Ecotoxicology and Environmental Safety 102 (2014) 42–47

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Potential environmental toxicity from hemodialysis effluent

Carla Keite Machado [a], Luciano Henrique Pinto [b], Lineu Fernando Del Ciampo [c], Luciano Lorenzi [a], Cláudia Hack Gumz Correia [d], Donat Peter Häder [e], Gilmar Sidnei Erzinger [f,*]

[a] Department of Biology, Rua Paulo Malschitzki, 10, Campus—Industrial Zone, PO Box 246, CEP 89219-710, Joinville, SC, Brazil
[b] Department of Pharmacy, Rua Paulo Malschitzki, 10, Campus—Industrial Zone, PO Box 246, CEP 89219-710, Joinville, SC, Brazil
[c] Inovaparq, Rua Paulo Malschitzki, 10, Campus—Industrial Zone, PO Box 246, CEP 89219-710, Joinville, SC, Brazil
[d] Laboratory of Ecotoxicology, Rua Paulo Malschitzki, 10, Campus—Industrial Zone, PO Box 246, CEP 89219-710, Joinville, SC, Brazil
[e] Neue Str. 9., 91096. Möhrendorf, Germany
[f] Department of Medicine and Pharmacy, Master's and PhD Program in Health and Environment, Rua Paulo Malschitzki, 10, Campus—Industrial Zone, PO Box 246, CEP 89219-710, Joinville, SC, Brazil

ARTICLE INFO

Article history:
Received 27 October 2013
Received in revised form
6 January 2014
Accepted 8 January 2014
Available online 1 February 2014

Keywords:
Bioassay
Hemodialysis effluent
Daphnia magna
Euglena gracilis
Environmental toxicity

ABSTRACT

Understanding the toxicity of certain potentially toxic compounds on various aquatic organisms allows to assess the impact that these pollutants on the aquatic biota. One source of pollution is the wastewater from hemodialysis. The process of sewage treatment is inefficient in inhibition and removal of pathogenic bacteria resistant to antibiotics in this wastewater. In many countries, such as Brazil, during emergencies, sewage and effluents from hospitals are often dumped directly into waterways without any previous treatment. The objective of this study was to characterize the effluents generated by hemodialysis and to assess the degree of acute and chronic environmental toxicity. The effluents of hemodialysis showed high concentrations of nitrites, phosphates, sulfates, ammonia, and total nitrogen, as well as elevated conductivity, turbidity, salinity, biochemical and chemical oxygen demand, exceeding the thresholds defined in the CONAMA Resolution 430. The samples showed acute toxicity to the green flagellate *Euglena gracilis* affecting different physiological parameters used as endpoints in an automatic bioassay such as motility, precision of gravitational orientation (*r-value*), compactness, upward movement, and alignment, with mean EC_{50} values of recalculate as 76.90 percent ($\pm$4.68 percent) of the undiluted effluents. In tests with *Daphnia magna*, the acute toxicity EC_{50} was 86.91 percent ($\pm$0.39 percent) and a NOEC value of 72.97 percent and a LEOC value 94.66 percent.

1. Introduction

Tarrass et al. (2010) reported that water is becoming a dwindling natural resource due to global warming, climate change, and frequent droughts; in fact, it is too valuable to be wasted. Hemodialysis uses large volumes of water. In a patient undergoing dialysis treatment three times per week for 4 h, about 18,000 L of dialysis fluid are used. Up to 25 percent of the water used for the treatment are discarded as waste.

As our planet's population continues to grow the number of dialysis patients increases. The annual increase is expected to be 6 percent, which will result in approximately 4 million patients by 2025. As the number of dialysis patients continues to grow, the amount of natural resources consumed and wastewater produced by dialysis facilities increases accordingly (Connor et al., 2010).

In the United States, 5000 dialysis clinics (which represent 26 percent of the global dialysis market) perform over 50 million dialysis treatments per year, consuming over 5 trillion liters of freshwater, based on its 325,000 patients in 2007. In Australia, an estimated 400 million liters or 400 olympic-sized swimming pools of water are used each year (Tarrass et al., 2010). In Brazil, in 2010, more than 105,000 patients were on hemodialysis, consuming more than 17 million liters of freshwater per year at hemodialysis facilities. Therefore, due to this huge water consumption, dialysis centers should focus on water conservation (Machado, 2013).

Agar (2012) conducted an extrapolation of data for the dialysis population currently estimated at ~2 million patients worldwide and concluded that a "world dialysis service" uses ~156 billion liters of water and discard around two thirds of that during reverse osmosis and one third at the end of the hemodialysis process.

Wastewater generated by hemodialysis may have a significant impact on the environment due to its high conductivity and salinity. However, the risk resulting from its discharge into bodies

* Corresponding author.
E-mail addresses: carla.keite@hotmail.com (C.K. Machado), lucianohp.pq@gmail.com (L.H. Pinto), lineudc@msn.com (L.F.D. Ciampo), llorenzi@univille.net (L. Lorenzi), clau.hgc@gmail.com (C.H.G. Correia), donat@dphaeder.de (D.P. Häder), gerzinger@univile.br, gerzinger47@gmail.com (G.S. Erzinger).

0147-6513/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2014.01.009

of water remains under-explored. It is evident that pollution can be reduced substantially through recycling of water. Moreover, limiting discharge can indirectly help in maintaining water quality (Tarrass et al., 2008, 2010; Tarrass and Benjelloun, 2010).

In addition to the discharge of mineral salts into the water, the lack of sufficient sewage treatment poses another risk, since microorganisms such as pathogenic bacteria, that are resistant to antibiotics, are released to the environment. In many countries, such as Brazil, during emergencies, sewage and effluents of hospitals are often discharged without any treatment (Emmanuel et al., 2005).

In 2008, in Brazil only 28.5 percent of the municipalities had wastewater treatment, and in the State of Santa Catarina, only 16.7 percent of the municipalities treated wastewater (IBGE, 2010). Given these facts, it is necessary to assess the potential environmental toxicology of effluents produced during hemodialysis. In order to quantify the toxicity of the pollutants several short-term and long-term test were conducted with two classical bioassays using the green flagellate *Euglena gracilis* and the cladoceran *Daphnia magna*.

2. Material and methods

2.1. Sample collection

Out of the four dialysis centers in Joinville, Brazil, one was chosen at random. Samples were collected on four different days in the period from August 2012 to January 2013. Samples were collected 01 L using a peristaltic pump connected to the output of the dialysis machine. In total, samples were collected simultaneously from 14 hemodialysis machines in parallel, corresponding to 14 different patients.

2.2. Sample characterization

Chemical analyses were performed by colorimetric methods, using a Smart 3 instrument (Poly Control Analytical Instruments, ISO 9001 certified, in accordance with the U.S. Environmental Protection Agency), measuring nitrite, nitrate, phosphate, silica, and sulfate. Chemical oxygen demand (COD) was analyzed according to the methodology described in Standard Methods (1998) by spectrophotometry (HACH Instruments, Model DR 4000 and biochemical oxygen demand BOD 5.20) was quantified according to standard methods.

2.3. Culture of Euglena gracilis

Tests were conducted with the F1 strain of *E. gracilis*, obtained from the collection of the Friedrich-Alexander University, Erlangen, Germany. The cultures were grown in mineral medium (Checcucci et al., 1976) in an incubator under fluorescence light at an irradiance of 20 W m^{-2} for 12 h and 12 h darkness, at a temperature of 18 °C.

2.4. Motility and orientation analysis of Euglena gracilis

For the experiments performed with *E. gracilis* we used the New Generation Ecotox (NGTOX) (Erzinger et al., 2010). This equipment is an evolution of an instrument called ECOTOX developed by Tahedl and Häder (2001). It is an automated bioassay in which a peristaltic pump driven by a stepper motor, transfers a cell suspension of the flagellate *E. gracilis* to an observation chamber after being automatically mixed with the pollutant. The images of the moving cells are detected and recorded in infrared by a CCD (charge-coupled device) camera connected to a microscope. The video images are displayed on a computer monitor. The software ImagingTox (Ciampo et al., 2012) determines the motion parameters and analyzes motility (percentage of motile cells), precision of gravitational orientation (*r*-value), swimming velocity and shape of the cells, and stores all this information in a database. Five different toxin concentrations produced in automatic serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32) were evaluated in sequence.

The system operates in real time and tracks a virtually unlimited number of cells in parallel. The software uses the vectors of the tracks to calculate various parameters. The motility parameter gives the percentage of cells moving at a velocity equal to or faster than the minimum velocity set in the program. The parameter velocity gives the swimming speed of the cells in $\mu m\,s^{-1}$. The cell compactness (form factor) describes the shape of the cell and has the lowest value of 1 when the cell has an absolutely round shape and increases as the cell increases in length. The parameter "upward" gives the percentage of cells that are moving upward ($\pm 90^{\circ}$ around the vertical direction). The *r*-value is a statistical parameter that describes the precision of gravitactic orientation of the cells and ranges from 0 (when the cells are moving in random directions) to 1 (when all the cells are moving in a the same direction). For hardware and more details about ECOTOX see Tahedl and Häder (1999).

The filling time of the observation chamber was set to 100 s and the rinsing time was 45 s. The duration of all tracking was 3 min. The minimum area for objects to be included in the vector analysis was set to 400 μm^2 and the maximum area to 2000 μm^2. Minimum speed at which the cells were considered motile was set to 15 $\mu m\,s^{-1}$. In order to avoid any effects of light (which could induce e.g. phototaxis), the cells of *E. gracilis* were incubated in darkness for 30 min before making measurements and the recordings were done using an infrared monitoring light.

2.4.1. Daphnia magna

The cultivation of the cladoceran *D. magna* was performed according to ISO 6342 (2012). Containers with a capacity of 500 mL culture medium (M4 medium) were used for growth of the organisms. Organisms were fed daily with an algal culture of *Scenedesmus subspicatus*, grown according to ISO 8692 (2012).

The methodology for acute (short-term) tests with the test organism *D. magna* followed the standard NBR 12713 protocol (ABNT, 2003; EPA, 2002). The samples collected were tested based on the exposure of neonates of *D. magna*, 2–26 h old, in dilutions of the sample for a period of 48 h (Flohr et al., 2005).

The chronic (long-term) toxicity tests were performed in accordance with ISO-10706 (2000) with modifications according to Bianchini and Wood, 2002, and Knops et al. (2001) for 21 days.

2.5. Measurement procedures

The size of the female Daphnias was estimated at the beginning and at the end of the test to evaluate the relationship between EL and BL (see below), using a stereomicroscope (Nikon SMZ 1500) with magnifications of 32× and 57×. The precision of the measurements was 15 μm for 32×, and 8.5 μm for 57×. BL is defined as the distance from the top of the head until the base of the carapace spine, and EL as the distance on the central axis from the base to the top of the first exopodite of the second antenna (Pereira et al., 2004). Measurements were made in a total of 350 animals. Somatic growth of females was calculated using Eq. (1):

$$g = \frac{\ln(l_f) - \ln(l_o)}{\Delta t(\text{days}^{-1})} \quad (1)$$

where l_f is the length of the body (mm), l_o the initial length (mm), and Δt the time interval (days).

2.6. Statistical analysis

Statistical analysis of data was performed by repeated measures one-way ANOVA. The significance level was set at 5 percent and 95 percent confidence. Statistical analysis was performed using SPSS v14.0 statistical software (SPSS, Chicago, IL).

To determine the lethal concentration for *E. gracilis*, the EC_{50} was used in Eq. (2) interpreting the experimental data (Tahedl and Häder, 1999):

$$y = \frac{y_0}{1 + (c/EC_{50})^b} \quad (2)$$

where *y* is the response variable (percentage of dead organisms), *c* is the concentration of the substance, y_0 is the response when the concentration tends to infinity, and *b* is a scaling factor.

The data were processed using the program SigmaPlot v12 (Systat Software Inc). This model corresponds to Eq. (3), proposed by Emmens (Tahedl and Häder, 1999) to interpret the concentration-effect relationships:

$$SS = \sum_{i=1}^{n} (y_i - \bar{y}_i)^2 \quad (3)$$

The software calculates the values of a nonlinear regression. The program Sigma-Plot was used to determine the sigmoidal curve, using the Levenberg-Marquardt algorithm (Eq. (2)) and to calculate the parameters of the independent variables that give the best fit between the equation and the data. This algorithm determines the parameters iteratively so that the sum of the squared differences between the observed values and predicted values of the dependent variable are minimized. EC_{50}, *b* and y_0 (see Eq. (3)) are optimized. The confidence intervals of the set of optimized parameters are calculated from the covariance matrix with an error level of 5 percent.

To determine the values of 48-h EC_{50} in *Daphnia* the statistical Probit Method (Weber, 1993) was used for parametric data and the Trimmed Spearman–Karber Method (Hamilton et al., 1977) for nonparametric data.

For the determination of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for different treatments were compared by the Kruskal–Wallis nonparametric test. When significant differences were found, the Mann–Whitney *U* test was used, with the significance level set for the