Dietary tissue cadmium accumulation in an amazonian teleost (Tambaqui, *Colossoma macropomum* Cuvier, 1818)

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(With 4 figures)

Abstract

Understanding the effects of metal contamination in the Amazon basin is important because of the potential impact on this region of high biodiversity. In addition, the significance of fish as the primary source of protein for the local human population (living either alongside the Amazon River or in the city of Manaus) highlights the need for information on the metal transfer through the food chain. Bioaccumulation of metals in fish can occur at significant rates through the dietary route, without necessarily resulting in death of the organism. The goal of this work was to expose an economic relevant species from the Amazon basin (tambaqui, *Colossoma macropomum*) to dietary cadmium (Cd) at concentrations of 0, 50, 100, 200, and 400 $\mu g.g^{-1}$ dry food. Fish were sampled on days 15, 30, and 45 of the feeding trials. Tissues were collected for analysis of Cd concentration using graphite furnace atomic absorption spectrophotometry. Cd accumulation in the tissues occurred in the following order: kidney > liver > gills > muscle. Relative to other freshwater fish (e.g., rainbow trout, tilapia), tambaqui accumulated remarkably high levels of Cd in their tissues. Although Cd is known to affect Ca^{2+} homeostasis, no mortality or growth impairment occurred during feeding trials.

Keywords: dietary cadmium, tissue accumulation, *Colossoma macropomum*, Amazon, soft water.

Acúmulo dietário de cádmio em tecidos de um teleósteo Amazônico (Tambaqui, *Colossoma macropomum*)

Resumo

O entendimento dos efeitos da contaminação por metais na Bacia Amazônica é importante devido ao potencial impacto sobre esta região de elevada biodiversidade. Além disso, a relevância dos peixes como fonte primária de proteína para a população humana local (tanto nas comunidades ribeirinhas ao longo do rio Amazonas, quanto na cidade de Manaus), ressalta a necessidade de informação sobre a transferência de metais através da cadeia alimentar. Bioacumulação de metais em peixes pode ocorrer em taxas significativas através da dieta, sem necessariamente resultar na morte do indivíduo. O objetivo deste estudo foi expor cronicamente uma espécie de importância comercial nativa da Amazônia (tambaqui, *Colossoma macropomum*) a dietas enriquecidas com cádmio (Cd) em concentrações de 0, 50, 100, 200, and 400 $\mu g.g^{-1}$ alimento seco. Os peixes foram amostrados nos dias 15, 30 e 45 do tratamento experimental. Os tecidos foram coletados para análise quanto a concentração de Cd por meio de espectrofotometria de absorção atômica acoplado a forno de grafite. O acúmulo de Cd nos tecidos ocorreu na seguinte ordem: rim > fígado > brânquias > músculo. Comparando-se com outras espécies de peixes de água doce (por exemplo, truta arco-íris, tilápia), o tambaqui acumulou níveis de Cd extremamente mais elevados em seus tecidos. Apesar do Cd ser conhecido por afetar a homeostase do Ca^{2+}, não houve mortalidade ou retardo no crescimento durante os testes dietários.

Palavras-chave: Cádmio, bioacumulação tecidual, *Colossoma macropomum*, Amazônia, água doce.

1. Introduction

In the Amazon basin, natural waters are highly susceptible to metal contamination because of the intense industrial activity occurring in urban areas. Cadmium (Cd), a non-essential metal, can be extremely toxic to fish, interfering primarily with calcium (Ca^{2+}) homeostasis (Verbost et al., 1989). Estimates of Cd release into Amazonian waters from industrial sources are not available, but the intensity of industrial activity in Amazonas, Brazil, is a likely source of this metal. For example, the formation water, associated with petroleum extraction processes along the Amazon River, has elevated Cd levels (10 $\mu g.L^{-1}$; Oliveira, 2003), and the release or spillage of this effluent to the aquatic environment constitutes a major concern.

Cd contamination represents a significant threat to Amazonian fish, particularly because of the soft nature of the water bodies in the basin. Calcium (Ca^{2+}), one of the major hardness cations, is usually present at concentrations < 180 μmol.L^{-1} in Amazonian waters (Furch, 1984). It is widely known that the toxic effects of metals like Cd to fish increase in soft water compared to hard water, because of the low availability of hardness cations to out-compete the toxic forms for binding sites on the gills (e.g., Playle et al., 1993).

We previously determined the sensitivity of a native Amazonian fish (tambaqui, *Colossoma macropomum*) to waterborne Cd toxicity in soft water (Matsuo et al., 2005). Because Cd accumulation in fish can be proportionally higher through dietary exposure than through waterborne exposure (e.g., Szebedinszky et al., 2001; Berntssen et al., 2003; Baldisserotto et al., 2005), the goal of the present study was to assess the chronic accumulation of Cd in the tissues of tambaqui following exposure to Cd-enriched diets. We also measured the specific growth rates (SGR) during the feeding trials to assess the possibility of growth impairment following dietary Cd exposure.

2. Material and Methods

2.1. Experimental animals

Juvenile tambaqui (8.52 ± 0.43 g) obtained from Embrapa (Empresa Brasileira de Pesquisas Agropecuárias, Manaus, Amazonas, Brazil) were kept in 100 L polyethylene tanks in aerated, soft wellwater for at least 3 weeks before we began experimentation. Soft water consisted of wellwater with an average composition of: Ca^{2+} = 11, Na^+ = 34, Cl^- = 28, Mg^{2+} = 0.8, K^+ = 15 (all in μmol.L^{-1}); pH 6.3; dissolved organic matter (DOM) = 0.9 mg C L^{-1}; background Cd = 0.3 μg.L^{-1}; temperature = 28 °C.

2.2. Experimental diets

To determine the influence of dietary Cd on tissue metal burden, we exposed tambaqui to long-term ingestion of substantially contaminated food (30 fish per tank, N = 2 replicates for each treatment). Experimental diets were prepared based on the methodology of Szebedinszky et al. (2001). Commercial dry fish food pellets (27% crude protein, 10% crude fat, 43% crude carbohydrate, 0.8% Na^+, 1% Ca^{2+}) were ground to a fine powder using a blender. To obtain the desired Cd concentrations in the experimental diets, we dissolved $Cd(NO_3)_2$. $4H_2O$ in deionized water, and then added this solution to the ground food. For example, to obtain a diet of 100 μg of Cd g^{-1} dry food, we dissolved 100 mg of Cd in 500 mL of deionized water and added this solution to 1 kg of the ground fish food. Experimental diets were then homogenized manually and mechanically, by passing it through a meat grinder several times before extruding the final pellets. The pellets were arranged on trays and allowed to air-dry for 24-48 hours. Once dried, the pellets were broken down manually to fit the mouth size of the experimental fish and stored at 4 °C in labeled containers. The control diet was prepared without any added Cd solution. Tambaqui were initially trained to feed on control food for at least 3 weeks prior to being fed the experimental diets.

Each set of replicates was fed a diet containing nominal 0 (control), 50, 100, 200, or 400 μg of Cd g^{-1} dry food (Table 1). Tambaqui were fed the experimental diet at approximately 2% body weight once daily. Tanks were siphoned within 30 minutes after the daily feeding to remove residual food and fecal material from water. The daily routine during feeding trials also included approximately 95% of the water renewal in the tanks. Exposure to Cd enriched diets did not result in mortality during the feeding period.

2.3. Sampling

On days 15, 30, and 45 of the feeding period, 10 fish from each tank were collected randomly and killed using an overdose of anesthetic (1 g.L^{-1} MS-222, Sigma) and tissues (gill, muscle, liver, and kidney) were sampled for analysis of Cd content. Tissue samples were vigorously rinsed in deionized water, blotted dry, and transferred to pre-weighed vials. Samples were digested in 1 N HNO_3 at 70 °C for 24-48 hours. Tissue digests were quickly spun and supernatant was diluted 1000 times in 0.1 N HNO_3 for analysis of Cd concentration using graphite furnace atomic absorption spectrophotometry (AAnalyst 800-GF, Perkin Elmer). Tissue samples from the control fish (fed Cd-free diets) did not require dilution for Cd concentration analysis.

2.4. Specific growth rate

To assess the possibility of growth impairment in tambaqui exposed to Cd-enriched diets, we calculated the specific growth rate (SGR) following Szebedinszky et al. (2001). SGR, in % day^{-1}, was estimated based on tank biomass measurements (N = 2 replicates for each treatment) over the feeding period, as follows:

$$SGR = ([\ln(w_2) - \ln(w_1)] \cdot t^{-1}) \times 100 \quad (1)$$

where w_1 and w_2 represent the bulk weight of fish (g) at the start and the end of the feeding period, respectively, and t is the length of the feeding period (d).

Table 1. Cd concentration in diet and SGR values

Nominal [Cd] (μg.g^{-1} dry food)	Measured [Cd] (μg.g^{-1} dry food)	Specific growth rate (SGR) (%/day)
0 (control)	2.4 ± 0.7	2.72 ± 0.29
50	60.6 ± 3.3	2.34 ± 0.12
100	115.2 ± 4.9	2.27 ± 0.24
200	241.1 ± 6.5	3.05 ± 0.38
400	383.1 ± 12.3	2.58 ± 0.15

2.5. Statistical analysis

Results are presented as mean ± SEM for each treatment. Statistical analysis used ANOVA followed by Dunnett's multiple-comparison tests (SPSS Software, version 10.0). The significance level was fixed at $\alpha < 0.05$ throughout the study.

3. Results

3.1. Tissue Cd accumulation

Chronic exposure of tambaqui to dietary Cd resulted in a very high accumulation of the metal in tissues in the following order: kidney > liver > gills > muscle (Figures 1-4). Cd accumulation in sampled tissues generally increased over exposure time, and relative to the concentration of the metal in the diet. Tambaqui muscle tissue, used frequently as a protein source by the local population of the Amazon, had maximum Cd concentrations of up to 3.0 $\mu g.g^{-1}$ wet tissue during the 45 d feeding trial (Figure 1). Detoxification sites, particularly liver and kidney, had maximum Cd concentration levels of approximately 140 and 500 $\mu g.g^{-1}$ wet tissue (Figures 3-4).

3.2. Specific growth rate

Despite the high accumulation of Cd found in sampled tissues, no mortality occurred during the feeding period. There was also no apparent impairment in growth

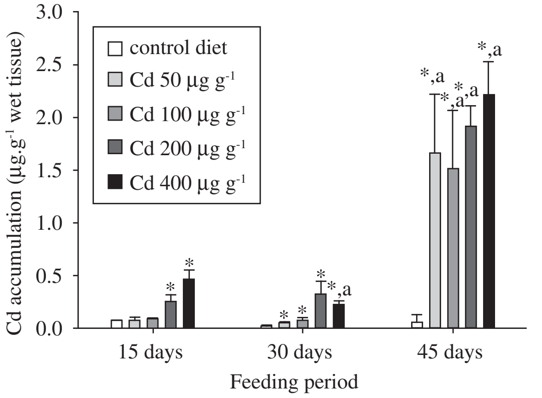

Figure 1. Muscle Cd concentrations (mean ± SEM, N = 10) in tambaqui chronically fed Cd-enriched diets. Asterisk (*) indicates significant difference relative to muscle samples from fish fed control diet (0 $\mu g.g^{-1}$ dry food) at each sampling day, whereas (a) indicates significant differences in Cd concentrations over the feeding period, relative to the initial 15 d ($\alpha < 0.05$).

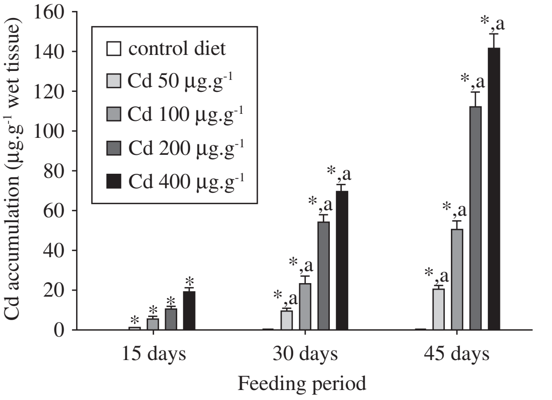

Figure 3. Liver Cd concentrations (mean ± SEM, N = 10) in tambaqui chronically fed Cd-enriched diets. Asterisk (*) indicates significant difference relative to liver samples from fish fed control diet (0 $\mu g.g^{-1}$ dry food) at each sampling day, whereas (a) indicates significant differences in Cd concentrations over the feeding period, relative to the initial 15 d ($\alpha < 0.05$).

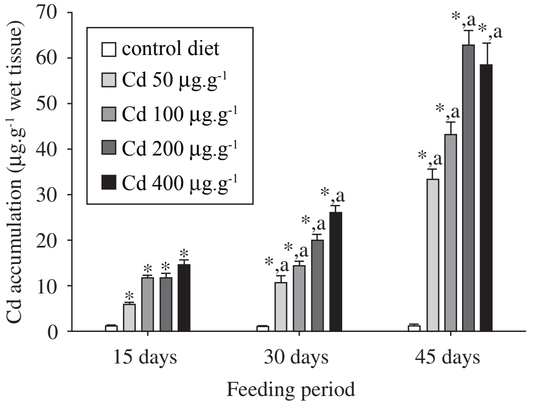

Figure 2. Gill Cd concentrations (mean ± SEM, N = 10) in tambaqui chronically fed Cd-enriched diets. Asterisk (*) indicates significant difference relative to gill samples from fish fed control diet (0 $\mu g.g^{-1}$ dry food) at each sampling day, whereas (a) indicates significant differences in Cd concentrations over the feeding period, relative to the initial 15 d ($\alpha < 0.05$).

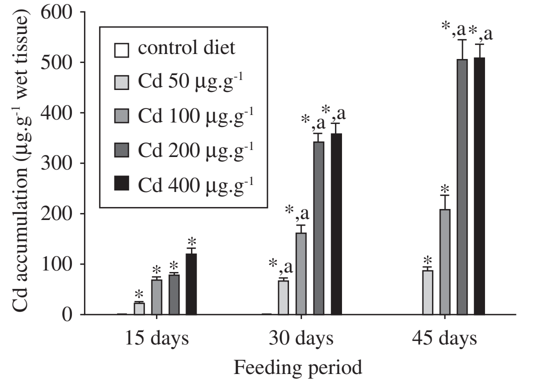

Figure 4. Kidney Cd concentrations (mean ± SEM, N = 10) in tambaqui chronically fed Cd-enriched diets. Asterisk (*) indicates significant difference relative to kidney samples from fish fed control diet (0 $\mu g.g^{-1}$ dry food) at each sampling day, whereas (a) indicates significant differences in Cd concentrations over the feeding period, relative to the initial 15 d ($\alpha < 0.05$).

during exposure to Cd-contaminated diets. Specific growth rate (SGR, %/day) in tambaqui fed on different diets did not differ significantly among treatments (Table 1).

4. Discussion

Tambaqui is highly susceptible to waterborne Cd toxicity, as indicated by the persistent inhibition of Ca^{2+} uptake following acute exposure to the metal (Matsuo et al., 2005). Fish chronically fed Cd-enriched diets, however, did not show obvious signs of toxicity or growth impairment (Table 1), even though Cd accumulation in vital tissues (kidney, liver, gills) was high (Figures 2-4). Specific growth rates (SGR) were similar among treatments, as has been reported in rainbow trout (*Oncorhynchus mykiss*) fed diets containing Cd (Szebedinszky et al., 2001; Baldisserotto et al. 2005). The increased tolerance to dietary Cd toxicity in fish appears to involve increased induction of various cytosolic proteins with Cd-binding potential, the best known of these being the metallotioneins (MT) (e.g., Dang et al., 2001).

Chronic dietary Cd exposure in tambaqui resulted in accumulation of Cd in the kidney > liver > gills > muscle (Figures 1-4), which agrees with the pattern of accumulation reported for rainbow trout (Szebedinszky et al., 2001). Quantitative differences relative to published studies, however, were remarkable: Cd accumulation in tissues of tambaqui through the dietary route was extremely high compared to values reported for rainbow trout (Szebedinszky et al., 2001; Baldisserotto et al., 2005). For example, kidney Cd accumulation in tambaqui had levels of up to 356 $\mu g.g^{-1}$ wet tissue when fed a Cd-enriched diet of 400 $\mu g.g^{-1}$ dry food for 30 d (Figure 1), whereas in rainbow trout, kidney Cd accumulation was only 11 $\mu g.g^{-1}$ wet tissue in fish fed diets up to 1,500 $\mu g.g^{-1}$ dry food for 36 d (Szebedinszky et al., 2001).

Interspecific differences in Cd tissue burden suggest that Cd accumulation from the diet may occur at much higher rates in tambaqui relative to rainbow trout. Apparently, a great portion of Cd ingested by rainbow trout is eliminated through the feces. Szebedinszky et al. (2001) documented very high Cd concentrations in feces ($\leq$ 244 $\mu g.g^{-1}$) collected from the posterior rectum of rainbow trout fed diets containing Cd from 15 to 1,500 $\mu g.g^{-1}$ dry food. Assuming that no significant absorption of Cd occurs from this intestinal segment on, we may also infer that a large portion of Cd fed to rainbow trout is eliminated through the feces. Similar results were found in tilapia (*Oreochromis niloticus*) fed diets containing Cd from 5 to 100 $\mu g.g^{-1}$ dry food, in which Cd content in feces were $\leq$ 279 $\mu g.g^{-1}$ (Nogami et al., 2000).

Although high concentrations of Ca^{2+} in either the water or the diet clearly have ameliorative effects on waterborne Cd toxicity in fish (e.g., Hollis et al., 2000; Zohouri et al., 2001; Baldisserotto et al., 2005), it is unclear whether the low water Ca^{2+} concentration in the holding tanks (~10 $\mu mol.L^{-1}$) contributed to amplify the dietary Cd accumulation in tambaqui. The sensitivity of tambaqui to waterborne Cd has been reported in a previous study (Matsuo et al., 2005), and the effect of soft water on dietary Cd accumulation still needs further investigation.

Cd concentrations in tambaqui muscle samples during our feeding trials were much lower (Figure 1) relative to other sampled tissues (Figures 2-4). The economical importance of tambaqui as a protein source among the population living in the Amazon should be emphasized in the context of metal bioaccumulation and transfer to consumers through the food chain.

We conclude that the tambaqui tolerate extended exposure to very high concentrations of dietary Cd, and they accumulate remarkably high levels of the metal in their tissues, particularly in the kidney and liver. Despite the high tissue Cd burden, the species does not show reduced survival or impaired growth, at least during the 45 d feeding period we tested.

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