EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 16(1) 2023 87-98
DOI 10.14456/ea.2023.8
ISSN 1906-1714; ONLINE ISSN: 2586-8861

Effects of Sodium Phytate on the Phytotoxicity and Arsenic (V) Absorption Performances of Water Hyacinth Grown in the Synthetic Wastewater as Acid Mine Drainage from Gold Mining

Wannipa Guayjarernpanishk[1] and Pantawat Sampanpanish[2,3,4*]

[1] Inter-department of Environmental Science, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand
[2] Environmental Research Institute, Chulalongkorn University, Bangkok 10330, Thailand
[3] Research Unit of Green Mining Management, Chulalongkorn University, Bangkok 10330, Thailand
[4] Research Program of Toxic Substance Management in the Mining Industry, Center of Excellence on Hazardous Substance Management, Bangkok 10330, Thailand

*Corresponding author: pantawat.s@chula.ac.th
(ORCID ID: orcid.org/0000-0002-2561-7459)
Received: November 18, 2022; Revised: December 8, 2022; Accepted: December 20, 2022

Abstract

In this study, the effects of sodium phytate on the phytotoxicity and heavy metal absorption performances of water hyacinth grown in acidic synthetic wastewater from gold mining were studied in four experiments. The studies included the following: the experiment on phytotoxicity. Treatment 1) by varied sodium phytate (1, 5, 10, and 15 mg/L); 2) by varied pH 4 - 6 in the experiment of synthetic wastewater containing 0.25 mg/L of Arsenic(V) and 1 mg/L of copper without sodium phytate; 3) the experiment with arsenic, copper, varied pH 4 - 6, and sodium phytate: copper at the mole ratio of 1:3 and 4) the experiment similarly as treatment 3 but the mole ratio was increased to1:6. Samples were collected at 7, 15, 22, and 30 days. Arsenic and copper accumulations were analyzed in the plant's roots and shoots (stem and leaves). The results found that the addition of sodium phytate showed low phytotoxicities in the water hyacinths, except for a concentration of 10 – 15 mg/L at 30 days, where the plants had significantly reduced chlorophyll contents. The experiments with both sodium phytate ratios at all pH values showed that the relative growth rates (RGRs) were better than those for the experiment without sodium phytate. Moreover, the addition of sodium phytate affected the absorption and accumulation of arsenic and copper in the water hyacinths. The water hyacinth roots had the highest ability to absorb and accumulate arsenic in the experiments with pH 4 at 22 days of the experiment, with values of 600.12 ± 120.47 mg/kg. The shoot had the highest ability to absorb and accumulate arsenic in the experiments with pH 4 and sodium phytate at the ratio of 1: 6 mol at 30 days of the experiment, with values of 23.03 ± 3.98 mg/kg. The root and shoot of water hyacinths had the highest ability to absorb and accumulate copper in the experiments with pH 6 at 7 days of the experiment, with the values of 12,038.61 ± 994.67 mg/kg and 172.70 ± 62.15 mg/kg, respectively. Therefore, this study concluded that water hyacinth has a high ability to increase their arsenic and copper uptake and accumulation when sodium phytate is added, which affects the non-phytotoxicity of water hyacinths.

Keywords: Arsenic; Copper; Sodium phytate; Water hyacinth; Acid mine drainage

1. Introduction

Lands around the world have various resources in current conditions, especially widely distributed mineral resources, such as copper, gold, lead, silver, and zinc (Ushakova *et al.,* 2022). The mining process involves digging out these various minerals for use. However, mining often produces acidic waste in the form of slag, tailings, and acid mine drainage (AMD), among others. Notably, acidic effluents result from the oxidation of sulfur and sulfite compounds that react with water and air and become acidic sulfuric compounds. They can well dissolve heavy metals with various toxicities (Skousen *et al.,* 2017; Wang *et al.,* 2021), causing high concentrations of heavy metals in wastewater. Heavy metal contamination occurs in a mining area's soil and water, which primarily pollutes surface water and groundwater (Sun *et al.,* 2015).

Many types of heavy metal contaminants include arsenic, manganese, lead, cadmium, and copper. In particular, arsenic and copper are abundantly found in the water sources of potential gold deposit areas. They are heavy metal substances that often cause phytotoxicity to the quality of the environment. A very popular remedy for this problem is phytoremediation. In this process, living plants are treated, removed, and rehabilitated in the area of metallurgical sludge ponds and acidic mine reservoirs from mining processes contaminated with arsenic and copper (Roy *et al.,* 2021). Phytoremediation is expected to reduce the distribution and phytotoxicity of arsenic and copper, which may contaminate the environment and affect humans. It is also a simple, low-cost method with no complicated steps (Ekta *et al.,* 2018). However, each plant has a unique mechanism for eliminating toxins. For example, plants can store, accumulate, decompose, or convert toxins into volatile substances (Seth, 2012; Ali *et al.,* 2013). Plants have good absorption and tolerance capabilities for each type of heavy metal, which is important for heavy metal pollution remediation (Sampanpanish, 2015), and the study of relative growth rates (RGRs) can assess the potential of the applied plants (Fu *et al.*, 2012; Sampanpanish *et al.,* 2018).

This study presents the use of synthetic wastewater containing arsenic and copper with pH values of 4, 5, and 6, representing acidic mine water, and selects water hyacinth to be a medium for absorbing and accumulating heavy metals. Water hyacinth is a plant with abundant biomass and is fast-growing, durable, and easily nursed. It has a high potential for heavy metal accumulation (Mishra *et al.,* 2008; Tananonchai and Sampanpanish, 2014). Moreover, as the efficiency of heavy metal absorption and accumulation must be increased, a complex compound, sodium phytate ($C_6H_6Na_{12}O_{24}P_6$) (the salt of phytic acid), is used. Phytic acid's structure can help the molecular binding of two or more positively charged minerals, such as copper, zinc, and manganese (Bohn *et al.,* 2008). Thus, the addition of phytic acid can help bind bonds with the copper substance (Bloot *et al.,* 2021). Therefore, it is expected to result in more arsenic uptake in the studied plants. Copper is found to have high stability in forming complex compounds with phytic acid, especially at different pH values (Bretti *et al.,* 2012; Nissar *et al.,* 2017; Marolt *et al.,* 2020). and copper could bind to the ions in the three phosphate groups (ratio of 1:3 mol) or the six phosphate groups (ratio of 1:6 mol) (Turner *et al.,* 2002). Thus, it was applied to determine the molar ratio of sodium phytate with 1: 3 and 1: 6 in this study. Therefore, this research aims to study the effect of sodium phytate on the phytotoxicity and heavy metal absorption of water hyacinth growing in acidic synthetic wastewater from gold mining for applications in the effective, concrete, and sustainable heavy metal recovery or problem-solving in areas contaminated with acidic water from mining.

2. Materials and Methods

2.1 Experiment preparation

The researchers used 5-L plastic containers in the experiments, which were soaked in 10% nitric acid for one night and then washed again with distilled water. The experimental plants were *Eichhornia crassipes* (Mart.) Solms or water hyacinths from natural rivers and were cultivated in plant nurseries to ensure similarities in age, petiole size, root, and weight for the experiments.

A plant sampling method was used to analyze arsenic and copper accumulation. The acidic synthetic wastewater from mining in this experiment consisted of the arsenic compound disodium hydrogen arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$), the copper compound copper sulfate ($CuSO_4$), and the chelating agent sodium phytate ($C_6H_6Na_{12}O_{24}P_6$).

The studies were divided into four experiments: the experiment on phytotoxicity. Treatment 1) by varied sodium phytate (1, 5, 10, and 15 mg/L); 2) by varied pH 4-6 in the experiment of synthetic wastewater containing 0.25 mg/L of Arsenic(V) and 1 mg/L of copper without sodium phytate; 3) the experiment with arsenic, copper, varied pH 4-6, and sodium phytate: copper at the mole ratio of 1:3 and 4) the experiment similarly as treatment 3 but the mole ratio was increased to 1:6. One water hyacinth was cultivated in a container with 5-L water, and each experiment was replicated three times. Water and plant samples were collected at 7, 15, 22, and 30 days of the experiments (throughout the experiments, there were pH adjustments every 7 days). The chlorophyll content of the water hyacinth leaves was measured and recorded using a Chlorophyll Meter SPAD-502 Plus machine. The plant samples were then analyzed for their RGRs and the amount of arsenic and copper accumulation in different parts of the water hyacinth.

2.2 Sample and data analysis

1) Water samples from each container were collected in two 100-ml vials. There were no additives in the first vial for the analysis of basic properties: pH value (pH), electrical conductivity (EC), and oxidation–reduction potential (ORP). The second vial was added with two or three drops of 65% nitric acid and analyzed for arsenic (As) and copper (Cu) using the USEPA Method 3015A (USEPA., 1998) with microwave digestion and inductively coupled plasma–optical emission spectrometry.

2) Plant samples were collected by washing them with tap water three to four times first and then washing them with distilled water once and drying them at room temperature for 2 – 3 h. The plant samples were divided into shoots (stem and leaves) and the underwater part (roots). Each part was weighed, incubated at 105 °C for 24 – 48 h, and weighed dry separately. Afterward, the sample plants were ground to powder separately to analyze the arsenic and copper accumulation amount according to the USEPA Method 3052 (USEPA., 1996).

3) RGR data were collected by recording fresh and dry weights of water hyacinth in each experiment period to calculate the amount of biomass and the RGR as the following formula (Hunt, 2002):

$$RGR = [\ln(W2) - \ln(W1)] / (t2 - t1)$$

where RGR is in units of g/d, t1 is the pre-experimentation time (d), t2 is the post-experimentation time (d), W1 and W2 are the plant DW (g) at t1 and t2, respectively, and ln is the natural logarithm.

4) The data analysis included the calculation and analysis of the variance of the data and arsenic and copper accumulation from water hyacinth cultivation in acidic synthetic wastewater using one-way analysis of variance to analyze significant differences at a 95% confidence level and compare the differences of the data under Duncan's new multiple range test. The statistical data were analyzed using the Statistical Package for Social Sciences, a statistical package program.

3. Results and Discussion

3.1 Effect of sodium phytate on phytotoxicity and RGR of water hyacinth

The sodium phytate concentrations of 1, 5, 10, and 15 mg/L were added to study the phytotoxicity and growth of water hyacinth by measuring chlorophyll content at 7, 15, 22, and 30 days of the experiment. The chlorophyll contents tended to slowly decrease at all periodic experiments with higher concentrations of sodium phytate. The chlorophyll content was highly reduced in the experimental plants by the sodium phytate at a concentration of 15 mg/L at 30 days. This might have been caused by the resulting excessive phosphorus, a component of sodium phytate, resulting in phytotoxicity or poisoning symptoms, such as yellowish leaves, old leaf apexes, and yellowish or brownish leaf margins that could be seen clearly. This phytotoxicity was consistent with the measured amount of chlorophyll (there was an obvious reduction) (Kasemsap, 2006; Ruamrungsri, 2001), as shown in Figure 1.

The chlorophyll content could be shown to correlate with the phytotoxicity performances of the study plants. When the plants showed poisoning symptoms, chlorophyll was reduced in the plants (Pavlovic *et al.,* 2014). Chlorophyll can construct complex forms with some heavy metal ions, which causes the color change of plant leaves (Yilmaz and Gokmen, 2016). This reduces the amount of chlorophyll and causes the accumulation of heavy metals in plants (Li *et al.,* 2015). Plants can also exhibit yellowish or brownish leaf margins because of destructible chlorophyll. Therefore, the chlorophyll content in the experiment with pH 4 tended to decrease at 15 days; the chlorophyll content was 11.9 ± 10.23 in this study. The chlorophyll contents in the experiments with pH 4 and sodium phytate (sodium phytate to copper at the ratios of 1:3 and 1:6 mol) tended to decrease 22 days into the experiments; the chlorophyll contents of both sodium phytate ratios were 1.50 ± 7.92 and 17.40 ± 6.98, respectively. However, 30 days into the experiments, all experiments found that the chlorophyll content increased. The chlorophyll content in the experiments with pH 5 and 6, including the experiment with sodium phytate (sodium phytate to copper at the ratio of 1:3 mol), slightly increased throughout the experiments, as shown in Figure 2. This was consistent with the properties of water hyacinth, which could grow well at pH 4 – 10 (Permpul and Boonkaewwan, 2001). Water hyacinth is also a hyperaccumulator with a high ability of heavy metal accumulation, where the plant absorbs heavy metal substances through the root cells, whereas some heavy metals are entrapped in the cell wall or transported into the epidermis of the plant tissue and the cortex in the root. Then, the heavy metals transfer from the root to the shoot part, which is induced by the transpiration process. When the heavy metals move to the apical cells, the plants then attempt to reduce the phytotoxicity of the heavy metals, where they store the heavy metals in harmless positions for critical processes at the cellular level (Ariyakanon, 2015). In addition, this study also found that water hyacinths were adaptable to various conditions. It could recover, grow up, and put forth leaves normally when the experimental duration increased.

For the RGR results for the water hyacinths grown in acidic synthetic wastewater with the addition of arsenic, copper, and sodium phytate, the experiments with pH 4 and

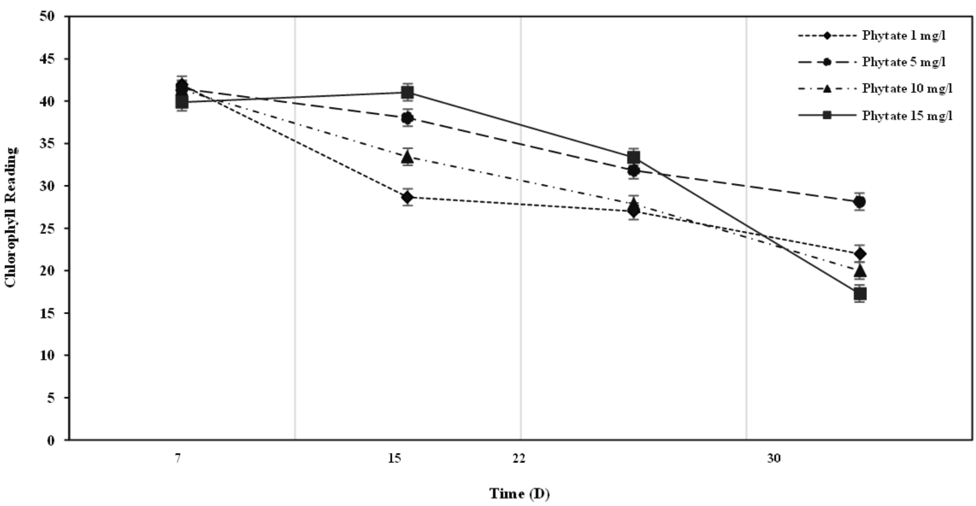


Figure 1. Chlorophyll content of water hyacinth in the experimental set containing sodium phytate at various concentration levels

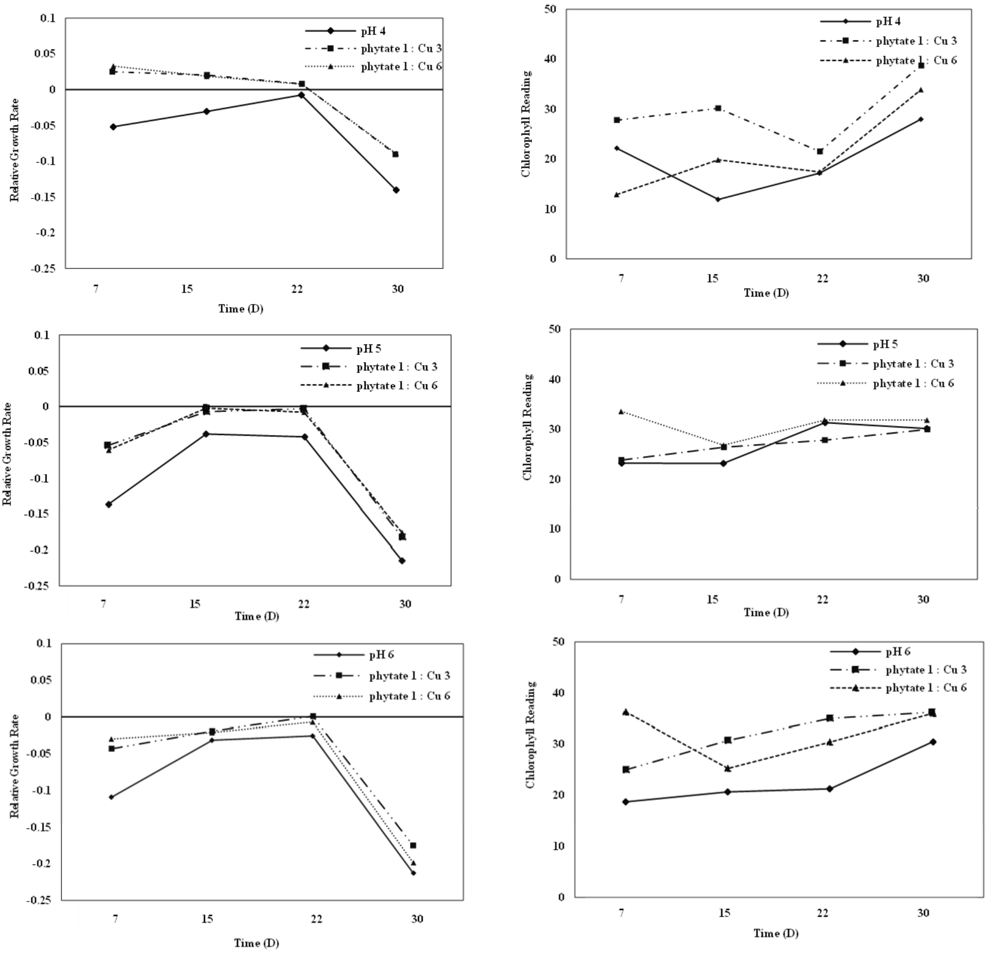


Figure 2. The relative growth rate (RGR) and chlorophyll content of water hyacinth samples at pH 4, 5, and 6 and with sodium phytate addition (sodium phytate to copper at the ratios of 1:3 and 1:6 mol)

those with both sodium phytate ratios of 1:3 and 1:6 mol showed a high RGR 7 days into the experiments. The water hyacinths showed an increase in the RGR 15 and 22 days into the experiments with pH 5 and 6, including the experiments with both sodium phytate ratios, as shown in Figure 2. Water hyacinth accumulated arsenic and copper in both the upper-water and underwater parts combined in high volume; however, the value decreased when the experimental duration increased. The experiment at 30 days had the highest decrease of all experiments. This was consistent with the study of Khang *et al.* (2012), who investigated the effects of arsenic on experimental plants and found that arsenic decreased the plant biomass, RGR, and accumulation of arsenic in plants. Therefore, when plants were exposed to arsenic, a nonessential nutrient to plant growth, the plant structure could not function under the metabolic process. In addition, high levels of arsenic in water with acidic pH could also damage cells and chloroplast structure in plant tissues, which reduces the rate of photosynthesis and biomass such as roots, stem height, branching, and yield, resulting in ultimate plant death. Thus, the results of this study were consistent with the report of Kongmuang and Sampanpanish (2010) that the accumulation of heavy metals increased when the concentration of heavy metals increased and that the growth rate of plants decreased with increasing concentrations of heavy metals. This study can also conclude that all experiments with sodium phytate had higher RGRs than those experiments without sodium phytate. The statistical analysis and comparison found that all experiments with synthetic wastewater at pH 4, 5, and 6 and those experiments with added sodium phytate had statistically significant differences ($P < 0.05$).

The analysis of the RGR and phytotoxicity of water hyacinth demonstrated that sodium phytate addition increased the RGR of water hyacinth to values higher than those in the experiments without sodium phytate. The sodium phytate addition could reduce the phytotoxicity from arsenic and copper by considering a few changes in the amount of chlorophyll. However, the experiments with the added sodium phytate had less accumulations of arsenic and copper. When the experimental duration increased, water hyacinth could also increase the accumulation of arsenic and copper in synthetic wastewater with acidic pH; moreover, water hyacinth could grow and put forth leaves better than the plants in synthetic wastewater without sodium phytate.

3.2 Effect of sodium phytate on the chemical properties of synthetic wastewater

Water hyacinths were grown in synthetic wastewater with arsenic and copper at pH 4 – 6 and with sodium phytate addition (sodium phytate to copper at the ratios of 1:3 and 1:6 mol). There were rectords of pH value, EC, and the ORP of the synthetic wastewater samples from all experiments. The EC of the experiments with pH 4 and both sodium phytate ratios were higher after the samples were collected at 7, 15, 22, and 30 days (Figure 3). However, a weekly pH adjustment increased the charges in the water (Kongmuang, 2010) when the duration of the experiment was increased, causing the dead leaves of the water hyacinths to fall into the water, which produced the decomposition of plants in synthetic wastewater. Moreover, the addition of organic matter charges more than the plants' rate of charge absorption, thus causing the amounts of electrolytes to increase (Pramoon, 2005). The ORP differences increased in all experiments that presented tendencies for acquiring electrons in acidic water, which could increasingly dissolve arsenic and copper in solution form; consequently, the potential differences in water were higher (Tuntoolavest Munsin, 2002).

3.3 Effect of sodium phytate on the arsenic absorption and accumulation of the water hyacinths

This section presents the effect of sodium phytate on the heavy metal absorption and accumulation of the water hyacinths grown in acidic synthetic wastewater containing arsenic of 0.25 mg/L. The plants could absorb and accumulate arsenic in the underwater part (roots) in higher quantities than in the shoots (stem and leaves) in all experiments, as shown in Figures 4(a), 4(c), and 4(e). This was consistent with the study of Mishra *et al.* (2008), which investigated the removal of mercury and arsenic in coal mine water using three plants: *E. crassipes, Lemna minor*, and *Spirodela polyrrhiza.* It found that *E. crassipes* had the best arsenic uptake and accumulation potential in all experimental plants, the roots accumulated arsenic better than did the leaves. This was also consistent with the study of Rahman and Hasegawa (2011), which studied the potential of floating plants for heavy metal treatment, where water hyacinth was a high potential plant. It could accumulate more arsenic in the roots than that in the leaves. Therefore, this study found that the experiment with pH 4 had the highest accumulation of arsenic in the underwater part (roots) of water hyacinth and could highly absorb and accumulate arsenic at 22 days of the experiment, with values of 600.12 ± 120.47 mg/kg, which was higher than those in the other experiments. The experiments with pH 5 and 6 found that water hyacinth absorbed and accumulated the highest amounts of arsenic in the roots 7 days into the experiments at 378.02 ± 208.57 and 285.97 ± 82.76 mg/kg, respectively. The experiment with pH 4 with sodium phytate at the ratio of 1:6 mol found that water hyacinth absorbed and accumulated the highest amounts of arsenic in the roots 15 days into the experiment at 266.48 ± 103.31 mg/kg.

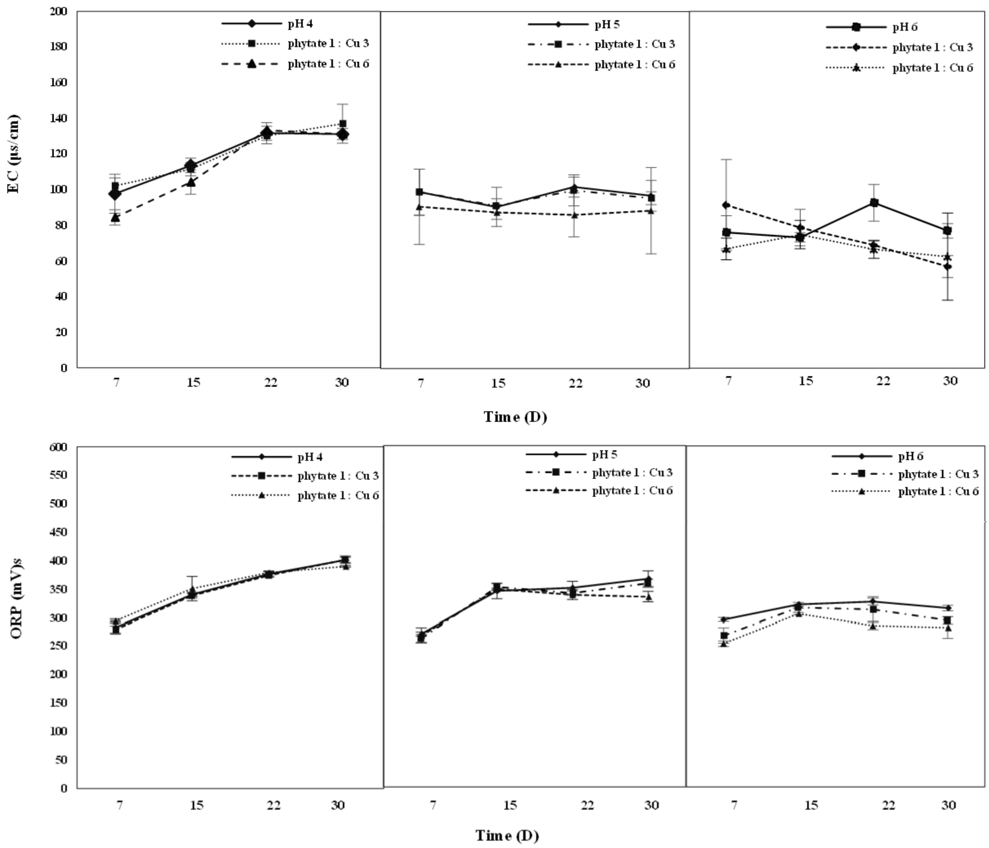
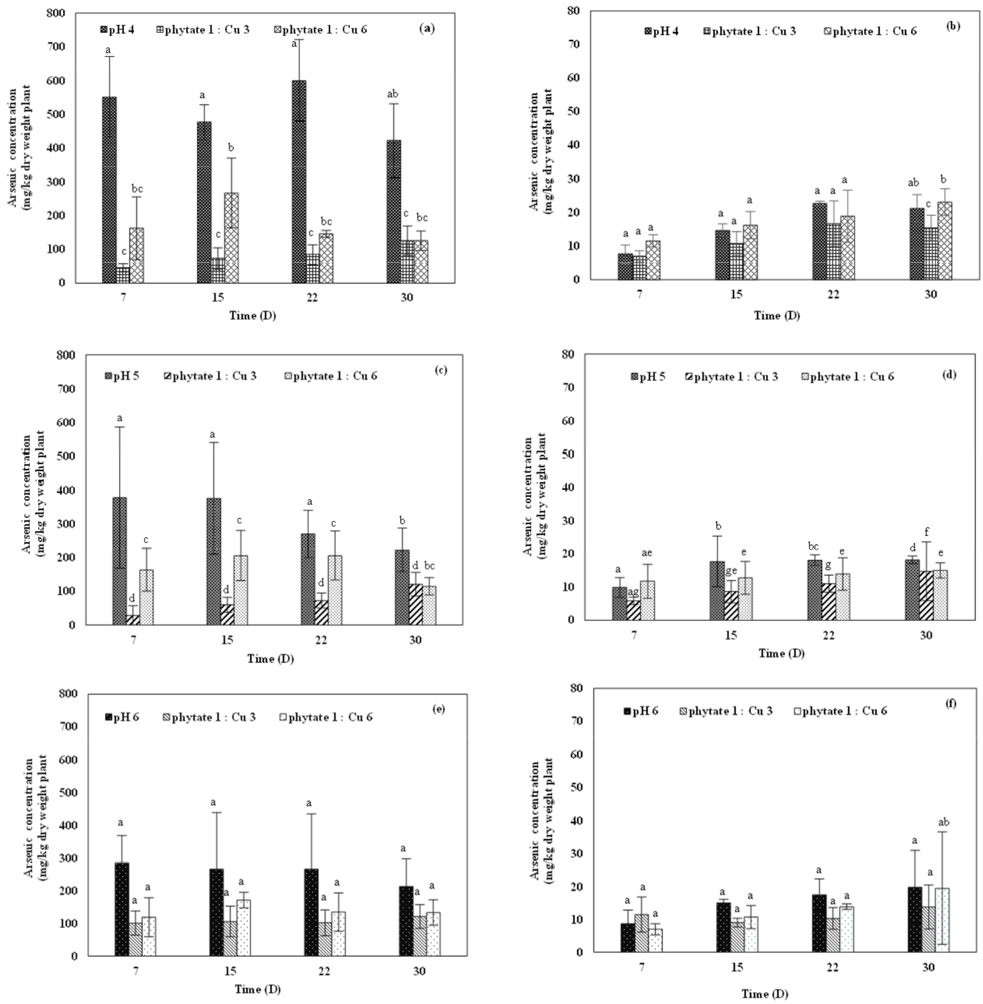


Figure 3. Electrical conductivity (EC) and oxidation–reduction potential (ORP) of the synthetic wastewater samples at pH 4, 5, and 6 and with sodium phytate addition (sodium phytate to copper at the ratios of 1:3 and 1:6 mol)

The experiment with pH 4 and sodium phytate at the ratio of 1:3 mol found that water hyacinth absorbed and accumulated the highest amounts of arsenic in the roots 30 days into the experiment at 124.33 ± 28.90 mg/kg. The comparison of the statistical values found that the experiment at pH 4, 5, and 6 with both sodium phytate ratios (1:3 and 1:6) had statistically significant differences ($P < 0.05$).

Furthermore, the experiment with pH 4 and sodium phytate at the ratio of 1:6 mol found that water hyacinth absorbed and accumulated the highest arsenic in the shoots (stem and leaves) 30 days into the experiment, having values of 23.03 ± 3.98 mg/kg. The experiments with pH 4 and the experiment with pH 4 and sodium phytate at the ratio of 1:3 mol found that water hyacinth absorbed and accumulated the highest amounts of arsenic in the stems and leaves 30 days into the experiment at 22.64 ± 0.62 and 16.53 ± 6.82 mg/kg, respectively, as shown in Figures 4(b), 4(d), and 4(f). However, the statistical values analysis and comparison found that the experiments with pH 4 and both sodium phytate ratios (1:3 and 1:6) had statistically significant differences ($P < 0.05$).

The result of this research showed that the sodium phytate structure consisted of six groups of phosphoric acid (H_3PO_4), which were phosphate groups $[PO_4]^{3-}$ with negative charges; therefore, they could well bind to heavy metal ions with two or more positive charges (Nissar *et al.,* 2017; Marolt *et al.,* 2020). Therefore, this study found that copper in synthetic wastewater could bind to the ions in the six phosphate groups (ratio of 1:6 mol) (Turner *et al.,* 2002) and form many bonds between the phosphate groups and copper. As a result, plants could increasingly absorb arsenic in the plant's roots, stems, and leaves.



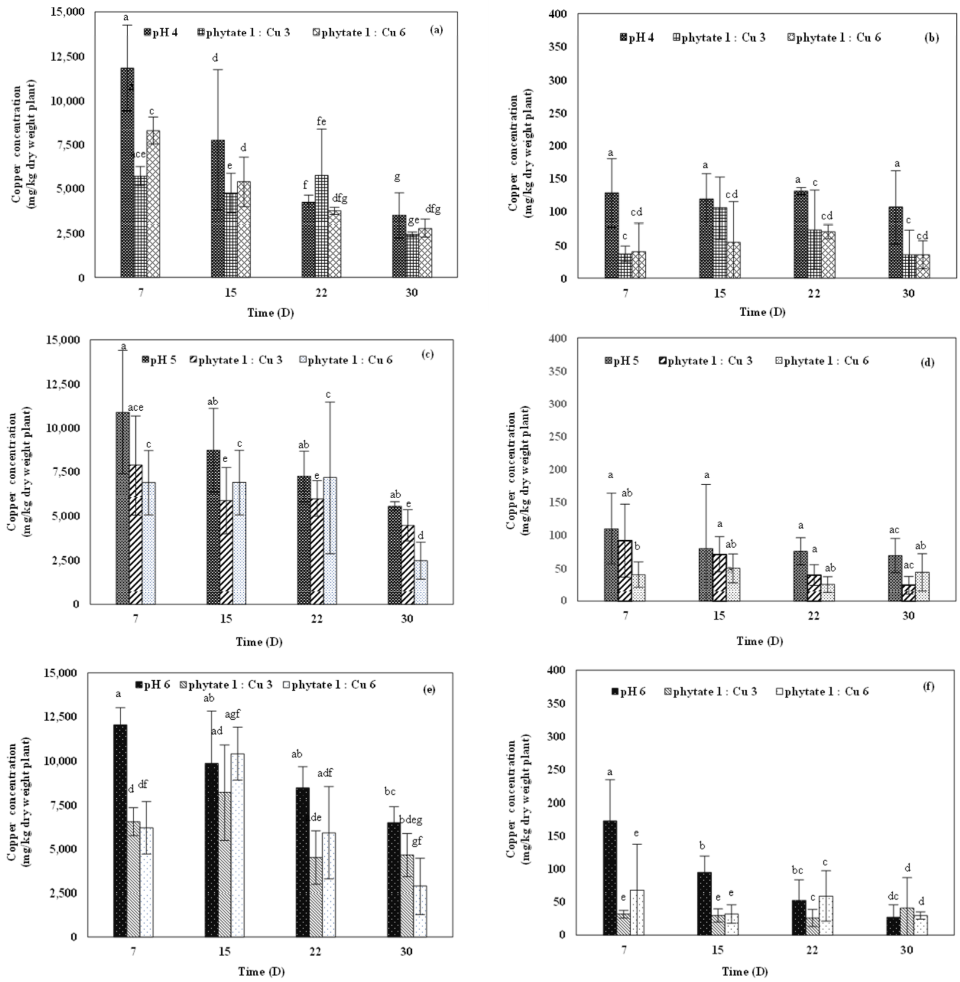
(a,b) pH 4, pH 4 + sodium phytate:Cu (1:3 and 1:6) in (a) roots and (b) shoots (stem and leaves),
(c,d) pH 5, pH 5 + sodium phytate:Cu (1:3 and 1:6) in (c) roots and (d) shoots (stem and leaves),
(e,f) pH 6, pH 6 + sodium phytate:Cu (1:3 and 1:6) in (e) roots and (f) shoots (stem and leaves)

Figure 4. Arsenic accumulation in the roots and shoots (stem and leaves) of water hyacinths from sodium phytate addition at various pH levels

3.4 Effect of sodium phytate on the copper absorption and accumulation of the water hyacinths

The addition of sodium phytate affected the absorption and accumulation of copper in the water hyacinths. The plants could absorb and accumulate copper in the underwater part (roots) in higher amounts than in the shoots (stem and leaves) in all experiments. The experiment with pH 6 had the highest accumulation of copper in the underwater part (roots), as shown in Figures 5(a), 5(c), and 5(e); the plants absorbed and accumulated the highest amounts of copper 7 days into the experiment at 12,038.61 ± 994.67 mg/kg, which was higher than those in the other pH experiments. The experiments with pH 4 and 5 found that the water hyacinths absorbed and accumulated the highest amounts of copper 7 days into the experiments at 11,827.90 ± 2,415.59 and 10,889.80 ± 3,506.11 mg/kg, respectively. The experiment with pH 6 and sodium phytate at the ratios of 1:3 and 1:6 mol found that the water hyacinths absorbed and accumulated the highest amounts of copper in the roots 15 days into the experiments at 10,415.39 ± 1,501.09 and 8,210.49 ± 2,704.86 mg/kg, respectively. The comparison of statistical values found that all experiments with pH 4, 5, and 6 had statistically significant differences ($P < 0.05$). This result was consistent with the study of Liao and Chang (2004), which found that water hyacinth could accumulate more copper in the roots than in the leaves, whereas the study of Rahman and Hasegawa (2011) reported that floating plants could also accumulate more heavy metals in their roots than in their leaves.

Furthermore, the experiment with pH 6 and the experiment with pH 6 and sodium phytate at the ratio of 1:6 mol found that the water hyacinths absorbed and accumulated the highest amounts of copper in the shoots (stem and leaves) 7 days into the experiments at 172.70 ± 62.15 and 67.41 ± 69.95 mg/kg, respectively. The experiment with pH 4 and sodium phytate at the ratio of 1:3 mol found that the water hyacinths absorbed and accumulated the highest amounts of copper in the stems and leaves 15 days into the experiments at 106.10 ± 46.85 mg/kg, as shown in Figures 5(b), 5(e), and 5(f). The comparison of the statistical values found that all experiments had statistically significant differences ($P < 0.05$), except for the experiments at pH 4 and 6 with both sodium phytate ratios, the experiment at pH 5, and the experiment at pH 5 with sodium phytate at the ratio of 1:6 mol.



(a,b) pH 4, pH 4 + sodium phytate:Cu (1:3 and 1:6) in (a) roots and (b) shoots (stem and leaves),
(c,d) pH 5, pH 5 + sodium phytate:Cu (1:3 and 1:6) in (c) roots and (d) shoots (stem and leaves),
(e,f) pH 6, pH 6 + sodium phytate:Cu (1:3 and 1:6) in (e) roots and (f) shoots (stem and leaves)

Figure 5. Copper accumulation in the roots and shoots (stem and leaves) of water hyacinths from sodium phytate addition at various pH levels

4. Conclusion

This study concludes that the addition of sodium phytate affects the uptake and accumulation of arsenic and copper in water hyacinths. Arsenic speciation at pH 4 - 6 was found in the form of $H_2ASO_4^-$, or $HASO_4^{2-}$or As (V), and copper was in the Cu^{2+} form. The study found that water hyacinth roots and shoots had the highest ability to absorb and accumulate arsenic in the experiment at pH 4 and the experiment at pH 4 with sodium phytate. The experiment at pH 6 without sodium phytate and the experiment at pH 6 with sodium phytate at the ratio of 1:6 mol found that the water hyacinths had the highest ability to absorb and accumulate copper in roots and shoots (stem and leaves). The RGR of the water hyacinths was highly reduced. It tended to decrease continuously because the water hyacinths were exposed to high levels of arsenic and copper and had an acidic pH, thus affecting plant growth. However, the water hyacinths could adapt to various conditions when the experimental duration increased. It could further grow up and put forth stems and leaves. Moreover, the water hyacinths grown in the experiments with sodium phytate addition could lower the accumulation of arsenic and copper and have a high RGR. The sodium phytate reduced the arsenic and copper phytotoxicity better than did the experiments without sodium phytate addition in all pH values. Nevertheless, the plants in the experiment with sodium phytate at the ratio of 1:3 mol (sodium phytate to copper) showed the lowest phytotoxicity in all pH experiments. Therefore, the results of this study can be used as a guideline for studying the effectiveness of sodium phytate substances in treating or restoring arsenic- and copper-contaminated areas and AMD. In addition, the study's results can also be applied to the study of other plants' phytotoxicities.

Acknowledgment

The authors thank the Interdisciplinary Program in Environmental Science, Graduate School, Chulalongkorn University, the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), Chulalongkorn University for the Research Unit of Green Mining Management. We would like to express our sincere thanks to the Environmental Research Institute (ERIC) for their support in terms of science equipment and facilities.

References

Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals-concepts and applications. Chemosphere 2013; 91: 869–881. Doi: https://doi.org/10.1016/j.chemosphere.2013.01.075

Ariyakanon N. Phytoremediation. 1st ed. Chulalongkorn University, Bangkok, Thailand. 2015.

Bretti C, Cigala RM, Stefano CD, Lando G, Sammartano S. Interaction of Phytate with Ag^+, CH_3Hg^+, Mn^{2+}, Fe^{2+}, Co^{2+}, and VO^{2+}: Stability Constants andSequestering Ability. Journal of Chemical and Engineering Data 2012; 57(10): 2838–2847. Doi: https://doi.org/10.1021/je300755y

Bloot APM, Kalschne DL, Amaral JAS, Baraldi IJ, Canan C. A Review of Phytic Acid Sources, Obtention, and Applications. Food Reviews International 2021; 1–20. DOI: https://doi.org/10.1080/87559129.2021.1906697

Bohn L, Meyer AS, Rasmussen SK. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. Journal of Zhejiang University Science B 2008; 9(3): 165-191. Doi: https://doi.org/10.1631/jzus.B0710640

Ekta P, Modi NR. A review of phytoremediation. Journal of Pharmacognosy and Phytochemistry 2018; 7(4): 1485-1489. Available from: https://www.phytojournal.com/archives/2018.v7.i4.5139/a-review-of-phytoremediation

Fu H, Yuan G, Cao T, Ni L, Li W, Zhu G. Relationships between relative growth rate and its components across 11 submersed macrophytes. Journal of Freshwater Ecology 2012; 27(4): 471–480. Doi: https://doi.org/10.1080/02705060.2012.684102

Hunt R, Causton DR, Shipley B, Askew AP. A Modern Tool for Classical Plant Growth Analysis. Annals of Botany 2002; 90: 485-488. Doi: https://doi.org/10.1093/aob/mcf214

Kasemsap P. Biology 2 Science and Mathematics Textbook Project, The Promotion of Academic Olympiad, and Development of Science Education Foundation under the patronage of Her Royal Highness Princess Galyani Vadhana Krom Luang Naradhiwas Rajanagarindra. Darnsuthapress Company Limited. Bangkok. 2006.

Khang HV, Hatayama M, Inoue C. Arsenic accumulation by aquatic macrophyte coontail (*Ceratophyllum demersum L.*) exposed to arsenite, and the effect of iron on the uptake of arsenite and arsenate. Environmental and Experimental Botany 2012; 83: 47–52. Doi: https://doi.org/10.1016/j.envexpbot.2012.04.008

Kongmuang K. Effect of EDTA and Citric Acid on Cadmium uptake by Water Hyacinth. Master's Thesis. Environmental Science. Graduate School, Chulalongkorn University. 2010.

Kongmuang K, Sampanpanish P. Effect of EDTA and citric acid on cadmium uptake by water hyacinth. In proceedings of the Mae Fah Luang Symposium 2010, On the Occasion of the 12th Anniversary Mae Fah Luang University Following the Legacy of H.R.H. The Princess Mother. (p 1) November 19-20,2010, Mae Fah Luang Chiang Rai University, Chiang Rai, Thailand. 2010.

Li X, Liu X, Liu M, Wang C, Xia X. A hyperspectral index sensitive to subtle changes in the canopy chlorophyll content under arsenic stress. International Journal of Applied Earth Observation and Geoinformation 2015; 36: 41-53. Doi: https://doi.org/10.1016/j.jag.2014.10.017

Liao SW, Chang WL. Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. Journal of Aquatic Plant Management 2004; 42: 60-68.

Marolt G, Gricar E, Pihlar, B., and Kolar, M. Complex Formation of Phytic Acid with Selected Monovalent and Divalent Metals. Frontiers in Chemistry 2020; 8: 582746. Doi: https://doi.org/10.3389/fchem.2020.582746

Mishra VK, Upadhyay AR, Pathak V, Tripathi BD. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes. Water, Air, and Soil Pollution 2008; 192: 303-314. Doi: https://doi.org/10.1007/s11270-008-9657-4

Nissar J, Ahad T, Naik H, Hussain S. A review phytic acid: as antinutrient or nutraceutical. Journal of Pharmacognosy and Phytochemistry 2017; 6: 1554–1560. Available from: https://www.phytojournal.com/archives/2017/vol6issue6/PartV/6-6-208-319.pdf

Pavlovic D, Nikolic B, Djurovic S, Waisi H, Andjelkovic A, Marisavljevic D. Chlorophyll as a measure of plant health: Agroecological aspects. Pestic. Phytomed. (Belgrade) 2014; 29(1): 21-34. Doi: https://doi.org/10.2298/PIF1401021P

Permpul K, Boonkaewwan S. Water Hyacinth Management Guide. Regional Environment office 5. Nakhon Pathom Province. 2001.

Pramoon W. Cadmium accumulation in water spinach grown in cadmium contaminated water. Master's thesis, Environmental Management, Graduate school, Chulalongkorn university. 2005.

Rahman MA, Hasegawa H. Aquatic arsenic: phytoremediation using floating macrophytes. Chemosphere 2011; 83(5): 633-646. Doi: https://doi.org/10.1016/j.chemosphere.2011.02.045

Roy D, Sreekanth D, Pawar D, Mahawar H, Barman KK. Phytoremediation of arsenic contaminated water using aquatic, semi-aquatic and submerged weeds. Biodegradation. IntechOpen; 2021. Doi: https://doi.org/10.5772/intechopen.98961

Ruamrungsri S. Soilless culture for horticultural crops. Odeon Store. Bangkok. 2001.

Sampanpanish P. Phytoremediation. 1sted. Thailand: Chulalongkorn University, Bangkok, Thailand; 2015: 232.

Sampanpanish P, Suwattiga P, Tippayasak K. Phytoremediation of Arsenic and Manganese from Tailing Storage Facility at Gold Mining. KKU Science Journal 2018; 46(1): 58-67.

Seth CS. A review on mechanisms of plant tolerance and role of transgenic plants in environmental clean-up. The Botanical Review 2012; 78: 32–62. Doi: https://doi.org/10.1007/s12229-011-9092-x

Skousen J, Zipper CE, Rose A, Ziemkiewicz PF, Nairn R, McDonald LM, Kleinmann RL. Review of Passive Systems for Acid Mine Drainage Treatment. Mine Water and the Environment 2017; 36: 133–153. Doi: https://doi.org/10.1007/s10230-016-0417-1

Sun W, Xiao T, Sun M, Dong Y, Ning Z, Xiao E, Tang S, Li J. Diversity of the sediment microbial community in the Aha Watershed (Southwest China) in response to acid mine drainage pollution gradients. Applied and Environmental Microbiology 2015; 81(5): 4874–4884. Doi: https://doi.org/10.1128/AEM.00935-15

Tananonchai A, Sampanpanish P. Effect of EDTA and DTPA on Cadmium Removal from Contaminated Soil with Water Hyacinth. Applied Environmental Research 2014; 36(3): 65-76. Doi: https://doi.org/10.35762/AER.2014.36.3.6

Tuntoolavest MS, Tuntoolavest MR. Chemical in Water and Wastewater. Bangkok. Chulalongkorn University Printing House. 2002.

Turner BL, Paphazy MJ, Haygarth PM, McKelvie I D. Inositol phosphates in the environment. Philosophical Transactions of the Royal Society B: Biological Sciences 2002; 357(1420): 449 - 469. Doi: https://doi.org/10.1098/rstb.2001.0837

US EPA. Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. Method. 3052. Washington D.C., USA. 1996.

US EPA. Microwave Assisted Acid Digestion of Aqueons Samples and Extract.Method. 3015A. Washington D.C., USA. 1998.

Ushakova E, Menshikova E, Blinov S, Osovetsky B, Belkin P. Environmental Assessment Impact of Acid Mine Drainage from Kizel Coal Basin on the Kosva Bay of the Kama Reservoir (Perm Krai, Russia). Water 2022; 14(5): 727. Doi: https://doi.org/10.3390/w14050727

Wang Z, Xu Y, Zhang Z, Zhang Y. Review: Acid Mine Drainage (AMD) in Abandoned Coal Mines of Shanxi, China. Water 2021; 13: 8. Doi: https://doi.org/10.3390/w13010008

Yilmaz C, Gokmen, V. Chlorophyll. Encyclopedia of Food and Health 2016; 37-41. Doi: https://doi.org/10.1016/B978-0-12-384947-2.00147-1