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Monitoring and evaluation of alternative collective solution for water supply of public schools in Itatiba, SP

Monitoramento e avaliação da qualidade da água de solução alternativa coletiva de abastecimento de escolas públicas do município de Itatiba, SP

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ABSTRACT

Introduction: Security of water supply is extremely important for public health, especially for school-age children who can stay in schools for five to eight hours a day. Water can contain a variety of contaminants that, at high levels, have been linked to a range of diseases in children. **Objective:** To evaluate, during 12 months, the quality of well water used as collective alternative supply solutions in 10 public schools in the city of Itatiba (SP). **Method:** Samples of water from the wells, reservoirs and drinking water were collected, totaling 100 samples, and chemical, physical, organoleptic and microbiological parameters provided for in Consolidation Ordinance No. 5, of September 28, 2017, Annex XX, were analyzed; additionally, a research was done related to the presence of foreign matter and protozoa *Cryptosporidium* spp. and *Giardia* spp. **Results:** Only three schools presented results in accordance with the legislation; the other had the presence of microorganisms (29.0% of total coliform and 9.0% of *E. coli*, in the total of analyzed samples), protozoa (15.0%), and of results above the maximum permitted value (MPV) for apparent color (8.0%), turbidity (11.0%), Fe (12.0%), Zn and Pb (5.0%). **Conclusions:** The results in disagreement with the legislation interfere with the water quality offered in the schools, being associated with the lack of investment in wells infrastructure and chlorination of the water. It is also observed a differential in the school that has partnership with the water treatment company of the municipality. This partnership should be maintained and, if possible, expanded to other schools.

KEYWORDS: Water Potability; Protozoan; Polymerase Chain Reaction; Sanitation; Child

RESUMO

Introdução: A segurança do abastecimento de água é de extrema importância para a saúde pública, principalmente para crianças que, em idade escolar, podem permanecer de 5 h a 8 h por dia nas escolas. A água pode conter uma variedade de contaminantes que, em níveis elevados, têm sido associados ao aumento de uma série de doenças em crianças. **Objetivo:** Avaliar durante 12 meses a qualidade das águas de poços utilizadas como soluções alternativas coletivas de abastecimento em dez escolas públicas do município de Itatiba (SP). **Método:** Foram coletadas amostras de três pontos de coleta, direto do poço, reservatório e bebedouro, totalizando 100 amostras, sendo analisados parâmetros químicos, físicos, organolépticos e microbiológicos previstos na Portaria de Consolidação n° 5, de 28 de setembro de 2017, anexo XX. Adicionalmente foi realizada uma pesquisa quanto à presença de matérias estranhas, incluindo os protozoários *Cryptosporidium* spp. e *Giardia* spp. **Resultados:** Três escolas exibiram resultados em acordo com a legislação. As demais apresentaram presença de microrganismos como bactérias (29,0% de coliforme total e 9,0% de *Escherichia coli*, no total de amostras analisadas) e protozoários (15,0%), além de resultados acima do valor máximo permitido (VMP) para cor aparente (8,0%), turbidez (11,0%), Fe (12,0%), Zn e Pb (5,0%). **Conclusões:** Os resultados em desacordo com a legislação interferem na qualidade das águas oferecidas nas escolas, estando associados à falta de investimento na infraestrutura dos poços e cloração da água, sendo observado um diferencial na escola que possui parceria com a empresa de tratamento de água do município. Esta parceria deve ser mantida e, se possível, expandida para as demais escolas.

PALAVRAS-CHAVE: Potabilidade; Protozoário; Reação em Cadeia da Polimerase; Saneamento; Criança

INTRODUCTION

Water is an essential element to life, and access to good quality water in adequate quantity is directly related to the population's health, contributing to reduce the occurrence of several diseases[1]. Water can contain a variety of contaminants that, at high levels, have been associated with an increase in a number of diseases in children, including acute diseases, such as gastrointestinal, development effects, learning disorders, endocrine dysfunction, and cancer[2]. The guarantee of human consumption of drinking water, free of pathogenic microorganisms and chemical substances harmful to health, consists of an effective action to prevent diseases caused by water[3].

Educational institutes use water to prepare meals, juices, clean utensils and facilities, in addition to direct consumption through drinking fountains, and may represent a vehicle for contamination, if basic hygiene rules are not strictly observed[4]. For public health, the water supply security is extremely important, especially considering that school-aged children can stay from 5h to 8h a day in public elementary schools in Brazil[5] and, normally, children ingest more water in relation to their body weight than adults, tending to greater exposure to water contaminants[2].

The Drinking Water, Sanitation and Hygiene in Schools: Global baseline report 2018 global assessment supported by World Health Organization/United Nations Children's Fund (WHO/UNICEF) pointed that 69% of schools worldwide have some basic drinking water service, with coverage generally higher than peri-urban schools, which in turn have significantly better coverage than rural schools. Few countries have reported the quality of drinking water in schools but the available data shows that rural schools have lower coverage of basic drinking water services than urban schools. In Brazil, from 2010 to 2016, the percentage of schools without water treatment decreased from 23% to 17%, but the data provided are insufficient to assess the quality of drinking water provided in schools[6].

Studies on water quality, especially underground, are of great importance for public health, receiving the attention of the scientific community of many countries[4,5,7,8,9,10,11,12]. Several types of drinking water contaminants can be of concern for children's health, such as, for example: *Escherichia coli*, lead, arsenic, nitrates, organic chemicals, and disinfection by-products (chloroform)[2].

Among the microorganisms found in the water, the protozoa (*Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, among others) have a relevant occurrence and currently have emerged affecting individuals in epidemic outbreaks. Factors such as an increase in water contamination, consumption of contaminated food, use of alternative water sources for consumption, globalization of businesses and trips (among others) may be related to the increase of these pathogens[13].

Consolidation Ordinance nº 5, of October 28, 2017, of the Ministry of Health (MS)[14], annex XX, states that all water destined for human consumption, distributed collectively through a system or collective alternative of water supply, must be subject to water quality control and surveillance. To be considered potable, water destined to supply the human population must meet the quality characteristics that are under the permissible values of chemical, physical, organoleptic, and microbiological parameters[14]. The ordinance also highlights the monitoring of cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. at the water catchment point when an annual geometric mean greater than or equal to 1,000 *E. coli*/100 mL in the water is identified[14].

Considering the importance of the subject and the lack of data on water quality on Itatiba (São Paulo), the objective of the present study was to assess for 12 months the potability of water from wells used as collective alternative supply solutions (CAS) in ten public schools of this city. Chemical, physical, organoleptic, and microbiological parameters provided in the legislation were analyzed and, in addition, the presence of foreign matter and protozoa *Cryptosporidium* spp. and *Giardia* spp.

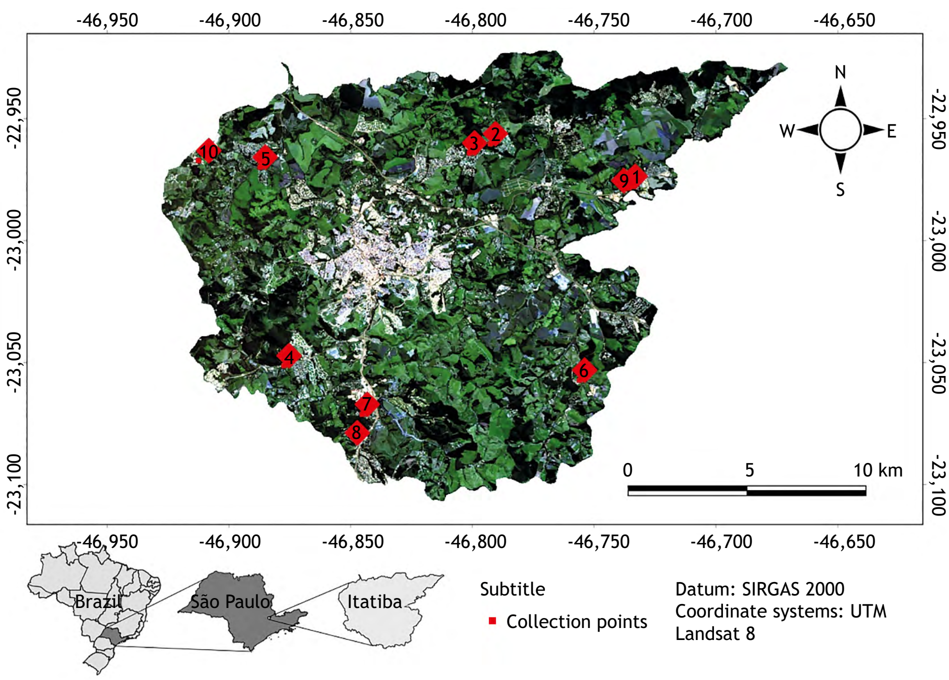
METHOD

Study area

Itatiba is a city in the state of São Paulo (latitude 23° 07′ 02″ South and longitude 46° 33′ 01″ West), part of the Metropolitan Region of Campinas, with an estimated population of 120,858 inhabitants[15]. The city has 62 public schools[16], being the ten schools evaluated in this study serving approximately 2,000 students which correspond to approximately 11% of the total students enrolled in 2017 elementary education in the city (public and private schools)[17]. The Figure shows the distribution of schools within the city.

Sampling

In 12 months, between July 2016 and May 2017, the Sanitary Surveillance (Visa) of Itatiba conducted four collections of water samples (identified as: fall, winter, spring, and summer) in ten schools that used wells as CAS. Schools were identified as S1 to S10. The collection kits (containing three bags for



Source: Elaborated by the authors, 2020.

Figure. Map with the collection points in the city of Itatiba (SP). The numbers represent, respectively, schools S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10.

microbiological tests, three for physical-chemical, one gallon for the protozoa, and two bottles for the metal ones) were supplied by the laboratory, decontaminated, and preserved according to the characteristics of each test, throughout the scheduled collections.

Three collection points were determined: straight from the well, reservoir, and drinking fountain, established for the physical-chemical (PC) and microbiological analysis. The straight from the well point was also established for the collection of microscopic tests and inorganic elements.

In this study, a total of 100 samples were analyzed, with 40 samples being collected at the drinking fountain, 40 in the reservoir, and 20 straight from the wells. This difference in sampling in the wells occurred due to the fact that some of the schools did not have access to wells (taps) and, in those cases, the determinations of inorganic elements and microscopic analysis were carried out at the reservoir point. The straight from the well sampling was not carried out during winter in schools S3 and S4; spring in schools: S1, S2, S3, S4, and S9; summer in schools: S1, S2, S3, S4, S8, and S9; fall in schools: S1, S3, S4, S5, S6, S8, and S9. Schools S7 and S10 did not provide access to the wells during the study.

Determination of parameters

To facilitate the disposition of the results and discussions, the parameters studied were divided into four groups: PC Analysis: apparent color, turbidity, total dissolved solids (TDS), total hardness, iron (Fe), chloride, sulfate, pH, ammonia, nitrite, and nitrate; Analysis of inorganic elements: Aluminum (Al), Antimony (Sb), Arsenic (As), Barium (Ba), Cadmium (Cd), Lead (Pb), Copper (Cu), Chromium (Cr), Manganese (Mn), Nickel (Ni), Selenium (Se), Sodium (Na), and Zinc (Zn); Microbiological analysis: total coliforms (TC) and *E. coli*.; and Microscopical analysis: foreign matter and research of *Cryptosporidium* spp. and *Giardia* spp.

PC Analysis

Approximately 1,000 mL of water was collected in a sterile and disposable bag, model Whirl-Pak®. The sample was analyzed as described in Standard Methods for The Examination of Water and Wastewater (SMEWW)[18] for apparent color (visual comparison method 2120 B), hardness (EDTA titrimetric method 2340 C), turbidity (nephelometric method 2130 B), pH value (electrometric method 4500-H^+ B), fluoride (ion-selective electrode method 4500-F^- C), nitrate (Ultraviolet spectrophotometric screening method 4500-NO_3^- B), nitrite (Colometric method 4500-NO_2^- B), Fe (phenanthroline method 3500-Fe B), and chloride (argentometric method 4500-Cl^- B). The ammonia parameter was determined by the photometric method with Merck's Spectroquant® kit. The parameters TDS (method 204/IV, conductivimetric) and sulfate (method 214/IV) followed the techniques described in "Physical-chemical methods for food analysis"[19].

Determination of inorganic elements

Sampling for inorganic elements testing was carried out in polypropylene flasks of 500 mL, previously washed, decontaminated, and added with HNO_3 to preserve the samples, as described in the

SMEWW[18]. Inorganic elements Al, Ba, Cd, Cu, Cr, Mn, Ni, Na, and Zn were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) (PerkinElmer, model Optima 8300). Elements As, Pb, and Se were analyzed by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) (PerkinElmer, model AAnalyst 600), using methods 3120 B and 3113 B, described on SMEWW[18].

Microbiological analysis

For the microbiological analysis, 100 mL of water was collected in a sterile and disposable bag, model *Thio-Bag Whirl-Pak*®, with sodium thiosulfate. The sample was evaluated for the presence or lack of TC and *E. coli* by the chromogenic/enzymatic substrate method (Colilert system, Idexx Laboratories/USA), method 9223 B described in SMEWW[18].

Microscopic analysis

The sample for investigation of foreign matter and protozoa was collected in a 20 L plastic gallon previously sanitized with a 20% sodium hypochlorite solution. The first step of the analysis consisted of filtering the sample in closed containers, previously autoclaved (30 min at 120 Kgf/cm^2), using 122 mm in diameter membranes and under the pressure of 0.7 kgf/cm^2.

After filtration, the membrane was transferred to a sterile Petri dish of 145 mm in diameter, to which 5 mL of ultrapure water was added, and then it was scraped with the aid of a cell scraper (cel scrap - Kasvi®). The material obtained was collected with the aid of a Pasteur pipette and transferred to a 15 mL falcon tube. The scraping procedure was performed again with an additional 5 mL of ultrapure water. The material obtained (10 mL) was centrifuged at 2,270 g for 5 min, with the supernatant (approximately 9 mL) discarded and the sediment material collected for the preparation of the slides and DNA extraction.

Foreign matter research: carried out by reading slides containing the sedimented material, stained with lugol, and subjected to analysis in a bright field microscope to identify foreign matters, including protozoa (oocysts and cysts).

Cryptosporidium spp. and *Giardia* spp research: 200 µL aliquots of the sedimented sample obtained from the previous step were treated with 40 µL of proteinase K and maintained for 18 h under the agitation of 500 oscillations per minute. After the end of the incubation, DNA extractions followed the protocol of the manufacturer of the QIAamp DNA Mini *Kit*-Qiagen® kit. The polymerase chain reactions (PCR) were performed using a commercial kit (GoTaq®Green Master Mix - Promega). Each 12.5 µL of the mix containing a unit of Taq DNA polymerase in 10 mM Tris-HCl, pH 8.5; 50 mM KCl; 1.5 mM $MgCl_2$ and 200 mM of each of the triphosphate deoxynucleotides (dATP, dGTP, dCTP, dTTP). Each reaction was performed by adding 5 µL of the target DNA and 10 pmol of each primer, in a final volume of 25 µL. Amplifications were performed using the Veriti® Thermal Cycler thermocycler (Applied Biosystems®). The molecular primers used are described in Table 1.

The amplified products were separated in a horizontal electrophoresis system in 1.2% agarose gel 1x TBE buffer (45 mM Tris-Borate and 1 mM-EDTA, pH 8.0), containing ethidium bromide at 0.5 g/ml. The molecular weight marker with multiple fragments of 100 bp was added to all gels and the runs were carried out at 100 Volts for 1 h (source PS 1006, Apelex, France). The samples were visualized and photographed on a Gene Genius transilluminator (Gel Capture Pro software, version 4.5.3) of ultraviolet at a wavelength of 302 nm.

RESULTS AND DISCUSSION

At each collection carried out, the Visa recorded important information for the conclusion of the results obtained, such as the type of well, chlorine water treatment, field analysis (pH and free residual chlorine), and rain occurrence in the 24 h before collection, as well as helping each school with the results. One of the actions resulted in the interdiction of the S2 school well in the scheduled fall collection, and this water supply started to be carried out by a water truck. Due to the interdiction, the collection was not performed at the straight from the well point of this school.

PC Analysis

Laws[14] establish a recommendation range for the pH between 6.0 and 9.5, and maximum permitted values (MPV) for the remaining 11 PC parameters evaluated in this study. The results of the sulfate parameter was lower than the method limit of quantification (LOQ) of 50 mg/L, and the pH values ranged from 5.4 to 7.9 for the 100 samples analyzed, these values do not

Table 1. Description of the molecular primers for conventional polymerase chain reaction used to identify protozoa *Cryptosporidium* spp. and *Giardia* spp.

Protozoa	Conventional PCR			
	Molecular primers	Sequence 5' - 3'	Expected product (bp)	Reference
Cryptosporidium spp.	CryIAL1(F)/ CryIAL2(F)/ CryIAL3(R)	TACCTACGTATGTTGAAACTCCG AGGATACGAAATATCAGGAAAGC TGTATATCCTGGTGGGCAGACC	703/531	This study
Giardia spp.	G7(F)/ G376(F)/ G759(R)	AAGCCCGACGACCTCACCCGCAGTGC CCATAACGACGCCATCGCGGCTCTCAGGAA GTCGTCTCGAAGATCCAGGGCGGCCTC	753/384	Cacciò and Ryan[20]

Source: Elaborated by the authors, 2020.
PCR: polymerase chain reaction.

compromise water quality in terms of human consumption, as they only reflect the geological composition of the soil[21]. Table 2 presents mean values, standard deviation, maximum and minimum found for the other PC parameters of each school, without differentiating the collection point or seasonality. The results of schools S2, S4, S5, S6, S7, S8, S9, and S10 were below the MPV for all PC parameters.

S1 presented a result above the MPV for Fe (0.76 mg/L) in the summer collection at the straight from the well point. As described in the legislation, the eventual occurrence of results above the MPV must be analyzed together with the history of water quality control and not in a timely manner[14]. Thus, this punctual result does not affect water quality, as regards this parameter, since the other points (reservoir and drinking fountain) and collections (spring, fall, and winter) are under the legislation.

In S6, a fluoride result of 0.51 mg/L was observed in the single sample collected at the straight from the well point, a value 2.5 times below the average (1.18 ± 0.26 mg/L) of the samples collected in other points. Comparing the results of the reservoir and drinking fountain water with the well water, there is evidence of water fluoridation or the use of a different source of supply from this school.

S7 declared a partnership with the Basic Sanitation Company of the State of São Paulo (Sabesp) for the treatment of water, an additional benefit to students and school staff, being the average result of fluoride (0.67 ± 0.09 mg/L), characteristic of fluoridated water, in accordance with Resolution SS-250, of August 15, 1995, of the São Paulo State Department of Health[22]. In the fall collection of S2, the average fluoride result was 0.59 ± 0.01 mg/L, while the other collections showed results below the limit of quantification (0.20 mg/L), confirming the origin of the water truck analyzed water.

For S3, the samples from well and reservoir points showed average results above the MPV for the apparent color (20.00 uH), turbidity (13.54 uT), and Fe (1.66 mg/L) parameters. At the drinking fountain point, the apparent color parameter presented a result above the MPV only in the fall collection (25.30 uH), the last one to be performed, indicating a possible saturation of the drinking fountain filter. The turbidity (average of 6.60 uT) and Fe (average of 0.95 mg/L) parameters were above the MPV in the winter, spring, and fall collections of the S3 school.

The apparent color parameter above the MPV in water causes consumer rejection and is related to the presence of organic matter, metals (Fe and Mn), and colored industrial waste. Turbidity, on the other hand, is the result of the presence of solid suspended materials (algae, plankton, organic matter, and other substances, such as Zn, Fe, Mn, and sand), resulting from the natural erosion process or from domestic and industrial waste, which reduce its transparency[1,23]. Both the color and turbidity of the water may be related to the high concentration of Fe, where its presence may favor the development of "iron-bacteria", which are not harmful to health but give color and odor to water[1,24]. The presence of iron can occur due to the natural interaction between groundwater and rock[21]. The excessive consumption of iron can cause hemochromatosis, which is characterized by the deposit of this metal in tissues of organs such as the liver, pancreas, heart, and hypophysis[24].

In the study by Scorsafava et al.[7] in the period from 2005 to 2008, 1,759 water samples (wells and mines) in 100 cities of the state of São Paulo were evaluated, and the authors found that 7.5% of the well water showed values above the MPV for apparent color, 5.0% for turbidity, and 8.5% for Fe. Soto et al.[8] evaluated the water quality of 50 public schools in the rural zone of the Ibiúna (SP) city, finding results above MPV for color (6.0%), turbidity (8.0%), and Fe (2.0%). These results are comparable to the 12.0% of samples above the MPV found in the present study (8.0% for apparent color, 11.0% for turbidity, and 12.0% for Fe).

In general, the results from school S3 showed a gradual decrease in the values obtained in the straight from the well, reservoir, and drinking fountain points, probably related to the precipitation of organic matter at the bottom of the reservoir and the retention in the drinking fountain filter[25]. These results may occur due to natural contamination or to wells built without technical criteria, with corroded or cracked coating, and without maintenance[26].

Determination of inorganic elements

Of the 12 elements studied, As, Cd, Cr, Ni, and Se were below LOQ (0.002 mg/L; 0.003 mg/L; 0.010 mg/L; 0.020 mg/L, and 0.003 mg/L, respectively) for the 40 samples analyzed. Table 3 presents mean values, standard deviation, maximum and minimum found for the seven quantified elements (Al, Ba, Pb, Cu, Mn, Na, Zn). In general, the results were below the MPV established in the legislation, except for Zn (S3) and Pb (S8).

The average Zn concentration in the S3 samples was 4.20 ± 1.19 mg/L, the lowest concentration (3.24 mg/L) in the summer collection, and the highest (5.89 mg/L) in the spring collection. The Zn concentration above 3mg/L tends to make the water look opalescent, develops an oily filter when boiled, giving an undesirable astringent taste to the water[27]. The Environmental Company of São Paulo State (CETESB)[28] establishes an intervention value for Zn in groundwater of 1.8 mg/L and the National Environment Council (Conama), through Resolution nº 420, December 28, 2009[29], an investigation value of 1.050 mg/L for Zn in waters for human consumption, values lower than that established by the legislation followed in this study (MPV of 5 mg/L)[14]. According to CETESB, concentrations above the intervention values, "indicate the need for actions to safeguard risk recipients and must follow the procedures for managing contaminated areas"[30].

Pb in S8 was detected only in the straight from the well point, and in the fall collection its concentration was of 0.022 mg/L, above the MPV, and in the summer collection it was 0.006 mg/L. This patter, concentration of Pb above LOQ at the straight from the well point, was also observed in schools S2 and S9, characterizing possible precipitation of this element at the bottom

Table 2. Mean values (X̄), standard deviation (SD), minimum and maximum (Min/Max) of the PC parameters found in school waters and the MPV.

School		Apparent color (uH)	Turbidity (uT)	TDS (mg/L)	pH	Total hardness (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Iron (mg/L)	Chloride (mg/L)	Fluoride (mg/L)
S 1	X̄ ± SD	< 5*	-	13.90 ± 4.14	6.08 ± 0.26	9.11 ± 1.80	-	0.64 ± 0.11	< 0.003*	-	20.56 ± 4.36	< 0.20*
	Min/Max		< 0.10*/2.10	9.75/24.90	5.40/6.35	6.09/13.13	< 0.01/0.13	0.44/0.76		< 0.10*/**0.76**	14.90/26.57	
S 2	X̄ ± SD	< 5*	-	38.70 ± 5.16	6.47 ± 0.36	27.44±5.78	< 0.01*	2.51 ± 0.84	< 0.003*	-	19.23 ± 13.66	-
	Min/Max		< 0.10*/073	34.45/47.12	6.12/7.12	21.21/36.54		0.92/2.96		< 0.10*/0.14	4.92/45.26	< 0.20*/0.59
S 3	X̄ ± SD	**20** ± 13	**13.54** ± 16.73	112.00 ± 6.61	7.23 ± 0.31	97.00 ± 8.24	< 0.01*	< 0.22*	< 0.003*	**1.66** ± 1.73	20.30 ± 4.23	0.50 ± 0.05
	Min/Max	5/**50**	0.89/**62.00**	104.60/123.30	6.82/7.64	86.87/113.70				0.16/**2.38**	14.90/29.80	0.42/0.54
S 4	X̄ ± SD	< 5*	-	48.20 ± 19.66	7.25 ± 0.13	34.69 ± 9.14	< 0.01*	< 0.22*	< 0.003*	-	27.32 ± 16.60	0.40 ± 0.03
	Min/Max		< 0.10*/0.44	33.74/79.26	6.95/7.35	27.41/53.54				< 0.10*/0.16	9.84/61.01	0.30/0.45
S 5	X̄ ± SD	< 5*	-	47.3 0.00 ± 1.88	7.11 ± 0.19	38.13 ± 2.13	< 0.01*	1.47 ± 0.08	< 0.003*	< 0.10*	21.26 ± 11.87	-
	Min/Max		< 0.10*/0.36	45.05/50.32	6.72/7.34	35.35/40.60		1.39/1.61			3.94/41.33	< 0.20*/0.20
S 6	X̄ ± SD	< 5*	-	50.20 ± 7.22	7.16 ± 0.55	18.56 ± 5.53	< 0.01*	0.59 ± 0.10	-	-	26.44 ± 9.40	1.18 ± 0.26
	Min/Max		< 0.10*/1.06	43.80/57.92	6.21/7.87	12.12/27.41		0.36/0.71	<0.003*/0.014	< 0.10*/0.21	13.91/38.38	0.51/1.34
S 7	X̄ ± SD	< 5*	-	82.70 ± 30.05	7.69 ± 0.06	47.33 ± 1.97	< 0.01*	0.30 ± 0.02	< 0.003*	< 0.10*	23.53 ± 13.00	0.67 ± 0.09
	Min/Max		< 0.10*/0.38	62.76/131.40	7.64/7.80	44.66/49.49		0.28/0.33			14.76/54.12	0.60/0.80
S 8	X̄ ± SD	< 5*	-	79.30 ± 34.62	7.13 ± 0.43	56.58 ± 4.98	< 0.01*	2.28 ± 1.20	-	< 0.10*	20.01 ± 3.79	< 0.20*
	Min/Max		< 0.10*/0.46	53.58/143.60	6.60/7.62	51.52/64.65		0.72/4.05	< 0.003*/0.004		14.76/26.57	
S 9	X̄ ± SD	< 5*	-	11.80 ± 2.41	5.95 ± 0.32	7.71 ± 1.79	< 0.01*	0.66 ± 0.07	< 0.003*	-	26.15 ± 12.38	< 0.20*
	Min/Max		< 0.10*/1.23	9.51/17.45	5.37/6.39	6.09/11.20		0.57/0.77		< 0.10*/0.14	15.74/49.20	
S 10	X̄ ± SD	< 5*	-	62.80 ± 5.90	6.98 ± 0.16	31.12 ± 7.29	< 0.01*	3.90 ± 0.06	< 0.003*	-	28.60 ± 7.22	0.50 ± 0.13
	Min/Max		< 0.10*/0.49	57.00/69.03	6.86/7.20	23.35/45.45		3.82/3.95		< 0.10*/0.1	17.71/37.39	0.30/0,60
MPV		15	5.00	1,000.00	6.00-9.50	500.00	1.50	10.00	1.000	0.30	250.00	1.50

Source: Elaborated by the authors, 2020.
SD: standard deviation; PC: physical-chemical; MPV: maximum permitted value by Consolidation Ordinance n° 5, Annex XX.
- mean not calculated, some results are below the limit of quantification; * Value below the limit of quantification; Bold: values above MPV

Table 3. Mean values ($\bar{X}$) in mg/L, standard deviation (SD), minimum and maximum (Min/Max) of the inorganic values quantified in the ten schools, and the MPV.

School		Al	Ba	Pb	Cu	Mn	Na	Zn
S1	$\bar{X}$ ± SD	< 0.05*	< 0.08*	-	-	-	2.20 ± 0.77	< 0.05*
	Min/Max			< 0.002*/0.005	< 0.05*/0.15	< 0.01*/0.03	1.76/3.36	
S2	$\bar{X}$ ± SD	-	-	-	< 0.05*	< 0.01*	4.35 ± 1.67	< 0.05*
	Min/Max	< 0.05*/0.06	< 0.08*/0.17	< 0.002*/0.005			3.43/6.86	
S3	$\bar{X}$ ± SD	< 0.05*	< 0.08*	< 0.002*	< 0.05*	0.02 ± 0.02	11.50 ± 0.36	4.20 ± 1.19
	Min/Max					0.01/0.05	11.11/11.95	3.24/**5.89**
S4	$\bar{X}$ ± SD	< 0.05*	< 0.08*	< 0.002*	< 0.05*	< 0.01*	5.39 ± 0.93	0.22 ± 0.13
	Min/Max						4.57/6.39	0.07/0.39
S5	$\bar{X}$ ± SD	< 0.05*	0.09 ± 0.01	< 0.002*	< 0.05*	< 0.01*	6.23 ± 0.23	< 0.05*
	Min/Max		0.09/0.10				5.89/6.38	
S6	$\bar{X}$ ± SD	< 0.05*	< 0.08*	< 0.002*	< 0.05*	-	17.93 ± 2.88	< 0.05*
	Min/Max					< 0.01*/0.02	15.02/20.91	
S7	$\bar{X}$ ± SD	< 0.05*	< 0.08*	< 0.002*	< 0.05*	< 0.01*	10.52 ± 0.27	< 0.05*
	Min/Max						10.26/10.90	
S8	$\bar{X}$ ± SD	-	0.12 ± 0.01	-	< 0.05*	< 0.01*	4.73 ± 1.98	< 0.05*
	Min/Max	< 0.05*/0.06	0.11/0.13	< 0.002*/**0.022**			2.37/6.79	
S9	$\bar{X}$ ± SD	< 0.05*	< 0.08*	-	< 0.05*	< 0.01*	1.80 ± 0.10	< 0.05*
	Min/Max			< 0.,002*/0.010			1.69/1.92	
S10	$\bar{X}$ ± SD	< 0.05*	0.15 ± 0.01	< 0.002*	< 0.05*	< 0.01*	13.24 ± 0.78	< 0.05*
	Min/Max		0.14/0.16				12.50/14.00	
MPV		0.20	0.70	0.010	2.00	0.10	200.00	5.00

Source: Elaborated by the authors, 2020.
SD: standard deviation; MPV: maximum permitted value; Al: aluminum; Ba: barium; Pb: lead; Cu: copper; Mn: manganese; Na: sodium; Zn: zinc.
- not calculated, some results are below the limit of quantification; * Value below the limit of quantification; Bold: values above MPV

of the reservoirs[31,32]. However, caution is needed when evaluating these results, as previously mentioned, any occurrences of results above the MPV should be analyzed together with the history of water quality control and not promptly.

The studied by Campos et al.[33] and Monte Blanco et al.[34] showed results above the MPV for Pb for 100.0% and 10.0%, respectively, of the samples analyzed. CETESB, in the 2013-2015 triennium, evaluated groundwater in 282 different points (among tubular and spring wells), checking lead concentrations above the MPV to the potability standard in five wells (2.0% of total wells), in different parts of the state of São Paulo. In this same study, wells located in the cities of Marília and Guarulhos showed Zn concentrations higher than the investigation value of Conama Resolution nº 420/2009 and the CETESB List of Guiding Values of 2014[35].

In the present study, from the 40 samples analyzed, only two (5.0%) had a concentration of the inorganic elements studied above the MPV. However, due to the risks to human health, the concentrations of the elements Zn and Pb, that exceeded or were close to the permitted limits, (S3, S8, and S9) demonstrated the need for continuous monitoring, mainly for Pb, as it is a toxic and cumulative metal that affects several systems of the human body, including the neurological, hematological, gastrointestinal, cardiovascular, and renal systems. Children are particularly vulnerable to the neurotoxic effects of Pb, and even relatively low levels of exposure can cause severe and, in some cases, irreversible neurological damage[36].

Microbiological analysis

In the four collections, the presence of TC or *E. coli* was not observed in schools S1, S3, S4, and S7 samples. This result shows the effectiveness of water treatments informed in the data from the field analysis for free residual chlorine provided by the Visa. Table 4 shows the results of the six schools with TC and/or *E. coli* presence.

According to the legislation[14], all water for human consumption, supplied collectively, must undergo a process of disinfection or chlorination and the presence of TC can indicate the inefficiency of the water treatment and or lack in the integrity of the water distribution system. Therefore, schools S5, S6, S9, and S10 showed TC in their samples, being an indicator of the lack or inefficiency of water treatment.

Samples collected in spring and summer in school S2 revealed the presence of TC and *E. coli* at the three studied points, indicating that the water supplied had not been previously treated and that the contamination originates from the well itself. School S8 revealed the presence of TC and *E. coli* in the water of the straight from the well point during the summer and at the reservoir point in the collections carried out in spring and in summer, the drinking fountain point, on the other hand, did not reveal the presence of *E. coli*, indicating an effective action from this school's filter.

E. coli has been used as the main indicator of thermotolerant coliforms and, although this microorganism is found in the

Table 4. Results of samples with the presence of TC and *E. coli*.

School	Collection point	Microbiological parameters			
		Winter	Spring	Summer	Fall
S2	Straight from the well	NC	TC and *E. coli*	TC and *E. coli*	NC
	Reservoir	TC	TC and *E. coli*	TC and *E. coli*	S
	Drinking fountain	TC	TC and *E. coli*	TC and *E. coli*	TC
S5	Straight from the well	NC	NC	NC	S
	Reservoir	S	S	S	S
	Drinking fountain	S	S	TC	S
S6	Straight from the well	NC	NC	NC	S
	Reservoir	TC	TC	TC	TC
	Drinking fountain	TC	TC	S	S
S8	Straight from the well	NC	NC	TC and *E. coli*	TC
	Reservoir	TC	TC and *E. coli*	TC and *E. coli*	TC
	Drinking fountain	S	S	TC	S
S9	Straight from the well	NC	S	S	S
	Reservoir	S	S	TC	TC
	Drinking fountain	S	S	TC	TC
S10	Straight from the well	NC	NC	NC	NC
	Reservoir	S	S	S	TC
	Drinking fountain	S	S	S	TC

Source: Elaborated by the authors, 2020.
TC: total coliforms; S: Satisfactory sample; NC: Not collected.

intestinal contents of men and warm-blooded animals, several strains have virulent attributes[37,38]. *E. coli* is one of the three main pathogenic bacteria transmitted by food and water in the world, being clinically important, as its infection can lead to a wide range of clinical manifestations, including asymptomatic infections, mild diarrhea, or severe diseases, such as hemorrhagic colitis and hemolytic-uremic syndrome. An outbreak caused by this pathogen can reach epidemic proportions, causing considerable economic burden[39].

In the fall collection (the last performed), schools S2 and S8 showed the absence of *E. coli* in the water, featuring an improvement in the quality of their water. This improvement was due to the Visa's action which, based on the results obtained in the previous collections, interdicted S2's well, passing the water supply to be carried out by a water truck, and at S8 there was a maintenance of the well and cleaning of the reservoir and water tanks. However, the presence of TC in school S2 drinking fountain and the S8 well and reservoir indicates that the measures taken were not totally efficient, suggesting more effective maintenance such as filter changes, water supply pipes verification, and even the replacement of the well since the lack of well maintenance can trigger conditions favorable to the development and survival of microbial pathogens[35].

In summary, 29.0% of total samples analyzed showed the presence of TC and in 9.0% of samples the presence of *E. coli* was confirmed (concentrated in two schools). Previous studies have been showing the same problems of microbial contamination in water supplied in Brazilian schools[40,41,42,43]. The presence of thermotolerant coliforms in these studies varied from 10.0% to 33.0% of the samples analyzed, an average percentage higher than that found in this study, and the authors highlighted the need of implanting sanitation measures and emphasized that the presence of these microorganisms can be related to the lack of hygiene in water tanks and pipes, the lack of effective chlorination, and the inefficiency of drinking fountain filters[25,40,41,42,43].

These pieces of evidences are aligned to the results and observations of this study, where six schools need to implement and keep a minimum treatment, such as chlorination, to improve the quality of water supplied to students. The evaluation and reforms of the physical structures of the wells are also necessary to assure the hygienic-sanitary conditions of the environment and, consequently, protect the supply source.

Microscopic analysis

Foreign matter research

In general, the water samples analyzed (straight from the wells and/or reservoirs) revealed the presence of foreign matter in suspension and deposit, identified as: amoeba cysts and free-living protozoa cysts, amorphous foreign matter, earthy material, free-living nematodes (phytoparasite), crystals, vegetable fibers, insect eggs, bird barbs, among others. The occurrence of these foreign matters is indicative of failures in good practices and structural problems in wells and/or reservoirs, which may allow dirt to enter.

In the winter collection of sample S2, carried out at the reservoir point, the microscopic analysis revealed the presence of *Eimeria* spp. cysts which, despite being a protozoan, is not considered a risk to human health, however, it is indicative of contamination by the feces of birds or ruminants[44]. Given these results, it is necessary to adopt appropriate hygiene and maintenance measures of the reservoirs, including a step of water filtration before use and consumption.

Cryptosporidium *spp. and* Giardia *spp.*

Table 5 shows the results of the *Giardia* spp. and *Cryptosporidium* spp. research in the 10 water samples from the ten studied schools. In all, 15.0% of samples analyzed revealed the presence of protozoa related to human health risks, and the results highlight the occurrence of protozoa in schools S2, S3, S5, S6, S9, and S10 in only one of the collections.

Giardia spp. is one of the most common and scientifically known protozoa, mainly because it causes diarrhea episodes, its cysts are resistant to chlorine, although they are less resistant than *Cryptosporidium* spp., both being more resistant than bacteria, surviving the levels and concentrations of chemical products routinely used for water treatment[45].

Among the several forms of water contamination by protozoa, some authors reported the participation of flies, especially coprophages, in the mechanical dissemination of protozoa, transporting them to water bodies in their exoskeleton and in the digestive tract[46,47], factors that can explain the occurrence observed in this study. Barçante et al.[48] analyzed 414 household wells in the city of Lagoa Santa (Minas Gerais) and detected *Giardia intestinalis* in 14.04% of the samples, associating the fact with the poor structural conditions of the reservoirs.

The occurrence and the detection of these protozoa in water samples collected in wells is little explored. The analysis of protozoa in water brings a series of scientific and technological challenges since the internationally recognized methodologies are highly specific. In Brazil, the research of protozoa in water by specific and sensitive techniques is still not widespread, considering aspects such as the specificity and cost of the techniques, allied to the difficulties in the detection and possible presence of interferents in the matrices.

The legislation establishes a relation between the occurrence of *E. coli* and the monitoring of *Giardia* spp. and oocysts of *Cryptosporidium* spp.[14]. Therefore, Table 6 brings a summary of all collections, the occurrence of protozoa, and the other parameters analyzed per school.

Comparing the results of microbiological analyzes with those of protozoa, we could observe that schools S1, S4, and S7 presented satisfactory results for microbiological and microscopic tests.

In schools S3, S5, S6, S9, and S10 the presence of *Giardia* spp. DNA was detected by the PCR and the presence of *E. coli* was not observed, suggesting that there is no correlation between these two parameters.

These results may be related to several factors, such as type of well, physical structures of the reservoirs, and/or the effectiveness of water treatment by chlorination. The PCR test is a more sensitive technique than the chromogenic/enzymatic substrate method (Colilert system) used to determine the presence or absence of TC and *E. coli* where, in cases where there was a water treatment, these microorganisms may have been inactivated by chlorine (as well as the protozoa), not being detected by the Colilert system.

In the S2 sample, *Cryptosporidium* spp. was detected and the presence of TC and *E. coli* was revealed during the spring collection, confirming the bad water quality in this well. In school S8, which revealed the presence of TC and *E. coli* in the spring and summer collections, the presence of protozoa was not detected, highlighting that, in this study, the correlation between the presence of *E. coli* (or TC) and protozoa in the water analyzed was not observed.

CONCLUSIONS

In the ten studied schools, only three (S1, S4, and S7) presented results in accordance with Consolidation Ordinance No. 5/2017, Annex XX, regarding tests performed. In the others, the presence of microorganisms, protozoa, and/or results above the MPV for apparent color, turbidity, Fe, Zn, and Pb

Table 5. *Cryptosporidium* spp. and *Giardia* spp. in water samples collected in different seasons

School	Collection point	Winter	Spring	Summer	Fall
S1	Reservoir/Well	ND[a]	ND[b]	ND[b]	ND[b]
S2	Reservoir/Well	ND[a]	Cryptosporidium spp.[b]	ND[b]	ND[a]
S3	Well	ND[b]	ND[b]	ND[b]	Giardia spp.[b]
S4	Well	ND[b]	ND[b]	ND[b]	ND[b]
S5	Reservoir/Well	ND[a]	ND[a]	Giardia spp.[a]	ND[b]
S6	Reservoir/Well	ND[a]	ND[a]	ND[a]	Giardia spp.[b]
S7	Reservoir	ND[a]	ND[a]	ND[a]	ND[a]
S8	Reservoir/Well	ND[a]	ND[a]	ND[b]	ND[b]
S9	Reservoir/Well	ND[a]	ND[b]	ND[b]	Giardia spp.[b]
S10	Reservoir	Giardia spp.[a]	ND[a]	ND[a]	ND[a]

Source: Elaborated by the authors, 2020.
ND: Not detected.
[a] collection in the reservoir; [b] collection in the well

Table 6. Summary of results obtained outside the recommended and positive limits found in the schools studied.

School	Number of samples analyzed		Number of samples with results above MPV or positive			
	PC/Microbiology	Protozoa/Element	PC	Element	Microbiology	Protozoa
S1	11	4	1 (Fe)	-	-	-
S2	10	4	-	-	6 (TC e *E. coli*) 3 (TC)	1 (*Cryptosporidium* spp.)
S3	12	4	11 (color, turbidity, and Fe)	1 (Zn)	-	1 (*Giardia* spp.)
S4	12	4	-	-	-	-
S5	9	4	-	-	1 (TC)	1 (*Giardia* spp.)
S6	9	4	-	-	6 (TC)	1 (*Giardia* spp.)
S7	8	4	-	-	-	-
S8	10	4	-	1 (Pb)	3 (TC and *E. coli*) 4 (TC)	-
S9	11	4	-	-	4 (TC)	1 (*Giardia* spp.)
S10	8	4	-	-	2 (TC)	1 (*Giardia* spp.)
Total	100	40	12	2	29	6
Total (%)			**12**	**5**	**29**	**15**

Source: Elaborated by the authors, 2020.
MPV: maximum permitted value; PC: physical-chemical; TC: total coliforms; Fe: iron; Zn: zinc; Pb: lead.

that interfere in the quality of the water supplied to schools was verified. The detection of protozoa in this study denotes the importance of monitoring the quality of the water supplied in schools, especially considering that children may be more susceptible to food and waterborne infections, thus preventing the occurrence of epidemic outbreaks. In this study, it was observed that water quality is associated with factors such as lack of investment in well infrastructure, water chlorination, and lack of professionals responsible for water control and maintenance.

The quality of the water supplied to children and employees of schools requires public policy strategies by health and environment-related agencies, to guarantee its quality and safety. We highlight the differential of the school that has a partnership with the cities' water treatment company to guarantee disinfection and fluoridation, resulting in a higher quality of the water supplied. This partnership must be maintained and, if possible, expanded to other schools with collective alternative solutions of water supply in the city to mitigate the risks in the other schools studied.

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Authors’ Contributions

Fioravanti MIA, Pereira PHL, Marciano MAM, Mazon EMA - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. Sanches VL – Analysis. Ferreira COF – Planning, collection, and analysis. All authors approved the final version of the work.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study’s political or financial peers and institutions.