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Article

Using Algal Indices to Assess the Ecological Condition of the Aras River, Northwestern Iran

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Abstract: This work is the first in a series, and its purpose is the comprehensive assessment of the ecological state of the Aras River using biological indicators of water quality by diatoms based on species' ecological preferences, pollution indices, statistics, and ecological mapping. Samples of diatoms and soft algae and measurements of water quality were analyzed at sixteen sampling sites (between 2020 and 2022) along the Aras River. The impact of anthropological activity on the river was monitored concerning water quality, river health, and ecosystem function. The physical and chemical characteristics of the water were measured. The biological properties of the algal periphyton communities, including species composition, were also measured. Based on the studies conducted in this research, 280 species were identified. The most prosperous species were *Diatoma vulgaris*, *Amphora ovalis*, *Cocconeis placentula*, *Rhoicosphenia abbre-viatae*, *Cymbella helvetica*, *Brevisira arentii*, *Navicula tripunctata*, *Nitzschia linearis*, *Microcystis botrys*, *Microcystis aeruginosa*, *Pseudanabaena limnetica*, *Scenedesmus obliquus*, and *Pleurosira laevis* (a pollution-resistant and salinity-resistant species first found in aquatic habitats in the Aras River). As a result, the empirical data and algal indices showed the river's lower reaches to be in poor condition. Exploration of the algal assemblage and water chemistry data using computationally unconstrained ordination techniques such as principal component analysis (PCA) and canonical correspondence analysis (CCA) indicated two strong gradients in the data sets. The results support that water body classification is a function of water chemistry and biological and hydrological characteristics, as it is necessary to include pollutant effects on biota since the nature of the receiving waters influences the river's water quality.

Keywords: bioindication; water quality; phytoplankton; phytoperiphyton; Aras River; Iran

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1. Introduction

Historically, water quality monitoring programs have focused on water chemistry criteria. Today, they are more likely to focus not only on water chemistry but also on biological and hydrological characteristics as well. This is because it is essential to demonstrate the effect of pollutants on biota as the effect on water quality is also influenced by the nature of the receiving waters. Water in rivers can be classified, based on biology, hydrology, and quality, into different ecological categories of conditions such as bad, poor, moderate, good, or high [1–3]. Water pollution can be defined as the contamination of water bodies by the entrance of large amounts of materials/substances into those bodies, resulting in physical or chemical changes to the water, modifying the natural features of the water, degrading the water quality, and adversely affecting humans and the environment [1,2]. Water pollution is a phenomenon in which the chemical, physical, and biological quality of natural waters is harmfully affected by the discharge of waste materials such as sanitary sewage [2,3], urban runoff [4], industrial waste [5], agricultural waste [6], animal husbandry effluents [7], vehicle- and traffic-related organic pollutants [8], and other industrial, urban, and agricultural waste materials [9]. These pollutants have many adverse effects on the environment [10] and human life [11–13], and the restoration of the environment requires much time, cost, and effort.

There has been a marked increase in freshwater demand brought about by rapid population growth in regions such as Africa [14–17], as well as the rapid expansion of industrialization [18,19] and agricultural development [20,21]. The limitation of water resources, the cheap price of water, the lack of proper agricultural management, the age of industrial processes and their pollution emittance, and the absence or inefficiency of water pollution control and monitoring systems are among the factors that require more attention [22].

Diatoms have long been used as powerful and reliable environmental indicators [23] in a variety of aspects, e.g., river pollution [24], lotic systems [25], sediments [26], eutrophication [27], palaeoecology [28], lake water level [29], and surface-water acidity [30]. Diatoms in aquatic environments can be investigated using precise statistical techniques. River systems are important ecosystems as they provide water for many human needs [31–35]. Since rivers are exposed to the most complex environmental systems in the world, it is necessary to estimate the conditions and trends of rivers where physical [36], chemical [37], and biological [38] approaches are often considered to be basic monitoring needs since they can provide comprehensive information for water management. However, it is very difficult to monitor all possible physical and chemical variables, as biological conditions are expensive to monitor, variable, and often have little reflection on the environment; therefore, monitoring of biology has been proven to be complementary to monitoring chemical and physical approaches. Algae are the simplest organisms with chlorophyll and are single-celled organisms from the protozoan and eukaryote series [39].

Algae are one of the leading indicators of water quality in their habitat [40–42] as each species is abundant in a certain environment, which is a sign of the natural condition of that area [43]. The abundance [44], biomass [45], translocation [46], and species composition [47] of algae are used in developing diatom-based systems of bioindicators, which may extend even to covering climate [48]. Aquatic organisms such as diatoms can act as biomarkers for responses to complex environmental conditions. They provide an ecological overview of the current situation from waterways and streams, and where possible, they are connected to the beds and respond to environmental changes faster than higher-level organisms. The high diversity and wide geographic distribution of diatoms [49] prove their superiority over other algal groups in the quality monitoring of aquatic ecosystems. Diatoms are one of the important criteria for measuring the ecological conditions of the aquatic environment [50–53] seen by the EU 2000 Water Framework Directive (WFD) as a complete determinant of the 'ecological status' which is "an expression of the quality of the structure and functioning of aquatic ecosystems" [54]. Diatoms react directly to many physical, chemical, and biological changes in water ecosystems, such as temperature and concentration of nutrients [55]. They also respond to chemical changes in the water, seasons, and physical variables [56–59]. Diatom species have very strong relationships with nutrients such as salinity, alkaline conductivity, and total phosphate, which can be measured at different stations. The composition of diatoms is affected by anthropogenic changes in water quality [60] which is one of the high-level factors that causes the formation of diatom compounds in water systems.

Diatoms are affected by time changes as they evaluate temporal changes in water quality [61], and act as a tracer of water sources and hydrological connectivity [62]. They are strongly influenced by the main controlling factors such as hydrological features, biogeochemical processes, temperature, and surface runoff caused by precipitation in these systems [63–69]. This shows that diatom community dynamics are a function of natural factors such as temporal variability [70–72] as well as anthropogenic factors such as pollutant emissions [58,73–75]. Diatoms are seen in brown and gold colors due to xanthophyll pigment [76], and photosynthetic pigments are bioactive compounds. They are not only responsible for the absorption of solar energy for photosynthesis [77–79], but also play a role in the process of light protection [80,81] and antioxidant activity [82] that help produce biomass and oxygen [83]. Diatoms are indicators of the impact of acid rain [84], which along with acidic sewage [85] is the main source of acidic river water [86] and metal pollution [87,88]. Diatoms are very sensitive to the pH of water [89–91] as changes in pH have a great effect on the mass of diatoms [92–96]. Diatoms play an important role in the food chain due to their high reproduction rate. Many diatoms have ecological tolerance and are suitable indicators for different environmental conditions [97]. Determining water quality using algal communities is a quick and cheap method [98]. The main aim of this research is to use diatom indices to assess the ecological status of various sampling stations of the Aras River in northwest of Iran. Furthermore, we present the results using diatom indices, which provide evidence that changes in the community composition of benthic diatoms can be used to infer changes in biological properties. Furthermore, using algal communities, we evaluated the relationships between water quality, species composition, biomass, chlorophyll *a* concentration, and ecosystem function along the river.

2. Materials and Methods

2.1. Study Area

The 1072 km long Aras River, called the Araz Chay River or Aras Chay in Azerbaijani Turkish, is known by different names in different sources, including Araks, Arax, Araxes, or Araz. The Aras River is a transboundary river between Iran, Turkey, Armenia, and Azerbaijan (Figure 1a). The Aras River originates from the Bingol in eastern Turkey. This turbulent river is born in the evergreen mountains of Minguldag and Bingol in the Arpacha region of Anatolia. The Aras River is fed by snow melting at the heights of these two mountains. After flowing into eastern Turkey, the Aras flows to the Turkish–Armenian border. The river continues its route until it crosses the Turkish–Armenian border, reaching Nakhchivan, and then the Iranian–Nakhchivan border. The Aras then joins the Iranian–Armenian border by passing international routes. Finally, it enters the Republic of Azerbaijan. The river eventually flows into the Kura River and joins the Caspian Sea (Figure 1b).

2.2. Selection of Sampling Stations

To identify the ecological conditions and diatom species, sampling was conducted at 16 stations (Table 1). Sampling of diatom communities, chlorophyll *a*, and river water was conducted for water chemistry. Each of the studied stations had different environmental conditions, and the amount of water and water turbidity differed during different seasons. In this study, for the purpose of studying the water chemistry and diatom flora of the Aras River, sampling was conducted with 3 replicates from each sampling station. The sampling sites were also chosen to ensure the understanding of the impact of regulated flow regimes on the physical, chemical, and biological environment downstream. Site descriptions were carried out at each selected sampling site. This inventory comprised the physical character and algal periphyton following standard field inventory protocols [99].

Figure 1. Map of sampling sites in the Aras River, NW-Iran. (**a**) The Aras River is a transboundary river between Iran, Turkey, Armenia, and Azerbaijan. (**b**) Sampling stations along the Aras river in northwest of Iran.

Table 1. Location of the 16 sampling stations along the Aras River with site GIS coordinates.

Code	Site Name	Latitude	Longitude	Altitude (m)
S1	Poldasht	45.06581333	39.34696033	787
S2	Before Aras Dam	45.22557378	39.19943588	770
S3	After Aras Dam	45.35672307	39.13794955	764
S4	Jolfa	45.62766533	38.93720633	707
S5	Jolfa to Siah Rud	46.10739022	38.86254289	615
S6	10 km to Siah Rud	46.17854387	38.84061849	553
S7	Sfiah Rud	46.00456867	38.86773967	648
S8	Nurdouz	46.20829367	38.83874067	669
S9	Tatar Oliya	46.77221433	39.04058333	360
S10	10 km Tatar Oliya	46.78872228	39.05588419	359
S11	Ving village	46.83488333	39.01158833	339
S12	Before Khodafarin Dam	47.35944405	39.41834632	142
S13	After Khodafarin Dam	47.33519688	39.40097177	146
S14	Aslandooz	47.41018800	39.44133100	157
S15	Oltan	47.76384033	39.60737133	63
S16	Pars Abad	47.91895151	39.64515549	45

2.3. Laboratory Processes

To investigate the Aras River's water chemistry and algal community structure, sampling was conducted with three replicates from each station. To check water chemistry, samples were taken from running water at each station. Samples were taken from rocks, sediments, and plants. The sampling methods of diatoms are completely different according to their habitats, such as rocks, sediments, and plants. After sampling, algae samples were placed in 200 mm plastic containers with tight lids. Lugol's solution [100,101] was used to maintain the natural shape of the diatoms until the laboratory treatments. All sample containers were placed inside a cool box. Along with algal samples, water samples were also taken in one-liter containers for analysis of nitrate, ammonium, sulfate, phosphate, dissolved oxygen, temperature, electrical conductivity, salinity, pH, electrical conductivity (EC), and total dissolved solids (TDS). In situ measurements of temperature (TEMP, °C), pH, electrical conductivity (μS cm^{-1}), turbidity (NTU), and dissolved oxygen (DO, mg L^{-1}) were obtained using a Hanna multi-meter HI9811-5N (Hanna Instruments, Smithfield, RI, USA). Permanent slides were prepared to determine the composition of species and count different groups of algae. The relative abundance of different algal groups (green algae, cyanobacteria, diatoms, and other algae) was calculated by placing 1 mL of each sample in a Sedgewick-Rafter chamber [102]. To identify and count diatom species, samples were prepared by the Battarbee method [103–105]. The samples were digested with 35% hydrogen peroxide in a cup with a temperature of 90 °C in a heater for 2 h, and then 5 cc of 35% hydrochloric acid was added to it. The washing step was performed 5 times. Seven hundred microliters of the washed sample were placed on a glass slide and allowed to dry in the open air using permanent Naphrax [103]. After the final control of the prepared slides and ensuring the complete preparation of the samples of all the stations, a detailed analysis of each is possible. Since diatoms generally have a minimal number and cannot be seen even with a 40 lens, all examinations were conducted with a 100 lens. As a result, a drop of immersion oil was placed on the center of each slide to study. Both soft algae and diatoms were identified in the laboratory using global and regional algal identification keys [104–108]. Cells were identified to species level, or where this was not possible, to the lowest taxonomic level possible. Sampling was conducted separately to measure dry mass (DM), ash-free dry mass (AFDM), and chlorophyll a. To determine the dry mass, the samples were dried in an oven for 24 h at 60 °C and weighed. In the next step, the samples were heated in the furnace for 4 h at a temperature of 525 degrees Celsius and weighed again.

Ash-free dry mass was estimated as the difference in mass before and after combustion and was expressed as mg cm^{-2} [109–111]. To measure chlorophyll *a*, the samples were transferred to laboratory tubes containing 10 cc of 95% ethanol [112–115]. The samples were kept overnight in the freezer. After reaching room temperature, the absorbance of the supernatant was determined at the wavelength of 665 nm before and after adding 0.1 normal HCl [116] which had been derived from Nusch's equation [114]. The relevant handbooks, monographs, and individual articles were used to determine species. The list of revealed diatoms in the Aras River was updated with algaebase.org. The total abundance was found for each species of planktonic and periphyton diatom species as abundance scores.

2.4. Data Analysis and Interpretation

The ranking was used for the relationship between the spread of diatoms and their diversity in the Aras River and water quality. For this, the relative abundance data of diatom taxa, along with environmental data, were entered into CANOCO software 4.5 [117,118]. A high numerical value indicates that the water body has a higher biological health. Shannon's diversity index can take values between 1 and 5, and any amount of a low index number indicates higher pollution, while Simpson's diversity index has values between 0 and 1, and the closer it is to 0, the less diverse it is. Uniformity values are between zero and one, which gives us information about the population structure; for example, the closer the value is to one, the more similarity there is between the abundance of species.

3. Results

Average Annual Water Chemistry in the Aras River

The physical and chemical variables were different in the studied stations, and their results are given in Table 2. The pH in the Aras River is mostly alkaline. The highest and lowest pH were 9.8 and 7.5, which were reported in station 6 and station 2, respectively. The value of EC was variable in sampling stations and the highest value of electrical conductivity is related to station 16 with EC = 4.3 μS cm^{-1}. The changes in nutrients and heavy metals were analyzed for the sampled stations and their results are shown in Table 3 and Figure 2.

Table 2. Scores of diatom indices from the sampled stations. Trophic Index Turkey (TIT); Trophic Index (TI); Eutrophication and/or Pollution Index-Diatom (EPI-D); Diatom Species Index Australian Rivers (DSIAR), Trophic Diatom Index (TDI), Pollution Sensitivity Index (IPS).

	EPI-D	TI	TIT	DSIAR	TDI	IPS
S1	0.75	0.88	1.08	80	35	4.1
S2	0.78	0.94	1.15	74	42	4.26
S3	0.91	1.1	1.21	75	41	4.50
S4	0.89	0.98	1.08	65	46	3.27
S5	0.88	1.1	1.99	55	55	3.55
S6	1.9	2.5	3.84	51	58	4.07
S7	1.5	2.56	2.66	52	59	3.28
S8	1.8	2.8	2.74	53	65	2.50
S9	1.5	2.6	3.01	42	72	2.48
S10	1.3	2.1	2.64	48	75	3.22
S11	1.6	1.21	2.65	48	76	3.66
S12	1.5	1.23	2.51	46	78	4.00
S13	1.5	1.54	2.48	45	81	3.19
S14	1.5	2.01	2.61	38	85	2.68
S15	1.8	1.47	2.19	25	89	3.08
S16	1.4	1.08	2.10	20	86	3.47

Table 3. Physical and chemical water quality characteristics on the Aras River.

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
pH		9.06	8.06	8.53	10.6	9.8	11.1	10.06	10.06	9.06	9.46	9.1	8.17	9	9	9.6	9.5
Temperature	°C	8.1	8.1	8.1	8.1	8.9	9.1	9.74	8.97	8.7	8.7	8.7	8.7	8.3	8.9	9.6	9.4
Conductivity	µS cm^{-1}	1.56	1.58	1.55	1.2	1.3	1.2	1.23	1.22	1.54	1.55	1.55	1.08	1.12	1.1	1.2	4.3
Turbidity	NTU	1.47	1.47	1.47	1.47	1.5	0.9	1.4	2.2	5.1	5.1	5.1	5.1	5.2	6	7.6	12
Dissolved oxygen	mg L^{-1}	8	8	8	8	8.7	8.9	8.7	8	8	8	8	8	7.006	7.5	10.4	6.7
Total dissolved solids	mg L^{-1}	8	7.5	0.8	0.72	0.73	0.72	0.76	0.72	0.66	0.71	0.75	0.58	0.54	0.6	0.7	2.2
Total suspended solids	mg L^{-1}	187	187	187	187	124	162	69.45	71.55	463	463	463	463	475	474	475	465
Mg^{2+}	mg L^{-1}	1.2	1.2	1.7	1.7	2	2.2	2.2	2.1	2.5	2.6	2.6	3.3	3.3	3.3	3.3	3.3
Zn	mg L^{-1}	5	5	5	5	6.3	8	11.6	13.6	51.8	51.8	51.8	51.8	48.4	43.5	55.1	52.1
K^{+}	mg L^{-1}	5.29	5.29	5.29	5.29	5.7	5.7	5.1	5.2	4.6	4.6	4.6	4.6	4.09	5.3	5.3	5.6
CL^{-}	mg L^{-1}	22	25	23	25	31	31	32	32	33	28	28	29	29	28	28	28
Ca^{2+}	mg L^{-1}	1.6	1.6	1.6	1.8	1.8	2.2	2.2	3.3	3.2	3.3	3.1	3.2	3.4	3.4	3.6	3.6
$So_4{}^{2-}$	mg L^{-1}	0.45	0.45	0.52	0.52	0.46	0.46	0.48	0.48	0.55	0.54	0.62	0.55	0.42	0.42	0.56	0.56
NH_3	mg L^{-1}	0.1	0.1	0.1	0.1	0.09	0.09	0.09	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.09	0.09
Total phosphorus	mg L^{-1}	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Total oxidized nitrogen	mg L^{-1}	0.06	0.08	0.07	0.08	0.08	0.08	0.05	0.05	0.07	0.06	0.06	0.08	0.08	0.08	0.08	0.08
Total nitrogen	mg L^{-1}	0.2	0.2	0.3	0.2	0.3	0.3	0.4	0.5	0.5	0.6	0.7	0.7	0.8	0.8	0.8	0.8

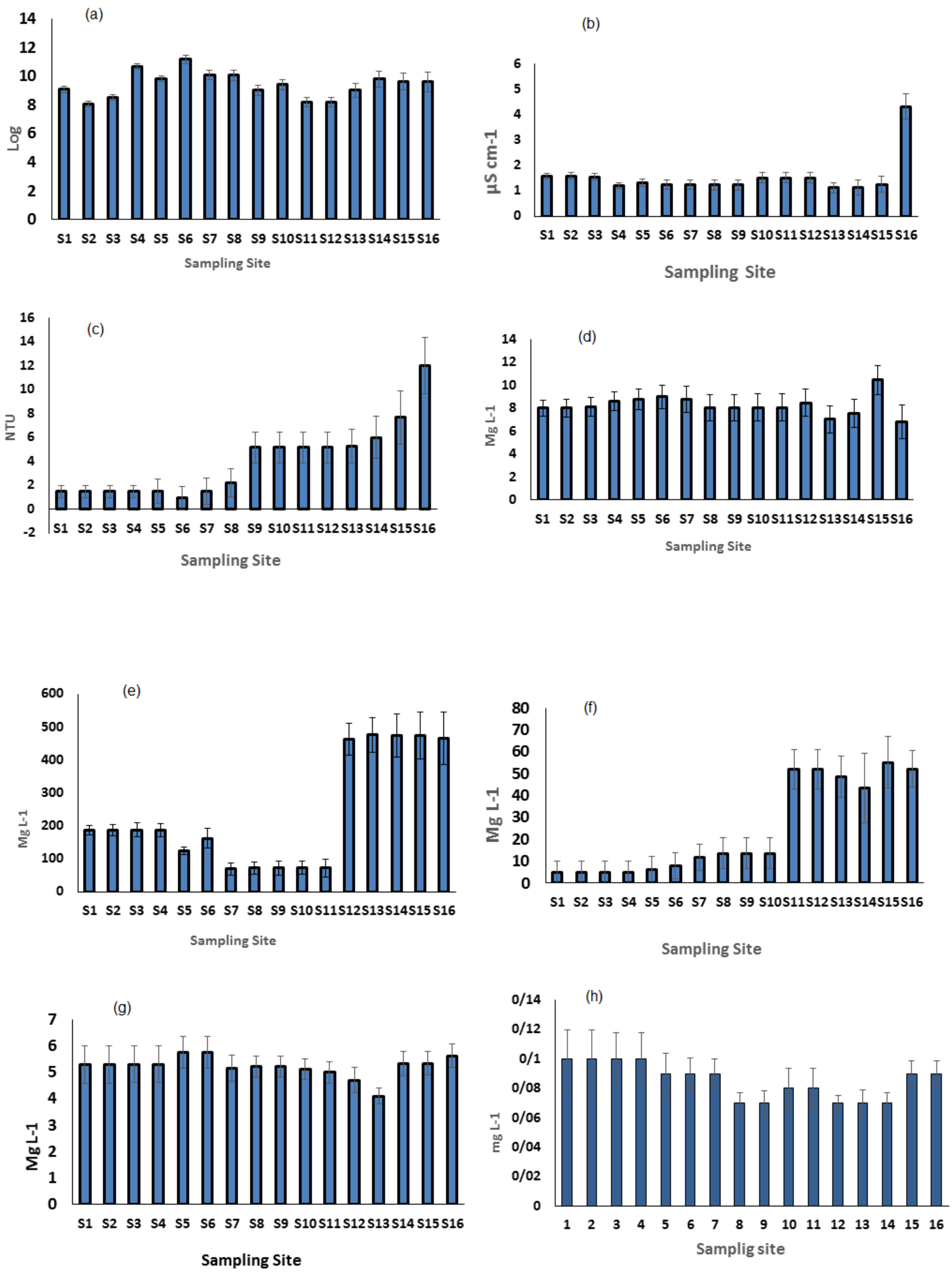

Figure 2. Changes in heavy metals and mineral elements along the Aras River: (**a**) pH; (**b**) electrical conductivity; (**c**) turbidity; (**d**) dissolved oxygen; (**e**) total suspended solids; (**f**) Zn; (**g**) K^+; (**h**) NH_3.

Several diatom indices, such as EPI-D, IPS, and DSIAR, were conducted to evaluate the ecological status of the Aras River (Table 2). Changes in chlorophyll-a concentration demonstrated a general increase from upstream to downstream (Figure 3). The accumulation of dry mass, AFDM, and chlorophyll-a concentration were measured. The accumulation of dry mass was typically greater in the downstream sites. The accumulation of AFDM also typically increased from upstream to downstream (Figure 4).

According to the investigations, the algal flora of Aras River consists of alkaline species. In this study, 246 species of diatoms (105 genera), 13 species of green algae (9 genera), 10 species of cyanobacteria (9 genera), and 11 other algae (9 genera) from the samples collected in 16 stations during one year. The diatom community (combination of species) was the most abundant, and it showed a high diversity in all Aras River stations. Meanwhile, the relative proportion of green algae and cyanobacteria was lower than diatoms and increased in the middle areas. According to the numbers, the density of diatoms and types of species was high in all stations. *Diatoma vulgaris*, *Ulnaria ulna*, *Cocconeis placentula*, *Gomphoneis olivaceum*, *Microcystis botrys*, *Microcystis aeruginosa*, *Pseudanabaena limnetica*, *Scenedesmus obliquus*, and *Rhoicosphenia abbreviata* were dominant species in autumn and all stations. Regarding diversity and density of diatoms, Station 1 has 37 species. According to the counts, the most common species are *Nitzschia supralitorea*, *Rhoicosphenia abbreviata*, *Gomphoneis olivaceum*, *Ulnaria ulna*, and *Diatoma vulgaris*. Station 5 has 35 species. These species are related to *Cyclotephanos dubius*, *Navicula novaesiberica*, and *Cymbella neogena*. Station 6 has 35 species. According to the counts, the most common species are *Diatoma vulgaris*, *Ulnaria ulna*, *Cocconeis placentula*, *Navicula tripunctata*, *Gomphoneis olivaceum*, *Rhoicosphenia abbreviata*, *Tetracyclus rupestris*, and *Nitzschia supralitorea*. In terms of diversity and density, Station 7 has 36 species. According to the counts, the most common species are *Navicula tripunctata*, *Gomphoneis olivaceum*, *Rhoicosphenia abbreviata*, *Cymbella tumida*, *Surirella brebissonii*, *Diatoma mesodon*, and *Nitzschia supralitorea.* Station 8 has 35 species. According to the counts, the most common species are *Amphora ovalis*, *Navicula tripunctata*, *Gomphoneis olivaceum*, *Rhoicosphenia abbreviata*, *Nitzschia recta*, *Surirella brebissonii*, *Cymbella dorsenotata*, and *Cymbella neocistula*. Station 12 has 37 species. These species are *Melosira varians*, *Brevisira arentii*, *Diatoma vulgaris*, *Ulnaria ulna*, *Cocconeis pseudolineata*, *Amphora ovalis*, *Navicula tripunctata*, *Nitzschia linearis*, *Cymatopleura solea*, *Bacillaria paxillifera*, and *Amphora aequalis*. In terms of diversity and density, Station 13 has 39 species. According to the counts, the most common species are *Melosira varians*, *Brevisira arentii*, *Diatoma vulgaris*, *Amphora ovalis*, *Nitzschia palea*, *Cymatopleura solea*, and *Bacillaria paxillifera*. Station 14 has 38 species. According to the counts, the most common species are *Brevisira arentii*, *Cocconeis placentula*, *Rhoicosphenia abbreviata*, *Navicula cryptotenelloides*, *Cocconeis pseudolineata*, *Navicula tenelloides*, *Nitzschia adamata*, *Amphora mongolica*, and *Navicula seibigiana*. Station 15 has 37 species. According to the counts, the most common species are related to *Diatoma vulgaris*, *Cocconeis placentula*, *Rhoicosphenia abbreviata*, *Navicula cryptotenelloides*, *Cocconeis pseudolineata*, *Navicula tenelloides*, *Nitzschia adamata*, *Amphora mongolica*, and *Navicula seibigiana*. Station 16 has 36 species. The most common species are *Melosira varians*, *Ulnaria ulna*, *Cocconeis placentula*, *Bacillaria paxillifera*, *Nitzschia acidoclinata*, *Navicula cryptotenella*, and *Fragilaria gracilis* (Table 4, Figure 5).

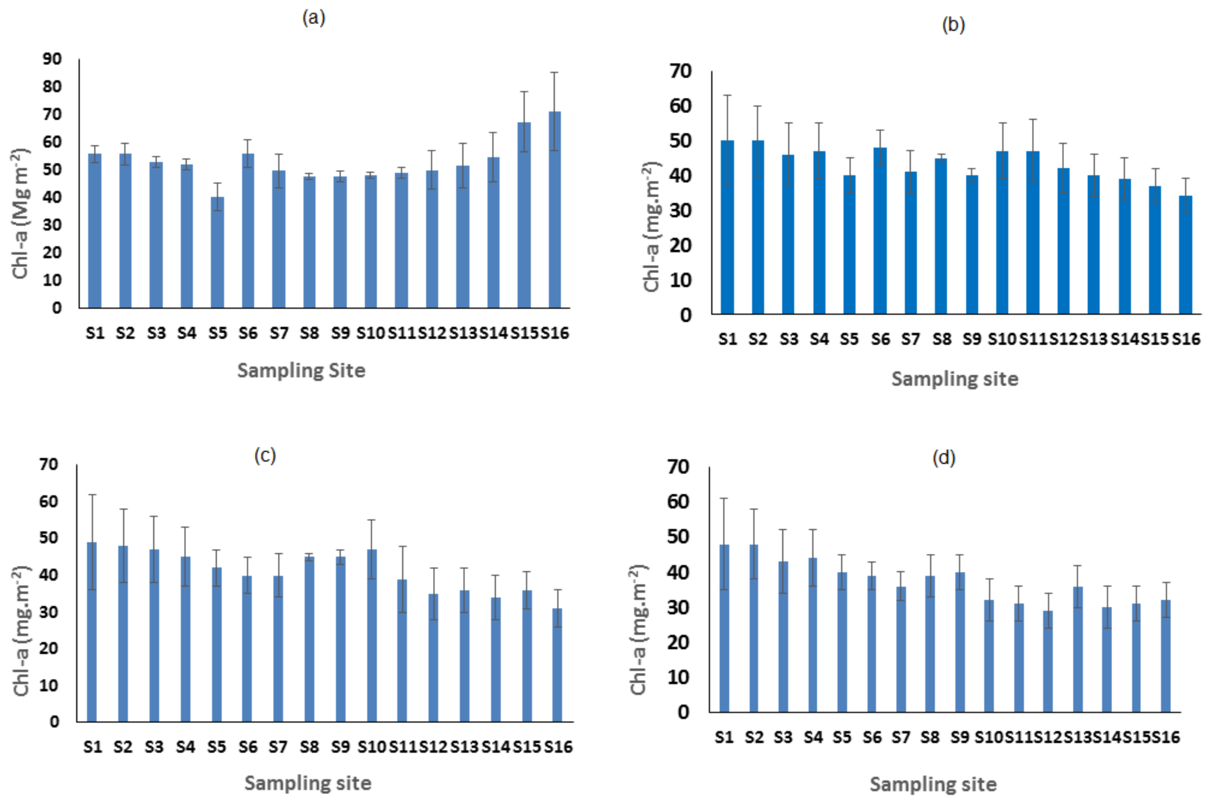

Figure 3. The concentration of chlorophyll *a* at each of the Aras River sites: (**a**) December 2022; (**b**) February 2022; (**c**) May 2022; (**d**) EC October 2022.

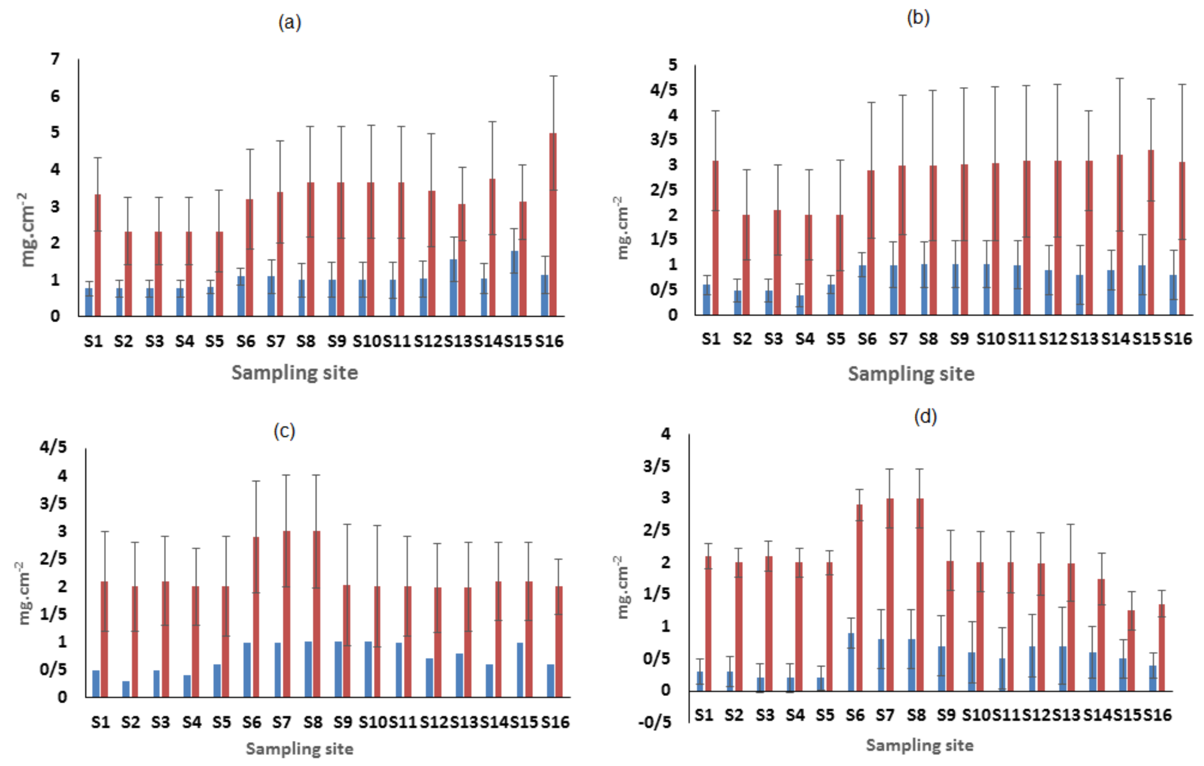

Figure 4. The accumulation of dry mass of the sampling stations along the Aras River: (**a**) December 2022; (**b**) February 2022; (**c**) May 2022; (**d**) October 2022. Ash-free dry mass (blue columns); dry mass (red columns).

Table 4. Algae list according to genus and number of species in each genus in the Aras River, northwestern Iran.

Bacillariophyta	Taxa No.	Chlorophyta	Taxa No.	Cyanobacteria	Taxa No.	Other Groups	Taxa No.
Amphora	15	*Chlorella*	1	*Anabaena*	2	*Chara*	1
Aneumastus	3	*Cladophora*	1	*Chroococcus*	1	*Ceratium*	2
Asterionella	2	*Chlorococcum*	1	*Microcystis*	2	*Cryptomonas*	1
Anomoeoneis	2	*Chlamydomonas*	1	*Nostoc*	1	*Euglena*	1
Achnanthidium	2	*Oedogonium*	1	*Oscillatoria*	1	*Gymnodinium*	1
Aulacoseira	1	*Scenedesmus*	5	*Phormidium*	1	*Peridinium*	1
Adlafia	1	*Schroederia*	1	*Spirulina*	1	*Staurastrum*	2
Amphipleura	1	*Stigeoclonium*	1	*Tolypothrix*	1	*Spirogyra*	1
Brebissonia	1	*Ulothrix*	1			*Staurodesmus*	1
Brachysira	3						
Brevisira	1						
Bacillaria	1						
Berkeleya	1						
Brockmanniella	1						
Ctenophora	1						
Cymbopleura	1						
Craticula	1						
Chamaepinnularia	1						
Campylodiscus	1						
Cymbella	20						
Caloneis	9						
Cocconeis	7						
Cymatopleura	4						
Cyclotella	3						
Cyclostephanos	1						
Caloneis	1						
Denticula	7						
Diatoma	6						
Didymosphenia	3						
Diploneis	2						
Discotella	1						
Diploneis	1						
Dimeregramma	1						
Delicata	1						
Eunotia	8						
Entomoneis	2						
Encyonopsis	2						
Epithemia	1						
Eucocconeis	1						
Fragilaria	9						
Surirella	13						
Nitzschia	35						
Pinnularia	18						
Gomphonema	25						
Geissleria	4						
Stauroneis	9						
Gyrosigma	2						
Gomphosphenia	2						
Halamphora	8						
Total	246		13		10		11

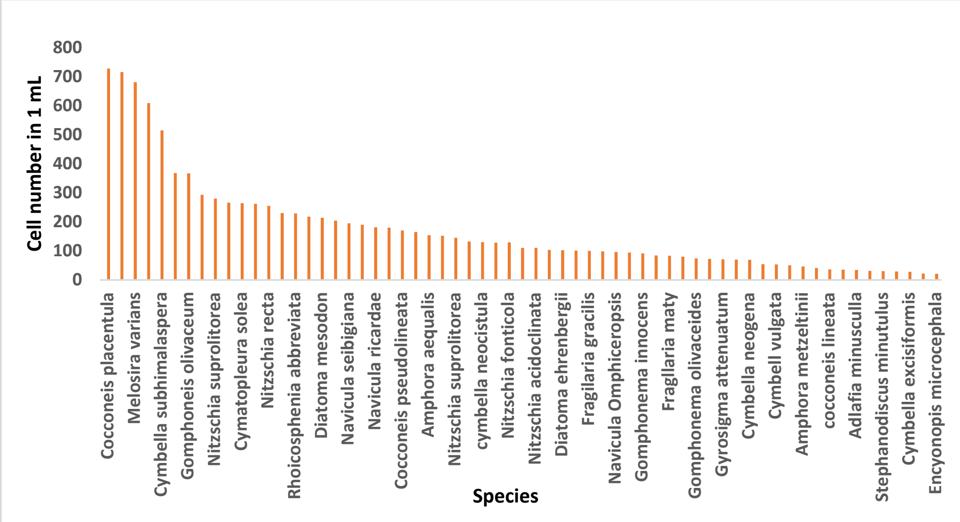

Figure 5. Algae count in the studied stations. The number of diatoms in 700 micro samples.

According to Figure 6 and the results obtained from the Aras River, the relative abundance of algal groups is different based on the reach of the river. The relative abundance of diatoms was high in all stations. Filamentous green algae and green algae were abundant in station 5 and station 14. On the other hand, cyanobacteria were abundant in station 5, station 13, and station 14. Other algae, such as *Euglena*, *Gymnodinium*, and *Peridinium*, varied slightly between sites (Figure 6).

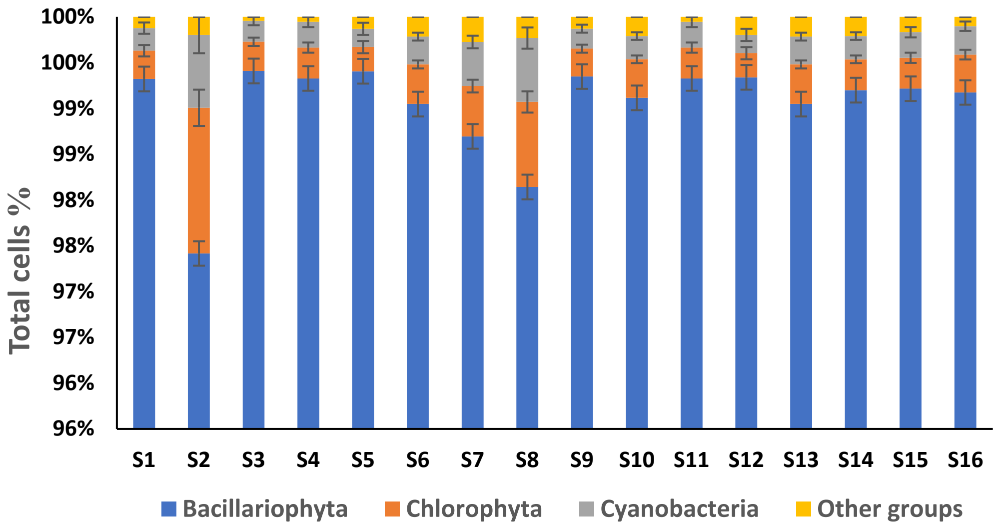

Figure 6. The results of the relative abundance of algae types along the sampling stations of Aras River. Some diatom species grow in higher electrical conductivity. Of these species abundantly found in station 16, we can mention *Geissleria schoenfeldii*, *Cymbella cymbiformis*, *Gomphonema augur*, *Caloneis silicula*, *Diploneis oblongella*, *Cocconeis pseudolineata*, *Navicula cari*, *Stauroneis anceps*, and *Nitzschia dissipata*. The WA regression results confirmed the high electrical conductivity of these species. Several diatom species that are abundant in stations 4, 5, 6, and 7 and are associated with NO_3^- include *Nitzschia palea*, *Navicula veneta*, *Cymbella neocistula*, *Gomphonema angustatum*, *Surirella ovalis*, *Nitzschia parvula*, and *Amphora ovalis*, which grow more in waters with higher NO_3^-. According to the CCA diagram, several diatom species are related to copper, primarily found in stations 8 and 13. Among these types, we can mention *Gomphonema angustatum*, *Navicula veneta*, *Nitzschia parvula*, and *Amphora ovalis*. According to the CCA diagram, the species *Nitzschia flexa*, *Navicula gregaria*, *Hantzschia amphioxys*, and *Rhoicosphenia abbreviata* have increased in stations 6, 7, 12, 13, 14, 15, and 16 with the increase in copper.

The PCA indicated that upstream species, such as *Navicula tripunctata*, were associated with high DO. In contrast, downstream species, such as *Tryblionella salinarum*, were related to increased turbidity, total suspended solids (TSS), conductivity, TN, Si, pH, and TDS. The CCA results showed that the most important variables were Cu, TN, TDS, temperature, TSS, turbidity, pH, and conductivity. Furthermore, the results revealed TP, temperature, and DO negatively correlate with turbidity, conductivity, and TSS on the second axis (Figure 7).

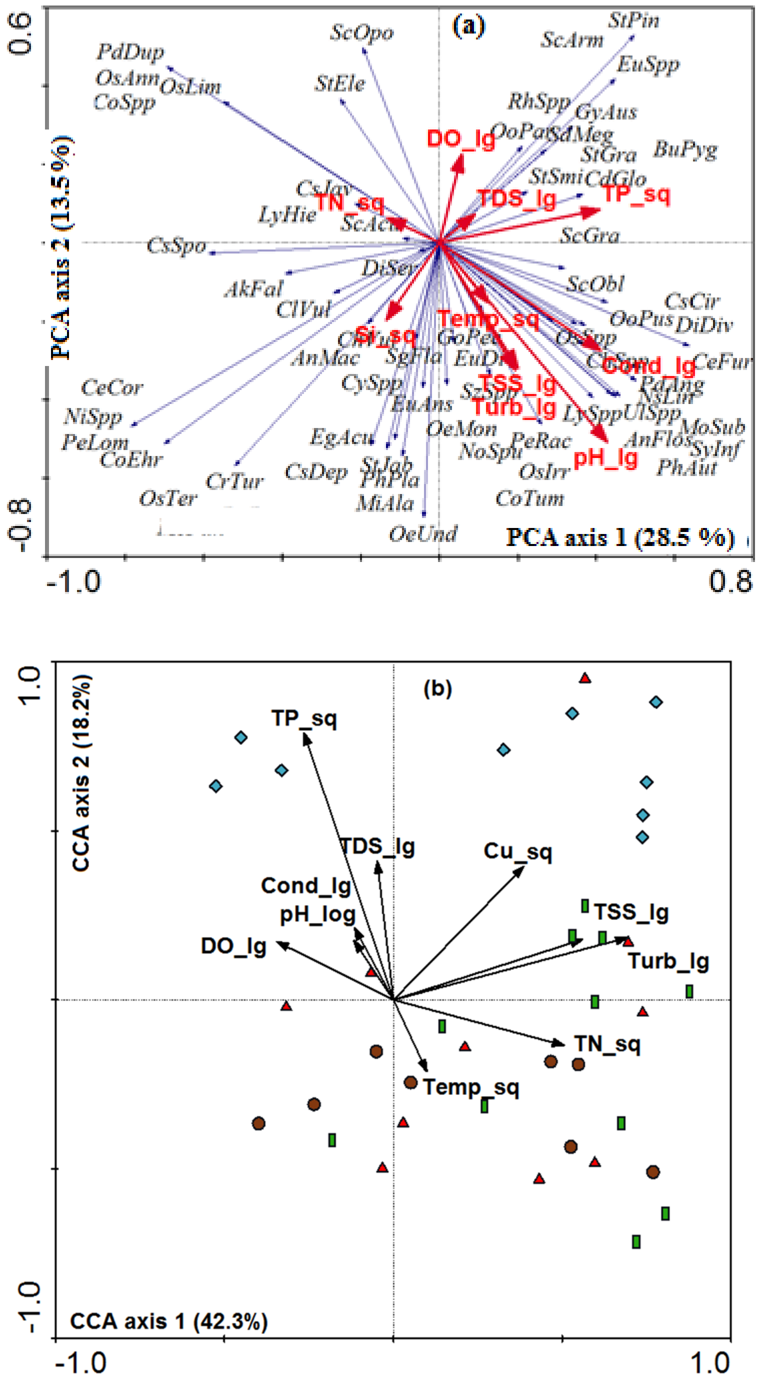

Figure 7. Principal component analysis (**a**) and canonical correspondence analysis (**b**) biplot for the relationship of environmental data and algal diversity.

4. Discussion

It is necessary to ensure water quality before using it for drinking, agricultural, and industrial applications. Hence, river water quality assessment is a prominent element in water resources assessment. Aquatic ecosystems are natural receivers of heavy metals. This study showed that heavy metals are high in the stations downstream of the Aras River. The high concentration of these variables is due to the entry of urban and industrial wastewater into the Aras River [43,119,120]. The generic content also changed down the river, from the richest genera *Cymbella*, *Cyclostephanos*, *Navicula*, and *Cocconeis* in the upper part, to *Diatoma*, *Nitzschia*, *Surirella*, and *Melosira varians* in the middle, and *Melosira varians*, *Bacillaria paxillifera*, *Navicula*, and *Nitzschia* in the lower part.

Downstream of the Aras River, salinity increases and salinity-resistant species are more numerous. The results showed that the increase in salinity and turbidity in the stations downstream of the Aras River causes a decrease in water oxygen saturation.

According to the calculations, diatom density and species diversity were high in all stations. *Diatoma vulgaris*, *Ulnaria ulna*, *Cocconeis placentula*, *Gomphoneis olivaceum*, and *Rhoicosphenia abbreviata* were the dominant species in autumn and in all stations. *Rhoicosphenia abbreviata* is one of the indicator species of water polluted with agricultural runoff. It was observed in all stations in the study area.

In stations 1 to 5, simultaneously with the significant reduction in *Cocconeis* populations, the abundance of *Nitzschia* and *Navicula* species suddenly increases. These results indicate an increase in the concentration of pollutants.

The observation of some deformities in *Cocconeis* members in large amounts is caused by the entry of heavy metals such as zinc into the river water. However, in the downstream stations, the size of *Cocconeis* populations increased, and the frequency of *Melosira varians* and *Brevisira arentii* species increased suddenly [121]. This shows that food availability in the downstream stations has caused the growth of these diatoms. On the other hand, the presence of *Melosira varians* species, which suddenly increased in the downstream stations, shows that the waters in the downstream stations have many minerals and organic pollutants [122,123].

From an ecological point of view, this species is observed in oligotrophic lakes with low to medium electrolytes, so it can be assumed that the water of the Aras River does not have acidic properties and is not a favorable environment for acid-loving diatoms [124]. In other words, the waters of this region belong to the so-called alkaline group. The significant problems and cost of measurements, fluctuation, and low repeatability of the physical and chemical characteristics of water, and the necessity of constant measurement of these characteristics, have led some biologists to analyze the species composition of different animal and plant groups instead of using water characteristics to evaluate the quality of surface water. Unlike measuring the physical and chemical properties of water, biological monitoring of water quality can result from the combined effects of various factors [125]. Different species of algae show different reactions to unfavorable situations. At its lowest level, biological monitoring is interpreted as checking the presence or absence of a species against a specific type of pollutant. However, before all species disappear, other more precise effects, such as the presence or absence of a particular species, can be studied to monitor population changes. Therefore, it is possible to mention the positive points of using biological indicators, including accuracy, considering the long-term effects of pollutants, being cheap, easy to use, and understood by non-specialists. Measuring, analyzing, and interpreting the biological quality data of aquatic ecosystems regularly makes it possible to adopt correct and appropriate management practices and gradually reduce river pollution to move towards standard quality [126].

5. Conclusions

Looking at the findings obtained from this study, the water quality of the Aras River in the studied area is not in a suitable condition in terms of many of the important variables mentioned. It seems that the existence of extensive agricultural activities and the emptying of industrial wastes are the most prominent factors of the poor water quality of the Aras River. According to the obtained results, the distribution of diatoms in the Aras River is affected by various factors, including climatic, geological, and hydrological factors, and human interventions. The results of this survey identified the main factors as natural factors and human interventions. The presence of heavy metals in rivers changes the shape of diatoms. The presence of pollutants and heavy metals in the middle and lower reaches of the Aras River has caused the growth and deformation of different species of diatoms. The middle and downstream parts of the Aras River contain much pollution and many heavy metals, which has caused a decrease in the growth of diatoms adapted to favorable conditions and an increase in the growth of pollution-resistant diatoms in the middle and downstream stations. Overall, the lower parts of the Aras River were in poor condition in terms of water quality, stream condition, and river health. However, environmental flows changed the water quality and stream condition, and these elements improved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11101867/s1.

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