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Planktonic Invertebrates in the Assessment of Long-Term Change in Water Quality of the Sorbulak Wastewater Disposal System (Kazakhstan)

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Abstract: The multicomponent composition of wastewater makes it challenging to assess its quality objectively, but the last one is a prerequisite for the safe re-use of wastewater. The solution to this problem should be aimed at finding criteria that make it possible to increase the objectivity of assessing the water quality of reservoirs with multicomponent pollution. This work analyzes the water quality of the Sorbulak wastewater disposal system in the summer of 2017, based on chemical variables and zooplankton structure and assess the long-term changes in the water quality of Sorbulak. According to the Kruskal–Wallis test, in 2017, the differences between the studied water bodies in the content of nutrients and heavy metals were mostly insignificant. From 2000–2002 to 2017, nitrate, nitrite nitrogen, and heavy metals in Sorbulak significantly decreased. Zooplankton communities consisted of a relatively small number of eurybiontic species resistant to environmental factors. The variability of the quantitative variables of zooplankton was associated with the nutrients. Males dominated the population of the cyclopoid copepods *Acanthocyclops trajani*. In 2000–2002 individuals with morphological anomalies were found in cyclopoid copepods populations, but were absent in 2017. The appearance of individuals with morphological anomalies was associated with copper or lead. The chemical data and structure of zooplankton communities indicated that the toxic pollution of Sorbulak decreased by 2017 compared to 2000–2002. Our results demonstrate that the structural variables of zooplankton communities could be successfully used to assess the water quality of water bodies with mixed pollution. We recommend using not only the traditional set of biological variables (abundance, biomass, diversity indices, and the average mass of an individual), but also data on the structure of species dominance, the sex structure of copepod populations, and the presence of individuals with morphological anomalies for monitoring of water bodies with mixed pollution.

Keywords: wastewater; zooplankton structure; sex ratio; morphologic abnormalities

1. Introduction

Due to socio-economic, demographic, and climatic reasons, the share of re-use wastewater for irrigation increases from year to year in different parts of the world [1,2] and especially in arid areas. However, such a policy of using water resources poses a serious threat to public health, due to the accumulation of toxic compounds in food [3–5], the risk of contracting infectious diseases [6], and the

genotoxic properties of wastewater [7]. Water quality assessment [1,2,4–7] identifies the benefits and risks of recycling wastewater. The solution to this problem is fraught with specific difficulties, primarily due to the multicomponent composition of wastewater. Along with nutrients, wastewater contains a wide range of toxic compounds, including heavy metals [8,9], pesticides, surfactants [10], phenols, petroleum products [11].

A complete chemical analysis of all hazardous compounds that make up wastewater is impossible both physically and economically. Moreover, it is challenging to predict chemical reactions between pollutants [12]. As a result, it is impossible to obtain objective information on the wastewater hazard only based on chemical methods. In the Water Framework Directive [13], it is recommended to use an integrated approach to assess aquatic ecosystem health. It is based on a combination of chemical and biological methods, and it is increasingly being used in environmental research [14–16]. The informative value of the biological assessment is associated with the integrated response of living organisms to the entire complex of external conditions, including natural and anthropogenic factors [17–20].

The need for wastewater re-use also exists in Kazakhstan, located mainly in the zone of insufficient moisture. Sorbulak, together with ponds (referred to as "the Sorbulak wastewater disposal system"), is one of the largest disposal systems in Kazakhstan and the world. Treated municipal and industrial wastewater from an area with a total population of 2.5 million people is discharged mainly into Sorbulak [21]. The ponds are designed to divert part of the wastewater when there is a threat of overflow from Sorbulak. In this case, water is discharged through a canal from the ponds into the Ili river and then enters Lake Balkhash, the largest fishing water body in Kazakhstan, after the Caspian and Aral Seas. The content of many pollutants in treated wastewater exceeds the maximum permissible concentration [22]. According to calculations [21], about 80 tons of suspended solids, 1.4 thousand tons of iron, 45 tons of copper, 29 tons of chromium, 1 ton of lead, 27 tons of cadmium, and the same amount of zinc and strontium entered Sorbulak with wastewater from 1973 to 1998.

The volume of wastewater entering Sorbulak has decreased in recent decades, due to socio-economic reasons [21]. However, in some years, there is a threat of a breakthrough in the barrier dams and the inflow of a large volume of polluted water into the fishery water bodies located below, including Balkhash Lake and the Ili River [8,23–25]. A comprehensive assessment of the water quality of Sorbulak reservoir and ponds is required to select the ways of re-using the large volume of water accumulated in these water bodies. Until now, such studies have not been carried out in Kazakhstan. Published articles provide fragmented data on the content of the primary pollutants in water, soil, the muscle tissue of fish [8,9,22,26–28], and the structure of zooplankton communities in Sorbulak [8,26–28]. Information on the pollution of wastewater ponds is absent, although, given their role, assessing the ecological state of these ponds is an urgent task.

A traditional set of biological variables is currently used to assess the organic pollution of aquatic ecosystems [17–20]. It includes data on the species composition, abundance, biomass, diversity indices, less often data on the size structure of aquatic communities. Criteria for the biological assessment of water bodies with toxic or mixed pollution have not been established. We assumed that, in addition to the above biological variables, data on the structure of species dominance, sex structure, and the appearance of individuals with morphological anomalies in populations of copepods could increase the efficiency of assessing the water quality of reservoirs with mixed pollution. We tested our hypothesis in the Sorbulak wastewater disposal system, which is characterized by multicomponent pollution.

The work aimed to analyze the water quality of Sorbulak and the ponds of the disposal system in the summer of 2017 based on the chemical variables and an expanded set of structural variables of zooplankton, as well as assess the long-term changes in water quality in Sorbulak in 2000–2017.

2. Materials and Methods

2.1. Description of Study Area

2.1.1. Climate

The territory is a part of a semi-arid zone that is characterized by a continental climate. Summers are dry and hot, with a maximum air temperature of up to +45 °C and an average July temperature of +25.5 °C. Winter is cold, with a minimum temperature of up to −40.0 °C. Frequent winds are characteristic. The average annual precipitation is 100–200 mm, with a maximum in April–May [29].

2.1.2. Physical and Geographical Characteristic of Sorbulak Wastewater Disposal System

Sorbulak is located 40–50 km north of Almaty (South-East Kazakhstan) (Figure 1). The reservoir was constructed in 1973 by filling the natural depression of the relief with wastewater that was treated at a sewage treatment plant. Wastewater enters Sorbulak through the main sewage canal. It originates at the wastewater treatment plant located 12 km northwest of Almaty and enters into the southeastern part of the reservoir. Domestic wastewater share accounts for up to 35–40%, industrial wastewater—up to 11–35%, atmospheric precipitation—up to 19%, groundwater—up to 9% of the total water volume [21].
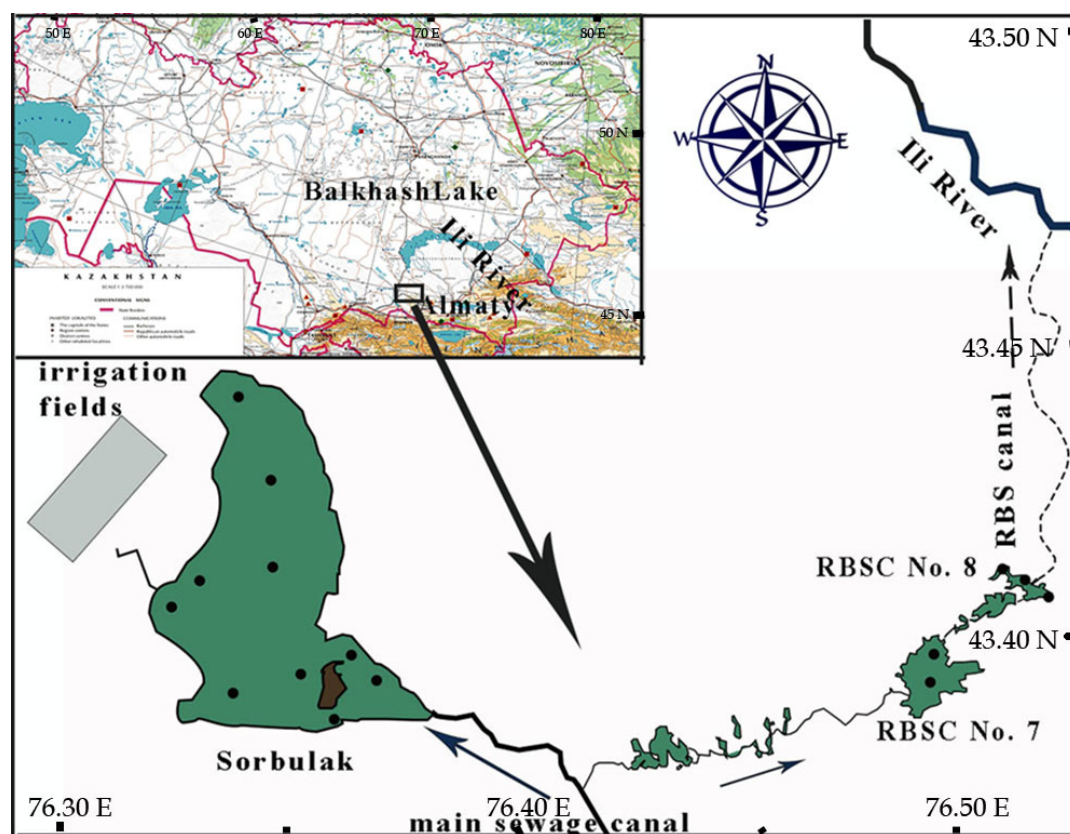

Figure 1. Schematic map of sampling stations in the Sorbulak wastewater disposal system (South-Eastern Kazakhstan).

Wastewater was planned to be used for irrigation of about 22.8 thousand hectares of land to prevent overflow and breakthrough of the barrier dam of Sorbulak; less than 3.0 thousand hectares remained by 2000. Irrigated fields are situated several kilometers northwest of the shoreline. The watering of fields is carried out, due to the canal, which originates in the western bay of Sorbulak. For an emergency, the Right-Bank Sorbulak Canal (RBSC) with a length of 63 km was additionally built in the 1990s.

There are eight ponds on the canal referred to as RBSC ponds. Pond No. 7 is the largest. Pond No. 8 is the last in the system. In case of an emergency (for example, when the volume of water in Sorbulak approaches the maximum permissible level), part of the sewage is discharged through the canal into ponds, bypassing Sorbulak. Then, from the last pond, water is released into the Ili River through the RBS canal.

Sorbulak is shaped like an irregular triangle. RBSC ponds are with an indented shoreline. According to the average depth (Table 1), Sorbulak and ponds belong to the type of shallow water bodies [30]. The water area and volume of RBSC ponds vary significantly and depend on the discharged wastewater volume. Sorbulak is characterized by relatively high water transparency, while the values of this variable in ponds are low.

Table 1. Physical and geographical characteristics of the Sorbulak wastewater disposal system. RBSC—Right-Bank Sorbulak Canal.

Variable	Sorbulak	RBSC No. 7	RBSC No. 8
altitude above sea level, m	620	618	615
water area, km^2	58	3.5	0.4
max water volume, km^3	1000	17.5	1.2
depth max, m	20.0	6.0	7.0
depth average, m	7.2	5.0	2.9
transparency, m	1.5	0.2	0.5
temperature, °C	27.0	28.5	27.8

2.2. Field Sampling

The studies of Sorbulak were performed in the summer of 2000, 2001, 2002, and 2017. RBSC ponds were studied only in the summer of 2017. A total of 45 zooplankton samples were taken altogether with samples to determine nutrients and heavy metals. The measures of the temperature and pH of the surface water layers were taken in the field environment by using the Hanna HI 98129 instrument in parallel with sampling. The transparency of the water was measured using a Secchi disk. Coordinate referencing of the stations was done by Garmin eTrex GPS-navigator.

Zooplankton samples were collected using a small Juday plankton net (input diameter 12 cm, mesh size 64 μm) by pulling it from the bottom to the surface. Surface water samples were taken at the same stations to determine the total dissolved solids (TDS), total hardness, nitrites, nitrates, ammonia, phosphates, iron, manganese, fluorine, silicon, heavy metals, and permanganate index [31]. We collected chemical and biological samples according to the state water quality monitoring program, approved in the Republic of Kazakhstan [32] for shallow unstratified water bodies [33]. Samples for analysis of TDS were taken in 1-L plastic containers. Samples for determining the nutrients were taken in 0.5 L glass containers and fixed with 1 mL of chloroform. Samples for determining the amount of easily oxidizable organic matter (permanganate index PI) were taken in 0.3 L glass containers and fixed with two ml concentrated sulfuric acid. Water samples for analysis of heavy metals were taken in 0.5 L plastic containers and fixed with concentrated chemically pure nitric acid. From 2000 to 2002, only four heavy metals (Cd, Cu, Pb, and Zn) were determined, while in 2017, additionally Cr and Ni. All samples were stored in a refrigerator and delivered to the laboratory within 1–2 days after collection.

2.3. Laboratory Analysis

Conventional methods of chemical analysis of water samples were used [34,35]. All water samples were analyzed in triplicate or quadruplicate. Nitrite nitrogen, nitrate nitrogen, ammonium nitrogen, phosphates, silicon, manganese, and iron were determined photometrically. Depending on the type of analysis, Griss's or Nessler's reagents, metallic cadmium, ammonium molybdate in combination with ascorbic or sulfosalicylic acid were used. The permanganate index (PI) was determined in acidic conditions by the Kubel method. Method sensitivity is 0.002 mg/dm^3; method accuracy is ±4%.

The total hardness was determined by the volumetric complexometric method with black eriochrome or black chromogen.

Analysis of samples for heavy metals was carried out in the analytical laboratory "KAZEKOANALIZ" (accreditation certificate No. KZ.I.02.1017) according to the Interstate standard [36]. Heavy metal measuring was performed by mass spectrometry with inductively coupled plasma using Agilent 7500 A manufactured by Agilent Technologies, Santa Clara, USA (National Standard RK ISO). Abundance Sensitivity of Agilent 7500 A: Low Mass $< 5 \times 10^{-7}$, High Mass $< 1 \times 10^{-7}$.

Zooplankton samples were processed with standard methods [37,38] using guides to the species identifications [39–42]. A sample was brought to a certain volume (150–500 cm^3) to calculate the quantitative variables of planktonic invertebrates. After thorough mixing, three sub-samples were taken from the sample using a 1 mL stamp-pipette. In this sub-sample, all encountered individuals and age stages of certain species (the most numerous) were counted in Bogorov's cell. Then the sample was concentrated to a volume of 125–150 cm^3. Three sub-samples were retaken from it, where less abundant age stages or species were counted. The whole procedure was repeated once more, while the sample was concentrated to a volume of 50 cm^3. In the end, the sample, with its volume of 20–25 mL, was viewed in its entirety for counting large and rare species of planktonic invertebrates. The results of counting individuals are recalculated per 1 m^3 using the formula (separately for each sample dilution):

$$N = \frac{n \times (V1/V2)}{V3} \tag{1}$$

where N is the abundance (ind./m^3), n is the number of individuals in a portion (specimens), V1 is the dilution volume (cm^3), V2 is the subsample volume (cm^3), V3 is the filtered water volume (m^3). The filtered volume of water was calculated by the formula:

$$V3 = h \times \pi r^2 \tag{2}$$

where h is the length of the net pulling (water column height), and r is the radius of the inner ring of the Juday net.

The total abundance was found for each species of planktonic invertebrates. For each crustacean species, the total abundance was calculated by summing the abundance of individual age and size stages. The total abundance of zooplankton was determined by summing up the abundance of all species found in the sample.

For all species of planktonic invertebrates encountered, the mass of an individual was calculated according to formulas specific to each species [38]. We counted the number of mature females, females with eggs, and males separately to describe the sex structure of copepod populations.

2.4. Statistical Analysis

We applied statistical methods to describe the structure and assess the similarity of the species composition of zooplankton communities in the studied water bodies. Statistical analysis of the relationship between ecological and biological variables makes it possible to assess the indicator role of planktonic invertebrates in assessing the water quality of aquatic ecosystems.

The number of species per sample, an average individual mass of an organism, and Δ-Snannon were calculated to describe the zooplankton structure. An average individual mass of an organism (mg) was calculated as the total biomass divided by the total abundance of zooplankton for each sample. Shannon index was calculated both based on the abundance and the biomass of species in the sample [43,44] using Primer 6 Software (https://primer.software.informer.com/6.0/) [45]. The first version of the index is designated as Shannon Ab (bit/ind.), the second one as Shannon Bi (bit/mg) for the convenience of distinguishing them. We calculated the values of Δ-Shannon as an arithmetical difference between Shannon Bi and Shannon Ab. Δ-Shannon characterizes the structure of the dominance of species. In large-sized communities, the distribution of species by abundance is more

uniform than by biomass. Accordingly, the Shannon Ab values are higher than the Shannon Bi, and Δ-Shannon is positive [46–52]. In disturbed communities, small species are dominated [53]; the Shannon Bi is higher than the Shannon Ab, and the values of Δ-Shannon are negative.

The calculation of community composition similarity was doing as the network analysis in JASP (Jeffrey's Amazing Statistics Program) 0.9.0.0 (https://jasp-stats.org/) on the botnet package in R-Statistica (https://www.r-project.org/) [54]. JASP plot was created as a calculation result of the percentage of an abundance of species on the level similarity 50% and as significant only when $p < 0.05$. The line thickness between stations reflect the correlation value; blue is positive, red is negative.

Statistical data analysis was performed using the Statistica 10 Software [55]. We calculated mean values with standard errors for all variables (pH value, Total Dissolved Solids, hardness, PI, Si, Fe, Mn, Cd, Cr, Cu, Ni, Pb, N-NO$_2$, N-NO$_3$, N-NH$_4$, PO$_4$, abundance, and biomass of Rotifera, Cladocera, Copepoda, total zooplankton, Shannon Ab, Shannon Bi, Δ-Shannon, species number, average individual mass), excluding the sex ratio, because females or males of copepods were absent in some samples. The sex ratio was calculated for each water body for each sampling date by dividing the average abundance of males by the average abundance of females. Differences in the mean values of chemical and some biological variables between the periods 2000–2002 and 2017 were performed using the Kruskal–Wallis test.

We calculated the Spearman correlation coefficient (r_S, $p < 0.05$) to assess the relationship between the environmental (pH value, Total Dissolved Solids, hardness, PI, Si, Fe, Mn, Cd, Cr, Cu, Ni, Pb, N-NO$_2$, N-NO$_3$, N-NH$_4$, PO$_4$) and biological variables (abundance and biomass of Rotifera, Cladocera, Copepoda, total zooplankton, Shannon Ab, Shannon Bi, Δ-Shannon, species number, average individual mass).

Principal component analysis (PCA) was performed using CANOCO 4.5 Software (https://canoco.software.informer.com/4.5/) based on 2000–2002 and 2017 [56]. We used PCA as a linear indirect gradient analysis because our data is represented by short lists of biological and environmental variables. PCA is also used to identify critical environmental gradients from environmental datasets. The identified gradients became the basis for a concise description and visualization of different taxa preferences of the habitat (niches) using the ordination diagram. For multivariate analysis, we took the external factors (temperature, depth, TDS, PI, N-NO$_2$, N-NO$_3$, N-NH$_4$, PO$_4$, PI, Si, Fe, Mn, Cd, Cr, Cu, Ni, Pb) and the structural variables of zooplankton communities (quantitative variables of Rotifera, Cladocera, Copepoda, total zooplankton, Shannon Ab, Shannon Bi, Δ-Shannon, species number, average individual mass). Moreover, we used the abundance of dominant planktonic invertebrates (Daphnia galeata, D. magna, D. pulex, Bosmina longirostris, Acanthocyclops trajani, Cyclops vicinus) and the sex ratio in the populations of cyclopoid copepods A. trajni and C. vicinus.

3. Results

3.1. Hydrochemical and Toxicological Characteristics of the Sorbulak Wastewater Disposal System

According to the chemical data (Table 2), in the summer of 2017, the water was alkaline, soft, and fresh in ponds, brackish and medium hardness in Sorbulak. The maximum concentrations of easily oxidizable organic matter (PI), silicon, nitrite nitrogen, nitrate nitrogen, phosphates, copper, and zinc were recorded in pond No. 7; iron in pond No. 8; ammonium nitrogen in Sorbulak. The content of manganese, cadmium, chromium, nickel, and lead in all water bodies was at a very low level. According to the Kruskal–Wallis test, statistically significant differences were found between the average content of nitrite nitrogen in Sorbulak and pond RBSC No. 7 ($p = 0.002$), and between pond RBSC No. 7 and No. 8 ($p = 0.036$).

Table 2. The environmental variables in the Sorbulak wastewater disposal system, mean values with standard error, summer 2017.

Variable	Units	Sorbulak	RBSC No. 7	RBSC No. 8
pH		9.0 ± 0.6	8.5 ± 0.01	9.4 ± 0.06
TDS	mg/dm^3	1234.3 ± 37.8	517.5 ± 20.0	584.1 ± 40.1
Hardness	mg-eq. dm^3	4.9 ± 0.03	2.3 ± 0.01	2.8 ± 0.3
PI	mg O_2 dm^3	11.2 ± 0.04	21.8 ± 0.1	10.9 ± 0.9
Si	mg/dm^3	2.1 ± 0.4	8.7 ± 0.1	8.3 ± 0.1
$N\text{-}NO_2$	mg/dm^3	0.012 ± 0.004	0.322 ± 0.02	0.006 ± 0.002
$N\text{-}NO_3$	mg/dm^3	0.151 ± 0.016	1.046 ± 0.045	0.078 ± 0.023
$N\text{-}NH_4$	mg/dm^3	0.453 ± 0.070	0.362 ± 0.045	0.275 ± 0.050
PO_4	mg/dm^3	0.15 ± 0.07	0.80 ± 0.27	0.28 ± 0.02
Fe	mg/dm^3	0.70 ± 0.14	0.93 ± 0.19	1.20 ± 0.40
Mn	mg/dm^3	0.005 ± 0.001	0.0 ± 0.0	0.003 ± 0.001
Cd	mg/dm^3	<0.00001	<0.00001	<0.00001
Cr	mg/dm^3	0.007 ± 0.0003	0.007 ± 0.0001	0.006 ± 0.0006
Cu	mg/dm^3	0.001 ± 0.0008	0.043 ± 0.042	0.001 ± 0.0001
Ni	mg/dm^3	0.0051 ± 0.0003	0.006 ± 0.003	0.005 ± 0.0001
Pb	mg/dm^3	0.0001 ± 0.0001	0.0001 ± 0.0001	0.0001 ± 0.0001

In Sorbulak, TDS of water in 2017 remained at the 2000–2002 level (Table 3). According to the Kruskal–Wallis test, the content of easily oxidizable organic matter ($p = 0.0001$) and ammonium nitrogen ($p = 0.001$) increased and the content of Pb ($p = 0.01$), Zn ($p = 0.006$), Cu ($p = 0.0009$), Cd ($p < 0.001$), nitrite nitrogen ($p < 0.02$), and nitrate nitrogen ($p < 0.002$) decreased significantly from 2000–2002 to 2017.

Table 3. The environmental variables of Sorbulak (mg/dm^3), mean values with standard error, summer of 2000–2002.

Variables	June–July 2000	June–July 2001	June–July 2002	July–August 2002
TDS	1079.5 ± 42.7	1240.8 ± 5.1	1166.5 ± 8.3	1129.9 ± 9.0
PI	-	6.4 ± 0.8	5.6 ± 0.6	5.7 ± 0.3
$N\text{-}NO_2$	-	0.150 ± 0.010	0.006 ± 0.0001	0.036 ± 0.005
$N\text{-}NO_3$	-	1.400 ± 0.180	1.020 ± 0.010	0.590 ± 0.030
$N\text{-}NH_4$	-	0.250 ± 0.020	0.070 ± 0.010	0.170 ± 0.020
PO_4	-	0.130 ± 0.010	0.070 ± 0.020	0.100 ± 0.020
Cd	0.004 ± 0.0002	0.005 ± 0.0001	0.078 ± 0.005	0.007 ± 0.001
Cu	0.007 ± 0.001	0.005 ± 0.0004	0.002 ± 0.0003	0.024 ± 0.001
Pb	0.006 ± 0.001	0.030 ± 0.002	0.022 ± 0.002	0.047 ± 0.006
Zn	0.047 ± 0.002	0.023 ± 0.005	0.014 ± 0.003	0.030 ± 0.004

3.2. The Structure of Zooplankton Communities

Forty-six species were recorded in the zooplankton, including twenty-three rotifers, fifteen cladocerans, and eight copepods (Table 4). In Sorbulak, the species richness of planktonic invertebrates in 2017 and 2000–2002 was the same, and higher than in the RBSC ponds. Rotifers *Hexarthra mira*, *Keratella quadrata dispersa*, *Polyarthra* sp., cladocerans *Bosmina longirostris*, *Chydorus sphaericus*, *Daphnia galeata*, *Daphnia magna*, *Daphnia pulex*, copepods *Acanthocyclops trajani*, *Cyclops vicinus*, and *Sinodiaptomus sarsi* were found in zooplankton during all study periods.

Table 4. Taxonomic composition of planktonic invertebrates in the Sorbulak wastewater disposal system, summer of 2000–2002, and 2017.

Taxon Name	Sorbulak 2000–2002	2017		
		Sorbulak	RBSC No. 7	RBSC No. 8
Phylum Rotifera				
*Bdelloida gen.*sp.		+		
*Notommatidae gen.*sp.		+		
Asplanchna girodi (Guerne)	+	+		
Brachionus angularis (Gosse)			+	
Brachionus calyciflorus dorcas Gosse	+	+	+	
Brachionus plicatilis longicornis (Fadeev)	+	+		
Brachionus quadridentatus Hermann	+			
Brachionus rubens Ehrenberg	+			
Brachionus urceus (Linnaeus)	+			
Brachionus variabilis (Hempel)		+		
Euchlanis dilatata (Ehrenberg)		+		
Euchlanis sp.	+			
Hexarthra mira (Hudson)	+	+	+	+
Keratella quadrata dispersa Carlin	+	+		+
Lecane luna (Muller)		+		
Lecane sp.	+			
Polyarthra major Burckhardt	+			
Polyarthra sp.	+	+	+	
Pompholyx sulcata (Hudson)	+	+		
Synchaeta kitina (Roussel.)		+		
Synchaeta stylata (Wierzejski)		+		
Synchaeta sp.	+			
Trichocerca sp.	+			
Phylum: Arthropoda				
Superorder Cladocera				
Alona quadrangularis (O.F. Muller)		+		
Alona rectangula (Sars)		+		
Alona sp.	+			
Biapertura affinis (Leydig)	+			
Bosmina (Bosmina) longirostris (O.F. Muller)	+	+		+
Ceriodaphnia pulchella (Sars)		+		
Chydorus sphaericus (O.F. Muller)	+	+	+	+
Daphnia (Ctenodaphnia) magna (Straus)	+	+	+	+
Daphnia (Daphnia) galeata (G.O. Sars)	+	+		+
Daphnia (Daphnia) pulex (De Geer)	+	+	+	+
Daphnia (Daphnia) longispina (O.F. Muller)	+			
Diaphanosoma mongolianum (Veno)	+	+		
Leydigia leydigii (Schoedler)		+		
Macrothrix laticornis (Jurine)	+			
Simocephalus sp.	+			
Subclass Copepoda				
Acanthocyclops trajani (Mirabdullayev et Defaye)	+	+	+	+
Cyclops scutifer (Sars)		+		
Cyclops vicinus (Uljanin)	+	+		+
*Cyclopoida gen.*sp.		+		
Limnocletodes behningi (Borutzky)		+		
Sinodiaptomus sarsi (Rylov)			+	+
Acanthodiaptomus denticornis (Wierzejski)	+			
Ergasilus sieboldi Nordmann	+			
Total No. species:	31	29	9	10

According to the JASP network plot (Figure 2), the highest similarity was found between planktonic invertebrates from ponds No. 7 and 8 in 2017 and between pond No. 8 in 2017 and Sorbulak in 2000 (blue lines). A significant similarity was also established between the species composition of zooplankton in Sorbulak in 2001 and 2002. In 2017, the zooplankton species composition in Sorbulak was somewhat similar to all other sampling sites. The most significant differences were revealed between the species composition in pond No. 7 in 2017 and Sorbulak in 2000 (red line). Differences

in the species composition were mostly recorded between the community of pond No. 7 and other water bodies. Accordingly, pond No. 7 can be marked as a critical point for all calculated relationships, which shows some specific features of this water body.
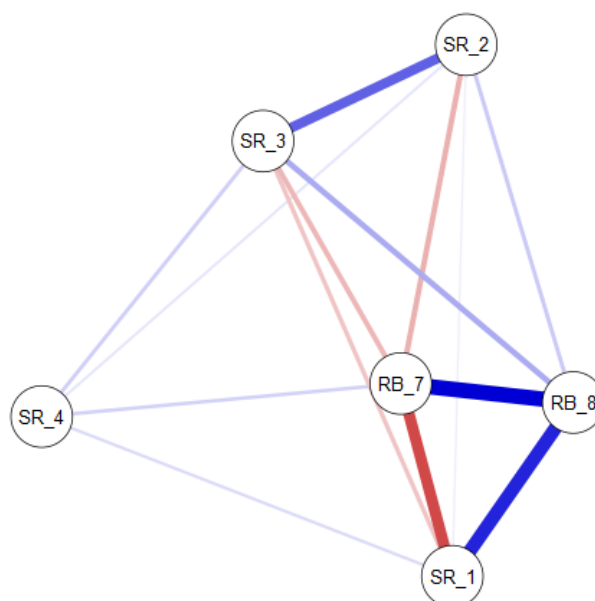

Figure 2. The JASP network plot of the similarity (in R-statistics) of the composition of planktonic invertebrate in the Sorbulak wastewater disposal system, summer 2000–2002, 2017. Abbreviations: SR_1–Sorublak, 2000, SR_2–Sorublak, 2001, SR_3–Sorublak, 2002, SR_4–Sorublak, 2017, RB_7–pond RBSC No. 7, 2017, RB_8–pond RBSC No. 8, 2017. The line thickness between stations reflect the correlation value; blue is positive, red is negative.

The abundance of zooplankton had more than a fourfold range of fluctuations depending on the water body and research period (Table 5). The highest zooplankton abundance was in Sorbulak in 2001 and pond No. 7 in 2017. The average zooplankton biomass varied by 13.0 times. Its maximum values were recorded in 2017 in pond No. 7. Copepoda mostly dominated. Rotifera or Cladocera were subdominants. Cladocera dominated in biomass, with Copepoda playing a minor role.

Table 5. The quantitative variables of zooplankton in the Sorbulak wastewater disposal system, mean values with standard error, summer 2000–2002, and 2017.

Water Body	Year	Rotifera	Cladocera	Copepoda	Total
			abundance, thousand ind./m^3		
	2000	73.1 ± 61.1	68.4 ± 14.4	97.9 ± 20.3	190.7 ± 34.3
Sorbulak	2001	174.2 ± 35.8	157.8 ± 22.8	305.5 ± 47.4	637.4 ± 76.9
	2002	49.2 ± 8.3	12.9 ± 1.3	84.5 ± 34.9	146.7 ± 28.6
	2017	175.8 ± 86.6	27.7 ± 7.7	102.0 ± 22.3	305.4 ± 91.8
RBSC No. 7	2017	58.0 ± 43.3	64.1 ± 11.9	535.2 ± 113.8	657.4 ± 102.4
RBSC No. 8	2017	24.0 ± 10.4	16.7 ± 2.2	146.0 ± 35.9	186.7 ± 40.9
			biomass, g/m^3		
	2000	0.10 ± 0.08	5.21 ± 1.20	1.12 ± 0.38	6.41 ± 1.31
Sorbulak	2001	0.49 ± 0.09	4.89 ± 0.77	1.89 ± 0.27	7.31 ± 0.98
	2002	0.10 ± 0.08	1.01 ± 0.28	0.20 ± 0.05	1.29 ± 0.38
	2017	0.18 ± 0.07	2.32 ± 0.63	0.14 ± 0.02	2.65 ± 0.06
RBSC No. 7	2017	0.05 ± 0.04	15.2 ± 4.85	1.78 ± 0.16	17.00 ± 5.00
RBSC No. 8	2017	0.02 ± 0.01	5.45 ± 1.56	0.64 ± 0.37	6.11 ± 1.50

The composition of the dominant species in the zooplankton slightly varied over the years (Table 6). Most often, cyclopoid copepods *Acanthocyclops trajani*, cladocerans of the genus *Daphnia*,

and the rotifers *Hexarthra mira* dominated. The ratio of the above species in quantitative variables of zooplankton varied depending on the water body. In Sorbulak, the proportion of *Hexarthra mira* and *Daphnia pulex* was higher than in the zooplankton communities of RBSC ponds. Simultaneously, the dominance of *Acanthocyclops trajani* and *Daphnia magna* in ponds was more pronounced than in Sorbulak. In some years, rotifers *Polyarthra*, *Asplanchna girodi*, and *Keratella quadrata* were also included in the dominant complexes of the surveyed water bodies.

Table 6. Composition of dominant species in zooplankton communities in the Sorbulak wastewater disposal system, summer 2000–2002, and 2017.

Species Name	Abundance, %	Biomass, %	Species Name	Abundance, %	Biomass, %
Sorbulak, 2000			*Cyclops vicinus*	29.7	13.1
Polyarthra sp.	23.3	0.8	*Acanthocyclops trajani*	27.9	4.9
Daphnia galeata	21.9	56.9	Sorbulak, 2017		
Daphnia longispina	10.9	22.7	*Hexarthra mira*	15.5	1.3
Cyclops vicinus	17.3	11.8	*Polyarthra* sp.	12.2	0.8
Acanthocyclops trajani	28.7	5.5	*Daphnia magna*	0.3	10.6
Sorbulak, 2001			*Daphnia pulex*	4.1	72.7
Asplanchna girodi	5.9	5.9	*Acanthocyclops trajani*	30.3	2.9
Keratella quadrata	15.4	0.8	RBSC No. 7, 2017		
Hexarthra mira	6.4	0.3	*Hexarthra mira*	7.1	0.3
Bosmina longirostris	14.5	11.1	*Daphnia magna*	2,8	76,0
Daphnia galeata	5.9	13.2	*Daphnia pulex*	3.0	11.7
Daphnia pulex	0.1	41.1	*Acanthocyclops trajani*	79.7	10.3
Acanthocyclops trajani	42.9	23.1	RBSC No. 8, 2017		
Sorbulak, 2002			*Hexarthra mira*	6.7	0.2
Keratella quadrata	20.4	1.4	*Daphnia magna*	4.0	67.6
Polyarthra major	12.2	1.3	*Daphnia pulex*	3,8	21,2
Daphnia pulex	5.8	66.7	*Acanthocyclops trajani*	77.0	10.2

The abundance of the dominant species *Acanthocyclops trajani* varied significantly over the years of research, with extremums in pond No. 7 in 2017 and Sorbulak in 2001 (Table 7). Males dominated the population of *A. trajani*, with the minimum values in the RBSC ponds in 2017. The abundance of *Cyclops vicinus* was relatively low. *Cyclops* was not found in the RBSC ponds in 2017. Except for 2002, the sex ratio in the population of this species was equal or female-dominated.

Table 7. The total abundance and sex ratio in the populations of *Acanthocyclops trajani* and *Cyclops vicinus* from the Sorbulak wastewater disposal system, mean values with standard error, summer 2000–2002, and 2017.

Water Body	Year, Month	Total	♀♀	♀♀ov	♂♂	♂/♀
		Acanthocyclops trajani				
Sorbulak	2000	58.7 ± 26.8	0.4 ± 0.2	0.08 ± 0.03	2.8 ± 1.6	5.8
	2001	360.3 ± 69.1	2.6 ± 0.6	0.6 ± 0.3	14.0 ± 7.8	4.4
	2002	75.9 ± 16.6	3.8 ± 1.3	0.3 ± 0.2	8.7 ± 3.0	3.3
	2017	92.7 ± 23.0	0.05 ± 0.03	0.02 ± 0.01	0.3 ± 0.1	4.3
RBSC No. 7	2017	523.9 ± 125.2	10.8 ± 1.0	0.5 ± 0.4	12.8 ± 4.9	1.1
RBSC No. 8	2017	143.6 ± 35.0	2.5 ± 2.1	0.6 ± 0.5	4.6 ± 4.3	1.5
		Cyclops vicinus				
Sorbulak	2000	39.8 ± 14.8	0.8 ± 0.5	0.6 ± 0.3	1.0 ± 0.2	0.7
	2001	26.8 ± 8.5	0.8 ± 0.4	0.3 ± 0.1	1.2 ± 0.9	1.0
	2002	43.6 ± 17.2	0.2 ± 0.1	0.05 ± 0.01	1.1 ± 0.8	4.4
	2017	8.1 ± 3.0	0.01 ± 0.01	0.01 ± 0.01	0.008 ± 0.005	0.9
RBSC No.7	2017	0	0	0	0	0
RBSC No.8	2017	0.01 ± 0.01	0	0	0	0

Remarkably, that individuals with morphological anomalies were recorded in the populations of *Cyclops vicinus* and *Acanthocyclops trajani* only in 2000–2002. Most often, there was a shortening of one

of the furcal rami, as well as shortening and deformation of the furcal setae (Figure 3). The abundance of such individuals was low, no more than 10–40 ind./m^3, but they were found in 50–60% of the samples during the indicated research period. In the summer of 2017, individuals with morphological abnormalities were absent in copepod populations.
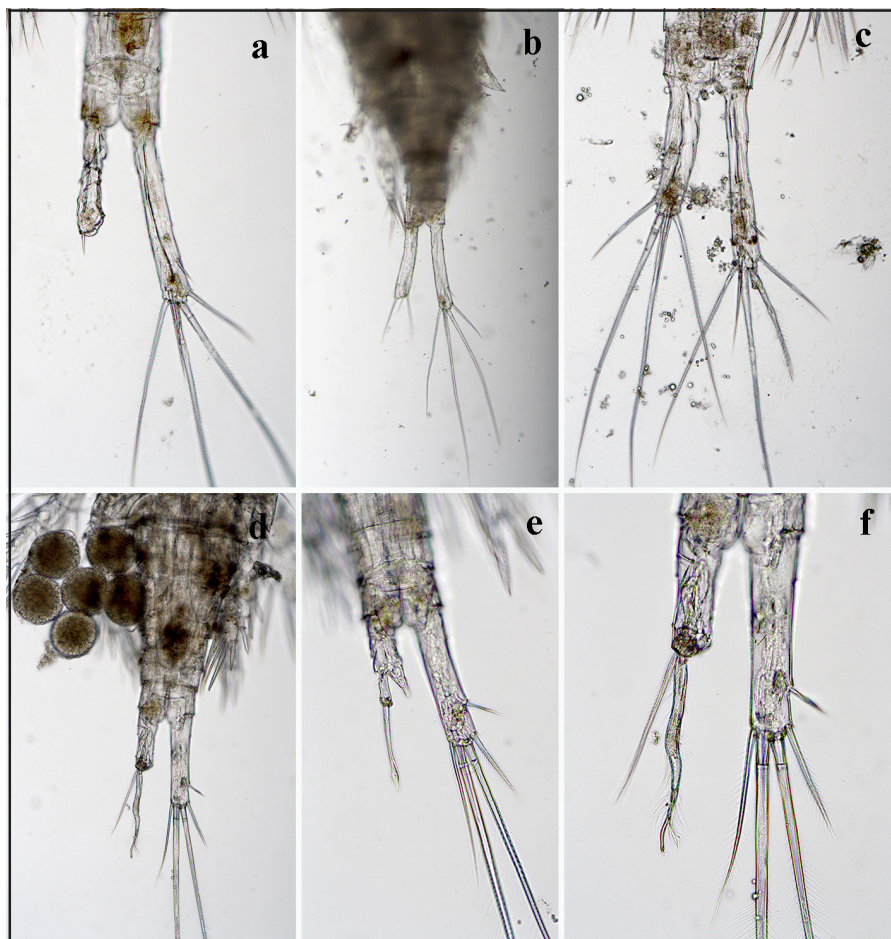

Figure 3. Individuals with morphological anomalies in the populations of *Cyclops vicinus* (**a**–**c**) and *Acanthocyclops trajani* (**d**–**f**) from the Sorbulak wastewater disposal system, 2000–2002. Photo by E.G. Krupa.

The average number of species of planktonic invertebrates per sample changed approximately twofold (Table 8). The maximum values of the variable were recorded in the zooplankton of Sorbulak in 2017. According to an average individual mass, the zooplankton communities were large in body size, especially in Sorbulak in 2000 and pond No. 8 in 2017. According to the Kruskal–Wallis test, the Shannon Ab values in RBSC ponds were statistically significantly lower than in Sorbulak. The average values of the Δ-Shannon index in the zooplankton of all water bodies were positive.

Table 8. Structural variables of zooplankton in the Sorbulak wastewater disposal system, mean values with standard error, summer 2000–2002, and 2017.

Water Body	Year	Species Number	Average Individual Mass, mg	Shannon Ab	Shannon Bi	Δ Shannon
	2000	8.7 ± 1.5	0.0343 ± 0.0047	2.18 ± 0.16	1.57 ± 0.22	0.62 ± 0.19
	2001	12.1 ± 2.0	0.0117 ± 0.0011	2.41 ± 0.18	2.09 ± 0.16	0.32 ± 0.11
Sorbulak	2002	9.0 ± 1.0	0.0096 ± 0.0030	2.21 ± 0.13	1.70 ± 0.17	0.50 ± 0.28
	2017	16.1 ± 0.7	0.0182 ± 0.0054	2.48 ± 0.07	1.77 ± 0.30	0.71 ± 0.35
RBSC No 7	2017	8.5 ± 1.5	0.0253 ± 0.0036	1.19 ± 0.26	1.16 ± 0.30	0.03 ± 0.04
RBSC No 8	2017	7.7 ± 0.7	0.0379 ± 0.0139	1.26 ± 0.20	1.17 ± 0.31	0.10 ± 0.42

3.3. Nonparametric Correlation Analysis

The values of Spearman's rank correlation coefficients (r_S) between environmental and biological variables are presented in Table 9. For rotifers, positive relationships were recorded with nitrite nitrogen, nitrate nitrogen, and TDS; negative relationships with copper and zinc content. For cladocerans, positive associations were revealed with the nitrite, nitrate, ammonium nitrogen, phosphates; negative relationships with TDS, manganese, cadmium, and lead content. For copepods, positive associations were established with nutrients, copper, zinc, and lead. The total quantitative variables of zooplankton showed a positive relationship with the content of nutrients and silicon; the relationship with the cadmium concentration was negative. The average mass of an individual increased with depth, as the r_S values show. Positive relationships were recorded between the Δ-Shannon and Shannon Ab indices and depth, transparency, and TDS; the relationship between the Shannon Ab index and silicon was negative.

Table 9. Spearman's rank correlation coefficients (r_S) between environmental and biological variables in the Sorbulak wastewater disposal system, summer 2000–2002, 2017, at $p < 0.05$.

Biological Variable	Environmental Variable	r_S	Biological Variable	Environmental Variable	r_S
Rotifera Abundance	N-NO$_2$	0.356	Copepoda Biomass	N-NO$_2$	0.560
	N-NO$_3$	0.438		N-NO$_3$	0.380
	TDS	0.501		PO$_4$	0.341
	Cu	−0.621		Cu	0.405
	Zn	−0.516	Copepoda Biomass	Zn	0.479
	TDS	−0.455		Pb	0.460
Cladocera Abundance	N-NO$_2$	0.600	Total Abundance	N-NO$_2$	0.580
	N-NO$_3$	0.379		N-NO$_3$	0.462
	PO$_4$	0.468		N-NO$_2$	0.589
Cladocera Biomass	N-NO$_2$	0.464	Total Biomass	PO$_4$	0.440
	N-NO$_3$	0.353		Si	0.535
	PO$_4$	0.405		Cd	−0.389
	Mn	−0.537	Average Mass	Depth	0.362
	N-NH$_4$	0.372		Depth	0.415
	Cd	−0.435	Δ-Shannon	Transparency	0.415
	Pb	−0.401		TDS	0.401
	Transparency	−0.351		Depth	0.466
Copepoda Abundance	N-NO$_2$	0.630	Shannon Ab	Si	−0.709
	N-NO$_3$	0.409		TDS	0.705
	PO$_4$	0.475		Transparency	0.476

3.4. PCA Analysis

The PCA biplot (Figure 4) shows that total zooplankton biomass and Cladocera biomass were mainly associated with nitrite nitrogen, phosphates, to a lesser extent with depth, and PI; relationship with TDS was negative. The content of nitrate nitrogen partially determined Copepoda biomass. The values of the average mass of an individual and Δ-Shannon index were most closely related to ammonium nitrogen content and PI. Rotifera biomass and Shannon Bi values were negatively correlated with ammonium nitrogen, depth, and partially PI. The distribution of zooplankton abundance in the gradient of external factors was similar to the distribution of biomass.

Concerning toxic pollution (Figure 5), Copepoda and Rotifera biomass was most closely associated with copper and zinc and negatively correlated with cadmium. Cladocera biomass and the average mass of an individual in zooplankton communities were in a negative correlation with the content of lead, cadmium, and TDS. On the contrary, Shannon Bi was positively associated with lead and partially with depth.
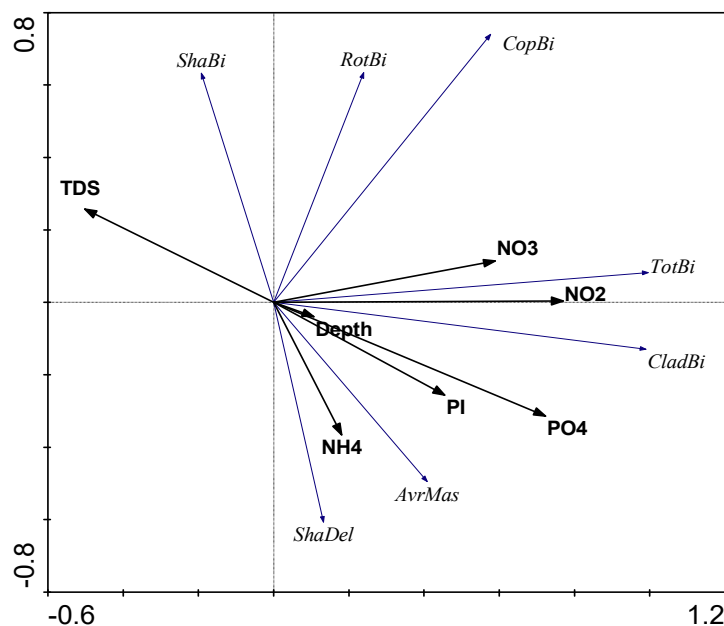
Figure 4. Principal component analysis (PCA) biplot of relationships between the content of nutrients, easily oxidizable organic substances and the structural variables of zooplankton communities in the Sorbulak wastewater disposal system, summer of 2000–2002, and 2017. The following abbreviations are used: RotBi—Rotifera biomass, CladBi—Cladocera biomass, CopBi—Copepoda biomass, TotBi—total zooplankton biomass, ShaBi—Shannon Bi, ShaDel—Δ-Shannon, AvrMas—an average mass of an individual. Black arrows are environmental factors; blue arrows are structural variables of zooplankton communities.
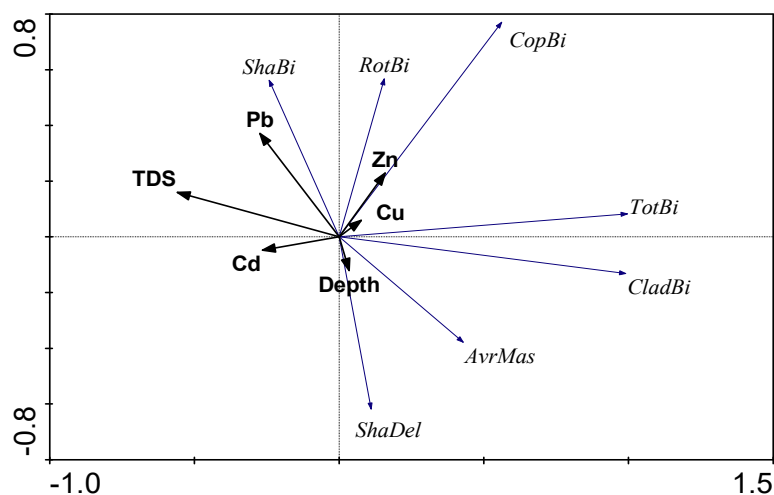
Figure 5. PCA biplot of relationships between the content of heavy metals, depth, TDS, and structural variables of zooplankton communities in the Sorbulak wastewater disposal system, summer of 2000–2002, and 2017. The following abbreviations are used: RotBi—Rotifera biomass, CladBi—Cladocera biomass, CopBi—Copepoda biomass, TotBi—total zooplankton biomass, ShaBi—Shannon Bi, ShaDel—Δ-Shannon, AvrMas—an average mass of an individual. Black arrows are environmental factors; blue arrows are structural variables of zooplankton communities.

As Figure 6 demonstrates, the abundance of *Bosmina longirostris*, *Daphnia pulex*, *D. magna*, *D. galeata*, and *Cyclops vicinus* were negatively associated with ammonium nitrogen. The relationship between *Acanthocyclops trajani* and nitrites as well as nitrates was positive, and the negative one was with depth and TDS. The abundance of *D. magna* was predominantly associated with phosphates and partly with ammonium nitrogen.
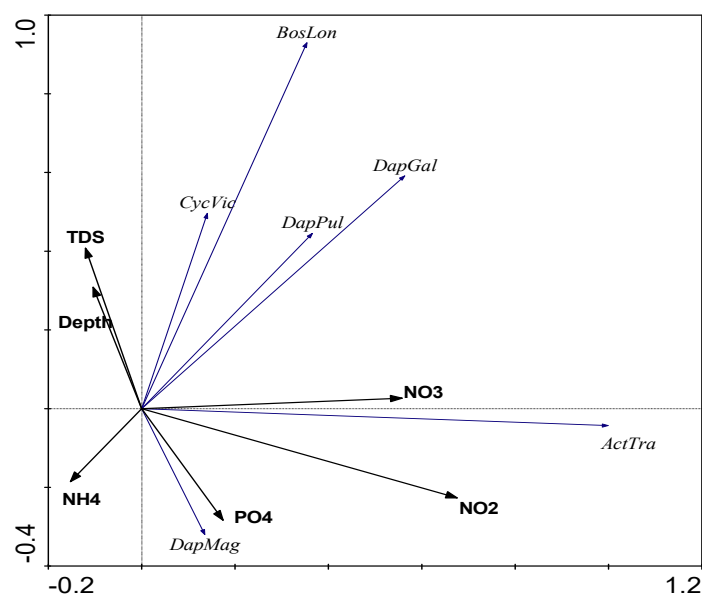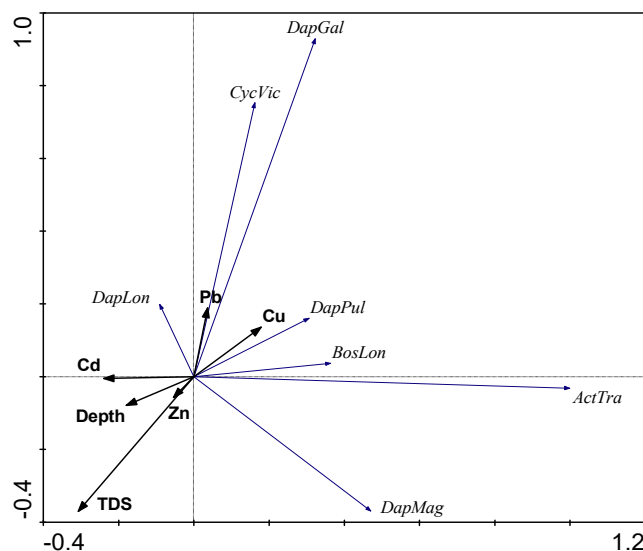
Figure 6. PCA biplot of relationships between nutrient content, depth, TDS, and the abundance of dominant planktonic invertebrates in the Sorbulak wastewater disposal system, summer of 2000–2002, and 2017. The following abbreviations are used: BosLon–*Bosmina longirostris*, DapPul–*Daphnia pulex*, DapMag–*Daphnia magna*, DapGal–*Daphnia galeata*, ActTra–*Acanthocyclops trajani*, CycVic–*Cyclops vicinus*. Black arrows are environmental factors; blue arrows are the abundance of dominant species of planktonic invertebrates.

The PCA biplot shows (Figure 7) that the abundance of *Daphnia longispina*, *D. galeata*, and *Cyclops vicinus* was predominantly associated with lead; there was a negative connection between the last one and zinc. The abundance of *D. pulex* and *Bosmina longirostris* was correlated with copper. The abundance of *D. magna* showed a negative dependence on cadmium and lead; *A.trajani*—on cadmium content.

Figure 7. PCA biplot of relationships between the content of heavy metals, depth, TDS, and the abundance of dominant planktonic invertebrates in the Sorbulak wastewater disposal system, summer of 2000–2002, and 2017. The following abbreviations are used: BosLon–*Bosmina longirostris*, DapPul–*Daphnia pulex*, DapMag–*Daphnia magna*, DapGal–*Daphnia galeata*, ActTra–*Acanthocyclops trajani*, CycVic–*Cyclops vicinus*. Black arrows are environmental factors; blue arrows are biological variables.

Figure 8 demonstrates that the abundance of mature individuals, individuals with morphological anomalies, and the dominance of males in the populations of *A. trajani* was associated with lead, and partially copper; the connection with TDS was negative. The total abundance and abundance of mature individuals in *C. vicinus* populations were associated with lead and zinc, but the abundance of individuals with morphological abnormalities was influenced by copper.
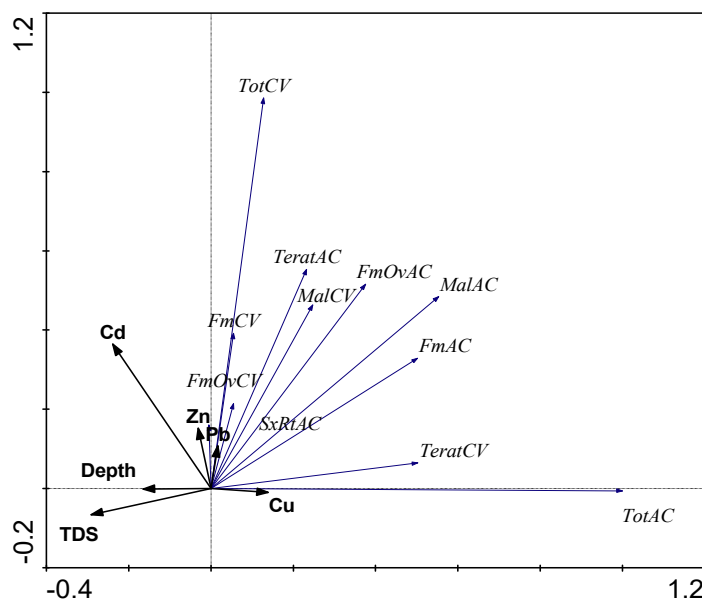

Figure 8. PCA biplot of relationships between the content of heavy metals, depth, TDS, and the structure of populations of cyclops *Acanthocyclops trajani* and *Cyclops vicinus* in the Sorbulak wastewater disposal system, 2000–2002, and 2017. The following abbreviations were used: TotAC—total abundance of *Acanthocyclops trajani*, including FmAC—mature females, FmOvAC—females with eggs, MalAC—males, SxRtAC—ratio of males to females, TeratAC—abundance of individuals with morphological anomalies; TotCV—total abundance of *Cyclops vicinus*, including FmCV—mature females, FmOvCV—females with eggs, MalCV—males, SxRtCV—the ratio of males to females, TeratCV—bundance of individuals with morphological anomalies. Black arrows are environmental factors; blue arrows are biological variables.

4. Discussion

4.1. Chemical Variables

According to chemical data, in 2017, the content of pollutants in the water of pond No. 7 was higher than in Sorbulak, but the differences in mean values were mostly insignificant. With the same composition of wastewater entering all reservoirs, some chemical parameters variability can be associated with the natural processes of self-purification of water bodies. The large volume of the water mass of Sorbulak [21] contributes to the more substantial dilution of the incoming pollutants in comparison with shallow-water ponds. The accumulation of contaminants in the bottom sediments of Sorbulak [9] leads to a decrease in their concentrations in the water column, as was observed in other large water bodies [57].

In the long-term aspect, the toxic pollution of Sorbulak varied significantly, which is generally typical for such water bodies [58]. Therefore, the content of Pb, Cd, Zn, Ni, and Co in water in 1985 did not exceed the maximum permissible level [26]. In 1997–2000, the content of heavy metals increased: Zinc up to 0.017–0.056, copper up to 0.004–0.029, lead up to 0.034–0.082, cadmium up to 0.004–0.009 mg/dm^3 [59]. According to the Kruskal–Wallis test, from 2000–2002 to 2017, the content of nitrate, nitrite nitrogen, and heavy metals in Sorbulak water decreased significantly, which may be explained by socio-economic reasons. Despite the growth in Almaty and its environs, during the period under review, the volume of discharged wastewater decreased from 135.7 to 101.5 million m^3 [21].

The share of industrial wastewater also decreased from 35% in 1997–1998 up to 11% in 2004, due to the closure of many industrial enterprises in Almaty. Moreover, the high abundance and biomass of phytoplankton in wastewater reservoirs (5891.7–6287.3 million cells/m^3 and 4.8–5.6 g/m^3) in the summer of 2017 [51] were one of the significant factors contributing to the purification of the water column of Sorbulak and ponds from heavy metals. It is known that the absorption of heavy metals by algae can be more intense than the accumulation in bottom sediments [60,61].

4.2. Biological Variables

4.2.1. Species Composition and Quantitative Variables

Zooplankton communities of the Sorbulak disposal system were characterized by relatively low species richness and high quantitative variables. This zooplankton structure is typical for water bodies with a high level of pollution [17,62–65]. Cyclopoid copepods *Acanthocyclops trajani* (in some periods together with *Cyclops vicinus*), Cladocera *Bosmina longirostris*, *Daphnia pulex*, *Daphnia galeata*, *Daphnia magna*, rotifers *Hexarthra mira*, *Asplanchna girodi*, *Keratella quadrata* dominated.

The eurybiontic species listed above are resistant to the influence of various external factors. *A. trajani* is common species in the water bodies of Kazakhstan with a high level of organic and toxic pollution [28,52,59,66,67]. Species of the genus *Bosmina* are resistant to industrial pollution [68]. The sensitivity of *Daphnia* species to heavy metals is species-specific and inversely proportional to body size [69]. *D. pulex* can physiologically adapt to a wide range of external conditions [70], including toxic pollution [71]. *D. magna* is the most tolerant of toxic effects among the species of this genus [72]. *A. trajani*, *D. pulex*, and *D. magna* dominated zooplankton in different periods of research, starting from the construction of Sorbulak [26], which confirms their high ecological plasticity.

In addition to toxic pollution, Cyanobacteria have a pronounced effect on the species composition of zooplankton. Filamentous cyanobacteria disrupt food collection in *Daphnia* by mechanically acting on the filter apparatus with long trichomes [73]. The intensity of this intervention depends on the water temperature, and body size of *Daphnia*. *D. longispina* is able better to break apart cyanobacterial trichomes when the water temperature rises. D. magna morphologically adapts to high-trophic water bodies dominated by filamentous blue-green algae, due to the thickening of the filter apparatus bristles [74]. Species of the genus *Microcystis* affect both the phytoplankton community itself, due to the deterioration of illumination and the zooplankton and ichthyofauna, due to the release of toxins and changes in pH [75]. The reaction of *Daphnia* to toxins of blue-green algae is species-specific [76]. Experimental and field studies have shown that *Daphnia* thrives in the presence of green algae, cryptophytes, chrysophytes, or diatoms [77–79].

In 2017, cyanobacteria were the absolute dominants in Sorbulak with average biomass of 4.9 g/m^3 [51]. The biomass of green algae was almost fifteen times less (0.33 g/m^3). In pond No. 7, in addition to blue-green algae with biomass of 4.7 g/m^3, dinophytic algae were also represented with biomass of 0.55 g/m^3. In pond No. 8, phytoplankton contained only cyanobacteria, with biomass of 4.8 g/m^3. These differences in the composition of microalgae can be the reason for the change in the proportion of species of the genus *Daphnia* in the zooplankton of the surveyed water bodies. The share of *D. magna* in the zooplankton biomass increased from 10.6% in Sorbulak to 67.6% in pond No. 8 and 76.0% in pond No. 7. The share of *D. pulex* decreased from 72.7% in Sorbulak to 21.2% and 11.7% in ponds No. 8 and No. 7, respectively. It can be assumed that *D. pulex* is more sensitive to the influence of cyanobacteria, as its role in zooplankton decreased in ponds, where the dominance of blue-green algae was almost absolute. A significant decrease in the proportion of *D. galeata* in zooplankton by 2017 may be associated with the high sensitivity of this species to blue-green algae [80].

Bosmina longirostris was a part of the dominant complexes in zooplankton of Sorbulak until 2002. The decrease in this species' role in zooplankton by 2017 is probably associated with the competition with large *Daphnia magna*. The maximum abundance of B. longirostris in Sorbulak was recorded in 2000–2001 when *D. magna* was absent. With an increase in the abundance of *Daphnia* by 2017,

the number of B. longirostris decreased by 8.7 times. In the RBSC ponds, where *D. magna* was one of the dominant species in 2017, no *Bosmina* was found. In 1985, when *D. magna* dominated in zooplankton, *Bosmina* was also absent [26]. *D. magna* is able to minimize the food stress caused by the filamentous cyanobacteria by setae thickening [74]. Although B. longirostris is common species in cyanobacterial blooms [81], it is probably unable to compete with such a large cladoceran species as *D. magna*.

4.2.2. Structural Variables

According to the Shannon index values (1.74–2.41 bit/ind. and 1.70–2.09 bit/mg in Sorbulak and 1.19–1.26 and 1.16–1.17 in ponds), the diversity of zooplankton of the surveyed water bodies varied from low to moderate levels [17]. The Shannon index values were similar to those established for zooplankton communities in water bodies with a pronounced bloom of cyanobacteria [82].

An average individual mass of an organism is traditionally used to assess the organic pollution of aquatic ecosystems. The values of this variable (Table 8) corresponded to the level of eutrophic (Sorbulak, 2002–2002, 2017) or mesotrophic (Sorbulak, 2000; Sorbulak and ponds, 2017) water bodies [17].

Disturbances in the structure of species dominance can be additional indicators of stress factors for zooplankton. The structure of species dominance in communities can be described using Clarke's W-statistics [83–85] or Δ-Shannon index [46–52]. In communities with large-sized species, the distribution of species by abundance is more uniform than by biomass. Accordingly, the Shannon Ab values are higher than the Shannon Bi, and Δ-Shannon is positive [46–52]. In disturbed communities, small species are dominated [53,83–85]; the Shannon Bi is higher than the Shannon Ab, and the values of Δ-Shannon are negative. Positive values of the Δ-Shannon index (Table 8) indicated the absence of pronounced stress for zooplankton communities in the surveyed wastewater water bodies. This is due to the predominance of planktonic invertebrates resistant to a wide range of organic and toxic pollution. The convergence of the biomass and abundance curves, with a value of the Δ-Shannon index of 0.03 (Table 8), was only recorded in the zooplankton of pond No. 7 in 2017. It can be associated with a very high copper content in the water in this period (Table 2).

4.2.3. The Influence of External Factors on the Quantitative Variables and Structure of Zooplankton Communities

Correlation and PCA analysis showed that the main factors of the spatiotemporal variability of the quantitative variables of zooplankton communities were the content of nutrients. This is because nutrients affect the composition and quantity of planktonic algae [86,87]; the latter, in turn, are food for planktonic invertebrates, especially *Daphnia* [88,89]. Copepoda was associated with copper and zinc content, which generally indicated their resistance to these metals. However, as a more toxic element [90], cadmium had a negative effect on copepods.

The response of planktonic invertebrates to toxic pollution is species-specific. *Acanthocyclops trajani* and *Cyclops vicinus* showed resistance to heavy metals. Our results confirm the literature data on the more excellent resistance of cyclopoid copepods to heavy metals compared to *Daphnia* [91]. In experiments on the assessment of chronic toxicity [92], cadmium suppressed the development of the cladocera *Moina monogolica* Daday at a concentration of 0.003 mg/dm^3. In the period up to 2002, the cadmium content in Sorbulak was higher than the given value. However, cadmium, like lead, did not adversely affect *Daphnia longispina*, *Daphnia pulex*, and *Daphnia galeata*. The absence of a pronounced toxic effect of heavy metals on cladocerans in the wastewater reservoirs of the RBSC can be explained by various reasons, including the multidirectional interaction of pollutants with each other [12], the protective effect of the chemical composition of water [93] and food availability [94].

The influence of external factors on the sex structure of planktonic invertebrates is poorly studied. Typically, females are slightly more numerous than males in populations of cyclopoid copepods [95–97]. This is explained by the fact that one male can fertilize several females, and there is no need for an equal sex ratio [39,98]. Deviations from the normal sex ratio in crustacean populations are induced by stress factors, including changes in water temperature, TDS, and pH [99–101]. We have

found that males' dominance in populations of cyclopoid copepods reaches an extremely high level (from 3–5 to 50 males per female) under conditions of intense organic and toxic pollution of the environment [28,59,66,67,86,102]. Considering the reference data, the recorded dominance of males in the Acanthocylops trajani population indicated the presence of stress factors for this species during all periods of the study of the wastewater reservoirs. According to the PCA biplot, disturbances in the sex structure in the *A. trajani* populations were predominantly associated with heavy metals concentrations. According to sex theory [100], it can be considered an adaptive response of A. trajani to stressful environmental conditions.

Individuals with morphological anomalies were recorded continuously in *Acanthocylops trajani* and *Cyclops vicinus* populations from Sorbulak and ponds in 2000–2002 (Figure 3) and 1998 [62]. They were completely absent in the summer of 2017. According to the PCA biplot, the appearance of individuals with morphological abnormalities in the cyclopoid populations was associated with copper or lead. Copper is highly toxic to most living organisms [12,103,104]. For cyclopoid copepods, copper is more harmful than zinc, chromium, and nickel [105].

4.2.4. Indicator Role of Zooplankton in Assessing the Water Quality of Water Bodies with Multicomponent Pollution

Abundance, biomass, diversity indices, and size structure of zooplankton are traditionally used to evaluate organic pollution of water bodies [17]. A positive relationship between the quantitative variables of zooplankton communities and nutrients was also recorded in our studies. Heavy metals or other toxicants contained in wastewater partially neutralize the stimulating effect of nutrients on primary producers [106], and as a consequence, on planktonic invertebrates. The ratio of taxonomic groups, for example, cladocerans and copepods, can be used to assess the ecological state of water bodies with mixed pollution. We established a positive relationship between cyclopoid copepods and the content of heavy metals in water bodies of the Sorbulak system. The relationship between cladocerans and heavy metals was negative. Thus, the dominance of cyclops, which are the most resistant to toxicants [91], is one indicator of stress for zooplankton communities.

We can also use the structure of species dominance, the sex structure of copepod populations, and the presence of individuals with morphological anomalies to monitor water bodies with mixed pollution. We have previously shown that the structure of species dominance is a more sensitive indicator compared to the species composition [46,107]. This is because external factors first cause intrapopulation rearrangements (for example, changes in the ratio of age stages in crustacean populations and the size of adults). Then the percentage of species in the quantitative variables of communities changes, i.e., the structure of their dominance. The species composition changes when the gradient of external factors goes beyond a particular species tolerance limits.

The composition of species dominating in the zooplankton of the surveyed water bodies has been relatively constant, since the formation of Sorbulak. The positive values of the Δ-Shannon index in the zooplankton of the studied water bodies were due to the dominance of species resistant to multicomponent pollution.

The cyclopoid copepods with morphological anomalies were presented in Sorbulak in 2000–2002, and they were absent in 2017. A revealed association between these individuals and heavy metals can indicate the toxic pollution of water bodies investigated, as was shown earlier [67,86,105,106]. These results corresponded to the chemical analysis data on the decrease in the level of toxic pollution of wastewater reservoirs of the Right-Bank Sorbulak Canal in 2017 and demonstrated the interannual increase of water quality in it.

Thus, the comprehensive studies carried out allow us to conclude that the level of toxic pollution of the Sorbulak wastewater disposal system has decreased over the past fifteen years. In 2017, Sorbulak and ponds were mainly characterized by organic pollution. Sorbulak water can be used for irrigation as previously planned. Given the significant variability of all parameters, constant monitoring of the Sorbulak wastewater disposal system is required.

5. Conclusions

Our results demonstrated that zooplankton communities of water bodies with multicomponent pollution consist of a small number of eurybiontic species. The variability of the quantitative variables of zooplankton is associated with nutrients, and as a consequence, algal communities. In particular, Cyanobacteria can affect the species composition of cladocerans. The most resistant species to cyanobacterial bloom is, obviously, *Daphnia magna*. It can be assumed that the ratio of species of the genus *Daphnia* may be an indicator of cyanobacterial bloom impact, although additional studies are required for the conclusion. The dominance of cyclopoid copepods as the most resistant to a wide range of external factors is one of the indicators of stress for zooplankton, and in particular, the indicator of toxic pollution. Moreover, the dominance of males and the appearance of individuals with morphological anomalies in copepod populations is an indicator of chronic stress associated, among other things, with toxic environmental pollution. Hence, our results demonstrate that the zooplankton structure can be successfully applied to assess the water quality of reservoirs with mixed pollution. We recommend using not only the traditional set of biological variables (abundance, biomass, diversity indices, and the average mass of an individual), but also data on the structure of species dominance, the sex structure of copepod populations, and the presence of individuals with morphological anomalies.

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