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THE EFFECT OF MERCURY, COPPER AND CADMIUM DURING SINGLE AND COMBINED EXPOSURE ON OXYGEN CONSUMPTION OF *ONCORHYNCHUS MYKISS* WAL. AND *CYPRINUS CARPIO* L. LARVAE

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ABSTRACT. The oxygen consumption of common carp and rainbow trout larvae exposed to mercury, cadmium and copper was measured. The experiment was performed on seven-month-old common carp (0.9-1.39 g) and one-month-old rainbow trout larvae (1.2-1.5 g) reared under laboratory conditions. The fish were treated for one hour with solutions of a single metal or mixtures of Hg+Cd, Cd+Cu or Hg+Cu. The concentrations of single metals or mixtures were 0.025, 0.05, 0.1 and 0.2 mg l^{-1}. Both single metals and mixtures reduced oxygen consumption in a concentration-dependent way. The results indicate that the oxygen consumption rate is a reliable indicator of metal toxicity to fish. The levels of oxygen consumption decrease indicate that the rankings of metal toxicity for the given nominal trace metal concentration are Hg+Cu > Cu > Cd+Cu > Cd+Hg > Cd for common carp and Hg+Cu > Cu > Cd+Hg > Hg for rainbow trout. The results show that copper is most toxic to both fish species. The mixtures are more toxic than single metals are and cause a greater reduction in oxygen consumption. Common carp larvae are apparently more sensitive to cadmium, while rainbow trout is more sensitive to mercury.

Key words: *CYPRINUS CARPIO, ONCORHYNCHUS MYKISS*, FISH, HEAVY METALS, OXYGEN CONSUMPTION, METAL MIXTURES

INTRODUCTION

Most studies of the effect of metals on fish address exposure to a single metal. Polluted water bodies, however, usually contain elevated levels of various metals. Therefore, the results obtained for exposure to a single metal in laboratory studies are hardly comparable with those from natural conditions.

To explain the interactions among various metals it is necessary to compare their effects separately and in mixtures. Theoretically, they may be antagonistic, additive or synergistic.

The results of survival tests indicate that metal mixtures are usually more toxic than single metal solutions and their action is synergistic (Lewis 1978, Khangarot 1981, Khangarot et al. 1981, Roy and Campbell 1995, Petrauskienė and Daniulytė 1996, Marr et al. 1998). However, the results obtained by Verma et al. (1982) showed that various mixtures of zinc, chromium and nickel were toxic to striped dwarf catfish *Mystus vittatus* in a synergistic or antagonistic way.

It seems that interactions among various metals are related to their competitive uptake from the environment and to different distribution in fish tissues. Many data show that certain metals affect the accumulation of other metals in fish (Allen 1994, 1995, Pelgrom et al. 1992, 1994, 1995, Ribeyre et al. 1995).

According to Reader et al. (1989), the mixture of Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn reduced survival of brown trout, *Salmo trutta* L. larvae, lowered levels of body Na^{+}, K^{+}, and Ca^{2+} ions and impaired bone calcification more than exposure to single metals did. Reduced body ion level usually results from their inhibited uptake by the gills. The gills are obviously very susceptible to waterborne metals, and often show various metal induced lesions. This leads not only to osmotic imbalance but may also impair respiratory functions. According to Petrauskienè and Daniulytè (1996), rainbow trout respiration rate was one of the parameters which were most sensitive to lethal and sublethal intoxication with metal mixtures. Vosylienè et al. (1999) also reported that respiratory functions were very susceptible to metal intoxication. Sublethal exposures often result in a reduced oxygen consumption rate (Skidmore and Tovell 1972, Radhakrishnaiah et al. 1993, De Boeck et al.1995).

Therefore, it was interesting to determine if the differences in single and mixed metal toxicity could be evaluated by measuring the oxygen uptake of exposed fish. The aim of the present study was to compare the effect of mercury, cadmium and copper used in single and mixed solutions on the oxygen consumption of common carp and rainbow trout larvae.

MATERIAL AND METHODS

The experiment was performed on common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* Wal.) larvae. The fishes were obtained from the Inland Fisheries Institute, Poland.

Incubation took place under conditions described by Ługowska and Jezierska (2000) for common carp and by Jezierska and Sarnowski (1999) for rainbow trout larvae. Seven-month-old common carp larvae (0.9-1.39 g) were held before the experiment under laboratory conditions at 22°C and one-month-old rainbow trout larvae (1.2-1.5 g) at 18°C.

Dechlorinated tap water was used (dissolved oxygen (DO) saturation about 90%, hardness 210 mg $CaCO_3$ l^{-1}, pH 6.7).

The measurements were done in 1 l respirometers containing tap water or heavy metal solutions. The solutions of Hg, Cd, and Cu were made using $HgCl_2$, $CdCl_2$, and $CuSO_4$, respectively. The same concentrations of heavy metals 0.025, 0.05, 0.1 or 0.2 mg l^{-1} for single metals and Hg+Cd, Cd+Cu and Hg+Cu mixtures were used. Preliminary studies showed that 96 LC_{50} values for carp larvae were Hg – 0.13, Cd – 0.7, and Cu – 0.5 mg l^{-1}. Therefore, the concentrations used in the present experiment were below these lethal levels. The fishes were exposed in the respirometers for one hour. After the experiments, each fish was weighed individually to the nearest 0.01 g. The exposure in each concentration of toxicant was carried out in 10 replicates. The dissolved oxygen content was measured to the nearest 0.01 mg l^{-1} using a Hanna HI 9143 DO-meter. The oxygen consumption of each fish was calculated from the difference of DO levels in the respirometers at the beginning and at the end of exposure, and expressed per g of body weight.

Statistical analysis was done using the Statistica package and the significance of differences was tested using one way ANOVA ($P \leq 0.05$).

RESULTS

The oxygen consumption of common carp and rainbow trout larvae in various metal solutions are shown in Table 1. No statistically significant differences were observed at the concentration of 0.025 mg l^{-1}, but mercury and cadmium-exposed common carp larvae took up slightly more oxygen and those treated with copper took up slightly less than the controls. Oxygen consumption by rainbow trout larvae at this concentration was similar to that of the control.

The mixtures Cd+Hg, Cd+Cu, and Hg+Cu at concentrations of 0.0125 + 0.0125 mg l^{-1} did not significantly change oxygen consumption, but the fish exposed to Hg+Cu used slightly less oxygen in comparison to the control.

At all the concentrations from 0.05 mg l^{-1} the oxygen uptake rate was reduced in both fish species. Of the single-metal treatments, copper seems to be the most toxic as it caused the greatest reduction in respiratory rate.

All the metal mixtures also reduced the oxygen uptake by common carp and rainbow trout. The highest decline was observed for the Hg+Cu mixture. Common carp larvae used only 0.34 mg O_2 h^{-1} g^{-1} in comparison to 0.52 mg O_2 h^{-1} g^{-1} in the control,

TABLE 1

Average oxygen consumption rates [mg O_2 h^{-1} g^{-1}] of metal – exposed common carp and rainbow trout larvae (* - significantly different from the control, $P \leq 0.05$).

Species	Control	Hg	Cd	Cu	Cd + Hg	Cd + Cu	Hg + Cu
		Concentration: 0.025 mg l^{-1}			0.0125 + 0.0125 mg l^{-1}		
Common carp	0.53	0.69	0.68	0.46	0.58	0.55	0.49
Rainbow trout	0.74	0.72	0.76	0.70	0.72	0.74	0.68
		Concentration: 0.05 mg l^{-1}			0.025 + 0.025 mg l^{-1}		
Common carp	0.52	0.49	0.54	0.45	0.42	0.40	0.34*
Rainbow trout	0.72	0.65	0.70	0.64	0.63	0.68	0.61*
		Concentration: 0.1 mg l^{-1}			0.05 + 0.05 mg l^{-1}		
Common carp	0.52	0.45	0.53	0.38*	0.38	0.36*	0.31*
Rainbow trout	0.72	0.59*	0.68	0.54*	0.56*	0.67	0.52*
		Concentration: 0.2 mg l^{-1}			0.1 + 0.1 mg l^{-1}		
Common carp	0.65	0.58	0.47*	0.26*	0.34*	0.32*	0.28*
Rainbow trout	0.72	0.50*	0.61	0.45*	0.46*	0.58	0.40*

while rainbow trout larvae used 0.61 mg O_2 h^{-1} g^{-1} in comparison to 0.72 mg O_2 h^{-1} g^{-1} in the control. Both differences were statistically significant.

Among the 0.1 mg l^{-1} solutions, copper was again the most toxic, significantly reducing oxygen uptake by common carp and rainbow trout larvae. It was followed by mercury, which reduced the respiratory rate of rainbow trout from 0.72 mg O_2 h^{-1} g^{-1} (control) to 0.59 mg O_2 h^{-1} g^{-1}.

All the mixtures caused significant reductions of oxygen consumption in the fish, but the common carp larvae were most affected by both the copper-containing mixtures Cd+Cu and Hg+Cu. Oxygen consumption declined to 0.36 and 0.31 mg O_2 h^{-1} g^{-1}. For rainbow trout, the mercury-containing mixtures Hg+Cu and Cd+Hg were the most toxic, reducing their oxygen uptake to 0.52 and 0.56 mg O_2 h^{-1} g^{-1}, respectively.

The 0.2 mg l^{-1} solutions caused further reduction of oxygen consumption by the fish. Common carp larvae took up considerably less oxygen in all the treatments in comparison to the control, and significant differences occurred in Cd and Cu solutions. Similar results were obtained for rainbow trout, but the differences were significant for Cu and Hg treatments. All the mixtures at concentrations of 0.2 mg l^{-1} caused significant reductions in oxygen uptake by common carp. It is noteworthy that in Cd+Cu and Hg+Cu treatments reduction exceeded 50%. Rainbow trout larvae also consumed much less oxygen than they did in the 0.1 mg l^{-1} mixtures, and reduction was insignificant only in Cd+Cu treatment, although the difference was also high – 0.58 mg O_2 h^{-1} g^{-1} in comparison to 0.72 mg O_2 h^{-1} g^{-1} in the control. In the other mixtures oxygen consumption was 0.46 mg O_2 h^{-1} g^{-1} (Hg+Cd) and 0.40 mg O_2 h^{-1} g^{-1} (Hg+Cu).

DISCUSSION

The results of the present study show that mercury, cadmium and copper reduced oxygen consumption by common carp and rainbow trout larvae in a concentration-dependent way. This confirms data obtained by other authors. A decline of oxygen uptake by common carp larvae after 1, 15 and 30-day exposures at 0.1 mg l^{-1} of mercury was reported by Radhakrishnaiah et al. (1993), and after two weeks of treatment with 0.22 mg l^{-1} of copper by De Boeck et al. (1995). According to Kazlauskienè et al. (1996), 0.25 mg l^{-1} of copper reduced oxygen consumption by rainbow trout. Skidmore (1970) reported a decrease of oxygen uptake rate in rainbow trout exposed to 40 mg l^{-1} of zinc.

Average oxygen consumption at each metal concentration as a percent of control values was calculated, which allows the effects of single and mixed treatments and the sensitivity of both fish species to be compared. Metal mixtures affected oxygen consumption of carp and rainbow trout considerably more than single metals. Copper combined with mercury was most toxic for both species, and these metals acted in a synergistic way; their joint effect on oxygen uptake was greater than the sum of the single effects. Metal mixtures at a concentration of 0.05 mg l^{-1} reduced oxygen consumption by 35% for carp and by 15% for rainbow trout, in comparison to the control. At 0.1 mg l^{-1} oxygen consumption rates were lower by about 40 and 28%, respectively. The highest studied concentration, 0.2 mg l^{-1}, caused a decrease of oxygen consumption by about 57 and 44% for carp and rainbow trout, respectively. Copper exposure alone affected oxygen consumption only at the highest concentration of 0.2 mg l^{-1} and the reduction was similar to that observed in Cu+Hg treatment. The Cu+Cd mixture significantly reduced oxygen consumption only in common carp, also in a synergistic way. In rainbow trout, however, the action of these metals was antagonistic. Cd+Hg treatment reduced oxygen uptake, but synergism was observed only at the concentration of 0.2 mg l^{-1}.

The data of the present study allow the treatments to be arranged according to their effect on oxygen consumption rate in each fish species, for common carp it was Hg+Cu > Cu > Cd+Cu > Cd+Hg > Cd and for rainbow trout it was Hg+ Cu > Cu > Cd+Hg > Hg.

The results indicate that copper exerted the strongest influence on oxygen consumption by common carp and rainbow trout larvae. The very high toxicity of copper (single and in mixtures) was reported by Marr et al. (1998) for carp, by Kazlauskiene et al. (1999) for rainbow trout and by Lewis (1978) for longfin dace, *Agosia chrysogaster*. According to Sayer et al. (1991), copper-containing metal mixtures caused the highest mortality, the greatest reduction of Na^+, K^+, and Ca^{2+} ions and the strongest impairment of bone calcification in brown trout, *Salmo trutta* L. Their results indicate that copper disturbed gill ion uptake. Pelgrom et al. (1995) and Laurént and McDonald (1986) also observed the reduction of sodium uptake in copper-exposed fish.

The strong effect of copper on oxygen uptake observed in the present study also suggests gill disorders. Metals may induce various disturbances in fish gills. Excessive secretion and coagulation of mucus impair gas exchange across the secondary lamellae epithelium (Pärt and Lock 1983, Handy and Eddy 1989). Metal accumulated in the epithelium may also reduce oxygen uptake (Youson and Neville 1987). Metal-induced gill lesions such as thickening and lifting of respiratory epithelium result in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult (Matthiessen and Brafield 1973, Evans et al. 1988, Benedetti et al. 1989, De Boeck et al. 1995, Dalzell and MacFarlane 1999).

The data presented in this paper show that short-term copper exposure resulting in a strong decline of oxygen consumption by the larvae of common carp and rainbow trout might have resulted from copper uptake by the gills and copper-induced epithelial lesions. Changes in oxygen consumption may be a reliable indicator of metal toxicity to fish. Mixtures of copper, cadmium and mercury are more toxic to common carp and rainbow trout than each metal alone, and among these metals copper is the most toxic one.

The results of our study also revealed interspecific differences in sensitivity to various metals. Common carp larvae are more sensitive to cadmium than rainbow trout, but the latter are more sensitive to mercury.

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STRESZCZENIE

WPŁYW RTĘCI, MIEDZI I KADMU NA ZUŻYCIE TLENU PRZEZ LARWY PSTRĄGA TĘCZOWEGO *ONCORHYNCHUS MYKISS* I KARPIA *CYPRINUS CARPIO*

W prezentowanej pracy badano zużycie tlenu przez 7-miesięczne larwy karpia (0,9-1,39 g) i 1-miesięczne larwy pstrąga tęczowego o podobnej masie (1,2 – 1,5 g). Ryby były eksponowane przez 1 godzinę w środowisku zawierającym jony rtęci, kadmu i miedzi pojedynczo lub w mieszaninach: Hg+Cd, Cd+Cu, Hg+Cu. Stężenia pojedynczych metali i ich mieszanin wynosiły odpowiednio: 0,025, 0,05, 0,1, 0,2 mg l^{-1}.

Metale działając pojedynczo, jak i w mieszaninach obniżają zużycie tlenu tym bardziej im większe jest ich stężenie. Uzyskane wyniki wskazują, że zużycie tlenu jest dobrym wskaźnikiem toksycznego działania metalu na ryby (tab. 1).

Na podstawie obniżenia zużycia tlenu można ustalić następujący ranking toksyczności metali: Hg+Cu > Cu > Cd+Cu > Cd+Hg > Cd dla karpia i Hg+Cu > Cu > Cd+Hg > Hg dla pstrąga. Wyniki wskazują, że miedź jest najbardziej toksyczna dla obydwu gatunków ryb. Mieszaniny są bardziej toksyczne niż pojedyncze metale i powodują większe obniżenie zużycia tlenu. Larwy karpia są wyraźnie bardziej wrażliwe na działanie kadmu, a pstrąga na działanie rtęci.

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