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PRELIMINARY STUDY OF HEAVY METALS IN WATER LILY PLANTS AROUND KOTA SAMARAHAN AREA

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Preliminary Study of Heavy Metals in Water Lily Plants around Kota Samarahan Area

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DECLARATION

I hereby declare that no portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualifications of this or any university or institution of higher learning.

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List of Abbreviations

Pb	Lead
Hg	Mercury
Ni	Nickel
As	Arsenic
Zn	Zinc
Cu	Copper
BAF	Bioaccumulation Factor
AAS	Atomic Absorption Spectroscopy
FIMS	Flow Injection Mercury System
ANOVA	Analysis of Variance
HNO_3	Nitric Acid
HCl	Hydrochloric Acid
ICP-MS	Inductively Coupled Plasma-Mass Spectrophotometry
FIA	Flow Injection Analysis
BDL	Below Detection Limit

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Abstract

Water lily plants have many uses such as fragrance essence, cuisines, and also act as phytoremediation agent for heavy metals in aquatic ecosystems. The objectives of this study were to determine the concentrations of selected heavy metals; Lead (Pb), Mercury (Hg), Nickel (Ni), Arsenic (As), Zinc (Zn) and Copper (Cu), and to find out the differences of these metal Bioaccumulation Factor (BAF) in water lily plants. Three species of water lily were found in this study and were identified as *Nymphaea lotus*, *Nymphaea pubescens* and *Nymphaea capensis.* Three study locations were chosen in Kota Samarahan area. Three samples were taken which were water lily plant, sediment and water. Plant samples were separated according to their parts prior to analyse (*e.g.* flower, stem, root and leaves). Heavy metals content were analysed using Atomic Absorption Spectroscopy (AAS) and Hg was analysed using Flow Injection Mercury Systems (FIMS 400) analyser. All types of heavy metals were detected in plant samples except Pb that only detected in sediment samples. In general, highest concentration of heavy metals detected in root (Zn; 113.33 mg/kg; *N. capensis* samples; Lake B), stem (Zn; 86.11 mg/kg; *N. pubescens* samples; Ditch C) and leaves (Cu; 0.23 mg/kg; *N. lotus* samples; Lake B). Comparison of level of pollution between three study locations was done by comparing the heavy metals that content in the sediment and water samples. Metal concentrations between study locations followed the order of Ditch C > Lake B > Lake A. The concentration of heavy metals content in sediment and water samples of all study locations were below toxicity level. BAF value were different between the water lily plant part and species. BAF values were only obtained for Ni and Zn because the concentration for Pb, Hg, As and Cu were Below Detection Limit (BDL). The highest percentage of BAFs for Ni was 187.86% in root part of *N. capensis* while for BAFs of Zn, the highest percentage was 369.18% in leaves of *N. capensis.*

Keyword: Heavy Metals, Water Lily, AAS, FIMS 400 analyzer, Bioaccumulation Factor

Abstrak

Bunga teratai mempunyai banyak kegunaan seperti pati minyak wangi, makanan, dan juga agen phytoremediasi bagi logam berat di dalam ekosistem akuatik. Tujuan kajian ini adalah untuk mengenalpasti kepekatan logam berat yang dipilih iaitu Plumbum (Pb), Merkuri (Hg), Nikel (Ni), Arsenik (As), Zink (Zn), dan Kuprum (Cu), dan untuk mengenalpasti perbezaan Faktor Bioakumulasi logam di dalam pokok bunga teratai. Tiga spesies telah dijumpai dalam kajian ini dan telah dikenalpasti sebagai Nymphaea lotus, Nymphaea pubescens dan Nymphaea capensis. Tiga lokasi kajian telah dipilih terletak di kawasan Kota Samarahan. Tiga sampel telah diambil iaitu pokok bunga teratai, tanah dan air. Sampel pokok telah diasingkan mengikut bahagian sebelum dianalisis (bunga, batang, akar dan daun). Kandungan logam berat dianalisis menggunakan Spektroskopi Serapan Atom (AAS) dan Hg dianalisis menggunakan Sistem Suntikan Aliran Merkuri (FIMS 400). Semua jenis logam berat dikesan di dalam sampel tumbuhan kecuali Pb yang hanya dikesan di dalam sampel tanah. Secara general, kepekatan logam berat tertinggi dikesan di dalam sampel akar (Zn; 113.33 mg/kg; sampel N. capensis; Tasik B), batang (Zn; 86.11 mg/kg; sampel N. pubescens; Parit C) dan daun (Cu; 0.23 mg/kg; sampel N. lotus; Tasik B) . Perbezaan kadar pencemaran di antara lokasi kajian dijalankan dengan membuat perbezaan logam berat yang terkandung di dalam sampel tanah dan air. Turutan kepekatan logam berat di lokasi kajian adalah Parit C > Tasik B > Tasik A. Nilai BAF adalah berlainan mengikut bahagian tumbuhan dan spesis. Nilai BAF hanya diperolehi untuk Ni dan Zn kerana kepekatan untuk Pb, Hg, As dan Cu adalah di bawah limit pengesanan. Peratusan BAF tertinggi untuk Ni ialah 187.86% di bahagian akar bagi N. capensis manakala untuk BAF Zn, peratusan tertinggi adalah 369.18% di bahagian daun bagi N. capensis.

Kata Kunci: Logam Berat, Teratai, AAS, penganalis FIMS 400, Faktor Bioakumulasi

1.0 Introduction

Water lily is herbaceous plants that grow in stagnant, calm and enclosed waters. This plant can easily found in lakes, ponds and ditch. This hydrophyte plant is floating on water surface with its big leaves support the floatation mechanism (Fayed and Abdel-shafy, 1985; Fulekar, 2005). Water lily has many colours such as pink, white and purple complete with sepals, stamen, carpels and petals (Skinner, 2006). This plant is closely associated with Chinese and Indian community, especially in their cooking. For examples, the roots (potato-like tubers) are extensively used in both Chinese and Indian cuisines. The leaves are commonly used as food wrappers by some people. Apart from being the food wrappers, the leaves are also very important to provide a cool and shady habitat for fishes. The flowers are widely used as essence in fragrances.

However, the number of pollutants entering the aquatic environments especially the ponds, lakes, ditch and swamps have increased greatly in recent years due to increasing populations, industrialisation, agricultural practices and transportation activities. Heavy metals are one of the toxic pollutants that entering the aquatic environment. In addition, the aquatic plants, such as water lily is used as an agent for phytoremediation which can absorb harmful pollutants such as heavy metals into their biomass (Shuaibu and Nasiru, 2011).

Heavy metals have molecular mass >5.0 g cm^{-3} and can exist in variety of physical and chemical forms, mostly in particulate and dissolved forms. The dissolved metals are defined as the fractions of metals that pass through a 0.4 µm or 0.2µm pore sizes filters

(Bruland and Lohan, 2003). The concentrations of heavy metals in ponds or lake reflect the combined effects of weathering, floodplain, anthropogenic inputs, and water chemistry. Human activities especially which close to the water systems are the main heavy metals contributions in the ponds or lakes (Abdel-Baki *et al.,* 2011). Heavy metals have the great tendency to accumulate in various aquatic organisms including aquatic plants, which may enter into human body through consumption, thus can cause serious health problems. Heavy metals cannot be degraded (Linnik and Zabenko, 2000) but can change into the different redox forms. They can be accumulated in the food chain leading to chronic disease in humans. Cumulative effects of metals and chronic poisoning may occur as result of long-term exposure of low concentration metals (Mitra *et al.,* 2012). In general, accumulation depends on several factors such as metal concentrations, exposure time, metal uptake, environmental condition (*e.g.* temperature, pH, salinity) and intrinsic factor (Jesierska and Witeska, 2006).

Therefore, it is very important to monitor heavy metals concentrations in water lily plant, particularly around Samarahan area which is still scarcely investigated, by applying these two proposed objectives:

(1) to determine the concentrations of selected heavy metals (Pb, Hg, Ni, As, Zn, and Cu) in different parts of water lily plants (*i.e.* flowers, leaves, stems and roots (rhizome)).

(2) to study the bioaccumulation factor (BAF) of heavy metals in water lily plants.

2.0 Literature Review

2.1 Water lily plant

Water lily is an aquatic flowering plant that belongs to family Nymphaeaceae and the species that commonly found in tropical country is categorized under *Nymphaea* sp. (Slocum, 2005). Water lily has eight genera (*e.g.Nuphar*, *Nymphaea*, *Victoria*) and consist about 70 species. For example, for genus *Nymphaea* comprise of 35 species.

The morphology (Figure 1) of the root is restricted to rhizomotous form and the leaves also known as (lily pads) lay on the surface of the water. The leaves shapes are particularly round and the colour is from primary medium to deep olive green. The leaves are like another terrestrial plant leaves in which capturing sunlight for photosynthesis and gas exchange. Water lily's stems are projected into the sediment and connected with the root that important to transportation process of mineral such as nitrogen (Les *et al.,* 1999). When focusing to water lily plant, the most interesting part is the flower. For tropical species, the flowers usually bloom during the day (usually in the morning) and it may have as many as 24 petals for a single flower. The colour of the flowers are vary, such as pink, white, purple and others with some species have a good smell and even suitable for fragrance making (Mauseth, 2003).

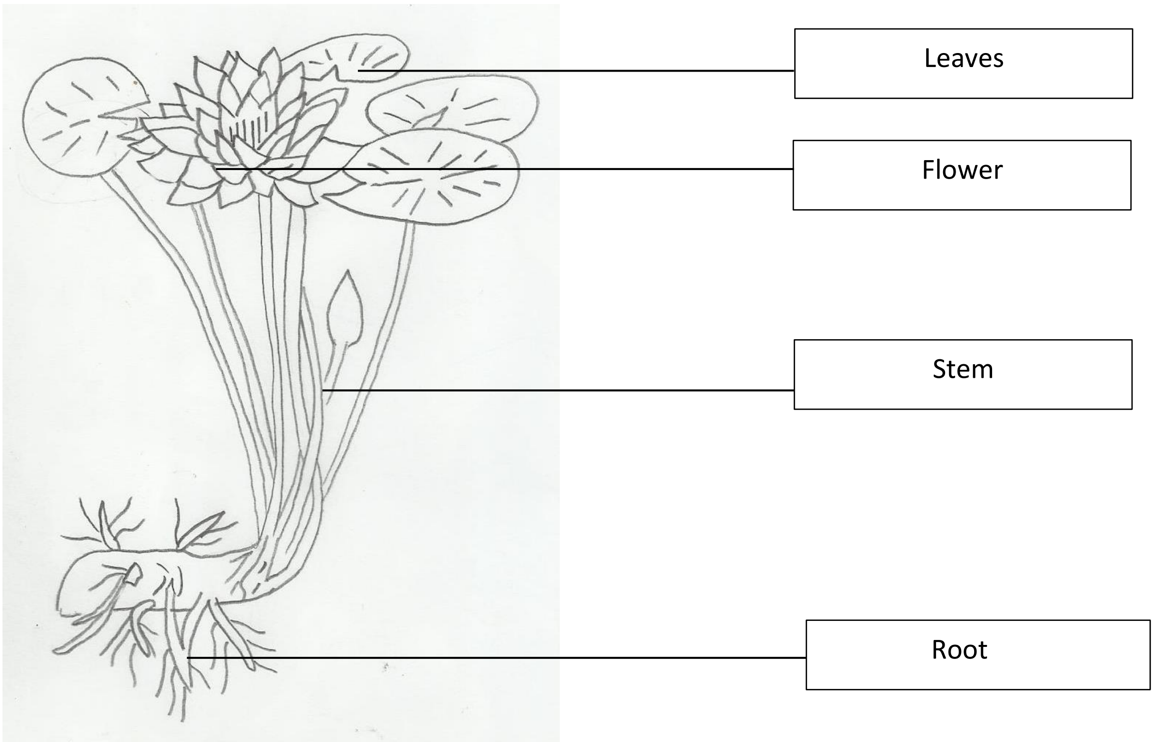

Figure 1 Morphology of water lily plant

2.2 Heavy metals effects to other living organism

Certain heavy metals, for examples Cu and Zn, are important for the plant growth in a very small amount. However, these essential metals can be harmful to the plant in the greater amount. Non-essentials metals, such as Pb, Hg, Ni, and As are toxic metals even in a small concentrations.

2.2.1 Lead (Pb)

Pb is well distributed in cells of organism. The toxicity properties of Pb is basically contributed by its ability to bind with biological molecules such as enzyme. Binding of Pb will interfere enzymes function and consequently resulting in malfunction and also adverse effect. Mechanism of Pb binding includes binding of the Pb at sulfhydryl and amide group then altering the enzyme configuration and prevent the enzyme to conduct its role in organisms' body system. Frequent problem that related with Pb toxicity is reduction in haemoglobin synthesis among children (Jose *et al.*, 2006).

2.2.2 Mercury (Hg)

Hg is one of the most toxic heavy metal that can accumulate in aquatic environment including organism, water and sediment. High concentration of Hg will significantly exist in high acidity surface water. Aquatic organism will convert Hg content from its surrounding into methyl-mercury and the absorption rate is very high. This heavy metal can lead to many bad effects and one of it is nerve damage (Singh, 2005). The biggest history of Hg poisoning is Minamata disease that occurs at Minamata Bay, Japan (Kurland *et al.*, 1960). It could also cause damages of animal's kidney, stomach and intestine and reproduction failure. In plant, Hg caused DNA alteration (Frausto and Williams, 1991).

2.2.3 Nickel (Ni)

Ni can easily exposed to living organisms. The sources can come from smoke including tobacco, exhaust, combustion of fossil fuels and many more. Adverse effect that commonly occurs in human was due to Ni is allergic reaction. This allergic reaction can cause skin rash and sometimes it cause asthma to more sensitive person. Furthermore, Ni also can cause lung problems such as bronchitis and prevention of lung from functioning. Long exposure to Ni can cause lung cancer (Singh, 2005).

2.2.4 Arsenic (As)

As exists in natural environment in very low levels. There are two forms of As that differentiates by using its binding characteristics which are inorganic As compound and organic As compounds. Inorganic As compounds mostly present in compounds that has oxygen, chlorine and sulphur molecules. In animals and plants, inorganic As combine with the hydrogen and carbon molecules. As is labelled as carcinogenic heavy metal that can contribute to cancer such as lung and respiratory tract cancer. Instead of that, intake of food that contaminated with As can increase probability of skin cancer, and tumour occurrence at certain organ for examples bladder, kidney and liver (Singh, 2005).

2.2.5 Zinc (Zn)

Zn is characterised with bluish-white shiny metal that widely used in many industries like paint making, ointments sector and also coating purpose. Its toxicity can be divided into two groups which are acute toxicity and chronic toxicity. Acute toxicity includes dryness of respiration systems, energy depletion, fever, stomach pain and also vomiting while chronic toxicity lead to pancreas problems, anaemia and also lowering the level of high-density lipoprotein cholesterol (HDL-the good form of cholesterol) living organism body (Singh, 2005).

2.2.6 Copper (Cu)

Cu is an essential trace nutrient that need by more than 30 enzymes to function. Although it has nutritional value, its labels as heavy metal when the level exceed permissible amount that suitable for living organism body needed (David *et al.*, 2002). Toxicity of Cu in aquatic environment comes to great concern because aquatic organism such as fish and crustaceans are very sensitive to Cu than mammals. Instead of that, algae and aquatic plant also very sensitive to Cu and it sensitivity may reach 1000 times more sensitive than mammal (Forstner and Witmann, 1979). Existence of Cu will cause mortality to all of the sensitive organisms.

2.3 Heavy metals in water lily plants

According to the study on heavy metals contents in water lily plants that had been conducted by Shuaibu and Nasiru (2011), water lily plants have higher ability for heavy metals uptake. The plants have high tendency to selectively bio accumulate Zn and Pb faster than Cd and Fe. In other study, Azizah (2010) found that the highest heavy metals contents were observed at the root, followed by the stems and the leaves of in water lily plant. The concentration of Zn and Pb were the highest followed by As, Cu, Ni and Hg.

2.4 Heavy metals in pond, lake and ditch water

Heavy metals are essential for the biological system in small quantities but to some extent they are very toxic that consequently will cause serious health problem and even fatality when the concentration exceeded of the certain limit (Al-Weher, 2008; Irwandi and Farida, 2009; Nanda and Abraham, 2013; West and Nurnberg, 1988).

According to Adriano (2011), there were many processes that contributed to heavy metals abundances in aquatic ecosystems such as deposition of acid and neutral compounds, anthropogenic activities, run-off and evaporation, buffering system including geochemical reaction and others. Heavy metals in water, aquatic plant and sediment in ponds, lakes and ditch have a complicated behaviour than in ocean because they are directly influenced by many input sources such as riverine systems, atmospheres, mixing processes and also suspension of sediment (Santschi, 1988).

2.5 Heavy metals in sediment

Sediment are the loose sand, clay, silt or even other soil particles that present at the bottom of the water body (Davies and Abowei, 2009). Accumulation of the sediment at the bottom of the water body is due to the erosion of the bedrock and soils that is past or continuous processes. Heavy metals in sediments may come from variety of activities which may include natural activities of geological phenomena such as formation of ore, weathering of rocks and leaching of the rocks weathering particle into the water bodies. Anthropogenic activities also contributed to a large portion of the total amount of heavy metals that exist in sediments. Some examples of anthropogenic activities are growing of human population, industrial sectors, agriculture based practices and exploration of natural resources that lead to exploitation of it (Ajayi and Osibanjo, 1981).

According to Praveena *et al*. (2007), existence of heavy metals in sediment of lake sources from biological and geochemical mechanisms will have very significant toxic effects especially to the sediment-dwelling organisms and also fish. Uptake of the heavy metals will caused many effects like reduced growth, impaired reproduction, low species diversity and even death to the aquatic organisms. So, monitoring the contamination in soil or sediment was very importance since the contaminant that present will affect the whole aquatic ecosystems including water resources such as the groundwater and surface water, aquatic plants, aquatic animals and also human population that have close relationship with the affected aquatic environment (Suciu *et al*., 2008).

3.0 Materials and Methods

3.1 Study location

The sampling was conducted at three different locations which were two lakes in UNIMAS old campus and one ditch at Kampung Rembus that located along Jalan Dato' Mohd Musa in Kota Samarahan between November 2013 and December 2013. The A and B represent the sampling location in UNIMAS old kampus while C represents the sampling location at Kampung Rembus (Figure 2).

Figure 2 Map of the study locations

3.2. Pre-sampling treatment

Double resealable zipper plastic bags were used for storage of the water lily plant and sediments, while 500 ml water samples bottles were used to store water samples for the heavy metals analysis. 500 ml water samples bottles, and petri dishes were thoroughly soaked into the detergent for one day and then transferred into the 10% acid wash solution for another one day. Then, the apparatus were rinsed using distilled water and transferred into laminar flow bench to dry it. After the drying process, the petri dishes and 500 ml water sample bottles stored in cleaned resealable zipper plastic bags to reduce the potential of contamination.

3.3 Sampling and sample storage

Water lily plant samples were collected and kept in polyethylene resealable zipper plastic bags and labelled. Sediment samples were collected by using plastic corer and then stored in labelled resealable zipper plastic bags (Govindasamy *et al.*, 2011). Water lily plant and sediment sample were kept in the ice box before stored into the freezer at temperature -20^{O}C in the laboratory. Water samples were collected in 500 ml acid-washed bottles with labels then were kept in the ice box before transferred to the laboratory. At Lake A, samples were taken from three different sites, while at Lake B samples were taken from two different sites and only one site at Ditch C. The numbers of sites were depended to the size of the lake or ditch and also the availability of samples. For each site, each type of samples was taken in triplicates.

3.4 Identification of species

Samples identification was conducted by taking several measurements of every parts of the plant (*e.g.* size of leaves, number of flower sepal and number of flower petals) and species identifications were conducted followed identification key prepared by Slocum (2005).

3.5 Sample preparation for heavy metals analysis

3.5.1 Water lily and sediment

Water-lily plant samples were defrosted and rinsed three times with distilled water. Then, the samples were cut by using plastic-ceramic knife based on interest parts which were flower, stem, root and leaves and placed in different petri dishes before dried in the ESCO ISOTHERM oven model OFA-32-S at 60℃ until reach constant weights. Each samples were prepared in triplicates. The dried samples then grinded by using non-metal pestle and mortar.

3.5.2 Sediment samples

Sediment samples were thawed and dried in ESCO ISOTHERM oven model OFA-32-S at 60℃ before grinded into powder form by using non-metal pestle and mortar. Each samples were prepared in triplicates.

3.5.3 Water samples

The water samples were acidified within 24 hours from sampling time by using 0.5ml 2M HCl for 500ml water samples (APHA, 1998). The samples were stored in clean container prior to analysis.

3.6 Acid digestion procedure of water lily plants and sediments samples

Water lily plant and sediment samples were digested using hot plate digestion method. For each samples, 0.5g dry weight of samples were weighed using AND analytical balanced model GH-252 in a weighing board. The samples then transferred into the conical flask followed by addition of 37% hydrochloric acid (HCl) and 65% of nitric acid (HNO_3) with ratio 3:1 (v/v) respectively. The conical flasks were placed on the hot place and heated at 200ºC about three hours until all the sample completely digested. The digestion process was done inside the fume chamber to avoid inhaling of the vapour released.

Then, each samples were filtered using Whatman filter paper 47µm and diluted until 50 ml using 0.1% HNO_3 and placed inside 50ml vials prior to heavy metals analysis.

3.7 Heavy metals analysis

Total heavy metals of Pb, Ni, As, Zn, and Cu that contain in the samples were analyzed by using Atomic Absorption Spectroscopy (AAS) Thermo Scientific, AAA iCE3500 in term of dry weight (Otchere, 2003). Standard solution of every metal element was prepared and analyzed prior to sample analysis. The analysis of samples were repeated in triplicate and the result were expressed on dry weight basis (mg/L). Detection Limit of AAS for heavy metals were showed in Table 1.

Hg concentration in samples were analyzed by using Flow Injection Mercury System (FIMS 400). The samples were analyzed in triplicate and the results were expressed as µg/L

Table 1 AAS detection limit for heavy metal analysis

Metals	Wavelength (nm)	Detection Limit (mg/L)	Calibration solution (mg/L)
Pb	217.0	0.013	1, 3, 5
Ni	232.02	0.008	1, 3, 5
As	193.7	0.12	3, 6, 10
Zn	213.9	0.0033	0.2,0.5,1.0
Cu	324.8	0.0045	2, 4, 6

3.8 Heavy metals concentration

The metal concentration calculated using equation 1:

Concentration (mg/kg) = (A x B)/C...................................(Equation 1)

Where,

A: Concentration of AAS/FIMS analysis (mg/L)

B: Volume of sample (L)

C: Sample weight (kg)