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Exploring the Potential of Utilizing Aquatic Macrophytes for Enhanced Phytoremediation of Zinc in Artificial Wastewater: Characteristics and Parameter Studies

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Abstract: Heavy metal pollution due to industrialization can threaten the surrounding environment and living organisms. Phytoremediation is a green technique that uses hyperaccumulator plants to eliminate or decrease heavy metals in polluted water bodies. The aim of this study was to investigate the changes in morphology of *Pistia stratiotes* (water lettuce) and *Eichhornia crassipes* (water hyacinth) before and after phytoremediation of zinc (Zn) by using scanning electron microscopy (SEM), electron dispersive X-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR). The SEM images showed the formation of small granular aggregates on the surfaces of the leaf and root. EDX results confirmed the uptake of Zn metal, especially in the plant roots. The FTIR spectra showed the Zn metal binding with several characteristic functional groups (O-H, C-H and C=O bonds). Different parameters were also studied to optimize the Zn uptake rate. Water lettuce achieved 80.1% phytoremediation of Zn after 5 days at optimum conditions (10 ppm of Zn, 6 ppm of sodium chloride and natural solution pH). Meanwhile, water hyacinth reached up to 88% when increasing the sodium chloride up to 9 ppm. In conclusion, Zn phytoremediation using both plants can be a potential remediation method for improving the quality of water.

Keywords: phytoremediation; zinc; water lettuce; water hyacinth; characteristics; parameter studies

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1. Introduction

Environmental pollution is slowly escalating to become a worldwide threat which requires proper mitigation and prevention. To date, fast-paced industrialization and urbanization have contributed to the pollution of water, forcing around 40% of the global population to face water scarcity [1]. According to the United States Environmental Protection Agency [2], metals or metalloids with a density of 5 g/cm^3 and above are classified as heavy metals, which includes aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), selenium (Se), mercury (Hg) and zinc (Zn). These heavy metals are capable of leaching into drinking water from household plumbing pipelines, natural mineral deposits, municipal waste disposal facilities and industrial activities which include mining operations, electronic manufacturing, petroleum refineries and the pharmaceutical industry. Once the polluted water is consumed by humans, even at a low concentration, the health problems imposed will be fatal. For instance, Al can cause neurotoxic effects, Pb can cause impaired mental and physical development among children, Cr can potentially damage the liver and cause vomiting, while Cd can be carcinogenic and cause multiple diseases such as endocrine disruption and osteoporosis [3].

Environmental monitoring agencies in various countries have developed approved limits for the heavy metal levels in consumable water in lieu of their detrimental effects on the surrounding environment [4]. In general, industrial wastewater treatment technologies can be divided into physical, chemical, physiochemical and biological methods, each with their own pros and cons [1]. Physical treatment methods include membrane filtration, screening and sedimentation and are generally cheaper and easier to operate but have a low treatment efficiency. Chemical methods such as chemical precipitation, ion exchange and solvent extraction can be metal-selective with a high treatment capacity but suffer from high maintenance cost and the production of unwanted sludge. Meanwhile, physiochemical methods include electrodialysis, coagulation and flocculation, as well as photocatalysis; they can also be metal-selective and highly efficient, but the drawbacks are the relatively expensive operation cost and that it is labor-intensive [1,5,6].

As a green alternative, biological treatment, which can extract harmful heavy metals in a more eco-friendly manner, is strongly recommended by researchers [4]. In this context, carrying out phytoremediation of heavy-metals-polluted water by using green plants has become one of the research hotspots in the area of green remediation technology [7]. Phytoremediation is a technique that directly use various types of plants to adsorb/absorb, accumulate or detoxify, reduce harmful effects and minimize heavy metal contamination in any water sources or soils through biological, chemical and physical processes. It is a cost-effective, long-term sustainable method that is less destructive to the surrounding environment and highly suitable for under-developed and developing countries [8]. This process uses green plants known as hyperaccumulator plants to remove heavy metal pollutants from the polluted environment through different techniques such as phytovolatilization, rhizofiltration, phytostabilization and phytoextraction [4]. Phytovolatilization refers to the conversion of toxic metals into less harmful volatile forms before their release into the atmosphere via the foliage system. On the other hand, rhizofiltration involves the removal of pollutants from contaminated water through processes such as adsorption onto roots or absorption by roots. Phytostabilization aims to immobilize heavy metals, reducing their bioavailability within the food chain. Meanwhile, phytoextraction entails the uptake, translocation and accumulation of contaminants in the aerial parts of plants [9]. According to An et al. [9], a hyperaccumulator plant is a plant species which can accumulate significant levels of heavy metals within their aerial parts without exhibiting phytotoxic symptoms. In contrast to non-hyperaccumulator plants, hyperaccumulator plants can bioaccumulate up to 100 times the amount of heavy metals under similar conditions. In recent years, studies have shown that the research focus has been slowly shifting from the discovery of hyperaccumulator plant species to the internal growth and metabolisms of the plants. This is because a research focus on uncovering the underlaying characteristics of the plants can help us understand and maximize the efficacy of phytoremediation as a solid and greener choice among all the conventional wastewater treatment methods.

However, in order for the phytoremediation process to be as efficient as possible, the selection of suitable plant species is imperative. Favorable characteristics of suitable phytoremediation plants include having a high biomass yield, the ability to absorb and tolerate copious amounts of heavy metals during its life cycle by transporting the absorbed heavy metals to the aerial parts of the plant and a fast growth rate [4]. Apart from favorable characteristics for phytoremediation purposes, external factors such as climate, temperature, pH, light irradiation, salinity and nutrient availability can also influence the growth of plants and phytoremediation performance. *Pistia stratiotes* (water lettuce) and *Eichhornia crassipes* (water hyacinth) are two of the most common hydrophytes used for the phytoremediation treatment of heavy-metals-laden wastewater due to their widely spread habitat, ease of maintenance and economical nature. Water lettuce, also known as water cabbage or shellflower, belongs to the Araceae and is mostly found in lakes, ponds and streams. It has a rapid growth rate and is also capable of surviving under high metal stress by accumulating metals in its roots and leaves, making it suitable for phytoextraction purpose [10]. Meanwhile, water hyacinth is closely related to the Liliaceae and can be easily found in large quantities throughout the year due to its inundation. The root of water hyacinth is unique in that it can accumulate and extract large amounts of heavy metals such as Cd, Cu, Pb, Zn and Hg. Similar to water lettuce, water hyacinth is highly recommended for the phytoremediation treatment of industrial effluent containing heavy metals due to its high biomass production rate and tolerance towards extreme surrounding environments, as well as its high metal uptake rate [11]. Water lettuce and water hyacinth were chosen for the Zn phytoremediation study due to their high growth rate, short growth cycles, low energy demands and high availability in Malaysia. To date, reports on the change in the surface characteristics of these two plants before and after performing phytoremediation when treating Zn-containing wastewater is rarely found in the literature. Although work with a similar idea has been conducted by Zheng et al. [12], in which the competitive interaction between the Cu and Cd metal sorption on water hyacinth plant roots was studied, the whole experiment was conducted based on the adsorption–desorption of metals on dried plant roots, rather than the phytoremediation process. In addition, it was reported in the literature that the phytoremediation period required to remove heavy metals was usually about 1–4 months [13–16]. Thus, the novelty of this study is the focus on the differences in the surface characteristics before and after phytoremediation, as well as the comparison of phytoremediation performance between water lettuce and water hyacinth in a rather short period. The aim of the present study was to analyze the important parameters (phytoremediation duration, Zn concentration, pH and salinity) affecting the phytoremediation performance of both plants with regard to the Zn-contaminated wastewater through the one-factor-at-a-time (OFAT) optimization method.

2. Materials and Methods

2.1. Chemicals and Materials

Fresh water lettuce and water hyacinth were purchased from the same local supplier to ensure uniformity. Reagent-grade zinc nitrate hexahydrate ($Zn(NO_3)^2 \cdot 6H_2O$, 98%) was obtained from Sigma-Aldrich (St. Louis, MO, USA) as the zinc source. It has a molecular weight of 297.49 g/mol and is highly soluble in water. Hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH, 99%) and sodium chloride (NaCl, 99%) were obtained from Merck (Darmstadt, Germany). All the chemicals were used as received and distilled water was used throughout the study.

2.2. Preparation of Plants

The fresh plants purchased were selected to have a weight of between 20 and 30 g each with green young leaves. The plants were first washed with running tap water to remove residual soil particles, dirt and insect larvae that had grown on them. These plants were then transferred to water tanks containing only distilled water with light exposure in a 10 h light/14 h dark cycle to allow them to acclimatize to their new surroundings. After one week of acclimatization, the plants were harvested and patted dry using clean paper towel, before being used in the subsequent experiment.

2.3. Preparation of Zn Stock Solution and Sodium Chloride Stock Solution

A total of 1000 ppm stock solution of Zn and sodium chloride was prepared by dissolving the zinc salt and sodium chloride, respectively, in distilled water. The stock solutions prepared were stored at room temperature and shielded from light exposure to prevent any degradation in concentration.

2.4. Phytoremediation of Zn

The experiment was carried out in a glass beaker filled with 1 L of synthetic Zn-containing wastewater solution under natural light irradiation. The solution was stirred by using a magnetic stir bar on a hotplate prior to the phytoremediation experiment to ensure a uniform dispersion of the Zn content. Next, the acclimatized plants were transferred to beakers filled with 1 L solution containing 5 ppm of Zn each in order to study the effect of the duration of phytoremediation. Afterwards, 1 L solutions with different concentrations of Zn (5, 10, 15, 20 and 25 ppm) were prepared from the as-prepared Zn stock solution. This range of Zn concentrations was selected based on the US EPA standard's regulated maximum contaminant level of Zn in drinking water and the Malaysia national water quality standard, as well as the limits for sewage and industrial effluent regulated under the Environmental Quality Act 1974 [17–19]. After that, 1 M of hydrochloric acid and 1 M of sodium hydroxide solutions were prepared and used to adjust the pH of the solution with specific Zn concentrations. Finally, 1 L solutions of specific Zn concentrations with specific pH and different salinity (3, 6, 9, 12 and 15 ppm) were prepared by using the as-prepared sodium chloride stock solution. At least 3 replicates were obtained to calculate the errors shown in the figures provided for the parameter studies. At the end of the experiment, the treated plants were harvested and dried in an oven at 100 °C overnight, cut into small pieces and stored in properly labeled polythene bags for further characterization analysis.

2.5. Plants Characterization

Both fresh and treated plants were washed and dried in an oven at 90 °C overnight. The dried samples were cut and separated into leaf and root parts. The separated parts were then finely cut into tiny pieces and passed through a No. 40 mesh sieve to obtain uniformly sized samples for further characterization [20]. All characterization studies were conducted using three replicates of both fresh and treated plant parts. The scanning electron microscopy (SEM) images of the fresh and treated dried plants were studied by using a Hitachi SEM S-3400N scanning electron microscope that was operated at 15 kV. The samples were mounted on the aluminum holder with carbon-conductive tape. The electron dispersive X-ray spectroscopy (EDX) analysis was conducted using Ametek EDAX software. The Fourier transform infrared spectroscopy (FTIR) spectra of the fresh and treated dried plants were analyzed by using the Thermo attenuated total reflection-FTIR with a scan number of 64 through a range of wavenumbers between 400 and 4000 cm^{-1}.

2.6. Analysis of Liquid Samples

After phytoremediation experiment, a certain volume of liquid sample was withdrawn from the solution and a syringe filter with a pore size of 0.45 μm was used to filter out the root hairs and other solid particles from the extracted liquid samples. The concentrations of Zn in the liquid samples were determined by using Perkin Elmer inductively coupled plasma–optical emission spectrometry (ICP-OES), model Optima 7000 DV. The removal efficiency of Zn by the plants was calculated as follows:

$$\text{Removal efficiency (\%)} = (C_0 - C_t)/C_o \times 100\% \quad (1)$$

where C_0 is the initial concentration of Zn before phytoremediation and C_t is the concentration of Zn at any reaction time t (days).

3. Results and Discussion

3.1. Characterization of Water Lettuce and Water Hyacinth before and after Phytoremediation of Zn

The functional groups of water lettuce and water hyacinth at their respective leaves and roots before and after phytoremediation of Zn were studied by using FTIR analysis. The results are shown in Figures 1 and 2, respectively.

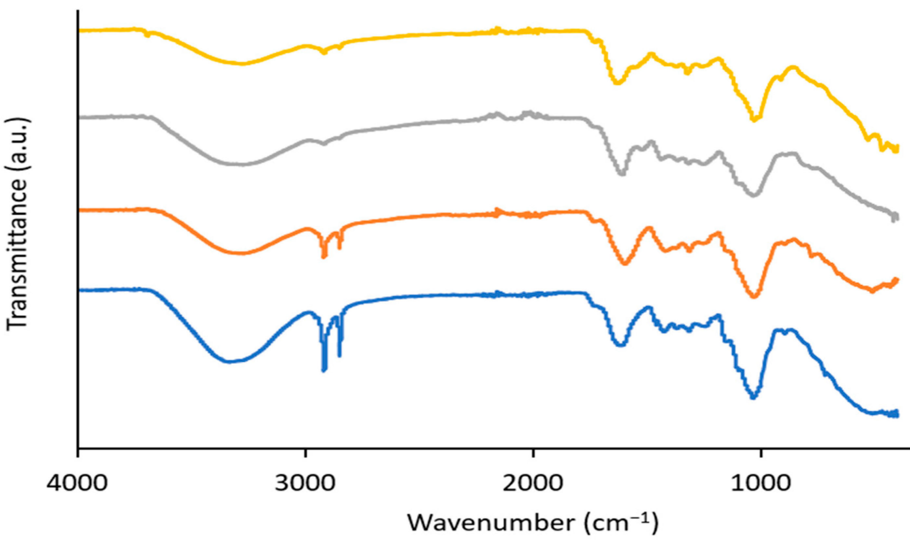

Figure 1. FTIR spectra for (a) leaf without Zn, (b) leaf with Zn, (c) roots without Zn and (d) roots with Zn for water lettuce.

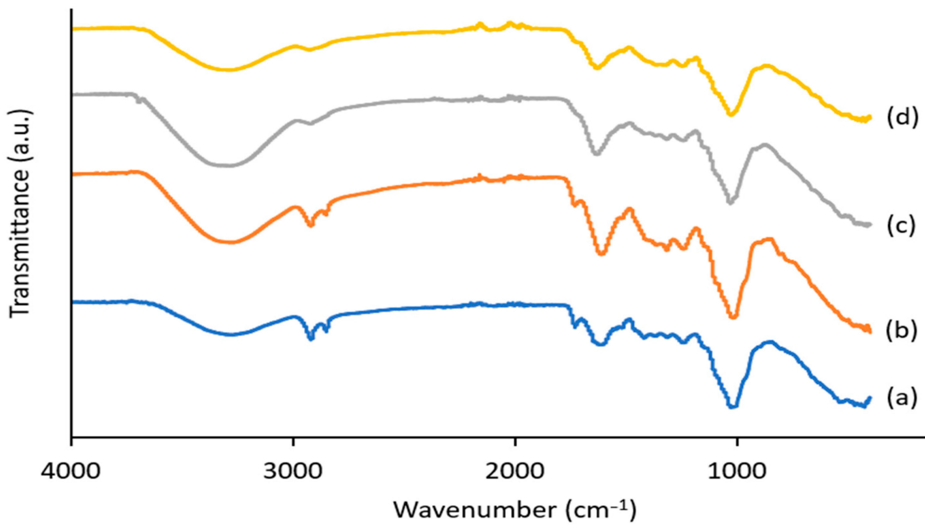

Figure 2. FTIR spectra for (a) leaf without Zn, (b) leaf with Zn, (c) roots without Zn and (d) roots with Zn for water hyacinth.

Figure 1 shows the FTIR spectra of both the water lettuce leaf and roots. As illustrated in Figure 1 (a) to (d), a wide and strong absorption band at 3340 to 3290 cm^{-1} could be observed, which corresponded to the existence of stretching vibrations of O-H bonds due to the presence of water remaining within the plant itself. As Zn was absorbed by the water lettuce, it could be observed that the band shifted from 3340 to 3290 cm^{-1} and from 3300 to 3290 cm^{-1} for the leaf and roots, respectively. The shifting of the band could arise from the cationic interaction between Zn^{2+} ions with the hydroxyl group for metal oxygen binding [14]. Meanwhile, for the leaf of water lettuce before and after Zn uptake, two sharp peaks could be observed at 2910 and 2850 cm^{-1}, which were associated with the stretching vibrations of C-H bonds within the cellulose structure of the water lettuce leaf. According to [15], these bands can be attributed to the presence of asymmetric methylene (CH_2) stretching around 2930 to 2910 cm^{-1} and symmetric CH_2 stretching around 2860 to 2840 cm^{-1}, which suggested the presence of aliphatic materials within the leaf cuticle such as wax and cutin. In contrast, the presence of C-H bonds around 2910 and 2850 cm^{-1} was very minimal for the water lettuce roots, as evidenced by the insignificant intensity of both peaks for Figure 1 (c) and (d). Additionally, a large absorption peak at 1600 to 1620 cm^{-1}

could also be observed in Figure 1 (a) to (d), which corresponded to the presence of C=O bonds. As shown in Figure 1, upon Zn uptake, the FTIR spectra displayed alterations, particularly in the bands associated with O-H, C-H and C=O. These changes suggested the participation of cationic elements such as Na^+ and K^+ in the adsorption of Zn^{2+} ions onto the plant surfaces through proton and metal exchange processes [16]. According to [21], the interaction between metals with functional groups of water lettuce acting as active sites could result in the alteration of shape, shifting of position and change in intensity of the characteristic peaks.

Figure 2 illustrates the FTIR spectra for both leaf and roots of water hyacinths. Similar to the water lettuce, a broad absorption band at around 3300 cm^{-1} could be observed in all the leaf and roots of water hyacinth before and after Zn uptake, which suggested the presence of O-H stretching vibration mode. Next, two smaller absorption bands at 2920 and 2850 cm^{-1} could only be found in the leaf of the water hyacinth which corresponded to the stretching vibration of C-H bonds. As mentioned beforehand, the presence of these aliphatic contents (symmetric and asymmetric CH_2 stretching band) was exclusive to the structure of the cuticles [15]. On the other hand, two absorption peaks were also found at 1730 and 1600 cm^{-1} for the water hyacinth leaf before and after Zn uptake, which were attributed to the stretching vibration of C=O bonds. However, the characteristic peak for C=O bonding could only be observed at around 1620 cm^{-1} for the roots of water hyacinth before and after phytoremediation of Zn. It could be observed that the intensity of the characteristic peak for C=O bonding changed as the water hyacinth underwent Zn phytoremediation, indicating possible interactions between the characteristic functional group and Zn metals [14].

In short, the FTIR spectra showed that both water lettuce and water hyacinth contained characteristic functional groups for most polysaccharides such as O-H, C-H and C=O bonds. According to [20], the presence of these diverse functional groups could potentially act as a binder for Zn ions. In this context, the binding interactions between the characteristic functional groups and Zn metals during phytoremediation, which resulted in the alteration of these characteristic peaks, were in good agreement with the results obtained by [14].

The surface morphology of the leaf and roots of water lettuce and water hyacinth before and after phytoremediation of Zn were investigated by using SEM analysis. The results are shown in Figures 3 and 4. Figure 3a,b show the SEM images of the leaf of water lettuce before and after the uptake of Zn, respectively. The rod-like structure with a folded shape of the leaf represented the trichomes of the plant, which were able to trap tiny air bubbles and keep the water lettuce floating on the water surface. After the phytoremediation of Zn, a cluster of small granules was observed around the voids between the trichomes as indicated by the red arrow shown in the figure, which were absent in the leaf prior to Zn uptake. According to [22], this could be associated with the formation of Zn metal complexes that subsequently immobilized the complexes within the voids. On the other hand, Figure 3c,d represent the SEM images of the water lettuce roots before and after Zn phytoremediation, respectively. Prior to the absorption of Zn, the surface of the plant roots was smooth and linear. Upon the Zn uptake, small aggregates and bulges alongside a creased surface morphology could be observed on the plant roots. The altered appearance of the plant roots could be attributed to the interactions between functional groups present on the roots' surface and the cationic Zn metal, leading to the formation of stable metal-chelating complexes [23].

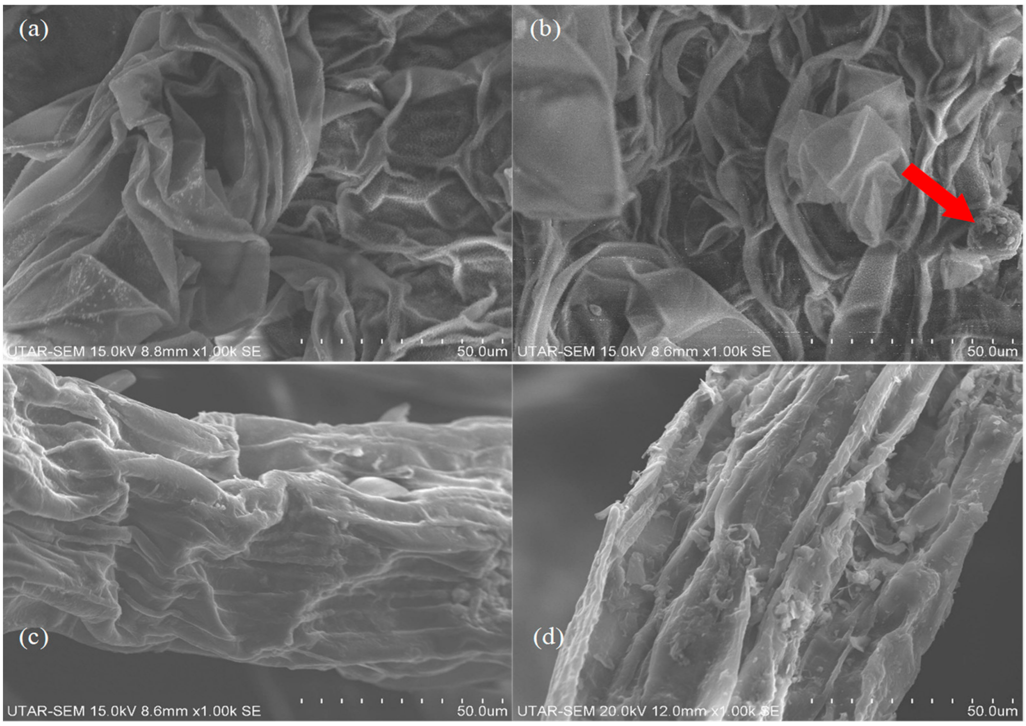

Figure 3. SEM images for (**a**) leaf without Zn uptake, (**b**) leaf with Zn, (**c**) roots without Zn and (**d**) roots with Zn for water lettuce.

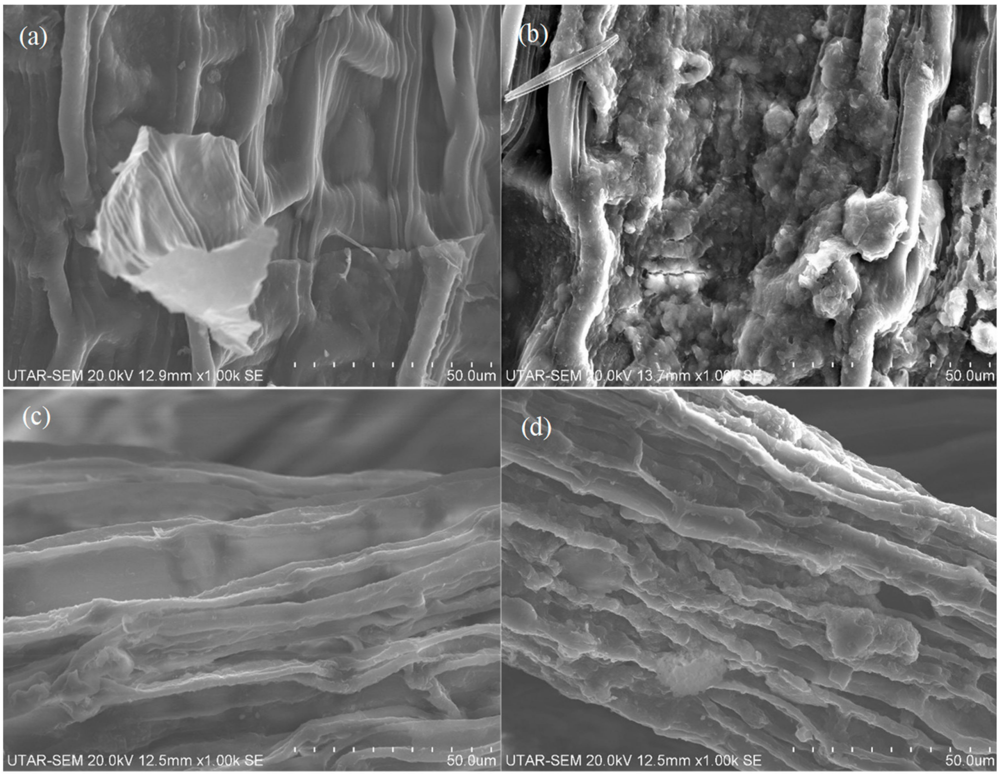

Figure 4. SEM images for (**a**) leaf without Zn uptake, (**b**) leaf with Zn, (**c**) roots without Zn and (**d**) roots with Zn for water hyacinth.

Figure 4a,b present the SEM images of the water hyacinth leaf before and after Zn uptake. In general, the surface morphology of the leaf was linear and covered with a copious amount of evenly distributed stomata. After the water hyacinth was subjected to a solution containing Zn metal, the stomata on the epidermis were saturated with aggregates and the surface had swollen. The presence of aggregates suggested the binding between the Zn metal and the carboxylic groups and other functional groups present to form stable metal chelates [21]. On the contrary, Figure 4c,d show the SEM images for the water lettuce roots before and after absorption of Zn. The epidermal cells of the water hyacinth roots were originally oblong and closely packed. When Zn was absorbed, the oblong-shaped roots broke into tiny fibrous roots which were also covered with small individual granules and agglomerates. The dissociation of roots and formation of tiny granules on the surface is in accordance with the result obtained by [22], where different features of plant surfaces such as carboxylic groups, esters and lignin were highly involved in the absorption of metals via the chelation of metal complexes.

Figures 5 and 6 represent the EDX mapping of water lettuce and water hyacinth leaf and roots after Zn phytoremediation. Figure 5 confirms the presence of Zn in both the leaf and roots of water lettuce, as indicated by its distribution across the plant tissues. Figure 5a shows a lower density of Zn distribution within the leaf of the water lettuce compared with its root counterparts that are shown in Figure 5b. Similarly, the distribution pattern of Zn within both the leaf and roots of water hyacinth, as shown in Figure 6a,b, followed the same trend, with a higher density of Zn distribution in the roots compared with the aerial part of the plant.

The EDX results for all the plant samples are shown in Table 1. The EDX analysis of the plant leaf and roots for both water lettuce and water hyacinth before Zn uptake consist of carbon (C), oxygen (O), sodium (Na), calcium (Ca) and potassium (K) elements. The Na and Ca were only observed in the roots and leaves of both plants, respectively, before the Zn uptake. Meanwhile, prior to the uptake of Zn, K was detected in the leaf and roots of water lettuce and water hyacinth. Ca and K were sources of nutrients for the growth and sustainability of the plants. For instance, Ca was responsible for the development of cell walls to resist diseases, and K was used to strengthen the plants during early growth and for the retention of water. On the other hand, Na was not a necessary nutrient for the growth and development of the plants, and a high amount of Na can induce salt stress, which is unbeneficial to the plants [24]. The atomic percentage of K in the leaves of water hyacinth was lower than its roots counterpart, which is in accordance with the findings reported by [25]. The presence of these mineral elements (Na, Ca and K) was in part due to the intake of nutrients in the previous surrounding environment during the growth of the water lettuce and water hyacinth prior to the application of Zn metal for phytoremediation.

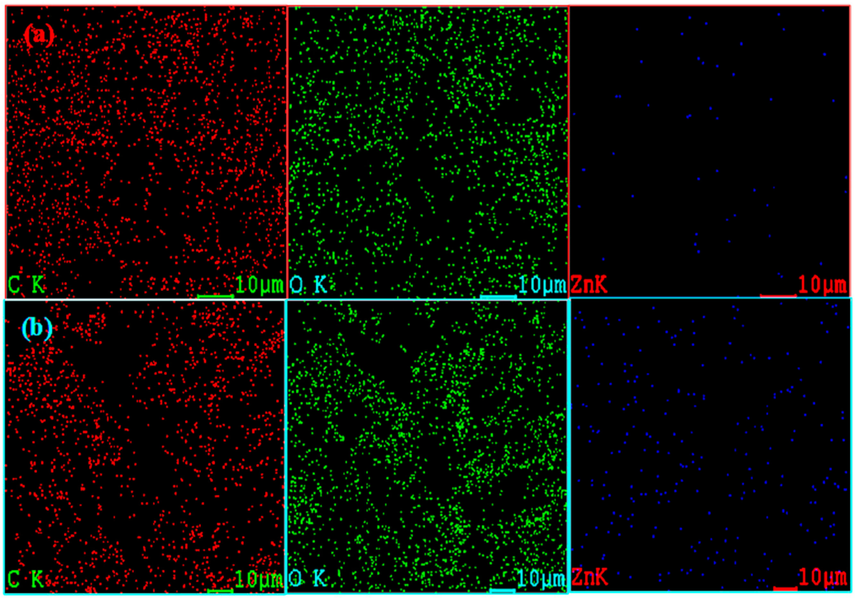

Figure 5. EDX mapping of (**a**) leaf and (**b**) roots after Zn uptake by water lettuce.

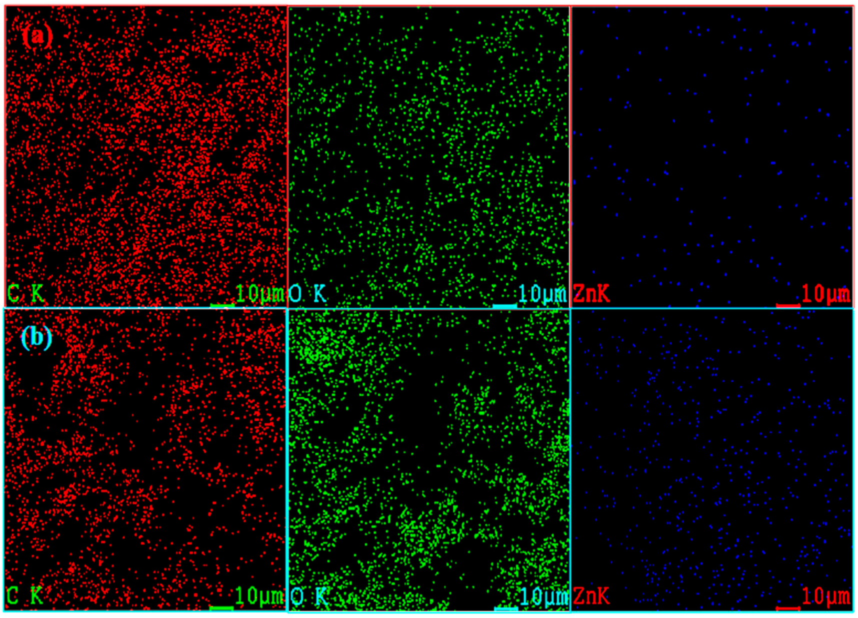

Figure 6. EDX mapping of (**a**) leaf and (**b**) roots after Zn uptake by water hyacinth.

Table 1. Elemental atomic percentage of all samples extracted from EDX analysis.

Plant Sample		**Elemental Atomic Percent (%)**					
		C	**O**	**Zn**	**Na**	**Ca**	**K**
Water lettuce	Leaf (without Zn)	43.3	33.7	-	-	16.2	6.8
	Leaf (with Zn)	57.1	41.5	1.4	-	-	-
	Roots (without Zn)	44.4	35.7	-	4.1	7.6	8.2
	Roots (with Zn)	40.6	52.1	7.3	-	-	-
Water hyacinth	Leaf (without Zn)	36.2	37.2	-	-	18.4	8.2
	Leaf (with Zn)	77.1	20.7	2.2	-	-	-
	Roots (without Zn)	48.7	46.8	-	1.6	-	2.9
	Roots (with Zn)	46.8	43.8	9.4	-	-	-

It was observed that as the plants were subjected to the solution containing Zn metal, the atomic percentage of Zn was increased. For both types of plant, the atomic percentage of Zn was found to be higher in the roots compared with their respective leaf counterparts. Not only that, the atomic percentage of Zn in the leaf and roots of water hyacinth after phytoremediation of Zn was also greater than that of the water lettuce. This finding could be related to the higher contaminant reduction capability of water hyacinth, which resulted in a greater amount of Zn being accumulated within the plant biomass [26]. After the uptake of Zn, the mineral elements were not detected through EDX analysis due to their release to the solution as a result of the stronger chelation ability of Zn^{2+} with the negatively charged functional groups found on the plant roots, replacing the originally bounded cationic mineral elements [12]. Meanwhile, the absence of mineral elements in the plant leaves after Zn uptake indicated the utilization of the mineral elements to facilitate cellular activities such as sequestration, compartmentalization and efflux of excess metals to reduce the stress induced by metal [27].

3.2. Parameter Effect

3.2.1. Effect of Duration of Phytoremediation

The duration of phytoremediation of Zn by water lettuce and water hyacinth was studied extensively, and the results are illustrated in Figure 7. The study on the duration of phytoremediation of Zn by both plants was conducted over a period of a week (7 days) to determine the time required to achieve maximum Zn removal efficiency by both plants.

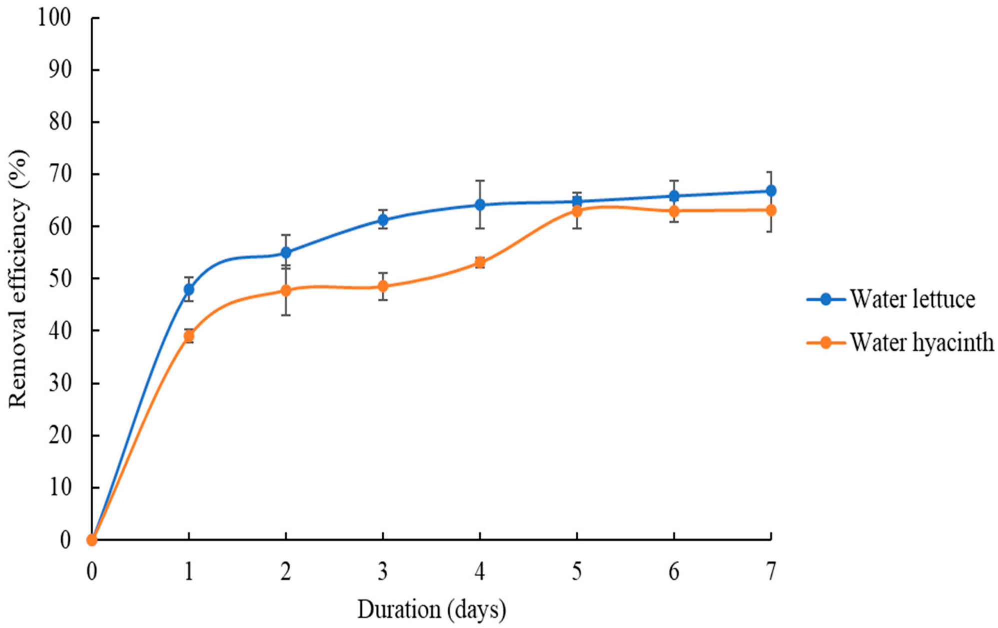

Figure 7. Duration of phytoremediation of Zn by water lettuce and water hyacinth.

Based on Figure 7, both plants exhibited the highest rate of removal efficiency during the first day of phytoremediation, where the water lettuce and water hyacinth recorded a 49% and 39% Zn removal efficiency, respectively. Nevertheless, the efficiency of removal of Zn by the water lettuce showed a continuous uptrend over the first four days of phytoremediation treatment, before reaching a constant removal rate on day 5 and onwards. The removal rate of Zn achieved by using water lettuce in the present study was greater than the one reported by [27].

On the other hand, water hyacinth experienced a steady increase in Zn removal efficiency during the first five days, and therefore took a slightly longer period of time to achieve a constant removal rate on day 6. The slight difference in time taken to achieve a constant rate of Zn removal between the two plants might be due to the faster growth rate of water lettuce, which could potentially absorb more Zn as one of the important nutrients required for plant growth in terms of metabolic and physiological mechanisms [28]. Having said that, both water lettuce and water hyacinth clearly showed a significant Zn removal efficiency within the first five days, which was indicative of the quick attainment of the saturation state. Upon reaching the saturation state, the plants were displaying a certain degree of difficulty with further absorbing Zn from the solution, even though the Zn concentration had reduced with the passage of time. In this context, a similar result was also obtained by [29], where the water hyacinth and water lettuce showed similar performances in terms of removing Zn. In this study, 5 days of phytoremediation duration was selected for subsequent studies, as it was found to be the most optimum timeframe for both plants to achieve their respective highest attainable Zn removal efficiency before saturation.

3.2.2. Effect of Zn Concentration

The phytoremediation of different concentrations of Zn by water lettuce and water hyacinth were studied. The results of the study are shown in Figures 8 and 9. For water lettuce, the Zn removal efficiency at different concentrations showed an uptrend for the first four days of the experiment. As discussed previously, at 5 ppm Zn concentration, the removal efficiency by water lettuce had reached its saturation point, with an almost stagnant removal rate of 65% on day 5 of the study. Meanwhile, the Zn removal efficiency continued to escalate throughout the whole five days of study for 10 ppm and 15 ppm Zn concentrations. The 10 ppm of Zn, in particular, had achieved the highest removal efficiency of 72% on day 5 compared with other concentrations of Zn. The removal efficiency for 15 ppm of Zn was recorded at 59% on the final day of study, which was lower than both the 5 and 10 ppm Zn concentrations. Nonetheless, the rate of removal for the 15 ppm Zn concentration was still increasing even on the fifth day of the study, indicating a possibility of achieving a higher Zn removal efficiency with a longer phytoremediation duration.

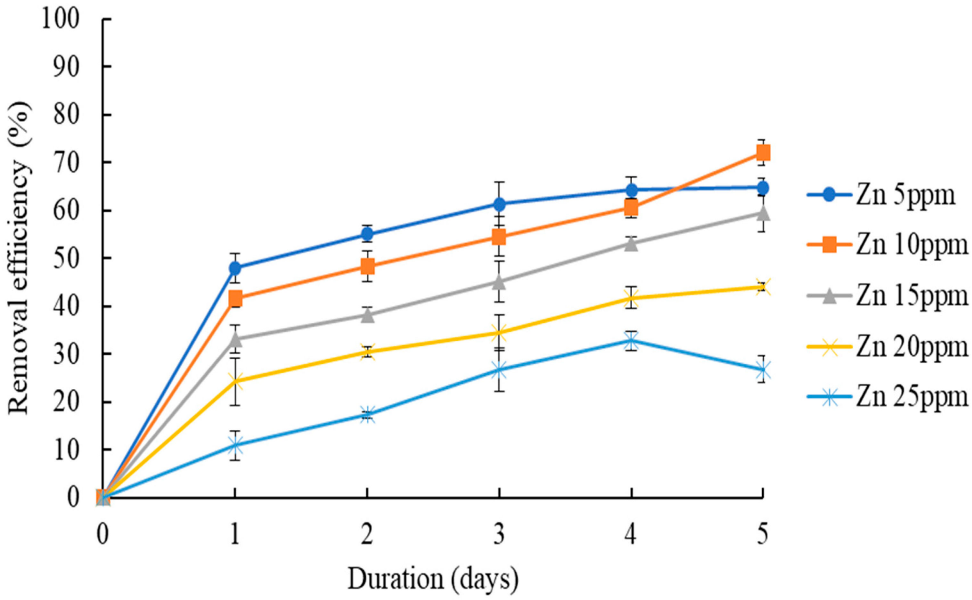

Figure 8. Effect of Zn concentration on the removal efficiency of Zn by water lettuce (duration = 5 days).

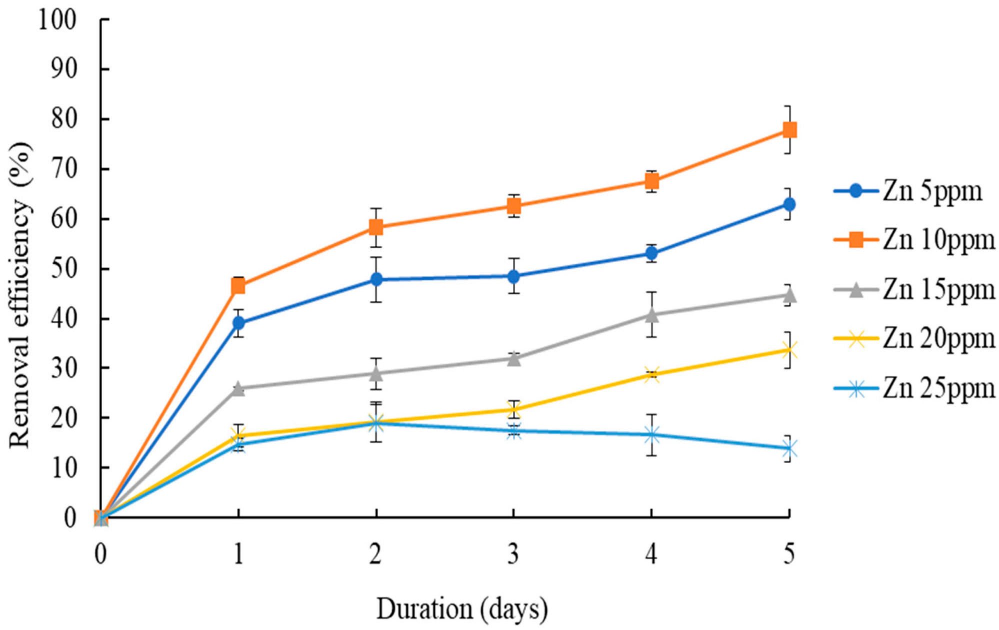

Figure 9. Effect of Zn concentration on the removal efficiency of Zn by water hyacinth (duration = 5 days).

On the other hand, the removal efficiency in higher concentrations of Zn at 20 and 25 ppm by water lettuce did not cross the 50% mark throughout the study period. At a Zn concentration of 20 ppm and above, the rate of removal was increasing slowly and reached a final removal efficiency of 44.1% on the fifth day of the study. Although the removal efficiency at 20 ppm of Zn was increasing at a very slow pace, the obtained data suggested that similar to the cases of Zn concentrations at 10 ppm and 15 ppm, the water lettuce could potentially remove more Zn contaminant if subjected to a longer study period. In the meantime, instead of showing a slowdown in terms of removal rate, the removal efficiency in 25 ppm of Zn on day 5 of the experiment was found to be lower than that on day 4, which indicated a possible re-release of absorbed Zn contaminants by the wilting plant. It was also observed that the plant leaves started to turn yellowish on day 4, which suggested the chlorosis of the plant leaves and overall necrosis of the plant due to exposure to a high concentration of Zn. This finding is in line with the result reported by [30]. In their study, it was observed that a high concentration of Zn had reduced the expression of photosynthetic pigments and interfered with the photosystem, as evidenced by the chlorosis of the fronds.

Moreover, [31] also reported that the growth of plants was retarded when exposed to a higher concentration of Zn due to the toxicity imposed onto the plant itself, subsequently causing the plant to suffer from wilting. Although hyperaccumulator plants such as water lettuce were capable of defending themselves from phytotoxicity induced by toxic heavy metals, excessively high concentrations of heavy metals could still endanger a plant's overall metabolic and physiological mechanisms. According to [32], reactive oxygen species (ROS) could be produced as a consequence of excessive accumulation of toxic heavy metals within the cytosol. The production of ROS could then lead to oxidative damage to the plant cells and damaging of DNA, as well as hampering of the cell antioxidant and homeostasis mechanisms. In addition, ROS could also damage the structure of the plant cells including the cell membrane, chloroplast and photosynthetic pigments, ultimately causing serious retardation to the plant's growth [33].

Based on Figure 9, water hyacinth showed an overall uptrend in terms of removal efficiency of Zn at all Zn concentrations except for 25 ppm of Zn. Similar to water lettuce, the water hyacinth's efficiency of removal of Zn was the highest at 78%, which was achieved when the plant was exposed to 10 ppm of Zn concentration. The second highest removal efficiency of Zn (63%) was attained when the Zn concentration was at 5 ppm. The difference in the removal efficiency with increasing Zn concentration could be associated with the higher number of Zn^{2+} ions available in the solution that can be absorbed by the plant. As the bioavailability of Zn was higher with an increased Zn concentration, the phytoremediation efficiency of Zn by water hyacinth was also increased due to more Zn^{2+} ions being available at the uptake sites. However, as the Zn concentration was further increased to 15, 20 and 25 ppm, the rate of removal of Zn by water hyacinth began to drop in a similar fashion to that of water lettuce. The final removal efficiencies of Zn at these concentrations were 44.7%, 33.7% and 13.9%, respectively, in ascending order. It could be observed that the removal rates of Zn at concentrations of 5, 10, 15 and 20 ppm were showing a continuing pattern even on the final day of the study, although the removal rate at 5 ppm of Zn by water hyacinth was found to be reaching a stagnant condition on day 6 and onwards in our earlier study. Thus, it is highly likely that the Zn removal efficiency by water hyacinth at these concentrations could reach even higher levels if given a longer time to perform phytoremediation. On the other hand, the rate of removal of Zn was almost identical during the first two days of the study when the plant was exposed to 20 and 25 ppm of Zn. Despite that, the similarity in their removal rate began to deviate starting from day 2 onwards, where the removal efficiency at 25 ppm of Zn was declining from its peak value of 18.9% to 13.9%. The reason behind the reducing Zn removal efficiency by water hyacinth at a concentration of 25 ppm was also similar to that of the water lettuce. During the study, the fronds of the water hyacinth were facing chlorosis at the relatively high concentration of Zn, whereby the color of the plant leaves turned from green to yellow. This chlorosis symptom was a direct result of the phytotoxicity experienced by the plant at such a high concentration of Zn. Based on Figures 8 and 9, it can be seen that water lettuce and water hyacinth demonstrated a great absorption and removal efficiency when subjected to 10 ppm of Zn. The high removal rate of Zn by these two plants might be associated with their special characteristics such as broad leaves and rapid growth rate, as well as their highly vascular and fibrous root system. Notwithstanding their special features, the Zn removal efficiency of both plants showed a decline at concentrations of 15 ppm and above, which could be attributed to the saturation of active sites for Zn absorption and the lower tolerance of plants to such high concentrations of Zn [34]. Thus, the concentration of Zn at 10 ppm was selected for subsequent studies, and the treated Zn concentration also falls within the permissible limit of effluent discharge into water bodies as stated by [35].

3.2.3. Effect of pH Solution

The effect of the solution's pH (pH 2, 4, 6, 8 and 10) on the water lettuce's and water hyacinth's removal efficiency of Zn was studied, and the results are shown in Figures 10 and 11, respectively. Based on Figure 8, pH 6, which was the natural solution pH, led to the highest removal efficiency of Zn by water lettuce (75%). No removal was observed at a solution pH of 2, even on the final day of study.

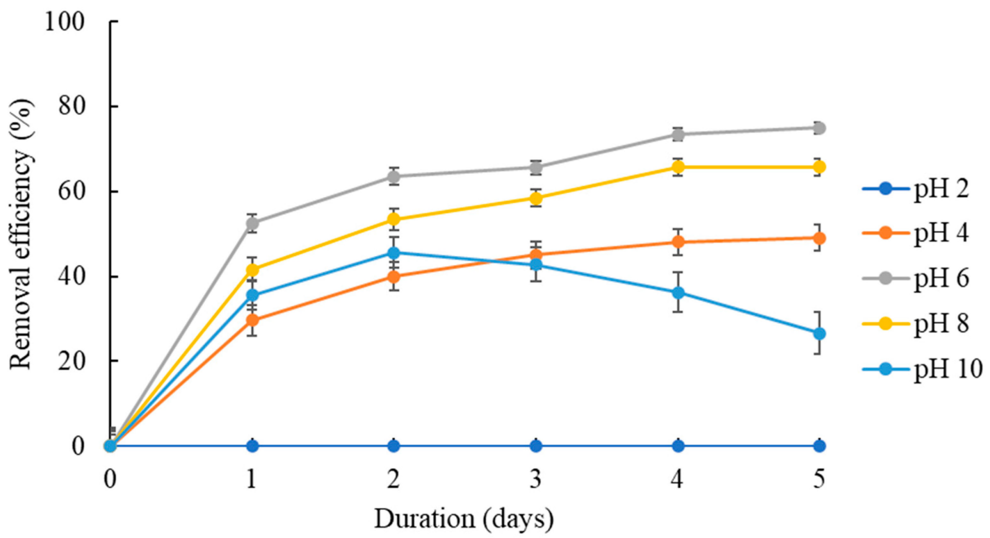

Figure 10. Effect of solution pH on the removal efficiency of Zn by water lettuce (duration = 5 days and Zn concentration = 10 ppm).

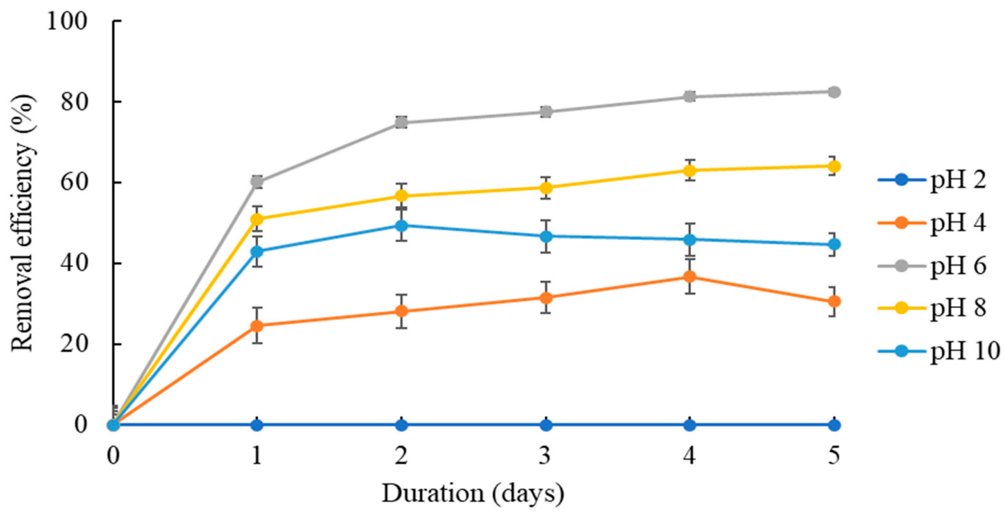

Figure 11. Effect of solution pH on the removal efficiency of Zn by water hyacinth (duration = 5 days and Zn concentration = 10 ppm).

The solution's pH plays a crucial role in the phytoremediation of Zn by accumulator plants. It is one of the external environmental factors which can significantly affect the growth of a plant. According to [36], the pH of a solution is dictated by the concentration or percentage of hydrogen ions (H^+). The solution pH can influence the toxicity of contaminants, and it also governs the form of substance in the solution. Not only that, hydrolysis, precipitation, complexation and redox reactions are also pH-dependent, which can directly affect the availability and speciation of heavy metals for absorption. Consequently, water lettuce was not able to remove Zn in an effective manner at solution pH 2, while at this pH, water lettuce was simultaneously showing symptoms of chlorosis, death and curling of plant leaf tips on the second day of the study.

When the solution pH was increased to pH 4, maintained at its natural solution pH (pH 6) or increased to pH 8, water lettuce was able to absorb Zn metal, showing removal efficiencies of 49.1%, 75% and 65.7%, respectively. At a slightly acidic condition (pH 4), the presence of H^+ ions potentially caused the competition with the Zn^{2+} ions for the available uptake sites of the plant roots. As a result, the amount of Zn metal removed from the solution was lower in an acidic condition. This finding also aligns with [37], where the removal efficiency of Zn by water lettuce was found to be the lowest (49.1%) at pH 4, suggesting the binding of metal ions with inorganic acid which ultimately leads to the formation of complexes and reduces the metal uptake. In this study, hydrochloride acid (HCl) was used to adjust the solution pH to its acidic condition, and thus, the reaction between Zn^{2+} ions and Cl^- ions might produce the chloride complex, which resulted in a lower availability of Zn for plant uptake during the phytoremediation process [38]. On the other hand, when water lettuce was subjected to Zn-containing solution at pH 8, we recorded and overall increment in the rate of removal of Zn throughout the whole study period, with it reaching the saturation state on day 4 of the study.

Nevertheless, when the solution pH was further increased to pH 10, the overall removal efficiency of Zn was poorer than for a solution pH of 8. The reduction in removal efficiency of Zn at increasingly alkaline conditions could be associated with the presence of OH^- ions, which might react with the Zn^{2+} ions, thereby causing the formation of $Zn(OH)_2$ precipitate and subsequently leading to a lower availability of Zn^{2+} ions. As a result, the formation of Zn precipitate at high-pH conditions could reduce the availability of Zn^{2+} ions to be absorbed by the active sites on plant roots during phytoremediation [39]. At a solution pH of 10, the removal efficiency of Zn also started to show a decline after the second day of the study, resulting in a final removal efficiency of only 26.6%. The decrement in the removal efficiency of Zn after its peak value on day 2 (45.6%) at solution pH 10 demonstrated that the re-release of absorbed Zn contaminants was evidenced by the yellowing of green leaves and necrosis of the plant during the second day of the study. According to [28], the ideal pH for healthy growth of water lettuce was neutral, and it was able to tolerate a range of pH values from 4 to 8. Therefore, the decaying water lettuce at both extreme conditions of solution pH (pH 2 and pH 10) observed in this study has verified this result.

Based on Figure 11, water hyacinth exhibited the highest removal efficiency (82.5%) of Zn at a natural solution pH of 6, while the removal efficiency of Zn was the lowest (−30.5%) at a solution pH of 4. Meanwhile, there was no Zn removal by water hyacinth at the solution pH of 2. From the obtained result, it can be observed that a lower removal efficiency of Zn occurred at a higher and lower pH than the natural solution pH (pH 6).

At solution pH 4, the removal efficiency of Zn by water hyacinth was increasing over the first four days of the study, before showing signs of plant necrosis and chlorosis, which ultimately led to a removal efficiency of 30.5% on the final day of the study. The same situation could be observed when water hyacinth was subjected to a Zn-metal-laden solution at a pH of 10. Under this highly alkaline condition, the water hyacinth only managed to survive up until the second day of experiment, before experiencing curling and yellowing of young leaves as well as necrosis, which caused the re-release of absorbed Zn contaminants and the natural decline in the efficiency of removing Zn. The water hyacinth ended the study with a removal efficiency of 44.7% at a solution pH of 10. Nonetheless, unlike solution pH 2, water hyacinth was able to survive better in solution pH 4 and 10 without immediate necrosis and chlorosis upon the beginning of the study. This finding is in accordance with the observation stated by [40], where water hyacinth was capable of surviving within the range of pH 4 to 10 by adjusting the solution pH to its requirements.

Meanwhile, water hyacinth did not experience necrosis and chlorosis when subjected to a solution pH of 8. As in the case of the natural pH solution, the Zn removal rate at a solution pH of 8 was in an overall increasing pattern throughout the study period, with a final Zn removal efficiency of 64%. The lower removal efficiency of Zn at solution pH 8 compared with the natural solution's pH could be ascribed to the reduced availability of Zn^{2+} ions for the plant's uptake, since these reacted with the OH^{-} ions present in the alkaline solution and subsequently generated the $Zn(OH)_2$ precipitate [39]. In short, both water lettuce and water hyacinth exhibited the highest removal efficiency of Zn at the natural solution pH of 6. As the ideal range for optimal growth of both plants was in between pH 6 and 7, and the pH of the permissible discharge of glove industry effluent was also between 6.5 and 7.5 [35], the natural solution pH of 6 was chosen as the optimum solution pH for the following study.

3.2.4. Effect of Salinity Concentration

Salinity is one of the most important environmental conditions and could significantly affect the metal phytoremediation performance of plants. According to [41], salinity stress could negatively affect the growth, respiration and photosynthesis mechanisms of a plant. In this context, sodium chloride was used as a source to alter the salinity of the solution. The effect of salinity concentrations (3, 6, 9, 12 and 15 ppm) on the removal efficiency of water lettuce and water hyacinth when removing Zn were studied, and the results are shown in Figures 12 and 13, respectively. The plants' efficiency when removing Zn was observed to be highly dependent on the salinity of the solution.

Based on Figure 12, the water lettuce's removal efficiency of Zn was the highest (80.1%) when the salinity concentration was at 6 ppm, while the lowest removal efficiency of Zn (5%) occurred at a salinity concentration of 15 ppm. According to [8], an increasing salinity concentration could lead to a direct and detrimental effect on the growth of a plant. This statement was clearly valid in conjunction with the results obtained from the present study. As the concentration of salinity was increased from 6 ppm to 15 ppm, a noticeable drop in removal efficiency of Zn could be observed. This was due to the excessive presence of sodium and chloride ions under high-salinity conditions, which could impair the growth and development of the plant through cytotoxicity. In addition, the high salinity concentration could also induce the generation of ROS, which leads to oxidative stress, distortion of genomic stability and damage of DNA [42]. The resulting damage imposed onto water lettuce at such high salinity concentrations (12 and 15 ppm) could be observed, as the removal efficiency of Zn started to reduce from its peak value on the second day of the study.

Nevertheless, the overall removal efficiency of Zn was in a constantly increasing pattern when the salinity concentration was increased from 3 to 9 ppm, which hinted at the possibility of achieving a higher removal efficiency with extended phytoremediation duration. In addition, the efficiency of removal of Zn also increased with a salinity concentration increase up to 9 ppm. The positive effects of low salinity concentrations on the water lettuce suggested that the cellular metabolisms and antioxidant activity, as well as a root-to-shoot translocation of nutrients, were not adversely affected [43]. In addition to that, the formation of Zn-chloride salt complexes in the presence of a tolerable amount of sodium chloride could potentially lead to a higher Zn removal rate by water lettuce. This was in accordance with the study conducted by [44], which suggested that the ability of accumulator plants to tolerate a low level of salinity stress could directly translate into an increased translocation rate of salt from the surroundings to the aerial plant parts.

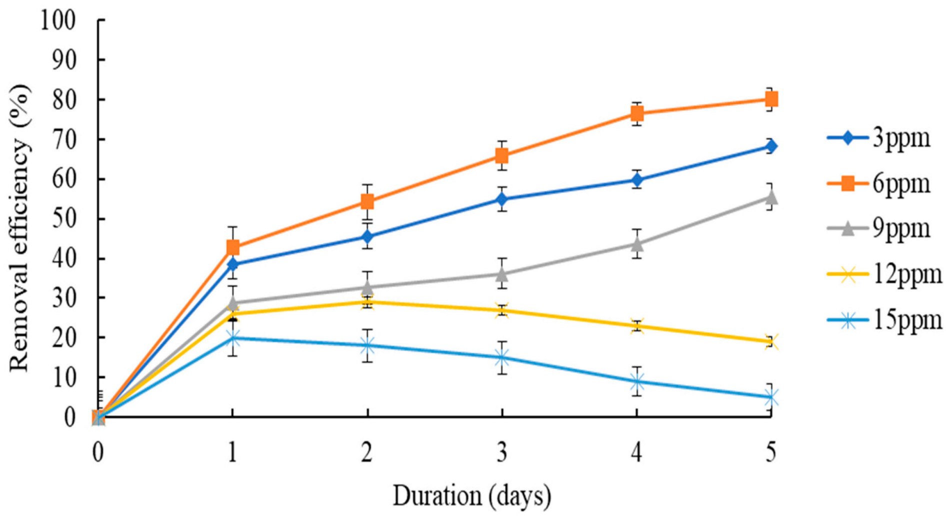

Figure 12. Effect of salinity concentrations on the removal efficiency of Zn by water lettuce (duration = 5 days, Zn concentration = 10 ppm and natural solution pH = pH 6).

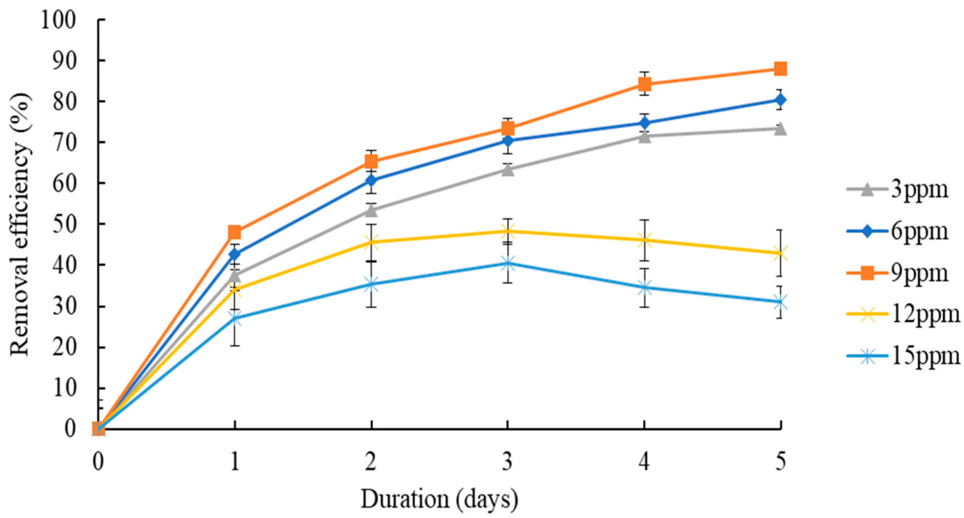

Figure 13. Effects of salinity concentrations on the removal efficiency of Zn by water hyacinth (duration = 5 days, Zn concentration = 10 ppm and natural solution pH = pH 6).

Based on Figure 13, the removal efficiency of Zn by water hyacinth was found to be the highest (88%) at a salinity concentration of 9 ppm, where a slightly higher tolerance towards salinity could be observed compared with water lettuce. On the other hand, identical to water lettuce, water hyacinth also displayed the lowest removal efficiency of Zn (31%) at a salinity concentration of 15 ppm. The overall trend of removal efficiency of Zn across different salinity concentrations for water hyacinth was also highly analogous to that of the water lettuce. Nevertheless, the most noticeable difference between these two plants could be observed at a salinity concentration of 9 ppm. Unlike the water lettuce, water hyacinth clearly showed its higher tolerance towards salinity in terms of the threshold limit for salinity concentration. When the salinity concentration was increased from 3 to 9 ppm, the reaction between positively charged Zn^{2+} ions and the salt-derived anions could form stable Zn-chloride ligands with enhanced mobility. As a result, when the ligands arrived at the root surfaces, Zn could then be absorbed by the plant roots via disassociation from the ligands [45]. However, as the salinity concentration was further increased up to 15 ppm, the effect of the enhanced Zn mobility was quickly outweighed by the negative effects caused by high salinity, such as the closure of stomata, lower transpiration rate, growth inhibition and oxidative damage caused by ROS generation [46]. Symptoms of necrosis and chlorosis could also be observed in water hyacinth at such high salinity concentrations, with the plant suffering from curling leaves, premature fall of leaves and discoloration of green young leaves. In conclusion, the optimum salinity concentration for water lettuce and water hyacinth was selected to be 6 and 9 ppm, respectively.

3.3. Possible Phytoremediation Strategies of Zn

The phytoremediation process of Zn-contaminated solution involves several mechanisms such as rhizofiltration, phytoextraction and phytostabilization, as shown in Figure 14. Rhizofiltration involves the absorption and sequestration of metal contaminants from water bodies. In this context, plants with a long and fibrous rhizosphere can potentially absorb a large amount of water, thereby allowing vast amounts of metal contaminants to be absorbed and adsorbed on the plant roots concurrently as well. According to [47], the rhizofiltration mechanism is more suitable than other available phytoremediation mechanisms when treating heavy metals from contaminated aqueous solutions. Aquatic plants such as water lettuce and water hyacinth are able to take up and translocate metals by utilizing their roots as active sites for adsorption on root surfaces or absorption into and sequestration within the roots prior to translocation to the aerial parts of the plant.

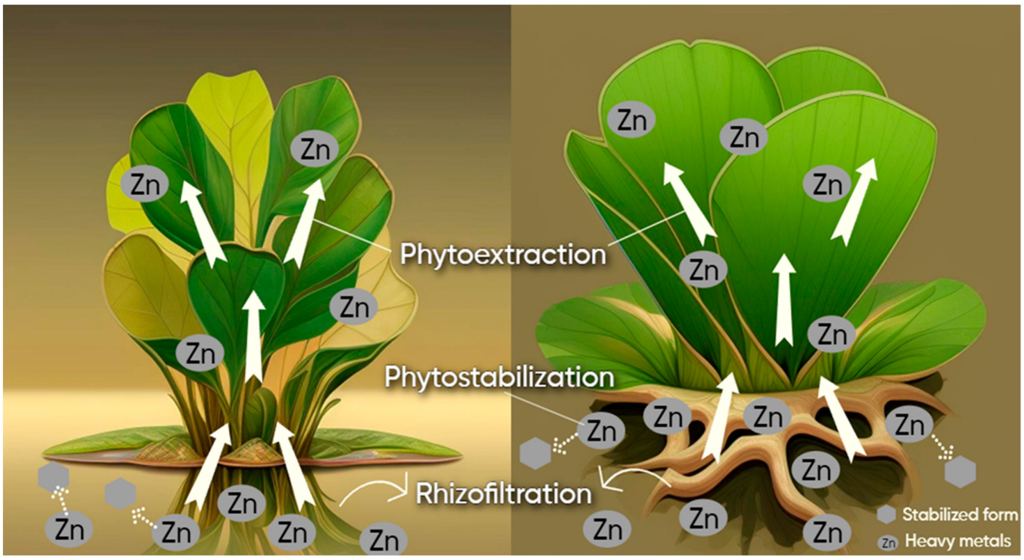

Figure 14. Mechanisms involved in the phytoremediation of Zn-contaminated solution by water hyacinth (**left**) and water lettuce (**right**).

Phytoextraction is defined as the process of accumulating heavy metals from the contaminated surrounding via the roots and transferring them towards the aboveground plant tissues, where the heavy metals are accumulated [48]. Due to the convenience of harvesting just the plant shoots, which are metal-enriched, phytoextraction by cultivating the plants directly on the contaminated sites is deemed the most commercially viable method of phytoremediation. According to [49], the phytoextraction mechanism requires the candidate plants to be tolerant towards metal-induced stress and capable of producing a high quantity of biomass. Apart from that, the in situ phytoextraction treatment also allows subsequent post-treatment handling methods such as composting, compaction and thermal decomposition to further minimize the risk of handling biomass with high levels of metal contaminants [50]. Plants can also establish phytostabilization, which is capable of constraining the movement of metal contaminants within the vadose zone through immobilization or root accumulation. Phytostabilization is technically a process used to stabilize the metal contaminants and reduce their mobility in order to prevent further intrusion into the food chain. In this context, [51] pointed out that both chemical (metal availability) and biological (metal plant uptake, ecotoxicological essays) tests should be carried out to monitor the metal contaminants if phytostabilization is chosen as the method for remediation.

3.4. Comparative Results and Literature Review

Table 2 lists several studies conducted by various researchers on the phytoremediation efficiency of various plants relating to different types of heavy metals. Based on Table 2, it can be noticed that most of the phytoremediation experiments were conducted with at least 30 days of duration. For instance, during the experiment conducted by Shehata et al. [52], both the Kenaf and Flax plants were cultivated for 8 weeks on the contaminated soil, consisting of several heavy metals such as Cr, Co, Cd and Mn, before harvesting. The phytoremediation efficiencies achieved by the Kenaf and Flax were between 12 and 36.5% and 9.4 and 45.2%, respectively. On the other hand, in another experiment conducted by Zhang et al. [53], Ryegrass, which is a hyperaccumulator plant, was incubated in sediment consisting of Cd, Pb and Zn at a temperature of 25 °C and a light/dark cycle of 12 h/12 h for a period of 30 days. After the incubation period, the phytoremediation efficiencies recorded were 47.8% for Cd, 37.2% for Pb and 42.5% for Zn. Meanwhile, Spiny pigweed was chosen by Njoku and Nwani [54] to be planted in pots containing heavy-metal-contaminated soil sourced from a local refuse dump site situated in Lagos. After 12 weeks, the plant was able to absorb 50.5, 49, 43.3 and 47.4% of the Cd, Cu, Pb and Zn, respectively. In the current work, both plants demonstrated highly efficient Zn removal rates, with water lettuce achieving 80.1% removal and water hyacinth reaching 88% removal within a remarkably short period of just 5 days.

Table 2. Phytoremediation efficiency of various plants for various heavy metals.

Plants Used	Targeted Heavy Metals	Phytoremediation Duration	Removal Efficiency	Reference
Hibiscus cannabinus L. (Kenaf) and *Linum usitatissimum* L. (Flax)	Cr, Co, Cd and Mn	8 weeks	Cr—34% Co—36.5% Mn—17.1% Cd—12% Mn—45.2% Cr—21.2% Co—17% Cd—9.4%	[52]
Clidemia sericea D. Don (Michelang)	Cd, Hg and Pb	12 weeks	Cd—49.2% Hg—18.4% Pb—32.3%	[55]
Lolium perenne L. (Ryegrass)	Cd, Pb and Zn	30 days	Cd—47.8% Pb—37.2% Zn—42.5%	[53]
Typha orientalis (Bulrush)	Cd, Cu, Ni and Pb	60 days	Cd—80% Cu—60% Ni—68.9% Pb— 8.6%	[56]
Cannabis sativa (Indian hemp)	Cu, Fe, Mn, Ni, Pb and Zn	60 days	Cu—75.9% Fe—88.6% Mn—70.8% Ni—78.7% Pb—83.9% Zn—39%	[57]
Amaranthus spinosus (Spiny pigweed)	Cd, Cu, Pb and Zn	12 weeks	Cd—50.5% Cu—49% Pb—43.3% Zn—47.4%	[54]
Pistia stratiotes (water lettuce) and *Eichhornia crassipes* (water hyacinth)	Zn	5 days	Zn—80.1% Zn—88%	Current work

4. Conclusions

This study demonstrated that both water lettuce and water hyacinth are capable of removing Zn contents via a phytoremediation process. SEM images revealed small granular aggregates on the surface of the plants as a result of the formation of stable metal-chelating complexes after phytoremediation. Meanwhile, EDX results proved the existence of Zn metal within the plants after the phytoremediation process, where the roots of both plants were observed to be saturated with Zn. On the other hand, FTIR spectra showed the shifting in bands for both plants after the phytoremediation process, which could be attributed to the interaction between the cationic Zn^{2+} and the hydroxyl group for the metal oxygen binding. The presence of several characteristic functional groups such as O-H, C-H and C=O bonds in both plants suggested the potential binding interaction between the functional groups and Zn metal ions, which could occur while keeping the chemical composition of both plants unchanged. In this context, the water hyacinth has a higher overall Zn content than the water lettuce, possibly due to its larger metal tolerance and phytoremediation capability. These characterization results could prove useful by shedding some light on the understanding of the underlaying complex mechanisms of the interactions between hyperaccumulator plants and heavy metals. By studying the changes in the morphological characteristics of both plants before and after phytoremediation, the phytoremediation performance can be enhanced via modification of the plants, such as through the transgenic method. Until now, to the best of our knowledge, transgenic plants with improved metal tolerance and phytoremediation efficiency have been based on the enhancement of the genes that are involved in the biosynthesis pathway of metal-binding proteins as well as the conversion of toxic ion into a less toxic state [58].

After conducting experimental studies on the phytoremediation of Zn by using both plants under different parameters, the optimum conditions for the highest removal efficiency (80.1% for water lettuce, 88% for water hyacinth) were achieved at a phytoremediation duration of 5 days, Zn concentration of 10 ppm and natural solution pH of 6, as well as salinity concentrations of 6 and 9 ppm for water lettuce and water hyacinth, respectively. Hence, both plants were proven to be strong contenders for phytoremediation of Zn in wastewater. Additionally, this information can be useful for enhancing the phytoremediation performance of plants, especially when selecting suitable hyperaccumulator plants for wastewater remediation applications. However, proper disposal and handling of plant biomasses after phytoremediation also require paying equal attention to fully maximizing the potential of heavy metal phytoremediation without compromising the environmental safety in the aftermath. While some studies have focused on post-phytoremediation plant biomasses, more efforts are still necessary to identify their potential applications. For example, phytomining, which involves recycling precious metals absorbed by hyperaccumulator plants, could offer insights into unlocking the largely untapped capabilities of hyperaccumulator plants and subsequently increasing their economic and scientific value.

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