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Fresh-Water Macroalgae In Monitoring of Water Pollution by Toxic Metals in Near-Border Territories.

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ABSTACT

Authors set a goal to find biomonitors of toxic elements for areas where monitoring of the trans-border transport of pollutants has not been performed. Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Hg, and Pb in macroalgae of water bodies located on the border with Mongolia and China were measured. Analysis of content of heavy metals in algae showed spatial differences in concentrations of chemical elements. Macroalgae show higher concentration of Cu, Zn, etc. in areas exposed to contamination. Statistical analysis proved a correlation between the concentrations of toxic elements in algae gathered in one place. We have offered *Tribonema* sp., *Cladophora fracta*, *Spirogyra spp*., and *Ulothrix zonata* species of macroalgae as interchangeable monitoring objects in the areas of specific concern.

Keywords: biomonitor, toxic metals, contamination, macroalgae

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INTRODUCTION

Mining industry often creates wastewater containing heavy metals that flow into natural waters. Biological indicators have been widely used to monitor and to characterize the status of environmental pollution in fresh water [1, 2, 3] and in marine water [4, 5, 6]. The use of biomonitors is more attractive, because they not only accumulate heavy metals from the environment, but also keep them in their body for a long period of time [7]. This allows to monitor condition of ecosystems in a continuous manner.

Problem of monitoring of toxic elements in trans-border territories (reports on transport) is the most serious. The problem of trans-border transport of pollutants is being actively discussed, because contamination by toxic elements poses a potential health hazard to both animals and humans [8]. Many studies have reported that macroalgae have a very high capacity for binding with metals due to the presence of polysaccharides, proteins, or lipids on the surface of cell walls. These contain functional groups such as aminos, hydroxyls, carboxyls, and sulfates, which can act as binding sites for metals [9, 10]. Macroalgae are good indicators of increased concentrations of heavy metals. Easiness of algae collection and cheap analysis makes macroalgae a good target for monitoring of pollution in the trans-border water bodies.

The studied Russian territory borders with Mongolia and China. In the upper reach of the Onon River basin the water flows from the territory of Russia onto the territory of Mongolia, and later returns from the territory of Mongolia onto the territory of Russia. The border with People's Republic of China passes through the midstream of the Argun River. In the area between the Molokanka Village and the Priargunsk Village no water inflows from the territory of Russia. The water of the Argun River in this area characterizes the run-off from the territory of China.

MATERIALS AND METHODS

Description of the Area of Research

The studied water bodies are located in the territory with diverse climatic conditions [11] and different anthropogenic impact on ecosystems. Therefore, samples collected on the studied area characterize territories with different degree of anthropogenic changes. Mainly rivers with low water consumption flowis on this territory under a slight moistening [12]. Areas of the Tyrin River, Banny Stream, and Lake Kenon are directly influenced by water contaminated by heavy metals. The waters of Lake Arey, Kadalinka and Byrtsa Rivers are not contaminated by industrial effluents.

Industrial and agricultural activity on the catchment basin of the trans-border rivers contributes to the high load on their ecosystems. In the studied territory deposits of non-ferrous metals are located [13]. Mining over more than 300 years has led to the dangerous pollution of areas surrounding mining and processing plants and camps by toxic metals [14]. Ajacent water bodies and streams are also contaminated. Over the last decade the Argun River is included in the list of the most polluted rivers in Russia; the Trans-Baikal Hydrometeorological Service registered that on the Molokanka-Kuti site maximum permissible concentrations of Mn and Cu are exceeded [15, 16]. In the summer of 2010 the Government of Mongolia addressed to the RF Ministry of Natural Resources a request to stop the pollution of the Ashinga River and later the Onon River by the Balja gold mining organization [17]. Toxic elements arrive into the aquatic ecosystems in places where population and production facilities are concentrated. Lake Kenon is located in the center of the largest metropolitan area (the City of Chita, 324.5 thousand of people) in the studied region. The main source of pollution is a thermal power plant [18].

Sokhondinsky State Nature Biosphere Reserve and Dauria State Nature Biosphere Reserve are located in the near-border area.

Description of Material

Samples of algae were collected in the period from 2011 to 2014 at 37 stations of Verhneamursky Basin (Fig. 1). Samples consisted of several species, less frequent they were represented by one species. To identify seasonal differences in the accumulation of toxic elements at Stations No. 1-4 samples were collected at the beginning (June) and in the end of the vegetation development (September-October) of 2011-2012.

Samples were collected from water bodies not polluted by industrial waste water in order to estimate the background content of toxic elements. Determination of species composition was performed on living specimens in a laboratory under a Nicon Eclipse 200 microscope. On the plant bodies of *C. fracta*, we could observe a large amount of epiphytic algae, mainly *Bacillariophyta*, and small epiphytic algae such as *Chlorophyta, Cyanophyta*. On the plant bodies of *Spirogyra spp*., less species and amounts of epiphytic algae were observed, but it is often determined with other representatives of *Zygnemataceae*.

In our studies GPS AQUAMETER AM-200 determined TDS (mg/dm^3) and pH. Algae samples at the sites of collection were washed by water from the water body to remove captured soil particles, epiphytes, and epizoites. Then algae samples were dried in air to absolute dry weight and then pulverized. Analysis of concentrations of heavy metals in the water and algae was performed by inductively coupled plasma-mass spectrometry (Elan DRC II; PerkinElmer). Accuracy of the measurements was checked by using Baikal *Elodea canadensis* Michx. (1803) (SRM, Canadian pond weed, EK-1, registration number COOMET CRM 0065-2008-RU) for plant samples as a standard reference material. The certified, measured and recovery values are presented (Kuklin, Matafonov, 2014). Recovery values were in the range of 68 % – 141 %. Cu, Zn, Hg, Cd was undervalued and As was overvalued for EK-1, Pb was overvalued for EK-1 (Kuklin, Matafonov, 2014). The detection limits were the following elements: Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Sn, Sb, Hg - 0.001-0.01 ppb; Sr, Mo, W, Pb 0.0001-0.001 ppb. The data were processed statistically using STATISTICA for Windows (Release 10, Copyright© Statsoft, Inc.).

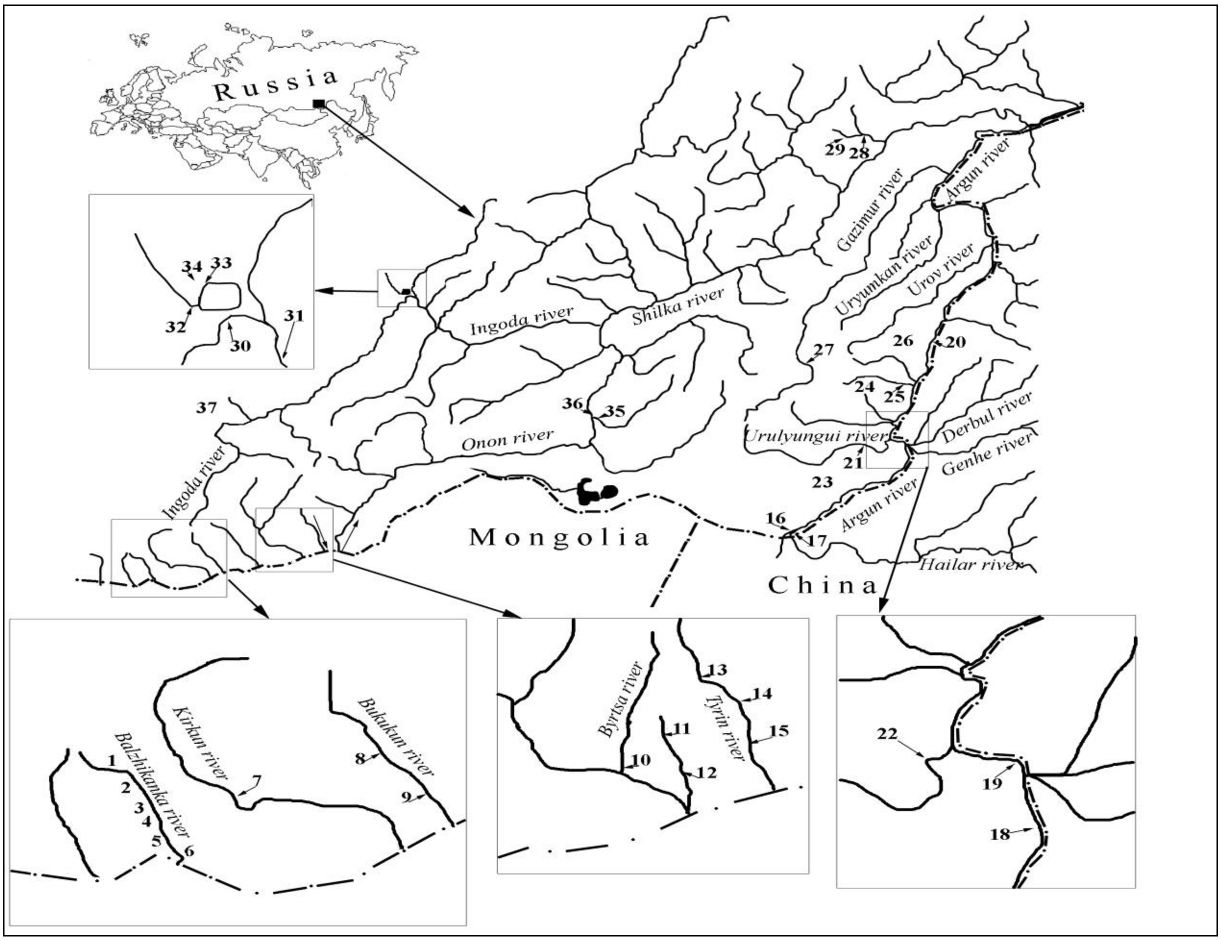

Fig. 1 Location of the Study Sites

RESULTS AND DISCUSSION

We do not consider the problem of the differences in the values of standardized indicators (Table 1) and their non-conformity between the countries in the article.

Table 1. Concentrations of toxic metals in water (2012-2013)

Site	Element μ/l												
	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Sr	Mo	Cd	Hg	Pb
St. 1	4,55	2.33	82.50	0.09	2.62	20.65	20.44	0.37	92.47	0.74	0.05	ND	0.80
St. 5	3.86	8.92	162.84	0.12	2.73	2.49	4.37	0.37	177.02	0.67	0.03	0.00	0.69
St. 7	3.43	6.65	112.96	0.09	2.15	1.97	11.58	0.69	47.18	0.36	0.03	0.00	0.92
St. 9	0.47	3.53	87.39	0.07	0.35	2.75	4.59	3.11	30.68	0.53	0.01	0.01	0.23
St. 10	ND	107.9	669.51	0.32	ND	ND	ND	4.27	190.08	0.83	0.00	0.03	ND
St. 11	ND	308.3	1435.31	0.90	1.98	ND	1.95	167.2	1457.5	2.45	0.01	0.02	2.21
St. 12	ND	38.25	500.96	0.34	1.19	ND	ND	8.60	906.19	2.47	0.01	0.01	0.81
St. 14	ND	37.91	581.72	0.22	ND	1.02	28.02	5.54	179.01	0.43	0.28	0.01	7.89
St. 16	ND	40.50	551.45	0.19	0.01	2.96	17.11	4.40	172.84	1.10	0.27	0.01	0.23
St. 17	ND	434.6	202.51	0.17	ND	0.39	5.68	2.75	263.93	2.97	0.01	0.00	ND
St. 18	ND	192.8	307.00	0.19	ND	0.76	17.20	3.90	206.98	0.93	0.01	0.01	0.32
St. 20	ND	34.00	320.81	0.20	ND	ND	ND	2.67	173.56	0.79	0.03	0.01	ND
St. 22	ND	20.34	318.82	0.20	0.56	ND	2.55	6.81	739.44	1.67	0.03	0.01	ND
St. 25	10.50	171.0	4993.9	3.66	15.6	65.8	101.72	7.28	331.62	3.36	0.25	0.02	13.07
St. 27	ND	52.33	282.12	0.16	0.37	ND	ND	1.51	241.04	0.85	0.02	0.01	ND
Standard of China	50 (Cr^{6+})	100	500	-	-	10	100	50	-	-	5	0.1	10
Standard of Mongolia	-	-	-	-	-	10	10	10	-	-	5	-	10
MAC	70 (Cr^{3+}) 20 (Cr^{6+})	10	100	10	10	1	10	50	400	1	5	0.01	6
US EPA CCC	75 (Cr^{3+}) 11 (Cr^{6+})	-	1000	-	52	9	120	150	-	-	0.25	0.77	2.5

ND - not detected, MAC - maximum admissible concentration for fishery waters (Russia) [20], US EPA CCC maximum concentration of the element in water for permanent aquatic communities to occur without harmful impact [21], Class III Standard of China is mainly applicable to the second class of protected areas for centralized sources of drinking water, protected areas for the common fishing and swimming areas [22], Standard of Mongolia [23].

The highest values exceeding standards in the near-border watercourses are shown by Mn, Fe, Cu, Zn, and Pb. In some cases, this is explained by the mineral composition of rocks (St. 1), and in other cases by the pollution resulting from mining (St. 14, St. 11, St. 25).

Algae Ecology

Mainly rivers with low water consumption flow on the studied territory under slight moistening [[12]]. In this water streams freshwater macroalgae grow in large quantities. The most frequently encountered in the studied territory are *Ulothrix zonata* (Weber & Mohr) Kützing, *Cladophora fracta* (Müll. Ex Vahl.) Kützing, species of the *Spirogyra* and *Tribonema* genera. We see these species as potential biomonitors of toxic metals in the region.

C. fracta grows under a wide range of environmental conditions from sea to fresh water and everywhere provides habitat and food for numerous organisms. It may be the most common freshwater macroalgae in the world [[24]]. In the studied area *C. fracta* grows in small and medium rivers and in the surf zone of lakes, located in the forest-steppe and steppe landscapes. The mineralization of water in places of growth ranges from 55 to 836 mg/l, averaging 305 mg/l. In taiga rivers it abundantly grows at contamination of water by nitrogen and phosphorus compounds. The species accumulates phytomass with up to 880 g/m^2 of wet weight [25]. *U. zonata* in the studied territory grows in small and medium rivers and in the surf zone of lakes, located in taiga and forest-steppe landscapes. Most often it is found in streams with salinity ranging from 55 to 900 mg/l, 305 mg/l on the average. It is frequently observed in areas of groundwater discharge into the mainstream, where it forms pure mass. Biomass can reach 100 g/m^2 in wet weight under favourable auspices [26].

Spirogyra spp. in the studied territory grows in small and medium-sized rivers, located in the forest-steppe and steppe landscapes. As well as Cladophora, *Spirogyra spp.* grows abundantly in areas of anthropogenic pollution. Species of the *Spirogyra* genus occur in a wide range of salinity (55-713), 234 mg/l on the average. Species often form pure, without admixture of other types of phytomass, accumulations with wet weight amounting up to 200 g/m^2 [25, 26].

Species of the *Tribonema sp.* genus on the studied territory grow in places of subsurface water discharge, often in streams from under the rock dumps and tailings dams regardless of the landscape at mineralization ranging from 55 to 900 mg/l. In favorable conditions phytomass of accumulations reaches up to 200 g/m^2 of wet weight [26].

Average concentrations of metals and their ranges in macroalgae are shown in Table 2. When calculating the average concentrations stations with statistically significant exceedance ($p<0.05$) over the average contents were excluded out of general set of samples. In general we can see some similarity in content of chemical elements in *Spirogyra spp.*, *U. zonata*, *C. fracta*, and *Tribonema sp.* algae in places of increased concentration of toxic elements (runoff from the tailings storage, rock dumps). These species are characterized by higher concentrations of chemical elements. In general during study of average contents abundance of chemical elements is observed in *Spirogyra spp.* (Fe Mn Sr Zn Cu As Ni Cr Pb Co Mo Cd Hg), *U. zonata* (Fe Mn Sr Zn Cu As Cr Pb Ni Cd Co Mo Hg), *C. fracta* (Fe Mn Sr Zn As Cu Pb Cr Ni Co Mo Cd Hg) and *Tribonema sp.* (Fe Mn Zn Pb Sr As Cu Cr Ni Co Cd Mo Hg). It was found out that *Spirogyra spp., U. zonata, C. fracta* in general contain similar elements while *Tribonema sp* shows sharp increase of Pb and Zn in comparison with other toxic metals.

Table 2 Concentrations of toxic metals (Mean±SE (SD)/min-max, µg g^{-1} in d.w.) in macroalgae

Element	N	*Spirogyra spp.*	N	*Ulothrix zonata*	N	*Cladophora fracta*	N	*Tribonema sp.*
Cr	19	3.7±1.3 (2.7) (0.00-27.1)	8	4.17±1.58 (1.89) 1.5-24.5	23	5.6±1.1 (2.6) 1.0-20.3	5	10±5.6 (4.5) 4.3-36.9
Mn*	20	0.6±0.3 (0.7) 0.07-14.9	8	0.18±0.1 (0.1) 0.04-1.1	28	0.9±0.3 (0.7) 0.16-15.4	6	0.7±0.8 (0.8) 0.1-5.4
Fe*	18	1.6±0.7 (1.3) 0.25-18.2	8	2.3±1.1 (1.4) 0.75-11.9	25	2.6±0.6 (1.5) 0.7-15.2	6	8.3±6.5 (6.2) 3.0-38.7
Co	18	2.8±0.84 (1.7) 0.37-24.62	7	0.66±0.23 (0.25) 0.3-5.2	27	1.8±0.4 (1.1) 0.3-14.2	6	3.5±2.9 (2.7) 0.8-19.2
Ni	18	5.3±1.5 (3.0) 1.1-34.1	7	3.2±2.06 (2.2) 1.5-11.8	27	5.3±0.7 (1.9) 2.5-28	6	8.9±4.9 (4.6) 2.2-21.0
Cu	16	8.0±2.0 (3.8) 0.3-29.9	6	8.8±5.2 (5.0) 0.9-40.9	26	8.3±2.3 (5.7) 0.8-114.2	6	40.7±33.9 (32.3) 9.2-1916.5
Zn	18	64±16.7 (33.6) 122-340	8	44.2±24.8 (29.7) 14.4-3521.6	28	45.7±10.6 (27.2) 6.9-938.4	6	351.1±472.1 (449.9) 52.6-2497.6
As	17	6.3±0.9 (1.8) 3.5-18.8	7	8.7±4.5 (4.8) 5.1-28.7	28	13.2±3.9 (10.1) 1.1-272.6	4	43.4±43.2 (27.2) 12.7-289.6
Sr	19	119.1±32.8 (67.98) 25-1198	7	125±36.7 (39.9) 74.4-207.3	24	166.5±33.8 (79.9) 78.9-859.1	6	86.3±36.6 (34.9) 33.4-279.8
Mo	20	0.7±0.14 (0.3) 0.14-6.53	8	0.3±0.17 (0.2) 0.2-2.3	22	0.9±0.2 (0.5) 0.2-5.4	5	0.6±0.3 (0.3) 0.3-1.1
Cd	18	0.6±0.2 (0.42) 0.12-3.86	8	0.6±0.5 (0.6) 0.1-13.2	29	0.4±0.1 (0.2) 0.1-9.5	5	2.4±3.6 (2.9) 0.2-15.0
Hg	19	0.01±0.003 (0.01) 0.00-0.03	7	0.01±0.0045 (0.005) 0.0007-0.0216	22	0.006±0.002 (0.005) 0.001-0.061	6	0.026±0.009 (0.008) 0.015-0.077
Pb	21	3.2±1.07 (2.34) 0.6-45.5	8	3.2±3.0 (3.56) 1.2-219.2	31	5.7±2.5 (6.8) 1.1-513.4	6	184.1±269.2 (256.6) 5.6-1004.7

* mg/g^{-1}

It is known that content of heavy metals in algae is species-specific and is determined by the structure and physiological peculiarities of an organism [[9]]. Table 3 shows coefficients of pair correlation between species of macroalgae from Lake Arey suffering no antropogenic impact and macroalgae from Tyrin River (St. 14) washing the tailings storage facility of the mining and processing complex. The lowest correlation was received for *Aegagropila linnaei* Kützing (growing in Lake Arey at depth exceeding 3 m on silts, geochemical conditions of which differ from the surf zone sands). *Chaetophora lobata* Schrank, *Spirogyra* spp. and *C. fracta* were growing on sands; this is the reason of similarity in concentration of elements.

Table 3 Correlation coefficient for macroalgae species

Lake Arey				
	C. fracta	*Spirogyra sp.*	*Ch. lobata*	*A. linnaei*
C. fracta	1			
Spirogyra spp.	0.81	1		
Ch. lobata	0.96	0.93	1	
A. linnaei	0.76	**0.23**	**0.55**	1

Tyrin River				
	C. fracta	*Tribonema sp.*	*U. zonata*	*Ch. lobata*
C. fracta	1			
Tribonema sp.	0.98	1		
U. zonata	0.82	0.81	1	
Ch. lobata	0.97	1.00	0.80	1

Thus, algae species of different taxonomic divisions similarly react to water pollution by toxic elements. The magnitude of the differences between species growing in pure water and in water contaminated by toxic metals is shown in Figure 2.

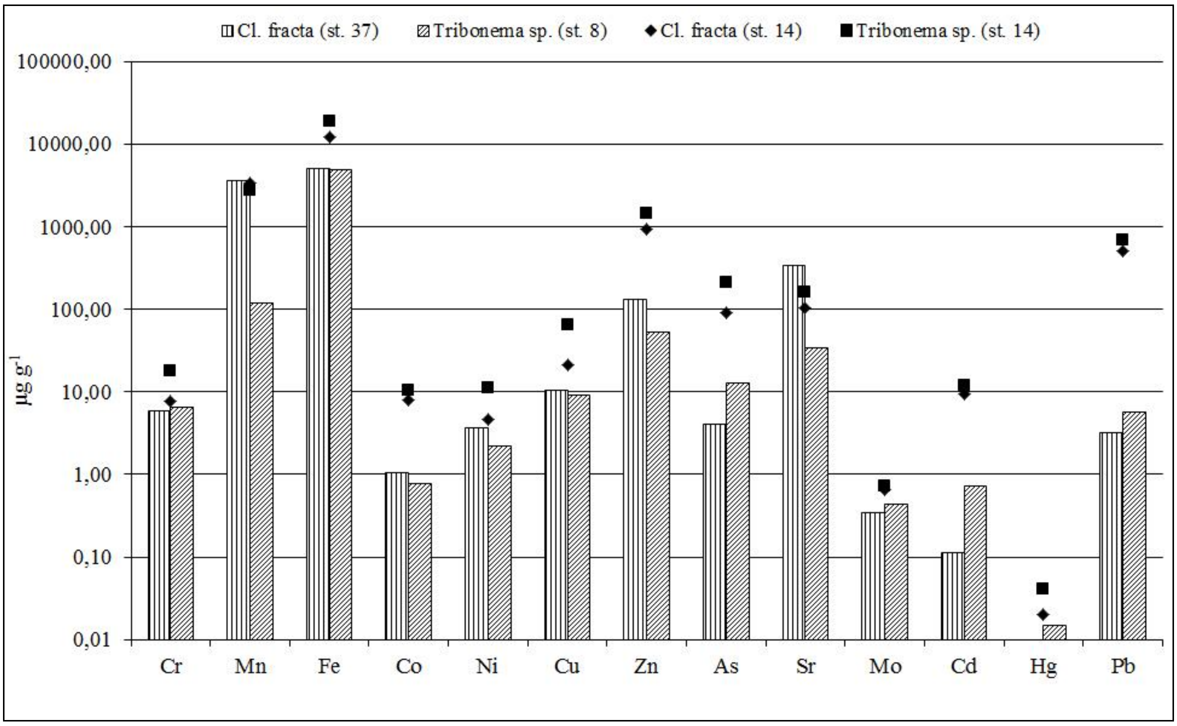

Fig. 2. Concentration of toxic elements in *Tribonema sp*. and *C. fracta* in clean water and contaminated water

Table 4 shows a comparison of the received values with previously obtained data.

Table4 Concentrations of toxic metals (mg kg^{-1} in d.w.) in macrophyte algae

Species	Elements						Author
	Cu	Zn	As	Pb	Cd	Hg	
U. zonata	6.4±3.8	27.2±11.0	6.7±2.9	1.9±0.9	0.4±0.1	0.01±0.009	[[26]]
	8.8±5.2	44.2±24.8	8.7±4.5	3.2±3.0	0.6±0.5	0.01±0.0045	Current studies
	2	25.7	4	0.8	0.2	-	[[28]]
	9.6±1.9 9.0±1.8	21.0±4.0 55±11	-	2.3±0.6 8.2±2.1	0.4±0.2 0.6±0.3	-	[[29]]
Zygnema sp.	9.2-17.4 177.1-309.3	31.9-55.4 49.1-60.4	-	0.1-10.0 10.6-42.9	0.1-0.7 0.7-3.8	-	[[2]]
Spirogyra spp.	0.6–16.0	0.0–12.9	-	ND	ND	-	[[1]]
	8.0±2.0	64.0±16.7	6.3±0.9	3.2±1.1	0.6±0.2	0.01±0.003	Current studies
Zygnemataceae	10.3±4.6	58.9±19.7	8.5±3.1	3.41±2.6	0.88±0.5	0.01±0.007	[[26]]
C. fracta	4.4	34	-	3	0.24	0.033	[[29]]
	9.13±3.6	73.1±64.6	35.4±28.4	4.3±3.8	0.94±1.1	0.02±0.015	[[26]]
	8.3±2.3	45.7±10.6	13.2±3.9	5.7±2.5	0.4±0.1	0.006±0.002	Current studies
Cladophora glomerata (L.) Kütz.	7 15	105 54	7.6 11.6	3.3 5.4	0.49 0.21	0.075 0.13	[[3]]
	4.7-40	-	-	0.3-2	0.1-0.7	-	[[31]]
	10.5	129	-	35.4	1.51	-	[32]
Ulotrichaceae	2.9-71.5	54.2-310	-	-	0.24-1.5	0.09-0.93	[[32]]
Zygnemataceae	3.1-73.4	31.3-251	-	-	0.25-1.5	0.09-0.87	
Cladophoraceae	3.3-81.2	45.8-295	-	-	0.19-1.9	0.1-0.75	
Non-polluted objects	2.5-60	80-230	0.03-0.8	0.3-20	0.1-0.5	0.04-0.3	

Note: in denominator – under contamination

Concentrations of such elements as Cu and Zn are within known limits for unpolluted rivers. In *U. zonata* Cd and Pb exceed the known values. *C. fracta* showed Pb values comparable to the concentrations in algae growing in polluted waters. Possible reason of this is the abundance of polymetallic deposits in the south-east of the Trans-Baikal territory. Received data for Pb characterize current natural background of the territory used over more than 300 years for mining of non-ferrous metals. The values obtained from Tribonema sp. characterize conditions of long-term permanent pollution by toxic elements.

Comparison of concentrations of toxic elements in algae with average contents for watercourses of the near-border area determines the most contaminated water streams. Among the watercourses crossing the boundary of Russia and tributaries of larger rivers the most polluted are the Tyrin River (St. 14) (*C. fracta*, n times higher than Mean+SE) Fe 6, Mn 5, Zn 27, Cu 4, As 10, Cr 2, Pb 162, Co 6, Cd 33, Hg 5; Srednyaya Borzya River (St. 25) Mn 2, Sr 2, Cu 2, Pb 4, Cr 4, Co 2, Mo 9, Cd 3; Kalga River (St. 24) Fe 2, Mn 13, Zn 2, Cu 3, Cr 3, Pb 2, Co 6; Dunda-Khongorun River (St. 11) Ni 5, As 3, Cd 2, Hg 2; Boguziya River (St. 28) Cu 3, Zn 2; and Tyrin River (St. 15) Mn 4, Zn 51, Cu 3, As 2, Pb 35, Co 6, Cd 13, Mo 5 (*U. zonata,* n times higher than Mean+SE) . Among water courses located at a considerable distance from the border the most polluted is Ingoda River (*Cl. fracta*) Fe 2; Sr 3; littoral of Lake Kenon in the area of TPP No.1 Cr 5; Mn 4; Fe 7; Co 7; Ni 6; Cu 19; Zn 14; As 3; Sr 5; Mo 3; Cd 2; Hg 5; Pb 4.

High concentrations of Pb, Zn and Cd in algae from the Tyrin River are caused by arrival of metals from non-reclaimed tailings storage facility of Khapcheranga mining and processing complex. Increased concentrations of Mo, Pb and Cd in Srednyaya Borzya River are caused by inflow of untreated waste water of placer gold mining. Increased As level in organisms of the Dunda-Hongorun River is caused by admixture of arsenopyrite in veins of gold. We can explain increased Sr content by discharge of bottom-ash waste into the water bodies as a result of emergency releases.

CONCLUSIONS

Algae have shown good repeatability of data during analysis of clear and contaminated water; therefore, they can be used as monitoring objects. Received average concentrations of Cu and Zn can be used as background values for unpolluted water streams. Studied algae can be used as biomonitors of pollution by toxic metals in the near-border territories. Tyrin River and Srednyaya Borzya River are the watercourses, which pose a potential danger of the transboundary transport of contaminants.

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