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Original Research Article

Short-term effects of produced water on microbial activity in semiarid soil

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A B S T R A C T

Produced water (PW) is derived from the oil extraction process, and has been suggested as an alternative irrigation source; however, the water constituents might have reflects on soil function. We evaluated the short-term effects of PW on the microbial activity of soil cultivated with bioenergy plants (sunflower cv. BRS 321 and castor bean cv. BRS Energia) in a semiarid environment. Plant species were irrigated at rates according to their requirements using three types of water: (1) groundwater (GW) from Açu aquifer, (2) produced water treated with simple filtration (PWF), and (3) produced water treated with reverse osmosis (PWRO), along with uncultivated areas, serving as the control. Cultivable bacteria and fungi were evaluated from the surface soil of the 2-biofuel crops during one production cycle. In addition, the levels of various soil biological attributes were recorded. Irrigation with GW and PWF resulted in the proliferation of soil microorganisms and increased dehydrogenase activity, whereas the PWRO treatment reduced the population of culturable bacteria after 7 weeks of planting both crops, and filamentous fungi after 11 weeks of planting castor beans. The irrigation with PWF and PWRO, in short-term, negatively affects the proliferation of microorganisms and dehydrogenase activity in soil supporting bioenergy crops.

K e y w o r d s

Microbial activity;
Wastewater reuse;
Biofuel crops;
Soil quality.

Introduction

Given the need to reduce the use of oil, in parallel to the growing interest in technologies that mitigate associated environmental impacts, the viability of alternative types of renewable energy, such as biodiesel, is being considered (Charles et al., 2007). In 2004, Brazil initiated a program to encourage the production and use of biodiesel, mainly for its high availability of agricultural land for oil producing plantations. In northeast Brazil, the castor bean (*Ricinus communis*

L.) is widely produced due to this plant having low production costs and resistance to water stress, which in turn promotes the social development (MME, 2004). Besides, for that region, sunflower (*Helianthus annuus* L.) planting has also been considered, for the same purpose (IICA, 2007).

Losses from oil production might also be reduced through the reuse of produced water (PW), which is the primary residue of the petrochemical industry (Veil et al., 2004). Of note, the improper disposal of this water, or failure to correctly treat it, risks polluting the soil (Nwaugo et al., 2007), surface water bodies (Gregory et al., 2011) and underground water (Hamawand et al., 2013). Some studies on wastewater treatment have been proposed, intended for use in irrigation (Melo et al., 2010).

PW is characterized by a mixture of water and oil and usually contains organic and inorganic salts, in addition to heavy metals (Andrade et al., 2010). PW is generated in large quantities in active oil fields, and is typically re-injected into wells (Ahmadun et al., 2009), or used for industrial purposes, such as dust control, vehicle cleaning, and fire control (Veil et al.,2004). Other reuse option includes crop irrigation (Andrade et al., 2010; Igunnu and Chen, 2012), which may prove advantageous in semiarid regions where water is scarce, especially for the cultivation of bioenergy plants, in order to avoid competition for water resources with food crops.

However, to apply PW to bioenergy crops, studies are needed to assess how this water affects various soil parameters, as well as the development of agronomic crops. This is because pollution from oil is known to have a number of negative effects on the growth and development of plants (Karamalidis et al., 2010). Doran and Zeiss (2000) stated that it is necessary to select attributes sensitive to handling and related to soil function when evaluating the impact of soil characteristics. Niemi and McDonald (2004) suggested that biological parameters serve as good indicators of soil quality, reflecting microbiota conditions, in addition to providing information about ecosystem complexity.

The current study aimed to reduce the environmental liability of oil companies and develop techniques for the reuse of water in potential bioenergy crops grown under semiarid conditions. Specifically, an irrigation experiment was set up using castor bean and sunflower cultivars to assess the short-term effects of PW on microbial activity in semiarid soil.

Materials and Methods

The study area was located at Belém farm (4°44 44.96 S and 37° 32 18.42 W) in Aracati, Ceará State, Brazil. The soil was characterized as Haplic Arenosol (IPECE, 2007). The regional climate is mild warm tropical semiarid, with an average annual air temperature of 27°C, and a short rainy season (Jan to Apr) (IPECE, 2012). The experimental field was planted with sunflower (*Helianthus annuus* L.) cultivar BRS 321 and castor bean seedlings (*Ricinus communis* L.) cultivar BRS Energia, after disking and fertilizing land plots following the methods of Raij et al. (1996) and Diniz Neto et al. (2009).

Two assays were performed in the experimental area during a single production cycle, using: 1) the sunflower crop and 2) the castor bean crop. Both crops were irrigated with three types of water:1) groundwater (GW) captured from the Açu aquifer, in FZB wells (250 m depth), 2) produced water treated by simple filtration (PWF), and 3) and produced water treated by reverse osmosis (PWRO). Irrigation was applied daily and water depths were calculated based on estimated crop evapotranspiration and drainage losses collected in small-scale lysimeters (diameter = 0,4 m and depth = 0,7 m). In addition to water management**,** uncultivated and non-irrigated control plots were used. All plots measured 400 m^2 and were arranged in a completely randomized design with 3 replications. The PW used for irrigation was supplied by FZB through its treatment plan. The PW was processed by separating the oil with a density treatment, followed by filtration with cationic resin cartridges (PWF), and subsequently ultrafiltration followed by reverse osmosis (PWRO).

Composite soil samples were collected from the surface layer (0–10 cm), along rows with plants spaced at1m intervals. Soil samples from sunflower plots were collected on 3 different occasions: (1) before planting the seedlings (pre-planting: April 2012), (2) at the vegetative growth and (3) at the flowering stage (2 and 7 weeks after planting: August 2012). Besides these three sampling periods, a fourth soil sampling was done in the castor bean plots, at final of flowering stage, 11 weeks after planting the seedlings (September 2012). After sieving, all soil samples (2-mm mesh) were taken to the laboratory of Embrapa, in Fortaleza, and stored in a refrigerator at 5 °C until microbiological analysis.

The samples were evaluated for total organic carbon content (TOC) following the method of Silva (2009), microbial biomass (MB) following that proposed by Mendonça and Matos (2005), and basal respiration (BR) and metabolic quotient (qCO_2), following the methods of Silva et al. (2007). The population densities of culturable bacteria and filamentous fungi were assessed, according to the method of Pepper and Gerba (2005). Dehydrogenase enzyme activity was assessed following the method of Casida et al. (1964).

The results of the analyses were assessed by adopting the split plot design, in which the main treatments are represented by water management and secondary treatments are represented by the timing of each culture, using the GLM (General Linear Models) release of SAS ® (SAS, 2000). The treatment means were compared by the Tukey test, at a 5% probability.

Results and Discussion

Short-term impacts of PW on microbial activity and other soil biological attributes were examined. We observed that PW affects the biological attributes of soils used to cultivate both sunflower (Table 1) and castor bean (Table 2) plants. There was only a significant interaction between major water management treatments and the period of times for the population density of culturable bacteria and dehydrogenase for both cultures, and of filamentous fungi in the cultivation of the castor bean.

The irrigation at rates according to plant species requirements using different types of water positively affected the number of microorganisms by the second week after planting the crops, increasing the number of colony forming units (CFU) of culturable bacteria and filamentous fungi when compared to the control (uncultivated and without irrigation). The interaction between the water management treatments and the periods (in which the soil was collected and assessed) was also significant for dehydrogenase activity for both crops. Dehydrogenase activity increased in treatments irrigated with GW and PWF, and remained at the same level of control in PWRO. All other soil biological attributes that were analyzed showed no significant interaction. However, period itself affected some parameters; specifically, RB, TOC, and MB for the sunflower crop (Table 1), and RB, TOC, and $qCO_2$for the castor bean (Table 2). Temporal variation in these parameters was related to pre-planting practices; namely, the physical manipulation of the soil and use of organic fertilizers. Differences among water management treatments and periods were not observed for qCO_2 in the plots with sunflower; however, qCO_2 levels changed in the castor bean plots between the pre-planting stage (which had the highest qCO_2 and RB levels) and the subsequent 3 stages when soil was collected. All water management treatments had similar TOC levels, which decreased across the study period; however, there were no significant differences among periods for MB in plots of castor bean.

Numbers of culturable bacteria and filamentous fungi we know represent a small fraction of soil microbes, but they reflect substantially on microbial activity. In our findings, PW affected microbial activity and other biological attributes in soil cultivated with sunflower and castor bean. In addition, the GW captured at 250 m depth in FZB and deemed fit for irrigation favored the proliferation of soil microorganisms during the fast plant-growing stage, two weeks after planting the crops. According to Geisseler et al. (2011), water availability has a strong impact on the presence and activity of microorganisms in the soil. The presence of plants, particularly the root system, might also influence the number of microbiota, as plants exude root compounds (Macek et al., 2000).

In previous studies using PW derived from oil wells at Belém farm, Melo et al. (2010) recommended a wastewater filtration in an ultrafiltration membrane and reverse osmosis apparatus, before its reuse, to meet the water quality standards. Thus, Melo et al. (2010) determined the efficacy of the PW by reverse osmosis within a pilot study for the purposes of subsequent use in crop irrigation.

Dehydrogenase activity is considered an important indicator of the microbiological activity, being particularly sensitive to environmental changes, especially in soils receiving long-term (> 50 years) the waste water coming from oil fields (Nwaugo et al. 2007) and soils treated with polluted water or effluents (Chen, et al. 2008). The increase observed in dehydrogenase activity under the PW treatment might arise due to the possible contribution of the organic substances, which were absent in the PWRO treatment following the sophisticated filtering process.

According Chatzakis et al. (2011), effluents provide nutrients and organic carbon to the soil; thus, stimulating the growth and activity of microorganism communities. Chatzakis et al. (2011) studied sunflower and castor bean irrigated with fresh water and sewage effluent wastewater. The data showed small differences in dehydrogenase activity, with higher values of effluent irrigation; however, plant species did not affect pH

Table.1 Effects of water management treatments on soil biological attributes during the cycle production of sunflower.

Irrigation treatment	Times (pre, and weeks post planting)			
	Pre	Two	Seven	Averages
Culturable bacteria (log CFU g^{-1} dry soil)				
GW	5.658Ab	6.507Aa	6.558ACa	6.251
WPF	5.817Ab	6.700Aa	6.646Aa	6.388
WPRO	5.727Ab	6.526Aa	6.060Bab	6.104
Control	5.941Aa	6.035Ba	5.813Ba	5.930
Averages	5.786	6.442	6.277	
Filamentous fungi (log CFU g^{-1} dry soil)				
GW	3.825	3.622	3.664	3.704A
WPF	3.608	3.702	3.645	3.652A
WPRO	3.638	3.657	3.610	3.635A
Control	3.537	3.482	3.023	3.347A
Averages	3.652a	3.616a	3.486a	
Dehydrogenase (μL H g^{-1} dry soil)				
GW	2.922Ab	10.286Aa	5.165ABab	6.124
WPF	3.656Ab	10.702Aa	8.812Aab	7.723
WPRO	3.791Aa	2.005Ba	2.017Ba	2.604
Control	3.388Aa	2.615Ba	1.368Ba	2.457
Averages	3.440	6.402	4.340	
Total organic carbon (g C kg^{-1} dry soil)				
GW	15.560	8.780	9.670	11.337A
WPF	15.900	7.340	8.910	10.717A
WPRO	14.810	8.780	10.850	11.480A
Control	18.510	15.270	17.620	17.133A
Averages	16.195a	10.043b	11.763b	
Microbial biomass (mg C-MB kg^{-1} dry soil)				
GW	156.360	130.910	109.090	132.120A
WPF	176.360	109.090	123.640	136.363A
WPRO	180.000	132.730	112.730	141.820A
Control	245.450	134.550	141.820	173.940A
Averages	189.543a	126.820b	121.820b	
Basal respiration (mg C-CO_2 kg^{-1} dry soil h^{-1})				
GW	1.098	0.427	0.671	0.732A
WPF	1.371	0.621	0.871	0.954A
WPRO	1.221	0.677	0.748	0.882A
Control	1.427	0.601	1.006	1.011A
Averages	1.279a	0.582b	0.824c	
qCO_2 (mg C-CO_2 g^{-1} C-MB h^{-1})				
GW	7.20	3.63	624	5.69A
WPF	8.75	5.26	824	7.42A
WPRO	7.49	5.69	722	6.80A
Control	6.85	5.50	954	7.30A
Averages	7.57a	5.02a	7.81a	

Groundwater (GW), produced water treated by simple filtration (PWF) and by reverse osmosis (PWRO), and non-irrigated control soil. Means followed by the same uppercase letter in columns and lowercase in rows for different soil attributes were not significantly different using the Tukey test (5%).

Table.2 Effects of water management treatments on soil biological attributes during the cycle production of castor bean.

Irrigation treatment	Times (pre, and weeks post planting)				
	Pre	Two	Seven	Eleven	Averages
Culturable bacteria (log CFU g^{-1} dry soil)					
GW	5.672Bb	6.456Aa	6.534Aa	6.785Aa	6.362
PWF	5.909ABb	6.559Aa	6.627Aa	6.692Aa	6.447
PWRO	5.697ABb	6.516Aa	5.932Bb	6.414ABa	6.140
Control	6.079Aab	5.828Bbc	5.525Bc	6.220Ba	5.913
Averages	5.839	6.340	6.154	6.528	
Filamentous fungi (log CFU g^{-1} dry soil)					
GW	3.659Aa	3.644Aa	3.701Aa	3.805ACa	3.702
PWF	3.766Aab	3.671Ab	3.611Ab	4.067Aa	3.779
PWRO	3.719Aa	3.541ABa	3.609Aa	3.669BCa	3.634
Control	3.685Aa	3.276Bb	3.261Bb	3.440Bab	3.415
Averages	3.707	3.533	3.545	3.745	
Dehydrogenase (μL H g^{-1} dry soil)					
GW	4.163Ab	7.093Aab	4.658Bb	9.776Aa	6.423
PWF	2.308Ab	6.831Aa	9.773Aa	8.173Aa	6.771
PWRO	2.636Aa	2.241Ba	2.665Ba	1.816Ba	2.339
Control	2.992Aa	2.017Ba	1.896Ba	2.252Ba	2.289
Averages	3.025	4.545	4.748	5.504	
Total organic carbon (g C kg^{-1} dry soil)					
GW	16.930	10.687	11.677	10.893	12.547A
PWF	16.523	11.670	11.470	11.100	12.691A
PWRO	16.113	11.277	9.603	13.043	12.509A
Control	18.783	8.913	11.330	14.153	13.295A
Averages	17.088a	10.637b	11.020b	12.298b	
Microbial biomass (mg C-MB kg^{-1} dry soil)					
GW	210.909	165.455	147.273	170.909	173.636A
PWF	205.455	212.727	212.727	152.727	195.909A
PWRO	267.273	121.818	156.364	161.818	176.818A
Control	200.000	130.909	105.455	245.455	170.455A
Averages	220.909a	157.727a	155.455a	182.727a	
Basal respiration (mg C-CO_2 kg^{-1} dry soil h^{-1})					
GW	1.528	0.426	0.684	0.733	0.842A
PWF	1.277	0.822	0.819	0.686	0.901A
PWRO	1.130	0.611	0.677	0.593	0.753A
Control	1.399	0.488	0.909	0.933	0.932A
Averages	1.334a	0.587b	0.772b	0.736b	
qCO_2 (mg C-CO_2 g^{-1} C-MB h^{-1})					
GW	8.01	2.58	4.80	4.44	4.96A
PWF	9.14	3.73	3.83	4.84	5.39A
PWRO	4.38	5.22	4.82	3.98	4.60A
Control	7.69	4.32	8.57	4.42	6.25A
Averages	7.30a	3.96b	5.51ab	4.42ab	

Groundwater (GW), produced water treated by simple filtration (PWF) and by reverse osmosis (PWRO), and non-irrigated control soil. Means followed by the same uppercase letter in columns and lowercase in rows for different soil attributes were not significantly different using the Tukey test (5%).

nor dehydrogenase activity of the soil. In the present work, the low dehydrogenase activity in the control soil might have been caused by high temperature, low humidity, and the presence of fewer microorganisms in the surface layer, as these factors affect soil microbial activity. Dry soil tends to have lower microbial activity and enzyme activity compared to moist soils, because it has lower water content and higher rates of oxygen diffusion and redox potential (Wolińska and Bennicelli, 2010), which may reduce dehydrogenase activity. The PWRO treatment had a low ionic concentration, in addition to the biocide Glutaraldeid at 10% (outlet 0.198 mg/l) (Melo et al., 2010), which might have affected the ionic balance of the soil pH and the proliferation of natural soil microorganisms.

Changes in the soil biological attributes such as RB, TOC, MB and qCO2 were expected to occur from the second week after planting, as the irrigation system alters soil properties and organic matter content (Hobbs et al., 2008). Such changes modify the structure of microorganism communities, and hence soil microbial activity (Helgason et al., 2009). The application of fertilizers on soil might have caused the proliferation of microorganisms during the pre-planting phase. These changes might have influenced the TOC, MB, and RB values of the soil, so that initially increased, but then decreased over the growing season of the plants. According Araújo et al. (2007), organic and chemical fertilization increases microbial activity and biomass. TOC level reductions encountered here supports the results obtained by Dantas et al. (2012) that observed a decrease in this parameter associated with the conventional management system, due at increase in consumption of readily available carbon by microbial biomass (Jakelaitis et al., 2008). The lack of response of MB to soil management treatments has also been reported by Kummer et al. (2008). According to Silva et al. (2010) that phenomenon is generally attributed to the recent planting of horticultural crops.

On base of results obtained in the present study we conclude the produced water, treated by filtration and reverse osmosis, in short-term, negatively affects the proliferation of microorganisms and dehydrogenase activity in soils supporting the castor bean and sunflower crops in semiarid environment. Soil attributes, such as TOC, BM, RB, and qCO_2 could not be used to evaluate the effects of irrigation with produced water during the first cycle of bioenergy plant cultivation. The produced water treated by filtration process might potentially be used for bioenergy crop irrigation; however, further studies are needed beyond a single crop cycle to investigate functional groups of microorganisms sensitive to water produced in order to elucidate its impact on the biological quality of soils in semiarid region.

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