



**UNIVERSIDADE FEDERAL DE SANTA MARIA  
CENTRO DE CIÊNCIAS NATURAIS E EXATAS  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:  
BIOQUÍMICA TOXICOLÓGICA**

**PARÂMETROS TOXICOLÓGICOS EM JUNDIÁS  
(*Rhambdia quelen*) EXPOSTOS AO CÁDMIO**

**DISSERTAÇÃO DE MESTRADO**

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**Santa Maria – RS, Brasil  
2008**

**PARÂMETROS TOXICOLÓGICOS EM JUNDIÁS (*Rhamdia  
quelen*) EXPOSTOS AO CÁDMIO**

por

**Alexandra Pretto**

Dissertação apresentada ao Programa de Pós-Graduação em Ciências  
Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa  
Maria (UFSM, RS), como requisito parcial para obtenção do grau de  
**MESTRE EM BIOQUÍMICA TOXICOLÓGICA**

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**Santa Maria – RS, Brasil**

**2008**

**Universidade Federal de Santa Maria  
Centro de Ciências Naturais e Exatas  
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica  
Toxicológica**

A Comissão Examinadora, abaixo assinada, aprova a Dissertação de  
Mestrado

**PARAMETROS TOXICOLÓGICOS EM JUNDIÁS (*Rhamdia quelen*)  
EXPOSTOS AO CÁDMIO**

elaborada por  
**Alexandra Pretto**

como requisito parcial para a obtenção do grau de **Mestre em  
Bioquímica Toxicológica**

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Santa Maria, 21 de agosto de 2008.

## DEDICATÓRIA

Aos meus pais Azair e Zélia por sempre estarem ao meu lado aconselhando, apoiando, incentivando e acreditando no meu esforço. Vocês sem dúvida são um exemplo de honestidade, caráter e de doação que seguirei.

À minha irmã Ana Paula pelos ensinamentos de irmã mais velha, pela amizade, pelo incentivo nos estudos e pelo apoio em minhas decisões.

Aos meus irmãos Jean, Alexia e Guilherme pela amizade, alegria e compreensão.

À minha querida avó Jandira pela dedicação, esforço incondicional e compreensão em todos os momentos.

## **AGRADECIMENTOS**

A Deus, que é a energia espiritual que me traz força para enfrentar os desafios, por permitir esta conquista.

À minha família que é meu porto seguro e está sempre pronta para acolher, aconselhar e entender as minhas dificuldades, expectativas e acreditar nas minhas conquistas.

À professora Dr<sup>a</sup>. Vania Lucia Loro, pela oportunidade e acolhida em seu laboratório ainda no início da minha graduação, pela disponibilidade em ensinar e aos conhecimentos passados ao longo dos anos de orientação, pelo auxílio prestado nas atividades, pela amizade e pela confiança depositada em mim.

À professora Dr<sup>a</sup>. Vera Maria Morsch pelos anos de orientação, pela amizade, confiança, disponibilidade em ensinar e pelo auxílio no desenvolvimento deste trabalho.

Ao professor Bernardo Baldisserotto, pela paciência, disponibilidade em ensinar e colaborar para a realização deste trabalho.

À professora Maria Amália, pela disponibilidade e auxílio prestado no desenvolvimento deste estudo.

Às queridas amigas Bibiana e Charlene, pelos anos de convivência, amizade e trabalho conjunto no laboratório. Obrigada pelo companheirismo, ensinamentos e colaboração. Tenho em vocês um exemplo de dedicação e competência.

Às colegas e amigas Roberta e Bárbara, pela alegria contagiante, felicidade e simplicidade que transpassam além da cooperação e auxílio sem medida de esforços na realização das atividades.

Às colegas e amigas Cândida e Adriana pelo carinho, amizade, convivência e colaboração.

Às amigas Lissandra, Rita, Aracéli, Denise, Milene e Alice pelas palavras carinhosas, incentivos, apoio e grande amizade.

Aos amigos de longa data Carolina, Joseânia, Rafael, Fábio e Viviane pelo apoio, carinho e ensinamentos adquiridos.

Às minhas colegas de apartamento Ketlin, Mônica, Daiane, Carla, Mariane e Adriane, pela amizade, alegria, paciência, compreensão nos momentos difíceis e pelos ensinamentos proporcionados pela convivência com vocês.

Às minhas amigas e ex-colegas de apartamento Ana Paula, Vanessa, Alice, Magali, Graziela, Alexania, Giovana, Denise, Juciléia pela amizade, carinho e apoio recebido.

Aos professores Luis Antonio de Avila e Maribel Antonelo Rubin pela disponibilidade de ler esta dissertação e compor a banca examinadora deste trabalho.

À UFSM e aos demais professores e funcionários do PPGBTOX pela colaboração e disponibilidade em ajudar.

À CAPES pela Bolsa de Pós-Graduação concedida.

Aos demais amigos aqui não citados, mas que de alguma forma colaboraram para esta realização, com palavras de incentivo que me impulsionaram a não desanimar com as dificuldades encontradas, meu sincero agradecimento.

*"Para realizar grandes conquistas, devemos  
não apenas agir mas também sonhar;  
não apenas planejar, mas também acreditar."*

*(Anatole France)*

## RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica  
Universidade Federal de Santa Maria, RS, Brasil

### **Parâmetros toxicológicos em jundiás (*Rhamdia quelen*) expostos ao cádmio**

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Orientadora: VANIA LUCIA LORO

Co-Orientadora: VERA MARIA MORSCH

Data e Local da Defesa: Santa Maria, 21 de agosto de 2008

Os ecossistemas aquáticos podem estar recebendo constantemente metais provenientes de descargas industriais e da agricultura geradas por atividades humanas. Desta forma, os organismos presentes no meio aquático podem ser alvo destes contaminantes. O objetivo deste estudo foi verificar os efeitos do cádmio sobre o metabolismo de jundiás (*Rhamdia quelen*). Neste trabalho primeiramente verificou-se a concentração letal média (CL<sub>50</sub>) do cádmio para o jundiá, obtendo-se o valor de 1,7 mg/L. Em seguida juvenis de *R. quelen* foram expostos por sete e 14 dias à 10% e 20% do valor da CL<sub>50</sub>. Ao final da exposição, os peixes foram submetidos a períodos de recuperação de sete e 14 dias. Os parâmetros analisados foram a atividade da enzima acetilcolinesterase (AChE) (cérebro e músculo), ocorrência de estresse oxidativo através de parâmetros como proteína carbonil (fígado), formação de espécies reativas ao ácido tiobarbitúrico (TBARS) (cérebro, fígado, rim, brânquias) e as enzimas antioxidantes catalase, glutathione S-transferase e superóxido dismutase (fígado, rim e brânquias). Além disso, parâmetros gerais relacionados ao metabolismo como glicogênio, glicose, lactato, proteína, aminoácidos e amônia (fígado, músculo e plasma) e acumulação de metais (cérebro, músculo, fígado, rim e brânquias) foram determinados. A atividade da AChE cerebral não foi alterada após sete dias de exposição mas foi observada significativa inibição após 14 dias de exposição e recuperação. Após os períodos de exposição, a AChE muscular foi aumentada (sete dias) e reduzida (14 dias). Ocorreu aumento de proteína carbonil após sete dias de exposição e redução após sete dias de recuperação. O mesmo período de exposição provocou aumento de TBARS no cérebro e brânquias e redução no rim. Seguindo a recuperação por sete dias ocorreu aumento de TBARS no fígado e redução nas brânquias. Após a exposição de 14 dias foi observado aumento de TBARS em todos os tecidos analisados, e somente no rim os valores retornam aos níveis do controle após a recuperação de 14 dias. A atividade da catalase aumentou no fígado e reduziu nas brânquias após os períodos de exposição. Na recuperação (sete dias) ocorreu redução da atividade da enzima nos dois tecidos. No rim nenhuma alteração foi observada na atividade desta enzima. A atividade da glutathione S-transferase aumentou no fígado após a exposição por sete dias e reduziu após o mesmo período de recuperação. Nas brânquias ocorreu um decréscimo significativo após exposição (sete e 14 dias) e a atividade só retornou aos valores do controle depois de 14 dias de recuperação. A atividade da superóxido dismutase, analisada no fígado, reduziu após os períodos de exposição e foi recuperada somente após 14 dias em água sem adição de cádmio. Os parâmetros relacionados ao metabolismo apresentaram alterações de



acordo com o tecido considerado e tempo de exposição. No fígado ocorreu aumento de lactato, proteína e aminoácidos e redução de glicose na exposição ao cádmio por sete dias. Na recuperação por sete dias, os níveis de glicogênio aumentaram e os demais parâmetros retornaram aos valores do controle. Seguindo 14 dias de exposição, a concentração de proteína hepática reduziu enquanto os níveis de aminoácidos e amônia aumentaram. Após a recuperação de 14 dias, aminoácidos e amônia permaneceram aumentados, lactato também aumentou e glicogênio reduziu neste período. No tecido muscular ocorreu redução de proteína e aumento de aminoácidos, amônia, glicogênio e glicose após exposição (sete dias). Na recuperação por sete dias, glicose permaneceu aumentada e proteína reduzida. Após 14 dias de exposição também foi verificada redução de proteína, mas ocorreu aumento de glicose e neste período de recuperação somente os níveis de amônia apresentaram aumento. No plasma, glicose, lactato e proteína foram alterados após exposição ao cádmio. Lactato aumentou após sete dias e depois de 14 dias expostos, jundiás demonstraram redução de glicose, lactato e proteína. Lactato reduziu após sete dias em recuperação e os níveis de proteína continuaram reduzidos após a recuperação por 14 dias. De maneira geral a acumulação de cádmio foi maior no rim, fígado e brânquias em comparação com o cérebro e músculo. Desta forma, as alterações observadas indicam que o cádmio afeta o metabolismo de jundiás. Os parâmetros analisados podem ser bons indicadores de toxicidade para cádmio.

*Palavras-chave:* cádmio; jundiá (*Rhamdia quelen*); AChE; estresse oxidativo; metabolismo; bioacumulação.

## **ABSTRACT**

Master Dissertation  
Graduating Program in Biological Sciences: Toxicological Biochemistry  
Federal University of Santa Maria, RS, Brazil

### **Toxicological parameters in silver catfish (*Rhamdia quelen*) exposed to cadmium**

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Date and place of defence: August, 21<sup>th</sup>, 2008, Santa Maria

The aquatic ecosystems may be constantly receiving metals from industrial discharges and agriculture generated by anthropogenic activities. Thus, the organisms in the aquatic environment may be the target of these contaminants. The aim of this study was verify cadmium effects on silver catfish (*Rhamdia quelen*) metabolism. First was verified the lethal concentration (LC<sub>50</sub>) 96 hours of cadmium for silver catfish and was obtained the value of 1.7 mg/L. Then juvenile of *R. quelen* were exposed for seven and 14 days to 10% and 20% of LC<sub>50</sub>. At the end of exposure periods the fish were subjected to recovery periods of seven and 14 days. The parameters analyzed were the activity of the enzyme acetylcholinesterase (AChE) (brain and muscle), oxidative stress through parameters such as protein carbonyl (liver), TBARS (brain, liver, kidney, gills) and the antioxidant enzymes catalase, glutathione S-transferase and superoxide dismutase (liver, kidney and gills). Furthermore, general parameters related to metabolism as glycogen, glucose, lactate, protein, amino acids and ammonia (liver, muscle and plasma) and metal accumulation were verified. The activity of brain AChE was not changed after seven days of exposure but significant inhibition was observed after 14 days of exposure and recovery. After the exposure periods, the muscle AChE was increased (seven days) and inhibited (14 days). Increase in protein carbonyl occurred after seven days of exposure and reduction was found after seven days of recovery. The same exposure period caused an increase in TBARS in the brain and gills and reduction in kidney. Following the recovery for seven days, an increase of TBARS occurred in the liver and reduction in the gills. After the exposure for 14 days TBARS increase was observed in all tissues tested. In the kidney values return to the control levels after the recovery of 14 days. The activity of liver catalase increased and reduced in gills after the exposure periods and the recovery for seven days caused reduction of the enzyme in the two tissues. In the kidney no change was observed in the activity of this enzyme. The activity of liver glutathione S-transferase increased after exposure for seven days and reduced after the same recovery period. In the gills a significant decrease occurred after exposure (seven and 14 days) and activity only returned to the control values after 14 days of recovery. The liver superoxide dismutase activity reduced after exposure periods and was recovered only after 14 days. The metabolic parameters presented changes according to tissue and time of exposure. In the liver occurred increase of lactate, protein and amino acids and reduction of glucose after exposure to cadmium for seven days. In the recovery period of seven days, the glycogen levels increased and the other parameters returned to the control values.

Following 14 days of exposure the liver protein concentration reduced however, the amino acids and ammonia levels increased. After the recovery of 14 days, amino acids and ammonia remained increased, lactate also increased and reduced glycogen in this period. In the muscular tissue protein reduction and increase of amino acids, ammonia, glycogen and glucose were observed after exposure (seven days). In seven days of recovery, glucose remained increased and protein reduced. After 14 days of exposure also was observed reduction of protein, but there was an increase of glucose. In the recovery of 14 days only the ammonia levels had increased. In plasma, glucose, lactate and protein were altered after exposure to cadmium. Lactate increased after seven days and silver catfish showed glucose, lactate and protein reduced after 14 days. Lactate reduced after seven days in recovery and the protein levels remained low after the recovery for 14 days. In general the cadmium accumulation was higher in kidney, liver and gills as compared to the brain and muscle. Thus, the changes observed indicate that cadmium affects the silver catfish metabolism. The parameters analyzed can be good indicators of cadmium toxicity.

*Key-words:* cadmium; silver catfish (*Rhamdia quelen*); AChE; oxidative stress; metabolism; bioaccumulation.

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## LISTA DE ABREVIATURAS

ACh: acetilcolina  
AChE: acetilcolinesterase  
AcSCh: acetiltiocolina  
Acetil-CoA: acetil coenzima A  
ATP: adenosina trifosfato  
BChE: butirilcolinesterase  
CAT: catalase  
CDNB: 1-chloro-2,4-dinitrobenzene  
CL<sub>50</sub>: concentração letal média  
DNPH: 2,4-dinitrophenylhidrazine  
DTNB: 5,5' dithio-bis(2-nitrobenzoic acid)  
EROs: espécies reativas do oxigênio  
GF AAS: absorção atômica em forno de grafite  
GPx: glutationa peroxidase  
GSH: glutationa reduzida  
GST: glutationa transferase  
H<sub>2</sub>O<sub>2</sub>: peróxido de hidrogênio  
LPO: peroxidação de ácidos graxos poliinsaturados  
MDA: malondialdeído  
SD: standard deviation  
SDS: lauril sulfato de sódio ou duodecil sulfato de sódio  
SOD: superóxido dismutase  
TBA: 2-ácido tiobarbitúrico  
TBARS: substâncias reativas ao ácido tiobarbitúrico  
TCA: ácido tricloroacético

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## APRESENTAÇÃO

Esta dissertação apresenta os resultados na forma de manuscritos, os quais encontram-se nos itens **MANUSCRITOS CIENTÍFICOS**. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios manuscritos e representam a íntegra deste trabalho.

No final desta dissertação encontram-se os itens **DISCUSSÃO** e **CONCLUSÕES**, nos quais há interpretações e comentários gerais sobre os manuscritos científicos contidos neste estudo.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO**, **REVISÃO BIBLIOGRÁFICA**, **DISCUSSÃO** e **CONCLUSÕES** desta dissertação.

## 1 INTRODUÇÃO

Os ambientes aquáticos como rios, lagos e reservatórios recebem constantemente resíduos industriais, urbanos e da agricultura, gerados pela atividade humana. Atualmente, a contaminação dos ecossistemas aquáticos por metais pesados é um problema ambiental crítico, pois estes poluentes podem causar efeitos adversos à biota que ali se encontra (ALMEIDA *et al.*, 2001; ROMANI *et al.*, 2003; RIBEIRO *et al.*, 2006). Os metais pesados estão presentes em concentrações baixas no ambiente, as quais podem ser tóxicas aos organismos (MONTEIRO *et al.*, 2005). Nos sistemas de água doce, alterações ocorridas em peixes são consideradas indicativos importantes da poluição por agentes tóxicos (RASHED, 2001).

Vários poluentes ambientais, entre eles os metais e os compostos orgânicos, são capazes de induzir estresse oxidativo em organismos aquáticos, incluindo os peixes (LIONETTO *et al.*, 2003; GIODA *et al.*, 2007). Enzimas como a catalase, a superóxido dismutase, a glutatona peroxidase e a glutatona S-transferase fazem parte do sistema antioxidante presente nas células para proteger o organismo contra os danos causados por espécies reativas do oxigênio (EROs). As atividades destas enzimas quando alteradas em peixes podem ser indicadores de exposição a poluentes aquáticos, bem como ferramentas utilizadas em avaliações toxicológicas (ALMEIDA *et al.*, 2001; MONTEIRO *et al.*, 2006). O aumento na formação de EROs pode causar danos em muitos constituintes celulares, incluindo proteínas, ácidos nucléicos e lipídios (ALMROTH *et al.*, 2005; BAGNYUKOVA *et al.*, 2005). Danos aos lipídios celulares podem ser verificados pelo aumento do malondialdeído (MDA), o principal produto da peroxidação de ácidos graxos poliinsaturados (ROMÉO *et al.*, 2000; ÜNER *et al.*, 2005). Os danos causados às proteínas pelas EROs podem ser avaliados pela medida de carbonilação de proteínas (ALMROTH *et al.*, 2005; PARVEZ & RAISUDDIN, 2005).

Os peixes tendem a desenvolver várias respostas bioquímicas e fisiológicas frente aos xenobióticos que entram no organismo. Estas respostas podem resultar na adaptação do organismo ao poluente ou induzir efeitos negativos na sobrevivência e condições de saúde do peixe (DE SMET & BLUST, 2001; BEGUM,

2004). A exposição a poluentes como metais pode alterar o metabolismo de proteínas, carboidratos e hematologia (DE SMET & BLUST, 2001). Entre as alterações decorrentes da toxicidade destes elementos, as mudanças no metabolismo energético dos organismos podem ser verificadas através da medida dos níveis de glicogênio, glicose e proteína em diferentes tecidos (HAMILTON *et al.*, 1998; ALMEIDA *et al.*, 2001). Outro parâmetro utilizado para avaliar a intoxicação por metais é a medida da atividade da enzima acetilcolinesterase (AChE). As colinesterases são enzimas que desempenham papéis importantes na neurotransmissão colinérgica central e periférica, hidrolisando ésteres de colina. Alterações na atividade da enzima acetilcolinesterase em resposta à exposição de peixes a xenobióticos podem afetar a locomoção e o equilíbrio dos mesmos (NEMCSÓK *et al.*, 1984, LIONETTO *et al.*, 2003; ROMANI *et al.*, 2003).

O cádmio é um dos metais que pode estar presente em águas contaminadas, podendo ser tóxico aos organismos deste ecossistema. Este metal é um elemento não essencial, que exerce uma série de efeitos patológicos em peixes, afetando o crescimento, a reprodução, as funções respiratórias e a osmorregulação (ALMEIDA *et al.*, 2001; WU *et al.*, 2007). A presença de cádmio na água, mesmo em baixas concentrações, pode provocar mortalidade em peixes. Isto decorre de danos causados ao fígado, onde ocorrem alterações enzimáticas, no tamanho do órgão e no conteúdo de glicogênio. Além disso, uma situação de hipocalcemia pode se estabelecer devido à redução na absorção de cálcio causada pelo cádmio (BALDISSEROTTO *et al.*, 2005). A absorção deste metal pelos peixes ocorre diretamente através da água (pelas brânquias), ou indiretamente, através da cadeia alimentar (HAMILTON *et al.*, 1998).

O jundiá (*Rhamdia quelen*) é uma espécie endêmica da parte sul da América do Sul. Na região sul do Brasil é uma espécie de interesse comercial, que sobrevive às temperaturas de inverno e tem um rápido crescimento no verão, além de se reproduzir em cativeiro (GOMES *et al.*, 2000; BARCELLOS *et al.*, 2004). Existem poucos estudos no Brasil relacionando a toxicidade de metais com espécies nativas como o jundiá. Neste sentido, os estudos envolvendo estes animais e contaminantes freqüentemente encontrados no ambiente, como o cádmio, são de grande interesse para avaliar questões referentes às respostas dos peixes a águas contaminadas com metais.

## 2 OBJETIVOS

### 2.1 Objetivo geral

Verificar os efeitos de concentrações subletais de cádmio sobre parâmetros metabólicos e de estresse oxidativo em jundiá (*Rhamdia quelen*).

### 2.2 Objetivos específicos

- Verificar a concentração letal média (CL<sub>50</sub>) do cádmio para o jundiá.
- Determinar a bioacumulação do metal em diferentes tecidos do jundiá.
- Avaliar o efeito da exposição subletal ao cádmio sobre a atividade da enzima acetilcolinesterase cerebral e muscular de jundiás.
- Investigar a ocorrência de estresse oxidativo medindo-se TBARS (em cérebro, fígado, rim e brânquias), carbonilação de proteínas (no fígado) e uma possível resposta antioxidante, medindo-se a atividade das enzimas catalase, glutathione S-transferase e superóxido dismutase em fígado, rim e brânquias deste peixe.
- Verificar o efeito do cádmio sobre os níveis de glicose, de proteína e de lactato plasmáticos.
- Estudar a resposta metabólica dos peixes através da medida de glicose, lactato, glicogênio, proteína, aminoácidos e amônia em diferentes tecidos de jundiás.
- Verificar se as alterações observadas irão persistir após igual período de recuperação em água livre de metal.

### 3 REVISÃO BIBLIOGRÁFICA

#### 3.1 Cádmió e o potencial de contaminação ambiental

Ambientes aquáticos podem constantemente estar sendo contaminados por metais. Nestes sistemas aquáticos geralmente são encontrados altos níveis de mercúrio, cromo, chumbo, cádmio, cobre, zinco e níquel (ZHOU *et al.*, 2008). As principais causas do aumento da presença destes contaminantes são as descargas geradas por atividades industriais e atividades relacionadas à agricultura (uso de fertilizantes) (LANGE *et al.* 2002; ROMANI *et al.* 2003; DAUTREMEPUITS *et al.*, 2004). Desta forma, as águas contaminadas podem atingir os sistemas de água doce e ambientes marinhos prejudicando os organismos que ali vivem. Alguns metais incluindo mercúrio, cromo, cádmio, níquel, cobre e chumbo podem apresentar alta toxicidade sobre os organismos aquáticos (ZHOU *et al.*, 2008).

O cádmio é um metal relativamente raro na crosta terrestre, presente em baixas concentrações e geralmente associado ao zinco na forma de depósitos de sulfito (ROMAN *et al.*, 2002; BURGER, 2008). É um metal de cor prata claro, dúctil, mole e maleável. Apresenta peso molecular 112,41 e número atômico 48. Tem ponto de fusão e ebulição, respectivamente, de 321°C e 767,2°C, e densidade de 8,64g/m<sup>3</sup>. Emite vapores, mesmo quando em temperaturas inferiores ao seu ponto de ebulição e em seu estado sólido, sendo insolúvel na água e nos solventes orgânicos usuais e oxidando-se em presença de ar e de umidade (ROMAN *et al.*, 2002).

O cádmio entra no ambiente através de fontes naturais e antropogênicas, por meio de atividades de fundição, produção de baterias, revestimentos eletrolíticos de metais, tintas, fertilizantes e estabilizadores plásticos. Entre as fontes naturais inclui-se o vulcanismo, liberação de vapores do oceano e queima de florestas (ROMAN *et al.*, 2002; ATLI & CANLI, 2007; BURGER, 2008). Este metal caracteriza-se por ser um elemento não essencial aos seres vivos, um metal pesado persistente, não biodegradável e conhecido por ser muito tóxico até mesmo em baixas concentrações (BHAKTA & MUNEKAGE, 2008; SOARES *et al.* 2008).

A captação de contaminantes pelos organismos aquáticos, entre eles os peixes, pode ocorrer diretamente pela água, sedimentos, partículas em suspensão ou através da cadeia alimentar (LIVINGSTONE, 2001; ALQUEZAR *et al.*, 2006a). Quando metais penetram sobre os organismos da biota aquática, pode ocorrer uma série de respostas a nível molecular, fisiológico ou comportamental (LINDE-ARIAS *et al.*, 2008).

Peixes têm sido freqüentemente usados como indicadores de poluição em ambientes aquáticos. Eles podem ser encontrados em quase todo meio aquático e exibem um grande papel ecológico, pois são considerados o topo da cadeia alimentar aquática (LINDE-ARIAS *et al.*, 2008). Logo, mudanças fisiológicas e bioquímicas em peixes podem servir como marcadores de poluição ambiental (ALMEIDA *et al.*, 2001; DAUTREMEPUITS *et al.*, 2004).

Quando expostos a concentrações subletais de cádmio, os peixes podem demonstrar efeitos em parâmetros hematológicos e alterações histológicas e morfológicas em tecidos como fígado, rim, brânquias e intestino (DE LA TORRE *et al.*, 2000; ALMEIDA *et al.*, 2001). Os principais órgãos alvo do cádmio nos peixes são o rim e o fígado, onde podem ser afetados vários sistemas enzimáticos (CATTANI *et al.*, 1996; DE LA TORRE *et al.*, 2000). Os metais pesados também podem afetar a reprodução em peixes influenciando o número de indivíduos nas populações e a estrutura do ecossistema (ALQUEZAR *et al.*, 2006b).

Nos peixes, as brânquias representam uma extensa superfície em contato direto com a água. Elas possuem três funções principais que são as trocas gasosas, regulação de íons e excreção de produtos do metabolismo. As brânquias são o primeiro alvo dos poluentes aquáticos por estar em constante contato com o meio externo (WONG & WONG, 2000). Logo, neste tecido além de acumulação de metais podem ocorrer várias alterações histopatológicas no epitélio branquial. O cobre inibe a atividade da enzima  $\text{Na}^+/\text{K}^+$ -ATPase levando a distúrbios osmorregulatórios (MONTEIRO *et al.*, 2005). Na exposição ao cádmio ocorre decréscimo na atividade da enzima  $\text{Ca}^{2+}$ -ATPase branquial, podendo levar a uma situação de hipocalcemia. A partir desta situação podem resultar deformidades esqueléticas e alterações no balanço de cálcio nos animais (WONG & WONG, 2000; WU *et al.*, 2007).

A toxicidade do cádmio pode ser influenciada por fatores físico-químicos da água como alcalinidade, pH, dureza, níveis de cálcio e substâncias húmicas (BENADUCE *et al.*, 2008). Peixes vivendo em um lago contendo água com pH ácido

e concentração de 0,1 µg/L de cádmio apresentaram uma concentração renal de 123 mg/Kg, um valor semelhante aos valores encontrados para peixes que vivem em áreas mais poluídas (CINIER *et al.*, 1999). Existem evidências de que o cádmio é menos tóxico em águas com dureza elevada e que a acumulação ou toxicidade diminuem com o aumento do pH (BENADUCE *et al.*, 2008). De acordo com BENADUCE *et al.* (2008) foi observado um maior número de ovos com superfície irregular, na espécie *Rhamdia quelen* (jundiá), após exposição a 18 µg/L de cádmio em 63 mg/L CaCO<sub>3</sub> comparado a concentração de 92 mg/L CaCO<sub>3</sub>. Nesse estudo também foi verificado que alguns parâmetros como a taxa de sobrevivência, comprimento, peso e altura da cabeça das larvas diminuía com a aumento nas concentrações de cádmio na água (4,5 a 18 µg/L) em 63 mg/L CaCO<sub>3</sub>. No entanto estas diferenças e relações não foram encontradas em larvas mantidas em 92 mg/L de CaCO<sub>3</sub>. A presença de cálcio na água tem um efeito protetor contra a toxicidade do cádmio. Isto se deve a competição entre os íons cálcio e cádmio pelos sítios de ligação na enzima Ca<sup>2+</sup>-ATPase nas células de cloreto branquiais (CINIER *et al.*, 1999; BALDISSEROTTO *et al.*, 2004). Já, a disponibilidade do cádmio na água pode ser modificada por interações do metal com ligantes, levando a formação de complexos. Ácidos húmicos são conhecidos por associarem-se com metais pesados e reduzir sua disponibilidade aos peixes (CINIER *et al.*, 1997).

A toxicidade do cádmio varia também com relação às espécies. Salmonídeos geralmente são mais sensíveis aos níveis de cádmio (BURGER, 2008). Para juvenis de truta (*Onchorhynchus mykiss*) obteve-se um valor de 0,12 mg/L de cádmio para a CL<sub>50</sub> de 48 horas em água com dureza 6 mg/L CaCO<sub>3</sub>. Em água com dureza 100 mg/L de Ca<sup>2+</sup> obteve-se valores de CL<sub>50</sub> 96 horas de 4,3 e 17.1 mg/L de cádmio, respectivamente, para larvas e alevinos de *Cyprinus carpio*. Em juvenis de *Oreochromis niloticus* foi encontrado o valor de 18.58 mg/L de cádmio para a CL<sub>50</sub> de 96 horas (ALMEIDA *et al.*, 2001) e 50 mg/L de cádmio para a CL<sub>50</sub> 48 horas em *Oreochromis mossambicus* (BASHA & RANI, 2003).



### 3.2 Jundiá (*Rhamdia quelen*)

Jundiá é o nome comum dado aos peixes pertencentes ao gênero *Rhamdia*. Este gênero é classificado dentro da família Heptapteridae, ordem Siluriformes, série Teleostei e classe Osteichthyes (Figura 1). No Brasil o jundiá também é conhecido por jundiá-tinga, jandiá, jandiá-tinga, mandi e sapipoca e na Argentina este peixe é conhecido como bagre, bagre-negro, bagre-sapo e bagre-sul-americano. O jundiá é encontrado do sudeste do México ao centro da Argentina (GOMES *et al.*, 2000).

O jundiá é um peixe de couro, cuja coloração varia de marrom-avermelhado claro a cinza, com a parte ventral do corpo mais clara. Ele pode variar a coloração do corpo de acordo com o ambiente que se encontra, pois quando colocado em ambientes claros, o jundiá tende a ficar mais claro e o inverso ocorre quando este peixe se encontra em um ambiente escuro. O jundiá apresenta barbilhões localizados junto à boca, que provavelmente possuem receptores de gosto para ajudar na localização do alimento e na percepção da qualidade da água (BALDISSEROTTO & RADÜNZ NETO, 2004).

Nos primeiros anos de vida o jundiá tem um grande crescimento e este crescimento é maior em machos do que em fêmeas até o terceiro ou quarto ano de vida. Daí em diante as fêmeas passam a ter um crescimento mais rápido. Porém em criação artificial observa-se um menor crescimento dos machos em função da precoce maturação sexual. O crescimento máximo das fêmeas pode chegar a 66,5 cm e dos machos aproximadamente 52,0 cm. As fêmeas também apresentam um maior tempo de vida, cerca de 21 anos, enquanto os machos podem teoricamente chegar a 11 anos (GOMES *et al.*, 2000).

O jundiá vive em lagos e poços fundos dos rios e tem preferência por ambientes de águas mais calmas com fundo de areia e lama, próximo às margens e vegetação. Escondem-se entre pedras e troncos, de onde saem à noite para procurar alimento (BALDISSEROTTO & RADÜNZ NETO, 2004). Uma acentuada aversão à luz e busca por locais escuros foi observada em experimentos realizados com larvas e alevinos desta espécie em cativeiro (PIAIA *et al.*, 1999). Exemplos adultos de *R. quelen* são omnívoros com uma clara preferência por peixes, crustáceos, insetos, restos vegetais e detritos orgânicos. As larvas de jundiás

alimentam-se de zooplâncton (BALDISSEROTTO & RADÜNZ NETO, 2004; GUEDES, 1980).

O jundiá é uma espécie nativa adaptada a diferentes ambientes, pois apresenta bons resultados em relação à criação principalmente nas regiões mais frias. É uma espécie rústica, de rápido crescimento nos períodos mais quentes e suporta bem as baixas temperaturas ocorridas na região Sul do País. Além disso, o jundiá é um peixe que apresenta excelente aceitação pelo mercado consumidor, tanto para a pesca quanto para a alimentação, sendo uma espécie com excelentes características para o processamento (BARCELLOS *et al.*, 2001; BARCELLOS *et al.*, 2003).

No Brasil poucos estudos analisaram os efeitos de metais sobre espécies nativas como o jundiá.



Figura 1: Exemplar de jundiá (*Rhamdia quelen*).

### 3.3 Enzima Acetilcolinesterase (AChE)

Acetilcolinesterase (AChE, EC 3.1.1.7) assim como butirilcolinesterase (BChE, EC 3.1.1.8) pertencem à classe das colinesterases, uma classe de enzimas que hidrolisam ésteres de colina em colina e ácido acético (Figura 2). AChE tem preferência por hidrolisar o neurotransmissor acetilcolina e não hidrolisa butirilcolina. Além disso, a atividade da AChE é inibida pelo excesso de substrato (CHUIKO, 2000). Em peixes, nos tecidos cerebral e muscular encontra-se principalmente AChE

enquanto a BChE está presente em maior concentração no fígado e plasma (ÜNER *et al.*, 2006).

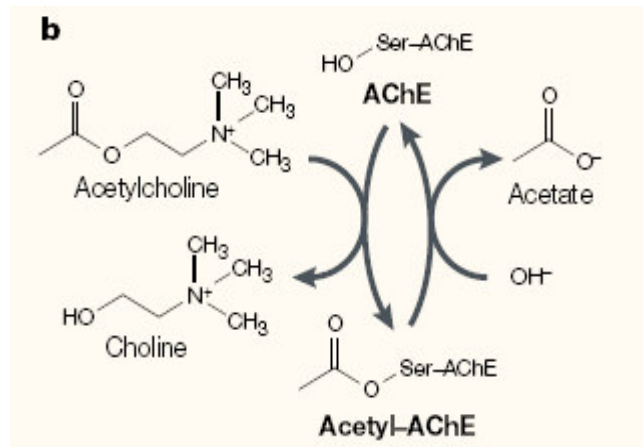


Figura 2: Reação catalisada pela enzima acetilcolinesterase (AChE) (adaptado de SOREQ & SEIDMAN, 2001).

AChE é conhecida por exibir o maior papel na neurotransmissão colinérgica hidrolisando o neurotransmissor acetilcolina nas sinapses colinérgicas (STEFANO *et al.*, 2008). A inibição da AChE está diretamente ligada aos mecanismos de ação tóxica de pesticidas organofosforados e carbamatos (DE LA TORRE *et al.*, 2002; LINDE-ARIAS *et al.*, 2008; STEFANO *et al.*, 2008). No entanto, um crescente número de estudos tem evidenciado que a atividade da AChE pode ser afetada por outras classes de pesticidas, metais pesados e outras misturas de contaminantes (LIONETTO *et al.*, 2003; GLUSCZAK *et al.*, 2006; MORAES *et al.*, 2007; FONSECA *et al.*, 2008).

Ao estudar a atividade da AChE em duas espécies de peixes, *Cnesterodon decemmaculatus* e *C. carpio*, em dois sítios experimentais em um rio na Argentina, DE LA TORRE *et al.* (2002) verificaram alterações na atividade da enzima. No sítio experimental menos poluído, *C. carpio* apresentaram redução na AChE cerebral mas em outro ponto experimental mais poluído foi observado mortalidade total das carpas. A inibição da AChE neste estudo foi atribuída a presença de altas concentrações de metais pesados encontrados na água. Em outro estudo conduzido por LIONETTO *et al.* (2003) foi verificada a atividade da AChE em *Mullus barbatus*. Os peixes apresentaram inibição na atividade da AChE no cérebro, provavelmente devido a presença de metais pesados pois eles foram coletados em locais próximos a áreas urbanas.

Outros resultados são demonstrados em estudos realizados com peixes criados em laboratório. Ao expor peixes da espécie *Sparus auratus* por 20 dias a concentrações de 0,1 e 0,5 mg/L de cobre, ROMANI *et al.* (2003) encontraram significativo aumento na atividade da AChE cerebral e muscular. Num estudo “in vitro”, zinco e cádmio não alteraram a atividade da AChE cerebral na espécie *Danio rerio* (zebrafish). As concentrações de metais usadas variaram de 0,05 a 1 mM (SENGER *et al.*, 2006). Larvas de truta (*O. mykiss*) expostas a crescentes concentrações do inseticida carbaril (188, 375 e 750 µg/L) demonstraram redução de até 38% na AChE cerebral após 24 horas de exposição e de até 41% após um período de 96 horas. No entanto, no mesmo estudo a exposição ao cádmio (2,5 e 5,0 µg/L) não alterou a atividade da AChE no tecido cerebral nesta espécie (BEAUVAIS *et al.*, 2001). Quando juvenis de carpas (*C. carpio*) foram expostas à concentração de 1,6 mg/L de cádmio por 14 dias, DE LA TORRE *et al.* (2000) também não encontraram alterações na AChE cerebral nesta espécie.

No entanto, a relação entre a presença de compostos tóxicos no meio aquático e as alterações na atividade desta enzima em espécies de invertebrados e vertebrados aquáticos tem sido usada como um biomarcador de poluição ambiental (MONSERRAT *et al.*, 2007). O estudo da AChE em tecidos como o cérebro que é um dos tecidos mais vulneráveis do organismo em vista da sua composição, se torna extremamente importante pois pode indicar injúrias teciduais ou mudanças comportamentais que podem vir a afetar a sobrevivência de peixes (DE LA TORRE *et al.*, 2000; BEAUVAIS *et al.*, 2001; MIRON *et al.*, 2005).

### **3.4 Estresse Oxidativo**

Todos os organismos aeróbicos dependem do oxigênio presente no ambiente para produção de energia e desta forma manutenção da vida. Assim, as reações de oxidação fazem parte do metabolismo normal das células que leva à formação de ATP. Neste processo, as moléculas de oxigênio são reduzidas à água pelos complexos que compõe a cadeia respiratória e podem ser formados vários produtos do metabolismo do oxigênio chamados espécies reativas do oxigênio (EROs) (MASELLA *et al.*, 2005; LUSHCHAK & BAGNYUKOVA, 2006). Entre as EROs estão

incluídos o anion superóxido ( $O_2^{\cdot-}$ ), peróxido de hidrogênio ( $H_2O_2$ ) e radical hidroxila ( $\cdot OH$ ).

O ânion superóxido, formado pela adição de um elétron ao oxigênio molecular, é uma espécie menos reativa, pois ele perde a habilidade de penetrar as membranas lipídicas e permanece no compartimento onde foi produzido. Diferentemente, a molécula de peróxido de hidrogênio é altamente capaz de penetrar as membranas biológicas assim como o radical hidroxila que possui forte reatividade com biomoléculas. O radical hidroxila é formado a partir do peróxido de hidrogênio em uma reação catalisada por íons metálicos ( $Fe^{2+}$  ou  $Cu^+$ ) conhecida como a reação de Fenton (Figura 4) (NORDBERG & ARNÉR, 2001).

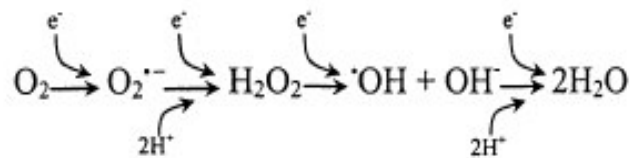


Figura 3: Esquema das reações de formação das EROs (adaptado de NORDBERG & ARNÉR, 2001).

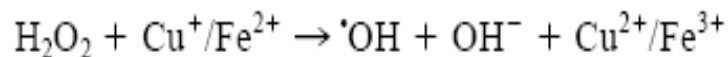


Figura 4: Reação de Fenton e formação do radical hidroxila (adaptado de NORDBERG & ARNÉR, 2001).

O estado de estresse oxidativo é estabelecido quando o nível de EROs excede a capacidade antioxidante da célula devido uma depleção do sistema de defesa antioxidante ou uma acumulação excessiva de EROs ou ambas as situações (MONTEIRO *et al.*, 2006; MASELLA *et al.*, 2005). Sob condições normais as EROs são detoxificadas e retiradas continuamente da célula pelo sistema de defesa antioxidante, constituído de enzimas e pequenas moléculas livres (NORDBERG & ARNÉR, 2001; SONG *et al.*, 2006). No entanto quando os organismos são expostos a contaminantes, a produção de EROs pode aumentar, prevalecer sobre sua degradação e levar a situação de estresse oxidativo (LUSHCHAK & BAGNYUKOVA, 2006). LIVINGSTONE (2001) revela que muitos contaminantes aquáticos como

benzeno e bromobenzeno e metais como alumínio, mercúrio, cádmio e cromo podem estimular a produção de EROs e resultar em danos oxidativos aos organismos aquáticos. As EROs podem provocar danos a componentes celulares por atingir proteínas, lipídios e ácidos nucleicos (Figura 5) (SIES, 1993; LUSHCHAK & BAGNYUKOVA, 2006; MÉNDEZ-ARMENTA & RÍOS, 2007; STURVE *et al.*, 2008).

Duas perturbações bioquímicas resultantes do estresse oxidativo são a peroxidação lipídica e a formação de proteína carbonil (ALMROTH *et al.*, 2005). Peroxidação lipídica é um processo que ocorre nas membranas celulares devido às reações entre as EROs e os ácidos graxos poliinsaturados (ORUÇ & USTA, 2007). Neste processo são formados vários produtos da degradação lipídica como malondialdeído (MDA) e outros aldeídos. O MDA é bem caracterizado por ser um produto da oxidação de ácidos graxos poliinsaturados podendo reagir com o ácido tiobarbitúrico (TBA) produzindo um intermediário colorido (TBARS) que é usado para quantificar a peroxidação lipídica (ROMÉO *et al.*, 2000; ALMROTH *et al.*, 2005).

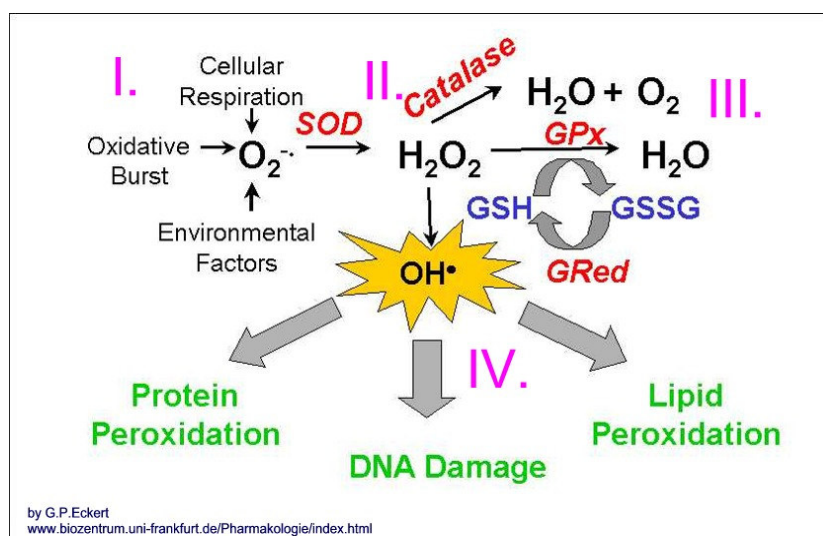


Figura 5: Danos oxidativos e enzimas antioxidantes. Disponível em: <http://www.biozentrum.uni-frankfurt.de/Pharmakologie/EU-Web/Goethe.htm>. Acesso em 02 jun. 2008.

Diversos estudos têm verificado alterações nos níveis de TBARS em peixes expostos a contaminantes ambientais. GIODA *et al.* (2007) encontraram aumento de TBARS em cérebro, fígado e músculo de *Leporinus obtusidens* (piava) expostos a 2,3 e 4,6 mg/L de zinco por um período de 45 dias, no entanto, concentrações de

cobre (0,02 e 0,04 mg/L) reduziram níveis de TBARS cerebral e hepático nesta espécie. Juvenis de *O. mykiss* demonstraram elevados níveis de TBARS nas brânquias após exposição a 10 mg/L de cromo. O maior aumento foi verificado após dois dias de exposição ao metal com retorno aos níveis basais após três semanas de exposição. Neste estudo também ocorreu aumento de TBARS no tecido hepático (ROBERTS & ORIS, 2004). Após exposição a diferentes concentrações de tório (25,3; 69,2; 209,5 e 608,7 µg/L) por 15 dias, jundiás exibiram significante decréscimo nos níveis de TBARS em brânquias, fígado e músculo (CORREA *et al.*, 2008). A exposição ao cádmio (100 e 500 µM) causou leve aumento nos níveis de TBARS (3 e 22%) em células renais de *Dicentrarchus labrax* enquanto menores concentrações de cobre (5 a 200 µM) induziram grande aumento neste parâmetro (ROMÉO *et al.*, 2000).

O processo de peroxidação lipídica influencia a fluidez das membranas e também a integridade de biomoléculas associadas à membrana como proteínas e colesterol (ALMROTH *et al.*, 2005). Os lipídios de membrana altamente oxidados, resultantes da peroxidação lipídica, podem atuar sobre proteínas próximas causando a formação de um excesso de proteínas carboniladas (DALLE-DONNE *et al.*, 2003; ALMROTH *et al.*, 2005). A formação de proteína carbonil também pode ocorrer devido a presença de EROs nas células, as quais agem sobre grupos amino de proteínas e desta forma alteram a estrutura ou a função de proteínas (ALMROTH *et al.*, 2005). Os grupos carbonil são produzidos sobre as cadeias de proteínas principalmente quando são oxidados os aminoácidos prolina, arginina, lisina e tirosina (DALLE-DONNE *et al.*, 2003). A formação de proteínas carboniladas é um processo irreversível que causa mudanças conformacionais, decréscimo na atividade catalítica em enzimas e pode resultar na quebra de proteínas devido ao fato de estas moléculas estarem mais susceptíveis a ação de proteases (ALMROTH *et al.*, 2005).

A investigação do conteúdo de proteína carbonil nas células é um biomarcador de estresse oxidativo muito utilizado em estudos humanos (PARVEZ & RAISUDDIN, 2005). Em peixes poucos estudos têm relacionado a exposição a xenobióticos com possíveis danos à proteínas e conseqüente formação de proteínas carboniladas. Após exposição aguda (48 horas) de *Channa punctata* a pesticidas como deltametrin (0,75 µg/L), endosulfan (10 µg/L) e paraquat (5 mg/L) ocorreu aumento de proteína carbonil em brânquias, rim e fígado (PARVEZ & RAISUDDIN,

2005). ALMROTH *et al.* (2008) observaram altos níveis de proteína carbonil no plasma de *Symphodus melops* quando os peixes foram coletados de um sítio com elevadas concentrações de metais pesados (cobre, zinco e ferro) na água e sedimentos. Os autores concluem que a carbonilação de proteínas ocorreu em função do estresse oxidativo provocado pela exposição dos peixes aos metais pesados.

### 3.5 Enzimas Antioxidantes

As células possuem um complexo sistema de defesa que neutraliza a formação de EROs e seus efeitos negativos sobre proteínas e lipídios celulares. Desta forma, o sistema de defesa antioxidante como é chamado, é constituído de antioxidantes de baixo peso molecular como glutathiona e ácido ascórbico e enzimas antioxidantes. Algumas enzimas que fazem parte do sistema de defesa antioxidante são a superóxido dismutase (SOD), glutathiona peroxidase (GPx), glutathiona S-transferase (GST) e catalase (CAT) (LIVINGSTONE, 2001; LUSHCHAK & BAGNYUKOVA, 2006; STURVE *et al.*, 2008).

A SOD (EC 1.15.1.1) é uma importante enzima antioxidante que se encontra tanto no citosol (CuZn-SOD) quanto no interior das mitocôndrias (Mn-SOD). Esta enzima catalisa a transformação de duas moléculas de ânion superóxido até peróxido de hidrogênio e oxigênio molecular (Figura 6) (BARATA *et al.*, 2005; ÜNER *et al.*, 2005; MONTEIRO *et al.*, 2006). Na mitocôndria o radical superóxido é formado em altas concentrações devido o extravasamento de elétrons da cadeia respiratória (NORDBERG & ARNÉR, 2001).

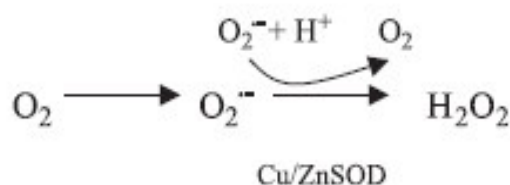


Figura 6: Reação catalisada pela enzima superóxido dismutase (SOD) (adaptado de HO, 2004).



A CAT (EC 1.11.1.6) é outra componente importante do sistema de defesa antioxidante. Esta enzima está localizada principalmente nos peroxissomos celulares e reduz o peróxido de hidrogênio até água e oxigênio molecular (Figura 7) (BARATA *et al.*, 2005; ORUÇ & USTA, 2007; SAMPAIO *et al.*, 2008). O peróxido de hidrogênio é produzido no metabolismo de ácidos graxos nos peroxissomos e pode penetrar através de todas as membranas biológicas inativando diretamente algumas enzimas (ATLI *et al.*, 2006).

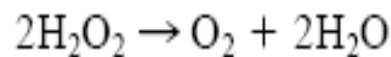


Figura 7: Reação catalisada pela enzima catalase (CAT) (adaptado de NORDBERG & ARNÉR, 2001).

Diferentes respostas da CAT têm sido observadas em animais expostos a metais, pois a atividade desta enzima mostra indução ou inibição dependendo da concentração do metal e espécie em estudo. Após exposição a 0,1; 0,5; 1,0 e 1,5 mg/L de prata, cádmio, cobre, cromo e zinco por 96 horas, *O. niloticus* apresentaram diferentes respostas na atividade da CAT em fígado, rim, brânquias, intestino e cérebro. No tecido hepático, os metais cádmio, cobre, cromo e zinco induziram aumento da CAT, enquanto nos tecidos renal e branquial, cádmio e zinco não afetaram a atividade da enzima. No cérebro, a concentração de 1,0 mg/L de zinco aumentou em cerca de 126% a atividade da CAT, enquanto 1,5 mg/L de prata reduziu 54% a atividade da enzima (ATLI *et al.*, 2006). Piavas expostas a concentrações de zinco (2,3 e 4,6 mg/L) e cobre (0,02 e 0,04 mg/L) por 30 e 45 dias aumentaram a atividade da CAT hepática em ambos os períodos e concentrações de metais testados (GIODA *et al.*, 2007).

A GST (EC 2.5.1.18) pertence ao sistema da glutationa (GSH). A GST pode ser encontrada no citosol, interior de mitocôndrias e microsossomos, por isso é classificada em três famílias (MASELLA *et al.*, 2005). A GST catalisa a conjugação de GSH com vários compostos eletrofílicos. Desta forma a enzima exibe um papel

protetor contra danos oxidativos por conjugar produtos da oxidação de lipídios como aldeídos e hidroperóxidos à GSH (BARATA *et al.*, 2005; MASELLA *et al.*, 2005).

A evidência de estresse oxidativo em peixes pode ser encontrada em vários estudos observando alterações na atividade de enzimas antioxidantes em animais expostos a poluentes, em experimentos conduzidos em laboratório ou realizados em sítios contaminados. SAMPAIO *et al.* (2008) conduziram um experimento com *Piaractus mesopotamicus* (pacu) expostos aos efeitos simples ou combinados da exposição ao cobre e condição de hipóxia. Neste estudo foi verificado decréscimo na atividade das enzimas CAT e SOD em músculo vermelho de pacus em resposta a condição de hipóxia e combinação de hipóxia com 0,4 mg/L de cobre. No fígado a resposta da CAT foi similar a do músculo, porém ocorreu redução da enzima também em resposta a simples exposição a 0,4 mg/L de cobre. Neste tecido a SOD foi induzida após exposição simples ao cobre e combinação com a situação de hipóxia. A exposição de jundiás a concentrações de tório (25,3; 69,2; 209,5 e 608,7 µg/L) causou alterações na atividade da GST em brânquias, fígado e músculo. No tecido branquial foi verificada redução da GST em todas as concentrações testadas, no fígado esta resposta ocorreu após exposição às concentrações 25.3 e 69.2 µg/L, porém, os peixes expostos a maior concentração demonstraram significativo aumento na atividade da GST (76,5%). No tecido muscular, foi observada inibição da enzima após exposição à concentração mais alta de tório e aumento da GST nas demais concentrações testadas (CORREA *et al.*, 2008). Alguns contaminantes acumulam-se fortemente nos tecidos sem induzir efeitos tóxicos, enquanto outros são caracterizados por elevada toxicidade mesmo a baixos níveis de exposição (AHMAD *et al.*, 2006). Os peixes respondem a exposição a poluentes alterando ou adaptando suas funções metabólicas. A resposta das enzimas antioxidantes parece depender da concentração dos metais, do tecido analisado e da espécie em estudo.

### 3.6 Parâmetros Metabólicos

A liberação de poluentes dentro do ambiente aquático pode refletir sobre os organismos presentes e desencadear mudanças em nível de parâmetros metabólicos. Respostas bioquímicas e fisiológicas podem ocorrer quando um tóxico

entra no organismo, como uma aclimatação do organismo ou uma situação de toxicidade podendo afetar a sobrevivência dos animais (DE SMET & BLUST, 2001). O estresse é um mecanismo fisiológico compensatório que o organismo adota em resposta a fatores físicos ou químicos, os agentes estressores, que podem afetar parâmetros bioquímicos a nível sanguíneo ou a nível de órgãos (VAN VUREN *et al.*, 1994). O consumo de oxigênio, o metabolismo de lipídios, conteúdo de lactato e glicogênio tecidual e níveis plasmáticos de glicose podem ser alterados depois da exposição a um xenobiótico (DE SMET & BLUST, 2001). Assim, estes parâmetros bioquímicos são sensíveis a concentrações subletais de agentes tóxicos.

Os peixes são uma importante fonte de alimento, são componentes importantes do ecossistema aquático e podem ser bons indicadores da toxicidade de poluentes porque suas respostas bioquímicas são similares às encontradas em mamíferos (GLUSCZAK *et al.*, 2006). Em peixes geralmente os maiores órgãos alvo de contaminantes como pesticidas e metais são o fígado e rim, que são os órgãos ativos na metabolização e eliminação de xenobióticos. Por isso, a análise de parâmetros bioquímicos pode ajudar a identificar os órgãos alvo de toxicidade e o estado geral de saúde do animal (ALMEIDA *et al.*, 2001). Neste estudo nós verificamos parâmetros gerais referentes ao metabolismo de carboidratos como glicogênio, glicose e lactato e níveis de proteína, aminoácidos e amônia, relacionados ao metabolismo de proteínas, para analisar os efeitos tóxicos do cádmio sobre jundiás, pois são limitados os estudos que analisam o impacto de metais pesados sobre o estado metabólico de peixes.

Segundo CATTANI *et al.* (1996) as concentrações de glicogênio e glicose podem refletir o estado metabólico dos tecidos. Organismos em situação de estresse geralmente aumentam sua demanda energética o que pode refletir em quebra de glicogênio hepático e muscular e aumento de glicose sanguínea. Aumento da produção de lactato também pode ocorrer em função de uma associação entre a demanda de energia e uma condição de hipóxia tecidual (GIMENO *et al.*, 1994; SANCHO *et al.*, 1998). Proteínas são as maiores constituintes no metabolismo dos animais e agentes tóxicos como metais pesados podem provocar mudanças no metabolismo destas moléculas como estimulação da síntese ou da quebra (DE SMET & BLUST, 2001).

### 3.7 Acumulação de Metais

Peixes acumulam metais pesados em seus tecidos em concentrações maiores às encontradas no ambiente. As principais formas de captação de metais são via ingestão de alimento contaminado ou por absorção branquial (ALQUEZAR *et al.*, 2006a; SOARES *et al.*, 2008). Os metais acumulam-se em tecidos metabolicamente ativos como rim, fígado, brânquias e trato digestivo (SOARES *et al.*, 2008). No caso do metal cádmio os principais órgãos alvo de acumulação são o rim e fígado (DE LA TORRE *et al.*, 2000), no entanto as brânquias por estarem em constante contato com a água contaminada também são descritas como um tecido bastante sensível a este metal (WU *et al.*, 2007). Nestes tecidos podem ocorrer significantes alterações bioquímicas, metabólicas e fisiológicas devido à presença do metal (ASAGBA *et al.*, 2008). Poucos estudos analisam a acumulação de cádmio no tecido muscular, porém esta análise é importante do ponto de vista da saúde humana (HAMILTON *et al.*, 1998; CINIÉR *et al.*, 1999).

Um experimento conduzido por Cinier *et al.* (1999) verificou o potencial de acumulação e eliminação de cádmio em tecidos de *C. carpio*. Os peixes foram expostos às concentrações de 53 e 443 µg/L por 127 dias seguindo um período de recuperação de 43 dias. A concentração de cádmio no fígado e rim aumentou de acordo com as concentrações do metal na água. Após 127 dias de exposição às concentrações de metal no rim foram 86 e 215 mg/kg e no fígado chegaram a 24 e 131 mg/kg. No tecido muscular foi observada baixa acumulação chegando a um valor médio de 2 mg/kg após o período de exposição. Depois de 43 dias em água sem metal, a perda de cádmio acumulado no músculo foi rápida e imediata ao contrário do rim e fígado onde não foi observada perda do metal. De acordo com CINIÉR *et al.* (1997) a exposição ao cádmio resulta em significativa captação de metal pelos órgãos, mas o padrão de captação varia com o tipo de órgão. Fígado e rim parecem ser os órgãos mais importantes na captação de cádmio (CINIÉR *et al.*, 1999). Nestes tecidos a concentração de cádmio aumenta rapidamente a um nível de saturação relacionado à sua capacidade de estocagem. A acumulação no músculo é estimulada quando a capacidade de estocagem do fígado e rim atinge o nível de saturação (CINIÉR *et al.*, 1997).

Outro estudo realizado por ASAGBA *et al.* (2008) verificou que a concentração tecidual de cádmio em *Clarias gariepinus* seguiu o padrão rim > brânquias > fígado > músculo após 21 dias de exposição a concentrações de cádmio (0,1 a 0,4 mg/L). Alguns estudos revelam que a acumulação de cádmio varia de acordo com o tecido considerado e os efeitos sobre alguns parâmetros bioquímicos dependem do tempo de exposição e concentração testada (CINIER *et al.*, 1999; ASAGBA *et al.*, 2008).

Desta forma, a presença de metais no ecossistema aquático pode afetar os organismos, resultando em alterações fisiológicas e bioquímicas. Alguns parâmetros que podem ser utilizados para verificar os efeitos da exposição a metais são a enzima AChE, enzimas do sistema de defesa antioxidante e intermediários metabólicos.

## **4 MANUSCRITOS CIENTÍFICOS**

Os resultados inseridos nesta dissertação apresentam-se sob a forma de manuscritos científicos, os quais se encontram aqui estruturados. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios manuscritos. Os manuscritos estão dispostos da mesma maneira que foram submetidos.

4.1 Atividade da acetilcolinesterase e respostas metabólicas em jundiás expostos ao cádmio.

4.1.1 Manuscrito I

**Acetylcholinesterase activity and metabolic responses in silver catfish  
(*Rhamdia quelen*) exposed to cadmium**

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Silveira Moraes<sup>1</sup>, Roberta Cattaneo<sup>1</sup>, Cândida Toni<sup>1</sup>, Lucélia Hoehne<sup>2</sup>, Valderi  
Dressler<sup>2</sup>, and Vania Lucia Loro<sup>1,3\*</sup>**

Acetylcholinesterase activity and metabolic responses in silver catfish (*Rhamdia quelen*) exposed to cadmium

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**Abstract**

Cadmium is a metal with no biological function in superior organisms and it is very toxic even at very low concentrations. Then, the objective of this study was to verify some toxicological parameters in silver catfish (*Rhamdia quelen*) exposed to cadmium. In this study, silver catfish was exposed to 0.0 (control), 0.2 and 0.4 mg L<sup>-1</sup> of cadmium for 7 and 14 days, followed by the same periods for recovery. The effects of cadmium on acetylcholinesterase (AChE) activity, parameters of protein and carbohydrate metabolism and metal accumulation in brain and muscle were verified. Thiobarbituric acid-reactive substances (TBARS) formation was evaluated in brain tissue as some metabolic parameters in plasma. Increase of TBARS levels was verified after exposure and recovery periods and brain AChE activity was reduced after 14 days of exposure. These parameters did not return to control values after recovery period. In the muscle AChE was altered in both exposure periods. In addition, fish exposed to cadmium showed changes in liver and muscle tissues concerning carbohydrate metabolism and similar results on protein metabolic profiles. Alterations in AChE activity and intermediaries metabolic may be good indicators of cadmium contamination in *R. quelen*.

**Keywords:** *Rhamdia quelen*, cadmium, AChE, metabolism, TBARS.

## 1. Introduction

Cadmium is a biologically non-essential metal and its distribution is relatively rare in the Earth's crust. Natural and anthropogenic sources as battery manufacture, smelting, paints, corrosive coatings and fertilizers are the main sources of cadmium entrance in the environment (Burger, 2008). Besides not being essential to any organism, cadmium is also a non-biodegradable and persistent type of heavy metal, having high toxic potential on live organisms. Another important adverse property of cadmium is its ability of accumulation in the sediments and in the aquatic organisms (Cinier et al., 1999; Bhakta and Munekage, 2008).

Fish accumulate heavy metals as cadmium in their tissues mainly through the ingestion of contaminated food or by absorption through the gills. The metal accumulation occurs mainly in gills, liver, kidney and digestive tract, which are metabolically active tissues (Cinier et al., 1999; Soares et al., 2008). Studies on cadmium accumulation in fish muscle were also performed and may be important from the point of view of human health (Cinier et al., 1999). In fish cadmium affects several enzymes impairing basic physiological and biochemical process like neurotransmission, transepithelial transport, immune system, intermediary metabolism and mixed function oxidases (De La Torre et al., 2000).

Acetylcholinesterase (AChE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in acetate and choline. AChE is reported as specific biomarker of exposure to some pesticides including organophosphorus and carbamates. These pollutants are known to selectively inhibit AChE activity (De La Torre et al., 2002; Lionetto et al., 2003). Inhibition of AChE can result in an excessive stimulation of cholinergic nerves, resulting in behavioral alterations as tremors, convulsions and

erratic or lethargic swimming (Beauvais et al., 2001; Fernández-Vega et al., 2002; Miron et al., 2005). However, exposure of fish to metals causes different responses to AChE activity as inhibition (Lionetto et al., 2003), increase (Romani et al., 2003), or no change (Beauvais et al., 2001; Senger et al., 2006). Moreover, few studies have correlated exposure to cadmium with effects on AChE activity in fish tissues. Alterations can also occur at the biochemical and physiological level, such as in the carbohydrate and protein metabolism and hematology (De Smet and Blust, 2001). We evaluated a possible stress situation caused by fish exposed to cadmium through of general parameters as glycogen, glucose, lactate, protein, ammonia and free amino acids levels in the tissues.

*Rhamdia quelen* (silver catfish) was chosen for this study because it is a native teleost from South America and is an economically important species of southern Brazil (Barcellos et al., 2004). In addition, there is no information available, regarding the biochemical response of silver catfish after exposure to cadmium. Thus, the objective of this study was to verify possible alterations in tissues of silver catfish after exposure to cadmium, assessing AChE activity, TBARS formation and metabolic parameters as toxicity indicators and if these alterations persist after transferring fish to clean water.

## **2. Materials and methods**

### *2.1. Chemicals*

All chemicals reagent grade were purchased from Sigma (St. Louis, MO, USA).

## 2.2. Fish

Silver catfish juveniles ( $23.9 \pm 3.1$  g and  $13.4 \pm 0.5$  cm) were obtained from a commercial fish farm near Santa Maria (Rio Grande do Sul, Brazil). Fish were acclimated to laboratory conditions for 15 days. They were placed in continuously aerated 250 L tanks (100 individuals per tank) with a static system under a natural photoperiod (12 h light-12 h dark) and receive feed once a day (commercial fish pellets with 42% crude protein, Supra, Brazil). In this period, the water quality was as follows: temperature  $23.7 \pm 1.0$  °C, pH  $7.6 \pm 0.2$ , dissolved oxygen  $6.8 \pm 0.5$  mg L<sup>-1</sup>, non-ionized ammonia  $1.23 \pm 0.05$  µg L<sup>-1</sup>, nitrite  $0.04 \pm 0.01$  mg L<sup>-1</sup>, hardness  $12.0 \pm 1.15$  mg L<sup>-1</sup> CaCO<sub>3</sub> and alkalinity  $39.0 \pm 3.2$  mg L<sup>-1</sup> CaCO<sub>3</sub>.

## 2.3. Experiment I - lethal concentration (LC<sub>50</sub>) 96 hours determination

After acclimation, groups of six fish (in duplicate) were transferred to 30 L boxes for LC<sub>50</sub> determination. Physico-chemical characteristics of LC<sub>50</sub> experimental water were as follows: temperature  $23.6 \pm 0.2$  °C, dissolved oxygen  $6.6 \pm 0.5$  mg L<sup>-1</sup>, non-ionized ammonia  $1.17 \pm 0.3$  µg L<sup>-1</sup>, nitrite  $0.03 \pm 0.01$  mg L<sup>-1</sup>, hardness  $18.6 \pm 2.3$  mg L<sup>-1</sup> CaCO<sub>3</sub> and alkalinity  $36.3 \pm 3.6$  mg L<sup>-1</sup> CaCO<sub>3</sub>. Concentrations used for cadmium (CdCl<sub>2</sub>.10H<sub>2</sub>O) were (mean ± standard deviation):  $1.0 \pm 0.005$ ,  $1.2 \pm 0.007$ ,  $1.45 \pm 0.008$ ,  $1.7 \pm 0.017$  and  $1.9 \pm 0.007$  mg L<sup>-1</sup>. Metal was added to the water only at the beginning of the experiment and the water was not changed throughout the experiment. Animals were not fed during the experimental period. Mortality from each metal concentration was recorded to estimate the LC<sub>50</sub>. The LC<sub>50</sub> for silver catfish was 1.7 mg L<sup>-1</sup> cadmium.

## 2.4. Experiment II - exposure to cadmium concentrations

Fish were transferred to plastic aquarium (45 L) with controlled aeration and temperature. The experiments were conducted as a random blocks design with two replications, in a factorial scheme. The factor A were the cadmium concentrations and the factor B were exposure/recovery periods. The cadmium concentrations were 0.0 (control), 0.2 and 0.4 mg L<sup>-1</sup> and the exposure/recovery periods were 7 and 14 days. In each aquarium were transferred 12 individuals, six to be used to evaluate the effect of exposure and six to evaluate the recovery. Stock solutions were prepared by dissolving cadmium chloride in the water and after added to the experimental aquaria to reach the desired concentration. In the exposure period of 7 days, water was not changed during the experiment therefore metal was added to the water only at the beginning. In the exposure period of 14 days, water was changed in the middle of exposure (seventh day) and metal was added again to replace those removed during change of water. Uneaten food and feces were siphoned daily, and a biofilter was used to kept water quality. Fish were fed in excess once a day with commercial fish pellets. There was no mortality throughout the experimental periods (seven and 14 days). Physico-chemical characteristics of water were as follows: temperature 23.8 ± 0.2 °C, pH 7.9 ± 0.1, dissolved oxygen 6.9 ± 0.3 mg L<sup>-1</sup>, non-ionized ammonia 3.42 ± 0.4 µg L<sup>-1</sup>, nitrite 0.04 ± 0.01 mg L<sup>-1</sup>, hardness 15.0 ± 2.1 mg L<sup>-1</sup> CaCO<sub>3</sub> and alkalinity 45.5 ± 2.8 mg L<sup>-1</sup> CaCO<sub>3</sub>.

### *2.5. Water and tissue ion content analysis*

In order to conduct analysis of cadmium accumulation by the fish, four fish were removed from each box after the exposure and recover periods. The brain, liver and part of the dorsal muscle were removed, washed in distilled water, dried with filter paper, weighed and packed in plastic vials and kept at -70 °C until analysis.

Tissue samples were digested (3 times for 1 min) in a microwave oven after to be added 2 mL of 65% HNO<sub>3</sub> and remained overnight. The resulting solutions were subsequently diluted to 10 ml with ultra pure water. The total content of cadmium in tissues and water samples was measured by graphite furnace atomic absorption spectrophotometry (GF AAS) and expressed as  $\mu\text{g g}^{-1}$  tissue and  $\text{mg L}^{-1}$ , respectively.

## 2.6. *Sampling*

After each period of exposure, six fish per aquaria were sampled. Blood was quickly collected for determination of plasma parameters. The fish were killed by excision of spinal cord behind operculum and tissues from brain, liver and muscle were removed, quickly placed on ice and frozen at  $-70\text{ }^{\circ}\text{C}$  for analysis of AChE activity and metabolic parameters. Six fish were kept in water without the addition of cadmium for seven and 14 days to recover (after 7 and 14 days of exposure, respectively). Control fish were sampled at each experimental period. The blood and tissues samples were collected as reported above to determine biochemical parameters.

## 2.7. *AChE assay*

AChE activity was measured as described by Ellman et al. (1961) and modified by Miron et al. (2005). Brain and muscle were weighed and homogenized in a Potter-Elvehjem glass/Teflon homogenizer with 50 mM sodium phosphate buffer, pH 7.2 (with 1% Triton X-100). The homogenates were centrifuged for 15 min at 3000g at  $5\text{ }^{\circ}\text{C}$  and the supernatant was used as the enzyme source. Aliquots of supernatant (50 to 100  $\mu\text{L}$ ) (brain and white muscle respectively) were incubated at

30 °C for 2 min with 0.1 M phosphate buffer pH 7.5, 10 mM DTNB as chromogen. After 2 min, the reaction was initiated by the addition of acetylthiocholine (0.5 mM) as substrate for the reaction mixture. The final volume was 2.0 mL. Absorbances were determined at 412 nm during 2 min. Enzyme activity was expressed as  $\mu\text{mol}$  acetylthiocholine (AcSCh) hydrolyzed  $\text{min}^{-1}$   $\text{mg}$  protein $^{-1}$ . Protein levels for AChE and TBARS were estimated by the method described by Bradford (1976), using bovine serum albumin as standard.

### *2.8. Lipid peroxidation levels (TBARS)*

Peroxides produced was estimated by a TBARS assay. This was performed by a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which is optically measured. Brain samples were homogeneized in NaCl 150 mM. Homogenate aliquot (400  $\mu\text{L}$ ) was added to 10% trichloroacetic acid (TCA) and 0.67% TBA to adjust to a final volume of 1.0 mL. The reaction mixture was placed in a microcentrifuge tube and incubated for 15 min at 95 °C. After cooling, it was centrifuged at 5000g for 10 min and optical density at 532 nm was determined. TBARS level was expressed as nmol MDA/mg protein according to Buege and Aust (1978).

### *2.9. Metabolic parameters*

Liver and muscle glycogen levels were determined according to Bidinotto et al. (1998) after KOH and ethanol addition for hydrolysis and precipitation of glycogen. For protein analysis, the tissues were heated at 100 °C with KOH and centrifuged at 1000g for 10 min. Supernatant was used to estimate the total protein level according to method described by Lowry et al. (1951). For lactate, soluble sugar and ammonia

determination, tissue samples were homogenized by adding 10% TCA using a motor-driven Teflon pestle and centrifuged at 1000g for 10 min for flocculation of the proteins. The completely deproteinated supernatant was used for lactate determination (Harrower and Brown 1972), soluble sugar (Park and Johnson 1949) and ammonia (Verdouw et al. 1978). For amino acid quantification, tissues (liver and muscle) were mechanically disrupted by adding 2 mL phosphate buffer 20 mM, pH 7.5 and the homogenates were centrifuged at 1000g for 10 min. The neutral supernatant extracts were used for colorimetric amino acid determination according to Spies (1957). Plasma glucose was measured by the glucose oxidase method with Bioclin test Kit. Plasma was dissolved in 10% TCA (1:20 dilution) and lactate was estimated according to Harrower and Brown (1972). Plasma total protein levels were measured according to Lowry et al. (1951) using bovine serum albumin as standard.

#### 2.10. *Statistical analysis*

The mean lethal concentration (LC<sub>50</sub>) for 96 hours was calculated using probit analysis as described by Finney (1971). The biochemical parameters data were compared using two-way analysis of variance followed by Duncan's multiple range test ( $P \leq 0.05$ ). Results obtained ( $n=8$ ) were expressed as mean  $\pm$  standard deviation (SD). Relation between AChE and TBARS levels were tested with linear regression. Statistical tests were done using GraphPad PRISM software (San Diego, CA).

### **3. Results**

No alterations were observed in brain AChE activity of silver catfish after 7 days of cadmium exposure and after the same period in water without addition of



cadmium (Fig. 1A). However, longer exposure period (14 days) caused significant inhibition in the brain AChE activity at both concentrations, and this inhibition remained even after 14 days of recovery (Fig. 1B). In the white muscle of silver catfish the AChE activity was increased after 7 days of exposure at 0.4 mg L<sup>-1</sup> cadmium and in the recovery period the values of the AChE activity in white muscle returned to control values (Fig. 2A). After 14 days of exposure the AChE activity in the white muscle was reduced at 0.4 mg L<sup>-1</sup> cadmium. When fish were removed from cadmium exposure and transferred to clean water for 14 days, a significant recovery of white muscle AChE activity was observed (Fig. 2B).

Fish exposed to both cadmium concentrations tested presented significantly higher TBARS levels in the brain in the two periods of exposure. An increase of approximately 37% in TBARS levels of fish exposed to both concentrations studied was observed after 7 days of exposure. In the recovery period, TBARS levels of fish exposed for 7 days to cadmium returned to control values following 7 days in water without the addition of cadmium (Fig. 3A). Following 14 days of exposure the increase in TBARS levels reached 75% and 55% in fish exposed to 0.2 and 0.4 mg L<sup>-1</sup> cadmium, respectively. Brain TBARS levels remained elevated in fish exposed for 14 days to cadmium (100% - 0.2 mg L<sup>-1</sup> and 130% - 0.4 mg L<sup>-1</sup>) after the recovery period of 14 days (Fig. 3B). In our study was observed a significant relation between brain AChE inhibition and TBARS increase in silver catfish exposed during 14 days to sublethal concentrations of cadmium (Fig. 4A) ( $r^2 = 0.8584$  and  $P < 0.0001$ ). This correlation was also observed after the recovery period of 14 days (Fig. 4B) ( $r^2 = 0.9662$  and  $P < 0.0001$ ).

Silver catfish exposed to cadmium showed changes in the accumulation of this metal in brain and muscle. After exposure of 7 days, little accumulation was observed

in brain however a higher concentration of cadmium was verified in muscle. The highest cadmium accumulation in these tissues was observed after 14 days of exposure. Brain exhibited a high potential of accumulation for cadmium after exposure of 14 days when compared to muscle. The accumulation in brain was 2-fold higher than that in muscle. The elimination of cadmium in the tissues was similar, and about 3-fold lower concentrations were found in the brain and muscle 14 days after transference to water without the addition of cadmium (Table 1). In the liver was observed higher accumulation when compared to brain and muscle. In this tissue, after both exposure periods, was verified increase in cadmium concentration but when fish were transferred to clean water for 7 days an increase even higher was found. After 14 days in water without the addition of cadmium was no observed elimination of cadmium in liver.

In this study, silver catfish exposed for 7 days to 0.2 and 0.4 mg L<sup>-1</sup> cadmium exhibited a significant reduction in the glucose levels and significant increase in the lactate levels in the liver. However, these parameters were not altered after 14 days exposure (Table 2). It was observed a significant increase in the protein levels in the liver after exposure for 7 days to 0.4 mg L<sup>-1</sup> cadmium, but a significant reduction in this parameter following 14 days exposure. Amino acids and ammonia values in the liver did not present changes when silver catfish were exposed for 7 days to cadmium. However, fish exhibited a significant increase in these metabolic parameters following 14 days exposure period to 0.4 mg L<sup>-1</sup> cadmium. Hepatic glycogen levels remained unaltered after both exposure periods. Following 7 days in water without the addition of cadmium, the parameters like glucose, lactate, protein, amino acids, and ammonia values returned to control levels. Only liver glycogen levels increased significantly in both groups. The percentage of increase in this

parameter reached 38% and 78.2% in fish that had been exposed to 0.2 and 0.4 mg L<sup>-1</sup> cadmium, respectively. After the recovery period of 14 days, liver glycogen level decreased to about 37% value of silver catfish exposed to 0.4 mg L<sup>-1</sup> cadmium. An increase was observed in the hepatic lactate concentration at 0.2 and 0.4 mg L<sup>-1</sup> cadmium (27% and 33%) and ammonia values (42% and 36%, respectively). In this period, amino acids levels increased 6% in fish exposed to 0.4 mg L<sup>-1</sup> cadmium and protein and glucose values did not differ significantly from control values.

Glycogen levels increased (150 and 137% at 0.2 and 0.4 mg L<sup>-1</sup> cadmium, respectively) in silver catfish muscle after 7 days exposure, but reduced after 14 days (Table 3). Muscle glucose values also increased after 7 days of exposure (24 and 33% at 0.2 and 0.4 mg L<sup>-1</sup>, respectively) but there were no changes in this parameter after 14 days exposure. Fish presented a significant decrease (21%) in protein levels when exposed to 0.4 mg L<sup>-1</sup> cadmium for 7 days and similar decrease (about 24%) was observed in fish maintained at both concentrations for 14 days. We observed an increase in amino acids and ammonia content in the white muscle of silver catfish after 7 days exposure at 0.4 mg L<sup>-1</sup> cadmium, but there were no changes in these parameters following 14 days exposure. Lactate levels were not altered by cadmium exposure.

As observed in the hepatic tissue, most metabolic parameters investigated in the muscle recovered after 7 days in water without cadmium. In this tissue lactate, glycogen, amino acids and ammonia concentrations returned to the control values (Table 3). But increasing glucose (40%) in muscle tissue remained at 0.4 mg L<sup>-1</sup> cadmium concentration still after recovery period. The protein values continued decreased at concentrations of 0.2 and 0.4 mg L<sup>-1</sup> cadmium (9% approximately for both concentrations) following recovery for 7 days in water without the addition of

cadmium. After 14 days of recovery the parameters altered in the muscle in the exposure period as glycogen and protein returned to control values and only ammonia levels were enhanced at 0.4 mg L<sup>-1</sup> cadmium (about 31%).

As seen in the Table 4, parameters studied in plasma of silver catfish had alterations that were time and metal concentration dependent. After 7 days exposure to 0.2 and 0.4 mg L<sup>-1</sup> cadmium, lactate levels significantly increased (38 and 47%, respectively), but protein and glucose levels remained unaltered. However, when fish were exposed for 14 days to 0.2 and 0.4 mg L<sup>-1</sup> cadmium, significant reduction in glucose, lactate and protein levels were observed. Following 7 days in water without the addition of cadmium, silver catfish showed reduction in lactate levels (42% in 0.2 mg/L and 26% at 0.4 mg L<sup>-1</sup>) and no significant alteration was verified in plasma glucose and protein values. After 14 days recovery period of silver catfish exposed to 0.4 mg L<sup>-1</sup> cadmium, plasma glucose and lactate levels return to control levels, but protein levels remained significantly lower (23%).

## Discussion

In this study the LC<sub>50</sub> obtained for silver catfish exposed to cadmium was 1.7 mg L<sup>-1</sup>. Other species are more resistant. In water with hardness of 100.5 mg L<sup>-1</sup> were obtained values of LC<sub>50</sub> of 4.3 and 17.1 mg L<sup>-1</sup> to cadmium for carp fry and fingerlings, respectively (Suresh et al., 1995). The determined 96 hours LC<sub>50</sub> of cadmium for juveniles *Oreochromis niloticus* was 18.58 mg L<sup>-1</sup> (Almeida et al., 2001). The 48 hours LC<sub>50</sub> for *Oreochromis mossambicus* was 50 mg L<sup>-1</sup> (Basha and Rani, 2003). Indigenous species like silver catfish appear to be more sensitive to metals than exotic species.

The results of the present investigation indicated that exposure of silver catfish to waterborne cadmium decreased significantly brain and muscle AChE activity after 14 days of exposure. The same results were not found following 7 days of exposure, where it was observed only an increase in the muscle AChE activity. Inhibition of AChE activity is reported mainly in studies performed with pesticides, but some investigations have correlated exposure to metals with effects on fish brain AChE activity. Lionetto et al. (2003) observed reduction in brain AChE activity in *Mullus barbatus* sampled in different places along the Italian coast contaminated with several heavy metals. In other study performed by De La Torre et al. (2002) brain AChE activity of juveniles *Cyprinus carpio* showed inhibition when fish were collected from contaminated rivers of Argentina. However, other studies showed different responses in AChE activity after exposure to cadmium or other metals. When rainbow trout (*Oncorhynchus mykiss*) larvae were exposed for 24 and 96 hours to 2.5 and 5.0  $\mu\text{g L}^{-1}$  cadmium no alteration was observed in the brain AChE activity (Beauvais et al., 2001). However, Romani et al. (2003) observed a significant increase in brain and muscle AChE activity of *Sparus auratus* in the presence of 0.5  $\text{mg L}^{-1}$  copper during 20 days. Organisms have several potential adaptations to changes in cholinesterases as increased synthesis of cholinesterases or a decreased synthesis of acetylcholine (ACh).

In our study the major effects was AChE inhibition probably due to cadmium toxicity. Heavy metals such as cadmium, at neuromuscular junctions, interferes with calcium-mediated neurotransmitter release. In the brain, this metal inhibits enzymes such as  $\text{Mg}^{2+}$ -ATPase and  $\text{Na}^+$ - $\text{K}^+$ -ATPase, causing metabolic effects and disrupting neurotransmitter uptake (Beauvais et al., 2001). In several situations acetylcholine is not broken and accumulates within synapses causing physiologic impairments and

alterations in fish swimming behavior (Miron et al., 2005; Gluszczak et al., 2006). The inhibition and activation in AChE activity of muscle reported in our study could be interpreted as changes in the enzyme due to binding metals with functional active AChE site. The cadmium also could interfere with calcium-mediated neurotransmitter and causes undesirable effects at neuromuscular junctions.

Exposure of silver catfish to cadmium for 7 and 14 days caused a significant increase in TBARS contents and cadmium accumulation in the brain. TBARS formation could be linked to cadmium accumulation. The fish metal accumulation was observed in several fish species. Gioda et al. (2007) found an increase in brain TBARS levels in *Leporinus obtusidens* exposed to zinc (2.3 and 4.6 mg L<sup>-1</sup>), and exposed to copper (0.02 and 0.04 mg L<sup>-1</sup>). Brain can be susceptible to oxidative damage since it contains a large amount of polyunsaturated fatty acids and consumes a high percentage of the body oxygen (Song et al., 2006). According to Livingstone (2001) lipid peroxidation (LPO), measured as TBARS, has been used as effective biomarker of toxic pollutants in fish exposed to copper, cadmium and iron. The increase in LPO seems to be linked to the inhibition of brain AChE activity. Relationship between LPO and AChE inhibition has been reported in some studies. This correlation was significant in *Oreochromis niloticus* exposed for 7 and 15 days to pesticide diazinon (1 and 2 mg L<sup>-1</sup>) (Üner et al., 2006). Pari and Murugavel (2007) also found changes in the activity of AChE and LPO in brain of rats exposed to cadmium. When the animals were exposed to metal (3 mg kg<sup>-1</sup> day<sup>-1</sup> subcutaneously) for 3 weeks, a significant decrease in the AChE activity was observed and in the same way a significant increase in the LPO levels. In our study, changes in the AChE activity can be associated with TBARS levels in brain. A significant relation was observed between brain AChE inhibition and TBARS increase in silver catfish. The

increase in brain TBARS levels can indicate damage to molecules like lipids and can lead to enzymatic alterations in this tissue.

Fish accumulate heavy metals in their tissues in higher concentration than the environment, and these metals can produce reactive oxygen species (ROS), resulting in LPO and antioxidant enzymes alterations (Soares et al., 2008). Some studies investigated metals accumulation and distribution in organs as liver, kidney, gills (Wu et al., 2007), brain, and muscle (Romani et al., 2003). In our study cadmium accumulation was high in brain and muscle following 14 days of exposure and the brain tissue accumulated about two-fold cadmium than muscle. In this period AChE activity in the brain was significantly inhibited and higher levels of LPO were observed in this tissue. The liver, when compared to brain and muscle presented the highest concentrations cadmium and in the period of 7 days was found the highest accumulation in this tissue. Several studies showed that liver and kidney appear to be the most important fish organs in cadmium sequestration (Cinier et al., 1999; Wu et al., 2007). In these organs cadmium concentration increased rapidly to the saturation level. When the storage capacity limits of the liver and kidney are reached, cadmium accumulation in other organs as muscle is stimulated (Cinier et al., 1997).

In this study different alterations were observed in the metabolic parameters in the liver and muscle tissue. There was a significant reduction in hepatic glucose but no changes were observed in the liver glycogen storage following exposure for 7 days to cadmium. Liver glucose was possibly utilized for maintenance of normal levels of plasma glucose, since glucose values were not altered either after exposure and recovery periods. Some studies have related glycogen depletion frequently accompanied by an increase of glucose in tissues (liver and muscle) or in the blood as a common response in fish exposed to heavy metals and stressful pollutants

(Gimeno et al., 1994; Cattani et al., 1996). In our study depletion of muscle glycogen was verified after 14 days of cadmium exposure, but muscular glycogen and glucose content increased in silver catfish exposed for 7 days to cadmium. Similarly *Oreochromis niloticus* also exhibited an increase of glucose concentration in muscle after 7 days of exposure to sublethal cadmium concentrations (0.32-2.56 mg L<sup>-1</sup>), but no alteration was observed in glycogen content in this tissue (Almeida et al., 2001). The sum of results showed that fish adapt metabolic state to survive in water cadmium contaminated and maintain glucose in the tissues.

Tissue protein content depends on the dynamic equilibrium between the rates of its synthesis and degradation. Considering protein metabolism in liver and muscle tissue, in this study the main effects were found when silver catfish were exposed to 0.4 mg/L cadmium. A reduction of protein levels associated with increasing amino acids and ammonia levels were found in this study. Probably the intensive proteolysis is due to cadmium toxicity. Some studies indicate a decrease in total protein content during heavy metal exposure. Cadmium concentrations (0.32, 0.64, 1.28 and 2.56 mg L<sup>-1</sup>) significantly decreased total protein levels in liver and white muscle of *Oreochromis niloticus* following 7 days in water with cadmium (Almeida et al., 2001). After 60 and 120 days exposed to zinc (2.4 mg L<sup>-1</sup>), *Channa punctatus* also exhibited reduction in liver and muscle protein levels (Hamilton et al., 1998). To overcome the stress caused by pollutants the animals require high energy. This energy demand might have led to the stimulation of protein catabolism (De la Torre et al., 2000). According to Almeida et al. (2001) the enhanced levels of glucose coupled with the maintenance of glycogen stores and the depletion of protein concentrations suggest that protein depletion enhanced the potential for gluconeogenesis in white muscle. Also, according to Almeida et al. (2001) cadmium may lead to biochemical changes



as observed when fish follow food deprivation resulting in broken molecules to energy obtaining. De Smet and Blust (2001) found increased amino acids concentrations in gills, liver and kidney in *Cyprinus carpio* exposed for 4 days to 0.8, 4 and 20  $\mu\text{M}$  cadmium. Protein catabolism release free amino acids and these are mobilized in order to provide the extra energy demands under stressful conditions caused by toxics (De Smet and Blust, 2001; David et al., 2004). An increase in the ammonia concentration in the liver and muscle of silver catfish exposed to 0.4  $\text{mg L}^{-1}$  cadmium might be due to elevated protein catabolism in these tissues. These results have been observed by other authors in fish tissues after exposure to toxicants (Begum, 2004; Gluszczak et al., 2007).

Lactate and amino acids are considered the main gluconeogenic precursors in mammals and could be in fish. In our study increase in lactate levels occur in the liver and plasma of fish exposed to cadmium for 7 days. An increase in lactate levels indicates metabolic disorders and may suggest a severe respiratory stress in the tissue (Begum and Vijayaraghavan, 1990). Gluszczak et al. (2007) found higher lactate levels in liver and white muscle of silver catfish in response to commercial formulation Roundup<sup>®</sup>. The elevation of lactate levels found in our study indicates metabolic disorders and a response against energy depletion. Reduction in lactate, protein and glucose levels in plasma after 14 days of exposure was showed in this study. These results can to mean a state of the energy failure for metabolic functions and for cadmium detoxification process in the silver catfish. These alterations could be an adaptive response of fish tissues to presence of cadmium in water.

In conclusion, the results obtained indicate that waterborne cadmium affects more strongly brain AChE activity and appears to be related with increase of TBARS levels in this tissue. The protein and carbohydrate metabolism in the liver and muscle

tissue is altered by cadmium exposure. The changes in metabolic parameters were reversible however, may affect fish health making them more sensitive to environmental changes. Thus the evaluated parameters can be used to monitor metal toxicity in silver catfish.

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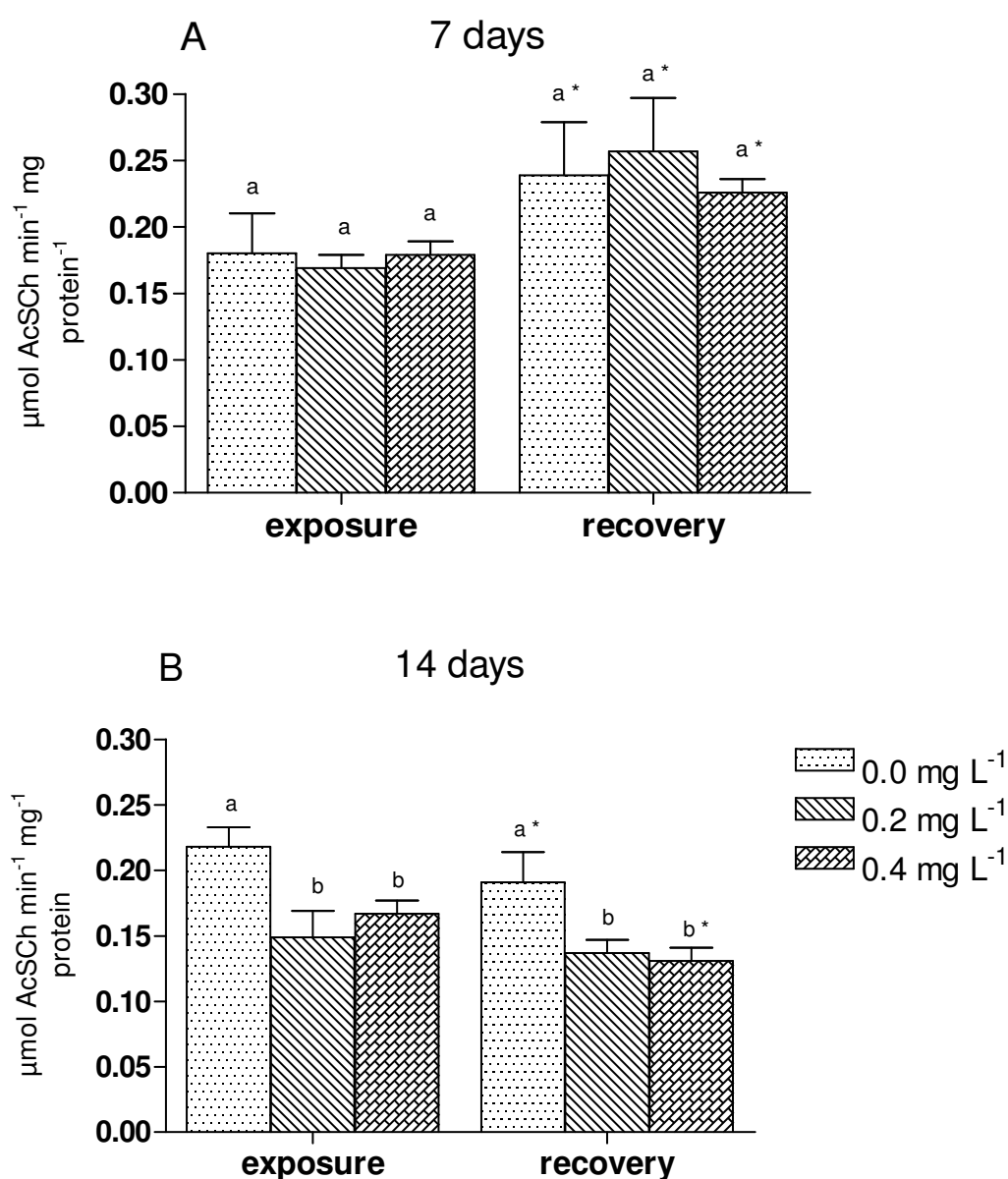


Figure 1. Specific AChE activity in brain of *Rhamdia quelen* after 7 days of exposure to cadmium and recovery (A) and after 14 days of exposure to cadmium and recovery (B). Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

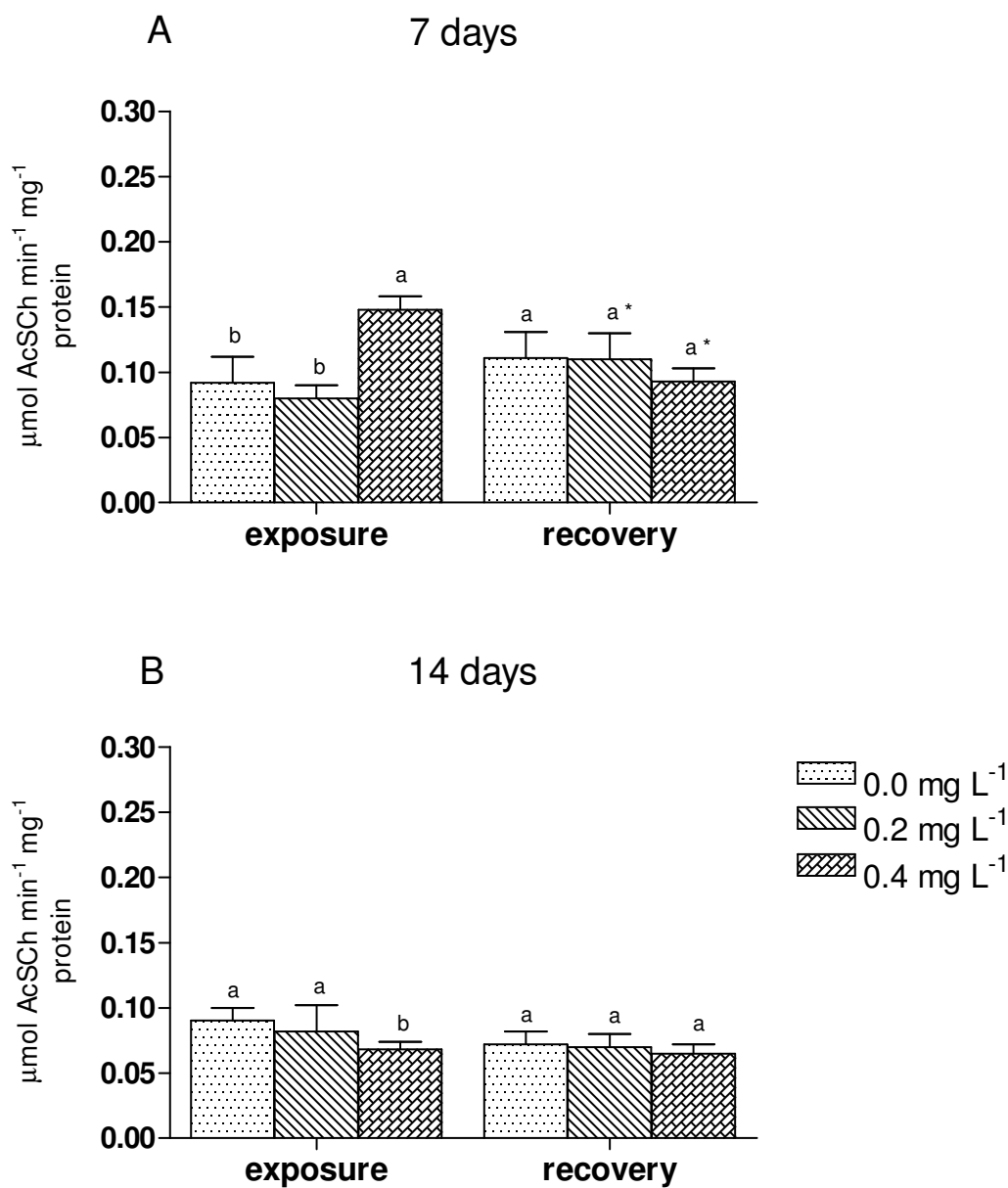


Figure 2. Specific AChE activity in muscle of *Rhamdia quelen* after 7 days of exposure to cadmium and recovery (A) and after 14 days of exposure to cadmium and recovery (B). Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

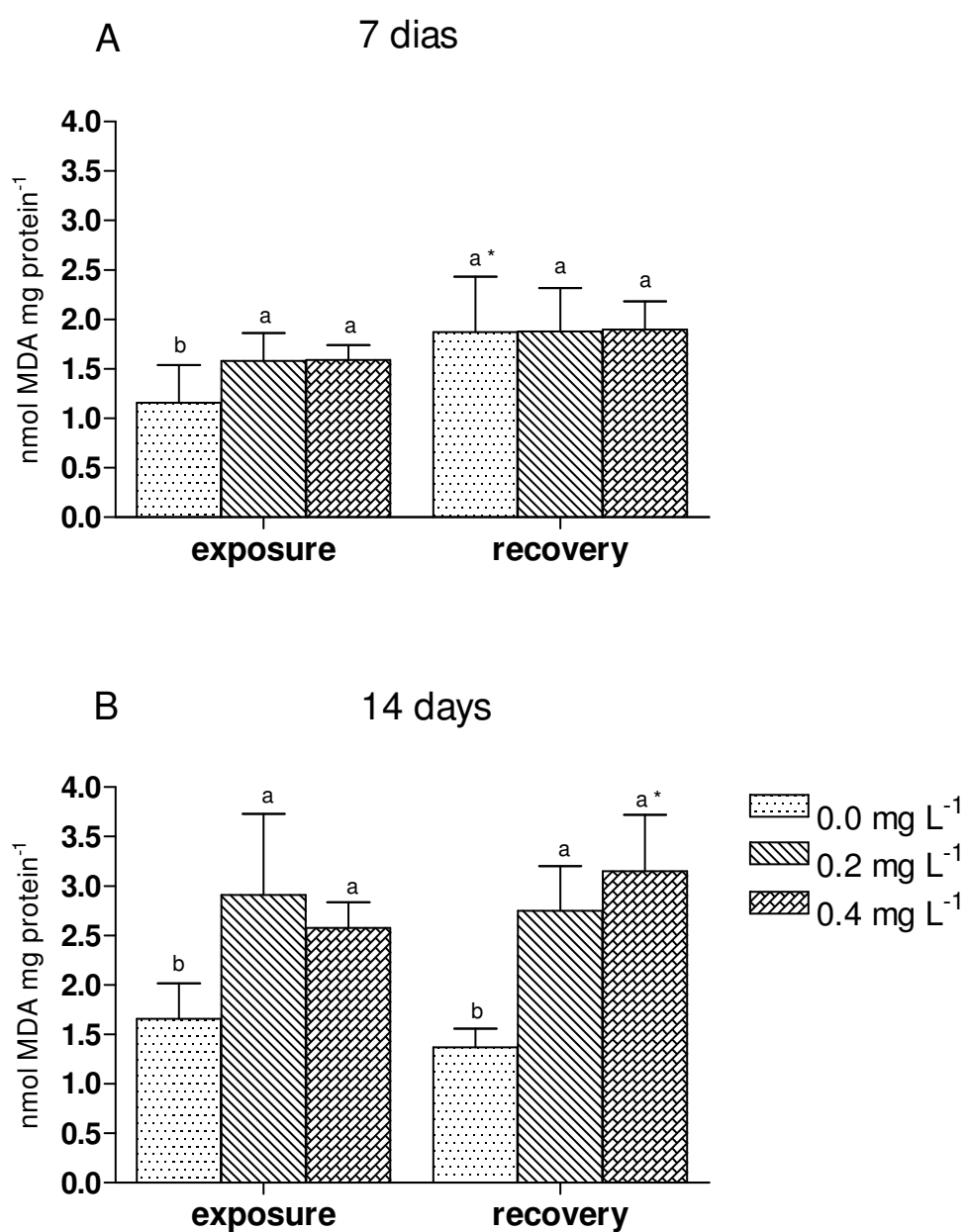


Figure 3. TBARS levels in brain of *Rhamdia quelen* after 7 days of exposure to cadmium and recovery (A) and after 14 days of exposure to cadmium and recovery (B). Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

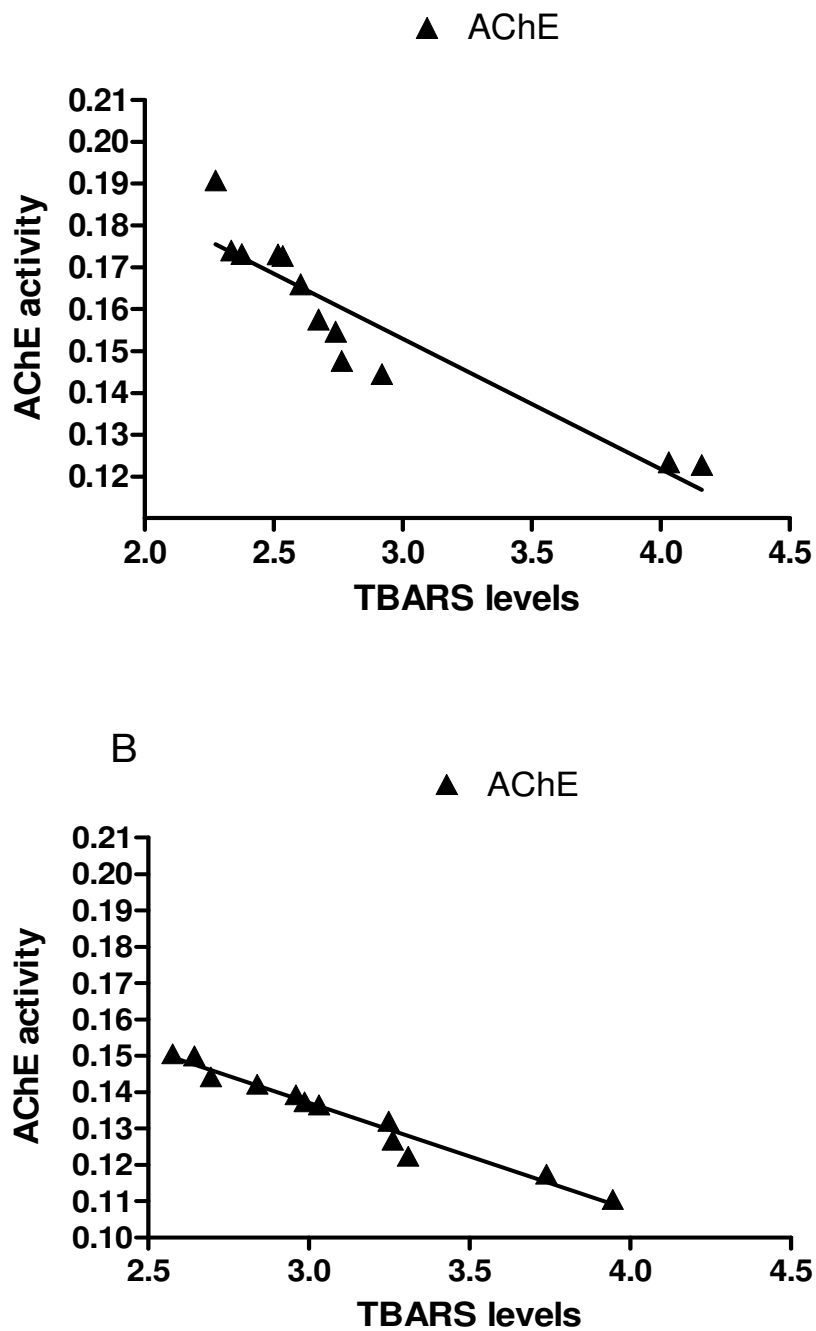


Figure 4. The relationships between the TBARS levels and the specific AChE activity in the brain of *Rhamdia quelen* after periods of 14 days in exposure (A) and recovery (B) to cadmium.

Table 1

Cadmium accumulation ( $\mu\text{g g}^{-1}$  tissue) in brain, muscle and liver of *Rhamdia quelen* after exposure to cadmium and recovery

Cadmium concentration in water $\text{mg L}^{-1}$	Exposure (days)		Recovery (days)	
	7	14	7	14
Brain				
0.0	< 0.006 <sup>a 1</sup>	0.006 <sup>a</sup>	< 0.006 <sup>a</sup>	0.037 $\pm$ 0.007 <sup>a*</sup>
0.2	0.006 <sup>a</sup>	0.209 $\pm$ 0.015 <sup>b</sup>	0.006 <sup>a</sup>	0.071 $\pm$ 0.015 <sup>b*</sup>
0.4	0.09 $\pm$ 0.029 <sup>b</sup>	0.255 $\pm$ 0.024 <sup>c</sup>	0.006 <sup>a*</sup>	0.097 $\pm$ 0.01 <sup>c*</sup>
Muscle				
0.0	< 0.006 <sup>a</sup>	0.058 $\pm$ 0.010 <sup>a</sup>	< 0.006 <sup>a</sup>	0.008 $\pm$ 0.001 <sup>a*</sup>
0.2	0.042 $\pm$ 0.008 <sup>b</sup>	0.091 $\pm$ 0.011 <sup>b</sup>	0.037 $\pm$ 0.004 <sup>b</sup>	0.024 $\pm$ 0.004 <sup>b*</sup>
0.4	0.081 $\pm$ 0.011 <sup>c</sup>	0.094 $\pm$ 0.014 <sup>b</sup>	0.043 $\pm$ 0.007 <sup>b*</sup>	0.046 $\pm$ 0.015 <sup>c*</sup>
Liver				
0.0	0.04 $\pm$ 0.006 <sup>a</sup>	0.170 $\pm$ 0.05 <sup>a</sup>	0.175 $\pm$ 0.06 <sup>a</sup>	0.117 $\pm$ 0.001 <sup>a</sup>
0.2	1.70 $\pm$ 0.14 <sup>a</sup>	10.40 $\pm$ 1.13 <sup>b</sup>	7.80 $\pm$ 1.13 <sup>b</sup>	4.80 $\pm$ 0.56 <sup>b</sup>
0.4	34.63 $\pm$ 9.89 <sup>b</sup>	14.30 $\pm$ 1.84 <sup>b</sup>	56.90 $\pm$ 1.20 <sup>c*</sup>	7.40 $\pm$ 1.50 <sup>c</sup>

<sup>1</sup>Data are reported as mean  $\pm$  standard deviation ( $n = 4$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 2

Effect of exposure and recovery of *Rhamdia quelen* to different concentration of cadmium on liver's glycogen, glucose, lactate, protein, amino acids and ammonia

Cadmium concentration in water mg L <sup>-1</sup>	Exposure (days)		Recovery (days)	
	7	14	7	14
Glycogen (μmol g <sup>-1</sup> tissue)				
0.0	144.7 ± 8.7 <sup>a1</sup>	165.1 ± 38.6 <sup>a</sup>	122.8 ± 17.1 <sup>c</sup>	103.7 ± 23.9 <sup>a*</sup>
0.2	158.6 ± 15.0 <sup>a</sup>	182.5 ± 25.9 <sup>a</sup>	169.5 ± 14.0 <sup>b</sup>	99.2 ± 19.7 <sup>a*</sup>
0.4	149.2 ± 5.6 <sup>a</sup>	210.4 ± 41.8 <sup>a</sup>	218.9 ± 41.6 <sup>a*</sup>	65.3 ± 12.1 <sup>b*</sup>
Glucose (μmol g <sup>-1</sup> tissue)				
0.0	91.1 ± 11.7 <sup>a</sup>	90.4 ± 16.9 <sup>a</sup>	89.0 ± 18.0 <sup>a</sup>	71.1 ± 5.7 <sup>a*</sup>
0.2	58.0 ± 6.8 <sup>b</sup>	85.7 ± 10.6 <sup>a</sup>	90.4 ± 10.8 <sup>a*</sup>	70.6 ± 9.7 <sup>a*</sup>
0.4	65.1 ± 9.4 <sup>b</sup>	86.3 ± 8.0 <sup>a</sup>	97.5 ± 11.3 <sup>a*</sup>	73.4 ± 5.1 <sup>a</sup>
Lactate (μmol g <sup>-1</sup> tissue)				
0.0	5.7 ± 1.7 <sup>b</sup>	6.4 ± 0.6 <sup>a</sup>	5.2 ± 1.3 <sup>a</sup>	4.9 ± 0.8 <sup>b</sup>
0.2	7.9 ± 1.6 <sup>a</sup>	5.8 ± 0.5 <sup>a</sup>	6.0 ± 1.3 <sup>a*</sup>	6.2 ± 0.7 <sup>a</sup>
0.4	7.5 ± 1.1 <sup>a</sup>	6.0 ± 0.9 <sup>a</sup>	5.5 ± 1.2 <sup>a*</sup>	6.5 ± 0.9 <sup>a</sup>
Protein (mg g <sup>-1</sup> tissue)				
0.0	89.4 ± 13.7 <sup>b</sup>	171.1 ± 52.1 <sup>a</sup>	160.1 ± 14.1 <sup>a*</sup>	117.9 ± 39.9 <sup>a*</sup>
0.2	105.1 ± 20.0 <sup>b</sup>	211.2 ± 38.5 <sup>a</sup>	147.9 ± 26.1 <sup>a*</sup>	119.9 ± 28.9 <sup>a*</sup>
0.4	113.2 ± 11.3 <sup>a</sup>	113.6 ± 10.6 <sup>b</sup>	168.2 ± 30.1 <sup>a*</sup>	94.1 ± 20.4 <sup>a</sup>
Amino acids (μmol g <sup>-1</sup> tissue)				
0.0	75.9 ± 14.1 <sup>b</sup>	101.6 ± 4.9 <sup>b</sup>	60.8 ± 1.93 <sup>a</sup>	137.4 ± 7.6 <sup>b*</sup>
0.2	71.2 ± 9.9 <sup>b</sup>	104.7 ± 5.4 <sup>b</sup>	74.3 ± 8.5 <sup>a</sup>	140.4 ± 3.4 <sup>b*</sup>
0.4	113.7 ± 20.0 <sup>a</sup>	127.4 ± 8.1 <sup>a</sup>	74.6 ± 16.3 <sup>a*</sup>	145.9 ± 3.8 <sup>a*</sup>
Ammonia (μg g <sup>-1</sup> tissue)				
0.0	28.2 ± 2.0 <sup>a</sup>	23.4 ± 3.5 <sup>b</sup>	38.1 ± 2.2 <sup>a*</sup>	22.9 ± 5.1 <sup>b</sup>
0.2	26.3 ± 3.5 <sup>a</sup>	27.6 ± 3.8 <sup>b</sup>	37.5 ± 1.9 <sup>a*</sup>	32.5 ± 6.3 <sup>a</sup>
0.4	28.2 ± 4.4 <sup>a</sup>	29.2 ± 3.7 <sup>a</sup>	40.5 ± 3.3 <sup>a*</sup>	31.1 ± 5.8 <sup>a</sup>

<sup>1</sup>Data are reported as mean ± standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 3

Effect of exposure and recovery of *Rhamdia quelen* to different concentration of cadmium on muscle's glycogen, glucose, lactate, protein, amino acids and ammonia

Cadmium concentration in water mg L <sup>-1</sup>	Exposure (days)		Recovery (days)	
	7	14	7	14
Glycogen ( $\mu\text{mol g}^{-1}$ tissue)				
0.0	3.74 $\pm$ 0.96 <sup>b 1</sup>	3.84 $\pm$ 0.22 <sup>a</sup>	3.60 $\pm$ 0.53 <sup>a</sup>	4.85 $\pm$ 0.80 <sup>a</sup>
0.2	9.36 $\pm$ 0.49 <sup>a</sup>	3.25 $\pm$ 0.22 <sup>b</sup>	3.86 $\pm$ 0.80 <sup>a*</sup>	4.20 $\pm$ 0.58 <sup>a</sup>
0.4	8.87 $\pm$ 1.51 <sup>a</sup>	3.23 $\pm$ 0.40 <sup>b</sup>	3.62 $\pm$ 0.43 <sup>a*</sup>	4.77 $\pm$ 0.60 <sup>a*</sup>
Glucose ( $\mu\text{mol g}^{-1}$ tissue)				
0.0	0.40 $\pm$ 0.05 <sup>b</sup>	0.40 $\pm$ 0.09 <sup>a</sup>	0.24 $\pm$ 0.04 <sup>b*</sup>	0.41 $\pm$ 0.03 <sup>a</sup>
0.2	0.53 $\pm$ 0.06 <sup>a</sup>	0.42 $\pm$ 0.09 <sup>a</sup>	0.24 $\pm$ 0.04 <sup>b*</sup>	0.37 $\pm$ 0.04 <sup>a</sup>
0.4	0.49 $\pm$ 0.05 <sup>a</sup>	0.46 $\pm$ 0.08 <sup>a</sup>	0.33 $\pm$ 0.05 <sup>a*</sup>	0.39 $\pm$ 0.04 <sup>a</sup>
Lactate ( $\mu\text{mol g}^{-1}$ tissue)				
0.0	6.85 $\pm$ 0.92 <sup>a</sup>	6.49 $\pm$ 0.77 <sup>a</sup>	4.96 $\pm$ 0.55 <sup>a*</sup>	6.12 $\pm$ 0.25 <sup>a</sup>
0.2	7.83 $\pm$ 0.99 <sup>a</sup>	6.11 $\pm$ 1.47 <sup>a</sup>	5.48 $\pm$ 0.65 <sup>a*</sup>	5.91 $\pm$ 1.26 <sup>a</sup>
0.4	6.82 $\pm$ 1.15 <sup>a</sup>	7.41 $\pm$ 0.49 <sup>a</sup>	5.20 $\pm$ 0.53 <sup>a*</sup>	6.39 $\pm$ 0.75 <sup>a</sup>
Protein (mg g <sup>-1</sup> tissue)				
0.0	100.1 $\pm$ 16.5 <sup>a</sup>	115.1 $\pm$ 12.4 <sup>a</sup>	105.4 $\pm$ 6.6 <sup>a</sup>	152.6 $\pm$ 21.3 <sup>a*</sup>
0.2	93.6 $\pm$ 14.8 <sup>a</sup>	86.7 $\pm$ 15.1 <sup>b</sup>	95.9 $\pm$ 8.3 <sup>b</sup>	147.7 $\pm$ 28.7 <sup>a*</sup>
0.4	79.08 $\pm$ 9.6 <sup>b</sup>	87.3 $\pm$ 11.9 <sup>b</sup>	96.5 $\pm$ 3.6 <sup>b</sup>	167.6 $\pm$ 16.1 <sup>a*</sup>
Amino acids ( $\mu\text{mol g}^{-1}$ tissue)				
0.0	18.8 $\pm$ 2.8 <sup>b</sup>	24.6 $\pm$ 7.1 <sup>a</sup>	19.0 $\pm$ 2.6 <sup>a</sup>	28.3 $\pm$ 4.8 <sup>a</sup>
0.2	20.3 $\pm$ 2.1 <sup>b</sup>	18.29 $\pm$ 2.9 <sup>a</sup>	21.1 $\pm$ 2.1 <sup>a</sup>	27.5 $\pm$ 3.9 <sup>a*</sup>
0.4	23.6 $\pm$ 4.3 <sup>a</sup>	19.1 $\pm$ 2.9 <sup>a</sup>	19.4 $\pm$ 2.8 <sup>a</sup>	25.1 $\pm$ 4.3 <sup>a*</sup>
Ammonia ( $\mu\text{g g}^{-1}$ tissue)				
0.0	8.73 $\pm$ 0.82 <sup>b</sup>	5.76 $\pm$ 1.27 <sup>a</sup>	7.17 $\pm$ 0.62 <sup>a*</sup>	6.27 $\pm$ 0.92 <sup>b</sup>
0.2	10.15 $\pm$ 1.25 <sup>b</sup>	7.34 $\pm$ 1.48 <sup>a</sup>	6.36 $\pm$ 0.55 <sup>a*</sup>	6.64 $\pm$ 0.87 <sup>b</sup>
0.4	11.33 $\pm$ 1.75 <sup>a</sup>	6.65 $\pm$ 1.02 <sup>a</sup>	6.65 $\pm$ 1.10 <sup>a*</sup>	8.19 $\pm$ 0.77 <sup>a*</sup>

<sup>1</sup>Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 4

Effect of exposure and recovery of *Rhamdia quelen* to different concentration of cadmium on plasma's glucose, lactate and protein

Cadmium concentration in water mg L <sup>-1</sup>	Exposure (days)		Recovery (days)	
	7	14	7	14
Glucose (mg dL <sup>-1</sup> )				
0.0	50.5 ± 12.0 <sup>a 1</sup>	53.4 ± 7.7 <sup>a</sup>	57.6 ± 16.4 <sup>a</sup>	43.3 ± 10.2 <sup>a</sup>
0.2	58.9 ± 9.2 <sup>a</sup>	35.5 ± 2.5 <sup>b</sup>	68.9 ± 16.6 <sup>a</sup>	42.5 ± 8.6 <sup>a</sup>
0.4	50.9 ± 13.8 <sup>a</sup>	39.3 ± 6.2 <sup>b</sup>	57.4 ± 13.6 <sup>a</sup>	46.5 ± 9.3 <sup>a</sup>
Lactate (μmol mL <sup>-1</sup> )				
0.0	3.08 ± 0.62 <sup>b</sup>	2.53 ± 0.58 <sup>a</sup>	2.84 ± 0.55 <sup>a</sup>	1.69 ± 0.17 <sup>a*</sup>
0.2	4.27 ± 0.59 <sup>a</sup>	1.84 ± 0.34 <sup>b</sup>	1.64 ± 0.41 <sup>b*</sup>	1.48 ± 0.19 <sup>a</sup>
0.4	4.53 ± 1.00 <sup>a</sup>	1.51 ± 0.55 <sup>b</sup>	2.08 ± 0.42 <sup>b*</sup>	1.80 ± 0.25 <sup>a</sup>
Protein (mg mL <sup>-1</sup> )				
0.0	32.3 ± 5.7 <sup>a</sup>	32.4 ± 2.4 <sup>a</sup>	25.4 ± 2.9 <sup>a*</sup>	30.3 ± 2.4 <sup>a</sup>
0.2	28.3 ± 3.2 <sup>a</sup>	28.2 ± 2.6 <sup>b</sup>	26.4 ± 3.3 <sup>a</sup>	28.2 ± 2.8 <sup>a</sup>
0.4	28.4 ± 3.6 <sup>a</sup>	29.1 ± 3.8 <sup>b</sup>	28.7 ± 1.9 <sup>a</sup>	23.3 ± 1.9 <sup>b*</sup>

<sup>1</sup>Data are reported as mean ± standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (ˆ) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).



4.2 Efeitos de concentrações de cádmio na água sobre a bioacumulação e vários parâmetros de estresse oxidativo em *Rhamdia quelen*.

4.2.1 Manuscrito II

**Effects of water cadmium concentrations on bioaccumulation and various oxidative stress parameters in *Rhamdia quelen***

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Effects of water cadmium concentrations on bioaccumulation and various oxidative stress parameters in *Rhamdia quelen*

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## Abstract

In the present study, the effects of sublethal cadmium concentrations on the parameters of oxidative stress were evaluated in silver catfish (*Rhamdia quelen*). The fish were exposed to 0.0, 0.2 and 0.4 mg L<sup>-1</sup> cadmium for 7 and 14 days following a recovery period of 7 and 14 days. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST), and indicators of oxidative stress such as thiobarbituric acid reactive species (TBARS) and protein carbonyl, were subsequently verified in fish tissues (liver, kidney and gills). In addition, the accumulation of cadmium was also evaluated in these tissues. Our results indicate that CAT and GST levels were reduced in the gills after exposure periods associated with an increase in TBARS levels while some variations remained during the recovery periods. In hepatic tissue, CAT, GST, TBARS, and protein carbonyl levels increased after exposure periods while SOD activity was reduced. During the recovery periods, some variations persisted in the liver. Cadmium accumulation was most significant in the kidney, followed by the liver and the gill. These results indicate that the sublethal cadmium concentrations studied invoke a stress response in silver catfish and the biochemical parameters measured herein may be suitable indicators of toxicity in this species.

**Keywords:** Cadmium, fish, liver, gill, oxidative stress, accumulation.

## 1. Introduction

Cadmium is one of the most toxic metals in the environment, serving no beneficial biological function in higher organisms, and is extremely toxic even at very low concentrations (Soares et al., 2008). Cadmium is also a prominent xenobiotic and cumulative pollutant found in aquatic ecosystems, making fish particularly vulnerable to cadmium exposure (Bhakta and Munekage, 2008). Fish accumulate cadmium in their tissues primarily through ingestion of contaminated food or via absorption along the gill surface from a polluted environment. The accumulation appears to occur predominantly in metabolically active tissues such as the kidney, the liver, the gills and throughout the digestive tract (Cinier et al., 1999; Soares et al., 2008). Studies exploring bioaccumulation and biochemical parameters in fish tissues can help elucidate the toxic effects of metals on aquatic organisms.

Several studies have associated heavy metals with the ability to produce reactive oxygen species (ROS), resulting in lipid peroxidation and variations in the level of antioxidant enzymes culminating in oxidative stress (Roméo et al., 2000; Sanchez et al., 2005). In the intracellular environment, the presence of many enzymatic and non-enzymatic antioxidant defense mechanisms maintains ROS at a low level. However, when these systems are overcome, a condition known as oxidative stress, affected organisms suffer oxidative damage to lipids, proteins and even to chromosomal DNA (Sies, 1993; Monserrat et al., 2007). Biomarkers that indicate oxidative stress in various organisms have been proposed to serve an important role in the biomonitoring of toxic metal pollution in aquatic ecosystems. Lipid peroxidation and protein carbonyl formation are two perturbations that are a consequence of oxidative stress. Lipid peroxidation results in the production of lipid radicals and in the subsequent formation of a complex mixture of lipid degradation

products (malondialdehyde and other aldehydes) that react with thiobarbituric acid (TBA) yielding a colored intermediate that is commonly used to quantify damage to cellular lipids (Almroth et al., 2005; Gioda et al., 2007). ROS are known to modify amino groups of proteins and thereby alter protein structure or function (Almroth et al., 2005). Protein carbonyl content is the most general indicator and the most commonly used marker of protein oxidation (Dalle-Donne et al., 2003). However, this biomarker has been used most commonly in research on oxidative stress in humans (Almroth et al., 2005; Parvez and Raisuddin, 2005) and few reports are available regarding its use in fish exposed to toxic chemicals such as metals.

All cells in eukaryotic organisms contain powerful antioxidant enzymes. The three major classes of antioxidant enzymes are superoxide dismutases, catalases and glutathione peroxidases. However, there are numerous specialized antioxidant enzymes that react to detoxify oxidant compounds (Sies, 1997). The activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) are parameters that are frequently studied when fish are exposed to metals. These enzymes belong to the antioxidant defense system that has evolved to protect the organism by directly scavenging oxygen intermediates as superoxide radicals and hydrogen peroxide, converting them to less reactive species (Sampaio et al., 2008). GST and GPx belong to glutathione (GSH) system that is one of the most important antioxidant systems in fish (Zirong and Shijun, 2007). GST catalyzes the conjugation of GSH with a variety of electrophilic metabolites that are involved in the detoxification of both reactive intermediates and oxygen radicals (Monteiro et al., 2006).

Silver catfish (*Rhamdia quelen*) can be found in waters from Southern Mexico to Central Argentina, and their husbandry is spreading across Southern Brazil

(Gomes et al., 2000), since these fish can survive cold winters and grow rapidly in the summer (Barcellos et al., 2003). This species shows good productivity and it is acceptable to consumers, however, there are only data about cadmium effects from *R. quelen* larvae (Benaduce et al., 2008). Laboratory or in situ fish exposure studies are often conducted to assess the lethal and sublethal effects of toxins, however few of these studies focus on the adaptation and/or the recovery capacity of these biomarkers when waterborne toxic exposure is terminated. The objective of this study was therefore to investigate possible consequences of cadmium exposure and the degree to which it accumulates in tissues by observing some oxidative stress parameters in tissues of silver catfish.

## **2. Materials and methods**

### *2.1. Chemicals*

All chemicals reagent grade were purchased from Sigma (St. Louis, MO, USA).

### *2.2. Fish*

Silver catfish juveniles from both sexes were obtained from a commercial fish farm near Santa Maria (Rio Grande do Sul, Brazil). Fish (weight,  $23.9 \pm 3.1$  g; length  $13.4 \pm 0.5$  cm) were acclimated in tanks (250 L) under laboratory conditions for 15 days. The fish were subsequently placed in continuously aerated water with a static system under a natural photoperiod (12 h light, 12 h dark) and received feed once a day (commercial fish pellets with 42% crude protein, Supra, Brazil). Water parameters were measured every day during the acclimation period and were as

follows: the temperature was  $23.7 \pm 1.0$  °C, pH  $7.6 \pm 0.2$ , dissolved oxygen concentration of  $6.8 \pm 0.5$  mg L<sup>-1</sup>, non-ionized ammonia  $1.23 \pm 0.05$  µg L<sup>-1</sup>, nitrite  $0.04 \pm 0.01$  mg L<sup>-1</sup>, hardness  $12.0 \pm 1.15$  mg L<sup>-1</sup> CaCO<sub>3</sub> and alkalinity  $39.0 \pm 3.2$  mg L<sup>-1</sup> CaCO<sub>3</sub>.

### 2.3. *Experimental design*

A previous experiment obtained an LC<sub>50</sub> 96 h of 1.7 mg L<sup>-1</sup> cadmium for silver catfish. Fish were transferred to plastic aquaria (45 L) with controlled aeration and temperature. Groups of 12 fish per aquarium (duplicates) were exposed to 0.2 or 0.4 mg L<sup>-1</sup> (approximately 10% and 20% of the LC<sub>50</sub>) for 7 and 14 days and the control groups (duplicate) were kept in water in the absence of the metal. Stock solutions were prepared by dissolving cadmium chloride (CdCl<sub>2</sub>.10H<sub>2</sub>O) in water and added to the experimental aquarium. During the exposure period of 7 days, the water was not changed during the experiment. Metal was then added to the water only at the beginning of the experiment. However, for the exposure period of 14 days, water was changed in middle of exposure (seventh day) using water with the same cadmium concentration. In this study, a biofilter was utilized in each aquarium to maintain the water quality. There were no mortalities throughout the experimental periods (7 and 14 days). The physico-chemical characteristics of water were as follows: temperature  $23.8 \pm 0.2$  °C, pH  $7.9 \pm 0.1$ , dissolved oxygen  $6.9 \pm 0.3$  mg L<sup>-1</sup>, non-ionized ammonia  $3.42 \pm 0.4$  µg L<sup>-1</sup>, nitrite  $0.04 \pm 0.01$  mg L<sup>-1</sup>, hardness  $15.0 \pm 2.1$  mg L<sup>-1</sup> CaCO<sub>3</sub> and alkalinity  $45.5 \pm 2.8$  mg L<sup>-1</sup> CaCO<sub>3</sub>. After each exposure period, six animals per aquarium were sampled. The fish were killed by excision of spinal cord behind the operculum and the tissues to be assayed (gills, liver and kidney) were removed and quickly placed on ice and frozen at -70 °C for analysis of oxidative stress parameters.

The remaining fish were maintained in the water without metal addition for 7 and 14 days to recover (after 7 and 14 days of exposure, respectively). Control fish were also sampled for each experimental period.

Four fish were sampled from each aquarium after exposure and recovery periods to perform analysis of cadmium accumulation in various fish tissues. The gills, liver and kidney were removed, washed in distilled water, dried with filter paper, weighed, and stored in plastic vials at  $-70\text{ }^{\circ}\text{C}$  for subsequent analysis. Tissue samples for the cadmium analysis were digested overnight for 1 min in a microwave oven after the addition of 2 mL of 65%  $\text{HNO}_3$ . The resulting solutions were subsequently diluted to 10 mL with ultra pure water. The total content of cadmium in the liver, the kidney, the gills and in the water samples was measured by graphite furnace atomic absorption spectrophotometry (GF AAS) and expressed in units of  $\mu\text{g g}^{-1}$  tissue and  $\text{mg L}^{-1}$  water, respectively.

#### 2.4. *Antioxidant enzymes*

All enzyme activities were measured spectrophotometrically. Samples of frozen tissue (liver, kidney and gills) were quickly weighed and homogenized in 20 mM potassium phosphate buffer (pH 7.5) using a Potter-Elvehjem glass/Teflon homogenizer. Samples were then centrifuged at 10 000  $g$  for 10 min at  $4\text{ }^{\circ}\text{C}$  and assayed for antioxidant enzymes activity. The protein content in the liver and in the kidney was measured using the Bradford assay (1976) while the protein content in the gills was determined using the protocol described by Lowry et al. (1951) with bovine serum albumin serving as the protein standard.

The CAT (EC 1.11.1.6) activity in liver and kidney tissues was assayed according to the work of Nelson and Kiesov (1972). The assay mixture consisted of



2.0 mL potassium phosphate buffer (50 mM, pH 7.0), 0.05 mL H<sub>2</sub>O<sub>2</sub> (0.3 M) and 0.05 mL of the homogenate. In order to determine catalase activity in the kidney, 0.1 mL H<sub>2</sub>O<sub>2</sub> (0.3 M) was used. The change in the H<sub>2</sub>O<sub>2</sub> absorbance was measured at 240 nm. Catalase activity was expressed in units of  $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$ .

The GST (EC 2.5.1.18) activity was measured in the liver and the gills using a procedure described by Habig et al. (1974) that involved 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The assay mixture contained 1 mM CDNB (in ethanol), 10 mM GSH, 20 mM potassium phosphate buffer (pH 6.5) and the tissue homogenates. Enzyme activity was calculated from the changes in absorbance at 340 nm using a molar extinction coefficient of  $9.6 \text{ mmol}^{-1} \text{ cm}^{-1}$ . One unit of GST activity was defined as the amount of enzyme required to catalyze the conjugation of 1  $\mu\text{mol}$  of CDNB with GSH per minute at 25 °C.

Measurements of SOD activity were performed for liver tissue based on the inhibition of the radical superoxide reaction with adrenalin as described by Mc Cord and Fridovich (1969). In this method, SOD present in the sample competes with the detection system for radical superoxide. A unit of SOD is defined as the amount of enzyme that inhibits the rate of oxidation of adrenalin by 50%. The oxidation of adrenalin leads to the formation of the colored product, adrenochrome, which is detected using a spectrophotometer. SOD activity is determined by measuring the rate of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mM, pH 10) and adrenalin (1 mM).

### 2.5. TBARS determination

The formation of peroxides can be quantified using a TBARS assay. This assay is performed by reacting malondialdehyde (MDA) with 2-thiobarbituric acid

(TBA), which can subsequently be optically measured. Tissue samples (liver, kidney and gills) were weighed and homogenized as described above in 20 mM potassium phosphate buffer (pH 7.5) using a Potter-Elvehjem glass/Teflon homogenizer. Samples were then centrifuged at 10 000 *g* for 10 min at 4 °C and analyzed using the TBARS assay. Aliquots of the homogenate aliquots were added to a solution containing 10% trichloroacetic acid (TCA) and 0.67% TBA and were adjusted to a final volume of 1.0 mL. The reaction mixture was placed in a microcentrifuge tube and incubated for 15 min at 95 °C. After cooling, the reaction was centrifuged at 5000 *g* for 10 min, after which the optical density at 532 nm was determined. The TBARS level is expressed in units of nmol MDA mg protein<sup>-1</sup> according to Buege and Aust (1978). In addition, the protein levels were estimated by the Bradford method (1976), again using bovine serum albumin as the protein standard as described above.

#### *2.6. Carbonil assay*

The liver tissue was homogenized in 10 volumes (w/v) of 10 mM Tris-HCl buffer pH 7.4 using a glass homogenizer. Protein carbonyl content was assayed by a procedure described previously by Yan et al. (1995) with slight modifications. Soluble protein (1.0 mL) was reacted with 10 mM DNPH in 2 N hydrochloric acid and incubated for 1 h in darkness at room temperature. A 0.5 mL aliquot of a denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3.0%), 2.0 mL of heptane (99.5%) and 2.0 mL of ethanol (99.8%) were then added sequentially to the mixture, which was vortexed for 40 s and centrifuged 3000 *g* for 15 min. The protein isolated from the interface of the aforementioned reaction was washed twice by resuspension in ethanol/ethyl acetate (1:1), and was then suspended in 1 mL of denaturing buffer. The carbonyl content was then measured spectrophotometrically

(Femto Scan spectrophotometer) at 370 nm. This assay was performed in duplicate and two blank tubes incubated with 2 N HCl in the absence of DNPH was included for each sample. The total carbonylation was calculated using a molar extinction coefficient of  $22.000 \text{ M}^{-1} \text{ cm}^{-1}$ .

### *2.7. Statistical procedures*

The data for each of these biochemical parameters were compared using the two-way analysis of variance followed by Duncan's Test. Results obtained ( $n=8$ ) were expressed as mean  $\pm$  standard deviation (SD). The differences were considered to be significant at a probability level of ( $P \leq 0.05$ ) between groups (exposure and recovery groups).

## **3. Results**

The mean values of cadmium concentrations (in  $\text{mg L}^{-1}$ ) in water samples collected from the first day of exposure were  $0.0004 \pm 0.00002$ ,  $0.236 \pm 0.004$  and  $0.414 \pm 0.006$  for control, 0.2 and 0.4  $\text{mg L}^{-1}$  groups, respectively. During the recovery periods (7 and 14 days), the metal concentrations means ( $\mu\text{g L}^{-1}$ ) were determined to be  $0.4 \pm 0.02$ ,  $0.4 \pm 0.02$  and  $0.55 \pm 0.06$  for those same groups. These results indicate that an increase in the waterborne cadmium concentration resulted in a progressive increase of cadmium in the liver, the kidney and the gills of silver catfish after exposure periods of 7 and 14 days (Table 1). The highest concentration of cadmium that accumulated in the liver was found to be  $0.4 \text{ mg L}^{-1}$  after an exposure and recovery period of 7 days. In the kidney, the accumulation pattern was similar to that found in the liver, which demonstrated a significant

increase after exposure periods. The cadmium accumulation increased in the liver and the kidney even after silver catfish were transferred to clean water for 7 days (Table 1). The cadmium concentration in the gills was similar for both exposure periods, with a significant increase observed with a concomitant increase in the waterborne cadmium concentration. After a recovery period of 14 days, we did not observe any elimination of cadmium from the liver. However, we did observe a reduction of cadmium in the kidney by roughly 38%. In the groups of fish exposed to cadmium and subsequently allowed to recover for periods of 7 or 14 days, we observed roughly a 46% and 50% elimination of cadmium, respectively, from gill tissues.

Fish exposed to cadmium exhibited an increased in CAT activity in the liver at both cadmium concentrations tested (0.2 and 0.4 mg L<sup>-1</sup>) after 7 (70-56%) and 14 days (45-49%) (Table 2). However, after a 7 day period of recovery, CAT activity in this tissue decreased by roughly 38% in fish that had been exposed to 0.4 mg L<sup>-1</sup> cadmium compared to the unexposed control fish. Similarly, CAT activity was observed to decrease in gill tissues of silver catfish after 7 and 14 day periods of exposure to cadmium. This inhibition reached 68% at 0.2 mg L<sup>-1</sup> and 83% at 0.4 mg L<sup>-1</sup> cadmium after exposure for 7 days and about 63% after 14 days (Table 2). After a recovery period of 7 days, CAT inhibition in gills remained (41-63%), but the activity of this enzyme was not altered compared to the control values after 14 days of recovery. There were no changes in CAT activity in the hepatic tissue after a recovery period of 14 days. In the kidney, we observed that CAT activity did not change between the assays performed during the exposure and the recovery periods.

Silver catfish maintained at 0.2 and 0.4 mg L<sup>-1</sup> cadmium for 7 days demonstrated a significant increase in the liver GST activity (69.8% and 36%, respectively) (Table 3). In addition, when these fish that had been exposed to 0.4 mg L<sup>-1</sup> were transferred to water without cadmium for 7 days, we observed a 34% decrease in GST activity. After exposure and recovery periods of 14 days, the liver GST activity returned to levels observed in the control fish liver tissues. In the gill tissue, the GST activity was significantly reduced after the exposure periods (7 and 14 days). Exposure to 0.2 and 0.4 mg L<sup>-1</sup> cadmium for 7 days appears to have inhibited this enzyme by roughly 63% and a 45 to 52% of the inhibition range that was observed following experiments with exposure times of 14 days. Even after the recovery period of 7 days, the GST activity in gills remained inhibited (about 44%), but these values recovered after 14 days in water in the absence of metal (Table 3).

In the present study, changes in the liver SOD activity were also observed. After 7 days in water with cadmium (0.4 mg L<sup>-1</sup>), silver catfish exhibited a reduction of 22% in the activity SOD and this reduction remained constant even after a recovery period of 7 days (Figure 1A). Following an exposure period of 14 days, SOD was inhibited at both metal concentrations (about 34% at 0.2 mg L<sup>-1</sup> and 29% at 0.4 mg L<sup>-1</sup> cadmium). However, the SOD values returned to values observed with the control fish after a 14 day recovery period (Figure 1B).

Exposure to cadmium (0.2 and 0.4 mg L<sup>-1</sup>) altered TBARS levels in different tissues of silver catfish. The liver did not show any changes in the levels after a seven day exposure period (Table 4). However, a significant increase in TBARS levels was observed following 14 days of exposure, with an increase of TBARS of 29% and 62% in fish from the two test groups exposed to 0.2 and 0.4 mg L<sup>-1</sup> cadmium, respectively. Following 7 and 14 days in water without cadmium, the

increase was 37% and 22%, respectively. In fish exposed to 0.4 mg L<sup>-1</sup> cadmium, we observed a reduction in the level of TBARS in the kidney by 32% after 7 days, but interestingly, a significant increase (127 to 150%) was verified after an exposure period of 14 days at both concentrations tested (Table 4). After both recovery periods, we observed that the TBARS levels returned to that observed for the control experiments for this tissue. The higher values of gill TBARS levels were obtained when silver catfish were exposed for 7 days to waterborne cadmium (increases of 296% and 273% at 0.2 and 0.4 mg L<sup>-1</sup> respectively). After a recovery period of 7 days, a reduction of approximately 40% in TBARS levels was observed in fish exposed to 0.4 mg L<sup>-1</sup> for 7 days. After 14 days of cadmium exposure there was a two-fold increase in TBARS levels in fish exposed to 0.2 mg L<sup>-1</sup> of cadmium. This increase persisted even after the recovery period of 14 days (Table 4).

Protein carbonyl formation was observed in the liver and few changes in these levels were observed in this study. After fish were exposed to cadmium (0.4 mg L<sup>-1</sup>) for 7 days, we discovered a 47% increase in protein carbonyl formation. In addition, a reduction in protein carbonyl levels in fish previously exposed to 0.2 mg L<sup>-1</sup> cadmium was observed following the recovery period of 7 days (Figure 2A). However, exposure and a recovery period of 14 days did not alter protein carbonyl levels in the liver of silver catfish (Figure 2B).

#### **4. Discussion**

The results of the present study indicated that cadmium exposure produces a significant accumulation of cadmium in tissues such as the kidney, the liver and the

gills. Pollutants rarely distribute uniformly in the animal body tissues, but can accumulate to varying degrees in particular target organs like the liver, the gill or the kidney (Cinier et al., 1999). At the end of the 7 and 14 day exposure periods, cadmium had accumulated most significantly in the kidney, followed by the liver and the gill tissues. These results are similar to those reported by Cattani et al. (1996) in *Dicentrarchus labrax* that were exposed to 0.5 and 5  $\mu\text{g L}^{-1}$  cadmium in seawater for 7 days. In studies performed in *Clarias gariepinus* that were exposed for 21 days to cadmium (0.1, 0.2 and 0.4  $\text{mg L}^{-1}$ ), cadmium accumulated preferentially in the kidney, followed by the gill tissue, the liver, and finally the muscle tissue (Asagba et al., 2008). Generally, the uptake of cadmium is tissue-specific and the concentration of cadmium in the tissues is influenced by the waterborne metal concentration. In addition, the gill, liver and kidney are the main sites of metallothionein production and metal retention. In these tissues, cadmium concentration increased due to the presence of these metallothionein proteins that are able to bind to the metal (Asagba et al., 2008). An increase in the liver and the kidney cadmium concentrations was observed in silver catfish after a recovery period for 7 days in cadmium-free water. Cinier et al. (1999) also observed that after an elimination period of 42 days, interestingly the cadmium concentration in the kidney of *Cyprinus carpio* had increased. According to Cinier et al. (1999), this unexpected increase may occur due to a redistribution of cadmium among the tissues before its ultimate excretion. For example, the metal may be transferred from the muscle tissues to the liver and kidney for excretion during the detoxification process. In our study, the uptake of cadmium could be influenced by metallothionein proteins content, water cadmium concentrations and certainly appears to be tissue specific.

Gills are known as a temporary target organ for cadmium accumulation after which the cadmium is transferred to organs such as the liver, kidney and intestines via the circulatory system or the enterohepatic circulation (Wu et al., 2007). Similar levels of cadmium accumulation was observed in the gills when silver catfish were exposed to this metal for 7 and 14 days and elimination was verified in this tissue following the recovery periods. These results indicated that cadmium accumulation occurs in the liver, the kidney and the gills of silver catfish and it appears that a long period of time is required for its elimination.

The induction of antioxidant enzymes like CAT, SOD and GST can be an important adaptation to pollutant-induced stress caused by cadmium. However, the activity of antioxidant enzymes can vary depending on the intensity and the duration of the chemical stress applied to the organism in addition to the susceptibility of the exposed species (Ballesteros et al., 2008). One example of this is the observation that CAT and GST activity in silver catfish tissues showed different responses to exposure to cadmium. In the liver, an increase in the levels of CAT and GST was accompanied by a decrease in SOD activity in this tissue during exposure periods. In the same period, the gill tissues showed a significant decrease in the CAT and GST activity. The changes observed in the liver and the gill antioxidant responses represented by the levels of CAT, GST and SOD, suggested that cadmium toxicity is a tissue-specific response.

Different concentrations of metals as copper, cadmium, zinc, and chromium (0.1 to 1.5 mg L<sup>-1</sup>) also increased the CAT activity in the liver of *Oreochromis niloticus* in an “in vivo” experiment performed over a period of 96 hours (Atli et al., 2006). In *Leporinus obtusidens*, exposure to zinc (2.3 and 4.6 mg L<sup>-1</sup>) and copper (0.02 and 0.04 mg L<sup>-1</sup>) for 30 and 45 days increased CAT activity (Gioda et al., 2007). The



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increase in CAT activity may indicate a compensatory response to the stress caused by cadmium exposure. On the other hand, exposure of *O. niloticus* to copper (0.1 mg L<sup>-1</sup>) and chromium (0.1-1.5 mg L<sup>-1</sup>) for 96 hours decreased CAT activity in the gills, while cadmium and zinc had no significant effects (Atli et al., 2006). Experiments exposing *Carassius auratus* to copper (0.01-0.25 mg L<sup>-1</sup>) for 40 days indicated that the liver CAT and GPx activities were inhibited (Liu et al., 2006). Asagba et al. (2008) discovered an increase in SOD activity in the kidney and a reduction of SOD activity in the gills in *Clarias gariepinus* exposed to cadmium (0.2 and 0.4 mg L<sup>-1</sup>) for 7 days. The reduction of antioxidant enzyme activity observed in our study can be explained by the high production of ROS induced by the metal. An increase in TBARS levels and protein carbonyl can lead to tissue damage and thus decrease the activity of the enzymes. In addition, some authors suggest that severe oxidative stress may suppress the activity of antioxidant defense enzyme due to oxidative damage and a loss of the compensatory mechanisms (Atli et al., 2006; Liu et al., 2006; Zhang et al., 2004).

The highest levels of cadmium were found in the kidney of silver catfish, but no changes in CAT activity were observed in this tissue during analysis of the experimental results. The TBARS levels were found to increase after exposure periods, but returned to control values after recovery periods in cadmium-free water. Conversely, an increase in TBARS levels occurred in both the exposure and recovery periods in both the liver and the gills. In this study, exposure to cadmium resulted in the suppression of the antioxidant enzymes and enhanced TBARS levels in some tissues, depending on the period. In *Dicentrarchus labrax*, cadmium caused a significant reduction of CAT activity while copper induced high MDA levels in kidney cells (Roméo et al., 2000). Gioda et al. (2007) reported an increase of TBARS

levels in the brain, liver and muscle of *L. obtusidens* exposed for 45 days to zinc (2.3 and 4.6 mg L<sup>-1</sup>), but copper (0.02 and 0.04 mg L<sup>-1</sup>) exposure for the same period reduced TBARS values in the liver and brain. Fish exposed to can pollutants adapt by changing their metabolic functions such as varying the expression of the antioxidant enzymes or increasing the TBARS levels. The formation of toxic hydroxyl radicals has direct consequences on cell integrity and cell function itself (Ballesteros et al., 2008). In the present study, the oxidative stress was verified in the liver and the gill tissues of silver catfish, and this stressful condition was followed by a tissue-specific antioxidant response.

In experiments related to TBARS determination, we also verified protein carbonyl formation as a parameter of protein damage caused by exposure to cadmium. Many previous studies have reported protein carbonyl content as a biomarker of oxidative stress in humans, but this biomarker has also been utilized to determine oxidative damage in fish (Parvez and Raisuddin, 2005). These authors have observed significant increases in protein carbonyl levels in tissues such as the liver, the kidney and the gill of *Channa punctata* exposed to deltamethrin pesticide (0.75 µg L<sup>-1</sup>) for 28 days. In our study, we observed an increase in liver protein carbonyl in fish exposed to 0.4 mg L<sup>-1</sup> cadmium, followed by a reduction after 7 days in metal free-water. Almroth et al. (2008) verified a similar increase in plasma protein carbonyl levels in fish captured in places where water and sediments were known to be contaminated with heavy metals. According to Almroth et al. (2005) a decrease in protein carbonyl levels may indicate that the susceptibility to proteolytic degradation has been increased by mild oxidation of proteins and an increase in this biomarker would indicate that normal protein metabolism is altered with accumulation of damaged molecules. The measurement of the induction in protein carbonyl levels

may serve as a useful indicator of the exposure to oxidative stress-inducing chemical contaminants in fish (Parvez and Raisuddin, 2005). In the present study, a stress condition indicated by protein carbonylation was observed only after seven days of exposure. In addition, after these fish were transferred back to cadmium-free water, protein carbonyl levels returned to levels observed in untreated control fish. Thus, this parameter is not a reliable indicator of cadmium toxicity.

## **5. Conclusion**

In general, our results indicated that tissues such as the liver and the gills in silver catfish were highly affected by cadmium, likely due to the liver's role as the main detoxification organ and to the fact that the gills interface directly with the external environment. These organs exhibited some differences in the oxidative stress markers analyzed. Thus, parameters as TBARS, CAT, GST, and SOD have been shown to be effective markers for cadmium exposure in silver catfish.

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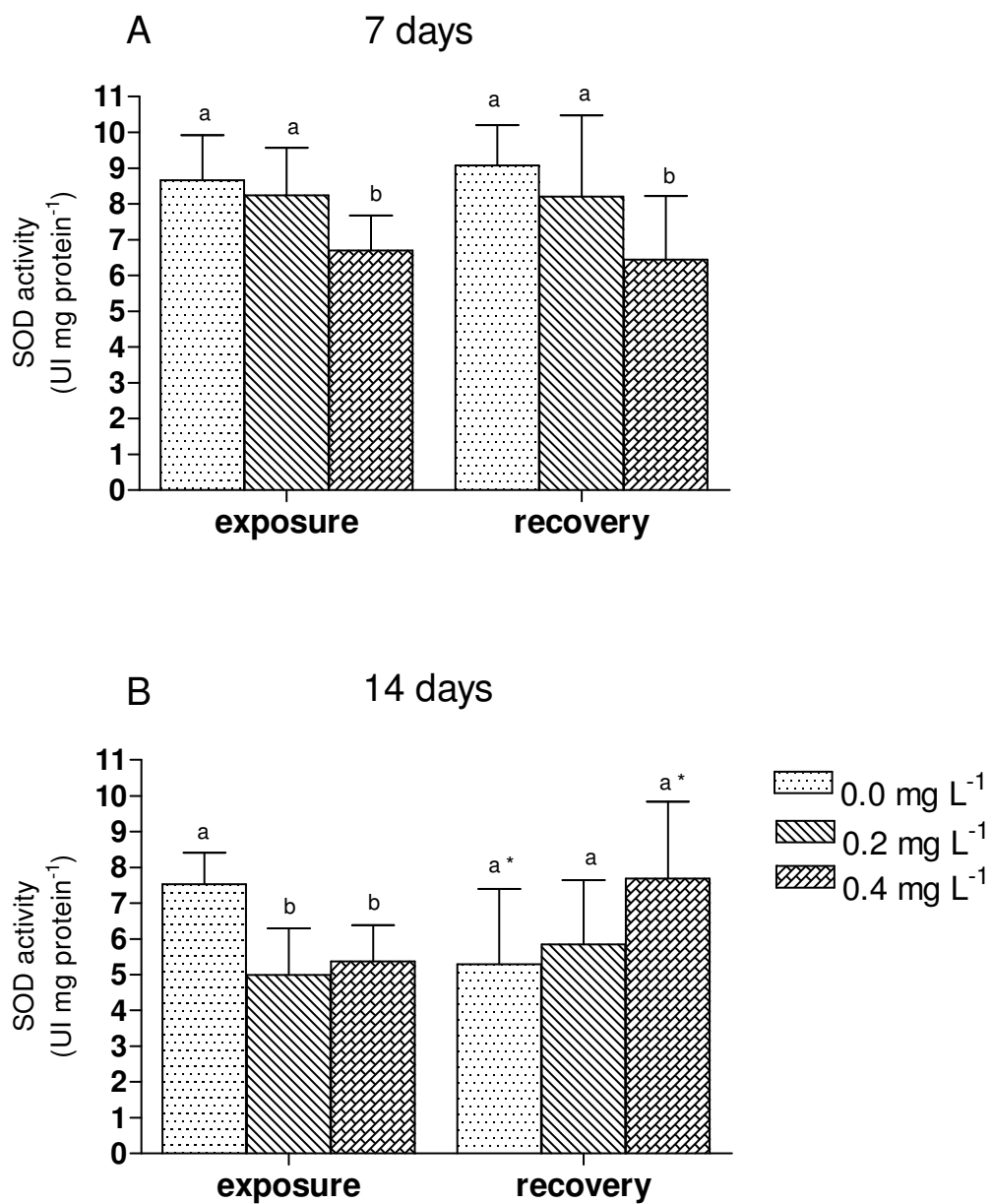


Figure 1. SOD activity in liver of *Rhamdia quelen* after seven days of exposure to cadmium and recovery (A) and after 14 days of exposure to cadmium and recovery (B). Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).



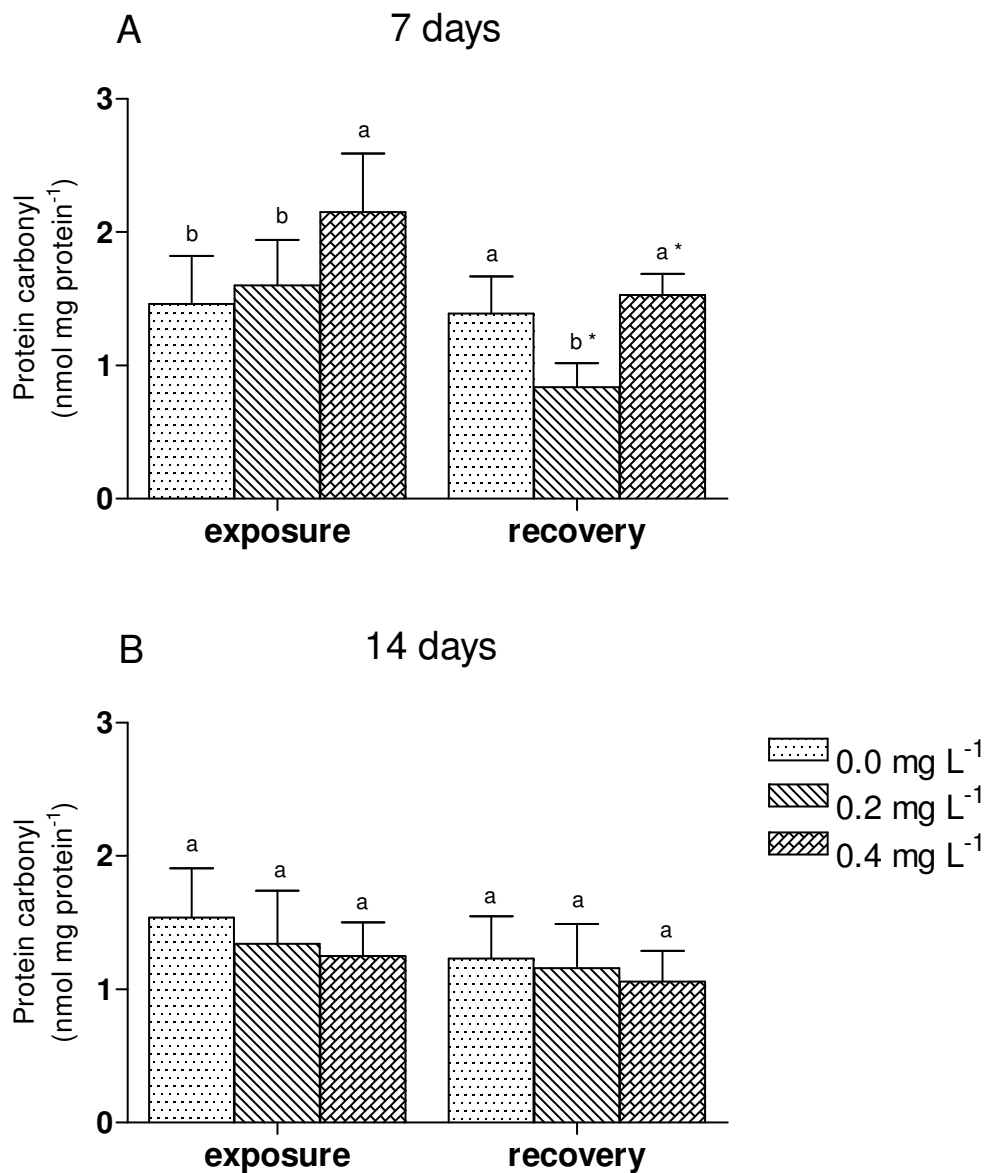


Figure 2. Protein carbonyl levels in liver of *Rhamdia quelen* after seven days of exposure to cadmium and recovery (A) and after 14 days of exposure to cadmium and recovery (B). Data are reported as mean  $\pm$  standard deviation ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 1

Cadmium accumulation ( $\mu\text{g g tissue}^{-1}$ ) in the liver, the kidney and the gills of *Rhamdia quelen* after exposure to cadmium and subsequent recovery

Tissue	Exposure (days)		Recovery (days)	
	7	14	7	14
Liver				
0.0 mg L <sup>-1</sup>	0.04 ± 0.006 <sup>b 1</sup>	0.170 ± 0.05 <sup>b</sup>	0.175 ± 0.06 <sup>c</sup>	0.117 ± 0.001 <sup>c</sup>
0.2 mg L <sup>-1</sup>	1.70 ± 0.14 <sup>b</sup>	10.40 ± 1.13 <sup>a</sup>	7.80 ± 1.13 <sup>b</sup>	4.80 ± 0.56 <sup>b</sup>
0.4 mg L <sup>-1</sup>	34.63 ± 9.89 <sup>a</sup>	14.30 ± 1.84 <sup>a</sup>	56.90 ± 1.20 <sup>a*</sup>	7.40 ± 1.50 <sup>a</sup>
Kidney				
0.0 mg L <sup>-1</sup>	0.043 ± 0.01 <sup>c</sup>	0.342 ± 0.106 <sup>b</sup>	0.427 ± 0.10 <sup>b</sup>	0.349 ± 0.13 <sup>c</sup>
0.2 mg L <sup>-1</sup>	4.30 ± 0.28 <sup>b</sup>	11.70 ± 5.66 <sup>a</sup>	11.80 ± 2.12 <sup>a*</sup>	8.47 ± 0.91 <sup>b</sup>
0.4 mg L <sup>-1</sup>	23.10 ± 0.10 <sup>a</sup>	17.90 ± 4.52 <sup>a</sup>	14.55 ± 4.59 <sup>a*</sup>	11.05 ± 0.07 <sup>a*</sup>
Gill				
0.0 mg L <sup>-1</sup>	0.271 ± 0.08 <sup>c</sup>	0.247 ± 0.02 <sup>c</sup>	0.169 ± 0.02 <sup>b</sup>	0.166 ± 0.001 <sup>b</sup>
0.2 mg L <sup>-1</sup>	1.02 ± 0.01 <sup>b</sup>	1.18 ± 0.35 <sup>b</sup>	0.93 ± 0.06 <sup>a</sup>	0.95 ± 0.10 <sup>a</sup>
0.4 mg L <sup>-1</sup>	1.77 ± 0.06 <sup>a</sup>	1.83 ± 0.49 <sup>a</sup>	0.95 ± 0.11 <sup>a*</sup>	0.92 ± 0.14 <sup>a*</sup>

<sup>1</sup>Values are expressed as the mean ± SD ( $n = 4$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (ˆ) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 2

Catalase activity in the liver, the kidney and the gills ( $\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$ ) of *Rhamdia quelen* after exposure to cadmium and subsequent recovery

Tissue	Exposure (days)		Recovery (days)	
	7	14	7	14
Liver				
0.0 mg L <sup>-1</sup>	0.138 ± 0.026 <sup>b1</sup>	0.128 ± 0.032 <sup>b</sup>	0.124 ± 0.041 <sup>a</sup>	0.123 ± 0.036 <sup>a</sup>
0.2 mg L <sup>-1</sup>	0.235 ± 0.076 <sup>a</sup>	0.186 ± 0.024 <sup>a</sup>	0.154 ± 0.045 <sup>a*</sup>	0.123 ± 0.021 <sup>a*</sup>
0.4 mg L <sup>-1</sup>	0.215 ± 0.041 <sup>a</sup>	0.191 ± 0.056 <sup>a</sup>	0.077 ± 0.016 <sup>b*</sup>	0.130 ± 0.009 <sup>a*</sup>
Kidney				
0.0 mg L <sup>-1</sup>	0.016 ± 0.002 <sup>a</sup>	0.020 ± 0.009 <sup>a</sup>	0.024 ± 0.011 <sup>a</sup>	0.023 ± 0.006 <sup>a</sup>
0.2 mg L <sup>-1</sup>	0.020 ± 0.005 <sup>a</sup>	0.020 ± 0.005 <sup>a</sup>	0.020 ± 0.011 <sup>a</sup>	0.017 ± 0.003 <sup>a</sup>
0.4 mg L <sup>-1</sup>	0.012 ± 0.003 <sup>a</sup>	0.016 ± 0.005 <sup>a</sup>	0.019 ± 0.005 <sup>a</sup>	0.015 ± 0.004 <sup>a</sup>
Gill				
0.0 mg L <sup>-1</sup>	0.536 ± 0.171 <sup>a</sup>	0.336 ± 0.091 <sup>a</sup>	0.403 ± 0.196 <sup>a*</sup>	0.307 ± 0.062 <sup>a</sup>
0.2 mg L <sup>-1</sup>	0.172 ± 0.063 <sup>b</sup>	0.122 ± 0.049 <sup>b</sup>	0.237 ± 0.099 <sup>b</sup>	0.224 ± 0.079 <sup>a</sup>
0.4 mg L <sup>-1</sup>	0.090 ± 0.039 <sup>b</sup>	0.128 ± 0.083 <sup>b</sup>	0.148 ± 0.062 <sup>b</sup>	0.367 ± 0.124 <sup>a*</sup>

<sup>1</sup>Values are expressed as the mean ± SD ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 3

Glutathione S-transferase activity in the liver, the kidney and the gills ( $\mu\text{mol mg protein}^{-1}$ ) of *Rhamdia quelen* after exposure to cadmium and subsequent recovery

Tissue	Exposure (days)		Recovery (days)	
	7	14	7	14
<b>Liver</b>				
0.0 mg L <sup>-1</sup>	0.361 ± 0.073 <sup>b1</sup>	0.262 ± 0.046 <sup>a</sup>	0.286 ± 0.019 <sup>a</sup>	0.341 ± 0.037 <sup>a*</sup>
0.2 mg L <sup>-1</sup>	0.613 ± 0.104 <sup>a</sup>	0.347 ± 0.065 <sup>a</sup>	0.310 ± 0.018 <sup>a*</sup>	0.329 ± 0.039 <sup>a</sup>
0.4 mg L <sup>-1</sup>	0.475 ± 0.075 <sup>a</sup>	0.315 ± 0.075 <sup>a</sup>	0.189 ± 0.038 <sup>b*</sup>	0.310 ± 0.051 <sup>a</sup>
<b>Gill</b>				
0.0 mg L <sup>-1</sup>	0.433 ± 0.147 <sup>a</sup>	0.400 ± 0.105 <sup>a</sup>	0.332 ± 0.094 <sup>a</sup>	0.204 ± 0.045 <sup>a*</sup>
0.2 mg L <sup>-1</sup>	0.163 ± 0.065 <sup>b</sup>	0.220 ± 0.070 <sup>b</sup>	0.184 ± 0.053 <sup>b</sup>	0.270 ± 0.080 <sup>a</sup>
0.4 mg L <sup>-1</sup>	0.167 ± 0.045 <sup>b</sup>	0.190 ± 0.085 <sup>b</sup>	0.192 ± 0.026 <sup>b</sup>	0.277 ± 0.076 <sup>a</sup>

<sup>1</sup>Values are expressed as the mean ± SD ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

Table 4

TBARS levels in the liver, the kidney and the gills (nmol MDA mg protein<sup>-1</sup>) of *Rhamdia quelen* after exposure to cadmium and subsequent recovery

Tissue	Exposure (days)		Recovery (days)	
	7	14	7	14
Liver				
0.0 mg L <sup>-1</sup>	0.53 ± 0.06 <sup>a1</sup>	0.69 ± 0.13 <sup>b</sup>	0.78 ± 0.17 <sup>b*</sup>	0.64 ± 0.10 <sup>b</sup>
0.2 mg L <sup>-1</sup>	0.55 ± 0.12 <sup>a</sup>	0.89 ± 0.09 <sup>a</sup>	1.06 ± 0.21 <sup>a*</sup>	0.79 ± 0.17 <sup>a</sup>
0.4 mg L <sup>-1</sup>	0.62 ± 0.14 <sup>a</sup>	1.12 ± 0.22 <sup>a</sup>	1.07 ± 0.20 <sup>a*</sup>	0.78 ± 0.05 <sup>a*</sup>
Kidney				
0.0 mg L <sup>-1</sup>	0.43 ± 0.06 <sup>a</sup>	0.22 ± 0.06 <sup>b</sup>	0.32 ± 0.05 <sup>a*</sup>	0.26 ± 0.05 <sup>a</sup>
0.2 mg L <sup>-1</sup>	0.40 ± 0.05 <sup>a</sup>	0.50 ± 0.12 <sup>a</sup>	0.28 ± 0.07 <sup>a*</sup>	0.29 ± 0.05 <sup>a*</sup>
0.4 mg L <sup>-1</sup>	0.29 ± 0.08 <sup>b</sup>	0.55 ± 0.18 <sup>a</sup>	0.24 ± 0.09 <sup>a</sup>	0.23 ± 0.05 <sup>a*</sup>
Gill				
0.0 mg L <sup>-1</sup>	2.16 ± 0.48 <sup>b</sup>	3.09 ± 1.31 <sup>b</sup>	2.26 ± 0.66 <sup>a</sup>	3.09 ± 1.04 <sup>b</sup>
0.2 mg L <sup>-1</sup>	8.56 ± 0.66 <sup>a</sup>	6.19 ± 2.18 <sup>a</sup>	2.11 ± 0.84 <sup>b*</sup>	5.11 ± 1.08 <sup>a</sup>
0.4 mg L <sup>-1</sup>	8.06 ± 1.36 <sup>a</sup>	3.50 ± 0.95 <sup>b</sup>	1.37 ± 0.36 <sup>b*</sup>	4.09 ± 1.51 <sup>b</sup>

<sup>1</sup>Values are expressed as the mean ± SD ( $n = 8$ ). Letters indicate significant differences among concentrations within the same exposure and recovery time and (\*) show differences between exposure and recovery period by the Duncan's Test ( $P \leq 0.05$ ).

## 5 DISCUSSÃO

O cádmio é um metal geralmente encontrado em baixas concentrações no ambiente. Porém, a liberação de poluentes industriais pode resultar no aumento de cádmio nos corpos d'água. No meio aquático este metal pode afetar vários organismos invertebrados e vertebrados. Atenção especial tem sido dada aos peixes por serem constituintes importantes do ecossistema aquático e fonte de alimentação humana (HAMILTON *et al.*, 1998; ALQUEZAR *et al.*, 2006a; BURGER, 2008). O jundiá é uma espécie nativa com ótimo potencial de desenvolvimento nas condições climáticas regionais. Mas, em rios localizados próximo a áreas industriais esta espécie pode ser afetada por metais como o cádmio. Esse estudo é relevante, pois no Brasil poucos estudos relacionam a toxicidade deste metal com espécies nativas como o jundiá e os resultados contribuem para os estudos com esta espécie.

Em peixes o cádmio tem efeitos sobre o crescimento e reprodução. Este metal pode acumular-se nos tecidos e causar uma série de alterações fisiológicas e metabólicas (WU *et al.*, 2007; DE LA TORRE *et al.*, 2000). Sendo assim, verificou-se se entre outros parâmetros, se o cádmio afeta a atividade da enzima acetilcolinesterase (AChE) em cérebro e músculo de jundiás e pode acumular-se nestes tecidos. De acordo com os resultados obtidos, observou-se uma inibição na atividade da AChE cerebral após 14 dias de exposição ao cádmio. Esta inibição persistiu depois de 14 dias de recuperação. No tecido muscular foi encontrado aumento da AChE após 7 dias de exposição. Poucos estudos têm relacionado a atividade da AChE com a exposição a metais pesados e os resultados encontrados são variáveis. De acordo com DE LA TORRE *et al.* (2000) a atividade da AChE cerebral de *Cyprinus carpio* não foi alterada após exposição ao cádmio. O mesmo fato foi reportado por BEAUVAIS *et al.* (2001) em *Oncorhynchus mykiss*. No entanto, aumento da AChE ocorreu no cérebro e músculo de *Sparus auratus* expostos ao cobre (ROMANI *et al.*, 2003). No nosso estudo, cádmio acumulou-se no músculo após ambos os períodos de exposição e no cérebro a acumulação ocorreu após a exposição por 14 dias, com uma concentração de metal neste tecido maior que no músculo. O cádmio acumulado no cérebro e músculo pode interferir na liberação de neurotransmissores e causar efeitos indesejáveis nas junções neuromusculares,

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podendo afetar algumas atividades nos peixes (BEAUVAIS *et al.*, 2001). As alterações observadas na atividade da AChE em nosso estudo podem estar relacionadas com a acumulação do cádmio nos tecidos pois, o metal pode provocar mudanças na conformação da enzima ao se ligar à ela ou se ligar diretamente ao sítio ativo da enzima, alterando sua função.

É descrito na literatura que o cádmio, assim como outros metais, pode aumentar a produção celular de espécies reativas do oxigênio (EROs) que causam danos a lipídios e proteínas celulares (ATLI *et al.*, 2006; SOARES *et al.*, 2008). A peroxidação lipídica pode ser verificada através dos níveis de TBARS (substâncias reativas ao ácido tiobarbitúrico). Em nosso estudo no cérebro dos peixes ocorreu aumento de TBARS após ambos os períodos de exposição a até mesmo depois de 14 dias em recuperação. De acordo com nossos resultados, o aumento nos níveis de TBARS acompanha a redução observada na atividade da AChE no período de 14 dias. De acordo com ÜNER *et al.* (2006) a peroxidação lipídica, verificada pelo aumento dos níveis de TBARS, causa danos celulares e pode estar relacionada à inibição da AChE cerebral. Os resultados referentes à inibição da AChE no cérebro podem ser devido a peroxidação lipídica observada no mesmo período para este tecido.

Com a inibição da AChE e aumento dos níveis de acetilcolina, pode ocorrer aumento na liberação de catecolaminas, que são relacionadas ao metabolismo energético (ÜNER *et al.*, 2006). Então, verificamos parâmetros relacionados ao metabolismo de carboidratos (glicogênio, glicose e lactato) e proteínas (proteína total, aminoácidos e amônia) para avaliar as respostas metabólicas do jundiá frente ao cádmio. Neste estudo encontramos redução de glicose hepática e aumento de glicose e glicogênio muscular após sete dias de exposição. A glicose pode estar sendo liberada pelo fígado a fim de manter a glicemia, já que o estoque de carboidratos muscular não foi utilizado. ALMEIDA *et al.* (2001) também observou aumento na concentração de glicose muscular em *Oreochromis niloticus* expostos ao cádmio por sete dias. Com relação ao metabolismo de proteínas, as maiores alterações encontradas no fígado e músculo foram em jundiás expostos a concentração 0.4 mg/L. No músculo ocorreu redução de proteína associado a aumento de aminoácidos e amônia. A quebra de proteínas musculares resultou num aumento de aminoácidos neste tecido e a metabolização dos mesmos aumentou a concentração de amônia. Um aumento de aminoácidos e proteínas foi encontrado

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no fígado de jundiás neste período e pode significar uma exportação de aminoácidos do músculo para síntese de proteínas hepáticas. As alterações nos níveis de lactato (plasma e fígado) e aminoácidos podem significar a utilização destes precursores na via gliconeogênica para síntese de glicose. Nossos resultados estão de acordo com os resultados encontrados por ALMEIDA *et al.* (2001). Podemos inferir que após sete dias de exposição ao cádmio, jundiás alteram seu metabolismo protéico e de carboidratos a fim de obter energia para as funções vitais e processos de detoxificação do metal.

Após o período de 14 dias de exposição poucas alterações foram observadas nos parâmetros relacionados ao metabolismo de carboidratos. Ocorreu quebra de glicogênio muscular após a exposição e quebra de glicogênio hepático depois do período de recuperação possivelmente para manter os níveis de glicose no sangue. ALMEIDA *et al.* (2002) e CATTANI *et al.* (1996) encontraram redução dos níveis de glicogênio muscular quando *O. niloticus* e *Dicentrarchus labrax* foram expostos ao cádmio, respectivamente. No entanto, neste período, as maiores alterações foram observadas no metabolismo protéico. Ocorreu redução de proteínas no músculo após a exposição e recuperação de 14 dias. No fígado além da redução de proteínas, jundiás demonstraram também aumento de aminoácidos e amônia. Neste tecido, o aumento nos níveis de aminoácidos e amônia indica o uso de aminoácidos no metabolismo energético, tanto formando glicose como resultando em acetil-CoA para produção de ATP. Em ambos os períodos de exposição (sete e 14 dias) os peixes parecem favorecer o metabolismo das proteínas do que os carboidratos para manutenção de seu metabolismo. Essa resposta adaptativa também ocorreu em *O. niloticus*, onde em um período curto de exposição (sete dias) os peixes mobilizaram proteínas e não carboidratos, porém neste estudo após 60 dias de exposição ocorreu uma mudança para o metabolismo de carboidratos a fim de manter as reservas de proteína (ALMEIDA *et al.*, 2001; ALMEIDA *et al.*, 2002).

Com relação à acumulação do metal, as maiores concentrações de cádmio foram encontradas no rim, fígado e brânquias de jundiás. Logo, nestes tecidos a presença do metal pode levar ao aumento na produção de EROs (LIVINGSTONE, 2001). Desta forma, nós verificamos alguns parâmetros indicadores de estresse oxidativo como TBARS, carbonilação de proteínas e enzimas antioxidantes como catalase (CAT), glutathione S-transferase (GST) e superóxido dismutase (SOD) em fígado, rim e brânquias de jundiás.



O fígado e as brânquias foram os tecidos mais sensíveis ao estresse oxidativo gerado pelo cádmio. Nestes tecidos ocorreu alteração nos níveis de TBARS e na atividade das enzimas antioxidantes após os períodos de exposição. No entanto, essas alterações foram dependentes do tempo e do tecido considerado. No fígado foi verificado aumento da CAT e GST e redução da SOD associado à carbonilação de proteínas. BASHA & RANI (2003) encontraram aumento na atividade de enzimas antioxidantes no fígado e rim em *Oreochromis mossambicus* expostos ao cádmio. Resultados similares foram encontrados por SANCHEZ *et al.* (2005) em peixes expostos ao cobre. Sob uma situação de estresse oxidativo ocorre indução das defesas antioxidantes a fim de proteger o organismo da ação das EROs (BASHA & RANI, 2003; ATLI & CANLI, 2007). Em nosso estudo, o aumento das enzimas CAT e GST representa uma resposta do sistema de defesa antioxidante contra a toxicidade gerada pelo cádmio. No entanto, no fígado, também foi observado depleção na atividade das enzimas antioxidantes juntamente com elevados níveis de TBARS. O aumento do TBARS hepático pode ser a causa da ausência de resposta ou redução na atividade das enzimas antioxidantes. Nas brânquias a resposta mais freqüente foi aumento de TBARS e inibição das enzimas CAT e GST. Neste tecido as concentrações de cádmio encontradas foram similares após os períodos de exposição. No rim, mesmo observando-se altos níveis de metal a enzima CAT não aumentou em resposta as alterações nos valores de TBARS. Em *Clarias gariepinus* expostos ao cádmio ocorreu redução da SOD no rim e brânquias. LIU *et al.* (2006) observou redução da CAT no fígado quando *Carassius auratus* foram expostos ao cobre. Um aumento nos níveis de EROs e produtos da peroxidação lipídica pode levar a danos teciduais e perda da atividade de enzimas antioxidantes (LIU *et al.*, 2006; ASAGBA *et al.*, 2008). Neste estudo pode-se observar que a presença do metal nos tecidos causa alterações no sistema antioxidante, com respostas que variam de acordo a concentração do metal, o tecido e parâmetro considerado.

## 6 CONCLUSÕES

Através dos resultados experimentais obtidos podemos chegar às seguintes conclusões:

1. A  $CL_{50}$  do cádmio para o jundiá é 1.7 mg/L;
2. O cádmio acumulou-se nos tecidos analisados, principalmente no rim, fígado e brânquias em comparação com o cérebro e músculo e o tempo de recuperação não foi suficiente para a total eliminação do metal;
3. A atividade da AChE foi alterada para as concentrações utilizadas de cádmio indicando que esta enzima pode ser um bom marcador da exposição de peixes a metais;
4. Os níveis de TBARS e a atividade das enzimas antioxidantes foram alterados em jundiás demonstrando uma situação de estresse oxidativo. Esses parâmetros são marcadores confiáveis a serem analisados em peixes expostos a metais. A carbonilação de proteínas não mostrou uma resposta clara e neste caso não foi um marcador eficaz;
5. Os níveis de glicose, lactato e proteína no plasma foram alterados após a exposição ao cádmio significando um efeito compensatório devido a toxicidade do metal;
6. Os parâmetros relacionados ao metabolismo de carboidratos e proteínas foram alterados no fígado e músculo demonstrando os mecanismos adotados pelo peixe em contato com o metal;
7. De maneira geral, os parâmetros relacionados ao estado de estresse oxidativo somente foram recuperados após 14 dias. Já, a maioria dos parâmetros metabólicos recuperou-se após sete dias.

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**PROTOCOLADO**

**UNIVERSIDADE FEDERAL DE SANTA MARIA  
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA  
COMITÊ DE ÉTICA E BEM ESTAR ANIMAL**

**Emissão de Parecer  
33/2007**

Nº Processo:	23081.010370/2007-82
Título:	Parâmetros toxicológicos em jundiás ( <i>Rhamdia quelen</i> ) expostos ao cádmio
Pesquisador Responsável:	Profª. Vânia Lúcia Loro
Instituição:	UFSM
Área:	→ DEP. Química
Data de Entrada:	27/07/2007
Data do Parecer:	06.08.2007

**Termo de compromisso com o bem estar animal:**

- Termo de compromisso assinado pelo Coordenador e outros executores do projeto  
 Termo de compromisso assinado pelo Coordenador se responsabilizando pelos demais executores do projeto  
 Termo de compromisso assinado unicamente pelo Coordenador  
 Termo de compromisso ausente no processo

**Adequação e relevância do projeto:**

- Problema científico relevante  
 Hipótese adequada  
 Objetivos relevantes  
 Metodologia adequada

**Caráter do projeto/ Linha de pesquisa quanto aos resultados esperados e benefícios potenciais, para a área em estudo e/ ou setores de aplicação da sociedade brasileira:**

- Inovador / novo conceito  
 Incremental (novas informações)  
 Confirmatório  
 Pouco relevante

**Necessidade da utilização de animais na experimentação:**

- O projeto necessita de animais para responder a pergunta científica  
 O projeto não necessita de animais e pode ser realizado com metodologia in vitro ou similar

**Análise do número de animais a ser utilizado no projeto:**

- Adequado  
 Inadequado

Base científica para classificar como inadequado e sugestão do número de animais que deverá ser utilizado:

**Currículo do pesquisador em relação à área de abrangência do projeto:**

- Adequado  
 Inadequado

**Avaliação global do projeto:**

- Aprovado *Tales*  
 Aprovado com ressalva \_\_\_\_\_

- Reprovado (a proposta não está de acordo com a ética e bem estar animal)

**Detalhe os pontos relevantes que o(a) levaram a avaliar negativamente o projeto:**

Santa Maria, 06 de agosto de 2007.

Declaramos que o projeto Parâmetros toxicológicos em jundiás (*Rhamdia quelen*) expostos ao cádmio registrado no Comitê de Ética e Bem Estar Animal sob número 23081.010370/2007-82, coordenado pelo pesquisador Prof<sup>a</sup>. Vânia L. Loro, cumpriu todas as exigências em relação ao Bem Estar Animal.

*Tales Branda*  
\_\_\_\_\_  
Prof. Tales de Moura Branda  
Presidente do Comitê de Ética e Bem Estar Animal

**OBS: Entregar relatório ao final da execução/ao término do projeto**