CARLA ROLIM FERRARI

Avaliação de Efluentes Radioativos da Unidade de Tratamento de Minério das Indústrias Nucleares do Brasil e de Bacia de Drenagem sob sua influência, com Ênfase na Caracterização Químíca e Ecotoxicológica

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

Área de Concentração: Biotecnologia

Orientadora: Dra. Heliana de Azevedo Franco do Nascimento

Versão Original

São Paulo

2017

CATALOGAÇÃO NA PUBLICAÇÃO (CIP)

Serviço de Biblioteca e informação

Biomédica

do Instituto de Ciências Biomédicas da Universidade de São

Paulo

Ficha Catalográfica elaborada pelo(a) autor(a)

Rolim Ferrari, Carla

Avaliação de Efluentes Radioativos da Unidade de Tratamento de Minério das Indústrias Nucleares do Brasil e de Bacia de Drenagem sob sua influência, com Ênfase na Caracterização Químíca e Ecotoxicológica / Carla Rolim Ferrari; orientadora Heliana de Azevedo Franco do Nascimento. -- São Paulo, 2017.

140 p.

Tese (Doutorado)) -- Universidade de São Paulo, Instituto de Ciências Biomédicas.

1. Mineração de Urânio. 2. Efluentes Radioativos . 3. Drenagem Ácida de Mina. 4. Caracterização Química e Ecotoxicológica. 5. Bioindicadores. I. de Azevedo Franco do Nascimento, Heliana, orientador. II. Título.

CARLA ROLIM FERRARI

Evaluation of the radioactive effluents from the Mineral Treatment Unit of the Brazilian Nuclear Industries and the Drainage Basin under their influence, with emphasis on the chemical and ecotoxicological characterization

Thesis presented to the Postgraduate Program in Biotechnology of the Biomedical Sciences Institute of the University of São Paulo, Brazil, to obtain the title of Doctor in Sciences.

Area of Concentration: Biotechnology

Orientadora: Dra. Heliana de Azevedo Franco do Nascimento

Original version

São Paulo

2017

UNIVERSIDADE DE SÃO PAULO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS

Candidata: Carla Rolim Ferrari

Titulo da Dissertação/Tese: Avaliação de Efluentes Radioativos da Unidade de Tratamento de Minério das Indústrias Nucleares do Brasil e de Bacia de Drenagem sob sua influência, com Ênfase na Caracterização Químíca e Ecotoxicológica

Orientador: Heliana de Azevedo Franco do Nascimento

A Comissão Julgadora dos trabalhos de Defesa da Tese de Doutorado, em sessão publica realizada a **........./......../..........**, considerou o(a) candidato(a):

(   ) Aprovado(a)(   ) Reprovado(a)

Examinador(a):Assinatura: ....
Nome: ....
Instituição: ....

Examinador(a):Assinatura: ....
Nome: ....
Instituição: ....

Examinador(a):Assinatura: ....
Nome: ....
Instituição: ....

Presidente:Assinatura: ....
Nome: ....
Instituição: ....

UNIVERSIDADE DE SÃO PAULO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS

Cidade Universitária "Armando de Salles Oliveira"
Av. Prof. Lineu Prestes, 2415 – CEP. 05508-000 São Paulo, SP – Brasil
Telefone :(55) (11) 3091-7733 - telefax : (55) (11) 3091-8405
e-mail: cep@icb.usp.br

Comissão de Ética em Pesquisa

CERTIFICADO DE ISENÇÃO

Certificamos que o Protocolo CEP-ICB Nº **666/14** referente ao projeto intitulado: "*Avaliação ecotoxicológica de efluentes radioativos da Unidade de Tratamento de Minério das Indústrias Nucleares do Brasil (UTM/INB) e de suas bacias de drenagem, com ênfase na toxicidade de metais para organismos planctônicos*" sob a responsabilidade de **Carla Rolim Ferrari,** foi analisado na presente data pela CEUA - COMISSÃO DE ÉTICA NO USO DE ANIMAIS e pela CEPSH- COMISSÃO DE ÉTICA EM PESQUISA COM SERES HUMANOS, tendo sido deliberado que o referido projeto não utilizará animais que estejam sob a égide da lei 11.794 de 8 de outubro de 2008, nem envolverá procedimentos regulados pela Resolução CONEP nº466 de 2012.

São Paulo, 27 de maio de 2014.

PROF. DR. WOTHAN TAVARES DE LIMA
Coordenador da CEUA - ICB/USP

PROF. DR. PAOLO M.A ZANOTTO
Coordenador da CEPsh - ICB/USP

Dedico essa tese de Doutorado a todas as pessoas que contribuíram para que esse trabalho se tornasse realidade, mas em especial a minha mãe Evany, pelo seu exemplo de vida e seu apoio constante na minha carreira como profissional, ao meu marido Gustavo, a minha irmã Renata e a tia Cecília. Pessoas especiais e queridas!!!

I dedicate this doctoral thesis to all those who contributed to making this work become reality, but especially to my mother Evany for her life example and constant support of my professional career, to my husband Gustavo, my sister Renata and my aunt Cecília. Special and much loved people!!!

Agradecimentos

-Agradeço primeiro a Deus, pela vida e pela saúde.

-A Dra. Heliana de Azevedo Franco do Nascimento pela orientação, pela oportunidade que sempre me proporcionou para o desenvolvimento desta tese e especialmente pela sua confiança, estímulo, auxílio, amizade e pelos valiosos ensinamentos ao longo desses dez anos, que com certeza me ajudaram muito a me tornar a profissional que sou hoje.

-Ao técnico, Armando Luis Bruschi do Laboratório de Radioecologia da CNEN, pela amizade, por todos os ensinamentos ao longo desses anos e pelo auxílio nas análises laboratoriais e coletas de campo, sendo de suma importância para os resultados deste trabalho.

-As minhas queridas amigas e estagiárias Emília Gabriela Costa Silvério, Tayná Cunha de Souza e Amanda Zavanin Trevisan pela ajuda e auxílio nas etapas desta tese, com certeza vocês foram essenciais.

-A minha colega e grande amiga Luciene Prakque por lavar e cuidar de todas as vidrarias utilizadas na pesquisa.

-Ao Dr. Marcos Roberto Lopes do Nascimento e ao Dr. Rodrigo Leandro Bonifácio chefes do Laboratório de Química Análitica e a todos os técnicos que fazem parte deste laboratório, pelo auxílio na realização das análises químicas.

-Ao técnico do Laboratório de Radioecologia Cláudio Vitor Roque pela amizade e pelos valiosos ensinamentos ao longo desses anos.

-Ao colega de trabalho Heber Luiz Caponi Alberti e Élis Lima pela elaboração do mapa.

-A Profa. Dra. Suzelei Rodgher pelo auxílio na interpretação de resultados relativos aos testes de toxicidade.

-A todos os funcionários da CNEN que participaram deste trabalho pela colaboração e ajuda.

-A minha querida mãe Evany que sempre acreditou e esteve ao meu lado em todos os momentos de nervosismo, incerteza, medo, insegurança me apoiando e me dando forças para seguir em frente.

-Agradeço ao meu marido Gustavo pela paciência, apoio, carinho e compreensão, durante todo esse tempo.

-Agradeço a tia Cecília e a minha irmã Renata por estarem sempre presentes em todos os momentos da minha vida.

-Agradeço também a Dani, ao Renato e a querida Anna Clara.

-A Comissão Nacional de Energia Nuclear CNEN pela infraestrutura oferecida para realização deste trabalho.

Agradecimento

Agradeço em especial ao Departamento Municipal de Eletricidade de Poços de Caldas DMEd e à Agência Nacional de Energia Elétrica ANEEL, pelo apoio financeiro concedido ao presente estudo - Processo nº: 01346.00019/2014-11.

Acknowledgements

-Firstly I am thankful to God for my life and health.

-To Dra Heliana de Azevedo Franco do Nascimento for her orientation, for the opportunity she always provided me to develop this thesis, and especially for her confidence, stimulus, aid, friendship and valuable teaching over the last ten years, which greatly helped me to become the professional I am today.

-To the technician Armando Luis Bruschi of the Radioecology Laboratory of CNEN for his friendship, for all the teaching over the years and for help in the laboratory analyses and field collections, all of great importance to the results of this work.

-To my friends and trainees Emília Gabriela Costa Silvério, Tayná Cunha de Souza and Amanda Zavanin Trevisan for their help and aid in the different steps of this thesis – you were certainly essential.

-To my colleague and great friend Luciene Prakque for washing and taking care of all the glassware used in this research.

-To Dr. Marcos Roberto Lopes do Nascimento and Dr. Rodrigo Leandro Bonifácio, heads of the Analytical Chemistry Department and to all the technicians who make up this laboratory, for their help in carrying out the chemical analyses.

-To Cláudio Vitor Roque for the friendship and valuable teaching over the years.

-To my work colleagues Heber Luiz Caponi Alberti and Élis Lima for elaborating the map.

-To Prof. Dr. Suzelei Rodgher for help in interpreting the results related to the toxicity tests.

-To all the employees of CNEN who took part in this work for their collaboration and help.

-To my dear mother Evany who always believed in me and stood by me in all moments of nervousness, uncertainty, fear and insecurity giving me support and strength to keep going.

-I am grateful to my husband Gustavo for his patience, support, care and understanding throughout this time.

-I am grateful to my aunt Cecilia and sister Renata for being present in all moments of my life.

-I am also grateful to Dani, Renato and dear Anna Clara.

-To CNEN – The Brazilian National Nuclear Energy Commission for the infrastructure offered to carry out this work.

-To the Foundation of the Municipal Department of Electricity and Distribution, DMEd for conceding financial resources.

Acknowledgement

I am grateful to the Poços de Caldas Department of Electricity and the Distribution/ National Electric Energy Agency by finnancial support to the presenty study – Process no: 01346.00019/2014-11.

"Na vida tudo tem seu apogeu e seu declínio. É natural que seja assim; todavia, quando tudo parece convergir para o que supomos o fim, eis que a vida resurge triunfante e bela...Novas folhas, novas flores, na indefinida benção do recomeço" Chico Xavier

RESUMO

FERRARI, C. R. **Avaliação de Efluentes Radioativos da Unidade de Tratamento de Minério das Indústrias Nucleares do Brasil e de Bacia de Drenagem sob sua influência, com Ênfase na Caracterização Quimíca e Ecotoxicológica.** 2017. 115 f. Tese (Doutorado em Biotecnologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2017.

A primeira mineração de urânio do Brasil está localizada nas dependências da Unidade de Tratamento de Minérios das Indústrias Nucleares do Brasil (UTM/INB). Atualmente, essa área mineradora encontra-se em fase de descomissionamento e a drenagem ácida de mina (DAM) constitui o principal passivo ambiental desse site, devido ao grande volume de efluentes radioativos gerados, os quais após tratamento são lançados na represa das Antas (RA). Neste contexto, o presente estudo avaliou a qualidade da água através da caracterização química e ecotoxicológica em amostras de efluente *in natura* (Cava da Mina)e efluente tratado (P41-S) procedente da UTM/INB, bem como em amostras de água da RA, sob influência dessa mineradora. Adicionalmente testes de toxicidade aguda com os metais urânio e manganês também foram realizados, além da determinação da especiação química do urânio e sua relação com a toxicidade para dafinídeos. De acordo com os resultados amostras do efluente *in natura* e tratado apresentaram maiores concentrações de espécies químicas, sendo consideradas agudamente tóxicas para os dafinídeos. Tais resultados indicaram que o sistema de tratamento do efluente *in natura* realizado pela mineradora foi impróprio e ineficiente. Em novembro de 2014 na RA valores de dureza extremamente elevados (543,55 mg L^{-1}) indicaram efeito protetor para os dafínideos ($P<0,05$), quando maiores concentrações dos metais F^{-} (4,5 mg L^{-1}) U (0,082 mg L^{-1}), Mn (1,125 mg L^{-1}) e Al (1,55 mg L^{-1}) foram registradas. O metal Mn apresentou potencial de causar toxicidade, uma vez que concentrações de Mn registradas no presente estudo e na literatura estiveram acima do valor de CE_{50} (5,93 mg L^{-1} Mn) registrado para *C. silvestrii*. Para U, mudanças nos valores de pH nos ensaios de *D.magna* estiveram associadas a alterações na especiação desse metal, com maiores concentrações das espécies potencialmente tóxicas para a biota, ou seja, UO_2^{2+} e UO_2OH^{+} sendo registradas em pH 7.Também a espécie nativa *Ceriodaphnia silvestrii* mostrou ser muito mais sensível ao metal urânio, quando comparada a *D. magna*, de acordo com os valores de CE_{50} registrados que foram: 0,07 e 0,56 mg L^{-1} U, respectivamente.

Palavras chave: Mineração de urânio. Efluentes radioativos. Drenagem ácida de mina Caracterização química e ecotoxicológica. Bioindicadores.

ABSTRACT

FERRARI, C. R. **Evaluation of the radioactive effluents from the Mineral Treatment Unit of the Brazilian Nuclear Industries and the Drainage Basin under their influence, with emphasis on the chemical and ecotoxicological characterization.** 2017. 115 p. Doctorate Thesis (Biotechnology) – Institute of Biomedical Science, University of São Paulo, São Paulo, 2017.

The first uranium mine in Brazil is located in the grounds of the Ore Treatment Unit of the Brazilian Nuclear Industries (UTM/INB). Currently this mining area is in the decommissioning phase and acid mine drainage (AMD) constitutes the main environmental liability of this site due to the great volume of radioactive effluent generated. The acid effluent is treated by physicochemical processes and released into the Antas reservoir. Thus, by way of ecotoxicological and chemical characterizations, the present study aimed to evaluate the effects of the discharge of the *in natura* (mine pit) and treated (P41-S) effluents proceeding from UTB/INB, into the Antas reservoir (CAB, P41-E, P14) under the influence of this mining company. In addition, acute toxicity tests with the metals of interest, uranium and manganese, were carried out, and the uranium speciation and its relationship with toxicity to daphnids determined. According to the results, samples of both the *in natura* and treated effluents presented high concentrations of some chemical species, and were considered acutely toxic to daphnids. These results indicate that the system used by the mining company to treat the *in natura* effluent was improper and inefficient. Also in November, 2014, Antas reservoir (AR) samples, showing very high hardness values (543.55 mg L^{-1}) showed positive correlation with the decrease in toxicity for daphnids ($P<0.05$), when higher concentrations of metals such as: fluoride (4.5 mg L^{-1}) uranium (0.082 mg L^{-1}), manganese (1.125 mg L^{-1}) and aluminum (1.55 mg L^{-1}) were registered. The metal manganese showed the potential to cause toxicity since the concentrations recorded in the present study and in the literature were above the EC_{50} value (5.93 mg L^{-1} Mn) registered for *C. silvestrii*. When the pH value was decreased from 8 to 7 the speciation of the metal uranium was shown to change, with greater concentrations of the species UO_2^{2+} and UO_2OH^+, considered potentially toxic to the biota, being registered at pH 7. Also the native species *Ceriodaphnia silvestrii* was shown to be much more sensitive to the metal uranium when compared to *D. magna*, according to the EC_{50} values recorded, which were 0.07 and 0.56 mg L^{-1} U, respectively.

Keywords: Uranium mining. Radioactive effluents. Acid mine drainage. Chemical and ecotoxicological characterization. Bioindicators.

LIST OF ILLUSTRATIONS

LIST OF TABLES

SUMMARY

THESIS PRESENTATION

The mode of presenting this Doctoral thesis in chapters favored publication of the results obtained in international scientific journals. Thus in relation to this form of presentation, some considerations should be taken into account. The thesis was divided in the following way: 1) Introduction and Justification; 2) General and Specific objectives; 3) Materials and Methods/Results and Discussion composed of four chapters; 5) General conclusions and Appendixes.

It should be highlighted that the Results obtained and Discussion were written in chapters in the form of scientific articles, as follows: **Chapter 3.1** Effects of the discharge of uranium mining effluents on water quality in the reservoir: an integrative limnological and ecotoxicological assessment; **Chapter 3.2** Acute toxicity of manganese for *Ceriodaphnia silvestrii* and *Daphnia magna* in bioassays and the potential toxicity of this metal in uranium mine effluents; **Chapter 3.3** An approach to the speciation and acute toxicity of uranium on the Daphnids *Daphnia magna* and *Ceriodaphnia silvestrii*; and **Chapter 3.4** Evaluation of the treatment system of the uranium mine effluent, from the chemical characterization and acute toxicity potential carried out using *Ceriodaphnia silvestrii* and *Daphnia magna*.

The chapters of the present thesis were written and formatted according to the norms of the scientific journals of interest, that is: **Chapter 3.1** - Scientific Reports, **Chapter 3.2** - Mine Water and the Environment; **Chapter 3.3** and **Chapter 3.4 -** Archives of Contamination and Toxicology. Since the majority of scientific journals require that the papers be written in English, all the chapters were written in this language, in order to make it easier to submit them to the above cited international journals of interest.

The elaboration of the thesis in this format is justified by the fact that it makes the publication of the articles in scientific journals quicker and less onerous, although, due to the independence of each chapter in relation to the others, some repetitions are inevitable.

1 INTRODUCTION AND JUSTIFICATION

1.1 Contextualization of the proposed study

Mining activities have contributed significantly to the growth and development of various countries, including Brazil, since the importance of this sector for the Brazilian economy is significant, with emphasis on the production of iron, niobium, manganese, and aluminum (bauxite), amongst others (IBRAM, 2008). Although the production of uranium in Brazil is not significant in terms of volume, it is important to point out that it is an integral part of the nuclear fuel cycle, and is therefore considered to be strategic for the country. It should also be pointed out that Brazil belongs to a restricted group of countries that dominate the nuclear technology referring to the fuel cycle, as from extraction of the mineral up to the production of nuclear electricity.

Nevertheless the expansion and continuation of mining activities in Brazil and in the world depends on the incentive and compromise of the large mining companies with respect to the preservation and recovery of the explored environment, so as to conform to the legal demands of the control organs, such as: The Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), The National Water Agency (ANA) and pertinent state and municipal organs such as The National Nuclear Energy Commission (CNEN), responsible for controlling and managing nuclear installations.

With respect to concerns about the impacts caused by mineral exploration, the question of water and water resources is frequently one of the most important conflicts involving mining and the society (REBOUÇAS; BRAGA; TUNDISI, 2006). According to Antunes et al. (2007), mining activities can involve great changes to the contour of the landscape, since they are related to chemical, physical and biological alterations to the environment explored.

In general, mining activities can cause significantly negative impacts to the environment, since in the majority of cases the development of such activities implies in: suppression of vegetation, soil exposure and erosion, alterations in the quality of the surface and subterranean water resources, in addition to causing air pollution, amongst other negative aspects associated with these activities (MECHI; SANCHES, 2010). Depending on the geological characteristics of the deposit of interest, some mining activities can also result in acid mine drainage (AMD), as well as generating mineral tailings and increasing the turbidity and silting of water bodies, which can directly affect the water resources in the region where they occur. AMD has been considered one of the main negative environmental impacts resulting from mining activities, due to its dynamics and persistence (SANTOS; LADEIRA, 2011). Another negative aspect that can result from mining activities is related to the occurrence of possible modifications in the natural structure of local communities, and also reduce the local biodiversity in freshwater bodies close to or under the influence of these sites (CISZEWSKI et al. 2013; DURÁN; RAUCH; GASTON, 2013; HOLOPAINEN et al. 2003; LEFCORT; VANCURA; LIDER, 2010).

In this context, the first uranium mine to be explored in Brazil (Uranium Osamu Utsumi Mine) is located in the Poços de Caldas plateau region (Minas Gerais, Brazil), where the activities started in 1982, with a total production of 1.030t U by 1995 (CIPRIANI, 2002). This mining area is located in the dependencies of the Mineral Treatment Unit of the Brazilian Nuclear Industries (UTM/INB), and since it is situated in a region characterized by presenting the anomaly of elevated natural radioactivity associated with volcanic rock and mineral uranium deposits, it has aroused the interest of researchers, originating characterization studies mainly in the fields of Physics, Chemistry, Geology, Radiology and Biology.

At the UTM/INB site, low uranium ore tailings in the sterile piles associated with iron pyrite sulfide (FeS_2), in the presence of water and oxygen, provide adequate conditions for the occurrence of AMD, leading to the formation of radioactive acid effluents. The occurrence of AMD is very common in uranium mining areas, since the majority of the U is associated with pyrite, which, under favorable environmental conditions (principally exposure to air and water) forms H_2SO_4 and $FeSO_4$ and then $Fe(OH)_3$, the latter being responsible for the red or orange color indicating the occurrence of AMD (MKANDAWIRE, 2013).

The radioactive effluents continuously produced at UTM/INB present relevant concentrations of the following chemical species: fluoride, sulfate, manganese, zinc, uranium, aluminum and others (NASCIMENTO; FUKUMA; HORTELLANI, 1998), constituting a complex metal mixture. The chemical *in natura* UTM/INB effluent treatment involves the use of calcium hydroxide, calcium oxide and barium chloride to raise the pH value, providing conditions to precipitate the stable and radioactive metals (NÓBREGA; LIMA; LEITE, 2008). After the chemical treatment, the stable and radioactive metals are precipitated in decantation basins, and the supernatant subsequently discharged into water bodies in the region, that is, into the Antas reservoir.

The UTM/INB site is currently in the closing down phase, but Brazil does not have the technology or systematized experience for the remediation and decommissioning of uranium mines and plants. Hence the regulatory organs, both nuclear and environmental, have the legal responsibility to analyze their safety so as to determine the long and short term consequence to humans and to the environment. With respect to protection of the environment, the current approach for the radiological protection of non-human species foresees determination of the effects of metal and radionuclide dose and concentration rates on plants and animals (ICRP-91, 2003; ICRP-108, 2008). Considering this focus, one applied analytical tool could be the eco-toxicity tests, which are used to detect and evaluate the inherent capacity of the toxic agent to cause deleterious effects on live organisms.

Thus the results of the ecotoxicological tests and chemical analyses generated in the present study could contribute to a better long and short term evaluation and forecast of possible impacts of uranium mining activities on the aquatic biota of tropical aquatic ecosystems, including the Antas reservoir. This is a proposal of an applied nature, making it possible to apply the results to other situations where contamination by stable and radioactive metals resulting from uranium mining activities has occurred. Hence with a view to contributing to knowledge concerning the above, the general objectives of the present study were to evaluate the water quality of samples of *in natura* and treated effluent originating from uranium mining with acid mine drainage (UTM/INB) by way of an integrated chemical and ecotoxicological approach, applying the same approach to water samples taken from the reservoir under the influence of this mining company.

1.2 Ecotoxicology and the Cladocerans

The toxicity tests are based on the fundamental principal that the identification of the response of live organisms to exposure to toxic agents is dependent on the quantity (exposure level) of these agents (HOFFMAN et al., 1995). According to Cooney (1995), the quantification of toxic stress on the aquatic biota depends mainly on methods based on acute and chronic tests. Such tests have the objective of evaluating the inherent effects of one or more substances and/or elements of interest (for example: environmental samples and/or metals) on the test species in a given time period (GHERARDI-GOLDSTEIN, 1988). Acute toxicity tests are generally defined by their short duration period (usually 2 to 4 days), and are carried out to quantify the effect of toxic agents that lead to immobility and lethality to the organisms during a short period in their life cycle. On the other hand, in chronic toxicity trials the more frequently observed evaluation criteria are concerned with reproduction (embryo, larva, juvenile and adult), covering the more sensitive life phases of the organisms (ABNT, 2009; ABNT, 2010; APHA, 1995).

Toxicity trials with aquatic organisms should be carried out with selected taxonomic groups considered representative of the aquatic ecosystems. Standardization of the use of test species is recommended, since the requisites for their use in toxicity tests are well known and established. In this context, the daphnids stand out amongst the invertebrates of sweet waters most frequently used as test organisms, since they are widely used in acute and chronic ecotoxicological evaluations (ABNT, 2009; ABNT, 2010; ASTM, 1992c; e; i). The daphnids are small freshwater micro-crustaceans commonly known as "water fleas", belonging to the Class of Crustacea, Order of Cladocera and Family of Daphniidae, which includes the *Daphnia* spp. (*D. pulex*, *D. magna*) and the *Ceriodaphnia* spp. (*Ceriodaphnia dubia*), ubiquitous in temperate freshwaters (BERNER, 1986). On the other hand the freshwater species *Ceriodaphnia silvestrii*, ubiquitous in tropical regions, is also widely used as a test organism in toxicity trials (ABNT, 2010; FONSECA; ROCHA, 2004). In freshwater habitats the daphnids *Daphnia* spp. and *Ceriodaphnia* spp. are considered ecologically relevant, since they convert the phytoplankton, the bacteria and other suspended particles into animal protein, as well as contributing with a significant part of the diet of many fish (COONEY, 1995).

The use of daphnids in ecological tests is related to some important intrinsic characteristics of these organisms, such as a relatively short life cycle, easy to cultivate and handle in the laboratory, parthenogenetic reproduction with the production of clones, wide distribution in freshwater bodies, and sensitivity to a wide range of aquatic contaminants (COONEY, 1995; LILIUS; HÄSTBACKA; ISOMAA, 1995).

The cladoceran undoubtedly most used in toxicity trials of ecotoxicological tests is the species *Daphnia magna* (ADEMA, 1978) due principally to the large size of the newborn, making them easy to observe. On the other hand the distribution of this species is restricted to temperate environments with high and medium northern latitudes, where the natural waters contain large amounts of carbonate and are therefore characterized as hard (MITCHELL; HALVES; LAMPERT, 2004; TERRA; FEIDEN, 2003). The majority of ecotoxicological studies in freshwater bodies are carried out in temperate regions, and therefore it is natural that the majority of the standard organisms used belong to these regions, such as *Daphnia magna*, since regions from tropical and arctic regions are rare amongst the recommended standard organisms (NIKINMAA, 2014). However the use of native species in tropical regions such as Brazil has recently been strongly recommended (ABNT, 2009; 2010; FONSECA; ROCHA, 2004; FREITAS; ROCHA, 2010), since such species are considered ecologically more relevant, reflecting local conditions more closely. In addition the use of indigenous species can explain differences in water quality, such as soft water, characteristic of tropical regions (HARMON; SPECHT; CHANDLER, 2003), where *D. magna* is considered exotic.

1.3 Uranium mining activities and AMD

Uranium mining has the potential to liberate radionuclides in addition to other stable metals in aquatic bodies close to the area, causing changes in the chemical and biological characteristics of the environment (ANTUNES et al., 2007b). According to Elbaz-Poulichet et al. (1999), uranium mining activities, as also other processes associated with this activity, can lead to metal and uranium enrichment in waters and rivers downstream from the sites, mainly due to inappropriate discharge (e.g. discharge of radioactive effluent into aquatic bodies). AMD frequently occurs in uranium mines due to the presence of metal sulfides, generally pyrite (FeS_2), associated with rocky matrixes, which, under favorable environmental conditions, promote AMD, which is related to the production of elevated amounts of radioactive effluent. It should be emphasized that the radionuclides can enter the biota present in the fresh or salty water systems by absorption from the water, sediment and food. The radionuclides tend to accumulate at the bottom of sediments, but can enter the organic debris of the reservoirs after the death of plants and animals, which can sequester the radionuclides in their tissues when still alive. Thus the resuspension or dissolution of the debris allows the radionuclides to be remobilized to other areas within the system, or even enter the food chain (COONEY, 1995). With respect to the toxicity of the metals, including the radioactive metal uranium, it should be mentioned that this can vary according to the aquatic species used, due to the intrinsic characteristics of each one and/or different genotypes of the same species (BAIRD et al., 1991), as also the environmental conditions related to water quality (e.g. hardness, pH value, alkalinity, dissolved organic matter), which tend to greatly affect chemical speciation and hence the bioavailability of the metals in the environment in question.

Different international organizations have recognized the need to guarantee that man, as also the environment at its different organizational levels, be adequately protected against the effects of radioactive substances in environments impacted by nuclear installations (IAEA, 2006; ICRP-91, 2003; ICRP-108, 2008; OECD-NEA, 2007). In 2003, the “International Commission on Radiological Protection (ICRP)” published a document concerning the importance of the environment, considering the impacts of ionizing radiation. Thus studies are required to determine the safe dose and/or concentration rates of radionuclides for the maintenance of the biota in order to attend the interests of the radiological protection of non-human species (ICRP-108, 2008). This focus, aimed at protecting the environment from the noxious effects of ionizing radiation, finds support in the application of ecotoxicology, a science which studies the effects of different toxic agents on the organisms present in the ecosystems.

In this context, studies of aquatic systems located in uranium mining regions in Australia (RIPON; RILEY, 1996), in Portugal (ANTUNES; PEREIRA; GONÇALVES, 2007a), in Canada (PYLE; SWANSON; LEHMKUHL, 2001; 2002; ROBERTSON; LIBER, 2007) and in The Czech Republic (HUDCOVÁ; BADUROVA; ROZKOSNY, 2013) showed evidence that the results of ecotoxicological analyses complemented the physical, chemical and radiological data, contributing to a better evaluation and forecast of the risks caused by discharging uranium mining debris into the environment. According to Sheppard et al. (2005), an evaluation of the risk of the impact caused by the majority of radionuclides is based on the dose rate received by the organism of interest. In addition, according to these authors, in the case of uranium, the risk coming from the chemical toxicity is more relevant than the risk of radiological toxicity. Thus the radioactive metal uranium is considered to be one of the greatest concerns in water bodies under the influence of uranium mines, since it is considered potentially toxic for freshwater biota (ANTUNES et al., 2007). According to the literature, studies have related the toxic and acute effects of this metal to freshwater invertebrates such as: the cnidarian *Hydra viridissima* (RIETHMULLER et al., 2001), the alga *Chorella vulgaris* (CHARLES et al., 2002; FRANKLIN et al., 2000), the macrophyte *Ceratophyllum demersum* (MARKICH, 2013), the worm *Tubifex tubifex* (LAGAUZÉRE; TERRAIL; BONZOM, 2009), the mosquito *Chironomus tetans* (MUSCATELLO; LIBER, 2009) and the cladoceran *Daphnia magna* (MASSARIN et al., 2010; POSTON; HANF; SIMMONS, 1984; ZEMAN et al., 2008).

In Brazil, in studies developed at the Antas reservoir (located in the hydrographic sub-basin of the Ribeirão das Antas river), which is under the influence of a uranium mine (UTM/INB), Ronqui et al. (2010) related the low density of planktonic organisms in this reservoir to the discharge of treated effluent coming from the UTM/INB demonstrating the effect of radioactive effluent on the biota. It should be pointed out that in natural environments, although organisms can help in detecting the impacts, it is difficult to establish a cause and effect relationship for the qualitative and quantitative population changes that occur in these ecosystems. Thus the majority of the quality standards used to protect aquatic life were, and still are, established based on laboratory studies which simulate what can occur in aquatic ecosystems due to the introduction of toxic agents reasonably close (BERTOLETTI, ZAGATTO; 2006). In other studies carried out at the Antas reservoir by Azevedo et al. (2010), Ferrari et al. (2010) and Rodgher et al. (2013), concentrations of the chemical species (uranium, manganese, fluoride, zinc and aluminum) above the limits established by current legislation (CONAMA 357/2005) were detected. It should be emphasized that the values for U determined in samples of water taken from the hydrographic sub-basin of the Ribeirão das Antas river in the above-cited studies were above the limit considered safe (concentration without toxic effect equal to 0.005 mg L^{-1} U) for freshwater invertebrates, as proposed in a review by Sheppard et al. (2005). In another study also carried out in the hydrographic sub-basin of the Ribeirão das Antas river, the results registered that water samples with a concentration of 0.062 mg L^{-1} U) caused chronic toxicity in the species *Ceriodaphnia dubia* (ARCAL RLA01/10-2009 project).

1.4 Justification

In this context, the need to constantly maintain and amplify ecotoxicological studies allied with the chemical characterization of water systems influenced by nuclear installations, should be emphasized, aiming for a better evaluation and forecast of the long and short term impacts of these installations on the environment. In addition, further information concerning the toxicity of effluents or of natural waters contaminated with uranium and other metals on the aquatic biota in tropical environments is scarce. Thus additional studies are absolutely necessary to contribute to the determination of safe predicted concentrations of toxic metals such as uranium, for aquatic organisms.

This is a proposal of an applied nature, which would make it possible to use the results of the present study in other situations where contamination by metals caused by uranium mining activities occurs.

*REFERENCES

ADEMA, D. M. M. *Daphnia magna* as a test animal in acute and chronic toxicity tests. **Hydrobiologia,** v. 59, n. 2, p. 125-134, 1978.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM): **Standard guide for conducting renewal life-cycle toxicity tests with *Daphnia magna*.** Designation E 1193-87. Annual Book of ASTM Standards, Vol. 11.04:812-828, 1992c.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM): **Standard guide for conducting three-brood renewal toxicity tests with *Ceriodaphnia dubia*.** Designation E 1295-89. Annual Book of ASTM Standards, Vol. 11.04:973-991, 1992e.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (ASTM): **Standard practice for conducting static acute toxicity tests with *Daphnia*.** Designation E 4229-84 (Discontinued 1990). Annual Book of ASTM Standards, Vol. 11.04:47, 1992i.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. ABNT NBR12713: **ecotoxicologia aquática - toxicidade aguda - método de ensaio com *Daphnia* ssp (Cladocera, Crustacea)**. Rio de Janeiro, 2009.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. NBR13373: **avaliação da toxicidade crônica utilizando *Ceriodaphnia* ssp., (Cladocera, Crustacea).** Rio de Janeiro, 2010.

AMERICAN PUBLIC HEALTH ASSOCIATION; AMERICAN WATER WORK ASSOCIATION; WATER CONTROL FEDERATION. **Standard methods for the examination of water and wastewater**. 19 ed. New York, 1995.

ANTUNES, S. C.; PEREIRA, R.; GONÇALVES, F. Acute and chronic toxicity of effluent water from an abandoned uranium mine. **Archives of Environmental Contamination and Toxicology,** v. 53, p. 207-213, 2007a.

ANTUNES, S. C.; DE FIGUEIREDO, D. R.; MARQUES, S. M.; CASTRO, B. B.; PEREIRA, R.; GONÇALVES, F. Evaluation of water column and sediment toxicity from an abandoned uranium mine using a battery of bioassays. **Science of the Total Environment**, v. 374, p. 252-259, 2007b.

AZEVEDO, H.; COSTA, W. C.; OLIVEIRA, M. J. D.; NASCIMENTO, M. R. L.; MOURÃO, G. B.; SELEGHIM, M. H. R.; WISNIEWSKI, M. J. S.; ALBERTI, H. C.; BRUSCHI, A. L.; CAMPOS, M. B.; FERRARI, C. R.; ROQUE, C. V.; RODGHER, S.; RONQUI, L. B. **Diagnóstico ambiental da sub-bacia hidrográfica do Ribeirão das Antas - MG visando o gerenciamento integrado, o uso racional e sustentável dos recursos hídricos**. Relatório final FAPEMIG, processo APQ7807-5.04/07, 2010.

BAIRD, D. J.; BARBER, I.; BRADLEY, M.; SOARES, A. M. V. M.; CALOW, P. A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. **Ecotoxicology Environmental Safety,** v. 21, p. 257-265, 1991.

BRASIL. Resolução N^{o} 357, de 17 de Março de 2005. Resolução do Conselho Nacional de Meio Ambiente - CONAMA para a classificação de águas de acordo com sua composição e os teores máximos de substâncias potencialmente prejudiciais. 2005. Diário Oficial da União, Brasília, DF.

BERNER, D. B. **Taxonomy of *Ceriodaphnia* (Crustacea: Cladocera) in U.S. Environmental Protection Agency cultures. EPA/640/4-86/032.** Cincinnati: U.S. Environmental Protection Agency, 1986.

BERTOLETTI, E.; ZAGATTO, P. Aplicação dos Ensaios Ecotoxicológicos e Legislação Pertinente. In: Bertoletti, E.; Zagatto, P (Eds). **Ecotoxicologia Aquática. Princípios e Aplicações**. São Carlos: RiMa, 2006. cap. 15, p.347-379.

CHARLES, A. L.; MARKICH, S. J.; STAUBER, J. L.; DE FILIPPIS, L. F. The effect of water hardness on the toxicity of uranium to a tropical freshwater alga (*Chlorella* sp.). **Aquatic Toxicology**, v. 60, p. 61-73, 2002.

CIPRIANI, M. **Mitigação dos impactos sociais e ambientais decorrentes do fechamento definitivo de minas de urânio.** 2002. 332 f. Tese (Doutorado) - Faculdade de Engenharia, Universidade de Campinas, Campinas, 2002.

CISZEWSKI, D.; ALEKSANDER-KWATERCZAK, U.; POCIECHA, A.; SZAREK-GWIAZDA, E. Small effects of a large sediment contamination with heavy metals on aquatic organisms in the vicinity of an abandoned lead and zinc mine. **Environmental Monitoring and Assessment,** v. 185, p. 9825-9842, 2013.

COONEY, J. D. Freshwaters Tests Chapter 2. p. 71-102. In: RAND, G. M (Ed.). **Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment.** Washington: Taylor & Francis, 1995, 1125 p

DURÁN, A. P.; RAUCH, J.; GASTON, K. Global spatial coincidence between protected areas and metal mining activities. **Biological Conservation,** v. 160, p. 272-278, 2013.

ELBAZ-POULICHET, F.; MORLEY, N. H.; CRUZADO, A.; VELASQUEZ, Z.; ACHTERBERG, E. P.; BRAUNGARDT, C. B. Trace metal and nutriente distribution in an extremely low pH (2.5) river- estuarine system, the Ria of Huelva (South- West Spain). **Science of theTotal Environmental,** v. 227, p. 73-83, 1999.

FONSECA, A. L.; ROCHA, O. The life cycle of *Ceriodaphnia silvestrii* Daday, 1902, a Neotropical endemic species (Crustacea, Cladocera, Daphniidae). **Acta Limnologica Brasiliensia.,** v. 16, n. 4, p. 319-328, 2004.

FERRARI, C. R. **Avaliação de efeitos de efluentes radioativos de mineração de urânio sobre características físicas, químicas e diversidade da comunidade zooplanctônica na Unidade de Tratamento de Minérios, na Represa das Antas e Represa Bortolan, Poços de Caldas (MG).** 2010. 132 f. Dissertação (Mestrado). Universidade de São Paulo, São Paulo, 2010.

FRANKLIN, N. M.; STAUBER, J. L.; MARKICH, S. J.; LIM, R. P. pH-dependent toxicity of cooper and uranium to a tropical freshwater alga (*Chlorella* sp.). **Aquatic Toxicol,** v. 48, p. 275-289, 2000

FREITAS, E. C.; ROCHA, O. Acute toxicity tests with the tropical cladoceran *Pseudosida ramosa*: the importance of using native species as test organisms. **Archives of Environmental Contamination and Toxicology,** v. 60, p. 241-249, 2010.

GHERARDI-GOLDSTEIN, E. Testes de toxicidade de efluentes industriais. **Ambiente,** v. 2, n. 1, p. 33-38, 1988.

HARMON, S. M.; SPECHT, W. L.; CHANDLER, G. T. A composition of the Daphnids *Ceriodaphnia dubia* and *Daphnia ambigua* for their utilization in routine toxicity testing in the Southeastern United States. **Archives of Environmental Contamination and Toxicology,** v. 45, p. 79-85, 2003.

HOFFMAN, D. J.; BARNETT, A. R.; BURTON, G. A. Jr.; CAIRNS, J. Jr. Introduction. In: HOFFMAN, D. J.; RATTNER, B. A.; BURTON, G. A. Jr.; CAIRNS, J. Jr. (Eds.). **Handbook of Ecotoxicology.** 2nd edition, Lewis Publishers: London, Washington, D.C, 1995.

HOLOPAINEN, I. J.; HOLOPAINEN, A. L.; HÄMÄLÄINEN, H.; RAHKOLA-SORSA, M.; TKATCHEVA, V.; VILJANEN, M. Effects of mining industry waste waters on a shallow lake ecosystem in Karelia, north-west Russia. **Hydrobiologia,** v. 506-509, n. 1-3, p. 111-119, 2003.

HUDCOVA, H.; BADUROVA, J.; ROZKOSNY, M. Quality and mutagenicity of water and sediment of the streams impacted by the former uranium mine area Olsi-Drahonin (Czech Republic). **Journal of Environmental Radioactivity,** v. 116, p. 159-165, 2013.

INSTITUTO BRASILEIRO DE MINERAÇÃO (IBRAM). **Informações e Análises da Econômia Mineral Brasileira.** 4. ed., 2008. Disponível em: <http://www.ibram.org.br/sites.

INTERNATIONAL ATOMIC ENERGY AGENCY. Fundamental Safety Principles: Safety Fundamentals. ISSN 1020-525X, ISBN 92-0-110706-4. In: **IAEA Safety Standard Series No. SF-2.** International Atomic Energy Agency, Vienna, 2006.

INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION. ICRP-91. A framework for assessing the impact of ionizing radiation on non-human species. **Annals of ICRB**. Elsevier, Great Britain, 2003.

INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION. ICRP-108. Environmental Protection: the Concept and Use of Reference Animals and Plants. **Annals of ICRB**. Elsevier, Great Britain, 2008.

LAGAUZÉRE, S.; TERRAIL, R.; BONZOM, J. M. Ecotoxicity of uranium to *Tubifex tubifex* worms (Annelida, Clitellata, Tubificidae) exposed to contamined sediment. **Ecotoxicology and Environmental Safety**, v. 72, p. 527-537, 2009.

LEFCORT, H.; VANCURA, J.; LIDER, E. L. 75 Years after mining ends stream insect diversity is still affected by heavy metals. **Ecotoxicology,** v. 19, p. 1416-1425, 2010.

LILIUS, H.; HÄSTBACKA, T.; ISOMAA, B. A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. **Environmental Toxicology and Chemistry,** v. 14, n. 12, p. 2085-2088, 1995.

MARKICH, S. J. Water hardness reduces the accumulation and toxicity of uranium in a freshwater macrophyte (*Ceratophyllum demersum*). **Science of the Total Environmental,** v. 443, p. 582-589, 2013.

MASSARIN, S.; ALONZO, F.; GARCIA-SANCHEZ, L.; GILBIN, R.; GARNIER-LAPLACE, J.; POGGIALE, J. C. Effects of chronic uranium exposure on life history and physiology of *Daphnia magna* over three successive generations. **Aquatic Toxicology,** v. 99, p. 309-319, 2010.

MECHI, A.; SANCHES, D. L. Impactos ambientais da mineração no Estado de São Paulo. **Estudos Avançados,** v. 24, p. 68, 2010.

MKANDAWIRE, M. Biochemical behaviour and bioremediation of uranium in waters of abandoned mines. **Environmental Science and Pollution Research**, v. 20, p. 7740-7767, 2013.

MITCHELL, S. E; HALVES, J.; LAMPERT, W. Coexistence of similar genotypes of *Daphnia magna* in intermittent populations: response to thermal stress. **Oikos,** v. 106, p. 469-478, 2004.

MUSCATELLO, J. R.; LIBER, K. Accumulation and chronic toxicity of uranium over diferent life stages of lhe aquatic invertebrate *Chironomus tetans*. **Archives of Environmental Contamination and Toxicology,** v. 57, p. 531, 539, 2009.

NASCIMENTO, M. R. L.; FUKUMA, H. T.; HORTELLANI, M. A. **Projeto Itataia - Controle de processo na produção de ácidos fosfórico e urânio**. Poços de Caldas: INB, (Manual de Métodos e Análises Químicas). 1988. p. 143.

NIKINMAA, M. **An Introduction to Aquatic Toxicology**. Academic Press is an imprint of Elsevier: The Boulevard, Langford Lane, Kidlington, Oxford, USA, 2014. p. 240.

NÓBREGA, F. A.; LIMA, H. M.; LEITE, A. L. Análise de múltiplas variáveis no fechamento da mina-Estudo de caso da pilha de estéril BF-4, Mina Osamu Utsumi, INB Caldas, Minas Gerais. **Revista Escola de Minas Ouro Preto,** v. 61, p. 197-202, 2008.

ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT, NUCLEAR ENERGY AGENCY (OECD-NEA). Environmental Radiological Protection NEA No. 6167. **Scientific Issues and Emerging Challenges for Radiological Protection. Report of the Expert Group on the Implications of Radiological Protection Science,** Paris, ISBN 978-92-64-99032-6, 2007. Disponível em: http://www.nea.fr/html/rp/reports/2007/nea6167-egis.pdf

PYLE, G. G.; SWANSON, S. M.; LEHMKUHL, D. M. Toxicity of Uranium Mine-Receiving Waters to Caged Fathead Minnows, *Pimephales promelas*. **Ecotoxicology and Environmental Safety**, v. 48, p. 202-214, 2001.

PYLE, G.G.; SWANSON, S. M.; LEHMKUHL, D. M. Toxicity of uranium mine receiving waters to early life stage fathead minnows (*Pimephales promelas*) in the laboratory. **Environmental Pollution,** v. 116, p. 243-255, 2002.

POSTON, T. M.; HANF, R. W.; SIMMONS, M. A. Toxicity of uranium to *Daphnia magna*. **Water and Air and Soil Pollution**, v. 22, p. 289-298, 1984.

PROJETO ARCAL RLA01/10 Qualidade das Águas do Ribeirão das Antas. **Relatório.** 2009**,** p. 27.

REBOUÇAS, A. C.; BRAGA, B.; TUNDISI, J. G. **Águas Doces do Brasil**: Capital Ecológico, Uso e Conservação. 3. ed. São Paulo: Editora Escrituras, 2006, p. 748.

RIETHMULLER, N.; MARKICH, S. J.; VAN-DAM, R. A.; PARRY, D. The effect of true water hardness and alkalinity on the toxicity of copper and uranium to a tropical freshwater hydra (*Hydra viridissima*). **Biomarkers**, v. 6, p.45-51, 2001.

RIPPON, G. D.; RILEY, S. J. Environmental impact assessment of tailings dispersal from a uranium mine using toxicity testing protocols. **Water Resources Bulletin**, v. 32, p. 1167-1175, 1996.

ROBERTSON, E. L.; LIBER, K. Bioassays with caged *Hyalella azteca* to determine in situ toxicity downstream of two Saskatchewan, Canada, uranium operations. **Environmental Toxicology and Chemistry**, v. 26, p. 2345-2355, 2007.

RODGHER, S.; AZEVEDO, H.; FERRARI, C. R.; ROQUE, C. V.; RONQUI, L B.; CAMPOS, M. B.; NASCIMENTO, M. R. L. Evaluation of surface water quality in aquatic bodies under the influence of uranium mining (MG, Brazil). **Environmental Monitoring and Assessment** v. 185, p. 2395-2406, 2013.

RONQUI, L. B.; AZEVEDO, H.; NASCIMENTO, M. R. L.; MACACINI, J. F.; ROQUE, C. V. Radioecological evaluation (spatial and temporal variation) of the planktonic microorganisms in the Antas Reservoir, Caldas, Minas Gerais, Brazil. **Atoms for Peace-An International Journal**, v. 3, p. 33-49, 2010.

SANTOS, E. A.; LADEIRA, A. C. Q. Recovery of uranium from mine waste by leaching with carbonate-based reagents. **Environmental Science & Technology,** v. 45, p. 3591-3597, 2011.

SHEPPARD, S. C; SHEPPARD, M. I; GALLERAND, M. O; SANIPELLI, B. Derivation of ecotoxicity thresholds for uranium. **Journal of Environmental Radioactivity,** v. 79, p. 55-83, 2005.

TERRA, N. R.; FEIDEN, I. R. Reproduction and survival of *Daphnia magna* Straus, 1820 (Crustacea: Cladocera) under different hardness condition**. Acta Limnologica. Brasiliensia,** v. 15**,** p. 51-55, 2003.

ZEMAN, F. A; GILBIN, R.; ALONZO, F.; LECOMTE-PRADINES, C.; GARNIER-LAPLACE, J.; ALIAUME, C. Effects of waterborne uranium on survival, growth, reproduction and physiological processes of the freshwater cladoceran *Daphnia magna*. **Aquatic Toxicology**, v. 86, p.370-378, 2008.

2 OBJECTIVE

2.1 General Objective

The objective of the present study was to evaluate the water quality of *in natura* and treated effluent samples coming from a uranium mining site with acid mine drainage (UTM/INB), and of water samples taken from a tropical reservoir under the influence of this mine, using an integrated chemical and ecotoxicological approach. With a view to complementing this ample approach, acute toxicity tests with the metals of interest uranium and manganese were also carried out, and the chemical speciation of the uranium and its relationship to toxicity to daphnids determined.

2.2 Specific objectives

✓- Determine the sensitivity range of the species *Ceriodaphnia silvestrii* to the reference substance sodium chloride and of *Daphnia magna* to potassium dichromate under the cultivation conditions established in the Ecotoxicology Laboratory of LAPOC/CNEN, for use in the toxicity tests;

✓- Seasonally evaluate the chemical quality and acute toxicity potential of *in natura* and treated effluent samples from the UTM/INB, and also water samples from the Antas reservoir.

✓- Determine the acute toxicity (CE_{50}) of the metals manganese and uranium for *C. silvestrii* and *D. magna* using standard reference substances so as to evaluate the toxic potential of these metals as compared to the values registered in water samples taken from the Antas reservoir;

✓- Determine the speciation of the uranium at pH 7 and pH 8 for the species *D. magna* and at pH 7.5 for the species *C. silvestrii*;

✓- Evaluate the efficiency of the system used to treat the *in natura* effluent from UTM/INB by way of a chemical characterization and acute toxicity tests with daphnids;

✓- Determine the acute toxicity potential (CE_{50}) of the *in natura* effluent from UTM/INB.

4 GENERAL CONCLUSIONS

- ✓The cladocerans *C. silvestrii* and *D. magna* cultivated in the Ecotoxicology Laboratory of LAPOC/CNEN showed reliable conditions to performing acute toxicity bioassays, since that the cultivation conditions agreed with those of the international and national norms;

- ✓Samples of the *in natura* and treated effluents presented seasonally higher concentrations of chemical species in relation to the reference point at the Antas reservoir, and were considered acutely toxic to the cladoceran *Ceriodaphnia silvestrii and D. magna*;

- ✓Seasonally, a clearly protective effect to the acute toxicity of the chemical mixture in water from the Antas reservoir was provided to the cladocerans *C. silvestrii* and *D. magna* by very high hardness values;

- ✓Mn values registered in the literature for water samples from the Antas reservoir revealed toxicity potential for the indigenous specie *C. silvestrii*, as observed in the EC_{50} value registered that was of 5.92 mg L^{-1}Mn;

- ✓The bioassays showed that *C. silvestrii* (EC_{50} = 0.07 mg L-1 U) was more sensitive to uranium metal than *D. magna* (EC_{50} = 0.56 e 2.4 mg L^{-1} U at pH 7 and 8, respectively), a fact that indicated the importance of using indigenous species, since that it represent the local flora.

- ✓When the pH value was decreased from 8 to 7 the speciation of the metal uranium was shown to change, with greater concentrations of the species UO_2^{2+} and UO_2OH^+, considered potentially toxic to the biota, being registered at pH 7;

- ✓The acute toxicity results, indicated that the treatment of the *in natura* effluent from the PM pond carried out by the mining company (UTM/INB) was improper and inefficient, since the treated effluent samples from the P41-S pond were as toxic as the *in natura* effluent samples from the PM pond for both daphnids.

- ✓Samples of the *in natura* effluent from the pit mine pond presented very high toxicity potential for the *C. silvestrii* and *D. magna*, according to the extremely low values EC_{50} registered that were: 0.34<0.41%<0.52 and 4.77<5.41%<6.18, respectively;

APPENDIXES A

Chemical composition of the culture medium for the Cladoceran species *Ceriodaphnia silvestrii.*

Solutions	Reagent	Amount
1	$CaSO_4.2H_2O$	1500
2	KCl	200
	$NaHCO_3$	4800
	$MgSO_4.\ 7H_2O$	6100

Concentrations are given in mg/L
ABNT (2010)

APPENDIXES B

Chemical composition of the culture medium for the Cladoceran species *Daphnia magna*; Fonte: Elendt and Bias, 1990.

Medium		ISO	M 4
	Trace nutrients[a]		
B	(H_3BO_4)	–	0.5000
Fe	($FeSO_4 * 7\,H_2O$)[b]	–	0.2000
Mn	($MnCl_2 * 4\,H_2O$)	–	0.1000
Li	(LiCl)	–	0.0500
Rb	(RbCl)	–	0.0500
Sr	($SrCl_2 * 6\,H_2O$)	–	0.0500
Mo	($Na_2MoO_4 * 2\,H_2O$)	–	0.0250
Br	(NaBr)	–	0.0125
Cu	($CuCl_2 * 2\,H_2O$)	–	0.0063
Zn	($ZnCl_2$)	–	0.0063
Co	($CoCl_2 * 6\,H_2O$)	–	0.0025
J	(KJ)	–	0.0025
Se	(Na_2SeO_3)	–	0.0010
V	(NH_4VO_3)	–	0.0003
	EDTA * $2\,H_2O$[b]	–	2.5000
	Macro nutrients		
$CaCl_2 * 2\,H_2O$		293.80	293.80
$MgSO_4 * 7\,H_2O$		123.30	123.30
$NaHCO_3$		64.80	64.80
KCl		5.80	5.80
$Na_2SiO_3 * 9\,H_2O$		–	10.00
$NaNO_3$		–	0.27
KH_2PO_4		–	0.14
K_2HPO_4		–	0.18
	Vitamins		
Thiamine[c]		–	75.0
B_{12}[c]		–	1.0
Biotin[c]		–	0.75

Concentrations are given in (mg/l), unless otherwise stated

[a] Concentrations are given for the target element (source compounds are given in brackets)

[b] Stock solutions of $FeSO_4 * 7\,H_2O$ and $Na_2EDTA * 2\,H_2O$ are poured together and autoclaved immediately

[c] (µg/l)

APPENDIXES C

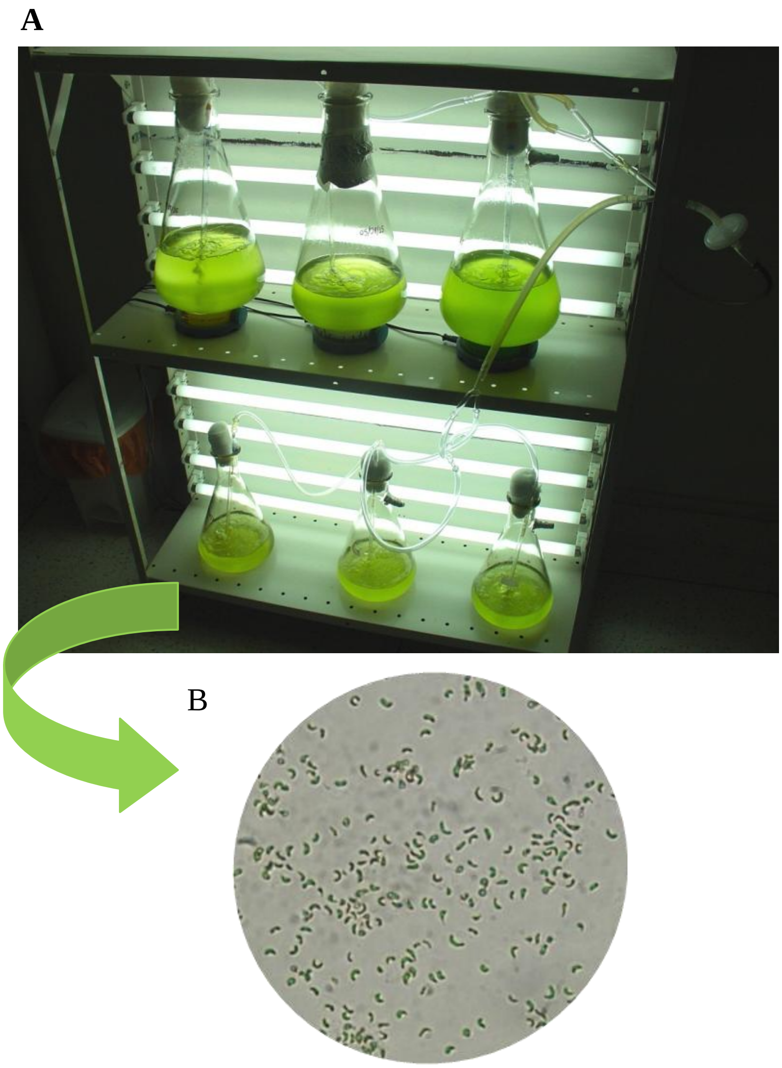
Chemical composition of the culture medium CHU for the alga *Raphidocelis subcapitata*.

CHU Medium	
Nutrients	
$Ca(NO_3)_2$	0.430
K_2HPO_4	0.050
$MgSO_4.7H_2O$	0.750
KCl	0.050
Na_2CO_3	0.200
$FeCl_3.6H_2O$	0.005

Concentrations are given in g/100mL
Müller (1972)

APPENDIXES D

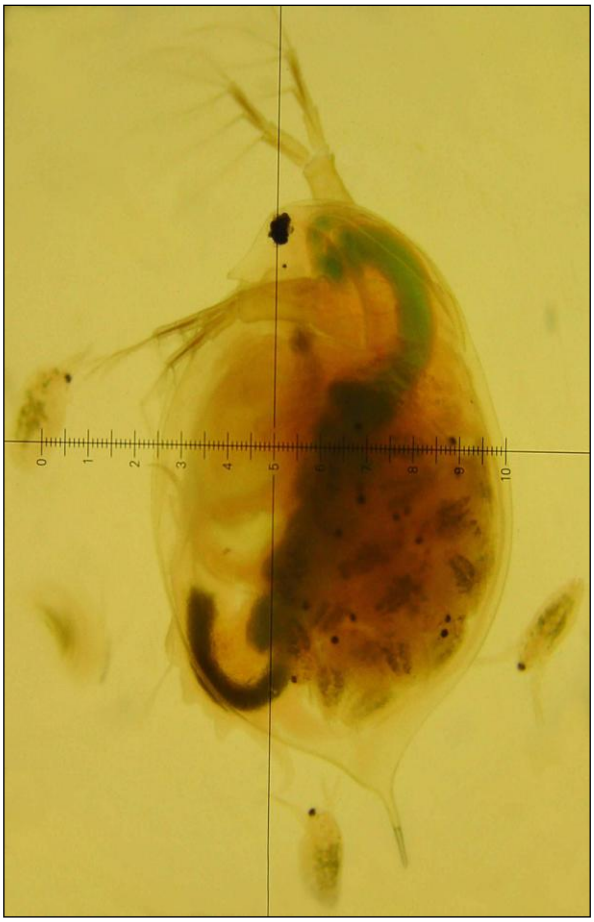
A: Photo illustrating the cultivation of the alga *Raphidocelis subcapitata* under axenic conditions in the Ecotoxicology Laboratory of LAPOC/CNEN; **B:** Photo of the microscopic field showing the cultivation of spread cells of *R. subcapitata*;



APPENDIXES E

A: Photo taken using a magnifying glass of an ovate adult female *Daphnia magna* (6.0 mm in length) cultivated in the Ecotoxicology Laboratory of LAPOC/CNEN; **B:** Photo taken using a magnifying glass of a male *Daphnia magna* (2.5 mm in length). Fotos: Ferrari, C. R.

A



B

APPENDIXES F

Photo carried out using the microscope (magnification x20) of an ovate adult female Ceriodaphnia silvestrii (1.1 mm in length) cultivated in the Ecotoxicology Laboratory of LAPOC/CNEN: Photo: Ferrari, C. R.

Table 3.4.1 Average values obtained for the

APPENDIXES G

Life cycle of *Daphnia magna*: Source: ISO:6341 (2012). Drawing: Lima, E. O. (2015).