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RESPONSE OF LEMNA MINOR AND SALNINIA NATANS AS PHYROREMEDIATION AGENTS TOWARDS FE, CU AND ZN TOXICITIES VIA IN VIVO MODEL SYSTEM

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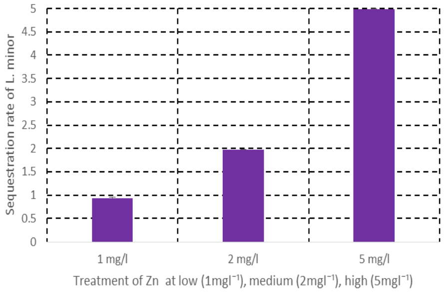
Graphical abstract



Abstract

A lack of aquatic plants in aquatic ecosystem may suggest a reduced population of wildlife whereas the absence of aquatic plants may indicate problems in water quality. However an overabundance of aquatic plants may due to excessive nutrients, organic or heavy metals interference. Aquatic plants are well known as a good accumulator for heavy metals in phyto-technologies approach as a green friendly since the last decades. Therefore this study aimed to assess heavy metals remediation 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 significant differences 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, high relationship 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 plant, heavy metal,phyto-remediation, phyto-technology, model system, green application

1.0 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 [1]. 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 [2]. Changes in water quality affect nutrients, sedimentation, temperature, pH, heavy metals, non-metallic toxins, organic and pesticides, and biological factors. These pollutants are globally persistent in the environment and can be transported long ranges to regions where they have never produced [3, 4]. Environmental Quality Report in 2009 showed that 46% of river water in Malaysia were polluted which is higher than previous years [5]. Based on the National Water Resources Study 2000 - 2050, the parameters which have exceeded Class III limits include NH_3-N, as the main pollutants result in low Water Quality Index (WQI), organic carbon, heavy metals, oil and grease [6]. The potential toxic elements such as copper (Cu), zinc (Zn) and iron (Fe) were essential elements to support biological process of plants [7].Furthermore, anthropogenic activities such as domestic sewage, tourism activities, land reclaiming and commercial activities were the main sources of heavy metals contaminant (Cu, Fe and Zn) increased up to 76% [7-8].

High concentration of Zn indicates the decrease growth and development, induction of oxidative damage to plants [8-9] whereas high concentration of Fe causes damages membranes structure, DNA and proteins [8]. On the other hand, high concentration of Cu affects plants germination, seedling length and number of lateral root (retard) [9] and becomes toxic to human being as well as aquatic life. On top of that, 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, an appropriate technology to absorb heavy metals pollutants in healthy way to treator remediate pollution in freshwater ecosystem is much needed.

Lack of technology development applied in many areas have many limitations due to costs and instruments such as ultra-filtration (UF) membrane [10], 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 ecology 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 [11]. 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 [12]. Sustainable approach and practice need to be emphasized and different strategies of green remediation need to be evaluated [13] sincethe 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 [14].

Many studies have reported that various types of aquatic plants have a great potential to accumulate trace elements through their roots, stems and/or leaves [15-20]. 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 [21]. 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 [22]. 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 [23-24].There are two methods that can be used in conducting this study, namely *in situ* (site sampling) or through artificial condition in controlled environment such as in the laboratory. In this study controlled environment or in vivo condition was selected as model system to study aquatic plants capabilities to sequester heavy metals contaminant. 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 interestas important tools in framing and studying biological processes [25, 26]. Modelling is an important tool for the comprehension of a complex ecosystem inspired from nature’s ecosystems with numerical functions and engineering optimization [27]. 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 [28]. In addition, 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 [15, 29-30].

Several studies reported that aquatic plants 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 as reported by Denga *et al*. [31] whereas the Lemnaceae such as *Lemna minor* and *Spirodela polyrrhiza* (duckweed) were observed as an excellent bio-accumulator for various type of heavy metals and toxic trace elements as well as to indicate abundance of nitrogen in contaminated aquatic ecosystem [32-34]. *Lemna minor*, *Lemna gibba* and *Lemna punctata* have been reported to show greater accumulation of zinc in roots when exposed to high levels of zinc on half-strength Hutner medium with Zn at 0.2, 3, 10, 30 and 100 mg l^{-1} in 10 days meanwhile *L. gibba* significantly accumulated zinc at low concentrations [35]. Meanwhile *Vallisneria spiralis* has been observed to accumulate high Cu and Cd in roots and shoots with different concentrations in prepared pot experiment contains of sediment within 21 days, however the plants shows a decrease in chlorophyll content [36]. Interestingly both living and dead of aquatic plants were reported and examined extensively as potential heavy metals accumulator from waste water [37]. A positive correlation was found between the level of metals in water and plants and /or between metals in soil and plants [16-17, 38-42]. Therefore, this 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) at different level of toxicities at different periods of time.

2.0 MATERIALS AND METHODS

2.1 Plant Selection

Two aquatic macrophytes *Lemna minor* (duckweed) and *Salvinia natans* (floating fern) were selected to assess their removal capacities for three heavy metals (Cu, Fe and Zn) from contaminated water via *in vivo* model system under laboratory conditions. Both macrophytes are perennial aquatic that carry out their entire lifecycle as free-floating plants. The selected plants species were maintained in a 5-L plastic bucket according to the procedure as detailed by Wang [43]. The plants were kept at a temperature of 24 ± 1°C and illuminated by cool daylight fluorescent tubes in 24-h light until further analysis [38].

2.2 Heavy Metals Preparation

Three heavy metals (Fe, Zn and Cu) with three different concentrations (1 mgl^{-1}, 2 mgl^{-1} and 5 mgl^{-1}) were added in each treatments. 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 was adjusted to 7.5. The experiment was carried out from week 1 until week 4 and 10 replicates were established for all treatments with 1 medium without plant species as control. All the aquatic plants developed through in vivo model system were harvested after week 1, week 2, week 3 and week 4.

2.3 Statistical Analysis

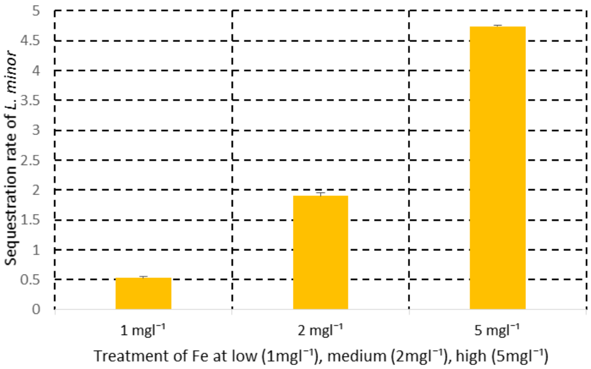
Analysis of variance (ANOVA) was established to test the validity and the significant of the data ($p<0.0001$) at three types of heavy metals (Fe, Zn and Cu) in three different concentrations (1 mgl^{-1}, 2 mgl^{-1} and 5 mgl^{-1}), at four different incubation periods of time and their interaction.

3.0 RESULTS AND DISCUSSION

Analysis of variance showed significant difference ($p>0.0001$) between heavy metals uptake, concentration range (1.0 mgl^{-1},2.0 mgl^{-1} and 5.0mgl^{-1}) and incubation period of time. Both aquatic macrophytes, *Lemna minor* (duckweed) and *Salvinia natans* (floating fern)accumulated and sequestered all the metals tested (Fe, Cu and Zn). The capabilities for metals absorption at different concentrations with increasing period of time could be explained clearly as indicated in Figures 1 to 6 in which this is in agreement with Dhir and Srivastava [23] and Misha and Tripathi[38].Another interesting part is *L. minor* and *S. natans* plants have different uptake mechanisms to specific metal as supported by Qian *et al*. [44]. In this findings, Fe sequestration rate efficiency by *L. minor* for 1mgl^{-1} (Figure 1) was approximately 65% at week one whereas for week four, Fe uptake increased up to 85 %. The treatment for 2.0 mgl^{-1} and 5.0 mgl^{-1} of Fe,Cu and Zn were observed more than 90%. A similar result was found by Miretzky *et al*. [45] which reported that *L. minor*sequestered 78.5% of Fe meanwhile Cu and Zn were sequestered more than 90% from a treated medium. In contrast, 72% of Zn was remediated by *L. minor* while Cu was absorbed at 99% [46]. If compared to another species, *L. minor* showed greater accumulation of Zn at high levels of toxicity than *L. gibba*[35].Based on the results,*S. natans* was detected with higher sequestration rate of 90%for all treatments (Figure 4, 5, 6). Previous report observed that *S. natans*capable to accumulate and sequester more than one heavy metals from multi solution up to 84 % and 73.8% of Zn and Cu respectivelyeven at high concentration but not Fe [23, 47]. 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 as submerged aquatic species [48].Another example, species such as *Centella asiatiaca* and *Eichhornia crassipes* had a maximum removal of Cu, whereas*Riccia fluitans* can sequester Mn, Zn and Pb at high toxicity [15, 49].

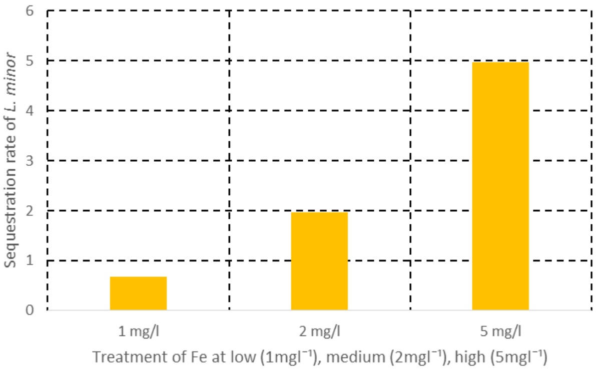
In order to exploit the metals accumulation by aquatic plants, several studies were reported that aquatic plants could be potential phyto indicator of industrial pollution from anthropogenic sources in the environment and become as essential micronutrients for plants [50, 51].However, in certain concentrations, those heavy metals can become inhibitory at the beginning and afterwards toxic. Several studies also mentioned that Zn exposure may cause toxic effect such as reduced growth and chlorosis whereas Cu responsible to plant cell alteration such as respiration and photosynthesis, decrease of biomass growth, disintegration of antioxidant system as well as induce stress in plant [51-53].Paradoxically an oxidation of Fe^{2+} (ferrous) to form Fe^{3+} (ferric) at aquatic roots cancreate a barrier to prevent toxic metals from entering plant root [8]. 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, it can be concluded that at high concentrations of Cu, *L. minor* had limited sequestration capacity, and low tolerance with this metal and similar results were reported by Prasad *et al*. [41]. Khellaf and Zerdaoui [54] also discovered that high concentration of Cu inhibited *L. gibba* growth due to high toxicity. In contrast, Cu and Ni also showed similar symptom of high toxicity to *Hydrilla vercillata*, *Elodea canadensis* and *S. natans* after 5 days [55].

A.



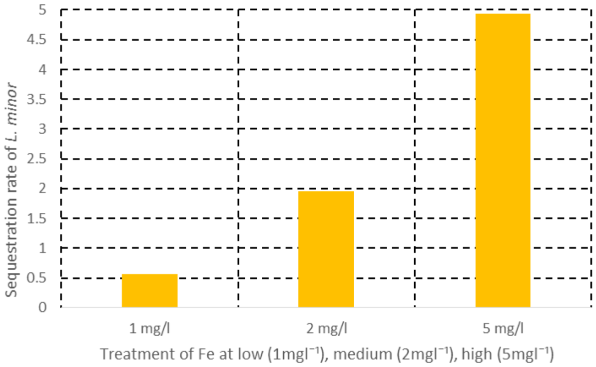
Fe sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 1

B.



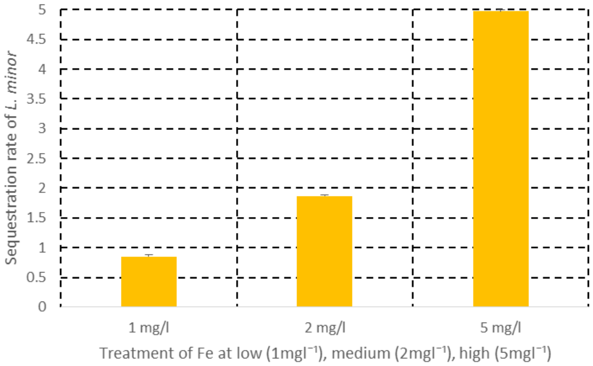
Fe sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 2

C.



Fe sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 3

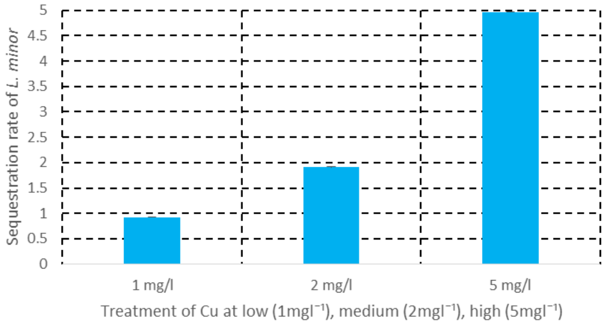
D.



Fe sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 4

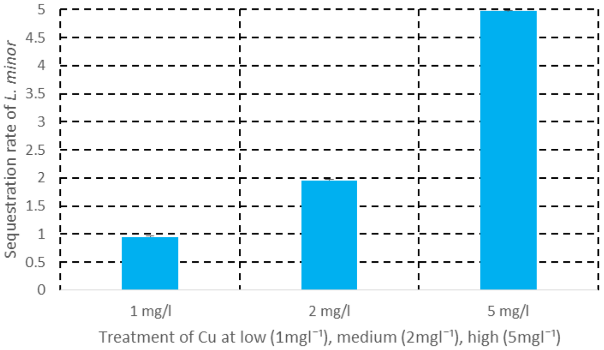
Figure 1 Assessment of Fe sequestration rate by *L. minor* in 1mgl^{-1}, 2mgl^{-1} and 5 mgl^{-1} at different incubation period at week 1, week 2, week 3 and week 4

A.



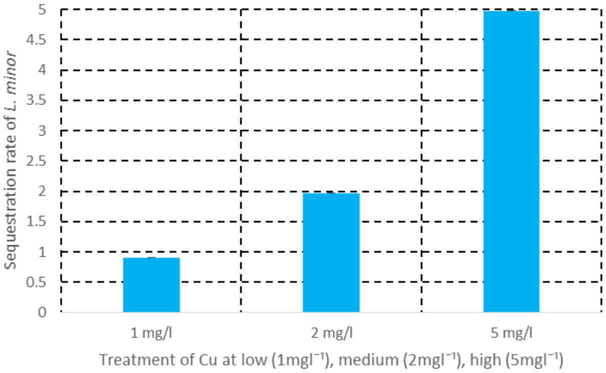
Cu sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 1

B.



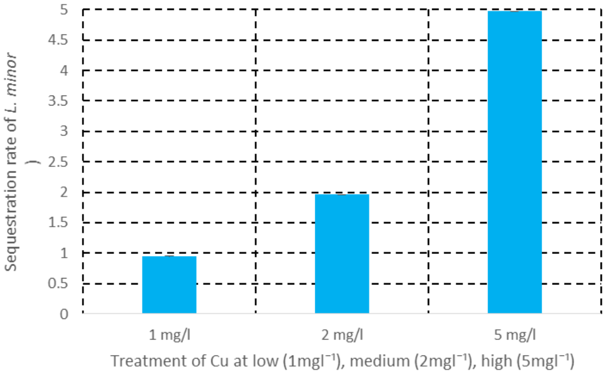
Cu sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 2

C.



Cu sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 3

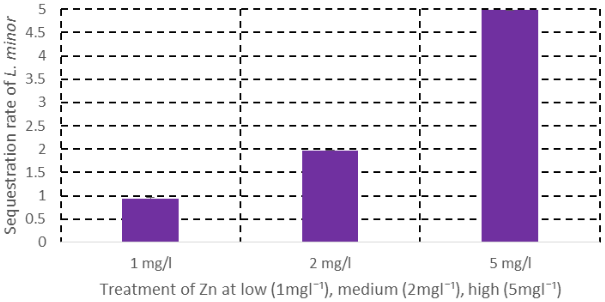
D.



Cu sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 4

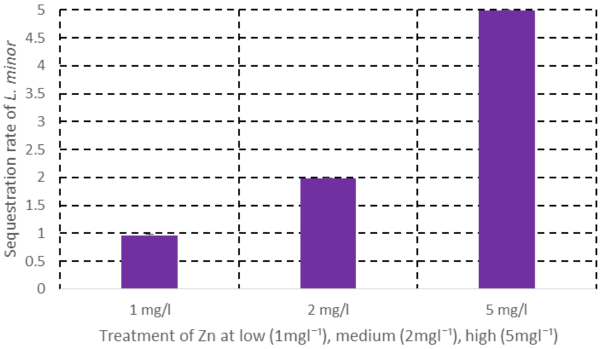
Figure 2 Assessment of Cu sequestration rate by *L. minor* in 1mgl⁻¹, 2mgl⁻¹ and 5 mgl⁻¹ at different incubation period at week 1, week 2, week 3 and week 4

A.



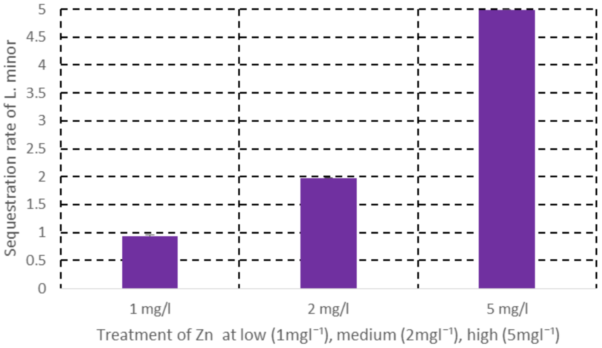
Zn sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 1

B.



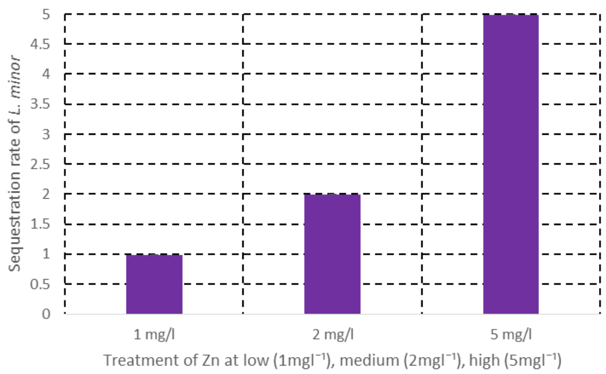
Zn sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 2

C.



Zn sequestration rate in 1.0 mgl⁻¹, 2 mgmgl⁻¹ and 5 mgl⁻¹ at week 3

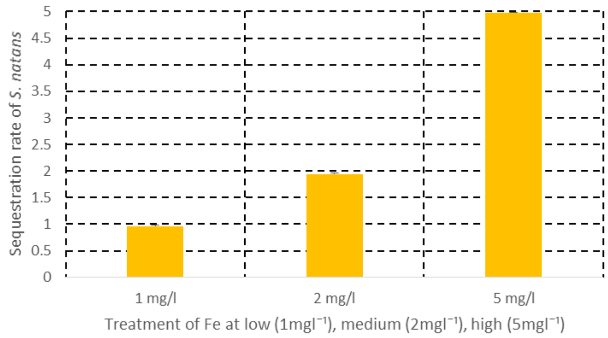
D.



Zn sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

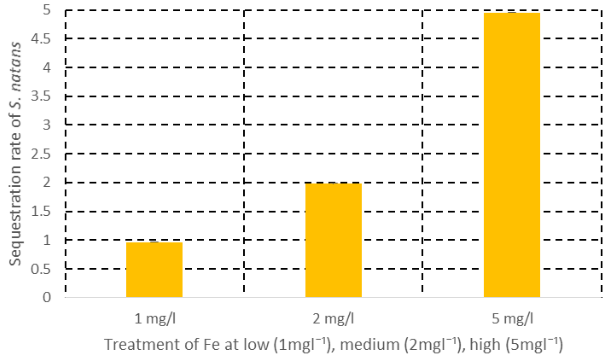
Figure 3 Assessment of Zn sequestration rate by *L. minor* in 1mgl^{-1}, 2mgl^{-1} and 5 mgl^{-1} at different incubation period at week 1, week 2, week 3 and week 4

A.



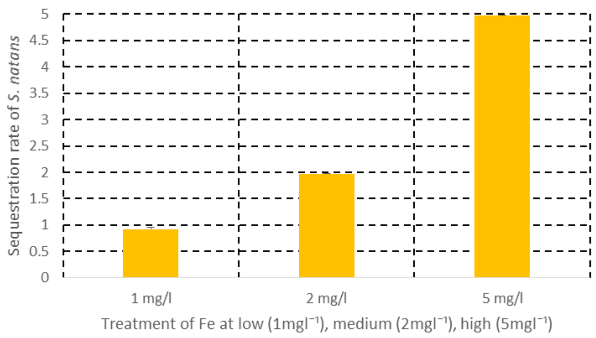
Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1

B.



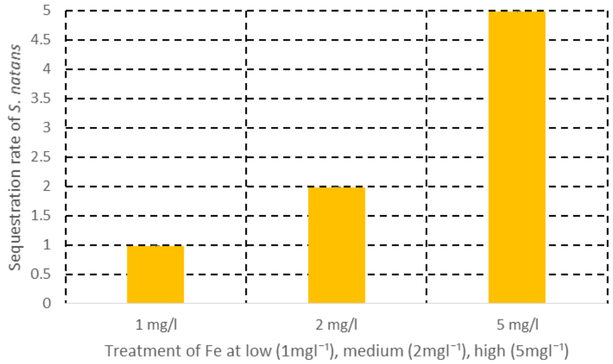
Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 2

C.



Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 3

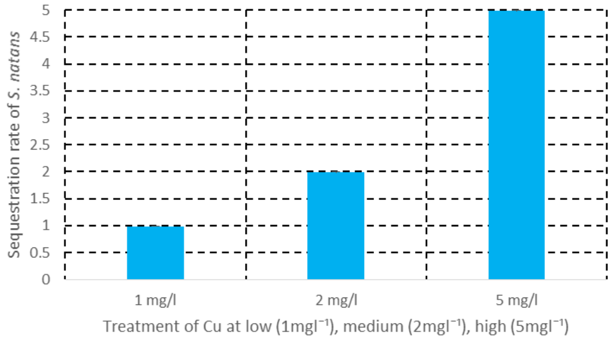
D.



Fe sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 4

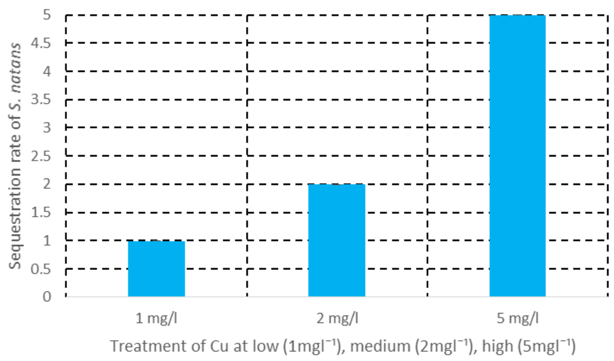
Figure 4 Assessment of Fe sequestration rate by *S. natans* in 1mgl^{-1}, 2mgl^{-1} and 5 mgl^{-1} at different incubation period at week 1, week 2, week 3 and week 4

A.



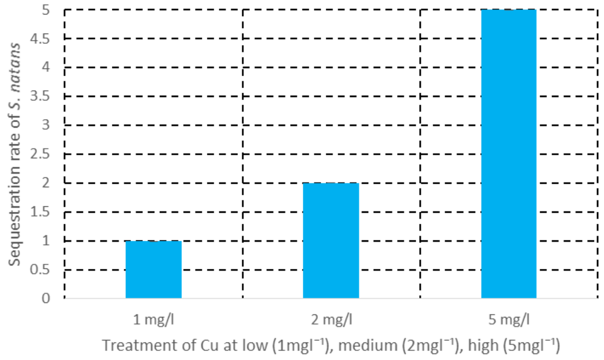
Cu sequestration rate in 1.0 mgl^{-1}, 2 $mgmgl^{-1}$ and 5 mgl^{-1} at week 1

B.



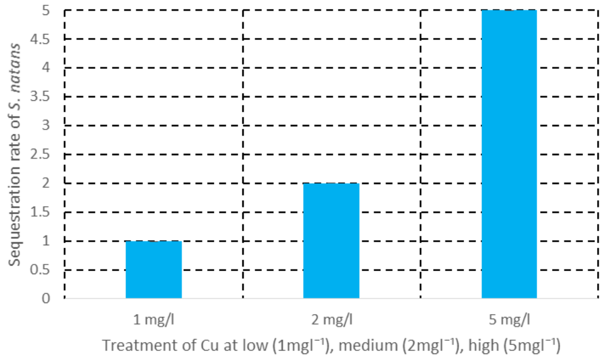
Cu sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 2

C.



Cu sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 3

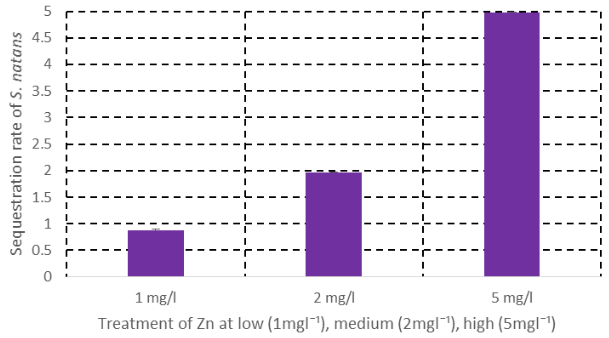
D.



Cu sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 4

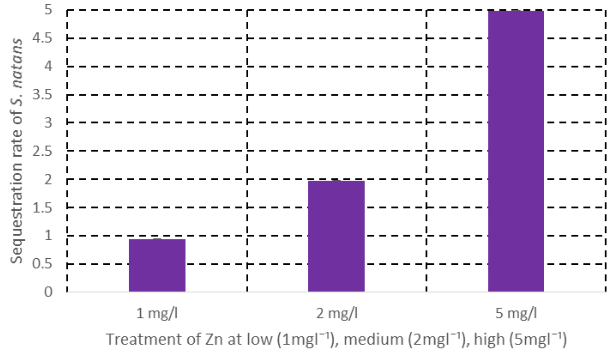
Figure 5 Assessment of Cu sequestration rate by *S. natans* in 1mgl^{-1}, 2mgl^{-1} and 5 mgl^{-1} at different incubation period at week 1, week 2, week 3 and week 4

A.



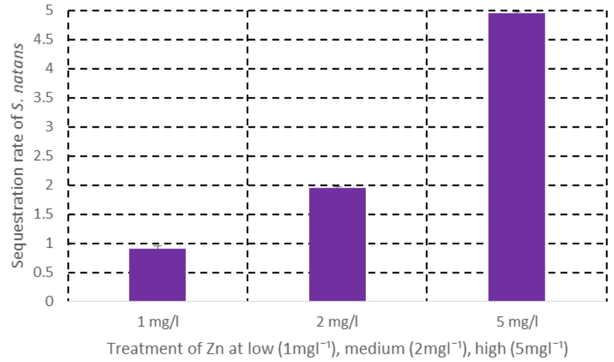
Zn sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 1

B.



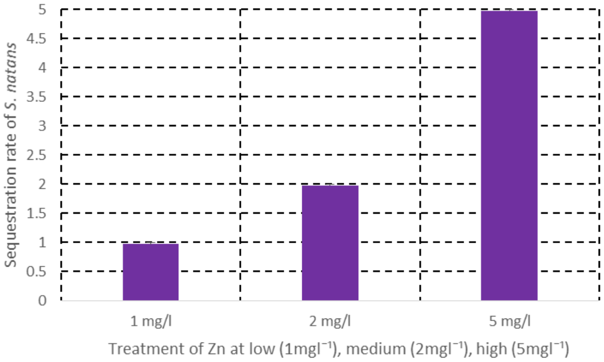
Zn sequestration rate in 1.0 mgl^{-1}, 2 mgmgl^{-1} and 5 mgl^{-1} at week 2

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

Figure 6 Assessment of Zn sequestration rate by *S. natans* in 1mgl^{-1}, 2$mgl^{-1}$and 5 mgl^{-1}at different incubation period at week 1, week 2, week 3 and week 4

4.0 CONCLUSION

In conclusion, *Lemna minor* and *Salvinia natans*were a potential phytoremediation agent to clean-up heavy metals pollutant in aquatic ecosystems. They are 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 phytoremediation 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 indicated that*L. minor* and *S. natans* were a great phytoremediation agents to sequester heavy metals at more than 90% of Cu, Fe and Zn at different period of time with three different concentrations (low, medium and high). *In vivo* model systems established in this study are proven as the best solutions to determine the plant capabilities to remove, sequester or accumulate heavy metals contaminants. Therefore, more studies are needed to manipulate these model system for further research the potential of aquatic plant based green technology system as well as to achieve optimum efficiency in the sequestration rate of heavy metals before large scale application is adopted and applied.

Acknowledgement

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