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INFLUENCE OF TEBUTHIURON AND VINASSE UNDER SOIL MICROBIOTA ACTIVITY IN SUGARCANE CROP

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ABSTRACT

Tebuthiuron is an herbicide used in sugarcane crops and can cause great environmental impact due to its high persistence and toxicity in soil. Thus, an organic compound addition influences the environmental behavior of this molecule and could increase its sorption and degradation. Vinasse is used in fertigation and this research aimed to evaluate microbial metabolism in soil cultivated with sugarcane with tebuthiuron and vinasse applications. Experimental design was completely randomized by 2x4 factorial scheme linked to tebuthiuron recommended dose (zero and 1.0x) and to vinasse volume generally used in crops (zero, 0.5x, 1.0x and 2.0x). Soil microbial respiration was performed in triplicate by Bartha and Pramer respirometric method and biodegradation activity was monitored by CO_2 determination weekly until 51-day evaluation period. Control treatment presented the lowest CO_2 production. The highest rate was observed when tebuthiuron and twice vinasse volume were present. However, results showed that there was no statistical difference between treatments with tebuthiuron independent of vinasse. Vinasse addition significantly increased the CO_2 production and it favored soil microbiota activity. Therefore, results suggested that tebuthiuron presence did not cause interference in soil microbial activity represented by CO_2 production. Moreover, microorganisms metabolic rate increased in vinasse presence especially in higher concentration.

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INTRODUCTION

The contribution of Brazil to world sugarcane production is 25% and the country is the largest producer of this crop. Consequently, Brazil also highlights with the world's largest sugar and ethanol production, which are main products of sugarcane industry. In 2018/19 harvest, this contribution amounts to approximately 660 million tons of sugarcane produced per year with 31.73 million tons of sugar and 32.31 billion liters of ethanol (CONAB, 2018). São Paulo state is responsible for the largest sugarcane production in country and concentrates a large part of powerplants as well as the largest planting areas.

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According to this, Brazil has a great advantage for its soil-climatic potential, which benefits agricultural production. However, although there is so much efficiency, there are still some factors that can cause impacts for production (CONAB, 2018). Among these factors, the presence of invasive plant species represents a great economic loss in Brazilian agricultural production due to their competition with crops in the most needed supplies such as light, nutrients and water. Moreover, they present release of allelopathic substances, act as pests and diseases host and cause problems during crop harvesting (Lamego *et al*., 2013). Thus, weed control must be done properly due to serious losses in crop productivity. A control practice previously adopted for weeds control was burning before sugarcane harvest. Nevertheless, this management was prohibited in Brazil because of its negative environmental impacts. Hence, there was a considerable increase in weeds growth in crops, responsible for production losses, and it was necessary the increase of herbicides use for their control (Toniêto *et al*., 2016). In this context, tebuthiuron (1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea) is presented, whose use in sugarcane cultivation in Brazil is widespread. It has pre-emergency action with photosystem II inhibition (MAPA, 2019). However, this herbicide presents high solubility and may persist in soil for up to two years or more after its application (Rodrigues and Almeida, 2011). Therefore, tebuthiuron can cause great environmental impact and its residual concentration is extremely relevant factor due to contamination potential. Another compound commonly used in sugarcane crops is vinasse in fertigation. As an alternative to the disposal of this by-product, it is used in fertigation because presents high organic matter content of nutritional richness - calcium, magnesium, potassium and sodium (Hidalgo, 2009). But this compound addition can also result in environmental impacts owing to high BOD, acid pH and high corrosivity (Lima *et al*., 2016). Thereby, dynamics control of agricultural pesticides depends on physical-chemical and biological characteristics of environmental matrix. These parameters affect persistence, chemical and microbial degradation rates, adsorption, volatilization, plant absorption, percolation, leaching and erosive processes (Andreu and Picó, 2004). The acceleration of herbicides degradation in soil can occur with adequate physical-chemical characteristics of environmental (humidity, pH, nutrients, etc.) to increase microbial activity by bioremediation process (Oliveira-Júnior *et al*., 2011). Microorganisms are indicators of soil quality and also are responsible for numerous processes and functions, such as compounds decomposition, nutrients cycling humic substances synthesis and soil aggregation (Burns *et al*., 2013). Enzymatic activity, respiration rate and microorganisms diversity allow to monitor environmental changes from agricultural use, besides evaluating and guiding management practices (Ferreira *et al*., 2017). Therefore, this study aimed to evaluate microbial metabolism in soil cultivated with sugarcane with tebuthiuron application in consortium with different vinasse volumes.

MATERIAL AND METHODS

Soil: The soil was collected from a sugarcane crop by conventional management - 21°21'50.2"S and 51°42'33.2"W - by five random points in approximately 100 m^2 extension. Remains of sugarcane straw and other debris were removed and samples were collected up to 15 cm deep. Afterwards, soil was transported to laboratory, sieved in 2.0 mm mesh and packed in a plastic box. The sample was classified as Distrofic Ultisol (Santos *et al*., 2018) and presented 86.7% of sand, 10.0% of clay and 3.3% of silt, which indicated sandy texture. Soil parameters revealed residual moisture of 0.6 g of water/100 g of soil; field capacity of 18.0 g of water/100 g of soil; overall density of 1.7 g cm^{-3} and bulk density 1.4 g cm^{-3}.

Tebuthiuron e vinasse: Herbicide tebuthiuron was purchased from a commercial company as Combine® 500SC - Dow AgroSciences Industrial Ltda. The vinasse was supplied by a sugarcane powerplant. Four liters of vinasse were collected in sterile glass bottles and conditioned at 4 °C. Table 1 shows the analytical results of composition and physical-chemical parameters for vinasse. It was observed that vinasse presented acid pH and high nutrients and organic matter concentrations (Table 1). Hence, it was highlighted the concentration of nitrogen, phosphorus and mainly potassium, whose contents favors its application as fertilizer in crops.

Table 1. Physical-chemical parameters of the vinasse

Parameters	Value	Unit	Parameters	Value	Unit
pH	4.4	-	Density	0.97	g mL^{-1}
Total Nitrogen	0.56	g L^{-1}	Organic Matter	9.51	g L^{-1}
Phosphorus	0.28		Total Carbon	5.28	
Potassium	1.42		SMR*	3.76	
Calcium	0.21		IMR*	0.19	
Magnesium	0.15		TMR*	3.95	
Sulfur	0.30		MOR*	13.27	

*SMR - Soluble Mineral Residue; IMR - Insoluble Mineral Residue; TMR - Total Mineral Residue; MOR - Mineral + Organic Residue.
Source: UNESP Soil Fertility Laboratory – Campus of Ilha Solteira, 2019.

Experimental design: The experimental design was completely randomized in 2x4 factorial scheme: tebuthiuron recommended dose (absence and presence) by Combine® 500SC for sandy soil; and vinasse volumes (absence and presence of three different amounts) generally used in sugarcane crop and determined based on potassium according to Technical Standard P4.231 (CETESB, 2015). The dose of Combine® 500SC was 2.0 L ha^{-1} (200 µL m^{-3}), which corresponds to 1.0 kg ha^{-1} of tebuthiuron. It represented values indicated for application in sandy and sandy-loam soils. In relation to vinasse, it was used the volume generally used by the sugarcane crops - 150 m^3 ha^{-1} (150 mL dm^{-3}), according to Technical Standard P4.231 (CETESB, 2015). Thus, it was used zero (H0) and 1.0x (H1) doses were used for tebuthiuron and four vinasse volumes: zero (V0.0), 0.5x (V0.5), 1.0x (V1.0) and 2.0x (V2.0). Treatments composition is shown in Table 2.

Table 2. Completely randomized experimental design in 2x4 factorial scheme according to tebuthiuron dose and vinasse volumes

Treatments	Herbicide	Vinasse
T1	H0	V0.0
T2	H1	V0.0
T3	H0	V0.5
T4	H1	V0.5
T5	H0	V1.0
T6	H1	V1.0
T7	H0	V2.0
T8	H1	V2.0

Source: Author, 2019.

For each treatment 2,500 cm^3 of soil was added to the respective volumes of tebuthiuron and vinasse (Table 1). Finally, soil field capacity was calculated between 60 and 70% and water amount required was added.

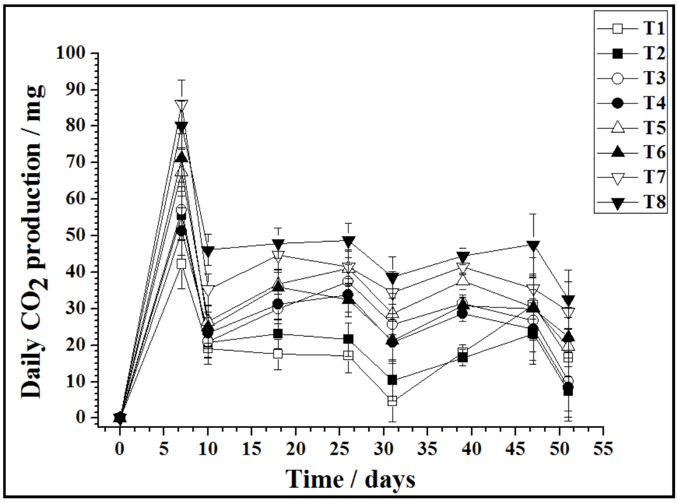
Biodegradation: Microbial respiration monitoring and consequent biodegradation rate in soil treatments were performed according to Bartha and Pramer respirometric method and Technical Standard L6.350 (CETESB, 1990). This procedure allowed inferring degradation behavior in treatments by CO_2 production of microbial metabolism. The Bartha and Pramer respirometer consists of a closed system with two connected chambers, where microbial activity is monitored by conductivity analysis in KOH solution, according to Faria *et al*. (2013). The transformation of conductivity values in mS cm^{-1} to mg of CO_2 was performed by the equation: "$GCO_2 = 151.7 - 9.599 * CT$" ($R^2 = 0.9999$); in which GCO_2 refers to carbon dioxide generation in mg and CT represents the conductivity result in mS cm^{-1} (Faria *et al*., 2013). Therefore, efficiency and lower risk of interference are guaranteed. Conductivity determinations and CO_2 quantifications in each respirometric system were performed weekly. After analysis, the respirometers were incubated at 28 ± 2 °C. Experiments were performed in triplicates.

Determination of tebuthiuron residues in soil: After soil natural attenuation period, a sample of each treatment containing the herbicide (T2, T4, T6, and T8) was sent to determine tebuthiuron concentration present in soil. This analysis was carried out at "Central Analítica de Resíduos e Contaminantes" from Embrapa (Brazilian Agricultural Research Corporation), according to Queiroz (2004) based on methanol extraction and high performance liquid chromatography - HPLC.

Accomplishment period and results analysis: The incubation period in microbial metabolism evaluation was 51 days in 2017. Hence, tebuthiuron residues concentration in soil samples at final evaluation time was evaluated (t51). Experimental data were analyzed by performing analysis of variance, Tukey test at 5.0% probability for means comparison using software Microcal Origin 8.0.

RESULTS AND DISCUSSION

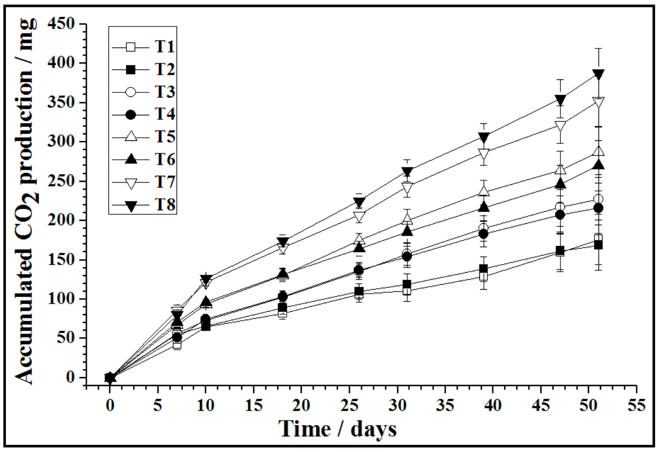
Biodegradation: Microbiological activity results in respirometers are presented in Figures 1 and 2 for daily and accumulated CO_2 production, respectively.



Source: Author – research data, 2019.

Figure 1. Daily CO_2 production during 51 days biodegradation by natural attenuation. Treatments composition (tebuthiuron-vinasse) - T1 (H0-V0.0); T2 (H1-V0.0); T3 (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); and T8 (H1-V2.0). (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); e T8 (H1-V2.0)

In Figure 1, there is a peak CO_2 production in first evaluation (t7) and subsequently there was a decrease and steady condition in microbial respiration rate of until the end of time (t51). Thus, this higher initial activity can be attributed to the presence of organic matter in soil and the highest values were in treatments with higher vinasse volume – V2.0 (T7 and T8). When organic matter was readily available, microbial metabolism was elevated at the beginning and reduced after decomposition over time. Furthermore, this study observed a decrease in CO_2 production at the beginning of incubation - after 96 h.



Source: Author – research data, 2019.

Figure 2. Accumulated CO_2 production during 51 days biodegradation by natural attenuation. Treatments composition (tebuthiuron-vinasse) - T1 (H0-V0.0); T2 (H1-V0.0); T3 (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); and T8 (H1-V2.0). (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); e T8 (H1-V2.0)

In Figure 2, it is possible to evidence the difference in accumulated CO_2 production based on vinasse volume in each treatment. Microbial respiration was directly proportional to vinasse volume used. Hence, results after 51 days separated the treatments into four groups according to vinasse: V0.0 (T1-T2), V0.5 (T3-T4), V1.0 (T5-T6) and V2.0 (T7-T8). Therefore, an increase of microbial degradation activity in presence of this residue was observed due to its composition (Table 1) with high concentration of organic matter and nutrients (nitrogen, phosphorus and potassium). Vinasse is composed by organic compounds easily decomposed from yeast lysis and partially fermented must (Hidalgo, 2009), whose composition represents an energy source readily available to soil microbiota. Agostinho *et al.* (2017) also reported that microbial respiration elevation after vinasse application in soil was generally attributed to high soluble carbon content and rapid assimilation. Table 3 demonstrates the accumulated CO_2 variance analysis generated in treatments after 51 days biodegradation. It was confirmed that vinasse addition to soil in any of the volumes (0.5, 1.0 or 2.0) significantly increased the CO_2 production in relation to soil without this compound (T1 and T2). This indicated that the vinasse benefited the soil microorganisms and increased their metabolic rate.

Table 3. Variance analysis in accumulated CO_2 production after 51 days of natural attenuation - Tukey test at 5.0% of significance. Treatments composition (tebuthiuron-vinasse) - T1 (H0-V0.0); T2 (H1-V0.0); T3 (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); and T8 (H1-V2.0). (H0-V0.5); T4 (H1-V0.5); T5 (H0-V1.0); T6 (H1-V1.0); T7 (H0-V2.0); e T8 (H1-V2.0)

Treatments	Accumulated CO_2 production / mg (51 days)	
T1	166.00 ± 31.99	f
T2	179.79 ± 25.73	ef
T3	235.53 ± 19.05	cde
T4	215.94 ± 9.64	def
T5	295.31 ± 11.74	bc
T6	267.01 ± 5.72	cd
T7	347.16 ± 9.29	ab
T8	377.37 ± 32.39	a

* lowercase letters represent significant difference in mean between treatments in column (Tukey test p <0.05 probability)
Source: Author – research data, 2019.

The CO_2 production in the treatments without herbicide that received 0.5x or 1.0x vinasse (T3 and T5, respectively) did not differ significantly as well as treatments with 1.0x or 2.0x (T5 and T7, respectively). Nevertheless, there was a significant difference between treatments that received 0.5x or 2.0x vinasse which indicated that soil respiration increased with greater volume of vinasse added to soil. Therefore, agroindustrial waste reuse in soil represents a sustainable alternative to its destination. Hence, vinasse application favored microbial activity (Table 3) and may directly influence tebuthiuron biodegradation as demonstrated in other studies with herbicides associated with agroindustrial residues in soil (Régo *et al*., 2017). Fertigation with vinasse increases organic matters and nutrients availability and favors exchange cations capacity (Hidalgo, 2009). Although the innumerable advantages, it is important to emphasize that its continuous and unplanned use can generate negative impacts due to composition, corrosivity and pH (Lima *et al*., 2016). In relation to treatments with tebuthiuron (T2, T4, T6 and T8), it was observed from Table 3 that there was no significant difference when comparing them with the assays without herbicide and same vinasse volume. In other words, treatments were grouped according to vinasse application: V0.0 (T1-T2), V0.5 (T3-T4), V1.0 (T5-T6) and V2.0 (T7-T8). Furthermore, higher amounts of vinasse in soil with tebuthiuron showed higher rates in microbial metabolism in relation to T2 (H1-V0.0). It was also observed that T4 (H1-V0.5) and T6 (H1-V1.0) did not differ significantly. However, the addition of double volume to soil in T8 (H1-V2.0) significantly increased CO_2 production compared to other treatments with herbicide (Table 3). Several studies reported herbicides impact on microbiota and their respiration in soil. In contrast to tebuthiuron results (Figure 2 and Table 3), imazethapyr and imazapyre (Souto *et al*., 2013) resulted an increase in CO_2 release by biodegradation of these molecules. However, high doses may cause toxicity to microorganisms and promoted less activity (Souto *et al*., 2013). On the other hand, negative impacts on microbial activity were observed when triasulfuron and prosulfocarb were present (García-Delgado *et al*., 2019). Changes in soil microbiology were demonstrated by lower microbial biomass and consequent reduced CO_2 release. In agreement with the present study (Figure 2 and Table 3), Pose-Juan *et al*. (2015) observed that mesotrione application had no effect on soil respiration.

Determination of tebuthiuron residues in soil: After 51 days of natural attenuation, HPLC analysis was performed to determine tebuthiuron residues in treatments with the herbicide (T2, T4, T6 and T8). T2, T4 and T6 samples obtained a result below the equipment quantification limit (10 $\mu g\ kg^{-1}$ soil). In T8 (H1-V2.0), soil presented 15 $\mu g\ kg^{-1}$ soil or 10.7% of the initial tebuthiuron amount. Note that herbicide leaching was not considered since the samples remained in plastic boxes and without rainfall simulation. Results suggested that tebuthiuron was degraded according to respiration rates (Table 3). Although the tebuthiuron half-life is approximately 15 months (Rodrigues and Almeida, 2011), changes in its persistence have been reported as a function of environmental conditions. As sandy soil was used in this study, it was demonstrated that low concentration of tebuthiuron was found after only 20 days in sandy soil and its half-life is also lower in sandy soils compared to clayey soils (Lourencetti et al., 2012). Another important factor with direct effect on molecule persistence was the vinasse addition. Thus, results may be associated with Lourencetti et al. (2012), whose experiment showed that vinasse presence influenced less persistence of tebuthiuron in soil. Furthermore, the historical on soil use and management may influence the organic compounds degradation. As soil sample was collected in sugarcane crop area with historical tebuthiuron application, microorganisms potentially capable of degrading the herbicide were probably pre-selected and present in soil. When an herbicide is added in soil, few microorganisms can perform its degradation and this initial step represents the adaptation period of microbiota, which produces essential enzymes to degrade the new substrate. Then, the microbial population increases and consequently the decomposition process is accelerated.

Conclusions

- The presence of tebuthiuron recommended dose had no effect on soil microbial activity, represented by CO_2 production;
- Microbial metabolic rate increased in vinasse presence especially when used in higher concentrations;
- Chromatographic analysis suggested that there was degradation of herbicide in treatments associated or not to vinasse.

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