Water Science & Technology

© 2021 The Authors Water Science & Technology Vol 84 No 10-11, 2903 doi: 10.2166/wst.2021.268

Relationships between abiotic and biotic variables in a maturation pond and their influence on *E. coli* removal

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ABSTRACT

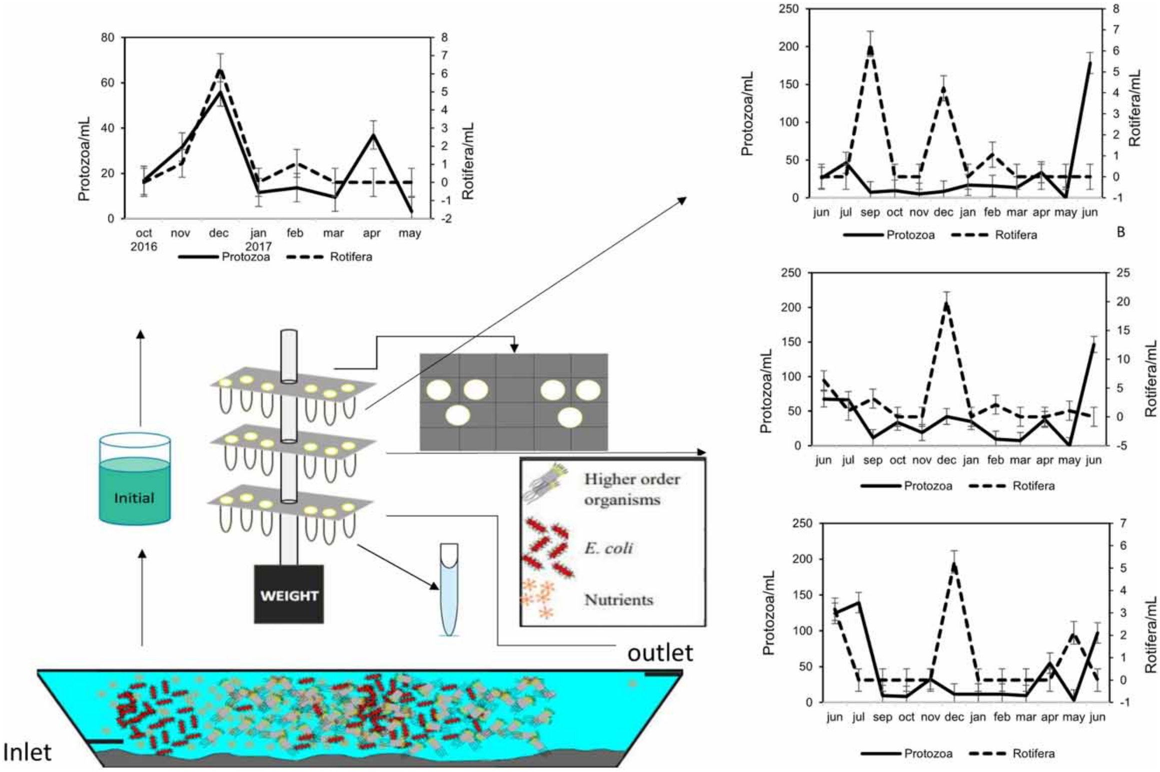
The effects of depth and climate seasonality on zooplankton, algal biomass, coliforms and *Escherichia coli* in a small full-scale shallow maturation pond receiving pre-treated domestic wastewater were evaluated during a tropical climatic seasonal cycle. The experiment revealed that the zooplankton community was dominated by rotifers and protozoans, and concentrations were influenced by seasonality. A negative correlation between zooplankton, and pH, dissolved oxygen, temperature and ultraviolet radiation, and chlorophyll-a and *Escherichia coli* were observed at all depths. The major driving forces influencing *Escherichia coli* were pH, dissolved oxygen, ultraviolet radiation and the zooplankton. A significant difference between *Escherichia coli* removal throughout the three different depths were observed. Both bacterial and zooplankton concentrations were greater closer to the bottom of the pond, therefore reinforcing the integral role of solar radiation on bacterial removal. These results give an insight on the dynamics of these groups in pond systems treating domestic wastewater, by correlating the variation of zooplankton with biotic and abiotic variables and seasonal changes in a tropical climate, where few studies have been performed on this topic.

Key words: biomass algae, chlorophyll-a, coliforms, *Escherichia coli*, waste stabilization pond, zooplankton

HIGHLIGHTS

- The variables pH, DO, zooplankton community and solar radiation are shown to be determining factors in reducing *E. coli* in a maturation pond.
- Zooplankton concentrations were greater closer to the bottom of the pond.
- *E. coli* removal varied between the three different depths reinforcing the role of solar radiation on bacterial removal rate.

GRAPHICAL ABSTRACT



INTRODUCTION

Treating vast volumes of wastewater is a challenge, especially in developing countries where lack of essential sanitation services is common. Investments in this sector are necessary even under financial limitations, since sanitation is crucial in preventing waterborne diseases (Clasen & Haller 2008). In this context, a sustainable wastewater treatment technology such as Waste Stabilization Ponds (WSPs) is a solution with low capital cost and low energy and maintenance requirements (Wallace *et al.* 2016a, 2016b). Brazil has the advantage of having a high availability of surface area and solar radiation, both necessary for this system (von Sperling 2016).

Researchers have repeatedly observed that artificial biological systems, such as the WSPs, can provide higher and more efficient disinfection results compared to conventional wastewater treatment technologies due to several interrelated processes (Shilton 2005; Liu *et al.* 2018). *Escherichia coli* (*E. coli*) bacteria are the most representative indicator of fecal contamination (Metcalf & Eddy Inc. *et al.* 2013) and pond systems, including maturation ponds, can achieve reduction efficiencies greater than 5 log units (von Sperling & Mascarenhas 2005; Buchauer 2007; Dias *et al.* 2014). Additionally, the bacterioplankton, phytoplankton and zooplankton present in WSP are important since they have different metabolic and ecological roles due to their physiological particularities. These communities increase wastewater treatment efficiency by molding the environment for aerobic decomposition by providing oxygen to the liquid, uptake and cycling of nutrients for growth, resulting in organic matter decomposition, and removal of nutrients and organic contaminants (Pu *et al.* 1998; Ansa *et al.* 2011).

Bacterial population control or removal in WSPs is driven by several biotic and abiotic factors. Among the abiotic factors, pH and DO are directly related to photosynthesis by algae (Liu *et al.* 2018) and water temperature differences lead to thermal stratifications events (even in shallow ponds), therefore decreasing *E. coli* abundance mainly in the surface layers (Liu *et al.* 2018). Greater bacterial population densities generally occur at the bottom of the ponds because of lower solar radiation intensity reaching the floor/base of the pond (Passos *et al.* 2019). Solar radiation is considered the most efficient form of coliform inactivation/disinfection/removal (Dias *et al.* 2017b), with ultraviolet radiation A (UVA) and ultraviolet radiation B

(UVB) contributing through three mechanisms: (1) direct damage to DNA; (2) indirect endogenous damage; and (3) indirect exogenous damage (Davies-Colley *et al.* 2000; Kadir & Nelson 2014; Liu *et al.* 2018). Other physical and hydraulic factors, such as attachment, starvation, hydraulic retention time, and pond depth, have been shown to also influence removal efficiencies (Ansa *et al.* 2011). Among the biotic factors that control bacterial populations in WSPs are predation by zooplankton or protozoa (Chabaud *et al.* 2006; Ansa *et al.* 2012) and competition for nutrients among heterotrophic bacteria (Ansa *et al.* 2011).

Maturation ponds following a facultative or anaerobic pond can act as a polishing phase of the effluent by removing pathogenic organisms through the abiotic and biotic factors previously described. To improve final effluent quality in order to reuse it for other practices (e.g., irrigation), it is essential to understand the multitude of synergetic inactivation and decomposition mechanisms, which are complex and still considered enigmatic due to the range of transformations that organic matter and other constituents undergo (Dias *et al.* 2017a).

The interactions among the abiotic and biotic variables in maturation ponds still require further investigation (Dias *et al.* 2017b). In the current study, the dynamics of zooplankton, phytoplankton (chlorophyll-a), and coliform bacteria communities were evaluated. Environmental conditions (dissolved oxygen (DO), pH, temperature, and solar radiation) were monitored to better understand the drivers of coliform and *E. coli* removal in this system. To the best of our knowledge, this is the first time that such an integrated approach has been performed to quantify the interaction of the plankton communities (zooplankton, algae, and bacteria) in this maturation pond.

METHODS

Study site and experimental design

The experiments were conducted at the Centre for Research and Training in Sanitation of the Federal University of Minas Gerais (CePTS-UFMG) (Figure 1), located in Belo Horizonte, MG, Brazil. CePTS is situated within the Arrudas Wastewater Treatment Plant (WWTP), a major sewage treatment plant operated by the state sanitation company of Minas Gerais – COPASA (19°53′42″ and 43°52′43″ and an altitude of 800 meters).

The experiments were conducted in the second maturation pond (44 cm depth) in a series of an upflow anaerobic sludge blanket (UASB) reactor, two maturation ponds, and a rock filter. The treatment line had recently undergone interventions to decrease land requirements and increase overall efficiency, as described by Dias *et al.* (2017a). The intervention in the second maturation pond resulted in a lowered hydraulic retention time of 1.8 days and a surface organic loading rate (median and

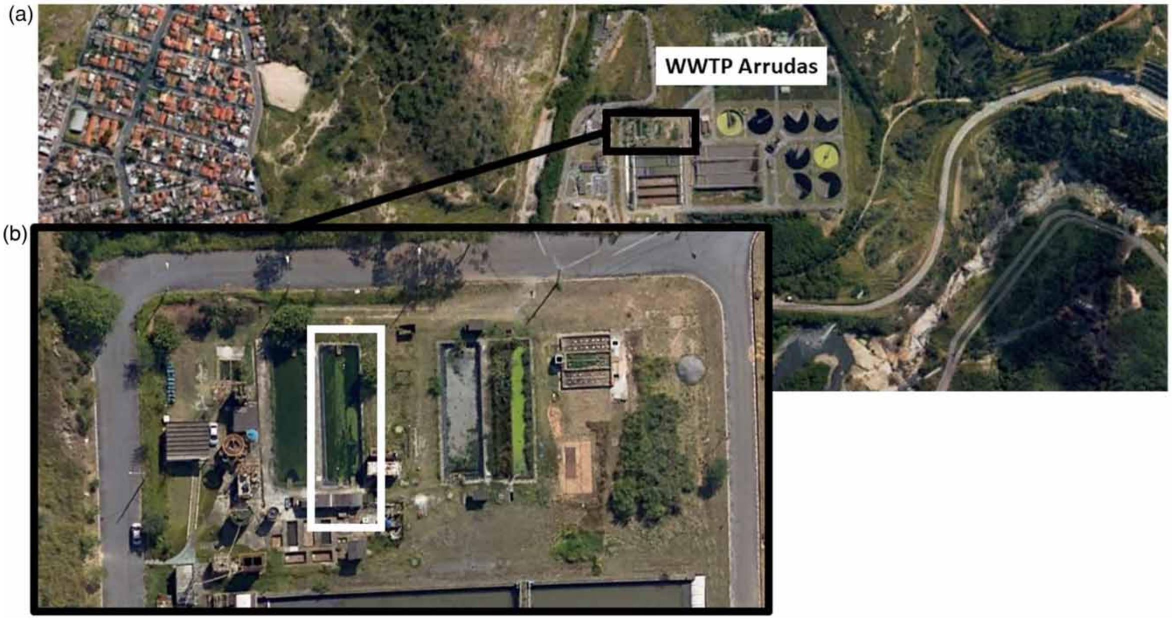


Figure 1 | Overview of Wastewater Treatment Plant (a) and Experimental Plant (CePTS) (b) with the WSPs and maturation pond of the study. White rectangle indicates the maturation pond used for the experiment. Retrieved from http://maps.google.com.br.

mean) of 147 and 161 kgBOD·ha^{-1}·d^{-1}. The system was monitored over several years, but, in this particular study, conditions were evaluated covering a complete seasonal cycle from June 2016 to May 2017, allowing evaluation of the dry (April to September) and rainy (October to May) seasons. *E. coli*, DO, pH, liquid solar radiation (RLQ) and global solar radiation (RGB) and zooplankton parameters were monthly analyzed. Chlorophyll was quantified during the same period, except for samples obtained in April and May 2017. Zooplankton community was also quantified, but data for the dry season were not obtained due to analytical artefacts. The study consisted of monthly experiments, in which an apparatus containing quartz tubes was filled with pond effluent and submerged at different depths in the maturation pond. In total, 12 experiments were performed on a monthly basis using the experimental apparatus shown in Figure 2. The quartz tubes (99.995% silicon dioxide) from ACTQUARTZO® were airtight and watertight, and were sterilized before each experiment. Each of the 18 quartz tubes was filled with 10 mL of effluent from the 1st pond of the series and submerged in the 2nd pond of the series at three different depths, hereby referred to as levels (L) 1 (10 cm deep), 2 (20 cm deep) and 3 (30 cm deep). As each tube was 10 cm in height, 10 cm layer 'slices' were characterized in the pond until 30 cm. Before filling the tubes, a fraction of 100 mL of the effluent was collected as a reference/control sample. The experimental apparatus was lowered into the 2nd pond, and remained submerged and exposed to solar radiation for 8 hours (9 am to 5 pm), acting as an isolated environment and therefore eliminating any other external interference (e.g., hydraulic stratification, sedimentation, and external environmental conditions). After 8 hours exposed to environmental conditions, the apparatus was removed from the pond and the samples were transported to the laboratory for analysis. Temperature, dissolved oxygen, and pH were quantified *in situ* with an YSI probe 556 multi-probe system during the experiments. Additional climatological data relating to average temperatures and solar radiation (RLQ and RGB) were obtained from the Centro de Desenvolvimento de Tecnologia Nuclear – CDTN (Center of Development for Nuclear Technology) at the UFMG.

Biological analyses

All samples were analyzed for the presence of *E. coli*, coliforms, chlorophyll-a and zooplankton. Analyses of total coliforms and *E. coli* were done following the enzymatic substrate technique (Colilert®), according to APHA (2012). In parallel, a portion of each sample was fixed using a 10% buffered formalin for quantification of zooplankton in an inverted microscope and settling chambers. Chlorophyll-a was extracted from filters using heated ethanol, then measured and quantified on a spectrophotometer at 665 nm wavelength, always on duplicate samples (Nusch 1980).

Statistical analyses

A principal component analysis (PCA) was performed (software PAST® vers. 2.17c) to identify the major environmental driving forces during the experiments. A Spearman correlation was performed for abiotic (temperature, DO, pH, RQL and RGB), and biotic (chlorophyll-a and *E. coli*) variables. The differences between dry and rainy seasons were assessed using the T test for each variable.

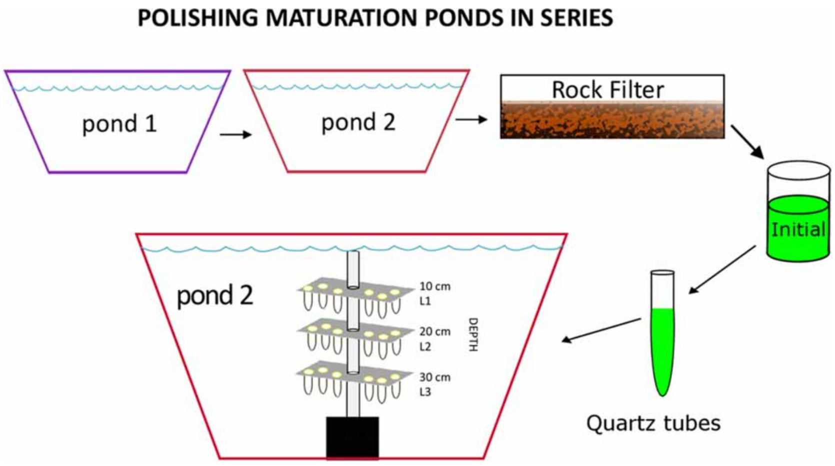


Figure 2 | Model of experimental apparatus with quartz tubes used for sampling with each depth. *Source*: Adapted from Dias 2016.

RESULTS AND DISCUSSION

Physical–chemical variables and chlorophyll-a

RLQ mean values ranged from 109.07 $W{\cdot}m^{-2}$ to 69.29 $W{\cdot}m^{-2}$ in the rainy and dry seasons, respectively (Figure 3(a)). The concentrations of DO were always high, and did not show a clear trend, with peaks in January and February (Figure 3(b)). Thus, DO concentrations were higher in the rainy (warmer) season.

The high values of DO were in accordance with Dias *et al.* (2017a), and are probably a result of solar radiation exposure with the fewer clouds during the warmer season. In the southeastern region of Brazil, where the study was performed, the rainy season is essentially in spring and summer, when higher insolation is also detected (Figure 3(a)), resulting in greater growth rates of the autotrophic community and consequently higher photosynthesis rates and DO concentration in the water. Subsequently, high photosynthetic activity with high chlorophyll-a levels were detected in the pond during the rainy season (Figure 4). However, no statistically significant pattern ($P > 0.05$) and variation between seasons and depths were observed according to the T-test for the initial sample ($P = 0.003$), L1 ($P = 0.05$), L2 ($P = 0.003$) and L3 ($P = 0.004$).

E. coli and coliforms

In general, *E. coli* densities were similar between the different depths (Figure 5). The lower reduction was observed in L3. The authors observed in most months a greater removal efficiency in the superficial layers, L1 (between 30.82–99.29% for coliforms, and 96.91–99.99% for *E. coli*) and L2 (between 14.89–99.98% for coliforms, and 69.1–99.99% for *E. coli*). The few times when the removal rate was greater in L3 (between 20.57–99.29% for coliforms, and 4.5–99.99% for *E. coli*), it was

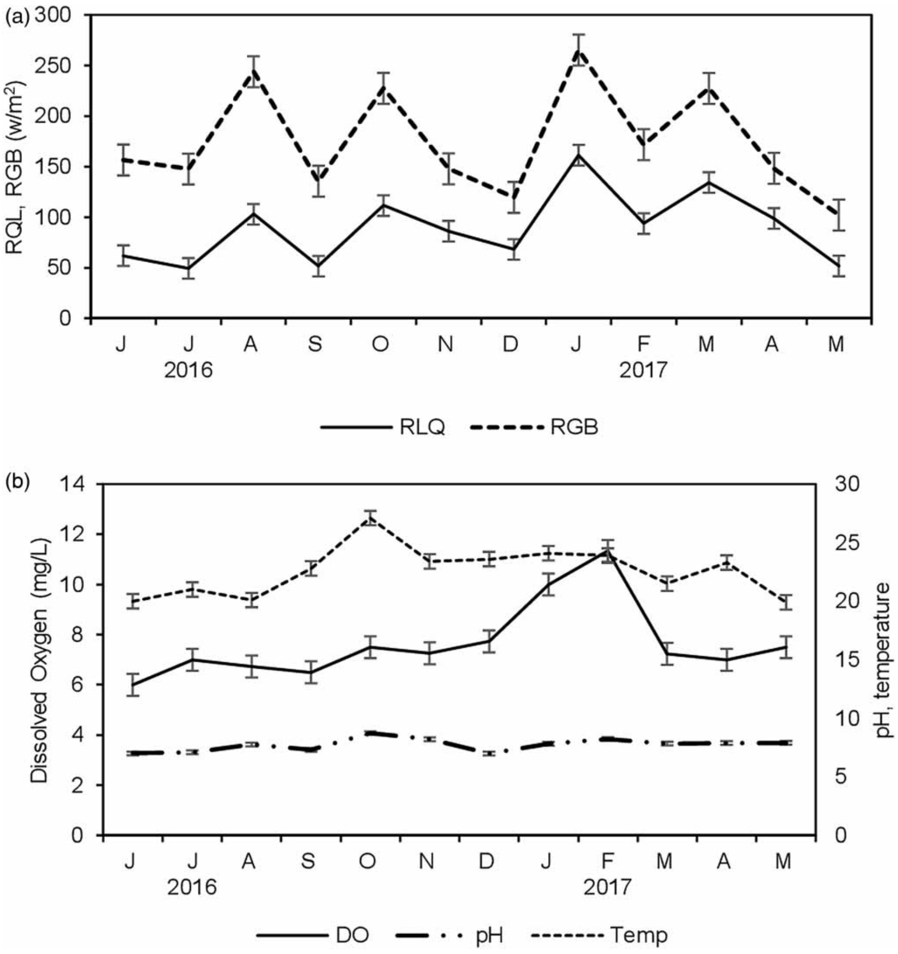


Figure 3 | Temporal variation (mean values) of Liquid Solar Radiation (RLQ) and Global Solar Radiation (RGB) (a) and DO, pH and temperature (b) along the study.

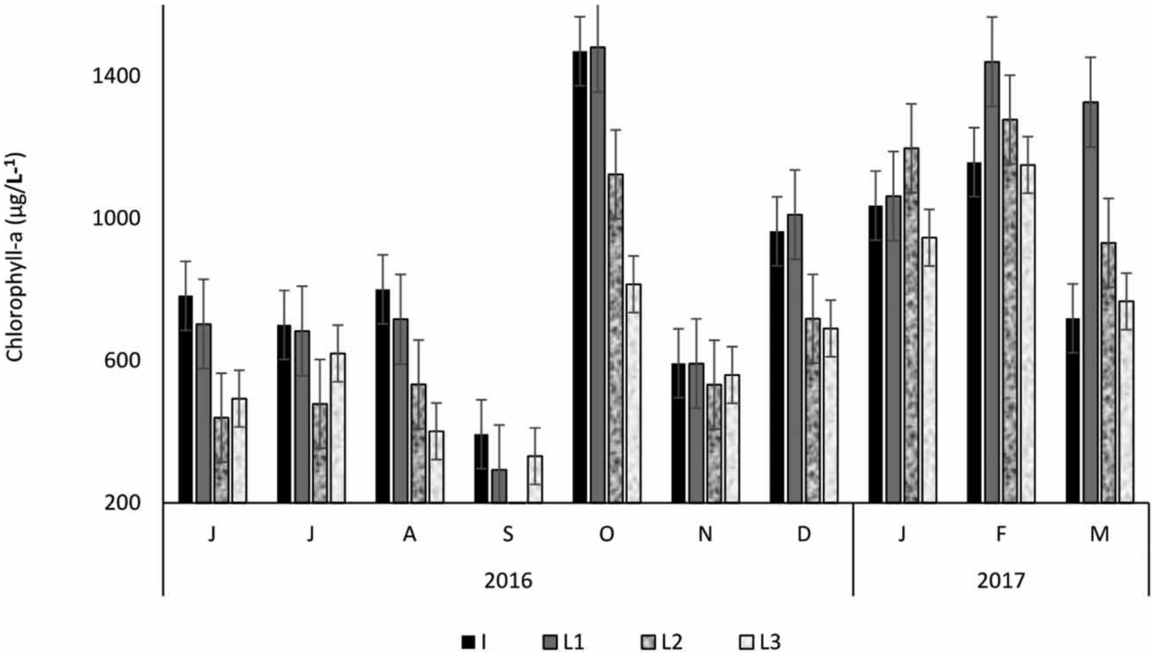


Figure 4 | Chlorophyll-a concentration in the second maturation pond from June 2016 to March 2017 in the initial sample (I) and at the three vertical levels (L1–L3).

in general, proportionally lower than that recorded for the upper layers. The results could be a consequence of UV-A and UV-B radiation only reaching a maximum depth of 10 cm (Dias *et al.* 2017b). Therefore, the incidence of UV was clearly an inhibitory factor for bacterial populations, as observed by Dias (2016) and by Dias & von Sperling (2017).

A statistically significant difference in *E. coli* concentrations by season was observed only between the surface (L1) and initial samples (control sample). During the seasons, the average concentration of *E. coli* in the control samples varied between 2.4×10^5 (dry season) and 2.7×10^8 (wet season).

Bolton *et al.* (2010) and Wallace *et al.* (2016a, 2016b) observed pH levels varying diurnally in WSPs, but almost always within a high range from 7.0 to 9.4. In the present study, the authors found similar results with the pH values varying between 7.0 and 8.7. It has been reported that these neutral to basic pH conditions contribute to removing indicator organisms (Liu *et al.* 2015).

To better understand the conditions and driving forces, a PCA procedure may be used to provide knowledge of the main processes occurring in WSPs and may act as a guide for monitoring and operating changes (Wallace *et al.* 2016a, 2016b). This multivariate statistical analysis using chlorophyll-a, UV radiation (RGB, RQL), DO, pH and temperature were evaluated to identify the driving forces responsible for the highest variance.

Observing Figure 6, it is possible to see that the first two axes together explained most of the total variance of the system (73.8%) and were related to pH (0.50) and chlorophyll-a (0.66). For each axis, the highest values indicated the most representative variables. Therefore, axis 1 explained 56% of the system's total variance and is related to pH, DO, temperature, and liquid (RLQ) and global solar radiation (RGB). Axis 2, with 17.8% of the total variance, was represented by chlorophyll-a, which was distant from the other variables.

Zooplankton dynamics

The highest densities of zooplankton organisms (protozoa and rotifers) were generally observed in June and July 2016 for all levels of the experimental apparatus (L1 > L2 > L3) (Figure 7). These months correspond to the first dry season the authors evaluate, but the same tendency was not observed for April and May 2017, which correspond to the second dry season evaluated in this study. The highest densities coincided with the lowest zooplankton diversity, showing that a few types of organisms were able to grow during this period.

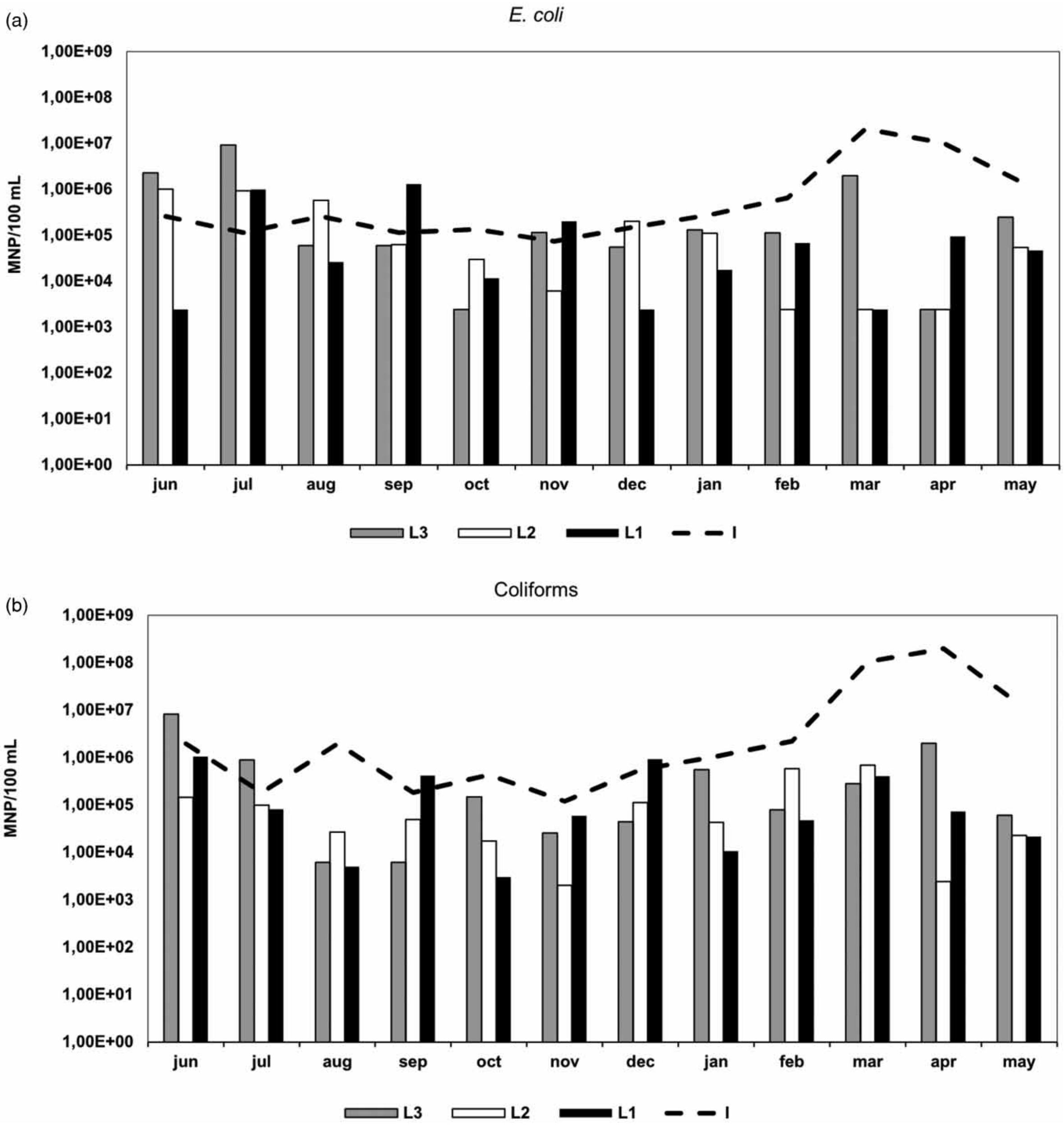


Figure 5 | (a) *E. coli* and (b) total coliforms concentration in WSP from June 2016 to March 2017 in the initial sample (I) and at three vertical levels (L1–L3).

Rotifer populations increased in L1 when compared to the initial sample, while the abundance of protozoans was relatively stable, except for L3 (Figure 7), where there would have been a high abundance of bacteria. In general, ciliates were dominant and frequently corresponded to the total or almost total protozoan community. Ciliates are generally the largest and most common protozoa in the microplankton and are important grazers of bacteria and algae in aquatic habitats. Thus, they participate in the transformation of organic matter by making it available to the upper trophic levels (Beaver & Crisman 1982, 1989).

Biological factors such as grazing by Protozoa and Rotifera have been shown to be an important mechanism in bacterial removal in systems like WSPs at deeper depths (Chabaud *et al.* 2006). With the multivariate analysis it was possible to observe that the highest densities of zooplankton organisms were related to the highest values of pH, DO, radiation and temperature,

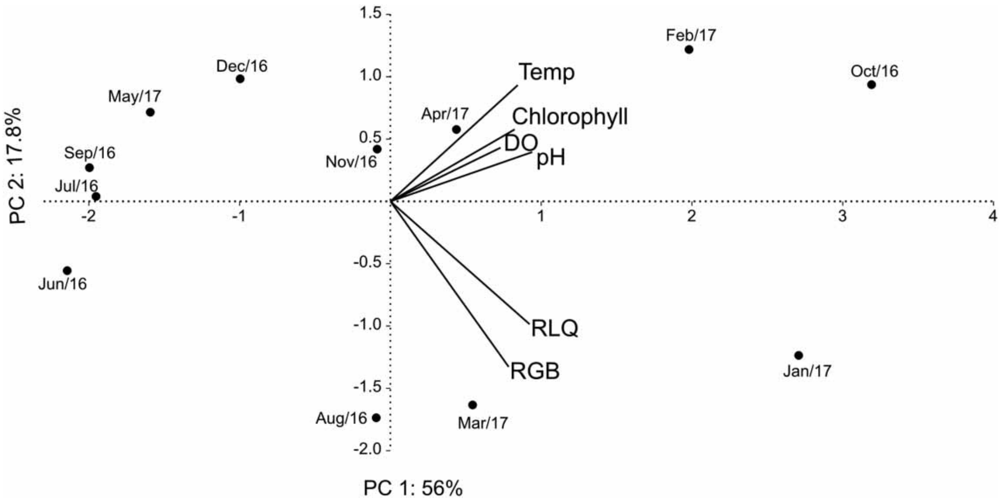


Figure 6 | PCA of abiotic variables measured in the maturation pond for Liquid Solar Radiation (RLQ) and Global Solar Radiation (RGB) from June/2016 to July/2017.

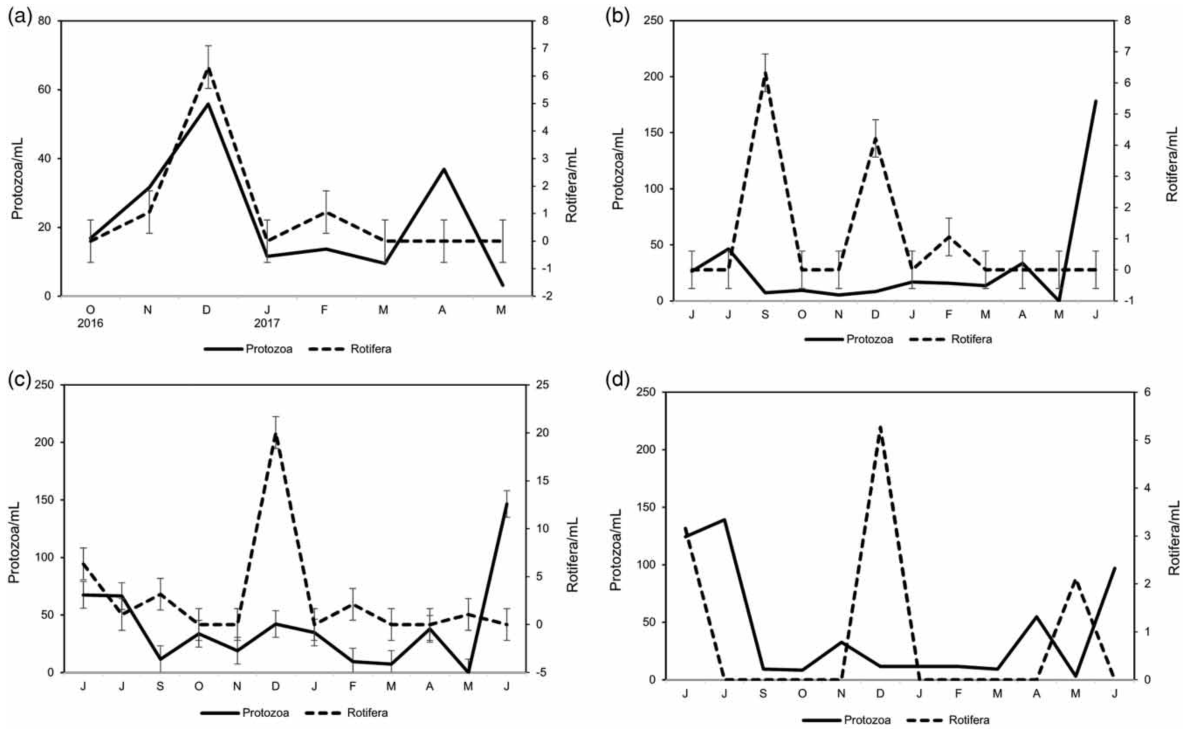


Figure 7 | Zooplankton community in the maturation pond from June 2016 to March 2017 in (a) initial sample (control sample – I) and at three vertical levels; (b) L1; (c) L2 and (d) L3. The zooplankton sample regarding August 2016 was lost due to contamination by fungi in a reagent used to fixate the samples.

showing an intimate connection between these biological community and the physical and chemical variables. Thus, these ecological interactions between zooplankton and microalgae also affected growth or decay of the coliform community, but the major driving factors were pH, DO and solar radiation.

CONCLUSIONS

A better understanding of the dynamics of pathogen removal in WSPs can assist in implementing more effective wastewater treatment in different geographical locations. As pH and DO together with solar radiation were shown to be determining factors in reducing *E. coli* in maturation ponds, this information can be used to guide wastewater treatments based on enhancing solar exposure and controlling algal growth. The interaction between different communities within the pond, allied with different environmental factors and physical attributes of the pond, govern its effectiveness and underlying mechanisms for disinfection. As shown, there is a magnitude of interactions occurring throughout the depth of the pond, with mechanisms presenting dominance during different seasons and environmental conditions. Identifying, investigating and understanding how these mechanisms and conditions play a role in the biological diversity of ponds would improve the knowledge on these systems, increase their overall efficiency, and make them more attractive as a low-cost solution, with the potential to present a highly graded effluent in bacteriological terms for different end-use purposes.

The authors highlighted the importance of ecological studies not only for understanding natural aquatic environments, but they could be performed in artificial systems and applied to important human activities. Here, the authors show that the ecological knowledge on a wastewater treatment can help us to better understand the processes and also could improve its efficiency. The more complete information can allow to improve services, assessment and monitoring operations, irrigation and anticipation of changes in loads, nutrients and bacterial communities providing sustainable solutions for developing countries such as Brazil.

ACKNOWLEDGEMENTS

The authors would like to thank CNPq, Capes, FAPEMIG, Finep and COPASA for supporting this research. The study was part of an international program funded by the Bill and Melinda Gates Foundation for the project 'Stimulating local innovation on the sanitation for the urban poor (SaniUP) in Sub-Saharan Africa and South-East Asia', coordinated by the UNESCO-IHE Institute for Water Education, Delft, the Netherlands.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 16 April 2021; accepted in revised form 26 June 2021. Available online 8 July 2021