Methods of Environmental Bioindication of Rivers Prone to Technogenic Salinization

M.A. Baklanov[1], P.B. Mikheev[1,2], O.I. Mikheeva[3], T.A. Sheina[1], E.A. Khayrulina[1]

[1]*Federal State Autonomous Educational Institution of Higher Education "Perm State National Research University", Perm;*

[2]*Khabarovsk Branch of the Federal State Budget Scientific Institution "Russian Federal Research Institute of Fisheries and Oceanography" ("KhabarovskNIRO"), Khabarovsk;*

[3]*Perm Branch of the Federal State Budget Scientific Institution "Russian Federal Research Institute of Fisheries and Oceanography" ("PermNIRO"), Perm*

Abstract

Indication of technogenic salinization in freshwater water bodies is an important tool for the detection of environmental degradation. Studies of the chemical composition of rivers of the Kama River basin prone to salinization were supplemented by an analysis of cyanobacterial-algal cenoses and communities of zooplankton macrozoobenthos and fish. In the most saline sections of the rivers, halophilic and euryhaline species of diatoms, zooplankton and zoobenthos dominate; fish are not found. Moreover, we have tested the parasitological and haematological analysis of fish for the possibility of using it in bioindication of lower and intermediate levels of salinization. The results show the possibility of using new markers of salinity found by means of parasitological and haematological analyses. The development and application of new methods of bioindication of technogenic salinization of rivers, along with the traditional chemical and biological techniques, are important for the assessment of the impact of anthropogenic stress factors on the biota.

Keywords: Bioindication, Technogenic Salinization, Fish Parasites, Fish Blood.

Introduction

Salinization of boreal freshwater water bodies is a serious problem due to the significant transformation of ecosystems in places of formation of contrasting technogenic geochemical anomalies with an increased content of water-soluble salts, as well as at a considerable distance from salinization sources. In various parts of the world, river ecosystems prone to anthropogenic salinization are characterized by degradation of biodiversity at all trophic levels, which leads to biotic imbalances and negative socio-economic consequences (Cañedo-Argüelles et al., 2013). Compared with other pollutants, salt is one of the least studied technogenic stress factors affecting boreal freshwater ecosystems (Vörösmarty et al., 2010; Bernhardt et al., 2017). At the same time, methods for environmental assessment of rivers using biological markers are an important tool in the indication of anthropogenic salinization.

There are several systems for bioindication of salinization in freshwater ecosystems based on the assessment of structure of diatoms, zooplankton, and macrozoobenthos communities. Under technogenic salinization of rivers, the species composition of biotic components changes from freshwater type to salt-tolerant type with a decrease in the overall level of biodiversity.

Findings

Russia is one of the world leaders in the production of potash and magnesium salts (Potash facts 2021). Extraction takes place within the Verkhnekamskoe Deposit of Potassium and Magnesium Salts (VDPMS, Perm Krai), the side effect of which is technogenic salinization of the environment. VDPMS contains about 30% of the world reserves of potash salts. Mining began in the 1930s, and during the period of the deposit's operation, about 270 million tons of halite waste and 30 million m^3 of clay-salt sludge have be stored on the surface (Khayrulina et al., 2018). In the areas affected by salt tailings piles, mineralization of natural water bodies reaches 50 g/L, the content of chlorides increases to 9.0 g/L, sodium - up to 3.0 g/L, potassium - up to 1 g/L; the content of calcium, magnesium, sulphates, as well as trace elements such as Mn, Pb, Sr, Rb, and Co increases (Liu, Lekhov, 2012). On the territory of the VDPMS, many rivers are prone to technogenic salinization, the indication of which was carried out on the basis of the chemical composition analysis of the rivers.

Studies of the chemical composition of the rivers of the Kama River basin prone to salinization were supplemented by the analysis of algal flora, zooplankton, macrozoobenthos, and fish. The changes in algal flora, zooplankton, macrozoobenthos and ichthyofauna were revealed. Thus, within river sections with the highest salt load, the green alga *Enteromorpha intestinalis* appears, indicating chloride pollution, along with the diatom *Actinocyclus normanii*, a representative species of the algal flora of the Caspian Sea (Martynenko et al., 2017). We observed significant changes in zooplankton: during salinization, the disappearance of cladocerans and a decrease in the species diversity of rotifers and copepods were noted. The basis of planktonic zoocenoses of rivers prone to salinization is made up of halophilic species, in particular *Brachionus plicatilis*, typical of shallow brackish water bodies (Krainev, 2014). An indicator of negative reaction of macrozoobenthos to salinity was also found: in areas with salt load, its biodiversity decreased by 1.5-3.0 times (Baklanov et al., 2019). Bentocenoses in saline zones significantly differed in Shannon's index, Balushkina chironomid index, as well as in the position of halophilic and euryhaline species in the structure of dominance of benthic invertebrate communities. Ichthyological studies showed that water salinization radically affects fish population structure. There are no fish in the area of intense salinization. Short-term visits of eurybiontic fish species from the Kama Reservoir were recorded in different years in the lower reaches of rivers (Site 3 at Table 1), where they are attracted by the presence of food items (Baklanov et al., 2019).

Along with traditional methods of bioindication based on the analysis of species composition of algae and invertebrates, such methods of bioindication as parasitological and haematological analyses of fish were tested for the first time. Quantitative and qualitative characteristics of blood elements

Table 1 *Average values of various parameters of algal cenoses, zooplankton and zoobenthos communities, as well as ichthyofauna of three sections of the Volim River drainage basin (Perm Krai, Russia), differing in the degree of anthropogenic salinization.*

Ecosystem's element	Parameter	Site 1	Site 2	Site 3
Potamophytoplankton (Martynenko et al., 2017)	N, K cells / L	719	452	684
	B, mg/L	1.592	0.555	3.310
	Shannon index	4.15	2.59	3.47
Zooplankton (Krainev, 2014)	N, ind. / m^3	9380	200	2020
	B, mg / m^3	29.83	0.23	0.69
	Species number	32	4	12
Macrozoobenthos (Baklanov et al., 2019)	N, ind. / m^2	1856	30663	12541
	B, mg / m^2	7.73	44.15	15.34
	Shannon index	1.21	0.31	0.53
Fish (Baklanov et al., 2019)	Species number	1	No fish	7
Total dissolved solids, g/L		0.57	41.99	8.68

reflect the physiological state of the fish organism. Analysis of the parasitic load makes it possible to assess the state of many ecosystem components since parasites' life cycle linking to various biotic elements. It was found that fish living under conditions of technogenic salinization showed a high degree of parasitic infections and a number of haematological parameters, potentially indicating a decrease in immunity. Thus, according to the results of the pilot project carried out in 2020, the ruffe *Gymnocephalus cernua* of the Volim River, exposed to the influence of the BKRU-3 waste disposal facility of Uralkali PJSC, had high level of helminth infection and a high proportion of erythrocyte morphological abnormalities. We identified values of infection extensity and intensity and determined the number of parasite species, which is more than double the background values in fish inhabiting unpolluted environment, in the Gaiva River in particular (Mikheeva, Mikheev, 2014) (Table 2). Erythropenia and significant morphological changes in erythrocyte series were also found in fish, which indicates an environmental hazard (Fig.). We also noted an increase in the rate of erythropoiesis (a greater number of young forms of erythrocytes) and an increase in the content of progenitor cells and monocytes in white blood (Table 3). It appears to be an active adaptive reaction of the ruffe to a stress factor.

According to literary sources, in most cases, fish living in polluted conditions have high helminths infection rate and morphological abnormalities of blood elements (Khan and Thulin, 1991; Kuperman, 1992; Katalay and Parlak, 2004; Marcogliese, 2005). The main mechanism that determines the increase in the number of helminthiases and structural abnormalities of blood elements in fish under conditions of pollution is immunosuppression, which manifests itself under negative influence of stress factors (Dunier, 1996). On the other hand, a number of studies describe a decrease in the parasitic load on fish under pollution compared with

Table 2 *Parasite infection of various organs of the ruffe of the Volim and Gaiva Rivers.*

Localization	Volim River	Gaiva River
	Parasite (IE; PA; II)	
stomach	*Bunodera luciopercae* 23%; 1,3; 1-2	
	Camallanus truncatus 12%; 1; 1-1	
	Triaenophorus nodulosus 35%; 2.5; 1-4	
intestine	*Ichthyocotylurus platycephalus* 12%; 8.3; 2-18	
	Bunodera luciopercae 15%; 2.3; 2-3	
	Camallanus truncatus 4%; 2; 2-2	
	Triaenophorus nodulosus 27%; 4; 4-4	
urinary bladder	*I. platycephalus* 8%; 6; 1-11	
ureters	*I. platycephalus* 100%; 12.9; 2-26	*I. platycephalus* 65%; 18.1; 7-74
kidneys	*I. platycephalus* 77%; 7.6; 2-18	
eye vitreous body	*Diplostomum spathaceum* 12%; 1.7; 1-2	
	Tylodelphys clavata 38%; 1.9; 1-2	*T. clavata* 4%; 1,0; 1
	Posthodiplostomum clavata 35%; 1.8; 1-4	
	P. brevicaudatum 8%; 1.0; 1	*Rhipidocotyle campanula* 11%; 1; 1
eye lens	*Diplostomum spathaceum* 88%; 2.3; 1-6	*D. spathaceum* 50%; 2.3;2-3
	Tylodelphys clavata 23%; 2; 2-2	

Note: IE – infection extensity; PA - parasite abundance; II - infection intensity.

Table 3 *Average values of the peripheral blood leukocytes ratio of the ruffe of the Volim and Gaiva Rivers.*

Indicator		Volim River	Gaiva River
Progenitor cells	Relative value, %	9,7	5,9
	Absolute value, thousand/1 µL	9,56	7,62
Neutrophils	Relative value, %	2,4	2,4
	Absolute value, thousand/1 µL	2,43	3,05
Eosinophils	Relative value, %	1,0	0,2
	Absolute value, thousand/1 µL	0,95	0,31
Monocytes	Relative value, %	5,9	2,7
	Absolute value, thousand/1 µL	5,86	3,34
Lymphocytes	Relative value, %	81,0	88,8
	Absolute value, thousand/1 mcL	79,67	113,08

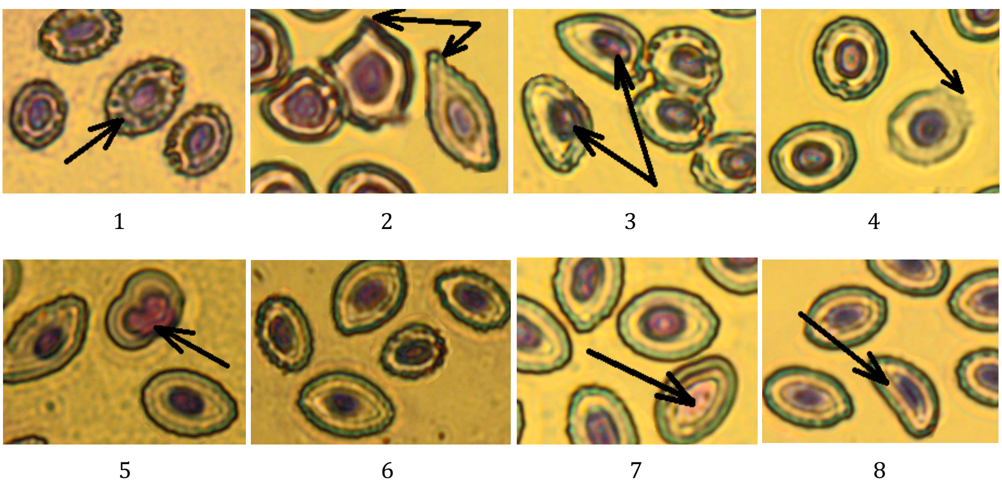

Fig. *Aberrant erythrocytes in ruffe of the Volim River prone to anthropogenic salinization: 1) cytoplasmic vacuolization, 2) poikilocytosis, 3) acentric location of the nucleus, 4) scalloped contour, 5) duplication of the nucleus, 6) anisocytosis, 7) chromatinolysis, 8) deformation of the nucleus.*

background data, which was determined by differentiated effect of pollutants on various types of parasites and by elimination of taxon-specific intermediate hosts sensitive to pollution (Blanar, 2009; Hanzelová et al., 2011). Since parasites are a connecting element of many components of biota, it is preferable to use an integrated approach to the study of the parasitological situation in water bodies prone to technogenic salinization. This requires an analysis of seasonal dynamics of the pollutant ions concentration in combination with studies of an abundance of intermediate hosts of fish parasites (Poulin, 1999; Marcogliese, 2005).

Conclusions

Examinations of the composition of algal flora, zooplankton, macrozoobenthos, ichthyofauna, indicators of infection and localization of parasites, as well as the characteristics of fish blood make it possible to indicate the level of salinity in rivers. Our results show the possibility of using new markers discovered by parasitological and hematological analyses. The development and application of new methods of bioindication along with the traditional analysis of the chemical composition of rivers is an important element in the analysis of the impact of anthropogenic stress factors on biota.

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References

Baklanov MA et al. (2019) Assessment of anthropogenic salinisation impact on the benthic invertebrates and fish in a small river – a tributary of the Kama Reservoir IOP Conf. Ser.: Earth Environ. Sci. 321 012060

Bernhardt ES, Rosi EJ, Gessner MO (2017) Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90. (doi:10.1002/fee.1450)

Blanar CA, Munkittrick KR, Houlahan J, Maclatchy DL, Marcogliese DJ (2009) Pollution and parasitism in aquatic animals: a meta-analysis of effect size. Aquatic toxicology (Amsterdam, Netherlands), 93(1), 18–28. https://doi.org/10.1016/j.aquatox.2009.03.002

Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz CJ (2013) Salinisation of rivers: an urgent ecological issue. Environ Pollut.;173:157-67. doi: 10.1016/j.envpol.2012.10.011. Epub 2012 Nov 29. PMID: 23202646.

Dunier M (1996) Water pollution and immunosuppression of freshwater fish, Italian Journal of Zoology, 63:4, 303-309, DOI: 10.1080/11250009609356150

Hanzelová V, Oros M, Scholz T (2011) Pollution and diversity of fish parasites: Impact of pollution on the diversity of fish parasites in the Tisa River in Slovakia. Species Diversity and Extinction. 265-296.

Katalay S, Parlak H (2004) The effects of pollution on haematological parameters of black goby (Gobius niger L., 1758) in Foça and Aliağa Bays. EU J Fish Aquat Sci 21: 113–117

Khan RA, Thulin J (1991) Influence of pollution on parasites of aquatic animals. Adv Parasitol.;30:201-38. doi: 10.1016/s0065-308x(08)60309-7. PMID: 2069073.

Khayrulina EA, Khomich VS, Liskova MYu (2018) Environmental issues of potash deposit development. Bulletin of the Tula State University. Geosciences, (2), pp. 112-126. (In Russ.)

Krainev EYu (2014) Raspredelenie zooplanktona reki Yaivy i nekotorykh eyo pritokov [Zooplankton distribution in the river Yaiva and some of its tributaries] Rybokhozyaistvennye vodoyomy Rossii: fundamental'nye i prikladnye issledovaniya [Fishery reservoirs of Russia: fundamental and applied research] (Saint-Petersberg: GosNOIRH Publ) pp. 459–469

Kuperman BI (1992) Parasites as bioindicators of the pollution of water bodies. Parasitology. No.6, pp. 479-482. (In Russ.)

Liu Y, Lekhov AV (2012) Modeling the change in the filtration parameters of gypsum rocks during the filtration of brines // Geoecology. Engineering geology. Hydrogeology. Geocryology. No. 6. P. 551 - 559.

Marcogliese DJ (2005) Parasites of the superorganism: are they indicators of ecosystem health? Int. J. Parasitol. 35, 705–716.

Martynenko NA, Pozdeev IV, Baklanov MA (2017) Struktura al'gocenosov rek Permskogo kraya v usloviyakh antropogennogo zacoleniya otkhodami kalii'nogo proizvodstva [Algaecenoses structure of the Permskiy krai rivers under anthropogenic salinization of potassium production wastes] Vestnik Permskogo universiteta. Seriya: Biologia [*Bulletin of Perm University. Biology*] 3 pp. 347–354

Mikheeva, O. I., Mikheev, P. B. (2014) Preliminary data on the parasitofauna of fish in the Kama reservoir basin. Part 1, 2. Proceedings of the Samara Scientific Center of the Russian Academy of Sciences, 16 (5-1), 575-587.

Potash facts (2021, Jan 15) Retrieved from https://www.nrcan.gc.ca/science-data/science-research/earth-sciences/earth-sciences-resources/earth-sciences-federal-programs/potash-facts/20521.

Poulin R (1999) The functional importance of parasites in animal communities: Many roles at many levels? Int J Parasitol.;29:903–14.

Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM (2010) Global threats to human water security and river biodiversity. Nature 467, 555–561. https://doi.org/10.1038/nature09440