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Public drinking water supply and egg laying by *Aedes aegypti*

ABSTRACT

OBJECTIVE: To evaluate the effect of the quality of publicly supplied water in domestic water tanks on egg laying by female *Aedes aegypti*.

METHODS: Laboratory study on immature *Ae. aegypti*, collected from water-tanks in the municipality of Potim, SP, Southeastern Brazil. Each cage contained three types of water in which eggs could be laid: Three choice per test were simultaneously used to deposit the eggs, ovipositor (A) with water collected from a water tank in Taubaté, ovipositor (B) with distilled water (control) and ovipositor (C) water collected from a water tank in the municipality of Potim. Physicochemical parameters were analyzed. The Kruskal-Wallis test was used to analyze the mean number of eggs in each water sample and the Dwass-Steel-Critchlow-Fligner test was used in making comparisons. To evaluate egg laying, an ovipositional activity index was adopted.

RESULTS: A significant difference in the number of eggs was found between the liquid solutions tested ($H = 45$; $p < 0.0001$). The number of eggs found in water tank samples originating from deep wells (C), was statistically superior to water samples from water tanks originating from superficial wells (A) ($p < 0.0001$) and from the Control (C) $p < 0.0001$. There was no significant difference between the number of eggs in Control (B) and the surface water (A). In all three tests, the first position was the most productive in all tested solutions. Only water sample (C) produced a positive index (0.54), i.e., attractive to egg laying.

CONCLUSIONS: Water quality influences egg laying by *Aedes aegypti*. The high concentrations of ammonium nitrate in public water supplies suggest that this chemical component was responsible for attracting pregnant female *Aedes aegypti* to lay eggs in these environments.

DESCRIPTORS: *Aedes*, growth & development. Water Physicochemical Characteristics. Surface Waters. Groundwater. Water Supply.

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INTRODUCTION

The *Aedes aegypti* mosquito is the principal carrier of the dengue virus and urban yellow fever, significant arboviruses which affect humans.

The females prefer to use artificial containers to lay their eggs.⁴ For Glasser & Gomes¹⁶ (2002) the distribution, population increase and density of this mosquito is directly influenced by human and environmental factors. The artificial breeding sites can be those abandoned in the open air as well as those used for storing water for domestic use, such as water tanks, tires, cans, vases etc. The females lay their eggs in these sites, in moist substratum close to water or in areas prone to flooding.

The selection of suitable locations to lay eggs by pregnant females is fundamental to the distribution and establishment of these populations. The insects' reproduction, aggregation and egg laying activities are governed by chemical signals. They are guided towards the source of the odor depending on the chemical and physical composition of the stimulus. Females use their sense of smell to select a location with the nutrients necessary for the development of the offspring. This factor is important, not just in increasing the chances of survival, but also in guaranteeing the emergence of a large number of adults.^{3,5}

Geier et al¹⁵ (1999) showed in a laboratory, that ammonium nitrate is a chemical component which attracts the *Aedes aegypti* mosquito to their host. That females are attracted to nitrogenous compounds, was also verified by Sunish et al¹⁹ (2003) in egg laying by the Japanese encephalitis vector *Culex tritaeniorhynchus*. The quantity of ammonium nitrate in the water strongly influences the egg laying of this mosquito.

Forattini & Brito¹² (2003) cited the presence of high concentrations of nitrogen in samples of water from water tanks for domestic use in the municipality of Potim, SP, Southeastern Brazil, and suggested that this chemical component may be responsible for attracting pregnant female *Aedes aegypti* to lay their eggs in these containers. This species has shown its capacity to develop not only in clean water, but also in water with different levels of pollution.²

The municipality of Potim, located in the *Vale do Paraíba* area of the state of São Paulo, has been the subject of monitoring and control activities for dengue, showing that breeding sites in the form of water tanks have high levels of *Aedes aegypti* activity, since 2001.¹² That this situation has remained unchanged in all these years suggests that these receptacles

are preferred for egg laying when compared with other possible sites inspected throughout the period.^a The drinking water in this municipality originates from the public water supply, the catchment of which is from the water table. The high levels of mosquito infestation culminated in dengue epidemics, for which control actions remain ineffective, as the breeding sites in question continue to be those most productive and positive for this mosquito. In Taubaté, SP, Southeastern Brazil, the catchment of the public water supply, which serves 100% of the population, originates in surface water. Although the municipality is completely infested by the mosquito, the most common breeding sites are characterized as small, disposable receptacles, according to data from the state of São Paulo, Department of Health of the *Superintendência de Controle de Endemias*, (Superintendence of Control of Endemic Diseases).

This study aimed to analyze the effect of the water quality in domestic publicly supplied water in domestic water tanks on egg laying by female *Ae. aegypti*.

METHODS

Samples of adult populations of *Ae. aegypti* were obtained from larvae and pupae collected in domestic containers, water tanks storing publicly supplied water in the municipalities of Potim and Taubaté, São Paulo state, between March and April 2009. The criteria for choosing these containers was based on the premises of: easy access as the visit would be repeated on subsequent days, and the presence of the head of the household at all inspections, as well as a guarantee by the municipality that these two locations chosen were not the subject of any control measures in this period. The water in one water container storing publicly supplied water from Potim, and another from Taubaté was analyzed.

The insectarium had a controlled temperature of 28°C ($\pm 5\%$), 80% RH ($\pm 5\%$) and photophase of 14:12 (L:D).

Each experimental unit was composed of 50 females F_1 . Individuals aged one to three days post-emergence were transferred, one by one, with the help of a handheld vacuum, to one of three cages (60x60x60 cm) and were fed daily with a solution of 10% honey and apple.

Females aged three days and over were provided with an anesthetized mouse for one hour a day, alternate days, for the duration of the experiment.

^a Secretaria de Saúde do Estado de São Paulo, Superintendência de Controle de Endemias, Serviço Regional de Taubaté. Relatório técnico. Taubaté; 2011.

Three substrata were available, simultaneously, in the cage for the purpose of laying eggs, denominated ovipositors. These were made of glass, 12x10 cm in diameter and height (and capacity of 160 ml), lined with filter paper, filled with 100 ml of the liquid to be tested: ovipositor (A) water collected from a water tank in Taubaté, ovipositor (B), distilled water (control) and ovipositor (C), water from a water tank in Potim.

The experiment was repeated 31 times, each time lasting 24 hours, after which the filter paper was removed and the eggs counted using a stereoscopic microscope. New ovipositors, with paper substrata and liquid solution were reintroduced into the cages and their position rotated in a clockwise direction.

The water samples were from water tanks, located in urban areas, previously determined by this study. Their interiors were cleaned with a vegetable fiber brush and a solution of 10% Sodium hypochlorite and sealed with a plastic cap. The plastic cap was placed over the lid with the aim of preventing insects getting in. Two weeks after this process, weekly water collections began (five in total). The water samples were collected weekly to fulfill two consecutive procedures:

1°) Immediate completion of tests for physicochemical characterization of different water sources based on the standards for drinking water established by the Ministry of the Health.^b

2°) Used to test their attractiveness to egg-laying *Aedes aegypti* females in the laboratory for a week.

The physicochemical parameters were analyzed on the first day the water was collected in the field, assessing: appearance, color, presence of chlorine, smell, pH, taste, clarity, conductivity, total dissolved solids, dissolved oxygen, total suspended solids, fixed suspended solids, volatile suspended solids, ammonium nitrate, nitrate, sulfate, phosphate, bacteriological tests for total and fecal coliforms. Each sample was stored in the laboratory, analyzed daily and used in tests throughout the week. This procedure was used to observe eventual alterations in concentrations of some chemical components (chlorine, ammonium nitrate and phosphate).

The analyses used the physical and chemical indices according to the Standard Methods for the Examination of Wastewater as a reference.¹¹

The Kruskal-Wallis test was used to analyze the mean number of eggs in the different water samples. The Dwass-Steel-Chritchlow-Flingner test, using Static Direct software, was adopted in making comparisons between samples.²¹

The Egg Laying Activity Index (EAI) was adopted to evaluate the egg laying response of the females to the different types of water.⁷

The results obtained from the EAI varied between -1 and +1 and can be interpreted in a positive or negative manner, i.e., substances which attract or stimulate the laying of eggs produce positive indices, whereas components which repel or inhibit produce negative indices.

$$IAO = \frac{N_t - N_c}{N_t + N_c}$$

In which: EAI = Egg Laying Activity Index, N_t = mean number of eggs to be tested and, N_c = is the mean number of eggs in the control.

The research was carried out according to the standards required by the Helsinki Declaration and approved by the Ethics Committee for Animal Experimentation of the *Universidade de Taubaté* (CEE/Unitau – Process n° 023/08).

RESULTS

Pregnant females laid eggs in the three types of water available, with a significant difference between the number of eggs in the solutions tested ($H = 45$; $p < 0.0001$) (Figure 1).

The number of eggs in ovipositor (C) was higher and statistically significant in relation to ovipositor (A) ($p < 0.0001$) and ovipositor (B) ($p < 0.0001$). There was no significant difference between the number of eggs in the control (B) and in ovipositor (A) ($p = 0.9978$).

A total of 95,299 eggs were collected, 64.4% in the sample of water from Potim (C), 19.1% from the control (B) and 17.51% from the sample of water from Taubaté (A).

The first laying was the most productive in all of the solutions tested in the three cages. The liquid solution from Potim had a mean number of 7,016 eggs from the sum of the three replications (34.5%). There was a decrease in the number of eggs on the subsequent days, which allowed a decreasing exponential curve until the end of the experiment to be verified (Figure 2).

Sample (C) produced a positive index (0.54), whereas the value was negative (-0.03) in sample (A). The results obtained from the EAI indicated that the water from Potim contained the presence of substances which acted to attract females to lay their eggs.

The ammonium nitrate content present in the samples of publicly supplied water in Potim (C) were found

^b Ministério da Saúde. Portaria n° 518, de 25 de março de 2004. Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade, e dá outras providências. Brasília (DF); 2005 [cited 2013 Jul 16]. Available from: http://portal.saude.gov.br/portal/arquivos/pdf/portaria_518_2004.pdf

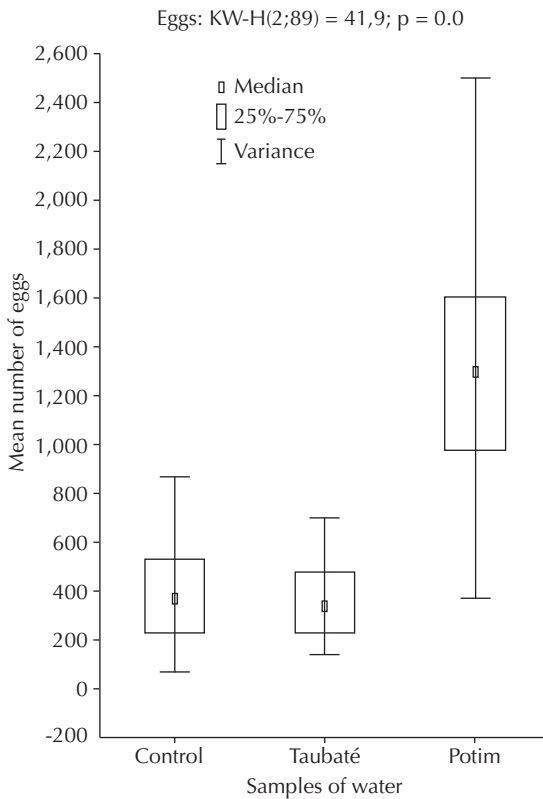


Figure 1. Number of *Aedes aegypti* eggs according to samples of the water tested, 2009.

to be above acceptable standards for drinking water, exceeding the values permitted by the Ministry of the Health^b (1.5 mg/l) and State Decree 12,486 (0.05 mg/l)^c (Tables 1 and 2). The parameters for chlorine, ammonium nitrate and phosphate were monitored daily to verify their volatilization during storage in the laboratory and their consequent decrease in concentration in the water used in the tests. The results show alteration in the values for concentrations of these components; however, they remained above the levels established in the abovementioned directives (Tables 1 and 2).

DISCUSSION

The higher number of *Aedes aegypti* eggs in water with high concentrations of ammonium nitrate suggests that this semiochemical may act as an attractant or stimulate at the scene of egg laying. This suggests that the water in Potim contains the presence of substances which attract females to lay eggs. The volatilization of NH_3 in the water at the breeding site may have been the chemical attraction responsible for guiding the flight of the pregnant females, as cited by Sunish et al¹⁹ (2003).

Different authors have indicated the effect of nitrogen in attracting female *Aedes aegypti* to lay eggs. Walker et al²⁰ (1997) found a positive, significant correlation between the larval productivity of *Aedes triseriatus* and the nitrogen coming from decomposing tree leaves in the water. Similarly to this study, Darriet & Corbel⁸ (2008), studying properties of attraction and physico-chemical changes in the water by various generations of *Aedes aegypti* larvae, concluded that this was more attractive to pregnant females than water which had never been colonized. The same authors investigated the effect of different concentrations of nitrogen, phosphorous and potassium based fertilizers on *Aedes aegypti* egg laying and showed that moderate levels were the most attractive.⁹

More recently, the abovementioned authors, studying the influence of decomposing plants and NPK fertilizer on *Aedes aegypti* biology, concluded that the combination of these components was the most attractive to egg laying females of this species, as well as allowing the mosquito larvae to develop.¹⁰

The high levels of ammonium nitrate found in this study may be due to the fact that the public water supply in Potim comes from the water table, at shallow depths of 300 and 350 meters underground in the Tremembé Geological Formation. Lithologic variations of this sediment have high levels of ammonium nitrate.^d Of the residences in the municipality, 92% use this water.

According to Alaburda & Nishihara¹ (1998), the presence of nitrogen compounds at different stages of oxidization is indicative of groundwater contamination and possible unsatisfactory sanitary conditions. Nitrogen participates in the formation of proteins and is a basic component of Biomass. Ammonium nitrate is an essential cellular component and may, in high concentrations, provide conditions for the excessive proliferation of organisms, with negative implications for the balance of the aquatic system. Such circumstances favor the proliferation of organisms such as micro plankton, algae and bacteria, essential to larval development. This process may influence the attraction exercised by the breeding site in question. The preference for laying eggs in water tanks may be associated with the strong, co-specific attraction exercised and serve to stimulate the female at the time of choosing an appropriate site for laying her eggs.

Sunish et al¹⁹ (2003) concluded that rice plantations fertilized using nitrogen based compounds in alkali soils favor the volatilization of these compounds, releasing ammonium nitrate and other substances.

^c São Paulo (Estado). Decreto Estadual nº 12.486, de 20 de outubro de 1978. Norma Técnica Alimentar - NTA 60. Águas de consumo alimentar. São Paulo; 1978 [cited 2013 Jul 16]. Available from: <http://www.labnacional.com.br/sites/all/themes/danland/legislacao/NTA60.pdf>

^d Instituto Pesquisa Tecnológica. Estudo das águas subterrâneas e elaboração da Carta Geotécnica do Município de Potim: relatório. São Paulo;1998.

Table 1. Results of the physicochemical analysis of samples from the public water supply. Potim, SP, Southeastern Brazil, collected 25th and 31st March and 8th, 14th and 22nd April 2009.

Parameters	Data/collection																	
	March, 25 (1 st Collection)			March, 31 (2 nd Collection)			April, 8 (3 rd Collection)			April, 14 (4 th Collection)			April, 22 (5 th Collection)					
Appearance	Clear			Clear			Clear			Clear			Clear					
Color (PtCo APHA)	8.0			12.0			7.0			29.0			13.0					
Chlorine (mg/L)	0.2			0.2			0.0			0.0			0.0					
Monitoring ^a	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<0.0	<0.0	<0.0	<0.0	<0.0	<0.0	<0.0	0.0	0.0	0.0
Odor	NO			NO			NO			NO			NO					
pH	7.8			7.7			7.6			7.9			7.7					
Taste	NO			NO			NO			NO			NO					
Clarity	NO			NO			NO			NO			NO					
Conductivity	NO			NO			NO			NO			NO					
Ammonium nitrate (mg/L)	1,9			1,9			1,8			1,8			2,0					
Monitoring ^a	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	1.8	1.5	1.5	1.9	1.9	2.0	1.9	1.8	1.8	1.8	1.7	1.7	1.4	1.1	1.7	1.7	1.7	
Nitrogen Nitrate (mg/L)	0.3			< 0.0			< 0.0			< 0.2			< 0.0					
Sulfate (mg/L)	< 7.0			< 7.0			< 7.0			< 7.0			< 7.0					
Phosphate (mg/L)	0.8			0.8			0.8			1.0			0.7					
Monitoring ^a	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	0.83	0.8	0.8	0.8	0.8	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.8	0.8	0.7	0.7	0.7	
Heterotrophic bacteria UFC/ml	Absent			1			Uncountable			411			8					
Col. total UFC/ml	Absent			Absent			Absent			Absent			Absent					
Col. fecal UFC/ml	Absent			Absent			Absent			Absent			Absent					
Total dissolved solids	NO			NO			NO			NO			NO					
Total suspended solids	< 1.0			< 1.0			< 1.0			< 1.0			< 1.0					
Total fixed solids	< 1.0			< 1.0			< 1.0			< 1.0			< 1.0					
Volatile suspended solids	< 1.0			< 1.0			< 1.0			< 1.0			< 1.0					

^a Analyses performed by HACH spectrophotometer model DR/2010 (Standard Methods). Parameters for chlorine, Ammonium Nitrate and Phosphate in Potim were monitored daily to verify the volatilization of these substances and the consequent decrease in the concentration in water used in the tests.

Culex tritaeniorhynchus was more common in such plantations than in those which did not use this type of fertilizer. In this study, it is probable that ammonium nitrate carried out the same mechanism in attracting the *Aedes aegypti* females to lay eggs.

Beserra et al⁵ (2010) observed that female *Aedes aegypti*, when confined, prefer anaerobically filtered effluent water to lay their eggs, develop and establish the population. In these conditions, hydro collection had 12.1 mg/l of ammonium nitrate -N, values higher than those found in this study. In the opinion of this author, *Ae. aegypti* do not prefer only clean water; there is variation in the water quality in which it can lay eggs and develop and establish the population.

Benzon & Apperson⁶ (1988) examined the contribution of bacteria in *Aedes aegypti* egg laying and stated that a suspended solution of *Acinetobacter calcoaceticus*, present in water in larval breeding sites, significantly induced this species to lay eggs. Serpa et al¹⁸ (2008) verified co-specific positive interference of water in larval breeding sites on female *Aedes aegypti* egg laying in laboratory conditions. High ammonium nitrate levels, allied to the presence of larvae of this species may have contributed to such circumstances.

The egg laying found in the water collected in the municipality of Taubaté was expected, as *Ae. aegypti* prefer to reproduce in clean water, although they can adapt to new situations which arise.¹²

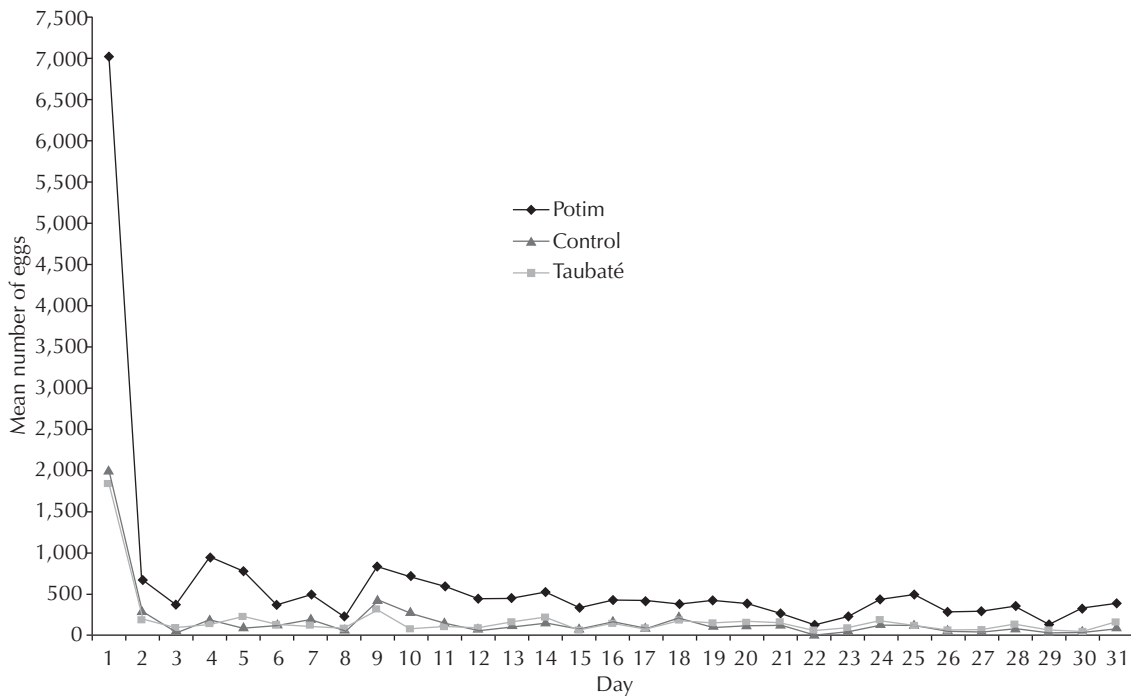


Figure 2. Mean daily number of *Aedes aegypti* eggs in the municipalities of Potim and Taubaté, Southeastern Brazil, and the Control, 2009. The number of eggs was in the water from the municipality of Potim, water from the municipality of Taubaté and distilled water used as the control.

Beserra et al³ (2006), studying the biology of adult *Ae. aegypti* at obtained a mean number of 271.9 eggs per female over a period of approximately 33 days. Such values are lower than those found in Potim in this study (mean 649.37 eggs/female over 31 days).

Gadelha & Toda¹⁴ (1985), in an approach on the biology and behavior of this species of mosquito cited that the first egg laying may reach 80 to 100 eggs, with a subsequent decrease in quantity to a mean 25 to 30 eggs. The former values are also inferior to those found in this study (217 eggs). On the other hand, the daily mean of eggs collected in the other ovipositors (14 eggs) was lower, compared with those reported by the same authors. These authors also cite that one single *Aedes aegypti* female can lay 12 to 15 times in her lifecycle. It is highly probable that the presence of ammonium nitrate stimulated egg laying, especially with regards to the first laying.

Gomes et al¹⁷ (2006) studied determination of daily patterns in female *Aedes aegypti* egg laying in the laboratory and observed that the highest percentage of eggs were deposited in the first laying compared with subsequent days.

The domestic water tank breeding site is the most commonly used and most productive container

for *Aedes aegypti* in the municipality of Potim. Conversely, their absence was verified in those residences whose water tank was replenished from shallow wells or ponds.^a

The results found here reinforce the importance of monitoring the water table used in the public water supply and produces concerns as to its protection and quality. Levels above those permitted may lead to unwanted situations for the organism, as well as attracting pregnant female *Aedes aegypti* to lay their eggs.

Freitas et al¹³ (2001) observed contamination, and high rates of water not fit for drinking in research carried out in the *Parque Fluminense*, Corumbá, RJ, Southeastern Brazil. According to them the extent of the problem is not just regional but takes on national proportions.

No other chemical component differed from the standards adopted for drinking water and, according to the literature,^{2,8-10,12,15,22} the discussion was aimed at differences in the concentration of ammonium nitrate found in the samples. Using pure solutions of this compound in known gradients of concentration to evaluate possible dose dependent effect, without including other compounds which may act as a stimulant in selecting sites to lay eggs is a methodological limitation of studies.

Table 2. Results of the physicochemical analysis of samples from the public water supply. Taubaté, SP, Southeastern Brazil, collected on 25th and 31st of March and 8th, 14th and 22nd April 2009.

Parameters	Data/collection																	
	March, 25 (1 st collection)			March, 31 (2 nd collection)					April, 8 (3 rd collection)			April, 14 (4 th collection)				April, 22 (5 th collection)		
Appearance	Clear			Clear					Clear			Clear				Clear		
Color (PtCo APHA)	6.0			4.0					4.0			2.0				6.0		
Chloride (mg/L)	0.4			0.4					0.4			0.2				0.3		
Monitoring	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–	–	–	–	–	–	–	
Odor	NO			NO					NO			NO				NO		
pH	7.5			7.4					7.5			7.6				7.4		
Taste	NO			NO					NO			NO				NO		
Clarity	NO			NO					NO			NO				NO		
Conductivity	NO			NO					NO			NO				NO		
Ammonium nitrate (mg/L)	< 0.0			< 0.0					< 0.0			< 0.0				< 0.0		
Monitoring	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	0.7	0.7	0.4	0.6	0.6	0.6	0.5	0.7	0.7	0.6	0.4	<0.1	<0.1	<0.1	0.6	0.6	0.5	
Nitrogen nitrate (mg/L)	0.8			0.7					0.7			0.7				0.7		
Sulfate (mg/L)	33.0			28.0					28.0			28.0				28.0		
Phosphate (mg/L)	0.5			0.4					0.2			0.3				0.4		
Monitoring	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	2 nd day	3 rd day	4 th day	5 th day	2 nd day	3 rd day	4 th day	
	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.2	
Heterotrophic bacteria UFC/ml	497.00			680.00					560.00			411				NO		
Col. total UFC/ml	Absent			Absent					Absent			Absent				Absent		
Col. fecal UFC/ml	Absent			Absent					Absent			Absent				Absent		
Total dissolved solids	< 1.00			< 1.00					< 1.00			< 1.00				< 1.00		
Total suspended solids	< 1.00			< 1.00					< 1.00			< 1.00				< 1.00		
Total fixed solids	< 1.00			< 1.00					< 1.00			< 1.00				< 1.00		
Volatile suspended solids	< 1.00			< 1.00					< 1.00			< 1.00				< 1.00		

The concentrations of ammonium nitrate found in the public water supply in Potim may have contributed to the choice of the mosquito to lay eggs here. It is, therefore, important to improve the quality of

this water, not only in order to reduce the mosquito infestation and consequent risk of transmitting dengue, but also for the health of the population in question.

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