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Evaluation of occurrence of NO_3^-, Coliform and atrazine in a karst aquifer, Colombo, PR

Avaliação da ocorrência de NO_3^-, coliformes e atrazina em um aquífero cárstico, Colombo, PR

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ABSTRACT

The vulnerability of karst aquifers to contamination by agrochemical compounds was studied. Such contamination occurs due to its geomorphological structure. Despite the fact, aquifers are important to provide potable water, there is a lack of research about karst aquifers in Brazil. The aim of this study was to evaluate the occurrence of nitrates, fecal coliform bacteria and atrazine in shallow and deep wells in the karst aquifer in the State of Paraná, which is affected by agricultural activities. This study was conducted in an intensive agricultural area located inside the basin of the Upper Iguassu/Ribeira in the Municipality of Colombo, Paraná in Brazil. The sampling campaigns were carried out between 2014 and 2015 in fifteen shallow wells and seven deep wells. Nitrates, total and fecal coliforms and atrazine were analyzed. Nitrates were found in higher concentration in all shallow wells, ranging from 0.14 mg L^{-1} and 40.22 mg L^{-1}. In deep wells, the lower concentrations were between 1.24 mg L^{-1} e 17.86 mg L^{-1}. The analysis of total and fecal coliforms showed the bias for nitrates. Atrazine was detected in five shallow wells and in four deep wells. Physico-chemical characteristics of atrazine as well as fractures of the karstic aquifer and high hydraulic conductivity can be considered as determining factors in the fate of pesticides.

Keywords: Groundwater; Pesticides; Shallow wells; Deep wells; Agriculture.

RESUMO

A vulnerabilidade de aquíferos do tipo cársticos à contaminação por atividades agropecuárias ocorre em função de sua estrutura geomorfológica. No entanto, existem poucos estudos desta natureza no Brasil. O objetivo deste estudo foi avaliar a ocorrência de nitrato, bactérias coliformes e atrazina em poços rasos e profundos no aquífero cárstico paranaense sob influência de atividades agrícolas. O estudo foi realizado em uma área de agricultura intensiva sobre aquífero cárstico da bacia hidrográfica do Alto Iguaçu/Ribeira no município de Colombo-PR. As campanhas amostrais foram realizadas no período entre 2014 e 2015 em 15 poços rasos e 7 poços profundos, para análise bacteriológica, de NO_3^- e de atrazina na água. A concentração de NO_3^- nos poços rasos variou entre 0,14 mg L^{-1} e 40,22 mg L^{-1}. Nos poços profundos, as concentrações foram menores, entre 1,24 mg L^{-1} e 17,86 mg L^{-1}. A análise de coliformes totais e fecais apresentou a mesma tendência da presença deNO_3^-. A atrazina foi detectada em 5 poços rasos e 4 profundos. Propriedades físico-químicas da atrazina, assim como, as fraturas do aquífero cárstico e alta condutividade hidráulica podem ser consideradas como fatores determinantes no destino deste pesticida.

Palavras-chave: Águas subterrâneas; Pesticidas; Poços rasos; Poços profundos; Agricultura.

INTRODUCTION

Agrochemical compounds, in the form of fertilizers and pesticides, have been identified in many studies as one of the main contaminant groups of groundwater and surface water (HILDEBRANDT et al., 2008; ANDRADE; STIGTER, 2009; KOMÁREK et al., 2010; KING et al., 2013; MADSEN; SOGAARD, 2014). Many of these compounds, due their physical-chemical properties, can be classified as carcinogenic, teratogenic and mutagenic, besides their having bioaccumulation capacities (HERNÁNDEZ et al., 2013).

Karst aquifers are characterized by their high vulnerability caused by their morphologic structure (HALAWANI et al., 1999; HATOUM, 2007). They are globally significant; being a source of drinking water for approximately 25% of the world population (GOLDSCHEIDER, 2005). Big urban centers in Europe, like Bristol, London, Paris and Vienna are totally dependent of this kind of aquifer (FORD; WILLIAMS, 1991).

The term "karst" means limestone rocks areas, when formations like caves, dolines (sinkholes) and giant underground rivers are formed by natural dissolution (MINEROPAR, 2001).

The karstification process begins with the combination of meteoric water or superficial water together carbon dioxide (CO_2) from the atmosphere or the soil. The result is water rich in carbonic acid (H_2CO_3) that promotes the dissolution of the ion carbonate of these types of limestone rocks (FORD; WILLIAMS, 1991). The formation process of the karst aquifer is not homogeneous. Many factors can interfere with homogeneity, such as: the variation of the chemical composition of rocks, the differences of the degrees of fracture, relative stratigraphic position and climate. These combined actions of then make some areas more susceptible than others and have more resistance for dissolution (FORD; WILLIAMS, 1991). Several studies (GOLDSCHEIDER, 2005; HAARSTAD; LUDVIGSEN, 2007; MAHLER; MASSEI, 2007; YU et al., 2015) are calling attention to the weakness of karst aquifer for possible contamination from pesticides and fertilizers. However, most of them are from Europe, Central and North America and China. Robert et al. (1999) identified a general pesticide contamination, detected in almost 100% of superficial water and 50% of deep wells. Younes and Galal-Gorchev (2000) and Arias-Estévez et al. (2008) indicated that, the contamination of groundwater from pesticides is the main way for human exposure to the toxicity of these contaminants. In Ireland, MacManus et al. (2014) analyzed for two years the behavior of several types of aquifers and concluded that karst reservoirs are the most vulnerable to contamination from these micropollutants. The studies regarding the effects due to pesticides generally are narrowed by the active principle, and the metabolic generated from the degradation of them are not considered (KOLPIN, 2013). There is a trend of these metabolites to be more stable and toxic than their original pesticide, on the environment (PUCAREVIC et al., 2002).

The karst aquifer, in the State of Paraná (PR), Brazil, were originated in the geologic formation Capiru of the Açungui Group (FIORI, 1992). This formation had its origin in the upper Neoproterozoic and has a range of 9 to 19 kilometers width with SW-NE direction. Covering partially or totally of some the cities of Metropolitan Region of Curitiba, Paraná (PR) (MINEROPAR, 2001). Calcitic and dolomitic metalimestones interleaving philites and quartzites, are prevalent with variable thickness, and with diabase dykes cutting these metasedimentary rocks (FIORI, 1992). This geologic structure is formed for conducts and fractures that eases the movement of the water infiltration in vertical flux (MAHLER et al., 2008). Inside karst aquifers, solute contaminants move through these connections in the groundwater (VESPER et al., 2001). The countryside of the city of Colombo, PR supply the Metropolitan Region of Curitiba, with vegetables and fruit and agricultural products, that includes the usage of agrochemical products. As this activity occurs in the area of the karst aquifer, there is a potentially high risk of contamination of its groundwater. Aquifers, in many places, when they are the only one are the main source of fresh water for human consumption and irrigation.

The karst aquifer of Paraná, in the Metropolitan Region of Curitiba, is a partial source and at times the only source of the water provision to cities of Colombo, Almirante Tamandaré, Bocaiúva do Sul, Campo Magro, Campo Largo and Itaperuçu (HINDI et al., 2013). It has properties that make the karst aquifer the most vulnerable to contamination by pesticides and nutrients in comparison of other kinds of aquifer (MAHLER et al., 2008; MILEK et al., 2011).

Nitrogen (NO_3^-) is present on the constitution of the NPK fertilizers and in urea. After the application to the soil, part is absorbed by the plants, another part is lost into the atmosphere by ammonification and denitrification process, and some parts are moved by the leaching (vertical) or superficial leaking (horizontal).

The synthetic organic pesticides are classified based on their function (herbicide, insecticide, fungicide), and their chemical structure (triazines, carbamates, pyrethroids, organochlorides, organophosporides).

Herbicides are the most utilized in agriculture, and among them atrazine is one of those destacted, from the triazine group (GARMOUMA et al., 1997). Atrazine (2-chlorine-4-(etilamine)-6-(isopropilamine)-s-triazine), is a systemic herbicide, utilized before and/or after the plague attack. It acts on the annual weed control on corn, sugar cane or sorghum (VELISEK et al., 2012). It also is applied near roads, highways and railway of similar function or purpose (ATSDR, 2003). Atrazine is often found in the world because of its efficiency in the combat of plagues and at low cost (HALLBERG, 1989; STOLTENBERG et al., 1990; ENVIRONMENT CANADA, 1993; JAYACHANDRAN et al., 1994; ZHANG et al., 2011; HILLEBRAND et al., 2014). According the Agricultural Defense Agency of Paraná (ADAPAR), It is the second most consumed pesticide in the Metropolitan Region of Curitiba (ADAPAR, 2015).

The atrazine is moderately soluble in water (S_w of 33 mg L^{-1}; K_{oc} of 100 mL^{-1}) and its movement in soil is limited by the adsorption of certain colloids that constitute the organic matter in soil (EPA, 2006). When it is applied, it tends to remain for many days in soil or in the target plant. However, when transported by leaching it can bioaccumulate inside aquatic organisms and the generated metabolite tends to be more toxic than the atrazine itself (ATSDR, 2003). The toxic effects of atrazine on a human being are associated with the alterations to the reproductive system (ATSDR, 2003). However, focusing basically on the rising of productivity of cultures, the application of pesticides research tends to neglect their ecotoxicological impacts (VIEL et al., 1998; MEYER et al., 2003; LEVARIO-CARRILLO et al., 2004).

The purpose of this study was evaluate the occurrence of nitrates, coliform bacteria and atrazine compounds along with assessing the risks of contamination in shallow and deep wells in the karst aquifer of Paraná, in areas where agriculture activity is potentially effected or impacted.

METHODS AND MATERIAL

Sampling

The sampling plan consisted of one month collecting of water samples (surface and deep) for nitrogen and coliform bacteria total and fecal, for a period of 9 months (July/2014 to March/2015) The wells were located in agricultural zones of Colombo, PR (Figure 1). There were 15 shallow wells (SW1, SW2, SW3, SW4, SW5, SW6, SW7, SW8, SW9, SW10, SW11, SW12, SW13, SW14, SW15), which varied in depth from 2m to 12m and in 7 deep tubular wells (DWF, DW3, DW4, DW5, DW3E, DW16, DWB), which the depths varied from 48m to 300m (Figure 2). The atrazine was analyzed using only one sampling, in September of 2015, in 11 shallow wells (SW1, SW2, SW3, SW6, SW8, SW9, SW10, SW11, SW13, SW14, SW15) and in 7 deep wells in December of 2015 (DWF, DW3, DW4, DW5, DWB, DW3E, DW16).

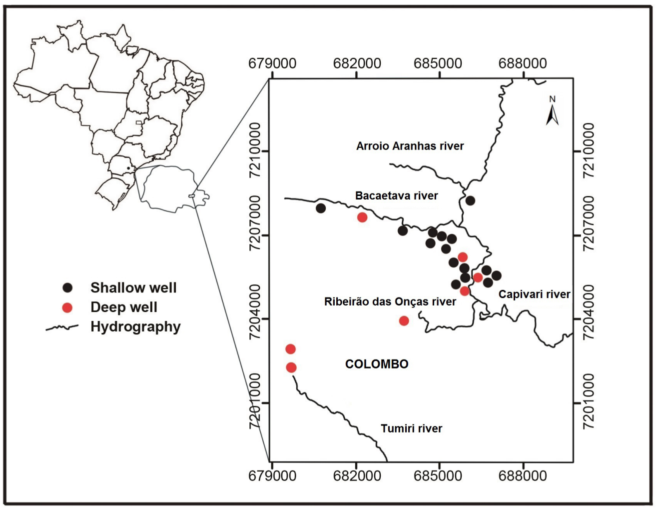

Figure 1. Localization of monitoring wells on the karst aquifer in the Capiru Formation of Açungui Group, Colombo, PR.

Nitrate and Coliform bacteria analysis

The nitrate analysis was performed using the reduction per cadmium criteria as described in Standard Methods for Water and Wastewater Examination (APHA, 2012), quantified by Visible Ultra-Violet Emission.

The bacterial analysis was obtained by employing the counting test of chromogenic subtract method, in which the Value Most Probable (VMP) of coliform bacteria in 100 mL of a water sample (APHA, 2012). Further, incubating the sample on 35.5 °C, for 24 hours. The yellow color of a sample indicated the confirmation of the total coliform bacteria in real-time. The development of fluorescence bright under a dark light indicated the presence of fecal coliform bacteria. Lastly, the transparence of sample analyzed indicated that the water didn't have any bacteria.

The statistical analysis of NO_3^- results, were achieved by using the methods for standard deviation, which is a generalization of the ANOVA analysis applied on generalized linear models. The regression used the concentration values of concentration x dynamic level of the wells. The adjustments were made to the model for the data using Gamma probability distribution and Normal distribution. The comparing of these treatments were then realized by the Tukey test (5%).

Atrazine analysis

The physical-chemical properties of atrazine are: solubility in water (S_W = 35 mg L^{-1}); partition coefficient octanol-water (Log K_{ow} = 2.7); coefficient of adsorption to organic matter;

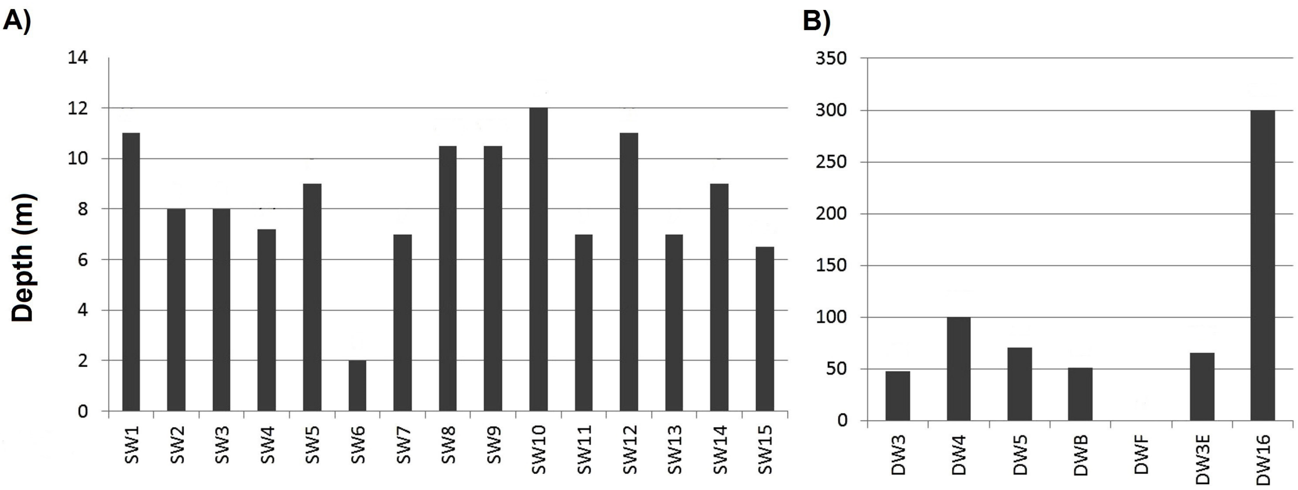

Figure 2. Depth (m) of shallow wells (SW1 to SW15) and deep wells (DW3 to DW16).

half-life time ($T_{1/2}$ = 86 days on water); Leaching potential index (GUS = 3.20); volatilization index ($K_H = 1.5 \times 10^{-4}$ m^3 mol^{-1}).

The atrazine was analyzed following the procedure of Lacorte et al. (2000), that consists on the extractions with ethyl acetate and further dilution to quantifying on Gas Chromatograph coupled with an Mass Spectrogram (Varian CG and MS 220), equipped with a capillary column of VF 1 ms (30 meters of length × 0.25 millimeters of intern diameter × 25 millimeters of thickness of film). The CG-MS was programmed to 70 °C for 4 minutes, heathen to 280°C during 52.5 minutes, reaching 4 °C min^{-1}. The chromatographic conditions of temperature of the interface and the ion source were 280 °C and 200 °C respectively. The ion trap mass spectrometer was operated by ionization by the impact of electrons, which included the ionization energy of 70 eV and emission of 300 mA. A constant flux of Helium gas of 1.0 mL min^{-1} was used as drag gas. The volume of injection was 1 µL in a mode of *splitless* (1 min) with the injector temperature on 250 °C. Beginning with the inducted dissociation per collision, 3 ions of the mass fragment were selected to the confirmation of atrazine.

Atrazine solutions were prepared as standard (99% of purity, Sigma-Aldrich., Missouri, USA) 3 µg L^{-1} and cholestane solutions were prepared as an internal standard (Sigma Chemical Company, St. Louis, USA) 10 mg mL^{-1} in ethyl acetate, maintained on –4 °C. Also 5 blank samples were prepared with natural mineral water (Serra da Graciosa).

For the identification of the compounds the Automatic Mass Spectral Deconvolution and Identification System program (AMDIS), 2.17 version of 2012 were utilized. The evaluation of atrazine concentration in the samples was captured through the ion m/z 200 at the time of retention, 29.7 minutes, since the ratio between the atrazine areas and the cholestane intern pattern, identified through the ion m/z 217 at the time of retention 59.3 minutes; according the below equation:

$$\text{Relative. Conc.} = \frac{[\text{Atrazine area}]_{\text{Standard}}}{[\text{Cholestane Area}]_{\text{Standard}}} \times 3 \qquad (1)$$

DISCUSSION AND RESULTS

Nitrate and coliform bacteria occurrence on groundwater

The temporal distribution and frequency of nitrates in shallow wells and in deep wells (concentration x months of sampling) are showed on Figure 3. The concentration of nitrates (nitrogen) in shallow wells was higher in January, February and March of 2015. This period normally has the higher ratio of rain, and only the highest value of nitrates was only in March of 2015 in deep wells. This period in March may have the higher concentrations of rain, also is the period of bigger recharge of the aquifer, this could be one reason for the high concentration of atrazine (DRAGON et al., 2015). There was also a difference in the concentration in shallow wells over the deep wells ($p < 0.05$). However, all the results were under the maximum value, allowed by the World Health Organization (WHO, 2011) that is 50 mg L^{-1} or 11 mg L^{-1} by N-NO_3^-.

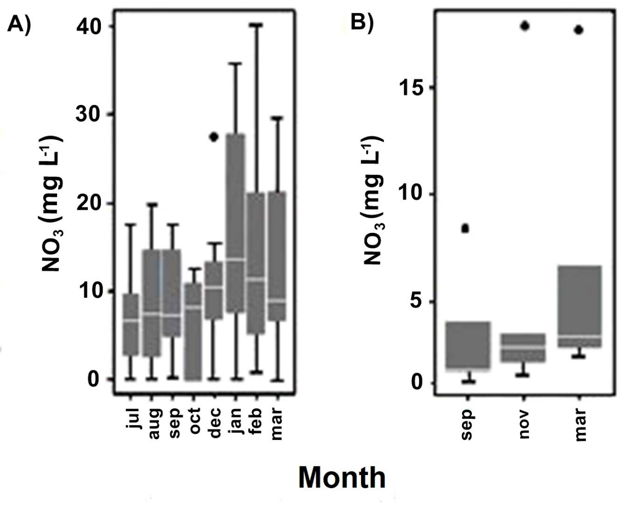

Figure 3. Average concentration of nitrate on shallow wells (A) and deep wells (B) of July, 2014 to March, 2015. Outlines represents the maximum values.

In the shallow wells the maximum value, considering the standard deviation, was 40.22 mg L^{-1}, and the minimum was near the detection limit of the used method. In the deep wells, the maximum value was 17.86 mg L^{-1}. The extreme values had a little representability when comparing with the average absolute values (Figure 3).

The spatial projection of nitrate average concentration (Figure 4) represents the composition of all of the water samples of shallow wells. It shows that the groundwater resources of the karst aquifer were vulnerable to nitrate contamination.

Figure 4 shows a higher nitrate concentration in the wells SW1, SW2, SW5, SW9, SW10 e SW11. The wells SW3, SW4, SW6, SW7, SW8 and SW12 presents the smallest nitrate concentrations.

From 91 samples of water of the shallow wells, 11 showed concentrations of nitrate between 20 to 40 mg L^{-1} and 80 samples showed values under 20 mg L^{-1}. In the wells SW13, SW14 e SW15 the nitrate concentration varied between 10 e 12 mg L^{-1}, although, they were located near the higher concentration wells. The higher concentrations were detected at the edges of the Capivari river (40.22 mg L^{-1}); SW2 (35.36 mg L^{-1}) and the Bacaetava river SW9 (29.61 mg L^{-1}) indicating anthropic contamination.

From the 25 samples of water of the deep wells, 15 indicated absence of nitrate or any value under the limit of the equipment detection, 8 samples showed concentrations between 5 and 10 mg L^{-1} and only on two, values above 17 mg L^{-1}. In these wells, the nitrate concentration follow this order: DWB, near the Bacaetava river (17.86 mg L^{-1}), DW3E, near downtown Colombo (8.18 mg L^{-1}), DW16 (6.63 mg L^{-1}), DWF (6.10 mg L^{-1}), DW5 (3.32 mg L^{-1}), DW3 (2.83 mg L^{-1}) e DW4, on the Capivari river flood plain (2.39 mg L^{-1}).

Nitrates when in excess, tends to percolate in soil with rainfall or irrigation, therefore contaminate the groundwater (JALALI, 2005). This behavior occurs because of nitrogen´s ionic structure. Once in contact with the particles in soil, where there is a dominance of negative charge, the electrostatic attraction doesn't occur, facilitating the leaching, promoting the nitrate

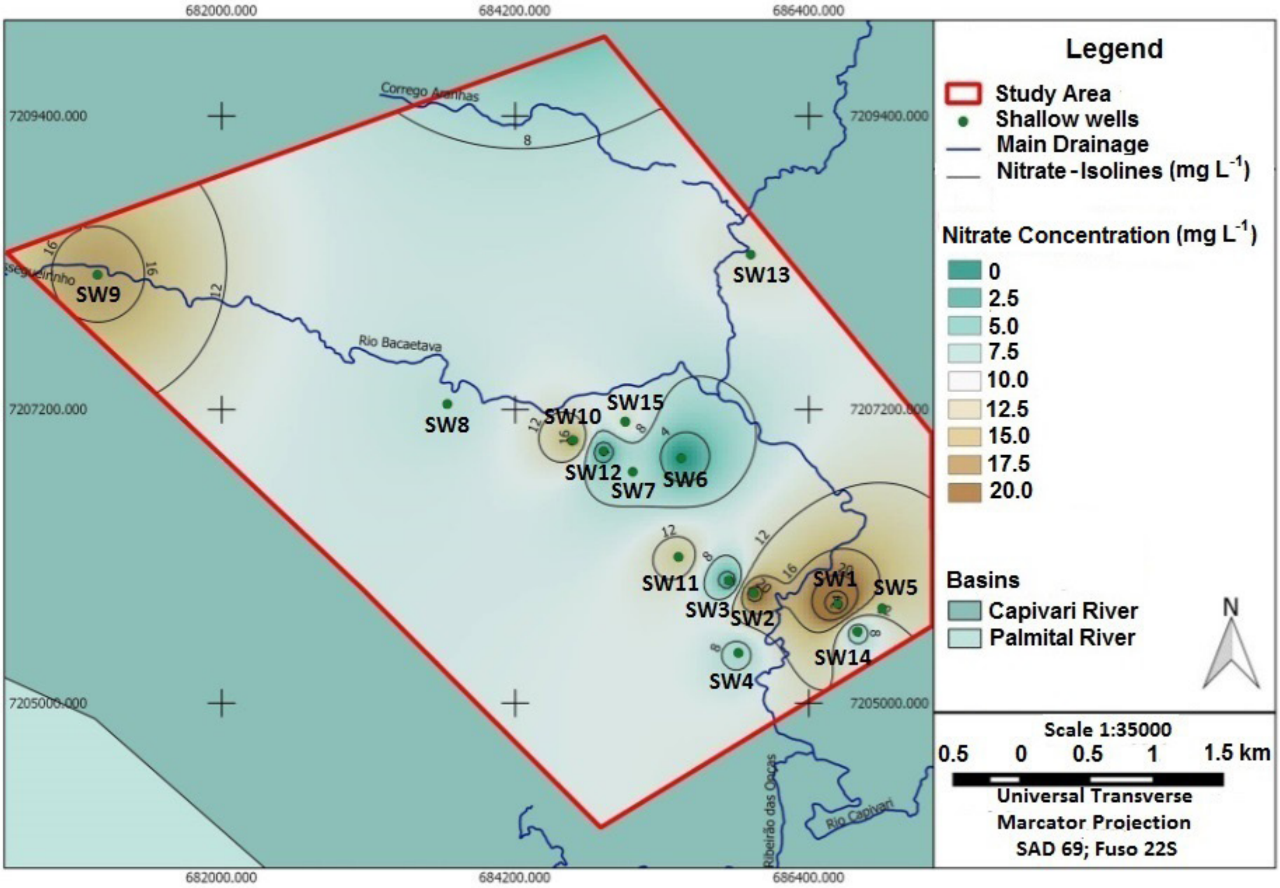

Figure 4. Nitrate distribution map on shallow wells. The experimental area is represented by the red contour.

concentration along the time (VASCONCELOS et al., 2013; HILLEBRAND et al., 2014).

The major part of the public supply of water regarding the study region came from the capitation of groundwater of the karst aquifer, by pumping in deep wells. For Bouman et al. (2002) the location of shallow wells and deep wells in intense agricultural areas justify the detection of nitrates. The Environmental European Agency considers that the agriculture contributes to 50% to 80% of total load of nitrate of groundwater (EEA, 2005). The literature shows, in global scale, that karst aquifers present higher nitrate concentration when comparing other types of aquifers. (VESPER et al., 2001; MAHLER et al., 2008). This are followed by the association between geology and shallow soils, preference vertical flux, dissolution fractures of carbonate rocks and doline formation (RICHARDS et al., 1996).

The depth of the wells was determinant for the concentration of nitrates in groundwater. In wells with depth bigger than 48 meters, the nitrate concentration was smaller than in shallow wells with depth less than 20 meters. Denitrification processes can justify these values. The reduction or the absence of nitrate concentration at the time can be an indicator of microbiologic processes along with the rise of N_2 concentration.

Total coliform bacteria and fecal coliform bacteria were present in all the samples of shallow wells and only in 2 deep wells (DW4 e DW3E), They showed total coliform bacteria in September and November of 2014, and a total absence of fecal coliform bacteria. The use of aviary manure as a fertilizer can justify this result. Although, the contribution of domestic (raw) sewage can't be discarded, considering that, the entrance of contaminants can be facilitated by the geomorphology of karst aquifers (CHESS, 1987). Besides, the superficial leaking of water, promoted by intense rainfall and agricultural irrigation, it tends to elevate the piezometric level of the free aquifer (FRITZSONS, 2003).

Atrazine on groundwater

The highest concentration of shallow wells was found in SW11, SW6, SW9, SW1, SW8 (Figure 5), and in deep wells on DW3, DW4, DW5, DWB (Figure 6). The maximum value allowed by EPA is 3 μg L^{-1} (EPA, 2009). In the wells SW2, SW3, SW10, SW13, SW14, SW15, DWF, DW3E and DW16, the atrazine wasn't found and the levels of concentration were below the detectable limit of the equipment.

Of the seven deep wells monitored, four presented chromatograph peaks with relative area of atrazine (DW3, DW4, DW5 e DWB). Of eleven shallow wells, five showed atrazine (SW1, SW6, SW8, SW9, SW11). The results show that the water of the karst aquifer was contaminated with atrazine, possibly with concentrations above the levels recommended for human consumption by the American legislation which is 3 μg L^{-1} (EPA, 2009).

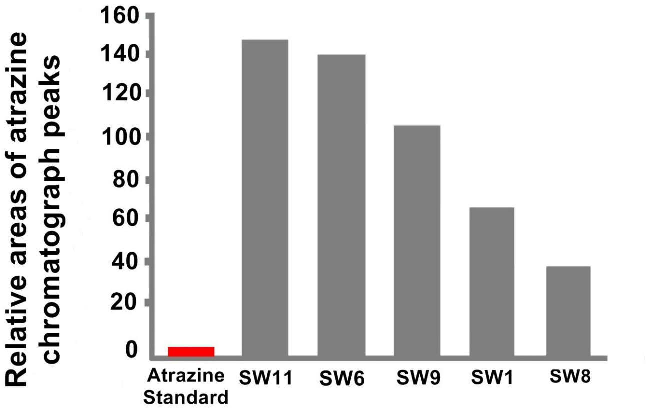

Figure 5. Relative areas of atrazine chromatograph peaks on the shallow wells.

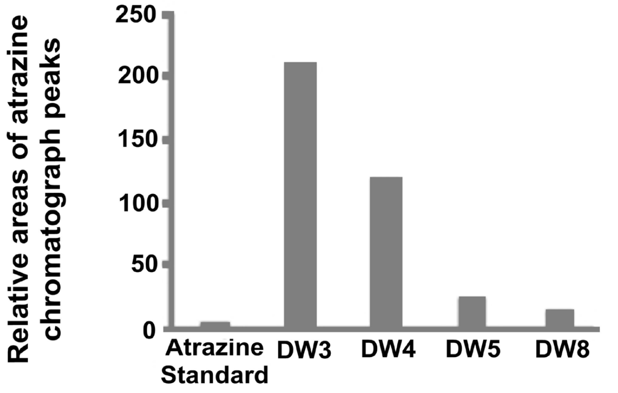

Figure 6. Relative areas of atrazine chromatograph peaks on the deep wells.

The physical-chemical properties of atrazine, such as the low rate of organic matter adsorption of the soil, the high potential of leaching in the soil and the long half-life time on water (THURMAN et al., 1992; MAHLER; MASSEI, 2007; MAHLER et al., 2008), associated with geomorphology of the karst aquifer and characterized by connections among fractures with high conductibility between then, all contributed to the groundwater contamination. When the water passes through the fractures and reaches the cavity underground, where the water is stored, the hydraulic conductibility tends to be smaller. So, the pesticide can be accumulated in these cavities (ATKINSON, 1977; REH et al., 2013).

Because the shallow wells are located in the yard of residents, in addition to the sampling, the sampling team provided environmental and educational training, demonstrating to the native population the importance of cleaning and regular disinfecting of wells to reduce the risk of contamination by coliform bacteria, and the need for appropriate planning of the allocation of agricultural planting, in an effort to reduce nitrate contamination.

CONCLUSION

The concentration of NO_3^- in every well tested (deep and shallow) were under the maximum values allowed by World Health Organization (50 mg L^{-1}). Higher values were found on shallow wells than deep wells, indicating contamination by fertilizers. Though, the presence of coliforms bacteria doesn't rule out the possibility of contamination from domestic (raw) sewage or the usage of manure as compost.

The qualitative method used for the atrazine analysis was chosen by the authors with the objective of identifying the atrazine presence in our distribution in shallow and deep wells located in a karst area with intensive agriculture. Atrazine was detected at the chromatographic peaks; Some with high intensity. The peaks exhibited larger atrazine levels relative to areas in the shallow and deep wells than to the standard atrazine relative area.

The geomorphological constitution of the karstic aquifer, such as fractures, channels in the rocks limestone can be associated with the atrazine presence on deep wells, since the fractures facilitate the direct transport of the pesticide to groundwater.

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Lucilia Maria Parron Vargas: Revisions of manuscript critically for improving the discussion of results.

Fabrício Augusto Hansel: Help with analytical methods for chromatography quantification of pesticides.

Sandro Froehner: Revision of manuscript and final approval of the version to be published.

Leonardo Tozine Palagano: Map elaboration, help in the field campaign, assisted in the text correction.

Ernani Francisco da Rosa Filho: Critical revision of the article and help in interpretation and discussion of data.