Journal of Hazardous Materials 419 (2021) 126410

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The effect of chelating agents on iron plaques and arsenic accumulation in duckweed (*Lemna minor*)

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ARTICLE INFO

Editor: Dr. C. LingXin

Keywords:
Arsenite
Arsenate
Aquatic plant
Fe plaques
Chelation

ABSTRACT

Iron plaques have been found to limit the phytoremediation efficiency by reducing iron solubility, while chelating agents can increase the bioavailability of iron from Fe plaques to numerous terrestrial plants. However, the effects of chelating agents on Fe plaques along the As accumulation in aquatic plants remain unknown. In this study, the effects of five chelating agents (EDTA, DTPA, NTA, GLDA, and CA) on the As (As(III) or As(V)), phosphate, and iron uptake by iron plaques and duckweed (*Lemna minor*) were examined. The results showed that the chelating agents increased the As accumulation in *L. minor* plants by desorbing and mobilizing As from Fe plaques. The desorption rates of As(V) (As(III)) from the Fe plaques by the chelating agents were 5.26–8.77% (8.70–15.02%), and the plants/DCB extract ratios of As(V) (As(III)) increased from 2.63 ± 0.13 (1.97 ± 0.06) to the peak value of 3.38 ± 0.21 (2.70 ± 0.14) upon adding chelating agents. Besides, the addition of chelating agents increased the uptake of P and Fe by *L. minor* plants. This work provides a theoretical basis for the remediation of As-contaminated waters by duckweed with the help of chelating agents.

1. Introduction

The presence of arsenic (As) in water is a threat to the ecosystem and human health, particularly when a dramatic increase in the population exposed to arsenic has been reported in recent studies (Shokoohi et al., 2021; Sovann and Polya, 2014). The activities, such as pesticide application (Rahman et al., 2019), fossil fuel combustion (Wang et al., 2021), and industrial utilization (Chen et al., 2015), are continuously contributing to the exacerbation of this situation. The maximum concentration of As allowed in drinking water recommended by the World Health Organization (WHO) is 10 μg/L (Gordon et al., 2011). However, As levels in the natural waters range from ≤ 1 μg/L to ≥ 5000 μg/L (Herath et al., 2016), which often remarkably exceed the threshold value (10 ug/L) proposed by the WHO, resulting in As poisoning. The symptoms of As poisoning may be acute or chronic, with long-term impact on human health, such as skin lesions (Wei et al., 2017), neurological complications (Mochizuki, 2019), and cancers (Singh et al., 2007). Therefore, restoration measures must be undertaken to reduce the As concentration in contaminated waters.

Various physico-chemical methods have been employed to remove As from water, including activated carbon adsorption, activated aluminum adsorption, chemical precipitation, and ion exchange process (Sodhi et al., 2019). However, most of them require huge capital and also produce secondary pollution (Ali et al., 2013). The chemical precipitation method, for instance, is more expensive and complicated for eradicating heavy metals due to the generation of a large amount of polluted slurry (Wang, 2015). Compared to the methods mentioned above, phytoremediation uses hyper-accumulative plants to remove the toxic substances in water, which is cost-effective, eco-friendly, *in situ* applicable, and does not require external energy (Mahajan and Kaushal, 2018). The accumulation of nutrients as well as the contaminants by the plants is a natural process, which ensures a cheap and green system for environmental clean-up, ease of operation, and can be applied to large areas (Peng et al., 2018a, 2018b). Duckweed is the smallest aquatic flowering plant in the world, which grows in multiple water ecosystems, such as streams, lakes, and ponds (Lam et al., 2014). Its yearly production has been documented to be 55 t/h dry biomass under suitable environmental conditions (Oron, 1994). In addition, duckweed survives over a wide range of pH (3.5–10.5) and temperature (7–35 °C) (Yang et al., 2021). Due to its extensive distribution, rapid growth, high multiplicity, and short lifespan, duckweed has been identified as a suitable biomaterial for the investigation of As metabolism and

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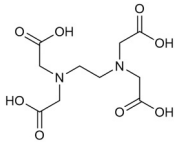
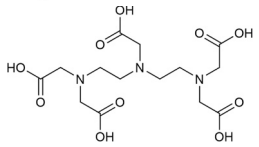
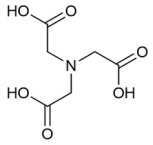
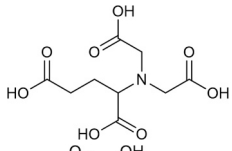
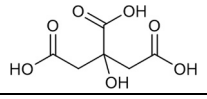
https://doi.org/10.1016/j.jhazmat.2021.126410
Received 9 February 2021; Received in revised form 11 June 2021; Accepted 12 June 2021
Available online 16 June 2021

Table 1
Basic properties of the biodegradable chelating agents.

Chelating agent	Molecular formula	Molecular weight	Water solubility	Toxicology (LD50)	Biodegradability	Eco-friendly	Safe to human health	Structural formula
EDTA	$C_{10}H_{16}N_2O_8$	292.24	0.5 g/L	2580 mg/kg (OIR)	×	×	×	
DTPA	$C_{14}H_{23}N_3O_{10}$	393.35	5 g/L	> 2000 mg/kg (OIR)	×	×	×	
NTA	$C_6H_9NO_6$	191.14	1.28 g/L	1470 mg/kg (OIr)	✓	×	×	
GLDA	$C_9H_9NNa_4O_8$	351.13	400 g/L	—	✓	✓	✓	
CA	$C_6H_8O_7$	192.13	750 g/L	6730 mg/kg (OIr)	✓	✓	✓	

EDTA, ethylene diamine tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; NTA, nitrilotriacetic acid; GLDA, N, N-bis(carboxymethyl)glutamic acid; CA, citric acid; OIR, orally in rabbit; OIr, orally in rat (Khalid et al., 2016; Mai et al., 2019; Nörtemann, 2005; OECD, 1992; Pinto et al., 2014; Sarwar et al., 2017).

phytoremediation (Ekperusi et al., 2019; Goswami et al., 2014).

Metal cycling and attenuation processes occurring in the environment involve complex mechanisms (Kumarathilaka et al., 2018). Generally, plants accumulate high levels of metals in roots (Xin et al., 2019). Iron plaques (Fe plaques) are found in plant rhizospheres and roots due to the deposition of iron hydroxide (Hossain et al., 2009; Cao et al., 2018). Iron plaques can be used as a buffer to reduce the solubility of As in water due to their strong adsorption of the metal ions (Peng et al., 2018a, 2018b; Liu et al., 2004). Moreover, Fe plaques within the rhizosphere also lead to Fe deficiency in plants, which limits their phytoremediation efficiency (Rahman et al., 2019). Therefore, mitigating the effects of Fe plaques is critical to the application of phytoremediation techniques.

Chelating agents can increase iron bioavailability by dissolving the deposited Fe. Aminopolycarboxylates, like ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA), are widely used in industrial, agricultural, and domestic applications (Khalid et al., 2016; Sarwar et al., 2017). However, the biodegradation of EDTA and DTPA is highly limited, and these can only be biodegraded by certain bacterial strains (Nörtemann, 2005), leading to their accumulation in aquatic systems. Thus, EDTA and DTPA do not meet the criteria set by the Organization for Economic Cooperation and Development (OECD, 1992) for biodegradability and persistence in the environment. Further, their bioaccumulation with toxic heavy metals poses a higher risk to the ecosystems and public health (Pinto et al., 2014). Therefore, researchers are now exploring chelating agents with lower toxicity or higher degradability, such as nitrilotriacetic acid (NTA), N, N-bis (carboxymethyl) glutamic acid (GLDA), and citric acid (CA) (Mai et al., 2019; Nörtemann, 2005).

In this context, this study investigated the influence of five common chelating agents (ETAD, DTPA, NTA, GLDA, and CA) on As deposition in duckweed, as well as the impacts of Fe plaques on the duckweed roots along with lower surfaces. Arsenic in the aquatic systems is present mainly in the form of inorganic trivalent arsenite species (As(III)) and pentavalent arsenate oxyanions (As(V)). Therefore, this work was aimed to investigate the elimination of As(III) and As(V). The novelty of this study is that we evidence how the restriction effect of Fe plaques on hyperaccumulation could be mitigated by suitable chelating agents. This study will help in understanding the hyperaccumulation mechanism of As in aquatic plants and improving the phytoremediation technologies.

2. Materials and methods

2.1. Materials and culture conditions

Lemna minor was originally collected from Dazu, Chongqing City, China (29°30′44″N, 105°45′55″E). The atpF-atpH intergenic sequence was adopted to identify the clones (Borisjuk et al., 2015). The clones were cultured in the Murashige and Skoog (MS) solid medium (Murashige and Skoog, 1962) containing 0.7% (wt/vol) agar and 1.5% (wt/vol) sucrose (pH 5.8) under 25 ± 1 °C, 16 h day/8 h night cycles, and light intensity of 40 μmol/m^2/s. The clones preserved on the MS medium were transferred to the complete strength Hoagland medium (Hoagland and Arnon, 1950) containing 1.5% sucrose to resuscitate. Then, the healthy clones were transferred to the 1/5 strength Hoagland medium for adaptive culture to accumulate enough biomass for subsequent experiments.

2.2. Induction of iron plaques

The method proposed by Liu and colleagues was used with minor modification to induce the formation of Fe plaques in *L. minor* roots as well as lower surfaces (Liu et al., 2005). Two grams (fresh weight, FW) of healthy and intact *L. minor* plants were collected from the Hoagland liquid medium and rinsed thrice with deionized water. The *L. minor* plants were then grown for 12 h in the deionized water (1 L) to prevent other elements from interfering with Fe. Later, two grams (FW) of *L. minor* plants were transferred to the MS solution (1 L) containing 0.36 mM Fe in the form of $FeSO_4{\cdot}7H_2O$. Meanwhile, 0.1 M HCl or KOH was used to set the solution pH to 5.8. After 24 h of growth, the Fe plaques,

visible as a reddish coating (Hossain et al., 2009), were formed onto *L. minor* plants. The weight of duckweed was determined using an electronic balance (GL124-1SCN, Sartorius, Gottingen, Germany) (Bergmann et al., 2000).

2.3. Arsenic treatment

After the formation of Fe plaques, all *L. minor* plants were washed thrice with deionized water and further grown for 3 days in 1/3 strength MS solution (500 mL) prior to As treatments (Liu et al., 2005). After 3 days, one gram (FW) of *L. minor* plants was transplanted into the 1/3 strength MS solution (500 mL) containing 6.0 μM As(III) or As(V), respectively. In this experiment, $NaAsO_2$ and $Na_2HAsO_4{\cdot}7H_2O$ were used to prepare the As(III) and As(V) solutions, respectively. The solutions were replaced every 2 days.

2.4. Application of chelating agents

After growing the *L. minor* plants for a week in the As-exposed solutions, As was adsorbed onto the Fe plaques or co-deposited with Fe onto the *L. minor* fronds and roots. Thereafter, every 0.2 g (FW) of *L. minor* plants in As(III) treatment or As(V) treatment were transferred into different 1/3 strength MS solutions (250 mL) containing 50 μM of DTPA, EDTA, GLDA, NTA, CA, or agent-free (control 1). Another 0.2 g (FW) of duckweed without arsenic treatment and agent-free were used as control (control 0). In this experiment, three replicates were set for every treatment. Table 1 displays the information of the chelating agents used in this work. The structures of the compounds were drawn in ChemDraw (v19.0.0.22).

2.5. Extraction of iron plaques

The Fe plaques formed in the roots and lower surfaces of *L. minor* plants were isolated using the dithionite–citrate–bicarbonate (DCB) extraction approach (Taylor and Crowder, 1983). All *L. minor* plants were washed thrice with deionized water, followed by the addition of DCB extract. All *L. minor* plants were subjected to 60 min of incubation with a mixture (40 mL) of 0.125 M $NaHCO_3$ and 0.03 M $Na_3C_6H_5O_7{\cdot}2H_2O$ at room temperature (20–25 ℃), and then one gram of $Na_2S_2O_4$ was added to the mixture. Thereafter, the resultant solution was diluted with deionized water to 50 mL. After DCB extraction, *L. minor* plants were dried in the oven for 24 h (ZRD-A7230, ZHICHENG, Shanghai, China) under 60 °C for obtaining the absolute weight of *L. minor* plants.

2.6. Sample preparation and assay

After the samples were dried, the graphite furnace digestion instrument (Z-2300, Hitachi, Japan) was used to digest approximately 100 mg of the samples (dry weight, DW) for 2 h under 150 °C (3 mL HNO_3, 2 mL H_2SO_4, 1 mL $HClO_4$). Deionized water was used to dilute the resultant digestion solutions up to 50 mL. Furthermore, the As and Fe contents in *L. minor* plants and DCB extraction were determined by the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Optima 8300, PerkinElmer, Inc., USA), the limits of detection (LOD) are 2 ppb (As) and 0.8 ppb (Fe). For ICP-OES detection, 2 mL of the test medium were filtered using a 0.45-μm syringe filter, and then measured by ICP-OES. Spectrophotometry was conducted to determine the total phosphate content (Lenore et al., 1998), LOD is 7 ppb.

2.7. Data analysis

The concentration of elements in *L. minor* plants and DCB extracts were determined on dry weight basis. The desorption or mobilization rates of As from the Fe plaques by chelating agents were calculated as follows:

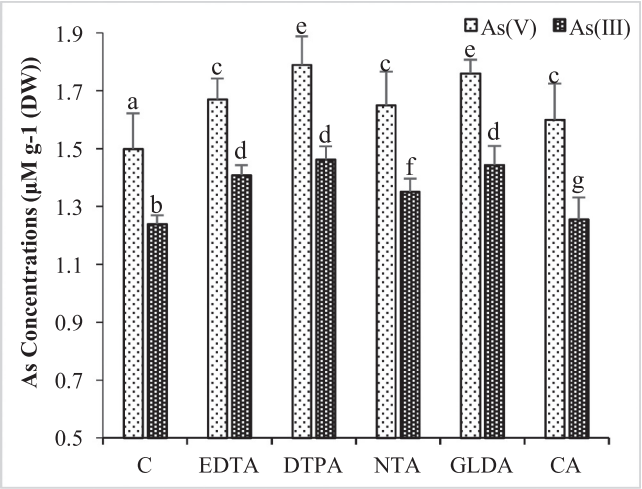


Fig. 1. Effect of chelating agents on arsenic uptake by *L. minor* plants. Values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars indicate significant differences ($p < 0.05$).

$$DR = \frac{C_C - C_T}{C_C} \times 100\%$$

DR: desorption or mobilization rates of As(V) from the Fe plaques by chelating agents (%); C_D: As content in DCB extract in the control group (μM g^{-1}(DW)); C_T: As content in DCB extract in the treatment group (μM g^{-1}(DW)).

The data were analyzed by one-way ANOVA and the significance of the difference was determined through Student's *t*-test using SPSS20.0. A difference of $p < 0.05$ indicated statistical significance.

3. Results and discussion

3.1. Effect of chelating agents on the uptake of arsenic by L. minor plants

As accumulation in *L. minor* plants remarkably increased with the addition of DTPA, EDTA, GLDA, NTA, and CA compared to that in control under the As(V) treatment and As(III) treatment. In addition, the As contents in *L. minor* plants after As(V) treatment were higher than those after As(III) treatment, irrespective of whether the chelating agent was added or not. On exposure to As(V) and As(III), there was an increase in the As uptake in duckweed by the chelating agents in the following order: DTPA > GLDA > EDTA > NTA > CA. Among the chelating agents, no significant differences were obtained between DTPA, GLDA, and EDTA under As(III) treatment, while no significant differences were obtained between DTPA, GLDA, and EDTA under As(V) treatment (Fig. 1).

The observation that the uptake of As(V) by *L. minor* plants is always higher than the uptake of As(III) (Fig. 1) conformed to a prior study (Liu et al., 2005), which reported higher As accumulation in the rice (*Oryza sativa* L.) shoots and roots grown in the As(V)-containing agar medium in comparison to those grown in the As(III)-containing solution. The same result was observed with another duckweed species *L. gibba*, which suggested that the As content in the plants increased after As(V) exposure relative to As(III) treatment (Mkandawire et al., 2004).

The different effects observed after the addition of different chelating agents might be attributed to the difference in the molecular structures of these chelating agents (Table 1). The ligands for the chelating agents are the nitrogen atom and the carboxylate ion (COO-) (Nörtemann, 2005). Generally, the greater the number of ligands, the stronger the interaction with metal ions. DTPA has three nitrogen atom and four COO-, GLDA has one nitrogen atom and four COO-, EDTA has two nitrogen atoms and four COO-, NTA has one nitrogen atom and three

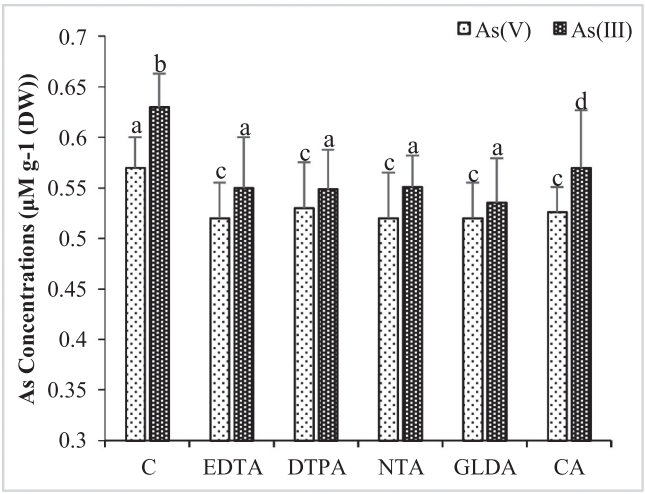


Fig. 2. Effect of chelating agents on arsenic uptake by the DCB extract. Values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars indicate significant differences ($p < 0.05$).

COO-, and CA has four COO-. The trend of increased As uptake and the number of chelating ligands is basically the same. The minor differences are due to the interaction with other ions. Moreover, there was no significant difference between DTPA and GLDA, indicating that GLDA is an ideal chelating agent for increasing the uptake of As due to its biodegradability and less biotoxicity. Wang et al. (2019) suggested that the addition of GLDA efficiently enhanced the soil bioavailability of Cd while promoting the phytoextraction rate of *Amaranthus hypochondriacus* L. Besides, GLDA has been utilized as an eco-friendly chelating agent to remove heavy metals from industrial sludges produced by a local battery company (Wu et al., 2015). GLDA is an NTA-free, environment-friendly, and biodegradable chelating agent obtained from a natural source, which proved to be highly effective in removing heavy metal from the soil in a wide pH range (Thinh et al., 2020). The environmental risk posed by metals has been reduced significantly, with most nutrients retained after GLDA application in soils (Wang et al., 2016). Based on the characteristics of GLDA reported and the results of this study, we may reasonably conclude that the addition of GLDA offered new insights into the effective removal of arsenic by duckweed (Wu et al., 2015). However, the field experiments are usually more complex owing to influences of environmental factors, such as pH, temperature, other heavy metals, and other pollutants. Therefore, we have to study the influence of possible environmental factors and determine the best field experiment application plan.

3.2. Adsorption of arsenic on Fe plaques and the effect of chelating agents on its uptake

The As(III) content in the DCB extract remarkably increased compared to that of As(V). In addition, their contents in the DCB extract declined after the addition of chelating agents into the solutions. The desorption or mobilization rates of As(V) from the Fe plaques by the chelating agents were calculated to be in the range of 5.26–8.77%. The desorption or mobilization rates of As(III) from the Fe plaques by the chelating agents were determined to be in the range of 8.70–15.02%. Among them, the As(III) contents desorbed by CA were the lowest. The desorption or mobilization rate from the Fe plaques by the chelating agent for As(III) was higher than that for As(V) (Fig. 2).

The adsorption of As by Fe plaques in plant roots leading to the decrease of As bioaccumulation in plants has been widely studied (Chen et al., 2005; Shilev et al., 2007; Hansel et al., 2002). The chelating agents were proven to promote the absorption of heavy metals from soil into the plants (Butcher, 2004; Chiu et al., 2005) as they increase the bioavailability of heavy metals (Wu et al., 1999). The chelating agents significantly increased As content in the terrestrial plants by dissolving more As derived from the soil (Chiu et al., 2005). However, the effect of chelating agents on the uptake and desorption of As in aquatic plants is still unclear. This study confirmed the same effect on As bioaccumulation in aquatic plants. In contrast, the As contents in the DCB extract declined after the addition of chelating agents into the solutions. Therefore, the chelating agents promoted As adsorption in *L. minor* plants, probably due to the solubilization of iron plaques. The mobilization and desorption of As from the Fe plaques increased the bioavailability of As in plants.

3.3. Effect of chelating agents on As transfer

For investigating the role of chelating agents on As transfer, its distribution on *L. minor* plants and the Fe plaques was measured. As shown in Fig. 3, the chelating agents increased the amount of As(V) and As(III) transferred from the Fe plaques to *L. minor* plants. When exposed to As (V), the chelating agents increased the plants/DCB extract ratio from 2.63 ± 0.13 to the peak value of 3.38 ± 0.21. When exposed to As(III), the chelating agents increased the plants/DCB extract ratio from 1.97 ± 0.06 to the highest value of 2.70 ± 0.14. Among the chelating agents, DTPA and GLDA showed the highest As content in *L. minor* plants

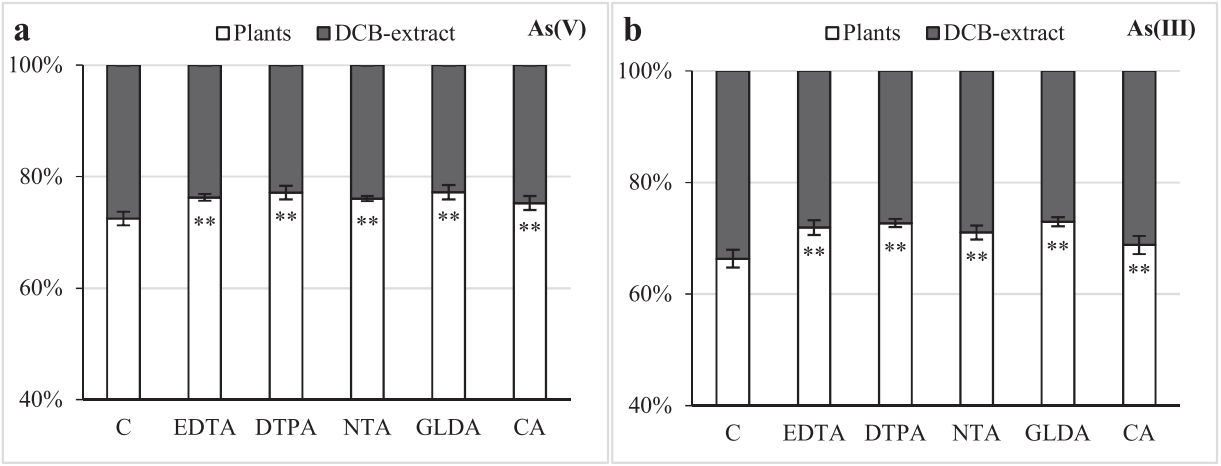


Fig. 3. Percentages of arsenic in *L. minor* plants and Fe plaques (means, n = 3). (a) Percentages of arsenic in *L. minor* plants and Fe plaques on treatment with As(V); (b) Percentages of arsenic in duckweed and Fe plaques on treatment with As(III). The data are expressed in mean ± standard deviation (n = 3). Asterisks indicate significant differences from control (*$p < 0.05$, ** $p < 0.01$).

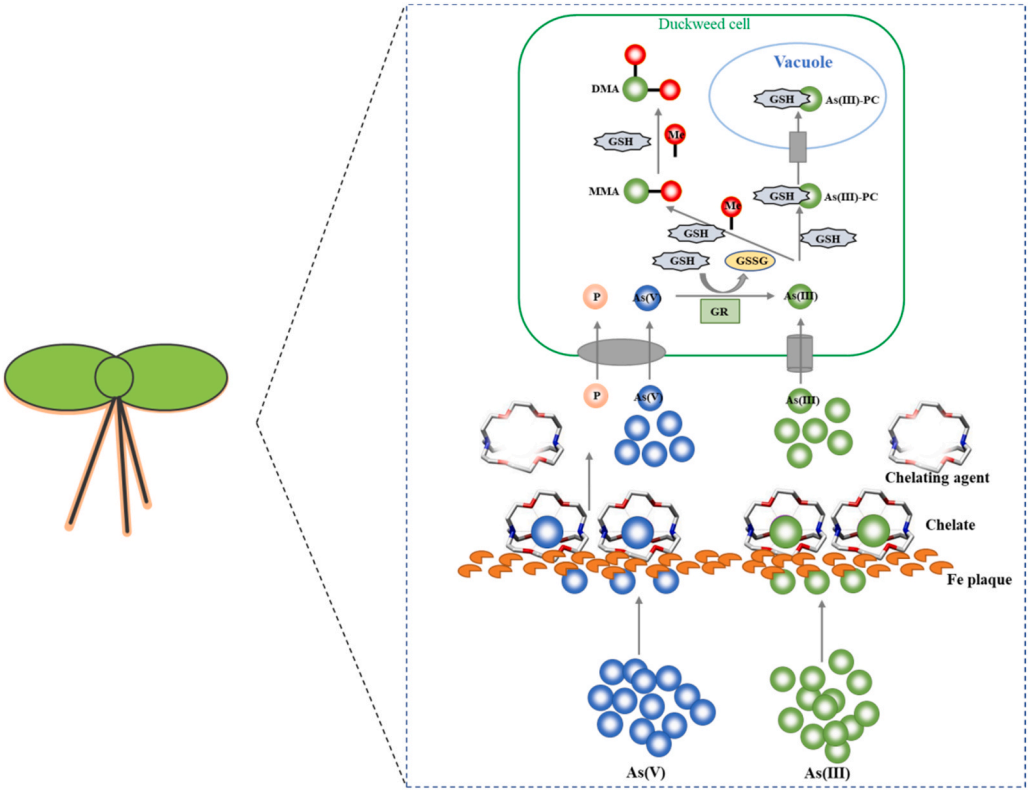


Fig. 4. Chelating agents facilitate the uptake by *L. minor* plants on resolving arsenic from the iron membrane. GSH, glutathione; GR, glutathione reductase; GSSG, oxidized glutathione; PC, phytochelatin; MMA, monomethylated arsenic; DMA, dimethyl arsenic.

(77.16% ± 1.21% and 77.19% ± 1.29%on exposure to As(V), 72.71% ± 0.74%, and 72.95% ± 0.81.21% on exposure to As(III)).

Several studies have reported the role of Fe plaques as barriers to the adsorption of toxic metals (Batty et al., 2000; Greipsson, 1994; Chen et al., 2005). This suggested that the Fe plaques on the duckweed rhizosphere and lower surfaces were the main obstacles toward As adsorption. The Fe plaques show a high affinity toward As(III) and As(V) (Raven et al., 1998),and can be adopted as the anti-As solubility buffer due to potent As adsorption (Liu et al., 2004). Therefore, the removal of Fe plaques is important for increasing the accumulation of As by plants. However, the addition of chelating agents increased the percentage of As, which indicated that the addition of chelating agents is useful for the desorption and mobilization of inorganic arsenic species from the Fe plaques for increasing the As bioavailability in plants.

Generally, plants would first reduce it to As(III) in the cytoplasm and then detoxify it after As(V) uptake, while plants detoxify As(III) directly after uptake (Panuccio et al., 2012). The reduction of As(V) to As(III) is coupled with the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) under the action of glutathione reductase (GR) (Zhao et al., 2009). There are two pathways for the detoxification of As(III) after uptake of As(III) or reduced from As(V) by plants. One is to be further methylated to less toxic monomethylated arsenic (MMA) or dimethyl arsenic (DMA). However, whether methyl-As is less toxic than inorganic As is still not clear (Dopp et al., 2010; Wei et al., 2017). Thus, whether duckweed can detoxify through this pathway remains to be further studied. The other one is the formation of phytochelatins (PCs, obtained from GSH-chelated As(III)), which are mainly preserved in the vacuoles (Zhang et al., 2002). It has been strongly suggested that the effect of PCs on As complexation accounts for a vital mechanism related to As detoxification in the plants. Besides, the exposure to As(III) or As(V) can trigger the potent responses toward PC accumulation and biosynthesis in the plants (Schulz et al., 2008; Srivastava et al., 2007). On the formation of As(III)-PC complexes, they are transferred to the vacuoles and sequestrated by the transporter (Song et al., 2010). The appropriate pH (about 5.5) in the vacuoles offers the appropriate condition for the stabilization of the As(III)-PC complexes (Zhao et al., 2009). As a result, the As(III)-PC complexes are sequestrated in the vacuoles, which is important for the mitigation of As(III) transfer in the duckweed cells.

Table 2
Phosphate concentrations in *L. minor* plants and DCB extracts.

Treatments	P (μMg^{-1} (DW))			
	L. minor plants		DCB-extracts	
	As (V)	As (III)	As (V)	As (III)
Control0 (without As or chelating agent)	0.101 ± 0.012a		185.74 ± 19.32a	
Control (without chelating agent)	0.052 ± 0.006b	0.047 ± 0.003c	87.84 ± 12.45b	92.46 ± 16.24c
EDTA	0.062 ± 0.011d	0.051 ± 0.004b	83.53 ± 11.71d	84.65 ± 12.03d
DTPA	0.061 ± 0.008d	0.053 ± 0.003b	82.64 ± 10.56d	87.82 ± 12.25b
NTA	0.062 ± 0.009d	0.050 ± 0.002e	81.44 ± 7.65d	88.45 ± 8.13b
GLDA	0.067 ± 0.010f	0.052 ± 0.005b	83.64 ± 10.01d	84.33 ± 9.35d
CA	0.065 ± 0.009f	0.050 ± 0.006e	81.39 ± 7.48d	89.12 ± 10.61b

Values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars stand for significant differences ($p < 0.05$).

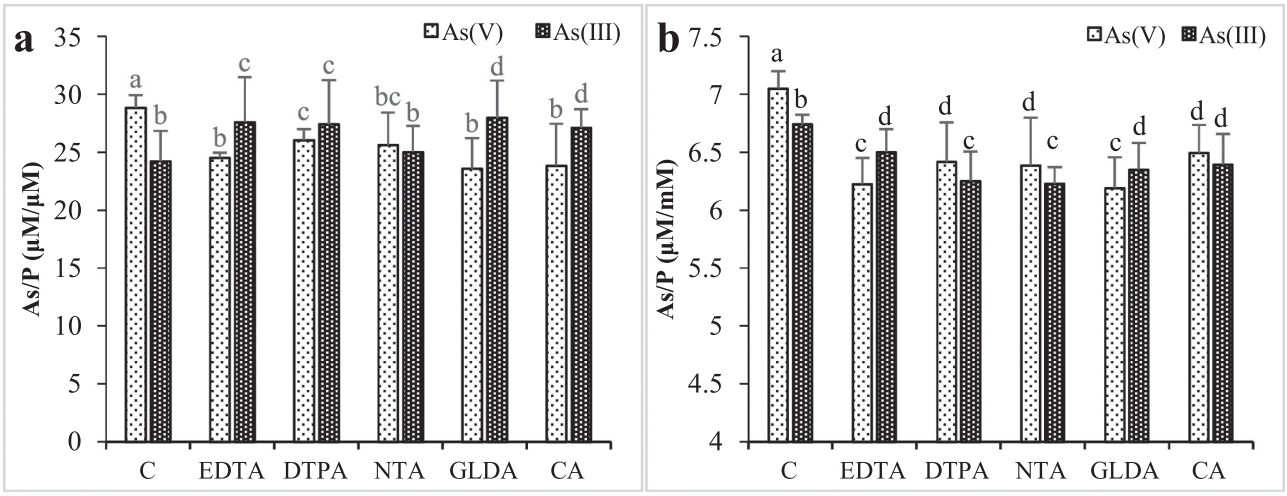


Fig. 5. As/P ratio in *L. minor* plants and DCB-extract. (a) As/P ratio in *L. minor* plants; (b) As/P ratio in DCB extract. Values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars indicate significant differences ($p < 0.05$).

Phosphate shows similar chemical features (acid dissociation constant, ion size, and symmetry) to those of As(V), and can compete against As(V) for adsorption sites on the surface of FeO (Hossain et al., 2009). The plant cells are able to absorb As(V) through the phosphate channel, resulting in a competition between the two ions for their uptake into plants (Abedin et al., 2002). In addition, the phosphate content also serves as a factor that affects the As(V) absorption into the plants. Nonetheless, As(III) is mostly absorbed via the plasma membrane aquaporin channel, but the phosphate absorption does not involve this channel (Jardine, 2003). Based on the above discussions, this study proposed a possible pathway for the absorption and detoxification of As by duckweed with the addition of chelating agents (Fig. 4). In addition, the concentrations of P on duckweed and Fe were determined to investigate the effect of P uptake caused by the chelating agents.

3.4. Effect of chelating agents on phosphate uptake

The As(III) and As(V) treatments significantly decreased the phosphorus levels within the DCB extract and *L. minor* plants in comparison to the controls (Table 2). Besides, the phosphorus concentrations within the DCB extracts were higher than those in *L. minor* plants. The addition of chelating agents in the solutions significantly elevated the absorption of phosphate into *L. minor* plants. Whereas, the phosphate concentrations in the DCB extracts on exposure to As(III) or As(V) greatly declined with the addition of chelating agents.

The As/P ratios in the DCB extract and *L. minor* plants (Fig. 5) suggested the As depletion or enrichment corresponding to the phosphate level. Specifically, the As/P ratios in *L. minor* plants after the As(V) treatment were higher than those after exposure to As(III) (Fig. 5a). Besides, the addition of chelating agents reduced the As/P ratios in *L. minor* plants after As(V) exposure, although the ratio significantly elevated after the As(III) treatment. The As/P ratios in *L. minor* plants treated with As(V) solution significantly declined, which suggested the depletion of As compared to that observed after As(III) exposure. Some articles reported that the reduction of As absorption in *L. minor* plants after exposure to As(V) was ascribed to the more potent surface chemical competition of phosphate than As (Panuccio et al., 2012; Xu et al., 2007). Phosphate serves as an effective competitive inhibitor for As(V) absorption due to the high membrane transporter selectivity of phosphate compared to As(V) (Panuccio et al., 2012). Therefore, although the As uptake in *L. minor* plants was increased by the addition of chelating agents, the As/P value was decreased, owing to the competition with P (Fig. 1 and Fig. 5a). The As/P ratios in the DCB extract were reduced after the addition of chelating agents in plants exposed to the As (III)- or As(V)-containing solutions (Fig. 5b). These results suggested that the As was desorbed from the Fe plaques of *L. minor* plants after As (V) or As(III) exposure (Huang et al., 2007).

Table 3
Fe concentrations in *L. minor* plants and DCB extracts.

Treatments	Fe (μMg^{-1} (DW))			
	L. minor plants		DCB-extracts	
	As (V)	As (III)	As (V)	As (III)
Control0 (without As or chelating agent)	62.56 ± 2.31a		896.8 ± 25.61a	
Control (without chelating agent)	63.22 ± 3.43a	64.14 ± 3.75a	614 ± 13.56b	612 ± 17.34b
EDTA	84.21 ± 5.31b	74.67 ± 3.33c	504 ± 10.37d	534 ± 13.36d
DTPA	79.36 ± 5.12d	73.98 ± 5.21c	482 ± 15.17e	511 ± 10.97b
NTA	83.02 ± 3.22b	71.20 ± 2.32c	503 ± 9.65d	529 ± 14.36b
GLDA	83.52 ± 4.56b	75.21 ± 3.63c	506 ± 11.63d	516 ± 13.77e
CA	84.25 ± 6.12b	73.98 ± 4.17c	476 ± 7.98e	501 ± 12.49b

The values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars stand for significant differences ($p < 0.05$).

3.5. Effect of chelating agents on iron uptake

The addition of chelating agents into the solutions promoted Fe absorption in plants, but the Fe contents in the DCB extracts declined (Table 3). Based on the reports by Hansel and colleagues (Hansel et al., 2002), the Fe plaques are formed due to radial oxygen diffusion as well as further oxidation of the ferrous ion. The Fe plaques formation will lead to the reduction of Fe availability in plants. In addition, As showed high adsorptive affinity toward Fe plaques, resulting in reducing As availability in the plants (Belzile and Tessier, 1990). Generally, siderophores were exuded from the plant rhizosphere microorganisms to the interface between the roots and Fe plaques (Liu et al., 2005). The siderophores formed complexes with Fe; thus, rendering the availability of Fe and As in plants. Our results suggested that the addition of chelating agents promoted the absorption of As and Fe by *L. minor* plants. This is because chelating agents increase availability in plants by possessing Fe on the plant surfaces, similar to the effect of siderophores.

The As/Fe ratios in *L. minor* plants (Fig. 6a) and DCB extracts (Fig. 6b) indicated the increased or decreased As concentration

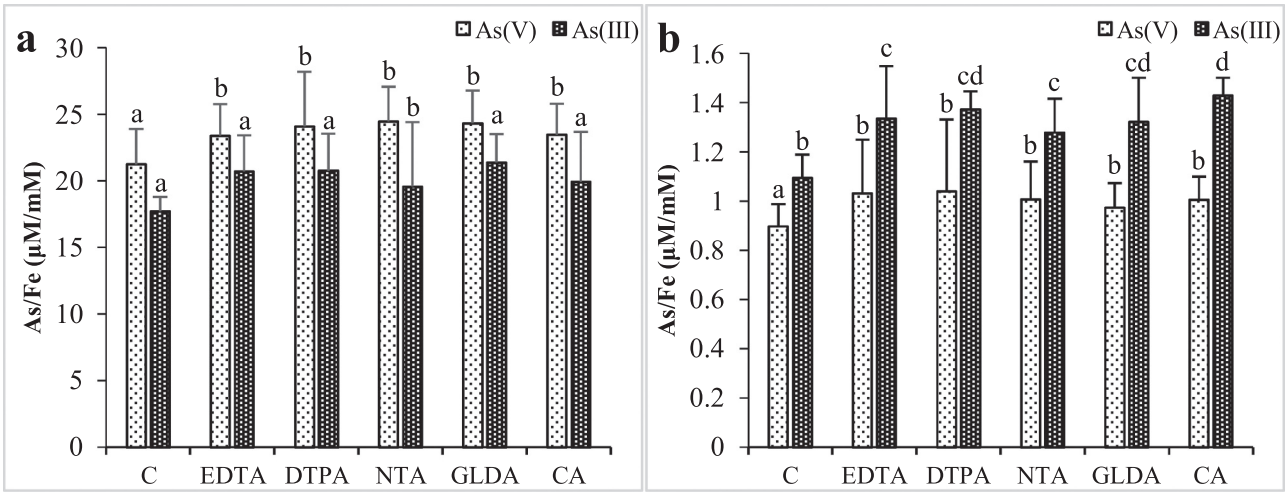


Fig. 6. As/Fe ratio in *L. minor* plants and DCB extract. (a) As/Fe ratio in *L. minor* plants; (b) As/Fe ratio in DCB extract. Values are presented as mean ± SD (n = 3). The lowercase letters and corresponding error bars show significant differences ($p < 0.05$).

compared to the Fe levels. In addition, the As/Fe ratios in *L. minor* plants after As(V) exposure were higher than those after As(III) exposure. In contrast, these ratios in the DCB extracts after As(V) exposure were lower than those after As(III) exposure. Such observations suggested that the uptake of As(III) by Fe plaques was higher than As(V) uptake. Further, significantly increased As/Fe ratios in *L. minor* plants were obtained after the addition of chelating agents, indicating that the chelating agents promoted more absorption of As into the plants compared to that of Fe.

4. Conclusions

In this study, the role of chelating agents on the Fe plaques and As accumulation in *L. minor* plants was systematically investigated. This study illustrated that the addition of chelating agents increased the arsenic accumulation in *L. minor* plants upon exposure to As(III) and As (V) by facilitating the mobilization and desorption of As from the Fe plaques. In addition, the chelating agents increased the uptake of beneficial elements (P and Fe) by *L. minor* plants. Among the chelating agents studied, the readily biodegradable GLDA offers new insights into the effective removal of As by duckweed. The findings of the present work further highlight the application of duckweed in the remediation of arsenic-contaminated water with the help of chelating agents.

CRediT authorship contribution statement

Gui-Li Yang: Conceptualization, Methodology, Writing - original draft. **Ming-Xing Yang**: Investigation, Methodology. **Shi-Ming Lv**: Methodology. **Ai-Juan Tan**: Supervision, Writing - reviewing & editing.

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

This work was supported by National Natural Science Foundation of China (No. 32001203 and No. 31760749); Guizhou Science and Technology Support Plan Project (No. Qian-Ke-He-Zhi-Cheng [2018] 2276); Guizhou University Research Project for Introducing Talent (No. Gui-Da-Ren-Ji-He-Zi [2019] 60).

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