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Acute toxicity from a prototype equipment of water separation through solar distillation on *Daphnia magna* Straus, 1820

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Abstract

The use of sustainable forms of energy should be encouraged, considering the damages that usual sources of energy can cause to our planet, which are often irreversible for decades or centuries. Despite the sun being the main source of energy on Earth, in tropical countries like Brazil, this kind of energy could be better exploited. The present work aims to evaluate the toxicity of purified water and a solution of $CuSO_4$, after decontamination in a prototype equipment of solar distillation of wastewater (due to separation of condensed water from non-volatile substances), in order to test the efficiency of the equipment. Thus, acute toxicity tests were performed with *Daphnia magna*. The EC_{50} – 48 h values (the median concentration of a substance that causes effect to fifty percent of organisms) for deionized water and solution of 1 mg L^{-1} of $CuSO_4$, both after distillation, were 19.61% and 17.08%, respectively. Results indicates that the final samples from the equipment were toxic to *D. magna*, and therefore, with a potential risk for the freshwater biota. Based on that, it is recommended improvements in the equipment structure, to reduce toxicity, since it occurred probably by contamination of the structures of the equipment itself.

Keywords: *Daphnia magna;* Ecotoxicology; risk assessment; solar distillation; wastewater treatment.

INTRODUCTION

Currently, waste production and management is a universal problem. According to Shutkin (2001), the United States produces about 113 trillion kilograms of general waste per year. The number of laboratories of academic institutions, private industries and pharmaceutical companies that generate large amount of residual substances is of great concern. At Universities, waste is produced in small amounts, but they have different sources and levels of toxicity (Nascimento & Tenuta-Filho, 2010), because of the different kinds of researches in progress, and, in most cases, are discarded in the environment, and may cause damage to natural ecosystems.

In Brazilian academic institutions, this problem can be even worse, because, usually, there are few discussions and institutional policies about the destination of wastewater from research and educational laboratories. In many cases, waste is stocked inappropriately and finally discarded in the sanitary drain or garbage (Jardim, 1998; Gerbase *et al.*, 2005; Imbroisi *et al.*, 2006). In Brazil, just about 67% of the sanitary sewer is treated before being dumped in rivers, lakes and reservoirs (Brazil, 2013).

Copper sulfate ($CuSO_4$) was used in present work as a simulation of wastewater. $CuSO_4$ has many uses: phytoplankton control (Padovesi-Fonseca & Philomeno, 2004), fungicide (Mirlean *et al.*, 2005), reference toxicant (Struewing *et al.*, 2015), and as analytical reagent for chemical and biomedical analysis (Pistorius *et al.*, 1996; Tsu *et al.*, 2004).

In the other hand, studies have shown that copper is toxic to several organisms, such as: fishes (Hernández *et al.*, 2006),

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cladocerans (Rodgher *et al.*, 2009), cnidarians (Markich & Camilleri, 1997) and algae (Franklin *et al.*, 2000; Rocha *et al.*, 2016). According to CONAMA, National Environment Council (Brazil, 2005), the maximum allowed concentration of dissolved copper in waters categorized as 'class 1', is 0.009 mg L^{-1}. Thus, it is important that the discarded of copper be appropriate and new ways of decontamination of this substance be studied.

In this context, it is important to develop methodologies for treating effluents from academic laboratories (or others) and at the same time monitor the possible impact of these effluents on aquatic biota.

Ecotoxicology offers important tools for evaluating the level of toxicity of discarded substances and predict what they might cause in natural environments. Chemical analysis are useful for identifying substances, but are not able to show the toxic effects of these substance on ecosystems (Magalhães & Ferrão-Filho, 2008) and to assess potential environmental risks. For this purpose, ecotoxicological evaluations are extremely necessary (Costa *et al.*, 2008).Cladocerans of Daphnidae family, e.g. *Daphnia magna* (Straus 1820), are included in national and international protocols for testing toxicants and environmental samples and have been extensively used in Ecotoxicological studies (ABNT, 2009).

It is well known that the study of new technologies that can provide more efficient ways of decontaminating different types of wastewaters is very relevant. According to SWERA (Solar and Wind Energy Resource Assessment) project, that studied solar energy resources from 1995 to 2005, Brazil has superior values of flux of global solar radiation than in countries of the European Union (Martins *et al.*, 2007). The states of Bahia (BA) and Santa Catarina (SC), have the maximum and minimum values of global daily radiation of the country, which corresponds to 6.5 kWh m^{-2} and 4.25 kWh m^{-2}, respectively (Echer *et al.*, 2006).

Based on this, it was created a unique prototype of solar distiller, which is, in theory, capable to decontaminate non-volatile substances of the water, using just solar radiation as energy for the whole process, in order to take advantage of the large solar radiation that occurs in Brazil. The present investigation was carried out to prevent pollution and suggest improvements on the equipment. Therefore, this paper aims to experimentally investigate the prototype equipment's final product, analyzing possible toxicity of the distilled samples, to evaluate the efficiency of the whole process**.** It was expected that the final products would not be toxic, since the substance tested must not condensate with the water at the experimental temperatures reached.

MATERIAL AND METHODS

Operation of the equipment.

The system of distillation presented in this paper is a low cost innovation product (Figure 1), that was design to separate volatile substances from non-volatile ones. The equipment is a combination of passive and active solar distillation and it was thought to be used in countryside communities.

The passive process consists of glass plate (Figure 1, A) that works as a condenser and has an inclination of 30°, oriented northwards to get the maximum solar light incidence in an entire day. The active portion consists of two solar plates (Figure 1, D) with small tubes filled with glycerin that works as heating medium.

After the entire process, the distilled water, separated from the non-volatile substances, flows into a channel (Figure 1, B) and is collected in a reservoir. The wastewater product, with these non-volatile substances can be removed from distiller by opening a tap (Figure 1, C). The capacity of the equipment is around 20 liters.

Organisms

Algal cultures

The algae *Pseudokirchneriella subcapitata* was cultured in L.C. Oligo medium (AFNOR, 1980), pH 6.0-7.0, previously

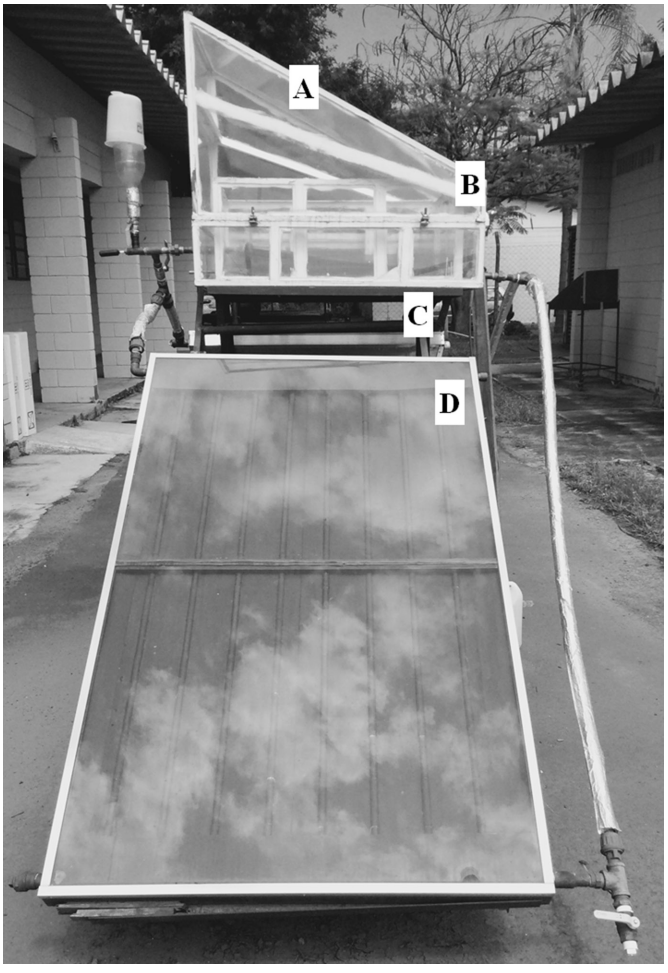

Figure 1. Representation of the prototype of solar distiller. Main structures: glass plate (A), condensate collection (B), wastewater output (C) and solar panel (D).

autoclaved at 121 °C and 1 atm for 20 min. The medium was inoculated with the algae in exponential growth phase, in aseptic conditions. Cultures were maintained at 25 ± 2°C, with a photoperiod of 12h/12h dark/light. For food preparation, the algae were centrifuged (Eppendorf 5702, Germany) at 2000 rpm for 10 minutes at 4 °C, and then the cells were re-suspended in reconstituted hard water. After that, the number of cells was estimated by using a Neubauer chamber, in a microscope (Leica DMLS, Germany) with a magnification of 400 times.

Zooplankton

Daphnia magna individuals were obtained from stock cultures maintained in the Experimental Aquaculture Station of the Department of Hydrobiology (21°58'55''S and 47°52'35''W), at the Federal University of São Carlos, Brazil.

The cladocerans were kept in an incubator cabinet (Nova Ética B.O.D. 411/D, Brazil) under static conditions at 25 ± 1 °C with photoperiod of 12h/12h; dark/light. The microcrustaceans were fed with suspensions of green algae *P. subcapitata* at a concentration of 3 x 10^5 cells mL^{-1} and they had also a complementary diet, based in suspension of dried baker's yeast.

Each *D. magna* clonal culture were started with juveniles (≤ 24 h-old), from third to fifth brood, isolated from parthenogenetic mothers and consisted of twenty adult organisms in a 2000 mL beaker, filled with 1500 mL of reconstituted hard water (ASTM, 2002) around 220-225 mg $CaCO_3$ L^{-1}, under pH 7.6-8.0, that was full renewed three times a week. The health and sensitivity of organisms was assured by tests with the reference toxicant sodium chloride (NaCl). All parameters for the cultures followed national standards (ABNT, 2009).

Acute toxicity tests

The acute tests with *D. magna* consisted in the exposure of 20 neonates (< 24 h-old) per treatment (N=4), therefore with 5 neonates in each replicate, in dilutions using reconstituted hard water, in the following sample concentrations: 100; 50; 25; 12.5 and 6.25%. The experiments were conducted in polystyrene flasks containing 15mL of test solution, kept in the dark and with no food supply.

After 24 h and 48 h, the immobile organisms were counted with the aid of a stereomicroscope (Leica MZ6, Germany). The EC50 (effective concentration, that causes immobility to 50% of test organisms) values were estimated by the Trimmed Spearman-Karber method (Hamilton *et al.*, 1977). The pH and conductivity measurements were taken at the beginning and the end of the experiments. Tests were validated when immobility in the control, with same number of replicates of other test solutions, did not exceed 10% (Abnt, 2009) and temperature during the tests do not deviate by more than 3° C.

We analyzed 3 samples, in the follow experiments:

Test 1: consisted of distilled water purified by reverse osmosis, used in the zooplankton cultures. This water was tested after passing through the process of solar distillation in the prototype equipment (Figure 1); in order to observe any toxicity related to the equipment that could be generated during the process, regardless the initial solution used in it. Pure culture water was used for the control group.

Test 2: the same purified water cited earlier, tested after process of solar distillation, this time in an equipment made 100% with glass, which was washed with hydrochloric acid (10%), and thus free of contamination, aiming to prove the non-contamination of the water used as the initial solution in the Test 1.

Test 3, solution of copper sulfate, in a concentration of 1 mg L^{-1}, tested after the same distillation process in the equipment of Test 1, aiming to simulate the possible decontamination of laboratory waste.

Metal determination

The total copper was determinate in the final distillate sample of Test 3, which had an initial test solution of 1 mg L^{-1} (nominal concentration) of $CuSO_4$. The total copper concentration was determinate by colorimetric method using spectrophotometry.

RESULTS AND DISCUSSION

All the experiments, including sensitivity and definitive tests were validated, since immobility in control groups did not exceed 10% (Figure 2), the temperature in chambers, during the tests, did not deviate more than 1 °C and pH (Table 1) did not vary more than 0.7 in the control groups.

Sensitivity tests performed corroborated the viability and health of the test organisms. Mean values of EC_{50} – 24 h and 48 h - were 6.41 and 5.93 g L^{-1} of NaCl, with standard deviation of 0.53 and 0.19, and coefficient of variation values of 8% and 3%, respectively. These results were similar to the LC_{50} – 48 h values found in literature: 5.55 g L^{-1} (Santos *et al.*, 2007); 4.77 g L^{-1} (Mount *et al.*, 1997) and 6.03 g L^{-1} (Cowgill & Milazzo, 1991).

According to Figure 2, the percentage of immobile organisms indicate that the samples on Tests 1 and 3 were toxic, after experimental process of decontamination. At Test 1, using purified water, after distillation, the EC_{50} – 48 h value was 19.61% (Table 2), which, is classified as very toxic (Bulich, 1982). Thus, this wastewater was not suitable for discharging in natural environments. Since the water used in the process was free of contamination, we can infer that some material that is part of the structure of the equipment was toxic, and thus the toxicity was transferred to the water.

After the collection of samples and the end of toxicity tests, it was observed that a part of the distiller that connects two hoses, which carry the distillate, was connected by a copper structure. This could be the reason for the toxicity, since copper can be released from pipes, becoming a source of metal in water

Table 1. Variables of ecotoxicology tests, measured at the beginning and end of experiments.

Test	Sample (%)	pH		Conductivity (µS cm^{-1})	
		initial	final	initial	final
1	Control	7.6	8.3	756.8	861.1
	6.25	7.4	8.3	713.7	813.6
	12.5	7.4	8.2	663.7	762.4
	25	7.4	8.1	583.6	670.3
	50	7.1	7.9	389.5	478.9
	100	6.0	7.3	3.858	38.12
2	Control	7.6	8.3	701.6	705.3
	6.25	7.2	8.3	655.4	662.7
	12.5	7.2	8.4	621.9	615.3
	25	7.2	8.2	542.1	543.1
	50	7.1	7.9	364.4	383.6
	100	7.0	7.3	29.78	51.40
3	Control	7.9	8.35	765	750
	6.25	7.8	8.38	693	659
	12.5	7.8	8.30	682	455
	25	7.79	8.30	614	401
	50	7.7	8.02	435	328
	100	5.65	7.22	2.9	48.1

(Rushing & Edwards, 2004). Nevertheless, this hypothesis does not exclude combined toxic effects related to other components of the equipment, such as painting and glue. Further evaluations need to be carried out to test these possibilities.

Since some organic compounds are easily volatile, they cannot be removed from the water by distillation process. A study of Fernández-Alba *et al.* (2002) demonstrated, for example, that some antifouling biocides added to painting, which are used in hulls of watercraft, were to toxic to *D. magna* and *Vibrio fischeri*. Thus, the painting used in the distiller's fabrication could be the cause of the toxicity pointed in Test 1, due to a possible contamination.

Test 2 was conducted in a glass container free of contaminants. The purified water was placed in the glass container, and after few days of solar radiation, the water was distilled and concentrated in a glass beaker inside the container. The EC_{50} – 48 h value of this concentrated could not be calculated (Table 2), therefore, the result was non-toxic.

This latter result proved that: (I) the water used in the laboratory of Plankton was adequate for the tests; (II) the process of distillation in a clean inert container did not cause toxicity in the samples; (III) and the ecotoxicological tests were effective in demonstrating non detected toxicity in the purified water.

Test 3 was performed with a solution of 1 mg L^{-1} of copper sulfate, after distillation, simulating the decontamination of wastewater. EC_{50} – 48 h of Test 3 was 17.08% (Table 2), a little higher than Test 1, and thus, the sample was classified as very toxic (Bulich, 1982).

Chemical determination showed a concentration of 0.27 mg L^{-1} of total copper in the final distillate (Test 3). Metal trace in the samples could not be explained by the initial test solution of copper, since this substance does not evaporate during the distillation process, once $CuSO_4$ boiling point is 650 °C (Weast, 1987).

The EC_{50}-48h of *D. magna* for copper is only 6.5 µg L^{-1} (Dave, 1984), classified as an extremely toxic substance (Zucker, 1985). This test indicated that the water was not suitable for dump in natural environments. According to Brazil (2005), resolution 357/2005, the allowed maximum value for dissolved copper in water is 0.009 mg L^{-1}, for freshwaters classified as 'class 1', which aimed to protect the aquatic communities.

Other values founded in literature for $CuSO_4$ to *D. similis* is 0.01 mg L^{-1} (EC_{50} – 48 h) (Tavares *et al.*, 2014) and 0.0826 mg L^{-1} (LC_{50} – 48h) for *D. magna* (Guilhermino *et al.*, 2000), which means that this genera is highly sensitive to this compound. Thus, the toxicity could be explained by the copper structures in the distiller, that possible leached the metal to the water, as mentioned in Test 1.

In addition to acute toxicity tests, chronic exposures need also to be performed in future studies, as in the work of Magdeburg *et al.* (2012). They analyzed survival and reproductive endpoints in *D. magna*, to test the efficiency of new steps for water decontamination in a wastewater treatment plant.

Other studies have also used cladocerans as test organisms, for testing: (1) the toxicity of pharmaceutical active compounds, detected chemically in wastewater treatment plants in Korea, by the acute and chronic toxicity tests. The authors concluded that the pharmaceutics indicated a low biological risk in that study area (Han *et al.*, 2006); (2) the toxicity of textile wastewater of a factory in Turkey, after processes of ozonation and coagulation, in order to decolorize and detoxify the water. The wastewater was toxic to *D. magna* (immobility as endpoint) even in the dilution of 150% (Selcuk, 2005).

In countryside communities, this prototype equipment could also be applied to obtain and reuse water for irrigation, for example. It is well known that wastewater irrigation can cause risks for human health, such as infections by helminths and organic or inorganic contaminants (Qadir *et al.*, 2010).

The purpose of the present work was to test a low cost equipment of water decontamination, which only demands solar energy to function, and could be used for professional and domestic purposes, being accessible for a large number of people, especially those in developing countries. This equipment has a high potential for use, once same parts of it could be replaced for others materials, since we showed that the process of distillation itself did not cause toxicity, demonstrated through the experiment in the equipment made of 100% of glass, in which the water had been condensate with no toxicity at the end of the process.. We believe that

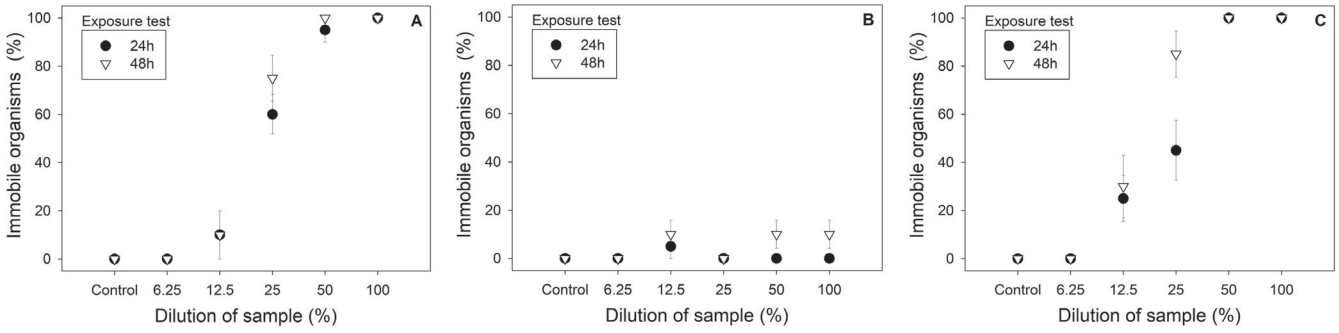

Figure 2. Detailed data of immobility for *D. magna*, for tests 1, 2 and 3, represented respectively, in A, B and C.

Table 2. Values of EC_{50} for *D. magna* and their respective level of toxicity, according to (Bulich, 1982).

Test	EC_{50} (%)	CI* (95%)	Exposure	Result
1	23.33	19.24 - 28.27	24 h	Very toxic
	19.61	16.66 - 23.09	48 h	
2	NC**	-	24 h	Non-toxic
	NC**	-	48 h	
3	21.76	17.74 - 26.70	24 h	Very toxic
	17.08	14.04 - 20.76	48 h	

CI*: confidence interval.
NC**: non-calculable.

this was the possible route of contamination in the prototype equipment of solar distillation, with contamination through the others structures of the equipment itself (that are not made of glass), evidenced by the concentrations of copper measured in the final condensed water.

CONCLUSIONS

The toxicity presented in the tested samples was caused by some material retained in the distiller, probably allocated after the process of condensation. Since the principle of solar distillation is that volatile substances evaporate, condense and then separate from the non-volatile ones, if there was a contamination before the condensation system, it would be decontaminated *a posteriori*. We conclude that the equipment is not ready for use yet, since its final remaining caused toxicity to the tested organism, *D. magna*. Since chemical analysis pointed copper in final samples of distillate, we suggest the replacement of the copper structure that carry the distillate, between two hoses, for a not toxic material, e.g. glass, polycarbonate or polystyrene. The long-term implications of this study will impact the design of the next distillers and in the selection of materials that will be used in their fabrication. After the improvements in the equipment, new ecotoxicology studies are needed before it can be used in large scale.

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