ACCUMULATION OF MACRO- AND MICROELEMENTS BY DUCKWEED (*LEMNA MINOR*) IN CONDITIONS OF LOW AND SIGNIFICANT ANTHROPOGENIC LOADING

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Abstract

Wide utilization of duckweed in phytoremediation of industrial and municipal wastewater supposes its significant role in maintaining ecological balance in polluted water bodies. Using ICP-MS method, peculiarities of macro- and microelements accumulation by Lemna minor L. in abundant flooded quarry near phosphorgypsum (PG) storage site and in unpolluted lake of Yoshkar-Ola was investigated. Duckweed biological accumulator factor (BAF) of macro-elements in conditions of elevated pollution was significantly lower than in unpolluted area. The same phenomenon was revealed for As, Sr, Cu and Ni contrary to Cr, Pb, V, Cd and Al with higher BAF values in conditions of increased pollution. The highest value of BAF (more than a million) was shown for Mn in unpolluted. During summer concentrations of P, Zn, Co, Cr. Cd, Ni, Al, Mn, Si, Cu and As in water flooded quarry decreased 13.25, 8, 4.2, 2.27, 2.0, 1.75, 1.75, 1.6, 1.44 and 1.41 times accordingly. Concentration of all microelements and heavy metals except Sr, Mn, Se and B also decreased. The period of intensive duckweed growth (summer) was accompanied by significant changes in BAF values: increase of macro-elements BAF; for Cr, Al, Pb, and V- intensive decrease of BAF values and among microelements intensive BAF increase only for Zn and Mn.

Keywords: *Lemna minor, phytoremediation, multifactorial pollution*

INTRODUCTION

Duckweed belongs to *Lemnaceae* family, and among other water plants is used most frequently for phytoremediation of industrial and municipal wastewater [1,2]. Duckweed growth is generally controlled by temperature and sunlight more than nutrient concentrations in the water. Under favorite conditions duckweed mass doubles in less than 2 days. Its growth rate is more than twice higher than growth rate of terrestrial plants [3]. Duckweed manifests itself as hyperaccumulator of Cd [4], Pb [5], B [6], Ni, Mn, P, Se [7], U, As [8,9] and Zn [1]. The intensity of water phytoremediation by duckweed exceeds that of algae and other aquatic macrophytes. The duckweed phytoremediation mechanism includes several processes: rhyzo-filtration, phyto-stabilization, phyto-extraction, formation of volatile compounds, and phyto-transformation [10]. Phyto-extraction is the most appropriate process in cleaning ponds from heavy metals. Phyto-transformation is typical for processes of pesticides, detergents, cyclic poly-aromatic compounds degradation caused by duckweed. Emission of volatile compounds may take place in conditions of Se or Hg excess in the environment.

The most effectively duckweed operates in conditions of low pollution levels, which determines the prospects of its use in reservoirs of protected areas [11] and natural parks [12]. There are many laboratory investigations on evaluation of duckweed utilization efficiency in purification of water from various contaminants [13-15]. More complex and less investigated is the question of water phytoremediation in natural conditions under multifactorial pollution. In this respect waterbodies in regions of opencast phosphorites mining and duckweed role in water systems stabilization are of special interest. Abandoned flooded carriers of opencast phosphorites mining in Voskresensk (Moscow region) are situated in the near vicinity of current phosphorgypsum (PG) storage site, as Voskresensk plant continues to produce mineral fertilizers from Khibiny appatity after natural deposites of phosphorites have been exhausted. PG - is the main waste in production of phosphate

fertilizers, and increased accumulation of minerals was registered earlier by mushrooms of this location [16].

The purpose of the present study was to assess the levels of water pollution in the flooded quarry, situated in the near vicinity of current PG storage site and to evaluate duckweed phytoremediation potential in these conditions.

MATERIALS AND METHODS

Sampling and samples preparation

Water and duckweed samples were gathered in May and September 2019 from Voskresensk flooded quarry (55°19′02″ N.L.. 38°39′09″ E.L.) and from the lake situated in the suburbs of Yoshkar-Ola (56° 38.0644' 0" N.L., 47° 53.9927' 0" E.L. (control). The latter reservoir demonstrated high biodiversity and the results of the appropriate analysis proved that its water quality was close to that described for quality of surface water in Bulgaria reserves [12] and Montenegro [11].

Water samples were kept in polyethylene bottles at +10°C before the analysis. Duckweed samples were washed with distilled water, dried at 70°C to constant weight and homogenized.

Bioaccumulation factor (BAF), which reflects the efficiency of plant accumulation of different pollutants from the environment, was calculate according to the equation:

$$BAF = (C_1/C_2),$$

where C_1- concentration of an element in duckweed, mg kg^{-1} d.w.; C_2- concentration of an element in water, mg L^{-1}

Concentrations of Cl^-, F^-, NH_4^+, $N0_3^-$ ions and pH were determined with the help of ion selective electrodes on ionometer Expert-001 (Econix inc., Moscow, Russia).

Total dissolved solids (TDS) were set using portable conductometer TDS-3A (HM Digital, Inc., Seoul, Korea).

Water hardness was evaluated via titration method using 0.05 M solution of EDTA [17].

Elemental analysis

Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Sr, V and Zn contents in water and duckweed powder samples were assessed using ICP-MS on a quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA), equipped with the seven-port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA) at the Biotic Medicine Center (Moscow, Russia). Each determination was performed in triplicate. Trace levels of Hg and Sn in samples were not taken into account and, accordingly, they were excluded from the Tables.

Toxicity assessment

The level of water integral toxicity was determined according to “Ecolum” bacterial test [18].

Statistics

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to the 0.05 probability level, using SPSS software version 21. Data expressed as a percentage were subjected to angular transformation before processing.

RESULTS AND DISCUSSION

Approaches to evaluate the quality of surface water vary from country to country and usually take into account water utilization purposes: as a drinking water, for technical needs or breeding of a concrete fish species. Taking into account sustainability of aquatic ecosystems, we compared water quality of

two reservoirs from unpolluted area (lake in Yoshkar-Ola suburbs) and of the abandoned flooded quarry situated in the near vicinity from PG storage site (Voskresensk).

Chemical composition and macro-elements content

Differences in water quality between these reservoirs may be easily detected via comparison of total dissolves solids (TDS), water hardness and other parameters (Table 1). Despite significantly higher concentrations of F^-, Cl^-, NH_4^+, $N0_3^-$, hardness value and mineralization in water of flooded quarry, evaluation of the toxicity levels revealed that the values determined were within the permissible range of ≤120 [18].

Table 1. Mineralization, hardness, pH, concentrations of $N0_3^-$, NH_4^+, Cl^-, F^- and toxicity of surface water in different ecological conditions

Parameter	Unpolluted area	Polluted area	
		Spring	Autumn
Mineralization (TDS), mg L^{-1}	131a	760b	869b
Hardness	2.65a	24b	25b
pH	8.7a	7.6b	8.4a
N03- mg L^{-1}	7a	29b	33b
$NH4^+$, mg L^{-1}	0.15a	2.7b	1.8c
Cl^-, mg L^{-1}	8.5a	50b	121c
F^-, mg L^{-1}	0.36a	1.3b	2c
Toxicity	<17a	120b	110b

Along each line values followed by the same letter do not differ statistically according to Duncan test at p≤0.05

Determination of macro-elements in water of control and polluted reservoirs revealed elevated anthropogenic loading in Voskresensk flooded quarry. The differences in P, Ca, K, Mg, and Na content between water of polluted and control reservoirs reached 16.5, 2.9, 55, 3.8 and 17.7 times accordingly. Data presented in Table 2 indicate high ability of duckweed to accumulate macro-elements both in control and polluted areas. Extremely high level of P in Voskresensk flooded quarry reflects significant phosphorous transfer from PG storage site to the environment despite the existence of wide bypass ditch round the PG storage site and utilization of Jurassic clay as the base for the storage of PG. Indeed, investigations of Martinuk et al.[19] demonstrated that about 69% P from PG were water extractable. According to Martinuk et al. data [19] P content in Voskresensk PG reached 1962 mg Kg^{-1}, that is 4 times lower that phosphorous content in duckweed.

Table 2. Macro-elements content in surface water and duckweed in conditions of low and elevated pollution

	Water, mg L^{-1}			Duckweed, mg kg^{-1}		
	Control	Polluted area		Control	Polluted area	
	spring	spring	autumn	spring	spring	autumn
P	0.45a	7.42b	0.56a	6466a	8344b	8023b
Ca	39.3a	114b	189c	25252a	45295b	150200c

K	0.88a	48.33b	43.3b	25775a	18650b	27499a
Mg	5.05a	21.63b	26.03b	6393a	3561b	7252a
Na	2.62a	46.43b	66.05b	2956a	1143b	2008c

Along each line separately for water and duckweed, values followed by the same letter do not differ statistically according to Duncan test at $p \leq 0.05$

Summer period increased accumulation of Ca by duckweed in flooded quarry 3.3 times, while P concentration in duckweed remained unchanged. Nevertheless among macro-elements phosphorous was the only one which concentration in water decreased 13 times during summer.

Calculation of bioaccumulation factor (BAF) revealed a general pattern: BAFs of macro-elements are higher in reservoirs with low anthropogenic loading and decrease significantly in reservoirs with multifactorial pollution (Fig.1). Thus, the highest duckweed BAF was registered for potassium (29290) and phosphorous (14370) in unpolluted reservoir, while the appropriate values did not exceed 386.5 and 1125 accordingly in case of flooded quarry.

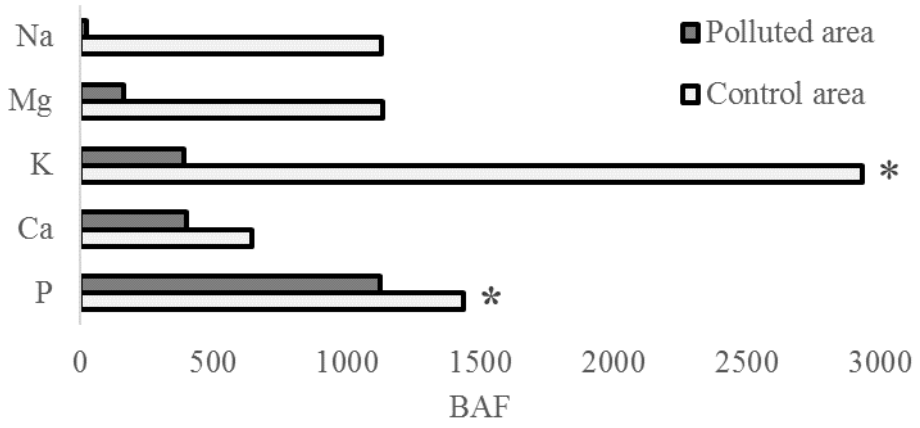

Fig. 1. BAF of macro-elements in duckweed in conditions of low and elevated pollution (*- values are decreased 10 times).

Despite the complexity of environmental pollution near PG storage site, the revealed phenomenon may be considered as an important feature of duckweed adaptability via regulation of the most important macro-elements accumulation.

Heavy metals

Voskresensk flooded quarry was characterized by significantly higher levels of all heavy metals tested and As compared to control reservoir (Table 3).Thus, the concentrations of Al, As, Cu, Pb, Sr, V in flooded quarry were 4 ,7, 90, 7, 9.3 times higher than in unpolluted area. Concentrations of Cr and Ni were elevated by 23.3 and 15 times accordingly compared to the appropriate data for control. In these conditions *Limna minor* demonstrated high phytoremediation potential, which was in accordance with the previous investigation conducted on *Lemna gibba* [2] and *Lemna minor* [15]. During the period of active *Lemna minor* growth (summer) in conditions of elevated anthropogenic loading water concentrations of Al decreased 1.6 times, As- 1.75 times, Cd and Ni- 2 times, Cr-1.4 times. Compared to heavy metals content in PG [19] duckweed accumulated 20 times higher levels of Ni, 1.5 times - of Cd, 5 times - of Pb and 2,5 times - of Cr. Literature data confirm hyperaccumulation of Cr by duckweed with the highest registered concentration of 3 mg kg^{-1} d.w.[20]. In conditions of PG storage site vicinity *Lemna minor* demonstrated Cr concentration of 99 mg kg^{-1} d.w.

Despite intensive increase in Sr accumulation by duckweed during summer the concentration of this element in water also increased (1.93 times) which may reflect intensification of environmental pollution as a result of temperature increase. Intensification of water pollution during this time seems to be connected with higher pollutants transition from PG storage site, decrease of toxic elements absorption by clay and organic matter of bottom sediments [21] at elevated temperatures and growth inhibition of *Lemna minor* due to anthropogenic loading.

Table 3. Water and duckweed heavy metals content in conditions of low and elevated pollution

	Water, Mg L^{-1}			Duckweed, mg kg^{-1}		
	Control	Polluted area		Control	Polluted area	
	area	spring	autumn	area	spring	Autumn
Al	0.02a	0.08b	0.05c	143a	674b	159a
As	0.001a	0.007b	0.004c	3.1a	5.76b	3.7a
Cd	0.0002a	0.0002a	0.0001b	0.07a	1.51b	0.94c
Cu	0.001a	0.09 b	0.004c	1.47a	4.43b	2.69c
Cr	0.003a	0.07b	0.05c	1.14a	96.69b	62.87c
Ni	0.004a	0.06b	0.03c	7.39a	99.19b	59.08c
Pb	0.0007a	0.005b	0.006c	0,34a	16.98b	5.39c
Sr	0.13a	1.21b	2.34c	105a	213b	624c
V	0.0005a	0.001b	0.002c	1,91a	5.1b	1.79a

Along each line separately for water and duckweed, values followed by the same letter do not differ statistically according to Duncan test at $P \leq 0.05$

Investigations of *Lemna minor* phytoremediation capacity on landfill leachate revealed that maximum concentrations of heavy metals take place at the 3-7 days of exposure, while later concentration of elements in duckweed declines [15]. We registered the same pattern in *Lemna minor* of the flooded quarry. Indeed, concentrations of Al, As, Cd, Ca, Ni, Pb and V decreased during summer 1.54 (Ni, As) - 4.2 (Al) times.

Calculation of BAF values for heavy metals revealed certain peculiarities of multifactorial pollution effect. Thus contrary to literature data [2] BAF of Al, Cd, V, Pb and Cr were higher in polluted area than in control reservoir, while only Ni, As, Sr and Cu demonstrated higher BAF values in conditions of low anthropogenic loading.

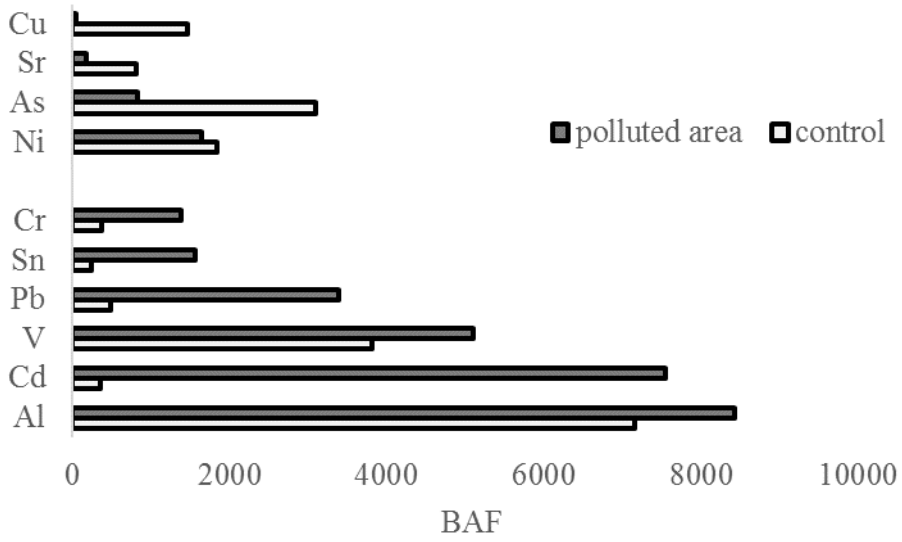

Fig. 2. BAF of heavy metals and As in duckweed in conditions of low and elevated pollution

Interesting but with similar Cd concentration in water BAF of this element in duckweed of flooded quarry was 20 times higher than in unpolluted area. Partly it may be explained by lower solubility of Cd at pH>8 [22], that decreases the level of Cd accumulation. Indeed pH of flooded quarry happened to be lower than water pH of Yoshkar-Ola lake (7.6 and 8.7 accordingly).

Trace elements

In spring concentrations of B, Co, Fe, Li, Mn, Se and Zn in flooded quarry exceed those for unpolluted area 3,10,4, 3, 6645, 3, 8 times respectively. Among microelements of flooded quarry, Mn attracts the most attention. Indeed, concentration of this element in Voskresensk surface water was 6645 times higher, than the concentration of Mn in Yoshkar-Ola lake. During the period of active duckweed growth (summer) Mn concentration in water decreased almost twice while Mn level in *Lemna minor* increased 3.4 times (Table 4). According to literature data, duckweed may accumulate up to 2800 mg Mn kg^{-1} [2]. Our data indicate that the value may be much higher (up to 7000 mg kg^{-1}).

As far as Fe is concerned, Moscow region and Yoshkar-Ola are known to possess high levels of Fe in groundwater, which indicates that Fe excess in Voskresensk flooded quarry may reflect both Fe transfer from PG and high content of the element in the environment. Earlier we demonstrated that high levels of Zn were typical for mushrooms grown near PG storage site reflecting elevated mobility of the element [16], which was in accordance with elevated concentrations of the element in water of flooded quarry. The period of intensive duckweed growth resulted in a significant decrease of Zn and Mn concentrations in water while intensive increase of B, Se and Mn content was registered in duckweed.

Concentration of B in duckweed in spring was close to that described in literature for *Lemna gibba* [23], but exceeded this value in autumn.

Table 4. Trace elements content in water and duckweed in conditions of low and elevated pollution

	Water, Mg L^{-1}			Duckweed, mg kg^{-1}		
	Control	Polluted area		Control	Polluted area	
	spring	Spring	autumn	spring	spring	autumn
B	0.01a	0.03b	0,05c	281a	1904b	2570c
Co	0.0002a	0.002b	0.002b	116a	35.22b	8.35c
Fe	0.24a	0.99b	1.07b	2138a	10611b	3003c
I	0.02a	0.01b	0.02a	11.8a	8.06b	10.44a
Li	0.003a	0.01b	0.02c	0.99a	0.65b	0.61b
Mn	0.002a	13.29b	7.57c	2024a	2077a	6995b
Se	0.0003a	0.001b	0.004c	0.15a	0.45b	0.53b
Si	3.33a	1.19b	0.82b	4.61a	84.45b	33.75c
Zn	0.01a	0.08b	0.01a	11.84a	75.65b	35.56s

Along each line separately for water and duckweed, values followed by the same letter do not differ statistically according to Duncan test at $P \leq 0.05$

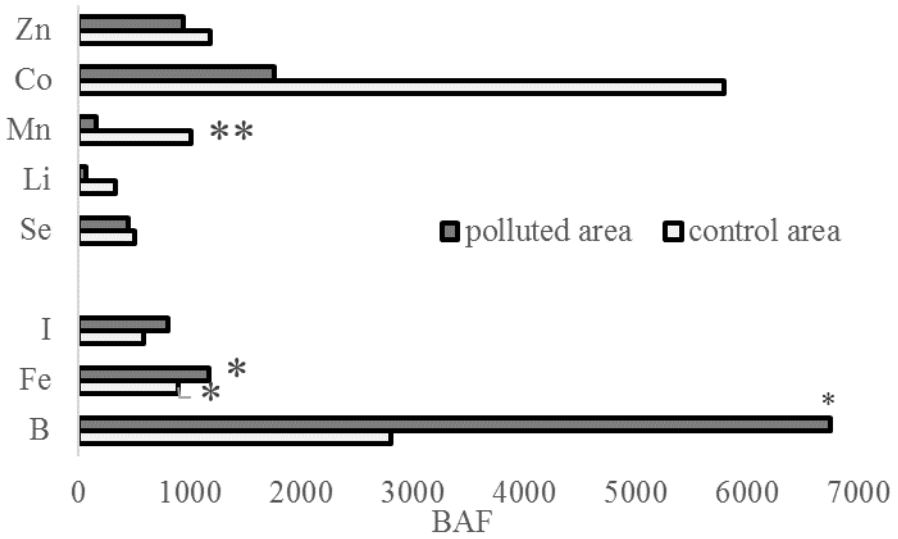

Fig. 3. BAF of microelements in duckweed in conditions of low and elevated pollution
(*- the values are decreased 10 times; **- the value is decreased 1000 times)

Among microelements tested, the highest values of BAF were registered for Mn in unpolluted area (more than a million) and B (63500) in conditions of multifactorial pollution. Higher BAF values in control reservoir compared to the polluted area were typical only for Zn, Co, Mn, Se and Li, but not for Fe, B and I (Fig.3). Among these elements Li and Co were found in trace amounts both in control and in polluted area.

According to Daud et al.[15] levels of heavy metals accumulation by *Lemna minor* decrease within 5-12 days with appropriate decrease of BAF. The authors described the phenomenon for laboratory conditions with rather minor time duration. The data of the present work indicate that changes in

duckweed elements content during the period of active duckweed growth (summer) may either be decreased or increased (Tables 2-5). Furthermore, in natural ecosystem of flooded quarry BAF values are also changed over the summer period. Fig.4 illustrates the main peculiarities of such changes. Thus, BAF of all macro-elements increases, as a result of intensive *Lemna minor* growth with the highest effect typical for phosphorous.

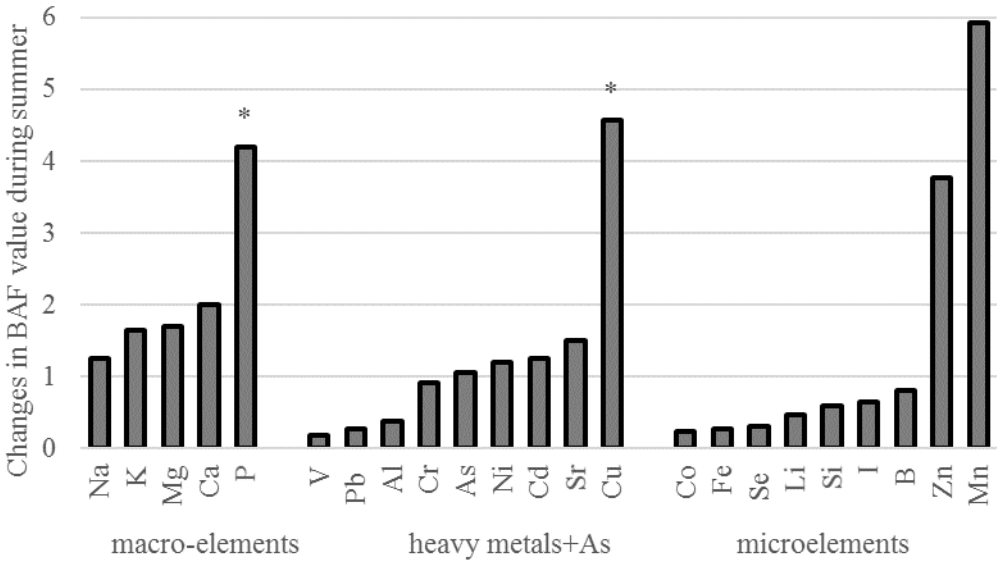

Fig. 4. Changes of BAF values during summer (*- values are decreased 3 times)

Among heavy metals and As tested only 55.6% of elements increase their BAF. They are Cu (the highest effect), Sr, Cd, Ni, As. Among microelements only 22.2% (Zn and Mn) demonstrated significant BAF increase.

The phenomenon seems to be typical for *Lemna minor* in conditions of elevated pollution loading. Nevertheless, additional investigations are necessary to reveal the extent of such patterns.

CONCLUSIONS

Investigation of duckweed phytoremediation capacity in conditions of low and elevated pollution revealed several peculiarities in relationship between BAF and concentrations of macro- and microelements in water while dynamics of BAF changes during summer demonstrated significant differences between macro-elements, heavy metals and microelements behavior. Lack of explanation of the phenomenon requires additional investigations.

ACKNOWLEDGMENT

This research did not receive any specific grant from public, commercial, or non-profit agencies.

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