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Title: Rio de Janeiro water crisis: a metagenomic approach.

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Abstract

In Rio de Janeiro's metropolitan area comprise, over 8 million inhabitants receive drinking water from Company responsible for water and sewage, capted by the Guandu river's water treatment station. Such water started to present unusual odour, taste and color in January 2020, as stated by the population itself. Results obtained through raw water microbiome metagenomic analysis inferred cyanobacteria presence, with *Planktothricoides* gender and *Planktothricoides* sp. SR001 being the most abundant gender and organism. Such do pose a risk to public health, as they are able to produce cyanotoxins that affect human health.

Keywords: cyanobacteria; water quality; consumption safety; public health.

Introduction

Cyanobacteria that are able to produce cyanotoxins, such as microcystin (hepatotoxin), saxitoxin and BMAA (neurotoxin), among others ^(1,2), do pose risk to human health, especially when present in water captation points for human consumption.

Brazil's actual legislation⁽³⁾ states that cyanobacteria monitoring must be performed at shallow spring water captation point. Also, should the cyanobacteria density exceed 20,000 cells/mL, a weekly-based cyanobacteria analysis must follow.

In early January 2020, more than 8 million inhabitants of the metropolitan area of Rio de Janeiro, who receive drinking water consumed by Company responsible for water and sewage (CWS), complained of unpleasant and unusual water taste, turbidity and odour^(4,5).

Therefore, this metagenomic study was proposed in order to evaluate CWS's raw water (water that is captured at the water treatment station, before any cleaning/filtering process) microbiome, so that any public health risk related to the presence of cyanobacteria might be inferred.

Methodology

Water samples used in this study (700 mL total volume) were collected at CWS's water treatment station barrage, at the exact raw water captation location. From such, 600mL were homogenized and submitted to ultracentrifugation for 2 hours (120,000 g) at 4°C. Supernant was discarded and the pellet was resuspended at the remaining volume. We were able to obtain a total of 6mL sediment volume, immediately allocated in two separate aliquots, named Raw water #1 and Raw water #2.

The obtained DNA from both samples was extracted using Quiagen's DNEasy kit. Libraries were built with Illumina's Nextera DNA FLEX 2x150 bp paired-end kit, following manufacturer's instructions. DNA quantification was performed at Qubit DNA HS and sample sizes were analyzed with DNA Sensitivity bioanalyzer, before sequencing.

Samples sequencing was performed at SENAI CETIQT Innovation Institute for Biosynthetic and Fibers sequencing facility⁽⁶⁾, using Illumina's NextSeq 550 (Illumina, INC, EUA). Sequencing data were analyzed at Stingray@Galaxy platform⁽⁷⁾.

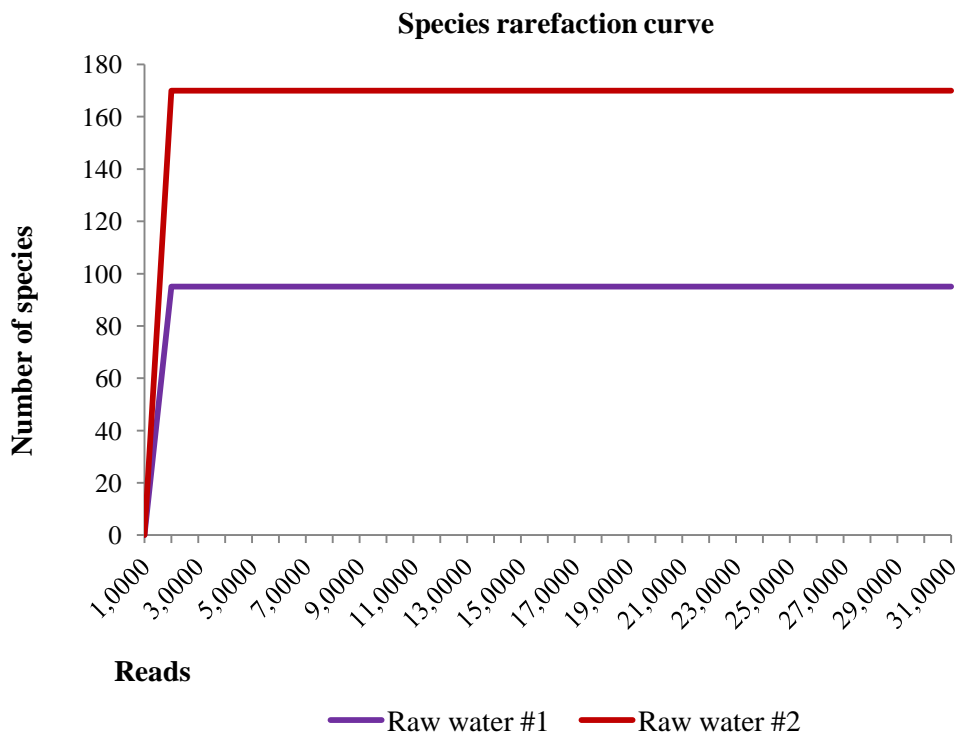
FastQC 0.67⁽⁸⁾ was used with standard parameters in order to infer sequencing data quality. No cleaning steps were required as both samples posed PHRED values greater than 30. Diamond 0.9.29 software⁽⁹⁾ was used to perform sequence similarity searches (e-value = 1e-05), against NCBI's 2019 RefSeq protein database (NCBI).

Obtained results were visualized through MEGAN6⁽¹⁰⁾ and KronaTools 1.1.0⁽¹¹⁾. We were able to plot both samples rarefaction curves, as well as to calculate Shannon-Weaver's alpha diversity index⁽¹²⁾, using MEGAN6⁽¹⁰⁾.

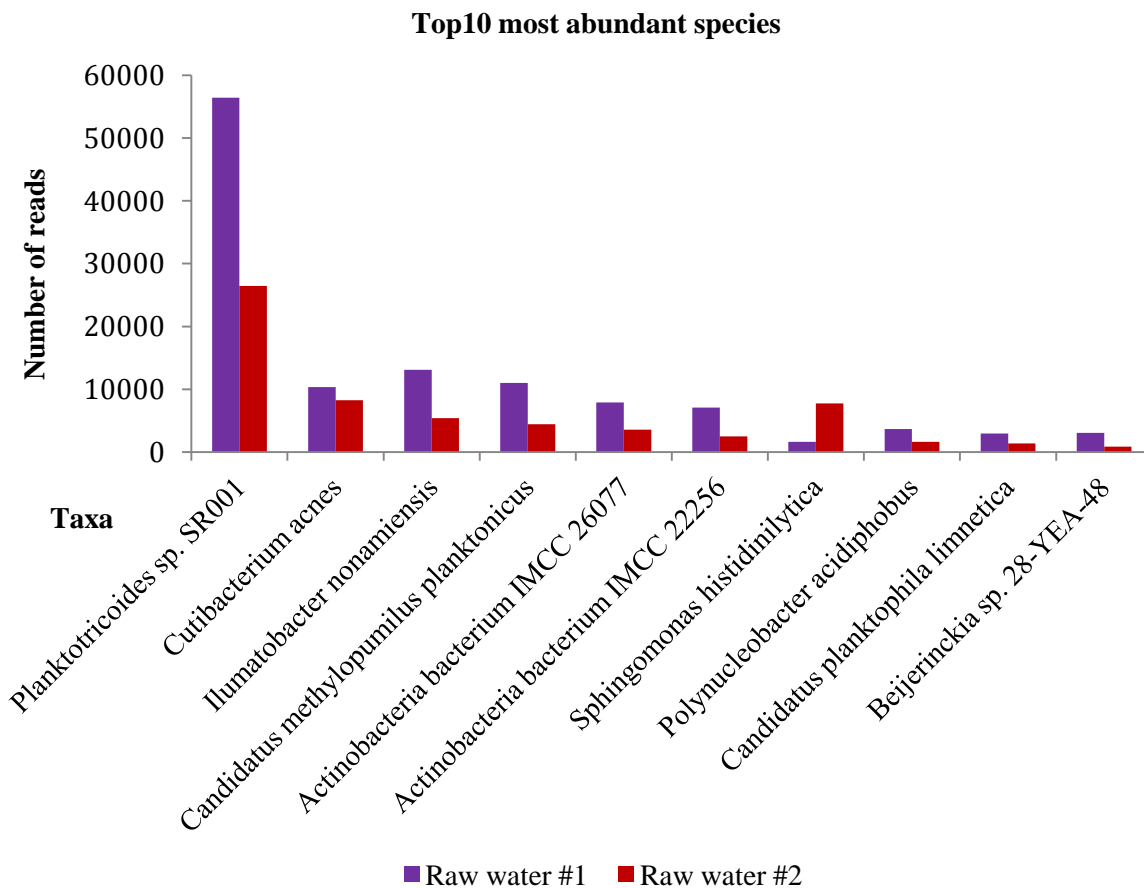
Results

DNA concentrations for each sample were 6ng/30 µL (Raw water #1) and 4.6ng/32 µL (Raw water #2). The values obtained for alpha diversity were 3.7 (Raw water #1) up to 4.8 (Raw water #2), which evidence low species diversity. Nevertheless, the rarefaction curves do manifest that both samples richness were explored to its full extent (Graph 1). The top 10 most representative species are depicted in Graph 2.

Graph 1. CWS's water treatment station raw water samples species rarefaction curves.



Graph 2. Top 10 most abundant organism species in CWS's water treatment station raw water samples.



The most abundant gender in both water samples was *Planktothricoides* and the most abundant species sequences are similar to NCBI's RefSeq *Planktothricoides* sp. SR001.

Planktothricoides gender include environmental filamentous cyanobacteria that belong to Oscillatoriales and Phormidiaceae families, which are capable of flowering in both brackish and fresh water⁽²⁾.

Although such gender was originally classified as *Planktothrix*, in Phormidiaceae family, due to its elevated morphologic similarity, recent 16S rRNA gene analysis⁽¹³⁾ were able to classify it as a new gender due to its phylogenetic distance between other cyanobacteria.

Such cyanobacteria are able to produce compounds that add abnormal and unpleasant odour and taste to fresh water, as geosmine and 2-methylisoborneole (2-MIB), as well as other substances which are known to be toxic to water biome⁽²⁾. Sivonen and Jones⁽¹⁴⁾ highlight that although cyanobacteria are from aquatic origin, terrestrial mammals are more endangered by such than the aquatic biome in which they flower. Therefore, contaminated raw water is a public health issue, especially regarding situations in which cyanobacteria may be present.

Constant water reservoir monitoring is required in order to avoid scenarios such as the ones in Itaparica, state of Bahia, Brazil, in 1988, with 88 human deaths and 2,000 intoxicated⁽¹⁵⁾; Caruaru, state of Pernambuco, Brazil, 1996, in which hemodialysis devices were treated with contaminated water and ended up killing 54 people⁽¹⁶⁾; and Pennsylvania, 1975, when 5,000 people suffered from gastrointestinal infections due to contaminated water⁽¹⁷⁾.

Planktothricoides sp. SR001 was sequenced and deposited in NCBI, being found and related to a fresh water supply in Singapore in 2017, which hydrographic bay suffered from industrial and residential waste⁽²⁾. Furthermore, such basin was characterized as being eutrophic, with elevated phosphor levels. This was the first isolated *Planktothricoides* sp. genome in tropical fresh water⁽²⁾ and authors do relate that such strain is able to produce 2-MIB, a compound that adds unpleasant taste and reduces fresh water palatability.

Besides *Planktothricoides*, the top 10 bacteria genders identified in our samples include: *Limnochabitans*, *Actinobacter*, *Polynucleobacter*, *Cuticterium*, *Pseudomonas*, *Massilia*, *Sphingnomonas*, *Ilumatobacter* and *Staphylococcus*.

Among the Ministry of Health⁽¹⁸⁾ list of public health-related cyanobacteria, we were able to identify *Cylindrospermum*, *Raphidiopsis* and *Hydrocoleum* genders (neurotoxin production related); *Microcystis*, *Nostoc*, *Synechocystis* and *Nodularia* (hepatotoxin production related); and *Aphanizomenon*, *Arthrospira*, *Oscillatoria*, *Phormidium*, *Planktothrix*, *Anabaena*, *Lyngbya* and *Cylindrospermopsis* (both neuro and hepatotoxin production related).

The lack of basic sanitation infrastructure in cities at the upstream to the spring water captation point creates a scenario in which a nutrient supply favours bacterial proliferation in

lagoons closer to raw water captation point. Therefore, Rio de Janeiro metropolitan area is actually consuming an indirect reuse water⁽¹⁹⁾.

As described in ABNT n°13969/1997⁽²⁰⁾, such is due to when effluents are discharged into the environment (upstream) and later reused at downstream, in its diluted form, in an unintentional and uncontrolled manner.

Also, due to cyanobacteria toxin production, treated or untreated effluent disposal with such toxins might drastically affect the water biome and change the food chain, as it inhibits other algae bloom⁽²¹⁾.

Finally, it was possible to infer that the raw water capted at Guandu water treatment station presented elevated algae bloom, with cyanobacteria which are able to produce cyanotoxins.

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