Lat. Am. J. Aquat. Res., 42(2): 381-385, 2014
"Proceedings of the 4to Brazilian Congress of Marine Biology"
Sergio O. Lourenço (Guest Editor)
DOI: 10.3856/vol42-issue2-fulltext-9

Short Communication

Influence of copper on *Euplotes* sp. and associated bacterial population

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ABSTRACT. The influence of copper on the ciliate *Euplotes* sp. and associated bacteria isolated from sediment samples of Guanabara Bay were investigated in bioassays. This region is highly affected by heavy metals such as copper, from solid waste constantly dumped in the bay and other sources such as industrial effluents, antifouling paints, atmospheric deposition and urban drainage, and even today there are few data on the metal toxicity to the ecosystem of the Bay of Guanabara. Bioassays were conducted to estimate the LC_{50}–24 h of copper, in order to determine the concentration of metal bearing 50% of the population mortality. The results indicated that the concentrations of 0.05 and 0.009 mg L^{-1} presented no toxicity to *Euplotes* sp. The associated bacteria are tolerant to copper concentrations used in bioassays, and suggest that they could be used as a potential agent in the bioremediation of areas affected by copper.

Keywords: *Euplotes* sp., bacteria, copper, heavy metals, Guanabara Bay, southeast Brazil.

Influencia del cobre sobre *Euplotes* sp. y la población de bacterias asociada

RESUMEN. La influencia del cobre sobre el ciliado *Euplotes* sp. y las bacterias asociadas aisladas de muestras de sedimentos de la bahía de Guanabara fueron investigados en bioensayos. Esta región está altamente afectada por metales pesados, como el cobre, a partir de residuos sólidos constantemente vertidos en la bahía y de otras fuentes, como los efluentes industriales, pinturas antiincrustantes, deposición atmosférica y drenaje urbano, y hasta hoy hay pocos datos sobre la toxicidad del metal para el ecosistema de la bahía de Guanabara. Se efectuaron bioensayos para estimar la CL_{50}-24 h de cobre para determinar la concentración de metales que lleva al 50% de la mortalidad de la población. Los resultados indicaron que las concentraciones de 0,05 y 0,009 mg L^{-1}, no representan toxicidad para *Euplotes* sp. Las bacterias asociadas son tolerantes a las concentraciones de cobre utilizadas en los bioensayos, lo que sugiere que podrían ser utilizadas como un agente potencial en la biorremediación de las zonas afectadas por el cobre.

Palabras clave: *Euplotes* sp., bacterias, cobre, metales pesados, bahía de Guanabara, sureste de Brasil.

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The microbial loop is mainly composed by bacteria, microflagelates and ciliates that have been studied primarily in oligotrophic environments. The main roles of microorganisms are recycling organic matter and, transforming dissolved organic matter (DOM) in particulate organic matter (POM) or vice versa (Paves & González, 2008). The DOM released by autotrophs is consumed by heterotrophic bacteria and transformed in growth. This DOM is rapidly converted into POM by bacteria, which is readily preyed by protists. So, nanoflagellates can control this process through pasture, converting the heterotrophic bacterial production in POM greater than 2.0 µm which is accessible to larger protists, as ciliates and dinoflagellates, and metazoans (Huppert *et al.*, 2004). Thus, protists such as ciliates and nanoflagellates act as an efficient link in the microbial loop and the classic food chain (Fenchel, 2008).

Pollution due to chemicals, including heavy metals, is a problem that may have negative consequences on the biosphere. Heavy metals as copper, particularly from industrial effluents, are constantly polluting our environments (Rehman *et al.*, 2006). These metals, depending on their concentration, may be considered harmful to exposed organisms, since they influence their growth processes, cell morphology, metabolism and function of plasma membrane and enzymes. Copper is considered a toxic element which may affect most of freshwater and marine invertebrates. However, it is also an essential micronutrient for bacterial metabolism, participating in energy production (Mayor *et al.*, 2013).

The ciliate *Euplotes* sp. is a dorsoventrally flattened organism placed in the Spirotrichea, with prominent ventral cirri and less conspicuous dorsal bristle cilia in the Order Hypotrichida (Lynn, 2007). The genus is broadly distributed as both symbionts and free-living forms that occupy a wide range of habitats from freshwater to brackish and marine, in sands and soils, and edaphic habitats. They are typically benthic substrate-oriented, and have also been recorded in tropical litoral habitats (Dragesco & Dragesco-Kernéis, 1986). Presently, we have few data about the copper effects on *Euplotes* sp. and the associated microbiota in eutrophic environments. This paper aims to study the influence of copper on *Euplotes* sp. and associated population of bacteria isolated from sediment samples in Guanabara Bay.

Study area and sampling

Guanabara Bay is a 384 km^2 eutrophic coastal bay in the southeast of Brazil, which receives domestic untreated sewage from at least 10 million people, most of the discharge occurring directly into the bay (Kjerfve *et al.*, 1997). Samples of *Euplotes* sp. were collected in sediment close to Ilha do Fundão (22°51'31"S, 43°13'14"W), in Guanabara Bay, and maintained in the laboratory to run toxicity tests in liquid medium containing natural seawater and rice grains as the carbon source (Dragesco & Dragesco-Kernéis, 1986). This culture medium was also used for maintenance and growth of associated bacterial microbiota.

Heavy metal exposure analysis

For the bioassay, 15 batches of 30 specimens of *Euplotes* sp. were prior maintained for 72 h in Petri dishes, with sterile seawater, without the addition of carbon source to reduce their microbiota and without food supply. After this period, healthy and motile free living individuals of *Euplotes* sp. were transferred to wells of in a sterile 24-well polystyrene plate. Each well was filled with 2.0 mL of seawater. The different treatments were prepared adding 0.001, 0.009, 0.05, 0.01 and 1 mg L^{-1} of a copper solution, which was taken from a stock solution of 1.0 M $CuSO_4$. No copper was added to the control, which had seawater only. The bioassay occurred in triplicate with 30 *Euplotes* sp. in each well, and the plates were incubated at 25ºC for 24 h, without supplementary nutrients.

To determine the LC_{50} three replicates were performed. Previously, 1.0 mL of each well was taken and 0.05 mL Bouin's fluid was added to fix cells (Foissner, 1991). Fixed and non damaged specimens of *Euplotes* sp. were quantified using a Sedgwick-Rafter counting chamber at 0 and 24 h, performed according to the standard CETESB (2005). The bacterial carbon was quantified in 1.0 mL of each well under an epifluorescent microscope, according to Kepner & Pratt (1994), and Carlucci *et al.* (1986).

Soluble metal concentration was measured using an inductive coupled plasma emission spectrometer (ICP-AES) sequential-multichannel Varian Ultra Mass, calibrated by direct comparison with standard solution of copper, considered as certified reference materials (Rauret *et al.*, 1999). The survival data was treated by Probit analysis in order to estimate LC_{50}-24 h of copper for *Euplotes* sp. (Diaz *et al.*, 2006).

After 24 h, the growth of *Euplotes* sp. occurred at 0.05 and 0.009 mg L^{-1} of copper, and had no growth with 0.1 and 1.0 mg L^{-1}of copper (Fig. 1a). The control group showed a slight decrease of *Euplotes* sp., with an increase in carbon of associated bacteria. The bacterial carbon ranged between 1.0x10^{-4} and 4.5x10^{-4} µg C cm^{-3} (Fig. 1b). The increase of *Euplotes* sp. number at 0.009 and 0.05 mg L^{-1} of copper favored the reduction of associated bacteria and there was no increase of bacterial carbon in presence of 0.1 and 1.0 mg L^{-1} of copper. The estimated rate of the Probit LC_{50} was 0.04 mg L^{-1} to the *Euplotes* sp. cells counting.

In the control group, predator and prey functioned as expected in a microbial loop (Fenchel, 2008). Despite the increasing number of *Euplotes* sp. in the concentrations of 0.009 and 0.05 mg Cu L^{-1}, bacterial carbon also increased, since the associated microbiota may use defense mechanisms under stressed environments, thus explaining the increase of the bacterial population in this case (Matz & Kjelleberg, 2005). Bacterial self-defense includes production of biofilm and changes in size proportions.

Biofilms enhance the bacterial proportions in a way that the aggregates may be larger than the *Euplotes* peristome. This mechanism/strategy protects bacteria from predators and enables them to increase their biomass (Matz & Kjelleberg, 2005). At concentrations of 0.1 and 1.0 mg L^{-1} of copper, the associated bacteria showed no increase of carbon, even in the absence of grazing and nutritional supplements. Therefore, it can be inferred that the microbiota associated with *Euplotes* sp. may be

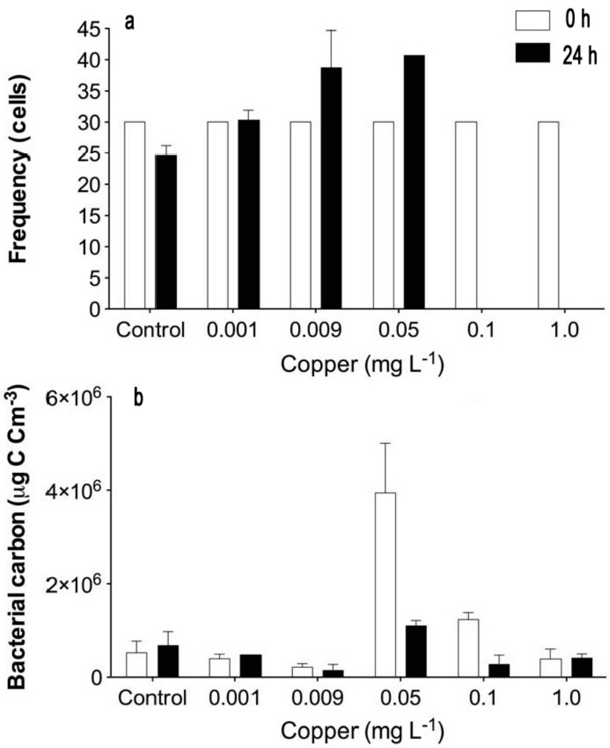

Figure 1. a) Number of individuals of *Euplotes* sp. under different Cu concentrations at the beginning (0 h) and the end (24 h) of the experiment, and b) bacterial carbon variation in function of different Cu concentrations at beginning 0 h and end 24 h of the experiment. Error bars denote standard deviation.

tolerant to the higher concentrations of copper used in bioassays.

According to Harrison *et al.* (2007), the ability of microorganisms to survive but not grow in the presence of metals, metalloids cations or oxyanions, either alone or in combination, defines tolerance. Bacteria may be able to grow vigorously and rapidly in the medium with copper (Okafor, 2007), using nitrogenous excreta from protists (Kirchman, 2000; Hahn & Höfle, 2001). This ability enhances the relative proportion of bacterial biomass and it influences the metabolic activity. The increased biomass improves the enzymatic contact and exchanges with the medium in a more intense way, making bacteria prone to absorb Cu quickly. Bacteria tend to invest in the maintenance of their structures to face a stressful environment. As a consequence, they increase their metabolism, but maintain their biomass (Harrison *et al.*, 2007).

After the addition of copper at concentrations of 0.05 and 0.009 mg L^{-1} a cell division occurred in *Euplotes* sp., since it seems to act as a micronutrient in lower concentrations, possibly contributing to maintain the predator-prey relationship. However, in higher concentrations, toxic effects may have occurred in *Euplotes* sp. The estimate LC_{50} Probit test indicates that the concentration would lead to a fatality of 50% of the population would be 0.04 mg L^{-1}. This concentration was below that stipulated by the National Council of the Environment to saline waters which may be intended for primary contact recreation, as CONAMA Resolution 274/2000 (CONAMA, 2011), 1.0 mg Cu L^{-1} for marine environments.

Analyzing other studies, we observed that the tolerated concentrations of metals depend on the study site and species examined. For example, throughout 1991-1992, 29 ciliate species have been isolated from an activated-sludge plant in Manchester, U.K., and showed resistance to 60 ppb of copper (Abraham *et al.*, 1997). In bioassays performed with heavy metals, the population of *Colpoda steinii*, a soil ciliated, was reduced by 50% in the presence of 0.10, 0.22, 0.25, and 0.85 mg L^{-1} of Ni, Cd, Cu and Zn respectively (Forge *et al.*, 1993). The copper concentration 1.58 mg L^{-1} inhibited 50% growth of the *Euplotes crassus* population in bioassays during 48 h, as well the resistance to *Euplotes vannus* with 0.2 mg L^{-1} (Kim *et al.*, 2011). According to Rehman *et al.* (2006), protozoa, specifically *Euplotes mutabilis* and *Tachysoma pellionella* used in media containing copper, lead, mercury and chromium were resistant to small amounts added daily (1.0 μg mL^{-1}). These results compared to our bioassays suggest that *Euplotes* sp. is resistant to 0.001 to 0.05 mg L^{-1} copper, because the ciliates continued to grow in the presence of the metal (Harrison *et al.*, 2007).

In the same way that copper is an important factor in the cells, it may also be harmful, promoting various deleterious effects such as changes in membrane due to depolarization and damage on receptor or carriers molecules, functional damage by their binding to macromolecules such as DNA and enzymes, forming protein damage or oxidative alterations of DNA and thereby causing multiple functional alterations (Bremner, 1998). In addition, it may cause cell damage due to the production of free radicals by Fenton reaction leading to a loss of cell integrity, decreased ATP synthesis, oxidation and lipid peroxidation, DNA damage and damage to vital organelles such as mitochondria and lysosomes (Bremner, 1998). A major concern is that environments such as Guanabara Bay are being threatened by the constant release of effluents containing heavy metals. In some sampling sites of Guanabara Bay concentrations of copper as high as 60 mg kg^{-1} have already been recorded, which undermine the self-sustaining of the ecosystem (Fonseca *et al.*, 2013). According to Meyer-Reil & Köster (2000), when a eutrophic ecosystem suffers a disturbance, it goes through a period of resilience that is the time (speed) that an ecosystem needs after disturbance to return to the initial status. However, in the case of the Guanabara Bay and other ecosystems, the resilience capacity seems to decrease in response to long-term disturbances, which can lead to an impairment of the natural environmental self-sustaining.

CONCLUSIONS

The Cu concentrations of 0.05 and 0.009 mg L^{-1} were not harmful to *Euplotes* sp., and according to Harrison *et al.* (2007) they might also confer resistance to copper, *i.e.*, the ability of a microorganism to continue growing in the presence of metals like copper. However, at concentrations higher than 0.05 mg L^{-1} this metal becomes toxic to *Euplotes* sp. The associated bacteria are tolerant to all copper concentrations used in bioassays, but keeping the bacterial carbon constant in the concentrations tested for the bacterial population of the Guanabara Bay sediments.

ACKNOWLEDGEMENTS

The authors acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support of the study.

REFERENCES

Abraham, J.V., R.D. Butler & D.C. Sigee. 1997. Ciliate populations and metals in an activated-sludge plant. Water Res., 31(5): 1103-1111.

Bremner, I. 1998. Manifestations of copper excess. Am. J. Clin. Nutr., 67: 1069S-1073S.

Carlucci, A.F., D.B. Craven, D.J. Robertson & P.M. Williams. 1986. Surface-film microbial populations: diel amino acid metabolism, carbon utilization, and growth rates. Mar Biol., 92: 289-297.

Companhia Estadual de Tecnologia de Saneamento Ambiental (CETESB). 2005. Fitoplâncton de água doce: métodos qualitativo e quantitativo (método de ensaio). Norma Técnica L5. 303. CETESB, São Paulo, 24 pp.

Conselho Nacional do Meio Ambiente (CONAMA). 2011. Resolução CONAMA Nº430. [www.mma. conama.gov.br/conama]. Reviewed: 10 December 2013.

Díaz, S., A. Martín-González & J. Carlos Gutiérrez. 2006. Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. Environ. Int., 32: 711-717.

Dragesco, J. & A. Dragesco-Kernéis. 1986. Ciliés libres de l`Afrique intertropicale. Introduction à connaissance et à l'etude des ciliés. Faune Trop., 26: 1-559.

Fenchel, T. 2008. The microbial loop-25 years later. J. Exp. Mar. Biol. Ecol., 366(1-2): 99-103.

Foissner, W. 1991. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Eur. J. Protist., 27(4): 313-330.

Fonseca, E.M., J.A. Baptista-Neto, C.G. Silva, J.J. McAlister, B.J. Smith & M.A. Fernandez. 2013. Storm water impact in Guanabara Bay (Rio de Janeiro): evidences of seasonal variability in the dynamic of the sediment heavy metals. Estuar. Coast. Shelf Sci., 130: 161-168.

Forge, T.A., M.L. Berrow, J.F. Darbyshire & A. Warren. 1993. Protozoan bioassays of soil amended with sewage sludge and heavy metals, using the common soil ciliate *Colpoda steinii*. Biol. Fert. Soils, 16(4): 282-286.

Hahn, M.W. & M.G. Höfle. 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol., 35: 113-121.

Harrison, J.J., C. Howard & R.J. Turner. 2007. Multimetal resistance and tolerance in microbial biofilms. Nat. Rev. Microbiol., 5(12): 928-938.

Huppert, A., R. Olinky & L. Stone. 2004. Bottom-up excitable models of phytoplankton blooms. B. Math. Biol., 66(4): 865-878.

Kepner, R.L. & J.R. Pratt. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental-samples: past and present. Microbiol. Rev., 58(4): 603-615.

Kim, S.-H., M.-Y. Jung & Y.-M. Lee. 2011. Effect of heavy metals on the antioxidant enzymes in the marine ciliate *Euplotes crassus.* Toxicol. Environ. Health Sci., 3(4): 213-219.

Kirchman, D.L. 2000. Microbial ecology of the oceans. John Wiley & Sons, New York, 552 pp.

Kjerfve, B., C.H.A. Ribeiro, G.T.M. Dias, A.M. Filippo & V.S. Quaresma. 1997. Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. Cont. Shelf Res., 17: 1609-1643.

Lynn, D.H. 2007. The ciliated Protozoa-Characterization, classification and guide to the literature. Springer, Ontario, 605 pp.

Matz, C. & S. Kjelleberg. 2005. Off the hook-how bacteria survive protozoan grazing. Trends Microbiol., 13(7): 302-307.

Mayor, D.J., N.B. Gray, J. Elver-Evans, A.J. Midwood & B. Thornton. 2013. Metal-macrofauna interactions determine microbial community structure and function in copper contaminated sediments. PLoS One, 8(5): e64940.

Meyer-Reil, L.A. & M. Köster. 2000. Eutrophication of marine waters: effects on benthic microbial communities. Mar. Pollut. Bull., 41: 1-6.

Okafor, N. 2007. Modern industrial microbiology and biotechnology. Science Publishers, New Hampshire, 530 pp.

Pavés, H.J. & H.E. González. 2008. Carbon fluxes within the pelagic food web in the coastal area off Antofagasta (23ºS), Chile: the significance of the microbial *versus* classical food webs. Ecol. Model., 212(3-4): 218-232.

Received: 22 May 2013; Accepted: 23 December 2013

Rauret, G., J.F. López-Sánchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure & P.H. Quevauviller. 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit., 1: 57-61.

Rehman, A., F.R. Shakoori & A.R. Shakoori. 2006. Heavy metal resistant ciliate, *Euplotes mutabilis*, isolated from industrial effluents can decontaminate wastewater of heavy metals. Bull. Environ. Contam. Toxicol., 76: 907-913.