sample (Hossan et al. 2020). Then, 0.25 mL of diphenylcarbazide reagent was added. After 5 minutes of incubation at room temperature, the absorbance was determined using a spectrophotometer at λ 540 nm (Pramono et al. 2013). The absorbance values obtained were converted to Cr^{6+} concentration using the Cr^{6+} standard curve line equation.

Cr^{6+} levels in plant organs analysis

The plants were cleaned with distilled water. The roots and leaves were separated and dried in an oven at 80°C for 48 hours. The dried plant samples were crushed and subjected to furnace treatment for about 6 hours. The weight of the resulting ash sample was measured. Cr^{6+} content was extracted by dissolving the sample in an aqua regia solution, which consisted of 7.5 mL of 2 M HCl and 2.5 mL of 1 M HNO_3, and then evaporating it until the volume was reduced to 5 mL. The extract was filtered using filter paper and distilled water was added until the volume reached 10 mL. Five mL of the extract was analyzed for its Cr^{6+} content using a diphenyl carbazide reagent (Pramono et al. 2013). This reagent was prepared by dissolving 0.125 mg of dyphenilcarbazide crystal in 25 mL acetone (Pramono et al. 2013). The sample was incubated at room temperature for 5 minutes, and the absorbance was determined using a spectrophotometer at λ 540 nm. The absorbance values obtained were converted to Cr^{6+} concentration using the Cr^{6+} standard curve line equation and then multiplied by the dilution factor and the weight of the sample ash.

Data analysis

The research data was analyzed using SPSS analysis. The homogeneity of all data was assessed, followed by a one-way ANOVA test. The Tukey test (Post hoc, significance level set at a <0.05) was performed to compare the mean values. The parameters that were tested included Cr^{6+} concentration in liquid media water hyacinth roots and shoots, bacterial growth, and water hyacinth growth parameters.

RESULTS AND DISCUSSION

***Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 growth**

During the study, the number of bacteria observed for growth is distinguished between *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9. The number of bacteria in all treatments containing *Microbacterium* sp. SpR3 increased from day 0 to day 7, reaching $1.26x10^6$-$2.15x10^6$ CFU mL^{-1} except for the R2M1 treatment. The highest number of *Microbacterium* sp SpR3 were R1M1, R0M1, R2M1, and R1M2 respectively (Figure 1). The decreasing number of bacteria starting on the 7th and 14th days is expected to occur due to growth inhibition caused by competition in using nutrients available in liquid media or around plant tissue.

The growth of *Micrococcus luteus* RT-9 (Figure 2) shows a different pattern from *Microbacterium* sp. SpR3. In the R1M0 treatment, bacterial growth occurred from the 14th day. On the 21st day, *Micrococcus luteus* RT-9 was found only in the R1M0 and R1M1 treatments. *Micrococcus luteus* RT-9 in treatments R1M0 and R1M1 is suspected to be capable of adapting to changes in environmental conditions and utilizing nutrient sources in liquid media. In the R2M1 and R1M2 treatments, *Micrococcus luteus* RT-9 did not grow and only found *Microbacterium* sp. SpR3. The results of Meitiniarti's research (2022) showed that the growth rate of *Micrococcus luteus* RT-9 (0.0205 h^{-1}) was lower than that of *Microbacterium* sp. SpR3 (0.850 h^{-1}). This difference in growth rates occurs due to competition or the adaptation of bacteria to environmental changes. *Micrococcus luteus* RT-9 did not compete using nutrients in liquid media or adapt to the environment. As a result, *Micrococcus luteus* RT-9 cells were not found on day 21 in the R2M1 and R1M1 treatments. Bacterial cells can produce extracellular matrix (ECM) to form architecturally complex biostructures called biofilms. ECM compounds against toxic stress, where the extracellular compounds are toxic to other species (Molina-Santiago 2021).

Based on the research results *Microbacterium* sp. SpR 3 and *Micrococcus luteus* RT-9 can survive by associating with water hyacinth plants in liquid media. Water hyacinth is a substantial component in wetland ecosystems and provides a large surface area to support microbial growth (Ashraf et al. 2018). Water hyacinth also provides a source of carbon and nutrients (such as free amino acids, protein, alcohol, vitamins, and hormones) through the roots so that bacteria can grow and carry out metabolism (Wickramasinghe et al. 2018). In addition, an increase in plant biomass can also lead to an increase in microbial populations (Arslan 2017).

Water hyacinth growth on Hoaglands media containing Cr^{6+} with *Microbacterium* sp. SpR3 and/or *Micrococcus luteus* RT-9

The growth rate of water hyacinth, based on dry weight, showed that there was no significant difference between the control and R1M1 treatment (Figure 3).

Table 1. Comparison of inoculum volume of Cr^{6+} reducing bacteria

Treatment	Repetition	The volume of Inoculum (mL)	
		***Microbacterium* sp. SpR 3**	***Micrococcus luteus* RT-9**
R1M0	4	-	50
R0M1	4	50	0
R1M1	4	25	25
R2M1	4	16.67	33.33
R1M2	4	33.33	16.67
R0M0 (Control)	4	-	-

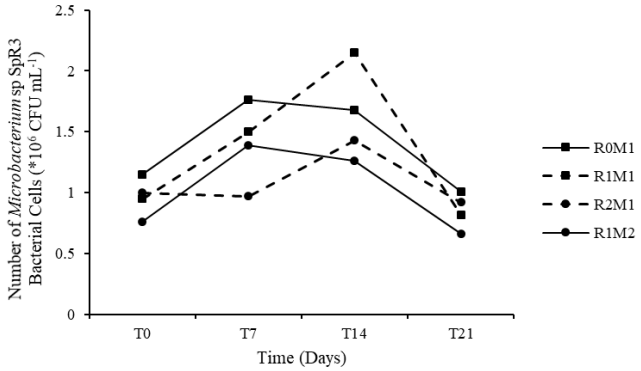

Figure 1. *Microbacterium* sp SpR3 growth in 21 days

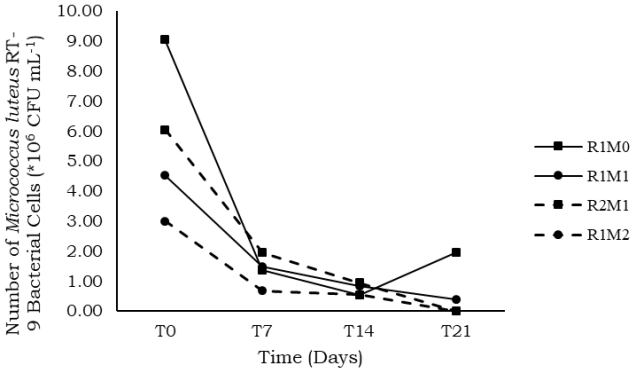

Figure 2. *Micrococcus luteus* RT-9 growth in 21 days

The growth rate of water hyacinth in the treatments showed differences between treatments. The accumulation of Cr^{6+} in water hyacinth in R1M0 and R0M1 treatments influenced the growth rate of water hyacinth in both treatment. Cr^{6+} accumulations in water hyacint R1M0 and R0M1 treatments were 96.60 and 187 µg.g^{-1} which were higher than R1M1, R2M1, R1M2 and control (Figure 6). The high accumulation of Cr^{6+} in the water hyacinth R1M0 and R0M1 treatments have caused low growth rate of water hyacinth in these treatments. Saha's research (2017) also showed that the accumulation of Cr^{6+} in water hyacinths can reduce the growth rate of water hyacinth. During 15 days of research, water hyacinth was planted in media with 5.0 mg.L^{-1} Cr^{6+} and was accumulated for 1.75x10^3 µg.g^{-1} and showed an average 11.14 cm growth rate on the stem, 8.8 cm on roots. Meanwhile, in media with 3.0 mg.L^{-1} Cr^{6+}, it was accumulated for 1.52x10^3 µg.g^{-1}, showing that the average growth of water hyacinth was 12.23 cm on the stem and on 9.25 cm on the roots. The number of *Microbacterium* sp SpR3 in R2M1 and R1M2 treatments from T0 to T21 was less than in R1M1 treatment. The number of *Micrococcus luteus* RT-9 bacteria in the R2M1 treatment decreased significantly from T0 to T21. The R1M2 treatment of *Micrococcus luteus* RT-9 bacteria at T0 to T21 was less than R1M1. This shows that the difference in the growth rate of water hyacinth in the R1M1, R2M1, and R1M1 treatments is caused by differences in the number of bacteria in each treatment. The results of research by Upadhyay (2017) with 49 days of research showed that the difference in the number of bacterial inoculations in the medium of 100, 150, and 200 mL inoculation had a plant weight of 6.10 g higher than with the addition of inoculation 150 (4.81 g) and 100 mL (4.70 g). Treatments R1M0 and R0M1 (single bacteria) showed lower water hyacinth growth compared to treatments R1M1, R2M1, and R1M2 (mixed bacteria), which means that the mixture of the two bacteria was more optimal in reducing the effect of Cr^{6+} toxicity on plants compared to single bacterium. The water hyacinth growth rate shows that the plants can still grow in media added with bacterial inoculation and suggests that *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 do not inhibit the growth of water hyacinth.

The root-shoot ratio is an important index for assessing plant health and as an indicator of plant stress caused by chemical or physical agents. In discussing the root-shoot ratio as potentially important in succession, the root is revealed to be the successor. The root-shoot ratio has an important role in ecological succession, where species have different strategies for growth above ground for light or competition below ground for water and nutrients (Agathokleous et al. 2019). A higher root-shoot ratio also reveals that water hyacinth plants can survive in harsher conditions due to the higher ability to store organic matter in stems and roots (Nazir et al. 2020). Measurement of the root-shoot ratio in this study showed that the root-shoot ratio in control decreased from 0.30 (on day 0) to 0.25 (on day 21), whereas all treatments increased (Figure 4). The increase in the root-shoot ratio indicates that the water hyacinth with the addition of inoculation with *Microbacterium* sp. SpR3 and/or *Micrococcus luteus* RT-9 can reduce plants' stress on Cr^{6+} so that they survive more under stressful conditions compared to control. Research by Nazir et al. (2020), showed that the rate of increasing in the root-shoot ratio in plants with the addition of bacteria is higher than without bacteria, namely 15 cm with bacteria and 7 cm without bacteria.

Effect of *Microbacterium* sp. SpR3 and/or *Micrococcus luteus* RT-9 on Cr^{6+} concentration in liquid media

Analysis of Cr^{6+} concentration in liquid media after 21 days revealed a decrease in all treatments, with significant differences observed between the treatments. The average decrease in Cr^{6+} concentration in liquid media from 5 mg.L^{-1} to 1.559 mg.L^{-1} was due to the bioremediation process by water hyacinth, *Microbacterium* sp SpR3, and *Micrococcus luteus* RT-9. The sequential decreases, from highest to lowest, were observed in the following treatments: R1M1, Control, R1M2, R2M1, R1M0, and R0M1 (Figure 5).

The decrease in Cr^{6+} concentration in the R1M1 treatment, which reached 88.06% with a final concentration of Cr^{6+} at 0.551 mg.L^{-1} indicates that the reduction in Cr^{6+} concentration is most significant when the two bacteria are mixed in a 1:1 (v/v) ratio compared to the control and the addition of bacteria either singly or in different volume ratios. The decrease in Cr^{6+} concentration in liquid media occur due to the reduction of Cr^{6+} by *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9.

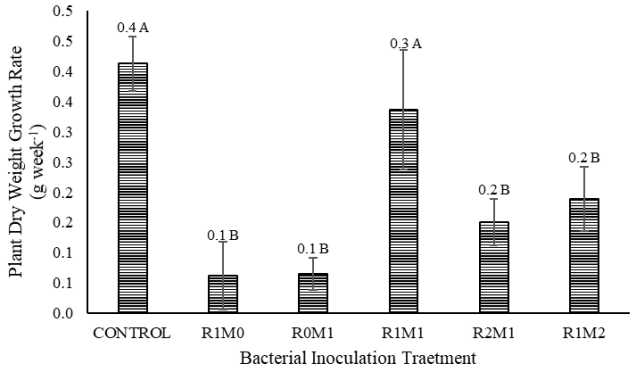

Figure 3. Water hyacinth dry weight growth rate in six treatments. * Numbers followed by the same letters mean that the test is not significantly different from the SPSS test at the 5% level

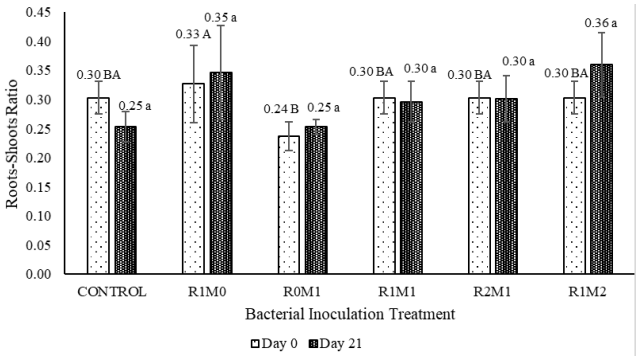

Figure 4. Water hyacinth roots-shoots ratio. * Numbers followed by the same letters mean that the test is not significantly different from the SPSS test at the 5% level. Capital letters for day 0, small letters for day 21

According to Innation et al. (2021), the addition of vermicompost containing *Microbacterium* sp. SpR3 can accelerate the decrease in Cr^{6+} concentration by 0.095 $mg.L^{-1}$ h^{-1} in the soil during seven days of study. Meitinarti et al. (2022) succeeded in isolating Cr^{6+}-resistant bacteria from the rhizosphere of the *Tagetes* sp plant, and one of these bacterial isolates, namely *Micrococcus luteus* RT-9, can reduce Cr^{6+}. Based on the results of this study, a mixture of the two bacteria *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 also showed the ability to reduce Cr^{6+} concentrations in liquid media.

In control, there was also a very high decrease, reaching 87.08%, with a concentration on the 21st day reaching 0.709 $mg.L^{-1}$. The significant decline in Cr^{6+} concentrations can be attributed to the presence of rhizosphere bacteria in water hyacinth, as no bacteria were identified in the control treatment. The root structure of water hyacinth provides a suitable environment for aerobic bacteria to obtain carbon sources and produce organic compounds for plants (Hasbi et al. 2020). The decrease in Cr^{6+} concentration in the control may be caused by several special characteristics of water hyacinths such as a dense fibrous root system, wide leaves, and fast growth (Saha et al. 2017). Absorption of Cr^{6+} and water through roots, because the root system induces significant cation exchange through the membrane cell enabling the mechanism of Cr^{6+} absorption into the root system (Hasbi et al. 2020). On the 21st day of treatment, the levels of Cr6+ in R2M1 and R1M2 were 1.94 $mg.L^{-1}$ and 1.44 $mg.L^{-1}$, respectively, which were lower than those in the R1M0 treatment at 2.181 $mg.L^{-1}$ and the R0M1 treatment at 2.528 $mg.L^{-1}$ (single bacteria). This is illustrated in Figure 5, showing that the combination of the two bacteria as more effective in reducing Cr^{6+} concentration in the liquid medium compared to single bacteria treatments. The R1M0 treatment showed a lower decrease than R0M1 because the number of bacteria in R1M0 on the 21st day was higher than in R0M1. These results indicate that the number of bacteria affects the reduction of Cr^{6+} in the liquid media. According to Christita and Iwanuddin (2017), the growth ability of Cr-resistant bacteria in media containing Cr^{6+} was directly proportional to their ability to reduce Cr^{6+}.

Microbes can reduce Cr^{6+} because bacteria have a gene that encodes the enzyme chromate reductase (Arslan et al. 2017). The enzyme chromate reductase can play a role in aerobic and anaerobic conditions. The enzyme chromate reductase plays a role in reducing Cr^{6+} to Cr^{3+}, a non-toxic form of chromium. In addition, bacteria can accumulate heavy metals with the help of protein and specific metal-binding peptides, which facilitate redox signaling processes after exposure to toxic metals in the context of Cr^{6+} exposure (Riva et al. 2019). Microbe's ability to reduce chromium is referred to as the bioprecipitation process, which is a chemical reaction that reduces heavy metals from toxic forms to non-toxic precipitates by microorganisms (Medfu Tarekegn 2020).

The low concentration of Cr^{6+} in the R1M1 treatment and the control led to the high growth rate of water hyacinth dry weight was caused by tolerance to Cr^{6+} stress in the environment. High concentrations of Cr^{6+} in the media require higher adaptability of water hyacinths, thereby reducing the growth process of water hyacinths (Worku et al. 2023). Research by Madan et al. (2017) showed that water hyacinth grown at a concentration of 50 $mg.L^{-1}$ only experienced a wet weight gain of 1.29 g day^{-1}, while water hyacinth grown at a concentration of 25 $mg.L^{-1}$ experienced a wet weight gain of 1.42 g day^{-1}.

Effect of Cr^{6+}-resistant bacteria on Cr^{6+} accumulation by water hyacinth

The accumulation of Cr^{6+} in water hyacinth was very high, reaching 42.40-187.20 $\mu g.g^{-1}$ dry weight (Figure 6). Saha's research (2017) showed that the accumulation rate of Cr^{6+} in water hyacinth reached 3500 $\mu g.kg^{-1}.day^{-1}$ dry weight in media containing 5 $mg.L^{-1}$ Cr^{6+}. Chakrabarty's research (2017) also showed accumulation of Cr^{6+} in water hyacinth after 7 days of treatment was 225.25 $\mu g.g^{-1}$, and 9642.75 $\mu g.g^{-1}$ after 15 days. Accumulation of Cr^{6+} was significantly different between treatments, between root and shoot parts (petiole and leaf). The results indicate that water hyacinth is a hyperaccumulator plant capable of absorbing Cr^{6+} in high concentration, even in environments with low level of contaminants which can be toxic to organisms.

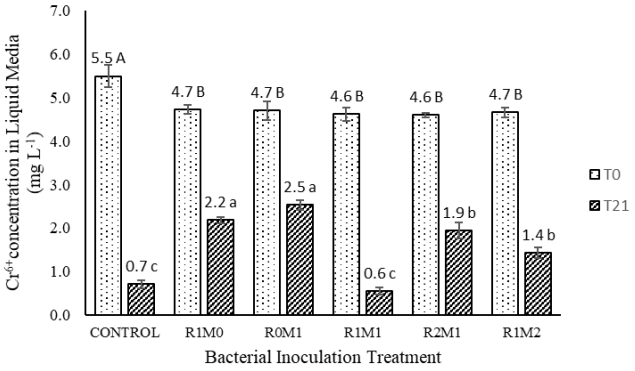

Figure 5. Cr^{6+} concentration in medium. * Numbers followed by the same letters mean that the test is not significantly different from the SPSS test at the 5% level. Capital letters for day 0, small letters for day 21

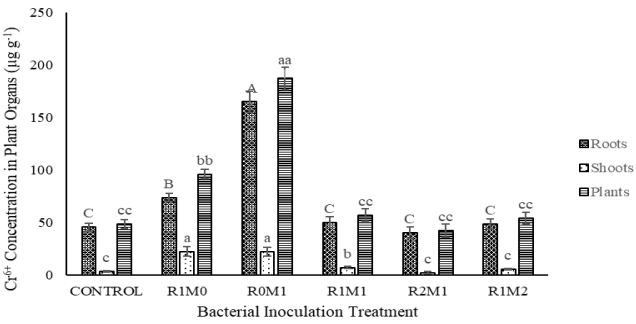

Figure 6. Cr^{6+} accumulation in plant organs. * Data labels followed by the same letters mean that the test is not significantly different from the SPSS test at the 5% level. Capital letters for roots, small letters for shoots, and double letters for plants

The average of Cr^{6+} accumulation in the roots reached 70.57 µg.g^{-1}, which was higher than in the shoots section, which was 10.37 µg.g^{-1}. The highest was in the R0M1 treatment, reaching 164.99 µg.g^{-1} (roots) and 22.25 µg.g^{-1} (shoots). Ashraf et al. (2018) showed accumulation of Cr^{6+} in the roots was 0.135 mg.g^{-1} greater than in shoots which was 0.04 mg.g^{-1}. The study results were caused by the uptake of Cr^{6+} in the media starting from the roots and further translocation from roots to shoots was limited due to protective mechanisms of the plants. In addition, rhizosphere microorganisms produce organic acids that can bind to the reduced form of Cr^{6+}, thus increasing Cr^{6+} bioavailability in roots (Ashraf et al. 2018).

The results showed that the rate of Cr^{6+} accumulation by plants and reduction in the media was inversely proportional. In the R0M1 treatment, the Cr^{6+} reduction rate in liquid media was the lowest at 0.72 mg.L^{-1}.week^{-1}, with the highest accumulation rate in plants at 61.34 µg.g^{-1}.week^{-1}. The linear relationship between Cr^{6+} concentration and Cr^{6+} accumulation rate by water hyacinth explains that higher Cr^{6+} concentration increases the rate of Cr^{6+} accumulation by water hyacinth. A study by Musdek et al. (2015) also showed that water hyacinth plants accumulated Cr^{6+} 1.60 µg.g^{-1} in media with a concentration of 5 mg.L^{-1} was higher than the accumulation of Cr^{6+} in media with a concentration of 1 mg.L^{-1} which was only 0.20 µg.g^{-1}.

Based on the research results, it can be concluded that the *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 can be used to reduce the concentration of Cr^{6+} in liquid media because the decrease in Cr^{6+} concentration in the R1M1 treatment was 88.06% higher than without the addition of inoculum (control), which was 87.08%. *Microbacterium* sp SpR3 and *Micrococcus luteus* RT-9 can also increase the growth rate of water hyacinth because in the R1M1 treatment the growth rate of dry weight is high. It is necessary to review the use of the combination of *Microbacterium* sp. SpR3 and *Micrococcus luteus* RT-9 in reducing the effect of Cr^{6+} toxicity in the growth of water hyacinth because the high accumulation of Cr^{6+} in water hyacinth in R1M0 and R0M1 reduces the growth rate of water hyacinth.

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