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Biological soil attributes in oilseed crops irrigated with oilfield produced water in the semi-arid region[1]

Atributos biológicos do solo cultivado com oleaginosas irrigadas com água produzida do petróleo em região semiárida

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ABSTRACT - Wastewater from oil is the main residue of the oil industry. Studies have shown that wastewater, or produced water, can be treated and used as an alternative source for the irrigation of oilseed crops. The aim of this work was to evaluate the effect of treated produced water on the biological properties of soil cultivated with the castor bean cv. BRS Energy and the sunflower cv. BRS 321 respectively, for two and three successive cycles of grain production. The first cycle in the sunflower and castor bean corresponds to the dry season and the second cycle to the rainy season. The third crop cycle in the sunflower relates to the dry season. The research was carried out from August 2012 to October 2013, in the town of Aracati, in the State of Ceará (Brazil), where both crops were submitted to irrigation with filtered produced water (FPW), produced water treated by reverse osmosis (OPW), or groundwater water from the Açu aquifer (ACW), and to no irrigation (RFD). The treatments, with three replications, were evaluated during the periods of pre-cultivation and plant reproduction for soil respiration (Rs), total organic carbon (TOC) and the population density of bacteria (Bact) and filamentous fungi (Fung) in the soil. In the sunflower crop, these soil attributes are sensitive to the irrigation water used. Irrigation of the castor bean affects soil respiration. Under the conditions of this study, irrigation with FPW may be a short-term alternative in the castor bean and sunflower crops.

Key words: Wastewater from the oil industry. Microbiological properties of the soil. Soil basal respiration.

RESUMO - A água residual do petróleo é o principal resíduo na indústria petrolífera. Estudos indicaram que água residuária ou água produzida pode ser tratada e usada como fonte alternativa na irrigação de culturas de oleaginosas. Este trabalho objetivou avaliar o efeito da água produzida tratada sobre atributos biológicos do solo cultivado com mamona cv. BRS Energia, e girassol cv. BRS 321, respectivamente, por dois e três ciclos sucessivos de produção de grãos. O primeiro ciclo de girassol e mamona corresponde à época seca e o segundo, à época chuvosa. O terceiro ciclo de cultivo de girassol se refere à época seca. A pesquisa foi conduzida durante o período de agosto de 2012 a outubro de 2013, no município de Aracati, Ceará (Brasil), onde ambas as culturas foram submetidas à irrigação com água produzida filtrada (APF), água produzida tratada por osmose reversa (APO), água do aquífero Açu (ACA) e sem irrigação (SEQ). Os tratamentos com três repetições foram avaliados em períodos de pré-cultivo, de reprodução das plantas, pela respiração edáfica (Re), carbono orgânico total (COT) e densidade populacional de bactérias (Bact) e fungos filamentosos (Fung) do solo. Esses atributos do solo são sensíveis às águas de irrigação usadas na cultura do girassol. A irrigação das mamoneiras afeta a respiração edáfica. Para as condições deste estudo, a irrigação com APF pode ser alternativa de curto prazo nas culturas de mamona e girassol.

Palavras-chave: Água residuária da indústria petrolífera. Atributos microbiológicos do solo. Respiração basal do solo.

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INTRODUCTION

The oil industry generates various waste products; one of the most important among these is wastewater from the exploitation of oil and gas (MOTTA *et al*., 2013). Water that is trapped in cracks in the rock and in subterranean holes together with the fossil fuels (AMINI *et al*., 2012), when extracted from the wells and separated from the oil at the industrial plant, is known as produced water (PW). This represents the greatest fraction of the liquid extracted from the rock, and can exceed 90% of the volume of oil obtained by the oil industry (MELO *et al*., 2010). Considering this proportion of oil to PW, the Fazenda Belém alone obtained more than 640,000 m^3 of this water in 2014. The Fazenda Belém is inserted in the Potiguar basin, in the northeast of Brazil, where there are several fields that generate more than 30 million m^3 of PW annually (ANP, 2015). The significant volume of PW justifies its being treated for the water to be reused.

Treatment of PW by nanofiltration (MOTTA *et al*., 2013), and treatment by reverse osmosis (MELO *et al*., 2010), have both been proposed for use in in irrigated agrosystems. The appropriate treatment of PW offers opportunities for the irrigated production of such oilseeds as the sunflower (*Helianthus annuus* L.) (SOUSA *et al*., 2016) and castor bean (*Ricinus communis* L.), whose products can be used to produce biodiesel. However, PW is usually rich in salts, which might compromise soil fertility. To maintain the quality of the soil, monitoring of the total organic carbon has been recommended (SILVA *et al*., 2012), together with soil respiration (SANTOS *et al*., 2011) and microorganisms (LOPES *et al*., 2014), as these indicators have a sensitive response to the management practices of the cropping system.

The impact of PW on the biological activity of the soil is little known. Lopes *et al.* (2014) evaluated the biological and biochemical attributes of soil irrigated with PW during one production cycle of the sunflower and castor bean. Those authors noted changes in the proliferation of microorganisms and in nitrogenase activity after irrigation with wastewater from oil. Ferreira, Weber and Crisóstomo (2015) also noted changes in the structure of the soil mesofauna during two production cycles of the same crops irrigated with produced water. It is known that poor quality water affects the way the soil functions, leading to the suggestion that the biological parameters of an agrosystem irrigated with PW should be monitored.

The aim of this study was to evaluate the effect of PW on the biological attributes of the rhizosphere of the sunflower cv. BRS 321 during three production cycles, and of the castor bean cv. BRS Energy for two two successive crop production cycles. Furthermore, the study aims to propose the use of biological quality indicators of the irrigated soil, including soil respiration (Rs), total organic carbon (TOC), and the population density of cultivable bacteria (Bact) and of cultivable filamentous fungi in the soil (Fung).

MATERIAL AND METHODS

Study of the sunflower cv. BRS 321 and castor bean cv. BRS Energy was carried out (2011 to 2013) in an area of an orthic Quartzarenic Neossol in the town of Aracati, at 4º44'45.6" S and 37º32'18.4" W. Based on the soil characteristics (SOUSA *et al* ., 2016) and the rainfall observed during the study (450 mm), which was concentrated from March to May (FERREIRA; WEBER; CRISÓSTOMO, 2015), a drip irrigation system was set up. Both the sunflower and castor bean crops were irrigated with filtered produced water (FPW), produced water treated by reverse osmosis (OPW) and groundwater water from the Açu aquifer (ACW) captured in wells at the Fazenda Belém (250 m deep), as well as one rainfed treatment (RFD), with three replications of 200 m^2 for each treatment. The irrigation water was characterised by Sousa *et al*. (2016).

In preparing the areas, conservation practices with the minimum of soil movement were adopted. Basic fertilisation consisted of the application of 75 Mg ha^{-1} for the first cycle and 25 Mg ha^{-1} for the second and third cycles. During cultivation of the crops under evaluation, the castor bean plants also received one supplementary fertilisation with urea (20 kg ha^{-1} N applied as base and 30 kg ha^{-1} N as topdressing), single superphosphate (80 kg ha^{-1} P_2O_5 applied as base) and potassium chloride (25 kg ha^{-1} K_2O applied as base). The sunflower plants received urea (50 kg ha^{-1} N applied as topdressing), single superphosphate (80 kg ha^{-1} P_2O5 applied as base) and potassium chloride (40 kg ha^{-1} K_2O applied as base). The doses of fertiliser for the sunflower and castor bean were based on suggestions made in Raij *et al*. (1996) and Dinis Neto *et al*. (2009), respectively.

Immediately prior to fertilisation and planting, and in the final stages of each oilseed, composite soil samples were collected from twelve experimental units for each crop. The soil was taken from the surface layer (up to 10 cm deep), always from areas close to the plants, along the eighteen central rows of each working plot (out of 20 rows). After collection, the soil samples were passed through a 2-mm mesh sieve for the removal of fragments of organic matter and roots, placed in labelled plastic bags and kept in a refrigerator at a temperature of approximately 5 °C until subjected to laboratory analysis.

To determine the Rs (soil respiration), the samples were balanced with distilled water to reach 65-70% of

field capacity, and incubated for up to 240 hours at room temperature (22 ± 5 °C); in calculating the C-CO_2 evolved from the soil, the procedures of Silva, Azevedo and De-Polli (2007) were followed. The TOC (total organic carbon) was measured using the method of wet oxidation, based on oxidation of the soil carbon by potassium dichromate in a heated acid medium, followed by titration with ferrous ammonium sulphate (SILVA, 2009). The populations of cultivable soil microorganisms were determined by the method of seeding onto solid media in Petri dishes; colony forming units (CFU) of the filamentous fungi were quantified in a Martin solid medium, and of the bacteria in a nutrient agar medium, as per APHA (2005).

The data were analysed separately for each crop, using a completely randomised design, with four main treatments (FPW, OPW, ACW and RFD) and the different periods of soil sampling. Those times corresponded to the periods of pre-cultivation and harvesting of the capitula in the first, second and third production cycles of the sunflower, and to the periods of pre-cultivation and harvesting of primary inflorescences in the first and second production cycles of the castor bean. In both experiments, those periods comprised measurements repeated over time; the MIXED procedure of the SAS® statistical software version 9.2 (ALISSON, 2010) was therefore adopted in the analysis. The populations of microorganisms were transformed in log(x) for normalisation. When noting a normal data distribution (FREITAS; FERREIRA; MOREIRA, 2011), the effects of the irrigation treatments and sampling times were evaluated, together with the interaction of these factors. Finally, nominal significances between treatments were estimated for the variables Rs, TOC, Bact and Fung in both oilseed crops.

RESULTS AND DISCUSSION

Values for evolved C-CO_2 from the soil samples tended to a gradual increase over the periods and cycles for both oilseed crops. Higher values were detected during Period 3 (May and June 2013) and Period 4 (October 2013) in the rhizosphere of the sunflower rows (Table 1), and during Period 3 (July 2013) in the rhizosphere of the castor bean (Table 2). This result was expected after the application of fertiliser and the irrigated cultivation of the oilseeds.

Organic and mineral fertilisers favour metabolic activity of the microbiota in a process of soil carbon mineralisation. The accumulation of organic matter in the soil generates more biological activity, with the consequent release of C-CO_2 (SILVA *et al*., 2012). Crop fertilisation also benefits growth of the microorganism communities in the rhizosphere by increasing the availability of nutrients and/or sources of labile carbon (MOREIRA; SIQUEIRA, 2006). The researchers Qiu, Huang and Lin (2014) investigated the effects of fertiliser application on communities of bacteria and fungi in an area cultivated with medicinal plants (*Camellia sinensis* [L.] Kuntze). They found high levels of soil nutrients, including organic matter (30.03%), as a function of the application of organic fertiliser, with an increase in the diversity of the soil bacterial communities, as well as significant changes in the structure of the soil fungal communities. Furthermore, Bonilla *et al*. (2012) investigated the effects of organic additives and soil management on the population-size of microorganisms in soil cultivated with avocado trees (*Persea americana* Mill.), and reported an increase in the population of certain microbial groups in the soil, due to the addition of fertiliser.

In the present work, a significant effect was seen on Rs from the irrigation treatments, the periods representing the cycles, and the interaction of these factors in the two oilseed crops (Table 3). The respiration resulting from the overall activity of the microbiota in soil cultivated with the sunflower plants (Table 4) revealed differences between treatments that received PW (FPW and OPW) and the control with no irrigation (p<0.0001) during periods 2 and 4 (pre-harvest period of the first and third cycles); the highest value for Rs however, was detected in non-irrigated experimental plots.

There was little change in the organic fertiliser from soil moisture (<5%) under rainfed conditions due to the low rainfall intensity in the area (FERREIRA; WEBER; CRISÓSTOMO, 2015). It is worth noting that oilseed production was only seen during the second cycle (April to July 2013), when a total of 448 mm of rain was registered. With the soil samples moistened during incubation and analysis in the laboratory, the labile fractions of organic compounds may have stimulated microbial activity and the consequent evolution of soil C-CO_2. The effect of soil moisture on soil respiration in the field has been reported by Geisseler, Horwath and Scow (2011) in California. Those authors found a positive correlation between soil respiration and soil moisture (p<0.001), i.e. respiration decreased with a reduction in water potential. Similar behaviour was reported by Araujo *et al*. (2008), who evaluated microbial activity through the production of C-CO_2 during the dry and rainy seasons in the semi-arid region of the State of Paraíba.

Pascual *et al*. (2007) evaluated the potential impact of soil moisture on microbial properties, including soil respiration, and on the biochemical properties of soil under semi-arid conditions and subjected to organic fertilisation with sewage sludge or mineral fertiliser. Those authors found that the water deficit affected soil respiration

Table 1 - Mean values and standard deviations for respiration activity, organic carbon, and populations of cultivable microorganisms in the rhizosphere of sunflower plants cv. BRS 321, for irrigation treatment and time of soil sampling

Girassol BRS 321				
Soil respiration (Rs) (mg kg^{-1} dry soil h^{-1})				
Treatment	P1[2]	P2	P3	P4
FPW[1]	1.188 ± 0.275	1.052 ± 0.704	2.103 ± 0.208	2.336 ± 0.235
OPW	1.338 ± 0.169	1.606 ± 0.666	2.597 ± 0.211	2.373 ± 0.309
ACW	1.098 ± 0.184	1.138 ± 0.012	2.634 ± 0.199	2.191 ± 0.068
RFD	1.218 ± 0.127	2.448 ± 0.152	2.922 ± 0.402	3.240 ± 0.152
Total organic carbon (TOC) (g OC.kg^{-1} dry soil)				
Treatment	P1[2]	P2	P3	P4
FPW	12.848 ± 1.260	9.311 ± 0.507	11.056 ± 4.507	5.211 ± 0.555
OPW	12.541 ± 1.884	7.802 ± 1.621	14.216 ± 4.097	10.069 ± 3.999
ACW	13.098 ± 3.826	7.817 ± 0.847	14.245 ± 3.596	5.860 ± 1.375
RFD	15.760 ± 2.563	15.317 ± 5.369	7.695 ± 1.810	7.459 ± 2.006
Population density of cultivable bacteria (log CFUg^{-1} dry soil)				
Treatment	P1[3]	P2	P3	P4
FPW	5.726 ± 0.245	6.060 ± 0.505	5.221 ± 0.267	-
OPW	5.816 ± 0.150	6.646 ± 0.073	5.600 ± 0.253	-
ACW	5.658 ± 0.150	6.588 ± 0.297	5.876 ± 0.221	-
RFD	5.941 ± 0.139	5.813 ± 0.107	5.520 ± 0.412	-
Population density of cultivable filamentous fungi (log CFU g^{-1} dry soil)				
Treatment	P1[3]	P2	P3	P4
FPW	3.638 ± 0.174	3.610 ± 0.100	2.988 ± 0.080	-
OPW	3.607 ± 0.083	3.645 ± 0.020	2.781 ± 0.677	-
ACW	3.825 ± 0.278	3.664 ± 0.141	3.017 ± 0.195	-
RFD	-	-	-	-

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); P4 (cycle 3 pre-harvest)

Table 2 - Mean values and standard deviations for respiration activity, organic carbon, and populations of cultivable microorganisms in the rhizosphere of castor bean plants cv. BRS Energy, for irrigation treatment and time of soil sampling

Castor Bean BRS Energia			
Soil respiration (Rs) (mg kg^{-1} dry soil h^{-1})			
Treatment	P1[2]	P2	P3
FPW1	1.157 ± 0.022	1.157 ± 0.248	2.458 ± 0.199
OPW	1.023 ± 0.142	0.950 ± 0.179	1.123 ± 0.109
ACW	1.402 ± 0.597	1.050 ± 0.185	2.410 ± 0.679
RFD	1.954 ± 1.081	1.835 ± 0.408	2.288 ± 0.163
Total organic carbon (TOC) (g OC.kg^{-1} dry soil)			
Treatment	P1[2]	P2	P3
FPW	13.880 ± 0.890	10.457 ± 1.246	10.119 ± 4.870
OPW	10.457 ± 1.167	10.089 ± 2.881	11.325 ± 1.601
ACW	10.119 ± 1.742	10.800 ± 0.876	7.643 ± 1.122
RFD	16.699 ± 2.986	12.897 ± 2.895	8.233 ± 0.737

Continuation Table 2

Population density of cultivable bacteria (log CFUg^{-1} dry soil)			
Treatment	P1[3]	P2	P3
FPW	5.910 ± 0.178	6.660 ± 0.051	5.441 ± 0.180
OPW	5.693 ± 0.081	6.232 ± 0.181	5.421 ± 1.267
ACW	5.670 ± 0.121	6.660 ± 0.044	5.587 ± 0.188
RFD	5.747 ± 0.500	5.873 ± 0.233	5.207 ± 0.191
Population density of cultivable filamentous fungi (log CFUg^{-1} dry soil)			
Treatment	P1[3]	P2	P3
FPW	3.767 ± 0.097	3.837 ± 0.252	2.733 ± 0.364
OPW	3.717 ± 0.114	3.641 ± 0.067	3.119 ± 0.092
ACW	3.657 ± 0.050	3.667 ± 0.058	2.855 ± 0.352
RFD	3.687 ± 0.040	3.355 ± 0.061	2.888 ± 0.368

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest)

Table 3 - *p*-values associated with the F-test for soil respiration (Rs) in the rhizosphere of sunflower and castor bean plants, for irrigation factor (I) and period of soil sampling (P)

Factor	DF	Rs		DF	Rs	
		F	*p*		F	*p*
		Sunflower			Castor bean	
I	3	24.88	0.0002*	3	44.99	<0.0001*
P	3	75.51	<0.0001*	2	9.05	0.0026*
I x P	9	5.38	0.0005*	6	6.78	0.0013*

*Significant by t-test at less than 5% probability

Table 4 - Difference estimation (D) and *p*-values associated with the t-test between mean values for soil respiration (Rs) in the rhizosphere of sunflower plants during the first, second and third production cycle, for irrigation treatment and period of soil sampling

Contrast	Treatment							
	P1[2]		P2		P3		P4	
	D	*p*	D	*p*	D	*p*	D	*p*
FPW1 x OPW	-0.1830	0.5807	-0.5543	0.1028	-0.4940	0.8765	-0.0363	0.9124
FPW x ACW	0.0903	0.7206	-0.0863	0.7325	-0.5317	0.1314	0.1453	0.5658
OPW x ACW	0.2733	0.2657	0.4680	0.0628	-0.0376	0.0125*	0.1817	0.4562
PW x ACW	0.1818	0.3284	0.1908	0.3055	-0.2847	0.9124	0.1635	0.3786
PW x RFD	0.0012	0.9945	-1.1828	<0.0001*	-0.4774	0.5658	-0.9397	<0.0001*
Contrast	FPW		OPW		ACW		RFD	
	D	*p*	D	*p*	D	*p*	D	*p*
E1 x E4	-1.1480	0.0012*	-1.0013	0.0002*	-1.0930	<0.0001*	-2.0217	<0.0001*

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest) * Significant by t-test at less than 5% probability

differently in soil that received organic or mineral fertiliser. In the soil with sewage sludge, soil respiration was lower when compared to that of the soil that received the application of mineral fertiliser. This result was attributed to the persistence of inactive organisms. The greater value for soil respiration with the application of mineral fertiliser was attributed to an increase in energy consumption due to the stress conditions.

In the castor bean crop (Table 5), the data for soil respiration revealed differences at the end of the second cycle (P3) between the treatments with FPW and OPW (p<0.0001) and those with OPW and ACW (p = 0.0010). As noted above, during this production cycle of the sunflower crop (Table 4), the lowest respiration occurred in soil that received OPW.

Soil respiration activity may have been inhibited due to the presence of the biocide glutaraldehyde, a product added to the water during the process of reverse osmosis to avoid the formation of bacterial film on filter membranes used at the industrial plant. Pereira *et al*. (2014) investigated the toxicity of glutaraldehyde at different organisation levels of aquatic organisms. Those authors observed a moderate toxic effect on different groups of organisms regardless of trophic level, with toxicity values that ranged from 3.6 mg/L (24 h EC_{50} *T. platyurus*) to 31.3 mg L^{-1} (72h EC_{50} *C. vulgaris*). Moreover, Ferreira *et al*. (2015) found a reduction in the reproduction rate of *Falsonia candida* in soil irrigated with glutaraldehyde (44.4 mg L^{-1}). It is important to note that faunal organisms, mainly mesofauna, feed on cellular structures and microorganisms, and could have their activities inhibited by glutaraldehyde.

During the crop cycles of the castor bean (Table 6), a reduction was also seen in the respiration activity of the soil irrigated with FPW for the pre-cultivation period and the second cycle (p<0.0001), these periods coinciding with the dry season and rainy season in the region.

The effect of the period of soil sampling on soil respiration was also seen by Diniz *et al*. (2014) when evaluating changes in microbiological attributes, including soil respiration, due to seasonal variation, in a forest area of native macauba palm trees (*Acrocomia aculeata*) in the Brazilian Cerrado. Those authors demonstrated that soil respiration was sensitive to seasonal variation, being higher during the rainy season.

The TOC content of soil cultivated with sunflower (Table 1) and castor bean (Table 2) demonstrated a certain trend towards a reduction in the values of this attribute of soil quality; that reduction is related to the mineralisation of organic fertiliser and the consequent availability of nutrients in the soil throughout the oilseed crop cycle. In the sunflower crop, the interaction of the factors irrigation and period significantly affected the organic carbon content of the soil (Table 6), with the most significant differences occurring between irrigated plots and those under rainfed conditions.

For the sunflower crop (Table 7), significant differences were detected in the TOC content of the soil that received PW (OPW and FPW) and the rainfed treatment during period 2 (p = 0.0027) and Period 3 (p = 0.0154); differences between the other contrasts were not considered. In soil irrigated with FPW, differences were found in TOC content between periods 1 and 4 (p =

Table 5 - Difference estimation (D) and *p*-values associated with the t-test between mean values for Rs in the rhizosphere of castor bean plants during the first and second production cycles, for irrigation treatment and period of soil sampling

Irrigation treatment x Period						
Contrast	P1[2]		P2		P3	
	D	*p*	D	*p*	D	*P*
FPW1 x OPW	0.1340	0.3439	0.2077	0.1507	1.3630	<0.0001*
FPW x ACW	-0.2450	0.4633	0.1077	0.7454	0.0480	0.8847
OPW x ACW	-0.3790	0.2540	-0.1000	0.7586	-1.3150	0.0010*
AP x ACW	-0.3120	0.3379	0.0038	0.9905	-0.6335	0.0634
AP x RFD	-0.7600	0.0800	-0.7824	0.0723	-0.3000	0.4703

Contrast P1 x P3	FPW		OPW		ACW		RFD	
	D	*p*	D	*p*	D	*p*	D	*p*
	-1.3007	<0.0001*	-0.0716	0.6401	-1.0077	0.0527	-0.3333	0.6035

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest), *Significant by t-test at less than 5% probability

Table 6 - *p*-values associated with the F-test for total organic carbon (TOC) in the rhizosphere of sunflower and castor bean plants, for irrigation factor (I) and period of soil sampling (P)

Factor	DF	TOC		DF	TOC	
		F	*p*		F	*p*
		Sunflower			Castor bean	
I	3	1.01	0.4375	3	1.97	0.1978
P	3	13.86	<0.0001*	2	17.19	0.0001*
I x P	9	3.46	0.0072*	6	1.71	0.1872

*Significant by t-test at less than 5% probability

Table 7 - Difference estimation (D) and *p*-values associated with the t-test between mean values for TOC in the rhizosphere of oilseed sunflower plants during the first, second and third production cycle, for irrigation treatment and period of soil sampling

Irrigation treatment x Period								
Contrast	P1[2]		P2		P3		P4	
	D	*p*	D	*p*	D	*p*	D	*p*
FPW1 x OPW	0.3063	0.8934	1.5087	0.5112	-3.1600	0.1753	-4.8577	0.0421*
FPW x ACW	-0.2507	0.9057	1.4940	0.4825	-3.1887	0.1410	-0.6490	0.7593
OPW x ACW	-0.5570	0.8185	-0.0146	0.9952	-0.0286	0.9906	4.2087	0.0923
AP x ACW	-0.4038	0.8375	0.7397	0.7075	-1.6087	0.4170	1.7798	0.3700
AP x RFD	-2.9312	0.1754	-7.0066	0.0027*	5.4772	0.0154*	-0.4118	0.8461
Contrast	FPW		OPW		ACW		RFD	
	D	*p*	D	*p*	D	*p*	D	*p*
P1 x P4	70.6363	0.0004*	2.4723	0.1348	7.2380	0.0012*	8.3017	0.0056*

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest) and P4 (cycle 3 pre-harvest); *Significant by t-test at less than 5% probability

0.0004). Differences between periods were also detected in lots that received ACW and those that were not irrigated, suggesting that the period of the year and the sunflower cycle affect the soil carbon content.

In the castor bean crop a significant effect for sampling period was only detected with TOC content (Table 8), with significant differences in this variable detected between periods 1 and 3 (p<0.0001). Furthermore, Diniz *et al*. (2014), when evaluating variations in the organic carbon content of the soil in a forest of macauba palm trees, noted seasonal variations, with values for organic carbon content higher during the period of greatest rainfall.

The increases in TOC contents between periods 1 and 3 may be due to applied fertilisers and the cycling of the cultural remains of the castor bean. This is consistent with the data obtained by Silva *et al*. (2012), who evaluated the TOC content of agricultural land and pasture in the 'Paraíba do Sul' valley (state of Rio de Janeiro), during the wet and dry seasons. The authors found higher levels of soil carbon in areas of forest and pasture, and associated them with the greater amount of crop residue produced by the various species, as well as the cycling of soil nutrients.

The Bact populations in the soil cultivated with sunflower and castor bean were influenced by irrigation treatment and sampling periods (Table 9), represented by the oilseed production cycles. The interaction of these factors was significant for Bact populations in the soil cultivated with sunflower; in the castor bean, only the effects of isolated factors were detected.

From Tables 1 and 2 it can be seen that in general the Bact populations was higher in the second crop cycle, coinciding with with rainy periods in the region (FERREIRA; WEBER; CRISÓSTOMO, 2015), and during vegetative growth, as a result of the ground cover. Lopes *et al*. (2014), studying the effect of produced water on soil microbial activity, found a higher population density for cultivable bacteria at the flowering stage of the first cycle of the sunflower cv. BRS 321 and the castor

Table 8 - Difference estimation (D) and *p*-values associated with the t-test between mean values for TOC in the rhizosphere of castor bean plants during the first and second production cycles, for irrigation treatment and period of soil sampling

Contrast	Period		Contrast	Irrigation treatment	
	D	*p*		D	*p*
FPW1 x OPW	-0.3051	0.7913			
FPW x ACW	0.6246	0.6200			
OPW x ACW	0.9297	0.2341	P1[2] x P3	5.4815	<0.0001*
AP x ACW	0.7771	0.3747			
AP x RFD	-1.2310	0.0750			

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); *Significant by t-test at less than 5% probability

Table 9 - *p*-values associated with the F-test for the population density of cultivable bacteria (Bact) in soil of the sunflower and castor bean, for irrigation factor (I) and period of soil sampling (P)

Factor	DF	Bact		DF	Bact	
		F	*p*		F	*p*
		Sunflower			Castor bean	
I	3	5.99	0.0193*	3	17.69	0.0007*
P	3	20.60	<0.0001*	2	14.39	0.0003*
I x P	9	3.44	0.0223*	6	1.64	0.2010

*Significant by t-test at less than 5% probability

bean cv. Energy BRS. The positive influence of the roots on the activity of microorganisms was also reported by Santos *et al*. (2011) when studying the effect of the presence and absence of halophytes on microbial activity in saline soil. Those authors found greater microbial activity in soil close to the plant roots.

For soil cultivated with the sunflower (Table 10), the Bact populations in the soil collected during period 2 differed between the treatments receiving FPW and OPW ($p = 0.0218$), FPW and ACW ($p = 0.0478$), and PW against the rainfed treatment ($p = 0.0026$), and during period 3 between the treatments with FPW and ACW ($p = 0.0171$) and PW against the rainfed treatment ($p = 0.0177$). No changes in Bact populations were detected between periods 1 and 4, considering the irrigation treatments and the control with no irrigation. It is believed that the differences between FPW, OPW and ACW are due to the physical and chemical characteristics of the water used, as well as to changes in the soil properties that may influence bacteria and other soil microorganisms.

Yet, in the castor bean crop (Table 11) there were no significant differences between irrigation treatments for Bact populations in the soil, except between PW and the rainfed treatment ($p = 0.0179$). There was significance by t-test between periods 1 and 3 at a level of 6.8%. The low level of significance may be due to the reduced number of cycles (first and second cycles), but interference from the castor bean plants in maintaining the populations of cultivable bacteria should also not be ruled out. In future studies, the relationship between plants and groups of functional microorganisms in an irrigated agrosystem should be explored using molecular metagenomic techniques.

In relation to the castor bean (Table 12), the sampling period had a significant effect on the Fung populations ; however, the irrigation treatments did not differ by t-test ($p = 0.1891$).

Fungal populations ranged from 10^2 to 10^4 CFU per gram of soil (Tables 1 and 2), reducing over the crop cycles; this reduction may be associated with moisture and the flow rates for energy and soil heat. The greater amount of Fung populations in period 1 and 2 (dry season) for most of the irrigation treatments can be explained by the lower level of water content in the soil and the consequent increase in heat flow rate, which favours soil filamentous fungi. This result confirms observations made by Rodrigues *et al*. (2011), who evaluated microclimate conditions on the populations of fungi in the soil of a wet tropical forest, and concluded that those microorganisms grew faster in the dry season. Period 3 coincided with

Table 10 - Difference estimation (D) and *p*-values associated with the t-test between mean values for Bact in the rhizosphere of oilseed sunflower plants during the first and second production cycles, for irrigation treatment and period of soil sampling

Contrast	Treatment					
	P1[2]		P2		P3	
	D	*p*	D	*p*	D	*p*
FPW1 x OPW	-0.0900	0.7012	-0.5853	0.0218*	-03793	0.1192
FPW x ACW	0.0680	0.7860	-0.5280	0.0478*	0.6553	0.0171*
OPW x ACW	0.1580	0.3585	0.0573	0.7360	0.2760	0.1181
AP x ACW	0.1130	0.5302	-0.2353	0.2002	0.4657	0.0177*
APs x RFD	-0.2073	0.2498	0.6181	0.0026*	0.0462	0.7935

Contrast	FPW		OPW		ACW		RFD	
	D	*p*	D	*p*	D	*p*	D	*p*
P1 x P3	0.5053	0.1105	0.2160	0.2317	-0.2180	0.3513	0.4213	0.0523

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); *Significant by t-test at less than 5% probability

Table 11 - Difference estimation (D) and *p*-values associated with the t-test between mean values for Bact in the rhizosphere of castor bean plants during the first and second production cycles, for irrigation treatment and period of soil sampling

Contrast	Period		Contrast	Irrigation treatment	
	D	*p*		D	*p*
FPW1 x OPW	0.2416	0.3763			
FPW x ACW	0.0313	0.1699			
OPW x ACW	-0.2102	0.4397	P12 x P3	0.3411	0.0688
AP x ACW	-0.0894	0.5122			
AP x RFD	0.3035	0.0179*			

[1]Filtered produced water (FPW) and water treated by reverse osmosis (OPW), water captured from the Açu aquifer (ACW) and the control with no irrigation (RFD); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); *Significant by t-test at less than 5% probability

Table 12 - *p*-values associated with the F-test for the population density of cultivable filamentous fungi (Fung) in soil cultivated with sunflower and castor bean plants, for irrigation factor (I) and period of soil sampling (P)

Factor	DF	Fung		DF	Fung	
		F	*p*		F	*p*
		Sunflower			Castor bean	
I	3	8.04	0.0085*	3	2.02	0.1891
P	3	29.69	<0.0001*	2	57.92	<0.0001*
I x P	9	1.81	0.1596	6	1.94	0.1363

*Significant by t-test at less than 5% probability

the rainy season in the region. Such climate conditions may have hindered development of the cultivable soil filamentous fungi. Souto *et al*. (2008) evaluated the populations of microorganisms and soil mesofauna in the semi-arid region of the state of Paraíba during periods with different rates of rainfall, and observed greater fungal growth and lower bacterial growth in the season with less rainfall. Those authors attributed the result to a reduction in O_2 due to the rainfall. In the present work no differences were noted in the Fung populations between irrigation treatments for either oilseed crops (Tables 13 and 14), but they varied throughout the soil sampling periods.

Table 13 - Difference estimation (D) and *p*-values associated with the t-test between mean values for Fung in the rhizosphere of sunflower plants during the first, second and third production cycle, for irrigation treatment and period of soil sampling

Contrast	Period		Contrast	Irrigation treatment	
	D	*p*		D	*p*
FPW1 x OPW	0.0678	0.6830	P12 x P3	0.7368	<0.0001*
FPW x ACW	-0.0898	0.2351			
OPW x ACW	-0.1577	0.3396			
AP x ACW	-0.1238	0.2064			

[1]Produced water (AP), filtered (FPW) and water treated by reverse osmosis (OPW), and water captured from the Açu aquifer (ACW); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); * Significant by t-test at less than 0.01% probability

Table 14 - Difference estimation (D) and *p*-values associated with the t-test between mean values for Fung in the rhizosphere of castor bean plants during the first and second production cycles, for irrigation treatment and period of soil sampling

Contrast	Period		Contrast	Irrigation treatment	
	D	*p*		D	*p*
FPW1 x OPW	-0.0464	0.6523	P12 x P3	0.8077	<0.0001*
FPW x ACW	0.0495	0.6750			
OPW x ACW	0.0960	0.2278			
AP x ACW	0.0728	0.4007			
AP x RFD	0.1350	0.1377			

[1]Produced water (AP), filtered (FPW) and water treated by reverse osmosis (OPW), and water captured from the Açu aquifer (ACW); [2]P1 (cycle 1 pre-planting); P2 (cycle 1 pre-harvest); P3 (cycle 2 pre-harvest); * Significant by t-test at less than 0.01% probability

CONCLUSIONS

1. Microbiological activity and soil organic carbon are sensitive to the irrigation of sunflower plants with oilfield produced water. In the castor bean crop, that irrigation affects soil basal respiration;
2. Irrigation with oilfield produced water and water treated by filtration may be a short-term alternative for the production of the castor bean and sunflower to obtain biodiesel. In future work, biological properties of the soil should be evaluated in the medium and long term, in order to make oilseed production more sustainable and viable in the semi-arid region.

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