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## Bio-indication in the Amur River, Russian Far East.

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### ABSTRACT

The technogenic accidents in Jilin province (China) in November 2005 and July 2010 have drawn attention to environmental problems of the Amur River basin. The lack of reliable information on the anthropogenic pressure of natural complexes in newly developing northern territories of China, namely, the Sungari Basin and the right bank areas of the Amur River, makes the efforts of the Russian authorities less efficient in conserving Amur ecosystem biodiversity and in reducing river water pollution. Studies of algal biodiversity and structural dynamics of the Amur River ecosystem reveals the impact of phenols on water quality which was followed till the river mouth. Bio-indication and statistics help us to reveal species-indicators and bio-sensors of pollutants. These algae are more influenced by phenols in low-mineralized unpolluted water. The ecosystem on the oligotrophic level is more impacted by the chemical pollutants and degrades from left riverside to right across the river especially after impact of the Sungari River input.

**Keywords:** Bio-indication, pollution, ecological assessment, large river, Amur River, Far East

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**INTRODUCTION**

The technogenic accident in Jilin province (China) in November 2005 and October 2010 has drawn attention to environmental problems of the Amur River basin. Freshwater rivers are essential sources necessary to study, protect, and improve their ecological state [1]. One of the priorities of the national environmental policy in many countries is assuring ecological security by solving various problems on the regional level. Our ecological investigations are focused on the protection of ecological complexes and their biodiversity, prevention of degradation and recovery of disturbed ecosystems, and their stable functioning. The main object of these ecological investigations is to observe biological systems of different organization levels and to measure their responses to environmental changes [2]. The sum of all toxicological factors influences aquatic populations of water environments, affecting their ability to be sustained and their biodiversity.

**Toxic substances found in the Amur River**

The analysis of seasonal pollution of the Amur River with stable polyaromatic hydrocarbons (PAH), containing 3-5 aromatic rings (phenanthrene, benzopyrene, and their homologs), showed that in summer the total content of these toxicants in some river passages in the lower Khabarovsk was 10 times higher than in winter [3,4]. This indicates that in summer increase number of anthropogenic sources of PAH like fires, which became very often in recent years.

The results of complex assessments of the Amur River ecological situation and water quality were obtained by the Institute of Water and Ecology Problems and the Institute of Tectonics and Geophysics of the Far East Branch of the Russian Academy of Sciences with a combination of bio-indication, physical and chemical methods (IR and UV- spectroscopy, liquid and gas-liquid chromatography, and atomic adsorption spectrometry). The sum of volatile nitrogen-containing substances, trimethylamine, histamine, DDT group pesticides, hexachlorocyclohexane (HCH), and ions of trace metals were analyzed in fish tissue. Also, a sanitary and microbiological assessment of fish muscles and gill contamination (Table 1) was conducted [4].

**Table 1: The Amur River pollution with polyaromatic hydrocarbons below the Sungari Juncture in July, 2005 [4]**

Station	Site	From left bank, m	Sampling sites	Phenanthrene, ng L <sup>-1</sup>	Chrysene, ng L <sup>-1</sup>	Benzo(b) Fluoranthene, ng L <sup>-1</sup>	Sum of 7 PAH, ng L <sup>-1</sup>
1	1a	200	Upper Sungari juncture (Amurzet village)	0	0	0	0.016
2	2a	100	Left bank, surface	0.004	0.002	0.002	0.027
		100	Left bank, bottom	0.011	0.004	0.003	0.050
	2b	300	Middle, surface	0.019	0.008	0.018	0.151
		300	Middle, bottom	0.013	0.008	0.030	0.090
	2c	500	Right bank, surface	0.011	0.003	0.084	0.113
		500	Right bank, bottom	0.011	0.005	0.054	0.094

Therefore, the following questions become most urgent. How did these toxicants influence life functions of aquatic inhabitants of phytoplankton? What is the spatial extent of their impact? What is the long-term effect for certain representatives of trophic chains, for biodiversity and stable functioning of the Amur and Sungari rivers ecosystem?

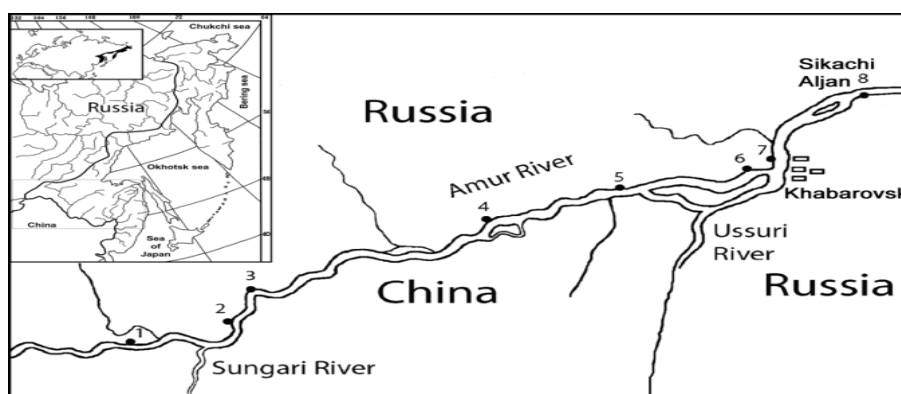
We monitored algal species diversity in the Amur River after the November 13, 2006 accident at the chemical plant in Jilin (China) by bio-indication methods and statistical analysis (CCA) to reveal the response of first trophic level populations on the river pollution anthropogenic impact.

The reaction to nitrobenzene attack of the Amur River ecosystem which is under impact of water of the Sungari River was studied on the station before mouth of the Sungari River as well as on few stations below it (Fig. 1).

**MATERIAL AND METHODS**

**Sampling and study site**

For our study we collected 29 samples of planktonic algae during the period from 24 June 2005 till 3 July 2006. In addition, we used data from our sampling trip in July-August 1997. The samples were collected at six designated sampling stations along the Amur River (Fig. 1): Station 1, Amur River, Amurzet village, above Sungary River, (230 km above Khabarovsk); Sea River mouth; Station 2, Amur River, 4 km below Sungary River (225 km above Khabarovsk); Burea River mouth; Station 3, Amur River, Nizhne-Leninskoe (180 km above Khabarovsk); Station 4, Amur River, Upper-Spasskoe (130 km above Khabarovsk); Station 5, Amur River, Fuyuan (60 km above Khabarovsk); Station 6, Amur River, 7 km above Khabarovsk; Station 7, Amur River, 5 km below Khabarovsk; Station 8, Amur River, Sikachi-Aljan (75 km below Khabarovsk). In selected stations samples were taken of the river profile from the left to right banks and recorded as a, b, c, and d.



**Figure 1: Sampling sites in the Amur River**

The qualitative samples of phytoplankton were obtained by scooping up with a plankton net, gas No. 74, placed in 15-ml plastic tubes, and fixing them in 4% formaldehyde. The quantitative samples was scooped as 1 liter and investigated by sedimentogravimetric method. Algae were studied with a dissecting Amplival microscope under magnifications of x400–1000 and were photographed with a digital camera. Diatoms were prepared using the peroxide technique [5] modified for glass slides [6].

In addition to our sampling, we used data from chemical analyses regularly performed by the Institute of Water and Ecological Problems FEB RAS expedition.

**Chlorophyll analysis**

Measurements of chlorophyll *a* concentration in the water were performed by the Center on Monitoring of Environmental Pollution GU Khabarovsk from 1 liter water samples which were placed in a dark bottle and kept cool [7,8], and concentrated with membrane filters of Whatman GF/C (0.5-1 μm). For retard degradation and enhanced filtration efficiency MgCO<sub>3</sub> was added. The samples were placed into a freezer to provide the adequate preservation of pigments. Homogenization and extraction was done using 90% acetone. The chlorophyll *a* was determined with the spectrophotometric method on 430, 630, 645, 663, and 750 nm. The calculation of chlorophyll *a* concentration was determined using the equation [9,10]:

$$C \text{ Chl } a = 11.64 \text{ Abs}_{663} - 2.16 \text{ Abs}_{645} + 0.10 \text{ Abs}_{630} \quad (4)$$

Where  $V_f$  is the volume filtered (L),  $V_e$  is the volume of extract (ml), and  $p$  is the path length (cm).

**Bio-indication**

Our ecological analysis is based on the list of algal species indicators [11,12], and revealed a grouping of freshwater algae according to the following environmental variables: pH, salinity, organic pollution, temperature, trophic state, type of nutrition, and rheophility. Each group was separately assessed to its

significance for bio-indications. Species that respond predictably to these variables can be used as bio-indicators reflecting the reactions of aquatic ecosystems to the abovementioned variables.

### Saprobic Index (S)

Saprobic Index (S) was calculated from the following formula (where S is the index of saprobity for algal community;  $s_i$  is the species-specific saprobity level;  $a_i$  is the frequency values [12]:

$$S = \frac{\sum_{i=1}^n (s_i \times a_i)}{\sum_{i=1}^n (a_i)}$$

The Saprobic Index S indicates the saprobic zone. Sládeček [13] adapted the classes of water quality based on the ecological classification widely used in European and Asian countries [12,14-16].

### Statistics

The Shannon's diversity index [17] was calculated as:

$$\bar{H} = -\sum_{i=1}^s \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

Where:  $N$  = common organisms abundance,  $l$ ;  $s$  = species number;  $n_i$  = species number of every species;  $\bar{H}$  = Shannon species diversity index, bit.

Statistical methods were used in comparative floristic approaches [18] for calculating similarity of algal communities in the sampling stations.

### CCA Analysis

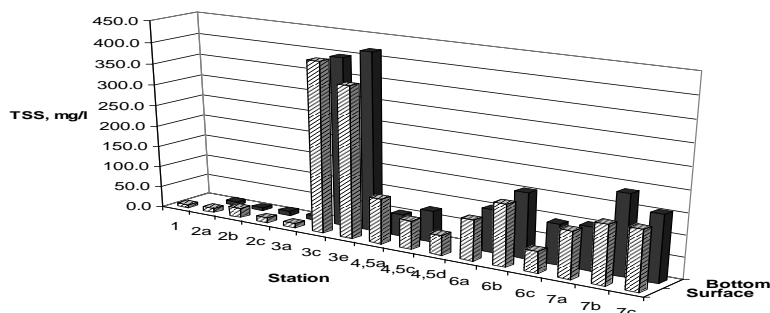
In order to determine the environmental conditions of algal assemblages, environmental parameters together with algal assemblages were analyzed using Canonical Correspondence Analysis (CCA) with CANOCO for Windows 4.5 package [19]. The CCA biplot represents the overlapping of species in relation to the combination of different environmental variables. Arrows represent environmental variables, with the maximal value for each variable located at the tip of the arrow [20].

## RESULTS AND DISCUSSION

### Chemical conditions

Chemical conditions of the Amur River water across all sampling stations at the time before impact are shown in the Table 2. As seen from the data, the Amur River water was low-alkaline with low to middle mineralized and low to middle organic pollution and color. Water variables measured after July's catastrophe impact (Table 3) show decreases of oxygen variables (BOD and COD), increases of ammonia concentration as well as the influence of phenols, which enriched the water over all of the river flow and increased during the year after the catastrophe (Table 4).

As revealed in a recent investigation [21], the distribution of nitrobenzene pollution spot over the river was relevant to the water stream. Many toxic elements (trace metals and stable organic substances) were included in the suspended matter and were discharged into the Amur with the Sungari runoff. This is shown in Fig. 2 in which bottom and surface TSS enrichments were similar and increased near the right bank of the river after impacted station 2. Suspended matter contains various toxic substances including stable organic pesticides, polyaromatic hydrocarbons and toxic elements. Saprophytes and pathogenic microorganisms, detritus formed from plant residues, and dead hydrobionts were transported together with the suspended matter.



**Figure 2: Spatial water pollution in the Amur River with suspended matter (mg/l) passing from Blagoveschensk to Khabarovsk (July 2005) in surface and bottom waters: 1-2 below Blagoveschensk (left bank, middle, right bank); 3 below the Sungari juncture (a-left bank, b-middle, c-right bank); 7-9 below Fuyuan (a-left bank, b-middle, c-right bank); 10-12 below Khabarovsk (a-left bank, b-middle, c-right bank).**

**Table 2: Environmental variables in the sampling stations of the Amur River in July-August 1997 at the surface**

Station	Site	Color, grad.	pH	TDS, mg L <sup>-1</sup>	NH <sub>4</sub> , mg L <sup>-1</sup>	NO <sub>2</sub> , mg L <sup>-1</sup>	NO <sub>3</sub> , mg L <sup>-1</sup>	PO <sub>4</sub> , mg L <sup>-1</sup>	Secchi, m	O <sub>2</sub> , mg L <sup>-1</sup>	O <sub>2</sub> %	CO <sub>2</sub> , mg L <sup>-1</sup>	BOD, mg O <sub>2</sub> L <sup>-1</sup>	COD, mg O <sub>2</sub> L <sup>-1</sup>
1	1	60	7.3-7.45	57.8	0.09-0.17	0.003	0.10-0.18	-	0.7	7.04	87	3.0	9.8-12.7	30.2-67.6
2	2	70	7.65	84.3	0.25	0.000	0.53	0.029	-	7.20	89	2.7	9.8	52.9
6	6a	40	7.35	60.0	0.10	0.001	0.02	-	0.5	7.35	92	4.5	9.0	50.0
	6b	45	7.30	62.4	0.11	0.001	0.02	-	0.6	7.68	94	5.0	9.9	39.4
	6c	45	7.20	57.1	0.17	0.001	0.28	-	0.6	8.00	95	6.0	8.1	36.9
7	7	30	7.35	57.5	0.94	0.003	0.65	-	-	-	-	-	11.1	-
8	8a	45	7.00	59.4	0.07	0.006	0.02	-	0.4	8.00	96	4.4	8.4	55.6
	8b	40	7.50	63.1	0.16	0.006	0.02	-	0.4	7.68	93	3.6	8.4	38.1
	8c	45	6.75	64.7	0.08	0.005	0.10	-	0.4	8.48	101	4.4	8.1	-

**Table 4: Environmental variables in the sampling stations of the Amur River in June 2006**

Station	From left bank, m	Site	Color, grad.	pH	Hardness, mg L <sup>-1</sup>	TSS, mg L <sup>-1</sup>	NH <sub>4</sub> , mg L <sup>-1</sup>	NO <sub>2</sub> , mg L <sup>-1</sup>	NO <sub>3</sub> , mg L <sup>-1</sup>	PO <sub>4</sub> , mg L <sup>-1</sup>	SO <sub>4</sub> , mg L <sup>-1</sup>	Phenols, mg L <sup>-1</sup>
1	200	1a-surface	58.0	7.31	0.47	35.6	0.40	0.014	0.29	0.040	4.6	0.002
	300	1b-surface	60.5	7.22	0.51	43.8	0.62	0.017	0.23	0.028	7.2	0.004
	400	1c-surface	60.0	7.21	0.47	37.6	0.40	0.044	0.27	0.036	7.4	0.005
	500	1d-surface	48.5	7.14	0.47	51.6	0.54	0.038	0.23	0.027	4.0	0.005
2	100	2a-surface	58.5	7.35	0.49	58.2	0.30	0.018	0.33	0.032	6.0	0.002
	300	2b-surface	58.5	7.24	0.47	41.2	0.30	0.017	0.35	0.026	7.8	0.002
	500	2c-surface	50.0	7.34	0.61	424.4	0.40	0.020	0.61	0.048	10.4	0.004
3	100	3a-surface	58.0	7.33	0.43	35.8	0.48	0.016	0.44	0.062	5.0	0.004
	200	3b-surface	45.5	7.30	0.42	40.0	0.96	0.015	0.46	0.055	6.0	0.005
	400	3c-surface	47.0	7.29	0.53	48.0	1.42	0.025	0.51	0.065	9.4	0.003
	600	3d-surface	47.0	7.33	0.59	78.6	0.54	0.028	0.73	0.122	7.9	0.004
		3d-bottom	45.5	7.42	0.74	101.8	0.40	0.038	0.55	0.155	10.8	0.004
	800	3e-surface	47.5	7.37	0.63	50.0	0.96	0.025	0.73	0.162	7.1	0.006
4,5	200	4a-surface	46.5	7.10	0.53	70.4	0.60	0.026	0.55	0.056	11.8	0.004
	400	4b-surface	44.0	7.34	0.51	106.0	0.62	0.030	0.61	0.086	11.6	0.006
	800	5c-surface	34.5	7.36	0.72	182.2	0.40	0.028	0.92	0.102	12.0	0.003
6	200	6a-surface	44.0	7.16	0.63	78.8	0.62	0.035	0.61	0.065	14.2	0.004
	400	6b-surface	41.0	7.22	0.66	135.4	0.40	0.032	0.95	0.103	14.0	0.003
	800	6c-surface	37.0	7.09	0.66	175.4	0.36	0.037	0.84	0.125	13.0	0.002

**Table 3: Environmental variables in the sampling stations of the Amur River in August 2005**

Station	From left bank, m	Site	pH	TSS, mg/l	Cond., mksm cm <sup>-1</sup>	Phenols, mg L <sup>-1</sup>	O <sub>2</sub> , mg L <sup>-1</sup>	BOD, mg O <sub>2</sub> L <sup>-1</sup>	COD, mg O <sub>2</sub> L <sup>-1</sup>	NH <sub>4</sub> , mg L <sup>-1</sup>	NO <sub>2</sub> , mg L <sup>-1</sup>	NO <sub>3</sub> , mg L <sup>-1</sup>	PO <sub>4</sub> , mg L <sup>-1</sup>
1	200	1a- surface	7.8	7.4	125.1	0.002	8.76	1.8	26	0.32	0.010	0.14	0.063
Sea River mouth		surface	7.54	9.0	49.4	0.002	7.70	1.07	25	0.30	0.010	0.17	0.042
2	100	2a-surface	7.49	10.4	55.6	0.002	7.87	1.34	23	0.30	0.010	0.18	0.045
	100	2a-bottom	7.46	9.60	55.8	0.002	8.05	1.10	23	0.30	0.010	0.17	0.054
	300	2b-surface	7.49	22.4	57.4	0.002	9.30	2.02	25	0.30	0.025	0.18	0.035
	300	2b-bottom	7.70	7.00	58.0	0.002	7.27	1.48	25	0.30	0.010	0.17	0.032
	500	2c-surface	8.02	11.2	99.5	0.002	8.16	1.52	28	0.30	0.011	0.22	0.052
	500	2c-bottom	8.01	10.2	101.3	0.002	7.76	1.47	22	0.30	0.010	0.33	0.044
Burea River mouth		surface	7.17	7.60	28.5	<0.002	7.40	1.67	22	0.30	0.010	0.33	0.030
		bottom	7.32	7.80	28.2	<0.002	8.16	1.91	34	0.30	0.016	0.44	0.032
Amurzet		surface	7.57	10.8	74.0	<0.002	7.29	1.66	28	0.30	0.010	0.14	0.045
		bottom	7.72	12.8	75.8	<0.002	7.98	1.89	26	0.30	0.016	0.17	0.040
3	100	3a-surface	7.92	10.2	76.9	0.002	7.16	1.43	26	0.30	0.010	0.40	0.035
	100	3a-bottom	8.09	11.0	78.9	0.002	6.26	1.68	34	0.30	0.010	0.44	0.035
	400	3c-surface	7.98	400.4	172.6	0.002	7.16	1.93	30	0.60	0.080	0.77	0.555
	400	3c-bottom	8.09	397.6	173.5	0.003	6.73	1.46	28	1.42	0.045	0.77	0.525
	800	3e-surface	7.66	355.6	170.4	0.002	7.22	1.04	28	1.20	0.065	1.16	0.528
	800	3e-bottom	7.89	417.8	171.4	0.003	7.16	1.38	29	1.12	0.082	1.33	0.224
4,5	200	4a-surface	7.92	106.2	107.8	<0.002	6.73	1.69	35	0.03	0.064	0.77	0.112
	200	4a-bottom	7.96	51.4	106.3	<0.002	7.16	1.20	25	0.05	0.042	0.44	0.092
	400	4b-surface	8.02	67.8	162.7	0.002	6.85	1.30	25	0.52	0.064	0.77	0.195
	400	4b-bottom	8.03	73.4	168.7	0.002	6.73	1.58	15	0.88	0.047	0.86	0.220
	800	5c-surface	8.14	46.8	163.8	0.002	7.44	1.14	15	0.60	0.053	0.92	0.096
6	200	6a-surface	8.07	95.2	129.3	<0.002	7.54	2.15	13	0.72	0.052	0.67	0.132
	200	6a-bottom	8.04	102.0	147.1	<0.002	7.10	1.46	16	0.70	0.050	0.67	0.155
	400	6b-surface	8.05	143.4	144.9	0.002	5.72	1.41	12	0.72	0.040	0.50	0.132
	400	6b-bottom	8.04	153.4	148.4	0.002	5.9	1.30	12	0.92	0.073	0.50	0.155
	800	6c-surface	8.09	50.4	155.2	0.002	6.85	1.84	13	0.88	0.053	0.44	0.173
	800	6c-bottom	8.06	94.4	157.2	0.002	5.72	1.00	15	0.30	0.048	0.44	0.179
7	200	7a-surface	7.95	109.8	121.4	0.002	6.79	2.18	15	0.30	0.042	0.32	0.097
	200	7a-bottom	7.89	100.8	125.5	0.002	6.73	1.52	12	0.36	0.029	0.30	0.145
	400	7b-surface	7.87	137.4	118.2	0.002	7.15	1.60	39.5	0.40	0.054	0.26	0.172
	400	7b-bottom	6.75	187.4	138.1	0.002	7.15	1.81	35.4	0.30	0.058	0.26	0.170
	800	7c-surface	7.47	139.4	100.7	0.002	8.58	2.08	34.3	0.30	0.049	0.30	0.086
	800	7c-bottom	7.09	154.2	101.2	0.002	7.86	1.30	34.3	0.30	0.016	0.26	0.100

A comparison of suspended solids between the periods of impact and after one year as well as across the river canal show that the impact of the Sungary River water was high during catastrophe and can be seen after one year after the catastrophe. Suspended matter enrichments can be seen mostly near the right bank (Fig. 3).

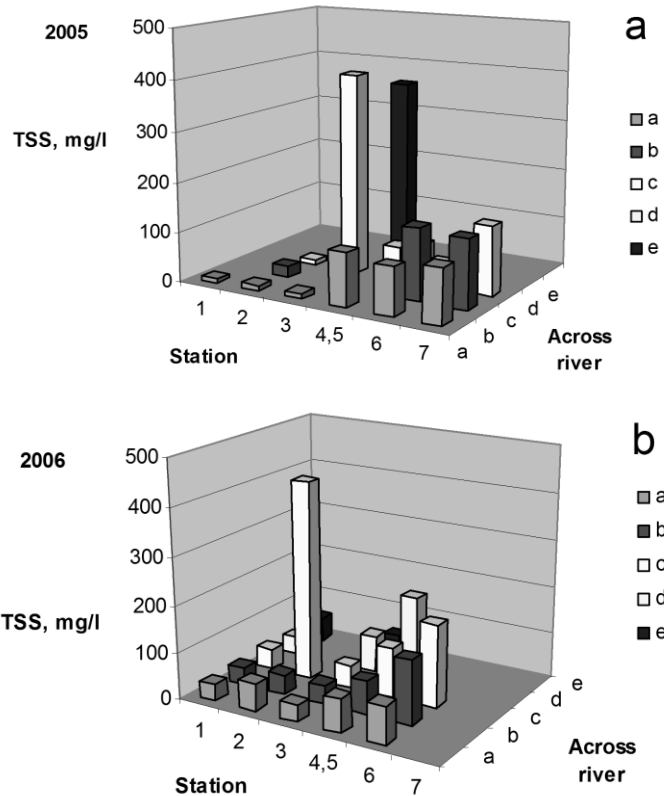


Figure 3: Spatial water pollution of suspended matter (mg/l) over stations cross section from left river banks of the Amur River in 2005 (after impact), and 2006 (one year after catastrophe).

Fig. 4 shows that the distribution of pollutants and suspended matters is similar and increased near the right bank of the river.

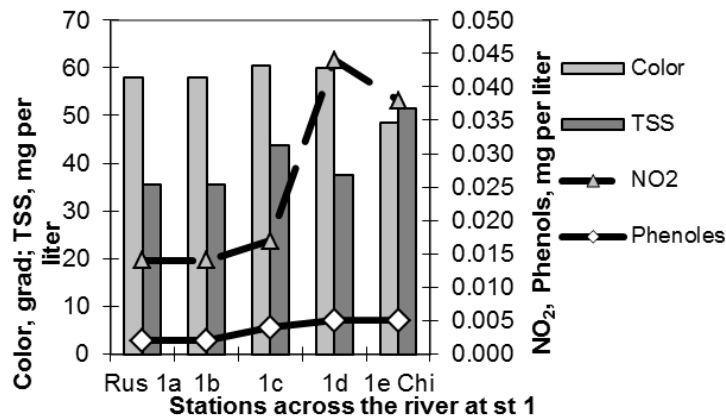


Figure 4: Cross section of water pollution in the Amur River by major pollutants in July, 2005.

### Algal species diversity and abundance

The full checklist of the Amur River and their tributaries, estuary, and lakes algal diversity contain 813 species from 211 genera, belonging to seven taxonomic divisions [22].

In 29 samples of plankton from 6 stations on the Amur River we distinguished 145 species belonging to 5 algal divisions: Cyanoprokaryota – 3, Dinophyta – 1, Chrysochyta – 5, Bacillariophyta – 114, and Chlorophyta – 23. The Bacillariophyta strongly prevail (Fig. 5).

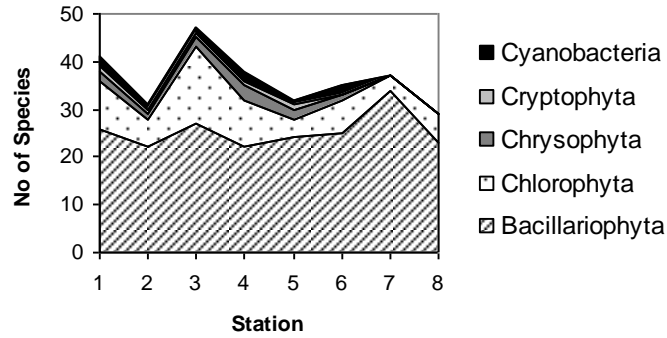


Figure 5: Spatial distribution of algal division over sampling stations of the Amur River

The dynamic of algal division over the stations (Fig. 5) shows a significant decrease in algal diversity at station 2 below the Sungari River mouth, from 42 to 29 species. At stations 3 and 4 we can see increases of algal diversity up to 48 species and after that it decreases. These dynamics represent the marked impact of the Sungari River polluted waters on the Amur River native algal community.

As can be seen in Fig. 6, species richness sharply decreased from left to right on the river banks when algal community content is represented only one species of *Eunotia* which is an acidity indicator. In station 2 diversity decreased, but distribution is the same, from *Actinastrum aciculare* with score 2 to *Eunotia* sp. with score 1. Using this data, we calculated the indicator species representation over the Amur River stations according to diverse indication systems.

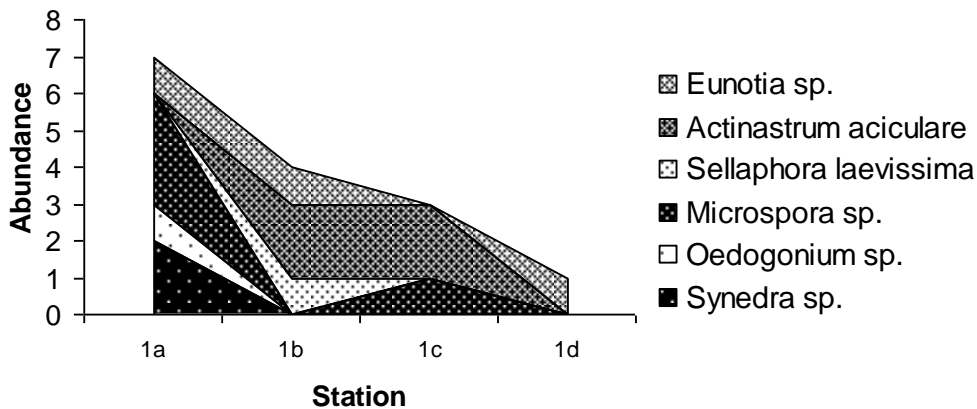


Figure 6: Spatial distribution of algal species over cross section on sampling station 1 of the Amur River

**Bio-indication analysis**

All indication systems are relevant to increases of the analyzing variable (major arrow in Fig. 7). We used eight systems to determine Amur River algal communities' responses to the ecological impact at the sampling stations. Distribution of each indicator group over sampling stations is shown in Fig. 7. Bio-indication shows that the Amur River water is temperate (Fig. 7a), slow streaming and intermediately oxygen enriched (Fig. 7b), low saline (Fig. 7c), low alkaline (Fig. 7d), and reflect the regional norm for silicate provinces [23].

Indicators of organic pollution belonged to II-III Classes of water quality (Fig. 7e, 7f). Nutrition type indicators show the impact on photosynthesis after station 2, where high ranked heterotrophic species (hce) enrich the algal community (Fig. 7g) and increase in followed stations. This situation reflects the toxic impact to the photosynthetic process of producers. The same situation can be seen in the trophic state system (Fig. 7h): indicators of mesotrophy start from station 2 and contain up to half of community.



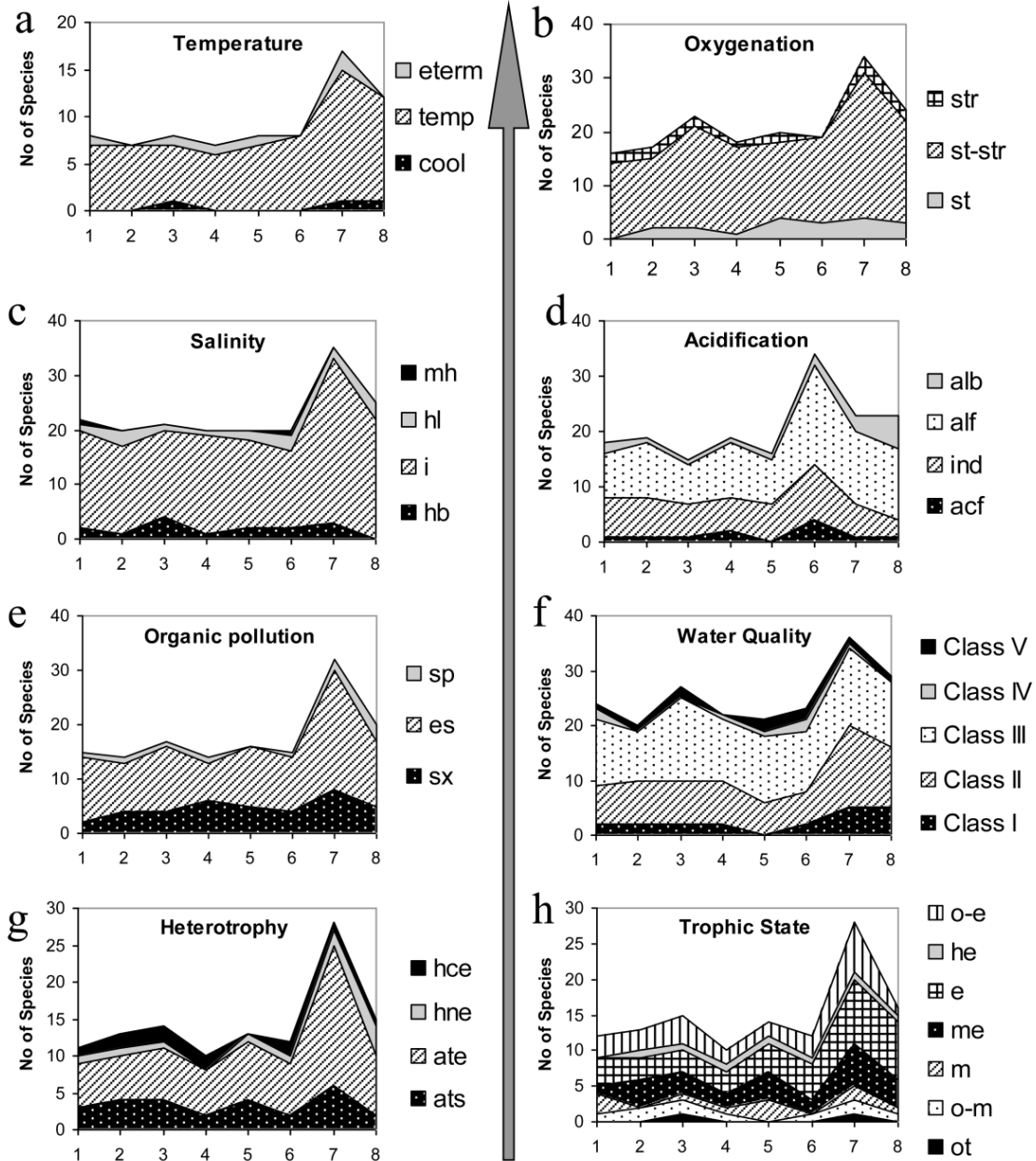


Figure 7: Bio-indication plots over sampling stations of the Amur River. Temperature

(a): cool, cool-water; temp, temperate; eterm, eurythermic. Oxygenation (b): st, standing water with low oxygenation inhabitants; st-str, low-streaming middle oxygenated water inhabitants; str, streaming water enriched by oxygen inhabitants. Salinity (c): hb, halophobes, i, indifferent; hl, halophiles; mh, mesohalobes. Acidification [24] (d): ind, indifferent; alf, alkaliphil; acf, acidophil; alb, alkalibiont. Organic pollution [25] (e): sx, saproxenes; es, eurusaprobies; sp, saprophiles. Class of water quality (f): I, clean water – V, heavy polluted water. Nitrogen uptake metabolism [26] (g): ats, nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate, nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne, facultative nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; hce, obligate nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen. Trophic state [26] (h): o-m, oligo-mesotraphentic; ot, oligotraphentic; m, mesotraphentic; me, meso-eutrathentic; he, hypereutrathentic; e, eutrathentic; o-e, oligo- to eutrathentic (hypereutrathentic).

We grouped the saprobity indicators of the Sládeček [27] method to five relevant Classes of water quality (Fig. 8). The number of species in each Class revealed organic load preferences for all Amur River communities. The histogram shows that the first three Classes of indicators prevail and are cut off by the standard deviation line. The summit of the trend line (polynomial) reveals the prevailing group of middle pollution indicators, which is evidence of low organic pollution of the Amur River as a whole. This tendency also revealed that the trend line (power) shows a decrease in the “polluted” species category. But the presence of 5th Class indicators revealed periodic impacts of pollution on the algal community.

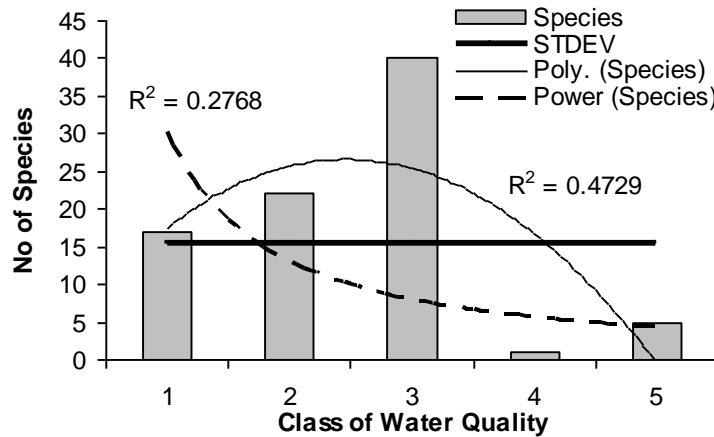


Figure 8: Distribution of species indicators of water quality of the Amur River over water quality Classes

**Dynamic of biological variables**

The saprobity indices, which were calculated for each of sampling stations (equation 1), varied from 1.43 to 2.12 for phyto- and from 1.34 to 2.01 for zooplankton (Fig. 9) and reflected the oligo- to beta-mesosaprobic self-purification zones, attesting to Class II of water quality at the outlet and Class II-III of moderately polluted waters at stations below the Sungari River (Table 5). The range of variations increases from upper stations down the river, peaking at station 6, which reflects the contribution of pollution from the Khabarovsk area. We compare dynamic of Index S with species richness and Shannon index (Fig. 9). Can be seen that impacted community of station 2 have sharply decreased parameters but it's restored till natural condition on the stations 4-5 above Khabarovsk. Subsequent dynamics shows the parameters decreasing below Khabarovsk to the mouth of the river.

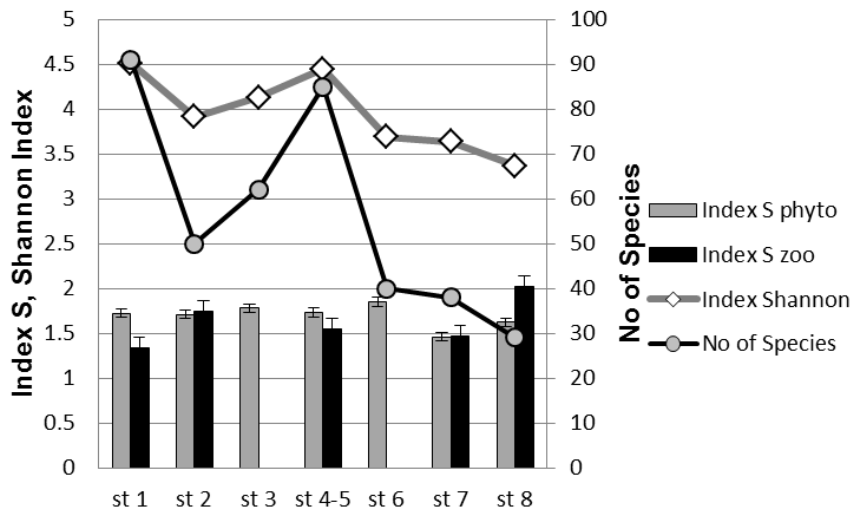


Figure 9: Dynamic of index saprobity S, Shannon Index and phytoplankton Species richness over sampling stations of the Amur River

The chlorophyll- $\alpha$  concentration in the river is correlated with the streaming rates [28] and the nutrient concentrations [29]. In the Amur River, chlorophyll- $\alpha$  concentration in 2005 was low in all sampling stations (Tables 5, 6), corresponding to the ultra-oligotrophic (before the Sungari River input) till the mesotrophic level (after the Sungari waters input). The data reflect the pollution influence, which comes with the Sungari waters.

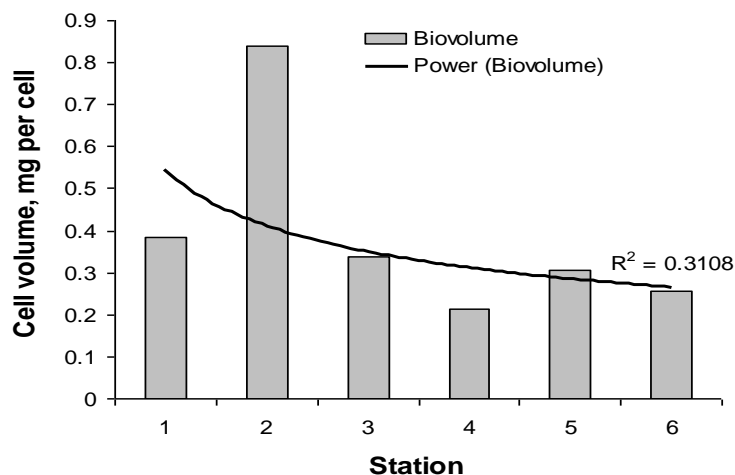
**Table 5: Biological indices, Class of water quality (according to [14]), and the trophic level (according to [30]) over stations of the Amur River in August 2005**

Station	Index S, phytoplankton	Class of water quality	Trophic level, Chl-a	Class Chlorophyll	Index S, zooplankton
1	1.63-1.89	III	Ultra-oligotrophic	1-3	1.17-1.50
2	1.56-1.82	III	Mesotrophic	3-6	1.56-1.93
3	1.43-2.02	II-III	-	-	-
4	1.64-1.90	III	Mesotrophic	3-5	1.55
5	1.52-1.95	III	Mesotrophic	-	-
6	1.70-2.12	III	Oligo-mesotrophic	2-3	1.30-1.64
7	1.47	II	-	3	1.88-2.15

**Table 6: Biological parameters of the algal species communities over stations of the Amur River**

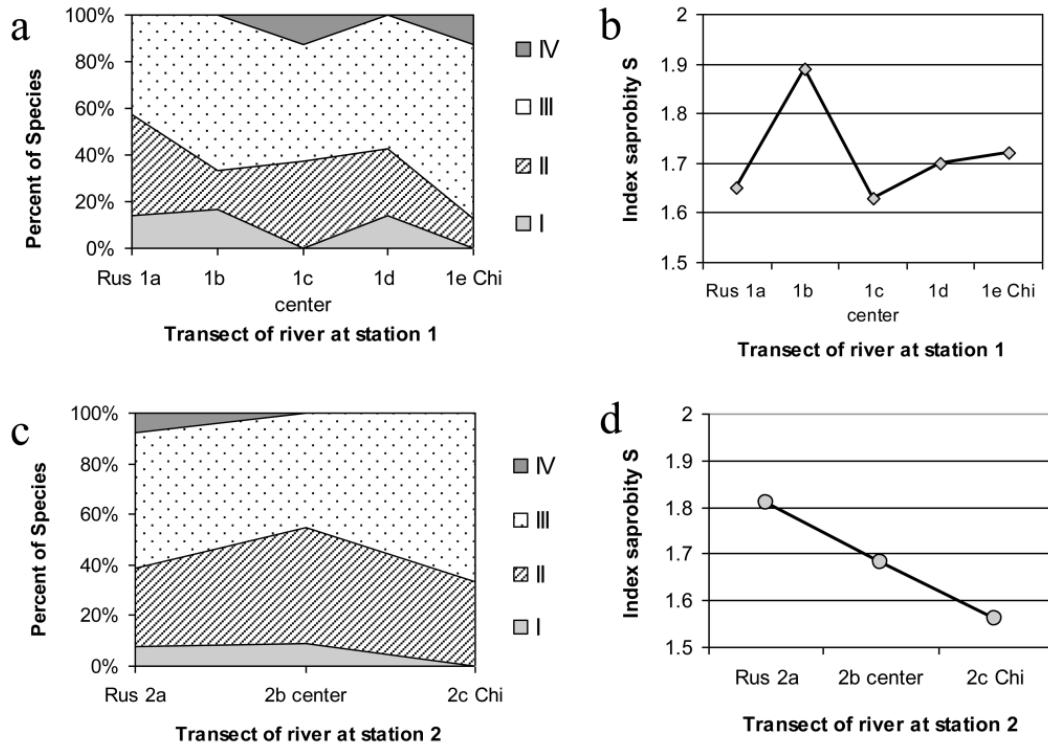
Station	No. of Species	No. of cells, cells 10 <sup>3</sup> L <sup>-1</sup>	Biomass, mg L <sup>-1</sup>	Average cell Biovolume, mkm <sup>3</sup>	Chl-a, mg L <sup>-1</sup>
1	91	501.3	0.383	0.00089	0.2-3.2
2	50	323.4	0.839	0.00195	4.7-52.3
3	62	865.8	0.339	0.00046	-
4	71	600.4	0.213	0.00033	6.6-25.2
5	53	906.6	0.307	0.00054	-
6	41	447.7	0.256	0.00078	2.5-4.6
7	38	-	-	-	3.3-9.2
8	29	-	-	-	-

Tables 5 and 6 show the algal community in station 2 is impacted by input from the Sungari River, which shows a decrease in species richness and phytoplankton abundance. On the other hand, we can see a stimulating effect on biomass production and Chl-a concentration. This effect can be seen in increases in mean algal cell volume. At the same time the increase in Chl-a concentration reflects change in the oligotrophic state of the river to mesotrophic from station 2. But decreasing saprobity indices in station 2 show the inhibitory effect on photosynthetic activity. These two last sentences both point to the complexity of water pollution that in the same time contents of organic matter and toxic pollutants which both impacted of producers. In any case, this complex effect is reduced in following stations of the Amur River, as can be seen in Fig. 10.



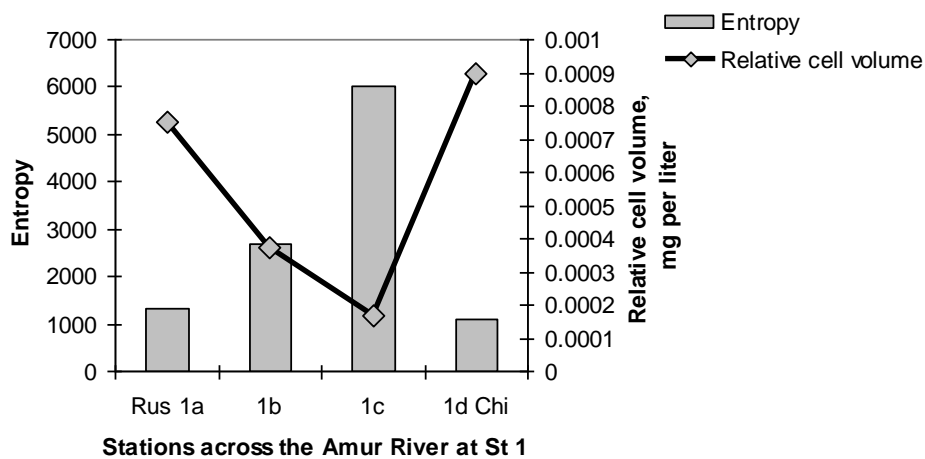
**Figure 10: Distribution of calculated mean cell size over sampling stations of the Amur River**

Fig. 11 show distribution of algal indicators of the water quality cross section over sampling stations 1 (Fig. 11a) before impact and station 2 (Fig. 11c) after impact of the Amur River ecosystem in 2005, and it can be seen that the structure of indicators decreased after the impact near China riverside. The index saprobity S dynamic also shows decreases in organic pollution after impact near the right riverside (Fig. 11b,d). Decreases in the Index S can also be related to the toxic impact of photosynthesis.



**Figure 11: Distribution of species indicators of water quality and index saprobity S cross section of over sampling stations 1 (before impact) and 2 (after impact) of the Amur River in 2005**

Volume of cells is a very important variable [31-33] that influenced the cell division rate as well as the ecosystem structure [34]. Our calculation of the relative cell volume cross section at station 1 in 2005 shows that minimal volume was present at the center of the river channel and increased on both sides, Russian and Chinese, and is opposite to the entropy of the river ecosystem (Fig. 12). This type of distribution demonstrated that ecosystem activity is high near both riversides. Whereas cell number increased down the river communities, the relative cell volume decreased (Fig. 13), which can be as a result of anthropogenic press to phytoplankton of the Amur River.



**Figure 12: Distribution of relative cell volume and calculated entropy of communities over sampling stations of the Amur River in 2005.**

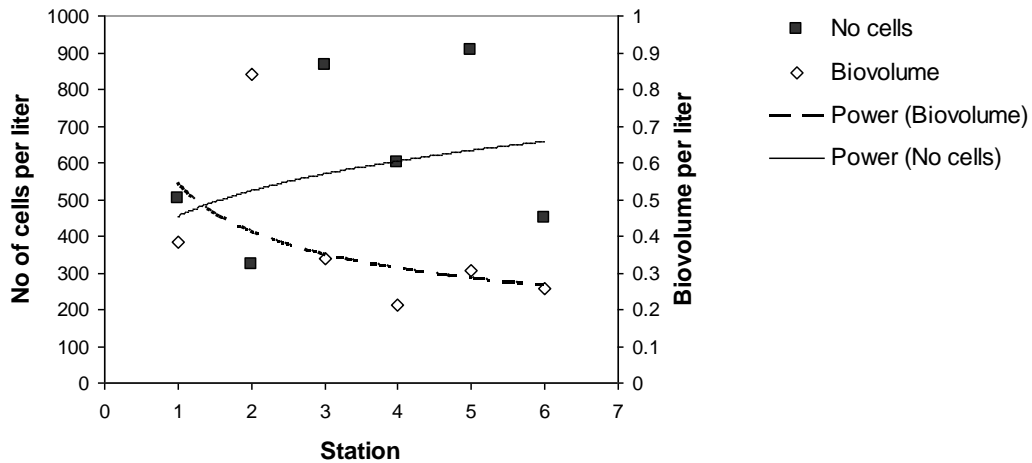


Figure 13: Distribution of algal cells abundance and calculated relative cell biovolume over sampling stations of the Amur River in 2005

CCA Analysis

Statistical analysis of relationships between species content in communities with environmental variables for the Amur River as a whole was calculated using the CANOCO program. CCA biplot (Fig. 14) shows that river water is enriched by studied macro-ions and phenols from one source because all arrows are grouped in one set. The right circles on the biplot include species that are under impact of these variables and therefore can be used as bio-sensor species, which are represents by mostly sensitive diatom species.

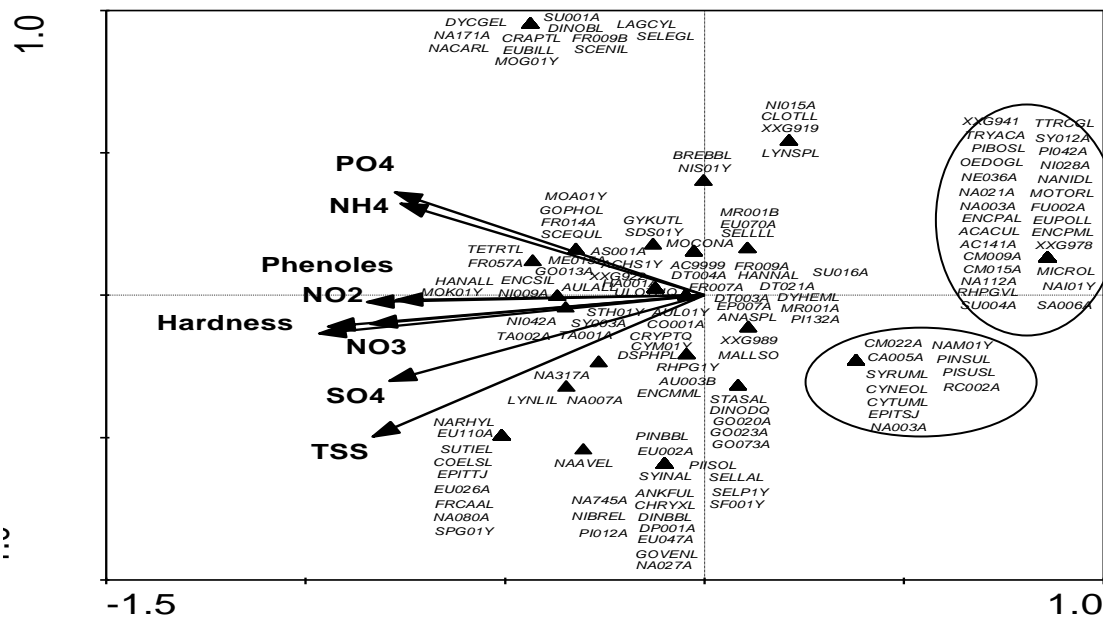


Figure 14: Canonical Correspondence Analysis of full list of algal species from qualitative and quantitative samples and environmental variables relationships for the Amur River in 2005

The same analysis based on phytoplankton communities only (Fig. 15) shows that macro-ions do not significantly impact to algal communities, but is important water acidity and phenols concentration in water. Sulfates and pH as well as nitrates and phenols show the opposite influence. This means that water pH has no connection to air pollution but related with influence of water from the tributaries. The second pair of variables shows that organic pollution (nitrates) and technogenic impact (phenols) come from different sources – river beds and river tributaries, respectively.

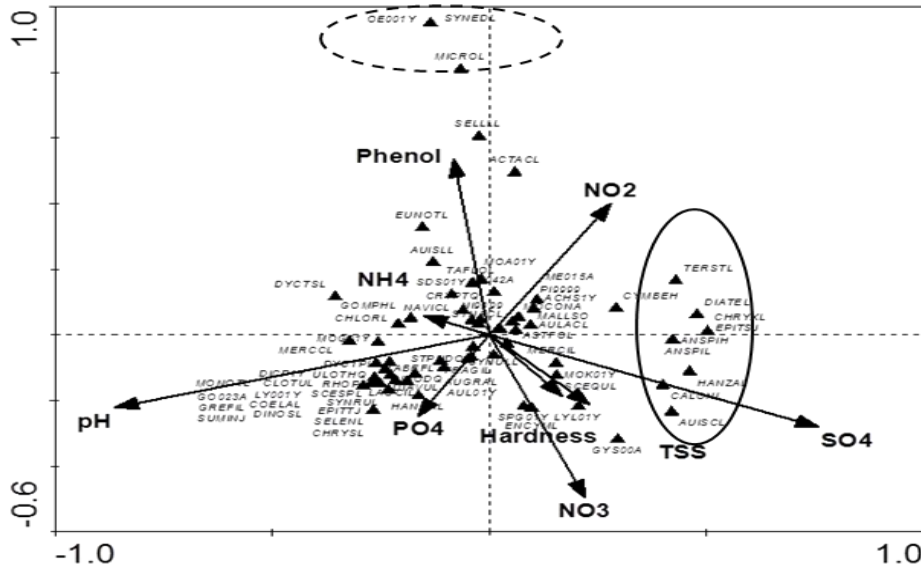


Figure 15: Canonical Correspondence Analysis of algal species from quantitative samples and environmental variables and their influence on the Amur River algal communities in 2005.

As we can see in Fig. 15, the major environmental parameters that influenced algal species diversity at sampling stations of the Amur River are insignificantly affected. In the right circle a few species inhabiting the fresh low-polluted water with neutral pH are marked. But several species (upper circle) can be bio-indicators for the presence of phenols. These species are: *Oedogonium* sp., *Synedra* sp., and *Microspora* sp.

Remarkably, the three mentioned above species of algae are more influenced by phenols in low-mineralized, unpolluted water. Therefore, the ecosystem on the oligotrophic level is more impacted by chemical pollutants.

**Comparative floristic**

A statistical comparison of species richness that was revealed in each sampling station shows that all algal diversity can be divided into three clusters with a similarity level of 40% (Fig. 16). The first cluster on the similarity tree shows species from stations 1, 2, 4, and 5, whereas species from stations 3 and 6, and 7 and 8 are in the second major cluster.

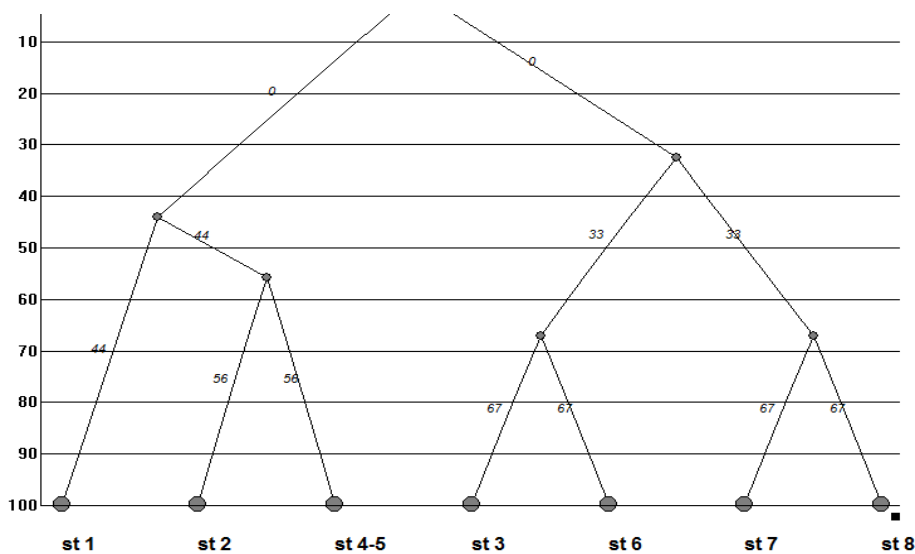


Figure 16: Tree of similarity of the phytoplankton communities in the sampling stations of the Amur River calculated on the basis of Sørensen-Czekanowski indices. At the similarity level of 40% three clusters are cut off.

A calculation of species overlapping studied river communities show a high similarity level for all sampling stations, which fluctuated between 35 and 62%. Dendrite in Fig. 17 reveals one core of species richness that are marked as large circles – stations 4-5 in which community similar to station 1. Therefore, results of comparative floristic show restoration of impacted phytoplankton diversity at the river part above Khabarovsk.

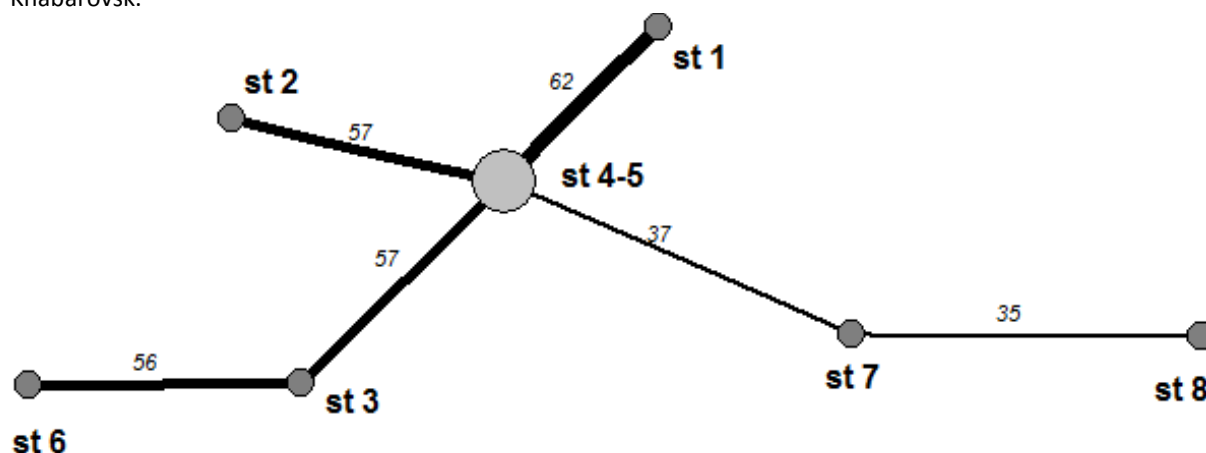


Figure 17: Dendrite of phytoplankton species richness overlapping in the sampling stations of the Amur River calculated on the basis of Sørensen-Czekanowski indices. The bold lines represent the most similar communities. Communities that included most of the species from the others are represented in large circle.

### CONCLUSION

Management of such a complex ecosystem as the Amur River cannot be carried out successfully without considering the transboundary impact of one of its main tributaries, the Sungari River, and defining its major control parameters. The lack of reliable information on anthropogenic pressure on natural complexes in the newly developing northern territories of China, namely, the Sungari Basin and the right riverside areas of the Amur River, makes the efforts of the Russian counterparts less efficient to conserve biodiversity of the Amur ecosystem and to reduce river water pollution. The impact of river discharge into the Okhotsk and Japanese seas is an extremely important ecological aspect, which has not been sufficiently addressed. The intensive anthropogenic impact on Far Eastern natural complexes (discharging untreated sewage, mining by-products, timber harvesting, and harmful chemicals from agriculture, etc., into the river) determines the essence and specifics of current ecological problems in Priamurje and might serve as indicators of a critical situation in the coastal sea areas.

Nowadays, studies of algal biodiversity and structural dynamics of ecosystems are not sufficient to assess water resource quality and its prospective regeneration.

Phytoplankton cell number increased down the river communities; the relative cell volume decreased, which is influenced by the anthropogenic impact on phytoplankton of the Amur River.

Indicators of organic pollution show Classes II and III of water quality. Nutrition type indicators point to the impact of photosynthesis after the Sungari waters input. The same situation is in the trophic state system indication - indicators of mesotrophy are starts from station 2 and contain up to half of community down the river.

Statistical analyses of relationships between algal communities and environmental variables show that organic pollution (nitrates) and the technogenic impact (phenols) come from different sources – river beds and river tributaries, respectively. Bio-indication shows that ecosystem activity is high near both riversides but decreased after the Sungari waters impact. Diversity of impacted community is restored during river flow until Khabarovsk.

Therefore, we revealed that the impact to the Amur River ecosystem is started from basic level of trophic pyramid – phytoplankton, of this large, important transboundary river. Sustainable development of the

Far East and the success of many social and economic programs targeted to secure safety and ecological risks reduction will depend by and large on joint efforts of many specialists in Russia, China, and experts from the international community in environment monitoring and conservation aquatic ecosystem functioning laws.

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#### REFERENCES

- [1] Zalewski M. *Ecological Engineering* 2000; 165: 1–8.
- [2] Kondratjeva LM. *Ecological Risk of Water Ecosystems Pollution*, Dalnauka, Vladivostok, 2005.
- [3] Kondratyeva LM, Rapoport VL, Zolotuchina GF, Vasiljeva LV. *Ecological problems of big rivers-3*. Institute of Ecology of Volga Basin RAS, Toljatti, 2003, pp. 125.
- [4] Kondratyeva LM, Zolotuchina GF, Rapoport VL. *Fundamental problems of study and use of water and water resources: Proceedings of Scientific Conference*, Institute of Geography SB RAS Press, Irkutsk, 2005, pp. 281–283.
- [5] Swift E. *Phycologia* 1967; 6: 161–163.
- [6] Barinova SS. *Paleontological J*, Moscow 1997; 31: 239–245.
- [7] Abakumov AV. *Handbook for hydrobiological monitoring of freshwater ecosystems*. Gidrometeoizdat, Sanct-Peterburg, 1992.
- [8] Carlson, Simpson 1996 Carlson RE, Simpson J. *A Coordinator's Guide to Volunteer Lake Monitoring Methods*. North American Lake Management Society, Madison, Wisconsin, 1996.
- [9] Parsons TR, Strickland JD. *J Marine Res* 1963; 21(3): 155–163.
- [10] Jeffrey SW, Humphrey GF. *Biochemie und Physiologie der Pflanzen* 1975; 167: 191–194.
- [11] Barinova SS, Medvedeva LA, Anissimova OV. *Diversity of algal indicators in environmental assessment*. Pilies Studio, Tel Aviv, 2006.
- [12] Barinova S. *Algal diversity dynamics, ecological assessment, and monitoring in the river ecosystems of the eastern Mediterranean*. Nova Science Publishers, New York, 2011.
- [13] Sládeček V. *Ergeb Limnol* 1973; 7: 1–128.
- [14] Romanenko VD, Oksijuk OP, Zhukinsky VN, Stolberg FV, Lavrik VI. *Ecological impact assessment of hydrotechnical constructions on water bodies*, Naukova Dumka, Kiev, 1990.
- [15] Whitton BA, Rott E, Friedrich G. *Use of algae for monitoring rivers*. Institut für Botanik University Press, Innsbruck, 1991.
- [16] The Directive 2000/60/EP of the European Parliament and of the Council establishing a framework for community action in the field of water policy. OJL 327.
- [17] Odum EP. *Science* 1969; 164: 262–270.
- [18] Novakovskiy AB. *Abilities and base principles of program module "GRAPHS"*. Komi Nauchnyy Center, Ural'skoe Otdelenie Russkoy Akademii Nauk, Syktyvkar 2004; 27: 1–28.
- [19] Ter Braak CJF, Šmilauer P. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5)*. Microcomputer Power Press, Ithaca, 2002.
- [20] Ter Braak CJF. *Vegetatio* 1987; 69: 69–77.
- [21] Berdnikov NV, Rapoport VL, Rybas OV, Pelykh TI, Zolotukhina GF, Zazulina VYe. *Tikhookeanskaya geologiya* 2006; 25(5): 94–63.
- [22] Medvedeva LA, Sirotskiy SYe. *Biogeochemical and geocological investigations of terrestrial and freshwater ecosystems 12*, Dalnauka, Vladivostok, 2002, pp. 130–218.
- [23] Meybeck M, Helmer R. *Palaeogeography, Palaeoclimatology, Palaeoecology (Global and Planetary Change Section)* 1989; 75: 283–309.
- [24] Hustedt F. *Abhandl Naturwiss Ver Bremen*, 1957; 34: 181–440.
- [25] Watanabe T, Asai K, Houki A. *Sci Total Envir* 1986; 55: 209–218.
- [26] Van Dam H, Mertens A, Sinkeldam J. *Netherlands J Aquatic Ecol* 1994; 28: 117–133.
- [27] Sládeček V. *Acta Hydrochem Hydrobiol* 1986; 14: 555–566.
- [28] Bourassa N, Cattaneo A. *J North American Benthological Soc* 1998; 17(4): 420–429.
- [29] Biggs BJF. *J North American Benthological Soc* 2000; 19: 17–31.
- [30] Dokulil MT. *Bioindicators and Biomonitors*, Elsevier, Oxford, 2003, pp. 285–327.





- [31] Stolte W, Riegman R. *Microbiology* 1995; 141: 1221–1229.
- [32] Finkel ZV, Beardall J, Flynn KJ, Quigg A, Alwyn V, Rees TVA, Raven JA. *J Plankton Res* 2010; 32(1): 119–137.
- [33] Reavie ED, Barbiero RP. *Phytotaxa* 2013; 127(1): 150–162.
- [34] Zhang Y, Yin Y, Wang M, Liu X. *Optics Express* 2012; 20(11): 11882–11898.