Chemosphere 73 (2008) 365–370

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Discriminating the pH toxicity to *Poecilia reticulata* Peters, 1859 in the Dunas Lake (Camaçari, BA, Brazil)

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ARTICLE INFO

Article history:
Received 26 September 2007
Received in revised form 28 April 2008
Accepted 27 May 2008
Available online 15 July 2008

Keywords:
Acidification
Acute Toxicity
Bioassays
Biomonitoring
Survival time

ABSTRACT

Toxic potential of the pH reduction to fingerlings of *Poecilia reticulata*, through acute toxicity bioassays, as well as the influence of increased pH on the toxicity were assessed. Acid lake samples (Dunas Lake) were collected during 19 months, and assessed with following treatments: water at local pH (±3.0) and samples with modified pH to 3.5, 3.8, 4.0, 4.3, 4.6, 5.0, 5.5, 6.0, and 6.5. Culture water samples with pH reduced to 3.0 were also assessed. Newborn *P. reticulata* were exposed during 96 h, and dead/immobile organisms were counted at various time intervals during exposure (short intervals in the beginning and long towards the end). Mean results of LT_{50} and confidence intervals from the Dunas Lake and control water with reduced pH were 1.36 (±0.48) h, and 1.03 (±0.50) h, respectively, with no statistical difference. Samples with increased pH showed a significant reduction in toxicity, with no toxicity detected at pH 6.0 and higher. Relationship between pH and lethal toxicity for fingerlings of *P. reticulata* demonstrated that pH exerted a strong effect on the survival of this species at the Dunas Lake, explaining about 80% of the toxicity observed.

1. Introduction

The knowledge about ecosystem acidification goes back to the mid-18th century (Gorham, 1998), but, over the past 30 years, scientists have been working to understand the causes and results of the acidification process, and how acidic deposition might influence different ecosystems (Driscoll et al., 2003). Acidification is one of the main causes of environmental degradation in aquatic ecosystems in the temperate regions, not only due to its toxicity, but also due to its effect on the speciation, mobility, and bioavailability of other toxicants (Lopes et al., 1999). For instance, many regions are severely impacted by acidic rain, acid mine drainage, volcanic activities or by other types of anthropogenic activities (Geller et al., 1998; Ribeiro et al., 2002), resulting in decreasing pH values, followed by increasing metal solubility (Geller et al., 1998). As a whole, acidification in the Brazilian ecosystems is little documented, although the impact caused on the biodiversity is very similar to other regions (Jesus, 1996). Rodhe et al. (1988) identified schematically areas in which acidification might represent a potential threat on the basis of expected emissions, population density, and soil sensitivity for south-eastern Brazil. More recently, Moreira-Nordemann et al. (1988); Ometto et al. (2005) and Martinelli et al. (2006) pointed out the risks involved in sugar-cane burning to the acidification of the same region. An acidified lake, and its ecotoxicity history, after rehabilitation, has been described by da Silva et al. (1999a, 2000).

The toxicity of the trace elements, especially metals, does not only depend on their concentration, but also on their bioavailability, which is higher under reduced pH conditions (Wren and Stephenson, 1991; Renoux et al., 2001). Therefore, the degradation of fish populations is explained primarily by the direct combined influence of low pH and aluminum ions, which causes biochemical and physiological disturbances (Moiseenko and Sharova, 2006). Besides, pH influences the ionic regulation of aquatic species, the rate of organic matter decomposition, and primary production (Abel, 1996; Geller et al., 1998). According to Driscoll et al. (2003), few fish species can survive in pH lower than 4.5. Indeed, most of the acidified waters throughout the world are fish-free environments (Van Sickle et al., 1996; Nixdorf et al., 1998); however some small South American fish can be found in acidic blackwater rivers (such as Rio Negro, Amazon, Brazil) whose pH can be around 3.5 (Mounier et al., 1999; Matsuo and Val, 2002; Aride et al., 2007). Gonzalez et al. (1998) suggested that exceptional acid tolerance is a characteristic of fish that inhabit acidic ecosystem. A tool adopted for preliminary environmental evaluation of acidification effects in aquatic ecosystems is the use of survival time as an endpoint, allowing the establishment of the species recolonization potential (Ribeiro et al., 2002). It is reasonable to suppose that, increasing

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0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.05.061

pH values should improve water quality of acidic systems, leading to previous or acceptable ecological conditions, and to reestablishment of previous food webs, therefore it is extremely relevant to assess biological and chemical conditions of acidified ecosystems (Tipping et al., 2002; Driscoll et al., 2003).

Poecilia reticulata (guppy) is a tropical species, very abundant in shallow waters of canals, rivers, lagoons and reservoirs in South America, and largely employed in ecotoxicological tests. The results of several studies showed that *P. reticulata* is a very sensitive species when exposed to toxicants (Gallo et al., 1995; Miliou et al., 1998; Polat et al., 2002; Yilmaz et al., 2004), and, therefore, adequate for biomonitoring programs (Widianarko et al., 2000) and is recommended as a standard ecotoxicological test organism (OECD, 1992; ABNT, 2002). The sensitivity of *P. reticulata* in comparison to other fish species, as *Brachydanio rerio* (zebra fish), *Cyprinus carpio* (common carp), *Lepomis macrochirus* (bluegill), *Pimephales promelas* (fathead minnow), *Salmo gairdneri* (rainbow trout), was studied by Vittozzi and De Angelis (1991). Additionally, *P. reticulata* has been used with success in the biomonitoring of an acidic lake for several years (da Silva et al., 1999a), including in *in situ* bioassays (Araújo et al., 2006). This species has been chosen as a test organism for the Dunas Lake survey, because it was a common species in the lake prior to the contamination episode.

According to da Silva et al. (2000), in the late 1980s large quantities (*ca*. 34 t) of both industrial and domestic solid waste including sulphur, iron, titanium dioxide and ilmenite residues were deposited on the dunes adjacent to a small lake (Dunas Lake, Camaçari, BA, Brazil). These wastes were leached out by rainwater percolating through the dunes, thus contaminating the ground- and surface-water. There was a decrease in pH to 1.8 of the ground- and surface-water and an increase in the concentrations of dissolved iron and sulphate, causing the precipitation of humic acids, leading to highly transparent waters with a concurrent disruption of the biological communities in the lake (da Silva et al., 1999b, 2000). It was not investigated if the toxicity of the water of the Dunas Lake was due to the direct effect of low pH value, about 3.0, or due to the ability of low pH to increase the bioavailability of toxic metals. The methodologies to discriminate a toxic component using serial dilutions or chelating agents can mask the ecotoxicity and alter the characteristics of the samples (Lopes et al., 1999; Ribeiro et al., 2002). In this context, this work aimed to discriminate, through bioassays with altered pH, the potential toxicity of the acidity to *P. reticulata*; identifying possible reduction in the toxicity due to increased pH; and to determine threshold survival for *P. reticulata* in relation to Dunas Lake pH.

2. Materials and methods

2.1. Study site

The acidic lake (Dunas Lake) is located in Camaçari (BA, Brazil) (Fig. 1) between geographic coordinates 12°48′09″ to 12°48′12.3″ S and 38°13′09″ to 38°13′14″ W, lying within a depression, forming a narrow and shallow body of freshwater between dunes along the Atlantic Ocean (da Silva et al., 2000). After contamination, a rehabilitation program was carried out (1992–1993) to recover ground- and surface water quality and reduce contamination (Gomes, 1994; da Silva et al., 1999b). Initially, the residues were partially removed and the contaminated dune was sealed with impermeable layers of clay and topsoil (hydraulically encapsulated). An additional action was to pump the groundwater to reduce the contaminated plume (Gomes, 1994; da Silva et al., 1999b, 2000). The results of the established biomonitoring program, including bioassays with guppy fingerlings (*P. reticulata*), were described by da Silva et al. (1999a, 2000) and Araújo et al. (2006).

2.2. Sampling

Monthly samplings ($n = 19$) of the Dunas Lake were carried out from March 2003 to November 2004, except for August and October 2004. Samples were transported to the laboratory and kept at 4.0 ± 1.0 °C until the next day, when the pH was measured. Immediately the samples were distributed in 5 l beakers to alter the pH. Samples were treated with NaOH 1 M to raise their pH, with the following treatments: original Dunas Lake pH (pH not altered – about 3.0), 3.5, 3.8, 4.0, 4.3, 4.6, 5.0, 5.5, 6.0, and 6.5. These treatments aimed to assess the toxicity reduction after increasing pH. To assess the toxic effect of pH, the control water pH (i.e. dechlorinated tap water) was reduced to the same pH value of the Dunas Lake, using H_2SO_4 1 M, because this was the main acid in the acidification process (da Silva et al., 1999b). A possible difference between the Dunas Lake water *in natura* and the control sample with reduced pH may be attributed to other factors potentially toxic present in the Dunas Lake that could interfere in the toxicity which may be influenced by pH.

2.3. Physical–chemical analysis

Dissolved oxygen content (WTW, Inolab Oxi Level 2), water hardness (APHA, 1998), pH (Digi-Sense, Cole Parmer) and conductivity (Hanna HI 9033) of the samples were evaluated at the beginning

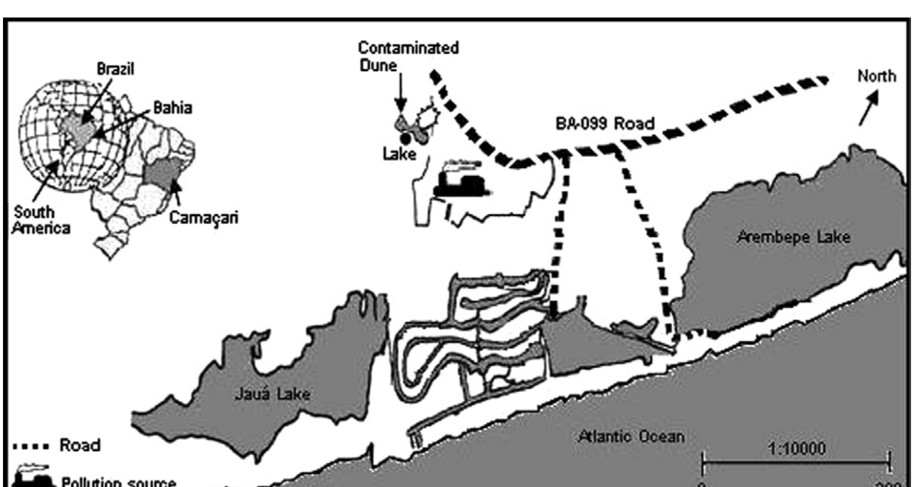

Fig. 1. Location of the study site.