

BEHAVIORAL RESPONSES IN RAINBOW TROUT *Oncorhynchus mykiss* AS INDICATORS OF SUBLETHAL EXPOSURE TO HEAVY METALS

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Abstract. Heavy metals are common pollutants of aquatic ecosystems and usually are found in mixtures of various combinations. At present the controlling of pollution of aquatic environment by heavy metals is performed basically by use of analytical investigations. However, biological control of effluent toxicity before effluents are discharged into receiving waters is still seldom used. Therefore, the search into suitable, easy available and maintainable test-objects, their rapid, sensitive, and non-specific test-functions which provide information not only about lethal but also about sublethal effects of pollutants is needed. Fishes are considered to be among the best test-objects. Alterations in a number of fish behavioral responses are sensitive indicators of sublethal exposure to heavy metals and other aquatic pollutants. These are no standardized procedures yet. Laboratory tests were conducted on rainbow trout *Oncorhynchus mykiss*. Behavioral responses, such as: leaving the nest, response to external stimuli and breathing rate in larvae as well as detection-avoidance, locomotor activity, gill ventilation frequency, and coughing rate in juveniles and adults were investigated. Obtained data showed that fish behavioral responses can be successfully applied in solving not only theoretical but also practical problems of aquatic toxicology, such as bioassay testing for hazard assessment of separate heavy metals and their mixtures, in industrial wastewaters as well as in natural inland waters.

Keywords: rainbow trout, behavior, toxicity, heavy metals.

1. Introduction

Heavy metals are common persistent pollutants of aquatic ecosystems entering them through numerous diverse anthropogenic and natural sources (Moore 1991).

In industrial wastewaters they are usually found in mixtures which are specific to a particular pollution source. At present the controlling of pollution of aquatic environment by heavy metals is performed basically by use of analytical investigations.

However, the evaluation of effluent quality by physical-chemical measurements does not provide information regarding the possible synergetic or antagonistic effects that complex effluents may have on aquatic life.

Therefore, it is necessary to perform biological control of effluent toxicity, before effluents are discharged into receiving waters. For rapid determination of wastewater toxicity level, bioassay testing is used.

Bioassay testing is defined as a rapid experimental evaluation of water toxicity level by use of biological objects and processes (Flerov 1989). Test-objects must be easily available and maintainable. Their test-

functions must be sensitive not only to acutely toxic effluent levels, but also provide information about sublethal concentrations.

Fishes are considered to be among the best test-objects. Alterations in fish behavioral responses are sensitive indicators of sublethal exposure to heavy metals and other pollutants (Giattina and Garton 1983; Atchison *et al.* 1987; Little and Finger 1990; Scherer 1992; Kane *et al.* 2005).

The aims of the present study were: (1) to generalize data on behavioral responses of rainbow trout at different stages of development (larvae, juveniles, adults) under the effect of heavy metals, (2) to perform comparative analysis of their sensitivity to the effect of heavy metals, and (3) to evaluate suitability of the responses studied for needs of bioassay testing of the waters containing heavy metals for hazard assessment.

2. Materials and methods

The tests were conducted on rainbow trout at different stages of development (larvae, juveniles, adults).

Test fish were obtained from hatchery and acclimated to laboratory conditions.

The effect of separate heavy metals and their mixtures of different composition were investigated.

A number of fish behavioral responses (leaving the nest, response to external stimuli, breathing rate in larvae, detection and avoidance, locomotor activity as well as gill ventilation frequency and coughing rate in juveniles and adults) were studied.

Deep-well water was used as the dilution water. Average hardness of the water was approximately 284 (271 – 296) mg/L as CaCO₃, alkalinity was 244 (232 – 256) mg/L as HCO₃⁻, pH was from 7.9 to 8.1, temperature was maintained at 10.5 to 11.5°C, and oxygen concentration was maintained at a range of 8 to 10 mg/l.

The effects of separate heavy metals and three model mixtures of different composition were investigated. The formation of model mixtures was carried out basing on: Maximum Permissible Discharges (MPD, accepted for Lithuania) of 5 common heavy metals into municipal sewage wastewater entering treatment plants (HMMM-1); available analytical data of average annual amounts of representative heavy metals in cooling wastewater discharged from the Ignalina Nuclear Power Plant into the Drūkšiai lake (HMMM-2); the available analytical data of average annual amounts of representative heavy metals in wastewater after treatment discharged from Vilnius city into the Neris river (HMMM-3) (Table 1).

The stock solutions of HMMM were prepared in distilled water the final concentration being recalculated according to the amount of heavy metal ion.

Nominal heavy metal concentrations were checked by atomic absorption spectrophotometry during blank tests using either AAS VARIAN-250 PLUS or SHIMADZU AA-6800 by flame or graphite furnace techniques. Mean measured concentrations were within 5 – 10 % of target.

The Sensitivity of the behavioral responses studied was evaluated by comparing their threshold values. Threshold-Effect-Concentration (TEC) was estimated by defining geometric mean between the Lowest-Observed-Effect-Concentration (LOEC) and the No-

Observed-Effect-Concentration (NOEC) (Van Leeuwen and Hermens 1995) and expressed as a part of 96-hour LC50 value.

3. Results and discussion

The data obtained showed that the sensitivity of fish behavioral responses to heavy metals varies significantly depending on fish stage of development and chemical origin of the metal or the composition of the mixture studied (Table 2).

Overall, the most sensitive rainbow trout behavioral responses were found to be avoidance, detection and locomotor activity in juveniles. Low sublethal concentrations of separate heavy metals and their mixtures corresponding to 0.00006 – 0.007 parts of their 96-hour LC50 values can be detected by use of these responses. (Svecevičius 2005; 2007; 2009).

Respiratory responses in juveniles and adults (coughing rate and gill ventilation frequency) were found to be less sensitive. By use of these responses such effective levels as 0.005 – 0.14 parts of 96-hour LC50 values can be estimated (Kazlauskienė and Vosyliene 1990; Svecevičius 2005; 2009).

In spite of that behavioral data indicates that they are much more sensitive than many fish biochemical – physiological parameters (Kazlauskienė and Vosyliene 2008).

Meanwhile, the least sensitive responses have been breathing rate, leaving the nest and response to external stimuli in larvae. They can be used to detect nearly lethal (0.12 – 0.5 parts of 96-hour LC50 value) heavy metal mixture concentrations (Kazlauskienė *et al.* 1995; Kazlauskienė 2004).

Although the literature on various types of fish behavioral responses to heavy metals and other aquatic pollutants is abundant they are still seldom used as ecotoxicological tools in the day-to-day management monitoring of the industrial wastewaters and/or toxicity of the aquatic environment.

Table 1. Composition of heavy metal model mixtures (HMMM)

Heavy metal (HM)	Source	Concentration of HM (mg/l) accepted as 1%		
		HMMM-1	HMMM-2	HMMM-3
Cu	CuSO ₄ ·5H ₂ O	0.005	0.0075	0.02
Zn	ZnSO ₄ ·7H ₂ O	0.01	0.064	0.06
Ni	NiSO ₄ ·7H ₂ O	0.005	0.0021	0.05
Cr	K ₂ Cr ₂ O ₇	0.025	0.0028	0.02
Fe	FeSO ₄ ·7H ₂ O	0.05	–	0.3
Pb	Pb(NO ₃) ₂	–	0.0142	0.03
Cd	Cd(CH ₃ COO) ₂ ·2H ₂ O	–	0.00018	0.003
Mn	MnSO ₄ ·5H ₂ O	–	0.0099	0.09

Table 2. Behavioral responses of rainbow trout to heavy metals and their mixtures

Test-object	HM or HMMM	Behavioral responses studied	TEC as a part of 96-hour LC50	Reference
Larvae	HMMM-1	Breathing rate (20-day exposure)	0.38	Kazlauskienė <i>et al.</i> 1995
		Leaving the nest	0.5	
		Responses to external stimuli	0.5	
Larvae	HMMM-2	Breathing rate (20-day exposure)	0.28	Vosylienė <i>et al.</i> 2003
Larvae	HMMM-3	Breathing rate (20-day exposure)	0.12	Vosylienė <i>et al.</i> 2003 Kazlauskienė. 2004
		Leaving the nest	0.12	
		Responses to external stimuli	0.12	
Juveniles	Copper	Avoidance	0.0015	Svecevičius 1999
Juveniles	HMMM-3	Avoidance	0.003	Svecevičius 2001
Juveniles	Zinc	Gill ventilation frequency (1-hour exposure)	0.092	Svecevičius 2002
		Coughing rate(1-hour exposure)	0.092	
		Avoidance	0.0008	
Juveniles	HMMM-1	Gill ventilation frequency(1-hour exposure)	0.05	Svecevičius 2004
		Coughing rate(1-hour exposure)	0.05	
		Avoidance	0.005	
Juveniles	HMMM-2	Detection	0.007	Svecevičius 2005
		Avoidance	0.006	
		Locomotor activity(1-hour exposure)	0.007	
		Gill ventilation (1-hour exposure) frequency	0.032	
Juveniles	Hexavalent chromium	Coughing rate (1-hour exposure)	0.14	Svecevičius 2007 Svecevičius 2009
		Avoidance	0.00006	
		Detection	0.002	
		Locomotor activity(1-hour exposure)	0.002	
		Gill ventilation (1-hour exposure) frequency	0.007	
Adults	Copper	Coughing rate (1-hour exposure)	0.07	Kazlauskienė and Vosylienė 1990
		Gill ventilation (24-hour exposure) frequency	0.05	
		Coughing rate (0.5-hour exposure)	0.005	

4. Conclusions

1. Obtained data showed that fish behavioral responses could be a powerful tool in identifying sublethal exposure to heavy metals.
2. By means of locomotor-avoidance responses very low even background levels of heavy metals can be detected. These responses can be used with no doubt in bioassay testing of natural slightly polluted waters.
3. Respiratory and specific larvae responses were found to be less sensitive. In spite of that they can be also safely used in bioassay testing of untreated wastewaters as well as waters after treatment before they are discharged into receiving waters.
4. The determination of water-quality criteria at present is based on conventional standard acute and chronic testing procedures. Fish behavioral tests seem sensitive, rapid, ecologically significant, and they could be easily standardized.

5. In solving practical problems of aquatic toxicology, such as bioassay testing, fish behavioral responses can be successfully applied for hazard assessment of industrial wastewaters before and after treatment as well as natural inland waters containing heavy metals, and perhaps other aquatic pollutants of different chemical origin or their complexes.

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