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FIRST INVESTIGATION ON BIOACCUMULATION POTENTIALITY OF *LEMNA GIBBA* L. FOR HEAVY METAL POLLUTION IN LEBANESE FRESH WATERS

H. GHANEM*[1, 2], S. BAYDOUN[3], H. ABOU HAMDAN[4], L. ITANI[5], L. CHALAK[2] AND S.I. KORFALI[6]

[1]*Biology Department, Faculty of Sciences, Beirut Arab University, Debbieh, Lebanon*
[2]*Plant Production Department, Faculty of Agronomy, Lebanese University, Beirut, Lebanon*
[3]*Research Center for Environment and Development, Beirut Arab University, Bekaa, Lebanon*
[4]*Life and Earth Sciences, Faculty of Sciences I, Lebanese University, Beirut, Lebanon*
[5] *Faculty of Health Sciences, Beirut Arab University, Beirut, Lebanon*
[6] *Natural Sciences Department, Lebanese American University, Beirut, Lebanon*

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ABSTRACT

Addressing heavy metal pollution is considered among the most serious challenges of fresh water bodies. Floating macrophytes such as *Lemna gibba* (duckweed species) have a great potential in the bioaccumulation of heavy metals alleviating these contaminants. This research aimed to characterise the quality of water habitat and to evaluate the natural bioaccumulation potential of native *L. gibba* growing naturally in polluted surface water bodies in Lebanon. A set of physiochemical properties with chlorophyll a (Chl a) and levels of heavy metals *i.e.* Cu, Cr, Zn, Ni, Cd, Mn, Pb and As were determined in seven surface water bodies with high relative abundance of *L. gibba* in order to have a minimum understanding of its habitat. Findings revealed mineralization, phosphates, Chl a, turbidity, As, Cd and Mn levels that exceed international limit values. Statistical analysis with PCA revealed three components with strong loadings of phosphates, nitrates, turbidity, total hardness, Mg hardness, Cl^- and pH. On the other hand, bioaccumulation factors (BAFs) of tested heavy metals were also determined in plant tissues using AA. Values of BAFs ranged between 35.25 and 1068 and followed the descending order of Zn>Cu>Mn>Ni>Pb>As>Cd>Cr indicating high accumulation capacity of heavy metals. Strong associations between the BAFs of As, Mn and Ni on one side and the factor scores of physiochemical variables on the other side were observed. This study confirms the ability of *L. gibba* to efficiently accumulate a large span of heavy metals prevailing in the Lebanese water bodies, and therefore its potentiality to be used effectively in the reduction of pollutants. Additional investigations are required to check the efficiency of this macrophyte for *in situ* phytoremediation of local aquatic environments contaminated by the anthropogenic activities.

KEYWORDS: Duckweed, Bioaccumulation factor, Lebanese polluted water, Heavy metals

INTRODUCTION

Heavy metal pollution is considered to be one of the most serious fresh water contaminations that have attracted global attention owing to its abundance, persistence and high environmental toxicity (Al Akeel, 2018 and Selvi *et al*., 2019). Heavy metals are listed as priority pollutants by the United States Environmental Protection Agency (EPA, 2016). According to their prevalence and the severity of their toxicity, lead, mercury, arsenic, and cadmium are ranked by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) among the top toxic substances. Both natural and anthropogenic activities are responsible for this challenge with the latter generating elevated levels that have significant adverse effects on aquatic ecosystems and humans. A large number of industries are emanating their metal containing effluents into fresh waters without any treatment. Heavy metals accumulate through the food chain and create environmental and health problems. They can also form harmful complex compounds which critically affect the functions and services of fresh aquatic ecosystems (Rai, 2018). Heavy metal pollution cannot be degraded by natural processes and persist in soil and sediments from where they are released gradually into water bodies. Alleviation of heavy metals requires special attention to sustain water quality and consequently human wellbeing. Conventional treatment systems have been developed for the abatement of heavy metal pollution from industrial effluents (Ali *et al*., 2013 and Rai 2018). Much of these conventional remediation methods are efficient but they are costly and economically non-feasible. Thus, there has been a growing interest to develop sustainable remediation systems through the introduction and promotion of novel biotechnological processes such as phytoremediation (Rai, 2008, 2009; Ali *et al*., 2013 and Ansari *et al*., 2014). Phytoremediation is the use of plants to remove environmental pollutants from soil or waterbodies (Pilon-Smits, 2005).

Various aquatic plants, also called macrophytes, naturally present in an aquatic ecosystem, are well-known for their ability to uptake nutrients, heavy metals and other pollutants (Herath and Vithanage, 2015 and Rai, 2018). Some aquatic plants have the ability to tolerate elevated levels of heavy metals and accumulate them in high concentration in their tissues.

The process of absorbing and accumulating heavy metals or excess nutrients in harvestable parts of plants, such as roots from aqueous substrates, is known as bioaccumulation. *Phragmites* sp., *Lemna* sp. and *Typha* sp are examples of macrophytes that are highly efficient in the uptake of heavy metals and nutrients through root systems and accumulate them in the biomass reducing therefore the concentrations of these polluting elements (Kastratovic *et al*., 2013; Kastratovic *et al*., 2015 and Morari *et al*., 2015).

Lemna gibba L, commonly known as duckweed, is a small-sized freshwater floating macrophyte from the family *Lemnaceae* (Ziegler *et al*., 2015) characterized advantage of growing under varied climatic conditions with rapid growth rates (Ziegler *et al*., 2015). The individual plant consists of a leaf-like structure or frond, connected to a fine rootlet (Landlot, 1986). The species inhabits stagnant to gently flowing surface waters and reproduces mainly vegetatively. *L. gibba* has been used in many countries across the world for the removal of nutrients and heavy metals from wastewater and constructed wetlands (Khellaf and Zerdaoui, 2009 and Radic *et al*., 2011). Several studies have shown that duckweed plants are able to efficiently remove and accumulate large amounts of nutrients (Zhao *et al*., 2015; Chen *et al*., 2018 and Ceschin *et al*., 2019) and heavy metals like nickel, copper, cadmium, zinc, manganese, boron, uranium and arsenic from domestic and agricultural wastewater (Zayed *et al*., 1998; Bianconi *et al*., 2013; Chuidioni *et al*., 2017 and Bokhari *et al*., 2019). These bioaccumulation properties make the *Lemna* sp. a very good contender for phytroremediation approaches of polluted water.

Lebanese water bodies are facing severe progressive deterioration of water quality (Daou *et al*., 2018 and Shaban and Hamzé, 2018) due to increased domestic, municipal, agricultural and industrial activities, and increase in environmental degradation resulting from urbanization (IRG, 2013). This has recently been intensified due to the sudden unprecedented population increase (30%) since the beginning of Syrian conflict in 2011 with around 1.5 million refugees crossing borders into Lebanon. This has resulted in high increases in the amount of domestic sewage, industrial effluent and agricultural runoff exacerbating a pre-existing pressure on natural water bodies (MoE/EU/UNDP, 2014). Major pollutants found in Lebanese surface waterbodies include nutrients, organic compounds, toxic metals, suspended solids and microbial pathogens. The high levels of heavy metals loads have resulted in severe pollution conditions in Lebanese waterbodies particularly Litani River Basin (Haydar *et al*., 2014 and Korfali *et al*., 2014). Haydar *et al*. (2014) showed that all investigated sites are characterized by moderate to heavily polluted by Cu, Pb and Zn, heavily for Cr and Ni in the sediments of Quaraoun Lake. Korfali *et al*. (2014) conducted a study to estimate the levels and sources of heavy metals in soils of Upper Litani Basin. Data revealed the following average levels of some heavy metals in soils with high percentage of samples exceeding the international guidelines notably Mn (593 mg/kg), Ni (98 mg/kg) and Cr (143 mg/kg). As well, Abi Saab *et al*. (2018) found that the heavy metal pollution (Cu, Pb, As and Ni) in the influent water of a constructed wetland on the Litani River significantly exceeded the range of the environmental limit values (EPA, 2000). The observed high levels of heavy metals and other pollutants are the outcome of the discharges of untreated wastewaters directly into rivers. Only a few operational wastewater treatment plants exist in certain areas of the country and the efficiency of the management is still considered inadequate (GEF/ World Bank/Plan Bleu, 2015).

Phytoremediation efforts are recently introduced in Lebanon represented in a pilot constructed wetland in the Upper Litani River basin (free water surface), the first of its type in the country, and another small wastewater treatment station (vertical subsurface flow) of a small town (Bcharreh) towards the north of Lebanon (pS-Eau, 2018). Both projects are based on the use of *Phragmites australis* as the primary macrophyte. Performance assessment studies have clearly showed high removal capacities of nutrients, heavy metals and various pollutants (LRBMS, 2012; Amacha *et al*., 2017 and Abi Saab *et al*., 2018). However, due to the rapid vegetative reproduction and colonization capacity of *P. australis*, the wetlands are becoming fully dominated by this species and negatively affecting the purification capacity of constructed wetlands. Frequent harvesting can mitigate this problem, but the removal of large amounts of biomass raise a serious concern of its management (Wang *et al*. 2018). Alternatively, *Lemna* species hold immense potential for both nutrient and heavy metals recovery and easier management through utilization as fodder or feed for livestock including cattle, poultry, and fish among others (Gupta and Prakash, 2014 and Shammout and Zakaria, 2015). The plant is recognized to have a nutritive value that is comparable to that of soybeans, to be a rich source of proteins (up to 45%, on dry mass basis) and to contain a good array of essential amino acids (Gupta and Prakash, 2014 and Shammout and Zakaria, 2015). Given this additional advantage, it becomes highly necessary to explore the potential of a native *Lemna* sp. such as *L. gibba* for phytoremediation purposes of Lebanese polluted fresh waterbodies being an indigenous species in Lebanese freshwater ecosystems (Tohme and Tohme, 2014). The species is found naturally growing mainly in stagnant and slow flowing rivers and ponds including some highly contaminated water bodies such as Litani River and other water bodies in Bekaa region (Ismail *et al*., 2009 and Abou-Hamdan *et al*., 2014). This is particularly important as the implementation of phytoremediation of contaminated water in semi-arid environments such as East Mediterranean countries, is believed to be less feasible than in humid regions with more favorable climate for plant growth (Piha *et al*., 1995 and Padmavathiamma *et al*., 2014). To date, constructed wetland pilot projects in Lebanon have been limited to the use of *P. Australis* not taking advantage of other indigenous species such as *L. gibba* L.

In this context, the current study was designed to characterize the aquatic habitat of Lebanese duckweed, *L. gibba* L. in terms of water quality and to assess its *in situ* bioaccumulation potential of heavy metals of polluted water surface fresh water under local field conditions. Findings will contribute to advancing knowledge of phytoremediation of water taking into consideration the site specificity of this sustainable and environmentally friendly water treatment technology.

MATERIALS AND METHODS

Study Sites

During the preparatory phase of this study (2016-2017), several field surveys on different surface waterbodies in Upper Litani River basin and Qaraoun reservoir where Lemna plants was previously found (Abou Hamdan et al., 2014) were conducted to visually assess the relative abundance of L. gibba according to the Braun-Blanquet scale (1932). Sites that had high relative abundance of >50-75% were selected for water and Lemna sampling. Figure 1 and figure 2 illustrate the names, location and coordinates of these sites. These sites are namely: Fayda (Site 1), Ghouzeil River (Site 2), Rawda (Site 3), Haouch ElHarimeh (Site 4), Khiara (site 5), Jebjanine constructed wetland (Site 6) and Qaraoun after dam area (Site 7) were selected for the study (Fig. 1). The identification of *Lemna species* was based on the morphological taxonomic keys of Landolt (1986) and Les *et al*. (2002). This identification was further confirmed through standardized molecular biology techniques (DNA sequencing) at the Biodiversity and Functional Genomics Laboratory, Faculty of Sciences, Saint Joseph University, Sciences and Technology Campus.

Upper Litani basin is situated in Bekaa region that is located between two mountain ranges, Mount Lebanon to the west and the Anti-Lebanon to the east. It consisted in main agricultural areas and natural areas; its fertile soil is the country's prime agricultural region. Climate in this region is characterized by typically Mediterranean climatic conditions. The average annual precipitation ranges from 600 to 800 mm in west Bekaa. Temperature is seasonal, hot in dry summer (average 35 °C) and cold during winter (average 7 °C). The relative humidity ranged from 50 % in summer and 70% in winter in average (Data Climatique, LARI, 2016).

Water habitat characterization

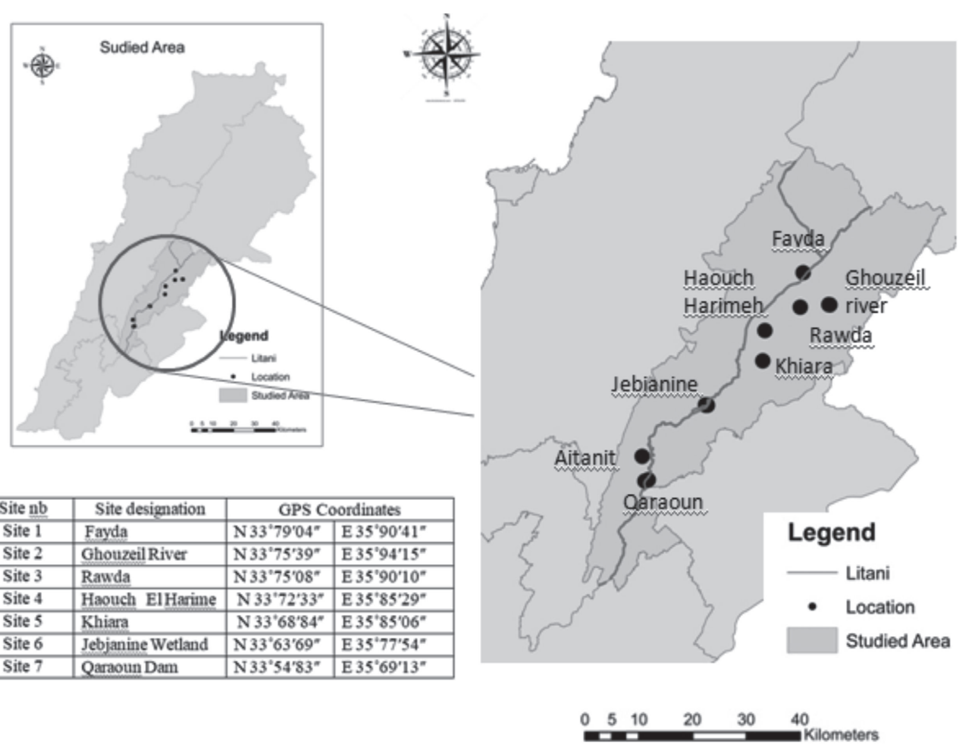
Three water samples were collected from each site for physical and chemical analysis, directly at subsurface points, in polyethylene bottles and then transported to the laboratory in cold storage. Similarly, three plant samples were collected directly from the water surface and kept in ice boxes to be transported to laboratory for analysis.

Upon arrival to the laboratory, water samples were filtered and divided in two parts, one for physico-chemical parameters analysis and the other (acidified with nitric acid) stored at 4 °C, for heavy metals (lead, cadmium, arsenic, chromium, copper, nickel, manganese and zinc) level determination. Analytical work followed the standard procedures and methods (APHA, 21st Edition, 2005). Each sample was analyzed in three replicates.

Temperature and dissolved oxygen were measured onsite using a dissolved oxygen/temp-meter (GondoEzodo PDO-408, Taiwan) as well as turbidity assessed by a turbiditymeter (LT Lutron TU 2016, Taiwan). Electrical conductivity (EC) was recorded using a SensIon 7 HACH conductivity meter (HACH, 2005). The pH is measured using a pH Meter SensIon 7 HACH, while alkalinity is determined by titration using sulfuric acid standard solution (0.02N). Calcium and magnesium hardness are also titrated by EDTA titration method. Nitrates, sulphates, phosphates and chlorides are analyzed using a spectrophotometer DR 2800 HACH. Chlorophyll a in water was extracted by dimethyl sulfoxide and acetone method and measured by spectrophotometry (Jeffry and Humphery, 1975 and Burnison, 1980).

Bioaccumulation analysis

Collected duckweed plants were washed thoroughly with distilled water, blotted, and oven-dried at 70°C for 48 h. Each sample was then digested with 10 mL ultrapure nitric acid. After digestion, the volume of each sample was diluted to 25 mL using deionized water. Subsequently, heavy metals (lead, cadmium, arsenic, chromium, copper, nickel, manganese and zinc) were determined by Thermal Atomic Absorption spectrometry (AA 6300 "Shimadzu"/ Graphite Furnace).



Site nb	Site designation	GPS Coordinates	
Site 1	Fayda	N 33°79'04"	E 35°90'41"
Site 2	Ghouzeil River	N 33°75'39"	E 35°94'15"
Site 3	Rawda	N 33°75'08"	E 35°90'10"
Site 4	Haouch El Harime	N 33°72'33"	E 35°85'29"
Site 5	Khiara	N 33°68'84"	E 35°85'06"
Site 6	Jebjanine Wetland	N 33°63'69"	E 35°77'54"
Site 7	Qaraoun Dam	N 33°54'83"	E 35°69'13"

Fig. 1. Study sites in the Upper Litani Basin and Qaraoun Lake.

Fig. 2. *Lemna gibba* growing naturally in polluted Lebanese waterbodies (site 4).

The bioaccumulation factor (BAF) of each element was calculated as the ratio between the metal concentration in plant tissue and its concentration in water (Zayed *et al*., 1998) following the below formula:

BAF = concentration of metal in plant tissue (mg/kg)/concentration of metal in water (mg/L).

Statistical analysis

The comparison of means of the considered variables between different sites is computed using one way analysis of variance (ANOVA). The Statistical Package for the Social Sciences statistical software package version 22 (IBM SPSS Inc., Chicago, IL, USA) was used for all computations and a p value < 0.05 was considered to be statistically significant.

To characterize the aquatic habitat of *L. gibba* L., water quality variables were elucidated by the principal component analysis (PCA) and the factor analysis (FA). Both were conducted to examine the underlying patterns or relationship for quality variables and reduce the dimensionality of data set into a smaller set of factors or components for prediction purposes on the most meaningful parameters sites (Tao *et al*., 2016).

In order to run the analysis, the correlation matrix among the physicochemical variables was statistically examined to justify undertaking factor analysis. The χ^2 of the measured values of parameters for the Bartlett test of sphericity was significant at $p<0.05$, and the Kaiser–Meyer–Olkin test showed a score of >0.6, indicating that the correlation among the variables was sufficiently strong for the PCA and the factor analysis (Tao *et al*., 2016). The obtained PCs were subjected to Varimax rotation (orthogonal transformation) with Kaiser's normalization to maximize difference between variables and facilitate smooth interpretation of data (Tao *et al*., 2016). Factor loadings indicated the strength and characteristics of water quality. Based on their absolute magnitude, factor loading values greater than 0.75, between 0.75-0.5 and 0.5-0.3 were classified as strong, moderate and weak, respectively (Tao *et al*., 2016). Positive factor loading shows that the contribution of the variables increases with the increasing factor loading in dimension. Negative factor loading on the other hand indicates a decrease of contribution of the variable with increasing loading.

To study the relation between the bioaccumulation factor of trace metals and water quality characterizing *L. gibba* habitat in study sites, Pearson correlation analysis was applied to assess the correlation of the bioaccumulation with factor scores of identified water quality components characterizing the *L. gibba* aquatic habitats in the tested sites.

RESULTS AND DISCUSSION

Water habitat characterization

Results of characterization of *L. gibba* habitat in terms of physiochemical and biological properties of water quality of the sites under study are given in Table 1. Statistically significant differences were revealed by ANOVA ($p<0.05$) to exist among the determined variables, which indicates a considerable variability in the aquatic habitat. The water collected from different sites exhibited significant diversity in EC values; the highest is for site 6 (1137.67 mS/cm) followed by site 5 (949.3 mS/cm) and site 3 (775.67 mS/cm) as presented in Table 1. These differences could be attributed to both natural biogeographic factors and anthropogenic pressure that structure habitats of the plant (Rameshkumar *et al*., 2019), which confirms the capacity of the plant to thrive over a wide range of habitat quality. The pH values significantly differ between sites and fell in the range between 6.63 in site 2 and 8.7 displaying a slight shift to moderate alkalinity in site 7 that could be mostly attributed to the presence of carbonates and bicarbonates in the bedrocks of tested waters (Saadeh *et al*., 2012 and Rameshkumar *et al*., 2019). High levels of EC and Cl^- ranging from 501.67±36.3 to 1137.67±79 mS/cm and 40.33±1.66 mg/L to 260.33±1.45 mg/L respectively were noted. These values exceeded the limit standards for irrigation water set by FAO (2011). This indicates the presence of high mineralization levels that may be originated from different anthropogenic pollution, agricultural runoff and wastewater sources in addition to natural bedrocks and sediments (Water Research Center, 2014). Elevated concentrations of EC and Cl^- in streams (values that exceeding 3 mS/cm and 10 mg/L respectively, FAO, 2011) can be toxic to some aquatic life. With respect to PO_4, prevailed concentrations varied between 0.24±0.06 mg/L and 6.81±0.40 mg/L with all values exceeding 0.1 mg/L, the maximum acceptable level to avoid accelerated eutrophication (FAO, 2011). Phosphorus originating from wastewater and treatment facilities, nutrient runoff from crop land and river bed sediment enacts a unique and important role in determining the trophic status of receiving waters. This aspect is justified by the high levels of Chl a (33.0±0.77 and 125.23±2.2 µg/L) and turbidity (2.75±0.07 and 57.07±2 NTU) at the studied sites (Table 1) indicating eutrophic to hypereutrophic conditions (Carlson and Simpson, 1996). The high turbidity levels ensued most probably from the high organic loads, the discharges of untreated domestic and agricultural wastewaters and by effluents of poorly treated wastewater from treatment facilities usually lead to major depletion of DO levels and increased oxygen demands, and reduction in the amount of light reaching water sediments (Rameshkumar *et al*., 2019). All these effects can inhibit photosynthesis and growth of submerged aquatic plants and result in further reduction in DO below 5 mg/L which adversely affects aquatic ecosystem (FAO, 2011). In the view of the above discussed water quality properties, it is evident that the water of the studied sites are encountering quality deterioration. This deterioration is most probably the outcome of domestic sewage, extensive agricultural activities and livestock farms that characterizes the Bekaa region coupled with the inadequate wastewater treatment efforts facing major challenges (Abbas *et al*., 2017). Most towns and villages in study area (Upper Litani Basin) dispose their untreated wastewater directly to nearby rivers or streams. The quality deterioration herein indicated are in line with those of previous reports confirming the quality deterioration of sampled water bodies (Saadeh *et al*., 2012; Haydar *et al*., 2014 and Amacha *et al*., 2015). Furthermore, the high temperatures and the low amount of precipitation coupled with intense evapotranspiration affect Upper Litani Basin during summer months (LRBMS, 2011a); salinity and other pollutants become more concentrated. This renders most waterbodies in the basin inappropriate for irrigation and unsuitable for the most aquatic living organisms. Nevertheless, macrophytes used in phytoremediation are recognized to tolerate high levels of different pollutants. The characteristics of water habitat of this study clearly reveal the high capacity of *L. gibba* to thrive in such highly eutrophic levels as discussed above (Table 1). This is in line with previous studies where they reported a high capacity for *Lemna* sp. to grow in water with relatively high levels of nitrogen, phosphorus, and potassium and to concentrate minerals and synthesize proteins (Gupta and Prakash, 2014 and Kastratovic *et al*., 2015). Specifically, phosphorus availability is recognized to positively influence the growth rate and biomass

Table 1. Mean values and standard errors of water quality parameters of the study sites.

Water parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Temp (ºC)	21.33±0.24^{bg}	19.1±0.18^{d}	21.33±0.30^{bg}	22.77±0.35^{c}	21.6±0.17^{bg}	19.8±0.07^{a}	23.5±0.15^{e}
DO (mg/L)	1.29±0.01^{d}	1.39±0.05^{e}	1.02 ±0.04^{bc}	1.08±0.04^{c}	0.98±0.02^{b}	0.76 ±0.03^{a}	0.87±0.01^{f}
Turb (NTU)	19.71±1.37^{d}	12.80±0.44^{b}	12.53 ± 0.13^{b}	2.75±0.07^{c}	4.34±0.31^{c}	57.07 ± 2.0^{a}	11.68±0.66^{bc}
Chl a (µg/L)	43.08±0.98^{d}	33.0±0.77^{e}	89.87±2.55^{b}	125.23±2.18^{c}	36.33±1.80^{e}	111.68±1.9^{a}	17.74±1.06^{f}
pH	6.7±0.09^{a}	6.63±0.0 ^{a}	6.74±0.03^{a}	6.88±0.01^{a}	6.81±0.02^{a}	6.9±0.07^{a}	8.70±0.04^{b}
E C (mS/cm)	646±4.04^{bd}	632.3±36.18^{d}	775.67±53.47^{b}	756±44.06^{bd}	949.3±27.97^{c}	1137.67±79.0^{a}	501.67±36.3^{e}
Tot hard (mg $CaCO_3$/L)	353±0.09^{a}	307±0.09^{d}	367±0.09^{ab}	383±0.07^{b}	467±0.09^{c}	357±0.07^{ab}	230±0.06^{e}
Ca^{2+} hard (mg $CaCO_3$/L)	150±0.10^{d}	250±0.00^{c}	287±0.03^{b}	263±0.09^{c}	323±0.09^{a}	333±0.09^{a}	173±0.07^{e}
Mg^{2+} hard (mg $CaCO_3$/L)	203±0.14^{d}	57±0.09^{abe}	80±0.10^{b}	120±0.15^{c}	143±0.09^{c}	23±0.03^{a}	57±0.03^{abe}
Alka (mg $CaCO_3$/L)	233±0.06^{c}	277±0.09^{d}	320±0.06^{b}	250±0.06^{cd}	266±0.09^{d}	420±0.20^{a}	127±0.07^{e}
Chloride (mg/L)	248±3.60^{cd}	195.33±8.67^{b}	199±6.03^{b}	260.33±1.45^{c}	40.33±1.66^{e}	126.83±2.9^{a}	220±13.43^{fg}
Sulfates (mg/L)	2.67±0.33^{c}	70±1.15^{e}	31.33±0.33^{b}	28.33±0.33^{b}	73.67±0.88^{d}	57.67±1.45^{a}	25±1.53^{f}
Phos (mg/L)	0.24±0.06^{c}	1.45±0.09^{c}	4.58±0.18^{b}	0.52±0.02^{c}	0.41±0.02^{c}	6.81±0.40^{a}	1.22±0.09^{c}
Nitrates (mg/L)	0.33±0.03^{c}	0.8±0.06^{a}	0.87±0.03^{a}	1.43±0.09^{b}	1.7±0.11^{b}	0.67±0.07^{a}	0.67±0.03^{a}

Superscript a, b, c, d, e, f, g indicate homogenous value subsets that significantly differ by ANOVA.

which may be the case of the study (Appenroth and Adamec, 2015 and Ceschin *et al*., 2019). However, nitrates in this study exhibited low to moderate levels (ranging from 0.33±0.03 to 1.70±0.11 mg/L) coinciding within the range of category on irrigation requirements according to FAO (2011). The low nitrate levels could be explained by the dynamics of nitrogen species favouring reduced nitrogen species under reducing conditions associated with the low levels of dissolved oxygen (Rai, 2009). Previous studies assessing nitrates, nitrites and ammonia in surface water bodies in Upper Litani River Basin have indicated that the high levels of N mainly inorganic species were due to the heavy agricultural and domestic pollution load in the basin (Haydar *et al*., 2014; Amacha *et al*., 2015; Amacha *et al*., 2017 and Abou Hamdan *et al*., 2014).

The PCA revealed three different components of pollution levels of the different *L. gibba* habitats, which collectively explained 77.9% of the variance. Factor loadings of the three components are shown in Table 2 noting that alkalinity and Ca hardness were excluded for their high correlation coefficient (r>0.8) (data not shown).

Component 1 accounting for 35.9 % of all variance was positively and highly associated with phosphates (loading +0.949) and turbidity (loading +0.910). This component was only moderately influenced by Mg hardness (loading -0.745). Such loadings may be associated with high agricultural runoff and soil erosive phenomena of natural flooding frequently occurring during wet seasons in sites having the highest scores on this component, site 3 (Rawda) and 6 (Jebjanine wetland) (Table 1) which are found in proximity of flood-prone area parcels.

Table 2. Factor loadings matrix for the three components derived from the physicochemical properties of the study sites. Bold font values > 0.75 and between 0.75-0.5 represent strong and moderate loadings, respectively.

Variables	Components		
	1	2	3
PO_4	**0.949**		
Turbidity	**0.910**		
Mg Hardness	**-0.745**		0.347
Chlorophyll a	0.417	0.241	
NO_3	-0.381	**0.850**	
Cl^-	-0.221	**-0.825**	
Total Hardness		**0.824**	0.418
E C	0.590	**0.722**	0.217
SO_4	0.267	**0.681**	
pH		-0.337	**-0.922**
Temperature	-0.516		**-0.738**
DO	-0.531	-0.367	**0.691**
% Variance	35.9 %	25.1 %	16.9 %

Component 2 accounting for 25.1 % of the total variance was strongly affected by nitrates (loading +0.850), total hardness (loading +0.824) and chloride (loading -0.825). This component was also moderately influenced by sulphates (loading +0.681) and conductivity (loading +0.722). This indicates that the water parameters characterizing the quality of *Lemna* habitat originates from organic and inorganic pollutants discharged from agricultural and domestic origins. This is confirmed by the sites having the highest scores on this component, site 4 and 5 having the highest levels of nitrates and the lowest levels of Cl^- (Table 1) which are receiving agricultural and domestic effluents.

Component 3 accounting for 16.9 % of the variance was influenced by high negative loading of pH (loading -0.922) and temperature (loading -0.738) and moderate association with DO (loading +0.691). This component accounts for the organic and biological characteristics of the water quality variables. The studied sites received organic effluents from domestic and municipal wastewaters; this input of organic material contributes to the alteration of water quality as indicated in sites 1 and 2 with high loading scores, having the highest levels of DO and lowest pH (Table 1).

It is worthwhile to stress that more systematically observed data should be used in this analysis to obtain higher certainty in the distinctions and explanation of the physical nature of significant variables (variables with high loadings) in the components (e.g. PO_4 and turbidity in component 1 in Table 2). Actually, the characterization of water quality herein was limited to one dry season campaign and a narrow samples size. However, the output still provides reliable information that indicates differences in ranking of the relative importance of the particular variables of every component of the *L. gibba* habitat compared to the classical approaches.

Levels of heavy metals in water

Levels of assessed heavy metals (i.e. Pb, As, Cd, Cr, Cu, Mn, Zn and Ni) presented in Fig. 3 demonstrate statistically significant spatial variations by ANOVA ($p <0.05$) among sites under study. This diversity may be attributed to both natural influences and the fluctuations in the amount of agricultural run-off discharge and sewage effluents released into the waters or the weathering of minerals and soils (Table 1). Among the assessed heavy metals, As, Cd and Mn exhibited high to moderate values (9.88±1.05 µg/L, 28.18±1 µg/L and 197.74±63.6 µg/L, respectively) (Fig. 3) that exceeded the limits set by the US-EPA (2000) for surface water and FAO (1994) for irrigation water. This may be associated with the excessive application of pesticides and fertilizers. Metals such as Cd and As are non-essential toxic elements and are major environmental pollutants. They are spread by industrial activities and agricultural fertilizers and pesticides (Mishra *et al*., 2019).

On the other hand, the levels of Zn, Ni and Cu contents fall within the permissible limits of FAO (1994) and EPA (2000), this could be attributed to being scavenged by precipitation and transfer to sediments (Matagi *et al*., 1998) or uptake by *L. gibba* and other aquatic plants as indicated in the high bioaccumulation levels shown herein (Fig. 5) and in other studies. Likewise, the occurrence of Pb may be associated with fumes emanating from vehicles on roads in proximity of the watercourses in sites 1, 3 and 4 having significantly higher levels when compared with other sites (Fig. 3). Some previous studies assessed the levels of heavy metals in the Litani River Basin watercourses. Haydar *et al*. (2014) investigated the water and sediments of Upper Litani Basin and Quaraoun Lake; moderate levels of Cu, Pb and Zn, and high levels of Cr and Ni were reported. Higher levels of heavy metals in our study were found and this is most probably due to the continuous heavy loading of pollution levels. In addition, the results of Korfali *et al*. (2006) revealed high levels of heavy metals in sediments of Quaraoun Lake, notably Pb (18.39 mg/kg), Cu (15.51 mg/kg), Zn (11.69 mg/kg) and Ni (10.3 mg/kg). Our results were comparable to the findings of a study conducted by the Litani River Basin Management Support Program (LRBMS, 2011b) that reports high levels of Mn (0.27 mg/L) and Cd (0.079 mg/L) exceeding the permissible thresholds set by the USEPA (2000). Similarly Jurdi *et al*. (2002) found high levels of heavy in water sampled from Quaraoun Lake, Zn (0.182 mg/L), Cr (0.042 mg/L), Cu 0.032 (mg/L) and As (0.025 mg/L).

Heavy metals in plant tissues

As shown in Fig. 4, plants of *L. gibba* growing naturally in the study sites accumulated different levels of heavy metals in their tissues suggesting different accumulation capacities of *L. gibba* for different heavy metals (Velichkova, 2019). The accumulation values varied between 4138.13 and 82.28 µg/kg and followed the descending order of Zn>Cu>Mn>Ni>Pb>As>Cr>Cd. The values recorded 4138.13±142.14 µg/kg for Zn, 939.4±9.45 µg/kg for Cu, 791.63±7.16 µg/kg for Mn and 391.01±6.43 µg/kg for Ni displaying higher values than normal averages in plants growing in uncontaminated natural environments (Kabata-Pendias and Pendias, 2001). Lead, As, Cd and Cr had the values of 335.92±18.3 µg/kg, 297.36±9.02 µg/kg, 113.07±2.14 µg/kg and 82.28±2.6 µg/kg, respectively. These results are in line with other studies confirming the capacity of *L. gibba* to accumulate heavy metals. As indicated by ANOVA, levels of each metal varied significantly between the studied sites (Fig. 4). These variations may be due to the difference of biotic and physical factors of the habitat that affect the mobility and bioavailability of heavy metals at the different sites (Favas *et al*., 2014).

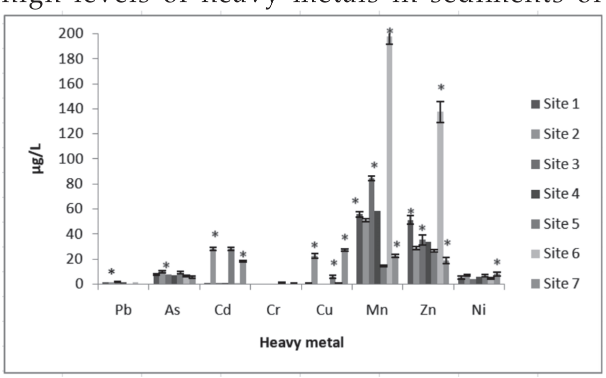


Fig. 3. Heavy metals contents in water samples in µg/L represented by the mean values and standard errors. Superscript * indicate significantly different by ANOVA.

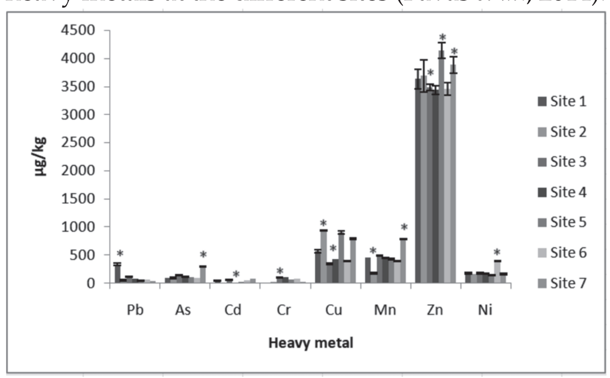


Fig. 4. Heavy metals contents found in *Lemna* plant tissues with values presented in µg/kg. Superscript * indicate significantly different by ANOVA.

Numerous studies in different countries under both field and laboratory conditions showed comparable levels of accumulation of the plant affirming its high uptake capacity and suitability for the phytoremediation of heavy metals in polluted surface water as well as industrial and mining wastewater (Ziegler *et al*., 2019). In a very recent study, high bioaccumulation levels of 427 mg/L for Ni, 293 mg/L for Pb, and 105 mg/L for Cd of municipal effluent in Pakistan were reported (Bokhari *et al*., 2019). According to the authors, the high biomass production of *L. gibba* during the remediation period in the mentioned study resulted in enhanced bioaccumulation and uptake of significant amounts of Ni, Pb, and Cd from both industrial and municipal effluents under study. Similar findings were also obtained with As and Cd growing in mining water in Turkey (Sasmaz *et al*., 2015; 2019).

Noteworthy, the plant tissues had accumulated considered levels of Zn, Cu, and Ni, and Pb in the plant tissues (4138 μg/kg, 939.4 μg/kg, 391.01 μg/kg and 335.92 μg/kg respectively) that may underline their low levels in water which fell within the permissible levels (Fig. 3). This finding is in accordance with the findings of Sasmaz *et al*. (2015) who reported Zn accumulation level at a 1146 magnitude in the plant as compared to that of water in mining water in Turkey.

The Pearson correlation analysis between the heavy metal levels in water and the concentrations in plant tissues (data not shown) revealed significant positive correlation with Cu ($r=0.731$, $p < 0.01$) and a negative significant correlation with As ($r=-0.497$, $p < 0.05$). Other positive/negative correlations, however not significant, with Pb, Cd, Cr, Mn, Zn and Ni were also found. This may suggest that *L. gibba* accumulation is obviously a characteristic provided by its capacity for the accumulation of each individual element (Favas *et al*. 2014).

Bioaccumulation of heavy metals in plant tissues

According to ANOVA analysis, statistically significant differences in the BAFs per metal are found between the studied sites (Fig. 5). The observed spatial differences could be attributed to variations in habitat characteristics and types and levels of pollution between sites affecting the bioavailability of heavy metals uptake (Mkandawire and Dudel, 2005). The values of bioaccumulation factor, representing the ratio of metal in plant tissues to that of water habitat and reflecting the phytoremediation potential of *L. gibba*, are presented in Fig. 5. A descending order Cu>Cr>Pb>Zn>Ni>Cd>As>Mn was observed. The highest BAF of Cu reached the value of 1068.95 in site 1 while the lowest BAF values were obtained for Mn (2.52) in site 6 (Fig. 5). This variability in bioaccumulation of heavy metals could be caused by the initial availability of the metal in water to the plant and to the biology and ecology of the plant species (Bokhari *et al*., 2016). In addition, in our study, the difference in the order of the metal levels in *L. gibba* compared to the sequence of their BAFs is noted further suggesting the different accumulation capacity of *Lemna* sp. for certain metals (Kastratovic *et al*., 2015). In a previous study the BAF of Mn of *L. gibba* in Bulgarian water reservoirs was found to be thousand times higher than that of several other metals (Velichkova *et al*., 2019). Similarly, high BAF values for Ni (24), Pb (23.5) and Cd (17.4) from industrial and municipal effluents in Pakistan were also reported (Bokhari *et al*., 2019). The authors implied that metal dynamics is primarily controlled by geochemical and biological factors affecting heavy metals mobility and bioaccumulation capacity by the plant (Bokhari *et al*., 2019). In addition, Hegazy *et al*. (2009) reported a rank of heavy metals BAF of Zn, Cr, Pb and Cu by *L. gibba* for according to the preference for bioaccumulation with a BAF of 13.9, 6.3, 5.5 and 2.5, respectively in industrial wastewater in Egypt.

Table 3 describes the associations of the factor scores of quality components as obtained by the PCA of water quality variables with the BAFs of heavy metals presented in Table 2 and Fig. 5, respectively. Scores of component 1 had a significant positive correlation (r= 0.899, at p< 0.05) with BAF of Ni suggesting that BAF of Ni is strongly influenced

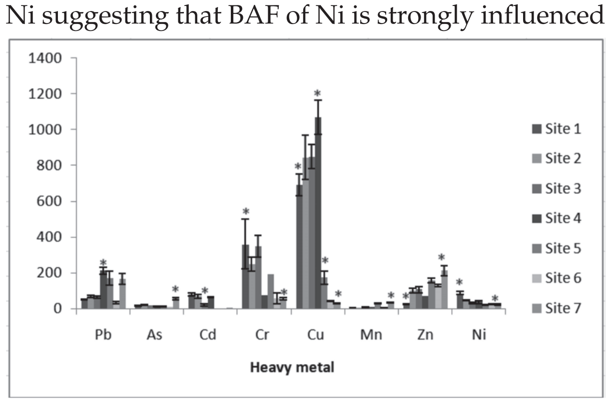


Fig. 5. Bioaccumulation factor of heavy metals ratios in plant samples represented by the mean values and standard errors. Superscript * indicate significantly different by ANOVA.

by the presence of phosphate and turbidity. Scores of component 3 characterized by strong-moderate loadings of pH and temperature and DO also had significant negative association with the BAFs of As and Mn (r=-0.920, at p< 0.01 and r= -0.776, at p< 0.05, respectively). Other correlations, however, not statistically significant were also found. This may apparently suggest that the accumulation of heavy metals by aquatic plants in natural conditions is influenced by a number of abiotic factors such as pH, presence of other cations, temperature, as well as biotic factors such as the hyperaccumulation capacity, tolerance and detoxification forms by a plant along with other factors (Matagi *et al*., 1998 and Mkandawire and Dudel, 2005).

Several studies elucidated the effect of physiochemical variables on the accumulation of heavy metals by duckweed. The arsenic bioavailability varies with the pH in the milieu, nature of minerals constituting the sediments, and presence of competing ions (Mkandawire and Dudel, 2005). Phosphate has been earlier shown to decrease the uptake of arsenic in *L. gibba* L. from aquatic environment (Mkandawire *et al*., 2004). Mkandawire and Dudel (2005) also reported that *L. gibba* increased its uptake of As with the increase in As in the medium but decreased with the increase of phosphorus in the medium. Rai (2009) stated that the increase in pH and prevailing reducing conditions (low DO levels) in the aquatic environment results of decrease in availability of heavy metals to plants. According to various authors (Kaur *et al.*, 2013; Verma and Suthar, 2015 and Velichkova, 2019), there is a correlation between the values of pH, temperature and the accumulation of heavy metals by *L. gibba*. As well, the measured pH (6.27-6.46) and temperatures (22-23 °C) and a concentration of 0.04-0.68 mg/L nitrate ions in water reservoirs increase the accumulation capacity of the *L. gibba* for heavy metals (de Souza *et al*., 2019 and Velichkova, 2019). Moreover, several authors indicated that optimum plant growth is obtained in the pH range of 6-7.5 where the plants were able to accumulate high levels of heavy metals and therefore reduced considerably the metals initial concentrations in the aqueous media (Mkandawire and Dudel, 2005; Khataee *et al*., 2012 and de Souza *et al*., 2019).

The above discussion confirms the importance and the high potential of *L. gibba* L. for the phytoremediation of heavy metals of polluted Lebanese waters under field conditions, *L. gibba* can be recommended in constructed wetland systems as an alternative or complementary component with *Phragmites australis*, already used in the phytoremediation pilot projects in Lebanon (LRBMS, 2012; Amacha *et al*., 2017 and Abi Saab *et al*., 2018). The rapid growth rate and easy management make the plant an ideal candidate. Its potential use as a feed source for livestock, poultry and aquaculture, as well as an energy source for biofuel production and metal recovery through phytomining can further support the multi-benefits and suitability for phytoremediation.

CONCLUSION

Although this study is limited to one dry season campaign and a narrow samples size, it describes comprehensively the habitat of *L. gibba* in several sites in Lebanon in terms of phosphates, turbidity and chlorophyll a, and investigates, for the first time in the country, the bioaccumulation capacity of this native duckweed species for heavy metals. This study showed that *L. gibba* subsists over a wide range of nutrient levels indicating its adaptability to various aquatic eutrophic ecosystems. Further studies are necessary to investigate the usefulness of *L. gibba* as ecological indicators that show direct response to metal concentrations present in aquatic ecosystem. Our findings also confirmed the ability of *L. gibba* to efficiently accumulate a large span of heavy metals prevailing in the Lebanese water bodies, and therefore its potentiality to be used effectively in the reduction of pollutants. Additional investigations are required to use practically this duckweed species, alone or in association with other macrophytes, for *in situ* phytoremediation of local

Table 3. Correlation between factor scores of the different patterns and bioaccumulation of heavy metals in *L. gibba* plant tissues. Superscript indicate significantly different by ANOVA, at the levels of *p < 0.05; ** p < 0.01.

Scores	Pb	As	Cd	Cr	Cu	Mn	Zn	Ni
Component 1	-0.530	-0.008	0.597	0.553	0.067	-0.416	-0.564	0.899*
Component 2	-0.105	-0.381	-0.179	0.265	-0.157	0.191	-0.015	0.060
Component 3	-0.242	-0.920**	0.332	0.001	0.429	-0.776*	-0.662	0.186

aquatic environments contaminated by the anthropogenic activities.

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