

## Is groundwater fauna impacted by swine effluent fertigation?

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### ABSTRACT

In this study, we tested whether the fertigation of swine effluent impacts the underground aquatic fauna of porous free aquifer. The physicochemical parameters of groundwater were determined and correlated with the fauna present in the aquifer on fertigated and non-fertigated areas with swine effluents treated in biodigester. Seasonality influences on water quality was also tested. For this purpose, groundwater samples from pre-existing farmer-owned water wells and piezometers using the bailer and 65-Micra mesh net for filtering organisms. The physicochemical results show that there may be some changes in quality parameters. We recorded twelve invertebrate taxa, with Acari and Copepoda being the most prevalent. Colonization of aquatic species may have been limited by the emergence of exotic organisms and water quality.

**Keywords:** Aquatic Life in the Subsurface; Stygofauna; Sedimentary Aquifer

### 1 INTRODUCTION

Groundwater is the main source of drinking water, the largest amount of which is carried by porous spaces and rock fractures. The groundwater system, which comprises the geological substrate, groundwater itself, and living organisms, is mainly fed by energy and matter allochthonous (DANIELOPOL, 1989; DANIELOPOL et al., 2003).

Groundwater research is progressing in many countries around the world, especially in North America and Europe, where groundwater ecosystem assessments are increasingly needed as part of environmental impact assessments (KORBEL et al., 2017). As regards in Brazil, the domain of groundwater organisms is still little known, the largest amount of information refers to karst relief fauna (BRANCELJ et al., 2013; GALLÃO & BICHUETTE, 2018).

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Knowledge of groundwater fauna is a useful indicator of aquifer environmental health (GRIEBLER et al., 2014; HUMPHREYS, 2009). For this, it is essential to have a detailed understanding of your biota and good biological sampling to monitor groundwater (KORBEL et al., 2017). However, to make a complete diagnosis of the underground aquatic system, it is necessary to analyze a fauna, hydrochemical and microbiological data set (MARMONIER et al., 2018; TOMLINSON et al., 2007). In Brazil, legislation requires an assessment of the chemical and ecological status of surface water while existing groundwater regulations in Brazil do not include the assessment of fauna during the environmental review process, considering the chemical approach sufficient to generate status information the Environmental health of aquifers (CONAMA 396/2008; Portaria n°5/2017).

Aquifer overload with agricultural pollutants (fertilizers and pesticides) affects groundwater quality, human health and induces drastic changes in the diversity of underground organisms (DANIELOPOL et al., 2003; DI LORENZO et al., 2018). Groundwater fauna communities can also be substantially altered over short distances, periods and lower depths of the water table by changing soil quality (HAHN, 2006; SCHMIDT & HAHN, 2012). Aquifer heterogeneity, water chemistry, and groundwater location also affect species diversity and groundwater abundance, which even in small numbers significantly influence sediment permeability through excavation activity and thus affect soil transport and distribution of matter (GRIEBLER & AVRAMOV, 2014). So, the evaluation of aquifer fauna assists in the development of conservation policies and research improvements (DANIELOPOL, 1989; DANIELOPOL et al., 2003; LOPEZ et al., 2017).

This study investigated the biodiversity and hydrochemistry of unconfined aquifer in the Midwest of Brazil with the aim of: (1) evaluate whether the occurrence of groundwater fauna correlates with free aquifer abiotic parameters in fertigated and non-fertigated areas with swine effluents treated in biodigester, (2) determining the physicochemical parameters of groundwater and correlate with groundwater fauna, and (3) test whether seasonality has an effect on groundwater quality.

## 2 MATERIALS AND METHODS

### 2.1 Study area

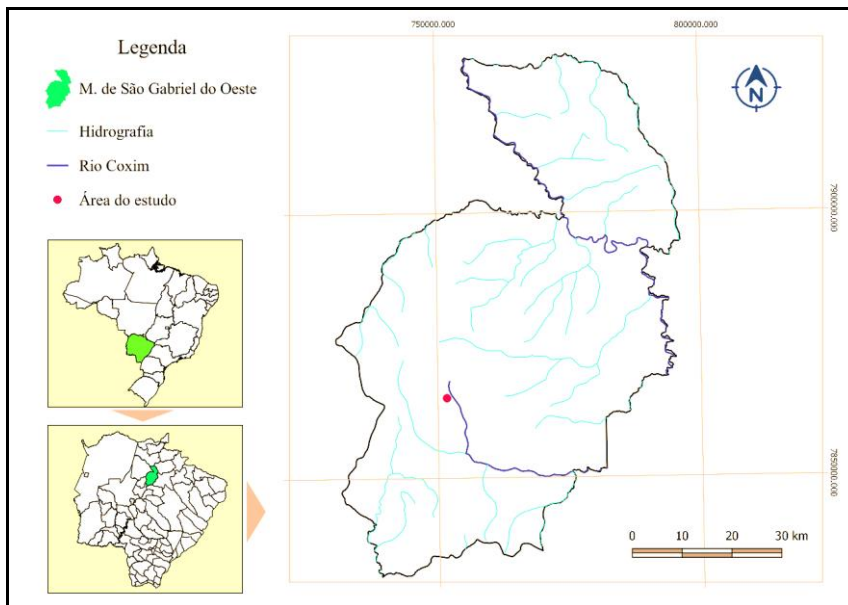
The study was carried out in the north-central region of Mato Grosso do Sul State, in the Midwest Brazil (Figure 1). The area is intensely used for mechanized agriculture (rice, soybean, cotton, corn, and sorghum) and activities of pig farming, livestock and ostrich breeding. In the study area, the deposits of the Debris-Lateritic coverage outcrops, from Tertiary-Quaternary age, in varying thickness. Such deposits are characterized by brownish red oxisols kaolinite and gibbsite, presenting in the most immature profiles very iron levels (CPRM, 2006). The predominant grain size in both surface (0-10cm) and subsurface (10-75cm) soils and sand, followed by the silt and clay fraction (FERRARO et al., 2015).

It is in the microregion of the upper Taquari River basin, upper Paraguay River Basin, covering the Taquari and Miranda River sub-basins, with areas of 88.5% and 11.5% respectively (SEMA, 2010) on the Cenozoic Aquifer System (CAS). The Cenozoic Aquifer System (CAS) consists of the sediment package, which covers the interest-Jurassic sandstones of the Botucatu Formation (Guarani Aquifer), in part of the area. The groundwater quality of CAS is classified as calcium magnesium bicarbonate (SOUZA et al., 2014).

In winter, the rainfall is lower than in summer. The climate is classified as AW – tropical climate, the average temperature and the annual average rainfall are 23.3°C and 1,507mm, respectively.

The agricultural activities in the settlement are composed of corn and soybean crop rotation systems (PAHL et al., 2018). In rural areas, it is very common human supply by CAS exploitation from shallow wells, maximum with 80 m deep (SOUZA et al., 2014). The water supply of the properties of the settlements is made exclusively by CAS exploitation. The sampling included two lots of Campanário rural settlement, where there are four monitoring wells and two supply wells, drilled in CAS.

Figure 1 - Map of the location of the study area, São Gabriel do Oeste municipality, in Mato Grosso do Sul



Source: authors

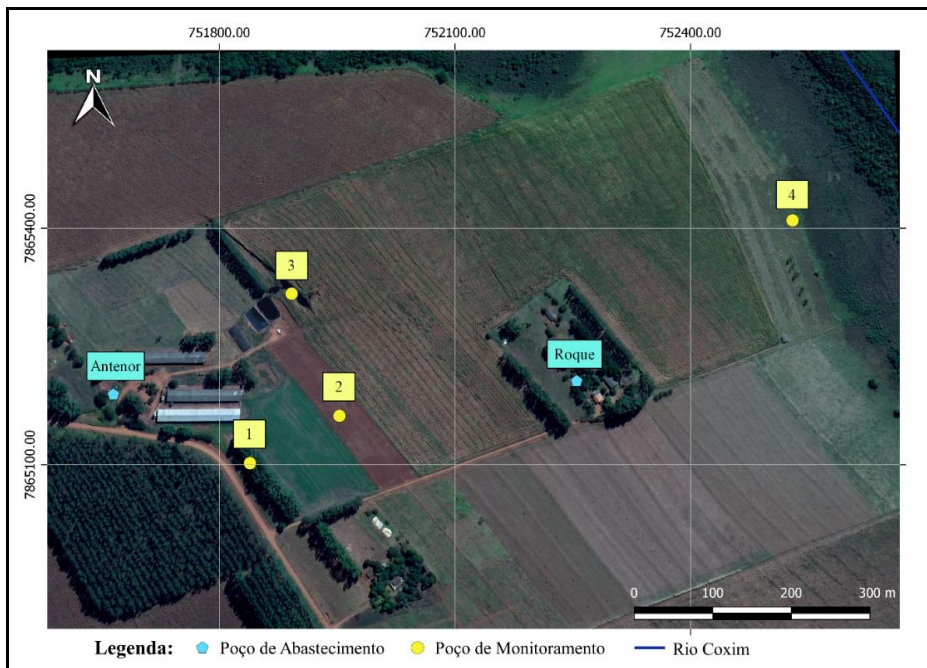
The study area has three pig-sheds installed and a swine effluent treatment system with biodigester. After effluent treatment, sprinkler fertigation is usually done in pasture and arable areas (FERRARO et al., 2015). The effluent from the second lagoon is periodically discharged into the area to be fertigated. Previous research conducted at this site related to the impacts of fertigation with swine effluent found soil and groundwater contamination through coliform investigation (PAHL et al., 2018), metal, effluent and soil analysis (SOUZA et al., 2014; FERRARO et al., 2015).

## 2.2 Sampling

Groundwater biota and water samples were collected in 6 boreholes (Figure 2), in May and again in September 2018. All the boreholes were permanently covered with a lid and without installed pumping structures. The sampling included two lots, where there are four monitoring wells (this term is synonymously used for “piezometers”, in this study) and two supply wells (tubular wells), drilled in the Cenozoic Aquifer. Two wells are located upstream of the area that is fertigated and the piezometers are located in the fertigated area and downstream. The wells that are used for drinking water supply are equipped with a permanent pump, in which the

wellbore collection was performed. For piezometers, bailer collectors were employed. Sampling was performed in two periods, dry (D) and wet (W).

Figure 2 – Location of sampling points in the study area (Campanário Settlement, São Gabriel do Oeste - MS)



Source: authors

Immediately after sample collection, they were filtered through a 68  $\mu\text{m}$  mesh net to collect groundwater fauna. Sampling schemes are illustrated in Table 1. After fixation in the field with 70% ethanol, specimens were sorted under a stereomicroscope and identified to class/order level in the laboratory. After the biological sampling, electrical conductivity (EC), pH, oxide-reduction potential and temperature of groundwater were measured by a multiparametric probe (Aquaread AP 700) in a vessel directly after pumping for three times.

Table 1 – Sampling scheme

SITE	PROCEDURES	ANALYSIS METHOD
Supply	150L water collection at permanent pump outlet	Use of a bucket and 68 $\mu\text{m}$ mesh net filtration
Monitoring	55L water collection with Bailer	68 $\mu\text{m}$ direct network filtration

Source: authors

Water samples to be tested for other chemical parameters in the laboratory were set aside after fauna had been removed. Samples were transported to the laboratory in a cooling box within a few hours after collection. Groundwater quality analyses were performed by the São Paulo State University (Unesp), Institute of Geosciences and Exact Sciences, Rio Claro. The parameters analyzed were the metals Cd, Cr, Pb, Ni, Sn, Co, Mn, Mo, V, Sr, Cu and Zn, and calcium, chloride, nitrate, nitrite, NH<sub>4</sub>, phosphate, siliceous, phosphorous, sodium and Mg.

## 2.4 Data analysis

All data were verified for normal distribution by the Kolmogorov-Smirnov test. All statistical analyses were conducted with a significance level ( $\alpha$ ) of 0.05. As the environmental parameters were on different measurement scales, they were normalized prior to the statistical analyses.

For a comparative graphical representation of study sites based on hydrochemical data, a multivariate principal component analysis (PCA) was performed. For this, the hydrochemical data used were first log-transformed ( $x+1$ ). Correlations were analyzed by using the Spearman-test for non-normal distributions and Pearson-test for samples with normal distributions, followed by the Tukey posttest.

## 3 RESULTS AND DISCUSSION

### 3.1 Hydrochemical

There was no significant difference in groundwater quality due to the seasonality (Spearman and Pearson tests:  $p \geq 0.05$ ). Mean values of groundwater quality variables (Table 2) in dry season were not significantly different from those in wet season.

Table 2. Mean and standard error of mean (SEM) values of the physicochemical parameters investigated in the Cenozoic Aquifer, in the dry (D) and wet (W) season.

	pH	ORP	EC	T	Ca	PO <sub>4</sub> <sup>3-</sup>	Si	P	Cl <sup>-</sup>	Mg	Mn	Na
D (n = 6)												
Mean	5,675	107,117	812,183	24,717	2,235	0,143	1,787	0,047	116,418	0,901	0,151	47,938
SEM	0,251	22,060	807,560	0,214	1,760	0,043	0,254	0,014	116,320	0,824	0,141	47,213
W (n = 6)												
Mean	5,567	32,367	1110,016	27,000	1,374	0,120	1,782	0,039	121,940	0,648	0,146	36,130
SEM	0,311	33,737	1104,000	0,639	1,078	0,333	0,319	0,011	121,820	0,582	0,114	35,375

The number of samples collected in each season is in brackets. ORP, redox potential (mV); EC, electrical conductivity ( $\mu\text{S}$ ); T, temperature ( $^{\circ}\text{C}$ ); Ca, calcium ( $\text{mg L}^{-1}$ ); PO<sub>4</sub><sup>3-</sup>, Phosphate ( $\text{mg L}^{-1}$ ); Si, silicon ( $\text{mg L}^{-1}$ ); P, phosphorus ( $\text{mg L}^{-1}$ ); Cl<sup>-</sup>, chloride ( $\text{mg L}^{-1}$ ); Mg, magnesium ( $\text{mg L}^{-1}$ ); Mn, manganese ( $\text{mg L}^{-1}$ ); Na, sodium ( $\text{mg L}^{-1}$ ); Ni, nickel ( $\text{mg L}^{-1}$ ); NO<sub>3</sub><sup>-</sup>, nitrate ( $\text{mg L}^{-1}$ ).

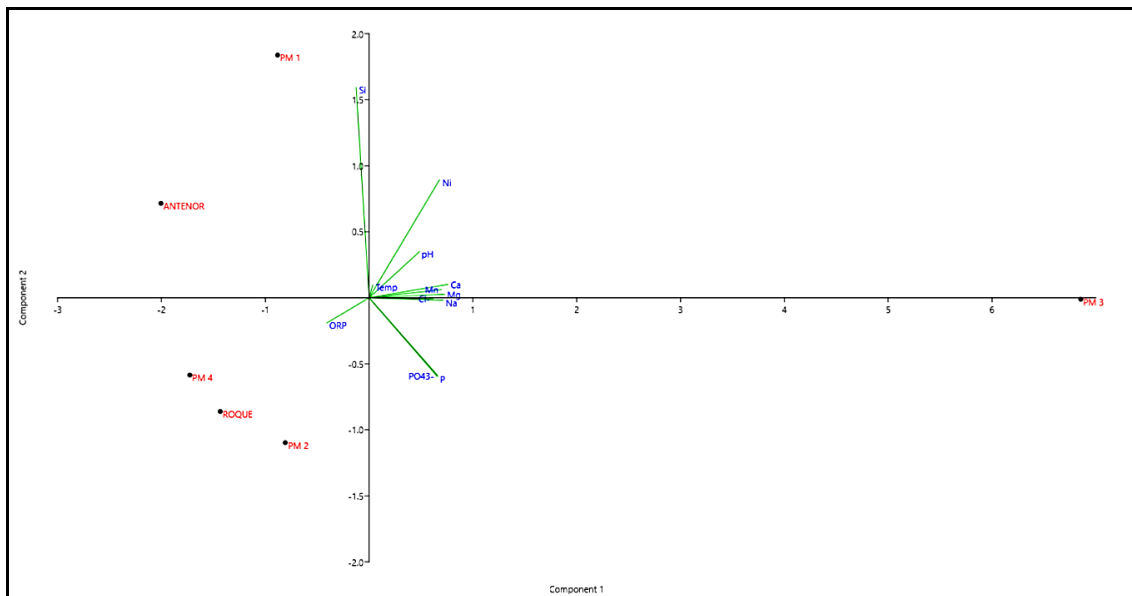
Source: authors

Regarding to the sample points, the PM3 compared to the other wells presented, in both sampling campaigns, the highest concentration values of Ba, Ca, Cl<sup>-</sup>, K, Mg, Mn, NH<sub>4</sub>, NO<sub>3</sub><sup>-</sup>, P, Sn, SO<sub>4</sub><sup>2-</sup>, Sr e V. Higher values were observed for point 3, near manure ponds. Electrical conductivity showed high values, while ORP presented low values. The pH values are below the standard for drinking water limits established by Portaria de Consolidação nº 5/2017 which is 6.0 to 9.5 (BRASIL, 2017).

Groundwater with high-temperature variability has a high exchange with surface water (HAHN, 2006). The high observed temperature values may be because of the increase in surface temperature outside the well and may be influenced by some delay in temperature registration. Previous work in the study area has shown that the temperature of the Cenozoic aquifer averages 25 $^{\circ}\text{C}$  (FERRARO et al., 2015; SOUZA et al., 2014). No resolution is setting the maximum value allowed for this parameter.

The results of the multivariate principal component analysis (PCA) data are shown in Figure 3 and 4.

Figure 3. Principal Component Analysis of groundwater physicochemical parameters (PCA) – Dry season (D).

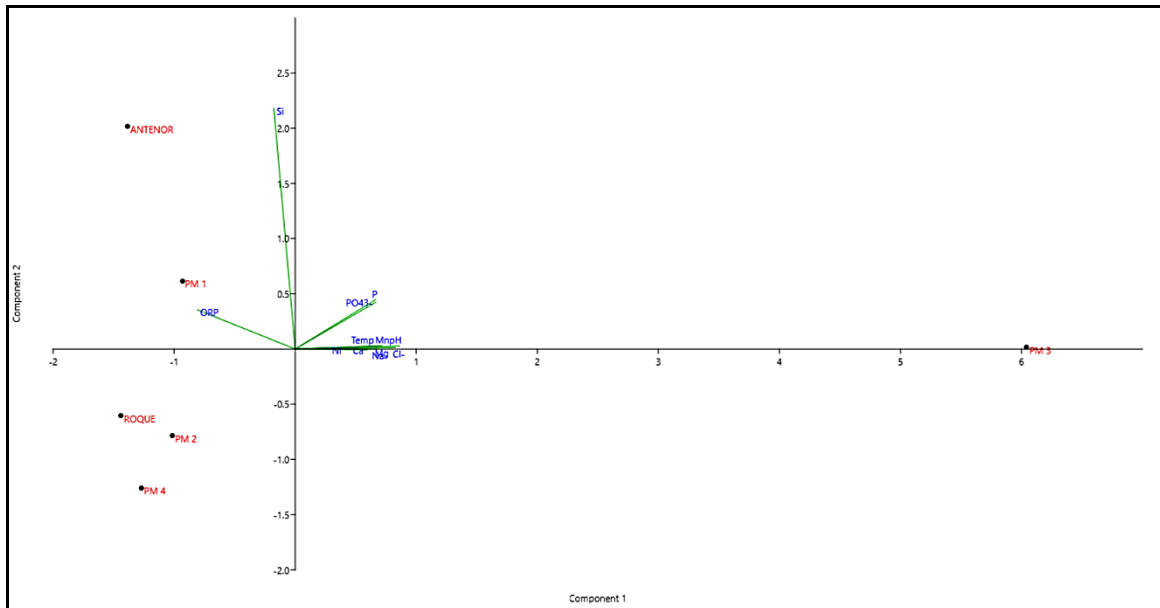


Source: authors

The highest values in Component 2 were associated with Silicon (Si), while in component 1, Calcium (Ca) and Magnesium (Mg). The value of Si variable associated with PM1 indicates that the PM1 and Antenor points had the highest Si concentrations, and low  $\text{Cl}^-$ , Mg and Ca concentrations. The PM3 presented higher values in the pH, ORP, Ca,  $\text{PO}_4^{3-}$ , P,  $\text{Cl}^-$ , Mg, Mn, Na and Ni, these elements that are associated to component 1. The points PM4, Roque and PM2 are plotted in an intermediate area, this means that the values obtained in water samples have no significant association with the evaluated elements. The values got in these 3 points show that the water quality is not as contaminated as PM3. As for the wells PM1 and Antenor, however, the chemical elements that were evaluated are not in high concentrations either. PM4, Roque and PM2 are not of as good quality as PM1 and Antenor (they are in the non-fertigated area), but not as bad as the water collected in PM3. In the dry season, there were higher concentration values of the physicochemical parameters, mainly of the ORP.



Figure 2. Principal Component Analysis of groundwater physicochemical parameters (PCA) -Wet(W) season.



Source: authors

Similarly occurs in the rainy season, with alteration only in the association of Si in the Antenor well.

### 3.2 Fauna

Organisms were present at all collection points as well as at both sampling periods. Table 3 contains fauna presence results for all sample wells. Results from both campaigns included Nematoda, Oligochaeta, Copepoda, and Ostracoda, as well as the other taxa. In total, three phyllo, six classes, and seven orders were collected. All organisms found are representatives of meiofauna.

Table 3. Biotic analysis results for both sample data.

Taxa	PM 1	PM 2	PM 3	PM 4	ROQUE	ANTENOR
DOMAIN EUKARYA						
<b>KINGDOM ANIMALIA</b>	0	0	0	0	0	0
<b>FILO NEMATODA</b>	0	1	23	22	9	2
	0	0	0	0	0	0
<b>FILO ARTHROPODA</b>	0	0	0	0	0	0
<b>SUBFILO Crustacea</b>	0	0	0	0	0	0
<b>Class Ostracoda</b>	0	0	1	0	0	3
	0	0	0	0	0	0
<b>Class Maxilopoda</b>	0	0	0	0	0	0

	SUBCLASS COPEPODA	0	0	0	0	0	0
1834)	Order Cyclopoida (Burmeister,	6	4	13	65	24	8
		0	0	0	0	0	0
	<b>Class Malacostraca</b>	0	0	0	0	0	0
	SUBCLASS EUMALACOSTRACA	0	0	0	0	0	0
1915)	Order Bathynellacea (Chappuis,	5	18	0	1	1	2
		0	0	0	0	0	0
	<b>SUBFILO Chelicerata</b>	0	0	0	0	0	0
	SUBCLASS ARACHNIDA	0	0	0	0	0	0
	Order Acari (Leach, 1817)	21	26	21	65	48	9
		0	0	0	0	0	0
	<b>SUBFILO Hexapoda</b>	0	0	0	0	0	0
	<b>Class Insecta</b>	0	0	0	0	0	0
	SUBCLASS PTERYGOTA	0	0	0	0	0	0
	<b>Infraclass: Neoptera</b>	0	0	0	0	0	0
	<b>Superorder: Exopterygota</b>	0	0	0	0	0	0
1836)	Order Thysanoptera (Haliday,	2	0	0	1	0	1
		0	0	0	0	0	0
	<b>Superorder: Endopterygota</b>	2	0	0	0	0	0
1758)	Order Coleoptera (Linnaeus,	0	0	0	0	0	1
1758)	Order Hymenoptera (Linnaeus,	3	1	0	0	0	12
		0	0	0	0	0	0
	<b>FILO ANNELIDA</b>	0	0	0	0	0	0
	<b>Class Clitellata</b>	0	0	0	0	0	0
	SUBCLASS OLIGOCHAETA	3	0	0	0	0	0
	<b>Total number</b>	42	50	58	154	82	38

Source: authors

The abundance of taxa in descending order was, in the dry season: Acari, Cyclopoida, Nematoda, Bathynellaceae, Hymenoptera and Ostracoda; in wet season: Cyclopoida, Acari, Bathynellaceae, Hymenoptera, Nematoda, and Ostracoda.

The greatest richness in both sampling campaigns was from the Antenor supply well, which is the deepest well; and lower richness in wells PM2, in the dry season, and PM1 and PM3 in the wet season. The organisms of Hexapoda and Coleoptera, Hymenoptera, and Thysanoptera were found, but accidentally, because they are common in the terrestrial environment. According to Hahn (2009), groundwater with the high exchange with surface water has higher proportions of accidental organisms, that are not frequent in groundwater. While hydrochemistry mainly reflects the

hydrogeological origin of waters, the variability in faunal communities reflects the interaction between surface water and groundwater (BORK et al., 2009).

The organisms found here corroborate other studies. The aquatic fauna is shaped significantly by hydrological interactions (HUMPHREYS, 2009), the organisms found are the crustaceans (Copepoda, Ostracoda, Amphipoda, Isopoda, Syncarida, Cladocera), but the Oligochaeta species of the phylum Annelida, Mollusca (snails and slugs), and Nematoda (worms) also live in groundwater (GALASSI et al., 2009; GIBERT et al., 1994; TOMLINSON et al., 2007).

The abundance and richness of the fauna were higher in the non-irrigated points (PM1 and Antenor), different from the data found by KORBEL et al. (2013), where the highest results were from irrigated sites. PM4 concentrated the largest abundance of organisms, with a greater abundance of Cyclopoida and Acari. The order Cyclopoida was present at all points and the Ostracoda class presented individuals only in the Antenor well. Brancelj et al. (2016) found Copepoda distributed in wells predominantly associated with higher K and Na concentrations.

Monitoring points 1, 2 and 3 show a smaller number of organisms. Possible reasons may be that the points have higher values for Ba, Cd, Mn, and Pb. In addition, PM3 had a higher impact, and 1 and 2 had slightly lowed ORP and electrical conductivity, but enough to change the environment.

## 5 CONCLUSION

There is groundwater fauna in the studied primary porous aquifer, the Cenozoic Aquifer System and it was represented by the Acari, Bathynellaceae, Coleoptera, Cyclopoida, Nematoda, Oligochaeta and Ostracoda taxa.

Regarding the physicochemical parameters of water samples, the piezometer 3 (PM3) presents significant difference in water quality, with values above the standards of electrical conductivity, mainly because of high values of Na, Cl<sup>-</sup> and K. These elements contribute to greater electrical conductivity of water.

There was no significant correlation between the values of the physicochemical parameters and the organisms found. However, they seem to be correlated to diminish the number of organisms in groundwater but is not possible to say they cause different fauna. Therefore, swine effluent fertigation could affect groundwater fauna, mainly in population distribution.

In relation to seasonality, groundwater samples are slightly higher mineralized in dry season than in wet season.

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