ASSESSING THE ALUMINIUM PHYTOACCUMULATION POTENTIAL OF *CAROLINA FANWORTH* SPECIES (*CABOMBA CAROLINIANA* A. GRAY) IN EXPERIMENTAL STRESS CONDITIONS

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Cabomba caroliniana submerged aquatic plants species were grown in experimental aquariums conditions, at different 0 - 1,000 mg/kg Al concentrations. The evolution of several water parameters was monitored and determinated of tissular aluminium and photosynthetic pigments concentrations were performed. Aluminium hyperaccumulation in Cabomba caroliniana species occured at background concentrations of 500-1,000 mg/kg (1,209-3,694 mg/kg, accumulation coefficient 2.42-3.69). High Al levels decreased carotenoids content and induced abnormal variations of total chlorophyll content and oxygen production, without significant biomass decrease. Water conductivity and total dissolved solids increased, probably due to a release of organic solutes. The obtained results emphasize that this aquatic species could act as a valuable aluminium bioaccumulator, for aluminium-polluted freshwater bioremediation purposes.

Keywords: phytoaccumulation, aluminium, *Cabomba caroliniana* A. Gray, submerged aquatic plants

1. Introduction

One of the ecological issues posing an increasing interest currently is that of heavy metals pollution, a threat to natural environments, crops, livestock and human health. Plants can be affected by heavy metals toxicity. Adaptation mechanisms are variate, including metal exclusion at root level (limiting radicular uptake), root sequestration (metals are absorbed, but not translocated towards

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more sensitive tissues) or bioaccumulation in various tolerant tissues. While hazardous in vegetable food production, phytoaccumulation can have important applications, such as phytoremediation of polluted environments and phytomining [1].

All soils and waters contain aluminium, usually in low amounts. While it is not considered an essential nutrient, it can stimulate plant growth. High concentrations, however, induce toxic effects. Depending on plants species and local *p*H conditions, such effects can occur even et 2-3 mg/kg [2].

Natural aluminium sources include the relatively abundant aluminosilicate minerals (feldspars, kaolins, micas, etc.). Industry is another important source of aluminium compounds. Soils contain an average of 70,000 mg/kg, of which a small fraction is made up of soluble forms. Its bioavailability highly depends on *p*H. In freshwater bodies, aluminium can amount up to 2 mg/kg, while in acid waters it can reach 40 mg/kg [3]. The upper limit set by Romanian laws for aluminium in drinking water is 0.20 mg/kg [4].

Aluminium occurs in water as particulate and monomeric fractions, with the latter including $Al(OH)_3$, AlF_3 and $Al_2(SO_4)_3$ and aluminium-containing organic compounds. Also, $Al_2(SO_4)_3$, together with polyaluminium chloride is also a common coagulant for water treatment [3].

There are multiple possible pathways for aluminium phytotoxicity, including water absorption inhibition, through root tissue damage, lowering nitrogen, phosphorus, calcium and magnesium uptake. Aluminium excess reduces root respiration, growth of lateral roots, thickens cell walls and damages root cortex. Aluminium also prevents normal thylacoid development in chloroplasts and inhibits the synthesis of photosynthetic pigments [2].

Various studies have shown that aluminium phytoaccumulation rarely occurs in nature, most known plants being „excluders", which limit radicular uptake of aluminium ions. They do this by secreting organic acids (citric, oxalic) [5].

Cabomba caroliniana A. Gray species, commonly called Carolina fanwort, Carolina water shield, fish grass, or Washington grass, is a member of the Cabombaceae family (order Nympheales). A herbaceous, submerged plant, it grows in shallow freshwater bodies (streams, small rivers, ponds, lakes, reservoirs, sloughs, ditches and canals). It grows well in high nutrient environments with low *p*H, but in more alkaline waters it tends to lose its leaves. High calcium levels inhibit growth and unlike other aquatic weeds, *Cabomba* can grow well in turbid water. It prefers a warm, humid climate with a temperature range of 13-27 ºC, but can survive also when the surface of the water body is frozen [6].

This stem has horizontal and erect stems, growing from underground rhizomes. Leaves are up to 5.5 cm long and very finely dissected (Fig. 1). Flowers

are up to 1.5 cm in diameter, white variate colors, white to purple-violet. Mature plant size is ussualy approximately 30-80 cm but may grow up to 10 m long. *Cabomba caroliniana* is an extremely persistent and competitive plant, growing quickly and crowding out other vegetation. It is known to exhibits chemical defense mechanisms against aquatic herbivores and pathogens [7].

A native of subtropical-temperate areas in eastern North and South America, it is widely grown as a decorative aquarium plant, due to its attractive leaves and flowers. It has become invasive in many parts of the world [8]. Studies emphasize that *Cabomba caroliniana* species presents some bioaccumulative properties, with valuable capacity of phytoaccumulating high levels of copper from the environment [9].

(a) (b)

Fig. 1. *Cabomba caroliniana* A. Gray plant species, in (a) decorative aquarium and (b) experimental aquariums

2. Materials and Methods

Cabomba caroliniana A. Gray plants were acquired from local aquaristic shop in Constanța, Romania. Plants were distributed in five experimental aquariums, with 15 g plant material in each (wet weight). Laboratory aquariums consisted of 5 L plastic vials filled with filtered lake water from the same added as $Al_2(SO_4)_3$ up to a total aluminium concentration of 0, 10, 100, 500, and 1,000 mg/kg, respectively.

The experimental period was 35 days. Water samples were analyzed, weekly for *p*H, total dissolved oxygen and electrical conductivity, using a water multiparameter device, *p*H-conductivity-dissolved oxygen-meter HI2020-01 Edge, Hanna Instruments and for total dissolved solids (TDS), using a 3WIN TDS-2A portable TDS-meter.

At the end of the experiment, plants were also taken for analysis. A sample of 0.1 g from each aquarium plant were ground in 80% acetone, filtered and analyzed using a WPA S106 UV-Vis spectrophotometer (absorbance at 470, 647, 663 nm wavelengths). The concentrations of chlorophyll *a*, chlorophyll *b* and total

carotenoids (xantophylls and carotins) were determined using trichromatic equations [10].

The rest of the plant samples were oven dried for three days at 80 ºC, for determining the dry biomass at the end of the experiment. A quantity of 0.25 g of each dry sample was digested overnight in 5 mL concentrated HNO_3 and boiled at 150 ºC. An aliquot of 2 mL H_2O_2 (30%) were added and boiled again. The resulting solution was diluted up to 50 mL (adding 2% NH_4Cl and 0.5% $CaCl_2$) [10-17]. Aluminium content was determined by atomic absorption spectrometry, HR-CS ContrAA700, Analytik Jena AG, Germany, acetylene-nitrous oxide flame technique, atomization temperature 2450 °C, reading at 396 nm wavelength [10, 11]. Aluminium concentrations were expressed as mg/kg.

The biological accumulation coefficients (BAC), were also calculated as ratios of tissular aluminium to water aluminium concentrations [18].

Triplicate biological and biochemical sampling was performed for each analysis. One-way ANOVA test was used to assess the significance of variations pigment concentration and metal accumulation.

3. Results

Table 1

Water physical-chemical parameters for each experimental aquarium

Water aluminium concentration (mg/kg)	*p*H	EC (µS/cm^2)	O_2 (mg/L)	TDS (mg/L)
0	9.76±0.20	1.37±0.10	8.81±2.92	186.80±30.94
10	9.35±0.24	1.30±0.11	9.01±2.76	198.40±19.88
100	5.13±0.19	1.47±0.14	8.42±1.36	230.60±29.65
500	4.10±0.12	2.56±0.26	21.39±2.25	419.40±49.60
1,000	3.85±0.17	3.73±0.42	24.20±0.65	659.20±82.23

Water *p*H, electrical conductivity, dissolved oxygen and total dissolved solids (TDS) are shown in Table 1. For comparison, control water samples (filtered lake water with no *Cabomba caroliniana* plants) have shown a 9.95 *p*H, 1.20 mS/cm^2 conductivity, 6.22 mg/L oxygen and 172 mg/L TDS. While at 0-10 mg/kg dissolved aluminium, water *p*H remained alkaline, acidification (*p*H 3.85-5.13) occured at higher concentrations. Increases in electrical conductivity (over 2 mS/cm^2) dissolved oxygen (over 20 mg/L) and total dissolved solids (over 400 mg/L) were noticed at 500-1,000 mg/kg aluminium.

The concentrations of chlorophylls and carotenoids in *Cabomba caroliniana* plant leaves are shown in Fig. 2. Chlorophyll *b* was the dominant pigment in all samples. Total chlorophylls ranged between 1,398-2,185 mg/kg, while for carotenoids, values were inversely proportional to aluminium concentration, ranging between 63-428 mg/kg.

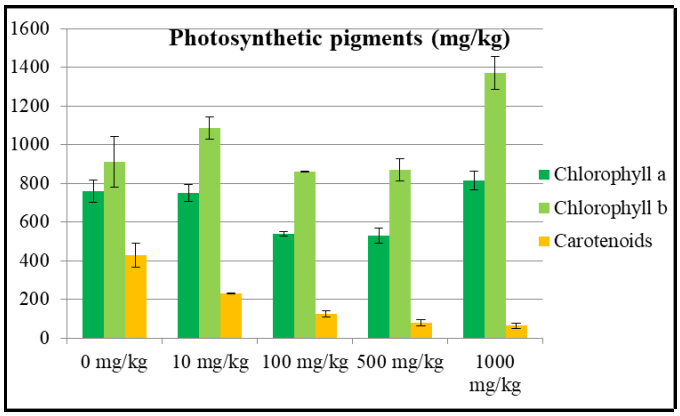

Fig. 2. Foliar concentrations of main photosynthetic pigments in *Cabomba caroliniana* plants after 35 days, for each water aluminium concentration.

Table 2

Aluminium accumulation in *Cabomba caroliniana* plants and dry biomass

Water aluminium concentration (mg/kg)	Average tissular aluminium concentration (mg/kg)	BAC	Average dry biomass (g)
0	4.64±3.33	-	0.63
10	27.78±13.58	2.78	0.69
100	350.50±28.99	3.51	0.83
500	1,209.33±392.21	2.42	0.54
1,000	3,694.00±254.56	3.69	0.63

Table 2 shows comparative aluminium concentration in tissular samples, biological accumulation coefficients (BAC) and final dry biomass. There were major differences between tissular aluminium concentrations, proportional to ambient aluminium levels. BAC and biomass showed no direct proportionality. ANOVA test results (Table 3) show significant differences among the five experimental variants concerning water parameters, foliar carotenoids concentration and aluminium uptake.

4. Discussion

All water studied quality parameters were influenced by the addition of aluminium sulfate. While the natural *p*H of Tăbăcăriei Lake water from Constanta, Romania, was an alkaline one, *p*H values decreased with the increase in $Al_2(SO_4)_3$ content, with no major differences over time. Electrical conductivity and total dissolved solids (TDS) showed increases with aluminium concentration, especially at 500-1,000 mg/kg water aluminium concentrations, with values also increasing over time.

Table 3

Results of one-way ANOVA test for selected parameters

Parameter		SS	df	MS	F	P-value	F crit
Water *p*H	Between groups	167.200	4	41.800	1190.889	<0.001	2.866
	Within groups	0.702	20	0.035			
	Total	167.902	24				
Water EC	Between groups	22.066	4	5.516	98.009	<0.001	2.866
	Within groups	1.125	20	0.056			
	Total	23.192	24				
Dissolved Oxygen	Between groups	1203.956	4	300.989	64.093	<0.001	2.866
	Within groups	93.921	20	4.696			
	Total	1297.878	24				
TDS	Between groups	818379.4	4	204594.9	89.313	<0.001	2.866
	Within groups	45815.2	20	2290.76			
	Total	864194.6	24				
Chlorophyll *a*	Between groups	216749.2	4	54187.3	3.349	0.055	3.478
	Within groups	161792.3	10	16179.23			
	Total	378541.6	14				
Chlorophyll *b*	Between groups	559236.6	4	139809.1	2.538	0.105	3.478
	Within groups	550696.8	10	55069.68			
	Total	1109933	14				
Carotenoids	Between groups	271432.6	4	67858.16	8.621	0.002	3.478
	Within groups	78710.06	10	7871.006			
	Total	350142.7	14				
Tissular aluminium	Between groups	21190127	4	5297532	113.407	<0.001	3.837
	Within groups	373700.2	8	46712.52			
	Total	21563827	12				
BAC	Between groups	2.635	3	0.878	1.040	0.440	4.757
	Within groups	5.067	6	0.844			
	Total	7.702	9				

In many cases, a decrease in TDS is linked to metal phytoaccumulation and removal from freshwater [19]. The increases found in this case can be attributed to the presence of *Cabomba caroliniana* plants and are probably due to an increase in organic solutes.

Dissolved O_2 concentrations were higher at 500-1,000 mg/kg aluminium, however they did not have the ascending evolution found at lower concentrations. This indicates an abnormal, accelerated photosynthetic process, due to physiological stress. At 10 and 100 mg/kg, the evolution of oxygen production was similar to that in the control aquarium.

The evolution of photosynthetic pigments seems to confirm a limited physiological stress: total chlorophylls showed a decrease at 100-500 mg/kg aluminium, followed by significantly higher levels at 1,000 mg/kg, while carotenoids constantly decreased with growing aluminium levels. Biomass, on the other hand, had its maximum at 100 mg/kg background aluminium concentration, while at the highest metal concentrations, they were similar to those in the control aquarium, suggesting a significant degree of physiological tolerance to high aluminium levels.

Regarding metal accumulation efficiency, there are four aspects to be dealt with. First, comparing tissular Al levels to those in the "standard reference plant" (average aluminium concentrations in known Global flora) all determined values (4.64-3,694.00 mg/kg) were significantly higher than the reference value of 0.20 mg/kg [20].

Second, the widely accepted minimal thresholds for defining aluminium hyperaccumulating plants are set at 1,000 mg/kg, while other authors propose a lower value, of 300 mg/kg [20]. In this case the only *Cabomba caroliniana* samples exhibiting higher values were those taken at 500-1,000 mg/kg, suggesting a minimal limit at which hyperaccumulation occurs, between 100-500 mg/kg ambient aluminium levels.

Third, the BAC can be used to define accumulation and hyperaccumulation. Sekabira et al. [21] use a BAC scale ranging from below 0.01 (non-accumulating plants), to 0.01-0.1 (low accumulators), 0.1-1 (moderate accumulators) and BAC>1 (hyperaccumulators). *Cabomba caroliniana* plants had BAC values constantly close to of above 1, showing a strong metal accumulation at all ambient concentrations. However, BAC have their limits as indicators. One of them is extrapolating this coefficient (primarly designed for plant-soil metal uptake) to a liquid environment, since the ratio would involve a dry mass (for plants) to water mass.

Some of the main factors enhancing metal sequestration are *p*H, which, at low values, solubilizes aluminium ions (as shown above, water samples with 100-1,000 mg/kg aluminium had a *p*H lower than 5.13) and plant exudates, which often contain organic acids and chelating factors [22]. As shown above, higher TDS values at 100-1,000 mg/kg aluminium might indicate increased production of such exudates.

Considering these values, *Cabomba caroliniana* species it is an aluminium bioaccumulator, even if not the most effective. *Cabomba pyauhyensis* (*Cabomba*

furcata) was found to accumulate up to 865.82 mg/kg tissular aluminium, but when growing at only 0.21 background concentration. *Hydrilla verticillata*, 280.82 mg/kg and *Egeria densa*, with 66.86 mg/kg were not found to be hyperaccumulators, but had notable BAC values at the same concentration [23]. *Ceratophyllum demersum* was found to have hyperaccumulative potential, with up to 1,000-4,000 mg/kg tissular aluminium at 3-9 mg/kg background concentration [24].

Thus, unlike other known aluminium hyperaccumulators, *Cabomba caroliniana* is less efficient, operating only at high background metal concentrations. However, it still fulfils five of the criteria stated by Thangavel and Subbhuraam [22] for selecting valuable hyperaccumulators: ability to grow outside the area of collection, high metal tolerance, fast growth, resistance to disease and pests and low risk of transferring metal through trophic chains.

5. Conclusions

Part of the analyzed water parameters changed over time (including dissolved oxygen, conductivity, TDS), pointing to an increase in organic solutes and to abnormal photosynthetic rates induced by high background aluminium levels. Total chlorophylls and carotenoids synthesis was affected by high aluminium concentrations, while final biomass did not show major differences, suggesting a limited phytotoxic effect and a degree of tolerance for this species.

Considering results on tissular aluminium accumulation in *Cabomba caroliniana* A. Gray plants was found at all background levels, with constantly high BAC. However, only at 500-1,000 mg/kg background concentrations surpassed the accepted minimal thresholds for aluminium hyperaccumulation. *Cabomba caroliniana* species can, thus, be considered an aluminium hyperaccumulator, but only at such environmental concentrations, while also having other qualities (such as fast growth and tolerance to a wide range of aquatic habitats) that make it suitable for phytoextractive purposes.

Thus, *Cabomba caroliniana* A. Gray species can be considered a valuable aluminium bioaccumulator, with possible applications in the bioremediation of aluminium-polluted freshwater bodies.

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