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Assessment of Bioconcentration Factor of Chromium by Instrumental Neutron Activation Analysis in *Argyrodiaptomus falcifer* Daday, a Subtropical Freshwater Copepod

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Abstract The objectives of this study were to determine the capacity of the freshwater calanoid copepod *Argyrodiaptomus falcifer* (Daday, 1905) to accumulate Cr from water, to know the bioconcentration factors in order to evaluate its potential as a biomonitor, and to compare this with data previously obtained with *Daphnia magna* Straus under identical conditions. By static bioassays using triplicates and a control, a pool of *A. falcifer* was exposed to three concentrations of Cr (VI): 150 μg/L (T1), 280 μg/L (T2), and 350 μg/L (T3) for 48 h to later determine by Instrumental Neutron Activation Analysis the amount of Cr accumulated. *A. falcifer* accumulated Cr in all the three concentrations tested. The comparison of T1, T2, and T3 and the control showed significant differences ($p<0.05$) but not between the treatments ($p>0.05$). On the other hand, *A. falcifer* accumulated more Cr than *D. magna*, but these differences were not significant ($p>0.05$). Almost no information is available about metal toxicity in freshwater copepods so the reported results are of high importance in order to detect good biomonitors of freshwater Cr-polluted environments.

Keywords *Argyrodiaptomus falcifer* · Bioconcentration factor · Biomonitor · Chromium · Freshwater copepod · Instrumental neutron activation analysis · Static bioassay

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1 Introduction

Concentrations of chemicals in organisms are regarded as being indicators of the bioavailable fraction of those substances in the environment. Delineating metal uptake in freshwater invertebrates is important for understanding metal bioaccumulation and toxicity and for setting appropriate water quality criteria. Zooplanktonic organisms are suitable for this purpose because of their short life span, their presence in any season, and their great capacity to accumulate several pollutants. Among zooplanktonic organisms, most of previous work has been performed with cladocerans, although accumulation capacity of some

species or collectives of marine copepods have been studied by different authors (Moraitou-Apostolopoulou 1982; Zauke et al. 1996; Wang and Fisher 1998, 1999; Bielmyer et al. 2006). Laboratory or field surveys have been performed with Cu, Zn, Cd, Co, Ni, and Pb (Verriopoulos et al. 1982; Kahle and Zauke 2002a, b, 2003; Das and Das 2005). A few studies analyze the effect of Cr (VI) on copepods (Hutchinson et al. 1994; Miliou et al. 2000), but information on Cr accumulation in freshwater copepods is very scarce in spite of the importance of Cr as a pollutant derived of various industrial processes.

Moreover, most toxicity bioassays with copepods have been performed to evaluate the effects of contaminated sediments (Kovatch et al. 1999; Turesson et al. 2007; Eka et al. 2007), but the importance of the dissolved phase in the toxicity to planktonic organisms such as calanoid copepods is of high relevance. For instance, the uptake of contaminants from water is crucial for copepods, as was demonstrated by Wang and Fisher (1999) who delineated the routes of various metals (Ag, Cd, Co, Se, Zn) in marine invertebrates. These authors concluded that, in suspension feeders such as copepods, the uptake from the dissolved phase and food ingestion can be equally important for metal accumulation.

Because of the great concentration capacity of the accumulator organisms, heavy metals may easily be measured, even when they are so diluted in the water that they cannot be analyzed by the commonly used methods. Bioconcentration factors (BCFs) have frequently been used to evaluate the potential of an organism for bioaccumulation of waterborne or particle-bound metals (Kahle and Zauke 2002b). In general, there are two different ways to calculate BCFs: (1) using the ratio of $k1$ and $k2$ from kinetic data not assuming that an equilibrium has been reached during the experiment or (2) using the ratio of metal concentrations in the test organism *CA* and the exposure concentration *CW* assuming that an equilibrium has been reached (Kahle and Zauke 2002a).

The aim of this study was to assess the capacity of the freshwater calanoid copepod *Argyrodiaptomus falcifer* (Daday, 1905) to accumulate Cr from water, to know the BCF in order to evaluate its potential as a biomonitor, and to compare this with data previously obtained with *Daphnia magna* Straus under identical conditions (Regaldo et al., in press).

2 Materials and Methods

A. falcifer is a very common species widely distributed in the floodplains of South American rivers such as Paraná, Paraguay, and Uruguay. The current taxonomic status of *A. falcifer* was established by Paggi (2006). This author states that *Diaptomus falcifer* should be re-assigned to the genus *Argyrodiaptomus* and that it is a senior synonym of *Argirodiaptomus argentinus* (Wright, 1938).

A. falcifer was collected from temporary ponds of the Paraná river floodplain, Santa Fe, Argentina, (31°37′ S; 60°41′ W), using a 45 μm zooplankton net. These being hypereutrophic temporary ponds, collected copepods were placed into Petri dishes in order to separate the specimens from the particulate organic matter. Adult males and gravid females were sorted under an Olympus Z TX-E dissection stereomicroscope using a plastic micropipette. Adults were randomly separated to provide 12 sets of specimens, 150 per chamber. Sorted specimens were acclimatized to synthetic water (2 g $NaHCO_3$; 2.24 g $CaCl_2$; 0.26 g K_2SO_4 in 10 l distilled water); conductivity, 0.05 mS cm^{-1}; dissolved oxygen, 7.8 mg l^{-1}; pH 7.6, in an incubator for 24 h, at test conditions of 20±1°C, 12:12 h (L/D), fed ad libitum with *Chlorella* sp., and used the following day.

Static bioassays were conducted in order to test the Cr accumulation of *A. falcifer*. Cr was added as chromium dichromate ($Cr_{77}K_2$). The pools of copepods were exposed to three concentrations of Cr (VI): 150 μg/L (T1), 280 μg/L (T2), and 350 μg/L (T3) for a period of 48 h. The toxicant was added to a freshly prepared and aired solution of synthetic water. No food was given to the test organisms during the experiment.

Bioassays were carried out in 250 ml glass beakers, each with 200 ml of synthetic aired water. Bioassays were conducted by triplicate per treatment and a control (without Cr) at identical conditions than acclimation phase. After 48 h, specimens were sieved and carefully washed twice with distilled water, dried at 60°C till constant weight to later determine the amount of accumulated Cr by Instrumental Neutron Activation Analysis (INAA).

Samples of approximately 1 mg were analyzed by INAA, together with certified reference material IAEA V-10. Four-hour irradiations were carried out at RA-3 reactor (thermal flux $6.10^{13} cm^{-2}\ s^{-1}$, 10 MW

nominal power), at the Ezeiza Atomic Center of the Argentine National Atomic Energy Commission. Measurements of ^{51}Cr (half-time 27.72 days, γ energy peak 320 keV) were done after 30 days of decay, using a 30% efficiency Ortec GeHP detector, coupled to a multichannel Ortec 919 E module. Gamma Vision software was used for spectra acquisition, and the concentrations were calculated using a software developed at the laboratory. The uncertainty informed is the combined uncertainty (k=1) calculated considering the measurement uncertainty and the uncertainty of the calibration standard (IAEA V-10 Hay powder) as in its certificate (IAEA 2000).

Quality assurance of analytical results was evaluated by analyzing the certified reference material MRCChEN-002, clam (*Venus antiqua*), provided by the Chilean Atomic Energy Commission. This reference material was analyzed by applying the same experimental conditions used in copepod samples analysis.

Observed BCF was calculated at the end of a 24-h exposure according to the following expression (Ravera 2001; Kahle and Zauke 2002a): BCF=concentration in the animal (C_A)/concentration in water (C_W).

Normality of log-transformed data was checked using the Kolmogorov–Smirnov goodness-of-fit test. Analysis of variance followed by the Tukey test was used to compare mean values (α<0.05) to check for significant differences between control and treatment groups. Standard deviation (s.d.) and variation coefficient (v.c.) were obtained from BCF data.

3 Results

Chromium results obtained for certified reference material MRCChEN-002 showed a good agreement with the certified value. Relative error was 6.8%, indicating the accuracy of the obtained results. Standardized difference or z-score value (Bode 1996), obtained for Cr was 0.53 (z <1), indicating that the result is satisfactory and in agreement with the certified value.

A. falcifer accumulated Cr in all the three tested concentrations (Fig. 1). Comparison of T1, T2, and T3 and the control showed significant differences (p<0.05) but not between the treatments (p>0.05). On the other hand, *A. falcifer* accumulated more Cr than *D. magna*, but differences were not significant (p>0.05).

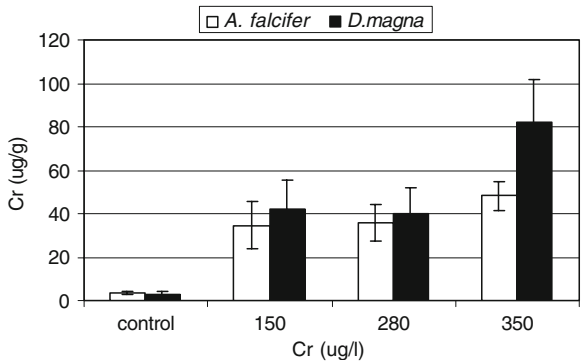

Fig. 1 Cr concentrations recorded in *A. falcifer* and *D. magna* tissues after exposure to the control (without Cr) and the three Cr (VI) concentrations tested: 150 (T1), 280 (T2), and 350 μg/l (T3). Measurements performed by Instrumental Neutron Activation Analysis. *Bars* indicate 1 Standard Deviation (S.D.)

An inverse relationship was observed between the Bioconcentration Factor (BCF, ratio tissues/water) and chromium concentration exposure level in *A. falcifer*. The highest BCF obtained was 231.07 (s.d.=71.74, v.c.=1.62) in T1, while the intermediate and the higher concentrations were very similar: 127.73 (s.d.=30.24, v.c.=1.66) in T2 and 137.40 (s.d.=18.82, v.c.=1.5) in T3. All these values were very similar to those recorded with *D. magna*: 281.67 (s.d.=86.64, v.c.= 0.29) in T1, 144 (s.d.=40.51, v.c.=0.11) in T2 and 234.67 (s.d.=54.81, v.c.=1.50) in T3.

4 Discussion

Our study suggests a net accumulation strategy for Cr in *A. falcifer*. Due to their central role in the food chain and the worldwide distribution of copepods, they are interesting candidates for bioaccumulation studies.

When considering the use of biomonitors to evaluate environmental quality, the sensitivity of prospective biomonitors to detect environmental changes is crucial. Short life span organisms may be used as accumulators specially for detecting short-term pollution. In this survey, 24 h exposure was shown to be enough to detect Cr accumulating capacity by *A. falcifer*.

Marchese et al. (2008) measurements of metal concentrations in the tissues of freshwater biomonitors reflected the bioavailability of metal in the environment. In a multispecies semistatic test, these

authors registered an inverse relationship between the bioconcentration factor (ratio tissues/water) and the exposure concentrations in all species. Although all the studied species studied could be proposed as biomonitors, *Ceratophyllum demersum*, *Limnodrilus udekemianus*, and *Zilchiopsis collastinensis* were the best biomonitors because of their higher capacity to accumulate Cr.

INAA was shown to be an appropriate technique to determine Cr in copepods as no sample preparation previous to the analysis is needed, and it has high accuracy and precision and low detection limits. Added to its characteristics, the methodological advantage of the INAA is permitting the quantification of chromium in a small amount of sample. The number of copepods collected under the experimental conditions (150 per replica) could seem low if compared with what is possible to capture in marine environments where zooplankton samples are obtained from the sieving of huge volumes of the water (impossible to get from our small water body) taken with vertically towed plankton nets with a mesh size of 500–700 μm and 1 m diameter. In these conditions, the number of animals is about 1,300 in each of the exposure treatments. On the other hand, the biomass required to perform more traditional bioaccumulation analysis, like graphite furnace atomic absorption spectrophotometry, is higher (of about 10 mg dried copepods, in terms of Zauke and Schmlenbach 2006) than that obtained in this study. In contrast, INAA is a fast and reliable procedure to routinely measure the abundances of up to about 35 elements even in small (<1 mg) samples (Koeberl 1993). Many species that may be considered good indicators of water quality are very small and sometimes scarce. The minimum biomass required for having a reliable result with this method is compatible with those concentrations usually found in eutrophic and polluted freshwater environments that are often lower than those obtained from marine environments.

INAA has been used for multiple applications: e.g., for the determination of Cr and Co in human serum (Versieck et al. 1978) to investigate the absorption of Hg in algal biomass (Mosulishvili et al. 2004), multi-elements in earthworms (Zheng et al. 1997), the distribution patterns of rare earth elements and their binding species with proteins in humans (Chen et al. 2001), heavy metals in different organs of fishes living in polluted environments (Wood et al. 1990; Veado et al. 2007), the impact of heavy metals from environmental tobacco smoke (Landsberger and Wu 1995), to characterize several biological reference materials and selected food items (Rao et al. 1995), for air pollution research (Chung et al. 1997; Jasan et al. 2004; Wannaz et al. 2008), and in the geological sciences, INAA is still considered the method of choice for multi-element studies requiring precision and accuracy (Koeberl 1993).

5 Conclusions

Our results show that the calanoid copepod *A. falcifer* can be used in biomonitoring studies to assess the quality of freshwater environments. This view is confirmed by a net Cr accumulation strategy recorded in comparison to the control. These findings agree with a net accumulation strategy. *A. falcifer* accumulates Cr and could therefore be proposed as biomonitor of contamination of freshwater environments. However, both *A. falcifer* and *D. magna* can be regarded as suitable biomonitors for the element under consideration. Finally, we can conclude that neither Cr accumulation nor BCF were different for *A. falcifer* and *D. magna*, so that a priori, we can assess that copepods would not be better biomonitors than cladocerans.

Almost no information is available about metal toxicity in freshwater copepods so the reported results are of high importance to detect good biomonitors of freshwater Cr polluted environments.

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References

Bielmyer, G. K., Grosell, M., & Brix, K. V. (2006). Toxicity of silver, zinc, copper, and nickel to the copepod *Acartia tonsa* exposed via a phytoplankton diet. *Environmental Science & Technology*, *40*, 2063–2068. doi:10.1021/es051589a.

Bode, P. (1996). *Instrumental and organizational aspects of a neutron activation analysis laboratory, PhD Thesis*. Delft, The Netherlands: Delft University of Technology.

Chen, C. H., Zhang, P., & Chai, Z. (2001). Distribution of some rare earth elements and their binding species with proteins in human liver studied by instrumental neutron activation analysis combined with biochemical techniques. *Analytica Chimica Acta*, *439*(1), 19–27. doi:10.1016/S0003-2670(01)01024-8.

Chung, Y. S., Jeong, E. S., & Cho, S. Y. (1997). Intercomparison and determination of environmental standard samples by instrumental neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry*, *217*(1), 71–76. doi:10.1007/BF02055351.

Das, B. K., & Das, N. (2005). Impacts of quicklime (CaO) on the toxicity of copper ($CuSO_4$, $5H_2O$) to fish andfish foodorganisms. *Chemosphere*, *61*, 186–191. doi:10.1016/j.chemosphere.2005.02.064.

Eka, H., Nilssona, E., Birgerssonb, G., & Davea, G. (2007). TNT leakage through sediment to water and toxicity to *Nitocra spinipes*. *Ecotoxicology and Environmental Safety*, *67*, 341–348. doi:10.1016/j.ecoenv.2006.10.011.

Hutchinson, T. H., Williams, T. D., & Eales, G. J. (1994). Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (*Cyprinodon variegatus*) and copepods (*Tisbe battaglial*). *Marine Environmental Research*, *38*, 275–290. doi:10.1016/0141-1136(94)90028-0.

IAEA. (2000). International Atomic Energy Agency, Reference Material IAEA-V10, Trace Elements in Hay Powder, Reference Sheet, January 2000.

Jasan, R. C., Verburg, T. G., Wolterbeek, H. T. H., Plá, R. R., & Pignata, M. L. (2004). On the use of the lichen *Ramalina celastri* (Spreng.) Krog. & Swinsc. as an indicator of atmospheric pollution in the province of Córdoba, Argentina, considering both lichen physiological parameters and element concentrations. *Journal of Radioanalytical and Nuclear Chemistry*, *259*(1), 93–97. doi:10.1023/B:JRNC.0000015812.46333.41.

Kahle, J., & Zauke, G. P. (2002a). Bioaccumulation of trace metals in the calanoid copepod *Metridia gerlachei* from the Weddell Sea (Antarctica). *The Science of the Total Environment*, *295*, 1–16. doi:10.1016/S0048-9697(01)01147-0.

Kahle, J., & Zauke, G. P. (2002b). Bioaccumulation of trace metals in the copepod Calanoides acutus from the Weddell Sea (Antarctica): comparison of two-compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology (Amsterdam, Netherlands)*, *59*, 115–135. doi:10.1016/S0166-445X(01)00245-4.

Kahle, J., & Zauke, G. P. (2003). Trace metals in Antarctic copepods from the Weddell Sea (Antarctica). *Chemosphere*, *51*, 409–417. doi:10.1016/S0045-6535(02)00855-X.

Koeberl, C. (1993). Instrumental neutron activation analysis of geochemical and cosmochemical samples: A fast and reliable method for small sample analysis. *Journal of Radioanalytical and Nuclear Chemistry*, *168*(1), 47–60. doi:10.1007/BF02040877.

Kovatch, C. E., Thomas Chandler, G., & Coull, B. C. (1999). Utility of a full life-cycle copepod. Bioassay approach for assessment of sediment-associated contaminant mixtures. *Marine Pollution Bulletin*, *38*(8), 692–701. doi:10.1016/S0025-326X(99)00029-6.

Landsberger, S., & Wu, D. (1995). The impact of heavy metals from environmental tobacco smoke on indoor air quality as determined by Compton suppression neutron activation analysis. *The Science of the Total Environment*, *173*, 323–337. doi:10.1016/0048-9697(95)04755-7.

Marchese, M., Gagneten, A. M., Parma, M. J., & Pavé, P. J. (2008). Accumulation and elimination of chromium by freshwater species exposed to spiked sediments. *Archives of Environmental Contamination and Toxicology*, *55*(4), 603–609. doi:10-1007/s00244-008 9139-0.

Miliou, H., Verriopoulos, G., Maroulis, D., Bouloukos, D., & Moraitou-Apostolopoulou, M. (2000). Influence of Life-History Adaptations on the Fidelity of Laboratory Bioassays for the Impact of Heavy Metals (Co^{2+} and Cr^{6+}) on Tolerance and Population Dynamics of *Tisbe holothuriae*. *Marine Pollution Bulletin*, *40*(4), 352–359. doi:10.1016/S0025-326X(99)00221-0.

Moraitou-Apostolopoulou, M. (1982). Acute toxicity of copper to a copepod. *Marine Pollution Bulletin*, *9*, 278–280. doi:10.1016/0025-326X(78)90612-4.

Mosulishvili, L. M., Belokobylsky, A. I., Khisanishvili, A. I., Frontasyeva, M. V., Kirkesali, E. I., & Aksenova, N. G. (2004). Application of epithermal Neutron Activation Analysis to Investigate accumulation and adsorption of Mercury by *Spirulina platensis* biomass. *Journal of Radioanalytical and Nuclear Chemistry*, *14*(35), 1–12.

Paggi, J. C. (2006). Redescription and re-evaluation of the taxonomic status of the neotropical copepod *Diaptomus falcifer* Daday, 1905 (Calanoida: Diaptomidae). *Studies on Neotropical Fauna and Environment*, *41*(1), 67–78. doi:10.1080/01650520500233687.

Rao, R. R., Holzbecher, J., & Chatt, A. (1995). Epithermal instrumental neutron activation analysis of biological reference materials for iodine. *Fresenius' Journal of Analytical Chemistry*, *352*(1–2), 53–57. doi:10.1007/BF00322296.

Ravera, O. (2001). Monitoring of the aquatic environment by species accumulator of pollutants: A review. *Journal of Limnology*, *60*(1), 63–78.

Turesson, E. U., Stiernstrom, S., Minten, J., & Adolfsson-Erici, M. (2007). Development and reproduction of the freshwater harpacticoid copepod *Attheyella crassa* for assessing sediment-associated toxicity. *Aquatic Toxicology (Amsterdam, Netherlands)*, *83*(3), 180–189. doi:10.1016/j.aquatox.2007.04.002.

Veado, M. A. R., Heeren, A. O., Severo, M. I., Grenier-Loustalot, M. F., Arantes, I. A., Cabaleiro, H. L., & Almeida, M. R. (2007). INAA and ICP-MSHS: Metal pollutants in fish tissues *Nile tilapia* (Oreochromic niloticus) in Pampulha Lake, Belo Horizonte city, Minas Gerais State, Brazil). *Journal of Radioanalytical and Nuclear Chemistry*, *272*(3), 511–514. doi:10.1007/s10967-007-0613-3.

Verriopoulos, G., & Moraitou-Apostolopoulou, M. (1982). Differentiation of the sensitivity to copper and cadmium in different life stages of a copepod. *Marine Pollution Bulletin*, *13*(4), 123–125. doi:10.1016/0025-326X(82)90368-X.

Versieck, J., Hoste, J., Barbier, F., & Michels, H. (1978). Determination of chromium and cobalt in human serum by neutron activation analysis. *Clinical Chemistry*, *24*(2), 303.

Wannaz, E. D., Harguinteguy, C. A., Jasan, R., Plá, R. R., & Pignata, M. L. (2008). Identification of atmospheric trace-element sources by passive biomonitoring employing PCA and variogram analysis. *International Journal of Environ-*

mental Analytical Chemistry, *88*(4), 229–243. doi:10.1080/03067310701727411.

Wang, W. X., & Fisher, N. S. (1998). Accumulation of trace elements in a marine copepod. *Limnology and Oceanography*, *43*, 273–283.

Wang, W. X., & Fisher, N. S. (1999). Delineating metal accumulation pathways for marine invertebrates. *The Science of the Total Environment*, *237–238*, 459–472. doi:10.1016/S0048-9697(99)00158-8.

Wood, C. M., McDonald, D. G., Ingersoll, C. G., Mount, D. R., Johannsson, O. E., Landsberger, S., et al. (1990). Effects of water acidity, calcium, and aluminum on whole body ions of brook trout (*Salvelinus fontinalis*) continuously exposed from fertilization to swim-up: A study by instrumental neutron activation analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, *47*(8), 1593–1603.

Zauke, G. P., & Schmalenbach, I. (2006). Heavy metals in zooplankton and decapod crustaceans from the Barents Sea. *The Science of the Total Environment*, *359*, 283–294. doi:10.1016/j.scitotenv.2005.09.002.

Zauke, G. P., Krause, M., & Weber, A. (1996). Trace metals in mesozooplankton of the North Sea: Concentrations in different taxa and preliminary results on bioaccumulation in copepod collectives (*Calanus finmarchicus/C. helgolandicus*). *International Review of Hydrobiology*, *81*, 141–160. doi:10.1002/iroh.19960810115.

Zheng, J., Goessler, W., Geiszinger, A., Kosmus, W., Chen, B., Zhuang, G., et al. (1997). Multi-element determination in earthworms with instrumental neutron activation analysis and inductively coupled plasma mass spectrometry: A comparison. *Journal of Radioanalytical and Nuclear Chemistry*, *223*(1–2), 149–155. doi:10.1007/BF02223376.