HEAVY METALS UPTAKE IN CLOSED-RECIRCULATING WATER SYSTEM FOR PHYTOREMEDIATION

(A STUDY TOWARDS AQUAPONIC AS BIOSENSOR TOOL FOR ENVIRONMENTAL BIOTECHNOLOGY)

EDWARD ENTALAI ANAK BESI

Report submitted in partial fulfillment of the requirements for the award of Bachelor of Applied Science (Honours) in Industrial Biotechnology

Faculty of Industrial Sciences & Technology

UNIVERSITI MALAYSIA PAHANG

2014

APPROVAL PAGE

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this project report and in my opinion this project is satisfactory in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Honours) in Industrial Biotechnology.

Signature :

Name of Supervisor : DR.RAMA YUSVANA

Position : SENIOR LECTURER

Date : 31st December 2014

STUDENT'S DECLARATION

I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :

Name : EDWARD ENTALAI ANAK BESI

ID Number : SB11037

Date : 31st December 2014

ACKNOWLEDGEMENT

First of all, praise to the Almighty Father, for His eternal blessing and guide until I able to accomplish this Final Year Projects as a partial fulfillment of the requirements for my undergraduate study. I would like to express my deep gratitude and appreciation to my supervisor Dr.Rama Yusvana, senior lecturer at Faculty of Industrial Science and Technology (FIST) of Universiti Malaysia Pahang (UMP), for all of his valuable supports and inspiring guidances.

I also want to thanks Mr. Fendi and Mr.Alim, who are Laboratory Assistants at FIST Laboratory, for all the helps they gave to me during my project's period. Moreover, I would like to give thanks to my friends and coursemates for sharing their knowledge and views on the number of issues related to my project. Last but not least, I want to thanks my family, especially my lovely mother, Madam Sawai, for all the love and care that they had gave to me.

CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

LIST OF SYMBOL

mg	Milligram
mg/L	Milligram per Litre
mm	Millimetre
cm	Centimetre
µg/mL	Microgram per Microlitre
mg/L	Milligram per Litre
g	Gram
nm	Nanometre
µL	Microlitre
M	Molar
ppm	Part per Million
rpm	Revolution per Minute
mM	Millimolar
oC	Degree Celcius
>	More Than
<	Less Than

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
HNO_3	Nitric Acid
HCL	Hydrochloric Acid
Cu	Copper
Cd	Cadmium
Ni	Nickel
Pb	Lead
Zn	Zinc
ANOVA	Analysis of Variance
H_0	Null Hypothesis
H_1	Testing Result
RGR	Relative Growth Rate
NAR	Net Assimilation Rate
LAR	Leaf Area Ratio
LWR	Leaf Weight Ratio
SLA	Specific Leaf Area
BCF	Bioaccumulation Coefficient Factor
TF	Translocation Factor
DO	Dissolved Oxygen
TDS	Total Dissolved Solid
Ca	Chlorophyll *a*
Cb	Chlorophyll *b*
C(x+c)	Carotenoid (Xanthophyll and Carotene)

ABSTRACT

Closed-artificial aquaponic system enables us to study the accumulation of heavy metals by plant in real simulation of the ecosystem, without endangering the environment. Heavy metals accumulation in the environment poses great risks to flora and fauna. Phytoremediation is applied to purify the heavy metals in the environment by using plant. In this study, the concentrations of heavy metals (Cu, Pb, Ni, Zn) accumulated by the wheatgrass *(Triticum aestivum)* are monitored by using Atomic absorption spectroscopy (AAS) and observing its effects to the plant. *Triticum aestivum* is suggested as a best candidate to be a accumulator plant for phytoremediation. Other than that, *Triticum aestivum* is also identified as a tolerant plant for phytostabilization. Plant can be used as the natural environmental biosensor by observing the changes in morphological characteristics of the *Triticum aestivum* after being exposed to the heavy metals. The percentage of phytotoxicity of *Triticum aestivum*, which depend on changes in length of shoots and roots of the *Triticum aestivum*, increases as the concentration of the heavy metals accumulated by the *Triticum aestivum* increases. The changes in anatomy of the plants due to the toxicity of the heavy metals are studied by using Scanning Electron Microscopy (SEM). Bioaccumulation Coefficient Factor (BCF) and Translocation Factor (TF) of heavy metals are studied to determine whether *Triticum aestivum* is a good candidate for accumulator plant and also tolerant plant in phytoremediation. Water quality measurement are monitored, and measured by using Portable Kit, to find the relationship between the conditions of the water and the level of metals toxicity and also the Bioaccumulation Factor (BCF) and Translocation Factor (TF) of the heavy metals. The effects of heavy metals to the biochemical properties of *Triticum aestivum*, such as content of chlorophyl (*a* and *b*), carotenoid, and flavonoid, are also being studied. Fishless cycling of aquaponic system is preferable to the study involving analysis of heavy metals, since the addition of heavy metals can lead to high mortality of the fish. Aquaponic is used as a biosensor tool to detect the presence of heavy metals in contaminated water for further research related to phytoremediation. Atomic absorption spectroscopy (AAS) methods are very suitable methods for monitoring the levels of heavy metals in natural water or plants. They provide accurate and rapid determinations. The data from the AAS analysis is analyzed based on graphs of concentrations against time. The energy source of an Atomic Absorption spectrometer will emits resonance line radiation. The AAS has more than one channel for simultaneous detection and determination of several elements. The absorbance of the heavy metals are directly proportional to the concentration of the heavy metals itself.

ABSTRAK

Sistem akuaponik tertutup membolehkan kita untuk menjalankan penyelidikan terhadap penyerapan logam berat oleh tumbuhan, di mana teknik ini tidak akan membahayakan alam sekitar. Penyerapan logam berat oleh alam sekitar boleh mengancam kehidupan flora dan fauna. Fitoremediasi digunakan untuk menapis logam berat yang terdapat dalam alam sekitar dengan menggunakan tumbuhan. Dalam penyelidikan ini, jumlah logam berat (Cu, Pb, Ni, Zn) yang diserap oleh rumput gandum *(Triticum aestivum)* akan ditentukan dengan menggunakan Atomic Absorption Spectroscopy (AAS) and kesan-kesan disebabkan penyerapan logam berat terhadap tumbuhan tersebut juga akan diperhatikan. *Triticum aestivum* diperkenalkan sebagai tumbuhan penyerap yang terbaik. Selain itu, *Triticum aestivum* juga diperkenalkan sebagai tumbuhan toleran untuk fitostabilasi. Tumbuhan digunakan sebagai biosensor alam sekitar yang semula jadi dengan menyelidiki perubahan yang berlaku terhadap ciri-ciri morfologi *Triticum aestivum* setelah menyerap logam berat. Peratusan fitotoksik *Triticum aestivum*, yang mana bergantung kepada perubahan yang berlaku kepada ukuran daun dan akar tumbuhan tersebut, akan meningkat sekiranya jumlah logam berat yang diserap oleh *Triticum aestivum* juga meningkat. Perubahan yang terjadi kepada anatomi tumbuhan tersebut diselidik menggunakan Scanning Electron Microscope (SEM). Faktor Bioakumulasi (BCF) dan Faktor Pemindahan (TF) logam berat oleh *Triticum aestivum* juga diselidiki, di mana objektifnya ialah untuk mengetahui sama ada *Triticum aestivum* sesuai menjadi tumbuhan penyerap dan tumbuhan toleran untuk fitoremediasi. Penyelidikan tentang kualiti air dijalankan dan disukat menggunakan alat penyukat mudah alih, untuk belajar tentang hubung kait diantara kondisi air dengan kadar toksik dan begitu juga dengan Faktor Bioakumulasi (BCF) dan Faktor Pemindahan (TF) logam berat. Perubahan yang terjadi kepada biokimia *Triticum aestivum*, seperti kandungan klorofil (*a* dan *b*), karotena, dan flavonoid, juga diselidiki. Kitaran akuaponik tanpa menggunakan ikan akan digunakan dalam penyelidikan yang melibatkan pengunaan logam berat, kerana kehadiran logam berat dalam air untuk sistem akuaponik boleh menyebabkan peningkatan kadar kematian ikan tersebut. Akuaponik digunakan sebagai peralatan biosensor menjalankan penyelidikan yang melibatkan penggunaan logam berat toksik, dan untuk mengesan kehadiran logam berat di dalam air yang tercemar untuk penyelidikan yang berkaitan dengan fitoremediasi. Atomic Absorption Spectroscopy (AAS) adalah salah satu teknik yang paling sesuai untuk menganalisis kehadiran logam berat dalam air semula jadi atau dalam tumbuhan. Teknik ini dapat memberikan keputusan analisis yang tepat. Data-data yang diperolehi daripada AAS ini akan dianalisis menggunakan graf kepekatan logam berat lawan masa. AAS merupakan teknologi yang sangat moden dimana ianya menggunakan lebih dari satu saluran yang membolehkan analisis melibatkan beberapa elemen. Kuantiti cahaya yang diserap oleh logam berat selari dengan jumlah logam berat tersebut.

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Aquaponic is defined from the integration of *"aquaculture"* and *"ponos"*. *Aquaculture* means growing fish in a re-circulating system, while *ponos* is the Greek word for growing plants with or without media. The system involves two methods which are re-circulating fish farming and hydroponic plant farming. In aquaponic system, fish are raised in the tank, which is positioned below the aquaculture plant. Water from the fish tank is pumped to the plants and running through the bio-filter where the bacteria are situated. The naturally occured bacteria root of the plants in aquaponic system is the nitrifying bacteria such as *Nitrosomonas sp* and *Nitrobacter sp*. The bacteria convert ammonia and nitrite to nitrate. This process is called as Nitrification. Plants will absorb the nitrate and the nutrient rich water.

Several criteria must be found in the plants are used in the aquaponic system such as easy to grow and able to grow faster. This is the reason why Wheatgrass *(Triticum aestivum)* is preferable for aquaponic because this species have those criteria. *Triticum aestivum* have a high nutritional value to human body. However, other plants species also can be used such as leguminous species (*Acacia confusa Merr*. and *Ormosia pinnata Dunn*.) could obviously absorb more heavy metals (Cu, Zn, Pb and Cd) from the landfill site leachate than non-leguminous species (Jiang et al. 2001), and vegetables such as cucumber, tomatoes, and cabbages (Li.et al, 1999). Wheatgrass juice contains more than 70% chlorophyll, which is an important blood builder. Besides that, chlorophyll of the *Triticum aestivum* is antibacterial and can be used inside and outside the body as a natural healer. The type of fish that going to be used in the system is strong and not easy to die due to sudden environmental changes such as Gourami and Tilapia fish. Filtered water is returned to the fish tanks and reused by the fish. Ammonia are produced from the fish waste. However, too much waste substance is toxic for the fish. Nitrites are degraded by the nitrifying bacteria (*Nitrosomonas sp and Nitrobacter sp.*). This process also known as bioremediation which is the process where microorganism (*Nitrosomonas sp and Nitrobacter sp.*) metabolizes harmful element to environmentally safe levels for consumption. The nitrates will be taken by the plants as nutrients. The nutrients are natural fertilizer which feeds the plant.

It takes around 14 days for the wheatgrass to fully grown. Then, water that being filtered through the growth medium in the grow beds will be very useful for the fish since the water contains nutrients. Oxygen enters the system due to the flushing of water from the upper part where the plants grow, then deliver to the fish tank. Siphon is used to drain the water into the fish tank. Once the water levels inside the grow bed reach the level limit, the siphon will automatically flushes the water inside the grow bed. Fishless cycling used in the treatment set of the aquaponic system because the analysis process involving addition of heavy metals. The Fishless cycling means that running the aquaponic system without adding the fish to avoid the death of the fish due to the heavy metals, and also simply means that instead of having fish excrete ammonia, the nitrifying bacteria will nitrify the nitrates from atmosphere via Nitrogen cycles. In 1885 Ulysse Gayon (1845-1929) and his assistants closed the cycle by identified the denitrifying bacteria that can reduce nitrates and, via Nitrogen oxide and Nitrous oxide, return Nitrogen to the atmosphere.

Plants can take up heavy metals by their roots, or even via their stems and leaves, and accumulate them in their organs. Plants take up elements selectively. This process is called as Phytoremediation. Most of the heavy metals are distributed in plants' root tissues which indicating that an strategy for metals tolerance exists in a plant. Phytoremediation uses only plants with great ability to concentrate elements from environment and remove it from contaminated sites (Gurbisu and Alkorta, 2003). These plants metabolize heavy metals in their tissues. One example of the plant is Wheatgrass (*Triticum aestivum L*). One reason for using plants for remediation concerns the relatively low cost and maintenance requirements (Cuningham and Berti 1993).

There are three types of group of phytoremediation which are phytoextraction, rhizofiltration, and phytoestabilization. Phytoextraction is the process where the plants are grew on contaminated sites and then harvested in order to remove the heavy metals from environment (Baker,1994; Salt,1995). Phytostabilization is where the metal tolerance plants are used to reduce the mobility of the metals and to avoid environmental degradation (Bradshaw,1980; Williamson,1982). Rhizofiltration is when the roots of the plants absorb heavy metals from polluted sites and later harvested to remove the metals (Miller, 1996; Lasat, 2000).

1.2 PROBLEM STATEMENT

Disposal of heavy metals to the environment affects the quality of the drinking water supply and the food sources for the other living organisms (Wang et al. 2001). There are many efforts that have been made by researchers throughout the world to reduce the heavy metal pollution, but none of the efforts successfully remediate heavy metals from environment with zero effects to the ecosystem. Nowadays, cleanup processes of heavy metal pollution are expensive and environmentally destructive (Nanda et al., 1995; Meagher, 2000). Most of the researchers, such as Gabriella Máthé-Gáspár and Attila Anton (2005), use the real natural ecosystem to study phytoremediation, which their site of field study is located in Gyöngyösoroszi, lower flooded area of Toka valley.

Despite that, a new technique is used, called aquaponic system, which are more environmental friendly and low cost. Aquaponic can be used as closed-artificial (man-made) ecosystems where all sorts of experiment can be carried out. The closed-artificial ecosystem is used to study the phytoremediation of heavy metals by using plant as natural

REFERENCES

Abdul Ghani, 2010, Toxic Effects of Heavy Metals on Plant Growth and Metal Accumulation in Maize (Zeamaysl), Iranian Journal of Toxicology, Volume 3, No.3, Autumn 2010, pp. 325-334.

Akio Enders, Johannes Lehmann, 2012, Comparison of Wet Digestion and Dry Ashing Methods for Total Elemental Analysis of Biochar, Communications in Soil Science and Plant Analysis, 43:1042–1052.

Alan R.Wellburn, 1994, The Spectral Determination of Chlorophyll a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution, J.Plant Physiol, Vol, 144, pp. 307-313.

Akpor O. B. and Muchie M., 2010, Bioremediation of polluted wastewater influent : Phosphorus and nitrogen removal, Scientific Research and Essays Vol. 5(21), pp. 3222-3230.

A. Mbiri, A. Onditi, N. Oyaro and E. Murago, 2011, Determination of Essential and Heavy Metals in Kenyan Honey by Atomic Absorption and Emission Spectroscopy, Jasgt Vol. 13(1) 2011, pp 107-115.

B. Buszewski, A. Jastrzębska, T. Kowalkowski, A. Górna-Binkul, 2000, Monitoring of Selected Heavy Metals Uptake by Plants and Soils in the Area of Toruń, Poland, Polish Journal of Environmental Studies Vol. 9, No. 6 (2000), 511-515.

Afshin Qishlaqi and Farid Moore, 2007, Statistical Analysis of Accumulation and Sources of Heavy Metals Occurrence in Agricultural Soils of Khoshk River Banks, Shiraz, Iran, American-Eurasian J. Agric. & Environ. Sci., 2 (5): 565-573, 2007, ISSN 1818-6769.

Biljana Bojovic, J.Stojanovic, 2005, Chlorophyll and Carotenoid Content in Wheat Cultivars As a Function of Mineral Nutrition, Arch. Biol. Sci., Belgrade, 57(4) : 283-290.

Dijana Grd, Jasminka Dobsa,Vesna Simunic-Meznaric and Teuta Tompic, 2012, Analysis of Heavy Metals Concentration in Wastewater along Highways in Croatia, Journal of Computing and Information Technology - CIT 20, 2012, 3, 209–215.

Fikriye Kirbag Zengin, Omer Munzuroglu, 2005, Effects of Some Heavy Metals on Content of Chlorophyll, Proline and Some Antioxidant Chemicals in Bean (Pharseolus vulgaris L.) Seedlings, Acta Biologica Cracoviensia Series Botanica, 47/12 : 157-164.

Hendrik Poorter, 1989, Plant Growth Analysis : Towards a Synthesis of the Classical and The Functional Approach Physiologia Plantarum, 75: 237-244.

Hendrik Poorter, Carlo Remkes, 1990, Leaf Area Ratio and Net Assimilation Rate of 24 Wild Species Differing in Relative Growth Rate, Oecologia (1990) 83 : 553-559.

Harmonization of Regulatory Oversight in Biotechnology, 1995, Consensus Document on the Biology of Triticum aestivum (Bread Wheat), OECD Environmental Health and Safety Publications, ENV/JM/MONO(99)8, pp48.

Joshua P. Adams, Ardeshir Adeli, Chuan-Yu Hsu, Richard L. Harkess, Grier P. Page, laude W. dePamphilis, Emily B. Schultz and Cetin Yuceer,2011, Plant Biotechnology Journal 2011 Society for Experimental Biology, Association of Applied Biologists and Blackwell Publishing Ltd, Plant Biotechnology Journal, 10, 207–216.

J.Nouri, N. Khorasani, B. Lorestani, M. Karami, A. H. Hassani, N. Yousefi, 2009, Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential, Environ Earth Sci (2009) 59:315–323.

Ladawan Rattanapolsan, Waranan Nakbanpotc, Piyaporn Saensouk, 2013, Metals Accumulation and Leaf Surface Anatomy of Murdannia spectabilis Growing in Zn/Cd Contaminated Soil, Environment Asia, 6(2) : 71-82.

Marisa Intawongse & John R. Dean, 2006, Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract, Food Additives & Contaminants, 23:1, 36-48.

M.O.Humpreys, 1984, Relationship Between Tolerance to Heavy Metals in Agrostis Capillars L. (A.Tenuis Sibth), New Phytol, 98 : 177-190.

Robert B.Lew, 1972, Atomic Absorption Analysis of Heavy Metals in Factory Water and Granulated Sugar, VOL. 17,To. 2, Oct. 1972, 144-153.

Y'kbal Kojuncu, Jozˇica Majda Bundalevska, U¨ mit Ay, Katarina C ˇ undeva, Trajcˇe Stafilov, and Go¨ksel Akcin, 2004, Atomic Absorption Spectrometry Determination of Cd, Cu, Fe, Ni, Pb, Zn, and Tl Traces in Seawater Following Flotation Separation, Separation Science and Technology, Vol. 39, No. 11, pp. 2751–2765.

Steve Diver, 2006, Aquaponics - Integration of Hydroponics with Aquaculture, ATTRA-National Sustainable Agriculture Information Service, pp 1-28.

Gabriella Máthé-Gáspár, Attila Anton, 2005, Phytoremediation study: Factors influencing heavy metal uptake of plants, Acta Biologica Szegediensis, Volume 49(1-2):69-70.

Isak Rajjak Shaikh , Parveen Rajjak Shaikh, Rafique Ahmed Shaikh and Alamgir Abdulla Shaikh, 2013, Phytotoxic effects of Heavy metals (Cr, Cd, Mn and Zn) on Wheat (Triticum *aestivum* L.) Seed Germination and Seedlings growth in Black Cotton Soil of Nanded, India, Vol. 3(6), 14-23.

Xuedong Yan, Fan Zhang, Chen Zeng, Man Zhang, Lochan Prasad Devkota and Tandong Yao, 2012, Relationship between Heavy Metal Concentrations in Soils and Grasses of Roadside Farmland in Nepal, Int. J. Environ. Res. Public Health 2012, *9*, 3209-3226.

Shuiping Cheng, Heavy Metals in Plants and Phytoremediation, 2003, ESPR - Environ Sci & Pollut Res 10 (5) 335 - 340 (2003).

Srivivasan Malar, Sahi Shivendra Vikram, Paula JC Kavas and Venkatachalam Perumal, 2014, Lead Heavy Metal Toxicity Induced Changes on Growth and Antioxidative Enzymes Level in Water Hyacinths [Eichhornia crassipes (Mart.)], Malar et al. Botanical Studies 2014, 55:54.

Mohammad Rezvani and Faezeh Zaefarian, 2011, Bioaccumulation and translocation factors of cadmium and lead in Aeluropus littoralis, Autralian Journal of Agricultural Engineering 2(4):114-119 (2011).

B. Lorestani, M. Cheraghi and N. Yousefi, 2011, Accumulation of Pb, Fe, Mn, Cu and Zn in Plants and Choice of Hyperaccumulator Plant in the Industrial Town of Vian, Iran, Arch. Biol. Sci., Belgrade, 63 (3), 739-745.

Surendra Vikram Ghavri, Kuldeep Bauddh, Sanjeev Kumar and Rana P. Singh, 2013, Bioaccumulation And Translocation Potential Of Na+ And K+ In Native Weeds Grown On Industrially Contaminated Soil, International Journal of ChemTech Research, Vol.5, No.4, pp1869 - 1875.

Kyle W. Tomlinson, Frank J. Sterck, Frans Bongers, Dulce A. da Silva, Eduardo R. M. Barbosa, David Ward, Freek T. Bakker, Martijn van Kaauwen, Herbert H. T. Prins, Steven de Bie and Frank van Langevelde, 2012, Biomass partitioning and root morphology of savanna trees across a water gradient, Journal of Ecology, 10.1111/j.1365-2745.2012.01975.x.

B. Shipley, 2006, Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis, Functional Ecology, 10.1111/j.1365-2435.2006.01135.x.

Allah Bakhsh Gulshan, Altaf Ahmad Dasti and Shazia Shakoor, 2012, Eco-Physiological Traits NAR and LAR Role in Determining The Relative Growth Rate in Competition, ARPN Journal of Agricultural and Biological Science, Vol.7 No. 10, ISSN 1990-6145.

Richard Taylor, EDd, and RDCS, 1990, Interpretation of the Correlation Coefficient: A Basic Review, Journal of Diagnostic Medical Sonography, 1: 35-39.

Afshin Qishlaqi and Farid Moore, 2007, Statistical Analysis of Accumulation and Sources of Heavy Metals Occurrence in Agricultural Soils of Khoshk River Banks, Shiraz, Iran, American-Eurasian J. Agric. & Environ. Sci., 2 (5): 565-573, ISSN 1818-6769.