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RESEARCH PAPERS

Consortium of Higher Aquatic Plants and Microalgae Designed to Purify Sewage of Heavy Metal Ions

N. R. Akmukhanova, B. K. Zayadan, A. K. Sadvakasova*, K. Bolatkhan, and M. O. Bauenova

Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, 050040 Republic of Kazakhstan

**e-mail: asem182010@gmail.com*

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Abstract—We selected higher aquatic plants (HAP) and microalgae possessing a high sorption capacity in respect to heavy metals to form a consortium designed to purify contaminated aquatic ecosystems. Accumulation of heavy metals Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} was investigated in plants *Pistia stratiotes*, *Elodea canadensis,* and *Lemna minor* and green microalgae *Chlorella vulgaris* BB-2, *Ankistrodesmus* sp. BI-1, *Chlamydomonas reinhardtii* B-4, and *Scenedesmus quadricauda* B-1. It was found that intense accumulation of metals occurs in cultures of HAP *Pistia stratiotes* and *Elodea canadensis*. These plants are macroconcentrators of zinc, lead, and copper and microconcentrators of cadmium. Out of the examined cultures of microalgae, effective bioaccumulators of heavy metals were *C. vulgaris* BB-2 and *Ankistrodesmus* sp. BI-1. It was shown that heavy metals are selectively taken up from the medium in the series $Zn^{2+} > Cu^{2+} > Cd^{2+} > Pb^{2+}$. In order to produce a consortium of higher aquatic plants and microalgae for purification of polluted aquatic ecosystems, we investigated interaction of HAP *P. stratiotes* and *E. canadensis* with microalgae *C. vulgaris* BB-2 and *Ankistrodesmus* sp. BI-1 in the course of their cocultivation. Neutral relations were detected between the cells of microalgae *C. vulgaris* BB-2 and *Ankistrodesmus* sp. BI-1 and HAP *E. canadensis*. At the same time, the cells of *Ankistrodesmus* sp. BI-1 and HAP *P. stratiotes* formed a symbiosis. Microscopic examination showed numerous points where the cells of microalgae *Ankistrodesmus* sp. BI-1 were attached to the roots of *P. stratiotes* plants. We tested an opportunity to employ the association between *P. stratiotes* and *Ankistrodesmus* sp. BI-1 for purification of simulated wastewater polluted with heavy metal ions. This consortium proved to be capable of eliminating contaminants from the sewage, reducing their level in the sewage to standard values, and active accumulation of heavy metal ions.

Keywords: consortium, higher aquatic plants, microalgae, sewage, heavy metals

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INTRODUCTION

Treatment of domestic and industrial wastes is an urgent ecological issue in many regions of our planet. In spite of all the measures taken and methods employed for sewage treatment, contaminants permanently come in water bodies. Heavy metals (HM) are among the most dangerous pollutants. It is known that, as a result of interaction with other substances, heavy metals can form high-toxic substances and accumulate in the “water–plants–animals–humans” food chain in the amount manifold exceeding their content in water bodies, which may cause various diseases of the nervous system and some other cases, including oncology [1].

In order to accelerate purification and restoration of spoilt aquatic ecosystems, it is necessary to use biological resources of bacteria and other communities involving organisms with different biochemical potential. Natural associations possess more rehabilitation functions, because they always comprise photosynthesizing organisms: higher plants, eukaryotic algae, and cyanobacteria [2].

Artificial systems of water purification resting on consortia of organisms belonging to different taxonomic groups, application of active strains of destructor microorganisms, isolation and use of microalgae resistant to contaminated water, and inclusion in purifying consortium of higher aquatic plants make it possible to produce a novel integral biotechnology of reclamation and rehabilitation of reservoirs contaminated with various pollutants, including heavy metals. It is necessary to obtain experimental data concerning quantitative relations between HM in the “environment–plant” system, which may form a scientific foundation of selecting cultures for bioremediation of contaminated water.

In this relation, it is important to look for and select active biological objects suitable to make up a consortium, reveal new types of relations between them in artificially produced associations, form a community

Abbreviations: HAP—higher aquatic plants; HM—heavy metals.

with a broad spectrum of sorption of heavy metals on this basis, and widely use it for bioremediation of natural water and sewage.

In this work, we have made an attempt to produce a consortium of higher aquatic plants (HAP) and microalgae and test its possible use for treatment of sewage contaminated with heavy metal ions.

MATERIALS AND METHODS

We used natural and collection strains of microalgae: *Chlorella vulgaris* BB-2, *Scenedesmus quadricauda* B-1, *Chlamydomonas reinhardtii* B-4, and *Ankistrodesmus* sp. BI-1 and higher aquatic plants: *Pistia stratiotes* (water lettuce), *Lemna minor* (duckweed), and *Elodea canadensis* (Canada water weed).

Before the experiment, the cells of microalgae were transferred from slant agar or liquid maintenance medium to the flasks with fresh nutrient medium of the same composition that will be subsequently used in the investigation. In the experiments where different cultures were compared, all the forms were preliminarily transferred on the same day and with the same cell density or biomass of microalgae. Cultures of microalgae were grown during 4–6 days at illumination of 60 W/m^2. Strains of microalgae were cultured on liquid and agar nutrient media (04, Tamiya, and L2-min) [3]. Microalgae were grown in 250–1000-mL cone flasks illuminated with daylight lamps (60 W/m^2) at 25–28°C.

When the effect of heavy metals on HAP was studied, the metals were added to the nutrient media as salts: $CuSO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $PbSO_4$, and $CdCl_2 \cdot 5H_2O$ at concentrations from 0.01 to 1 mg/L on the basis of 10 MPC for each metal ion. For *P. stratiotes,* four vessels containing 500 mL of distilled water were supplemented with Steinberg medium to achieve a final concentration of 5% [4]. Five large or medium size specimens of water lettuce with rosette diameter of 6–12 cm and weight of 4.0–13.0 g were selected for the experiments. Canada water weed plants were selected by identical morphologic characteristics. Apical whorls of Canada water weed were placed by five pieces in 500-mL beakers with settled tap water supplemented with 5% of Hoagland-Arnon medium [5] at 23–25°C and daylight. The plants of duckweed were grown on Steinberg medium in 500-mL glass beakers at 20–22°C and steady illumination with a luminescent lamp. The plants were accommodated in the beakers filled with the nutrient medium at a certain concentration of HM. The beakers were placed under a luminescent lamp with illumination intensity of 60 W/m^2 at room temperature. Plant material sampled for analysis was washed for 3 min in 0.01% Na-EDTA and then three times rinsed in distilled water for 3–5 min to remove metals absorbed on the surface.

When the effect of heavy metals on microalgae was investigated, we supplemented respective nutrient media (04, Tamiya, and L2-min) with the salts $CuSO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $PbSO_4$, and $CdCl_2 \cdot 5H_2O$ at concentrations from 0.01 to 1 mg/mL on the basis of 10 MPC for each metal ion. Microalgae were cultivated during 6 days in 250-mL cone flasks illuminated with daylight lamps (30–60 W/m^2) at 25–28°C and continuous aeration. After cultivation, biomass of microalgae was separated using a 5810 R centrifuge (Eppendorf, Germany) for 15 min at 3000 rpm.

The content of heavy metals in the tissues of examined plants and biomass of algae was determined by means of atomic absorption spectroscopy using an MGA-915MD spectrometer (Atompribor, Russia) after wet digestion in 70% HNO_3 of special grade [6].

In order to look into relations between higher aquatic plants *P. stratiotes* and *E. canadensis* and microalgae *C. vulgaris* BB-2 and *Ankistrodesmus* sp. BI-1, we grew them together in laboratory conditions. To this end, sterile beakers were filled with 250 mL of sterile nutrient medium, then culture of microalgae with initial density of 10^6 cells/mL was added, and five plants were accommodated therein. Concurrently with the experimental types of treatment (microalgae + HAP), control monocultures were examined under the same conditions. All the types of treatment were repeated three times. In 7 days, we compared growth and morphological changes in plants and microalgae of the experimental and control types of treatment.

In order to estimate purifying properties of the formed consortia, sewage waters taken from Sorbulak waste disposal plant near Almaty containing different mineral substances were supplemented with the salts of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}) at a concentration of 20 mg/L each; then ten specimens of HAP *P. stratiotes* were added per 10 L and *Ankistrodesmus* sp. BI-1to a final concentration of 2.5×10^7 cells/mL. Cultivation lasted for 48 h. After that, *P. stratiotes* and *Ankistrodesmus* sp. BI-1 were separated from the solution by means of filtration and centrifugation. Content of biogenic elements and ions of heavy metals under investigation was determined in supernatant.

RESULTS AND DISCUSSION

Selection of Higher Aquatic Plants (HAP) for Use in Purification of Aquatic Ecosystems Contaminated with Heavy Metals

By the extent of HM accumulation, aquatic plants are conditionally subdivided into macro-, micro-, and deconcentrators [7]. Upon different content of metals in bottom sediment, the same HAP species may be attributed to different classification groups. By the character of accumulation and distribution of metals depending on their level in the habitat, plants are also subdivided into three groups: (1) accumulators notable for an elevated content of metals in the organs irre-

Table 1. Accumulation of heavy metals by higher aquatic plants

Plant	Me^{2+}	Initial concentration of Me^{2+} in the medium, mg/L	Final concentration of metal in supernatant, mg/L	Concentration of Me^{2+} in plants, mg/L	Coefficient of accumulation of Me^{2+} in plants, %
Lemna minor	Zn	1	0.256 ± 0.001	0.74 ± 0.001	74
Pistia stratiotes	Zn	1	0.02 ± 0.001	0.978 ± 0.001	98
Elodea canadensis	Zn	1	0.139 ± 0.001	0,86 ± 0.001	86
L. minor	Cu	1	0.28 ± 0.00015	0.719 ± 0.00015	72
P. stratiotes	Cu	1	0.2 ± 0.0001	0.978 ± 0.0001	98
E. canadensis	Cu	1	0.07 ± 0.0001	0.931 ± 0.0001	93
L. minor	Pb	0.3	0.80 ± 0.0005	0.219 ± 0.0002	73
P. stratiotes	Pb	0.3	0.054 ± 0.002	0.246 ± 0.001	82
E. canadensis	Pb	0.3	0.045 ± 0.0001	0.255 ± 0.0003	85
L. minor	Cd	0.01	0.003 ± 0.0006	0.006 ± 0.0007	60
P. stratiotes	Cd	0.01	0.0020 ± 0.001	0.0080 ± 0.001	80
E. canadensis	Cd	0.01	0.0020 ± 0.001	0.0080 ± 0.001	80

spective of their concentration in the environment; (2) indicators that accumulate the metals in proportion to their concentration in the habitat; (3) excluders with intracellular concentration of the metal being maintained at a low level irrespective of external concentrations [8].

We investigated accumulation of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}) by *P. stratiotes, E. canadensis,* and *L. minor* plants at a metal concentration of 10 MPC. Analysis has shown that almost all heavy metals tended to accumulate in plant tissues.

Our experiments showed that, after cultivation of plants, concentration of the examined metals in the medium considerably decreased. Out of heavy metals under investigation, higher aquatic plants accumulated very much Zn^{2+}, with the content of HM in plant biomass ranging from 0.69 to 0.9 mg/g. The greatest accumulation of zinc in *P. stratiotes* was 98% and that in *E. canadensis* was 86% of the initially added concentration. *L. minor* accumulated less zinc, with the percentage of accumulated metal of 74%. Similar pattern was observed for copper ions. The greatest accumulation of Cu^{2+} was observed in *P. stratiotes* (97%), that in *E. canadensis* was lower (93%), and the least accumulation was observed in *L. minor* (71%). The quantity of accumulated Pb^{2+} was in the range from 0.219 to 0.255 mg/g dry wt, with its greatest content being found in *E. canadensis* (85%). Percentage of taken up Cd^{2+} turned out to be low in all the examined plants. The greatest accumulation (80%) was detected in *P. stratiotes* and *E. canadensis.*

Thus, we revealed differences in the pattern of accumulation of heavy metals in the examined species of HAP. The obtained data also suggest that accessibility of HM to HAP depends on their affiliation with biological groups of accumulators, excluders, or indicators, which (on condition that the quantity of HM is the same) determines their content, dynamics of accumulation, and absolute quantities. By the uptake of HM, HAP species we examined form series shown in Table 1.

Irrespective of their belonging to different ecological groups, aquatic plants may accumulate rather high levels of certain elements during their lifetime [9]. Study of HAP is a necessary component of the monitoring of water bodies, because the elements of the natural environment differently respond to man-caused intervention. Faculty for accumulation of chemical elements is very important for estimation of the quality of waters. Thus, accumulating much heavy metal in their biomass, HAP act as a powerful biofilter promoting self-purification of the aquatic ecosystem. We found that *P. stratiotes* and *E. canadensis* are macroconcentrators in respect to zinc, lead, and copper ions and microconcentrators of cadmium. *L. minor* is a microconcentrator of all the examined heavy metals. Our experiments showed that Zn^{2+} and Cu^{2+} are very much involved in migration cycles, and Pb^{2+} and Cd^{2+} are less involved. Such a faculty of Zn and Cu for selective accumulation in plant tissues is probably related to their participation in metabolic processes as components of pigments, vitamins, and enzymes.

Table 2. Series describing the uptake of heavy metal ions by higher aquatic plants

Plants	Heavy metals
Lemna minor	Zn > Pb > Cu > Cd
Pistia stratiotes	Zn > Cu > Pb > Cd
Elodea canadensis	Cu > Zn > Pb > Cd

Table 3. Accumulation of heavy metal ions by the cells of microalgae

Strain of microalgae	Accumulation of different ions of heavy metals by the cells of microalgae, mg/L			
	Cu^{2+}	Cd^{2+}	Zn^{2+}	Pb^{2+}
Chlorella vulgaris BB-2	0.921 ± 0.02	0.004 ± 0.0001	0.853 ± 0.01	0.105 ± 0.01
Ankistrodesmus sp. BI-1	0.892 ± 0.02	0.006 ± 0.0002	0.902 ± 0.03	0.097 ± 0.002
Scenedesmus quadricauda B-1	0.453 ± 0.01	0.004 ± 0.0001	0.877 ± 0.01	0.086 ± 0.002
Chlamydomonas reinhardtii B-4	0.303 ± 0.01	0.006 ± 0.0001	0.893 ± 0.02	0.090 ± 0.003

Selection of Microalgae for Use in Purification of Polluted Aquatic Ecosystems

In littoral ecosystems, algae predominantly accumulate metals belonging to a certain group (Table 2). Individual species of algae and higher aquatic plants can accumulate a specific group consisting of five or seven metals. For instance, algae from the order Ceramiales mainly accumulate Ti, Mn, Fe, and V [10]. At the same time, some algae are notable for a selective type of accumulation of toxic metals. It is known that a selective uptake of the elements by the algae and their tolerance are controlled at the genetic level. For instance, some algae are capable of selective accumulation of zinc. Among these are the species from the genus *Fucus*. At the Norwegian shore suffering from man-caused pollution, the content of zinc in *Fucus evanescens* was 2207 μg/g. Selective accumulation of heavy metals by the algae points to their important biogeochemical role in distribution of microelements and toxic metals between abiotic and biotic components of maritime ecosystems. This property characteristic of certain algae makes it possible to use them as components of biological filters and consortia designed for removal of the local contamination of aquatic ecosystems [11].

When cultural medium was supplemented with $CuSO_4 \cdot 2H_2O$ at a concentration of 1 mg/mL, the content of copper in the cells of *C. vulgaris* and *Ankistrodesmus* sp. sharply rose as early as in 6 h, and, subsequently, it gradually increased during 6 days. On the sixth day, accumulation of Cu by the cells of *C. vulgaris* was 0.92 mg/L and that by the cells of *Ankistrodesmus* sp. was 0.89 mg/L. In the cells of *C. reinhardtii* and *S. quadricauda,* accumulation of copper after 24-h-long culturing was 0.49 mg/L, and it decreased to approximately 0.37 mg/L by the sixth day of cultivation.

Dynamics of decrease in Zn from the cultural medium in the course of development of microalgal cultures showed that peak uptake of the metal by all the examined cultures occurred during the first 24 h after its addition to the medium. As early as in 6 h after the addition of Zn at a concentration of 1.0 mg/L, its level in the medium became much lower (0.75 mg/L on the average). In 24 h, the medium lost up to 0.85 mg/L of zinc ions, and microalgae accumulated up to 0.90 mg/L on the sixth day of culturing.

All the examined cultures of microalgae showed a high cumulative activity in respect to zinc, which apparently depends on its necessity for normal growth of photosynthesizing organisms (in particular, for electron transport and operation of many of the key enzymes) and its lower toxicity as compared with other examined metal ions [12]. In order to obtain Zn in a quantity sufficient for metabolism, the cells employ several types of proteins participating in its binding and transfer. It was shown that zinc may be translocated in the cells of algae and plants by the proteins belonging to CDF and ZIP families [13]. The proteins involved in the transfer of metals may show a high or low affinity for the translocated ions. A wide range of the genes participating in the synthesis of transport proteins suggests that some regulatory mechanisms should operate ensuring adaptation of microalgae and plants to high concentrations of HM in the environment.

Investigation of the dynamics of accumulation of cadmium ions in the cells of microalgae in the presence of cadmium in the medium at a concentration of 0.01 mg/L showed that the cultures of *C. reinhardtii* and *Ankistrodesmus* started to actively accumulate cadmium during the initial 6 h of culturing; during this time interval, concentration of cadmium in the supernatant decreased to 0.0068 mg/L on average and its level in the cells amounted to 0.0030 mg/L. On the sixth day of culturing, accumulation of the metal by the cells of these cultures ran to 0.0060 mg/L.

During the initial hours, the cells of *C. vulgaris* and *S. quadricauda* took up a little cadmium. However, upon a prolonged cultivation in the presence of Cd^{2+}, the intracellular level of these ions somewhat rose. Accumulation of cadmium by the cells of *C. vulgaris* and *S. quadricauda* was 0.0042 mg/L on average.

As to accumulation of lead by microalgae grown in the cultural medium containing this metal at a concentration of 0.3 mg/L, its uptake by the cells was much lower than accumulation of the rest HM. For instance, accumulation of lead by the cells of *Ankistrodesmus* sp. was 0.097 mg/L, that in *C. vulgaris* accumulation was 0.105 mg/L, that in *S. quadricauda* accumulated up to 0.086 mg/L, and that in *C. reinhardtii* was 0.090 mg/L, which amounted to 31% on average

Fig. 1. *Pistia stratiotes* before culturing with microalgae. Photographs show the root system of the plant free of the cells of microalgae (magnification ×40).

of the initially added concentration of metal with predominant amount of lead being taken up by 24 h of the experiment.

Thus, out of the examined microalgae, the most efficient bioaccumulators proved to be *C. vulgaris* and *Ankistrodesmus* sp. Preferable uptake of HM from the medium is described by the series: $Zn^{2+} > Cu^{2+} > Cd^{2+} > Pb^{2+}$ (Table 3).

These data point to a significant difference in the rate and degree of accumulation of heavy metals by the cells of individual microalgae.

Consortium of Higher Aquatic Plants and Microalgae Designed for Purification of Polluted Aquatic Ecosystems

Structural and functional features of the groups of algae and cyanobacteria in many respects depend on their development by themselves or in association with higher aquatic plants. In biotopes lacking macrophytes, application of nitrogen (400 mg/dm^3) caused an intense development of cyanobacteria with domination of the species *Oscillatoria planctonica*. At the same time, biotopes with macrophytes showed only a short-term activation of development of green algae followed by a resumption of domination of diatoms associated with almost total depletion of nitrogen (to 2.3 mg/dm^3). Thus, HAP appreciably influenced both hydrochemical regime of the reservoirs and algal communities [14].

In order to look into interaction between HAP *P. stratiotes* and *E. canadensis* and microalgae *C. vulgaris* and *Ankistrodesmus* sp., we grew them together under laboratory conditions. In 7 days, we analyzed changes in the number of microalgae upon their cocultivation with HAP (Table 4).

When microalga *C. vulgaris* was cultivated together with HAP, the growth of chlorella was slightly stimulated (by 2%) and its cells propagated freely without being attached to plants. Chlorella did not exert any adverse effect on HAP growth. A neutral type of relationships was detected between the cells of *C. vulgaris* and HAP. Similar pattern was observed when *E. canadensis* was cocultivated with the cells of *Ankistrodesmus* sp.

Meanwhile, cocultivation of *Ankistrodesmus* sp. and *P. stratiotes* revealed a symbiotic type of relationships. Microscopic examination showed numerous points of contact between the roots of *Pistia stratiotes* and the cells of microalgae *Ankistrodesmus sp.* BI-1. Figures 1 and 2 show distinct differences in the root system of *Pistia stratiotes* before and after cultivation with microalgae. In Fig. 2, arrows show the points of close contact between the cells of microalgae and the roots of plant observed upon their cocultivation (Table 5). When cultivated together, both the cells of microalga *Ankistrodesmus sp.* BI-1 and *Pistia stratiotes* grew actively with a slight increase in the number of the cells of microalgae occurring in the experiment as compared with control material.

Table 4. Effect of higher aquatic plants on the growth of microalgae

Strain of microalgae	Initial number of cells of microalgae, 10^6 cells/mL	Number of cells of microalgae without HAP, 10^6 cells/mL	Number of cells of microalgae in the presence of HAP, 10^6 cells/mL	
			Pistia stratiotes	*Elodea canadensis*
Chlorella vulgaris BB-2	1 ± 0.1	9.2 ± 0.04	9.1 ± 0.02	9.3 ± 0.01
Ankistrodesmus sp. BI-1	1 ± 0.2	9.1 ± 0.02	9.4 ± 0.01	9.0 ± 0.02

Fig. 2. *Pistia stratiotes* cocultured with *Ankistrodesmus* sp., BI-1. Arrows point to close contacts between microalgae and plant roots (magnification ×40).

Investigation of the Extent of Purification of Polluted Water Using a Consortium of HAP and Microalgae

At the initial stage of investigation, any method of purification should be tested using model (simulated) solutions. This makes it possible to reveal and describe the mechanisms of reactions going on in the course of treatment, identify the most probable products of transformation of the main pollutants, estimate dynamics of their accumulation, and optimize parameters of the treatment process [15].

As the main aim of our work was to make up a consortium of HAP and microalgae designed for treatment of sewage contaminated with HM ions, we looked into the possible use of association of *P. stratiotes* and *Ankistrodesmus* for this purpose. For purification, we used residential wastewaters supplemented with the salts of HM. Experimental wastewater had biochemical oxygen demand (BOD_5) of 62.2 mg/L, ammonia content of 13.7 mg/L, nitrites of 0.4 mg/L, nitrates of 0.8 mg/L, and phosphates of 4.46 mg/L. The content of HM ions (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}) was 20 mg/L each. Ten plants of *P. stratiotes* were accommodated in the simulated wastewater, *Ankistrodesmus* sp. was added to ensure density of 2.5×10^7 cells/mL, and cultivation lasted for 72 h. Then the plants and microalgae were separated from the wastewater by means of filtering. Table 5 shows that the efficiency of model wastewater treatment by this consortium was 98% for biogenic substances and 89–93% for ions of heavy metals.

Thus, we showed that it is feasible to use the formed consortium of HAP and microalgae for treatment of simulated wastewater polluted with HM ions. The obtained results suggest that the consortium of HAP *P. stratiotes* and microalga *Ankistrodesmus* sp. is more efficient for purification of model wastewater in laboratory conditions than plants and microalgae used

Table 5. Purification of model wastewater using a consortium of higher aquatic plants *P. stratiotes* and microalga *Ankistrodesmus* sp. during 72 h

Characteristic	Initial concentration of pollutant, mg/L	Final concentration of pollutant, mg/L		
		Ankistrodesmus sp.	*Pistia stratiotes*	Consortium (*P. stratiotes* + *Ankistrodesmus* sp.)
BOD_5, mg/L	62.2	25.6 ± 0.02	12.4 ± 0.03	4.6 ± 0.04
Ammonia	13.7 ± 0.3	1.2 ± 0.01	–	–
Nitrites	0.4 ± 0.01	–	–	–
Nitrates	0.8 ± 0.01	–	–	–
Phosphates	4.46 ± 0.2	–	–	–
Cadmium	20 ± 0.2	7.8 ± 0.02	7.1 ± 0.02	2.2 ± 0.02
Zinc	20 ± 0.3	6.1 ± 0.03	5.9 ± 0.03	1.4 ± 0.03
Copper	20 ± 0.3	7.3 ± 0.01	6.1 ± 0.02	3.62 ± 0.01
Lead	20 ± 0.2	7.2 ± 0.05	6.6 ± 0.03	2.0 ± 0.02

separately. Our data may serve as a basis for working out a biological method of treatment of waste discharged by manufacturing enterprises.

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