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Sublethal Toxicity and Accumulation of Cadmium in *Tilapia aurea*

S. E. Papoutsoglou and P. D. Abel

Department of Animal Production, Laboratory of Applied Hydrobiology, Agricultural University of Athens, 75 Iera Odos, Votanikos, 11855 Athens, Greece

Study of the sublethal toxicity of pollutants to fish has mainly been confined to North American and North European species, many of them of little direct commercial importance. Tilapia spp. are important food fishes in many countries and are increasingly important in aquaculture. Relatively little information exists on their susceptibility to many pollutants. A recent study (Abel & Papoutsoglou 1986) indicated that Tilapia aurea was among the more resistant species to cadmium, in terms of lethal toxicity of the metal. The present paper reports an investigation into the sublethal toxicity of cadmium in Tilapia aurea.

MATERIALS AND METHODS

Juvenile T. aurea from a commercial fish farm were acclimated in the laboratory for 2 weeks. Batches of 150 fish were drawn randomly from a population of mean weight 3.33g (s.d. 0.68g), placed in each of five glass aquaria of 250-L capacity, and allowed a further 2 weeks to acclimate to the experimental conditions before dosing with cadmium was begun. The characteristics of the dilution water were monitored daily during the first week of the experiment and thereafter three times per week, and are summarized in Table 1. This water was conditioned municipal tap water drawn from the laboratory 50-ton recirculating system (dechlorinated, filtered and UV-sterilised).

Cadmium was administered as $Cd(NO_3)_2.4H_2O$ (AR grade), at nominal concentrations of 0.1, 0.05, 0.02, 0.01 mg L^{-1} and control, under continuous-flow conditions. Dilution water was supplied to each tank at a rate sufficient to change 95% of the test solution within 20 hrs (see nomograph in Sprague 1969). Toxicant solution was supplied at an appropriate rate from stock bottles which were replenished daily. The toxicant delivery system was comprised of modified Marriotte bottles under pressure from the laboratory's aeration system, which supplies filtered oil-free compressed air at almost constant pressure. Cadmium concentrations in the test tanks were monitored daily for the

Send reprint requests to Prof. S.E. Papoutsoglou at the above address.

first 7 days and thereafter three times per week. Samples were taken in acid-washed polythene bottles, acidified with sufficient

Table 1. Characteristics of the dilution water.

Determinand	Mean	Range	Std. deviation	Coefficient of variation %
Temperature ^{o}C	21.4	19.24	1.47	6.87
pH	7.68	7.4-8.02	0.20	2.55
Total alkalinity (mg l^{-1} $CaCO_3$)	112	96-121	6.3	5.60
Hardness (mg L^{-1} $CaCO_3$)	145	134-158	8.3	5.65

AR grade HCl (a few drops) to reduce the pH to approximately 1, and measured using a Perkin-Elmer atomic absorption spectrophotometer. The accuracy of the determination was checked periodically by analysing ten replicate sub-samples of a solution containing 0.02 mg Cd L^{-1}. Overall the procedure gave a mean value of 96% of the nominal concentration, with a coefficient of variation of 2.6%. On a small number of occasions (between 2 and 4 per tank) when the dosing system failed, cadmium stock solution was added and mixed thoroughly to restore the test solution to its correct strength.

Fish were fed daily to satiation on commercial pelleted food, administered in three lots between 8 a.m. and 6 p.m. each day. Fish were not fed on days prior to weighing. The amount of food taken was approximately 8% of the total wet weight of fish per day. Fecal matter and excess food were removed by regular siphoning. Each tank was also fitted with a power-driven filter.

The experiment was continued for 16 weeks. Fish were individually weighed and measured every 2 weeks, and returned to the experimental tanks. At the end of the experiment, the following operations were carried out. Fish from each tank were stunned, sectioned and blood samples taken from the heart. Haematocrit was measured using standard microhaematocrit techniques (Baker & Silverton, 1976). Haemoglobin content was determined using a Coulter haemoglobinometer. Blood smears were taken and stained with Giemsa's stain for visual examination of blood cell morphology. Haematocrit determinations were also made on ten fish from each tank taken after ten weeks. A small number of blood samples were rejected, as the results indicated that they were contaminated with water or other body fluids. Ten

fish from each tank were used for each haematological determination.

Thirty fish from each tank were used to determine the cadmium content of the muscle tissue. Ten samples were obtained from each tank, each being a composite of three individuals. The semi-frozen fish were skinned, and entire muscle fillets removed from each side of each fish using plastic implements. The tissue samples were weighed and wet-digested in a 50:50 mixture of concentrated HNO_3 and hydrogen peroxide at 160°C (FAO/SIDA 1983). The digests were made up to constant volume with deionized water and analyzed by atomic absorption spectrophotometry.

Seventy fish from each tank were used to determine the total moisture, protein, fat and ash contents of the fish. Total moisture was determined by drying the homogenised fish tissue to constant weight at 70°C. Protein (N x 6.25) was determined by the standard Kjeldahl procedure (Pearson, 1976). Total fat was determined by the method of Folch et al (1957), and ash content by igniting dried tissue to constant weight at 500°C.

RESULTS AND DISCUSSION

Cadmium concentrations in the test tanks are summarized in Table 2 (the data excludes the few occasions when the dosing system failed). Since the measured concentrations are considerably less than the nominal concentrations, all cadmium concentrations hereafter referred to are the mean measured concentrations shown in Table 2.

Table 2. Levels of cadmium measured by atomic absorption spectrophotometry in the test tanks. No. of determinations = 20. All figures are $\mu g\ L^{-1}$ N.D. = not detectable. (Detection limit 1 $\mu g\ L^{-1}$).

Nominal	Measured concentration	
concentration	Mean	Range
100	52	41-64
50	28	23-32
20	14	11-17
10	6.8	4.9-8.1
Control	1.5	N.D-2.4

Mortalities among the test fish during the experiment were low (Table 3) and not related to poison concentration. The cadmium appeared to have no effect on the growth (Fig. 1, Table 3),

or on the moisture, fat, protein or ash content or haemoglobin concentration of the fish (Table 3). Haematocrit values of fish examined after 10 weeks exposure showed a general decline with increasing cadmium concentration, with a statistically significant reduction occurring at Cd concentrations between 14 and 28 g l^{-1} (Fig. 2). However in fish examined after 16 weeks' exposure, this trend was no longer apparent (Table 3). Examination of stained blood smears showed that cadmium-exposed fish had a generally higher proportion of degenerated erythrocytes (Table 3). These cells typically appeared as "ghost" cells, i.e. consisting of a cell membrane and nucleus, and empty of cytoplasm. However, because of the high variability between individuals in the frequency of occurrence of "ghost" cells, the increased incidence is not statistically significant. Levels of cadmium in muscle tissue at the end of the experiment showed an increase with concentration of cadmium in the water (Table 3). Of the variables measured, only the haematocrit value after 10 weeks' exposure and the muscle cadmium concentrations indicate the effects of cadmium at the concentrations tested.

Growth rate, in spite of its ecological and commercial importance, is frequently not a good indicator of sublethal toxic effect in fish (Sprague, 1971), and there are even some reports of pollutants causing increased growth rate (Pickering, 1968; McLeay and Brown, 1974), though the mechanism of this is not clear. Pickering and Gast (1972) reported that cadmium concentrations up to 110 µg L^{-1} had no effect on growth in Pimephales promelas, though lower concentrations exerted other adverse effects. Haematological parameters are widely used in assessing sub-lethal toxicity, though their biological significance is often unclear. For example, although cadmium appears to reduce haematocrit values in T. aurea, it is reported that in Salmo gairdneri 6.4 µg L^{-1} Cd caused an increase in haematocrit (Majewski and Giles, 1981). An obvious possibility is that the observed response may be dependent upon the duration of exposure. Clearly a reduced haematocrit value (or indeed any other sublethal response to toxicity) will take some time to manifest itself. Thereafter the fish may regain normal haematocrit levels owing to the effects of compensatory physiological mechanisms. In the present experiment, the observed reduction in haematocrit values which was evident after 10 weeks' exposure was not observable after 16 weeks' exposure.

As Tilapia species are exploited and cultured for human consumption in many countries, the importance of accumulation of cadmium in the muscle tissue is obvious. In many species, cadmium accumulates to higher concentrations in liver, gill and kidney tissue than in muscle (Alabaster and Lloyd, 1980). Consequently accumulation in muscle, which is important from the point of view of human health, has received little attention. In fact the muscle cadmium levels recorded in this experiment are sufficiently high to cause concern. Although few national or international authorities have so far set limits for cadmium in foodstuffs, for lack of accurate information, the World Health

Table 3. Values of some variables measured after 16 weeks. Figures in parentheses are 95% confidence limits.

Cd conc^{n} µg L^{-1}	52	28	14	6.8	Control
Mean weight g	7.60 (7.2-8.0)	7.91 (7.40-8.42)	7.33 (7.13-7.53)	7.74 (7.52-7.96)	7.28 (6.86-7.70)
Mean length cm.	7.66 (7.41-7.91)	7.61 (7.33-7.89)	7.52 (7.22-7.82)	7.92 (7.56-8.28)	7.48 (7.18-7.78)
% mortality	4.0	2.7	2.7	4.7	6.7
Mean moisture %	70.1	70.9	70.9	70.1	70.9
Mean fat % dry wt.	22.3	25.4	28.3	24.4	25.5
Mean protein % dry wt.	57.8	57.4	55.5	57.8	57.0
Mean ash % dry wt.	19.9	19.2	19.1	17.8	17.5
Haematocrit %	29.8 (23.5-36.1)	25.7 (20.6-30.8)	34.3 (28.5-40.1)	31.2 (28.8-33.6)	32.0 (26.7-37.3)
% damaged erythrocytes	9.2	3.2	5.0	3.9	1.0
Haemoglobin g $100mL^{-1}$	10.29 (9.25-11.33)	9.94 (8.26-9.42)	9.50 (8.58-10.42)	8.48 (7.06-9.90)	10.08 (8.66-11.50)
Muscle Cd mg kg^{-1} net weight.	0.92 (0.78-1.10)	0.72 (0.57-0.86)	0.23 (0.20-0.27)	0.12 (0.09-0.14)	0.06 (0.04-0.12)

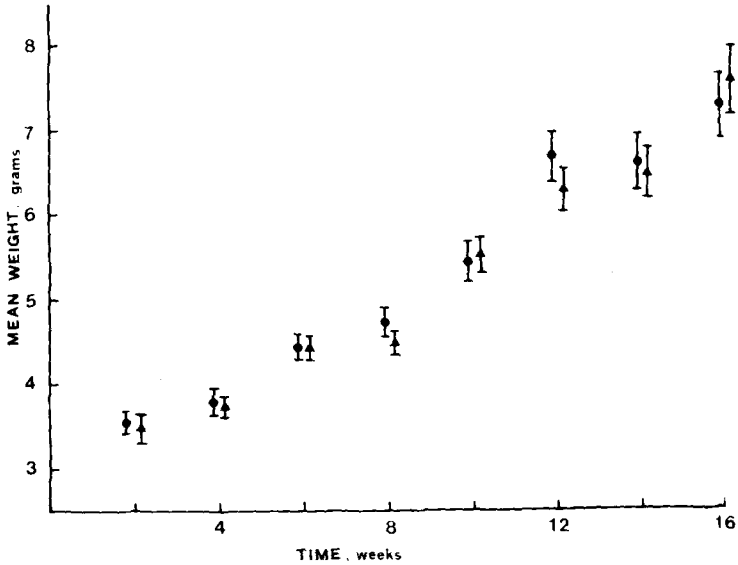

Figure 1. Growth of control fish (●) and of fish-exposed to 52 μg L^{-1} Cd during the experiment. No significant difference in mean weights is apparent at any stage of the experiment. Data for intermediate cadmium concentrations have been omitted for clarity. Vertical bars represent 95% confidence limits.

Organisation has proposed a provisional tolerable weekly intake of 0.4-0.5mg per individual (WHO 1972). It is reported (Dr. L.J. Saliba, personal communication) that two countries, the Netherlands and Norway, have set limits of 0.5 mg Kg^{-1} for cadmium in fish flesh. Table 3 shows that this level is exceeded in fish exposed to the two highest concentrations tested in the present experiment.

Thus a preliminary estimate of the maximum acceptable cadmium concentration for Tilapia would be between 14 and 30 μg L^{-1}. This is similar to comparable figures for other non-salmonid species, e.g. 37 μg L^{-1} for P. promelas (Pickering & Gast, 1972) and 31 μg L^{-1} for Lepomis macrochirus (Eaton, 1974), notwithstanding the apparent high resistance of T. aurea to lethal cadmium toxicity (Abel and Papoutsoglou, 1986). Although the suggested figure for Tilapia is based partially on tissue cadmium levels rather than solely on criteria of toxicity to the fish itself, in view of its importance as a food fish this approach is considered justifiable.

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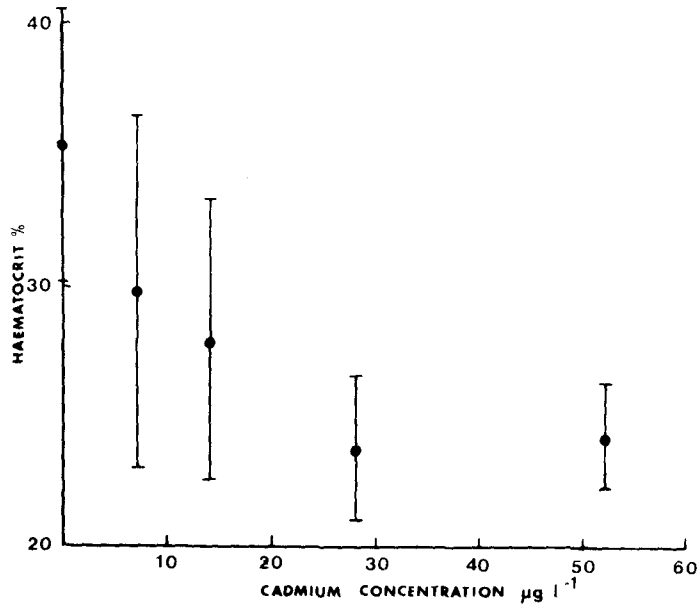

Figure 2. Haematocrit values (mean ± 95% confidence limits) for T. aurea exposed to various levels of cadmium for 10 weeks).

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