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DAPHNIA TEST – A SHORTCUT FOR HUMAN HEALTH PROTECTION ASSESSMENT

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Abstract

Daphnia magna toxicity testing was evaluated as a method for estimating the potential trace metals hazard to the environment. Electroplating whole effluent evaluation was used as case study. D. magna proved to be sensitive enough to Zn, Cr and to a certain extent to Ni to serve as a quick but reliable method for assessing possible human health hazard by bioconcentrated trace metals via freshwater fish consumption.

Key words: *Daphnia magna, metals, toxicity testing, effluent, human health.*

INTRODUCTION

Even today's toxicity tests on *Daphnia magna* are the only universal tests with freshwater invertebrates that are formally accepted as standard ones by all the most important in-ternatiomal organizations: EC, OECD, ISO, US EPA (Mark and Solbe, 1998). As such, they are included in the obligatory monitoring of waste and recipient waters, but also as a re-liable and quick method for evaluation of the impact of certain hazardous materials on life environment (Adams, 1995). A few compre-hensive studies (Dierickx and Bradael-Rozen, 1996; Lilius and Isoma, 1955 – quot. Marks and Solbe, 1998; Nelson and Roline, 1998), as well as the data from AQUIRE database (US EPA, 2000a) show that *D. magna* is one of the most sensitive freshwater organisms to inorganic pollution, especially to heavy metals. For that reason data on lethal concentrations of most metals have served as a basis for establishing environmental standards and criteria (US EPA, 1986).

By the processes of bioaccumulation and bio-concentration, metals from the water column get into the tissues of freshwater fishes, and, at elevated ambiental concentrations, even the nutritional consumption of the musculature may present a possible risk to human health (Teodorovic, 1999). The goal of this paper was, on the example of an electric equipment factory, to examine whether the relative sensibility of *D. magna* to metals may be used to serve as a quick, simple, but also reliable method for evaluation of the possible threats to human health by the toxic effects of Zn, Ni and Cr released into the recipient (Tikvesh reservoir).

MATERIALS AND METHODS

The laboratory culture of Daphnia magna was grown in standard conditions (US EPA, 1993): glass vessels with population concen-tration up to 100 units, hard synthetic stan-dard water (NaHCO3 192 mg/l, KCl 8mg/l and MgSO4×7H2O 245 mg/l dissolved in deionized water EC< 20? S/m2 and aerated for 24 h; added CaSO4 95mg/l separately dis-solved in deionized water), with no aeration, laboratory lighting, photo period 16 h of light/ 8 h of darkness, temperature 25±°C, feed – 3 times a week YCT combination (fish pellets, wheat and beer malt). One-off (instant) sample of recipient water for dilution was taken on the left bank of the Crna River, before one Kavadarci town sewerage outlet. The basic physico-chemical parameters were determined in standard and Tikvesh reservoir waters (Table 1).

Hardness and alkalinity were determined titrationally (APHA, 1995), whereas electro-conductivity, pH and O_2 electrochemically. The waste water sample from the electric equipment factory was taken as a daily composite, and a physico-chemical characterization was performed following standard method (APHA, 1995) (Table 2). Acute toxicity of the waste water was estimated using static test in duration of 96 h, on the neonatals of *D. magna* aged 24 h. The test was set with 5 units per test in a vessel of 50 ml volume, with 30 ml test solution in two runs. The dilutions were made parallely – with standard and with recipient water, in volume concentrations: 6.25, 12.5, 25, 50 and 100%, together with double control (recipient and standard water). The effect under observation was immobilization, or the units′ mortality. The condition for acceptance of the acute test was 90% control survival (US EPA, 1993). The results were processed using Dunnett test with variance analysis (ANOVA) (US EPA, 1991). LC/EC_{50} and $_{10}$ were determined by standard methods: Probit method with X^2 for hete-rogeneity (EPA Probit Calculation Program Version 1.5, US EPA, 1993) and Spearman Karber/Trimmed Spearman Karber method (EPA Trimmed Spearman Karber Version 1.5, US EPA, 1993).

Table 1. Values of basic parameters: standard and Tikvesh waters

Parameter	Standard Water	Tikvesh Water
Temperature (°C)	25	8
pH	8	7.8
Hardness (mg $CaCO_3$/l)	320	230
Alkalinity (mg $CaCO_3$/l)	245	220
Dissolved oxygen mg O_2/l	9	12
Saturation O_2%	85	97
- mS/m^2	700	500

RESULTS AND DISCUSSIONS

By physico-chemical characterization of the waste water (Table 2) the presence of Zn, Ni and Cr was established, which is expected, having in mind that it is a matter of effluents from the electrical equipment factory which, in its production process, has a line for gal-vanic processing of the metals. There were no other potentially hazardous materials, and the other examined parameters (all of them far beneath the MAC, Official Gazette of R. M., 2005) indicate waste water of low organic load.

Waste water toxicity was tested on *D. magna* in 6.25, 12.5, 25, 50 and 100% dilutions made with standard synthetic water as well as with the recipient (Tikvesh reservoir) water. The survival in both controls was 100%, which was expected regarding the favorable properties of the water (Table 2).

Table 2. Physico-chemical characteristics of the waste water

Parameter	Measuring unit	Value	Parameter	Measuring Unit	Value
Air temperature	°C	16	glowed residuum	mg/l	633
Water temperature	°C	16	loss by glowing	mg/l	201
$KMnO_4$	mg O_2/l	20.33	suspended material	mg/l	87
pH		8.4	greases and oils	mg/l	0.23
Draff materials	mg/l	1	surface active mat.	mg/l	0.86
HPK	mg O_2/l	180	Zn	mg/l	0.41
BPK_5	mg O_2/l	33	Ni	mg/l	3.6
Dry residuum at 105°C	mg/l	834	Cr (total)	mg/l	0.6

In the toxicity test where the Tikvesh reservoir water was used as a diluent (Table 3), 100% mortality was found at the 50% and 100% dilutions, which is also a statistically significant different survival compared to the control (one-way Dunnett test; $P < 0.05$). Since the mortality percentages did not monoto-nously grow together with the concentration of the effluent, LC_{50} was calculated using Trimmed Spearman-Karber′s method: Spear-man-Karber estimate 96-h LC_{50} 22.11% (95% trust interval: 15.21-32.14

In the test where standard synthetic water was used as a diluent (Table 4), after 96 h 100% mortality was noticed only in 100% waste water. However, statistically significant dif-ferent survival compared to the control was noted on the dilutions 50% and 100% (one-way Dunnett test;α =0.05).

The existence of partial mortality and the statistically important X2 test of heterogeneity (X2 calculated: 4.945; X2 – tabular value at 0.05: 7.815) prerequisited the use of Probit method for estimating LC/EC50 and 10:- LC/EC10: 11.181% (95% interval of trust: 14.288-17.319)-LC/EC50: 32.032% (95% interval of trust: 21.744-48.859)

Table 3. Results of the acute (96-h) effluent′s toxicity test (diluent – Tikvesh reservoir)

Dilution (%)	Nr. of orga-nisms per testvessel	Nr. of runs	Total nr. of organisms	Mean survival value	sd	cv (%)
Control	5	2	10	1.00	0	0.0
6.25%	5	2	10	0.90	0.1414	15.7
12.5%	5	2	10	0.70	0.4243	60.6
25%	5	2	10	0.60	0.0	0.0
50%*	5	2	10	0.0	0.0	0.0
100%*	5	2	10	0.0	0.0	0.0

statistically significantly different than the control – one-way Dunnett test (α =0.05)

Table 4. Results of the acute (96-h) effluent′s toxicity test (diluent – standard water)

Dilution (%)	Number of tested units	Number of dead	Proportion of dead	Probit calculation of deaths
control	10	0	0	0
6.25	10	1	0.1	0.0233
12.5	10	1	0.1	0.1259
25	10	2	0.2	0.3814
50*	10	7	0.7	0.7062
100*	10	10	1	0.9172

statistically significantly different than the control – one-way Dunnett test (α=0.05)

According to the results obtained, 96-h LC_{50} in Tikvesh water as a dilution was 22.11%, which means that the additive effect of Zn (0.09 mg/l), Ni (0.79 mg/l) and Cr (0.13 mg/l) was lethal for 50% of test organisms. But, in the tests with standard water as a dilution, 96-h LC_{50} was only 32.032%, or the mortality of 50% test units arouse as a response to the cumulative effect of 0.13 mg Zn/l, 1.1 mg Ni/l and 0.19 mg Cr/l. In the same test conditions, 96-h LC_{10} was 11.18% of the effluent, or 90% of the test units survived in the effluent with concentrations of 0.046 mg Zn/l, 0.4 mg Ni/l and 0.07 mg Cr/l. Obtained differences in the LC_{50} values in the tests with recipient and standard waters don′t surprise. It has been proven that the twovalent cations′ chemical form and bioavailability depends on pH, alkalinity and water hardness, or the metals′ toxicity and bioaccumulation drops with the increase of hardness and alkalinity (Leland & Kuwabara, 1985). Therefore the explanation for the reduced toxicity of the examined effluent in the standard water ought to be looked for in the water′s significantly greater hardness and alkalinity (320 and 245 mg $CaCO_3$/l) compared to the recipient water (230 and 220 mg $CaCO_3$/l) (Table 1).

The results obtained by these investigations agree with the literature data on toxicity of Zn, Cr and Ni on *D. magna.* Analyzing an effluent of the chemical industry, Tišler and Zagorc-Koncan (1994) identify the Zn as a direct cause of the recipient′s high toxicity downstream from the outlet and find that 48-h LC_{50} for *D. magna* is 0.8 mg Zn/l. The acute toxicity of the Zn 948-h LC_{50} for *D. magna* varies from 0.04 mg/l at the hardness of 50 mg $CaCO_3$/l, up to 5.5 mg Zn/l at 250 mg $CaCO_3$/l. The first signs of chronical tixicity were registered at 0.07 mg Zn/l (US EPA, 2000b). The acute toxicity of Cr (VI) for *D. magna* in soft water is 0.02 and in hard water 0.04 mg Cr (VI) /l, whereas the data on the toxicity of Cr (III) vary from 0.044 – 0.066 mg Cr (III) /l, depending on water′s hardness (US EPA, 1998). Based on available data (Kszos *et al.,* 1992), the toxic effects of Ni on *D. magna* in moderately hard water (100mg $CaCO_3$/l) appear not under 0.16 – 0.3mg Ni/l concentrations.

Because of the proven dependence of the toxicity and bioavailability of Zn, Ni and Cr on water′s hardness, at the establishing am-biental criterions for protection of the aquatic species and human health from the toxic effects of some metals, US EPA (1986) gives models following which MACs for individual hydroecosystems of different water hardness ought to be calculated.

Regarding Zn, freshwater organisms are con-sidered protected if 24-h average never ex-ceeds (0.83 [ln (water hardness as CaCO30] + 1.95), i.e. in our conditions 320 – 570 mg Zn/l (US EPA, 1986). Macedonian MACs (Official Gazette of RM, 2005) for I/II and III/IV classes of water are 0.2 or 1 mg Zn/l respectively, which is drastically above the lethal doses of Zn for D. magna, but at the same time is enough for protection of the human health. That′s why D. magna can be considered a suitable test organism for estimating a hydro-system′s pollution and the possible threat the latter poses to the people. US EPA (1986) prescribes a stricter ambiental concentration that safeguards human health′s protection from the toxic effects of Zn entered via eating fish: 47?g Zn/l. However, tests with D. magna can respond even to such strict demands, for LC10 calculated in this work is exactly 0.046 mg Zn/l.

Regarding Ni, freshwater organisms are considered protected if 24-h average (in μg Ni/l) does not exceeded (0.76 [ln (water hardness as CaCO3)] + 1.06) (US EPA, 1986), which in our conditions means 96 – 160 μg Ni/l (right on the limit MK MAC, Official Gazette of RM, 2005) and if the concentration (in μg Ni/l) never exceeds e (0.76 [ln (water hardness as CaCO3)] + 4.02), i.e. 1.8 – 3.1 mg Ni/l. And here as well D. magna may be called a suitable test organism, since according to MK MAC for III/IV water classes human health is not endangered by the toxic effect of Ni that enters the human organism by eating fish. According to US EPA, human health′s protection from the toxic effects of Ni entered via eating fish is secured at the ambiental concentration of 13.4 μg Ni/l. As a test organism D. magna is not able to meet such strict requirements since it isn′t sensitive enough to Ni.

Regarding sixvalent chromium, EPA (1998) thinks that freshwater organisms are protected if 4-days′ average does not exceed 11 μg Cr (VI) /l more often than once in three years and if 1-hour′s concentrations′ average does not exceed 16 g Cr (VI) /l more often than once in three years. The concentration of 50 μg Cr (VI) /l provides protection of human health from the toxic effects of Cr (VI) entered the human organism via eating fish (US EPA, 1998). D. magna is not able to meet such strict criterions, but it is a sufficiently sensitive test organism to MK MACs which presents no risks to human health via eating fish.

Regarding trivalent chromium, freshwater or-ganisms are considered protected if 4-days′ average (in μg CR (III) /l) does not exceeded (0.8190 [ln (water hardness as CaCO3) + 1.561) more often than once in three years (US EPA, 1998), which in our conditions means 210 – 370g Cr (III) /l; and if 1-hour′s concentrations′ average (in g Cr (III) /l) does not exceeded (0.8190 [ln (water hardness as CaCO3) + 3.688) more often than once in three years (US EPA, 1998), or 1700 – 3100 μg Cr (III) /l. It is considered that ambiental concentration of 170 μg Cr (III) /l provides human health′s protection from the toxic effects of Cr (III) entered via eating fish. Based on the results of these investigations and on literature sources, with its sensitivity to Cr D. magna is a suitable test organism for estimating the threat this metal poses to human health.

CONCLUSIONS

Based on all this, a conclusion can be made that the toxicity test on *Daphnia magna* may enable quick and reliable insight in the hydroeco-system′s pollution with metals, but also give a relevant estimation of the possible threat to human health posed by metals entered via eating freshwater fish.

In this particular examined case, *Daphnia magna′s* high mortality at high dilutions indicates that, even within the zone of total mixing of the waste and the recipient waters, the concentrations of Zn, Ni and Cr in the water can present danger to human health because by the processes of bioconcentration they are able to accumulate within certain fish tissues in significant a-mounts.

For this reason it would be necessary, from time to time, to check the contents of Ni, Cr and Zn in the muscles of the fish caught in that sector if in the vicinity of the examined waste water there is some sewerage system outlet or some outlet of any other organically loaded effluent (that attracts fish).

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