



Microbiological water quality monitoring of four ponds from Lagoa do Sino Farm located in São Paulo State, Brazil

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ABSTRACT. This study aimed to evaluate the microbiological quality of the water of four ponds used for irrigation on the Lagoa do Sino Farm, as well as to perform the genotypic characterization of virulence factors in *Escherichia coli* isolates. Sampling was conducted for 11 months, between 2015 and 2016. Samples were analyzed for the presence of thermotolerant coliforms, *E. coli* and heterotrophs. DNA was extracted from *E. coli* isolates, followed by genotypic characterization by polymerase chain reaction. Agricultural activities and pesticides used in the sampling period were documented in order to assess possible relationships between agricultural activities and microbiological water quality. The absence of suitable riparian vegetation around all the ponds was observed, benefiting the entry of organic matter and contaminants in the water body. A high index of thermotolerant coliforms in some months indicated the possibility of the transmission of pathogenic microorganisms in these ponds. The values found in some months were above the regulatory limits for water potability and water intended for irrigation. The agrochemicals used in the period seem to influence the results obtained. All 17 *E. coli* isolates showed at least one of the virulence genes *estA*, *stx1*, *stx2*, and *aatA*, indicating enterotoxigenic, enterohaemorrhagic or enteroaggregative nature. The presence of *E. coli* in the waters may be associated with the presence of animals. The water samples analyzed are not suitable for irrigation of vegetables that are consumed raw and/or low lying fruits ingested without skin removal. It is essential to broaden the control of the use of chemicals, as well as the preservation of riparian vegetation to improve the quality of water used in the farm's agricultural activities.

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Introduction

Water is a fundamental resource for life. This resource is used in different ways to meet human needs including domestic, leisure and recreation and also economic needs such as electricity generation, wastewater reception, waterway transportation, industrial use, fishing and aquaculture. Livestock and agriculture are responsible for 70% of the water consumed in Brazil (Freitas, Freitas, & Dias-Silva, 2019).

Agriculture, especially large-scale agriculture, uses products aiming to increase production, often resulting in improper soil treatment. This in turn is exposed to rainwater resulting in surface runoff. As a consequence, there is an increase of edaphic material to water sources in the proximity, carrying organic and inorganic matter and chemical compounds responsible for surface water contamination due to erosive processes as well as pollutant leaching due to unsustainable management (Steffen, Steffen, & Antonioli, 2011). It is noteworthy that water contamination is aggravated by the destruction of riparian vegetation (Jarek, Souza, Favaretto, & Ruaro, 2016).

The chemical and ecological changes caused in the aquatic system lead to an imbalance of fauna, flora and microbiota of water bodies, which results in economic losses, particularly those related to the treatment of drinking water (Harfuch et al., 2019).

Organic matter in the water allows multiplication of undesired microorganisms responsible for another important source of contamination. *Escherichia coli* is the main bacterium representing the group of thermotolerant coliforms and it is part of the normal mammalian gut microbiota. It is an accurate

indicator of water contamination by fecal material (Ishii & Sadowsky, 2008). Diarrhoeagenic *E. coli* may be associated with intestinal infection in both children and adults, especially in developing countries. They are classified into seven major pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffuse adhesion *E. coli* (DAEC) and adherent invasive *E. coli* (AIEC) (Almeida, Barros, Arouche, & Ferro, 2012; Schuroff, Lima, Burgos, Lopes, & Pelayo, 2014; Souza, Melo, & Melo, 2016; Yu et al., 2018).

Detection of *E. coli* in water indicates possible contamination by other important pathogens such as the bacterial *Salmonella* spp., *Shigella* spp. and *Campylobacter*, protozoa such as *Cryptosporidium* and *Cyclospora* and also viruses such as Hepatitis A, norovirus and even the recently identified SARS-CoV-2, the etiological agent of COVID-19 (Ashbolt, 2004; Stine, Song, Choi, & Gerba, 2005; Heller, Mota, & Greco, 2020). The presence of microorganisms in irrigation water subsequently contaminates the various agricultural products, especially those with large and irregular surface area (Silva, Lima, Queiroz, Jacome, & Jacome Junior, 2016). Thus, water and foodborne diseases represent an important public health problem.

According to the Food Disease Outbreak Surveillance System, during 2009-2015, among 2,953 outbreaks with a single confirmed etiology in the United States of America and Puerto Rico, norovirus was the most common cause of outbreaks and outbreak-associated illness, followed by *Salmonella*. Outbreaks caused by *Listeria*, *Salmonella* and EHEC were responsible for 82% of all hospitalizations and 82% of deaths reported in this period (Dewey-Mattia, Manikonda, Hall, Wise, & Crowe, 2018). In Brazil, of the 13,163 foodborne disease outbreaks notified during 2000-2018, at least 35.8% of the etiologic agent identified were bacterial with *E. coli* the second most commonly identified pathogen in the registered outbreaks (Finger, Baroni, Maffei, Bastos, & Pinto, 2019).

Monitoring of water quality used for different purposes, including irrigation, has been subject of several research studies (Scherer, Granada, Stülp, & Sperotto, 2016; Ramos, Mafra, Rech, Siegloch, & Rech, 2018; Melo & Queiroz, 2020; Nunes-Carvalho et al., 2020). It is worth mentioning that contamination of these waters by thermotolerant coliforms and/or *E. coli* has been frequently reported.

Therefore, this study aimed to monitor the microbiological water quality of pond water intended for irrigation use, over 11 months and to genotypically characterize isolates of *E. coli* for pathogenicity. The sampling took place in four ponds on the Lagoa do Sino Farm, located in the state of São Paulo, Brazil.

Material and methods

Studied area and sampling

Lagoa do Sino Farm is located in the municipality of Buri in the State of São Paulo, Brazil (latitude 23°35'58.01" S and longitude 48°31'46.66" W). It has an area of 643 ha, with central pivot irrigation in approximately 100 ha. Intensive production is concentrated in corn, soybean and wheat crops, which require large amounts of water, fertilizers and agrochemicals for production. To a lesser extent, beans, squash, cassava, vegetables, fruits are produced, all of which are consumed by farm workers and students. Furthermore, there is an agroforestry system (AFS). It is noteworthy that since 2013, the Center for Natural Sciences of the Federal University of São Carlos is located on the premises.

The water samples were collected from four of the nine existing ponds on the farm in order to represent the altitudinal gradient, testing the hypothesis that the agricultural activity on the farm promotes an accumulation of pollutants in the water system. All the reservoirs belong to the same watercourse (Figure 1). Thus, the ponds included in this sample design were P1; located at the highest altitude (latitude 23°36'48.00" S and longitude 48°33'9.76" W), followed by P2 (latitude 23°36'10.41" S and longitude 48°32'12.11" W), P3 (latitude 23°35'49.94" S and longitude 48°31'47.06" W) and P4 (latitude 23° 35'43.55" S and longitude 48°31'14.42" W) (Figure 1).

The composite samples were collected in sterilized glass bottles. Water was collected at three distinct points and then mixed to form a single homogenized sample. Water collection was superficial and occurred between 0 and 30 centimeters from the water depth. After collection, the material was refrigerated and stored in an isothermal box (Brandão, 2011).

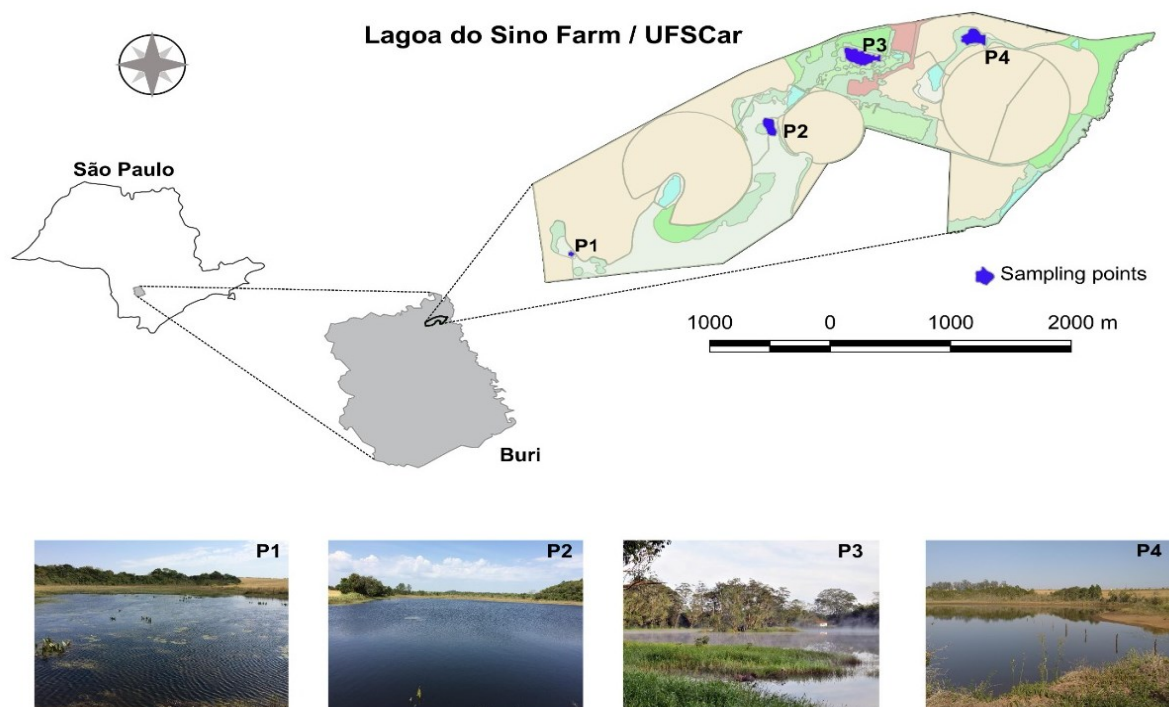


Figure 1. Location of the sampling points at Lagoa do Sino Farm.

Microbiological analysis

Microbiological analysis of water samples was performed as described (American Public Health Association [APHA], 2012). Briefly, for heterotrophic bacteria, serial dilutions were plated on Standard Method Agar (Himedia, India) and plates were incubated at 37°C for 48 hours. Total coliforms were assessed presumptively on Lauryl Tryptose Broth (Micro Med, Brazil) and confirmed on Brilliant Green Bile Broth (BGBB - Himedia, India) both incubated at 35°C for 48 hours. For thermotolerant coliforms, aliquots from BGBB positive tubes were transferred to tubes containing EC Broth (Himedia, India) following incubation for 48 hours at 44.5°C. Finally, *E. coli* was detected streaking aliquots from EC broth positive tubes on Eosin Methylene Blue Agar (EMB – TM Media, India) following incubation for 24 hours at 37°C.

Escherichia coli isolation

Escherichia coli typical colonies were confirmed by Gram dye and by polymerase chain reaction; detecting *uidA* gene codifying the β -glucuronidase enzyme. The primers *uidA1* (5'-TGTTACGTCCTGTAGAAAGCCC-3') and *uidA2* (5'- AAAACTGCCTGGCACAGCAATT-3'), were used, generating an amplicon of 153 pb. Confirmed isolates were transferred to Nutrient broth and after incubation for 18 hours at 35°C, they were stored at – 20°C in 20 % glycerol.

Detection of genes associated with virulence factors

DNA extraction from water isolates was performed according to Cocolin, Manzano, Cantoni and Comi (2001).

PCRs were performed in order to detect eight virulence codifying genes: *eltB* and/or *estA* (structural genes for LT and ST toxins, respectively, for ETEC); *stx1* and/or *stx2* (Shiga toxin 1 and 2 for EHEC); *eaeA* (intimin for EHEC and EPEC); *ial* (invasion associated locus in EIEC); *bfpA* (bundle-forming pilus structural gene for EPEC) e *aata* (anti-aggregation protein transporter from EAEC plasmid) (Nguyen, Le Van, Huy, Gia, & Weintraub, 2005). The primers are presented in Table 1.

PCRs were performed using GoTaq® PCR Master Mix (Promega Corporation, Madison, Wisconsin, USA) according to instructions provided by the manufacturer. Thermocycler (Applied Biosystems, Foster City, California, USA) was used under the following conditions: an initial denaturation cycle at 94°C for 2 min., 34 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 40 s and extension at 72°C for 40 s, with a final extension cycle at 72°C for 4 min. (Holland, Louie, Simor, & Louie, 2000). PCRs were mixed with UVView™ 6x Loading Dye (Bio-Rad Laboratories, California, USA) and subjected to 2% agarose gel electrophoresis in TBE

buffer (45 mM Tris-borate and 1 mM EDTA) prepared according to Green and Sambrook (2012). The 100 bp DNA Ladder marker (Ludwig Biotec, Brazil) was used to ascertain whether the size of the amplicons corresponded to the expected size for each fragment. The images were visualized under ultraviolet light emission (TFX - 35M, Vilber Loumart, France).

Table 1. Primers sequence used to amplify genes able to codify virulence factors in *Escherichia coli* (Nguyen et al., 2005).

Target gene	Primers	Sequence (5'-3')	Amplicon size (pb)
<i>eltB</i>	<i>eltB 1</i>	TCTCTATGTGCATACGGAGC	322
	<i>eltB 2</i>	CCATACTGATTGCCGCAAT	
<i>estA</i>	<i>estA 1</i>	GCTAAACCAGTA ^c _A GGTCTTCAAAA	147
	<i>estA 2</i>	CCCGGTACA ^c _A GCAGGATTACAACA	
<i>stx1</i>	<i>vt1 1</i>	GAAGAGTCCGTGGGATTACG	130
	<i>vt1 2</i>	AGCGATGCAGCTATTAATAA	
<i>stx2</i>	<i>vt2 1</i>	ACCGTTTTTCAGATTTT ^c _A CACATA	298
	<i>vt2 2</i>	TACACAGGAGCAGTTTCAGACAGT	
<i>eaeA</i>	<i>eaeA 1</i>	CACACGAATAAACTGACTAAAAATG	376
	<i>eaeA 2</i>	AAAAACGCTGACCCGCACCTAAAT	
<i>ial</i>	<i>ial 1</i>	CTGGTAGGTATGGTGAGG	320
	<i>ial 2</i>	CCAGGCCAACCAATTATTTCC	
<i>bfpA</i>	<i>bfpA 1</i>	TTCTTGGTGCTTGCGTGTCTTTT	367
	<i>bfpA 2</i>	TTTTGTTTGTGTATCTTTGTAA	
<i>aatA</i>	<i>pCVD 1</i>	CTGGCGAAAGACTGTATCAT	630
	<i>pCVD 2</i>	CAATGTATAGAAATCCGCTGTT	

Inventory of agricultural and agrochemical activities and weather conditions

During the sampling period, an inventory of agricultural activity such as soil crop types and agrochemical used was carried out in order to correlate the use of agrochemicals to changes in the water microbiological profile.

Data of air temperature (°C) and rainfall (mm) were obtained from the *Instituto Nacional de Pesquisas Espaciais* (INPE).

Statistics

Microbiological data were analyzed by descriptive statistics and presented by bar plots, box diagrams and time series charts to summarize the distribution of thermotolerant coliforms, *E. coli* and heterotrophics. Heterotrophic counts (CFU mL⁻¹) were log₁₀ transformed. The statistics Pearson's ϕ and Cohen's κ were calculated to infer about the correlation and concordance of the presence/absence of thermotolerant coliforms and *E. coli* (Warrens, 2008; Ranganathan, Pramesh, & Aggarwal, 2017).

All statistical analyzes and graphs were obtained by using the statistical R software (R Core Team, 2019).

Results

Microbiological water quality of ponds from Lagoa do Sino Farm

Throughout 11 months, the water quality of four pounds (P1, P2, P3, and P4) from Lagoa do Sino Farm were monitoring. P1 is devoid of adequate riparian vegetation and it is very close to a spring. It was observed that the region is influenced by neighboring farms agricultural activities. During sampling, the occurrence of thermotolerant coliforms and, consequently, the presence of *E. coli* in this sample location was verified in October and November 2015 and February 2016, especially the latter, with high detection (930 NMP. (100 mL)⁻¹) (Figure 2). In October, precipitation was equivalent to 171.1 mm with an average temperature of 26°C. In February, the precipitation was 205 mm with a temperature of 29°C.

In the whole study area, the soil is characterized as red latosol, lacking vegetation cover and adequate level curves for agriculture in several points which, according to Lima et al. (2013), favors erosive processes. With the exception of March 2016 (160 CFU mL⁻¹) all other months demonstrated heterotroph counts higher than 2,000 CFU mL⁻¹ (Figure 3). Heterotrophs are associated with organic matter.

P2 is also devoid of adequate riparian vegetation. During the sampling months, there was a decline in the MPN of thermotolerant coliforms between October and December 2015. From March to July 2016, the MPN for thermotolerant coliforms remained below the detection limit of the technique (<3 MPN (100 mL)⁻¹) (Figure

2). Apart from December, where thermotolerant coliforms were enumerated, *E. coli* was also present. For heterotrophic bacteria, the only months in which the count of this microbial group was below 500 CFU mL⁻¹ were April and May 2016 (Figure 3). According to the farm managers responsible for agricultural activities, the agrochemicals applied near P2 varied according to the period and type of crop (soy or corn). In March and April 2016, pesticides Losban, Galil, Orthene, Tracer and Bazuka were applied for pest control, Soberan and Atrazine for weed inhibition and Priori Extra for fungus control.

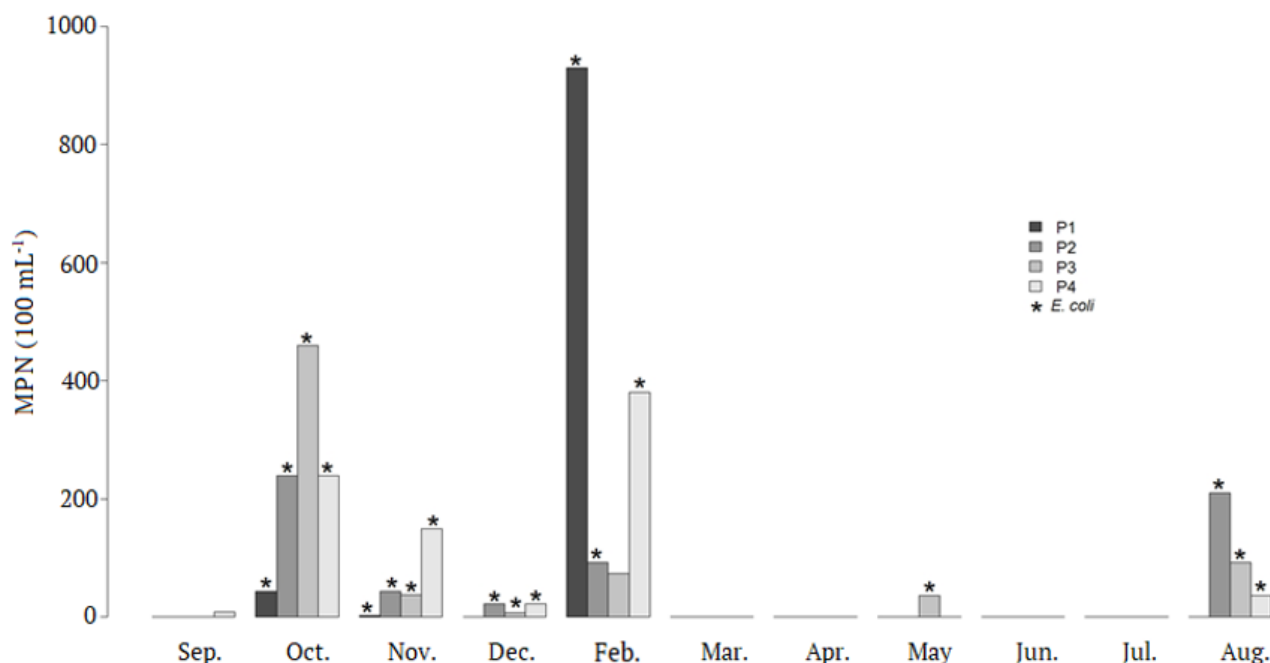


Figure 2. Thermotolerant coliforms distribution and presence of *Escherichia coli* in the water samples from four ponds on Lagoa do Sino Farm. The lower detection limit of the applied technique was 3 MPN (100 mL)⁻¹ and, therefore, absence of bars for the sampling period indicates values under lower detection limit and not necessarily absence of the microbial group. *E. coli* presence in the sample is qualitatively indicated by the * signal.

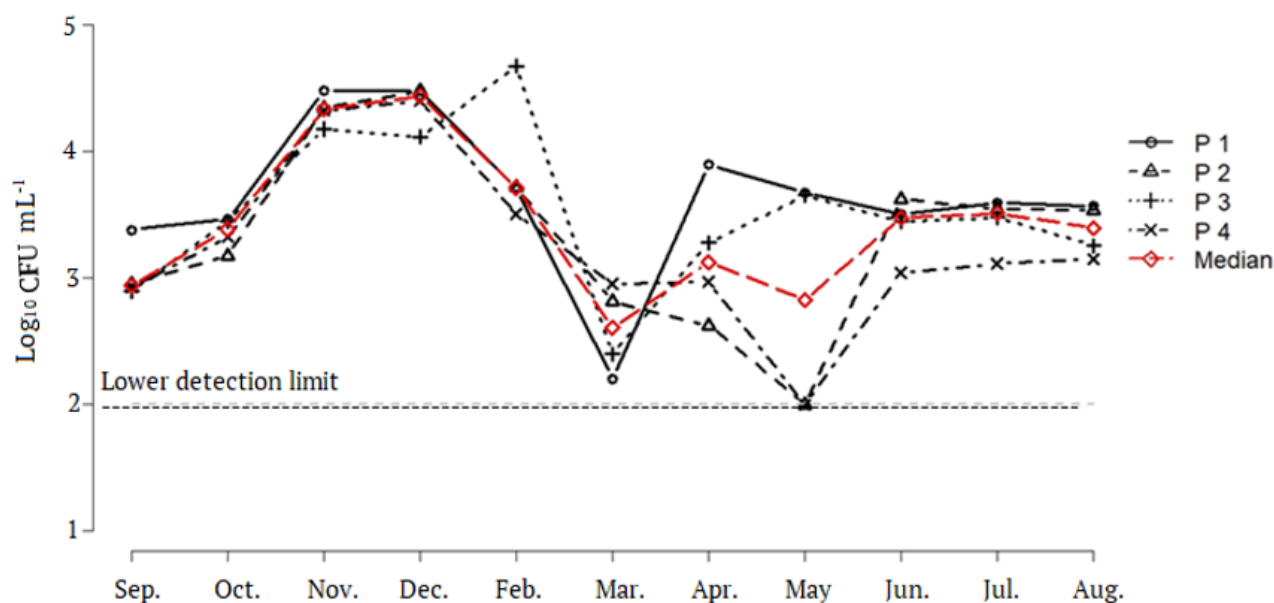


Figure 3. Heterotrophs counts in water samples from four ponds on the Lagoa do Sino Farm. The lower detection limit of the applied technique was 100 CFU mL⁻¹ (grey line) and, therefore, months where values reach the lower detection limit indicate values below the lower detection limit and not necessarily absence of the microbial group. The red line indicates the global contamination profile based on the median of the observed values per month for the four ponds. Lines connecting the points have been added to facilitate viewing.

P3 is influenced by nearby sheep and anthropogenic action, as since its location is easily accessible; an ecotourism trail lies nearby. Its surroundings are devoid of riparian vegetation and it receives effluents from

the previous ponds. The highest presence of thermotolerant coliforms was detected in October 2015 (Figure 2). Although in November, December, May and August the MPN of thermotolerant coliform in the water was below 100, *E. coli* was detected in all water samples. Heterotrophics remained at high concentrations in all sampled months, except for March, 250 CFU mL⁻¹. Among all the samples collected during the study period in the four sampling points, the waters of P3 in February reached the highest contamination level, at 47,000 CFU mL⁻¹ (Figure 3). In the months of May and June, rainfall ranged from 50 to 100 mm and the average temperature was 17°C.

Regarding agrochemicals applied near P3, in November and February there was heavy use of products designed for fungal control (Opera and Comet), pest control (Fastac, Imidacloprid, Bazuka Pirate and Game) and for weeds inhibition (Roundap, 2,4D and Soberan).

P4 is located approximately 4.2 km from P1, where runoff occurs on a sloping bed. At this point there are very close plantations and absence of adequate riparian vegetation in the surroundings. The reception of effluents at P4, from the previous ponds increases the amount of existing organic matter. By receiving effluents from all other collection points (P1, P2 and P3), P4 is a more unstable environment in comparison. In the rainy months (October, November, December and February), where precipitation ranged from 200 to 250 mm, the presence of thermotolerant coliforms and *E. coli* was notable. In September, thermotolerant coliforms were detected, but not *E. coli* (Figure 2). With the exception of March, where the concentration was below the detection limit (<100 CFU mL⁻¹), all other months had counts of heterotrophic bacteria above 500 CFU mL⁻¹.

The detectable levels of thermotolerant coliforms and the presence of *E. coli* in the 44 samples (Figure 2) were significant and positively correlated ($\phi = 0.911$, $\chi^2 = 32.92$, $p < 0.001$). The concordance between these detections was given $\kappa = 0.908$, i.e. almost perfect. Then, thermotolerant coliforms above the detection levels almost surely indicated presence of *E. coli* in the water samples.

Summarizing, while thermotolerant coliforms were less frequent at P1, in which only 27% of the analyzed samples had shown values above the lower detection limit, their presence were more frequent at the remains sample points (P2, P3 and P4) (Figure 4). On the other hand, distribution of heterotrophic counts was very similar among all the sampling points. Only two results of the 44 analyzed samples (4.5 % of results) were undetectable. All the other observations had shown Log₁₀ CFU greater than 2, which corresponds to counts superior than 100 CFU mL⁻¹ (Figure 4).

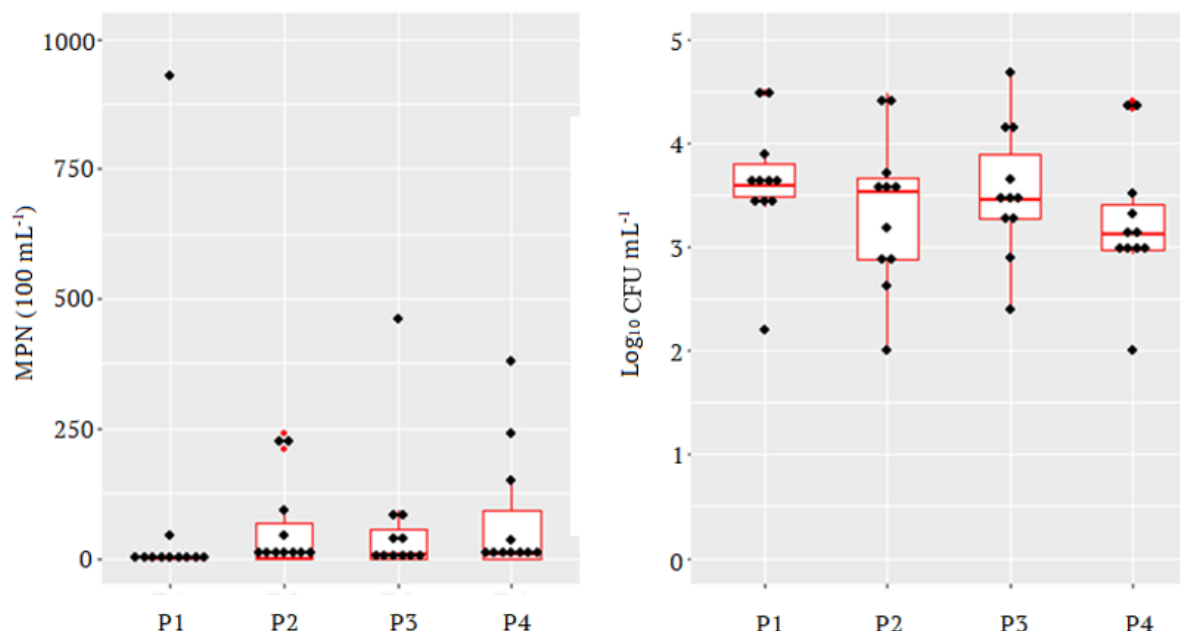


Figure 4. Distribution for thermotolerant coliforms (MPN (100 mL⁻¹)) and heterotrophics (Log CFU mL⁻¹) bacteria over 11 months of sampling.

Genotypical characterization of virulence factors associated to pathogenic *Escherichia coli*

From the four analyzed sampling points during 11 sampling stages (44 samples), 17 *E. coli* isolates were also confirmed by molecular detection of the presence of the constitutive gene *uidA*, whose product is the enzyme β -glucuronidase. Table 2 shows the positive isolates as well as their respective point and month of collection.

Table 2. Confirmed *Escherichia coli* isolates and its respective month point of sampling.

Month	Sampling point	Isolate reference number
Oct/2015	1	1
	2	2
	3	3
	4	4
Nov/2015	4	6
Dec/2015	1	9
	2	10
	4	14
Feb/2016	1	15
	1	16
	1	17
	2	18
	4	20
	4	21
	4	22
	4	23
	3	24

The ponds with the highest incidence of *E. coli* were P1 and P4, contributing 7 isolates (41%) and 5 isolates (29%), respectively. Three isolates (18%) were obtained from P2 while only 2 isolates (12%) were recovered from P3. The months with the highest number of positive *E. coli* isolates were October 2015 and February 2016.

Of the 17 *E. coli* isolates, all contained characteristic pathogenic *E. coli* virulence genes (Table 3).

Table 3. Distribution of virulence genes and classification of *Escherichia coli* isolated from four ponds of Lagoa do Sino Farm.

Isolate	<i>estA</i>	<i>stx1</i>	<i>stx2</i>	<i>aatA</i>	Category
1	+				ETEC
2	+				ETEC
3	+				ETEC
4		+	+		EHEC
6		+	+		EHEC
9	+				ETEC
10		+	+		EHEC
14		+			EHEC
15		+			EHEC
16		+	+		EHEC
17				+	EAEC
19		+			EHEC
20		+	+		EHEC
21		+	+		EHEC
22		+	+		EHEC
23		+			EHEC
24			+		EHEC

Twelve isolates (70.6%) were classified as EHEC due the presence of *stx1* and/or *stx2* genes, whose transcription and translation give rise to the Shiga toxin (Table 3). The presence of the *eaeA* gene confirms the detection of a typical EHEC isolate, although its presence is not essential. No isolate tested positive for the presence of *eaeA* gene, which encodes intimin, a membrane protein that mediates intimate adherence and contributes to the focusing of cytoskeletal proteins beneath bacteria (Jerse, Yu, Tall, & Kaper, 1990; Kaper, Nataro, & Mobley 2,004). Thus, among isolates classified as EHEC, 41.1% had the combined presence of *stx1* and *stx2* genes. However, while 23.5% of isolates had only the *stx1*, 5.9% of the isolates evaluated had only the *stx2* gene (Table 3).

The presence of the *eltB* and/or *estA* genes confirms the ability of the isolate to produce enterotoxin, characteristic of the ETEC pathotype. Although no isolates had the *eltB*, four isolates (23.5%) were classified as ETEC since they had the *estA* gene, confirming the isolate's ability to produce thermostable enterotoxin.

Among all *E. coli* isolates, only one (5.9%) was classified as EAEC based on the presence of *aatA* gene (Table 3).

The genes *eaeA*, *ial* and *bfpA* were not identified in any isolates evaluated and, therefore, no isolates were classified as EPEC or EIEC.

Discussion

According to Vieira, Atayde, Carvalho, Carvalho and Fonteles Filho (2008), the variation in the bacterial population occurs due to many factors, such as changes influenced by season, temperature, dissolved oxygen, conductivity, particulate matter, rainfall, among others.

It was found that all ponds do not have adequate riparian forest. Such deficiency benefits the entry of organic matter into the water body, as a consequence of an erosive process that contributes to water contamination (Jarek et al., 2016). It is important to note that in warmer months, there is an increase in fauna activity representing an increase in the impact on the microbiological quality of waters, expressly in remote areas, with little anthropogenic interference, such as P1, P2 and P4 (Moraes, Tauk-Tornisielo, & Ventorini, 2012).

The National Environmental Council (*Conselho Nacional do Meio Ambiente*), through Regulation No. 357 of March 17, 2005 (Brasil, 2005) classifies waters intended for irrigation into three categories based on the type of crop to be irrigated and establishes contamination limits for thermotolerant coliforms. Water intended for irrigation of vegetables that are consumed raw and low lying fruits; ingested without skin removal are classified as class 1 and may not exceed 200 thermotolerant coliforms per 100 mL of water. Class 2 encompasses water intended for irrigation of vegetables, fruit and park plants, gardens, sports fields and leisure with a limit of 1,000 thermotolerant coliforms per 100 mL of water. Finally, water used for irrigation of arboreal, cereals and forages is classified as class 3, with a limit of 4,000 thermotolerant coliforms per 100 mL of water (Brasil, 2005). According to this regulation, which is the current legislation with regard to irrigation water, all ponds had thermotolerant coliform counts below the limits established for water used to irrigate vegetables and fruit plants (class 2), arboreal, cereal and forages (class 3), those being the main groups cultivated at Lagoa do Sino Farm. On the other hand, on a smaller scale, there is the cultivation of vegetables that are consumed raw and, for their irrigation, in October 2015, P1, P3 and P4 presented contamination by thermotolerant coliforms above the limit established for class 1 water (200 thermotolerant coliforms per 100 mL). In February 2016, P1 and P4 also exceeded the limit set for irrigation of raw consumed vegetables as well as P2 in August 2016 (Figure 2).

The CONAMA (Brasil, 2005) regulation does not establish criteria for heterotrophs count limits. However, when assessing water quality this is an important parameter, as it is indicative of the presence of organic matter in water, that can be used as a source of energy by various microbial groups, including pathogens. The water quality monitoring agencies establish that excessive concentrations of heterotrophic bacteria can suppress coliform development and thus interfere with the recovery of this group (Maier, Pepper, & Gerba, 2009). Thus, counts above the detection limit are extremely worrying, as they favor the onset of waterborne diseases (Domingues et al., 2007).

The constant high levels of heterotrophic bacteria at all collection points (Figure 3), every month, indicated the presence of organic matter in the ponds, which is natural in green areas (Schuroff et al., 2014). Factors such as phosphorus and nitrogen addition through fertilizers can increase the multiplication of heterotrophic bacteria (Barreiros, Manaia, & Nunes, 2011). Importantly, within certain limits, the presence of this microbial group is expected, since heterotrophic bacteria are part of the aquatic biota, integrate the local fauna and, when in a low population, indicate low amount of organic matter (Guerra, Otenio, & Silva, 2006).

The decay of thermotolerant coliforms and *E. coli* in some sampling months may be related to the application of agrochemicals in the surrounding plantations. Such occurrence may have directly affected water quality, due to the accumulation of chemical agents in the soil.

Some products are considered extremely toxic, especially Atrazine, which is a potential water contaminant due to its high soil persistence, slow hydrolysis, low to moderate solubility and moderate absorption of organic matter and clay (Arantes et al., 2006). It is noteworthy that some bacteria present in the soil help in the degradation process of some types of agrochemicals. However, the amount of product applied as well as its toxicity may inhibit the microbial degradation activity leading to water course contamination and, consequently, changes in the local biota leading to death or resistance (Pereira & Freitas, 2012). Studies by Strong, Mctavish, Sadowsky and Wackett (2000) show that *E. coli* can potentially be employed in the biodegradation of this type of pollutant.

Balagué, Sturtz, Duffard and Duffard (2011) studied the in vitro effects of some herbicides on *E. coli* growth, finding that exposure to 1 mM 2,4-D decreased growth and total protein content in all *E. coli* strains tested. In addition, successive exposures to 0.01 mM 2,4-D produced a toxic effect interfering with cell growth. Studies conducted by Botelho, Froes and Santos (2012) evaluated the toxicity of the main herbicides used in

crops on *E. coli* growth. The herbicides considered desiccant (non-selective), Gliz, Roundup and Gramoxone, interfered with the exponential growth of the bacteria, particularly in the first 100 minutes of cultivation in the presence of agrochemicals.

Another factor that may be related to coliform decay is low rainfall, since the dilution of agrochemicals in these periods is not effective. With the reduction of rainfall and lower temperatures, the receipt of water from the ponds above its level also decreases, as well as the drainage of organic matter by washing the soil, such as P2, P3 and P4. The reduction of coliform contamination in the sample point P3 may also be associated with the removal of sheep from the area around this pond in May 2016.

The presence of wild boar has already been registered in the Farm (visual, footprints and feces), mainly in areas close to corn and soybean crops. Corn is the most consumed cereal by wild boars (Torres, Ambrósio, Lopes, Cancela, & Fonseca, 2012). Leal, Tonello, Dias and Mingoti (2017) by characterizing the springs of the Itanguá Stream basin (also located in the Southwest of São Paulo State) attributed the presence of wild boar, the water contamination (observing faecal and urine) and destruction of native vegetation due to trampling and feeding. Kaller, Hudson III, Achberger and Kelso (2007) found that the presence of wild boars near water bodies can compromise water quality due to contamination by microorganisms, in addition increased erosion at the banks due to wallowing behavior. Locality is a determining factor for such results, since the more remote the locality, as in the case of P1 and P4, the higher the incidence of such wild animals.

Interestingly, isolates confirmed as *E. coli* were detected in soybean (October and November 2015) and corn (February, March and April 2016) planting periods. This information supports the suggestion that contamination of the ponds water by *E. coli* may be related also to the presence of boars seeking for food. It is worth mentioning that seasonality also influences the results obtained as verified by studies conducted by Moraes et al. (2012) that related water contamination to the seasonal period.

Escherichia coli is widely used as a major bacterial indicator of fecal contamination due to its limited survivability in low organic matter environments, as expected from water intended for human consumption. However, recent studies have suggested the adaptive capacity of *E. coli* in adverse environments (Titilawo, Obi, & Okoh, 2015). Thus, it is suggested that the constant presence of agrochemical residues in the studied environment may induce mechanisms of resistance to such bacteria allowing them to remain longer in this type of environment.

Studies conducted by Drumond, Santiago, Moreira, Lanna, and Roser (2018) isolated from the surface waters of the Xopotó River Basin, in the Alto Rio Doce Region, Minas Gerais, 103 *E. coli* positive colonies. Of these, two isolates were classified as ETEC, two as EHEC and three as EPEC. These results confirm the contamination of surface waters with diarrheagenic *E. coli* categories, indicating risks associated with the use of these waters.

The EHEC pathotype, found in the waters of Lagoa do Sino Farm, causes hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans, and its major virulence factors include intimin and Shiga toxins (Drumond et al., 2018). Fruits and vegetables are the main foods involved in cases and outbreaks of this type of food infection (Mittelstaedt & Carvalho, 2006). Some studies have shown that strains hosting only *stx2* gene are potentially more virulent than those carrying the *stx1* gene or even strains carrying both genes (Nataro & Kaper, 1998).

This scenario is worrying, considering that the ETEC category is the most frequently isolated bacterial enteric pathogen in children under 5 years of age in developing countries. It accounts for approximately 300 million cases of diarrhea and 380,000 deaths annually, with food and water being the main routes of transmission (Vidal et al., 2005; Walk, Alm, Clahoun, Mladonicky, & Whittam, 2007). The presence of the *estA* gene associated with ETEC strains has also been reported by Ishii and Sadowsky (2008) as being common in surface waters and its origin comes from diarrhea events in humans and swine. This factor may be linked to P1 contamination, where there were reports by Lagoa do Sino Farm employees of the frequent wild boars sightings in their surroundings.

The EAEC category, identified in this study is considered an emerging pathogen and is associated with acute diarrhea in both developed and developing countries (Moura et al., 2012). They do not secrete toxins but cause chronic or persistent diarrhea. Antimicrobial resistance is considered one of the most relevant phenomena and contributes to the increase in mortality rates by this pathotype (Regua-Mangia, Gomes, Vieira, Irino, & Teixeira, 2009).

Conclusion

According to current Brazilian legislation, the water from the sampled points is only suitable for use in irrigation of vegetables, fruit, arboreal, cereals and forages. Control measures should be taken to recover the pondshore since the absence of riparian forest facilitates the entry of contaminants into the waters. The pathogenic *E. coli* incidence in the waters is a worrying factor due to the great risk associated to foodborne disease in consumers. It is also recommended that future work be carried out aiming to monitor the contamination by chemical agents in the waters, as these may accumulate in existing fauna.

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