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Lethal effects of Zn, Cu and their mixture on the medicinal leech (*Hirudo verbana*)

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Medicinal leech (*Hirudo verbana*) were exposed for 30 days to various concentrations of Zn (0.188, 0.375, 0.75, 1.5, 3.0, 6.0, 12.0, 24.0 and 48.0 mg/l), Cu (0.004, 0.008, 0.016, 0.032, 0.063, 0.125, 0.25, 0.5, and 1.0 mg/l) and their equitoxic mixture Zn + Cu (0.188 + 0.006, 0.375 + 0.012, 0.75 + 0.023, 1.5 + 0.045, 3.0 + 0.09, 6.0 + 0.18, 12.0 + 0.36, and 24.0 + 0.72 mg/l). The LC_{50} values were determined for the following exposures: 48 h, 96 h, 10 d, 20 d, and 30 d. Mortality of animals was observed during the whole 30-d period. Lethal effects of metals on leech were prolonged as compared to fish; therefore, long-term experiments for leech are needed. The sensitivity of leech to Cu was remarkably higher than to Zn. A partially additive interaction of Zn and Cu in the mixture was found. The additive effects were considerably higher during the shorter (96 h, 10 days) than during the longer exposures (20 and 30 days).

Key words: copper, medicinal leech, heavy metal mixture, long-term exposure, zinc

INTRODUCTION

The impact of heavy metals and their interactions in mixtures are being investigated using various species of invertebrates. Studies on the medicinal leech, which is a convenient tool for toxicological research, are still scarce (Флеров, 1989; Petrauskienė, 2003, 2004). The lethal effects of heavy metals and other chemicals on fishes are mostly pronounced during the first four days of exposure; usually, the concentration that caused a 50% mortality during a 96-h exposure period (96-h LC_{50}) is estimated as an index of acute toxicity. Many species of invertebrates were considered more tolerant to various toxicants since their 96-h LC_{50} was remarkably higher than that for fishes (Moore, Ramamoorthy, 1984). More recently it has been found that the lethal effects of various toxicants on invertebrates are prolonged, and mortality may occur after a 96-h exposure (Moles, 1998, 2001; Scarlett et al., 2007).

The aim of the present study was to determine the lethal effects of Zn, Cu and their mixture on the medicinal leech during a long-term exposure.

METHODS

Experiments were carried out on the medicinal leech (*Hirudo verbana*) bred under laboratory conditions. Animals under study were 22–24 months old with the body weight between 4 and 8 g. Leech had not been fed for 4 months before initiation of experiments. Animals were placed in pairs into 1–l glasses, half filled with a test solution of metals or with control water. Water and solutions in glasses were renewed every 24 h. Animals were exposed to solutions of Zn, Cu and their mixture for a 30-day period. Stock solutions were prepared in distilled water using chemically pure sulphates $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$. The final concentration was recalculated according to the content of heavy metal ions.

Deep well water in which leech were bred and constantly maintained served for dilution and for the control tests. Water specifications: hardness 270–300 mg/l as $CaCO_3$; alkalinity 244 mg/l as HCO^{-3}; pH = 7.9–8.1; dissolved oxygen 5–7 mg/l; temperature 19.5–20 °C. Animals were exposed to nine different concentrations of single metals: Zn 0.188, 0.375, 0.75, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0 mg/l; Cu 0.004, 0.008, 0.016, 0.032, 0.063, 0.125, 0.25, 0.5, and 1.0 mg/l. The Zn + Cu mixture was composed based on the 30-d LC_{50} values of single metals. Concentrations of Zn and Cu in the test mixture were as of follows (Zn + Cu): 0.188 + 0.006, 0.375 + 0.012, 0.75 + 0.023, 1.5 + 0.045, 3.0 + 0.09, 6.0 + 0.18, 12.0 + 0.36, and 24.0 + 0.72 mg/l. Twenty leech were used for each test concentration. The mortality of leech was recorded at 24-hour intervals. The LC_{50} values and their 95% confidence intervals were calculated by the trimmed Spearman–Kärber method (Hamilton et al., 1977) for the following exposures: 48 hours (h), 96 h, 10 days (d), 20 d, and 30 d. The mode of interactions of metals in a mixture was estimated using the mixture toxicity index (MTI) calculated according to the formula of Könemann (1981).

RESULTS

The data presented in Table 1 show that the duration of exposure influenced the LC_{50} values estimated for all types of solutions. LC_{50} values for Zn decreased from 33.94 mg/l (48-h exposure) to 3.0 mg/l (30-d exposure). The latter concentration was 11.1 times lower than that of 48-h LC_{50}. The values of LC_{50} for Zn were gradually decreasing during the 48-h and to 20-d exposures,

Table 1. **Estimated LC_{50} values and their 95% confidence intervals for medicinal leech exposed to heavy metal solutions at different duration of exposure**

Metal solution	Exposure duration	LC_{50}, mg/l	95% confidence intervals, mg/l
Zn	48 h	33.94	15.88–72.53
	96 h	15.83	11.36–22.08
	10 d	6.89	4.95–9.60
	20 d	3.22	2.59–3.99
	30 d	3.00	2.33–3.87
Cu	48 h	>1.00	–
	96 h	0.84	0.55–1.28
	10 d	0.38	0.27–0.53
	20 d	0.17	0.12–0.22
	30 d	0.09	0.07–0.13
Zn + Cu	48 h	20.89 + 0.63	14.76 + 0.44–29.58 + 0.86
	96 h	13.79 + 0.414	11.28 + 0.338–16.85 + 0.506
	10 d	6.89 + 0.207	5.36 + 0.161–8.87 + 0.266
	20 d	3.65 + 0.094	3.05 + 0.092–5.66 + 0.169
	30 d	2.80 + 0.084	2.26 + 0.067–3.47 + 0.104

while the 20-d LC_{50} and 30-d LC_{50} values were quite close, their ratio being only 1.07. In fact, there was no significant difference between these two values because their 95% confidence intervals were completely overlapped.

The range of the Cu concentrations applied did not allow to calculate the 48-h LC_{50} value. LC_{50} values for Cu were gradually decreasing during the 96-h and the 30-d exposure. The differences between all the estimated LC_{50} values for different exposures were significant because 95% confidence intervals either did not overlap (among 96-h, 10-d and 20-d LC_{50} values) or overlapped very slightly (between 20-d and 30-d LC_{50} values). The 30-d LC_{50} value was 9.33 times lower than that of 96 h.

The estimated LC_{50} values for a mixture at different exposures (Table 1) showed that in the majority of cases the concentrations of single metals in a mixture were lower than those in solutions of single metals. The mixture toxicity indices were 0.55, 0.37, 0.048 and 0.099 for the 96-h, 10-d, 20-d, and 30-d exposures, respectively (Table 2). Thus, MTI were more than 0 and less than 1. According to Könemann (1981), it means that the interaction of Zn and Cu in a mixture was partially additive. The additive effects were more pronounced in short-term tests, indicating a loss of the potential for additivity during a prolonged exposure.

Table 2. **Mixture toxicity index (MTI) at different duration of exposure**

Exposure	96 h	10 d	20 d	30 d
MTI	0.55	0.37	0.048	0.099

DISCUSSION

Mortality of leech exposed to Zn, Cu and to their equitoxic mixture was observed during the whole 30-d period of exposure. The estimated 30-d LC_{50} values for Zn and Cu were 11–9 times lower than those in acute toxicity tests (48-h or 96-h exposure). Thus, the lethal effects of metals on leech are considerably more prolonged than in fish. The mortality of fish in heavy metal solutions was mainly observed during the 96-h exposure (Petrauskienė, Daniulytė, 1996; Schüürmann, Market, 1998; Svecevičius, 1999; Jezierska, Witeska, 2001).

Copper was much more toxic to leech than Zn (up to 33.3 times after a 30-d exposure), i. e. the mode of toxicity of these two metals was the same as in fish (Jezierska, Witeska, 2001; Marčiulionienė et al., 2002). The toxicity of these two metals differs for various invertebrate species. Lethal toxicity caused by Zn was higher than that of Cu on young crab *Chasmagnathus granulata* (Ferrer et al., 2006). Cu was more toxic than Zn to amphipod *Allorchestes compressa* (Ahsanullah et al., 1998), to nauplii of brine shrimp *Artemia salina* (Taylor et al., 2005), to earthworm *Aporrectodea caliginosa* (Khalil et al., 1996), to larvae of the marine polychaete *Hydroides elegans* (Gopalakrishnan et al., 2007), to rotifer *Brachionus plicatilis* (Taylor et al., 2005), and to soil ciliates *Colpoda steinii*, *Colpoda inflata* and *Cyrtolophosis elongata* (Diaz et al., 2006). However, data on the toxicity of Cu and Zn to photobacterium *Vibro fischeri* are contradictory: according to Mowat and Bundy (2002), Zn was more toxic than Cu, while Tsiridis et al. (2006) found that the toxicity of Zn was lower than that of Cu.

The 96-h LC_{50} of Zn for adult rainbow trout estimated in our laboratory (in water with the same characteristics that were used in the present study) was 3.8 mg/l (Svecevičius, 1999; Marčiulionienė et al., 2002). Thus, the Zn acute toxicity test showed that rainbow trout was four times more sensitive than leech. However, 30-d LC_{50} for leech is 3 mg/l, therefore lethal effects on leech were observed at a concentration lower than the lethal concentration for rainbow trout. The median lethal concentrations of Cu in a short-term exposure were similar for rainbow trout and for leech: 96-h LC_{50} for rainbow trout was 0.65 mg/l (Marčiulionienė et al., 2002), while for leech it was 0.84 mg/l. However, the 30-d LC_{50} of Cu for leech determined in our study was 0.09 mg/l, i. e. the value was 7.2 times lower than the 96-h LC_{50} for rainbow trout, indicating a high sensitivity of leech to Cu. The mixture toxicity index determined in our study for leech showed that the interaction of Zn and Cu in their mixture was partially additive. Additive effects were more pronounced in short-term tests, indicating a loss of the potential for additivity during a prolonged exposure. The interaction of Zn and Cu in mixtures varied greatly for various invertebrate species. The interaction was synergetic for nematode *Caenorhabditis elegans* (Jonker et al., 2004), partially additive for zebra mussel *Dreissena polymorpha* (Kraak et al., 1993), antagonistic for periwinkle *Tympanotonus fuscatus* (Otitoloju, 2002), and synergistic for bacteria *Escherichia coli* and *Vibro fischeri* (Preston et al., 2000; Tsiridis et al., 2006).

In conclusion, the assessment of lethal toxicity of heavy metals and other chemicals, based on short-term tests, cannot provide enough information needed for predicting the environmental impact on invertebrates. As the present study and investigations of other researchers show (Moles, 1998, 2001, Scarlett et al., 2007), the lethal effects of toxicants on invertebrates are more prolonged as compared with those on fish; therefore, long-term experiments are necessary. The further investigations of the sublethal effects of Cu and Zn and their mixture are necessary to evaluate the sensitivity of leech and to determine the mode of action of metals in mixtures.

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References

1. Ahsanullah M., Mobley M. C., Rankin P. 1998. Individual and combined effects of zinc, cadmium and copper on the marine amphipod *Allorchestes compressa*. *Australian J. Marine Freshwater Res*. Vol. 39(1). P. 33–37.
2. Diaz S., Martín-Gonzállez A., Gutiérrez J. C. 2006. Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. *Environ. Int*. Vol. 32(6). P. 711–717.
3. Ferrer L., Andrade S., Asteasuain R., Marcoveccio J. 2006. Acute toxicities of four metals on the early life stages of the crab *Chasmagnathus granulata* from Bahía Blanca estuary, Argentina. *Ecotoxicol. Environ. Safety*. Vol. 65(2). P. 207–209.
4. Gopalakrishnan S., Thilagam H., Raja P. V. 2007. Toxicity of heavy metals on embryogenesis and larvae of the marine sedentary polychaete *Hydroides elegans*. *Arch. Environ. Contam. Toxicol*. Vol. 52. P. 171–178.
5. Hamilton M. A., Russo R. C., Thurston R. V. 1977. Trimmed Spearmen–Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol*. Vol. 11. P. 714–719.
6. Jezierska B., Witeska M. 2001. *Metal toxicity to fish*. University of Podlasie: Siedlice. 316 p.
7. Jonker M. J., Sweijen R. A. J. C., Kammenga J. E. 2004. Toxicity of simple mixtures to nematode *Caernohabditis elegans* in relation to soil sorption. *Environ. Toxicol. Chem*. Vol. 23(2). P. 480–488.
8. Khalil M. A., Abdel-Lateif H. M., Bayoumi M., van Straalen N. M. 1996. Analysis of separate and combined effects of heavy metals on the growth of *Aporrectodea caliginosa* (Oligocheta; Annelida), using the toxic unit approach. *Applied Soil Ecol*. Vol. 4(3). P. 213–219.
9. Könemann H. 1981. Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. *Toxicology*. Vol. 19. P. 229–238.
10. Kraak M. H. S., Lavy D., Schoon H. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. *Environ. Toxicol. Chem*. Vol. 13. P. 109–114.
11. Marčiulionienė D., Montvydienė D., Kazlauskienė N., Svecevičius G. 2002. Comparative analysis of the sensitivity of test-organisms of different phylogenetic level and life stages to heavy metals. *Environ. Chem. Physics*. Vol. 24(2). P. 73–78.
12. Moles A. 1998. Sensitivity of ten aquatic species to long-term crude oil exposure. *Bull. Environ. Contam. Toxicol*. Vol. 61. P. 102–107.
13. Moles A. 2001. Changing perspectives on oil toxicity evaluation. In *Proceedings of the 2001 International Oil Spill Conference (Global Strategies for Prevention, Preparedness, Response, and Restoration)*. Am. Petrol. Inst.: Washington DC. P. 435–439.
14. Moore J. W., Ramamoorthy S. 1984. *Heavy Metals in Natural Waters*. New York Inc: Springer Verlag. 268 p.
15. Mowat F. S., Bundy K. J. 2002. Experimental and mathematical / computational assessment of the acute toxicity of chemical mixtures fom the Microtox assay. *Advances Environ. Res*. Vol. 6(4). P. 547–558.
16. Otitoloju A. A. 2002. Evaluation of the joint-action toxixcity of binary mixtures of heavy metals against the mangrove periwinkle *Tympanotonus fuscatus* var. *radula* (L.). *Ecotoxicol. Environ. Safety*. Vol. 53(3). P. 404–415.
17. Petrauskienė L. 2003. Water and sediment toxicity assessment by use of behavioural responses of medicinal leech. *Environ. Int*. Vol. 28. P. 729–736.
18. Petrauskienė L. 2004. The medicinal leech as a convenient tool for water toxicity assessment. *Environ. Toxicol*. Vol. 19(4). P. 36–341.
19. Petrauskienė L., Daniulytė G. 1996. Lethal and sublethal effects of heavy metal mixture on rainbow trout. *Ekologija*. Nr. 1. P. 7–12.
20. Preston S., Coad N., Townend J., Killham K., Paton G. I. 2000. Biosensing the acute toxicity of metal interactions: are they additive, synergistic, or antagonistic? *Environ. Toxicol. Chem*. Vol. 19(3). P. 775–780.
21. Scarlett A., Rowland S. J., Canty M., Smith E. L., Galloway T. S. 2007. Method for assessing the chronic toxicity of marine and estuarine sediment-associated contaminants using the amphipod *Corophium volutator*. *Marine Environ. Res*. Vol. 63(5). P. 457–470.
22. Schüürmann G., Market B. (eds.). 1998. *Ecotoxicology: Ecological Fundamentals, Chemical Exposure, and Biological Effects*. Berlin: Spektrum, Akd. Verl. 900 p.
23. Svecevičius G. 1999. Acute toxicity of zinc to common freshwater fishes of Lithuania. *Acta Zool. Lituanica*. Vol. 9. P. 114–118.
24. Taylor R. L., Caldwell G. S., Bentley M. G. 2005. Toxicity of algal-derived aldehydes to two invertebrate species: Do heavy metal pollutants have a synergistic effect? *Aquat. Toxicol*. Vol. 4(1). P. 20–31.

25. Tsiridis V., Petala M., Samaras P., Hadjispyrou S., Sakellapropoulos G., Kungolos A. 2006. Interactive toxic effects of heavy metals and humic acidis on *Vibrio fischeri*. *Ecotoxicol. Environ. Safety*. Vol. 63(1). P. 158–167.
26. Флеров Б. А. 1989. *Эколого-физиологические аспекты токсикологии пресноводных животных*. Ленинград: Наука. 144 с.

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CINKO, VARIO IR JŲ MIŠINIO LETALUS POVEIKIS MEDICININEI DĖLEI (*HIRUDO VERBANA*)

Santrauka

Medicininės dėlės (*Hirudo verbana*) buvo veikiamos 30 dienų (d.) Zn, Cu ir jų ekvitoksinio mišinio tirpalais. Nustatytos LC_{50} šioms poveikio trukmėms: 48 val., 96 val., 10, 20 ir 30 d. Dėlės žuvo per visas 30 dienų. Metalų letalus poveikis dėlėms buvo labiau ištęstas laike, lyginant su poveikiu žuvims, todėl dėlių jautrumui įvertinti reikalingi ilgalaikiai bandymai. Dėlės buvo daug jautresnės variui nei cinkui. Cinko ir vario mišinyje nustatyta šių metalų iš dalies adityvi sąveika. Adityvus poveikis buvo ryškesnis esant trumpoms ekspozicijoms (48 val. – 10 d.), nei ilgoms (20–30 d.).

Raktažodžiai: cinkas, ilgalaikis poveikis, medicininė dėlė, sunkiųjų metalų mišinys, varis