



Removal of microcystin-LR from eutrophic water using solar distillation

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Received 15 April 2021; Accepted 21 January 2022

ABSTRACT

The aim of this study was to evaluate the removal of microcystin-LR (MC-LR) from eutrophic water produced in the laboratory, using a pyramid-shaped solar still to obtain drinking water that meets all potability standards recommended in ordinance 2914/11 of the Brazilian Ministry of Health. The methods used for the analysis of physicochemical parameters were according to APHA, 2012. The detection and quantification of MC-LR was performed using coupled high-performance liquid chromatography and mass spectrometry. The results showed turbidity removal of over 95%, and reduction of true color from 19 to 5 uH after the distillation process. As expected, there was also a decrease in pH and hardness in relation to the study water. Ammonia showed a relative increase in concentration but still remained within the maximum allowed by law. The results showed a mean concentration of 11.4 $\mu\text{g L}^{-1}$ of MC-LR in the studied water, while no cyanotoxin was detected in the distilled water. To further verify the removal of MC-LR, the samples were then injected in a mass spectrometer, and the results demonstrated that there was a concentration of MC-LR in the remaining water in the tray of the still, so that the final treated water was free of cyanotoxin.

Keywords: Water treatment; Microcystin-LR; Cyanotoxins; Solar energy; Liquid chromatography

1. Introduction

The increase in the release of nitrogen and phosphorus from various anthropic activities into water basins has modified the characteristics of these water bodies. A consequence of the greater amounts of these nutrients in water is the accelerated growth of producer microorganisms, predominantly cyanobacteria, due to their capacity for assimilation of the nutrients. The alteration that occurs in this enriched environment is part of the process of eutrophication, which affects the water quality of springs,

and it results in a high density of algae and cyanobacteria (10^6 – 10^8 cells mL^{-1}).

The presence of these microorganisms hampers water treatment for human consumption, resulting in increased costs. These microorganisms commonly produce substances that give an unpleasant taste and odor to water, in addition to producing toxins (cyanotoxins), depending on indeterminate environmental changes, which can be harmful to human health subject to the extent of exposure time.

Especially in Northeast Brazil, a predominantly semi-arid region, the water available for supplying the population comes mostly from wells and reservoirs, distributed in

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a limited and irregular way, and has a high salinity level (brackish water). In some regions, the lack of good water leads people to consume water with high levels of biological and chemical contamination (salts), with consequent harm to health [1].

There is a need for advanced or alternative technologies that can guarantee the quality of water that is produced mainly for domestic use. Brazil is strongly favored by natural sources of renewable energy, which can be used in alternative technologies; solar energy, in particular, dispenses with the use of fuel and electricity, making it economically and environmentally advantageous.

Solar energy combined with distillation is a promising way to obtain potable water. This process simulates on a small scale the natural cycle of water: water vapor production above the surface of liquids and transport by the wind, followed by air cooling and mixing, and then condensation and precipitation [2], that is, evaporating polluted water and then condensing the vapor, obtaining distilled water at the end of the process. Although it is a simple technology, the purification of water through solar distillation is quite effective, producing water with a high purity, superior to commercial bottled water.

Accordingly, solar distillation can be used diffusely, benefiting mainly rural communities, who tend to accept the idea because this technology is easy and low-cost. The project to use solar distillers in the treatment of contaminated water mainly benefits people in isolated communities or in places where there is a critical supply situation, providing drinking water at a low production cost and through renewable energy. Therefore, this study evaluated the operation and efficiency of a solar still in the removal of microcystin-LR in the production of potable water.

2. Material and methods

The experimental system was developed and monitored in the months of February and March of 2015 at the Experimental Station for Biological Treatment of Sanitation Sewers (EXTRABES) UEPB, where the solar still was installed. The Station is located in the city of Campina Grande – PB at latitude 7° 13' 11" 7° S and longitude 35° 52' 31" W, with an average altitude of 550 m above sea level.

2.1. Cultivation of the *Microcystis aeruginosa* strain

The *Microcystis aeruginosa* strain (Word Data Center Microorganisms 835), producer of microcystin-LR (MC-LR) was supplied by the Botany Department of the Federal University of São Carlos, São Paulo, Brazil. The bacteria were grown in sterile ASM-1 liquid medium, continuously aerated and maintained under controlled conditions of temperature (24°C), photoperiod (12 h) and light intensity (about 1,200 Lux using 40 W fluorescent tubes). Cell growth was monitored by counting the cells using an inverted microscope, following the sedimentation method [3] until reaching exponential growth, where a concentration of 10^7 cells mL⁻¹ was obtained after 15–18 d of culture. The cells were then subjected to freezing and thawing to achieve cell lysis and subsequent release of the intracellular toxin into the culture medium [4,5].

2.2. Obtaining study water (SW)

The study water (SW) used in the experiments was prepared with tap water from the public supply. After being collected, the water was reserved and allowed to stand for at least 24 h at room temperature and without exposure to sunlight to eliminate residual chlorine. After the water was dechlorinated, the SW preparation was completed with the addition of the microcystin-LR toxin, extracted from the *M. aeruginosa* culture lysate diluted 1:10 in water.

2.3. Overview of the experimental system

The solar still developed consisted of an ordinary glass cover with a thickness of 4 mm, in form of a pyramid for the best capture of radiation and with a 25° angle, as shown in Fig. 1.

The glass cover was connected to the tray, which was separated from the wooden table by a Styrofoam sheet to thermally insulate the bottom to minimize heat loss (Fig. 1). The distillation tray was constructed of stainless steel and consisted of two compartments, a trough for collection of distilled water, connected to the water outlet of the still, and an upper (SW) reservoir inside the tray, connected to the inlet of the feed system.

The trough was 4 cm wide and had a steep slope so that the distilled water would run off and be collected faster into the lower reservoir, avoiding re-evaporation. The inside of the reservoir had the entire bottom painted matte black to increase radiation absorption, and the size of the reservoir was 0.5 m² with a height of 6 cm. The size of the still was chosen for safety and practicality reasons when handling, since periodic cleaning was necessary.

The tray was fed continuously using a water dispensing system. To keep the sheet of water constant it was necessary that the entire system be properly leveled. The sheet of water in the tray had to be at the same height as the water column of the upper reservoir (SW), so that as the water evaporated from the tray, it was immediately replenished. The SW remained at a fixed height; thus, to increase the sheet of water, the height of the tray had to be lowered, and to decrease the sheet of water, the height of the tray had to be increased. The solar distiller was positioned in a strategic area where there was no shading, but rather a higher incidence of radiation throughout the day.

2.4. Meteorological data

Data were collected from a meteorological station installed a few meters away from where the experimental system was placed. The Davis Instruments Vantage Pro System 2, model 6152C weather station provided daily and hourly data on various parameters, including temperature, solar energy, wind speed, air humidity, atmospheric pressure and precipitation.

2.5. Analytical methods for characterization of studied water

The following parameters were determined to characterize the water before and after treatment: pH, turbidity and true color by the methods recommended by [6];

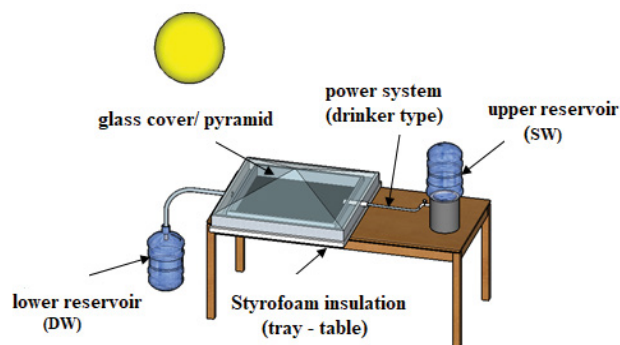


Fig. 1. Solar distillation experimental system.

chlorophyll-a by the absolute methanol (100%) extraction method according to [7] and hardness and ammonia by ion chromatography using a Thermo Scientific Dionex chromatograph, model ICS - 1100. The cation chromatography column was Dionex Ion Pac CS 12A with CS CG 12A pre-column, and an ASRS 300 2 mm cationic suppressor was used. The eluent for cation separation was 10 mM H_2SO_4 , with a flow rate of 0.25 mL min^{-1} , temperature at 35°C and run time of 17 min.

2.6. Validation of an analytical method for quantification of microcystin-LR by HPLC-MS

The identification and quantification of microcystin-LR were performed by coupled high-performance liquid chromatography and mass spectrometry (HPLC-MS), after validation of the method based on the literature and mainly by ANVISA [8], Resolution No. 833 of 2003, and by INMETRO [9], in document DOQCGCRE-008 [10] of 2016. The parameters used in the HPLC are presented in Table 1.

The validation of the method used for the detection and quantification of MC-LR was through the optimization of the

chromatographic parameters and conditions. Two mobile phases were tested: water and methanol, and water and acetonitrile.

To define the analytical method, the analytical conditions were determined in three sequential stages: (1) implementation of the method for detection and quantification of MC-LR, (2) acquisition of the analyte under study, and (3) concentration and semi-purification of microcystin-LR. The parameters analyzed for the validation of the quantitative methods were: specificity and selectivity, linearity, precision, limit of detection, limit of quantification, interval and accuracy.

3. Results and discussion

Table 2 shows the characteristics of SW that were obtained from the dilution of *M. aeruginosa* culture lysate in dechlorinated water.

SW had a pH close to neutral, high turbidity and true color with some fluctuations. Hardness and ammonia levels were well below the maximum allowed by law. Chlorophyll-a values corresponded to a natural environment, in accordance with the classification of the trophic state of lakes based on the extent of eutrophication.

3.1. Efficiency of water production

This study carried out with a solar distiller showed that the condensed water vapor in the glass cover drained to the end, because of the 25° inclination, so there were no drip losses in the tray. The results indicated that the water production in the system was directly related to solar energy; on days of higher radiation and higher temperatures the volume produced was greater (mean of 1.7 L $m^2 d^{-1}$). Low volumes (0.4 L $m^2 d^{-1}$) were observed on days when the maximum temperature did not exceed 29.4°C.

Regarding meteorological variables, only ambient temperature and solar energy showed a significant influence on

Table 1
Description of the parameters used in the analysis by HPLC-MS

Parameters	Description
Precolumn	Cartridge ULTRA C18 (3 × 2,1 mm)
Column	KINETEX C18 (2,6 μm × 100 mm × 2,1 mm)
Concentration gradient	50% H_2O and 50% de acetonitrile and 1% de formic acid (isocratic)
Total analysis time	5 min
Sample injection volume	100 μL
Gases used	Helium and nitrogen
Capillary temperature	350°C
Detector voltage	5 kV
Interface	Electrospray ionization (ESI) – positive mode
Monitored ions	Microcystin-LR: $m/z = 9,955,560 (M+1)^+$ in mode SIM
Scanning range m/z	0–5 min (800–1,050). Monitoring in positive ionization mode, ion scanning in fullscan, SIM for Microcystin-LR: $m/z = 9,955,560 (M+H)^+$
Time of accumulation of ions in the octapole	Microcystin-LR : 100 ms
Oven temperature of the column	50°C

Source: Author, 2018.

Table 2
Characteristics of studied water for MC-LR

Parameters	Minimum	Maximum	Average	N*
pH	7	7.7	7	15
Temperature (°C)	24.9	28.4	26.3	15
Turbidity (uT)	16.3	33.5	26.4	15
True color (uH)	8.7	26.1	19	15
Hardness (mg CaCO ₃ L ⁻¹)	182.2	224.2	209	8
Ammonia (mg NH ₃ L ⁻¹)	0.08	1.2	0.65	8
Chlorophyll-a (µg L ⁻¹)	2.3	32.7	14.2	15

*Average data of 15 and 8 measurements on different days.

system productivity. Analysis of the data of the experimental system correlated with wind speed, air humidity and atmospheric pressure, showed that these factors had no effect on the efficiency of the system.

In the experimental system, the water depth was a minimum of 1 cm and maximum of 2 cm, controlled by the leveling of the system. The same was observed by Rajamanickam and Ragupathy [11] who tested depths of 1.0, 2.5, 5.0 and 7.5 cm and obtained the best result with the shallowest, where the maximum distillate output was 3.07 L m⁻² d⁻¹.

3.2. Quality of treated water

After the solar distillation process, the parameters of pH, hardness, true color and ammonia were evaluated (Fig. 2). Turbidity and chlorophyll-a were also determined, and the results are described below.

3.2.1. pH

pH of the distilled water was lower (on average 6) compared to that of SW used to supply the system (on average 7.1) (Fig. 2). This was expected since distilled water is usually acidic. Water for human consumption is generally a solution, but distilled water in principle would be a pure substance. However, any water in contact with the atmosphere will dissolve carbon dioxide and thereby will have a pH below 7.0.

The Ministry of Health through Regulation 2914/11 [12] recommends that the pH of water intended for human consumption and provided by the public supply network be in the range of 6.0–9.5. Only 33% of the water samples analyzed had a pH lower than 6.0, which does not pose a problem, since the pH of water can easily be corrected with the addition of an alkaline solution.

When analyzing fifteen physical-chemical parameters in samples of water used to supply residents of the Gaza

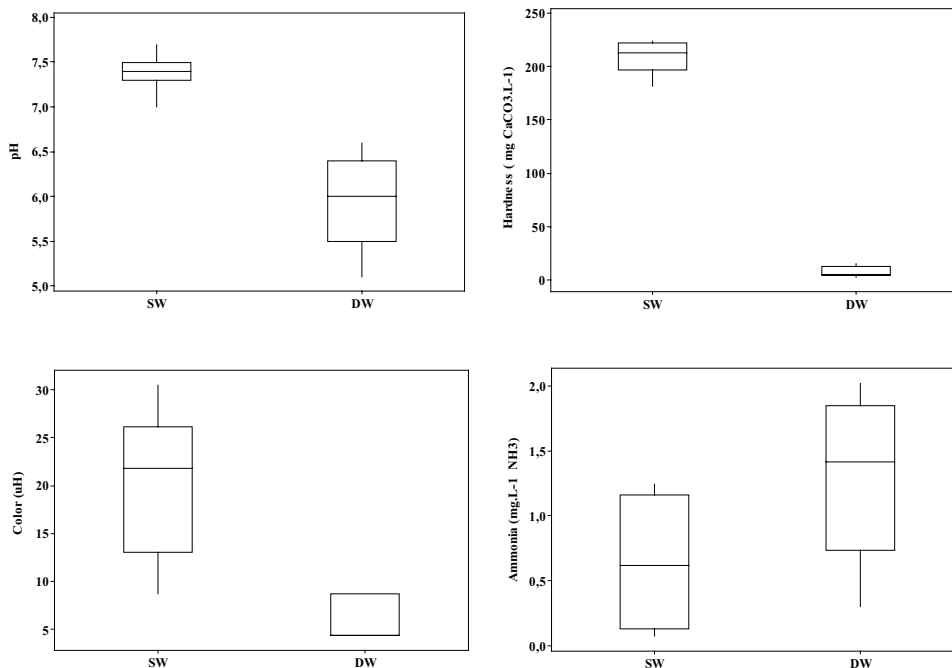


Fig. 2. Box plot of pH values and average concentrations of true color, hardness and ammonia in study water and distilled water.

Strip in Palestine [13], it was found that 44% of the 158 samples of desalinated water had a pH less than 6.5 (value recommended by WHO). In the desalting membrane, the pH of the permeate (desalinated water) is generally less than 6, the same as expected for distilled water. This is because carbon dioxide passes through the membranes and bicarbonate is blocked. Since the pH is regulated by the logarithm of the ratio of bicarbonate and carbon dioxide, the pH of the permeate is always low. The authors of that study also suggested a post-treatment in such case, where a simple pH correction could be made if truly needed, such as by mixing the water with limestone grains.

3.2.2. True color and turbidity

True color refers to color determination in samples without turbidity (removed by centrifugation, for example), where intensity can be measured in color units (uH) [6]. Natural waters generally have color intensities varying from 0 to 200 uH. To meet potability standards, water must have an apparent color intensity of less than 5 uH [14].

Before distillation of SW, the average true color was 20 uH (Fig. 2). For true color analysis, the samples were filtered through 0.45 μm membranes for turbidity removal, different from the determination of apparent color in which the samples are read directly in the spectrophotometer, but the SW values remained high. After solar distillation, true color was reduced to an average of 5 uH, indicating that there was removal of most of the organic matter dissolved in the water.

Turbidity is a physical characteristic of water, due to the presence of suspended particles, that is, suspended solids, finely divided or in colloidal state, and microscopic organisms [12]. The size of the particles responsible for the turbidity varies greatly, from coarse to colloidal, according to the level of turbulence of the water body. The presence of these particles causes the dispersion and absorption of light, leaving the water with a cloudy appearance, which may impair photosynthesis in algae and submerged aquatic plants. Considered a key parameter in the monitoring of surface water quality by its influence on the ecology of the aquatic environment [15], turbidity can decrease the penetration of light in the water column and thereby reduce its transparency.

The turbidity parameter showed removal efficiency with values over 95%. The values for SW varied between 16.3 and 33.5 uT, due to the *Microcystis aeruginosa* lysate added to the dechlorinated water, which had a great deal of organic matter, pigments and cell debris from the cyanobacteria. These values were significantly reduced after the distillation process to a mean of 0.37 uT.

3.2.3. Hardness

As a characteristic conferred to water by the presence of salts of alkaline earth metals (calcium, magnesium, etc.) and some metal salts to a lesser degree, hardness is measured in mg L^{-1} calcium carbonate. For drinking water, the potability standard sets the limit at $500 \text{ mg L}^{-1} \text{ CaCO}_3$ [16,17].

Total hardness, in terms of calcium carbonate, was determined by the ion chromatography method. SW was

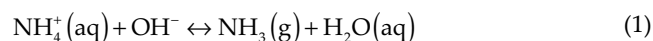
hard for all the samples analyzed, showing high concentrations of Ca^{2+} and Mg^{2+} , mostly over $200 \text{ mg CaCO}_3 \text{ L}^{-1}$. For the distilled water, the concentration decreased considerably to an average of $7.3 \text{ mg CaCO}_3 \text{ L}^{-1}$, as seen in Fig. 2. In the process of solar distillation by evaporation of the water, salts precipitate in the distillation tray, resulting in soft distilled water, with low concentrations of Ca^{2+} and Mg^{2+} .

Although it has been shown that water hardness does not have a serious impact on health, hard water is responsible for the formation of deposits in hot water heaters and home installations, as well as influencing the performance of various cleaning detergents [18]. In addition, a high concentration of magnesium in drinking water may impart a bitter taste [19], so hardness is an undesirable feature for drinking water.

3.2.4. Ammonia

The concentrations of ammonia for SW and distilled water showed significant changes. Initially, ammonia concentration in SW was below 1.5 mg L^{-1} , and after solar distillation, the level observed was higher than the recommended limit in at least half of the samples analyzed. The desorption of ammonia may have occurred. Ammonia desorption is a physical process of removing the gas phase from a liquid, mainly due to the increase in the total contact surface of the liquid phase with the surrounding (atmospheric) environment, so that drag and molecular diffusion effects promote the passage of ammonia into the air [20].

Ammonia in the aqueous phase is balanced in two forms, ionic (NH_4^+) and molecular gas (NH_3). Eq. (1) shows the equilibrium conversion of ammonium ion to ammonia gas.



The equilibrium conversion for the equation depends on the pH; for pH about 7.2, the equilibrium tends to be shifted to the left. With increasing pH, the equilibrium shifts to the right, and thus, there is a greater increase in the gas fraction. In the distillation tray, due to the increase in temperature, the pH of the water was around 8.5, which probably facilitated the volatilization of ammonia contained in the water in the tray. With the increase of the ammonia gas fraction, when mixing with the condensing water vapor in smaller volumes, there was an increase in the ammonia concentration in the liquid part.

3.2.5. Chlorophyll-a

In any aquatic environment, variations in biomass and algal diversity are responses to changes in the physical-chemical and biological quality of water. The determination of chlorophyll-a in SW showed variations, where the values observed were between 3.0 and $32.7 \mu\text{g L}^{-1}$. In the distilled water, it was not possible to detect chlorophyll-a, as it was well below the limit of detection for the method used.

3.3. Validation of the analytical methods for quantification of MC-LR

For the analysis of the experimental data, a calibration curve for microcystin-LR was generated. The toxin standard

was necessary for proper calibration of the equipment. The MC-LR standard was obtained and certified by the National Research Council of Canada (NRCC).

To construct the calibration curve, nine microcystin-LR concentration standards were injected in the HPLC-MS apparatus. For each standard, 6 replicates were run on the same day, and this procedure was performed three times, on three different days. Thus, each standard was injected for analysis 18 times.

The analytical curve obtained was considered linear, represented by Eq. (2), and it showed a correlation coefficient (r) of 0.99, indicating that the detector response in the analyzed interval was proportional to the concentrations of the MC-LR standards. According to the Brazilian Health Surveillance Agency (ANVISA), a correlation coefficient equal to or greater than 0.99 is acceptable.

$$y = 1,290.9x + 96.857 \quad (2)$$

The method was considered selective, since it complies with the recommendations of ANVISA [8]: Resolution RE No. 899 [10] of May 29, 2003, which considers selectivity through the separation of the compound of interest by chromatographic techniques, or the use of methods that are specific to the structure of the substance of interest, for example, mass spectrometry, nuclear magnetic resonance, infrared spectroscopy or specific bioassays.

The recovery levels were within the established criteria for the working range, that is, between 60% and 115%. The HPLC-MS technique proved to be very sensitive for the detection and quantification of the analyte, obtaining LD and LQ values below the maximum value allowed in water for human consumption ($<1 \mu\text{g L}^{-1}$) as required by Brazilian law. Precision was assessed by the determination of repeatability (intra-run precision) by agreement between the results within a short period of time, with the same operator and the same instrumentation, and values obtained showed less than 20% relative standard deviation with respect to the concentrations studied.

The range of an analytical method corresponds to the range from highest to lowest level that can be precisely and accurately determined using the linearity of the method. According to the curves obtained for two ranges, 0.1–50, and 1–50, determined by the LD and LQ, the proportionality between chromatogram peak and analyte concentration in the sample was practically the same. To evaluate accuracy, we calculated the mean and coefficient of variation. For the three concentration levels of this determination, 10, 30 and $50 \mu\text{g L}^{-1}$, the mean concentration obtained was $30 \mu\text{g L}^{-1}$. Transforming this mean concentration to weight/volume percentage concentration gives $3 \times 10^{-8} \text{ kg L}^{-1}$, which equals 0.000003%. According to the literature [10], the recovery of analyte as a function of concentration, for $0.000003\% \geq 0.000001$, is equivalent to an acceptable recovery range of 60%–115%. In this way, all concentration ranges described showed acceptable accuracy.

3.3.1. Microcystin-LR

After the identification of MC-LR using the validated standard method, analyses were carried out to measure its

concentration in the SW samples and the water distilled by the solar distiller.

SW had an initial average concentration of $11.4 \mu\text{g L}^{-1}$. After the solar distillation treatment, no MC-LR was detected in the water distilled by the system. To show that the cyanotoxin was removed or that it was not carried in the water vapor from the SW in the distillation tray, two further analyses were conducted on the SW remaining in the distillation tray 24 h, after evaporation/condensation.

The remaining water in the tray contained $23 \mu\text{g L}^{-1}$ MC-LR. This showed that as SW evaporated and the vapor condensed, the toxin was retained in the bottom of the distillation tray, increasing in concentration in the remaining water, thus demonstrating its removal from the outlet water. The second analysis was a direct injection of the distilled water samples into the mass spectrometer, no cyanotoxin was detected. The spectrum generated for the distilled water showed no cyanotoxin peak; the spectrum showed only noise for the MC-LR peak. Thus, the cyanotoxin was found to be concentrated in the water remaining in the tray, resulting in the final treated water being cyanotoxin-free.

4. Conclusion

Water treatment by solar distillation was efficient in greatly reducing turbidity, true color and hardness. However, attention should be paid to the final concentration of ammonia in the distilled water, which was higher than that in eutrophic water.

The method validated for analyzing MC-LR using HPLC-MS did not detect the cyanotoxin in the water produced by the solar still, showing the ability of the system to remove MC-LR at an initial concentration of $11.4 \mu\text{g L}^{-1}$, producing water free of MC-LR.

We found that solar distillation was effective in the purification of eutrophic water, since HPLC-MS showed the absence of cyanotoxin in the water after treatment and since all other organoleptic parameters were then in accordance with the law. Thus, the water obtained by solar distillation proved to be suitable for human consumption.

The proposed solar still showed potential to solve small scale water desalination problems, which occur mainly in the interior of Northeast Brazil, where the insolation index is high and rainfall low.

Acknowledgments

The authors wish to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Financiadora de Estudos e Projetos (FINEP) for financial support.

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