

Conference Paper

The Role of Heavy Metals-Resistant Bacteria *Acinetobacter* sp. in Copper Phytoremediation using *Eichhornia crasippes* [(Mart.) Solms]

Wahyu Irawati¹, Adolf Jan Nexson Parhusip², Nida Sopiah³,
and Juniche Anggelique Tnunay¹

¹Department of Biology, Teachers College, Universitas Pelita Harapan, M.H. Thamrin Boulevard 1100, Lippo Karawaci, Tangerang 15811, Banten, Indonesia

²Universitas Pelita Harapan, M.H. Thamrin Boulevard 1100, Lippo Karawaci, Tangerang 15811, Banten, Indonesia

³Institute for Environmental Technology, Agency for the Assessment and Application of Technology, Serpong, Indonesia

Abstract

Phytoremediation is a bioremediation process using plants and microorganisms to extract, sequester, or detoxify heavy metals. *Eichhornia crassipes* [(Mart.) Solms] is a well-known phytoremediating plant that has the ability to remove heavy metals from water by accumulating them in their tissues. *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 are copper resistant bacteria isolated from industrial waste in Rungkut, Surabaya. The aim of this research was to study the effect of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 inoculation in copper phytoremediation process using *Eichhornia crassipes*. Bacterial isolate with colony form unit of 10^8 was inoculated into the rhizosphere of *Eichhornia crassipes* in water containing $10 \text{ mL} \cdot \text{L}^{-1}$ and $20 \text{ mL} \cdot \text{L}^{-1}$ copper. Copper removal in contaminated water and copper accumulation in the plant roots was analyzed using atomic absorption spectrophotometer. The results showed that inoculation treatment enhanced the potency of the plant to reduce copper from 94 % concentration level in the medium without bacterial inoculation to 98.3 % and 97 % in medium inoculated with *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2, respectively. *Eichhornia crassipes* inoculated with *Acinetobacter* sp. IrC1 accumulated up to six fold higher copper concentrations in roots compared with un-inoculated controls. The roots of *Eichhornia crassipes* accumulated $596 \text{ mg} \cdot \text{kg}^{-1}$ and $391 \text{ mg} \cdot \text{kg}^{-1}$ in medium containing $5 \text{ mL} \cdot \text{L}^{-1}$ and $10 \text{ mL} \cdot \text{L}^{-1}$ copper without inoculation, while, the upper part of the plants accumulated up to $353.25 \text{ mg} \cdot \text{kg}^{-1}$ and $194.15 \text{ mg} \cdot \text{kg}^{-1}$ in medium inoculated with *Acinetobacter* sp. IrC1, respectively. The findings of the study indicated that *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 can improve the phytoremediation potential of *Eichhornia crassipes*.

Keywords: *Acinetobacter* sp.; copper; *Eichhornia crassipes*; phytoremediation, removal

Corresponding Author:

Wahyu Irawati

w.irawati3@gmail.com

Received: 9 June 2017

Accepted: 15 July 2017

Published: 11 September 2017

Publishing services provided
by Knowledge E

© Wahyu Irawati et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the NRLS Conference Committee.

OPEN ACCESS

1. Introduction

Heavy metal contamination in water as a result of industrial activities is a serious environmental problem. Industries often discharge waste waters either directly or indirectly which is endangering the life of organism [1]. Heavy metals are considered as the most dangerous elemental pollutants because of their toxicities to human health [2]. There are two types of heavy metals is essential and non-essential. Non-essential heavy metals are toxic because it is a carcinogen that can cause cancer and other dangerous diseases especially for children. Non-essential heavy metals is also toxic if it is in high concentration. [3]. One of the most immediate concern and abundant metal is copper. Copper contamination is usually caused by a variety of sources including agriculture, electropolating, mining, leather tanning, chemical industry, and the textile industry. [4].

Various methods have been used to treat heavy metal pollution, which is chemically treatment such as precipitation, adsorption, nanofiltration, and reverse osmosis, but these methods are expensive, require high energy, and not environmental friendly because it can not remove all the heavy metals from the environment. [5]. Therefore, it should be considered a method that is eco-friendly, inexpensive, and efficient to resolve the problem of heavy metal pollution.

Phytoremediation is a waste treatment method that is eco-friendly and can solve the problem of environmental pollution because some plants have the potential to remove heavy metals from the environment [6]. Phytoremediation is the use of plants and microorganisms to reduce the concentration of heavy metals in the environment [3]. Phytoremediation is a detoxification method which manipulate the characteristics of plants to accumulate heavy metals in plant biomass. It is resulting of decreasing in heavy metals concentration in the environment [7]. The success of phytoremediation process depends on the ability of plants to grow in heavy metals contaminated area and to remediate the pollutants through several mechanisms such as accumulation the heavy metals in the plant tissues.

Eichhornia crassipes [(Mart.) Solms] frequently used in phytoremediation process because it can grow and produce high biomass in an environment that contains high concentration of heavy metals without showing many of the symptoms of toxic [8]. *Eichhornia crassipes* is a hiperaccumulator plant that can accumulate heavy metal 100,000 times greater than other plants inside its tissues [9]. Therefore the use of *E. crassipes* as agents of phytoremediation can be a solution to overcome the metal pollution in the aquatic environment because the plant is not only hiperaccumulator plant but also easily available, low cost, effective, and eco-friendly methods [10]

Recently, phytoremediation associated with bacteria has appeared as a more successful approach to remove heavy metals from contaminated sites [11]. Plant growth

promoting rhizobacteria is microorganisms which growing in the root zone and closely associated with roots or a diverse group of free-living bacteria which grow in the root area of plants. The bacteria can improve the growth and development of the host when the plant was grown in heavy metal contaminated sites by reducing toxic effects of heavy metals on the plants. [12, 13]. Previous study showed that inoculation of *Bacillus licheniformis* [(Weigmann 1898) Chester 1901] in the rhizosphere of *Brassica juncea* [(L.) Vassiliï Matveievitch Czernajew] enhanced cadmium accumulation in plant tissues [14]. The addition of *Microbacterium arabinogalactanolyticum* [(Yokota et al. 1993) Takeuchi and Hatano 1998], *Sphingomonas macrogoltabidus* (Takeuchi et al. 1993), and *Microbacterium liquefaciens* [(Collins et al. 1983) Takeuchi and Hatano 1998] significantly increased the plant uptake of nickel compared with un-inoculated controls [15].

E. crassipes is found growing in almost all the aquatic environment in Indonesia. Some bacteria can be isolated from heavy metal contaminated sites and introduced into the rhizosphere of the plant to enhance phytoremediation processes. *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 are copper resistant bacteria isolated from industrial wastewater in Indonesia that have high potency to be accumulated [16]. The aim of this research was to study the effect of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 inoculation in increasing the efficiency of *E. crassipes* to remove and accumulate copper.

2. Materials and methods

2.1. Bacterial strains, media and growth

Acinetobacter sp. IrC1 and *Acinetobacter* sp. IrC2 are copper-resistant bacteria with the Minimum Inhibitory Concentration (MIC) of (6 to 7) mM CuSO_4 , under the accession number of JX009133 and JX 009134, respectively. Bacterial isolate was grown in Luria Bertani (LB) agar containing the following contents (per L): tryptone: 10 g, yeast extract 5 g, NaCl 10 g, and glucose 0.1 g. Cells were incubated at 37 °C on a 200 rpm shaker [16] (1 rpm = 1/60 Hz). Growth was monitored by measuring optical density at 600 nm. Amount of 100 μL cells culture with appropriate dilution were spreaded on agar medium to determine the value of colony form unit (CFU). Bacterial inoculum with CFU of 10^8 would be ready for inoculating treatment in phytoremediation processes.

2.2. Phytoremediation experiment

Some of *Eichhornia crassipes* was collected from Kelapa-Dua Lake, Tangerang, Indonesia. Approximately (250 to 300) g of each plants were selected and put into containers

containing 8 L water for acclimatization during 2 wk before being exposed to copper contaminants. Each plant was put into containers containing $10 \text{ mg} \cdot \text{L}^{-1}$, $20 \text{ mg} \cdot \text{L}^{-1}$, and without copper, with three replicates for each plants at each treatment, respectively. Amount of 800 mL of bacterial inoculums 10^8 Colony Form Unit (CFU $\cdot \text{mL}^{-1}$) was inoculated into the rhizosphere of *E. crassipes* in the container as inoculated treatment. The plants also were grown in water without copper addition as an uninoculated control. The volume of water in each container was kept to be constant and the change in volume due to evaporation was compensated by the addition of water. Water solution was collected every two weeks during 28 d for copper analysis. Physiological appearance of leaves was also being observed including color and chlorotic leaves for assessment the effect of copper toxicity toward the plant.

2.3. Copper content analysis

The root of plant was collected, washed with water, and dried in oven for 24 h at 70°C . Amount of 0.2 g of dried root sample was grounded and suspended in 10 mL of water. The suspension was disrupted with HNO_3 at 100°C . Copper content in dried root sample was analyzed using atomic absorption spectrophotometer for assessment the potency of *E. crassipes* to accumulate copper. Water sample from the container was also collected and disrupted with the same way for measuring the copper removal efficiency by *E. crassipes*. The removal efficiency was calculated as $(C_i - C_f / C_i) \times 100\%$ where C_i is the initial concentration and C_f is the remaining copper concentration in the solution [17].

3. Results and discussion

3.1. Bacterial inoculum

Acinetobacter sp. IrC1 and *Acinetobacter* sp. IrC2 are heavy metals-resistant bacteria isolated from industrial sewage in Rungkut, Surabaya, Indonesia. The bacteria have high resistance to some heavy metals such as copper, zinc, cadmium, mercury, and lead might be due to the selective pressure by pollution of industrial wastewater at Rungkut, the location from where the strain has been isolated. Chan et al. [18] reported that *Acinetobacter* can usually isolated from polluted environment. *Acinetobacter* species are important biotechnological tools, and have been utilized extensively in the synthesis of enzymes and other life-sustaining macromolecules and for degradation of recalcitrant compounds [18]. Genus *Acinetobacter* was a member of bacterial community in rhizosphere of *E. crassipes* and in wetland water samples [19]. It was expected that inoculation of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 to

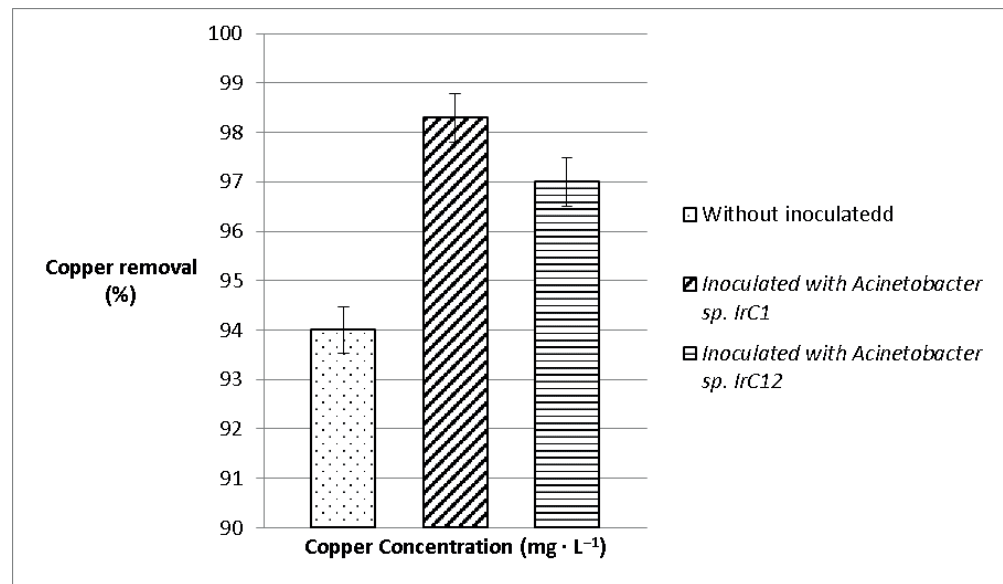


Figure 1: The effect of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 inoculation to remove copper in medium containing $10 \text{ mg} \cdot \text{L}^{-1} \text{ CuSO}_4$.

rhizosphere of *E. crassipes* improving the plant to accumulate and remove copper from solution.

3.2. Copper removal

The potency of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 to reduce copper concentration in water containing $10 \text{ mL} \cdot \text{L}^{-1} \text{ CuSO}_4$ can be seen at Figure 1. The results showed that bacterial inoculation treatment enhanced the potency of the plant to reduce copper from 94 % concentration level in the medium without bacterial inoculation to 98.3 % and 97 % in medium inoculated with *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2, respectively. Previous study showed that the ability of *E. crassipes* without bacterial inoculation to remove and accumulate cadmium, chromium, copper, lead, and nickel was 24 % to 80 % of total heavy metals [20]. Sharma et al. [21] reported that *Eichhornia crassipes* showed increased removal efficiency of heavy metals through the activity of its rhizospheric bacteria. The bacteria facilitate the uptake of essential elements.

According to Jing et al. [15], plant and bacteria can form specific associations in which the plant provides the bacteria with a specific carbon source that induces the bacteria to reduce the phytotoxicity of the contaminated sites. Rhizosphere plays a significant role in heavy metals phytoremediation in contaminated area. Microbial populations have potential to enhance phytoremediation processes. Bacteria can augment the remediation capacity of plants or reduce the phytotoxicity of the contaminated sites. Bacteria affect the mobility and availability of heavy metals in the plant through

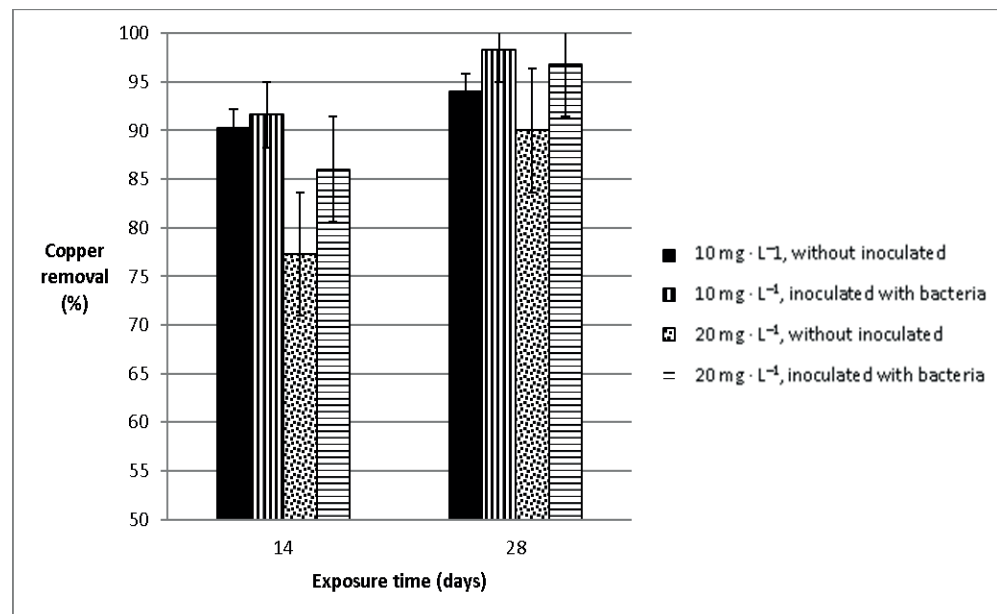


Figure 2: The potency of copper removal by *Eichhornia crassipes* in water containing 10 mL · L⁻¹ and 20 mL · L⁻¹ CuSO₄ without and with inoculation of *Acinetobacter* sp. IrC1.

release of chelating agents, acidification, phosphate solubilization and redox changes. Babalola [22] reported that plant roots provide exudate such as free amino acids, proteins, carbohydrates, alcohols, vitamins, and hormone for bacteria which are important sources for bacterial nutrient. These biochemical mechanisms increase the remediation activity of bacteria associated with plant roots [15].

According to Hussein [14] bacteria that interact specifically with plant roots in rhizosphere inducing phytoremediation either by forming symbiotic relationships with the plant or by microbial biotransformation of organic compounds, thereby indirectly facilitate their removal. It suggested that *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 induced copper phytoremediation in *E. crassipes* not only by forming symbiotic relationship but also by bacterial bioaccumulation. *Acinetobacter* sp. IrC2 showed that multiple resistance to heavy metals and had ability to accumulate copper, zinc, lead and cadmium [23]. Previous study showed that copper resistance mechanism of *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 were facilitated through the bioaccumulation of copper inside the cell up to 364.66 mg · L⁻¹ of copper [16] It also was reported that the bacterium removed copper up to 52.98 % [24].

Copper removal by *Eichhornia crassipes* inoculated with *Acinetobacter* sp. IrC1 in medium containing 10 mM and 20 mM increased during observation periode for 28 d (Figure 2).

The effect of *Acinetobacter* sp. IrC1 inoculation treatment in copper removal by *E. crassipes* can be seen at Figure 3. Copper removal of the plants with inoculation treatment was higher than without inoculation treatment. The copper removal percentage of

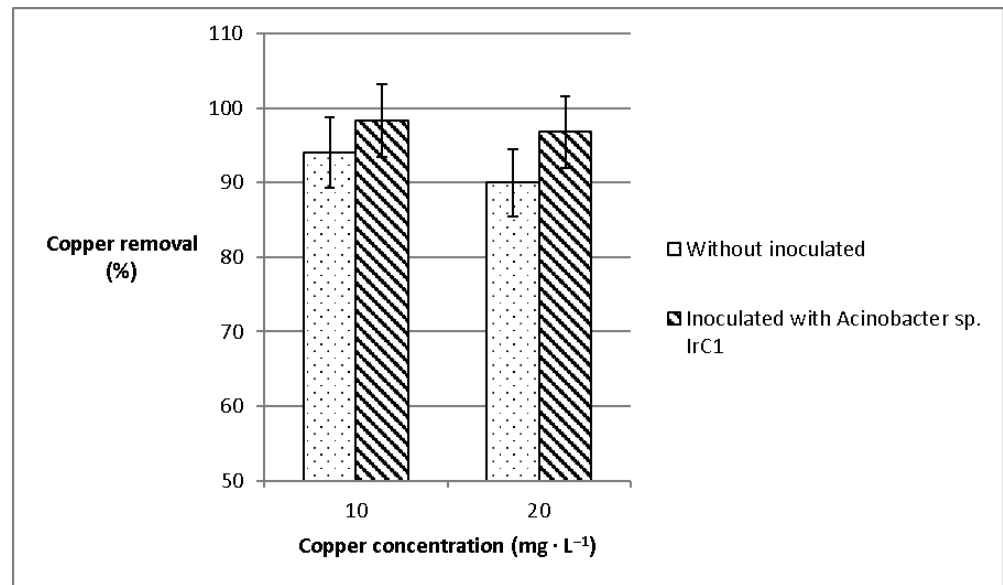


Figure 3: The effect of *Acinetobacter* sp. IrC1 inoculation treatment in copper removal by *Eichhornia crassipes*.

plants growing in medium containing 10 mg · L⁻¹ CuSO₄ were higher than that of 20 mg · L⁻¹ CuSO₄. The copper removal percentage of *Acinetobacter* sp. IrC1 and *E. crassipes* in medium containing 20 mg · L⁻¹ decreased might be due to the saturation of the available binding sites of the bacteria and the plants to remove copper. Increasing the concentration of heavy metals lowered copper removal percentage due to its toxic effects. The metal toxic influence the blocking of functional groups, displacement and substitution of essential metal ions from biomolecular, conformational modifications, denaturation and inactivation of enzymes as well as disruption of cellular and organellar membrane integrity [25]

3.3. Copper Accumulation

The effect of *Acinetobacter* sp. IrC1 inoculation to the ability of *E. crassipes* to accumulate copper in medium containing 10 mg · L⁻¹, 20 mg · L⁻¹, and without copper can be seen at Figure 4. Inoculation with *Acinetobacter* sp. IrC1 significantly enhanced the potency of *E. crassipes* to accumulate copper. *Eichhornia crassipes* inoculated with *Acinetobacter* sp. IrC1 accumulated up to six fold higher copper concentration in roots compared with un-inoculated controls. The roots of *Eichhornia crassipes* accumulated 596 mg · kg⁻¹ and 391 mg · kg⁻¹ in medium containing 5 mL · L⁻¹ and 10 mL · L⁻¹ copper without inoculation, while, the upper part of the plants accumulated up to 3 532.5 mg · kg⁻¹ and 1941.5 mg · kg⁻¹ in medium inoculated with *Acinetobacter* sp. IrC1, respectively.

Previous studies showed that bacterial inoculation enhanced the ability of *E. crassipes* to accumulate heavy metal. Application of cadmium or lead resistant microorganisms improved the ability of *Solanum nigrum* to accumulate heavy metals and increased

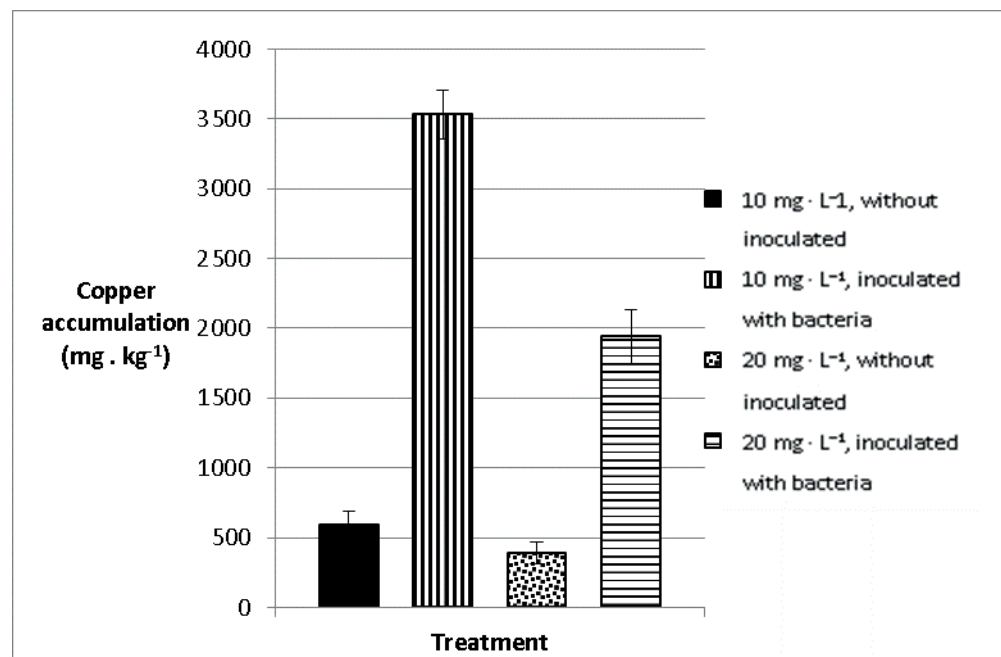


Figure 4: The effect of *Acinetobacter* sp. IrC1 inoculation to the ability of *Eichhornia crassipes* to accumulate copper in medium containing 10 mL · L⁻¹, 20 mL · L⁻¹, and without copper.

plant yield [26]. *Eichhornia crassipes* inoculated with *Ochrobacterium anthropi* and *Bacillus cereus* increased mangan accumulation in roots by 2.4 and 1.3 fold compared to un-inoculation, respectively [27]. Huang et al. [28] reported that inoculation of multiple heavy metals *Burkholderia* sp. strain LD-11 significantly enhanced copper and/or lead accumulation especially in the roots of the plants grown in the polluted sites. Inoculation with the bacteria improved germination, promoted elongation of roots, and enhanced the dry weight of the plants in the presence of copper. Plant-growth-promoting bacteria especially with the resistance to multiple heavy metals are helpful to phytoremediation processes. *Acinetobacter* sp. IrC1 is also a multiresistant bacteria so it must be very helpful for improving phytoremediation processes.

Beneficial effects of plant growth promoting bacteria in phytoremediation are to promote the plant to uptake and accumulate copper [29]. *Eichhornia crassipes* has affinity towards heavy metals because it contains high cellulose and functional groups such as amino (-NH₂), carboxil (-COO-), hydroxil (-OH-), sulfahydryl (-SH) that binds heavy metals. [30]. The efficiency of phytoremediation process enhance the plant-associated beneficial microbes by alternating the metal accumulation in plant tissues and by promoting the shoot and root biomass production. [31]. *Acinetobacter* sp. IrC1 might also promote copper phytoremediation processes in *Eichhornia crassipes* by absorbing the copper inside the cells. Previous study reported that copper resistance mechanisms of *Acinetobacter* sp. IrC1 was facilitated through the bioaccumulation of copper inside the cell which was marked by the alteration in the colour of the colonies into blue in high

concentration [16]. CopA gene encoding copper accumulation has been successfully amplified in plasmid of *Acinetobacter* sp. IrC1 [32].

A group of plants termed hyperaccumulators are considered to be the best candidates for taking up toxic metals, transporting them, and accumulating them [33]. The highest ability of *E. crassipes* to accumulate copper was $1941.5 \text{ mg} \cdot \text{kg}^{-1}$ in medium inoculated with *Acinetobacter* sp. IrC1 (Figure 5). Bacterial inoculation treatment enhanced the potency of the plant to accumulate copper more than $5.00 \text{ mg} \cdot \text{kg}^{-1}$ dry weight in plant roots. It indicated that inoculation of *Acinetobacter* sp. IrC1 affected *E. crassipes* to become a good accumulator. Kabeer, et al. [19] reported that the plant is recognized by a good accumulator because of its ability to take heavy metals up more than $5.000 \text{ mg} \cdot \text{kg}^{-1}$ dry weight and its ability to bioconcentrate the element in its tissues [19].

High copper concentration affected the physiological appearance of *E. crassipes* (data is not shown). *Eichhornia crassipes* with bacterial inoculation grew well in medium containing $20 \text{ mg} \cdot \text{L}^{-1}$ without significant changes in physiological appearance compared to un-inoculation controls, whereas, the leaves growing in medium without bacterial inoculation were becoming chlorotic. Hu et al. [34] reported that high concentration of copper caused chlorophyll degradation and induced oxidative stress in chloroplasts because of inhibition of chloroplast development and chlorophyll biosynthesis in the presence of a high level of copper. The result was similar with the previous study in sunflower plants. Total chlorophyll content decreased in sunflower plants under nickel and lead treatment [17].

Bioaccumulation of copper in *E. crassipes* could result in the loss of pigment concentration significantly. High concentration of copper can lead to chlorophyll degradation. Excess copper can induce oxidative stress in chloroplasts which brings damage to membranes by impacting the normal physiological function of proteins, lipids, and membranes [34]. Accumulation of heavy metals inside the plant at high concentration may induce toxicity by modifying essential protein structure or replacing essential elements. It can be seen from chlorotic symptoms on leaves, growth impairment, browning of roots, and disruption of the photosynthesis process [35]. Cellular enzymes and proteins contain several mercapto ligands that can structurally chelate metals, and thereby cause these proteins to lose their functional property. Heavy metals also generate oxidative stress that is mediated through the generation of free radicals [36].

4. Conclusions

The findings of this study indicated that *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 inoculation enhanced the efficiency of phytoremediation using *E. crassipes*. Inoculation treatment enhanced the potency of the plant to remove copper and accumulate it up to six fold higher copper concentration in roots compared with un-inoculated controls.

Bacterial inoculation induced the plant to grow well without physiological changes as a result of copper toxicity. *Acinetobacter* sp. IrC1 and *Acinetobacter* sp. IrC2 are promising bacterial inoculant to improve the phytoremediation processes using *E. crassipes*.

Acknowledgements

This research grant was funded by Direktorat General of Higher Education, Ministry of National Education, Indonesia through Hibah Bersaing Project with the contract number of DIPA: SP DIPA-042.04.1.400170/2016. We are grateful to Prof. Triwibowo Yuwono, Ph.D from Gadjah Mada University Indonesia in giving invaluable suggestion. We would like to thank Samuel Riak, Dorkas Neti, Jody Pangau, Erwin Natha Perdana, Lion Supriadi, student of Teachers College, Biology Education Department, Universitas Pelita Harapan for being very helpful and eager to learn about this project.

References

- [1] Dhote S, Dixit S. Water quality improvement through macrophytes—a review. *Environmental Monitoring and Assessment* 2009;152(1):149–153.
- [2] Boran M, Altınok I. A review of heavy metals in water, sediment and living organisms in the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences* 2010;10:565–572.
- [3] Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals—concepts and applications. *Chemosphere* 2013;91(7):869–881.
- [4] Rai PK. Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. *Critical Reviews in Environmental Science and Technology* 2009;39(9):697–753.
- [5] Machado MD, Soares HM, Soares EV. Removal of chromium, copper, and nickel from an electroplating effluent using a flocculent brewer's yeast strain of *Saccharomyces cerevisiae*. *Water, Air, & Soil Pollution* 2010;212:199–204.
- [6] Rezanía S, Ponraj, M, Talaiekhosani A, Mohamad SE, Din MFM, Taib SM, et al. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. *Journal of Environmental Management* 2015;163:125–133.
- [7] Denton, BP. Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi. *Basic Biotechnology eJournal* 2007;3:1–5.
- [8] Malar S, Sahi SV, Favas PJ, Venkatachalam P. Mercury heavy-metal-induced physiochemical changes and genotoxic alterations in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Environmental Science and Pollution Research* 2015;22(6):4597–4608.

- [9] Mishra VK, &Tripathi BD. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. *Bioresource Technology* 2008;99(15):7091–7097.
- [10] Rai PK, Singh MM. *Eichhornia crassipes* as a potential phytoremediation agent and an important bioresource for asia pacific region. *Environmental Skeptics and Critics* 2016;5(1):12–19.
- [11] Koo SY, Cho KS. Isolation and characterization of a plant growth-promoting rhizobacterium, *Serratia* sp. SY5. *Journal of Microbiology and Biotechnology* 2009;19(11):1431–1438.
- [12] Glick BR, Karaturović DM, Newell PC. A novel procedure for rapid isolation of plant growth promoting pseudomonads. *Canadian Journal of Microbiology* 1995;41(6):533–536.
- [13] BelimovAA, Kunakova AM, Safronova VI, Stepanok VV, Yudkin LY, Alekseev YV, et al. Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. *Microbiology* 2004;73(1):99–106.
- [14] Hussein HS. Optimization of plant-bacteria complex for phytoremediation of contaminated soils. *International Journal of Botany* 2008;4(4):437–443.
- [15] Jing Y, Zhen-li H, Xiao-e Y. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University SCIENCE B*2007;8(3):192–207.
- [16] Irawati W, Yuwono T, Soedarsono J, Hartiko H. Molecular and physiological characterization of copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. *Microbiology Indonesia* 2012;6(3):107–116.
- [17] Mokhtar H, Morad N, Fizri FFA. Phytoaccumulation of copper from aqueous solutions using *Eichhornia crassipes* and *Centella Asiatica*. *International Journal of Environmental Science and Development* 2011;2(3):205–210.
- [18] Chan KG, Atkinson S, Mathee K, Sam CK, Chhabra SR, Cámara M, et al. Characterization of N-acylhomoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: Co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. *BMC Microbiology* 2011;11:51.
- [19] Kabeer R, Varghese R, Kochu JK, George J, Sasi PC, Poulouse SV. Removal of copper by *Eichhornia crassipes* and the characterization of associated bacteria of the rhizosphere system. *Environment Asia* 2014;7(2):19–29.
- [20] El-Gendy AS. Modeling of heavy metals removal from municipal landfill leachate using living biomass of Water Hyacinth. *International journal of phytoremediation* 2008;10(1):14–30.
- [21] Sharma R, Sharma K, Singh N, Kumar A. Rhizosphere biology of aquatic microbes in order to access their bioremediation potential along with different aquatic macrophytes. *Recent Research in Science and Technology* 2013;5(1):29–32.

- [22] Babalola OO. Beneficial bacteria of agricultural importance. *Biotechnology letters* 2010;32(11):1559–1570.
- [23] Irawati W, Parhusip AJ, Sopiah N. Heavy metals biosorption by copper resistant bacteria of *Acinetobacter* Sp. IrC2. *Microbiology Indonesia* 2015;9(4):163–170.
- [24] Irawati W, Kusumawati L, Sopiah RN. The potency of acinetobactersp IRC2 isolated from industrial wastewater treatment plant in Rungkut-Surabaya as a bioremediation agent for heavy metals. *Asian Journal of Microbiology, Biotechnology, and Environmental Sciences Journal Papers* 2015;17(2):357–363.
- [25] Gadd GM. Interactions of fungi with toxic metals. *New Phytologist* 1993;124(1):25–60.
- [26] Gao Y, Miao C, Wang Y, Xia J, Zhou P. Metal-resistant microorganisms and metal chelators synergistically enhance the phytoremediation efficiency of *Solanum nigrum* L. in Cd- and Pb-contaminated soil. *Environmental Technology* 2012;33(12):1383–1389.
- [27] Abou-Shanab RAI, Angle JS, Van Berkum P. Chromate-tolerant bacteria for enhanced metal uptake by *Eichhornia crassipes* (Mart.). *International Journal of Phytoremediation* 2007;9(2):91–105.
- [28] Huang GH, Tian HH, Liu HY, Fan XW, Liang Y, Li YZ. Characterization of plant growth promoting effects and concurrent promotion of heavy metal accumulation in the tissues of the plants grown in the polluted soil by *Burkholderia* strain LD-11. *International Journal of phytoremediation*. 2013;15(10):991–1009.
- [29] Singh DK, Kumar S. Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. *Chemosphere* 2008;71(3):412–418.
- [30] Patel S. Threats, management and envisaged utilizations of aquatic weed *Eichhornia crassipes*: An overview. *Reviews in Environmental Science and Biotechnology* 2012;11(3):249–259.
- [31] Rajkumar M, Sandhya S, Prasad MNV, Freitas H. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnology Advances* 2012;30(6):1562–1574.
- [32] Irawati W, Yuwono T, Rusli A. Short Communication: Detection of plasmids and curing analysis in copper resistant bacteria *Acinetobacter* sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4. *Biodiversitas* 2016;17(1):296–300.
- [33] Saxena P, Misra AND. Remediation of heavy metal contaminated tropical land. *Soil Heavy Metals*, 2009;19:431–477.
- [34] Hu C, Zhang L, Hamilton D, Zhou W, Yang T, Zhu D. Physiological responses induced by copper bioaccumulation in *Eichhornia crassipes* (Mart.). *Hydrobiologia* 2007;579(1):211–218.

- [35] Göhre V, Paszkowski U. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 2006;223(6):1115–1122.
- [36] Seth CS, Chaturvedi PK, Misra V. The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. *Ecotoxicology and Environmental Safety* 2008;71(1):76–85.