EFFECT OF NUTRIENT AND INOCULATION ON METAL REMOVAL FROM SIMULATED WASTEWATER BY *Pistia stratiotes* AND *Eichhornia crassipes*

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FACULTY OF SCIENCE & NATURAL RESOURCES
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ABSTRACT

Heavy metals are recalcitrant in the environment and are considered as one of the major environmental problem. This has led to intensive research on their removal from wastewater by various technologies such as phytoremediation. The main aim of this study is to evaluate the potential of two locally available floating aquatic macrophytes, *P. stratiotes* (water lettuce) and *E. crassipes* (water hyacinth) in removing heavy metals Pb, Zn, Ni, Cd and Cr in single and mixed conditions at 0.2 mg/L, 0.5 mg/L and 1.0 mg/L from simulated wastewater using CVIF reactor for 15-day period. The concentration of metals in wastewater and plants were determined by ICP-OES. The heavy metals removal percentages of single and mixed were varied for different metals and initial concentration exposed. The removal of Pb in single exposure was found to be faster as compared to other metals at all concentration levels. This was further proven by the removal percentage of 98.8% at initial concentration of 1.0 mg/L after 7 days cultivation. The results also showed that *E. crassipes* was more tolerant towards Pb either in single or mixed as compared to *P. stratiotes* whereby the uptake amount was higher by 2 times. The uptake and distribution of single metals Pb, Zn, Ni, Cd and Cr were higher in roots by almost 5-fold as compared in leaves and by 15-fold in stalks respectively, whereas in mixed metals by almost 3.5-fold as compared in leaves and by 7.8-fold in stalks of *E. crassipes*. The plants cultivated in 0-fold nutrition level showed the highest Pb removal percentage. The percentage removal of Pb at 1.5 mg/L is shown in descending order as 0-fold (99.6%) > 1-fold (94.7%) > 2-fold (85.4%) nutrient levels. In comparison, the removal percentages of Pb at 2.0 mg/L is higher at 0-fold nutrient (83.5%) followed by 1-fold nutrient (58.9%) and 2-fold nutrient (37.8%). The distribution pattern of single Pb at 1.5 mg/L and 2.0 mg/L with addition 0,1 and 2-fold nutrient levels showed that Pb are highly uptake in roots by 2-fold as compared to leaves and stalks in both plants. The inoculation of Pb-tolerant *B. cereus* 1-NMeHI-Cr2 (T1) onto the roots of *P. stratiotes* and *E. crassipes* CVIF reactor had displayed a higher removal percentage with 99.6% followed by T2 with (99.4%). The attachments of Pb-tolerant *B. cereus* 1-NMeHI-Cr2 to roots of both plants have shown by SEM images. In addition, the inoculation of *B. cereus* 1-NMeHI-Cr2 (T1) using CVIF reactor had displayed an increase by 0.2 % in the

removal of Pb as compared to T2 (without inoculation). The efficiency of removal by CVIF reactor indicates the circulation of wastewater provides adequate aeration to the roots system of both plants thus enhance the microbial activity in the roots system. The results also shown that both plants have a similar distribution pattern of Pb in plant tissues at all treatments as roots > leaves > stalks (*E. crassipes*). However, both plants recorded a different uptake value (uptake capacity) whereby roots was higher by 3-fold as compared to leaves and stalks (*E. crassipes*). The physical changes such as roots become fragile or decay and yellowing and wilting of leaves for both plants were monitored throughout the experiments. Studies with regard to aquatic plants combination to be used in engineered wetland treatment ponds should be seriously undertaken for developing more efficient, natural and economic approach in removing heavy metals from contaminated water. The ability of *P. stratiotes* and *E. crassipes* to remove heavy metals especially Pb had indicates their potential in treatment of metal polluted water. This supports the idea that the interaction between aquatic plants and beneficial rhizospheric microbes can enhance the role of plants to remove heavy metal pollutants and are considered to be an important component in phytoremediation technology.

ABSTRAK

KESAN NUTRIEN DAN INOKULASI KE ATAS PENYINGKIRAN LOGAM DARIPADA AIR SISA SIMULASI OLEH Pistia stratiotes DAN Eichhornia crassipes

Logam berat merupakan satu komponen yang sukar didegradasi dalam alam sekitar dan merupakan satu masalah pencemaran yang utama. Pencemaran ini telah membawa kepada satu penyelidikan bagi menyingkirkan logam berat daripada air sisa dengan menggunakan pelbagai teknologi seperti fitoremediasi. Tujuan utama kajian ini adalah untuk menilai potensi dua makrofit akuatik, P. stratiotes (Kiambang air) dan E. crassipes (Keladi bunting)dalam menyingkirkan logam berat Pb, Zn, Ni Cd dan Cr dalam kondisi tunggal dan campuran pada kepekatan 0.2 mg/L, 0.5 mg/L dan 1.0 mg/L daripada air sisa simulasi dengan menggunkan reactor CVIF dalam tempoh 15 hari. Kepekatan logam berat dalam air sisa simulasi dan makrofit akuatik ditentukan dengan menggunakan ICP-OES. Kadar keupayaan penyingkiran logam berat dalam bentuk tunggal dan campuran adalah berbeza bagi setiap jenis logam berat dan kepekatan awal logam yang didedahkan. Penyingkiran logam berat Pb tunggal didapati lebih cepat berbanding dengan logam berat lain pada semua kepekatan awal yang didedahkan. Hal ini dibuktikan dengan peratusan penyingkiran 98.8% pada dedahan 1.0 mg/L selepas 7 hari tempoh eksperimen. Keputusan ini juga menunjukkan E. crassipes mempunya toleransi yang lebih baik terhadap Pb pada pendedahan tunggal dan campuran berbanding P. stratiotes di mana kadar pengambilan E. crassipes adalah 2 kali ganda banyak berbanding P. stratiotes. Pengambilan dan taburan logam berat Pb, Zn, Ni, Cd dan Cr di dalam kedua-dua tumbuhan makrofit adalah lebih 5 kali ganda di akar berbanding daun dan 15 kali ganda berbanding di batang pada pendedahan tunggal. Manakala pada pendedahan campuran, taburan di akar adalah 3.5 kali ganda banyak berbanding di daun dan 7.8 kali ganda banyak berbanding di batang tumbuhan E. crassipes. Peratusan penyingkiran logam Pb pada kepekatan 1.5 mg/L mengikut urutan menurun adalah pada gandaan 0 nutrien (99.6%) > 1-gandaan nutrien (94.7%) > 2-gandaan nutrien (85.4%). Sementara itu, penyingkiran logam Pb pada kepekatan 2.0 mg/L mengikut urutan menurun adalah 0-gandaan (83.5%) > 1-gandaan (58.9%) > 2-gandaan (37.8%).Corak taburan logam tungga lpada kepekatan 1.5 mg/L dan 2.0 mg/L dengan penambahan 0,1,2-gandaan nutrient menunjukkan akumulasi logam Pb adalah tertinggi di akar sebanyak 2 kali ganda berbanding dengan daun dan batang pada kedua-dua makrofit akuatik. Inokulasi dengan toleran-Pb B. cereus 1-NMeHI Cr2 terhadap akar P. stratiotes dan E. crassipes menunjukkan peratusan penyingkiran yang tinggi dengan 99.6% diikuti dengan peratusan tanpa inokulasi iaitu sebanyak 99.4%. Perlekatan toleran Pb B. cereus 1-NMeHI Cr2 pada akar kedua-dua makrofit akuatik ditunjukkan oleh imbasan SEM. Selain itu juga, inokulasi B. cereus 1-NMeHI Cr2 terhadap kedua-dua akar tumbuhan makrofit menggunakan CVIF reactor menunjukkan peningkatan sebanyak 0.2 % berbanding dengan akar tanpa inokulasi. Kecekapan penyingkiran oleh reaktor CVIF ini juga menunjukkan bahawa peredaran air sisa menyediakan pengudaraan yang icukup kepada sistem akar kedua-dua tumbuhan bagi meningkatkan aktiviti mikrob dalam sistem akar tersebut. Kajian ini juga menunjukkan kedua-dua makrofit akuatik mempunyai taburan yang sama bagi logam Pb di dalam tisu tumbuhan pada kesemua jenis rawatan mengikut urutan menurun akar>daun>batang (E. crassipes). Walau bagaimanapun, kedua-dua tumbuhan mencatatkan nilai pengambilan yang berbeza (kapasiti pengambilan) di mana akar adalah lebih tinggi sebanyak 3 kali ganda berbanding dengan daun dan batang (E. crassipes). Perubahan fizikal seperti akar menjadi rapuh atau mereput dan dan daun menjadi kuning dan layu pada kedua-dua tumbuhan dapat dilihat sepanjang eksperimen dijalankan.Kajian mengenai gabungan tumbuhan akuatik yang akan digunakan dalam kejuruteraan kolam rawatan tanah lembap perlu dipertimbangkan secara seriusbagi memperoleh pendekatan yang lebih cekap, semula jadi dan ekonomi dalam menghapuskan logam berat dari air yang tercemar. Keupayaan P. stratiotes dan E. crassipes untuk menyingkirkan logam berat terutama Pb menunjukkan potensi mereka dalam rawatan air. Hal ini menyokong idea bahawa interaksi antara tumbuhan akuatik dan mikrob rhizospheric boleh meningkatkan peranan tumbuhan untuk menyingkirkan logam berat dan dianggap sebagai satu komponen penting dalam teknologi fitoremediasi.

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
CVIF	Continuous Vertical Inlet Flow
CDF	Cation diffusion facilitator
COPT	Copper transporter
EDTA	Ethylenediamine tetra acetic acid
IAA	Indole acetic acid
ICP	Inductive Coupled Plasma
IRT	Iron regulated transporter
NA	Nutrient agar
NRAMP	Natural resistance macrophage protein
pH	hydrogen ion concentration
PDR	Pleiotropic drug resistance
PGPR	Plant Growth Promoting Rhizobacteria
ppm	parts per million
RC	Removal capacity
rpm	revolutions per minute
sp.	Species
ZRT	Zinc regulated transporter

LIST OF SYMBOLS

%	percentage
g	gram
mL	mililiter
mg/L	milligram per liter
ug/mL	microgram per mililiter
µm	micrometer
^{0}C	degree celcius
NO^{-3}	nitrate
O_2	oxygen
H_2O_2	hydrogen peroxide
OH	hydroxide
L	liter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Water is the most important element in the world. Over two thirds of the earth surface is covered by water. Among of that, 97% of the water on earth is salt water and the other 3% is freshwater. Water sources can be divided into two that is surface water and groundwater. The surface water is water that we can see everyday such as rivers, lakes, streams, reservoirs, and even the oceans. Other source is groundwater where the water precipitation sinks into the ground and goes into watersheds, aquifers and springs. The groundwater flows through layers of sand, clay, rock, and gravel and this clean the water.

Water quality can be described as the condition of the water including its physical, chemical and biological characteristics. The quality of water can be affected by substances like pesticides or fertilizers that can negatively affect human and aquatic life. The quality of non-polluted water must be followed the standards of USEPA, which consists of four basic elements that are designated uses of the water body, water quality criteria, water policy and general policies addressing implementation issues. These standards have been implemented to help protect and maintain water quality necessary to meet and maintain designated or assigned uses, such as swimming, recreation, public water supply, and aquatic life.

Pollution of water, air and soil with heavy metals is a major environmental problem (Srivastava & Purnima, 1998). Generally the water pollution can be categorized as direct and indirect contamination sources. Direct sources include effluent outfalls from factories, refineries, sewage sludge disposal, agriculture waste, and metals from smelting and mining activities (Kang, 2007). An indirect source comes from contaminant that enter water supply from soils or groundwater