A STUDY ON REMOVAL OF HEAVY METALS FROM WASTEWATER BY FLOATING PLANTS

BY

OLEY PHEARKEO

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE (ENGINEERING AND TECHNOLOGY)
SIRINDHORN INTERNATIONAL INSTITUTE OF TECHNOLOGY
THAMMASAT UNIVERSITY
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A Thesis Presented

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OLEY PHEARKEO

Submitted to
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Abstract

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by

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Heavy metal contaminated water is the major environmental problem and has been increasing with the discharge of industrial wastewater untreated or partially treated. They affect human, fauna, and flora systems. The cost effective and environmental friendly technology, phytoremediation, uses plants to remove heavy metals from wastewater or to render them harmless. Water hyacinth (WH), Water lettuce (WL), Creeping waterprimrose (CM), Floating moss (FM), and Common duckweed (CD) were selected to study the Cd and Pb uptake. Screening of plants for Cd and Pb hyperaccumulators was conducted at 4 and 10 mg/L, respectively. To study the effect of heavy metals concentration on plants uptake, the concentration of Cd was varied from 5, 10, 20, 30, 40, 60, 80 and 100 mg/L, and 10, 30, 50, 70, 100, 200, 300 and 400 mg/L for Pb. Moreover, the effect of nutrients was also investigated by adding 3, 10, 20 and 30% of Hoagland's nutrients solution to contaminated water with heavy metals. The heavy metals in water and plant samples were analyzed by ICP-OES. Based on the uptake, CM and WH were selected as Cd and Pb hyperaccumulator, respectively. FM was selected as both Cd and Pb hyperaccumulators. During the experiments, plants looked unhealthy with increasing metals concentration and exposure times (except WH at 10 and 30 mg/L of Pb, and

FM at 10 mg/L of Pb), due to Cd and Pb toxicity which caused the relative growth reduction. However, they could remove heavy metals within short duration (1day). In Cd contaminated water, CM and FM were able to remove Cd within 6-8 days when the concentration increased from 5 to 100 mg/L. The Cd removal efficiency decreased when the concentration increased. At 5 mg/L, CM and FM could remove Cd 76.1 and 89.2%, respectively, and 39.7 and 53.2% were removed at 100 mg/L, respectively. Although Cd removal efficiency of plants was high, the Cd remained in water was still higher than the standard of industrial wastewater effluent. However, biomass productivity was high at the studied Cd concentration (up to 92.1 and 93.9% for CM and FM, respectively). The Cd accumulation in CM and FM increased with increasing Cd concentration, except Cd accumulation in FM at 100 mg/L. BCFs for plants indicated as moderate accumulators at concentration from 5 to 10 mg/L for CM and at concentration from 5 to 60 mg/L for FM, respectively. For other concentration, plants were poor Cd accumulator. In Pb contaminated water, the highest Pb removal efficiency of WH was found at 10 mg/L (98.3%). It can be noted that Floating moss at concentration from 10 to 100 mg/L, Pb was almost removed 100%. The lowest Pb removal efficiency was 32.8 and 61.3 % at 400 mg/L for WH and FM, respectively. The results also show that plants had high biomass productivity which was over 78% for WH and 89% for FM. The Pb accumulation in WH and FM increased with increasing concentration, expect at 100 mg/L for FM. BCFs show that FM could be moderate accumulator at concentration from 10 to 400 mg/L. In contrast, WH was the moderate accumulator at concentration from 10 to 70 mg/L and poor accumulator at other concentration. Based on the high removal efficiency of plants at 10 mg/L of Pb (including screening experiment), Pb removed by WH, CM, FM and Common duckweed could be discharged to environment without any treatment. For the effect of nutrients, the results show that it did not have any effect on heavy metals uptake by plants. CM (for Cd), WH (for Pb) and FM (for Cd and Pb) were selected to study the effect of nutrients. Relative growth, biomass productivity, heavy metals removal efficiency, heavy metals accumulation and BCFs were not influenced by the amount of nutrients. A comparison of Cd and Pb at 30 and 100 mg/L, it indicates that Cd was more toxic than Pb. Based on all results, the floating plants can uptake Cd and Pb, and also can be used as bio-indicators of water, especially CM (for Cd), WH (for Pb) and FM (for both Cd and Pb).

Keywords: Cadmium, lead, uptake, floating plants, nutrients, phytoremediation, wastewater

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Chapter 1

Introduction

1.1. Problem statement

Heavy metals are persistent and non-perishable in environment, which are from increased discharged of untreated or partially treated wastes of industries such as metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and photographic industries (Wan Ngah & Hanafiah, 2008). According to Barakat (2011), heavy metals such as Cd, Cr, Cu, Ni, As, Pb, Fe and Zn are mostly released from chemical industries. The agricultural runoff, which is from pesticides and fertilizers, can also cause wastewater contaminating with heavy metals (Hou et al., 2007; Megateli et al., 2009). These heavy metals are transferred to aquatic environment through the food chain (Parlak et al., 2013), and can be easily transported and accumulated in tissues, especially the living organisms (Barakat, 2011; Wan Ngah & Hanafiah, 2008).

In the present, water contaminated by heavy metals is a major environmental problem in the world. Whether these heavy metals occur in river, stream, pond or ditch, they affect human health. The local people who stay nearby the contaminated zone are affected directly (Miretzky et al., 2004). For instance, a major past disaster ‘Itai-Itai’ caused by the contamination of Cd in the Jintsu river in Japan is well known (Förstner & Müller, 1973; Hiatt & Huff, 1975). Cd is also human carcinogens (Barakat, 2011). Axtell et al. (2003) reported that heavy metals can cause anemia, diseases of the liver and kidneys, brain damage and ultimately death. Their effects are not only on human but also on fauna, flora and ecological systems (Algarra et al., 2005; Gavrilescu, 2004; Nagajyoti et al., 2010). Even though at low concentration, it still toxic on living things (Nagajyoti et al., 2010). Therefore, heavy metals must be treated before discharge to the environment. According to Kamal et al. (2004) and Ning et al. (2011), heavy metals cannot vanish easily and the cleanup is usually required for their removal. Several technologies, chemical, physical and biological methods, have been widely used to remove heavy metals from environment, but these technologies are costly (Hou et al., 2007). It's more expensive if these technologies are used for large volumes of contaminated water or soil with low metal concentration, and when high standards of cleaning are required (Sasmaz & Obek, 2012). In contrast, phytoremediation has been considered the cost effective and eco-friendly technology for heavy metals removal from environment such as soil, surfaced water including groundwater (Ha et al., 2009; Hou et al., 2007).

Phytoremediation uses aquatic plants to remove heavy metals through the bio-sorption processes and metabolism-dependent bioaccumulation. These plants are quite effective at separating heavy metals from their surrounding water (Uysal & Taner, 2009). Heavy metals are accumulated by plant tissues (Lee & Yang, 2010) such as roots, stems, shoots and leaves (Raskin et al., 1997). The plant species being used for phytoremediation should be a hyperaccumulator and have high biomass (Tangahu et al., 2011). Research carried by Maine et al. (2001), Carranza-Álvarez et al. (2008), and Mishra and Tripathi (2008) have stated that plants with high growth rate, easy spreading, easy harvesting, tolerant to high nutrients, tolerant to heavy metals over long duration of exposure, and also with higher removal efficiency are an excellent choice for phytoremediation. In additional, plants with high bioconcentration factor would be qualified as hyperaccumulator. Many researchers have found that floating plants can be used to remove heavy metals from wastewater. Water hyacinth (*Echhornia crassipes*) has shown the ability to accumulate many heavy metals such as As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Zn (Agunbiade et al., 2009), and CN^- (Ebel et al., 2007). Water lettuce (*Pistia stratiotes*) can also remove Cd (Maine et al., 2001), Fe, Zn, Cu, Mn, Cr, Pb, Ni (Miretzky et al., 2004; Mishra & Tripathi, 2008; NurZaida & Piakong, 2011) from wastewater. According to Phetsombat et al. (2006) and Uysal and Taner (2009), Common duckweed (*Lemna minor*) has demonstrated the removal of heavy metals from wastewater, especially Pb. Floating moss (*Salvania cucullata*) can remove Cr (VI) (Baral et al., 2008), Cd and Pb (Phetsombat et al., 2006). However, Water hyacinth (*E. crassipe*), Water lettuce (*P. stratiotes*), Creeping waterprimrose (*Jussiaea repens* L.), Floating moss (*S. cucullata*), and *Common duckweed* (*L. minor*) are not explored much for removal of Cd and Pb, especially high concentration.

1.2. Objective of study

The purpose of the study is to examine the Cd and Pb removal from synthetic wastewater. The specific objectives are followed:

- To investigate the capacity of different floating plants for Cd and Pb uptake
- To investigate effect of Cd and Pb concentration, and nutrients on uptake by selected species

1.3. Scope of study

- Cd and Pb accumulation in the floating plants; Water hyacinth (*E. crassipe*),Water lettuce (*P. stratiotes*), Creeping waterprimrose (*Jussiaea repens L.*), Floating moss (*S. cucullata*) and *Common duckweed* (*L. minor*) in different initial concentration of Cd (5, 10, 20, 30, 40, 60, 80 and 100 mg/L) and Pb (10, 30, 50, 70, 100, 200, 300 and 400 mg/L), and also nutrients (3, 10, 20 and 30%) in synthetic wastewater.
- The capacity of floating plants for Cd and Pb uptake is investigated.
- The experiments are conducted until a constant uptake by floating plants is observed.
- Cd and Pb concentrations in wastewater and floating plants are analyzed by Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES).
- The relative growth, biomass productivity, heavy metals removal efficiency, heavy metals accumulation in plants and bioconcentration factor (BCF) are measured.

Chapter 2

Literature review

2.1. Heave metals

2.1.1. Heavy metal definition

Heavy metals refer to many elements with greater atomic number (>20) and have a high relative density (>4 g/cm^3).The most common heavy metals are lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag), etc. Heavy metals have toxicity on living things including human, even though at low concentration (Nagajyoti et al., 2010). According to Tangahu et al. (2011), heavy metals cannot be only biodegraded but can be also accumulated in living organisms causing various diseases and disorders. Akpor and Muchie (2010) reported that common heavy metals, As, Cu, Cd, Hg and Zn, have been identified in the polluted water. However, there are some metals are still necessary micronutrients to plant growth, especially Zn, Cu, Mn, Ni, and Co.

Lead (Pb)

Pb has an atomic number 82, atomic weight 207.19, and a specific gravity of 11.34. It is a bluish or silvery-grey with 327.5°C melting point and 1740°C boiling point at atmospheric pressure. The oxidations of Pb are +2 and +4. It is very poor solubility in water when the nitrate, chlorate , and chloride, most of the inorganic are salted with Pb^{2+} (Tangahu et al., 2011; WHO, 2001b).

Cadmium (Cd)

Cd has an atomic number 48, atomic weight 112.41. It is a soft, ductile, silver-white metal with 320.9°C melting point, 765 °C boiling point and relatively high vapor pressure. According the report of WHO (2001a), several compounds of inorganic cadmium are quite soluble in water such as acetate, chloride and sulfate. In contrast, cadmium oxide, carbonate and sulfide are almost insoluble.

2.1.2. Sources of heavy metals

Heavy metals found in the environment are from natural and anthropogenic sources. They are from natural source such as mineral, erosion and volcanic activity. However, the most heavy metals reaching to environment are from anthropogenic source. This includes mining, smelting, electroplating, agriculture (pesticides and fertilizers as well as bio-solids), sludge dumping, industrial discharge, etc (Ali et al., 2013). According to Özmen et al. (2004), the contaminated marine and fresh water of heavy metals, As, Cr, Cu, Mn and Ni are presented, come from domestic wastewater effluents. Heavy metals in Coal-burning power plants such as As, Hg and Se are in particular. For non-ferrous metal smelteries include Cd, Ni, Pb and Se. In the iron and steel plants are Cr, Mo, Sb and Zn. Moreover, in dumping of sewage sludge, As, Mn and Pb are presented. Table 2.1 gives anthropogenic sources of heavy metals in the environment.

Table 2.1: Anthropogenic sources of heavy metals in the environment

Heavy metals	**Sources**	**References**
Cd	Paints and pigments, mining, plastic stabilizers, electroplating, incineration of cadmium containing plastics, phosphate fertilizers, and automotive tires	Pulford and Watson (2003); Chaiyasith et al. (2006) and Hou et al. (2007)
Cu	Electroplating industries, mining	Baraket (2011)
Pb	Urban runoff, mining, smelting, paint, batteries, lead piping factories	Singh et al. (2012)
As	Mineral activities, laser manufactures semiconductors, glass industry, pharmaceutical products, and pigments, fertilizers	Alvarado et al. (2008)
Cr	Electroplating industries, milling, etching	Kurniawan and Sillanpaa (2010); and Baraket (2011)
Ni	Electroplating industries, milling, etching	Baraket (2011)
Hg	Electroplating industries, mining, coal-burning power plants	Özmen et al. (2004); and Algarra et al. (2005)
Zn	Electroplating industries, steel plants	Özmen et al. (2004); and Algarra et al. (2005)

Sources of Pb

Pb and its compounds are crucial toxic heavy metal reaching water through urban runoff or discharging from sewage treatment plants and industrial plants (Singh et al., 2012). According to Yongpisanphop (2005), Pb has been estimated to the disposal emission into the environmental media including the atmosphere. The worldwide emission of Pb in the fresh water was from different sources such as atmosphere (87,000-113,000 tons/year), manufacturing process metals (2,500-22,000 tons/year), chemical (400-3,000 tons/year), pulp and paper (100-900 tons/year), petroleum product (20-100 tons/year), dumping of sewage sludge (2,900-10,000 tons/year), domestic wastewater including central (900-7,200 tons/year), and non-central (600-4,800 tons/year), smelting and referring including nonferrous metal (1,000-6,000 tons/year), iron and steel (1,400-2,800 tons/year) and stream electrical production (200-1,200 tons/year).

Sources of Cd

Cd and its compounds are reported to be widespread heavy metal pollutant in natural and wastewater (Hou et al., 2007). They are found in plastics, paintings, enamels, inks, display devices and photovoltaic cells (Kolobov, 1996), as well as in batteries, alloys in electroplating, welding, electrical and nuclear fission applications (Fthenakis, 2009; Fthenakis, 2004; Tingsheng et al., 2002). In 2004, the worldwide production of Cd was estimated to23,000,000 tons (Fthenakis, 2004). Cd is found in many sources, for example, atmospheric deposition derived from mining, smelting, and fuel combustion (Suchismita et al., 2014), domestic wastewater and industrial discharge (Benavides et al., 2005).

2.1.3. Heavy metals contaminated water

In the present, heavy metals contamination in environment is the major global concern. This is caused by the rapid increasing industrialization, especially smelting, metal plating, battery manufacture, mining activities, petroleum refining, tanneries, paint manufacture, pesticides, printing and photographic industries. Due to having untreated or partially treated before discharge to environment, the industrial wastewater is detected the common heavy metals, particularly Zn, Hg, Cu, Cd, Pb and

Cr (Wan Ngah & Hanafiah, 2008). Furthermore, contaminated water of heavy metals also occurs in environment because of the erosion runoff of mine wastes, the dusts produced during the transported crude ores, the heavy metals corrosion and leaching soil and groundwater (Fillaudeau et al., 2006; Nagajyoti et al., 2010).

2.1.4. Effect of heavy metals

As known well that heavy metal are the most hazardous element such as Cd, Cr, Cu, Ni, As, Pb, and Zn. These heavy metals are almost from industrial wastes. Singh et al. (2012) stated that in the wastewater detection has found Cd, Pb, Co, Zn and Cr, they are presented at both low and very high concentration. Once these heavy metals reach the environment, the living organism can absorb by entering of food chain (Barakat, 2011). The heavy metals reach the food chain through plants and aquatic animals when they are presented in sediment. Any of heavy metals may cause acute or chronic toxicity (poisoning) on living things (Singh et al., 2012) including human health. According to Barakat (2011), heavy metals affect the growth and development of human body. They cause cancer, damage organ and nervous systems. It seriously affects when human get a large amount of heavy metals resulting in death. There are some diseases caused by As, Cd, Cr, Cu, Ni, Zn, Pb and Hg as shown in Table 2.2.

Axtell et al. (2003) demonstrated that Pb can produce anemia, diseases of the liver and kidneys, brain damage and ultimately death. Sharma and Dubey (2005) documented that the toxicity symptom in plants showing the stunted growth, chlorosis and blacking of root system is caused by excess Pb. not only this, Pb also inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability.

Cd is one of the most toxic metals affecting man. It accumulates in human body affecting kidney, bone, and also causes cancer (Ahluwalia & Goyal, 2007). For plants, Cd reduces the nitrate absorption and its transportation from root to shoot and also decreases the water content in plants resulting the inhibiting growth of plants (Nagajyoti et al., 2010).

Table 2.2: Heavy metals and their toxicities on health (Barakat, 2011)

Heavy metals	Toxicities
As	Skin manifestations, visceral cancers, and vascular disease
Cd	Kidney damage, renal disorder, and human carcinogen
Cr	Headache, diarrhea, nausea, vomiting, and carcinogenic
Cu	Liver damage, Wilson disease, and insomnia
Ni	Dermatitis, nausea, chronic asthma, coughing, and human carcinogen
Zn	Depression, lethargy, neurological signs and increased thirst
Pb	Damage the fetal brain, diseases of the kidneys, circulatory system, and nervous system
Hg	Rheumatoid arthritis, and diseases of the kidneys, circulatory system, and nervous system

2.2. Phytoremediation

Phytoremediation uses variety of plants to degrade, extract, contain, or remove contaminants from soil and water, including groundwater(Sharma & Pandey, 2014). These contaminants involve heavy metals and their compounds (Ali et al., 2013; Pulford & Watson, 2003; Singh et al., 2012). In case of heavy metals removal from wastewater, the aquatic plants are applied for this technology. Aquatic plants are able to accumulate heavy metals inside their parts (Lee & Yang, 2010) such as roots, stems and leaves(Raskin et al., 1997).

Phytoremediation mechanisms are phytoextraction, phytostabilization, phytostimulation,rhizofiltration, phytodegradation and phytovolatilisation (Ali et al., 2013; Pulford & Watson, 2003; Sharma & Pandey, 2014; Tangahu et al., 2011). These mechanisms are shown in Figure 2.1.

- Phytoextraction/phytoaccumulation/phytoabsorption/phytosequestration: This technique, plant roots absorb the contaminants from soil then translocate to the shoots or some parts of the plants.
- Phytostabilization: Plants have been used to remove the contaminants from soil, sediment, sludge and groundwater through absorption and accumulation.

The contaminations are absorbed into roots, or precipitation within the root zone. This technology can also prevent the contaminants migration in soil, as well as their movement by erosion and deflation.

- Phytostimulation/rhizodegradation: It uses plants to reduce the contaminated soil by their roots. This technology has some successes in treatment of organic chemicals, including petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, pesticides, polychlorinated biphenyls(PCBs), benzene, toluene, ethylbenzene, and xylenes (Etim, 2012; Sharma & Pandey, 2014).
- Rhizofiltration: It uses plants to clean up communal wastewater or contaminated wetland, surface water. The contaminants involve heavy metals or other inorganic compounds, e.g. Pb, Cd, Cu, Ni, Cr and Zn. These are adsorbed or precipitated by plant’s roots.
- Phytodegradation: This technique uses plants to degrade the organic pollutants from soil, sediment, or groundwater. It can also degrade the synthetic herbicides and insecticides. However, phytodegradation is limited to remove organic pollutants only. Therefore, it can not apply to remove heavy metals because heavy metals are non-biodegradable.
- Phytovolatilisation: This technique uses plants to absorb and transpire the contaminants or pollutants from the soil to the atmosphere by conversion them to volatile form. Phytovolatilisation is available for removal of organic pollutants and some heavy metals such as Hg and Se.

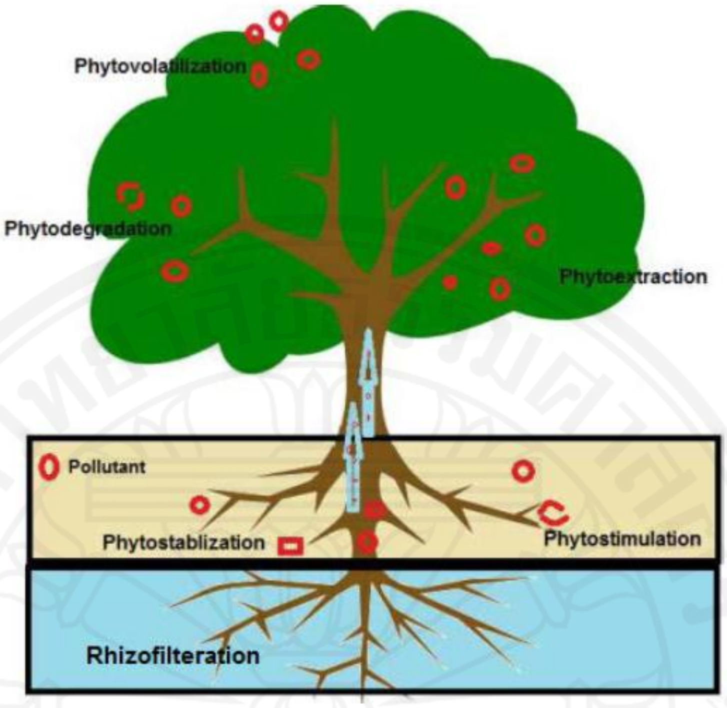


Figure 2.1: Phytoremediation mechanisms

2.2.1. Advantage and disadvantage of phytoremediation

Phytoremediation has been accepted for environmental treatment in a recently year. This technology is used for cleaning-up contaminated sites with metals, especially contaminated soil and water including groundwater. Additionally, it is the lower cost treatment than another technology. It is also the environmental friendly technology (Macek et al., 2000). Although the phytoremediation is the cleanest and cheapest technology, it still has limitation. This is caused by climatic and geologic conditions of the sites. The temperature, altitude, soil type and accessibility for agricultural equipment are considered as the limitation of phytoremediation (Schmöger et al., 2000). Phytoremediation takes time longer than another technology to treat the contaminants. The contaminants can be accumulated in fuel wood. Moreover, the collected contaminants in leaves can be released again into the environment during litter fall. The formation of vegetation may be limited by extreme environment toxicity (Macek et al., 2000; Schmöger et al., 2000). Table 2.3 is the summarizing of advantage and limitation.

Table 2.3: Advantages and limitations of phytoremediation (Singh et al., 2003)

Advantages	Limitations
1. Solar driven 2. In situ 3. Passive 4. Public 5. Cost 10-20 % of mechanical treatment 6. Transfer is more rapid than natural attention 7. Fewer secondary waste 8. Fewer air and water emission 9. Soil remain in place and are usable follow in treatment	1. Mass transfer limitations associated with other biotreatment 2. Limited to shallow soil, streams and groundwater 3. Hyperaccumulation of hazardous might be toxic for plants 4. Bioavailability and toxicity of degradative product is not known 5. Slower than mechanical treatment 6. Effect for only moderately hydrophobic 7. Potential for contaminants to enter food chain through animal consumption 8. Contaminants may be mobilized into the groundwater 9. Unfamiliar to many regulators

2.2.2. Effective Factors of uptake mechanisms

There are several factors affecting on heavy metals uptake. Plant species, properties of medium, the root zone, vegetative uptake, addition of chelating agent are presented as the factors of uptake mechanisms (Cheng, 2003; Tangahu et al., 2011) showing in Figure 2.2.

The plant species

The successful phytoremediation depends on the properties of selected plant species. Plants are able to be heavy metals hyperaccumulators, and also produce the high biomass productivity. Several common plants applying for phytoremediation are *Typha latifolia* (Sasmaz et al., 2008), *Echhornia crassipe* (Alvarado et al., 2008; NurZaida & Piakong, 2011), *Pistia stratiotes* (Bich & Kato-Noguchi, 2012), *Scirpus tabernaemontani* (Skinner et al., 2007), *Arabis paniculata Franch* (Tang et al., 2009), etc. The different species are able to absorb the different heavy metals as shown in Table 2.4.

Properties of Medium

The properties of medium are very important for heavy metals uptake by plants, especially pH, addition of chelators, fertilizers. pH, for instance, the soil containing the organic matter and the phosphorus affect the amount of Pb absorption of plants. To reduce the Pb containing in soil, pH of soil is adjusted with lime to 6.5-7 (Tang et al., 2009). Most of the plants need nutrients for their metabolism as fertilizer. Moreover, adding fertilizer helps plants to adapt in the new environment that use for phytoremediation. Many researchers use not only Hoagland's solution nutrients such as Megateli et al. (2009), Phetsombat et al. (2006) but also hydroponic fertilizer "10-6-16" with 1mL:1L of water (Kamal et al., 2004) to remove heavy metals from water.

The Root Zone

The root zone is special interesting in phytoremediation. It can absorb contaminants in soil or water and metabolizes the contaminants inside the plant tissue. This process is called translocation. The contaminants in the root are translocated to other parts of plants through the plasma membrane (Ebrahimpour & Mushrifah, 2008; Sasmaz et al., 2008). In general, the contaminants or heavy metals are found in roots higher than other parts. Soltan and Rashed (2003) demonstrated that the concentration of Cd and Pb was 100 mg/L, Water hyacinth's root accumulated 2,060 µg/g, and 325 µg/g was found in its aerial parts. The Pb was found 34,950 and 1,030µg/g in Water hyacinth's roots and its aerial parts, respectively. However, the leaves of some species accumulate heavy metals higher than their roots. For instance, the simulated wastewater with Ni and Cr at 0.5 mg/L, and the period of cultivation was 15 days. Ni was found 0.09 mg/g in Water lettuce's leaves, and 0.05 mg/g in its roots. Cr was also found in its leaves higher than roots, it was 0.13 mg/g in roots and 0.09 mg/g in leaves (NurZaida & Piakong, 2011).

Vegetative Uptake

The environmental conditions affect vegetative uptake of plants. The temperature reduces the growth of substances and root length. The success phytoremediation depends on a contaminated-specific hyperaccumulator. Furthermore, the uptake of heavy metals depends on the bioavailability of heavy metals in wastewater, as well as the other elements and substances interaction in the water. According to Soltan and Rashed (2003), heavy metals accumulation in Water hyacinth was affected by the external solutions. Water hyacinth was cultivated in different medias: distilled water, Nile water (Nile river in Egypt in September 1999), wastewater (from Kima drain wastewater, Aswan, Egypt), synthetic wastewater with supplement of heavy metals mixture of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn at 1, 3, 5, 7, 10, 50 and 100 mg/L, respectively, and also individual solutions with 100 mg/L of Pb and 100 mg/L of Cd. The results show that Water hyacinth was able to survive in heavy metals mixture in the concentration up to 3 mg/L and 100 mg/L of Pb individual solution. At the same time, it leads to rapid fading at the mixture heavy metals and individual concentration 100 mg/L of Cd as shown in Table 2.5.

Addition of Chelating Agent

The chelating agent adding, micronutrients cause plants to be faster uptake heavy metals and also have less expensive remediation periods. It was found that EDTA (Ethylenediaminetetraacetic acid) was used for plants cultivation for 2 weeks. Plants could improve their translocation of heavy metals in plants tissues as well as overall phytoextraction performance. However, using the synthetic chelating agent such as NTA (Nitrilotriacetic acid) and EDTA is the environmental risk, especially contaminated soil with heavy metals. Therefore, this should be concerned (Tangahu et al., 2011).

Table 2.4: Effect of external solutions (growth media) on the accumulation of metals in water hyacinth (Soltan & Rashed, 2003)

Media	Duration of Experiment (hr)	accumulation (mg/g dry weight)							
		Cd		Co		Cr		Cu	
		Roots	Aerial parts	Roots	Aerial parts	roots	Aerial parts	Roots	Aerial parts
Distilled water	240	65	5	52	26	135	115	164	109
Nile water	240	10	2	63	17	160	64	141	53
Wastewater	96	15	5	64	30	155	95	142	68
Heavy metal solution									
1 mg/L (mixture)	240	615	30	1300	37	450	52.5	1750	57
3 mg/L (mixture)	240	865	50	1530	49	1950	58	2110	8
5 mg/L (mixture)	192	630	95	1680	169	1550	48	2710	252
7 mg/L (mixture)	192	640	280	1355	570	2300	236	2750	1105
10 mg/L (mixture)	168	635	485	2000	800	2500	495	2900	700
50 mg/L (mixture)	96	1010	930	2680	2225	3000	1500	2950	15255
100 mg/L (mixture)	72	620	1,200	1915	2475	2150	3000	2800	1900
100 mg/L Pb^{+2}	240	25	15	117	40	165	45	268	89
100 mg/L Cd^{+2}	96	2060	325	58	10	140	54	129	47
		Mn		*Ni*		*Pb*		*Zn*	
Distilled water	240	1950	755	95	40	30	455	225	225
Nile water	240	1875	785	140	55	31	470	180	180
Wastewater	96	1945	1290	125	60	46	277	230	230
Heavy metal solution									
1 mg/L (mixture)	240	1950	635	1100	107	1800	85	1850	325
3 mg/L (mixture)	240	1965	765	1210	121	4900	125	4350	345
5 mg/L (mixture)	192	2110	1230	960	260	3800	90	3850	1000
7 mg/L (mixture)	192	1965	1155	900	560	3850	155	3850	2800
10 mg/L (mixture)	168	1990	915	1090	945	1600	95	4700	4000
50 mg/L (mixture)	9	2010	1840	1400	1300	2250	170	5000	4850
100 mg/L (mixture)	72	1995	1900	1035	1500	1090	105	3800	5400
100 mg/L Pb^{+2}	240	1915	1005	140	70	34950	1030	650	280
100 mg/L Cd^{+2}	96	1955	640	115	65	2400	80	700	245

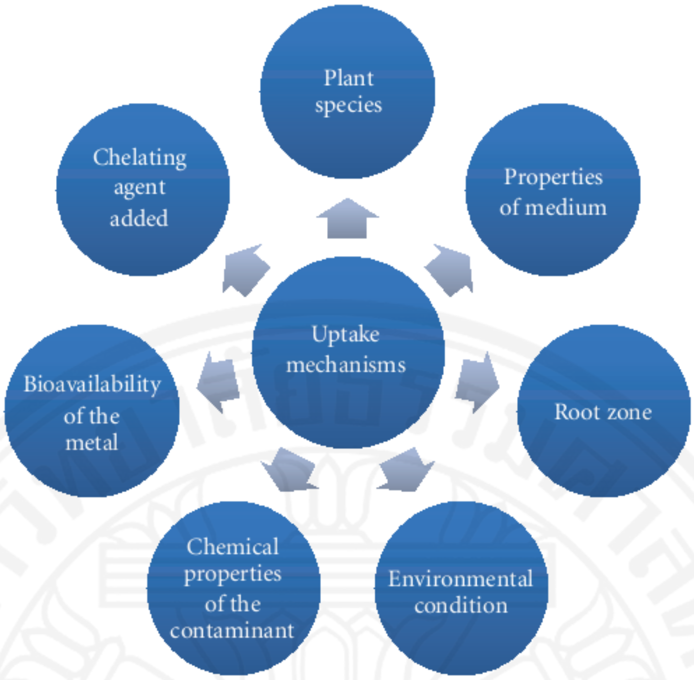


Figure 2.2: Factors which are affecting the uptake mechanisms of heavy metals (Tang et al., 2009)

2.2.4. Heavy metal absorption in aquatic plants

Heavy metals are removed from environment by aquatic plants through 3 patterns: 1) plants attach the heavy metals to their cell wall; 2) the roots accumulate heavy metals then translocate to the shoots; and 3) hyperaccumulation, heavy metals content in plant parts (Mishra & Tripathi, 2008). According to Keskinkan et al. (2004), *C. demersum* was able to accumulate many heavy metals, especially Zn, Pb and Cu. The aquatic submerged plant was an effective bio-sorbent for these heavy metals removal under dilute metal conditions. The study shows that *C. demersum* adsorption based on the Langmuir coefficients. The maximum absorption capacity was 13.9 mg/g for Zn, 44.8 mg/g for Pb and 6.2 mg/g for Cu. In additional, both *L. palutris* and *P. stratiotes* L. were examined for their capacities to remove heavy metals from contaminated water. Fe, Zn, Cu, and Hg were removed by these plants in a Solar Aquatic System treating municipal wastewater. The average removal efficiency for the three plant species were 99.8%, 76.7%, 41.6%, and 33.9% of Hg, Fe, Cu, and Zn, respectively. The removal of Zn and Cu were constant (0.5 mg/L/day for Zn and 0.2 mg/L/day for Cu), whereas Fe and Hg depended on the concentration of these elements in the contaminated water and ranged from 7.0 to 0.4 mg/L/day for Fe and 0.0787 to 0.0002 mg/L/day for Hg (Kamal et al., 2004).

2.3. Ecology of aquatic plants

The adaptation of plants to live in the aquatic environment including saltwater and freshwater, aquatic plants, occur permanently or seasonally in wet environment (wetland). Aquatic plants are referred to hydrophytes or macrophytes. These plants desire to submerge in water or float on surface water. The most common adaptation plants such as aerenchyma, floating leaves and finely dissected leaves are presented. These plants grow permanently in water or wetland with the suitable factors, especially ambient environmental conditions. This includes the temperature, light, turbidity, pH, dissolved organic carbon, nutrients, toxic chemical present (Pho-Eng & Polprasert, 1996), chlorophyll (related to phytoplankton biomass), and depth (Lacoul & Freedman, 2006).

Aquatic plants are classified to 4 functional groups (Lacoul & Freedman, 2006), which depend on their stage of growth or depth of water levels (Polprasert, 1986) as shown in the Figure 2.3.

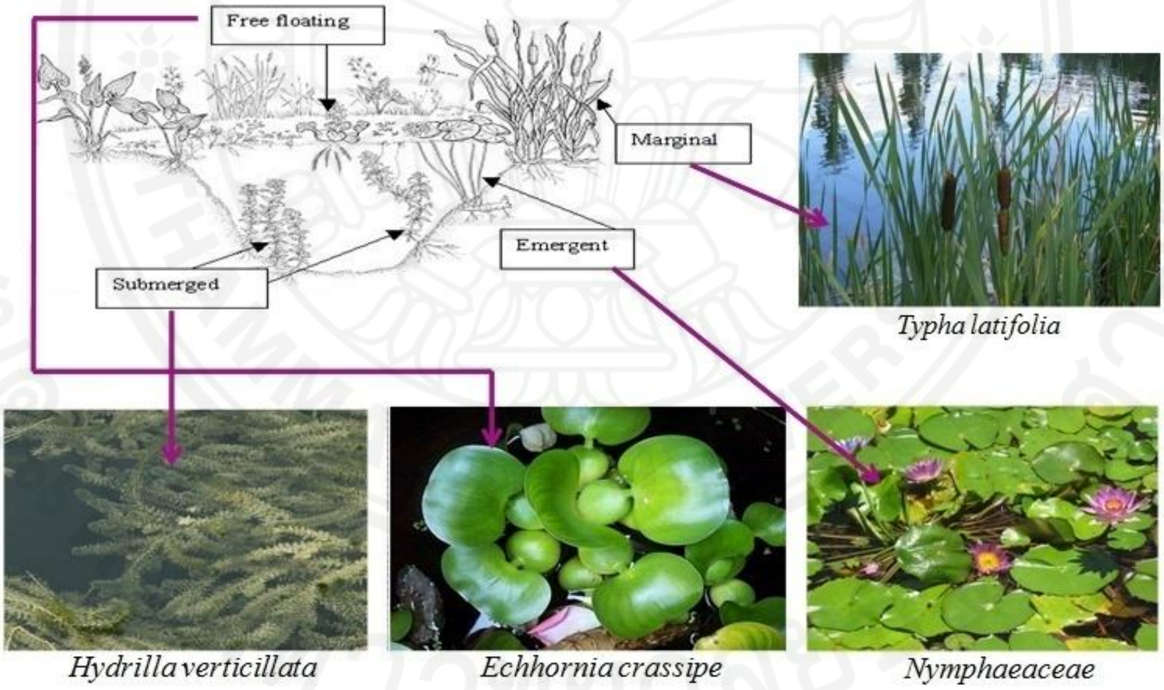


Figure 2.3: Four groups of aquatic plants

Submerged plants

Submerged plants are aquatic plants with roots submerge in the bottom substrate. Their leaves are normally underwater. They sometimes float on the surface. These plants usually occur at various depths in pond or lake. The common species found in these environment are Water-starwort (Callitrichaceae), Water-milfoil (Haloragaceae), Pondweed (Potamogetonaceae), and Plodea, Wild celery, and Frogbit (Hydrocharitaceae).

Emergent plants

Emergent plants are the plants with roots in the bottom substrate, but some parts such as leaves, stems and flowers (if the species has flowers) reach above the water surface. These plants, which mostly occur in shallow, include species of sedges and bulrush (Cyperaceae), rushes (Junceae), grasses (Poaceae), cattails (Typhaceae).

Marginal plants

Marginal plants are the plants with roots submerge into the sediment, but the leaves floated on the surface water. These plants are found at the moderate depth of water level and also commonly occur in the low visible water. Marginal plants refer to the species of Lotus (Nelumbonaceae) and Water lily (Nymphaeaceae).

Floating plants

Floating plants refer to the plants that whole body such as roots, stems, leaves and flowers float on the surface water. They are freely moved by wind and current water. Water lettuce (Araceae), Mosquito-fern (Azollaceae), Water hyacinth (Pontederiaceae), and Duckweed and Watermeal (Lemnaceae) are classified as floating plants.

2.4. Plants for phytoremediation

As well known the phytoremediation uses plants to remove the contaminants from environment. Aquatic macrophytes referred to aquatic plants are applied for wastewater treatment. Carranza-Álvarez et al. (2008) reported that the selected aquatic macrophyte should be fast growth and easy harvesting, have high biomass production and can accumulate high concentration of nutrients and heavy metals over a long exposure times. Furthermore, a perfect plant species for phytoremediation should have these criteria: 1) in low concentration, plants reveal high accumulation efficiency on heavy metals; 2) high accumulation of heavy metals in organs which are easily harvest; 3) plant is able to accumulate several kinds of heavy metals; 4) plants resist diseases and pests; and 5) it demonstrates some environmentally-friendly economic utilization (Cheng, 2003). Many species of aquatic plants can be applied for phytoremediation such as Indian mustard (*Brassica juncea* (L.) Czern), Sunflower (*Helianthus annuus* L.), Water hyacinth

(*Eichhornia crassipes*), Pennywort (*Hydrocotyle umbellate*), Duckweed (*Lemna minor*), Water velvet (*Azolla pinnata*) (Eapen et al., 2003). These plants are able to remove various heavy metals from wastewater by using their hair roots to absorb heavy metals (Dushenkov et al., 1995). Cu, Cd, Cr, Ni, Pb, Hg, Fe and Zn removed from wastewater by plants are presented (Dushenkov et al., 1995; Eapen et al., 2003; Kamal et al., 2004). The capacity of plant for each heavy metal removal depends on plant species as shown in Table 2.5.

Table 2.5: Floating plants for phytoremediation

Heavy metals	Plants		References
	Common names	**Scientific names**	
Ni, Pb	Common duckweed or Lesser duckweed	*Lemna minor*	Axtell et al. (2003) and (Alvarado et al., 2008)
Fe, Zn, Cu, and Hg	creeping primrose	*Ludwiigina palustris*	Kamal et al. (2004)
Hg, Cr, Ni, Pb and Cd	Floating moss	*Salvinia cucullata*	Banerjee and Sarker (1997) and Phetsombat et al. (2006)
As, Cd, Cr, Cu, Ni, Pb, Fe, Au and Zn	Gibbous duckweed	*Lemna gibba*	Sasmaz and Obek (2012), Parlak et al. (2013) and Megateli et al. (2009)
Cd, Cu, Pb, Zn, Hg, Cr and Ni	Water hyacinth	*Echhornia crassipe*	Alvarado et al. (2008) and NurZaida and Piakong (2011)
Cr, Cd, Zn, Ni and Pb	Water lettuce	*Pistia stratiotes*	NurZaida and Piakong (2011) and Singh et al. (2012)

2.5. Floating plants for phytoremediation

1) Water hyacinth (*Echhornia crassipes)*

Water hyacinth (*E. crassipes*) is floating macrophyte that originates from South America. In the present, it is widespread in all tropical climates (Agunbiade et al., 2009; Ebel et al., 2007; Romero-Guzmán et al., 2013). Water hyacinth, easily adaptable species, can survive in various aquatic environments and also propagates fast on the surface water. It grows fast in good conditions (Chunkao et al., 2012). Water hyacinth is the common plant found throughout the year in the drainage channel system and also in the irrigation fields (Singh et al., 2012).

According to Maine et al. (2001), the considered macrophytes to apply for treatment system should be a plant having rapid growth, easy spreading, relatively constant growth rate, high-pollutants uptake capacity, easy harvesting and preferably, and profitable later use. Moreover, Water hyacinth has high tolerant in water pollution (Alvarado et al., 2008; Ebel et al., 2007; Zhou et al., 2009) and greater heavy metals removal efficiency, high fibrous root system (Mishra & Tripathi, 2008). Based on these criteria, Water hyacinth is a perfect candidate for treatment wastewater.

Water hyacinth could accumulate heavy metals e.g. Cd, Cu, Pb, Zn, Hg, Cr and Ni (Alvarado et al., 2008). These heavy metals are passed through porous membranes and absorbed in all parts (roots, leaves, stalks and flowers) of the water hyacinth. The highest concentration of heavy metals are found in the Water hyacinth’s roots (Chunkao et al., 2012; Singh et al., 2012; Soltan & Rashed, 2003). NurZaida and Piakong (2011) found that the removal of various heavy metals in simulated wastewater with 0.5 mg/L of Pb, Zn, Ni, Cd and Cr, respectively. After 15 days of the experiment, Ni was the highest accumulation by Water hyacinth (0.4 mg/g), followed by Cd (0.3 mg/g), Pb (0.3 mg/g), Cr (0.3 mg/g), Zn (0.2 mg/g) as shown in Table 2.6.

Table 2.6: Distribution of heavy metals in Water hyacinth (NurZaida & Piakong, 2011)

Heavy metals	**Water hyacinth**					
	Initial accumulation (mg/g)			**Final accumulation (mg/g)**		
	Roosts	Leaves	Stalks	Roots	Leaves	Stalks
Pb	0.008	0.028	0.019	0.12	0.07	0.032
Zn	0.009	0.017	0.015	0.1	0.03	0.029
Ni	0.021	0.025	0.033	0.08	0.24	0.034
Cd	0.012	0.021	0.011	0.14	0.09	0.021
Cr	0.011	0.014	0.017	0.11	0.07	0.024

http://masteringhorticulture.blogspot.com/2010/08/eichornia-crassipes.html

Figure 2.4: Water hyacinth (*Echhornia crassipe*)

2) Water lettuce (*Pistia stratiotes*)

Water lettuce distributes worldwide in the tropics and subtropics as South America, Africa, Austria, the Netherlands, Portugal, Russia, Slovenia, Spain and Asia (Neuenschwander et al., 2009). It has widespread habitat and potential to grow in the rich nutrients environment and muddy water with low light intensities (Tewari et al., 2008; Zimmels et al., 2006). It is a common free floating freshwater macrophyte surviving in the areas with 15-35°C. It can also survive at least two months in cold water at 4°C and several weeks at -5°C. However, its optimum growth temperature is between 22 and 30°C (Neuenschwander et al., 2009). Water lettuce exists in the ponds, river (Šajna et al., 2007) having pH between 4 and 7 (Neuenschwander et al., 2009).

Water lettuce has high biomass crop with an extensive root system that is able to enhance the heavy metals removal from contaminated water with Cr, Cd, Zn, Ni and Pb (NurZaida & Piakong, 2011). These heavy metals are accumulated through roots and leaves. In the wetland at neutral condition (pH 7), Water lettuce could remove 99.3% of Pb and 65.9% of Cd (Singh et al., 2012). According to NurZaida and Piakong (2011), Water lettuce could remove Pb, Zn, Ni, Cd and Cr in simulated wastewater with 0.5 mg/L, respectively, within 15 days period. The result shows in Table 2.7. Water lettuce had accumulated heavy metals in plant parts (roots and leaves) which was ranged from high to low accumulation of Cr> Cd> Zn> Ni> Pb with amounts of them 0.22> 0.20> 0.19> 0.14> 0.13 mg/g, respectively. Water lettuce was able to accumulate Cd even at high concentration in the water. As a research of Maine et al. (2001), Water lettuce was placed in the plastic reactor with lake water and Cd concentration at 1, 2, 4, and 6 mg/L with 21 days period. The results show that the Cd containing in its roots increased with increasing the concentration of Cd. It was 2.1, 2.5, 3.8 and 4.4 mg/g, respectively. At the same time, the Cd concentration in the aerial parts also increased. It was 0.2, 0.4, 0.4 and 0.5 mg/g, respectively.

Table 2.7: Distribution of heavy metals in Water lettuce (NurZaida & Piakong, 2011)

Heavy metals	**Water lettuce**			
	Initial accumulation (mg/g)		**Final accumulation (mg/g)**	
	Roosts	**Leaves**	**Roots**	**Leaves**
Pb	0.03	0.011	0.09	0.04
Zn	0.016	0.017	0.11	0.08
Ni	0.008	0.003	0.05	0.09
Cd	0.001	0.011	0.12	0.08
Cr	0.006	0.013	0.09	0.13

http://upload.wikimedia.org/wikipedia/commons/a/af/Starr_071121-0019_Pistia_stratiotes.jpg

Figure 2.5: Water lettuce (*Pistia stratiotes*)

3) Floating moss (*Salvinia cucullata*)

Floating moss is a common free floating plant found in tropical and subtropical regions in the world (Baral et al., 2008). It is applied for heavy metals removal (Hg, Cr, Ni and Pb) from wastewater through roots and leaves (Banerjee & Sarker, 1997). Floating moss can also remove Cr (VI) from the aqueous system (Baral et al., 2008). Phetsombat et al. (2006) estimated accumulation of Cd and Pb in Floating moss by cultivation in 3 percentage of Hoagland's nutrients solution (5.6 pH was adjusted). It was placed in laboratory under control conditions: illuminate with a light intensity of 45 µmoles m^{-2}/s; in 12hr/12hr light and dark cycle; under the temperature of 25± 2ºC; contaminated water at 0.5, 1, 2 and 4 mg/L of Cd and 5, 10, 20 and 40 mg/L of Pb; and the period of the experiment was 8 days. The results are shown in Table 2.8 for Cd containing in the Floating moss. The amount of Cd accumulation in leaves was increased with increasing the concentration and exposure times. At 0.5, 1, 2 and 4 mg/L, the Cd containing in leaves was 3.8, 13.7, 189.2, and 679.2 µg/g (dry weight), respectively. Cd found in roots was 17.5, 84.6, 829.0 and 1,636.1 µg/g (dry weight), respectively. For the Pb containing in the Floating moss is shown in Table 2.9. At 5, 10, 20 and 40 mg/L, the Pb accumulation in leaves was found 273.4, 1040.7, 1340.3 and 3,982.6 µg/g and 1,861.8, 7,661.3, 10,064.7 and 14,305.6 µg/g dry weight in roots, respectively.

Table 2.8: Cd accumulation in Floating moss (Phetsombat et al., 2006)

Cd conc. (mg/L)	**Cd accumulation (µg/g dry weight)**									
	Day 0		**Day 2**		**Day 4**		**Day 6**		**Day 8**	
	roots	**leaves**	**roots**	**Leaves**	**Roots**	**Leaves**	**roots**	**Leaves**	**roots**	**leaves**
Control	0	0	0	0	0	0	0	0	0	0
0.5	0	0	1.4	0.5	5.1	1.4	16	2.1	7.5	3.8
1	0	0	29.8	5.5	60.9	6.6	83.1	12.5	84.6	13.8
2	0	0	320.2	125.5	590.6	138.4	789.2	154.4	829.1	189.2
4	0	0	767.2	223.1	830.2	290.6	1,530.3	310.1	1,636.1	679.2

Table 2.9: Pb accumulation in Floating moss (Phetsombat et al., 2006)

Pb conc. (mg/L)	**Pb accumulation (µg/g dry weight)**									
	Day 0		**Day 2**		**Day 4**		**Day 6**		**Day 8**	
	roots	**leaves**	**roots**	**leaves**	**Roots**	**Leaves**	**Roots**	**leaves**	**roots**	**leaves**
control	0	0	0	0	0	0	0	0	0	0
0.5	0	0	877.1	66.1	1,237.4	176.2	1,522.6	194.4	1,861.8	273.4
1	0	0	4034.4	580.9	5,652.5	831.7	6,939.7	882.5	7,661.3	1,040.7
2	0	0	5,302.1	632.3	7,241.4	837.1	9,429.3	945.4	10,064.4	1,340.3
4	0	0	9,388.9	2,005.6	12,432.5	2,308.99	13,009	3,453.5	14,305.6	3,982.6

http://rbg-web2.rbge.org.uk/thaiferns/factsheets/index.php?q=Salvinia_cucullata.xml

Figure 2.6: Floating moss (*Salvinia cucullata*)

4) Common duckweed *or* Lesser duckweed (*Lemna minor*)

Common duckweed is a free floating plant found in surface water, lakes, ponds, canals, wetland, etc. It is fast growth and adapts itself easily in various conditions (Singh et al., 2008). It is commonly found in almost every climate, except entirely waterless desert like the tundra (Romero-Guzmán et al., 2013). Common duckweed can survive in environment with pH between 3.5 and 10.5, and temperature between 5 and 35°C. However, its optimum growth temperature is from 20 to 31°C (Singh et al., 2012).

Common duckweed is demonstrated that it is able to remove heavy metals from polluted water (Cardwell et al., 2002; Hou et al., 2007). For instance, 91% of Pb was removed from wastewater at 20 mg/L initial concentration and pH 5 (Uysal & Taner, 2009). It was also able to remove Ni 85% at 5mg/L (Axtell et al., 2003).

http://www.aquaticplantcentral.com/forumapc/plantfinder/details.php?id=131

Figure 2.7: Common duckweed (*Lemna minor*)

5) **Gibbous duckweed** (*Lemna gibba*)

Gibbous duckweed, floating plant, is found in worldwide including the tropical to the temperate zones. It distributes widely in both freshwater and brackish estuaries. However, Gibbous duckweed is commonly found in tropic conditions (Sasmaz & Obek, 2012). It is widely distributed freshwater such as ditches, ponds and lakes, and also found in brackish estuaries (Parlak et al., 2013).

Gibbous duckweed has rapid reproductive rates, easy propagation and can also treat wastewater, especially heavy metals contaminated water. Thus, Gibbous duckweed can be a warning indicators to the environment (Böcük et al., 2013; Megateli et al., 2009; Parlak et al., 2013). The heavy metals that Gibbous duckweed is able to remove are As, Cd, Cr, Cu, Ni, Pb, Fe, Au and Zn (Sasmaz & Obek, 2012). Parlak et al. (2013) demonstrated that Cd accumulated in Gibbous duckweed is 0.8, 1.7, 1.5, 0.7 and 0.2 mg/kg (dry weight) at 0.05, 0.5, 5, 10, and 20 mg/L, respectively. According to the report of Megateli et al. (2009), it could remove 73% of Cd and 69% of Zn within 2 days at 10^{-4} and 10^{-1} mg/L, respectively.

http://commons.wikimedia.org/wiki/File:LemnaGibba%2BWolffiaArrhiza.JPG

Figure 2.8: Gibbous duckweed (*Lemna gibba*)

6) **Water fern** *(Salvinia natan*)

Water fern, a free floating aquatic weed, grows rapidly in ponds, lakes, ditches, and wastewater in Southern Asian countries (Dhir et al., 2011; Rahman et al., 2008). It has leaves in whorls of three, two elliptic leaves float on the water surface, and the third one is heavily dissected and submerged performing similar functions as roots (Jampeetong & Brix, 2009).

Water fern is tolerant to high metals concentration (Dhir et al., 2011) and can accumulate heavy metals like Cr, Co, Zn, Fe, Ni, Cu, Cd, Mn, and Pb (Dhir et al., 2011; Dhir & Srivastava, 2011). Due to its high growth rates, it can produce double biomass in the shorter time. For example, it had high productivity or density 5.8-11.4 g/m^{-2} within 2 days, when cultured in a chemically defined Hoagland medium, and around 20–120 kg/ha/day under natural conditions (Dhir et al., 2011).

According to Dhir et al. (2011), Water fern was potential to accumulate heavy metals at high concentration (35 mg/L) of Cr, Fe, Ni, Cu, Pb and Cd, respectively. The ranged heavy metals containing was from 6 to 9 mg/g dry weight, while accumulation of other heavy metals, Co, Zn and Mn, ranged from 3 to 4 mg/g dry weight as shown in Table 2.10.

Table 2.10: Heavy metal accumulation in Water fern (whole plant) measured after 48 hr of metal exposure (Dhir et al., 2011)

Metals	**Concentration (mg/L)**	**Accumulation rate (mg/g dry weight)**	
		Control	**Treated**
Cd	35	0.0117±0.002	6.487±0.8
Cu	35	0.0187±0.002	7.267±0.8
Ni	35	0.0237±0.004	9.087±1.3
Co	35	0.0027±0.003	3.947±0.5
Pb	35	0.0057±0.007	7.927±0.6
Fe	35	0.2937±0.05	9.727±1.0
Cr	35	0.0087±0.001	8.727±0.9
Mn	35	0.0217±0.005	4.387±0.5
Zn	35	0.0187±0.007	4.507±0.5

http://robinsyard.blogspot.com/2012/09/salvinia-natans.html

Figure 2.9: Water fern *(Salvinia natan*)

Chapter 3

Methodology

This chapter describes on Cd and Pb removal by5floating plants; Water hyacinth (*E. crassipe*), Water lettuce (*P. stratiotes*), Creeping waterprimrose (*Jussiaea repens* L.), Floating moss (*S. cucullata*) and Common duckweed (*L. minor*). Screening floating plants for Cd and Pb hyperaccumulators was conducted and 2 best species for high uptake of Cd and Pb were identified. Effects of these heavy metals concentration and Hoagland nutrients solution were also investigated. Cd and Pb were analyzed by Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES) for both wastewater and floating plants after digestion. All the experiments were conducted in duplicate and placed outdoor under the greenhouse. The overall research frame work is shown in Figure 3.1.

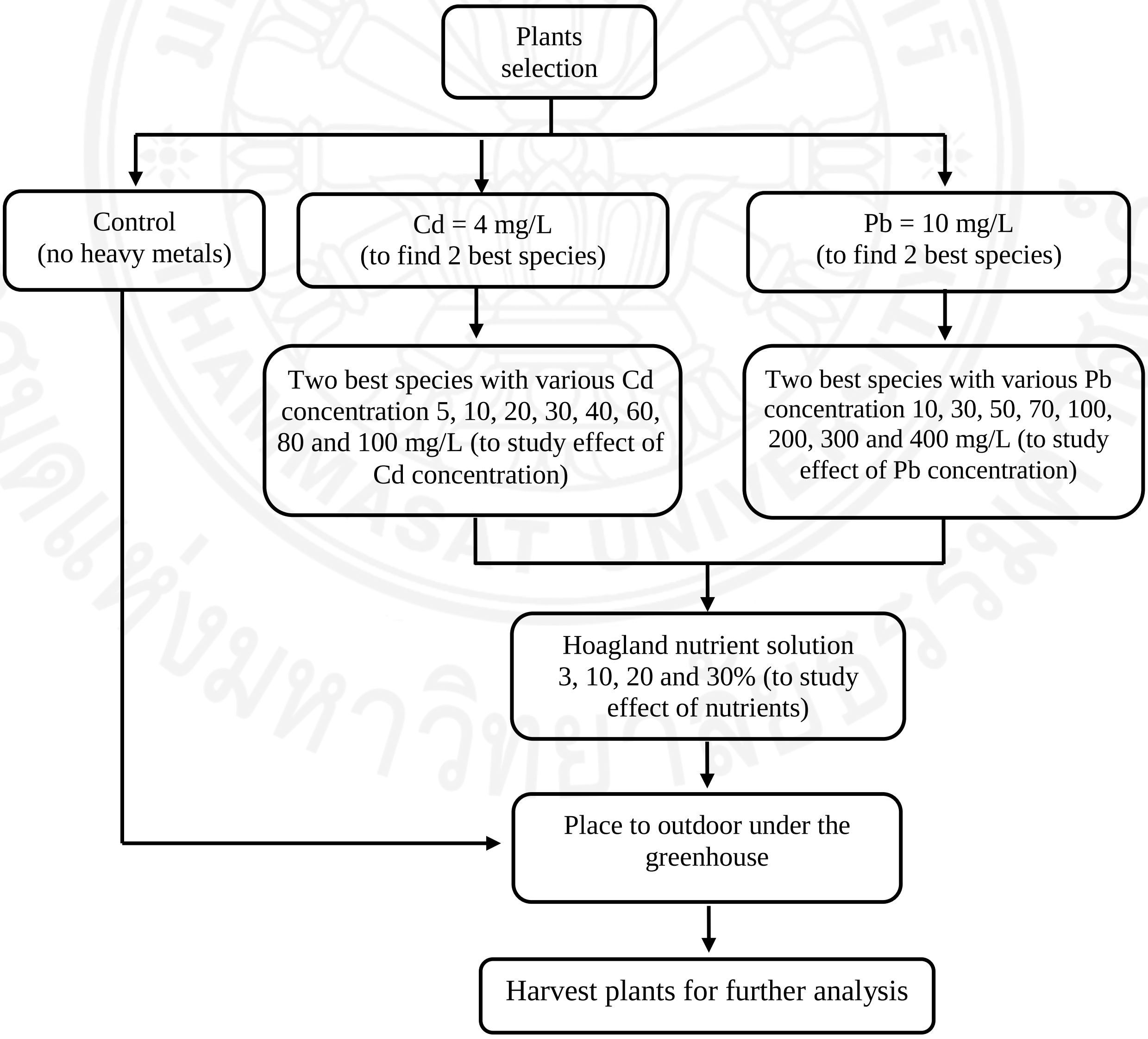


Figure 3.1: Overall research framework.

3.1. Experiment preparation

1) Reactors and Equipment.

Reactors were constructed from plastic material. The dimension of reactors is 18 x 16 x 9.5 cm (length, width, depth) that was used in the experiments. Each reactor was placed on the shelf. To eliminate possible contamination on the reactors and experimental equipment, they were soaked in 10% HNO_3 solution overnight before use in the experiments (Ebrahimpour & Mushrifah, 2008).

2) Floating plants

Water hyacinth, Water lettuce, Creeping waterprimrose and Common duckweed were collected from the channels around Thammasat University, Rangsit Campus. Floating moss was collected from the channels around Asian Institute of Technology (AIT). These plants were transferred to the laboratory in polyethylene bags and washed by tap water, then soaked in water overnight. Before cultivation, they were washed again by reverse osmosis (RO) filtered water to remove the dirt. The weight of plants was measured in g (fresh weight). About 39±2 g of Water hyacinth, Water lettuce, respectively, and 15±0.5 and 3 g for Creeping waterprimrose and Common duckweed, respectively were used for the experiments.

3) Reagents

The names lists of reagents used in the study are listed below:

Table 3.1: Reagents used in this study

No.	Chemical name	Chemical formula
1	Ammonium Molybdate	$(NH4)_6Mo_7O_{24}.4H_2O$
2	Boric Acid	H_3BO_3
3	Cadmium Nitrate	$Cd(NO_3)_2.4H_2O$
4	Calcium Nitrate	$Ca(NO_3)_2.4H_2O$
5	Cobalt Nitrate Hexahydrate	$Co(NO_3)_2.6H_2O$
6	Copper (II) Sulfate	$CuSO_4$
7	Hydrogen Peroxide	H_2O_2

8	Iron (II) Sulfate Heptahydrate	$FeSO_4.7H_2O$
9	Lead (II) Nitrate	$Pb(NO_3)_2$
10	Magnesium Sulfate Heptahydrate	$MgSO_4.7H_2O$
11	Manganese Sulfate Heptahydrate	$MnSO_4.7H_2O$
12	Monopotassium Phosphate	KH_2PO_4
13	Nikel Sulfate Heptahydrate	$NiSO_4.7H_2O$
14	Nitric Acid	HNO_3
15	Potassium Nitrate	KNO_3
16	Potassium Sulfate	K_2SO_4
17	Sodium Hydroxide	NaOH
18	Sodium Tungstate (Natri Volframat)	$NaWO_4.2H_2O$
19	Zinc (II) Sulfate	$ZnSO_4$

4) Stock solution of heavy metals

Stock solution of Cd and Pb (1,000 mg/L) were prepared by dissolving 2,750 mg of $Cd(NO_3)_2.4H_2O$ and 1,599 mg of $Pb(NO_3)_2$ in 1,000 ml of de-ionized water.

5) Hoagland's nutrients solution

The chemical composition of the Hoagland's nutrients solution used in this study is shown in the Table 3.2. After sterilization of this solution, pH was adjusted to 6.5 by NaOH (0.1 N) (Megateli et al., 2009).

Table 3.2: The composition of Hoagland's nutrients solution (Megateli et al., 2009)

Chemical composition	**Hoagland's medium (mg/L)**
$Ca(NO_3)_2.4H_2O$	118
KNO_3	5.055
$MgSO_4.7H_2O$	4.932
KH_2PO_4	0.68
$FeSO_4.7H_2O$	0.307
K_2SO_4	0.348
H_3BO_3	0.286
$MnSO_4.7H_2O$	0.155
$ZnSO_4$	0.022
$CuSO_4$	0.0079
$NiSO_4.7H_2O$	0.00478
$NaWO_4.2H_2O$	0.00179
$(NH4)_6Mo_7O_{24}.4H_2O$	0.0128
$Co(NO_3)_2.6H_2O$	0.0049

3.2. Screening of floating plants for Cd and Pb hyperaccumulators

- Control reactors (no heavy metals), five floating plants were separately cultured in the 3% of Hoagland's nutrients solution (Phetsombat et al., 2006). Water hyacinth, Water lettuce and Creeping waterprimrose were cultivated in 1.5 L of the solution and Floating moss and Common duckweed were cultivated in 0.5 L of the solution. These plants were placed outdoor under the greenhouse.
- Artificial wastewater: Water hyacinth, Water lettuce and Creeping waterprimrose were cultured in 1.5 L of artificial wastewater which was supplemented with 3% of Hoagland's nutrients solution and individual heavy metals concentration (4 mg/L of Cd and 10 mg/L of Pb, respectively). For Floating moss and Common

duckweed were cultured similarly, but the volume of artificial wastewater was 0.5 L. These experiments were done to finding 2 best Hyperaccumulators. Cd and Pb concentration was supplemented by Cd and Pb stock solution. Finally, all plants were carried out to the greenhouse outdoor.

- Additional experiment was set on contaminated water with Cd and Pb without the plants to check Cd and Pb adsorption on the reactors. pH and temperature were measured every 3 day of cultivation. The evaporative loss in each reactor was replaced every day by RO filtered water to control Cd and Pb concentration (Soltan & Rashed, 2003). To indicate the toxicity of heavy metals on plants, the physical plants were also observed on a daily basis.

3.3. Experiment of effect of Cd and Pb concentration

To study the effect of Cd and Pb concentration on the uptake, 2 hyperaccumulators were selected for Cd, and two for Pb based on the results of the screening. They were cultured at the different concentration varying from 5, 10, 20, 30, 40, 60, 80 and 100 mg/L of Cd, and 10, 30, 50, 70, 100, 200, 300 and 400 mg/L of Pb, respectively. The artificial wastewater was supplied with 3% of Hoagland’s nutrients solution. The uptake was compared to find the highest Cd and Pb uptake by floating plants.

3.4. Effect of nutrients on heavy metals uptake

Generally, plants need nutrients to support their health and survive in the environment. Therefore, Hoagland’s nutrients solution was selected for experiments. Hyperaccumulators were cultivated in different Hoagland’s nutrients (3, 10, 20 and 30%), and the heavy metals concentration 40 mg/L of Cd and 100 mg/L of Pb, respectively, to study the effect of nutrients on heavy metals uptake.

3.5. Floating plants and wastewater analysis

Floating plants and wastewater samples were analyzed as shown in Figure 3.2.

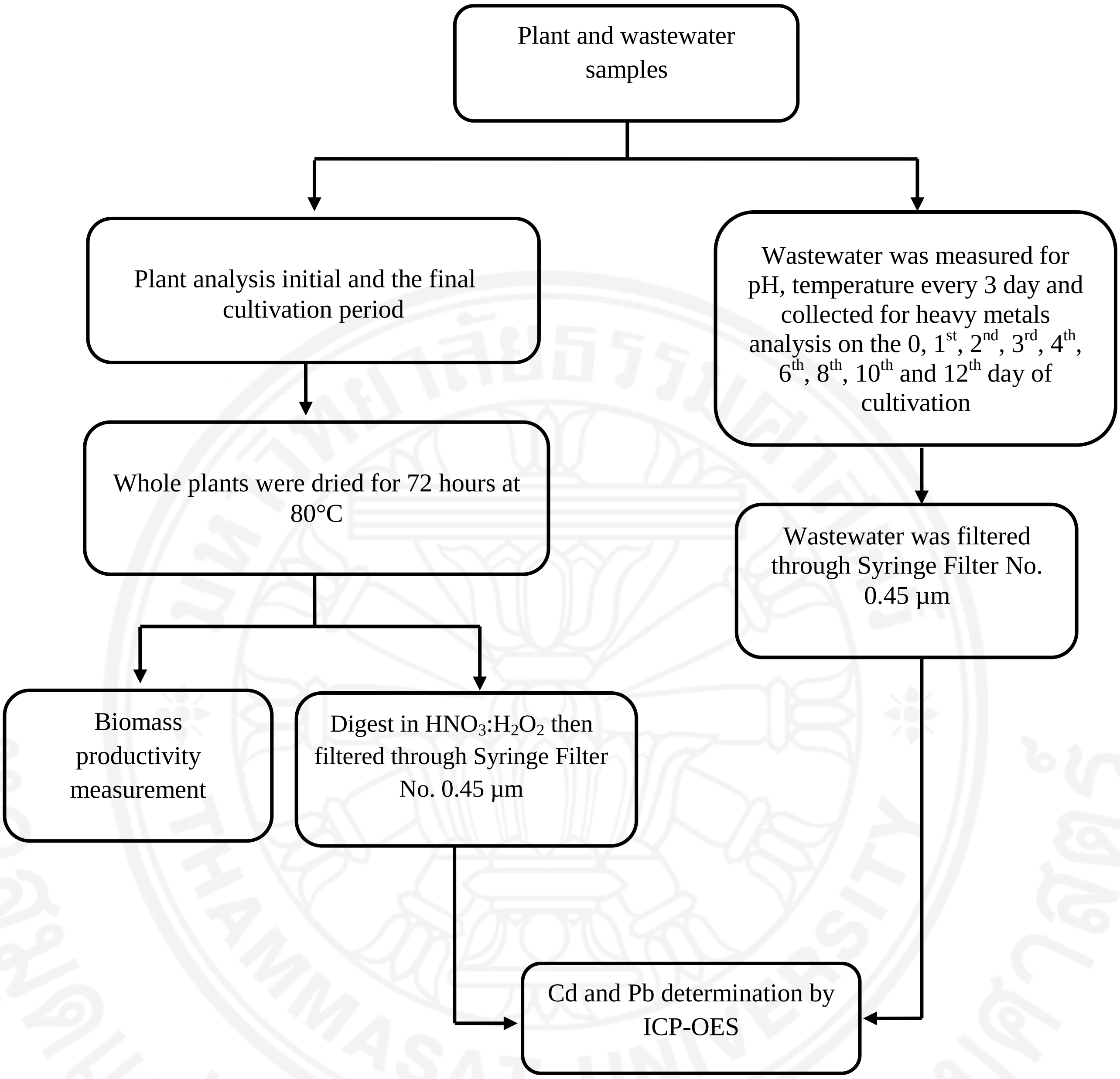


Figure 3.2: Diagram of floating plant and wastewater samples analysis.

3.5.1. Floating plant samples analysis

The process of plant samples analysis is shown in Figure 3.3. Floating plant samples were analyzed for heavy metals, at the start of the experiment and the last day of cultivation. All plant species were carefully washed by using tap water several times and rinsed with RO filtered water to remove all the debris, then dried to constant mass in an oven for 72 hours at 80°C. If the temperature is lower than 80°C, the water will not be removed from plants resulting in poor homogenization and an incorrect analysis. On the contrary, if the temperature is higher than 80°C, it may lead to thermal decomposition and reduction of dry weight (Campbell & Plank, 1998). The dried plant samples were cut to small size as possible by using scissors and mixed until homogenized, then digested by Microwave digestion as following the application of Milestone Ethos Pro at Center of Excellence on Hazardous Substances Management, Metallurgy and Materials Science Research Institute, Chulalongkorn University. The plant samples were digested with HNO_3:H_2O_2 (7:1 ml/ml) at high temperature (200°C) and 980 watt of Microwave power for 50 minutes, then cooled in a hood. After that, the solution was filtered through Syringe Filter No. 0.45 µm. The final volume was made to 50 ml using de-ionized water. Finally, the samples were analyzed in an Inductively Couple Plasma-Optical Emission Spectrometer, Perkin-Elmer Optima 8000 (ICP-OES) to examine the amounts of Cd and Pb. The determination by ICP-OES was performed in triplicate with correlation coefficient>0.98 for all of analysis.

Figure 3.3: The plants digestion process

3.5.2. Wastewater samples analysis

Water samples were collected from each reactor (10 ml) for Cd and Pb analysis on the 0, 1st, 2nd, 3rd, 4th, 6th and 8th day (the last day of cultivation) and the 0, 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th day, respectively. The samples were filtered through Syringe Filter

No. 0.45 µm and analyzed by ICP-OES in the same way as the plant analysis to find Cd and Pb concentration remained in water.

Figure 3.4: Heavy metals analysis by ICP-OES

3.6. Floating plant measurement

3.6.1. Relative growth of floating plants

The plant samples were weighed both before and after cultivation. The relative growth was calculated by the following equation:

$$RG = \frac{FFW}{IFW} \quad \text{......................................} \quad (1)$$

Where, RG is the relative growth, FFW is the final fresh weight, and IFW is the initial fresh weight

3.6.2. Biomass productivity

All plant species were dried in an oven for 72 hours at 80°C. The dried samples were weighted by electronic balance. The biomass productivity was expressed as a percentage decrease of biomass relative to control.

3.6.3. Heavy metal removal efficiency

The removal efficiency of each plant was calculated as following the equation:

$$R(\%) = \left[\frac{C_0 - C_t}{C_0}\right] \times 100 \quad \text{...............................} \quad (2)$$

Where, C_0 and C_t are the residual concentration of heavy metals at the beginning and at time t of the experiment, respectively.

3.6.4. Bioconcentration factor (BCF)

Bioconcentration factor of chemical by plant organism is generally expressed as the BCF, which is the ratio of substance's concentration absorbed by organism (mg/kg) to that dissolved in the surrounding medium (mg/L) (Walker, 1987). BCF was calculated by the following equation:

$$BCF = \frac{Concentration\ of\ metals\ in\ dried\ plant}{Initial\ concentration\ of\ metals\ in\ solution} \ldots\ldots\ldots\ldots\ldots (3)$$

The mean of heavy metal concentration from duplicate experiments in the artificial wastewater is reported in mg/L, and heavy metals containing in plants were reported in mg/g (dry weight).

Chapter 4

Results and discussion

This chapter describes the effectiveness of floating plants on Cd and Pb removal from artificial wastewater. During the experiment, the pH of Cd and Pb contaminated water was 5.8-6.8 and 5.3-6.5, respectively. The temperature was between 27 and 31ºC. Rhizofiltration is the main mechanism for phytoremediation. It is clean up or remediation technology using plants to remove inorganic compounds or heavy metals from groundwater, surface water or wastewater. The contaminants are absorbed by plant roots. Hence, the plant used for rhizofiltration should have a fibrous and large root and increasing root area (Etim, 2012). In rhizofiltration technology, the plant retains heavy metals within its root, especially Cd, Pb, Cu, Ni, Zn and Cr (USEPA, 2000). The results of Cd and Pb removed by selected floating plants are presented in this section.

4.1. Wastewater contaminated with Cd

4.1.1. Screening hyperaccumulators for Cd

1) Visual changes observed in floating plants at 4 mg/L of Cd

The plants were healthy during the cultivation in the Hoagland medium (control). In contrast, they looked unhealthy in Cd contaminated water with time increasing. The visual changes observed in all floating plants are summarized in Table 4.1 and Figure 4.1 to Figure 4.5. Water hyacinth looked unhealthy with some leaves turning yellow on the 3rd day while Common duckweed had yellow leaves after cultivation of only 1 day and almost dead on the 3rd day. From the observation of plants on the 2nd to the last day, Water lettuce looked unhealthy with some leaves turning yellow. Similarly, Floating moss had some leaves turning brown. For the observation of Creeping waterprimrose, it looked unhealthy with some leaves turning brown on the 2nd day and started looking unhealthy until the last day. From visual changes observed in plants, it can conclude that this was caused by Cd toxicity. Nagajyoti et al. (2010) reported that Cd affects plants because it reduces the nitrate absorption and its transportation from root to shoot, and it also decreases the water content in the plants. The study of Sooksawat et al. (2013) indicated that *Nitella opaca* died in Cd contaminated water at 0.5 mg/L.

Table 4.1: Visual changes observed in floating plants (4 mg/L of Cd)

Plant species	**Exposure times (day)**				
	0	1	2	3	4
Water hyacinth	H	H	H	UY	UY
Water lettuce	H	H	UY	UY	UY
Creeping waterprimrose	H	H	UY	UB	UB
Floating moss	H	H	UB	UB	UB
Common duckweed	H	UY	UB	UM	-

Note: H, the plant looked healthy with green leaves; UY, the plant looked unhealthy with some leaves turning yellow; UB, the plant looked unhealthy with some leaves turning brown; and UM, the plant looked unhealthy and almost dead.

Water hyacinth in Hoagland medium

0 day 2 days 4 days

Water hyacinth in Cd contaminated water (4 mg/L)

0 day 2 days 4 days

Figure 4.1: Visual changes observed in Water hyacinth at 4 mg/L of Cd

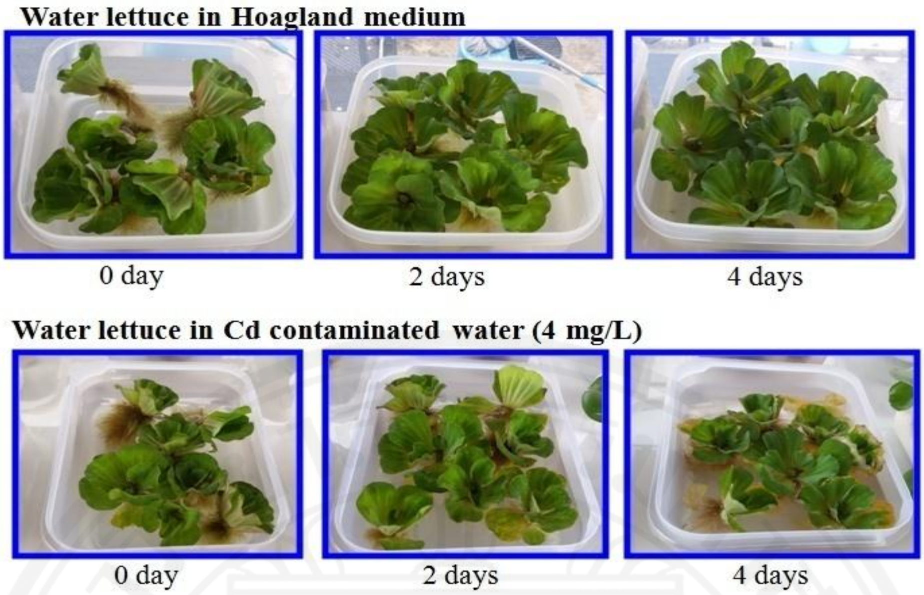


Figure 4.2: Visual changes observed in Water lettuce at 4 mg/L of Cd

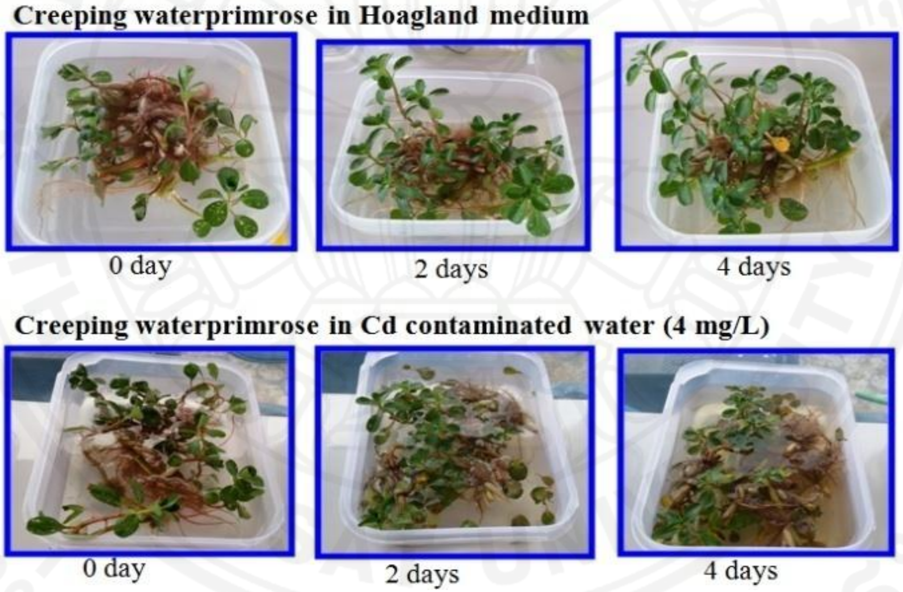


Figure 4.3: Visual changes observed in Creeping waterprimrose at 4 mg/L of Cd

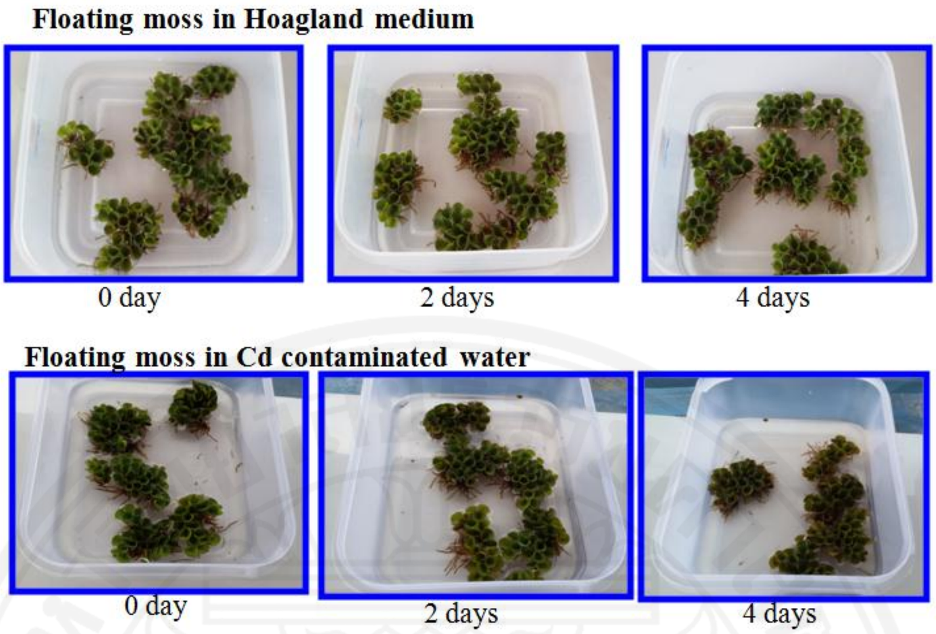


Figure 4.4: Visual changes observed in Floating moss at 4 mg/L of Cd

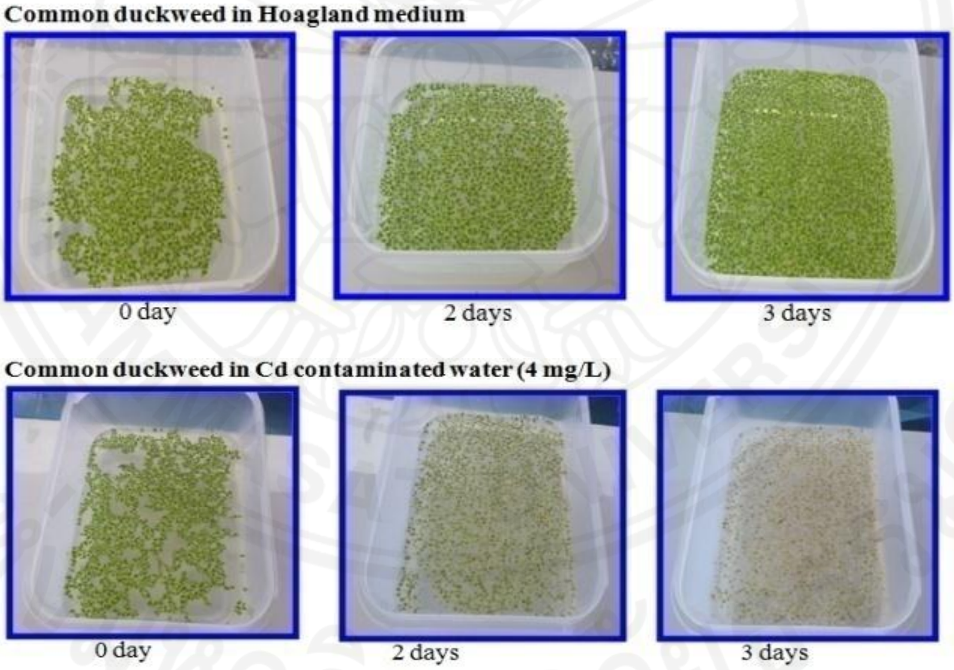


Figure 4.5: Visual changes observed in Common duckweed at 4 mg/L of Cd

2) Relative growth of floating plants at 4 mg/L of Cd

A comparison of plant relative growths in Hoagland medium and Cd contaminated water is shown in Figure 4.6. Relative growth of plants, Water hyacinth (0.93), Water lettuce (0.97) and Creeping waterprimrose (0.97), declined slightly showing the Cd tolerance of plants at 4 mg/L for 4 days. While, relative growth of Common duckweed decreased drastically. It was lower than the relative growths in Hoagland medium more than 2 times (0.63 in contaminated water and 1.62 in Hoagland medium). The reduction in Floating moss' relative growth (0.65) was also observed. This shows that Cd toxicity affects the relative growth of some plants. Lu et al. (2004) reported that Cd inhibits the growth of plants. For instance, 50% decrease in relative growth was observed in Common duckweed at 0.89 mg/L of Cd (Chaudhuri et al., 2014).

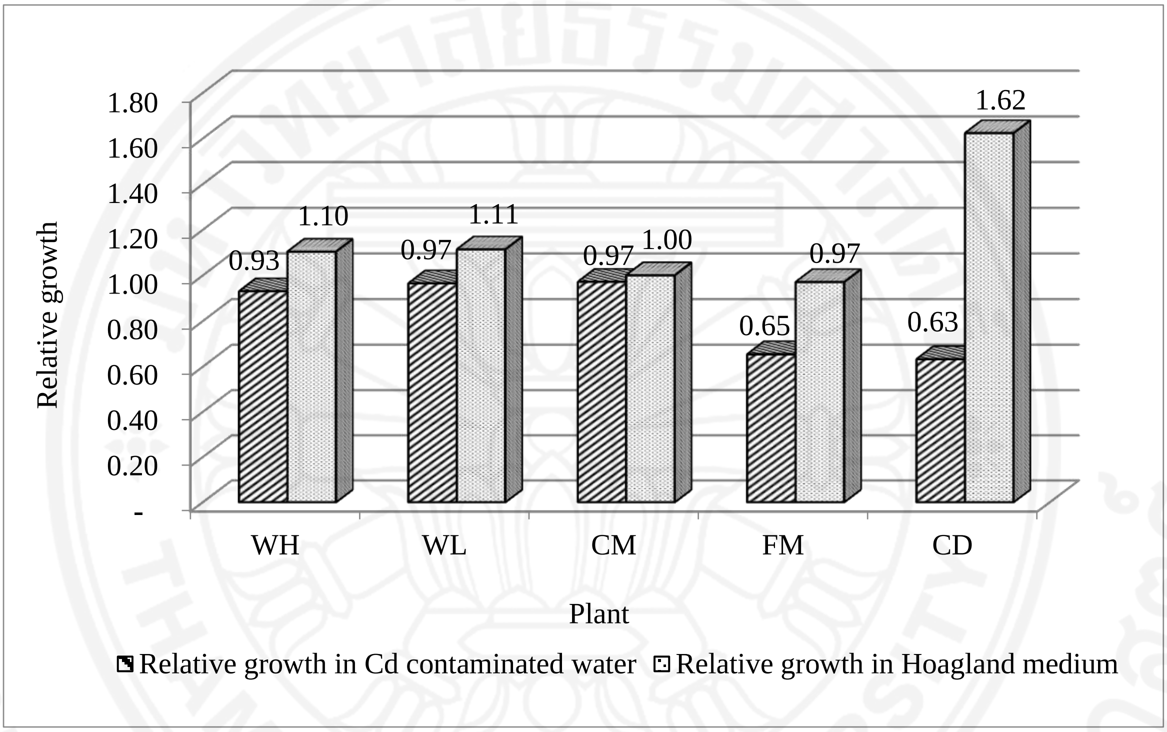


Figure 4.6: Relative growth of floating plants (fresh weight) as compared to control

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

3) Biomass productivity at 4 mg/L of Cd

The biomass productivity of plants for 4 days cultivation and Cd concentration at 4 mg/L was greater than 85%, except Common duckweed (Figure 4.7). The highest biomass productivity was found in Floating moss (99.4%). This disagrees with Phetsombat et al. (2006). They found that Floating moss had low biomass productivity (39.7%) at the same Cd concentration and period of cultivation as in the present study. The biomass productivity of Water hyacinth, Water lettuce and Creeping waterprimrose was found as 92.6, 85.7 and 98.8%, respectively.Carranza-Álvarez et al. (2008) stated that the high biomass production is a characteristic of plants for phytoremediation. Thus, the floating plants investigated in this study (except Common duckweed) can be candidates for phytoremediation. The biomass productivity of Common duckweed for 3 days cultivation was the lowest (37%). This might be caused by Cd toxicity. Common duckweed seemed to be sensitive to Cd. Therefore, Cd might affect the biomass productivity of some plant species. According to Suchismita et al. (2014), biomass productivity of Water hyacinth, and Water lettuce decreased because of the Cd toxicity. The study of Fayiga et al. (2004) also reported that the biomass productivity of *Pteris vittata* L. decreased with increase in Cd concentration.

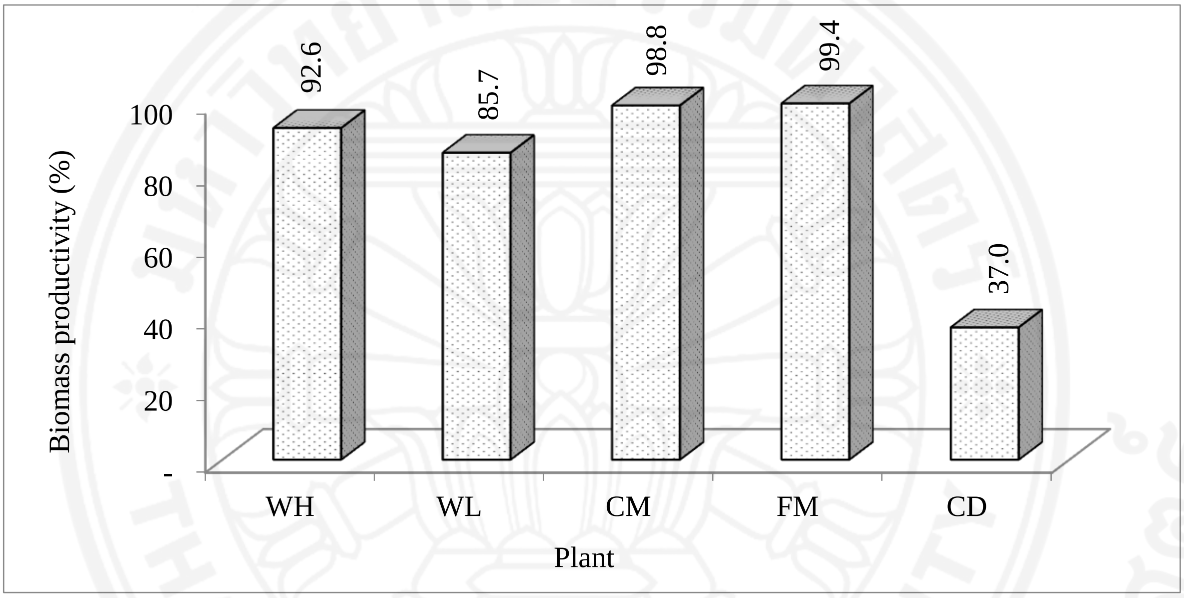


Figure 4.7: Biomass productivity of floating plants (dry weight)

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

4) Cd removal efficiency at 4 mg/ L of Cd

The initial concentration of Cd (4 mg/L) in each reactor decreased drastically within 1 day. It shows that all plants could remove Cd even in the short time of cultivation. On the 1st day of cultivation, Water hyacinth, Water lettuce, Creeping waterprimrose, Floating moss and Common duckweed were able to remove Cd 63.3, 78.2, 81.8, 89.8 and 70.4%, respectively. A Study by Maine et al. (2001) also reported that Water lettuce could remove Cd 72% within the first 24 hours of the experiment when it was cultivated in contaminated water with Cd at 4 mg/L. Figure 4.8 shows that Floating moss, Creeping waterprimrose and Water lettuce removed Cd slowly after 1st day, and became almost constant until the end of cultivation. At the same time, Common duckweed removed so fast from the 1st to 2nd day (from 70.4 to 87.9%) and it was almost constant on the last day of cultivation (on the 3rd day). Even though the Cd removal efficiency of Water hyacinth was the lowest on the 1st day, it could remove Cd gradually until the end of cultivation. However, the highest Cd removal efficiency on the last day of cultivation was found for Floating moss (94%), followed by Creeping waterprimrose (90.8%), Water lettuce (89.3%), Common duckweed (87.8%) and Water hyacinth (81%). The results were similar with the study of Mishra and Tripathi (2008). It was found that Water hyacinth and Water lettuce were able to remove Cd 77 and 70 % at the initial 5 mg/L, respectively. The high percentage of Cd removal caused by plants is due to high absorption and high uptake (Parlak et al., 2013). In addition, the result shows that no Cd absorption occurs on reactor wall. Therefore, the decreased in Cd concentration in water was mainly caused by plants absorption.

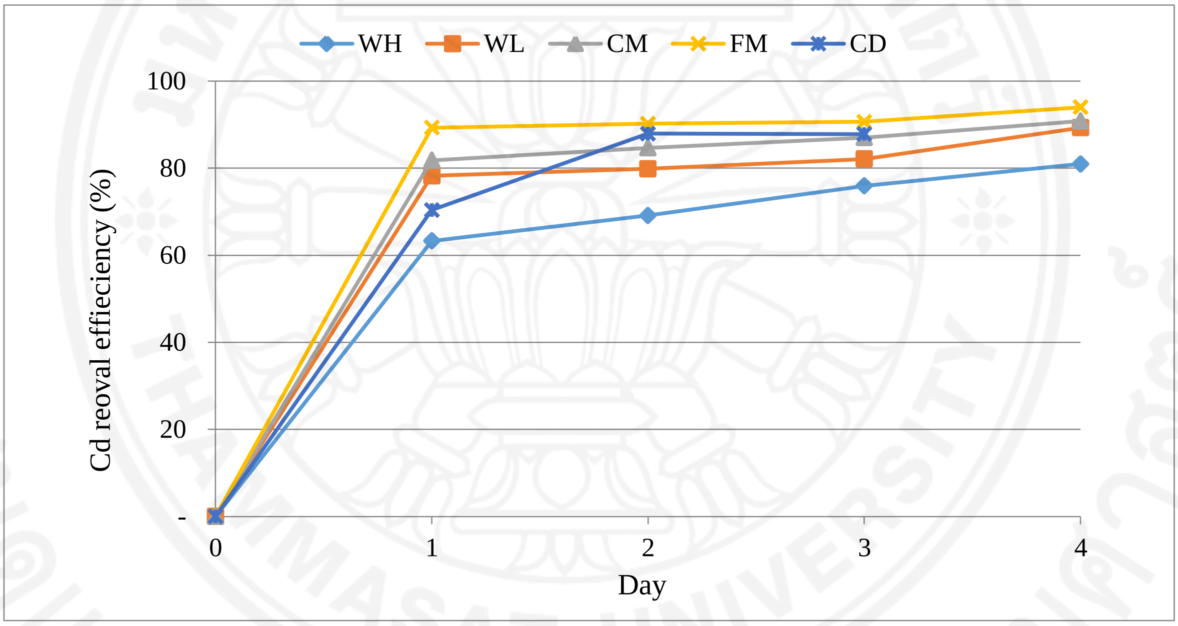


Figure 4.8: Cd removal efficiency by different floating plants at 4 mg/L

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

5) Cd accumulation in floating plants at 4 mg/L

The Cd accumulation in plants was determined at the start of the experiment. The results demonstrated that Cd accumulation in the floating plants used for the study was negligible. After the plants harvesting, the Cd accumulation in plants is shown in Table 4.2. The highest Cd accumulation in the plant was found in Common duckweed (19 mg/g dry weight). The lowest Cd accumulation in the plant (1.6 mg/g dry weight) was found in Creeping waterprimrose. It was also found that the Cd accumulation in Water hyacinth, Water lettuce and Floating moss was 2.7, 2.2 and 2.4 mg/g, respectively. The different Cd accumulation in each plant might be because of the effectiveness of plant species and amount of initial fresh weight used in the present experiment. Gupta and Sinha (2007) reported that the uptake of heavy metals and accumulation depends on the available concentration of heavy metals in water, sequential solubility and the plant species. Table 4.2 summarizes Cd accumulation in different plant species and is compared with this study. It is seen that the Cd accumulation in plant species is different. Variation may be due to different culture conditions and initial fresh weight of plants in water.

Table 4.2: A comparison of Cd accumulation by floating plants

Floating plants		Cd conc. (mg/L)	Cd accumulation (mg/g)				Period (day)	Sources
Common names	Scientific names		Roots	Leaves	Whole	Shoots		
Water hyacinth	*Echhornia crassipe*	4			2.7		4	This study
		5			0.3		15	Mishra and Tripathi (2008)
		4	2.04			0.11	12	Lu et al. (2004)
Water lettuce	*Pistia stratiotes*	4			2.2		4	This study
		5			0.3		15	Mishra and Tripathi (2008)
Creeping waterprimrose	*Jussiaea repens*L.	4			1.6		4	This study
Floating moss	*Salvinia cucullata*	4			2.4		4	This study
		4	1.6	0.7			8	Phetsombat et al. (2006)
Common duckweed	*Lemna minor*	4			19		4	This study
		3			3.4		22	Chaudhuri et al. (2014)
-	*Nitella opaca*	0.5			1.5		6	Sooksawat et al. (2013)

6) Bioconcentration factor at 4 mg/L of Cd

Bioconcentration factor (BCF) indicates the metal accumulation in plants which is calculated in dry weight basis. The BCF values for different plant species are shown in Figure 4.9. The maximum BCF was found in Common duckweed (4,859), indicating that Cd uptake is better than other species. Study by Chaudhuri et al. (2014) also found the

BCF value greater than 1,000 for Common duckweed indicating that it is an efficient accumulator of Cd. Sooksawat et al. (2013) reported that if BCF is greater than 1000, it can be qualified as good hyperaccumulator. By comparing the BCF values in this study, the BCF for Creeping waterprimrose appeared to be low indicating poor accumulator (398). For other species, BCFs indicated that they were the moderate Cd accumulators, except Common duckweed. The BCF for Water hyacinth, Water lettuce and Floating moss was 703, 554 and 613, respectively. Lu et al. (2004) reported BCF for Water hyacinth was 622 at 2 mg/L of Cd. Phetsombat et al. (2006) found the BCF for Floating moss was 578 at 4 mg/L of Cd. In this study, the BCF for Common duckweed was the highest, although, its biomass was the lowest as shown in Figure 4.7. Generally, the amount of Cd accumulation in plants depends on their biomass productivity also. As the removal efficiency was high, but biomass productivity was low, thus the bioconcentration factor per unit weight was high.

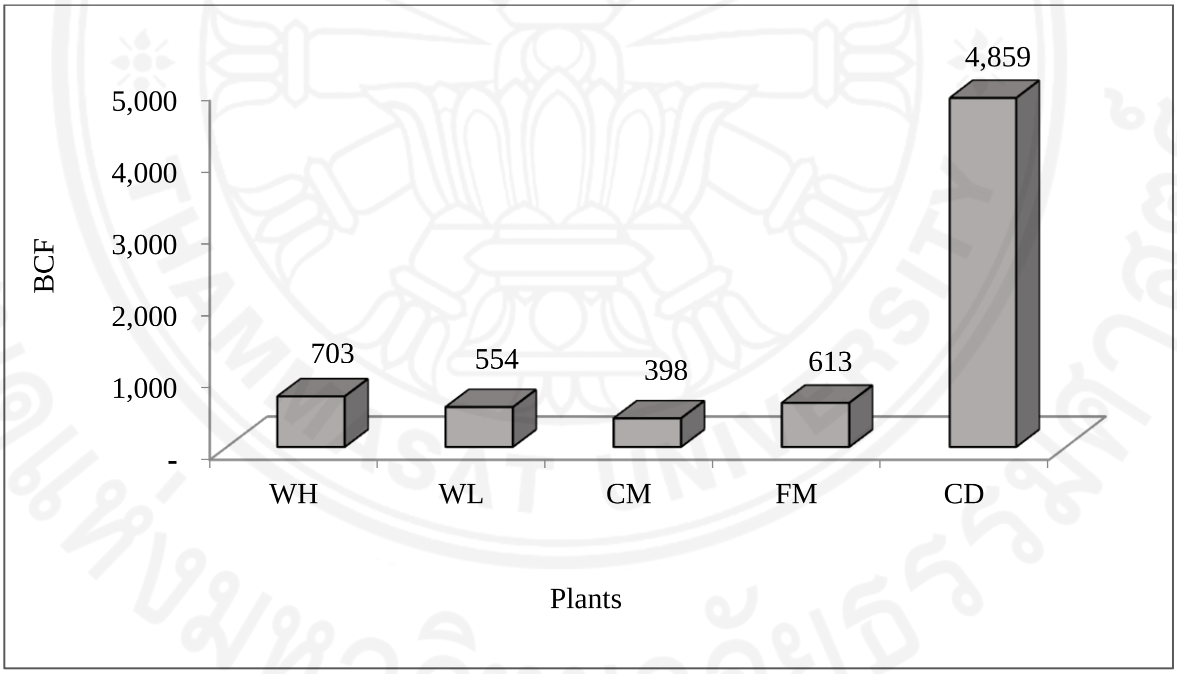


Figure 4.9: Bioconcentration factor of different floating plants

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

4.1.2. Effect of different Cd concentration on uptake

Based on the results of the screening experiment (4 mg/L of Cd), Creeping waterprimrose, Floating moss and Common duckweed were appropriate for the further experiments. However, Common duckweed was not selected to study the effect of different Cd concentration on uptake by plant. Even though it had the highest Cd accumulation and also highest BCF, it could not survive well as other species (only 3 days). Therefore, two hyperaccumulators of Cd, Creeping waterprimrose and Floating moss, were selected. They were tolerant with Cd toxicity as they look healthy with green leaves during cultivation for 4 days. They also had high biomass productivities and Cd removal efficiency.

To study the effect of different Cd concentration on uptake, Creeping waterprimrose and Floating moss were cultivated in Cd contaminated water at 5, 10, 20 and 30 mg/L for 6 days, and 8 days at 40, 60, 80 and 100 mg/L

1) Visual changes observed in floating plants at different Cd concentration

The plants looked unhealthy with increasing Cd concentration and exposure times. Table 4.3 and Figure 4.10 show the visual changes observed in Creeping waterprimrose at different Cd concentration. In the reactor with 5 mg/L of Cd, Creeping waterprimrose looked unhealthy and partial wilting was observed on the 2nd day of cultivation. Inhibition continued till the last day with some yellow leaves falling down in the reactors. On the 1st day, Creeping waterprimrose in each reactor looked unhealthy with partial wilting, except the plant in the reactor at 5 mg/L of Cd. When the Cd concentration increased from 10 to 30 mg/L, it looked similarly unhealthy and almost dead on the 6th day (the last day of cultivation). Table 4.3 also shows that Creeping waterprimrose looked unhealthy with some leaves turning yellow, felling down and turning brown when the concentration increased from 40 to 100 mg/L. Finally, it died completely on 8th, 7th, 6th and 6th day at 40, 60, 80 and 100 mg/L, respectively.

The visual changes observed in Floating moss is summarized in Table 4.4 and Figure 4.11. The plants looked unhealthy with increasing Cd concentration and exposure times. The plant at 5 mg/L was healthier than other concentration for 2 days, but it looked unhealthy with some leaves turning yellow on the 3rd day and some leaves turning brown on the last day (the 6th day). For other Cd concentration, plants looked unhealthy after short period (1 day), especially at increasing concentration from 60 to 100 mg/L. They looked unhealthy with some leaves turning yellow, brown with exposure times, and died completely on the 7th day. This is because of the Cd toxicity.

Based on the results, it can conclude that Creeping waterprimrose and Floating moss were quite tolerant at 5 mg/L of Cd for 1 day and 2 days, respectively. Moreover, Floating moss was healthy for 1 day at Cd concentration from 10 to 40 mg/L, but Creeping waterprimrose was unhealthy with partial wilt. On the last day of cultivation (8th day) at 40 mg/L, Floating moss looked almost dead, while, Creeping waterprimrose died completely. It was also observed that at concentration from 80 to 100 mg/L, Floating moss died completely on the 7th day, while, Creeping waterprimrose on the 6th day. This shows that Floating moss was a bit better than Creeping waterprimrose. The study of Li et al. (2008) found that Creeping waterprimrose could survive for 7 days at 40 mg/L. Suchismita et al. (2014) stated that plants looking unhealthy with some leaves turning yellow can be due to the chlorophyll synthesis inhibition. Their wilting might be cause by Cd. Cd induces the suppression of transpiration by stomatal closure and also reduces stomatal conductance which presumably affects metabolism and reduces photosynthesis.

Table 4.3: Visual changes observed in Creeping waterprimrose at different Cd concentration

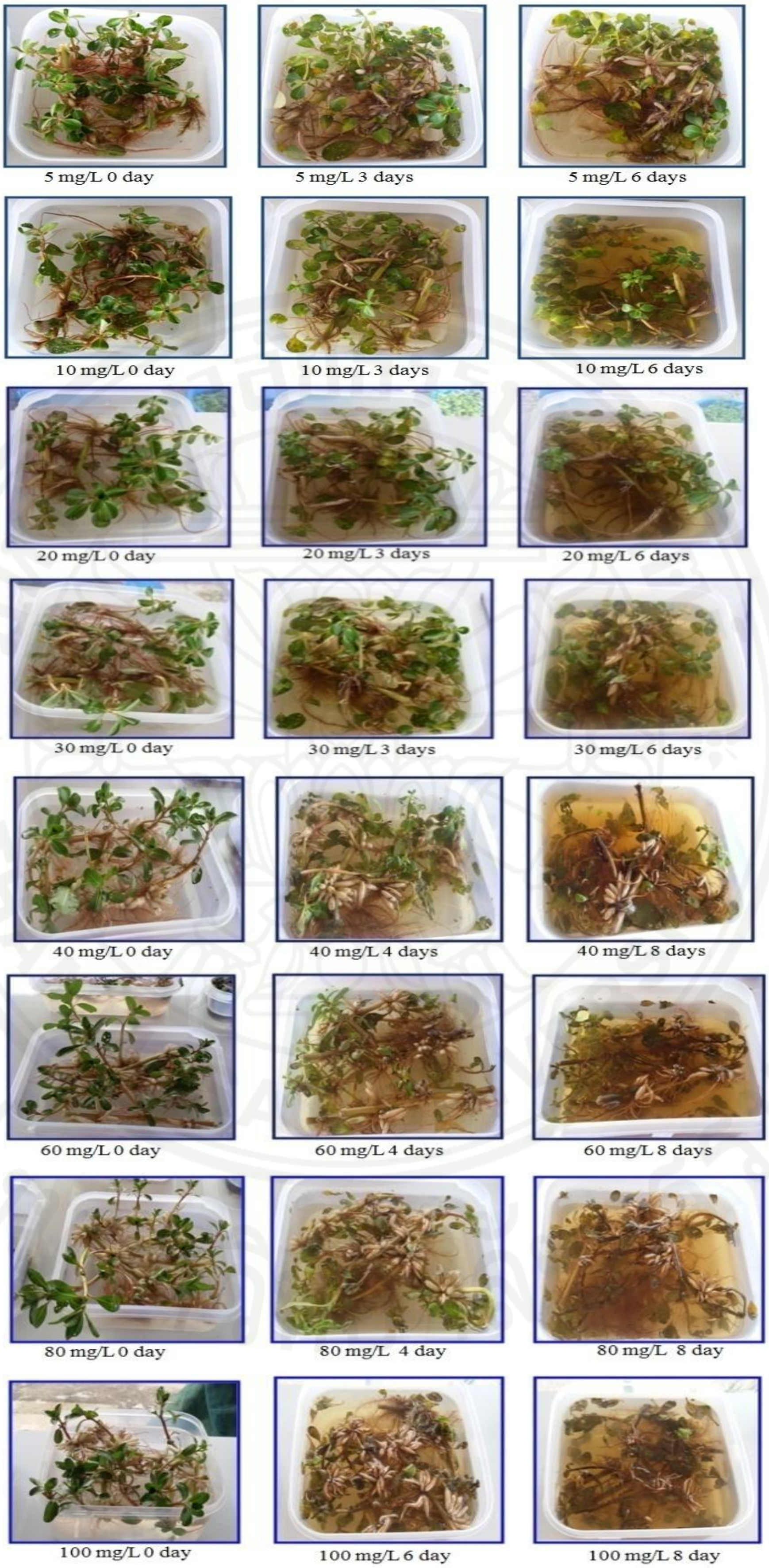
Cd Conc. (mg/L)	**Exposure times (day)**								
	0	1	2	3	4	5	6	7	8
5	H	H	UP	UY*	UY*	UY*	UY*	-	-
10	H	UP	UY	UY*	UB*	UM	UM	-	-
20	H	UP	UY	UY*	UY*	UM	UM	-	-
30	H	UP	UY	UY*	UM	UM	UM	-	-
40	H	UP	UP	UY*	UB*	UB*	UB*	UM	CD
60	H	UP	UP	UY*	UB*	UB*	UM	CD	CD
80	H	UP	UP	UY*	UB*	UM	CD	CD	CD
100	H	UP	UY*	UB*	UM	UM	CD	CD	CD

Table 4.4: Visual changes observed in Floating moss at different Cd concentration

Cd conc. (mg/L)	Exposure times (day)								
	0	1	2	3	4	5	6	7	8
5	H	H	H	UY	UY	UY	UB	-	-
10	H	H	UY	UY	UY	UB	UB	-	-
20	H	H	UY	UB	UM	UM	UM	-	-
30	H	H	UY	UB	UM	UM	UM	-	-
40	H	H	UY	UB	UM	UM	UM	UM	UM
60	H	UY	UB	UB	UM	UM	UM	CD	CD
80	H	UY	UB	UB	UM	UM	UM	CD	CD
100	H	UY	UB	UM	UM	UM	UM	CD	CD

Note: H, the plant looked healthy with green leaves; UY, the plant looked unhealthy with some leaves turning yellow; UB, the plant looked unhealthy with some leaves turning brown; UP, the plant looked unhealthy with partial wilting; UM, the plant looked unhealthy and almost dead; and CD, the plant looked completely dead.

*Yellow leaves fell down



5 mg/L 0 day 5 mg/L 3 days 5 mg/L 6 days

10 mg/L 0 day 10 mg/L 3 days 10 mg/L 6 days

20 mg/L 0 day 20 mg/L 3 days 20 mg/L 6 days

30 mg/L 0 day 30 mg/L 3 days 30 mg/L 6 days

40 mg/L 0 day 40 mg/L 4 days 40 mg/L 8 days

60 mg/L 0 day 60 mg/L 4 days 60 mg/L 8 days

80 mg/L 0 day 80 mg/L 4 day 80 mg/L 8 day

100 mg/L 0 day 100 mg/L 6 day 100 mg/L 8 day

Figure 4.10: Visual changes observed in Creeping waterprimrose at different Cd concentration

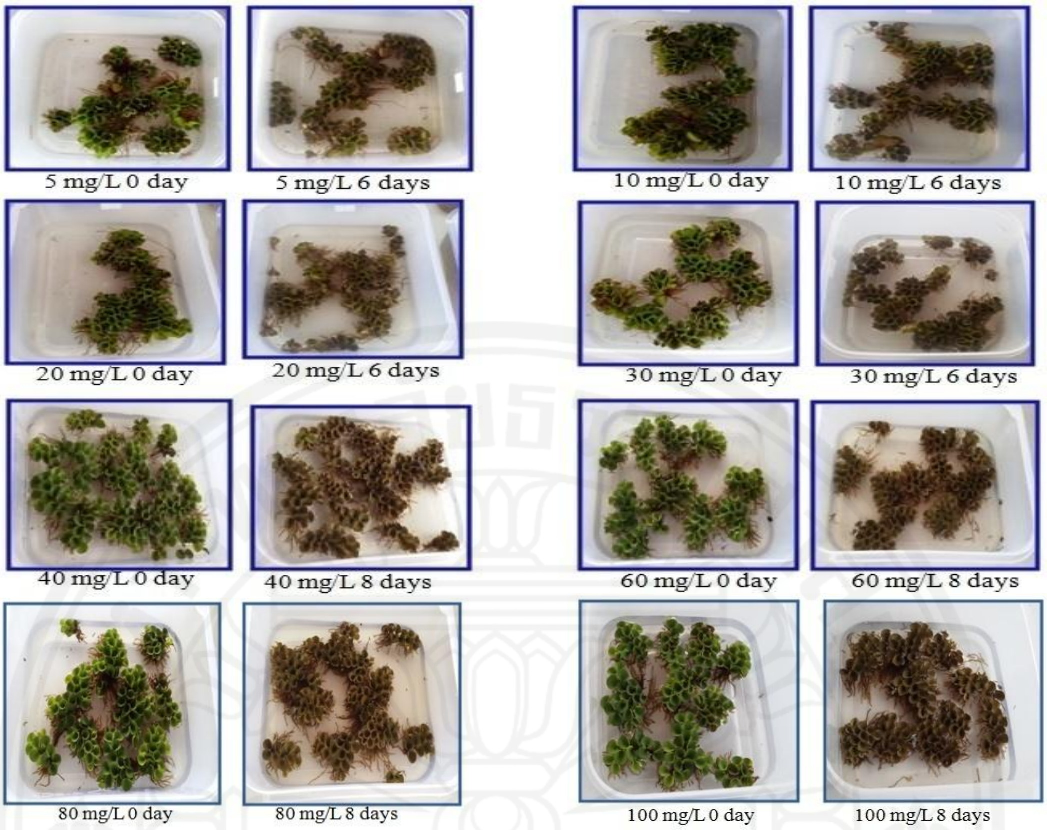


Figure 4.11: Visual changes observed in Floating moss at different Cd concentration

2) Relative growth of floating plants at different Cd concentration

The effect of Cd concentration on the relative growth of Creeping waterprimrose and Floating moss is shown in Figure 4.12. The relative growth of plants in each concentration was compared with the relative growth in Hoagland medium (control). For Creeping waterprimrose, the relative growth decreased gradually with increasing Cd concentration. The decreasing relative growth at each concentration ranged from 0.98 to 0.89 at concentration from 5 to 100 mg/L.

For the relative growth of Floating moss, it decreased slightly at concentration from 5 to 20 mg/L in range from 0.99 to 0.89, but it declined drastically at concentration from 30 to 100 mg/L in range from 0.8 to 0.64. Kay et al. (1984) reported that Cd reduces 10% relative growth of plants when compared with control. The results in current study also show that Cd affects the relative growth of some plants. It may depend on the water contained in the plants. Creeping waterprimrose has higher percentage of moisture than Floating moss, and shows higher relative growth. The reduction of relative growth also depends on the Cd concentration. As the Cd concentration at 5 mg/L, relative growth of

Floating moss was similar with the one in Hoagland medium, while it was almost 2 times decreasing at 100 mg/L.

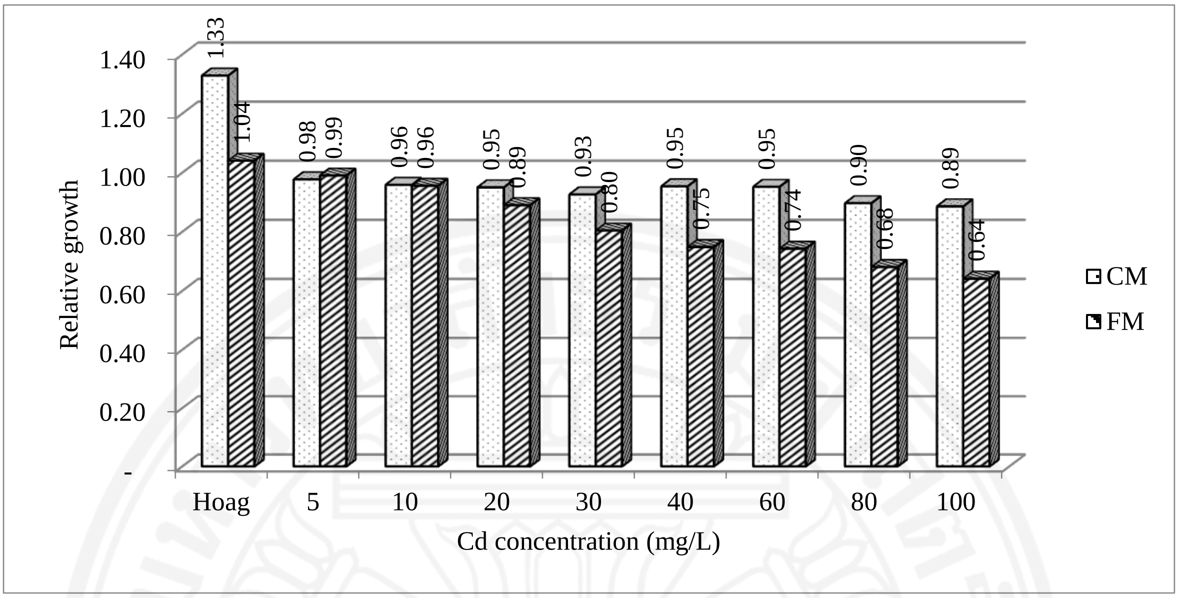


Figure4.12: Relative growth of Creeping waterprimrose and Floating moss at different Cd concentration

Note: Hoag, Hoagland’s nutrient solution; CM, Creeping waterprimrose and FM, Floating moss.

3) Biomass productivity of floating plants at different Cd concentration

The effect of variable Cd concentration on biomass productivity is shown in Figure 4.13. The biomass productivity decreased with increasing Cd concentration. The period of cultivation was 6 days at concentration from 5 to 30 mg/L and 8 days at concentration from 40 to 100 mg/L. For Creeping waterprimrose, it had the highest biomass productivity (92.1%) at 5 mg/L and lowest one was found at 100 mg/L (79.7%). Similarly, Floating moss had the highest biomass (93.9%) at 5 mg/L and the lowest one was found at 100 mg/L (81.4%). Biomass productivity decreased gradually with increasing concentration. The decrease in biomass productivity might be owing to disturbed carbohydrate and nitrogen metabolisms, and reduction in protein synthesis or low photosynthetic reactions as observed under metal stress conditions (Suchismita et al., 2014). Based on the result of this study, Creeping waterprimrose and Floating moss had high biomass productivity, indicating that the Cd toxicity did not affect much to biomass productivity of plants. Therefore, Creeping waterprimrose and Floating moss can be considered for phytoremediation and as hyperaccumulators.

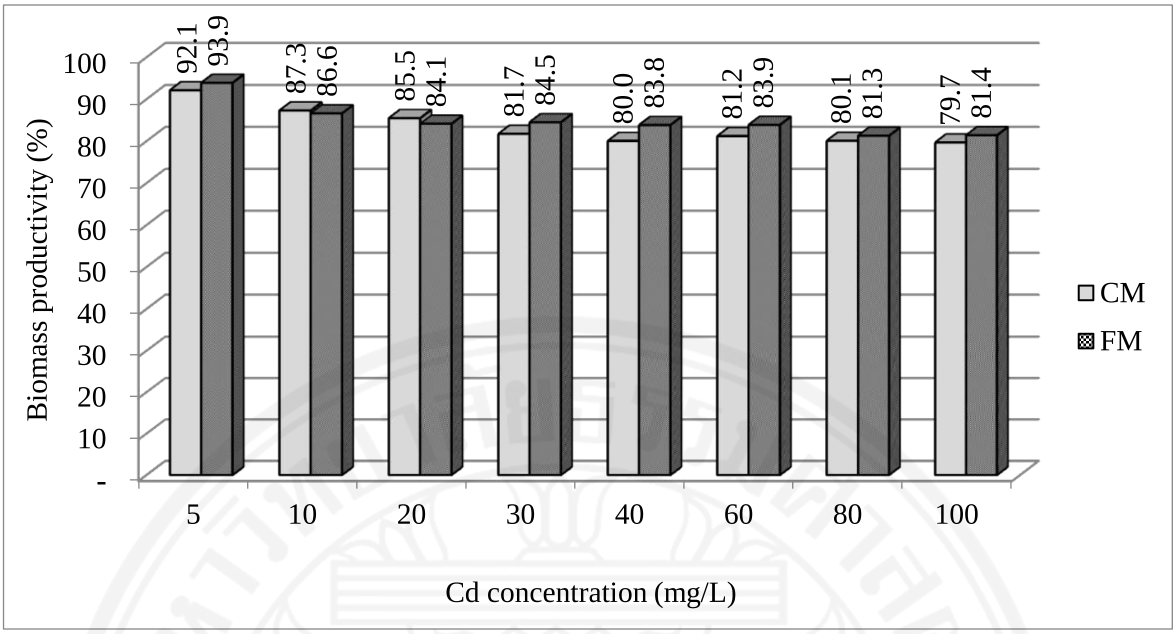


Figure 4.13: Biomass productivity of Creeping waterprimrose and Floating moss at different Cd concentration

Note: CM, Creeping waterprimrose and FM, Floating moss.

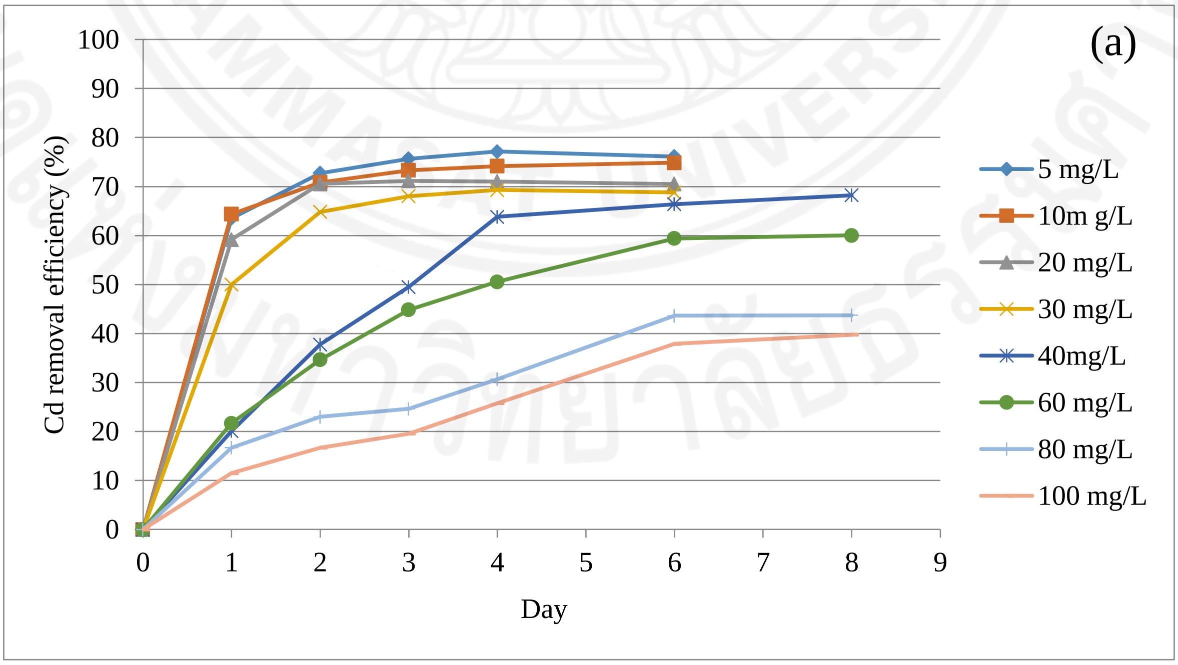
4) Cd removal efficiency of floating plants at different Cd concentration

The floating plants were cultivated for 6 days at Cd concentration 5, 10, 20 and 30 mg/L, and for 8 days at 40, 60, 80 and 100 mg/L. The Cd removal efficiency of floating plants increased with exposure times, but it decreased when the Cd concentration increased. Figure 4.14 (a) shows the Cd removal from artificial wastewater by Creeping waterprimrose. At Cd concentration from 5 to 30 mg/L, Creeping waterprimrose could remove Cd drastically within 1 day. It was constant from the 2nd day to the last day of cultivation. The Cd removal efficiency of Creeping waterprimrose declined from 76.1 to 68.8% with increasing concentration from 5 to 30 mg/L. Although the removal efficiency was low on the 1st day at 40 and 60 mg/L, Creeping waterprimrose was able to remove Cd gradually with increase in times. From the 6th to 8th day at 40 and 60 mg/L, the Cd remaining in wastewater was almost constant. It was found that Cd was removed 68.2 and 60% on the last day of cultivation, respectively. The results also show that the Cd at 80 and 100 mg/L seemed to be removed difficultly on the 1st day. Cd removal efficiency was only 16.7 and 11.5%, respectively, but the last day it increased to 43.7 and 39.7%, respectively.

Floating moss was able to remove Cd in a short period. Cd removal efficiency increased with exposure times, but it decreased with increasing concentration. At concentration from 5 to 100 mg/L on the 1st day, Cd was removed in range from 83.3 to

38.7% and from 89.2 to 53.2% on the last day of cultivation as shown in Figure 4.14 (b). The decreasing Cd removal efficiency with increasing concentration in this study is in line with the study of Maine et al. (2001). It found that Water lettuce could remove Cd from artificial wastewater 87.8, 86.8 and 86.6 % at 1, 2, and 6 mg/L, respectively. Thus, it can demonstrate that Cd removal depends on initial concentration.

Based on the Cd removal efficiency of Creeping waterprimrose and Floating moss at all studied concentration, the Cd remaining in contaminated water was still higher than standard of industrial wastewater effluent of Pollution Control Department (PDC, 2004), Ministry of Natural Resources and Environment in Thailand (Cd is not over than 0.03 mg/L allowed to discharge to environment). Comparing the Cd removal efficiency of both species, Floating moss could remove Cd from artificial wastewater more than Creeping waterprimrose even though the concentration was changed from 5 to 100 mg/L (Figure 4.15). At 5 mg/L, Floating moss could remove Cd 89.2%, while 76.1% was removed by Creeping waterprimrose. And also at 100 mg/L, Cd removal was 53.2% by Floating moss and 39.7% by Creeping waterprimrose. According to Rıdvan Sivaci et al. (2004), heavy metal removal depends on plant species. As their result shows that *Myriophyllum triphyllum* was more effective than *Myriophyllum spicatum* L. at 16 mg/L of Cd concentration. Chaudhuri et al. (2014) also revealed that *Spirodela polyrhiza* could remove 52.7% and *Lemna minor* was 40.7% at 3 mg/L of Cd, respectively.



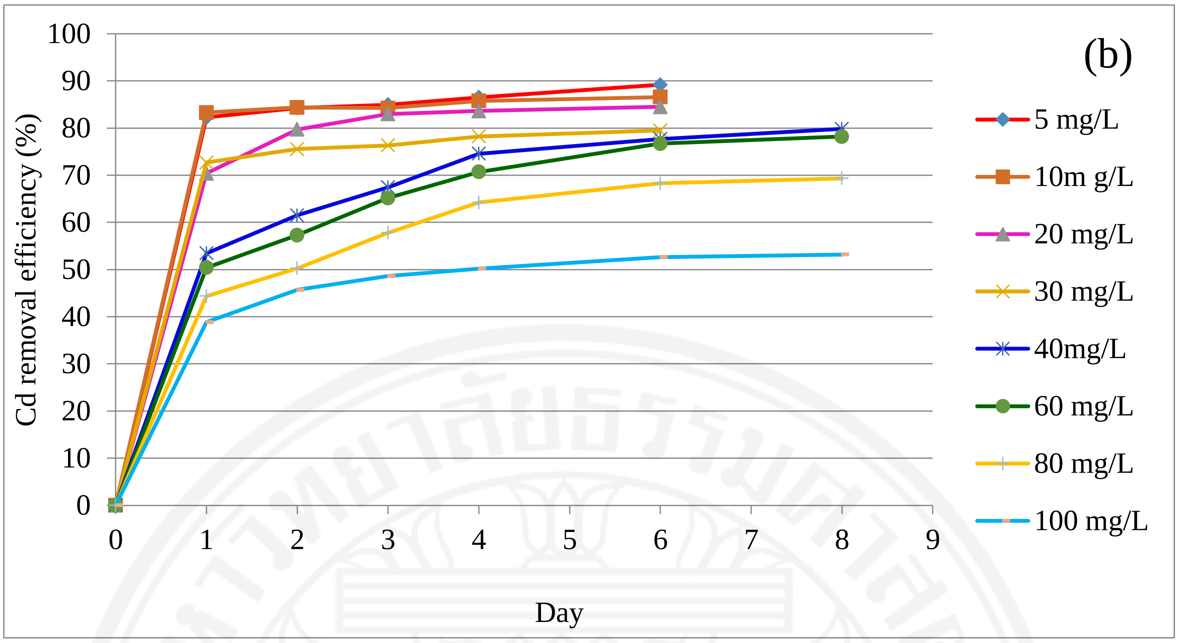


Figure 4.14: a) Cd removal efficiency of Creeping waterprimrose and b) Cd removal efficiency of Floating moss at different Cd concentration

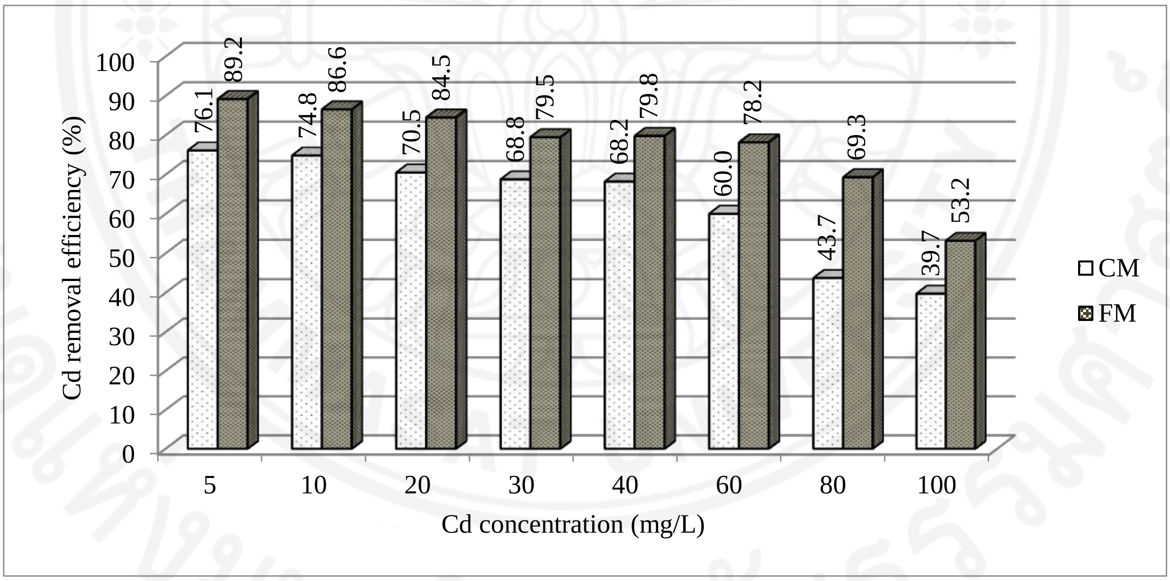


Figure 4.15: Comparison of Cd removal efficiency between Creeping waterprimrose and Floating moss at different concentration

Note: CM, Creeping waterprimrose and FM, Floating moss.

5) Cd accumulation in floating plants at different Cd concentration

Cd accumulation in plants at variable Cd concentration is shown in Figure 4.16. The Cd concentration used in this study was varied from 5 to 100 mg/L. The amount of Cd in given species (except Floating moss at 100 mg/L) increased with increasing Cd concentration. Cd accumulation in plants increased drastically from 2.1 to 24.7 mg/g for Creeping waterprimrose at 5 to 100 mg/L, and 2.8 to 29.9 mg/g for Floating moss at concentration from 5 to 80 mg/L. Cd accumulated by Floating moss decreased when concentration increased from 80 to 100 mg/L. It was in range from 29.9 to 23.4 mg/g. This demonstrates that Floating moss was able to accumulate Cd up to 80 mg/L. The decreasing Cd accumulation in plant tissues depends on the initial concentration. Chaudhuri et al. (2014) found that Common duckweed can accumulate Cd 1.6, 2.9, 4.0, 4.7, 3.9 and 3.4 mg/g at 0.5, 1, 1.5, 2, 2.5, and 3 mg/L, respectively. Phetsombat et al. (2006) also found that Floating moss accumulated Cd 17.5, 84.6, 829, and 1636 µg/g for roots and 3.8, 13.7, 189.2 and 679.2 µg/g for leaves at 0.5, 1, 2 and 4 mg/L, respectively. According to Zhu et al. (1999), the metal accumulated by plant is more than 5,000 mg/kg (5 mg/g) dry weight of a given element, it is a criterion to recognize a good accumulator. Hence, Creeping waterprimrose and Floating moss in present study were good accumulators at Cd concentration from 20 to 100 mg/L. Floating moss was good accumulator at 10 mg/L also.

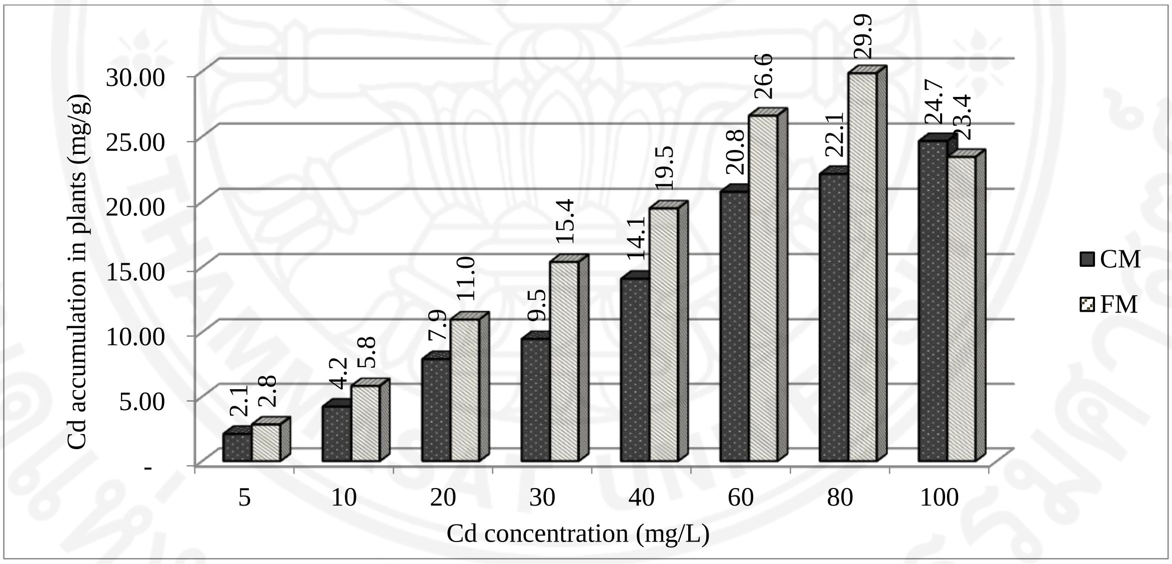


Figure 4.16: Cd accumulation in floating plants at different Cd concentration

Note: CM, Creeping waterprimrose and FM, Floating moss.

6) Bioconcentration factor at different Cd concentration

The BCF values for floating plants decreased gradually with increased Cd concentration, as shown in Figure 4.17. The increasing Cd concentration from 5 to 100 mg/L, BCF for Creeping waterprimrose decreased from 460 to 234. This shows that it was a moderate accumulator at 5 and 10 mg/L of Cd and it was a poor accumulator at other concentration. For Floating moss, BCF value also decreased from 579 to 223, indicating that Floating moss was a moderate accumulator at concentration from 5 to 60 mg/L and was poor accumulator at other concentration. Generally, the amount of metal accumulation in plants increases when the heavy metal in water increases, whereas, BCF value decreases with decreasing concentration. Lu et al. (2004) reported that BCF value for Water hyacinth decreased when Cd concentration in water was over 2 mg/L. Suchismita et al. (2014) also found that BCF values of Water lettuce decreased when Cd concentration increased. At 10, 15 and 20 mg/L, the BCF values were 1168, 635 and 373 for roots, and 8481, 384 and 150 for shoots, respectively. The decreased BCFs also depended on the growth inhibition of plants, especially in at high Cd concentration (Taner, 2010). Based on the visual changes observed in Creeping waterprimrose and Floating moss also confirms that BCFs were affected by Cd concentration. However, Floating moss was a bit better than Creeping waterprimrose on Cd toxicity indicting that Floating moss has higher BCFs then Creeping waterprimrose, except its BCF at 100 mg/L as shown in Figure 4.17.

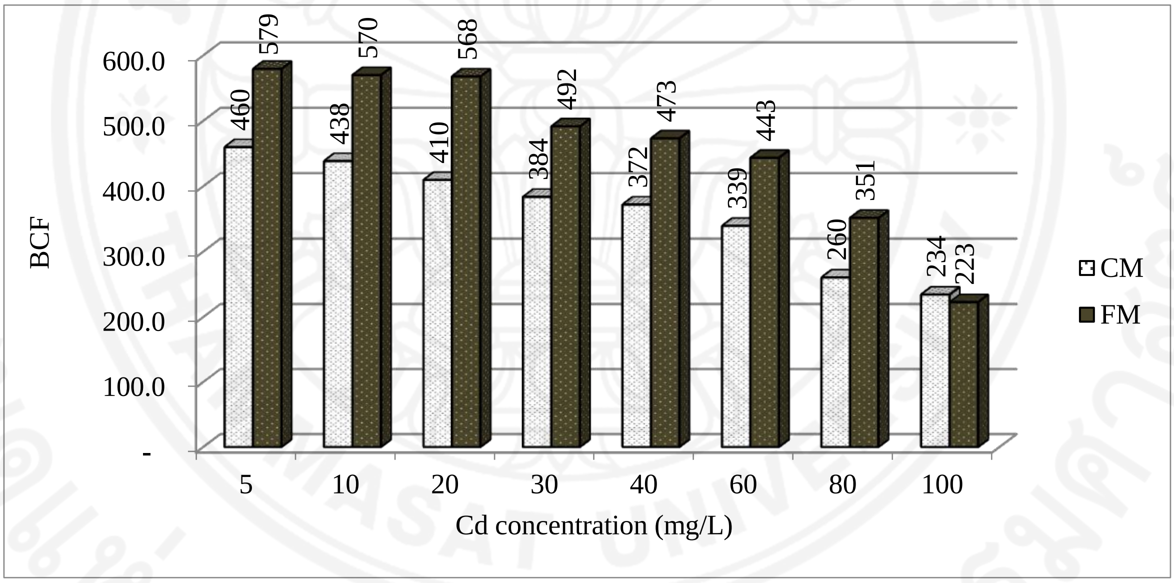


Figure 4.17: Bioconcentration factor at different concentration of Cd for Creeping waterprimrose and Floating moss

Note: CM, Creeping waterprimrose and FM, Floating moss.

4.1.3. Effect of different amount of Hoagland’s nutrients solution in Cd contaminated water

To study the effect of nutrients on Cd uptake by plants, the amount of Hoagland’s nutrients solution used in experiment was 3, 10, 20 and 30%. This was supplemented with 40 mg/L of Cd. The visual changes of Creeping waterprimrose and Floating moss were observed during cultivation for 8 days. They started looking unhealthy from the 2nd day and almost dead on the last day of cultivation. This might be caused by Cd toxicity. Hence, the relative growth and biomass productivity were reduced as shown in Figure 4.18 and 4.19, respectively. Despite the Cd concentration at 40 mg/L, Creeping waterprimrose could remove Cd over 20 and 69%, at the beginning (the 1st day) and the end of cultivation, respectively, as shown in Figure 4.20 (a). While on these days, Floating moss was able to remove Cd over 67 and 82%, respectively, as shown in Figure 4.20 (b). Table 4.5 shows that the amount of Cd in plants was in range from 14.5 to 15.4 mg/g for Creeping waterprimrose and from 18.5 to 19.4 mg/g for Floating moss. BCFs for plants are shown in Figure 4.21. BCF for Creeping waterprimrose indicated that it was a moderate Cd accumulator, but BCF for Floating moss showed a poor Cd accumulator. The result shows that the different amount of nutrients had no effects on Cd uptake by Creeping waterprimrose and Floating moss. As they had similar parameters of Cd uptake, such as relative growth, biomass productivity, Cd removal efficiency, Cd accumulation in plants and BCF value at the different amount of Hoagland's nutrients solution (Figure 4.18 to Figure 4.21, and Table 4.5). It is known that Cd affects plants growth reducing the nutrients absorption and its transport from roots to shoots (Benavides et al., 2005). This disturbs the chorolophyll synthesis and photosynthesis (Johna et al., 2009). It could be concluded that the plants refuse the nutrients after Cd was accumulated in their tissues (on the 1st day of cultivation). Based on results of this study, Creeping waterprimrose and Floating moss are not sensitive to rich nutrients. Therefore, they could be considered as good candidates for removal of Cd contamination in water containing high amounts of nutrients.

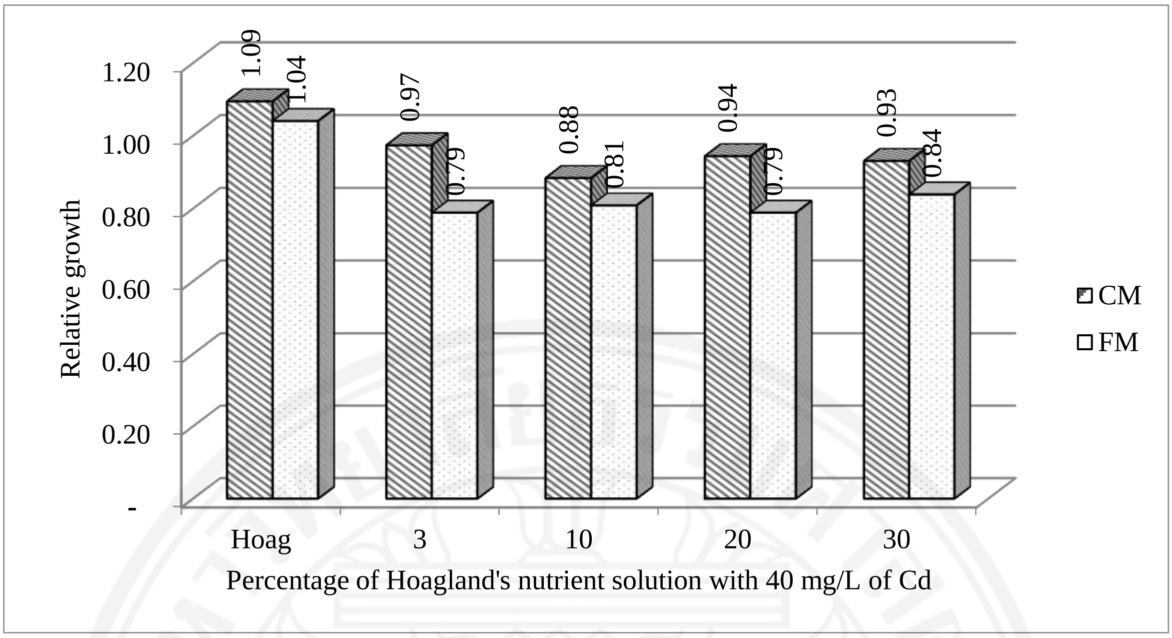


Figure 4.18: Relative growth of plants in different amount of Hoagland’s nutrients solution with 40 mg/L of Cd

Note: CM, Creeping waterprimrose and FM, Floating moss.

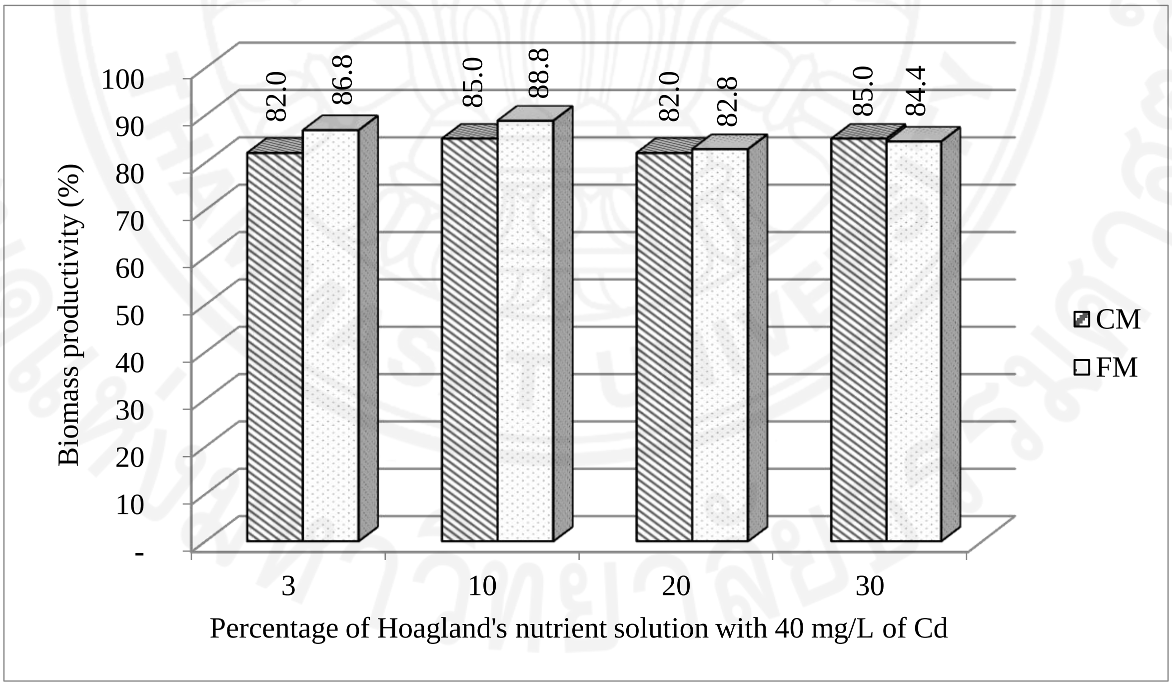


Figure 4.19: Biomass productivity of plants in different amount of Hoagland’s nutrients solution with 40 mg/L of Cd

Note: CM, Creeping waterprimrose and FM, Floating moss.

Table 4.5: Cd accumulation in floating plants in different amount of Hoagland's nutrients solution with 40 mg/L

Plant species	**Cd accumulation in plants (mg/g)**			
	3% Hoag	10% Hoag	20% Hoag	30% Hoag
CM	15.1	14.6	15.4	14.5
FM	19.4	18.5	19.1	19.3

Note: Hoag- Hoagland's nutrients solution with 40 mg/L of Cd

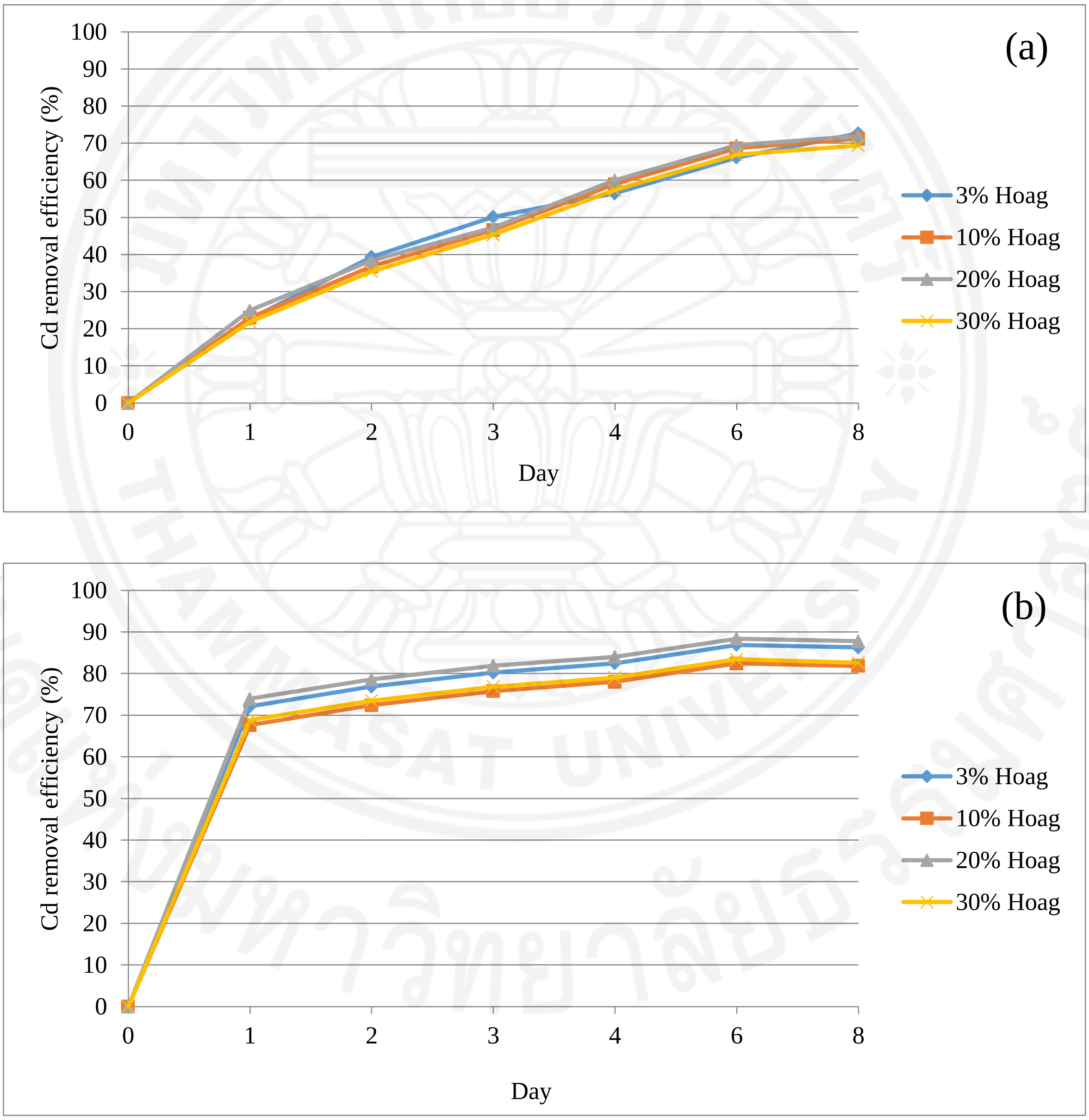


Figure 4.20: Effect of different amount of nutrients on a) Cd removal efficiency by Creeping waterprimrose and b) Floating moss at Cd 40 mg/L

Note: Hoag- Hoagland's nutrient solution with 40 mg/L of Cd

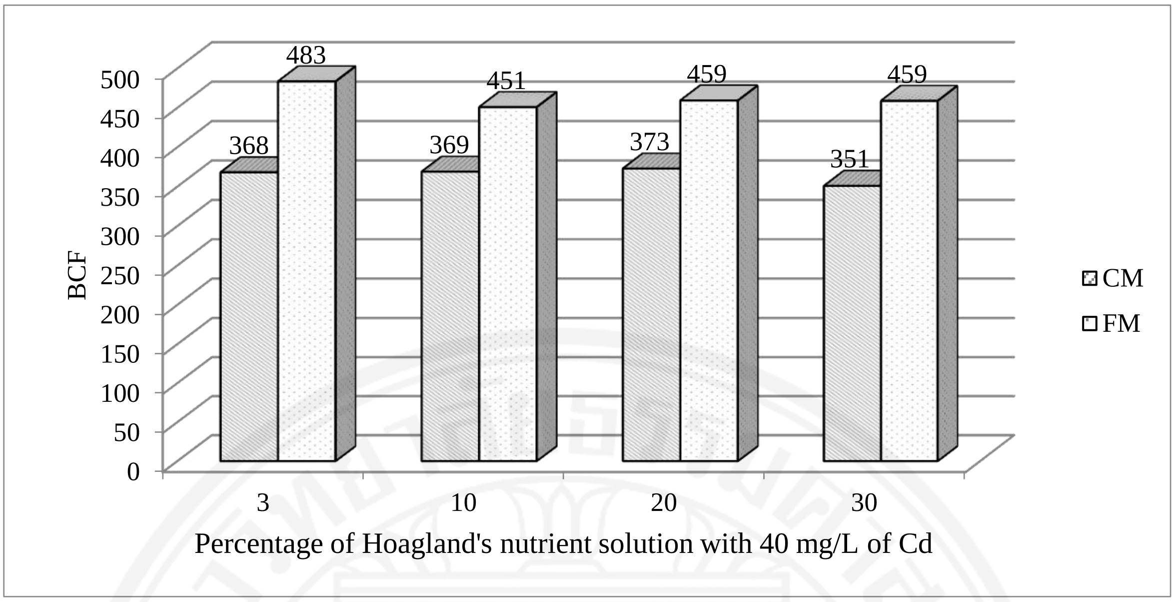


Figure 4.21: BCF for plants in different amount of Hoagland’s nutrients solution with 40 mg/L of Cd

Note: CM, Creeping waterprimrose and FM, Floating moss

4.2. Wastewater contaminated with Pb

4.2.1. Screening hyperaccumulators for Pb

1) Visual changes observed in floating plants at 10 mg/L of Pb

The plants were observed during the experimental period for 8 days as shown in Table 4.6 and Figure 4.22 to Figure 4.26. Water hyacinth, Creeping waterprimrose and Floating moss looked healthy with green leaves and also strong roots and stems (only roots and leaves for Floating moss) in Hoagland medium as well as in Pb contaminated water. These plants seemed to be tolerant floating plants at concentration 10 mg/L of Pb, and may be able to survive in contaminated water for longer than 8 days. The study of Soltan and Rashed (2003) found that Water hyacinth survived and looked healthy in contaminated water with 100 mg/L of Pb for 24 days. Floating moss was also tolerant floating plant and could survive in Pb contaminated water at 40 mg/L for 8 days (Phetsombat et al., 2006). In the present study, Water lettuce looked unhealthy even in Hoagland medium with leaves turning yellow on the 6th day. Water lettuce was also observed in contaminated water. Some of leaves turned yellow on the 3rd day and deteriorated with increase in exposure times. Similarly, Common duckweed looked unhealthy with leaves turning yellow on the 4th day and yellow color of leaves increased with time (8 days). From visual changes of studied plants, it can be concluded that it is probably caused by the Pb toxicity. Pb impairs plant growth, root elongation, chlorophyll production and water, and protein content (Kumar et al., 2013).

Table 4.6: Visual changes observed in floating plants at 10 mg/L of Pb

Plant species	Hoagland medium	Exposure times (day)							
	1-8 day	1	2	3	4	5	6	7	8
Water hyacinth	H	H	H	H	H	H	H	H	H
Water lettuce	H*	H	H	UY	UY	UY	UY	UY	UY
Creeping waterprimrose	H	H	H	H	H	H	H	H	H
Floating moss	H	H	H	H	H	H	H	H	H
Common duckweed	H	H	H	H	UY	UY	UY	UY	UY

Note: H, the plant looked healthy with green leaves; and UY, the plant looked unhealthy with some leaves turning yellow; *, some leaves turning yellow.

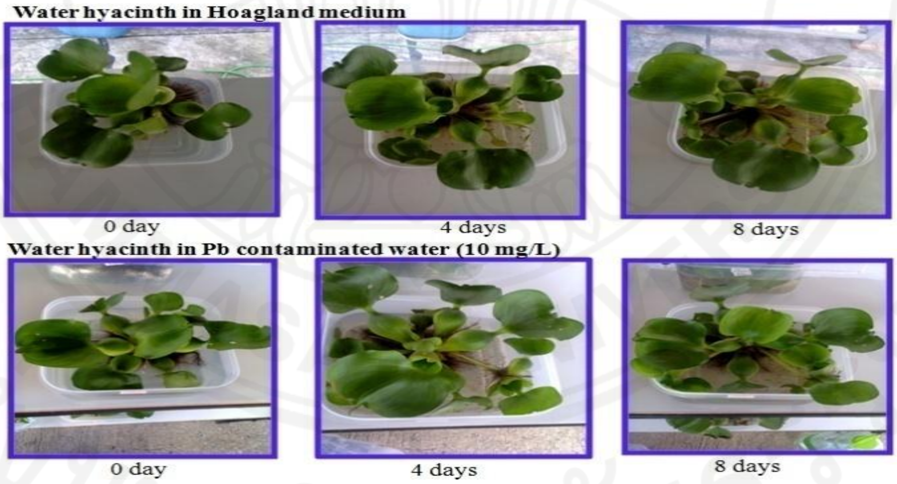


Figure 4.22: Visual changes observed in Water hyacinth at 10 mg/L of Pb

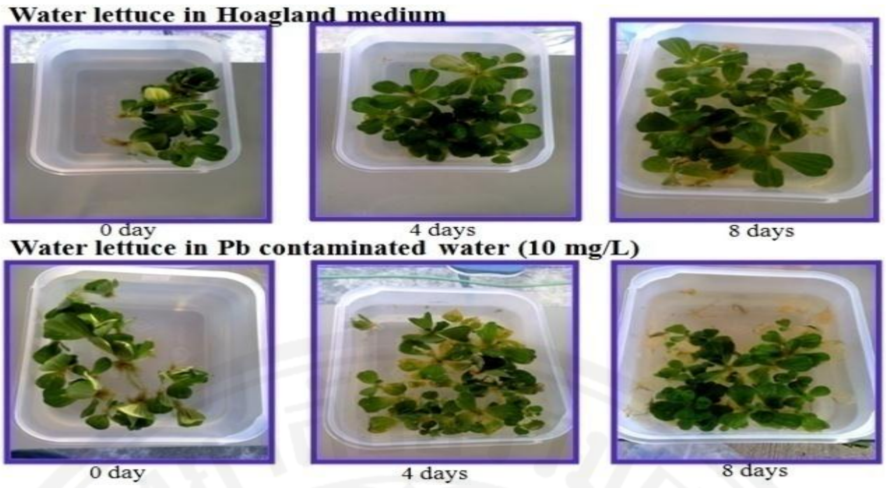


Figure 4.23: Visual changes observed in Water lettuce at 10 mg/L of Pb

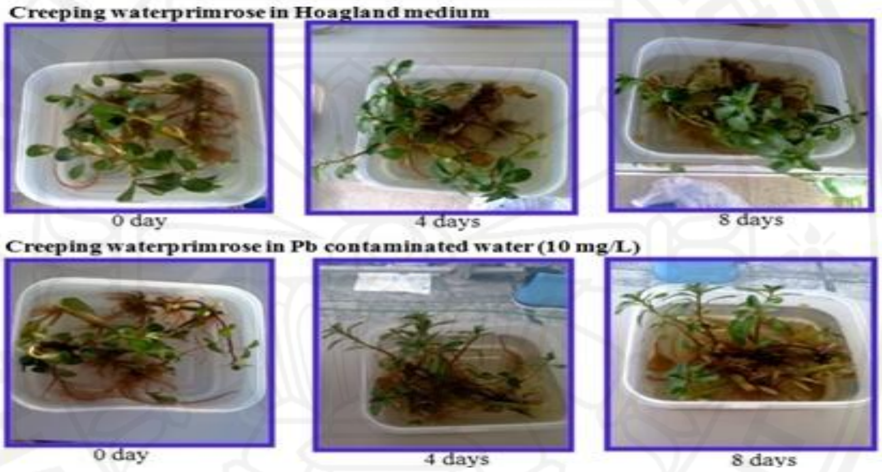


Figure 4.24: Visual changes observed in Creeping waterprimrose at 10 mg/L of Pb

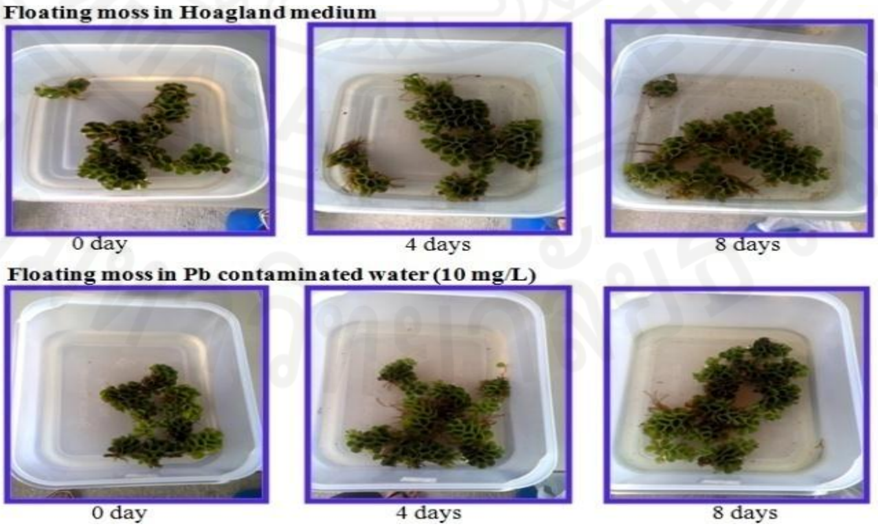


Figure 4.25: Visual changes observed in Floating moss at 10 mg/L of Pb

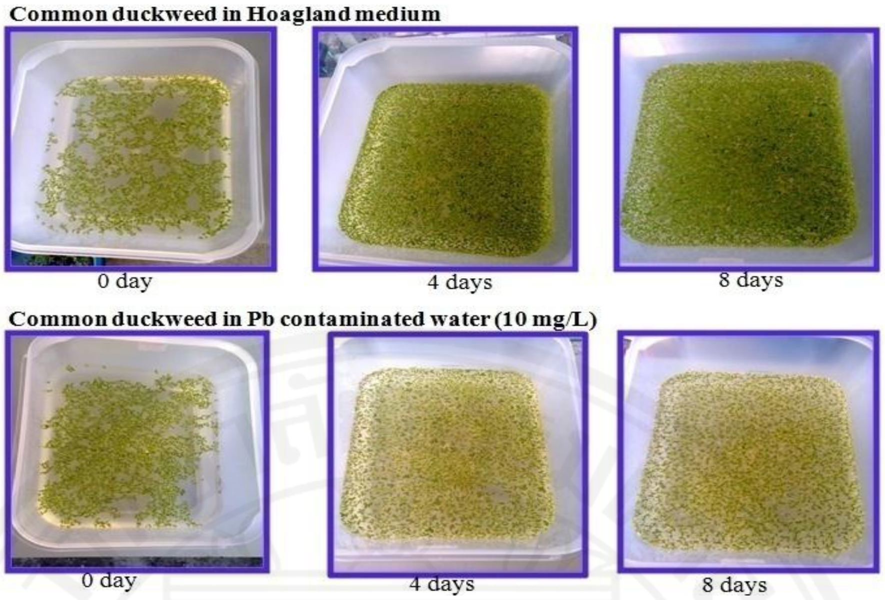


Figure 4.26: Visual changes observed in Common duckweed at 10 mg/L of Pb

1) Relative growth of floating plants at 10 mg/L of Pb

A comparison of relative growths of five floating plants in Hoagland medium and in Pb contaminated water is shown in Figure 4.27. The relative growth of Common duckweed, which was 1.89 in Hoagland medium and 1.57 in Pb contaminated water, was the highest amongst all species. Its relative growth is in agreement with Axtell et al. (2003). Common duckweed grows very fast as it can double its mass within a week under optimum conditions. The relative growth of Water hyacinth indicating the tolerant species on Pb at 10 mg/L was similar with the one in Hoagland medium. However, the relative growth of all plant species in Pb contaminated water decreased drastically, except Water hyacinth. The relative growth was 0.59, 0.77 and 0.46 for Water lettuce, Creeping waterprimrose, and Floating moss, respectively. This shows inhibition of growth due to Pb toxicity. The inhibited growth of many floating plant species due to Pb toxicity was reported in many research such as for *Potamogeton pectinatus* (Sasmaz & Obek, 2012), *Eichhornia crassipes* (Win et al., 2003), *Chara aculeolata*, and *Nitella opacai* (Sooksawat et al., 2013).

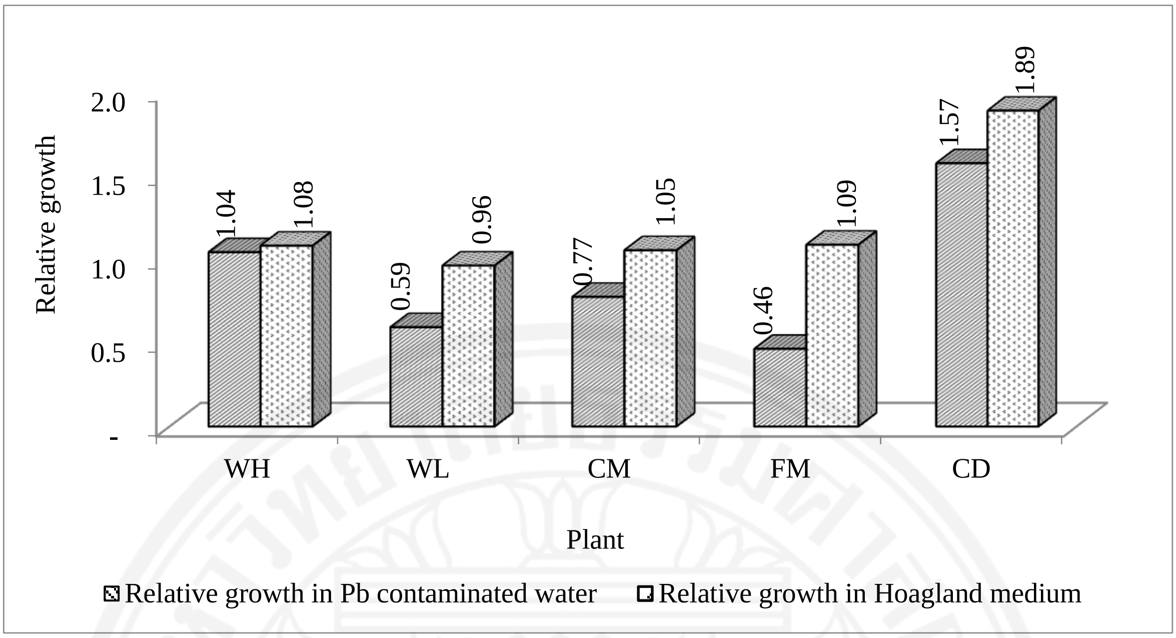


Figure 4.27: Relative growth of floating plants at 10 mg/L of Pb

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

2) Biomass productivity of floating plants at 10 mg/L of Pb

The biomass productivity of plants in Pb contaminated water decreased in order as Floating moss>Water hyacinth>Water lettuce>Creeping waterprimrose>Common duckweed as shown in Figure 4.28. During the experimental period (8 days), however, the difference in biomass productivity for plants was very little, except Common duckweed. It was found that at 10 mg/L of Pb in water had small effect on biomass productivity of studied plants, except Common duckweed. The biomass productivity of Common duckweed was the lowest (59.8%). This shows that Pb was toxic to Common duckweed. For other species had high biomass productivity (over 83.1%) and could also tolerate and survive well in Pb contaminated water, especially Water hyacinth, Creeping waterprimrose and Floating moss. Carranza-Álvarez et al. (2008) stated that the high biomass production of plants is the characteristic of plants for phytoremediation. Additionally, biomass productivity could be an indicator for the overall health of plant growth (Tangahu et al., 2013).

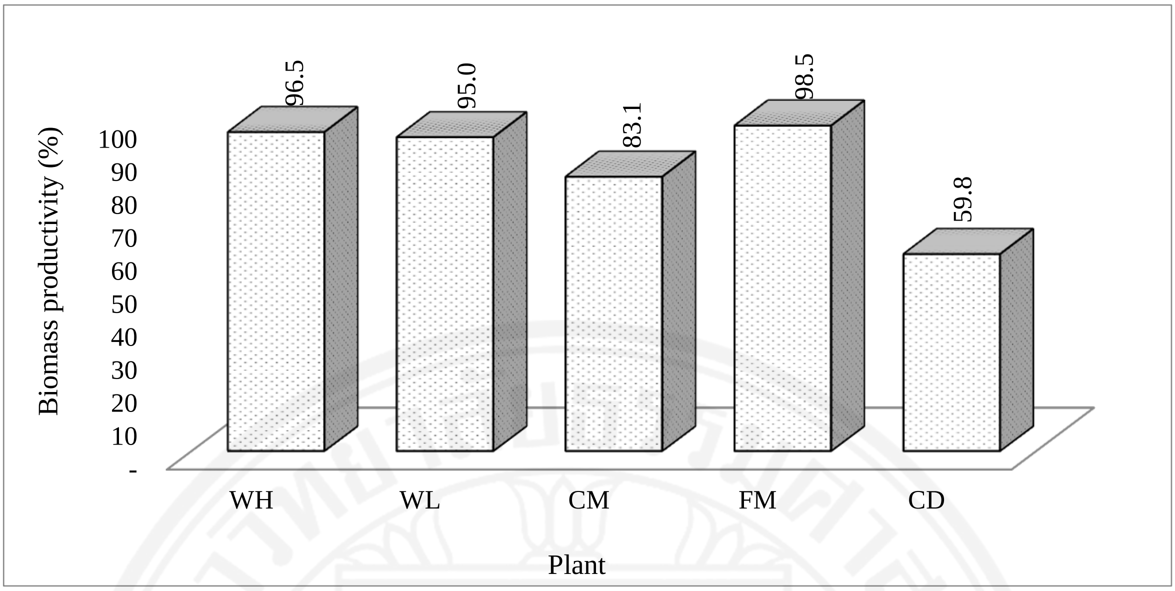


Figure 4.28: Biomass productivity of different plant species

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

3) Pb removal efficiency of floating plants at 10 mg/L

All floating plants were able to remove Pb from contaminated water at 10 mg/L as shown in Figure 4.29. Pb was removed from water within 1 day. Water hyacinth, Water lettuce, Creeping waterprimrose, Floating moss and Common duckweed could remove Pb 98.9, 94.4, 93.2 91.8 and 91%, respectively. At the end of cultivation (8th day), Pb was almost completely removed from water. Comparing Pb removal efficiency of each plant, the highest removal efficiency of Pb was found in Water hyacinth (99.9%), followed by Common duckweed (99.8%), Creeping waterprimrose (98.6%), Floating moss (97.9%) and Water lettuce (96.6%). Although the Water lettuce and Common duckweed did not look healthy as other species, their Cd removal efficiency was similarly to others. Common duckweed had the lowest biomass productivity, but could remove Cd as well. Miretzky et al. (2004) found that Water lettuce was able to remove Pb 99.7% and 98.5% from wastewater at initial concentration 4 mg/L and 1 mg/L, respectively. Common duckweed could remove 76% of Pb at 10 mg/L (Axtell et al., 2003). However, the results of current study indicate that all plant species were able to remove Pb in a short period. Based on the high removal efficiency of plants at 10 mg/L of Pb, Pb remained in all reactors (expect Water lettuce) could be discharged to environment without any future treatment. It was in range of the standard of the industrial wastewater effluent of Pollution Control Department (PDC, 2004), Ministry of Natural Resources and Environment in Thailand (Pb not over than 0.2 mg/L). For the wastewater in Water lettuce reactor, Pb contaminated in water was 0.3 mg/L, which was close to the standard.

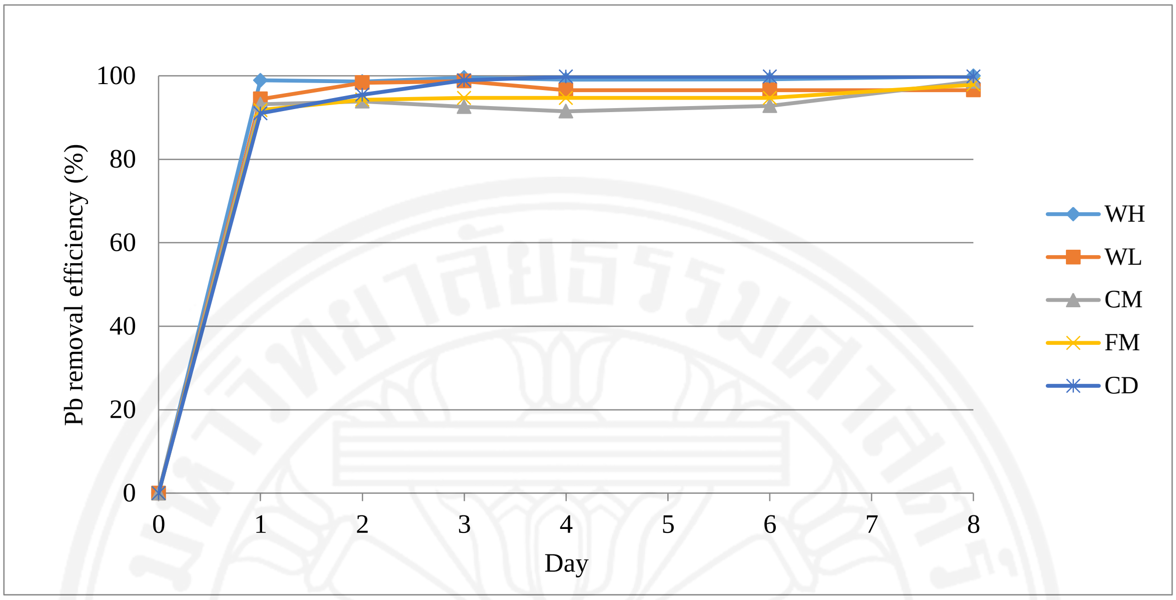


Figure 4.29: Pb Removal efficiency of different plant species

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

4) Pb accumulation in floating plants at 10 mg/L

The Pb accumulation in plants was determined at the start of cultivation. The results demonstrated that Pb accumulation in floating plants was negligible. The final Pb accumulation in plants is shown in Table 4.7. The highest Pb accumulation in plants was in Common duckweed (30.5 mg/g dry weight). While, the lowest one was found in Water hyacinth (4.9 mg/g dry weight). For Pb accumulation in Water lettuce, Creeping waterprimrose and Floating moss was 18.9, 6.6, and 7.8 mg/g (dry weight), respectively. The difference in Pb accumulation in each plant might be caused by the effectiveness of plant species used in the present experiment. Gupta and Sinha (2007) reported that the different heavy metals uptake and their accumulation in plants depend on the available concentration of heavy metals in water, sequential solubility and the plant species. Table 4.7 summarizes Pb accumulation in various plant species as compared with this study. All plants have the different accumulation of Pb. These might be because they were cultured in different conditions.

Table 4.7: Pb accumulation in Floating plants

Floating plants		Pb conc. (mg/L)	Pb accumulation in plants (mg/g)				Period (day)	Sources
Common names	**Scientific names**		**Roots**	**Stems**	**Leaves**	**Whole**		
Water hyacinth	*Echhorni acrassipe*	10				4.9	8	This study
		0.5	0.11	0.04	0.01		15	NurZaida and Piakong (2011)
		1, 4 and 16				2.2, 3.1 and 3.4	32	Espinoza-Quiñones et al. (2013)
Water lettuce	*Pistiastra tiotes*	10				18.9	8	This study
		1, 2 and 4				0.19, 0.214 and 0.01	15	Miretzky et al. (2004)
Creeping waterprim rose	*Jussiaear epens*L.	10				6.6	8	This study
Floating moss	*Salviniac ucullata*	10				7.8	8	This study
		5, 10, 20 and 40	1.9, 7.7, 10.1, and 14.3		0.3, 1.04, 1.3, and 4.0		8	Phetsombat et al. (2006)
Common duckweed	*Lemna minor*	10				30.5	8	This study
		0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 8.0 and 10				3.6, 4.9, 5.6, 7.0, 8.5, 12.9, 17.7 and 20	7	Dirilgen (2011)

5) Bioconcentration factor at 10 mg/L of Pb

The BCFs for different plant species are shown in Figure 4.30. The highest BCF was found in Common duckweed (3,322), indicating that Pb uptake was better than other species, followed by Water lettuce (2,054). The high value of BCF value indicates the ability of plant accumulation of Pb. According to Yongpisanphop (2005), the BCF value for *Hydrocotyle umbellata* was 1,915 (at 80 mg/L of Pb). Sooksawat et al. (2013) and Suchismita et al. (2014) reported that if BCF value is greater than 1000, it can be qualified as good hyperaccumulator. The BCF values for Water hyacinth (535), Creeping waterprimrose (721) and Floating moss (851) indicate them as moderate accumulators at 10 mg/L of Pb. Phetsombat et al. (2006) found that the BCF value for Floating moss was 870 (at 10 mg/L of Pb). Sooksawat et al. (2013) also found that BCF value for *Nitella* *opaca* was higher than *Chara aculeolata* at 10 mg/L of Pb. Therefore, it can be concluded that BCF value depends on amount of Pb accumulation in different plant species.

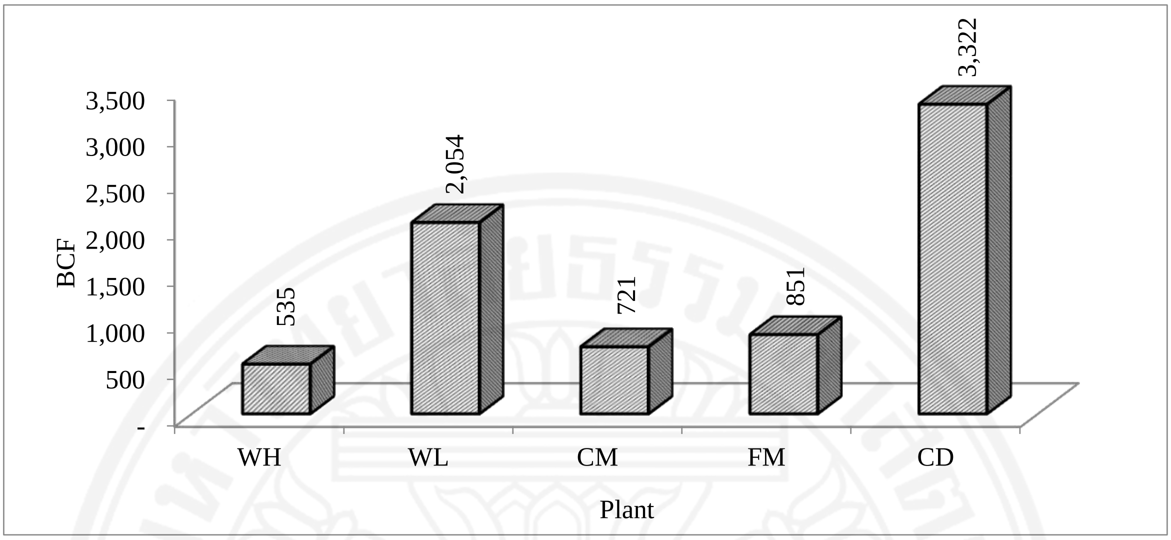


Figure 4.30: Bioconcentration factor for different plant species at 10 mg/L of Pb

Note: WH, Water hyacinth; WL, Water lettuce; CM, Creeping waterprimrose; FM, Floating moss; and CD, Common duckweed.

4.2.2. Effect of different Pb concentration on uptake

Based on the results of the screening experiment (10 mg/L of Pb), two species, Water hyacinth and Floating moss, were selected as hyperaccumulators. They were tolerant to Pb as they looked healthy with green leaves during cultivation. They also had high biomass productivities and Pb removal efficiency. Even though Common duckweed had the highest Pb accumulation and BCF value, it was not selected. This was because it was unhealthy with some leaves turning yellow during the cultivation. Water hyacinth and Floating moss were cultivated in Pb contaminated water at 10, 30, 50 and 70 mg/L for 10 days, and for 12 days at 100, 200, 300 and 400 mg/L.

1) Visual changes observed in floating plants at different Pb concentration

In general, plants look unhealthy with the time increase and Pb concentration, except Water hyacinth at 10 and 30 mg/L, and Floating moss at 10 mg/L. Table 4.8 summarizes the visual changes observed in Water hyacinth in this study. During the cultivation for 10 days, Water hyacinth looked healthy with green leaves at 10, 30 mg/L of Pb. This shows that it was tolerant to Pb. It also looked healthy at 50 and 70 mg/L, but it looked unhealthy with some leaves turning yellow on the 7th day. When the Pb concentration increased from 100 to 400 mg/L, it was unhealthy with exposure times. During the experimental period (12 days), it looked unhealthy with some leaves turning yellow, some leaves turning brown and almost dead with increase in exposure times. It was very important to note that Water hyacinth was unhealthy with some leaves turning yellow and partial wilting on the 2nd day and almost dead on the 7th day at 400 mg/L of Pb. This might be due to the strong Pb toxicity.

Visual changes observed in Floating moss are summarized in Table 4.9. Floating moss was healthy with green leaves during the period of cultivation at 10 mg/L of Pb for 10 days. On the other hand, it was unhealthy with increasing time and Pb concentration from 30 to 400 mg/L. Floating moss looked unhealthy with some leaves turning yellow and brown, and almost dead. Furthermore, it died completely on the 11th, 9th and 8th day at 200 mg/L, 300, and 400 mg/L, respectively. Sharma and Dubey (2005) documented that the toxicity symptom in plants, such as stunted growth, chlorosis and blacking of root system is caused by excess Pb. Pb also inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability.

When comparing the two species, Water hyacinth was more tolerant than Floating moss, especially at 30 to 400 mg/L of Pb as shown in Table 4.8 and Table 4.9. It can conclude that the health of plants depends on the Pb concentration and plant species.

Table 4.8: Visual changes observed in Water hyacinth at different Pb concentration

Pb Conc. (mg/L)	**Exposure times (day)**												
	0	1	2	3	4	5	6	7	8	9	10	11	12
10	H	H	H	H	H	H	H	H	H	H	H	-	-
30	H	H	H	H	H	H	H	H	H	H	H	-	-
50	H	H	H	H	H	H	H	UY	UY	UY	UY	-	-
70	H	H	H	H	H	H	H	UY	UY	UY	UY	-	-
100	H	H	H	UY	UY*	UY*	UB*	UY*	UY*	YB*	UB*	UB*	UB*
200	H	H	H	UY*	YU*	UB*	UB*	UB*	UB*	UM	UM	UM	UM
300	H	H	H	UY*	UY*	UB*	UB*	UB*	UB*	UM	UM	UM	UM
400	H	H	UY*	UB*	UB*	UB*	UB*	UM	UM	UM	UM	UM	UM

Table 4.9: Visual changes observed in Floating moss at different Pb concentration

Pb Conc. (mg/L)	**Exposure times (day)**												
	0	1	2	3	4	5	6	7	8	9	10	11	12
10	H	H	H	H	H	H	H	H	H	H	H	-	-
30	H	H	H	H	UY	UY	UY	UY	UY	UY	UY	-	-
50	H	H	H	UY	UY	UY	UY	UY	UY	UY	UY	-	-
70	H	H	H	UY	UY	UY	UY	UY	UY	UY	UY	-	-
100	H	H	UY	UY	UB	UB	UB	UM	UM	UM	UM	UM	UM
200	H	H	UY	UB	UB	UB	UB	UM	UM	UM	UM	CD	CD
300	H	H	UY	UB	UB	UM	UM	UM	UM	CD	CD	CD	CD
400	H	YU	UB	UB	UB	UM	UM	UM	CD	CD	CD	CD	CD

Note: H, the plant looked healthy with green leaves; UY, the plant looked unhealthy with some leaves turning yellow; UB, the plant looked unhealthy with some leaves turning brown; UM, the plant looked unhealthy and almost dead; and CD, the plant looked completely dead.* Partial wilting


10 mg/L 0 day
10 mg/L 10 days
30 mg/L 0 day
30 mg/L 10 days
50 mg/L 0 day
50 mg/L 10 days
70 mg/L 0 day
70 mg/L 10 days
100 mg/L 0 day
100 mg/L 12 days
200 mg/L 0 days
200 mg/L 12 days
300 mg/L 0 day
300 mg/L 12 days
400 mg/L 0 days
400 mg/L 12 days

Figure 4.31: Visual changes observed in Water hyacinth at different Pb concentration

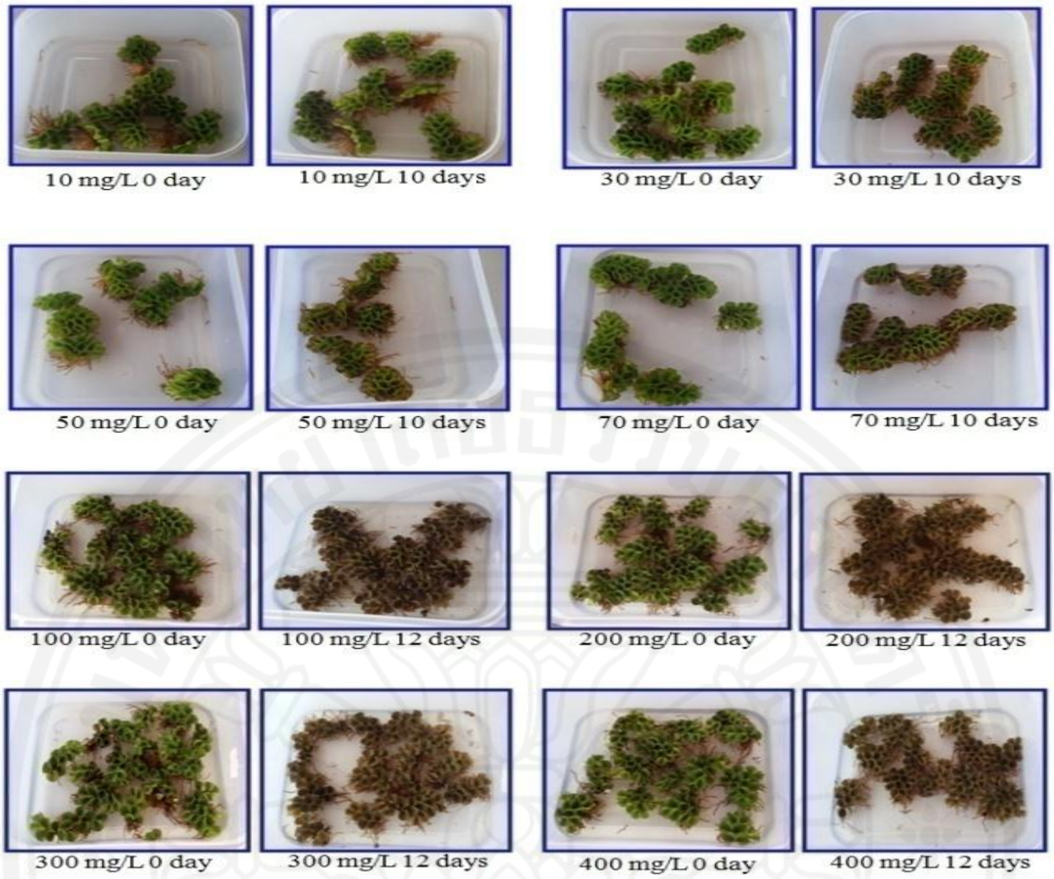


Figure 4.32: Visual changes observed in Floating moss at different Pb concentration

2) Relative growth of floating plants at different Pb concentration

Water hyacinth and Floating moss grow fast in suitable conditions and tolerate wastewater and thus were selected to study Pb removal from water environment. The Pb concentration was varied from 10 to 400 mg/L and the effect relative growth of plants was studied. The relative growth of plants in wastewater declined with increasing Pb concentration when compared with the one in Hoagland medium as shown in Figure 4.33. Water hyacinth’s relative growth declined slightly in each concentration from 1.03 to 0.91 at concentration from 10 to 400 mg/L of Pb. Thus, the results show that Water hyacinth was not much sensitive to Pb. On the other hand, the relative growth of Floating moss decreased drastically with increasing concentration. It decreased from 0.85 to 0.58 at concentration from 10 to 400 mg/L. It can demonstrate that the Pb affected the relative growth of Floating moss. Based on the comparison of two plant species, it can be said that Water hyacinth was more tolerant to Pb than Floating moss.

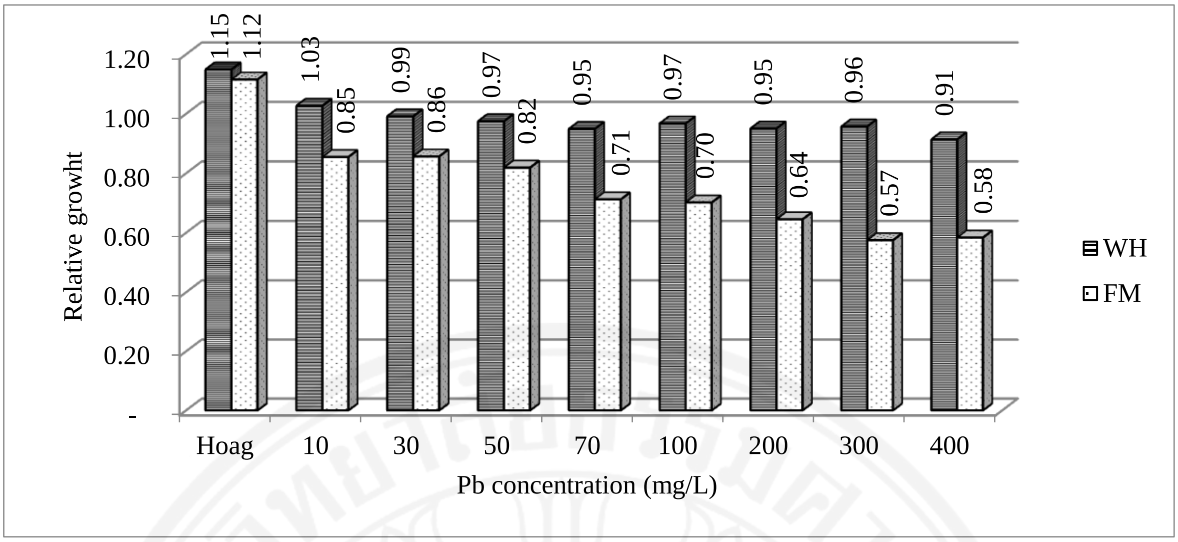


Figure 4.33: Relative growth of Plants at different concentrations of Pb

Note: WH, Water hyacinth: and FM, Floating moss

3) Biomass productivity of floating plants at different Pb concentration

Biomass productivity of plants was high and decreased gradually with increasing Pb concentration, especially the biomass productivity of Floating moss as shown in Figure 4.34. The biomass productivity of Water hyacinth and Floating moss decreased gradually from 94.3 to 78.7% and 97.2 to 89.3% with the concentration increased from 10 to 400 mg/L of Pb, respectively. Although Water hyacinth was more tolerant to Pb than Floating moss when compared for the visual change observation and relative growth, its biomass was lower. However, biomass productivity of Water hyacinth and Floating moss was quite high. Thus, Pb did not affect much to these species. Carranza-Álvarez et al. (2008) reported that the high biomass production of plants is the characteristic of plants for phytoremediation. Therefore, Water hyacinth and Floating moss could be considered as candidates for phytoremediation.

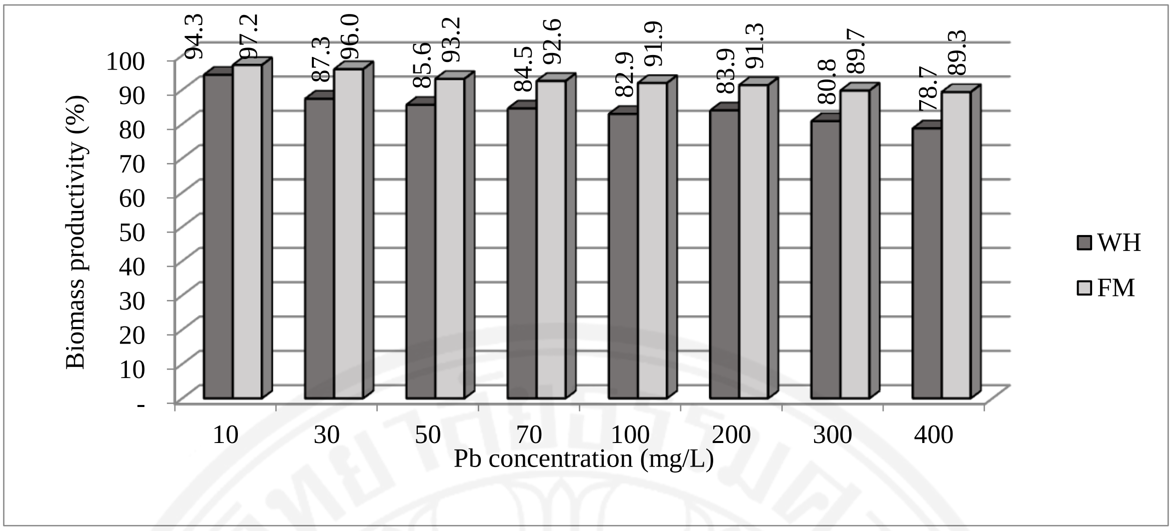


Figure 4.34: Biomass productivity of Plants at different concentration

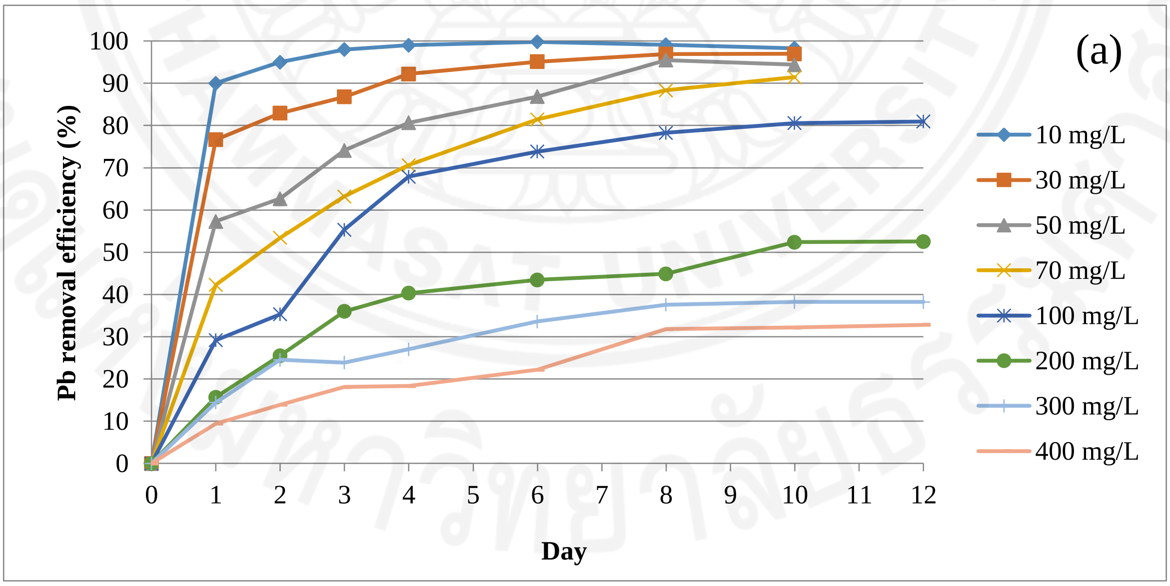
Note: WH, Water hyacinth: and FM, Floating moss

4) Pb removal efficiency of floating plants at different Pb concentration

The experiment was conducted for 10 days at 10, 30, 50 and 70 mg/L, respectively, and 12 days at 100, 200, 300 and 400 mg/L, respectively. Figure 4.35 (a) and (b) show that the Pb removal efficiency of plants increased with exposure times, but it decreased with the increase Pb concentration. Comparing two species, the Floating moss was able to remove more Pb than Water hyacinth. The Pb removal efficiency of Water hyacinth is shown in Figure 4.35 (a). The highest removal efficiency of Pb was found at 10 mg/L, on the 1st day (90%) and the last day of the cultivation (98.3%). Pb was also removed drastically within 1 day at increase concentration from 30 to 70 mg/L. It was constant from the 8th day to the last day of cultivation with decreasing Pb removal efficiency from 98.3 to 81%. At concentration 100 mg/L, it was noted that even though Water hyacinth removed Pb slowly for 2 days, it could remove Pb well on the 3rd to 4th day and continued removing till the last day (81%). For increasing concentration from 200 to 400 mg/L, the Pb removal efficiency decreased from 15.7 to 9.4% on the 1st day, and 52.5 to 32.8% on the last day of cultivation.

Floating moss was able to remove Pb well at concentration up to 100 mg/L. Pb was removed over 85% within 1 day. Pb remaining in water was almost constant from the 2nd till the last day of cultivation. On the last day of cultivation, Pb removal efficiency of Floating moss was almost 100% at concentration from 10 to 100 mg/L. At 200 mg/L, it can be seen that Pb was removed slowly (61.1%) on 1st day. It was increased gradually with exposure times and was constant on the 8th day to the last day of cultivation (97.3%). It demonstrates that Floating moss had high effectiveness to remove Pb from wastewater with high concentration (200 mg/L). Moreover, Floating moss could also remove 300 mg/L of Pb 43.3% on the 1st day and 81.6% on the last day. The Pb removal efficiency also increased with exposure times at the 400 mg/L of Pb, it was 37 and 61.3% on the 1st and last day of cultivation, respectively.

Based on the results, it can be inferred that Floating plants have capacity to remove Pb from wastewater at both low and high concentration. However, the Pb removal efficiency declined with the increasing concentration and exposure times. Tangahu et al. (2013) used *Scirpus grossus* to remove Pb from contaminated water in Pilot Reed Bed. Within 28 days of the experiment, the plant was able to remove Pb 100, 99.9 and 99.7 % at 10, 30 and 50 mg/L, respectively. Based on the high Pb removal efficiency of both Water hyacinth and Floating moss at 10 mg/L in this study, the treated wastewater after plants harvesting could be discharged to the environment without any treatment. Floating moss could also remove Pb very well at 30 and 50 mg/L. The Pb remaining in water was 0.35 and 0.38 mg/L, which is closed to the standard of industrial wastewater effluent of Pollution Control Department(PDC, 2004), Ministry of Natural Resources and Environment in Thailand (Pb not over than 0.2 mg/L).



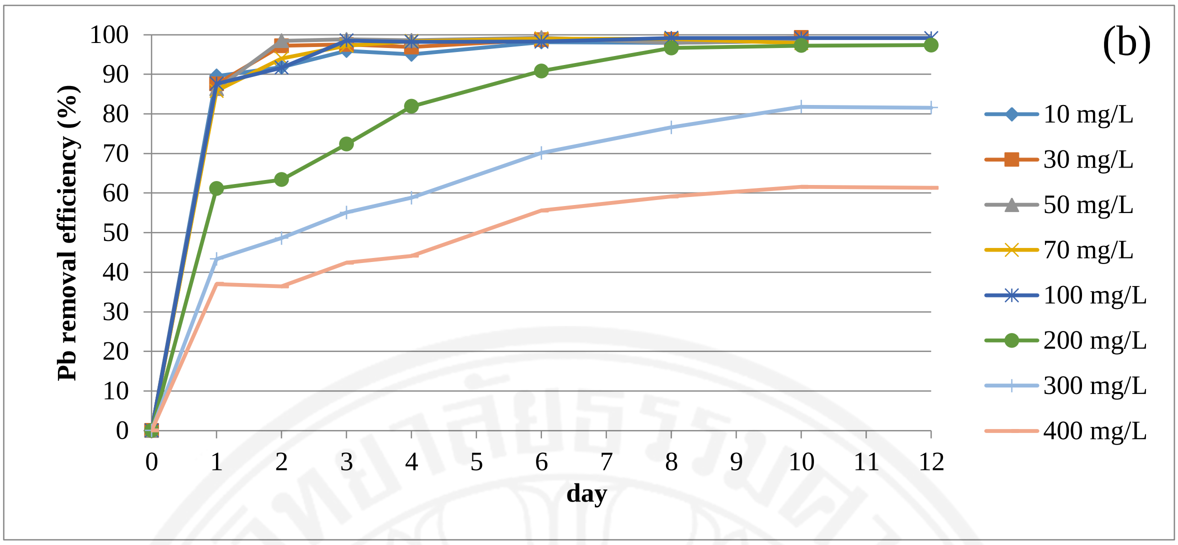


Figure 4.35: Pb removal efficiency at different concentration a) Pb removal efficiency of Water hyacinth and b) Floating moss

5) Pb accumulation in floating plants at different concentration

Pb accumulation in Plants increased with increasing concentrations, except at 400 mg/L for Floating moss as shown in Figure 4.36. Pb accumulated by Water hyacinth and Floating moss at concentration from 10 to 400 mg/L was in range from 4.9 to 69.7 mg/g and 7.1 to 181 mg/g, respectively. It can be seen that even though concentration increased from 300 to 400 mg/L, the capacity of Floating moss to accumulate Pb was similar. Dirilgen (2011) also found that the Pb accumulation in Common duckweed increased when increased the concentration. It was 3.6, 4.9, 5.6, 7.0, 8.5, 12.9, 17.7 and 20 mg/g at 0.1, 0.2, 0.5, 1, 2, 5, 8 and 10 mg/L, respectively. Zhu et al. (1999) reported that the metal accumulation in floating plant is more than 5,000 mg/kg (5 mg/g) dry weight of a given element, it is a criterion to recognize as a good accumulator. Thus, floating plants in present study could be the good accumulators of Pb at all concentration (at 10 to 400 mg/L), except at 10 mg/L for Water hyacinth.

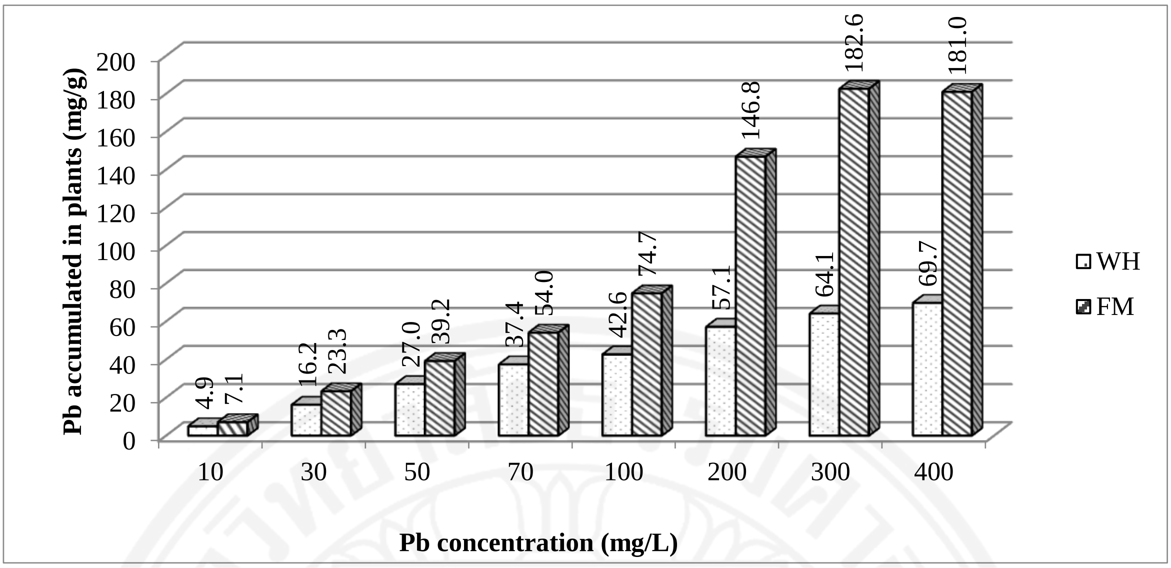


Figure 4.36: Pb accumulation in plants at different concentration
Note: WH, Water hyacinth: and FM, Floating moss

6) Bioconcentration factor for plants at different Pb concentration

BCF value is used for estimation Pb accumulation in plants, and also demonstrates the Pb toxicity on plants. It is calculated as the ratio of Pb in the plant (mg/kg dry weight) and the initial Pb concentration in contaminated water (mg/L). The results are presented in Figure 4.37. BCF values declined when the Pb concentration increased.

BCF value for Water hyacinth declined gradually from 546 to 523 with the increasing concentration from 10 to 70 mg/L, and declined drastically from 427 to 173 at concentration from 100 to 400 mg/L. BCF value for Floating moss also decreased slightly from 758 to 719 at concentration from 10 to 300 mg/L, and decreased drastically from 600 to 450 at concentration from 300 to 400 mg/L. This indicates that BCF values decreased when the Pb concentration increased. Dirilgen (2011) also found that BCF value for Common duckweed decreased with increasing Pb concentration. They were 5800, 5617, 4507, 3330, 2378, 2044, 1570 and 1330 at 0.1, 0.2, 0.5, 1, 2, 5, 8 and 10 mg/L pf Pb, respectively. According to the criteria of the good accumulator reported by Suchismita et al. (2014) and Zhu et al. (1999), BCF value should be greater than 1,000. Therefore, Floating moss in this study is the moderate accumulator of Pb up to the concentration 400 mg/L. Water hyacinth was also a moderate accumulator at concentration up to 70 mg/L, but it was a poor accumulator at other concentration.

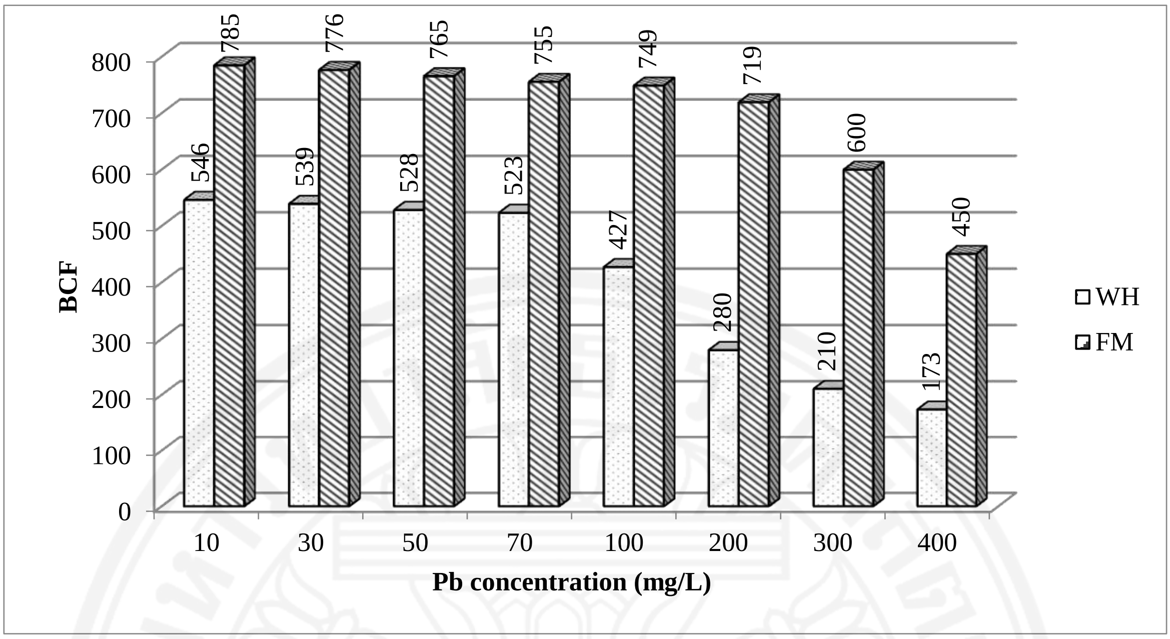


Figure 4.37: BCF values for Water hyacinth and Floating moss at different Pb concentration

Note: WH, Water hyacinth: and FM, Floating moss

4.2.3. Effect of different amount of Hoagland’s nutrients solution in Pb contaminated water

As known well that nutrients are essential for plants growth. The experiment was conducted to find the effectiveness of plants to remove Pb from artificial wastewater with different amount of nutrients. The amount of Hoagland’s nutrients solution using in this study was 3, 10, 20 and 30%. It was supplemented with 100 mg/L of Pb. Comparing effectiveness of Water hyacinth and Floating moss in this experiment, the relative growth, biomass productivity, Pb removal efficiency, amounts of Pb accumulation in plants, BCF vale for each plant were almost similar as shown in Figure 4.38 to Figure 4.42 and Table 4.10. The relative growth of plants did not reduce drastically comparing with the plants’ in Hoagland medium. The relative growth of Water hyacinth in Hoagland medium was in range 1.07-1.09 and 1.01-1.07 for Floating moss. Whereas, the relative growth of plants in treated wastewater was in range 0.94-0.95 for Water hyacinth and the Floating moss was 0.81-0.85. The high biomass productivity of Water hyacinth in this study was in range of 91.2-93.4% and Floating moss’ was 95-97.4%. Carranza-Álvarez et al. (2008) reported that the high biomass productivity is a criterion for phytoremediation. The Pb removal efficiency of Water hyacinth was in range of 28.7-31% on the 1st day, increasing gradually till the Pb concentration was constant (on the 8th day). It was in range 78.2-80.4% on the last day of cultivation. Floating moss seemed to absorb Pb more than Water hyacinth. It could remove Pb in range 80.9-85% on the 1st day, it was constant on the 2 nd day in range 93.6-96% and almost 100% on the last day of cultivation. Pb accumulation in plants is also very important to indicate the effectiveness of plants for Pb removal. Table 4.10 shows that the amount of Pb in Water hyacinth was in range 39.8-43 mg/g and 70.5-75.5 mg/g for Floating moss. This is consequence for moderate BFC value that it was in range 387-421 for Water hyacinth and 720-741 for Floating moss (Figure 4.32). Several researchers explored that Pb is one of the heavy metal with high persistence in environment and toxic to living things. Pb toxicity affects the seed germination, plant growth, chlorophyll production due to the inhibition of enzyme activities, nutrient absorption, water balance, and also change in hormonal status and alteration in membrane permeability (Sharma & Dubey, 2005). Based on the similar parameters of effectiveness of plants on Pb uptake at different amount of nutrients, it could conclude that Water hyacinth and Floating moss are not sensitive to nutrients concentration. Therefore, these species can be used to remove Pb in wastewater with high amount of nutrients.

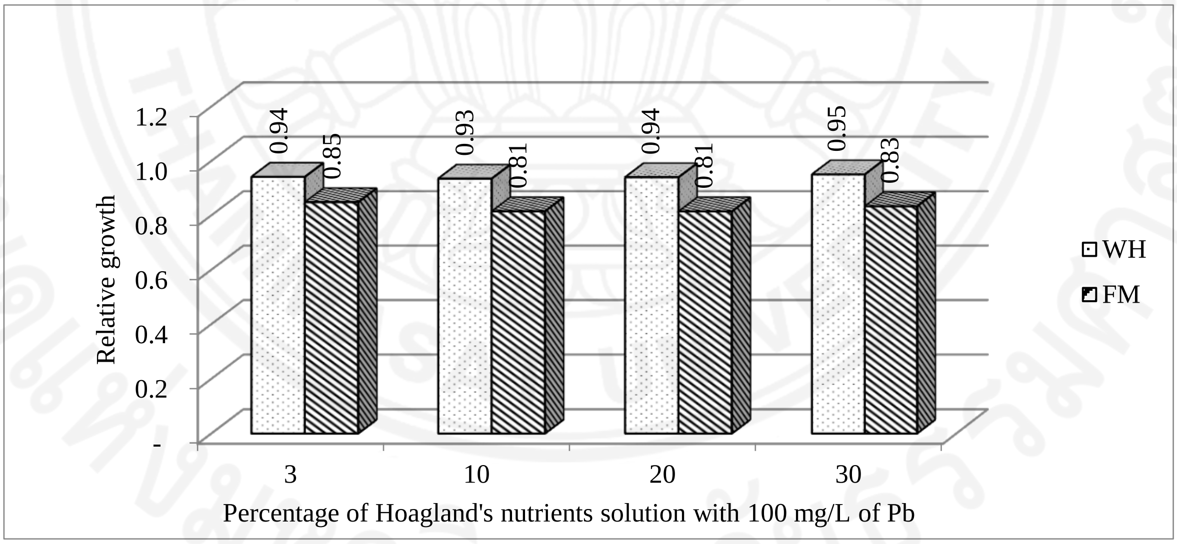


Figure 4.38: Relative growth of plants at different amount of Hoagland’s nutrients solution with 100 mg/L of Pb

Note: WH, Water hyacinth: and FM, Floating moss

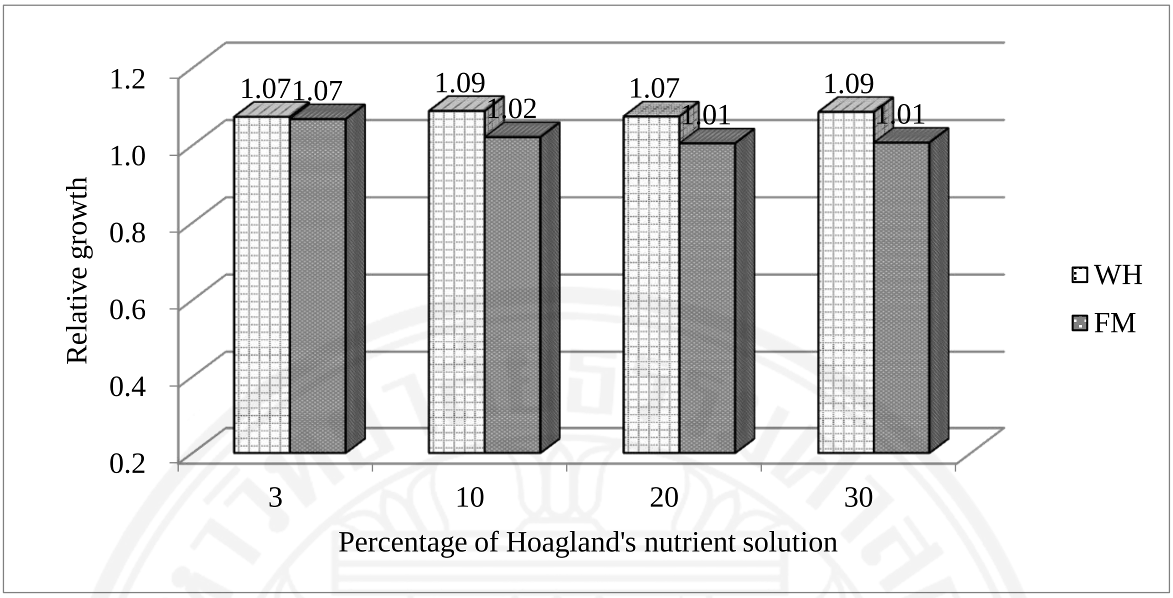


Figure 4.39: Relative growth of plants at different amount of Hoagland’s nutrients solution (control)

Note: WH, Water hyacinth: and FM, Floating moss

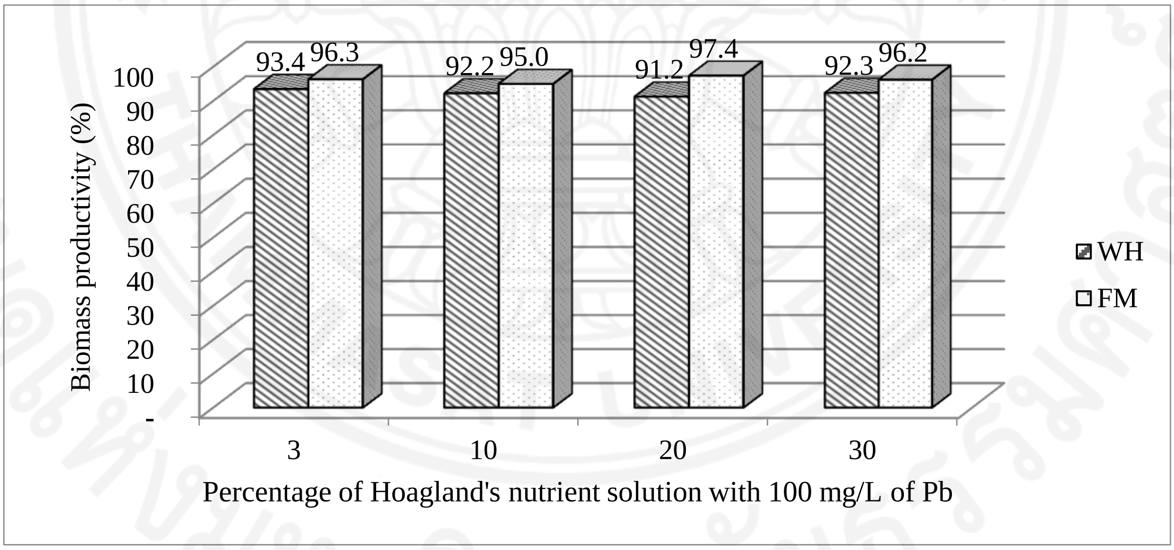
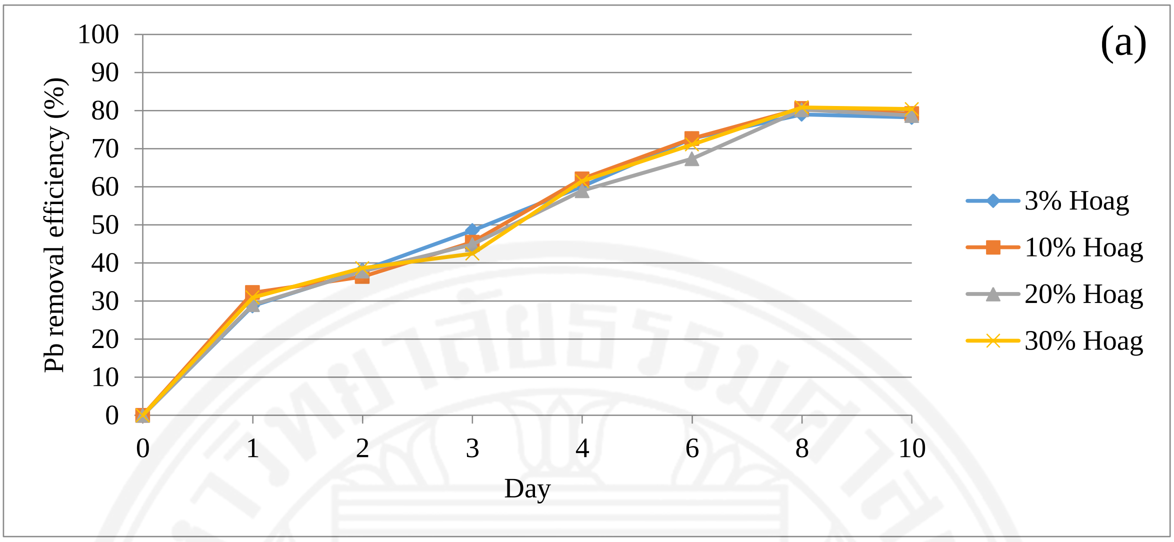


Figure 4.40: Biomass productivity at different amount of Hoagland’s nutrients solution

Note: WH, Water hyacinth: and FM, Floating moss



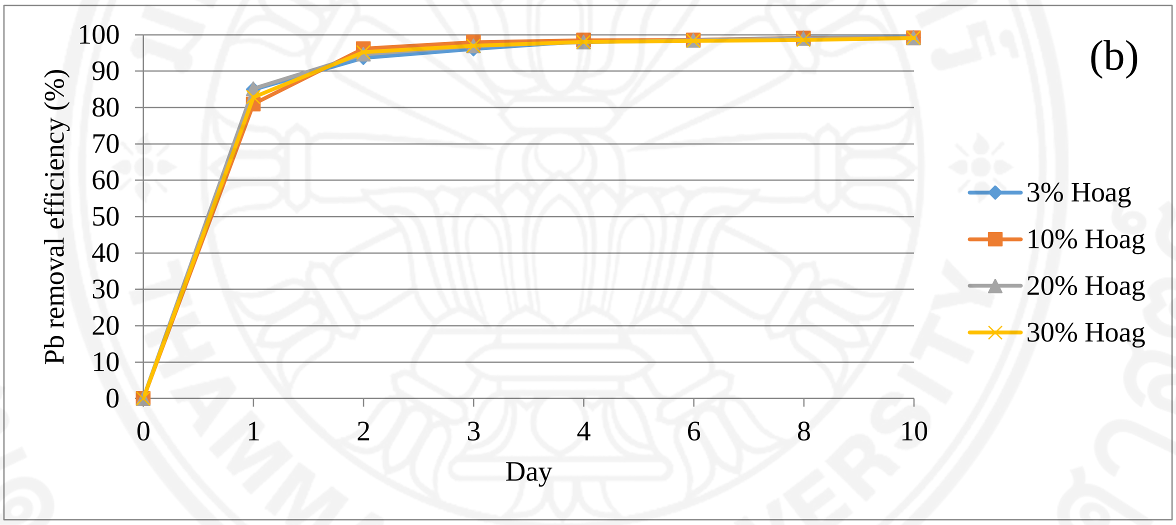


Figure 4.41: The effect of different amount of nutrients on a) Pb removal efficiency of Water hyacinth and b) Floating moss with100 mg/L of Pb

Note: Hoag- Hoagland’s nutrients solution with 100 mg/L of Pb

Table 4.10: Pb accumulation in floating plants at different amount of nutrients

Plant species	**Pb accumulation in plants (mg/g)**			
	3% Hoag	10% Hoag	20% Hoag	30% Hoag
WH	41.1	40.7	39.8	43
FM	73.6	70.5	75.5	73.7

Note: Hoag- Hoagland’s nutrients solution with 100 mg/L of Pb

WH, Water hyacinth: and FM, Floating moss

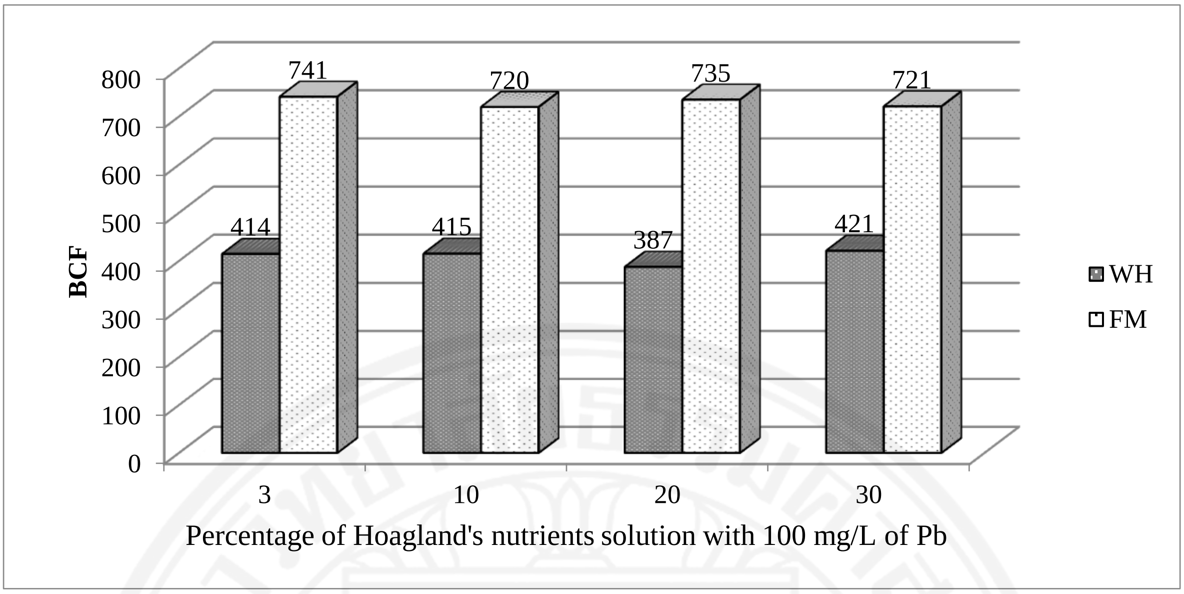


Figure 4.42: Biomass productivity of plants at different amount of nutrients with 100 mg/L of Pb

Note: WH, Water hyacinth: and FM, Floating moss

4.3. Comparison of effectiveness of Floating moss for Cd and Pb removal from wastewater

Floating moss was evaluated for Cd and Pb uptake at 30 and 100 mg/L. Relative growth, biomass productivity, Cd removal efficiency, Cd accumulation and BCF value of plant in contaminated water with Cd were lower than in contaminated water with Pb as shown in Table 4.11. Cd and Pb removal efficiency of Floating moss are shown in Figure 4.43. Whether the concentration of Cd and Pb was low (30 mg/L) or high (100 mg/L), Floating moss was able to remove these heavy metals. At 30 mg/L, Cd and Pb were removed 79.5 and 99.3%, respectively, and at 100 mg/L, they were removed 53.2 and 99.2%, respectively. This shows that Floating moss had lower Cd removal efficiency than Pb. The amount of Cd accumulation in plant was also lower than Pb at the same concentrations. For instance at 100 mg/L, the Cd accumulation in the plant was 23.4 mg/g, but it was 74.8 mg/g for Pb. According to Soltan and Rashed (2003), Cd accumulation in the roots of Water hyacinth was 2,060 µm/g and the aerial part was 325 µm/g, while, Pb was 34,950 µm/g in the root and 1,030 µm/g in the aerial part at 100 mg/L. Smolyakov (2012) found that Water hyacinth was able to remove heavy metals, especially Cd and Pb (low concentration). After treaded by Water hyacinth at 50 µg/L of Cd and 250 µg/L of Pb, Cd remaining in water was50%, but Pb was 26%. Soltan and Rashed (2003) confirmed that

Cd has stronger effect on Water hyacinth than Pb, resulting from inhibited growth. Thus, Floating moss in this study was tolerant to Pb and could be a good candidate to apply to remove Pb from wastewater more than Cd.

Table 4.11: Comparisons of effectiveness of Floating moss on Cd and Pb removal from artificial wastewater

Heavy metals	**Cd**	**Pb**	**Cd**	**Pb**
Concentration (mg/L)	30	30	100	100
Relative growth	0.80	0.86	0.64	0.70
Biomass productivity (%)	84.5	96.0	81.4	91.9
Heavy metals removal efficiency(%)	79.5	99.3	53.17	99.2
Heavy metals accumulation (mg/g)	15.4	23.3	23.4	74.8
BCF value	491	776	222.8	748.5
Period of experiment (day)	6	10	8	12

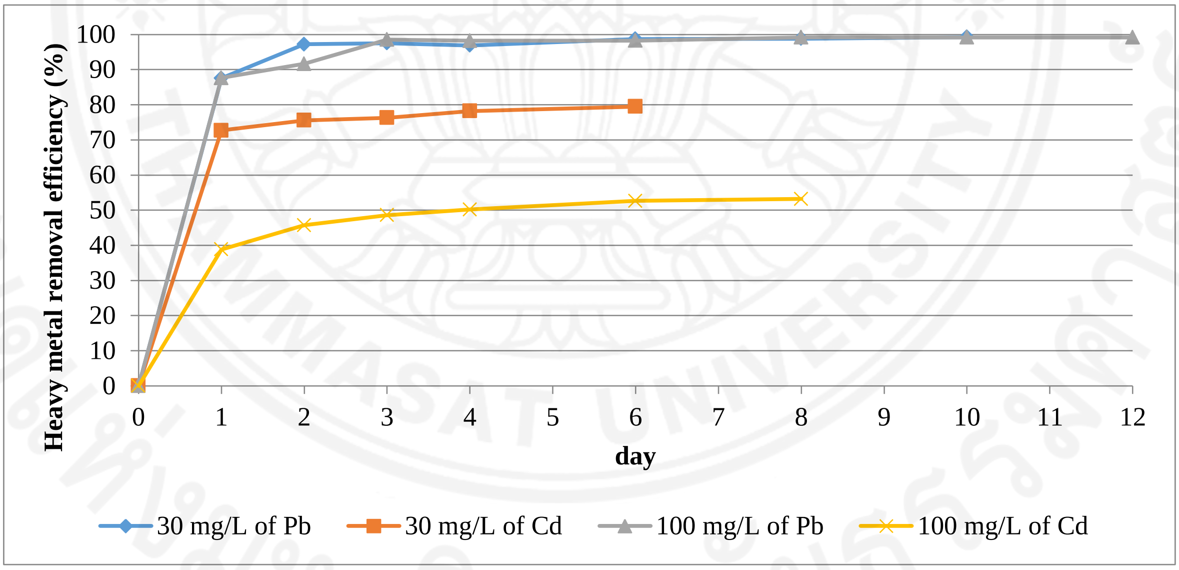


Figure 4.43: Comparison of heavy metals removal efficiency of Floating moss

4.4. Plants density of cultivation and harvesting

In the present study, the plants density of cultivation in heavy metal contaminated water was 26 g/L (26kg/m^3) for Water hyacinth, Water lettuce and Creeping waterprimrose. For Floating moss and Common duckweed was 30 and 6 g/L, respectively. Based on the results which showed high heavy metals removal efficiency, these plants densities are suitable for phytoremediation (rhizofiltration). In the Cd and Pb contaminated water, plants should be harvested whenever the heavy metals concentration in water is constant. The period of harvesting depends on the heavy metal concentration, their removal, and plant species.

The Cd contaminated water at 4 mg/L (Figure 4.8), Creeping waterprimrose and Floating moss can be harvested on the 2nd day of cultivation. Water hyacinth and water lettuce can be harvested when the Cd containing in water is constant. Common duckweed can be harvested within 3 days, otherwise, it might decompose and release Cd back to water again. Figure 4.14 (a) shows Cd removal efficiency of Creeping waterprimrose at different concentration. At concentration from 5 to 30 mg/L, Creeping waterprimrose can be harvested on the 4th day and the 8th day at concentration from 40 to 100 mg/L. For Floating moss, it needs to be harvested earlier than Creeping waterprimrose. The suitable days for harvesting are on the 2nd day at concentration from 5, 10 and 30 mg/L. It also can be removed from wastewater on the 3rd day at 20 mg/L and on the 8th day at concentration from 40 to 100 mg/L, as shown in Figure 4.14 (b).

For the Pb contaminated water at 10 mg/L (Figure 4.29), all plants can be harvested on the 2nd day. These plants had similar capacity on Pb removal based on the screening experiment. At different Pb concentration 10, 30, 50, 100, 200, 300 and 400 mg/L, Water hyacinth can be harvested on the 4th, 8th 10th, 12th, 10th, 10th, and 8th day, respectively. It can be seen that Water hyacinth at 70 mg/L can be harvested on the day of Pb remaining in water is constant, as shown in Figure 4.35 (a). Figure 4.35 (b) shows that the Pb remaining in treated wastewater by Floating moss. At concentration from 10 to 100 mg/L, Floating moss can be harvested on the 3rd day, and can be harvested on the 10th and 12th at 300 and 400 mg/L, respectively.

Chapter 5

Conclusions and recommendations

5.1. Conclusions

1) The effectiveness of plants on Cd uptake

Results from the study indicate that Cd was toxic to some plants and they looked unhealthy and could not survive in contaminated water (4 mg/L of Cd), especially Common duckweed. However, the plants could remove Cd well even in the short period of cultivation (within the 1^{st} day of cultivation). It can be seen that the Cd concentration in water decreased drastically. The percentage of Cd removal efficiency in decreasing order was as Floating moss>Creeping waterprimrose>Water lettuce>Common duckweed> Water hyacinth. The plants also had high biomass productivity, except Common duckweed. Although the biomass productivities were higher, plants looked unhealthy with partial wilting indicating that they were not able to survive in the Cd contaminated water. The Cd accumulation in Common duckweed was the highest (19 mg/g dry weight), and BCF value was also the highest (4,859) even though it looked unhealthy. The decreasing Cd concentration in water was mainly due to plants uptake. The results indicate that all plants can be used for Cd removal from wastewater and also act as a bio-indicator of the quality of water.

To study the effects of Cd concentration and amount of nutrients on plants uptake, Creeping waterprimrose and Floating moss were selected from the screening experiment because they had the highest Cd uptake. Plants looked unhealthy with increasing Cd concentrations and exposure times. Biomass productivity was high even though at 100 mg/L of Cd (79.7% for Creeping waterprimrose and 81.4% for Floating moss). Cd removal efficiency decreased with increasing concentration. At concentration 5 and 100 mg/L, the Cd removal efficiency of Creeping waterprimrose was 76.1 and 39.7%, respectively, and Floating moss's was 89.2 and 53.2 % respectively. With increasing Cd concentration, Cd accumulation in plants increased, except at 100 mg/L for Floating moss. The BCF values decreased with increasing Cd Concentration. BCFs for Creeping waterprimrose indicate that it is a moderate accumulator for Cd at 5 and 10 mg/L, and for other concentration is poor. While, BCF value for Floating moss indicates that it is moderate accumulator at concentration from 5 to 60 mg/L, and very poor accumulator at other concentration.

2) The effectiveness of plants on Pb uptake

In the screening experiment with 10 mg/L of Pb, all floating plants had capacity to remove Pb from contaminated water. Despite the short period of experiment, Pb was almost removed 100%, except for Water lettuce. Based on the high removal efficiency of Water hyacinth, Creping waterprimrose, Floating moss and Common duckweed, the treated water in all reactors can be discharged to the environment without any further treatment. Moreover, all plants had high biomass productivities and were tolerant to Pb (except Common duckweed). However, Common duckweed had the highest Pb accumulation (30.5 mg/g dry weight) and the highest BCF value (3322). Thus, all plants can be candidates for phytoremediation.

To study the effects of Pb concentration and amount of nutrients on plants uptake, Water hyacinth and Floating moss were selected from the screening experiment. The result of experiment of effect Pb concentration on uptake demonstrates that Water hyacinth and Floating moss looked unhealthy with increasing Pb concentration and exposure times, except Water hyacinth at 10 and 30 mg/L, and Floating moss at 10 mg/L. Even though the Pb concentration increased, plants still had high biomass productivity. At the Pb concentration from 10 to 400 mg/L, Water hyacinth had biomass productivity in range from 94.3 to 78.7%, and from 97.2 to 89.3% for Floating moss. The results show that the Pb removal efficiency decreased when the Pb concentration increased. At concentration from 10 to 400 mg/L, Pb was removed from 98.3 to 32.8% by Water hyacinth.

For Floating moss at concentration from 10 to 100 mg/L, Pb was almost removed 100%. After having treated at 30 and 50 mg/L by Floating moss, wastewater was closed to standard of discharged wastewater. The highest accumulation of Water hyacinth and Floating moss was found at 400 and 300 mg/L, respectively, and the lowest was found at 10 mg/L. Water hyacinth was found to be moderate accumulator at concentration from 5 to 70 mg/L, other concentration was poor accumulator. Based on BCF values, Floating moss can be classified as moderate accumulator at all concentration. Therefore, Creeping waterprimrose and Floating moss can be used for removal Pb from wastewater.

3) The effect of nutrients on Cd and Pb uptake

The results of this experiment including relative growth, biomass productivity, Cd removal efficiency, Cd accumulation in plants, and BCF value were almost similar when the percentage of Hoagland's nutrients solution was increased from 3 to 30%. The criteria of Pb removal from wastewater are also similar in each percentage of Hoagland's nutrients.

This means that amount of Hoagland's nutrients solution had no effect on Cd and Pb uptake by plant (Creeping waterprimrose for Cd, Water hyacinth for Pb and Floating moss both Cd and Pb).This indicates that plants are not sensitive to nutrients and can remove Cd and Pb even though the water contains additional nutrients.

4) Comparison of effectiveness of Floating moss on Cd and Pb removal

Comparing the toxicity between Cd and Pb on Floating moss at 30 and 100 mg/L, the plant cultivated in Cd contaminated water looked unhealthier than the plant in Pb contaminated water. The relative growth, biomass productivity, heavy metals removal efficiency, heavy metals accumulation and BCFs of Floating moss in Cd contaminated were lower than the one in Pb contaminated water. This shows that Cd has very strong effect Floating moss in comparison with Pb. Floating moss also has effectiveness of Cd uptake lower than Pb.

The results of this study indicate that all plants had high efficiency on Cd and Pb uptake, especially Creeping waterprimrose for Cd, Water hyacinth for Pb, and floating moss for both Cd and Pb. Therefore, they can be candidates for phytoremediation or use for wastewater treatment and also can be bio-indicator of the quality of water.

5.2. Recommendations

- Since the study was only done in laboratory with stimulated wastewater, it recommended to do research with real wastewater.
- Use Cd and Pb mix wastewater to study the impact of others on uptake.
- Extend time to study the changes of parameters that indicate effectiveness of plants on heavy metals uptake.
- Other locally available species should be explored.
- Experiment with pot culture need to bed explored as well.

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