UPTAKE OF NUTRIENTS FROM HOUSEHOLD WASTEWATER BY AQUATIC MACROPHYTES (*Eichhornia crassipes* and *Salvinia molesta*)

Nur Ilyana bt Narawi

A dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Science (Hons.)

Resource Chemistry Programme
Faculty of Resource Science and Technology
UNIVERSITY MALAYSIA SARAWAK
2008

DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application of another degree of qualification of this or any other university or institution of higher learning

____

(Debbie Deborah Paka)
Matric No: 13947
Date: 28 May 2008

Acknowledgment

I would like to take this opportunity to express my deepest appreciation and sincere thanks to my supervisor for listening to my problems in carrying out this project and coach me into the right direction. It has been a privilege to be under Assoc. Prof. Dr. Ling Teck Yee. Besides, I would also thank my most precious friends who constantly reminded me not to give up and were willing to share their knowledge and ideas with me. Also, special thanks to my dear parents for their continues support and concern on my final year project. Last but not least, I would like to thank those who have inspired me to take this task as a learning experience from which I have gained so much.

Table of Content

Contents		Page Number

List of Tables

List of Figures

EFFECT OF CADMIUM ON THE UPTAKE OF NITROGEN AND PHOSPHORUS BY WATER LETTUCE (*PISTIA STRATIOTES*)

Debbie Deborah Paka

Resource Chemistry Program
Faculty of Resource Science and Technology
University Malaysia Sarawak

Abstract

The aim of this research was to determine the effect of cadmium at different concentrations on the reduction of nutrients in wastewater by water lettuce and the uptake of nutrients. Water lettuce were cultured in wastewater containing various concentrations of cadmium (0, 3 and 6 mg/L) with each treatment having 3 replicates. Analysis on cadmium and nutrient content in the wastewater (ammonia-N, nitrate-N and orthophosphate) as well as the plant tissue (Total Kjeldahl Nitrogen and Total Phosphorus) were done. During the 15 days of batch culture, percentage of nutrients reduction in wastewater treated with water lettuce occurred in control, 3 mg/L Cd and 6 mg/L tank were 92.7%, 92.4% and 40.8% (ammonia-N); 89.8%, 82.7% and 45.2% (nitrate-N) and 83.9%, 55% and 36.9% (orthophosphate) respectively. Percentage of nutrients increase in water lettuce plant for control, 3mg/L Cd and 6 mg/L Cd tank were 428.9%, 223.3% and 79.9% (TKN) and 61.8%, 29.9% and 20.1% (TP) accordingly. The study shows that the presence of cadmium had interfered with the nitrogen and phosphorus uptake of the aquatic plant. There were also some toxicity symptoms observed on the water lettuce in the presence of cadmium such as inhibition of growth and root development of the water lettuce.

Keywords: Water Lettuce; Cadmium; Nitrogen; Phosphorus

Abstrak

Kajian dilakukan bagi tujuan untuk melihat kesan kepekatan cadmium yang berbeza pada pengurangan nutrisi di dalam air kumbahan/buangan dan pengambilan nutrisi oleh kiambang. Kiambang dikulturkan di dalam air kumbahan/buangan yang mengandungi kepekatan cadmium yang berbeza (0, 3 dan 6 mg/L) di mana setiap kepekatan mempunyai 3 replikasi. Analisis untuk kandungan cadmium dan nutrisi dalam air kumbahan/buangan (*ammonia-N, nitrate-N* dan *orthophosphate*) serta tisu tumbuhan (*Total Kjeldahl Nitrogen* dan *Total Phosphorus*) dilakukan. Semasa 15 hari pengkulturan, peratus pengurangan nutrisi di dalam besen air kumbahan/buangan *control*, 3 mg/L Cd dan 6 mg/L Cd yang dirawat menggunakan kiambang mengikut urutan adalah 93.4%, 92.8% dan 39.23% (*ammonia-N*); 90%, 85.7% dan 50% (*nitrate-N*) dan 76.9%, 40.2% dan 36.4% (*orthophosphate*). Peratus meningkatan nutrisi di dalam kiambang bagi *control*, 3 mg/L Cd dan 6 mg/L Cd mengikut urutan adalah 428.9%, 223.3% dan 79.9% (TKN) manakala 61.8%, 29.9% dan 20.1% (TP). Kajian juga telah menjumpai bahawa kehadiran cadmium telah mengganggu pengambilan nutrisi tumbuhan akuatik. Terdapat juga beberapa kesan toksik yang dilihat jika terdapat kehadiran cadmium seperti pertumbuhan dan pemanjangan akar tumbuhan akuatik yang terbantut.

Kata Kunci: Kiambang; Cadmium; Nitrogen; Fosforus

1. Introduction

Aquatic plants have been identified as a potential group for accumulating and bioconcentrating heavy metals because it can readily absorbed it. Realizing of this phenomenon, scientist came out with a new cleanup technology termed phytoremediation where selected aquatic plants that can accumulate metals are used for environmental remediation (Lasat, 2002). The effectiveness of this technique depends on the capability of the selected plants to grow and accumulate metals under the specific conditions of the site being remediated (Kulli *et al*., 1999).

Some aquatic plants which have been studied recently of their potential for phytoremediation are *Eichornia crassipes*, *Lemna minor* and *Pistia stratiotes* (Satyakala and Jamil, 1992). *Pistia stratiotes* were selected in view of their high growth rates. These plants grow rapidly under favorable conditions in a nutrient-rich environment such as domestic sewage (Zimmels, 2006). Heavy metals have become entrenched in the literature of environment pollution (Nieboer and Richardson, 1980). For example, cadmium is one of major concern that can cause pollution in aquatic system. It is highly toxic to animals and plants. High occurrence of cadmium is often link with human – derived contamination such as mining and smelting and also agricultural activities**.** The used of household liquids such as detergent also contributes to the source of heavy metals in household wastewater. In *Populus canescens*, exposure to cadmium causes reductions in photosynthesis, water and nutrient uptake (Schutzendubel *et al*., 2002). Hence, presence of high level of cadmium concentrations will interfere with the nutrient uptake of the aquatic plants, namely the uptake of nitrogen and phosphorus. Lack of nitrogen therefore always slows down the synthesis of protein and thus generally inhibits plant growth (Bergmann, 1992) while phosphorus deficiency leads to problems with maintenance of the cell structure in the plants (Bergmann, 1992).

Eventhough it is a success strategy of using the aquatic macrophytes in phytoremediation, scientist found out that high concentrations of some trace metals are known to cause deleterious effects in aquatic plants (Mertz, 1969). Various researches had been conducted concerning the effect of different kinds of heavy metals towards the aquatic plants like *Eichornia crassipes*, *Lemna minor* and *Pistia stratiotes* (Satyakala and Jamil, 1992) regardless of its biochemical or physiological changes. Most of the studies done are to investigate changes in the plant growth, contents of soluble protein and photosynthetic pigments as well as the activity of antioxidant enzymes caused by different types of heavy metals such as zinc, copper, nickel, cadmium (Hou *et al*., 2007). Pollution occurs more widely nowadays due to increase of human activities which contributes most to it. Due to the potential role of *Pistia stratiotes* in phytoremediation of contaminated aquatic environments, it is critical to monitor and understand the potential detoxification for this species when plants are exposed to a range of cadmium concentrations (Sivaci, 2007). Therefore, it useful to study the effects caused by heavy metals which pollute the household wastewater to the aquatic plants such as *Pistia stratiotes*, *Lemna minor* and *Eichornia crassipes* which can be used for phytoremediation purposes and those which could use household wastewater as their habitats.

Knowledge such as the amount of nitrogen and phosphorus uptake by the plants with and without presence of cadmium can be obtained from this research can be added as one of the biological changes in plants resulting from exposure of plants to high concentrations of cadmium (heavy metals). Furthermore, the study can give information on whether cadmium might interfere with the nutrients uptake by the aquatic plants. It is essential to study the range of cadmium concentration which is toxic to the aquatic plants namely water lettuce when these plants are used as the candidate for the phytoremediation of cadmium contaminated waterbody. The knowledge obtained will help to identify the suitable level of cadmium in waste water where water lettuce will be use to treat it.

The objectives of this research are:

i. To determine the effect of cadmium at different concentrations on the reduction of nutrients in wastewater treated with water lettuce.
ii. To determine the uptake of nutrients by water lettuce under different concentrations of cadmium.

2. Literature Review

2.1 Heavy Metals

According to the definition given in the dictionaries and encyclopedia of the scientific term, heavy metals refers to all metals having a specific gravity greater than 4, or 5 or greater than 5 (Nieboer and Richardson, 1980). Some heavy metals which are required by living organisms in a trace amount includes cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), strontium (Sr), vanadium (V) and Zinc (Zn), and they are referred to as essential heavy metals whereas non-essential heavy metals of particular concern in the environment are cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), and silver (Ag), (Kennish, 1992)

2.2 Sources of Heavy Metals in Waste

High concentrations of this metal occur in wastes from dyeing, tanning, explosives, ceramics, textiles, paints and paper industries (Satyakala and Jamil, 1992). Domestic wastewater effluents contain large amounts of trace metals such as copper (Cu), lead (Pb), zinc (Zn) and cadmium (Cd) from metabolic waste products, corrosion of water pipes and heavy metals from household products, such as detergent which contain iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), cobalt (Co), zinc (Zn), boron (B) and arsenic (Ar) (Csuros and Csuros, 2002). Wastewater treatment usually removes less than 50 percent of the metal content of the influent, leaving the effluent with significant metal loading (Jenkins, 1981). The sludge resulting from wastewater treatment is also rich in metals. Domestic wastewater and the dumping of domestic and industrial sludge are the major artificial sources of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb) mercury (Hg) pollution (Csuros and Csuros, 2002).

2.3 Cadmium

Heavy metals can be distinguished as essential and non-essential to plants as seen from the general plant biological and physiological point of view. Cadmium is universally acknowledged to be a harmful and dangerous heavy metal and is toxic for human and plants even at a low concentrations. Increasing amounts of cadmium are entering the biosphere from variety of sources, including the factories that use it, zinc smelting plants, diesel engines, particles from tyres, oil and coal-fired furnaces and sewage sludge and composted waste used in agriculture and market gardening (Bergmann, 1992). According to Bergmann (1992), fertilizers in Australian superphosphate had a mean cadmium content of 40 ppm which contributes to heavy metals contamination from agriculture sector.

2.4 Toxicity of Cadmium in aquatic plants

In aquatic systems, cadmium is most readily absorbed by organisms directly from the water in its free ionic form cadmium (II) (AMAP, 1998). The acute toxicity of cadmium to aquatic organisms is variable, even between closely related species, and is related to the free ionic concentration of the metal (Goyer, 1986). Aquatic plants have some kind of barrier to avoid uptake of heavy metals, but when metal concentrations become higher, this barrier loses its function, probably due to toxic action by the metals and the uptake massively increases (Baker, 1981). The known toxicity of cadmium is it antagonizes the uptake of essential elements such as copper and zinc, and irreversibly replaces them in enzymes reactions needed in RNA, DNA and protein metabolism. Other symptoms of cadmium toxicity in plants include inhibited growth, and interfere with the chlorophyll synthesis resulting in chlorotic blotches or discolouration of the leaves (Bergmann, 1992). The visible symptoms of injuries in plants for some heavy metals are shown in appendix 1.

2.5 Metal uptake and removal by plants in terrestrial and aquatic environment

Plants are able to take up metals in air and water as well as in soil and sediment. Higher plants take metals from water or air by the shoots, and their roots take up metals from soil or sediment, as well as from solution when they are cultured in it. In addition to the uptake, there is also a release of metals back into the water and soil from plant or to the air in gaseous form from the leaves. Therefore, metals accumulation depends on both uptake into the plant tissue and leakage into the surrounding medium. In some plants, heavy metals are being taken through its root but the exact locality of the metal uptake by roots varies depending on the element under consideration. Some metals in some plants are taken up primarily at the apical region while others may be taken up over the entire root surface. Leaves can also take up both essential and non-essential heavy metals. The absorption of heavy metals by the leaves depends on the metals species involved. For example, cadmium, zinc and copper show greater leaves penetration than lead which is mostly absorbed to the epicuticular lipids at the surface. Besides that, the uptake of heavy metals by leaves also influence by the plant species. Different species have different cuticles with different compositions of epicuticular and intracuticular lipids, and thus different permeability. In addition, metal uptake and its toxicity effect on plants also influence metal uptake by leaves. Cadmium uptake by the roots affects the formation of cuticle by forming epicuticular lipids which are shorter with higher polarity, hence resulting in higher permeability and increased in cadmium uptake by leaves (Orcutt and Nilsen, 1998).

During the past decade there has been increasing interest in the use of aquatic vascular plants for the removal of pollutants from domestic and sewage effluents as many research were conducted to determine the effectiveness of aquatic macrophytes on the removal of metals. This involves the use of plants to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes (Kulli *et al*., 1999). Surface water weeds like water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) have been shown to have great potential as biological filters for absorbing pollutants, including heavy metals, from wastewaters (Jamil *et al*. 1987)

Aquatic plants, such as water hyacinth (*Eichhornia crassipes*), pennywort (*Hydrocotyle umbellate*), and duckweeds (*Lemna minor*) (US EPA, 1988) are known to be effective in single pond wastewater treatment. Wastewater treatment systems using water hyacinth have been in practice in the states of California, Florida, Massachusetts and Texas (US EPA, 1988).

2.6 Mechanism of heavy metals uptake by aquatic plants

In a study conducted by Samardakiewicz and Wozny (2000), the first stage of the uptake when *Lemna minor* (duckweed) are exposed to heavy metals was detected exclusively in the outer layers of the root then later detected at the centre of the root (Wierzbicka, 1995). The uptakes of the heavy metals are initiated most often by a phase of rapid and passive adsorption to the cell wall, particularly in the walls of cells neighboring with intercellular spaces (Samardakiewicz and Wozny, 2000; Hunding and Lange, 1978). According to the study done by Samardakiewicz and Wozny (2000), lead which is used as the source of the heavy metals was found in the meristematic zone which are the protoderm, periblem and plerome of an aquatic plant (*Lemna minor*) although more lead accumulated in periblem cells than in the protoderm. This is because to in this region there are no mechanical barriers restricting apoplastic transport but this phenomenon still requires further investigation for further explanations. In other study conducted by Davies (1973), the uptake of zinc in the diatom *Phaeodactyium tricornutum* starts as a rapid adsorption onto the cell membrane followed by diffusion-controlled uptake rate and then by binding to proteins within the cell. The binding of zinc to protein may control the concentration in the cell. It is suggested that during the growth cycle the concentration of zinc reached a maximum and then decreased as the amount of protein in the cell declined. In addition, similar uptake patterns have been described for other metals such as nickel uptake in the same diatom species.

2.7 Importance of mineral nutrition for plant growth

2.7.1 Nitrogen

Nitrogen is one of the macronutrient in plants. It usually accounts for 1-5% of the organic substance in terms of dry weight (Frink *et al*., 1999). Nitrogen is absorbed by plants in ion form (NH_4^+ and NO_3^-) through plants roots and leaves (Nelson *et al*., 1980). A study conducted by Roger et al. (1977) states that nitrogen nutrients are absorbed by roots of aquatic plants and translocated to stems and leaves. They also noted a possible loss of N due to excretion from the stems or leaves. Problem occurs when the plants are having nitrogen deficiency where it inhibits protein synthesis and plants unable to remobilized the nitrogen in the nitrogenous cell constituents of older, growth would cease completely (Bergmann, 1992). Complete interruption of chlorophyll synthesis also regarded as the cause for the appearance of typical nitrogen deficiency symptoms. The inhibition of chloroplast and chlorophyll synthesis leads to well known pale to yellowish green appearance which gradually turns yellow when the deficiency becomes more severe. If a nitrogen deficiency materializes at a late stage of plant growth, the plant is able to sustain growth for some time by braking down the proteins in the plasma of the older leaves and translocating the amino acids to the growth centre in the meristemic tissue of the shoot (Maynard, 1979).

2.7.2 Phosphorus

Phosphorus generally accounts for some 0.1 – 0.5 % of the dry weight of plants, in which it is always present in its highest oxidation form. In these form, it is a constituent of essential cell components such as phosphoproteids and pholipoids. The cell components are important for the maintenance of cell structures. Phosphorus deficiency inhibits starch and cellulose synthesis and leads to abnormally high sugar levels. Lack of phosphorus merely inhibits or prevents growth and resembles small, tender, stunted shade plants, but have stronger stems (Bergman, 1992).

2.8 Water Lettuce *(Pistia stratiotes)*

Water lettuce is an aquatic plant belongs to the family Araceae. It is a monocotyledonous, free-floating aquatic plant. The leaves formed in rosettes are sessile, light dull green in colour, hairy and thick. The adventitious roots, which arise at the base in cluster, are light coloured and feathery. The stem is stoloniferous with monopodial branching. Stolon grows out from the leaf axils to give rise vegetatively to new plants. The flowers are inconspicuous and monoecious. The plant reproduces vegetatively and sexually. It is widely distributed in tropical and subtropical countries in lakes, rivers, ponds and ditches. (Schmitz *et al*., 1993).

2.9 Studies Related to Effect of Heavy Metals on *Pistia Stratiotes*

Previous study on chromium – induced biochemical changes in *Eichhornia crassipes* (Mart) Solms and *Pistia stratiotes* suggested that due to excess accumulation of trace metals in plant tissues, several biochemical processes, such as carbohydrate and nitrogen metabolism and the activity of certain metalloenzymes of the cell are affected (Satyakala and Jamil, 1992). Heavy metal accumulation in vascular plants is known to produce significant physiological and biochemical responses (Pahlsson, 1989). According to study conducted by Satyakala and Jamil (1992), when the concentration of chromium is as low as 10 ppm, there is no morphological changes shown by the plants but high concentrations of chromium bioaccumulation in plant tissues induced significant biochemical changes which were responsible for the inhibition of chlorophyll synthesis resulting in the loss of photosynthetic activity.

Another study done in India on toxicity effect of Hg(II) on changes in some biochemical parameter of *Pistia stratiotes* suggested that there are some changes in biochemical parameters of *Pistia stratiotes* at the exposure of 20 ppm of Hg(II) where at 20 ppm, Hg(II) lowered the chlorophyll by decreasing the synthesis of chlorophyll as well as possibly by increasing chlorophyllase activity besides decreasing the protein content in the shoot system while in root system the value remained more or less same as the control. It was also found out that in all treatment, free amino acid decreased in roots with increasing concentration of Hg(II) while the dry weight decreased in both roots and shoots which may be due to increased tissue permeability and loss of membrane integrity of plant tissue (Anil *et al.*, 1983). There are no significant changes in the parameters over control with time where the values were more or less as that in control. It was reported at 20 ppm concentration of Hg (II) promotes senescence of *Pistia* plants by general inhibition of the biosynthesis of cellular metabolites, impairing the degradation of the biochemical constituents and also interfering with the nucleic acid biosynthesis and protein synthesizing machinery in the plant, whereas in Hg (II) concentration below 20 ppm, the metabolic activities of the plant are least affected (Anil *et al.*,1983).

Water lettuce was also used in research of implication of heavy metals for phytoremediation. In the research conducted by Odjegba and Fasidi (2004), the toxicity of eight potentially toxic trace elements (Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn) to *Pistia stratiotes* was examined to determine if this plant showed sufficient tolerance and metals accumulation to be used to phytoremediate wastewater and/or natural water bodies polluted with these heavy metals. The study was done by spiking all the metals involved into the test media where the concentration of metals under studied are 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0 mM each for $AgNO_3$ (Ag), $Cd(NO_3)_2$ (Cd), K_2CrO_4 (Cr), $CuSO_4$ (Cu), $HgCl_2$ (Hg), $NiSO_4$ (Ni), $Pb(NO_3)_2$ (Pb) and $ZnSO_4$ (Zn) and the effect of heavy metals observed were measured according to their physiological changes. Through the study, they found out that root elongation as well as emergence of new roots decreased significantly with increase in metal concentrations besides a reduction in the rate of leaf expansion relative to metal type, their concentrations and the duration of exposure. The plant had the lowest and the highest tolerance indices for Hg and Zn respectively. A significant reduction in biomass production was observed in metal treated plants compared with the control plants. Overall, the relative growth rate of *Pistia stratiotes* was retarded by heavy metals under study (Odjegba and Fasid, 2004).

Many studies had been done related to *Pistia stratiotes* and the effect of heavy metals to the plant. Previous study also suggested that cadmium is said to be toxic to plants even at concentration as low as 1 mg/L (Metcalf and Eddy, 2003). Table 1 below shows some of the biochemical changes resulted from excess accumulation of heavy metals in plants.

Table 1: Biochemical changes resulting from excess accumulation of heavy metals in plants.

Effect	Study
Carbohydrate metabolism	Satyakala and Jamil, 1992
Nitrogen Metabolism	
Inhibition of chlorophyll synthesis	Satyakala and Jamil, 1992; Anil *et al*., 1983
Decrease of protein content	Anil et al., 1983
Dry weight of plants decreased	
Root elongation and emergence of new roots decrease	Odjegba and Fasid, 2004

Cadmium is one of the heavy metals which can cause deleterious effect to aquatic plants when it accumulates in excess in the plant tissue such as leaves and roots. Excess accumulation of heavy metals also often link to changes in biochemical properties in aquatic plants used in phytoremediation as stated in Table 1. Therefore, it is important to study the suitable concentration of heavy metals in which water lettuce will be use to treat the water body. This study provide the information on the effect of nutrients reduction from the wastewater by water lettuce and also the nutrient uptake by water lettuce in presence of cadmium at various concentration under studied (0, 3 and 6 mg/L Cd).

3. Materials and Methods

3.1 Aquatic macrophytes used for the experiment

Water lettuces (*Pistia stratiotes*) that were used as the plant in this experiment were collected from the drain in Kampung Semeba. Before the experiment, the plants were cultivated in basins of tap water for plant to grow and multiply and nutrient fertilizer in the form of tablets were added into the basin every week to provide sufficient nutrient for the development of plants.

Plants of similar sizes, shapes and heights were selected for the experiment. The size of the water lettuce that was selected for the experiment was approximately 15 cm in diameter (leaves) and roots for each plant in the basins was cut to a length of 10 cm. In preparation for the experiment, the leaves of the water lettuce which were seriously wilted were removed to prevent it from contributing nutrients to the wastewater during the experiment. Before the experiment, the fresh weights of the total plant in each basin were weighed.

3.2 Wastewater used for the experiment

For the experiment, household wastewater was collected from Kampung Sindang, Kota Samarahan. The wastewater was to supply adequate nutrient necessary for the plant growth. Before the experiment started, the sediment that might be present in the wastewater was allowed to settle down to the bottom of the basin for 1 day. The presence of sediments inside the wastewater was negligible due its very small amount and their presence in the same amount in every basins. The characteristics of the household wastewater collected were determined which includes the analysis of nitrate-nitrogen, ammonia-nitrogen and orthophosphate and cadmium content.

3.3 Experimental Setup (Completely Randomize Treatment with 3 replicates)

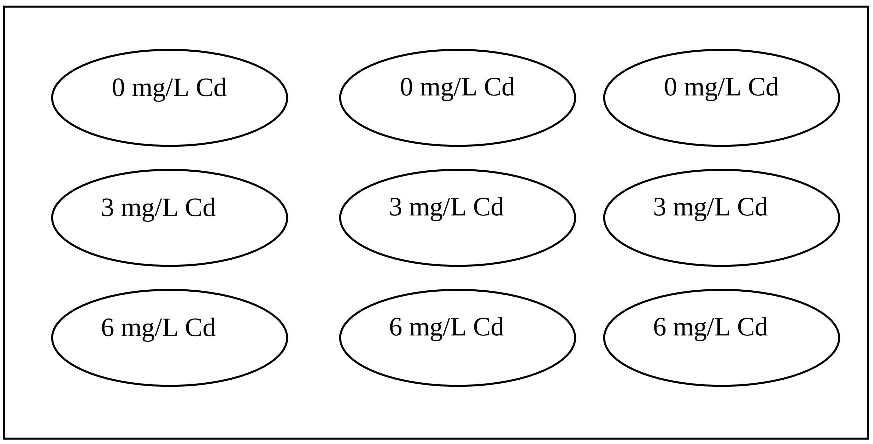

Figure 1: Three treatments of the experiment

In the experiment, the household wastewater collected was filled into nine round plastic basins (PVC), with diameter of 55 cm and depth of 23 cm. Every basin (tanks) was filled with 20 L of household wastewater and they were placed at a covered area at the ground floor of the Faculty of Resource Science Technology (FRST) building with sufficient sunshine exposure.

Three treatments were done for the experiment where the first treatment served as the control (no cadmium solution added) along with the second and third treatment were wastewater added with 100 mL cadmium solution of 3 mg/L and 6 mg/L respectively. The