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Effects of Ametryn on Microbial Activity in Soil with Biofertilizer Addition

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Abstract

In recent years, pesticide application has increased worldwide due to the need for increased food production. In this way, understanding of the effects that these xenobiotic molecules can result in the soil microbiota becomes fundamental. Thus, this work aimed to evaluate microbial enzymatic activity by FDA, as well as quantify bacteria and fungi contaminated soil by ametryn herbicide. In addition, biofertilizer was added as a form of microbial biostimulation in order to cause enrichment of the affected site. The ametryn herbicide was chosen because it is widely used in sugarcane crops in Brazil, in the control of weeds, and is moderately persistent in the environment. It can be evaluated that the biofertilizer was fundamental in increasing the microbial activity of the soil, even in the presence of ametryn, probably contributing to its biodegradation. The estimation of the enzymatic activity by FDA, was essential for the evaluation of the increase of the microbial action, contributing to the verification of the active microbiota in the soil. It is suggested the application of biofertilizer in soils contaminated by ametryn, as a way to mitigate affected sites.

Keywords: nutrients, herbicide, microrganisms

1. Introduction

Herbicides are the most commonly used groups of pesticides, which can act in contact with the plant or be translocated into the plant, which are more important to combat perennial weeds. Most pesticides applied to crops end up in water and soil, resulting in environmental contamination (Nicolopoulou-Stamati et al., 2016).

Ametryn is a herbicide belonging to the group of s-triazines widely used in sugarcane, corn and pineapple plantations, among others, in pre and post emergence periods for the control of weeds and grasses. It is a herbicide with a strong potential for contamination of surface and groundwater due to its environmental persistence, leaching and surface runoff (PPDB, 2018).

However, pesticides can positively or negatively influence soil microbiota dynamics, which in turn influence soil nutrient behavior (Nicolopoulou-Stamati et al., 2016).

Several studies in the literature such as Jilani et al. (2007), Souza and Resende (2003), Santos (1992) and Bettiol et al. (1998), have applied organic substrates to stimulate the local microbiota to aid in the biodegradation of the xenobiotic compounds discarded in the soil.

Biofertilizers are organic compounds rich in nutrients and contain microbial biomass, capable of nutritionally supplying the soil, aiding in the metabolic activity of the local community. Rêgo et al. (2014) when evaluating microbial respiration in soil contaminated with ametryn, observed that there was an increase in respiration rate, quantified by the accumulation of CO2 generation over time.

Thus, this work aims to evaluate the enzymatic activity by the FDA method and the quantification of bacteria and fungi, by the plating method, in soil contaminated by ametryn. As a form of microbial biostimulation, biofertilizer was applied in order to promote the enrichment of the affected soil.

2. Material and Methods

2.1 Ametryn Herbicide

For the purpose of this study, the herbicide ametryn of the brand Fluka (Germany), with 98.5% purity, in the granular form was used.

2.2 Biofertilizer

The Microge$^{o®}$ biofertilizer was provided by the company Microbiol Biotecnologia, in the municipality of Limeira-SP, which was patented by the same, with registration PI0207342-0 A2.

It was formed by fermented organic compounds containing living or latent cells of microorganisms (bacteria, yeasts, algae and filamentous fungi) and by their metabolites, in addition to organo-mineral chelates. Its production is based on composting (fermentation) in liquid medium, continuously and carried out in the same tank, through the use of a specially developed organic compound and the addition of extra inputs, without interruption of production (D´Andrea, 2010).

2.3 Soil Collection

The soil was collected in sugarcane plantation area, with a history of application of ametryn herbicide, in the region of Piracicaba, in state of the São Paulo, in Brazil, at 20 cm depth, using a spade and shovel. The soil granulometry used in the experiments was 42% sand, 33% clay and 25% silt, classified as clay-silty sand.

2.4 Soluble Fraction of Soil in Water

Weighed was 25 g of soil into 250 mL flask and 100 mL of distilled water were added and the flasks were shaken for 22 h ± 2 h. After this time, the vials were allowed to stand for 30 minutes. The soluble fraction was used in the quantification experiments of bacteria and fungi present in the soil.

2.5 Estimation of Microbial Activity of The Soil by the FDA Method

The total soil microbial activity was evaluated by the Fluorescein Diacetate Hydrolysis (FDA) method described by Boehm and Hoitinik (1992). Table 1 shows the experimental design used for the quantification of microbial activity.

Table 1. Samples performed out in the experiment to estimate microbial activity in soil, with application of ametryn and addition of biofertilizer

Samples	50 g of soil Dry base	Ametryn (µg/L)	Biofertilizer (%)
Control soil	+	-	-
8 µg/L ametryn +1% biofertilizer	+	8	1
8 µg/L ametryn +5% biofertilizer	+	8	5
8 µg/L ametryn +10% biofertilizer	+	8	10
12 µg/L ametryn +1% biofertilizer	+	12	1
12 µg/L ametryn +5% biofertilizer	+	12	5
12 µg/L ametryn +10% biofertilizer	+	12	10
8ug/L ametryn	+	8	-
12ug/L ametryn	+	12	-
1% biofertilizer	+	-	1
5% biofertilizer	+	-	5
10% biofertilizer	+	-	10

This methodology consists in quantifying the hydrolysis of fluorescein diacetate in the soil. The enzymes responsible for the hydrolysis of the FDA are abundant in the soil environment. Non-specific enzymes such as esterases, proteases and lipases have been shown to hydrolyze the FDA, being involved in the decomposition of many types of compounds. The hydrolysis ability of the FDA, appears to be widespread, especially among major decomposers, bacteria and fungi (Schnürer and Rosswall, 1982).

The concentration of FDA hydrolyzate (µg of FDA hydrolyzed per g-1 of dry soil per minute) was determined with the aid of the standard curve by correlation with the absorbance read in the sample and the FDA hydrolyzed. The calibration curve was obtained by adding FDA at concentrations of 0, 20, 40, 60 and 80 µg / g dry soil (0, 50, 100, 150, 200 µL) in 5 mL of potassium phosphate buffer, contained in test tube. The tubes were sealed and placed in a 100 ℃ water bath for one hour for total FDA hydrolysis. After hydrolysis, the FDA was added to a 250 mL Erlenmeyer flask containing 5 g of soil and 15 mL of potassium phosphate buffer. The Erlenmeyer flasks were shaken at 150 rpm at room temperature for 20 minutes. Then, 20 mL of acetone was added immediately into each vial to stop the reaction. The samples were then centrifuged at 2500 rpm for 15 min to determine the absorbance in a spectrophotometer at 490 nm.

For the analysis of the samples, 5 g of each soil sample were placed in duplicate in a 250 mL

Erlenmeyer flask, together with 20 mL of 60 mM potassium phosphate buffer (8.7 g K_2HPO_4, 1.3 g KH_2PO_4, 1000 mL water distilled; pH 7.6). The FDA hydrolysis reaction was initiated with the addition of 0.2 mL of the FDA stock solution (2 mg/mL acetone). The samples were shaken at 150 rpm and incubated at room temperature for 20 minutes.

The reaction was quenched by the addition of 20 mL of acetone P.A. per vessel, which remained capped until centrifuged. The samples were then centrifuged at 2500 rpm for 15 min. Afterwards, in a spectrophotometer, the absorbance of the centrifuges at 490 nm was determined.

2.6 Quantification of the Soil Microbiota

The soil microbial quantification was expressed in Colony Forming Units per gram of soil (CFU/g soil) of fungi and bacteria, using the Pour Plate method, using Plate Count Agar (PCA), and for fungi the Sabouraud medium, in duplicate.

The soluble fraction of the soil was diluted to the dilution 10-6, withdrawing the 1 mL aliquot. Dilutions 10^4, 10^5 and 10^6 were plated in Petri dishes in the 1 ml aliquot, and then the culture media were added. The plates were incubated for 48 hours at 35.5 ℃ for the heterotrophic bacteria, and for fungi were incubated for 72 hours at 28 ℃. After the incubation period, the colony forming units were read per gram of soil (CFU/g soil).

2.7 Analises Estatisticas

Statistical analyzes of the results were performed using Tykey test, considering a significant difference $p \leq 0.05$, in order to evaluate the different types of treatments, since the data are not distributed normal. The Analyzes Were Performed In Origin 9.0 Software.

3. Results And discussion

The table 2, expresses the result of the quantification of heterotrophic bacteria and fungi, and estimate of the enzymatic activity by the FDA method of soil contaminated by ametryn.

Table 2. Results of quantification of heterotrophic bacteria and fungi, and estimation of microbial activity by the FDA method

Samples	Heterotrophic bacteria (10^6) CFU / g soil	Fungi (10^6) CFU / g soil	µg FDA hydrolyzed/g dry soil
Control soil	1.55[a]	2.0[a]	26.20[a]
8 µg/L ametryn +1% biofertilizer	0.8[b]	1.8[a]	13.73[b]
8 µg/L ametryn +5% biofertilizer	1.2[b]	1.2[b]	24.78[a]
8 µg/L ametryn +10% biofertilizer	3.0[c]	2.0[a]	26.85[a]
12 µg/L ametryn +1% biofertilizer	0.1[d]	0.20[c]	13.78[b]
12 µg/L ametryn +5% biofertilizer	0.03[e]	0.70[d]	18.43[c]
12 µg/L ametryn +10% biofertilizer	17.0[f]	8.9[e]	18.72[c]
8ug/L ametryn	0.6[d]	0.22[d]	32.1[d]
12ug/L ametryn	0.06[e]	0.80[d]	30.75[d]
1% biofertilizer	0.105[d]	0.035[f]	22.48[a]
5% biofertilizer	0.045[e]	0.255[d]	47.93[e]
10% biofertilizer	3.0[c]	0.015[f]	51.45[f]

Means followed by the same letters do not differ from each other, by the Tykey test at 5% probability.

The addition of biofertilizer contributed to increase the quantification of heterotrophic bacteria and fungi in soil contaminated by ametryn. The application of carbon source helps in increasing the microbial activity, favoring the metabolization of organic compounds.

Probably, the carbon source from the biofertilizer, with easy access to the microorganisms, helped in its strengthening and enrichment, being verified by the increase of the estimate of the microbial activity by FDA, benefiting in the metabolism of the ametryn, since its biodegradation occurs by cometbolism .

The soil used in the experiments had sandy characteristics, with lower organic matter content. It is known that ametryn has a greater tendency to leach in most soils, but this factor can be maximized in sandy soils (Silva et al., 2012).

Also, the presence of triazine herbicides was observed by Armas et al. (2007) found that ametryn was at higher levels compared to atrazine and simazine. The ametryn presents greater solubility in water and less tendency of adsorption the particles of the ground. In Western Germany, in more than 100.000 samples collected from surface and groundwater,

80.7% of the contaminants were triazine (Beitz et al., 1994).

In this way, the addition of biofertilizer was essential for the increase of microbial activity in soil, as well as in the dissipation of ametryn, reducing its time of permanence in the environment.

4. Conclusions

In relation the methods used in the experiments, the microbial quantification by the plating method was simple and fast to obtain the results. However, it can quantify only a small portion of the microorganisms. The enzymatic quantification is more efficient because it quantifies a larger portion of the active microbiota in soil activities.

It is recommended the application of 10% of biofertilizer, for each 50 g of soil used with ametryn, in order to result in a greater increase of microbial activity. Also, it is suggested in future works to carry out chromatographic tests in order to confirm the biodegradation of the ametryn molecule, in order to contribute to the understanding of the dissipation of the herbicide in the environment.

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