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Ecological assessment of water quality in the Kabul River, Pakistan, using statistical methods

by

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Abstract

We identified 209 species of algae and cyanobacteria at 4 sites in the Kabul River. Green algae, diatoms, and charophytes dominated in the river, which reflects regional features of agricultural activity. Species richness and algal abundance increased down the river. The Water Quality Index characterizes the quality of water down the river as medium to bad. The index of saprobity S reflects Class III water quality. The Water Ecosystem Sustainability Index (WESI) shows contamination with nutrients. According to the River Pollution Index (RPI), waters in the river have low alkalinity and low salinity, and are contaminated with nutrients. Pearson coefficients showed that water temperature plays a major role in the total species richness distribution (0.93*) and in the green algae distribution (0.89*), while cyanobacteria were stimulated also by water salinity (0.91*). Stepwise regression analysis indicated water temperature as the major regional factor that determines riverine algal diversity. Surface plots and Canonical Correspondence Analysis (CCA) showed that salinity, nitrates, temperature, and Biochemical Oxygen Demand (BOD) can be defined as major factors affecting algal diversity. Dendrites mark the upper site of the Warsak Dam as the source of the community species diversity. Bioindication methods can give relevant and stable results of water quality and self-purification assessment that can be employed to monitor the regional water quality.

Key words: freshwater algae, river, water quality, statistical methods

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Introduction

As a rule, pollution of a given river depends on the number of people living on its banks and the economic growth. Chemical properties of the Kabul River water were determined with no available information on the anthropogenic load in the studied section of the river in previous studies (Yousafzai et al. 2010). There is no information about the aquatic biota in this part of the river.

Aquatic ecosystems in Pakistan are represented mostly by rivers rather than by lakes because the territory of the country is characterized by a high altitude. Algal diversity of Pakistan's river communities can help the ecological assessment of water quality, especially in the case of water used for agriculture. The algal community's diversity in the rivers of Pakistan has been studied only during the last two decades and requires further research. In this way, ecological studies of the algal flora in the Kunhar River were performed only once (Leghari et al. 2001). Algal diversity of the Meenachil River in the neighboring area of India was studied in respect of climatic seasonality (Sebastian 2016). Systematics and geographic distribution of some algal species, such as Chroococcus, was studied based on samples from the Pakistan river waters (Munir et al. 2016). Only one paper closely related to our study was found – the algal species diversity, ecology, and relationships with the environment of the Swat River in the Hindu Cush Mountains (Barinova et al. 2013). The importance of water quality resources assessment in Pakistan is now recognized as an actual problem. Thus, some economical methods are being researched to find solutions to this problem. One of the methods for water-resource quality assessment is bioindication. The algal bioindication approach was employed in Pakistan for algal communities in the Swat River (Barinova et al. 2013). The Swat River is an upper high mountain tributary of the Kabul River. Only algal biofouling of boats was investigated in the studied part of the Kabul River (Khuram et al. 2014). We decided to create an international team of scientists from different universities of Pakistan and Israel to find new statistical approaches in the water quality assessment of the Kabul River along with the most efficient and economic methods while determining significant factors for future monitoring of the water quality in Pakistan

The aim of the present study was to assess the water quality of the Kabul River on the basis of algal diversity, before and after the Swat River, with the help of statistical methods.

Description of the study site

Peshawar Valley

The Peshawar Valley is the most distinct region within the entire province of Khyber Pakhtunkhwa, Pakistan. This region was divided into five administrative units: Peshawar, Nowshera, Charsadda, Mardan, and Swabi Districts. The valley is located between 34°07′58′′N and 71°41′45′′E and has an altitude of about 335 m above sea level. The valley represents an irregular ellipse with its longer axis (116 km) extending from east to west and the shorter axis (84 km) extending from north to south (Salim and Khan 1973).

Physiography

Except for the eastern region, the valley is surrounded by mountains, which are offshoots of the Hindu Kush mountain ranges. In the east, these mountains have a small opening near Attock through which the Kabul River drains into the Indus River. The area of the valley does not include the tribal territories adjoining the districts, which is approximately 9583 sq. km, but with the tribal territories the area increases to about 13 087 sq. km (Government of Pakistan 1998; Salim and Khan 1973).

Geology

The geology of the Peshawar Valley is diverse, and its geological history is closely related to the Great Himalayan Mountains. The mountains surrounding the valley date from the Tertiary period. No fossils are known from this area. The Kabul River has a complex basin system. Sedimentary limestones and shales mostly represent the lower basin, while the headwaters of the main tributaries rise between very complex groups of igneous and metamorphic rocks (Government of Pakistan 1998; Salim & Khan 1973; Yousafzai et al. 2010).

Hydrography

The Peshawar Valley is fed by four main rivers: the Kabul River, the Swat River, the Bara River, and the mighty Indus River (Figure 1). The Swat River originates in Kalam and flows southward up to the place where it empties into the Kabul River. The Bara River originates in the Tirah Hills in the south and flows toward the northeast up to the place where it joins the Kabul River that originates in the Wonay Pass, Afghanistan, and flows from west to east in the center of the valley. The Kabul River flows into the mighty Indus River

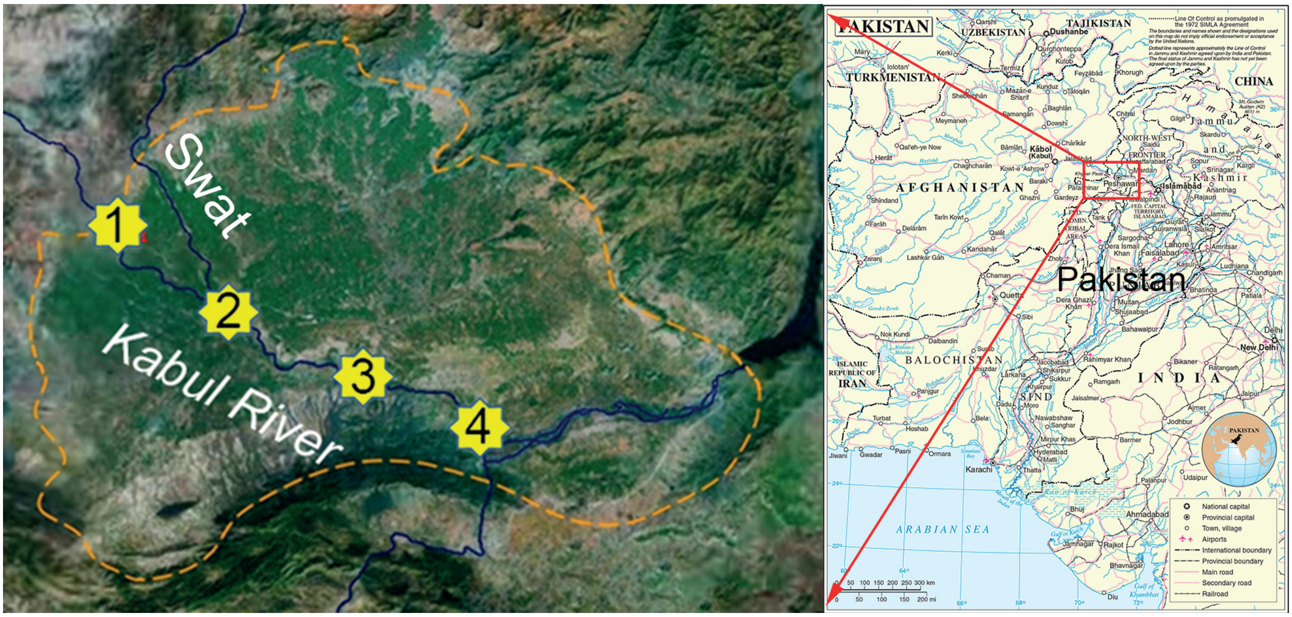

Figure 1

Peshawar Valley with the Kabul River sampling sites on the map of Pakistan (http://www.un.org/Depts/Cartographic/map/profile/pakistan.pdf)

(originating in Mansarwar, Tibet) in the east. The Indus River forms the eastern boundary of the valley and flows from north to south (Government of Pakistan 1998; Salim & Khan 1973).

Climate

The climate in the Peshawar Valley has been described as a modified Mediterranean type of climate. The summers are hot and the winters are cold. The range of temperature varies between 50°C (highest) and -3°C (lowest). Frosts may occur any time between December and February. Four seasons are recognized in the Peshawar Valley: autumn (September-November), winter (December-February), spring (March-May), and summer (June, July, and August) (Government of Pakistan 1998; Salim & Khan 1973).

The Kabul River

The Kabul River is a transboundary river between Afghanistan and Pakistan. It is 700 km long, 563.27 km of which is in Afghanistan and 136.79 km in Pakistan (Gresswell & Huxley 1965). It emerges in northeastern Afghanistan at the base of the Wonay Pass in the Paghman Range (Selsela-e-Kuh-e-Paghman) and ends in the Indus River near Khairabad, Attock District, northwest of Islamabad, Pakistan (Government of Pakistan 1998). The depth-width ratio is highly varied in different seasons and at different sites. Although no accurate data are available for Pakistan, the depth can vary from approximately 1.5 to 3.0 m and the width from 30 to 90 m. The average discharge at the Warsak Dam is 20.5×10^3 m^3 s^{-1}. The differences in the river flow result from seasonal rains, as well as from glacial and snow melting (Yousafzai et al. 2010).

The river splits into three branches in the place where the Peshawar Valley drains into the Kabul River. Two of these branches flow into the Peshawar district, while the main tributary flows into Charsadda. These three tributaries, i.e. Shah Alam, Naguman, and the main Kabul River can be seen while traveling by road from Peshawar to Charsadda. The three tributaries meet at Jangi Tapoo near Prang. The Swat River originates in Kalam, flows through the hills of the Swat Valley and enters the plains of the Peshawar Valley near Abazai, where it divides into two branches: Abazai and Khiali. The two branches meet and flow into the Kabul River near Jalabella, District Charsadda, forming a single mighty Kabul River that receives water from the Bara River and the Jindai River before emptying into the Indus River near Khairabad, Attock District (Government of Pakistan 1998).

Site Selection

To assess the algal diversity and ecology of the Kabul River, four study sites were selected in the Peshawar Valley (Figure 1). The sites included Warsak (01), Sardaryab (02), Nowshera (03), and Khairabad (04) as shown in the map. The quality of water in the Kabul River is affected by miscellaneous industrial and domestic wastewaters, and therefore the water is more polluted down the river (Ullah et al. 2013). In the process of sampling and analysis of samples, we assume that the pollution is higher at the Sardaryab, Nowshera, and Khairabad sites because they are densely populated areas. Furthermore, small mills (Mabel factories) are located at these sites and they directly dump their wastes into the river. The Warsak site is located upstream and can be considered as a reference.

Materials and Methods

Field sampling

Sampling was carried out during the autumn and spring seasons of 2014-2015. A total of 30 samples were collected by scratching all substrates at the sampling sites. Samples were collected within a 100-meter radius at each individual location. At each site, samples were collected from different habitats, including phytoplankton and periphyton (epiphyton, epilithon, and epipsammon). Samples were brought in standard specimen bottles to the Phycology and Tissue Culture Laboratory, Department of Botany, University of Peshawar.

Preservation, Labeling and Storage

Temporary preservation

The isolates were immediately preserved with neutral Lugol's iodine solution, 0.5 ml per 100 ml water sample, and stored for a short time (Edler and Elbrächter 2010). Lugol's iodine was prepared by dissolving 150 g of potassium iodide and 50 g of iodine in 980 ml distilled water, followed by addition of 20 ml of glacial acetic acid.

Permanent preservation

Samples were preserved in 3% FAA (Formalin, acetic acid, and alcohol) and kept for a long time to avoid spoilage (Edler and Elbrächter 2010). FAA was prepared by taking 850 ml (70%) of ethyl alcohol and adding 100 ml of formaldehyde (40%) and 50 ml of glacial acetic acid.

Vouchering and storage

The permanently preserved specimens were vouchered and submitted to the Department of Botany Herbarium, University of Peshawar, for storing.

Laboratory Processing

Microscopic morphology of the non-diatomaceous isolates was determined using the wet-mount staining method (Edler & Elbrächter 2010). This procedure was performed using a sterile micromanipulator to pick up algal filaments from temporarily preserved samples and then placed onto a clean glass slide on which a drop of distilled water was added beforehand. A drop of lactophenol cotton blue stain was added, and preparations were covered with clean cover slips. The slides were subsequently viewed under 10×, 20×, 40×, 60× and 100× Nikon Eclipse E200 microscope. Images of the taxa were taken with a BRESSER digital microscope.

The diatomaceous isolates were cleaned using a peroxide (H_2O_2) technique (Swift 1967). This process involves the oxidation of organic matter in a sample so that only silica walls of diatom cells remain. The empty frustules were then mounted and analyzed for their morphology.

Standard references were followed for taxonomic identification (Bellinger & Sigee 2015; Wehr 2002; Cox 1996; Prescott 1962; Desikachary 1959; Tiffany & Britton 1952; Transeau 1951; Collins 1909).

Water Quality Index

The Water Quality Index (WQI) was used to determine the class and status of the river (Mitchell and Stapp 1992).

$$WQI = \Sigma SIi$$

The 100-point index is divided into several ranges corresponding to the general descriptive terms shown in Table 1.

Saprobity Index S

Indices of saprobity S (Sládeček 1973; 1986) were calculated based on the identified species for each community and quantitative analysis of phytoplankton as:

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

where: S – Saprobity index of algal community; s_i – species-specific saprobity index; a_i – species abundance.

Table 1

The 100-point index ranges according to Mitchell and Stapp (1992)

Class	Range	Quality
1	90-100	Excellent
2	70-90	Good
3	50-70	Medium
4	25-50	Bad
5	0-25	Very Bad

River Pollution Index

The integral index of river pollution (RPI) was calculated for critical chemical variables over all sampling sites for each river, based on the integral method of Sumita (1986) and Watanabe et al. (1986), and developed by S. Barinova (Barinova et al. 2006; Barinova 2011). The integral index of river pollution is based on the pollution estimates (such as saprobity indices) for each of the sampling sites. The integral indices are calculated according to equation 2 (below):

$$RPI_d = \frac{\sum(Di + Dj) \times l}{2L} \quad \text{(Eq. 2)}$$

where: *Di*, *Dj* – saprobity indices for adjacent sites i, j; *l* – distance between two adjacent sites (km); *L* – total river length

RPIs are very conservative for stable ecosystems and can be used as reference variables for the river ecosystem stability assessment (Sumita 1986).

Water Ecosystem Sustainability Index (WESI)

The integral index of aquatic ecosystem sustainability (WESI) was constructed based on the results of our studies (Barinova et al. 2006; Barinova 2011). Calculations of the index are based on the water quality ranks as determined by Sládeček's saprobity indices and nitrate (or phosphates) concentrations.

$$WESI = \frac{Rank\ S}{Rank\ N\text{-}NO_3} \quad \text{(Eq. 3)}$$

where: *Rank S* – the rank of water quality according to the range of Sládeček's saprobity indices calculated for the sampling sites; *Rank* $N\text{-}NO_3$ – the rank of water quality according to the range of nitric-nitrogen concentrations.

At WESI ≥ 1, the photosynthetic level is positively correlated with the level of nitrate concentration. At WESI < 1, photosynthesis is suppressed (presumably due to toxic disturbances).

Determination of physicochemical properties of water

Physicochemical properties of the water from the sampling sites were measured in parallel with algal sampling. Temperature and water pH were measured at each site by using a HANNA HI98190 portable meter. Electrical conductivity and Total Dissolved Solids (TDS) were measured at each site by using a HANNA HI98192 meter. Turbidity was measured by using a HANNA HI98703 meter in the laboratory. Total Suspended Solids (TSS) values in the water samples were measured with a HACH TSS meter in the laboratory. Dissolved oxygen (DO) and Biochemical Oxygen Demand (BOD) were measured using a HANNA HI98193 meter in the laboratory. Alkalinity as $CaCO_3$ was analyzed by titration against standard sulfuric acid. Hardness as $CaCO_3$ was analyzed by complexometric titrations using EDTA (0.01M). Nitrites were analyzed by the spectrophotometric (colorimetric) method. Nitrates were analyzed by the spectrophotometric (colorimetric) method. Ammonia was analyzed by the spectrophotometric method using Nessler's reagent. Sulfates were analyzed by complexometric titrations using EDTA (0.01M). Phosphates (PO_4^{3-}) were analyzed by the spectrophotometric method using Nessler's reagent. Chlorides (Cl^-) were determined by titrating against silver nitrate (0.1N) using potassium chromate as an indicator.

Statistical analysis

Statistical analysis of the correlation between species diversity and the main water condition variables was calculated by distance-weighted least squares and Stepwise regression analysis using the Statistica 12.0 Program.

Statistical significance of variables was assessed using the Pearson correlation method from Wessa (2016).

Canonical Correspondence Analysis (CCA) from CANOCO for Windows 4.5 package (Ter Braak & Šmilauer 2002) was used to determine the relationships between species diversity in algal communities and their environmental variables.

Similarity between algal diversity and chemical variables was assessed with Sørensen indices using the GRAPHS program (Novakovsky 2004).

Results and Discussion

Chemical variables

The dynamic water variables over the sampling sites in the Kabul River are presented in Table 2, which shows that water temperature, pH, conductivity, TDS, TSS, salinity, chlorides, and alkalinity increased down the river. Concentrations of nitrates, nitrites, and ammonia dramatically increased at site 2 immediately after the Swat River input. At the same time, dissolved oxygen, total hardness, and sulfates decreased at site 2, whereas phosphates increased, which led us to conclude that the influence of the Swat tributary on the water quality of the Kabul River is high because its water was slightly enriched with major ions but brought more pollutants to the waters of the Kabul River. A similar conclusion was reached by Yousafzai et al. (2010) on the basis of a detailed chemical investigation conducted in the same part of the river.

Chemical variables in the Songhua River, from climatically similar regions of China (Barinova et al. 2016a), also fluctuated in small ranges and reflected fresh, low-alkaline, low-to-temperate temperature, and low-to-moderately polluted waters saturated with oxygen, while the index of saprobity S ranging from 1.57 to 1.79 defined the water quality as Class III.

Algal communities

Altogether 209 species of algae and cyanobacteria were found at 4 sites of the Kabul River (Figure 2, Tables 3, 4). Table 3 reflects ecological preferences of some algal species from Figure 2. Table 4 presents qualitative (the number of species) and quantitative (abundance scores) data for the four sites. Species richness and algal abundance similarly increased down the river. The greatest abundance in communities was mostly represented by filamentous charophytes (Table 4). The index of saprobity S, calculated based on the species abundance, increased from 1.55 to 1.59 after site 2 at Nowshera and Khairabad sites. The index reflects moderately-polluted water with developed communities. The analysis of the algal community revealed that the Swat River (the main left-hand high-mountain tributary of the Kabul River) was not particularly rich in species, and species richness was strongly correlated with water temperature (Barinova et al. 2013). Nevertheless, the community structure in the lower part of the Swat River (below 1400 m) was similar to the studied part of the Kabul River with the dominance of filamentous algae and diatoms. It is remarkable that Charophyta algae represent about a quarter of the algal flora in both rivers, and Spirogyra as well as other filamentous algae were the most

Table 2

Water variables over the study sites in the Kabul River

Parameter	Unit	Warsak	Sardaryab	Nowshera	Khairabad	RPI
Temperature	°C	18	19.6	20.8	22	20.2
pH		7.4	7.9	7.3	7.8	7.6
Conductivity	mSm cm^{-1}	263	268	377	471	340.6
Turbidity	NTU	3	4	9	7	6.1
T.D.S.	mg l^{-1}	110	135	212	190	168.1
T.S.S.		220	230	280	264	252.1
Salinity		0.2	0.6	1.3	2.0	1.0
Alkalinity	mg Eq l^{-1}	75	101	157	166	128.2
Hardness		167	144	155	180	157.4
D.O.	mg l^{-1}	6.5	5.59	6.78	6.5	6.3
B.O.D.	mg O_2 l^{-1}	0.8	1.4	5.4	6	3.5
NO_2^-	mg l^{-1}	0.00	0.03	0.03	0.38	0.1
NO_3^-		2.81	6.48	1.64	4.87	4.0
NH_3-N		0.00	0.13	0.54	1.92	0.6
S_2^-		0.16	0.33	0.36	0.80	0.4
SO_4^{2-}		26	23.25	25.6	59.5	30.6
PO_4^{3-}		0.14	0.26	0.15	1.14	0.4
Cl^-		7	8	12	18	11.0
Saprobity Index S		1.55	1.55	1.59	1.59	1.571
WESI Index		0.50	0.44	0.67	0.44	0.532
WQI Index		54.67	50.16	39.39	46.36	46.33

Table 3

Ecological preferences of the most distributed algal species-indicators in the Kabul River

Taxa	Hab	T	Oxy	Sal	Wat	Sap	Index S	Tro	Nutr
Cyanobacteria									
Chroococcus turgidus (Kützing) Nägeli	P-B, S	-	aer	hl	-	x-b	0.8	-	-
Bacillariophyta									
Cymbella affinis Kützing	B	temp	st-str	i	sx	o	1.1	ot	ats
Diatoma vulgaris Bory	P-B	-	st-str	i	sx	b	2.2	me	ate
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	B	-	st-str	i	es	o-a	1.9	me	ate
Chlorophyta									
Cladophora glomerata (Linnaeus) Kützing var. *glomerata*	P-B	-	st-str	i	-	o-a	1.9	-	-
Hydrodictyon reticulatum (Linnaeus) Bory	P-B	-	st	-	-	o-a	1.8	-	-

Table legend: Substrate preferences (Hab): P-B – planktonic-benthic, B – benthic, S – soil. Temperature preferences (T): temp – temperate. Oxygenation and streaming (Oxy): st – standing water, st-str – low streaming water, aer – aerophiles. Halobity degree (Sal): i – oligohalobes-indifferent, hl – halophiles. Saprobity groups according to Watanabe (Wat): es – eurysaprobes, sx – saproxenes. Species-specific Index of Saprobity (S). Self-purification zone preferences (Sap): x-b – xeno-beta-mesosaprobes; o – oligosaprobes; o-a – oligo-alpha-mesosaprobes. Trophic status (Tro): ot – oligotraphentic; me – meso-eutraphentic. Nitrogen uptake metabolism (Nutr): ats – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate – nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen

Table 4

Biodiversity data over the study sites in the Kabul River

Division	Warsak	Sardaryab	Nowshera	Khairabad
	Abundance, total scores			
Cyanobacteria	65	80	79	91
Bacillariophyta	46	58	64	63
Xanthophyta	25	25	35	35
Euglenophyta	4	7	5	5
Chlorophyta	92	135	142	149
Charophyta	203	236	231	248
Sum of Scores	435	541	556	591
	Number of Species			
Cyanobacteria	22	28	29	32
Bacillariophyta	26	34	36	36
Xanthophyta	5	5	7	7
Euglenophyta	3	6	5	5
Chlorophyta	24	36	37	39
Charophyta	47	58	56	60
No of Species	127	167	170	179

diverse groups. These characteristics can represent some regional features in the river basin with high agricultural activity. The common occurrence of green algae in the riverine community is correlated with the latitude of the river basin and therefore with sunlight intensity, as in the rivers in Ukraine (Bilous et al. 2012), China (Barinova et al. 2016a), and Israel (Barinova 2011; Barinova & Krassilov 2012).

Our bioindication approach to the Kabul River water quality assessment (Barinova et al. 2016b) shows differences between site 1 above the Swat River input and lower sites 2-4. Species indicators of salinity and organic pollution increased significantly after the Swat tributary.

Water Quality Index of the Kabul River

Water quality of the Kabul River in the Peshawar Valley was measured using the Water Quality Index (WQI). Water quality was medium at Warsak (54.67 points) and Sardaryab (50.16 points) sites (Table 2). The water quality decreased at Nowshera (46.36 points) and Khairabad (39.39 points) sites. WQI changes show also that pollutants carried by the Swat tributary, flowing into the Kabul River (before the Sardaryab site), contaminate the Kabul River, and thus the water quality continues to decrease down the river.

We have no other examples of the WQI calculations for regional rivers in Pakistan, but similar results were

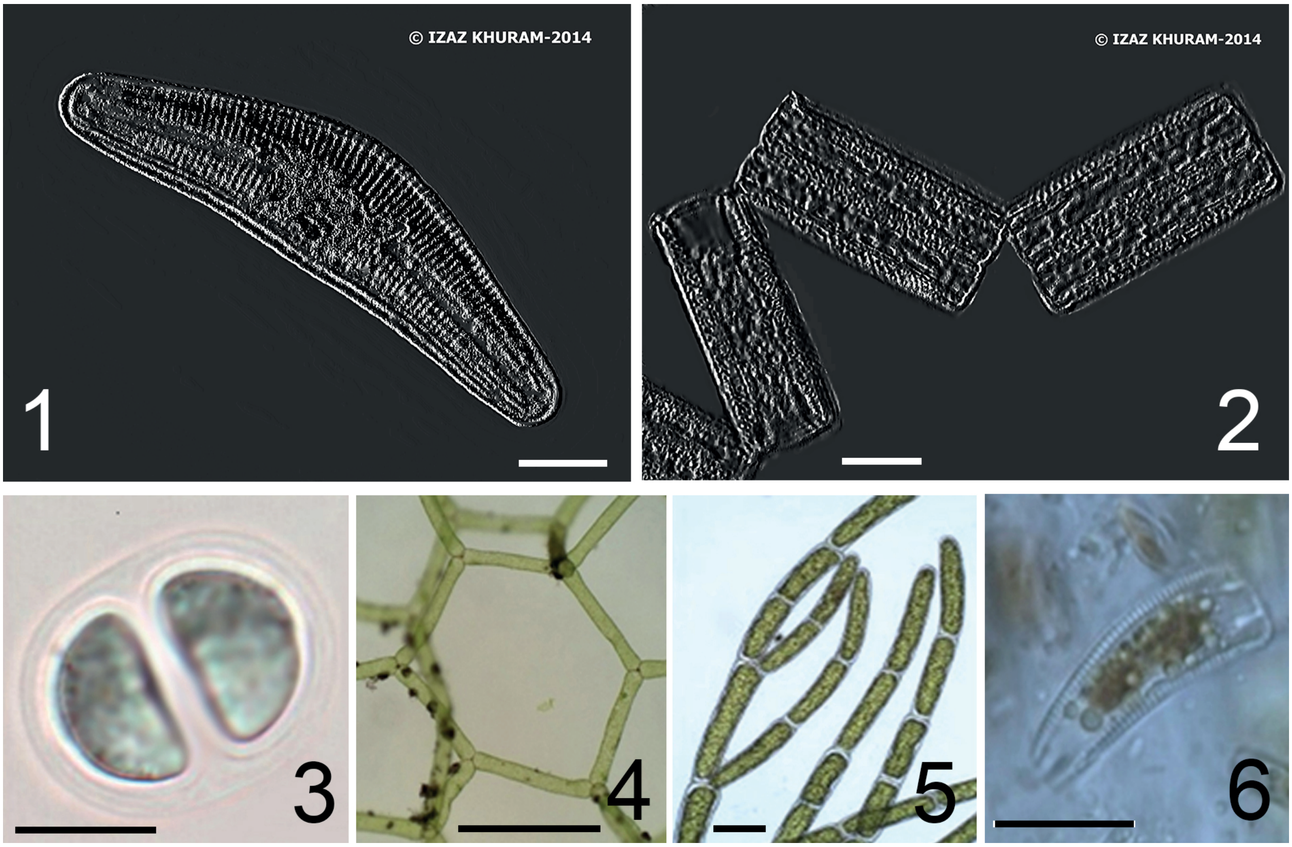

Figure 2

The most distributed algal species-indicators in the Kabul River communities with their ecological preferences: *Cymbella affinis* Kützing (benthic, temperature-temperate, low-streaming waters, pH-indifferent, saproxene, oligosaprobe, oligotraphentic, autotrophic) (1); *Diatoma vulgaris* Bory (planktonic-benthic, low-streaming waters, chloride-indifferent, saproxene, beta-mesosaprobic, meso-eutraphentic, autotrophic) (2); *Chroococcus turgidus* (Kützing) Nägeli (planktonic-benthic, aerophile, halophile, xeno-beta-mesosaprobic) (3); *Hydrodictyon reticulatum* (Linnaeus) Bory (planktonic-benthic, standing water, oligo-alpha-mesosaprobic) (4); *Cladophora glomerata* (Linnaeus) Kützing (planktonic-benthic, low-streaming waters, chloride-indifferent, oligo-alpha-mesosaprobic) (5); *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot (benthic, low-streaming waters, chloride-indifferent, eurysaprobe, oligo-alpha-mesosaprobic, meso-eutraphentic, autotroph) (6). **Scale bar:** fig. 2(1-4): 6-10 µm; fig. 2(5): 50 µm

published for the Yarqon River in Israel (Barinova et al. 2010b).

Statistical analysis of relationships between biological and chemical variables

Stepwise regression analysis

We used statistical methods to express the data on water chemistry (Table 2) as independent variables and community composition as dependent variables (Table 4) for the Kabul River sampling sites. Stepwise regression analysis results (Table 5) show (in step 1) that negative factors are not presented in the final step of calculation, but some effects of water conductivity, sulfate concentration, as well as ammonia and dissolved oxygen are negative factors in steps 2 and 3. Species richness as a whole, as well as cyanobacteria, green algae, and filamentous xanthophytes were dependent on water temperature. Species richness of euglenoids was determined by water pH. There were no other factors in the Kabul River that influenced the biological diversity.

The regression analysis conducted for the Swat River showed altitude and related water temperature as the major regulating factors for the riverine algal diversity (Barinova et al. 2013). The water temperature is also a regulating factor in the Kabul River. Therefore, the water temperature can be defined as a regional factor that determines the riverine algal diversity.

Table 5

Stepwise regression analysis results for the Kabul River sampling sites

Dependent variables	Step 1	Step 2	Step 3
Number of Species	T 0.85*	T, Cond 0.99**	T, Cond, Hard 0.99***
Cyanobacteria	T 0.94*	T, DO 0.99*	T, Sal, DO 0.99
Chlorophyta	T 0.80*	T, Cond 0.99*	T, **Cond**, Hard 0.99
Xanthophyta	T 0.89*	T, **SO_4** 0.88**	T, **SO_4**, **NH_4** 0.99
Euglenophyta	pH 0.39*	pH, Turb 0.71*	pH, **DO**, Turb 0.99

Negatively correlated variables are marked in bold. Statistical significance, p-value: *<0.05; **<0.01; ***<0.001

Pearson correlation

In Tables 2 and 4, water variables as a whole in the Kabul River fluctuated in small ranges. Nevertheless, we can see differences between the sites. The Pearson correlation calculated for environmental (Table 2) and biological variables (Table 4) shows that water temperature plays a major role in species richness distribution over the studied part of the river with a positive correlation of 0.93*. At the same time, it can be noted that only cyanobacteria had a Pearson coefficient of 0.97*, but other algal divisions were indifferent to temperature changes. Cyanobacteria were stimulated also by water salinity (0.91*), and this variable positively correlated also with species richness of green algae (0.89*). An increase in water TDS (0.88*) stimulated the number of diatom algae.

Therefore, our Pearson calculation confirms that water temperature plays a very important role in the riverine community in the Kabul River basin as a whole.

Surface plots

Surface plots were constructed from environmental and biological data (Tables 2 and 4). Figure 3 shows that abundance in algal communities, which was closely related to species richness, increased with temperature but was suppressed by water conductivity. On the other hand, the index of saprobity S, which reflects organic pollution, significantly increased with water conductivity but was indifferent to temperature. The BOD parameter that reflects organic pollution in the water also increased with temperature and water conductivity. Salinity is the most important part of the total ion content in the water that affected the algal community. The surface plot shows that salinity was the main factor determining the water ion content, which increased together with BOD but was indifferent to water temperature.

The role of salinity in the riverine and, especially, lacustrine communities was studied during the last few years (Barinova et al. 2004; 2009; 2010a; Klymiuk & Barinova 2016) and the results confirm our conclusion about the importance of salinity levels for species diversity and abundance in warm climatic regions.

Species-environment relationships

It was very important to determine the relationships between algal species in communities and the environmental factors at the studied sites. Two programs were used for calculations: CANOCO and GRAPHS.

A CANOCO plot (Figure 4a) was constructed on the basis of data on divisions and environmental variables with the number of species as species richness at each site. It shows that all environmental variables were grouped into two sets: 1) water pH and nitrate concentrations and 2) all other chemical variables. This analysis means that the water pollution is correlated with the nutrient input into the river water and, as a result, the biological processes increase the water pH. Algal species from the Charophyta division (rose circle) were impacted the most, whereas the most resistant Euglenophyta (green circle) species survived under the most favorable conditions.

The CANOCO biplot of environmental variables and phytoplankton abundance at the sampling sites (Figure 4b) shows that site 1 (Warsak, red circle) represents its own cluster that was different from cluster 2 and the others. The community at site 4 (Khairabad) showed adaptation to the impact. The most impacted communities were found at sites 2 (Sardaryab, blue circle) and 3 (Nowshera). This means that the pollution impact on the algal community started from site 2 where the river water is enriched with nutrients, and continued to the sites located at lower altitudes with adaptation observed at the site in Khairabad.

Therefore, our calculation of CCA shows the water nitrate concentration as one of the important variables, which together with temperature affects riverine diversity in the Kabul River. It can be seen that major nutrients come from agriculture in the Peshawar Valley, but not only with water of the Swat tributary.

A comparison of environmental variables and biological data on taxonomic division or species from the studied sites was performed using the GRAPHS Program.

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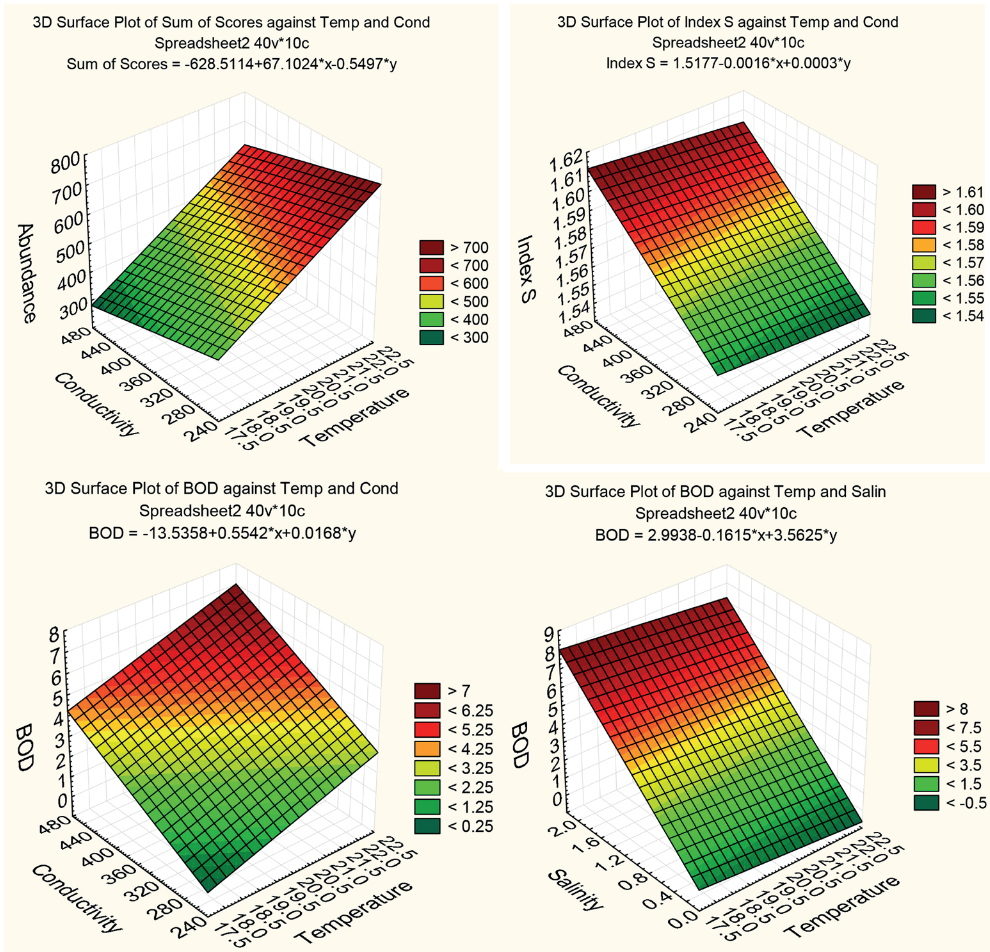

Figure 3

Surface plots of relationships between biological and chemical variables at the Kabul River sites

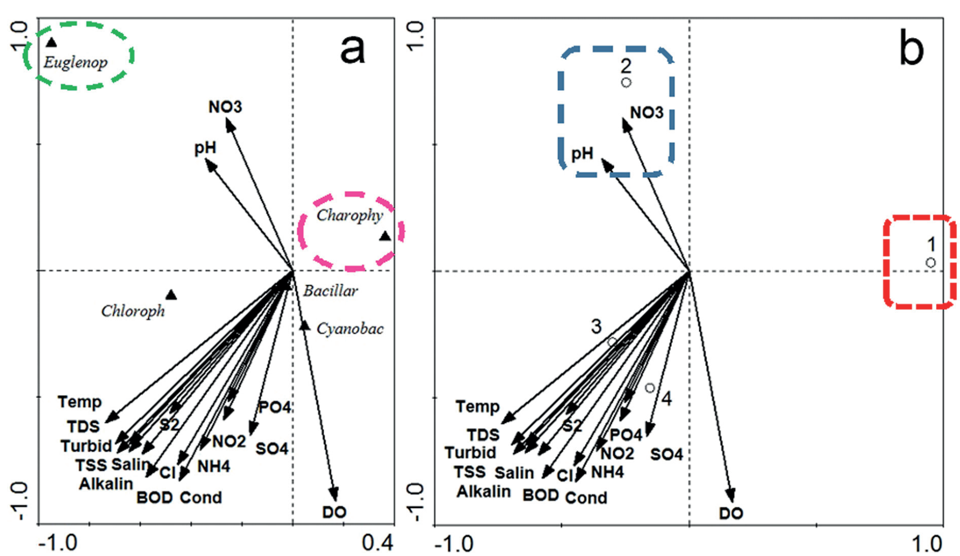

Figure 4

CCA Biplot of relationships between divisional diversity and environmental variables in the Kabul River (a); CCA Triplot of relationships between the sites and environmental variables in the Kabul River (b)

The dendrogram constructed on the basis of environmental variables (Figure 5a) reflects the similarity between environments of each study site. Two different clusters show that the studied part of the river is divided into upper (Warsak and Sardaryab) and lower (Nowshera and Khaidarabad) reaches.

The dendrogram of biological data similarity (Figure 5b) shows two different clusters: one of which represents the diversity of the upper site Warsak and the other one represents communities from the three other sites.

In contrast, the calculation of similarity between total biological and chemical variables at the studied sites shows two clusters, same as in the dendrogram of environmental variables only (Figure 5c). This means that environmental data composition, and not the algal community, was defined by water-quality changes in the studied part of the river.

The dendrite of the divisional structure similarity at the studied sites (Figure 6a) shows that most similar communities were at the lower sites (89%-99%), whereas the upper site of the Warsak community represents several different divisional structures.

The dendrite of the community structure similarity at taxonomic division levels (Figure 6b) at the studied sites shows high similarity, up to 100%, but the upper site of the Warsak community represents the source of the community structure and mostly included species from communities of other sites.

These results confirm that the Warsak site represents part of the Kabul River that is chemically and biologically different from the reaches below, which are affected by agriculture.

River Pollution Indices (RPI)

The River Pollution Indices (RPI) (Barinova 2011) were calculated for chemical and biological variables in the studied parts of the Kabul River. Table 2 shows that water in the river was low-alkaline, low-saline, fresh, but moderately organically polluted.

Our RPI calculations for a similar set of variables in other rivers such as the Songhua, the Lower Jordan, or the Southern Bug (Barinova 2011; Barinova & Krassilov 2012; Bilous et al. 2012; Barinova et al. 2010a; 2016a) show that the integral method for water quality assessment can yield relevant and stable results, which can be employed in the monitoring of regional water quality.

Water Ecosystem Sustainability Index (WESI)

The Water Ecosystem Sustainability Index (WESI) was calculated on the basis of the classification rank

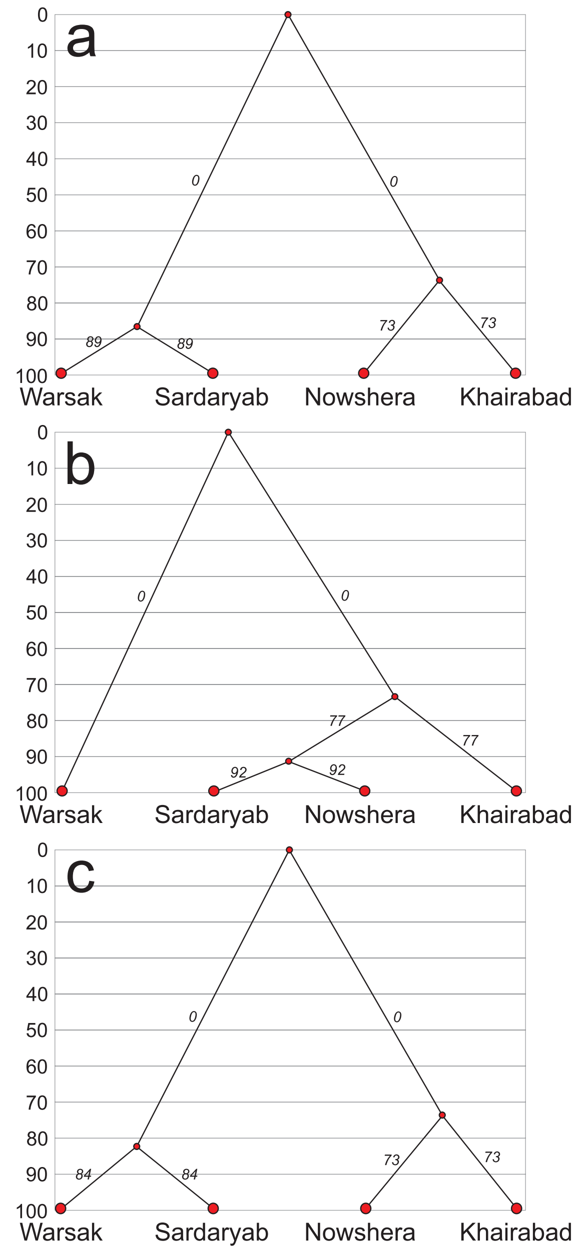

Figure 5

Dendrogram of all chemical variables at the Kabul River study sites (a); Dendrogram of all biological variables at the Kabul River study sites (b); Dendrogram of all variables at the Kabul River study sites (c)

of nitrates (Table 2) and the index of saprobity S (Table 2). As a result, WESI for each site as well as RPI-WESI were below 1.0. This calculation reflects the algal photosynthesis suppression and shows the Kabul River contamination with toxicants. Usually the concentration of phosphates in the rivers is unlimited, so was the case with the Kabul River. Therefore, we decide to

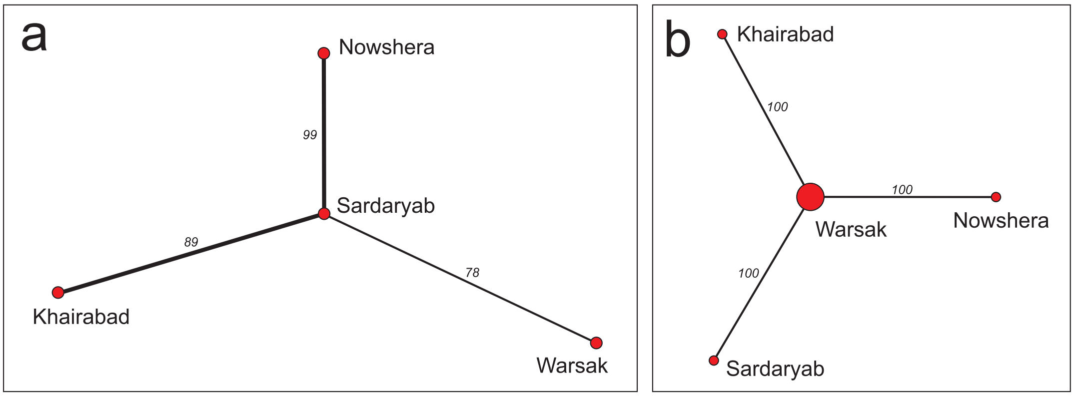

Figure 6

Dendrite of algal divisions' similarity at the Kabul River study sites (a); Dendrite of algal communities' similarity at the Kabul River study sites (b)

calculate the WESI for phosphates in the Kabul River. The results (Table 2) confirm our conclusion on nitrate concentration. The WESI (phosphates) ranged from 0.44 to 0.67 with RPI-WESI of 0.597, which is also below 1.0.

The impact of pollution on the small tributary in the Peshawar Valley was reported in 2009 (Ullah et al. 2013) near the Peshawar city and shows that the pollution level in the river is rising from upstream (at the city's entrance) to downstream (at city's exit) due to the discharge of domestic wastewater effluents, agricultural activities, and solid-waste dumping directly into the river. This site is outside our research range but reflects the situation in the region.

Based on chemical analysis, Yousafzai et al. (2010) concluded that salinity as well as nutrients reach the Kabul River from industrial and domestic sources. Many authors considered the Kabul River as a large subtropical river. According to this view, we compared the studied river with rivers from similar climatic regions. Therefore, the same domestic and agricultural impact on algal communities was found in the lower Jordan River in Israel as well as in the Songhua River in China (Barinova et al. 2010a; 2016a). The WESI values were below 1.0 in both rivers at the sites affected by salinity and agriculture non-point runoff, which reflects a low-toxicity impact of algal photosynthetic activity. On the other hand, indices of saprobity S at the sites of the studied part of the Kabul River show high self-purification capacity of algal communities.

Conclusion

As a result of our international team activity, we identified 209 species of algae and cyanobacteria at 4 sites of the Kabul River that flows across the Peshawar Valley in Pakistan. Israeli researchers used modern integral and statistical methods, and our Pakistan team collected all environmental and biological data. Our team concluded that algal communities studied for the first time in the lower part of the Kabul River increase in species richness down the river. This process was stimulated not only by the increasing water temperature but also by nutrient runoff from agricultural and domestic sources in the Peshawar Valley.

The Swat River is a left-hand high-mountain tributary, which brings fresh but organically polluted waters. The species richness of the algal community increased below the tributary's mouth as it is observed in rivers of climatically similar regions in Eurasia (Barinova et al. 2010a; 2016a). We discovered the dominance of green algae and diatoms in the studied algal community. The Charophyta species, such as filamentous *Spirogyra* and other filamentous algae, were diverse and can be representative of several other regional features in river basins with high agricultural activity.

Water salinity was determined as a major impact variable, which increased together with BOD. The nitrate concentration was also an important variable, which together with temperature affected riverine diversity in the Kabul River. Our analysis reveals that major nutrients come from agriculture in the Peshawar Valley, not only from the Swat tributary water. Statistical analysis shows that the accumulated data on the environment and algal community define the water quality changes in the studied part of the river. The upper site studied, Warsak, can be defined as a reference site for the future monitoring of the Kabul River. It is chemically and biologically different from the lower reaches, which are affected by agricultural and domestic waste in this area. We can conclude that the integral method used in the water quality assessment, which includes both chemical and biological variables, can yield relevant and stable results on the self-purification capacity assessment and hence it can be employed in monitoring of the regional water quality.

Acknowledgments

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