Heliyon 10 (2024) e26590

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Efficiency of sodium phytate in the remediation of As, Mn, and Cu contamination in acid mine drainage using water hyacinth

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ARTICLE INFO

*Keywords:*
Sodium phytate
*Eichhornia crassipes*
Arsenic
Manganese
Copper
Acid mine drainage (AMD)

ABSTRACT

The accumulation and uptake efficiency of heavy metals, including As, Mn, and Cu, in water hyacinth (*Eichhornia crassipes* (Mart.) Solms) grown in synthetic acidic wastewater supplemented with sodium phytate (SP) was examined. Three treatments were studied using synthetic acidic wastewater containing 0.25, 5.0, and 1.0 mg/L of As, Mn, and Cu, respectively, (SM + heavy metals) and having pH in the range of 4–6, which comprised of (1) control treatments using SM + heavy metals at pH 4, 5, 6 without SP, and treatments using SM + heavy metals at pH 4, 5, 6 with SP: Cu (2) in a 1:3 M ratio and (3) a 1:6 M ratio. The translocation factor (TF $<$ 1) indicated that plants had a lower capacity to transport heavy metals from the roots to the stems. The shoots of water hyacinth exhibited the highest capacity to absorb and store As in the pH 4-treatment with SP (SP:Cu1:3 mol), whereas the roots showed the greatest capacity at pH 4 without SP. The roots and shoots of the water hyacinth showed the greatest capacity to take up and store Mn in the pH 5-treatment with a 1:3 M ratio of SP:Cu. The roots showed the greatest capacity to take up and store Cu in the pH 6-treatment, and the shoots showed the highest capability in the pH 5-treatment with 1:3 M ratio of SP:Cu. Moreover, analysis of the chemical forms revealed that As accumulated in the arsenate form, whereas Mn accumulated in the divalent form.

## 1. Introduction

The mining industry includes various activities, such as explosions, smelting ores, transportation, and waste rock disposal. Each stage of the mining operation frequently causes environmental pollution issues affecting the air, water, and soil. Waste rock is the residual waste from the blasting process, which contaminates water. Waste rock contains large amounts of heavy metals. In particular, in potential gold deposit areas, waste rock frequently contains pyrite minerals, such as arsenopyrite (FeAsS) and pyrite ($FeS_2$). Sulfuric acid ($H_2So_4$) is produced when the remaining waste rock reacts with water and air, producing, which causes acid mine drainage (AMD). The acidic pH of water leads to elevated sulfate concentrations and readily facilitates the dissolution of large amounts of heavy metals [1–3]. Moreover, if leakage occurs in the mining and surrounding areas, it causes contamination by a variety of heavy metals,

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https://doi.org/10.1016/j.heliyon.2024.e26590
Received 27 July 2023; Received in revised form 7 February 2024; Accepted 15 February 2024

including As, Mn, Cu, Pb, and Cd [4]. Furthermore, the research report by Boonsrang et al. [5] and Charuseiam et al. [6], who studied and analysed the contamination from the leaching of waste rocks in the mine area, found that the leachate contained As, Mn, and Cu concentrations exceeding the surface water quality standards in Thailand. Therefore, if wastewater is contaminated with heavy metals, it can affect human health and other organisms in water sources [7]. Humans are exposed to heavy metals entering the body through inhalation, ingestion, and skin contact. If heavy metals enter the human body, they can affect human health. Heavy metal contamination in mining areas, especially with As, is very harmful to health, and As has been classified as a carcinogen. In particular, if humans are exposed to As for a long time, they develop lung, bladder, and skin cancers [8–11]. Long-term exposure to Mn mostly affects the nervous system. Damage to the nervous system causes behavioural changes such as decreased range of motion, twitching, and inability to speak normally, and in severe cases, symptoms similar to those of Parkinson's disease occur [12]. Therefore, it is necessary to resolve heavy metal contamination in AMD before it is discharged into the environment. Phytoremediation is an interesting approach to clean up contaminated environments. Plants rely on natural processes to absorb various elements, including heavy metals and pollutants from the environment. Phytoremediation is used to treat areas contaminated with heavy metals to reduce the distribution and phytotoxicity of contaminants to the environment and human health. This method is highly efficient, can be used in large, contaminated locations, has low operating costs, is environmentally friendly, and can be used in polluted areas without affecting the environment [13–17]. In addition, plants used for phytoremediation must be thriving, have a high ability to absorb and store heavy metals, have a short life cycle, produce a substantial amount of biomass, have a wide root system, reproduce well, and be easy to maintain [18]. Thus, *Eichhornia crassipes* (Mart.) *Solms*. (water hyacinth) was selected for treatment in this study. It has a high biomass, grows rapidly, is durable, easy to treat, and has good potential for heavy metal accumulation [19,20]. To simulate AMD conditions, water hyacinths were cultivated in synthetic wastewater at pH 4, 5, and 6, to which As, Mn, and Cu were added. This study involved various activities, including sample collection, analysis, and assessment of accumulation and distribution patterns of heavy metals, and attempts to improve the capacity of plants to absorb heavy metals. A commonly used approach to enhance absorption of heavy metals involves the use of complex compounds. Sodium phytate (SP) ($C_6H_6Na_{12}O_{24}P_6$), a salt of phytic acid, is a naturally occurring substance in legumes. The binding of mineral molecules to two or more positive charges is facilitated by their structures. The ionic strength of minerals is in increasing order of $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ [21–25]. Cu ions bind particularly strongly to phosphate groups in ratios of 1:3 and 1:6 mol, owing to the high stability value of complex compounds formed with phytic acid and the strength of the ions that bind with Cu. Cu can attach three to six ions with phosphate groups at molar ratios of 1:3 and 1:6 [26]. Therefore, SP: Cu molar ratios of 1:3 and 1:6 were used in this study. This was expected to increase the uptake of As and Mn by the studied plants at the different pH levels. In addition, the use of SP does not affect the toxicity of water hyacinth or wastewater treatment processes using living plants (phytoremediation) [27]. Therefore, this research aimed to study the heavy metal uptake and accumulation in water hyacinth from a synthetic acidic wastewater of mine activities supplemented with SP to effectively and sustainably apply heavy metal retrieval or treat contaminated regions of AMD from mines.

## 2. Materials and methods

### 2.1. Preparation of materials

Synthetic acidic wastewater from mining is composed of disodium acid arsenate heptahydrate: ($Na_2HAsO_4{\cdot}7H_2O$), manganese sulfate ($MnSO_4.H_2O$), and copper sulfate ($CuSO_4$) as heavy metal contaminants. Sodium phytate (SP, $C_6H_6Na_{12}O_{24}P_6$) was used as chelating agent.

For phytoremediation experiments, water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants were used. Plants were obtained from natural rivers and cultivated in a greenhouse for 3–4 weeks. The experimental plants were selected based on their age, stem and root sizes, and weight.

The 5-litre plastic bottles were used as the experimental containers. The plastic bottles was soaked in 10% $HNO_3$ overnight and rinsed thoroughly using distilled water (DI).

### 2.2. Experimental design

In this study, preliminary experiments were conducted with different concentrations of As (0.25, 0.5, and 1.0 mg/L), Mn (5, 10, and 20 mg/L), and Cu (1 and 2 mg/L) to determine the concentrations of the target heavy metals which were least toxic to water hyacinths and did not affect the plants' growth. From this experiment, the least toxic concentrations of As, Mn, and Cu were found to be 0.25, 5, and 1 mg/L, respectively. Therefore, these three concentrations were used in three combinations of wastewater with and without the chelating agent SP for phytoremediation experiments to grow water hyacinth (1 plant). The synthetic acidic mining wastewater containing 0.25 mg/L As, 5.0 mg/L Mn, 1.0 mg/L Cu (SM + heavy metals), at pH 4, 5, and 6 with and without SP was treated using three compositions as follows: 1) a control treatments using SM + heavy metals at pH 4, 5, 6 without SP addition; 2) treatments containing SM + heavy metals at pH 4, 5, 6 and 1:3 M ratio of SP: Cu; and 3) treatments containing SM + heavy metals at pH 4, 5, 6 and a 1:6 M ratio of SP: Cu. Plant samples were collected on Days 7, 15, 22, and 30. All experiments were repeated three times, including pH adjustments performed every seven days throughout the experimental period.

### 2.3. Sample collection and analysis accumulation and distribution

For analysis of As, Mn, and Cu accumulation in plant samples, the following protocol was followed. First, water hyacinth samples

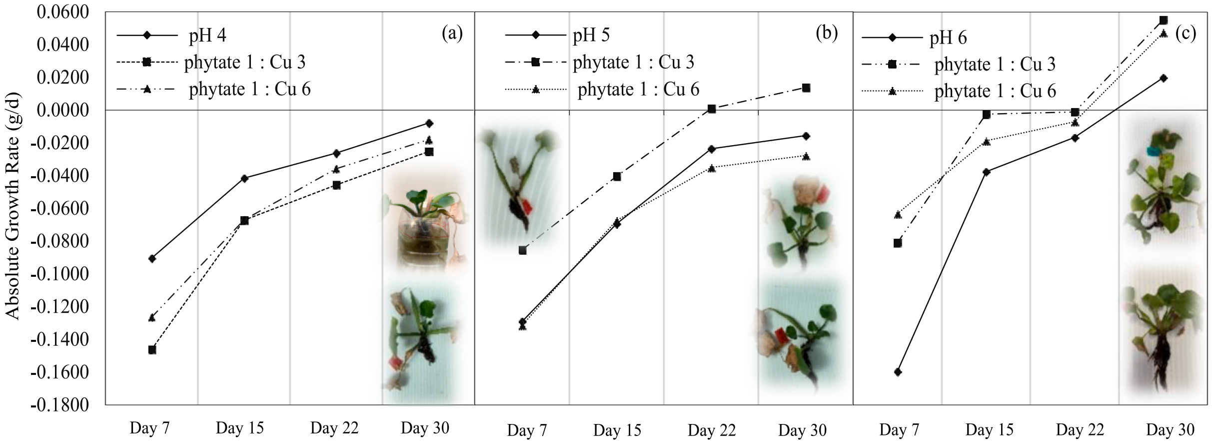


**Fig. 1.** AGR of water hyacinth; (a) pH 4 (b) pH 5 and (c) pH 6 treatments.

after each experimental duration were washed with tap water two to three times, rinsed with distilled water, and dried at room temperature. Then, plant samples were separated into submerged parts (roots) and shoots (stem leaves). Fresh weight of each plant part was measured and then each part was dried at 105 °C for 1–2 d, and weighed separately (dry weight). Finally, each part was analysed for absolute growth rate (AGR), which is a measure of the growth or change in total biomass per unit time per plant [28]. The AGR was calculated from the dry weight for each sampling period of the experiment [29] according to the formula shown in Equation (1).

$$\mathrm{AGR} = \frac{\mathrm{W_2 - W_1}}{\mathrm{T_2 - T_1}} \tag{1}$$

where AGR is in g/d, $T_1$ is the pre-experimentation time (d), $T_2$ is the post-experimentation time (d), $W_1$ is the dry weight (g) of the plant part at time $T_1$, and $W_2$ is the dry weight (g) of the plant part at time $T_2$.

Each fragmented sample (roots and shoots) was then ground to a fine powder to determine the cumulative content of total As, Mn, and Cu according to USEPA Method 3052 [30] and analysed by Inductively Coupled Plasma-optical Emission Spectrometry (ICP-OES), including assessment of the translocation and accumulation potential of plants to absorb and move heavy metals by calculating the translocation factor (TF) [31] using the formula shown in Equation (2).

$$\mathrm{TF} = \frac{\mathrm{Cshoot}}{\mathrm{Croot}} \tag{2}$$

where Cshoot is the metal concentration accumulated in the shoots (mg/kg), and Croot is the metal concentration accumulated in the roots (mg/kg).

The plant samples of each part were dried at 105 °C for 1–2 d were ground into a homogeneous mixture and analysed by X-ray absorption spectroscopy (XAS) with the 1.1 W beamline at the Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima Province. For Analysing As and Mn distribution and transport in plants, plant stems and root samples were prepared by cross-section technique, and the plant specimens were stored at −60 °C for two days and freeze-dried for one day. The prepared plant samples were examined by scanning electron microscopy (SEM) (FEI: QUANTA FEG 450) and energy-dispersive X-ray spectroscopy (EDS).

### *2.4. Statistical analysis*

Analysis of variance in the As, Mn, and Cu accumulated in plants in the phytoremediation experiment was performed using ANOVA, and the differences in the data were compared using Duncan's New Multiple Range Test (DMRT). The significance was determined at the 95% confidence level. ($P \leq 0.05$). The statistical program SPSS (Statistical Package for the Social Sciences) version 28 was used for all analyses.

## 3. Results and discussion

### *3.1. AGR and heavy metal translocation in plants*

The AGR of water hyacinths grown in synthetic acidic wastewater containing As, Mn, Cu, and SP (1:3 and 1:6 M ratios of SP: Cu) was studied. The results of the treatments indicated that during the initial growth stage, the plants were exposed to the acidic condition of the medium and simultaneously accumulated various heavy metals, which caused acute phytotoxicity that affected the

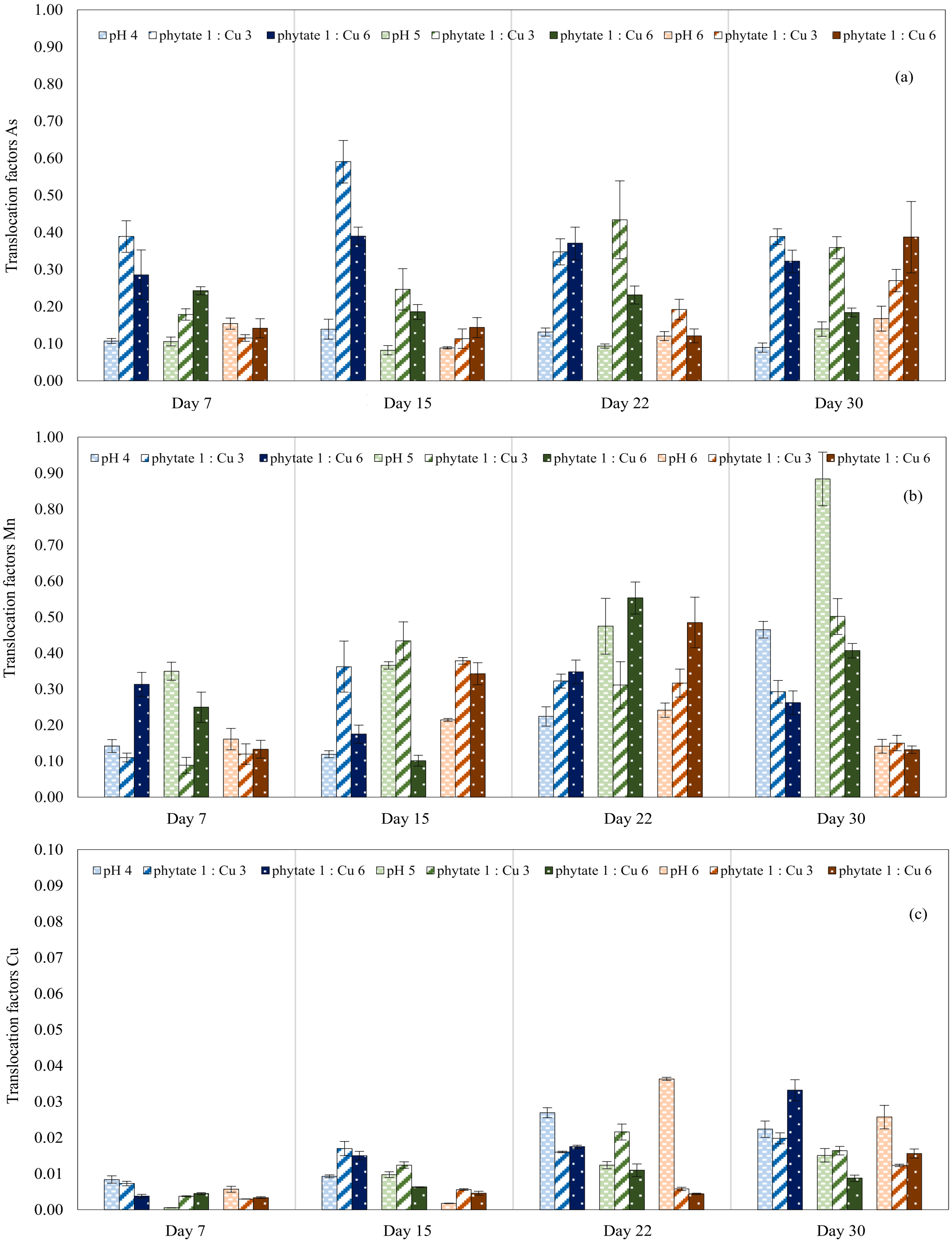


**Fig. 2.** TF and accumulation of (a) As (b) Mn and (c) Cu in water hyacinth.

photosynthesis of plants, resulting in brown leaves and withered plants. However, it was found that the pH 6-treatments with SP (1:3 and 1:6 M ratios) resulted in a higher AGR than the treatments without SP after a period of 7–15 days (Fig. 1(c)). The growth rate increased initially, remained constant from 7 to 22 days, and increased again after 30 days of treatment. This suggests that the plants adapted well to changing medium conditions. They were able to sustain, develop, and sprout leaves by spreading their roots more widely, as shown in Fig. 1. All treatments at pH 5 resulted in an increased AGR during the first sampling period, which then slowed down at 22–30 days of treatment (Fig. 1(b)). The treatment with SP (1:3 M ratio) resulted in the fastest growth rate. Treating water hyacinths at pH 4 in combination with SP at both 1:3 and 1:6 M ratios resulted in the lowest absolute growth rate among all treatments (Fig. 1(a)). This was due to more acidic conditions in this treatment than in the other treatments and a high accumulation of heavy

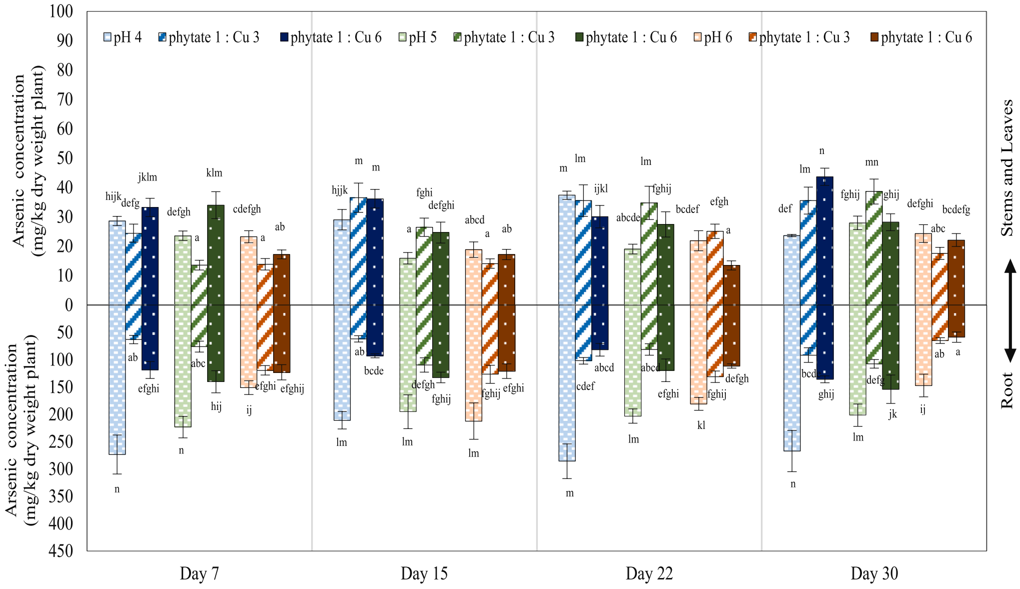


**Fig. 3.** As accumulation in the submerged parts (roots) and shoot parts (stems-leaves) of water hyacinth.

metals. This finding is consistent with the absorption of some heavy metals reported by Ingole and Bhole [32], who discovered that at low concentrations of heavy metals, plant growth was normal and heavy metal removal efficiency was higher. However, when the metal concentration increased, the plants began to wither, the heavy metal removal efficiency decreased by 39%, and the growth rate decreased. When plants are exposed to As, which is not an essential element for growth; it affects their physiology and functions in various systems (synthesis and metabolic processes in plants). In addition, this was consistent with the observations of Hua et al. [33], who investigated the ability of water hyacinth (*Eichhornia crassipes*), lettuce (*Pistia stratiotes* L.), and alligator weed (*Alternanthera philoxeroides*) to absorb Mn. They found that the growth rate decreased as the concentration of Mn in the plants increased. However, it can be concluded from this study that all SP treatments resulted in a higher AGR than non-SP treatments. Through statistical calculations and comparative analysis, it was found that all the experiments of synthetic acidic wastewater with pH 4 and 6 treatments produced results that were significantly different ($P < 0.05$).

The potential of plants to translocate and accumulate heavy metals was evaluated by calculating the TF, which is an indicator of the ability of a plant to move heavy metals from roots to stems [31]. The TFs were less than one in this study in the pH 4–6 treatments and SP (1:3 and 1:6 M ratios of SP: Cu). The results show that the plant had a lower ability to move As (Fig. 2(a)), Mn (Fig. 2(b)), and Cu (Fig. 2(c)) from the roots to the stems (TF < 1), resulting in more accumulation in the roots than in the stems, as shown in Fig. 2. These results agree with the findings of Zhang et al. [34], in their study on the concentrations of Zn, Cu, Cr, Pb, As, and Cd in various tissues of water hyacinths in Honghu Lake, China, and found that TF was less than 1. This result indicated that the majority of the absorbed water accumulated in the roots rather than in the stems. This is also consistent with the results of Hua et al. [33], who investigated the ability of three plants to absorb Mn: water hyacinth, lettuce, and alligator weeds. They found a TF of less than 1 at all concentrations of Mn.

## 3.2. *Uptake and accumulation of As, Mn, and Cu in plants*

### 3.2.1. *The uptake and accumulation of As in water hyacinth*

The uptake and accumulation of As in water hyacinth from SP-supplemented synthetic acidic wastewater showed that in all treatments, water hyacinths were able to uptake and store As within the submerged parts (roots) in a greater amount than the shoots of plants (stems, leaves), as presented in Fig. 3. This is consistent with the findings of Victor et al. [35], who investigated the absorption of Pb, Zn, Cd, Cu, and Cr and their bioaccumulation factors in *Eichhornia crassipes* and *Pistia stratiotes* grown in industrial wastewater. Both plants were found to accumulate more heavy metals in the roots than in the leaves, and TF was less than 1. This indicates greater heavy metal accumulation in the roots and less movement upwards in the stem. The present study found that after Day 22, the roots of water hyacinth could uptake and store 282.38 ± 26.23 mg/kg As in pH 4-treatment. Statistical comparisons showed a significant difference ($P < 0.05$) between all pH 4–6 treatments with SP (1:3 and 1:6 mol) and all treatments without SP addition, except for the pH 6 treatments after seven days. Yapoga et al. [36] examined the potential of water hyacinth to remediate four heavy metals found in industrial wastewater (Zn, Cd, Cu, and Cr). Their experiments showed that water hyacinth had the potential for remediation of all four heavy metals and could accumulate more heavy metals within the roots than within the leaves. Roy et al. [37] studied the potential of water hyacinths to treat As-contaminated wastewater and found As to accumulate in the roots rather than the stems and can be used by the plant in future.

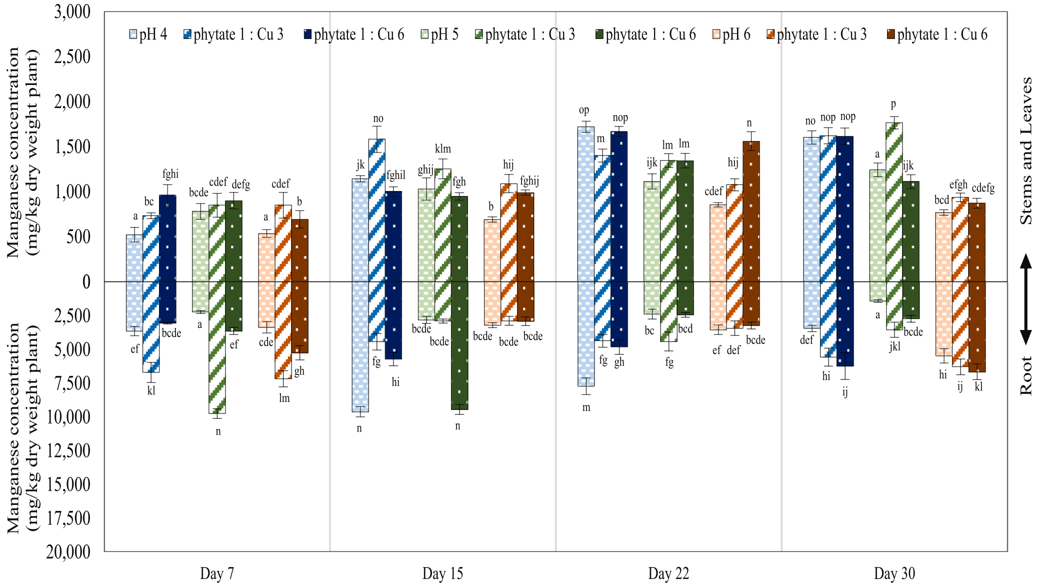


**Fig. 4.** Mn accumulation in the submerged parts (roots) and shoot parts (stems-leaves) of water hyacinth.

The pH 4-treatment with SP (SP:Cu 1:3 M ratio) resulted in accumulation of the most quantities of As (43.65 ± 2.96 mg/kg) within the shoots of water hyacinths (stem-leaf), and pH 5-treatment with SP (SP:Cu1:6 M ratio) resulted in the plants absorbing and accumulating the most quantities of As (38.64 ± 4.27 mg/kg) in the stem-leaf at the Day 30 of the treatments, as shown in Fig. 3. Nevertheless, the statistical, comparative, and analytical values indicated that the results of the pH 4- and pH 6-treatments with SP (1:3 and 1:6 mol) were significantly different ($P < 0.05$).

### 3.2.2. *The uptake and accumulation of Mn in water hyacinths*

The uptake and accumulation of Mn in water hyacinths in all experiments showed that the plants were able to uptake and store Mn in the submerged parts (roots) more than in the shoots (stems leaves). This is consistent with the findings of Zhou et al. [38], who examined the removal and accumulation of heavy metals, such as Mn, Pb, Zn, and Cd, in water hyacinths in hard water and discovered that floating plants had the greatest Mn removal capacity, including plants that had accumulated Mn in their roots in higher quantities than in their stems. Therefore, in this study, pH 5-treatment with SP (SP:Cu1:3 M ratio) resulted in the maximum accumulation rate of Mn in the submerged parts (roots) of floating plants, which absorbed and accumulated Mn in high quantities after seven days, with a value of 9764.19 ± 357.36 mg/kg, as shown in Fig. 4. This is consistent with the results of Jones et al. [39] who studied the removal of heavy metals by water hyacinths in rivers in England. Water hyacinths could remove up to 22% of the total Mn during the first day of exposure. The present study also found that plants could absorb and accumulate high quantities of Mn in the pH 4-treatment after 15 days, with a value of 9629.11 ± 383.84 mg/kg. The pH 5-treatment with SP (SP:Cu 1:6 M ratio) resulted in plant absorption and accumulation of high quantities of Mn at 15 days into the treatment at 9448.23 ± 362.71 mg/kg. This is also consistent with the results of Matindi et al. [40], who studied the uptake and accumulation of eight heavy metals, namely, $Pb^{2+}$, $Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $C^{2+}$, $Cd^{2+}$, and $Ni^{2+}$, in water hyacinths along the shoreline of Winam Bay in Victoria Lake, Kenya. Plants accumulate 3-fold more heavy metals in the roots than in the stems. This result was also consistent with the results of Alhaji et al. [41], who studied the potential of algae, water hyacinth, and lettuce for the uptake and accumulation of Mn, Ni, and Pb and found that heavy metals were increasingly accumulated within the roots more than within the stems, and water hyacinth produces considerable amounts of biomass that could be properly used in wastewater treatment. Statistical analysis of the values for Mn uptake and accumulation at different treatment durations indicated that the results for pH 4-treatments on Day 22, pH 5-treatments on Day 30, and pH 4-treatments with SP (1:3 mol) on Days 7 and 30 days were significantly different ($P < 0.05$) from those of all other treatments. The pH 5-treatment with SP (1:3 M ratio) resulted in the highest absorption and accumulation of Mn in the shoots (stems and leaves), as shown in Fig. 4. The floating plants had contained the maximum quantities of Mn from the uptake and storage at 30 and 22 days into the treatments, with values of 1764.69 ± 48.69 and 1721.87 ± 35.44 mg/kg, respectively. However, pH 4-treatment with SP (1:3 mol) resulted in a high absorption and accumulation of Mn 22 days into the treatment, with a value of 1667.41 ± 37.14 mg/kg. Statistical analysis showed that the results of pH 4- and pH 6-treatments after seven days, pH 4-treatment with SP (SP:Cu 1:3 M ratio) after 22 days, and pH 6-treatment with SP (SP: Cu 1:6 M ratio) after 22 days were statistically significantly different ($P < 0.05$) from those of all other treatments.

The studies by Nissar et al. [23] and Marolt et al. [24] described SP molecular structure contains phosphoric acid ($H_3PO_4$) and 6-phosphate groups, which produce phosphate $[PO_4]^{3-}$ anions; hence, they were able to bind to the heavy metals ions with two or more positive charges. Similarly, the results of this study showed that the Cu in synthetic acidic wastewater had a stronger ability to bind

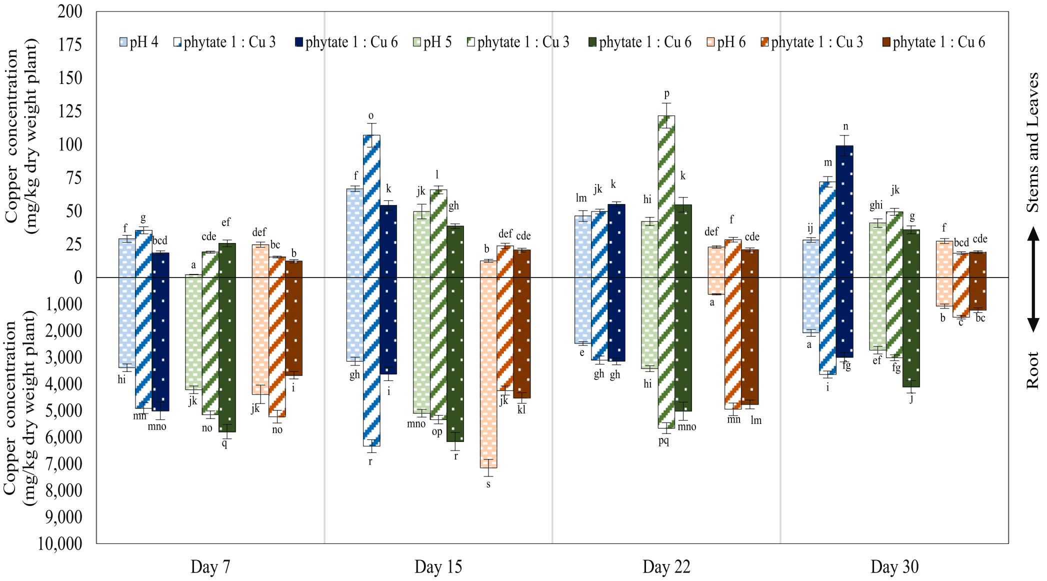
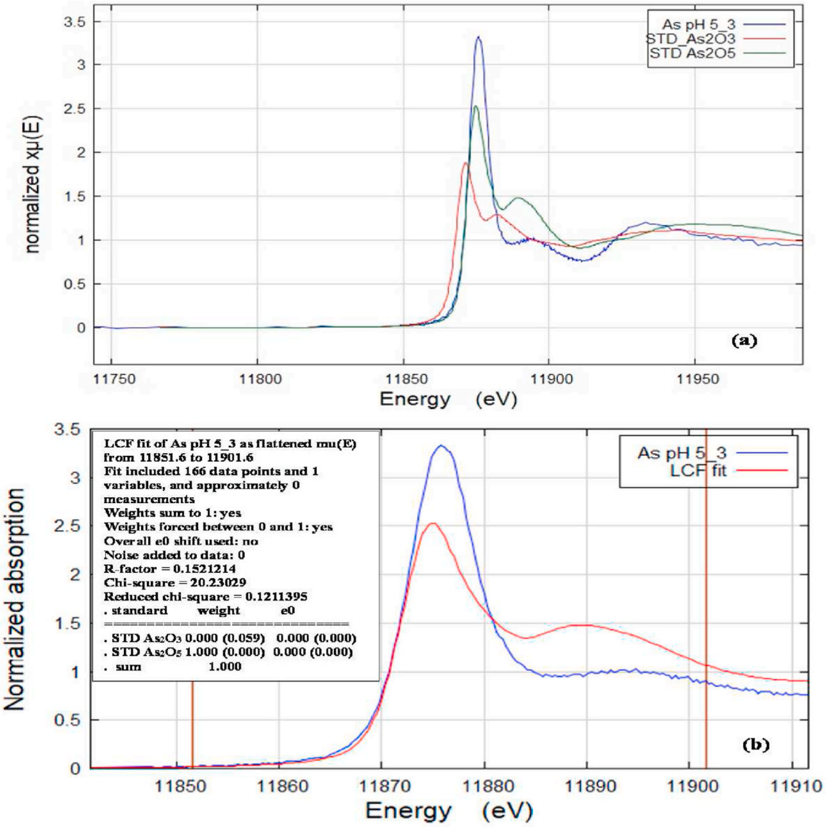


**Fig. 5.** Cu accumulation in the submerged parts (roots) and shoot parts (stem-leaf) of water hyacinth.



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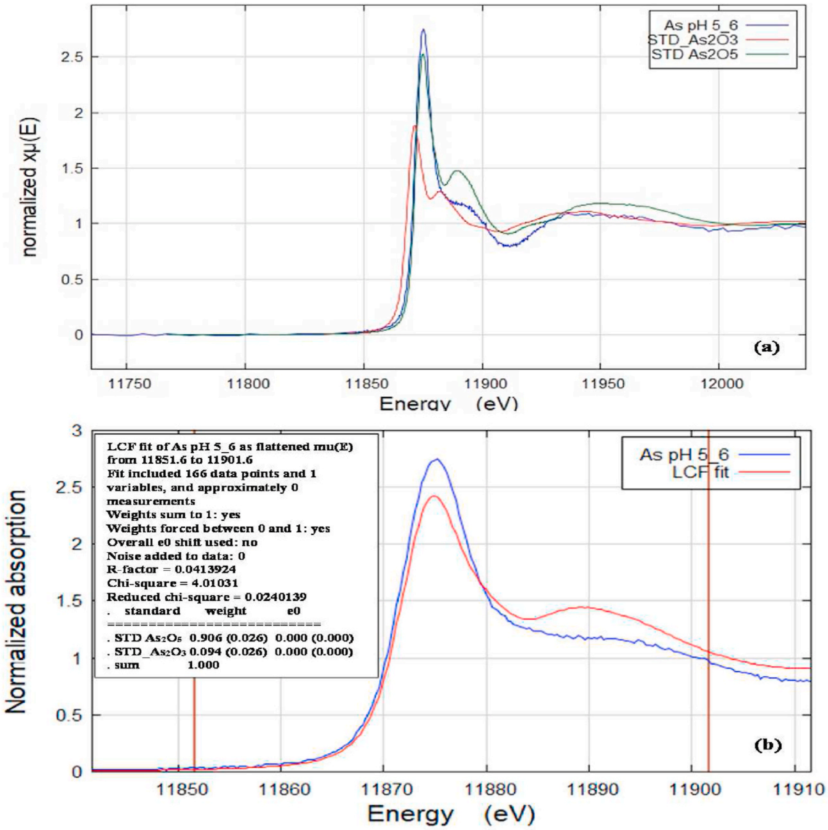
LCF fit of As pH 5_3 as flattened mu(E)
from 11851.6 to 11901.6
Fit included 166 data points and 1
variables, and approximately 0
measurements
Weights sum to 1: yes
Weights forced between 0 and 1: yes
Overall e0 shift used: no
Noise added to data: 0
R-factor = 0.1521214
Chi-square = 20.23029
Reduced chi-square = 0.1211395
. standard       weight        e0
=====================================
. STD As2O3 0.000 (0.059)   0.000 (0.000)
. STD As2O5 1.000 (0.000)  0.000 (0.000)
.  sum              1.000
```

**Fig. 6.** Chemical speciation of As in water hyacinths with SP addition; (a) the shoot parts (stems-leaves), and (b) the linear correlation of As in the pH 5-treatment with SP (1:3 mol) with the standard substances.



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LCF fit of As pH 5_6 as flattened mu(E)
from 11851.6 to 11901.6
Fit included 166 data points and 1
variables, and approximately 0
measurements
Weights sum to 1: yes
Weights forced between 0 and 1: yes
Overall e0 shift used: no
Noise added to data: 0
R-factor = 0.0413924
Chi-square = 4.01031
Reduced chi-square = 0.0240139
.   standard      weight         e0
================================
. STD As2O5  0.906 (0.026)  0.000 (0.000)
. STD_As2O3 0.094 (0.026)  0.000 (0.000)
. sum          1.000
```

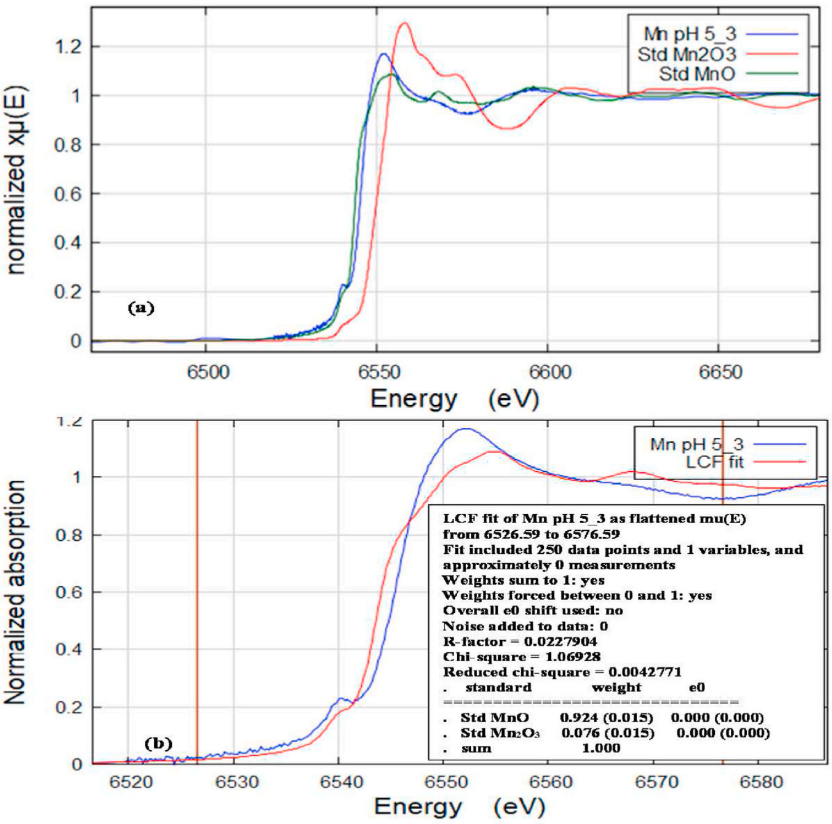
**Fig. 7.** Chemical speciation of As in water hyacinths with SP addition; (a) the submerged parts (roots), and (b) the linear correlation of As in the pH 5-treatment with SP (1:6 mol) with the standard substances.

with three phosphate anions of three phosphate groups (molar ratio of 1:3) than the ability to bind to six phosphate group ions (molar ratio of 1:6) [27], resulting in the formation of a large number of bonds between the Cu ions and phosphate groups. Consequently, plants were able to absorb As and Mn ions into their roots, petioles, and laminae.

### 3.2.3. The uptake and accumulation of Cu in water hyacinths

The uptake and accumulation of Cu in water hyacinths in all treatments showed that the floating plants had the ability to take up and store Cu in the submerged parts (roots) in greater amounts than within the shoots (stems, leaves), as shown in Fig. 5. This finding is corresponds to that of Hammad [42], who found that water hyacinths accumulated more Cu, Ni, and Zn in the roots than in the leaves. Moreover, pH 6-treatment resulted in water hyacinths accumulating the maximum quantities of Cu within the submerged parts (roots) at Day 15, with a value of 7149.69 ± 122.72 mg/kg that was more than the other treatments with different pH values. In addition, pH 4- and pH 5-treatments supplemented with SP (SP:Cu 1:3 and 1:6) resulted in the floating plants absorbing and accumulating large quantities of Cu at 15 days treatment duration, with values of 6331.09 ± 144.76 and 6155.83 ± 142.58 mg/kg, respectively. These results are consistent with those of Peng et al. [43], who studied the ability of water hyacinths to remove As, Cu, Cd, and Pb and found that plants had a greater ability to accumulate heavy metals within root tissues than within leaf laminas. These findings are also similar to the observations of Dadi-Mamud et al. [44] from the study on treatment of domestic wastewater collected from sewers in Makera and Chanchaga, Nigeria with water hyacinths and analysis of the accumulation of heavy metals such as Pb, Fe, Cu, Zn, Cr, and Mn in experimental plants. The results showed the highest accumulation of heavy metals in the plant roots. Statistical analysis indicated that pH 4- and pH 6-treatments at 22 and 30 days, pH 5-treatments with SP (SP:Cu1:6 mol) at seven days, and pH 6-treatments with SP (SP:Cu1:3 mol) at 30 days produced results that were significantly different ($P < 0.05$) from those of all other treatments.

The treatments that showed the highest Cu accumulation in the shoot parts (stems, leaves) were the pH 5-treatment with SP (1:3 mol), followed by the pH 4-treatment with SP (1:3 mol). These treatments led to absorption and accumulation of significant amounts of Cu within 22 and 15 days in water hyacinths at concentrations of 121.74 ± 9.43 and 107.03 ± 8.97 mg/kg, respectively. The pH 4-treatment with SP (1:6 mol) resulted in the floating plants absorbing and accumulating large quantities of Cu at 30 days treatment, with a value of 99.19 ± 7.77 mg/kg, as shown in Fig. 5. Statistical analysis indicated that all treatments produced statistically significant differences ($P < 0.05$). This study demonstrated that the SP molecular structure could bind better and formed many bonds between the Cu ions and phosphate groups than between the Mn ions and phosphate groups, causing plants to increasingly absorb Mn [22]. However, the plants absorbed and accumulated less than the plants accumulating Cu in the study by Guayjarernpanishk and



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LCF fit of Mn pH 5_3 as flattened mu(E)
from 6526.59 to 6576.59
Fit included 250 data points and 1 variables, and
approximately 0 measurements
Weights sum to 1: yes
Weights forced between 0 and 1: yes
Overall e0 shift used: no
Noise added to data: 0
R-factor = 0.0227904
Chi-square = 1.06928
Reduced chi-square = 0.0042771
.   standard          weight        e0
==================================
.  Std MnO       0.924 (0.015)   0.000 (0.000)
.  Std Mn2O3     0.076 (0.015)   0.000 (0.000)
.  sum                 1.000
```

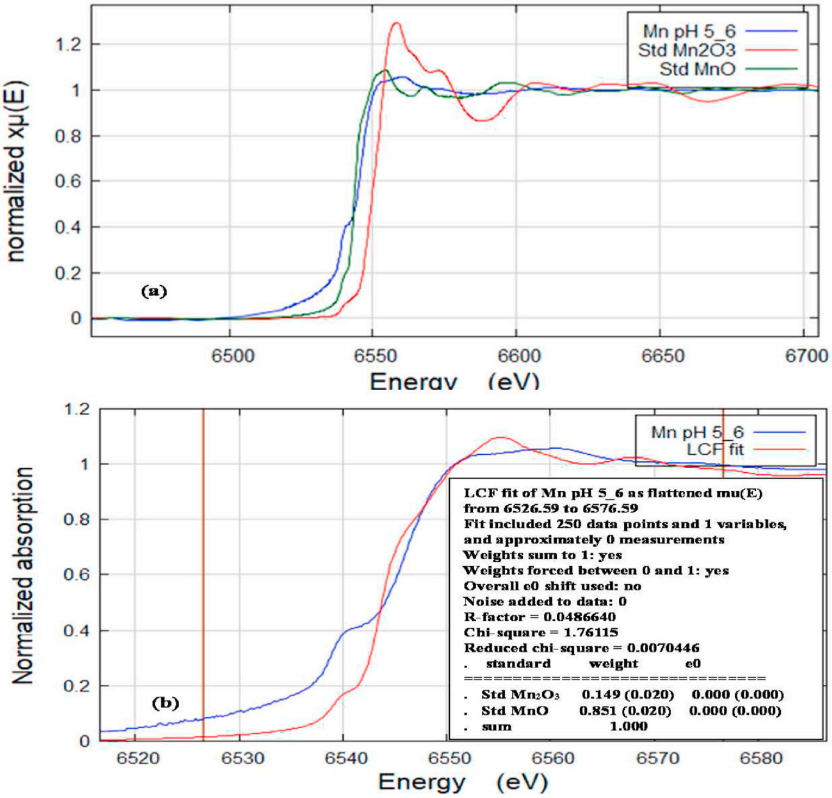
**Fig. 8.** Chemical speciation of Mn in water hyacinths with SP addition; (a) the shoot parts (stems-leaves), and (b) the linear correlation of Mn in the pH 5-treatment with SP (1:3 mol) with the standard substances.

Sampanpanish [27], who examined the effects of SP consequence to adsorb Cu of the floating plant could grow in acid mine drainage (AMD) from gold mining without Mn solution, which corresponded to the bonding of SP with the Cu substance.

From this study, after the experimental duration, it was found that the average concentrations of As, Mn, and Cu in the synthetic wastewater in the experiments with SP were in the range of 0.1790–0.2414, 0.1217–4.4826, and 0.0404–0.5389 mg/L, respectively. The efficiency of absorption, accumulation, and transport of As, Mn, and Cu in water hyacinths revealed that water hyacinth had the greatest efficiency in absorbing and accumulating As in treatments at pH 4, and in SP supplemented medium (SP:Cu 1:3 M ratio) on Day 15 of the experiment. The percentages of As accumulated in the submerged (roots) and shoots were 62.65 and 37.35, respectively. The water hyacinths shoots exhibited the highest efficiency for absorbing and accumulating Mn increasingly in the experiment at pH 5-treatment and in medium with SP:Cu at a ratio of 1:6 mol on day 22 of the experiments. The percentages of Mn accumulated in the underwater (root) and shoot parts were 64.48 and 35.57, respectively. However, the uptake and accumulation of Cu were greater in the underwater parts (roots) than in the shoots in all the experiments.

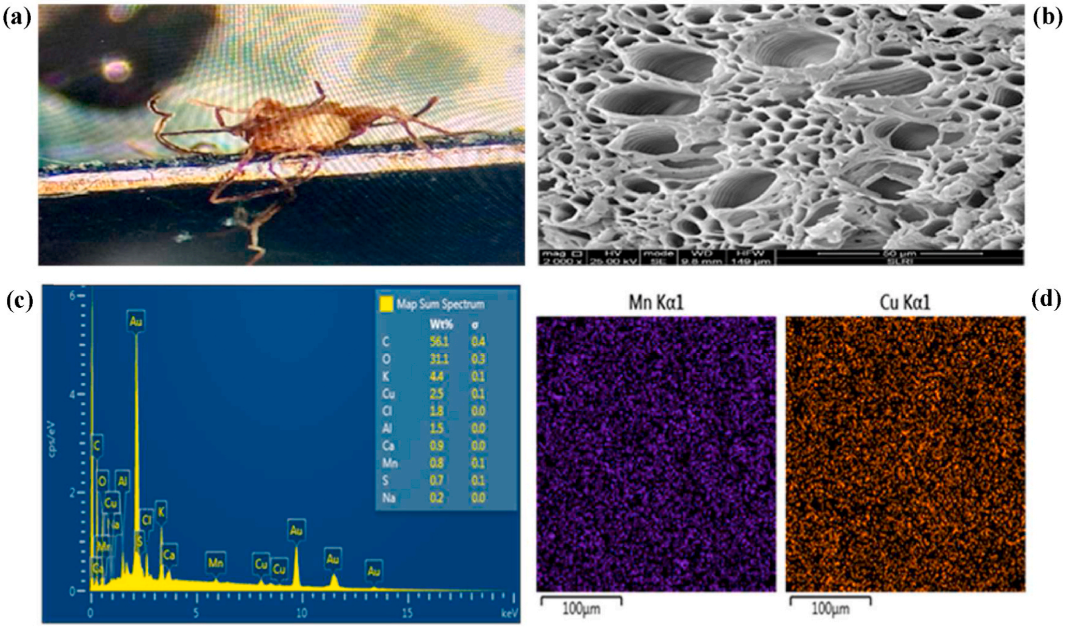
### 3.3. *Chemical speciation, distribution, and transport of As and Mn in water hyacinths by adding SP substance*

The chemical speciation of As and Mn accumulated in water hyacinth was examined using synchrotron light with the X-ray absorption near-edge spectroscopy (XANES) technique of beamline system 1 (BL1.1W) by applying synchrotron light with an X-ray energy range of 4–18 keV. Water hyacinth samples with the highest accumulation of As and Mn in their parts were specifically analysed using a linear correlation fit to determine the chemical form of the heavy metals that accumulated in the water hyacinths. In this study, the chemical forms of As were determined by analysing the samples and comparing with two standard reference substances, $As_2O_3$ and $As_2O_5$, with energies of 11,867.8 eV and 11,871.7 eV, respectively. The analysis of plant shoots (stems leaves) and submerged parts (roots) from the pH 5-treatments with both SP molar ratios (1:3 and 1:6) indicated that most of the As produced peaks in the spectrum as pentavalent (+5) or arsenate form (100%) with an energy value of 11,871.6 eV, as shown in Fig. 6(a and b) and Fig. 7(a and b). This form of As has poor mobility and is less toxicity than arsenite [45,46]. In the present study, SP addition facilitated water hyacinth to absorb and accumulate As in arsenate (As(V)) form, which passed through the same phosphate transporter. This is consistent with the results of Zhang et al. [47] who studied the speciation and distribution of As in hyperaccumulating plants before transformation into its arsenite form. It was also found that As was mobile in the stems and remained in its arsenate (As (V)) form, which is less toxic. Therefore, plants showed decreased phytotoxicity and continued to absorb As during growth. The plants could effectively adapt to



```
LCF fit of Mn pH 5_6 as flattened mu(E)
from 6526.59 to 6576.59
Fit included 250 data points and 1 variables,
and approximately 0 measurements
Weights sum to 1: yes
Weights forced between 0 and 1: yes
Overall e0 shift used: no
Noise added to data: 0
R-factor = 0.0486640
Chi-square = 1.76115
Reduced chi-square = 0.0070446
.   standard        weight          e0
==================================
.   Std Mn₂O₃     0.149 (0.020)   0.000 (0.000)
.   Std MnO       0.851 (0.020)   0.000 (0.000)
.   sum                  1.000
```

**Fig. 9.** Chemical speciation of Mn in water hyacinths with SP addition; (a) the submerged parts (roots), and (b) the linear correlation of Mn in the pH 5-treatment with SP (1:6 mol) with the standard substances.



| | Wt% | σ |
|---|---|---|
| C | 56.1 | 0.4 |
| O | 31.1 | 0.3 |
| K | 4.4 | 0.1 |
| Cu | 2.5 | 0.1 |
| Cl | 1.8 | 0.0 |
| Al | 1.5 | 0.0 |
| Ca | 0.9 | 0.0 |
| Mn | 0.8 | 0.1 |
| S | 0.7 | 0.1 |
| Na | 0.2 | 0.0 |

**Fig. 10.** Distribution and transport of Mn in the roots of water hyacinth in the pH 5-treatment with SP (1:3 mol); (a) the roots of water hyacinth, (b) SEM image of water hyacinth root (c) EDX analysis, and (d) elemental mapping of the element in the roots.

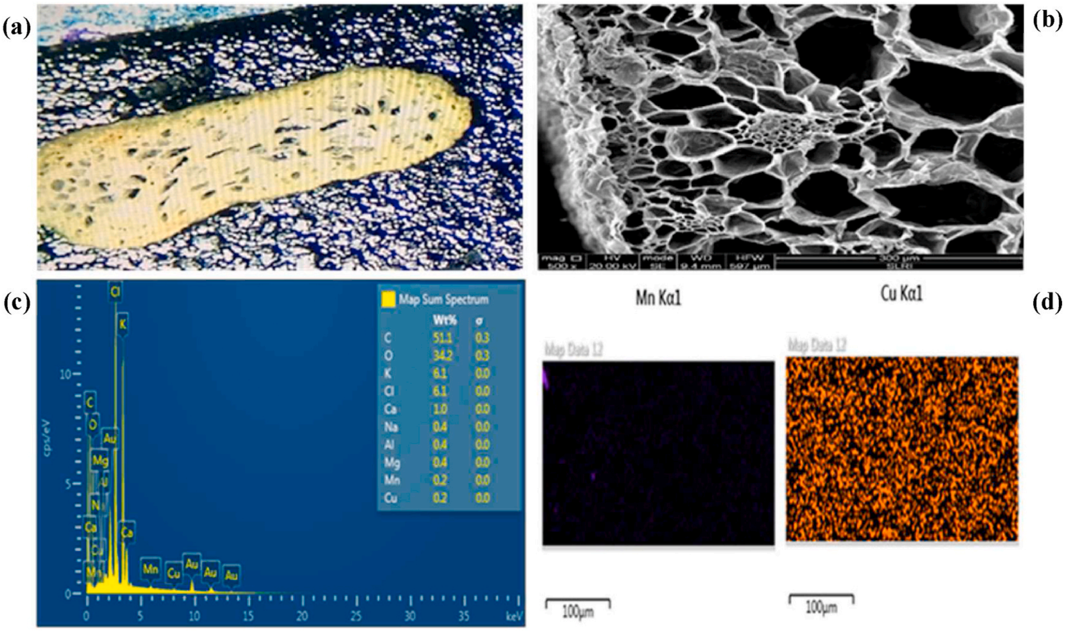


**Fig. 11.** Distribution and transport of Mn in the stems of water hyacinth in the pH 5-treatment with SP (1:3 mol); (a) the stem of water hyacinth, (b) SEM image of water hyacinth stems (c) EDX analysis, and (d) elemental mapping of the element in the stems.

produce shoots and leaves.

The chemical speciation of Mn was also analysed. A reference analysis was performed using two standard substances, MnO and $Mn_2O_3$, with energies of 6543.79 eV and 6550.30 eV, respectively. The results of the analysis of the plant shoots (stem-leaf) and submerged parts (roots) from the pH 5-treatments with both 1:3 and 1:6 M ratios of SP:Cu indicated that most of the Mn exhibited peaks in the spectrum as divalent Mn or $Mn^{2+}$ (stem-leaf = 92.4%, roots = 85.1%) with an energy value of 6546.59 eV, as shown in Fig. 8(a and b) and Fig. 9(a and b) [48,49]. This result demonstrated that plants could absorb Mn in the $Mn^{2+}$ form, which is in accordance with the research of Mousavi et al. [50], providing a general overview of the importance of Mn for crop production, as plants absorb Mn in the $Mn^{2+}$ form and transfer it to various tissues before transforming it into $Mn^{3+}$ and $Mn^{4+}$ forms. However, Mn is also essential for photosynthesis. It activates more than 35 enzymes involved in plant growth.

Moreover, distribution and transport of As and Mn in the roots and stems of water hyacinth samples with the highest accumulation of As and Mn in their parts were especially examined with scanning electron microscopy The distribution and transport of As and Mn in the roots and stems of water hyacinth samples with the highest accumulation of As and Mn concentrations were especially examined using scanning electron microscopy (SEM) at 20 and 25 kV, a magnification of 500x and 2,000x, and on a scale of 50 μm and 300 μm, respectively. Magnification was used in combination with energy-dispersive X-ray spectroscopy (EDS) of the pH 5-treatments with SP (1:3 mol), with values of 0.8% Mn in the roots of the water hyacinth, as shown in Fig. 10(a–d) and 0.2% Mn in the stems of water hyacinth, as shown in Fig. 11(a–d). However, As could not be detected because its accumulation in the sample was too low, and could not be measured. This finding is also similar to the research of Page and Feller [51], who analysed the transport and redistribution processes of heavy metals in crop plants. Manganese is readily transported from the xylem to the shoots. Such accumulation, with no or only minor redistribution, was observed for the micronutrient Mn [52,53].

## 4. Conclusion

The results of a study on the uptake and storage of As, Mn, and Cu in water hyacinths with SP addition at different pH levels showed that water hyacinths had the highest As uptake and accumulation in their submerged parts (roots) at pH 4-treatment. The shoot parts (stems leaves) of the plants accumulated the greatest amounts of As in the treatment with 1:3 SP:Cu molar ratio. The pH 5-treatment with SP:Cu (1:3 mol) resulted in floating plants having the greatest quantity of Mn absorbed and accumulated within both their above-water (stems-leaves) and submerged parts (roots). In the pH 6-treatment, water hyacinth was able to absorb and accumulate large quantities of Cu within the submerged parts (roots), whereas in pH 5-treatment with SP:Cu 1:3 mol, the plants exhibited the greatest capacity to absorb and accumulate Cu within the shoots (stems leaves). An assessment of As, Mn, and Cu translocation and accumulation potential showed that water hyacinth had less ability to move heavy metals from the submerged parts (roots) to the shoots (stem-leaf). The studied substances accumulated in greater amounts in the roots than in the stems. In addition, the treatments with SP showed that the AGR was better than in treatments without SP. However, all pH 5 and 6 treatments with 1:3 mol of SP:Cu resulted in less phytotoxicity than the other treatments. The chemical speciation of As in water hyacinth is mainly in the pentavalent (+5) or arsenate form, which is less toxic than the arsenite form. The chemical speciation of Mn in plants is mostly divalent Mn or $Mn^{2+}$ form, which is an essential nutrient for plant growth. Therefore, the results of this research can be used as a model to study the effectiveness of SP in the treatment or remediation of areas contaminated with As, Mn, and Cu, including acid mine drainage. Other factors or environments that may affect remediation should be considered and studied.

## Funding information

This study received financial support from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and the Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University, for the research unit.

## Data availability statement

Data included in article/supplementary material/referenced in article. No additional information is available for this article.

## CRediT authorship contribution statement

**Wannipa Guayjarernpanishk:** Writing – original draft, Methodology, Formal analysis, Data curation. **Pantawat Sampanpanish:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We gratefully thank the Environmental Research Institute (ERIC), the Research Unit of Green Mining Management (GMM), the Interdisciplinary Program in Environmental Science, Graduate School, Chulalongkorn University and We wish to thank the scientific staff at the beamline 1.1 W of the Synchrotron Light Research Institute (SLRI), Nakhon Ratchasima, Thailand, for assisting with the analysis of the XANES spectroscopy.

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