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Respiratory and hematological response of tench, *Tinca tinca* (L.) to a short-term cadmium exposure

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Abstract. The effects of 3 h exposure to 96hLC50 of cadmium (4.5 mg dm^{-3}) on oxygen consumption rate, and hematological parameters (RBC, WBC, erythrocyte and leukocyte pattern) of juvenile tench were evaluated. Oxygen consumption significantly decreased beginning from 24 h postexposure, and remained reduced until the end of the experiment (96 h postexposure). RBC gradually increased, together with the percentage of juvenile cells in circulation. On the other hand, cadmium induced damage to the red cells – the share of cellular anomalies significantly increased with time postexposure. They included abnormal cell shape, vacuolization, swelling, chromatin disintegration in the nucleus, and nucleus indentation. The exposed fish showed a gradual and significant decrease in WBC without a shift of lymphocyte/neutrophil proportion. No significant changes in thrombocyte count occurred. The results show that short-term exposure to cadmium reduced fish energetic metabolism, and suppressed immune abilities. The symptoms gradually developed after the end of exposure, and no recovery took place until 96 h.

Introduction

Fish gills comprise a large part of fish body that contacts the external environment, and they play an important role in the gas and ion exchange between the organism and environment. They are also an important way of uptake of heavy metal compounds into the organism. Thus the gills are the very first site where metal-induced lesions may occur which may result in an impaired gas and ion exchange. Subsequently, metal ions enter the blood in which they may affect the blood cells.

Cadmium is one of the most toxic heavy metals (after mercury and copper, according to Jones 1964; Hellawell 1989). It tends to accumulate in fish tissues, showing particular affinity to liver and kidney (Bentley 1991; Kock et al. 1996; Dallinger et al. 1997), and causes various physiological disturbances (review: Jezierska and Witeska 2001). Thus, even an incidental pollution of water with cadmium may pose a threat to fish.

The effects of cadmium on fish gills include excessive mucus secretion, changes in the chloride cells, various epithelial lesions, and the changes in lamellar blood circulation (Part and Svanberg 1981; Oronsaye and Brafield 1984; Karlsson-Norrgren et al. 1985; Yamawaki et al. 1986; Gill et al. 1988; Hofer et al. 1992; Lock et al. 1992; Battaglini et al. 1993; Pratap and Wendelaar Bonga 1993; Gargiulo et al. 1996).

From the gills, cadmium is taken up to the blood in which its level still increases after the end of exposure, when metal is no longer present in the water. In common carp exposed for 3 h to 10 mg dm^{-3} of Cd blood metal concentration reached the maximum (3.4 mg dm^{-3}) in 2 days postexposure (Witeska 2003), and in 4 days returned to the basal level.

In fish blood, cadmium causes damage to all types of blood cells, and induces plasma electrolyte imbalance. It produces morphological changes in red cells, affecting their cell membranes and nuclei (Gill and Pant 1986; Hofer et al. 1992; Witeska 2001; Witeska and Baka 2002; Witeska 2003). However, according to Houston and Keen (1984), cadmium has little effect upon circulating red cells but may profoundly reduce the ability to form new cells. Both, increased destruction and impaired production of red blood cells may result in anemia (Sjobeck et al. 1984; Beena and Viswaranjan 1987; Ruparelia et al. 1990; Saxena et al. 1992; Gill and Epple 1993). Cadmium almost always reduces the white blood cell count (Murad and Houston 1988; Ghazaly 1992; Saxena et al. 1992; Witeska 1998, 2003). It may increase lymphocyte mortality rate, and reduce the ability of phagocytes to intracellular killing (Witeska 2003).

Therefore, a short-term heavy metal exposure causes a rapid increase in metal levels primarily in the gills and blood, and in these tissues the earliest toxic effects may occur.

The aim of present study was to evaluate the acute effects of cadmium on oxygen consumption and hematological parameters of tench.

Materials and methods

The laboratory-reared 6 months old tench of 14.3 ± 5.9 g (mean $\pm$ SD) obtained from the hatchery of the Inland Fisheries Institute in Zabieniec were used in the experiment. Fifty fish were exposed for 96 h to the solutions of $CdCl_2$*$2.5H_2O$ of 0–20 mg dm^{-3} of Cd. The 96hLC50 was calculated using the probit method. For oxygen consumption measurements, 10 fish were exposed individually in 2.3 dm^3 aquaria for 3 h to 4.5 mg dm^{-3} of Cd (96hLC50). After the exposure the fish were quickly transferred to metal-free water. Oxygen consumption was measured using the Hanna Instruments HI 9314 dissolved oxygen meter, in 2.3 dm^3 closed respirometers. The measurements were done just before metal exposure (control), immediately after the end of it (Cd_0), and every 24 h postexposure, up to 96 h (Cd_{24}, Cd_{48}, Cd_{72}, Cd_{96}), in three series, keyed to A, B, and C. The series A included only the control, Cd_0, and Cd_{24} groups. The fish were starved during the experiment, and water temperature

was 19 ± 0.5 °C (mean ± SD). Each fish was individually weighed at the end of the experiment.

For blood analyses, 10 fish were exposed for 3 h to 4.5 mg dm^{-3} of Cd in 20 dm^3 aquaria, at 17 ± 0.5 °C. They were not fed during the experiment. Control fish were kept under similar conditions but in metal-free water. Blood (about 50 mm^3) was sampled from live fish by heart puncture immediately after the end of exposure (control, Cd_0), and then every 24 h postexposure, up to 96 h (Cd_{24}, Cd_{48}, Cd_{72}, Cd_{96}). Each fish was used only once. Red and white blood cell counts were calculated, and blood smears were done. The obtained results were subjected to the *U* test.

Results

No mortalities were observed during the experiment, and the fish did not show any visible symptoms of stress such as hyperactivity. No significant changes in oxygen consumption occurred during the cadmium exposures (Figure 1(a–c)). In all series, oxygen consumption by the control and Cd_0 fish was similar. A significant decrease was observed in 24 (series A and C) or in 72 (series B) hours from the end of exposure.

The results of hematological analyses (Table 1) show a gradual increase in red blood cell count (in Cd_{96} the value was significantly higher comparing to the control), accompanied by an increase in the percentage of juvenile cells (also in Cd_{96} significantly higher, comparing to the control). In metal-exposed fish (Cd_{96}) the frequency of erythrocyte cellular anomalies significantly increased (Table 1). They included (Figure 2): abnormal cell shape (a), often a 'spindle shape', vacuolization (b), 'bare nuclei' (c), 'empty cell'- swollen, with colorless cytoplasm, probably just before hemolysis (d), chromatin disintegration in the nucleus (e), nucleus indentation (f). Some of these changes increased the mortality rate of erythrocytes which is indicated by a significantly elevated percentage of hemolysed cells in circulation (Table 1, g in Figure 2).

White blood cell count gradually decreased beginning from Cd_{24}, and in Cd_{72} and Cd_{96} was significantly lower comparing to the control (Table 1). In the blood smears, lymphocytes and neutrophils were identified. The latter included mainly juvenile cells: myelocytes and metamyelocytes, very little mature polymorphonuclear forms were observed. No significant differences in proportions of these two cell types between the control and metal-exposed fish were found. The count of thrombocytes also did not significantly differ between the intoxicated and control fish (Table 1).

Discussion

In the present study, no significant changes in oxygen consumption were observed immediately after the end of exposure which might have been a

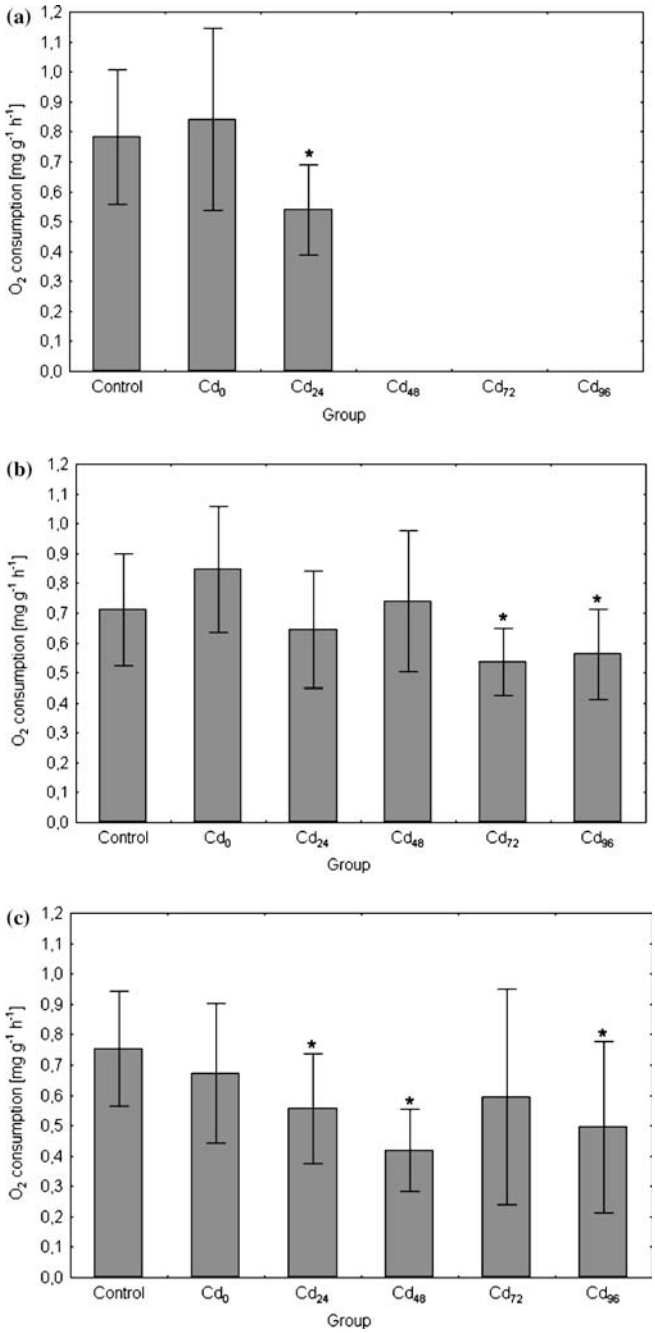


Figure 1. The effect of short-term cadmium exposure on oxygen consumption of tench measured in three series: A, B, and C (mean ± SD, $n = 10$, *values significantly different from the control, $p \leq 0.05$).

Table 1. The effects of short-term exposure to cadmium on hematological parameters in tench (mean ± SD, $n = 10$, *values significantly different from the control, $p \leq 0.05$).

Parameters	Experimental groups					
	Control	Cd_0	Cd_{24}	Cd_{48}	Cd_{72}	Cd_{96}
RBC (10^6 mm^{-3})	1.34 ± 0.33	1.28 ± 0.31	1.41 ± 0.38	1.50 ± 0.53	1.71 ± 0.25	1.89 ± 0.38*
Abnormal erythrocytes (%)	1.2 ± 0.7	3.3 ± 1.2*	2.6 ± 1.5*	2.8 ± 2.3	4.9 ± 2.1*	5.8 ± 2.1*
Hemolysed erythrocytes (%)	3.9 ± 3.5	3.9 ± 1.7	2.3 ± 1.7	0.8 ± 1.7	2.0 ± 1.0	7.8 ± 4.7*
Juvenile erythrocytes (%)	0.5 ± 0.4	0.1 ± 0.2	0.4 ± 0.6	0.8 ± 1.1	2.8 ± 4.6	5.0 ± 6.4*
WBC (10^3 mm^{-3})	44.6 ± 17.2	45.7 ± 19.9	30.0 ± 14.1	31.2 ± 14.4	25.0 ± 12.2*	23.5 ± 9.2*
Lymphocytes (%)	96.3 ± 4.5	95.8 ± 3.4	98.1 ± 2.5	96.0 ± 2.1	99.0 ± 1.6	98.0 ± 2.3
Neutrophils (%)	3.6 ± 4.6	4.2 ± 3.5	1.9 ± 2.6	4.0 ± 2.1	1.0 ± 1.6	2.0 ± 2.4
Thrombocytes (10^3 mm^{-3})	4.1 ± 5.5	7.2 ± 4.4	2.7 ± 2.6	4.3 ± 3.2	3.4 ± 3.0	6.1 ± 5.9

combined effect of both, increased oxygen demand and attempts of fish to meet it, and reduced ability of oxygen uptake due to the adverse effect of metal on gill epithelium. Slight increase or decrease of oxygen consumption by the fish in various series of the experiment might have resulted from these two reactions, and individual variability of the fish.

A significant decrease in oxygen consumption which indicates toxic effect of cadmium on respiratory functions took place in 24 (series A and C) or in 72 (series B) hours from the end of exposure. There are many data indicating that heavy metals may induce either an increase or a decrease in oxygen consumption by the fish (review: Jezierska and Witeska 2001). According to Atchison et al. (1987), and Diamond et al. (1990), metals may cause respiratory disturbances, often resulting in an increase in ventilation rate. This may be caused by agitation and hyperactivity in polluted environment that cause an increased oxygen demand. Heavy metal exposure may also induce epithelial lesions. Such disturbances were observed by various authors in the cadmium-exposed fish (Part and Svanberg 1981; Oronsaye and Brafield 1984; Karlsson-Norrgren et al. 1985; Yamawaki et al. 1986; Gill et al. 1988; Hofer et al. 1992; Lock et al. 1992; Battaglini et al. 1993; Pratap and Wendelaar Bonga 1993; Gargiulo et al. 1996). Reduction of oxygen consumption by *Channa punctata* during both, acute and chronic cadmium exposures was reported by Sastry and Shukla (1994).

A decrease of oxygen consumption in 24 h postexposure or later indicates that cadmium injured fish gills, and disturbances in oxygen uptake persisted and developed when cadmium was no longer present in the environment but was still present in the organism, even in 96 h after the end of exposure.

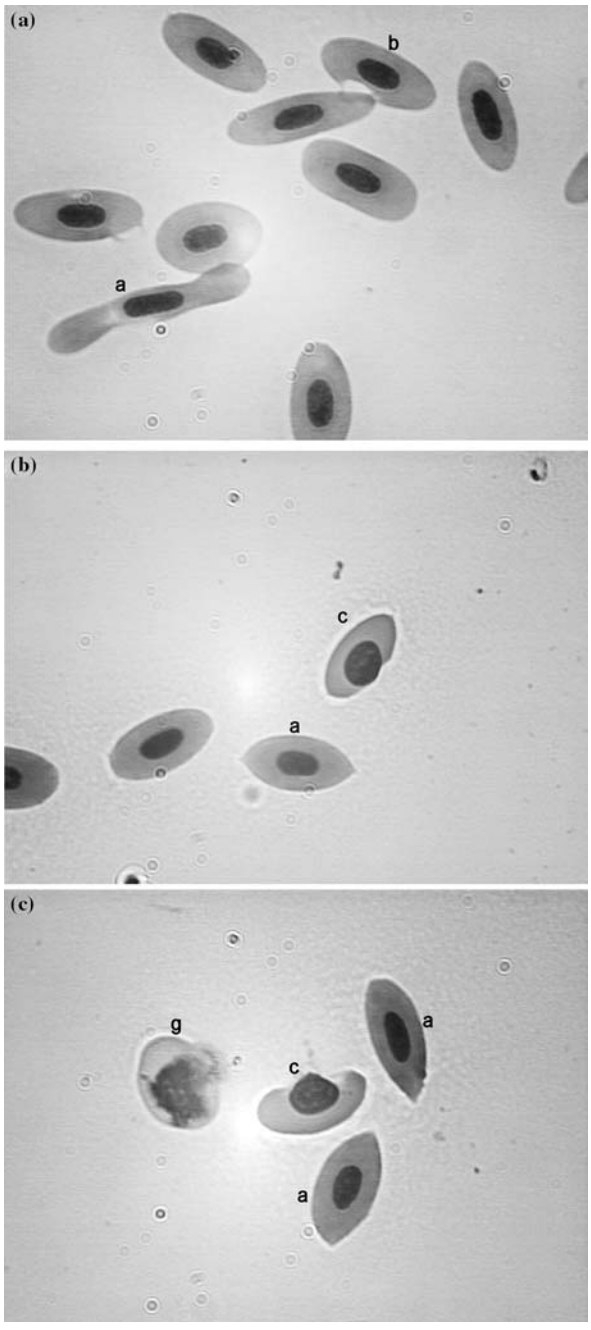
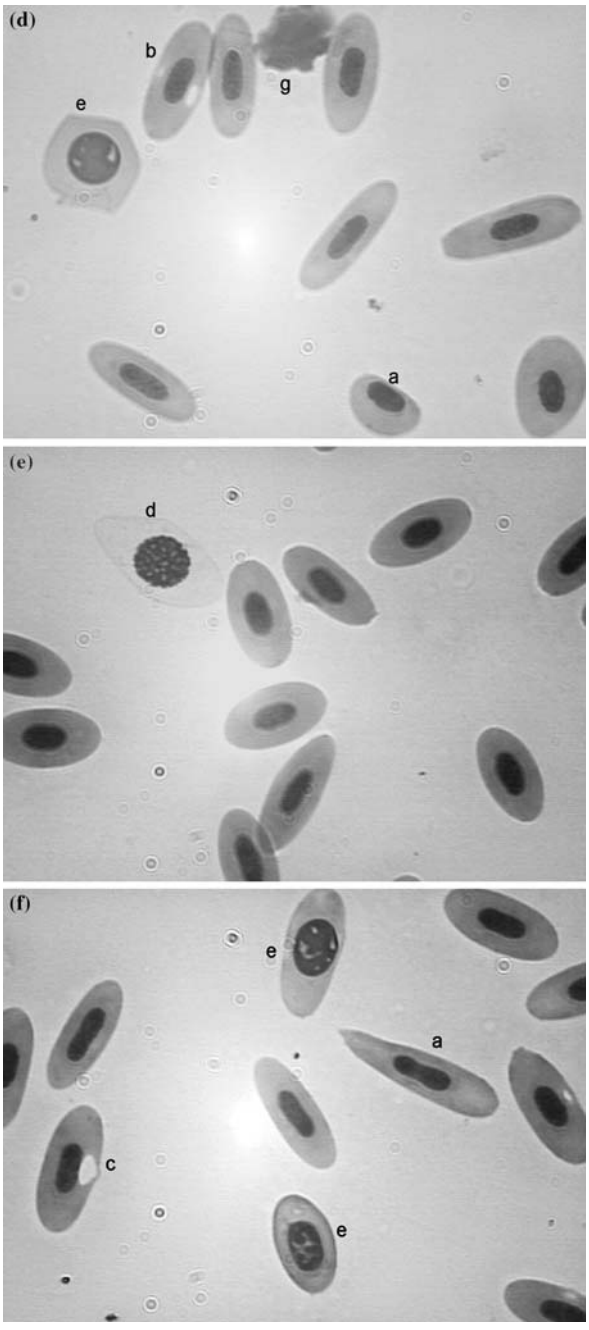


Figure 2. Cellular anomalies in erythrocytes of tench subjected to cadmium exposure (a – cell malformation, b – cytoplasm vacuolization, c – 'bare nucleus', d – cell swelling and hemoglobin disintegration, e – chromatin disintegration, f – nucleus indentation, g – hemolysis).



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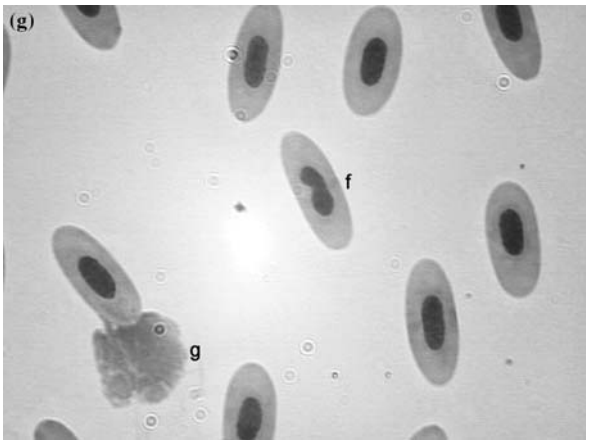

Figure 2. Continued.

The gradual increase in red blood cell count, together with the results concerning oxygen consumption, and an increase in proportion of juvenile red blood cells in circulation, indicate enhanced erythropoiesis due to hypoxia caused by metal-induced gas exchange impairment. At the same time, the pecentage of abnormal cells increased. Morphological changes in erythrocytes often occur in fish as a result of intoxication with various inorganic or organic poisons (Sorensen and Bauer 1983; Fletcher and White 1986; Gill and Pant 1987; Shandilya and Banerjee 1989; Boge and Roche 1996; Vosyliene 1996; Zeni et al. 2002). According to Vosyliene (1999) and Zeni et al. (2002), morphology of erythrocytes is one of the most specific and sensitive indicators of the effect of various environmental factors on fish. Own data (Witeska 2001, 2003) obtained for common carp subjected to 10 mg dm^{-3} of Cd for 3 h showed also considerable morphological anomalies in these cells (mainly nuclear malformations, cell malformations, and divisions). According to Gill and Pant (1986), the nucleus is the main target of metal action upon the cells. On the other hand, cadmium may substitute for calcium in cell membranes and probably render them more susceptible to destruction.

Cadmium usually induces anemia in fish (Beena and Viswaranjan 1987; Ruparelia et al. 1990; Saxena et al. 1992; Gill and Epple 1993; Mukherjee and Sinha 1993). However, according to Vosyliene (1999), the red blood cell system of fish is quite stable, and shows considerable compensatory abilities. In the present study an enhanced erythropoietic rate indicated by an increase in the percentage of juvenile erythrocytes probably induced by hypoxia, compensated for increased cell destruction by cadmium, and resulted in a gradual increase in red blood cell count. An increase in RBC in Cd-intoxicated fish was reported by Tort and Hernandez-Pascual (1990); Tort and Torres (1988); Morsy and Protasowicki (1990); Saravanan and Natarajan (1991); Ghazaly (1992), and

Witeska and Jezierska (1994), and an increase in proportion of juvenile erythrocytes in blood was observed by Srivastava and Mishra (1979), and Beena and Viswaranjan (1987).

The gradual decrease in white blood cell count indicates possible impairment of immune mechanisms in the intoxicated fish. Leukopenia is a commonly observed effect of cadmium intoxication (Murad and Houston 1988; Morsy and Protasowicki 1990; Ghazaly 1992, Saxena et al. 1992; Witeska 1998, 2003). Cadmium impairs also the activities of all white blood cells. Reduction of *Dicentrarchus labrax* phagocyte adherence by cadmium was reported by Lemaire-Gony et al. (1995), while at higher Cd concentrations production of reactive oxygen compounds was depressed both, *in vivo* and *in vitro* (Witeska 2003). A decrease in proliferation of mitogen-stimulated lymphocytes from Cd-exposed rainbow trout was observed by Thuvander (1989). According to Viale and Calamari (1984), cadmium slightly reduced humoral immune response in rainbow trout. Viola et al. (1996) reported Cd-induced suppression and increased mortality of *Ictalurus melas* NK cells *in vitro*.

In the present study no significant changes in thrombocyte count were observed. Similar result for Cd-treated eel was reported by Gill and Epple (1993), while other authors observed either thrombocytosis (Srivastava and Mishra 1979; Garofano and Hirshfield 1982; Sjobeck et al. 1984), or trombocytopenia (Murad and Houston 1988). Both reactions may be explained with stress – which usually causes an increase in thrombocyte count, and acceleration of hemostasis (Casillas and Smith 1977), but on the other hand – may probably promote cortisol-induced destruction of thrombocytes (Al-Akel and Shamsi 1996).

The obtained results show that oxygen consumption and hematological parameters did not change immediately after the end of acute cadmium exposure, but developed later, when metal was no longer present in the water. Cadmium probably injured fish gills and damaged erythrocytes which resulted in gas exchange disturbances. Increase in erythropoietic rate and red blood cell count did not fully compensate for a reduced oxygen consumption. Among the blood parameters of tench, red blood cell morphology and white blood cell count seem particularly sensitive to intoxication. At the same time, a substantial reduction in white blood cell count shows that cadmium might have caused a suppression of immune mechanisms. Thus, even a short-term water pollution with cadmium may result in severe physiological disturbances in fish organism that develop and persist when metal is no more present in the water.

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