PAK Publishing Group
Growing Knowledge for Future

Handbook on Economics, Energy,
Environment and Agricultural Sciences
ISBN: 978-969-9952-08-1

Response of *Lemna Minor* and *Salvinia Natans* as Bio-Sequester and Removal Agents Toward Fe, Cu and Zn Toxicities Through *in Vivo* Model System

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ABSTRACT

A lack of macrophytes in aquatic ecosystem may suggest a reduced population of wildlife whereas the absence of macrophytes may indicate problems in water quality. However an overabundance of macrophytes may due to excessive nutrients, organic or heavy metals interference. Aquatic macrophytes are well known as a good accumulator for heavy metals in phyto-technologies approach since the last decades. Therefore this study aimed to assess heavy metals sequestration rate of *Lemna minor* and *Salvinia natans* at three different concentrations ranging from low, medium and high (1 mgl^{-1}, 2 mgl^{-1} and 5 mgl^{-1}) of three types of heavy metal (Cu, Fe and Zn) at four different period of time (week 1 until week 4) through *in vivo* model system. The results established that there were significantdifferences between the sequestration rate of both species.*S. natans* ability and resistance over 3 types of heavy metal toxicity were much more higher and stable compared to *L. minor* and the capability of both species were varied and depending on the plant tolerance or resistance mechanism itself. Thus, the high correlation between metal removal in water and aquatic plant species indicates that those plants can effectively use for the removal of heavy metals from polluted or contaminated aquatic ecosystem of different concentrations.

Keywords: *Lemna minor*, *Salvinia natans*, aquatic macrophytes, heavy metals,bio-sequester, model system.

1. Introduction

In the last three decades, freshwater ecosystems have declined resulting in a threat of biodiversity due to water degradation. The population of freshwater species destroyed almost 50% on average; two-thirds greater than terrestrial and marine species (Jusoff, 2008). Even though the freshwater ecosystem consist of only 1% of the planet's surface, 12% of species live in freshwater and more than 25% of vertebrate species depend on freshwater ecosystems (UNEP, 2010). Changes in water quality affects nutrients, sedimentation, temperature, pH, heavy metals, non-metallic toxins, persistent organic and pesticides, and biological factors (Carr and Neary, 2008) and these pollutants are globally persistent in the environment and can be transported long ranges to regions where they have never produced (UNEP, 2009). According to Environmental Quality Report in 2009 showed that 46% of river water in Malaysia is polluted which is higher than previous years (DOE, 2011). Based on the National Water Resources Study 2000 - 2050, the parameters which have exceeded Class III limits include NH3-N, as the main pollutants result in low Water Quality Index (WQI), organic carbon, heavy metals, oil and grease (Al-Mamun and Zainuddin, 2013). The potential toxic elements such as copper (Cu), zinc (Zn) and iron (Fe) were essential elements to support biological process of plants (Nagajyoti et al., 2010). However, high concentration of Zn indicates the decrease growth and development, induction of oxidative damage to plants (Papanikolaou and Pantopoulos, 2005; WHO, 2004) whereas high concentration of Fe causes damages membranes structure, DNA and proteins (Papanikolaou and Pantopoulos, 2005). Meanwhile, high concentration of Cu affects plants germination, seedling length and number of lateral root (retard) (WHO, 2004) and becomes toxic to human being as well as aquatic life. Furthermore, both organic and inorganic pollutants in freshwater ecosystem would change the natural cycle and affect towards wildlife habitat as well as human health who become premier consumer to this untreated

freshwater ecosystem. Thus, the proper treatment for waste pollution in freshwater ecosystem need an appropriate technology to absorb heavy metals pollutants in healthy way.

Lack of technology development applied in many areas have many limitations due to costs and instruments such as ultrafiltration (UF) membrane (Juang et al., 2007), thus, the untreated wastes flow into freshwater ecosystem without filtration and treatment and destroy aquatic ecosystem cycles and threaten human life. Presently, phyto-technology is still a nascent technology that seeks to exploit metabolic capabilities and growth habits of higher plants. From landscape architecture perspectives, phyto-technology can create sustainable green space as well as provide a natural barrier for visual screening, reduce noise, and require less intense human interaction (ITRC, 2009). Phyto-technology refers an emerging cost effective and eco-friendly technology that use plant based to remove, sequester or transform a variety of contaminants in soil, water and sediment (Dhir et al., 2009). Sustainable approach and practice need to be emphasized and evaluate elements of cleanup project which gain from different strategies of green remediation (Pedron and Petruzzelli, 2011).The mechanisms of phyto-technology depend upon plant physiological process driven by solar energy, the rhizospheric process and available pioneer. It includes the accumulation of chemicals in plants to remove or degrade of organic and inorganic pollutants by decomposition of microorganism, absorption and volatilization and bioavailability of containment in environment (Paz-Alberto et al., 2014).

Many studies have reported that various types of aquatic macrophytes have a great potential to accumulate trace elements through their roots, stems and/or leaves (Jackson, 1998; Baldantoni et al., 2004; Mishra and Tripathi, 2009; Lesage et al., 2007).Macrophytes are aquatic plants which grow in or near water as emergent, submerged or floating whereas aquatic macrophytes refers to macroscopic forms of aquatic vegetation that encompasses macro algae (Rai, 2009). In addition, aquatic macrophytes are excellent indicators in polluted environment to respond with nutrients, light, toxic contaminant, metals, herbicides, turbidity, water level change and salt (Liu et al., 2007). Accumulation of metals by plants depend on type of soil, percentage of organic matter present in the soil and metals availability as well as soil acidity (pH) and the plant species that generally absorb by root and shoot system (Dhir and Srivastava, 2011; Afrous et al., 2011).There are two types of methods used in conducting the study, namely *in situ* (sampling) and experimental condition. The researcher selected experimental condition as the method to study aquatic plants and their capabilities because it easy to manipulate and manage small areas. There are various lab experiment techniques including greenhouse, hydroponic, pot and tank. However, there is no model system for small experiment.

The term 'model' refers to the scale of the modeller, meanwhile 'model system' is made to control the experimental environment that focuses only on a set of interactions being studied and its challenge is to provide a predictive value in a real system of interest (Basu and Schneider, 2006) as important tools in framing and studying biological processes (Peterson and Mrksich, 2007). Modelling is an important tool for the comprehension of a complex ecosystem inspired from nature's ecosystems with numerical functions and engineering optimization (Zhang et al., 2014). Meanwhile, plant growth models is a simplification of a complex system to structure and integrate available knowledge, test hypothesis as well as quantitative estimate of total plant mass, and above ground mass and/or yield (Poorter et al., 2013). Furthermore, through a modelling analysis, the prediction of chemical toxicity and potential mechanism for metabolism and toxicity of the pollutant can be performed. This approach offers a highly effective choice for risk assessment of metal pollution in aquatic ecosystems. For example, the modelling for cadmium exchange by aquatic moss completely fits the prediction results of other moss species (Zhou et al., 2008; Croisetiere et al., 2005).

Several studies reported that aquatic macrophytes from submerged, emergant and floating such as *Eichornia crassipes*(water hyacinth), *Pistia stratiotes* (water lettuce) and *Salvinia natans* (floating fern) can accumulate nutrients and toxic water pollutant (Denga et al., 2004) whereas the Lemnaceae such as *Lemna minor* and *Spirodela polyrrhiza* (duckweed) were observed as an excellent bioaccumulator for various type of heavy metals and toxic trace elements as well as to indicate abundance of nitrogen in contaminated aquatic ecosystem (Drost et al., 2004; Mkandawire and Dudel, 2007; Dunshenkov et al., 1995). In the experiment on *Lemna minor*, *Lemna gibba* and *Lemna punctata* have been kept on half-strength Hutner medium with Zn (0.2, 3, 10, 30 and 100 mg l^{-1}) in 10 days. The result revealed those species show greater accumulation of zinc in roots when exposed to high levels of zinc meanwhile *L. gibba* significantly accumulated zinc at low concentrations (Lahive et al., 2011). *Vallisneria spiralis* has been examined to Cu and Cd with different concentrations in prepared pot experiment contains of sediment within 21 days. The maximum accumulation of Cu has been found in roots and shoots, however the plants shows a decrease in chlorophyll content (Qian et al., 2010). Both living and dead of aquatic macrophytes were reported and examined extensively as potential heavy metals accumulator from waste water (Kuyucak and Volesky,

1989). A positive correlation was found between the level of metals in water and plants and /or between metals in soil and plants (Mishra and Tripathi, 2009, 2008; Bonanno and Giudice, 2010; Rahman et al., 2007; Wetzel, 2001; Prasad et al., 2001). Therefore, the study aimed to assess the efficiency of *Salvinia natans* and *Lemna minor* as potential bio-accumulator agent for Iron (Fe), Copper (Cu) and Zinc (Zn).

2. Materials and Methods

2.1. Plant Selection

Two aquatic macrophytes *Lemna minor* (duckweed) and *Salvinia natans* (floating fern) were selected to assess their heavy metals removal capacities for three heavy metals (Cu, Fe and Zn) from water under laboratory conditions. Both macrophytes are perennial aquatic that carry out their entire lifecycle as free-floating plants. The experimental plants were purchased from Bu Seng Chong Nursery Sdn. Bhd. (2º2'32"N 102º38"39E). The stocks were maintained in Herbarium Laboratory at the Kulliyah of Architecture and Environmental Design, IIUM, in a plastic bucket with 5-L capacity, according to the procedure reported by Wang (1986). These plants were grown in 792 experimental container (glass jars and sterile tubes) filled with 74 L of sterile deionised water. Three heavy metals (Fe, Cu and Zn) were added together in an amount that make their concentration in the experimental container 1 mgl^{-1}, 2 mgl^{-1} and 5 mgl^{-1} respectively (Mishra and Tripathi, 2008). The plants were kept at a temperature of 24 ± 1ºC and illuminated by cool daylightfluorescent tubes in 24-h light.

2.2. Heavy Metals Preparation

Three heavy metals Fe, Zn and Cu were added with different concentrations (1 mgl^{-1}, 2 mgl^{-1} and 5 mgl^{-1}) in each treatment test. Stock solutions of analytical grade heavy metals salt (FeSO4.7H20, CuSO4.5H2O and ZnSO4.7H2O) were prepared in deionized sterile water. The pH of the solution is 7.5. The experiment was carried out for from week 1 until week 4 and 10 replicates were considered for all treatments with 1 control medium without plant species. The test nutrient solution that exposed to heavy metals was harvested in week 1, week 2, week 3 and week 4.

2.3. Analysis of Heavy Metals in Water Samples

Water samples of 350 mL (35 mL x 10 replicate of sterile tube) with concentration were taken from all the experimental sets at week 1 (7 days) for heavy metals analysis. Heavy metals are analyzed with procedure adapted from "Standard Methods for Examination of Water and Wastewater" (APHA, 1999) which the approval of acceptance of method by United States Environmental Protection Agency (USEPA).

The metals analyses were carried out by means of Hach DR 5000 Spectrophotometer. Heavy metals were analyzed by using USEPA method accepted for water or waste water analysis. Three types of method had been applied for different heavy metals which are FerroZine® Method for iron (Fe) (Stookey, 1970), Porphyrin Method[1] for copper (Cu) (Ishii and Koh, 1979) and Zincon Method for zinc (Zn) (Federal Register, 1980). Water samples were filtered using vacuum filtration as scheduled (week 1, week 2, week 3 and week 4) from all the experimental sets at 24-h interval for heavy metal analysis.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was calculated to test the validity of the data and the significance of the variation in the data of three heavy metals studied Fe, Zn and Cu in different concentration for *Lemna minor* and *Salvinia natans* at different incubation period of time.

3. Results and Discussion

Analysis of variance showed significant difference ($p>0.001$) between heavy metals uptake, concentration range (1.0 mgl^{-1},2.0mgl^{-1} and 5.0mgl^{-1}) and incubation period of time. Both aquatic macrophytes, *L. minor* and *S. natans* accumulated and sequestered all the metals tested (Fe, Cu and Zn) in present investigation. The capacity for metals concentration with increasing period of time could be explained on the metal sequestration rate increased up to week four in different experimental sets (Figures 1 to 6) and this is in agreement with Mishra et al. (2008) and Dhir and Srivastava (2011). Another interesting part is *L. minor* and *S. natans* plants have different uptake mechanisms to specific metal as supported by Qian et al. (2009). In our findings, Fe sequestration rate was approximately 65% efficiency at week one whereas for week four, Fe uptake increased up to 85 % by *L. Minor* for 1mgl^{-1} (Figure 1). The treatment for 2.0 mgl^{-1} and 5.0 mgl^{-1} of Fe were observed more than 90% applied as well for Cu and Zn. A similar result was reported by Miretzky et al. (2004) who mentioned that *L. minor* was sequestered 78.5 % of Fe from

water meanwhile Cu and Zn were sequestered more than 90% from a solution medium. In contrast, 72% of Zn was remediated by *L. minor* while Cu was found similarly with this study where 99% of Cu was absorbed by *L. minor* (Azeez and Sabbar, 2012). As compare to another species, *L. minor* showed greater accumulation of Zn in high levels of toxicity than *L. gibba* (Lahive et al., 2011).

In this study, *S. natans* was detected with higher sequestration rate for approximately 90% efficiency in all treatments (Figure 4, 5, 6). Interestingly, *S. natans* have high capacity to accumulate and sequester more than one heavy metals from multi solution removal up to 84 % and 73.8 % of Zn and Cu respectivelyeven at high concentration for Cu and Zn except for Fe (Dhir and Srivastava, 2011; Dhir and Srivastava, 2013). The extent of heavy metal sequestration rate within aquatic plant species is known to vary significantly between species. As for example, the emergent aquatic plant species are usually sequestered lower amounts of metals than submerged aquatic species (Kamal et al., 2004). Species such as *Centella asiatiaca* and *E. crassipes* had a maximum removal of Cu in solution about 99.6% (Mokhtar et al., 2011).

In order to exploit the metals accumulation by aquatic plants, several studies were reported that aquatic plants could be indicator of industrial pollution from anthropogenic sources in the environment (Megateli et al., 2009) and become as essential micronutrients for plants (Teisseire and Guy, 2000). However, in certain concentrations, those heavy metals become first inhibitory and afterwards toxic. Several studies mentioned that Zn exposure may cause toxic effect such as reduced growth and chlorosis (Rout and Das, 2003) whereas Cu responsible to plant cell alteration such as respiration and photosynthesis, decrease of biomass growth, disintegration of antioxidant system, induce stress in plant (Teisseire and Guy, 2000; Clemens, 1996) as well as oxidation of Fe^{2+} (ferrous)to form Fe^{3+} (ferric).The formation of ferric at aquatic roots cancreate a barrier to prevent toxic metals from entering plant root (Papanikolaou and Pantopoulos, 2005).

Each plant species has different resistance and tolerance levels to different contaminants. Unfortunately, from our observation at 2.0 mgl^{-1} and 5.0mgl^{-1} of Cu, *L. minor* was found dead and bleached. Due to that, we could determine that at high concentrations of Cu, *L. minor* had limited adsorption capacity, accumulate and tolerate with this metal and similar report by Prasad et al. (2001). Meanwhile,Khellaf and Zerdaoui (2010) discovered that at high concentration of Cu inhibited *L. gibba* growth due to toxicity. In contrast, Cu and Ni also showed toxicity to *Hydrilla vercillata*, *Elodea Canadensis* and *S. natans* after 5 days (Begum and HariKrisna, 2010).

4. Conclusion

In conclusion, *Lemna minor* and *Salvinia natans* were a good and potential bio-sequester agent to clean-up heavy metals pollutant in aquatic ecosystems. They have been able to sequester all three heavy metals in a linear relationship with incubation period of time. The assessment of *L. minor* and *S. natans* as selected aquatic plant materials in this study successfully approved the hypothesis that both plants can be manipulated as bio-sequester agents in order to remove heavy metals contaminant in aquatic ecosystems as water treatment before the water is discharged into mangrove and marine ecosystems. The findings indicate that *L. minor* and *S. natans* were great bio-sequester agents to sequester heavy metals where more than 90% of Cu, Fe and Zn were sequestered at different incubation periods with different ranges. Experimental model system is among the best solutions to determine the plant capabilities to remove, sequester or accumulate heavy metals contaminants even though was limited to the natural environment (biotic and abiotic factor Therefore, more work is needed to optimise the design and management of aquatic plant based systems in order to achieve maximum efficiency in the sequestration rate of heavy metals before large scale application is adopted.

5. Acknowledgment

The authors would like to thanks Ministry of Higher Education (MOHE) and International Islamic University Malaysia (IIUM) for the Research Grant RACE140-020-0018 and FRGS13-052-0293.

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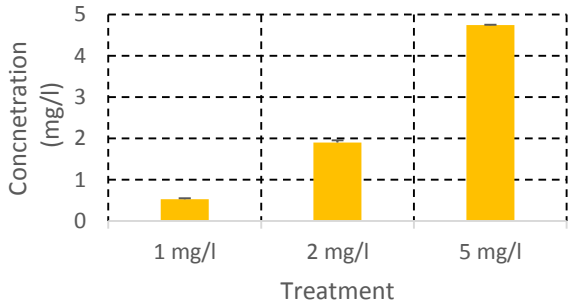

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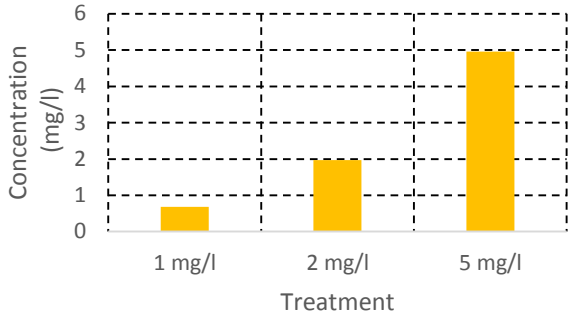

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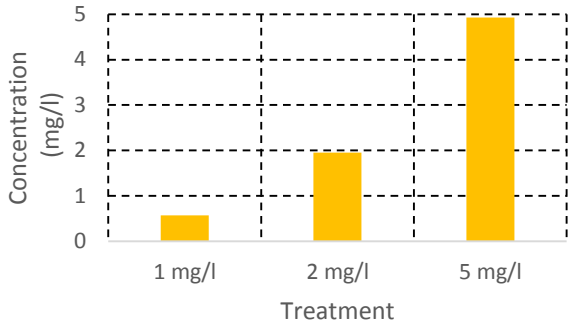

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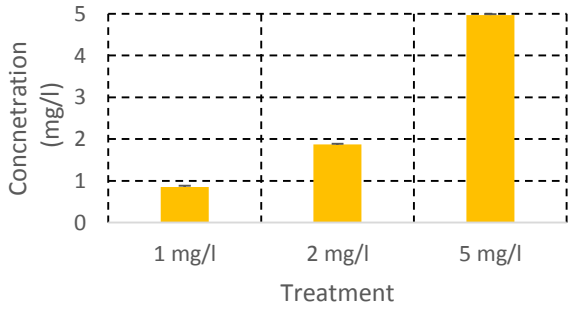
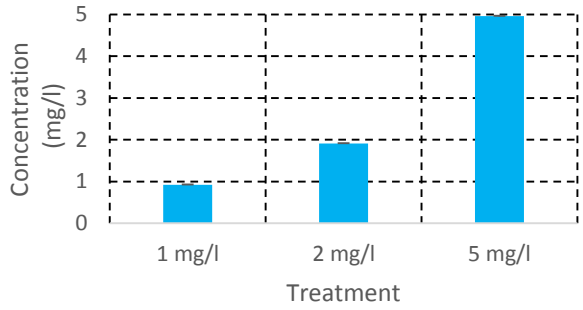


Figure-1. Assessment of Fe sequestration rate by *L. minor* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

A.



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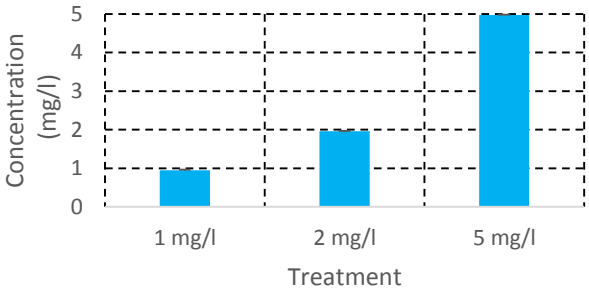

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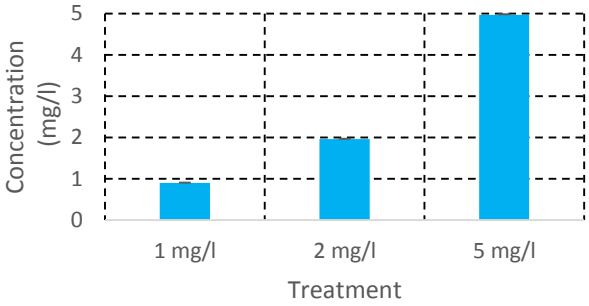

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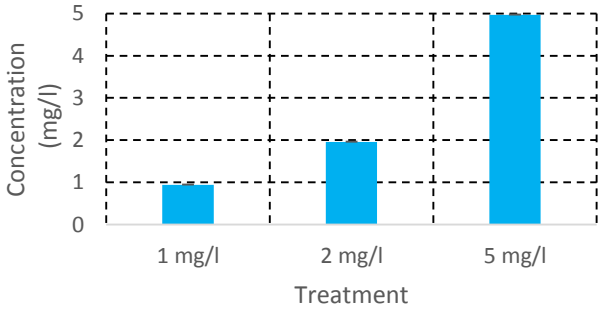
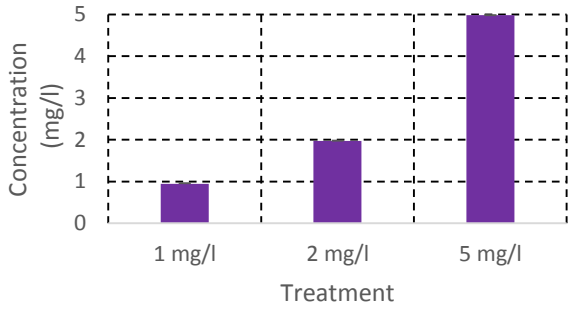


Figure-2. Assessment of Cu sequestration rate by *L. minor* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

A.



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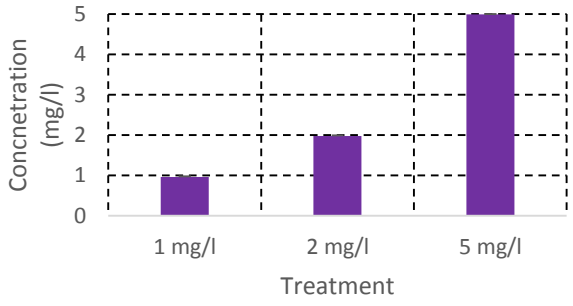

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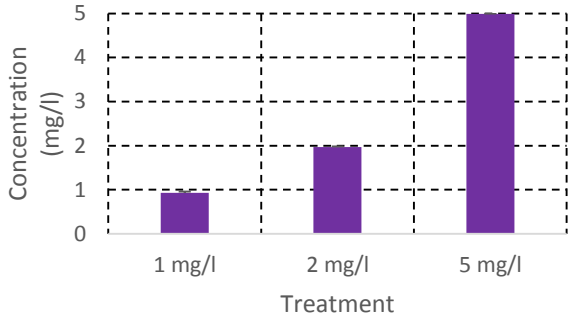

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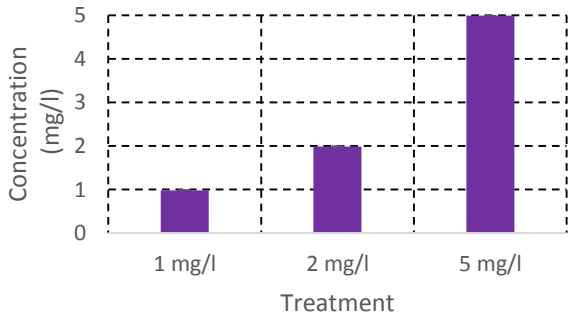

Figure-3. Assessment of Zn sequestration rate by *L. minor* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

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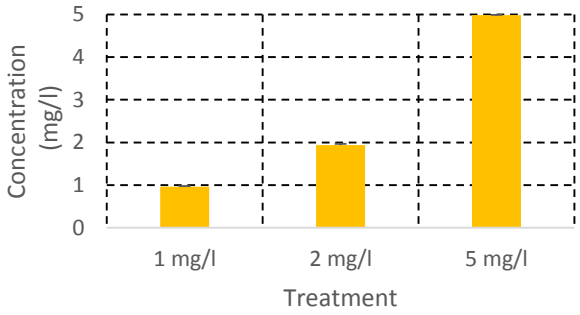

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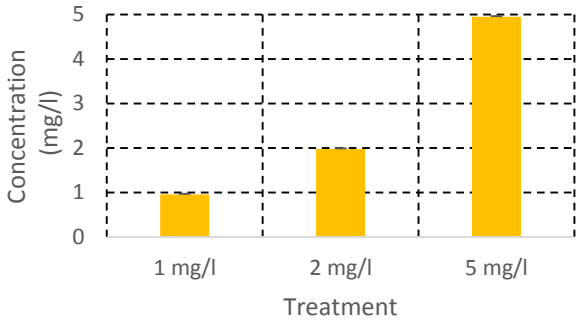

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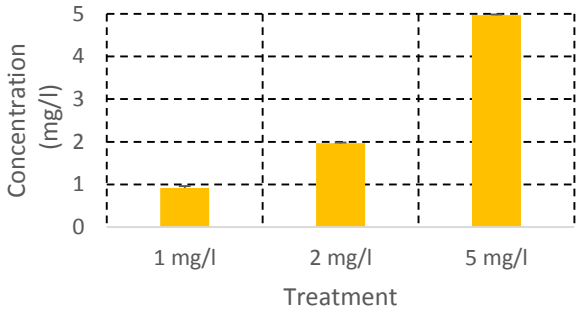

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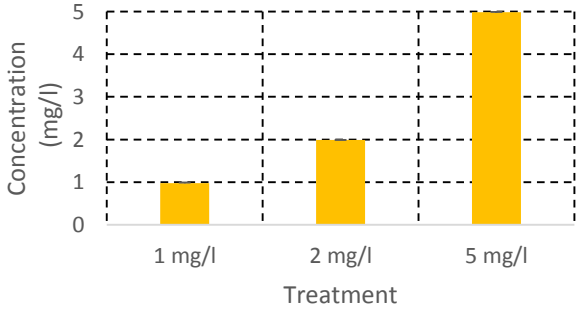

Figure-4. Assessment of Fe sequestration rate by *S. natans* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

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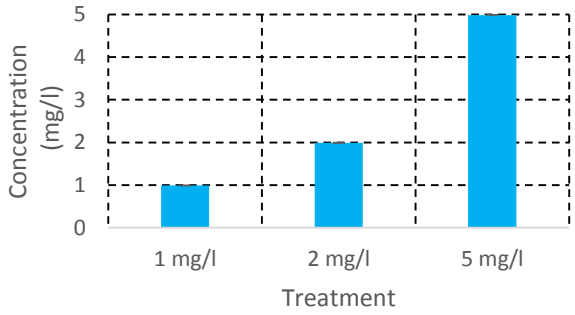

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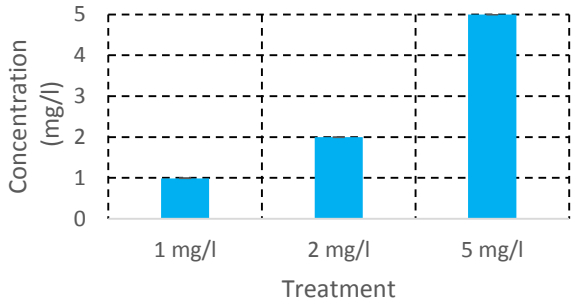

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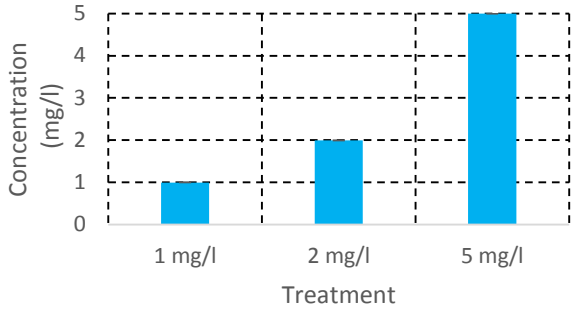

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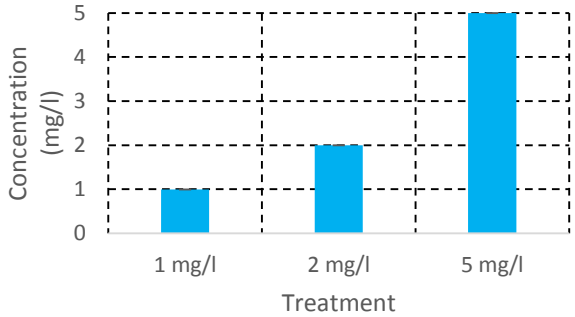

Figure-5. Assessment of Cu sequestration rate by *S. natans* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

A.

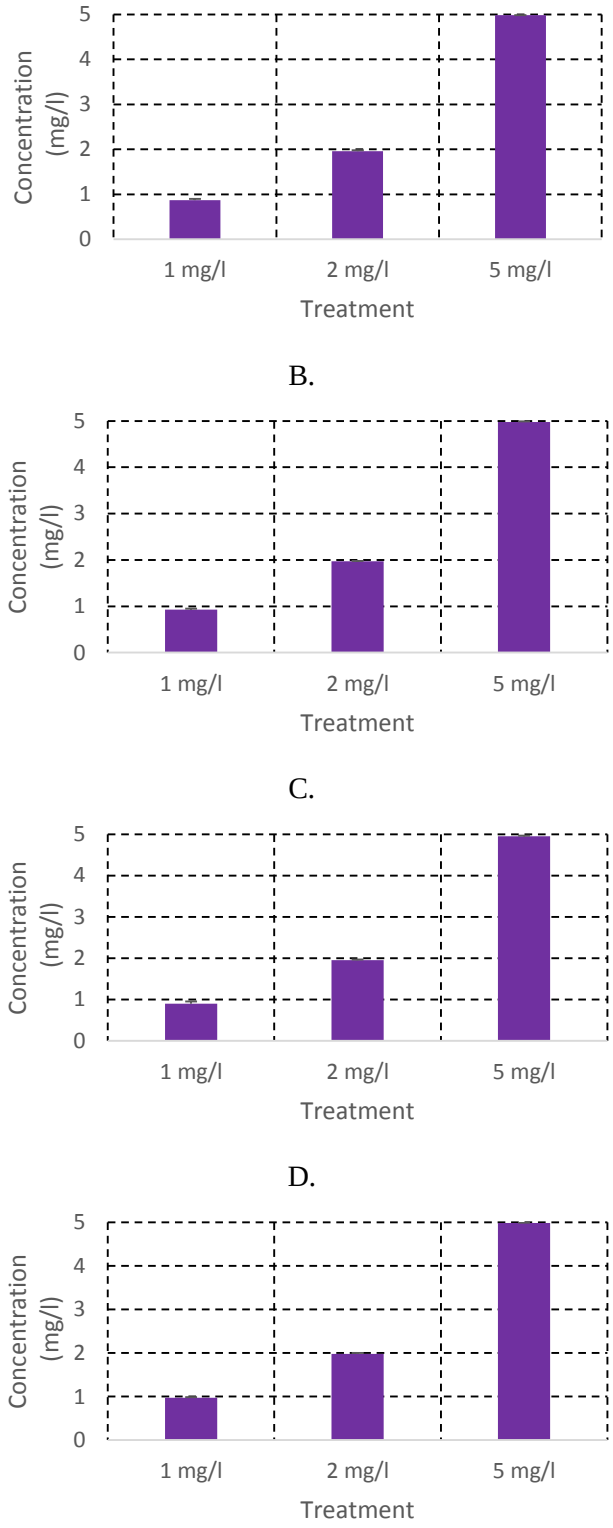

Figure-6. Assessment of Zn sequestration rate by *S. natans* in 1mg/l, 2mg/l and 5 mg/l at different incubation period at week 1, week 2, week 3 and week 4
A. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1
B. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2
C. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3
D. Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

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