Letter to Editor

Using Biological Methods to Determine Pollution Sources of the Vilnia River

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Abstract

The objective of the study was to investigate pollution sources in the Vilnia River and evaluate the toxicity of sewage, contamination of the Vilnia River water and bottom sediments, using a complex of biological methods. Toxicity was assessed by use of various indices of test-organisms: seed germination and root growth in *Lepidium sativum* L., and mortality and physiological indices in early ontogenesis (embryos and larvae) of *Oncorhynchus mykiss* L. Changes in biological indices of *Lepidium sativum* L. and embryos and larvae of *Oncorhynchus mykiss* L. demonstrated that sewage of an identified point of the pollution source and the water of the Vilnia River 300 m downstream from the pollution source, bottom sediments of the Vilnia River nearby the pollution source and 300 m downstream from the pollution source could be defined as toxic.

Keywords. test-organisms, Vilnia River water, sewage, bottom sediments, toxicity

Introduction

The Vilnia River is one of the most valuable rivers rich in the *Salmonidae* fish family. It is included in the list of the most important ichthyologic rivers, approved by the government of the Republic of Lithuania. This river is important for the restoration of salmon (*Salmo salar* and *Salmo trutta trutta*), as it is characterized by hydrological, physical and morphological indices favorable for the residence and spawning of *Salmonidae*. Earlier, *Salmonidae* fish were the primary species inhabiting the Vilnia River [19]. From 1970 untill the middle of the 1990s the Vilnia River was considerably polluted by effluents that led to the near extinction of *Salmonidae* sp. Pollution was stopped by the end of the 1990s. However, as the river flows through the environs of Vilnius, possibilities of fish residence and spawning are still connected with dispersed, spotted or occasional pollution.

As the *Salmonidae* family is very sensitive to water pollution, they are biological indicators of water quality. Therefore, restoration of *Salmonidae* resources is connected with the improvement of water quality [8, 27]. To organize proper restoration of these fish resources it is necessary to achieve as high quality of water and bottom sediments as possible. Their quality can be evaluated with the help of biological toxicity tests. The urban stream Store Vejleå (Denmark), which receives discharges of urban runoff, was investigated using a combination of biological toxicity tests and chemical analysis. The urban storm water and road runoff gave low, but statistically significant, effects on the reproduction of the alga *Pseudokirchneriella subcapitata* [7]. The toxicity of textile sludges and leachates was evaluated by a battery of toxicity tests carried out with bacteria, algae, daphnids, fish, earthworms and higher plants. The rank of biological sensitivity endpoints was: Algae ~ Plant biomass ~ Plant germination ~ Daphnids > Bacteria ~ Fish > Annelids [28]. Our previous studies have demonstrated that the

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plant *L. sativum* and *Salmonidae* fish *O. mykiss* are test-organisms that are very sensitive to the effects of different kinds of pollutants (some heavy metals, heavy metal model mixtures, orimulsion, crude oil, heavy fuel oil), sewage, polluted waters and bottom sediments [14, 16, 17, 24, 25, 30, 34]. Therefore, *L. sativum* and *O. mykiss* at different life stages as test-organisms are recommended to be included into a set of biotests for the assessment of the impact of heavy metals [17, 25]. In 2004-05 scientists of the Institute of Ecology of Vilnius University attended a project supported by the UNDP GEF SGP. The aim of the project was to restore the population of *Salmonidae* and to secure their preservation in the Neris-Vilnia River Basin. Also to prepare recommended methods for the selection of river riparian zones suitable for *Salmonidae* introduction and river stretches suitable for setting fish spawning grounds and improving ways of fish migration [13]. The present study is a part of this project.

The objective of our study was to investigate pollution sources in the Vilnia River and evaluate the toxicity of sewage, contamination of the Vilnia River water and bottom sediments, using a complex of biological methods.

Experimental Procedures

In 2005 a pollution source in the Vilnia River was tied to the company Statybos apdailos mašinos (Building Decoration Machinery). Chemical analysis of sewage of the identified pollution source was carried out and concentration of heavy metals was established: Ni – 0.02 mg/L; Cr – 0.06 mg/L; Zn – 0.07 mg/L; Fe – 0.17 mg/L, while the Maximum Permissible Concentration of these metals in Lithuanian inland waters are: Ni – 0.01 mg/L; Cr – 0.005 mg/L; Zn – 0.01 mg/L, Fe – 0.1 mg/L [1]. In the last five years the background concentration of heavy metals in the Vilnia is comparatively low (Cr concentration ranged from 0.0 to 2.7 μg/L; Zn from 7.04 to 32.10 μg/L; Ni from 0.0 to 2.6 μg/L) and do not exceed the Maximum Permissible Concentration [1]. Samples were collected in May 2005 (1 point – sewage of the pollution source; 2 point – water of the Vilnia River 300 m downstream the pollution source; 3 point – bottom sediments nearby the pollution source; 4 point – bottom sediments 300 m downstream the pollution source).

The toxicity of bottom sediments, sewage and the Vilnia River water was assessed by test-organisms:

Lepidium sativum L. was tested for seed germination and root growth (length) (OECD 208, 2003; Phytotoxkit). The test was carried out following a modified Magone [23] method. The level of toxic impact on *L. sativum* was assessed by the modified method of Wang [35]. According to the percent of root growth inhibition of 100-60%, 61-40%, 41-20% and lower than 19%, the toxic impact of the tested sample solution on *L. sativum* was classified as very strong, strong, moderate and weak, respectively [24].

Oncorhynchus mykiss L. was tested in early ontogenesis (embryos and larvae) for mortality throughout the test and physiological indices: heart rate (HR, counts/min), gill ventilation frequency (GVF, counts/min) after 5–10 days of exposure, growth (average body mass at the end of the test, mg), behaviour responses (scattering of larvae, making of nests), hatching parameters (the process of hatching), disorders in blood circulation of embryos and larvae. Long-term (30 days) toxicity tests with the rainbow trout (embryos and larvae obtained from the Žeimena hatchery) were conduced under semi-static conditions and two replications were done. Artesian water of high quality was used as the control. The average hardness of water was approximately 284 mg/L as $CaCO_3$, alkalinity was 244 mg/L as HCO_3^-, the mean pH was 8.0, temperature was equal to 9 ± 0.5 °C and the oxygen concentration ranged from 8 to 10 mg/L [15, 18].

Significance of all responses was verified by Student's t-test at $P \leq 0.05$, using the GraphPAD InStat programme (USA).

Results and Discussion.

<u>*Lepidium sativum* L.</u> Investigation of the toxic impact of sewage (1 pt.) and the River water 300 m downstream from the pollution source (2 pt.) on *L. sativum* seed germination revealed that after 2 days of exposure seed germination statistically decreased from 100% to 72% (1 pt.), decreased from 100% to 92% (2 pt.) and was similar to control (Table 1). Therefore, root length decreased from 100% to 57% (1 pt.) and to 78% (2 pt.) and was statistically different from the control (Table 1). Investigation of the toxic impact of bottom sediments near the pollution source (3 pt.) and 300 m downstream from the pollution source (4 pt.) on *L. sativum* seed germination and root growth revealed that after 2 days of exposure seed germination decreased from 100% to 94% (3 pt.) and was similar to unpolluted sediments (4 pt.), therefore root length decreased from 100% to 74% (3 pt.) and to 76% (4 pt.) and was statistically different from the control (Table 1). It is

Table 1. Effect of sewage, Vilnia River water and bottom sediments on test-object *Lepidium sativum* L.

Points	Biological indices	
	Lepidium sativum L.	
	Seed germination,%	Root length,%
1 pt. (sewage)	72 ± 4.6*	57 ± 4.4*
2 pt. (River water)	92 ± 4.8	78 ± 4.6*
3 pt. (sediments)	94 ± 4.8	74 ± 4.2*
4 pt. (sediments)	100	76 ± 4.4*
Control	100	100

* Values significantly different from controls ($P \leq 0.05$).

known that meristemic tissues of plants are very sensitive to the effect of different kinds of pollutants [4, 9, 10]. It is commonly known that [5, 23, 24] toxicants at concentrations not exceeding the levels producing toxic effects can stimulate plant metabolism as well as growth processes in plants and their cells. Nevertheless, metabolic products can disturb plant enzyme activity, and the degree of injuries depends on the intensity of metabolism [2, 6, 24].

The disturbances of seed germination and root growth of *L. sativum* demonstrated that sewage (1 pt.) could be defined as strongly toxic, Vilnia River water 300 m downstream from the pollution source (2 pt.), bottom sediments of Vilnia River nearby the pollution source (3 pt.) and 300 m downstream (4 pt.) could be defined as moderate toxic.

Oncorhynchus mykiss L. The fish was tested in early stages of development (embryos, larvae). Mortality of embryos and larvae in the Vilnia River water 300 m downstream from the pollution source (2 pt.) was significantly lower than that of the organisms affected by sewage (1 pt.), and was within the control range (Table 2). Furthermore, mortality of embryos in sewage was significantly lower as compared with mortality of larvae (Table 2). Hatched larvae are more sensitive to toxicants than embryos, as chorion (eggshell) of embryos provide some protection to develope organisms [3, 11, 15, 26].

In sewage (1 pt.) larvae of rainbow trout grew slower (at the end of the test the average body mass was significant lower), HR of embryos after 5 days exposure, HR and GVF of larvae after 10 days exposure were significantly lower than that in the water of the Vilnia River (2 pt.). Meanwhile, HR and GVF of larvae in the water of the River (2 pt.) were significantly lower than that in the control. However, HR of embryos in the water of the Vilnia River (2 pt.) was approximately the same as the control (Table 2).

Long-term (30 days) exposure to sewage not only reduced survival of embryos and larvae of rainbow trout, but also disordered the function of the most important vital systems, disturbed all early ontogenesis processes (formation of embryos, development, hatching of larvae, and their growth). Marked disorders were noticed in blood circulation: haemorrhages in different parts of the body (yolk sac; heart region; head); disorders in the process of hatching: head first hatching, disorders in the behaviour of larvae (larvae were scattered, more than 50% of larvae did not make nests). In our previous studies we evaluated biological consequences of sublethal effects of heavy metal model mixtures (HMMM) on rainbow trout in different stages of development by use of morphological, physiological and behavioural responses. Obtained data showed that low concentration of HMMM can produce negative effects on fish in early ontogenesis: disturb the activity of the most important physiological systems, change behavioural parameters, decrease the resistance of fish to the effects of environmental factors and decrease survival of fish in the later stages of development [12, 14, 15, 18].

Low concentrations of heavy metals cause changes in the development of eyes, jaw abnormalities, heart edemas. These changes disturb the feeding of fry and cause death of the organism [26]. Heart rate disorders may induce negative consequences for the respiratory process, development and hatching success. Changes in blood circulation in the yolk (blood concentrated in the yolk-sac, poorly developed blood circulation) also disturb the normal sequence of physiological processes, reduce respiration and feeding success, inhibit larvae growth rate, reduce biomass, retard development, and increase susceptibility to predation [12, 26, 29]. Changes which are heavily detected at the embryos stage are often seen as different functional disorders at the later stages of development (larvae, fry) [26]. Alterations in morphological, physiological, and behavioural responses that occur at the earlier stages of development of fish organisms can induce negative consequences not only for the well-being of an individual, but as well for the survival of the population in the future stages of development [12, 18].

The changes in biological indices of embryos and larvae of *Oncorhynchus mykiss* L. demonstrated that sewage (1 pt.) and the water of the Vilnia River 300 m downstream the pollution source (2 pt.) could be defined as toxic. Results imply, that the water could be toxic for native *Salmonidae* sp. fish (*Salmo salar, Salmo trutta*

Table 2. Effect of sewage and Vilnia River water on test-object *Oncorhynchus mykiss* L. in early stages of development (embryos, larvae).

Points	Biological indices					
	Oncorhynchus mykiss L.					
	Embryos		Larvae			
	Mortality,%	HR, counts/min	Mortality,%	Average body mass, mg	HR, counts/min	GVF, counts/min
1 pt. (sewage)	16.0	38.6 ± 3.4*	52.4	84.02 ± 0.04*	62.4 ± 2.6*	118.4 ± 2.8*
2 pt. (River water)	12.0	64.5 ± 4.2	18.2	96.11 ± 0.02	117.2 ± 4.2*	132.2 ± 4.6*
Control	4.0	65.3 ± 3.6	7.8	94.28 ± 0.03	130.2 ± 3.2	144.0 ± 3.6

* Values significantly different from controls ($P \leq 0.05$).

trutta, Salmo trutta fario) too, which environmental requirements and life strategy are similar to those of test salmonidae species *Oncorhynchus mykiss*. Until the mid 1990's the Vilnia was considerably polluted by effluents of factories situated in Naujoji Vilnia, a settlement situated several kilometres upstream from the sites of the present study. At that time *Salmonidae* sp. fish (*Salmo salar, Salmo trutta trutta, Salmo trutta fario*) were almost absent in the river [31]. However, after the collapse of the Soviet Union, downfall of centrally planned industry and subsequent direction of all wastewaters of Naujoji Vilnia to the wastewater treatment plant of Vilnius City, the situation has improved considerably. *Salmonidae* sp. fish returned to the river and started to increase in abundance. In addition, artificial rearing further supported populations of Salmo salar and Salmo trutta trutta. As a result, in 2005 the density of juveniles increased 35-fold, while that of spawning nests 26-fold comparing with 1998 [20, 32]. However, some indications of pollution are still present. For instance, the concentration of lead in the muscles of fish sometimes exceeds the Maximum Permissible Concentration of 0.2 mg/kg [33]. Additionally, the density of juveniles of *Salmonidae* sp. fish as well as the number of spawning nests is greater above the 1 pt. (sewage of the pollution source) and rather lower where the impact of sewage is less evident (dilution effect) as it was observed during the yearly monitoring of *Salmonidae* sp. fish [21, 22]. No spawning nests have been observed directly before the 1 pt. This strongly indicates a negative impact of the pollution source (even if it has local character); therefore, this place is not suitable for the release of *Salmonidae* sp. and preparation of spawning ground.

Conclusions

The disturbances of seed germination and root growth of *L. sativum* demonstrated that sewage (1 pt.) could be defined as strongly toxic, Vilnia River water 300 m downstream the pollution source (2 pt.), bottom sediments of the Vilnia River nearby the pollution source (3 pt.) and 300 m downstream (4 pt.) could be defined as moderately toxic.

The changes in biological indices of embryos and larvae of *Oncorhynchus mykiss* L. demonstrated that sewage (1 pt.) and the water of the Vilnia River 300 m downstream from the pollution source (2 pt.) could be defined as toxic.

The complex of the most sensitive indices of investigated *Lepidium sativum* L and *Oncorhynchus mykiss* L. can be successfully used for the assessment of toxicity of water, bottom sediments and sewage containing different kinds of pollutants. The obtained data permits us to predict the impact of pollution not only on the physiological status of aquatic organisms but also on their survival in natural water bodies.

Identification of the sources of pollution, using a complex of biological methods allows to choose river segments with lower level pollution, suitable for the release of *Salmonidae* sp., their spawning migration and preparation of spawning ground in the future, and to affect the natural abundance of *Salmonidae* fish population in the Vilnia River.

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