Can Tho University Journal of Science
website: ctujs.ctu.edu.vn

DOI: 10.22144/ctu.jen.2022.027

Effect of heavy metals on duckweed growth

Nguyen Duc Thang, Hoang Le Lan Anh, Pham Thi Thanh Van, Nguyen Minh Tuan, Nguyen Thanh Hang, Nguyen Huu Phuoc, Huynh Thuy Van Anh, Tran Thi Nhung, and Hoang Thi Nhu Phuong*

Faculty of Biology, Dalat University, Viet Nam

**Correspondence: Hoang Thi Nhu Phuong (email: phuonghtn@dlu.edu.vn)*

Article info.

Received 25 Aug 2022
Revised 11 Oct 2022
Accepted 17 Oct 2022

Keywords

Aquatic plants, duckweed, heavy metal, Lemna

ABSTRACT

In this study, three clones of the duckweed genus Lemna, collected from three different regions of Viet Nam, including Binh Thuan, Bac Giang and Ben Tre were used to evaluate the growth and development in a culture medium containing different concentrations of As^{3+}, $Cd^{2+,}$ or Pb^{2+}. During 14 days of incubation, the growth rate was determined daily by measuring the surface area of all plants of each sample. Experimental results show that the three Lemna clones could grow in nutrient medium contaminated by heavy metal at a concentration of 0.3-0.5 mg/L for As^{3+}, 0.15-0.3 mg/L for Cd^{2+}, and 0.15 mg/L for Pb^{2+}. At these concentrations, the division rate of the treated clones was several times higher than that of the controls. The highest increase in the surface area was recorded for Lemna BTN (68.47 times higher when cultured in medium supplemented in 0.3 mg/L Pb^{2+} than under control conditions). The surface area of Lemna BTR cultured in a medium supplemented with 0.3 mg/L As^{3+} was 54.65 times higher than in medium without arsenic. The obtained results showed that Lemna BGG and Lemna BTR can tolerate arsenic and cadmium pollution while Lemna BTN tolerates lead pollution.

1. INTRODUCTION

The process of industrialization and modernization has led to many serious environmental problems in the world. One of these issues is heavy metal pollution in water, which occurs not only in Viet Nam but also in many other regions of the world. Human activities such as the use of access pesticides, mining, industrial production, transportation, aquaculture, etc. release heavy metals into the soil and water environment. These activities have caused hazardous pollution of freshwater systems (Schwarzenbach et al., 2006). Metals, which cannot be transformed by microorganisms, accumulate in the water, soil, bottom sediments, and living organisms (Miretzky et al., 2004). Moreover, most heavy metals are toxic or carcinogenic and pose a threat to human health (Vinodhini, Narayanan, 2009). Arsenic (As), cadmium (Cd), lead (Pb), zinc (Zn), and nickel (Ni), are considered toxic because they cause deleterious effects on plants, animals, and humans. Arsenic is a major environmental pollutant and is usually released from agricultural products, especially pesticides and herbicides (Singh et al., 2007, Abdul et al., 2015). According to Abdul arsenic has been associated with various organs. Cadmium, derived mainly from paint, plastics, fertilizer, and pesticides, can cause high blood pressure, while lead is toxic to the kidneys and the nervous system (Abdul, 2015). *Lemna minor* is one of the ideal aquatic plants that can be used as a phytoremediator of various pollutants such as removing methylene blue (Imron et al., 2021). The maximum removal of Fe, Zn (88.18, 84.63 %) by *L. minor* at 100 ppm initial metal concentration after 20 days of incubation (Mohamed et al., 2021)

Many aquatic plants were reported that they can survive, grow and reproduce in contaminated environments. Metal-resistant organisms minimize the biological effects of metals in the soil or reduce the proteins to transport and absorb metals. Heavy metals are absorbed by root efflux transporters and promptly delivered to the vacuole or released out of the cell in non - accumulator species that can be exposed to metals. As a result, metal-resistant accumulator species detoxify metals in vacuoles via a tonoplast-carrying substance, whilst substantial amounts of metal are delivered to shoots and collected in ground-rising/collectible parts (Rascio & Navari-Izzo 2011; Lin & Aarts 2012).

Duckweeds are floating aquatic macrophytes, forming the family *Lemnaceae* a group of monocotyledonous flowering plants (Khellaf, 2009). Duckweeds can grow in an oxygen-deficient water environment and absorb essential nutrients such as PO_4 and NO_3, making them an effective wastewater treatment plant. Especially, duckweed has many advantages and potential over other groups of aquatic plants due to its very fast growth rate, small body size, and simple structure (Le et al., 2017). Therefore, duckweed is used to assess water quality and to remediate wastewater contaminated with heavy metals (Piotrowska et al., 2010). Almost all duckweed species can absorb and accumulate heavy metals such as Cd, Cr, Pb, etc. in their body. Therefore, they have great potential to treat wastewater contaminated by heavy metals that result from tanning technology, and mining, thus reducing the occurrence of these metals in the food chain. In Viet Nam, diverse duckweed species are distributed in most geographic regions of the country. So far, research on duckweeds as well as their applications for the treatment of water contaminated with heavy metals is limited.

Previous studies indicated that duckweed is effective phytoremediation for metal contamination in water, particularly effluent from industrial and agricultural processes. By increasing biomass and protein content in tissues, duckweed can cope with or reduce contaminants in wastewater (Mohedano et al., 2012; Saha et al., 2014). Increased protein concentration in duckweed could be due to increased synthesis of specific intense proteins (Sun et al., 2002), such as antioxidant enzymes, and heat shock proteins in response to a variety of intense conditions, including heavy metals and metal chaperones, which provide essential metal cofactors for targeting metalloproteins in various cellular compartments (Hall et al., 2002). This demonstrates that duckweed grows and develops using nutrients contained in wastewater. Furthermore, a plant is assumed to accumulate heavy metals as well as absorb them, while also having systems to protect itself against metal toxicity. After metal exposure, a rise in free amino acid concentrations has been linked to detoxification and metal reduction (Sharma & Dietz, 2006; Xu et al., 2012). Glycine, valine, methionine, phenylalanine, ornithine, proline, histidine, and glutamine accumulation in *Chlorella vulgaris* is linked to Cd complexity and tolerance (Chia et al., 2015). The accumulation of alanine, serine, phenylalanine, tyrosine, and threonine in the roots of *Solanum nigrum* revealed a high capacity for Cd accumulation and tolerance (Xu et al., 2012). Moreover, an increase in arginine synthesis causes an accumulation of polyamines, which are protective macromolecules such as polycations, negatively charged macromolecules in the cell (Alcázar et al., 2006). Organic acids, like citric and malic acids, are possible ligands for heavy metals and may thus play a role in cellular homeostasis tolerance, storage, and maintenance (Hall, 2002). It is also true in the case of heavy metal accumulation. Cd, Cu, and Zn are coordinated in hyperaccumulators by organic acids, which are often found in plant vacuoles (Küpper et al., 2009). The increased levels of malic and citric acid found in the leaves of the Cd-hyperaccumulator Solanum nigrum could be linked to the accumulation of Cd (Sun et al., 2006). Other metals, such as Pb, have no special function in plant physiological responses but can be taken by plants due to chemical properties that are comparable to important elements (Pais & Jones 2000). Additionally, arsenate and phosphate are chemically similar and arsenate is absorbed by plants using transporters that bear resemblance to phosphate transporters (Gusman et al., 2012). As a result, certain species improve their survival in the presence of arsenate by inhibiting phosphate transporters which alters the absorption kinetics (Meharg & Hartley, 2002).

Several studies showed heavy metal pollution in water, vegetables, and seafood in some areas of Viet Nam. Nguyen (2016) and Testuro (2014) found that Cd and Pb were the main pollutants in surface sediments in the Red River basin. While As, Cr, and Hg concentrations were higher than the allowable limit in the Mekong Delta. Nguyen (2013) detected heavy metals (As, Cd, Cr, Pb) in oyster tissue in coastal areas of Can Gio and Le (2013) found high concentrations in rock oysters and green mussels in coastal Do Son (cite from Nguyen, 2020)

Robert Anthony Martienssen said at the International Conference on Duckweed Research and Application that "We're interested in using or optimizing duckweed for use as a biomass for biofuel based on its ability to grow on wastewater and water in places which you would never imagine crops would grow". This message showed that duckweed could become the potential crop for di-purpose applications (wastewater treatment and biofuel biomass).

The present study investigates the effect of As, Pb, and Cd on the three *Lemna* clones collected from three different regions in Viet Nam to assess the tolerance to and survey their growth and development in water contaminated with these heavy metals.

2. MATERIALS AND METHOD

2.1. Plant material and culture medium

Three *Lemna* accession native to the Bac Giang (BGG), Binh Thuan (BTN), and Ben Tre (BTR) regions of Viet Nam were collected from natural ponds and placed in 350-mL sterile glass jars containing 150 mL medium and covered with a plastic film and grown at 25±2ºC, with a day-to-night cycle of 16:8 h (photon flux 40 µmol.$m^{-2}s^{-1}$) on N – medium (Appenroth et al., 1996). Different concentrations of arsenic (As^{3+}) (0.1; 0.3; 0.5; 0.7; 0.9 mg/L), cadmium (Cd^{2+}) and lead ions (Pb^{2+}) (0.1; 0.15; 0.2; 0.25; 0.3 mg/L) were added into N-medium. Four fronds of each *Lemna* accession were cultured on this medium for 14 days. These heavy-metal doses are environmentally relevant and were chosen to expose the duckweeds to low to moderate levels of As, Cd, and Pb. The stock solution (10mg/L) of As, Cd, and Pb was prepared by diluting As_2SO_3, $CdCl_2$, and $Pb(NO_3)_2$ (Sigma-Aldrich Co., USA) in distilled water, then the stock solution is added to the nutrient medium up to the mentioned concentrations.

2.2. Surface area measurement

The surface area based on the total area of all fronds of a sample was determined by image analysis using ImageJ software. Plants were collected for two weeks every day and photographed at the same time. The number of fronds is also counted on the images.

2.3. Replication and Statistical Analysis

All experiments were repeated 3 times. Data were analyzed by Microsoft Excel 2013 and Image J software.

3. RESULTS AND DISCUSSION

3.1. The effects of As^{3+}, Cd^{2+}, and Pb^{2+} on the growth of Lemna BGG

There was a significant increase in biomass of the accession *Lemna* BGG grown in the presence of As^{3+} and Cd^{2+} compared to the control. The concentrations of heavy metals for the *Lemna* BGG line were 0.5 mg/L for As^{3+}, 0.2 mg/L for Cd^{2+} and 0.15 mg/L for Pb^{2+}. At these concentrations, the surface area increased many times higher than that of the control. The 42.23-fold at 0.5 mg/L As^{3+} (from 0.430 to 18,158 cm^2), 40.81-fold at 0.2 mg/L Cd^{2+} (from 0.408 to 16,652 cm^2) and 32.19-fold at 0.15 mg/L Pb^{2+} (from 0.545 to 17,153 cm^2) (Table 2, chart 1). This proved that there was a positive effect of arsenic on the growth and development of *Lemna* BGG. The heavy metal adsorption capacity of different duckweed accessions has been studied previously (Goswami et al., 2014b; Teixeira et al., 2014; Mechora et al., 2015; Nguyen, 2016; Bokhari et al., 2019b). The obtained result from this study on *Lemna* BGG was similar to *L. minor* cultured in a medium containing 0.5 mg/L As^{3+} which yielded the best arsenic absorption within the range of concentrations investigated (Goswami et al., 2014b). Moreover, at concentrations higher than 0.2 mg/L Pb^{2+}, the growth of *Lemna* BGG was markedly inhibited. The absorption capacity for Pb^{2+} of different duckweed species has been studied and showed that each species has its absorption optimum, for instance, 1.51 mg/L for *L. gibba* (Bokhari et al., 2019b) or 0.2 mg/L for *L. minor* (Ucuncu et al., 2013). The obtained results for *Lemna* BGG are similar to that reported for *L. minor*.

The *Lemna* accession BGG could grow well at the concentration of As^{3+} and Cd^{2} above 0.5 mg/L. Thus, *Lemna* BGG can grow in water strongly contaminated with arsenic and cadmium indicated by the faster growth rate (increase in surface area) in supplemented heavy metals medium than that of control (Table 1 and Fig. 1)

Table 1. The increase in surface area (cm^2) of the Lemna BGG accession cultured in medium supplemented with As^{3+}, Cd^{2+}, and Pb^{2+} ions for 14 days

	As^{3+} (mg/L)						Cd^{2+} (mg/L)						Pb^{2+} (mg/L)					
Day	**0**	**0.1**	**0.3**	**0.5**	**0.7**	**0.9**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**
D1	0.519	0.452	0.470	0.430	0.470	0.530	0.375	0.410	0.450	0.408	0.455	0.486	0.400	0.455	0.545	0.517	0.460	0.477
D2	0.520	0.455	0.474	0.431	0.471	0.530	0.376	0.411	0.452	0.408	0.457	0.488	0.408	0.457	0.547	0.519	0.461	0.479
D3	0.635	0.511	0.694	0.589	0.501	0.601	0.381	0.512	0.589	0.561	0.549	0.610	0.532	0.594	0.501	0.612	0.564	0.508
D4	0.713	0.678	0.717	0.786	0.702	0.763	0.385	0.620	0.652	0.629	0.701	0.783	0.624	0.712	0.607	0.641	0.601	0.588
D5	0.963	1.391	2.218	1.955	1.937	1.542	1.002	1.609	1.965	1.703	1.527	1.581	1.044	1.670	1.651	1.685	1.591	1.964
D6	1.859	2.262	2.792	2.510	1.943	1.831	1.676	1.676	2.117	2.501	2.474	2.057	1.864	2.205	2.333	2.342	2.417	2.638
D7	2.738	3.260	3.872	4.524	3.240	2.555	2.752	3.116	3.728	4.466	2.562	2.529	2.632	2.993	3.425	3.262	3.072	3.284
D8	2.932	3.869	4.895	6.015	4.956	3.023	2.978	4.150	4.395	5.108	3.392	3.085	3.015	4.359	4.150	4.892	4.567	3.852
D9	3.650	5.106	6.962	8.060	6.690	4.814	3.422	5.536	6.039	6.587	4.982	4.671	3.422	5.360	6.039	6.886	5.991	4.085
D10	5.335	7.033	8.801	10.674	7.903	6.065	4.829	5.963	7.202	7.147	6.054	5.305	4.163	5.930	7.202	7.774	6.554	4.771
D11	5.864	8.832	9.802	12.770	8.920	7.868	5.163	7.041	8.816	9.015	7.482	6.752	5.557	7.071	8.816	9.869	7.798	5.584
D12	6.626	9.532	11.074	14.213	10.480	10.573	5.557	7.786	10.439	11.245	9.336	7.452	6.263	8.142	10.439	11.210	8.336	6.452
D13	6.965	11.151	12.989	15.719	13.092	12.739	7.304	9.179	11.998	14.258	10.624	9.822	7.795	11.179	13.798	14.125	11.024	7.442
D14	7.795	13.049	15.263	18.158	16.197	16.107	7.445	10.047	13.199	16.652	11.664	11.023	8.304	13.049	17.153	16.640	13.124	8.897

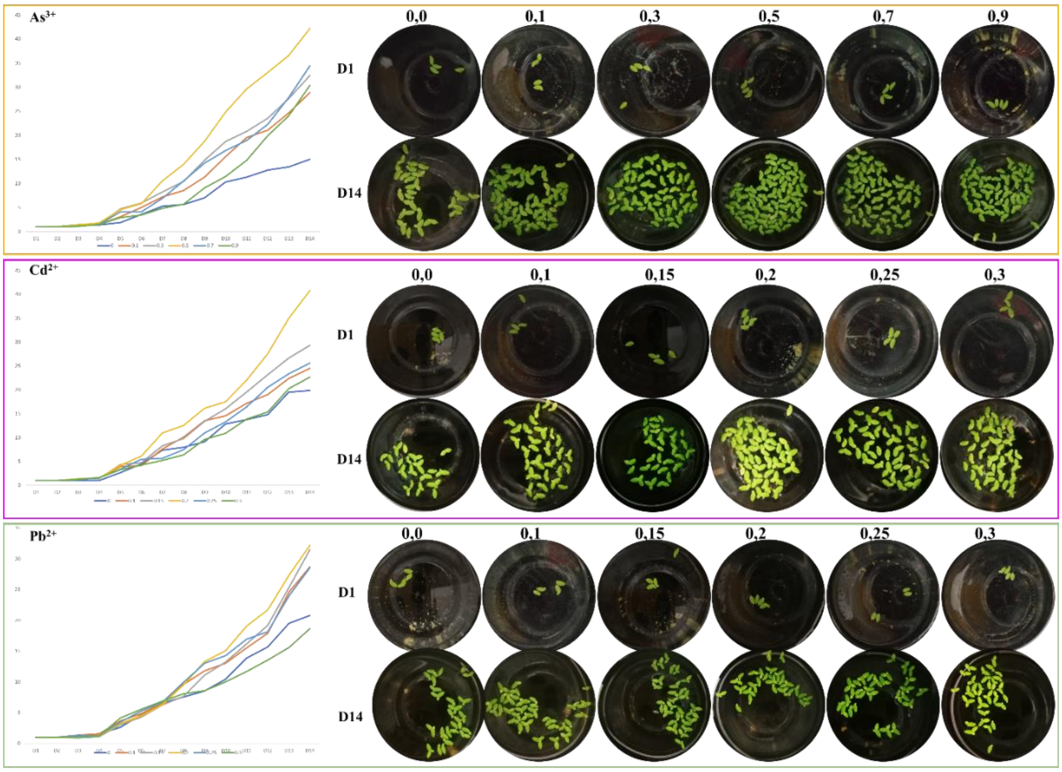

Figure 1. The increase in surface area (times) during 14 days and the growth of *Lemna* BGG cultured in medium supplemented with As^{3+}, Cd^{2+}, and Pb^{2+} ions (mg/L) on day 1 and day 14

Duckweed has a great ability to endure contaminants in the environment, as we can see by the minor damage (a small change in color and size of duckweed fronds (Fig.1)). In assessing the effectiveness of duckweed absorption and development in a contaminated environment, time length is also a significant consideration. Setting up an experiment for a short length of time can result in unfavorable developmental outcomes, however prolonging the time of the experiment can result in other outcomes (Ekperusi et al., 2019).

Metal removal was practically complete within the first 24 hours, according to earlier investigations (Axtell, 2003; Ucuncu, 2012). In a study of Pb accumulation in *L.minor*, Axtell discovered that the fastest accumulation (50–90%) occurred in the first 24 hours (Axtell, 2003). In Pb removal, investigated *L.minor* obtained a total of 95% Pb removal, with 85% performed in the first 24 hours (Rahmani & Sternberg, 1999).

L.gibba reported that can remove heavy metals from untreated industrial/city wastewater in about 21 days. An increase in Ni, Pb, and Cd concentrations of plant tissues was detected in experiments. The difference between the initial concentration (at inoculation) and the final concentration (at day 21) was significant ($p < 0.05$) (at harvest) (Syeda *et al.*, 2019). When the initial and final concentrations of Cd, Pb, and Ni in *L. gibba* tissues were compared, the final values were several times higher than the starting concentrations. Heavy metal accumulation in the tissues of *L.gibba* produced the same results as reported by Megateli (Megateli *et al*., 2009).

3.2. The effects of As^{3+}, Cd^{2+}, and Pb^{2+} on the growth of Lemna BTR

A similar growth rate between treatments was observed for the first 7 days, from the 8th day onwards, we noticed a large difference. Similar to the *Lemna* BGG, all treatments with arsenic ions yielded a higher growth rate than the control for *Lemna* BTR. The highest growth rate was observed at 0.3 mg/L (from 0.2 to 7.236 cm^2, around 36 times) while the control increased only 15 times (from 0.235 to 3.526 cm^2) after 14 days of cultivation (Table 3 and Fig 2).

After cadmium treatment, positive effects on the growth of *Lemna* BTR can be seen at 0.15 mg/L. After 14 days, the surface area increased 54.65 times (from 0.18 to 9,837 cm^2). At higher concentrations, there was no strong difference in growth rate compared to the control. A similar observation was reported for a growth rate of *L. minor* which was increased by 0.2 mg/L, while for *L. gibba* this was the case at 0.02 mg/L (Varga et al., 2013). Thus, the cadmium resistance of *Lemna* BTR was almost equivalent to that of *L. minor*.

For the lead treatments, the control plants showed the slowest growth rate (23.35 times) while 0.2 mg/L yielded the highest growth rate (from 0.251 to 10.837 cm^2=43.18 times). The growth rate of *Lemna* BTR decreased proportionally with further increasing lead concentration, but was still higher than that of the control. Our results on the *Lemna* BTR line are similar to those published for *L. minor* (Ucuncu et al., 2013).

Lemna BTR can grow well in water contaminated with arsenic and cadmium (Table 2 and Fig. 2)

Mohedano showed that the duckweed biomass level and protein content increased by 35% after being cultured in swine wastewater (Mohedano *et al.*, 2012) and Saha reported that duckweed after 21 days of experiment with wastewater from steel mills increased biomass by 30% (Saha et al., 2014). However, there were also contrasting results of pollutants, and heavy metals causing negative effects (Saha et al., 2014; Zhang et al., 2014a, b, c) or growth inhibition. (Wang et al., 2014a, b; Grijalbo et al., 2016; Wang et al., 2016) of duckweed depends on the concentration of pollutants and the toxicity level of the wastewater type. Furthermore, the success of heavy metal absorption by plants is associated with the ability of a specific phenotype and genotype of the plant to absorb and translocate heavy metals (Chen et al., 2013).

Table 2. The increase in surface area (cm^2) of the Lemna BTR accession cultured in medium supplemented with As^{3+}, Cd^{2+}, and Pb^{2+} ions for 14 days

	As^{3+} (mg/L)						Cd^{2+} (mg/L)						Pb^{2+} (mg/L)					
Day	**0**	**0.1**	**0.3**	**0.5**	**0.7**	**0.9**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**
D1	0.235	0.275	0.2	0.215	0.270	0.222	0.230	0.270	0.18	0.18	0.250	0.22	0.224	0.280	0.238	0.251	0.261	0.285
D2	0.286	0.277	0.201	0.217	0.273	0.224	0.261	0.274	0.182	0.18	0.264	0.220	0.285	0.287	0.238	0.252	0.262	0.288
D3	0.301	0.355	0.289	0.256	0.286	0.231	0.281	0.301	0.223	0.217	0.356	0.246	0.316	0.332	0.332	0.332	0.283	0.301
D4	0.387	0.435	0.313	0.330	0.394	0.292	0.312	0.423	0.338	0.298	0.410	0.359	0.394	0.490	0.490	0.490	0.363	0.338
D5	0.532	0.648	0.683	0.684	0.658	0.625	0.859	0.658	1.757	1.602	1.877	1.228	0.612	1.544	1.544	1.544	1.451	1.399
D6	0.677	0.861	1.053	1.038	0.922	0.958	1.659	0.862	1.899	2.243	2.221	1.451	1.235	1.893	1.893	1.893	1.742	1.786
D7	0.822	1.074	1.423	1.392	1.186	1.291	2.124	1.211	2.101	2.253	2.298	2.447	1.987	2.551	2.551	2.551	1.992	1.643
D8	1.862	2.301	2.532	2.894	2.562	2.826	2.683	2.121	2.921	2.672	2.699	3.057	2.214	2.981	2.981	3.101	2.101	1.918
D9	2.012	4.846	4.112	4.285	3.998	3.682	3.112	2.562	4.665	3.091	3.099	3.288	2.791	3.561	3.561	3.561	2.564	2.193
D10	2.650	5.526	4.991	5.236	5.356	3.982	3.845	3.854	5.843	3.510	3.500	3.788	3.281	4.164	4.164	4.164	3.245	3.115
D11	2.985	6.257	5.341	5.515	6.232	4.524	4.126	4.856	6.542	3.929	3.901	4.288	3.770	5.105	5.105	5.105	4.523	4.087
D12	3.024	7.315	5.972	5.799	6.888	4.998	5.147	6.652	7.498	5.398	5.442	5.465	4.260	5.856	6.041	6.574	6.024	5.874
D13	3.234	7.998	6.604	6.383	7.325	5.872	6.925	8.851	8.821	6.689	7.756	6.742	4.749	7.444	7.582	8.825	7.752	6.982
D14	3.526	8.429	7.236	6.968	7.847	6.124	7.547	9.741	9.837	7.576	9.021	7.442	5.239	8.112	8.264	10.837	9.125	9.011

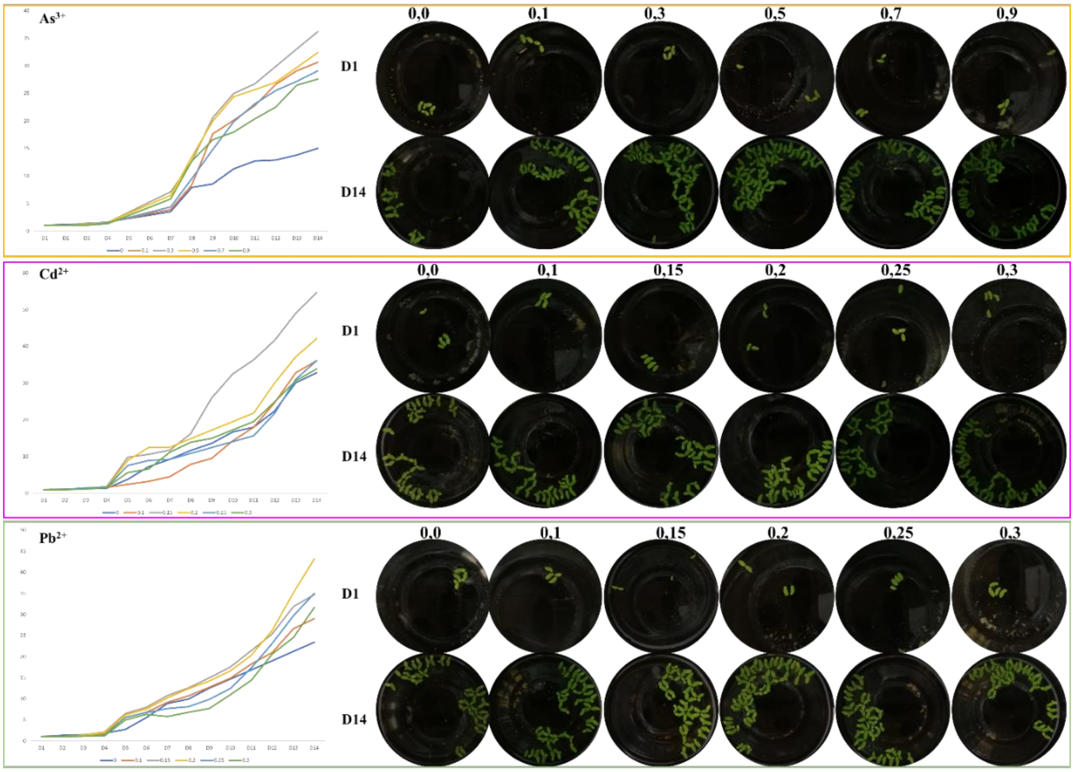

Figure 2. The increase in surface area (times) during 14 days and the growth of *Lemna* BTR cultured in medium supplemented with As^{3+}, Cd^{2+}, and Pb^{2+} ions (mg/L) on day 1 and day 14

Duckweed biomass and protein content increased by 35 percent after being grown in swine wastewater (Mohedano et al., 2012), or by 30% following 21 days of experiment with wastewater from steel mills (Saha et al., 2014). However, there were also negative consequences from pollution, heavy metals or growth suppression of duckweed (Wang et al., 2014a, b; Grijalbo et al., 2016; Wang et al., 2016) is dependent on the contaminants present, and the wastewater toxicity level.

3.3. The effects of As^{3+}, Cd^{2+}, and Pb^{2+} ions on the growth of Lemna BTN

Similar to experiments with the other two *Lemna* accessions, *Lemna* BTN also showed different responses to each concentration of metal ions added to the medium. The increase in surface area after treatments is shown in Table 4 and Fig. 3.

Figure 3 shows that the growth of *Lemna* BTN after heavy metal exposure differs from that of *Lemna* BGG and BTR.

The growth rate on media supplemented with arsenic did not change much compared to the control treatment. The maximum increase in the surface was achieved after the application of 0.5 mg/L arsenic which increased 47.48 times (from 0.213 to 10,113 cm^2) compared to the control which increased 34.88 times (from 0.201 to 7.011 cm^2) after 14 days of cultivation. Thus, the presence of arsenic (up to 0.9 mg/L) in the medium had only a little effect on the growth and development of *Lemna* BTN.

For the cadmium exposure, in the low concentration range (from 0.1 to 0.25 mg/L Cd^{2+}), the growth rate of *Lemna* BTN was significantly reduced and lower than that of the control. However, at a higher concentration (0.3 mg/L), the growth increased 39.12 times (from 0.255 to 9,976 cm^2), starting from day 7. The cadmium absorption for *L. gibba* species was recorded to be 0.74 mg/L. Therefore, the absorption capacity of metal ions of different *Lemna* accessions or species seems to be different (Bokhari et al., 2019b).

The *Lemna* accession BTN showed a was quite sensitive to lead and irregular surface increase with increasing concentration (similar to the medium with Cd^{2+}). At 0.1 mg/L Pb^{2+}, *Lemna* BTN showed increased growth compared to the control, followed by a decrease at 0.15 - 0.2 mg/L to the control level, and again an increase at 0.25 mg/L. At 0.3 mg/L

Pb^{2+}, the surface area of the *Lemna* BTN reached its peak with 18.349 cm^2 after 14 days (68.47 times than at day 1). At least, was no indication of growth inhibition by lead and this accession can easily live in environments with high lead concentrations. Similarly, Pb^{2+} absorption capacity was recorded for *L. gibba* at 1.51 mg/L (Bokhari et al., 2019b). Also, *Lemna* BTN has the potential to grow and absorb Pb^{2+} at high concentrations.

Axtell indicated *L. minor* ability to remove soluble Pb and Ni under different laboratory conditions (Axtell et al., 2003). The initial concentrations of Pb were 0.0, 5.0 and 10.0 mg/L and of Ni were 0.0, 2.5 and 5.0 mg/L in the experiment. Overall, *L. minor* removed an average of 76% Pb and 82% Ni from the medium. Similar results were reported while using duckweed (*L. minor* and *L. gibba*) in wastewater treatment to remove heavy metals under various laboratory tests (Loveson et al., 2013, Aurangzeb et al., 2014, Yilmaz & Akbulut, 2011). The removal efficiency of Cu, Ni, and Pb from wastewater was more than 60% for L. minor (58%, 68%, and 62% for Cu, Ni, and Pb respectively).

Basile determined that *L. minor* after 7 days treated with 10^{-4}M Zn could accumulate up to 58.800 mg/g metal in tissue, while with 10^{-4}M Pb can accumulate 22.533 mg/g metal, which is the reason that duckweed was supposed to be a potential subject for wastewater treatment (Basile et al., 2012). Syeda tested *L.minor* for Cd, Cu, Pb, and Ni accumulation from two wastewater types within 31 days. The results showed the accumulation of heavy metals in the plants and the reduced concentration of these elements in the wastewater. Great removal efficiency (94% Cd, 94% Cu, 97% Pb) for all metals and maximum removal of Ni (99%) from industrial wastewater (with an initial concentration in wastewater of Pb is 0.608 mg/L and Ni is 0.059 mg/L) (Syeda et al., 2016). In 2007, Keith exposed *L. minor* to an aqueous solution containing 125 mg/L Cu, 220 mg/L Cr, and 205 mg/L As in single and mixed forms, reported results showed that plants can remove 60% Cu, Cr and As when they are mixed, but when Cu (60 mg/L) is the only heavy metal in the solution, it could be removed up to 85%, over 7 days (Keith *et al*., 2007).

Even though duckweed is closely related and outstanding in the Lemnaceae family, different species have varying abilities to adapt to a variety of environmental contaminants or intensities. Although there may be a generic kind of metal absorption, absorption can also be species-specific. More research is needed to determine the absorption kinetics of various types of metal pollutants during macrophyte treatment. Due to a paucity of data to define the kinetics and reactions to certain metals in situ, it is unclear whether some plant species have an affinity for metal absorption (Ekperusi et al., 2019).

Table 3. The increase in surface area (cm^2) of the Lemna BTN accession cultured in medium supplemented with As^{3+}, Cd^{2+}, and Pb^{2+} ions for 14 days

	As^{3+} (mg/L)						Cd^{2+} (mg/L)						Pb^{2+} (mg/L)					
Day	**0**	**0.1**	**0.3**	**0.5**	**0.7**	**0.9**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**	**0**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**
D1	0.201	0.211	0.216	0.213	0.223	0.225	0.253	0.256	0.254	0.248	0.239	0.255	0.265	0.257	0.254	0.265	0.249	0.268
D2	0.221	0.226	0.252	0.245	0.265	0.265	0.318	0.321	0.345	0.358	0.284	0.301	0.322	0.325	0.314	0.295	0.301	0.311
D3	0.354	0.412	0.389	0.528	0.356	0.358	0.387	0.434	0.532	0.414	0.294	0.329	0.359	0.521	0.544	0.322	0.382	0.496
D4	0.776	0.652	0.685	0.701	0.472	0.498	0.428	0.547	0.587	0.71	0.301	0.557	0.578	0.607	0.599	0.485	0.432	0.648
D5	0.965	0.896	0.852	0.742	0.589	0.561	0.559	0.698	0.625	0.877	0.374	0.787	0.649	0.703	0.677	0.549	0.512	0.880
D6	1.123	1.121	1.011	0.986	0.923	0.828	1.193	2.154	1.732	1.719	1.269	1.410	2.140	3.367	2.328	2.017	2.586	2.782
D7	2.456	1.895	1.252	1.564	1.325	1.265	2.285	3.013	2.936	1.870	1.822	2.940	2.250	4.170	3.440	2.360	3.770	5.080
D8	3.012	2.735	1.985	2.811	1.842	1.859	3.159	4.270	4.075	2.748	2.603	4.019	3.281	6.214	4.911	3.496	5.648	7.269
D9	3.892	3.782	2.654	3.252	2.315	2.352	4.088	5.428	5.231	3.445	3.327	5.210	4.081	7.947	6.293	4.433	7.353	9.485
D10	4.514	4.856	3.825	4.125	3.556	3.113	4.567	6.585	6.386	4.141	4.051	5.353	4.882	9.681	7.674	5.371	8.790	11.701
D11	4.872	5.586	4.896	5.788	5.011	4.011	5.945	7.150	7.542	4.838	4.572	6.402	5.682	10.986	8.192	6.308	9.057	12.960
D12	5.556	6.565	6.052	7.652	6.004	5.562	6.245	7.743	8.012	5.534	4.775	7.593	6.483	11.414	9.056	7.246	10.762	13.917
D13	6.045	7.125	7.853	8.856	6.615	6.652	6.810	9.246	8.697	6.231	5.499	8.785	7.283	13.148	10.437	8.183	12.466	16.133
D14	7.011	8.245	9.251	10.113	7.898	7.526	7.393	10.121	9.853	7.748	6.223	9.976	10.806	14.881	11.819	10.132	14.171	18.349

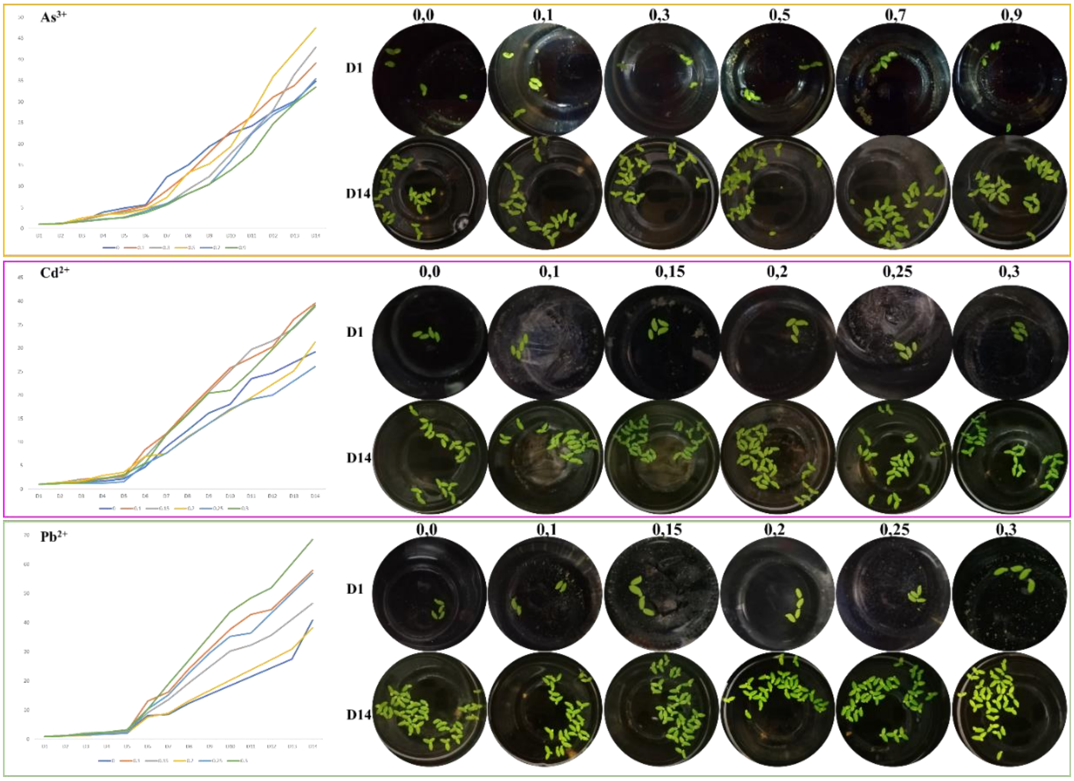

Figure 3. The increase in surface area (times) during 14 days and the growth of *Lemna* BTN cultured in medium supplemented with As^{3+}, Cd^{2+}. and Pb^{2+} ions (mg/L) on day 1 and day 14

Bonanno evaluated the removal of different heavy metals (As, Cd, Cl, Cu, Hg, Mn, Ni, Pb, and Zn) by twenty distinct macrophytes in a living and industrial wetland. They demonstrate that metal absorption or translocation does not follow a predictable pattern among plant species. Although removal rates differed widely across the twenty species, *L. minor* had the greatest elimination rate (Bonanno et al., 2018). The transit and status of inorganic contaminants in duckweed, particularly metals, although processes such as methylation, chelation, sequestration, and chemical binding are primarily responsible for metal detoxification and conversion from harmful to less toxic forms are still unclear (Chandra, 2015).

4. CONCLUSIONS

From obtained results of three duckweed clones (BTR, BTN and BGG) gave different reactions on As^{3+} (0.1; 0.3; 0.5; 0.7; 0.9 mg/L) or Cd^{2+}/Pb^{2+} (0.1; 0.15; 0.2; 0.25; 0.3 mg/L) treatments. In details, Lemna BGG line has the potential to grow in medium supplemented with 0.5 mg/L As^{3+} or 0.3 mg/L Cd^{2+}, while Lemna BTR line grew well in medium contained 0.3 mg/L As^{3+} or 0.15 mg/L Cd^{2+} or 0.2 mg/L Pb^{2+} and Lemna BTN line could grow well in medium contaminated by more than 0.3 mg/L of Pb^{2+}.

This is the first investigation on heavy metals resistance of duckweed lines collected from different regions along Viet Nam. The obtained data showed that duckweed in Viet Nam could be used for

wastewater treatment as reported by other international research groups. For further work, to survey the accumulation rate of these heavy metals in the three duckweed lines as well as increase the experiment scale to the pilot scale for practical application.

ACKNOWLEDGMENT

This work was supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) grant # 106.01-2020.33

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