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IMPACT OF OIL SHALE MINE WATER ON MICROBIOLOGICAL AND CHEMICAL COMPOSITION OF NORTH-EASTERN ESTONIAN RIVERS

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Numbers of different bacterial groups and physico-chemical water quality parameters were studied during four year period in two rivers receiving mine water from underground oil shale mine. Input of mine water causes increase of alkalinity, sulfate and calcium ion concentration in river water. Bacterial community structure of studied rivers was altered by mine water and had clear seasonal pattern. The results indicate that input of mine water increases number of viable heterotrophs and lipolytic bacteria in river water, especially during underground oil shale burning.

Introduction

The impact of oil shale mining on water resources may range from minimal through to severe. The effect depends on the location of the mine, the hydrology and climate of an area, and the physical and chemical properties of the coal [1].

The population of microorganisms reflects the condition of the water body and it also controls mineralization of organic matter and recovery of metabolic intermediates. As in other ecosystems, populations of river bacteria are controlled by a variety of abiotic and biotic forces. Abiotic variables, such as availability of nutrients and pollution, may determine bacterial abundance in river water [2]. Evidence is accumulating that bacteria are the pivotal group in the biodegradation and bioremediation of any ecosystem [3]. Thus, the degradation of contaminants in river water is essentially catalyzed by microbes and needs an active microbial community.

This paper considers the impact of oil shale underground mine water on chemical and microbiological characteristics of two rivers in north-eastern Estonia.

Materials and Methods

Sampling Sites

Water samples for chemical and microbiological analysis were collected from two rivers receiving oil shale mine water in north-eastern Estonia. Mine water is pumped into the River Jõuga and carried via the River Rannapungerja to Lake Peipsi. The River Rannapungerja receives most of the water from agricultural catchment area and inflow from the River Jõuga contributes ca 25 % of the total load. Distance between two sampling locations was 20 km. Twenty and twenty five samples were taken from the surface layer (about 30 cm from the surface) of the rivers Rannapungerja and Jõuga, respectively since April 1988 up to July 1991.

Bacterial Enumeration

The total number of bacteria was estimated by direct counts using brightfield microscopy. The spread-plate method was used to estimate count of viable heterotrophs on yeast extract peptone agar. Plates were incubated at 20 °C , and colony-forming units were counted on the 7th day. Numbers of denitrifying, lipolytic, cellulolytic and coliform bacteria were determined on selective media by using most probable number method [4].

Physicochemical Parameters

Temperature and oxygen concentrations were read at the site with a oxygen meter. pH was measured at room temperature with a pH meter. The following chemical parameters were assayed: total alkalinity, dissolved calcium and magnesium, dissolved chloride, dissolved sulfate, dissolved ammonium and nitrate, biochemical and chemical oxygen demand, inorganic carbon [5].

Numerical Analysis

Due to the irregular sampling intervals and small number of observations it was not possible apply time series analysis in data analysis. Multivariate statistics, primarily factor analysis and principal component analysis are a valuable tools in the study of population shifts in freshwater river bacteria [6]. Multivariate statistics allow to detect patterns in and to reduce the dimensionality of complex multivariate data. We applied principal component analysis (PCA) for extracting essential variance from a multivariate data sets. In the case of microbiological data the components were scored and dynamics of component scores was studied. Paired *t*-test was used to compare variables from two rivers.

Results

The mean water chemical parameters (Table 1) reflect the different environmental influences affecting two studied rivers. The River Jõuga maintains much higher concentrations of sulfate and calcium, compared to the River Rannapungerja throughout the studied period. At the same time the organic matter content is higher in the River Rannapungerja as reflected by chemical and biochemical oxygen demand values. There was no statistically significant differences in oxygen concentration and pH in studied two rivers.

Table 1. **Average Values and Standard Deviations of Water Chemical and Physical Parameters from Two Studied Rivers**

Variable	Site	
	River Jõuga	River Rannapungerja
Oxygen, mg l^{-1}	11.3 ± 0.7	10.8 ± 2.0
pH	7.8 ± 0.6	7.6 ± 0.5
BOD_5, mgO_2 l^{-1}	1.48 ± 0.67	2.36 ± 0.97
BOD_{20}, mgO_2 l^{-1}	3.05 ± 1.55	4.87 ± 1.66
COD_{Cr}, mgO_2 l^{-1}	26.1 ± 13.5	42.4 ± 19.4
COD_{Mn}, mgO_2 l^{-1}	8.6 ± 3.4	22.1 ± 10.1
NH_4^+, mg l^{-1}	0.36 ± 0.31	0.58 ± 0.27
NO_3^-, mg l^{-1}	1.23 ± 1.27	1.16 ± 1.08
Cl^-, mg l^{-1}	28.4 ± 8.9	15.5 ± 10.7
SO_4^{2-}, mg l^{-1}	207 ± 108	63 ± 44
HCO_3^-, mg l^{-1}	6.0 ± 0.8	3.7 ± 1.4
Ca^{2+}, mg l^{-1}	6.1 ± 1.9	3.6 ± 1.3
Fe^{3+}, mg l^{-1}	0.33 ± 0.09	0.71 ± 0.33
Load, m^3 s^{-1}	1.80 ± 0.68	6.00 ± 6.67

Data on microbiological parameters studied by us are given in Table 2. Although the mean of denitrifying and coliform bacteria were nearly twice higher in the River Jõuga, this difference was statistically not significant. In both rivers maximum numbers of heterotrophs (30×10^3-

-63 × 10^3 cells ml^{-1}) and lipolytic bacteria (95 × 10^3-250 × 10^3 cells ml^{-1}) were recorded just after the beginning of underground oil shale burning. The seasonal variation in the total bacterial number, number of viable heterotrophs, denitrifying and lipolytic bacteria was higher in the case of the River Rannapungerja as indicated by higher values of the coefficient of variation. From the bacterial groups studied only dynamics of cellulolytic bacteria had similar pattern in both rivers (Spearman $r = 0.65$, $p < 0.01$).

Table 2. **Average Values and Standard Deviations of Water Microbiological Parameters from Two Studied Rivers**

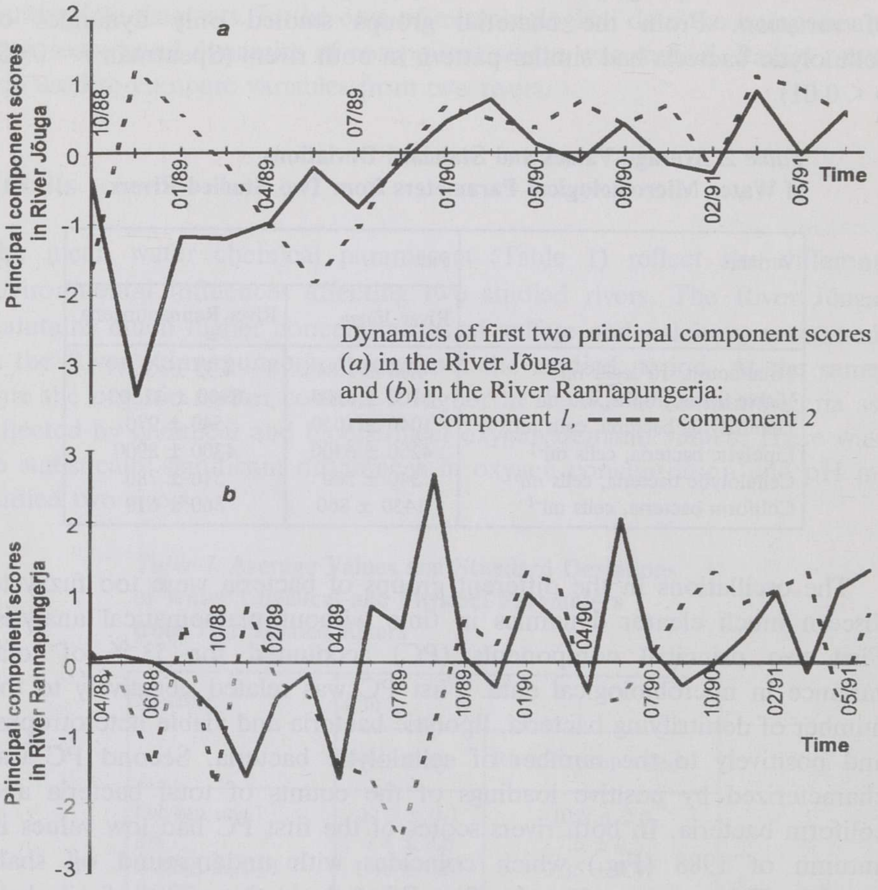
Variable	Site	
	River Jõuga	River Rannapungerja
Total count, 10^6 cells ml^{-1}	4.06 ± 1.84	4.52 ± 3.17
Viable heterotrophs, cfu ml^{-1}	8100 ± 6900	8900 ± 11500
Denitrifying bacteria, cells ml^{-1}	1040 ± 1010	540 ± 970
Lipolytic bacteria, cells ml^{-1}	4200 ± 6300	4300 ± 8900
Cellulolytic bacteria, cells ml^{-1}	340 ± 580	510 ± 780
Coliform bacteria, cells ml^{-1}	1430 ± 860	860 ± 610

The oscillations in the different groups of bacteria were too fuzzy to discern much clearer dynamics in time without mathematical analysis. First two principal components (PC) accounted for 53 % of total variance in microbiological data. First PC was related negatively to the number of denitrifying bacteria, lipolytic bacteria and viable heterotrophs, and positively to the number of cellulolytic bacteria. Second PC was characterized by positive loadings of the counts of total bacteria and coliform bacteria. In both rivers scores of the first PC had low values in autumn of 1988 (Fig.) which coincides with underground oil shale burning. This suggests that the first PC reflects the effect of oil shale mine water on the microbial community of the river. The second PC can be identified as domestic wastewater input.

Dynamics of the first PC scores exhibits clear seasonal dynamics. The score values are high during flood period in early spring and decrease towards summer months. During the summer and winter months when water input from catchment area is low the first PC scores are also low.

Discussion

The two rivers studied contrast greatly in their size and water input sources. Water from mine constitutes ca 75 % of the water load in the case of the River Jõuga. This high amount of mine water increases the hardness and gives rise to the consistently high sulfate and calcium concentration. The River Rannapungerja gets most of its water from surrounding agricultural lands and that accounts for the higher organic matter content in river water.



Dynamics of first two principal component scores (*a*) in the River Jõuga and (*b*) in the River Rannapungerja: —— component *1*, ---- component *2*

The microbial population of the River Jõuga showed less seasonal change than those of the River Rannapungerja. This could be the result of dampening effect of mine water on seasonal fluctuation of bacterial community of the River Jõuga. It has been shown that seasonality is one of the main factors influencing bacterial community structure in rivers [7]. Both rivers exhibit very high values of coliform bacteria due to input of domestic sewage into mine water before outlet to the River Jõuga.

To expand the causal interpretation of bacterial dynamics, the dynamics of microbial parameters were analyzed by using principal component analysis. The dynamics of major component scores extracted showed that in both rivers microbial population responded to the input of mine water. Studies in two other north-estonian rivers have shown that abundance of lipolytic and denitrifying bacteria correlates positively with the number of phenol-degrading bacteria [4]. The first PC was related to the number of lipolytic and denitrifying bacteria and this component can be considered as the predominant population of river microbial community involved in biodegradation of mine water constituents.

The effect of the mine water on the microbial community of the river is more pronounced in the River Jõuga as indicated by consistently low values of the first PC scores throughout the studied period and especially during summer months. The greatest upset of normal microbial community structure occurred during underground oil shale burning. As a result of oil shale burning process the concentration of phenolic compounds increased in the mine water (up to 0.40 mg l^{-1}), which seems to be the main cause of the changes in bacterial numbers. Increase of concentration of aromatic compounds in the river water stimulated the growth of viable heterotrophs and lipolytic bacteria. The microbial populations in studied rivers can be considered as preadapted to mine water constituents which lead to very rapid increase in biodegraders abundance in response to substrate input [8, 9].

Conclusions

Input of mine water into the river influences both water chemical and microbiological parameters. The analysis showed increased mineralization and sulfate concentration of the river water due to the addition of mine water. The bacterial population varied in the studied rivers according to the mine water input and season. In order to make direct connection between the mine water input and river water chemical and microbiological characteristics, the more advanced methods for studying the chemical composition of mine water and river microbial community should be used.

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