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Soils from microbasin of Espraiado, Ribeirão Preto City, Brazil: evaluation of microbial population from soil suspensions incubated with atrazine and 2,4-D
Les sols du microbassin de Espraiado, Ribeirao Preto, Brésil : évaluation de la population microbienne de suspensions de sols incubées avec l'Atrazine le 2,4-D

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INTRODUCTION

Since the late 60's the aim of the agricultural industry has been to improve crop yield by the intensive use of agrochemicals (pesticides, herbicides, fertilizers, etc.) and machinery. Harmful insects, mites, infectious fungi, bacteria and invasive weeds have been controlled by pesticides and herbicides involving a huge world market around 20 billions of dollars annually (TANAKA and OMURA, 1993).

This practice of long-term, sometimes indiscriminate and abusive use of agrochemicals all over the world is bringing concern to public authorities and to those involved with public health and sustainability of natural resources associated with agricultural practice, in consequence of actual analytical measurement of soil and surface and groundwater contamination (BELLUCK et al, 1991).

Brazil is the 5th highest world consumer of pesticides and herbicides used in sugarcane, soy, etc. plantation. The country has a diverse variety of unique and sensitive ecological systems claiming for studies on the impact of intensive agriculture.

Studies have identified a particular area with a high risk of groundwater contamination by agrochemicals, resulting from the geological formation of the region. There is a natural vulnerability of an aquifer just below, highly susceptible to infiltration due to continuous and excessive use of agrochemicals for sugarcane plantation, mainly. This site located in the region of Ribeirão Preto, State of Sao Paulo, is covered by the aquifer "Botucatu" which occupies more than 60% (116.000 Km^2) of the State, and represents the main reserve of State's groundwater. Around 60% of the urban cities in the State are supplied partially or totally by groundwater and in the Ribeirão Preto region, with more than 2 million inhabitants, practically 100% of the cities are exclusively supplied with groundwater. The aqüifer "Botucatu" extends to other North Western and

Southern Brazilian States and two other countries: Paraguay and Uruguay (CNPMA/EMBRAPA Annual Research Report, 1994).

A study on the overall impact of intensive agriculture on the environment is been done in a research area chosen as a model with the size of 4,141 ha, in a ridge watershed (the microbasin of Espraiado) mainly with sugarcane crop cover where the soil is very permeable. Results of the herbicides residues from the area and simulation anlyses were described (CERDEIRA et al, 1998). It is expected the data coming from the studies to be used in other regions in Brazil or even South America with similar conditions or potential problems. The data will also be used to adapt or create mathematical models that could be utilized for other regions.

This work aims to study the effects of herbicides on microbial community of soils from the Espraiado watershed. Problems in assessing the impact of herbicides on the soil microflora are numerous and complicated (SAHID et al, 1992). The interactions occurring simultaneously between microorganisms, soil and herbicides change, depending on soil characteristics. It is well known herbicides affect the density and composition of microbial community in soil, but those effects described in the literature are quite controversial (GROSSBARD, 1976).

So, we have decided to evaluate the microbial population of soil samples from the region and the effects of atrazine and 2,4-D on microbial population of soils samples in suspension by means of plate dilution technique, instead of treating the soil with herbicides. The soil samples were harvested monthly at 2 different depths from 9 points representing soils from the region under study.

MATERIAL AND METHODS

Soil samples

Soil samples from 9 selected points from Santa Maria farm, located in the microbasin of Espraiado were sampled monthly at 0-20 (since February, 1996) and 80-90 cm (since May, 1996) and immediately refrigerated and kept at 4^0C for analyses.

Characterization of microbial population from soil samples

Five grams (wet weight) of each fresh sample in 50 mL sterile water in 125mL-erlenmeyer were stirred for 3h at 28-30^0C. After stirring 8.0 mL of each soil suspension were transferred to a 50mL large-mouth tube containing 1.0mL 10 times concentrated Yeast Nitrogen Base (YNB) medium + 1.0 mL water. From these suspensions successive dilutions (10^{-2} and 10^{-3}) were made for the plating of 20 uL of each dilution in duplicate in YPGlucose medium. The plates were incubated at 30^0C and the colonies monitored by counting. The colony forming units (CFU) were calculated from the average of the best dilution and expressed per gram of wet soil.

Treatment of soil samples with atrazine and 2,4-D

Every month, the 18 fresh samples sampled from the selected points were suspended in sterilized water (5g/50ml) and incubated for 3h in a shaker at room temperature. Eight mL of each suspension was transferred to a 70mL culture tube containing 1.0mL yeast nitrogen base (YNB) plus 1.0mL water (control) or atrazine or 2,4-D to a final concentration of 1.0mg/mL and 0.5 mg/mL, respectively. The resulting 54 suspensions were kept with occasional stirring at room temperature for 21 days. The

plating of the suspensions (20uL per plate of 2 dilutions in duplicates) in yeast extract, peptone, glucose (0.1%) and agar medium (YPG) were made at days 1, 7, 14 and 21, after appropriate dilution to give a final colony count per plate of 30 to 500. The growth of the colonies were monitored until 7 days of incubation at 30^0C and one plate out of 4 was documented by photography. The results of colonies counting were expressed as CFU 10^{-7}/g wet soil. The quantitative effect of the herbicides were based on the counts of the growth kinetics of each curve compared to the control and the qualitative differentiation was visualized by colony morphology and microscopy.

RESULTS and DISCUSSION

Microbial population from soil samples

Soil samples were sampled monthly at 0-20cm (since February 1996) and 80-90cm depth (since May 1996) from 9 selected points covered with sugarcane plantation, and representing different soils from the region under study. These soils submitted to texture analysis were classified from clay to sandy in texture, with organic matter content of 0.41 to 3.36 at the surface and 0,13 to 1,1% at 80-90cm depth, determined by Wakley & Black method (1934). The pH (in water) was usually acidic, except for one point (P04) presenting pH closer to neutrality (ROCHA et al 1997).

In the conditions studied the results of the initial plating of the control suspensions have shown the samples from the surface (0-20cm) presented usually a microbial population around one order of magnitude higher than at the 80-90cm depth. Samples from July, 1996 had the lowest population, coinciding with the dry winter season. By colony analyses it was observed great biodiversity of the population in the medium employed (Table 1). Fungi colonies count were absent or very low in some months.

Plate dilution counting of the populations from suspensions incubated with atrazine and 2,4-D over a period of 21 days has shown the highest growth rate during the first week in the control and in the herbicide-treated soil samples (Figure 1).

To evaluate and compare the population density from different months, points and depth we have decided to calculate the total colony counts over the 21-day period, comprising 4 platings. From the total, the control of every soil sample submitted to atrazine and 2,4-D treatments was expressed as 100%. The plotting of % of control has revealed no effect or the extent of inhibition or stimulation provoked by each herbicide on each soil sample microbial population and the variation month by month. The Figure 1 shows the growth kinetics of the population from a soil sample, where atrazine has stimulated and 2,4-D inhibited the growth, compared to the control.

The results have shown the behavior of the populations submitted to the herbicides treatment has varied depending mostly on the month of soil harvest than in the differences of soil characteristics, although variations in response among different points were noticed.

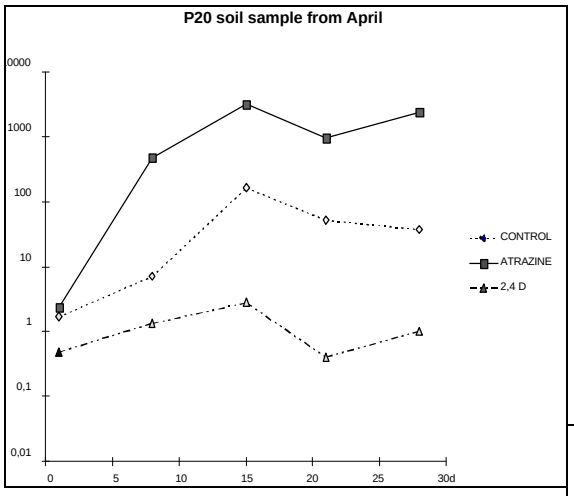


Figure 1 - Growth kinetics of microbial population from soil sample of point P20 (April) treated with atrazine (solid line) and 2,4-D (triangle, dashed line) compared to control (losangle, dashed line).

The microbial growth of soil samples of February to May (0-20cm), independent of the sample was inhibited by 2,4-D in 90% compared to atrazine, reaching sometimes 99.9% inhibition (Figure 2), while samples of August (0-20cm and 80-90cm) was stimulated by 2,4-D, showing density as high as 15 times the control (Figure 3).

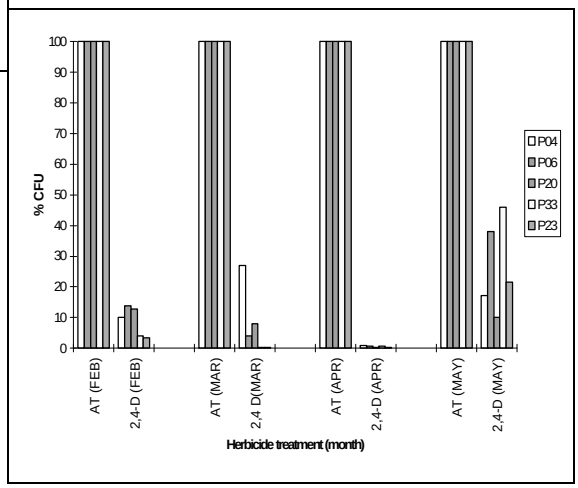


Figure 2 - Inhibition of microbial growth of soil samples of February to May by 2,4-D treatment (atrazine 100%).

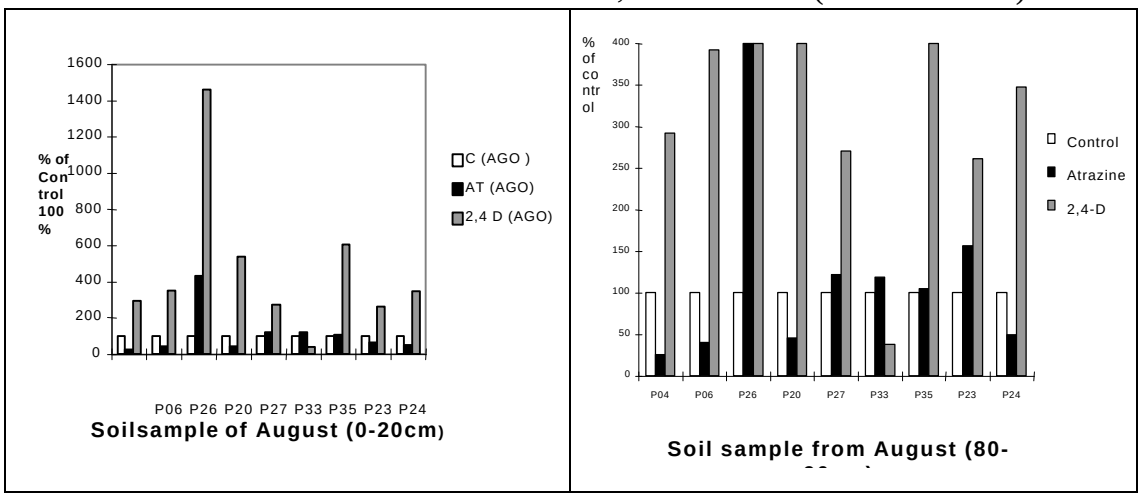


Figure 3 - Effect of atrazine and 2,4-D on population of August soil samples.

A dramatic change in response was observed with June (0-20cm) samples where atrazine turned, mostly, an inhibitor and 2,4-D stimulated growth in some samples (Figure 4). Atrazine has played as more potent inhibitor on soil microbial populations from 0-20cm samples of June, November (not shown), and from 80-90cm

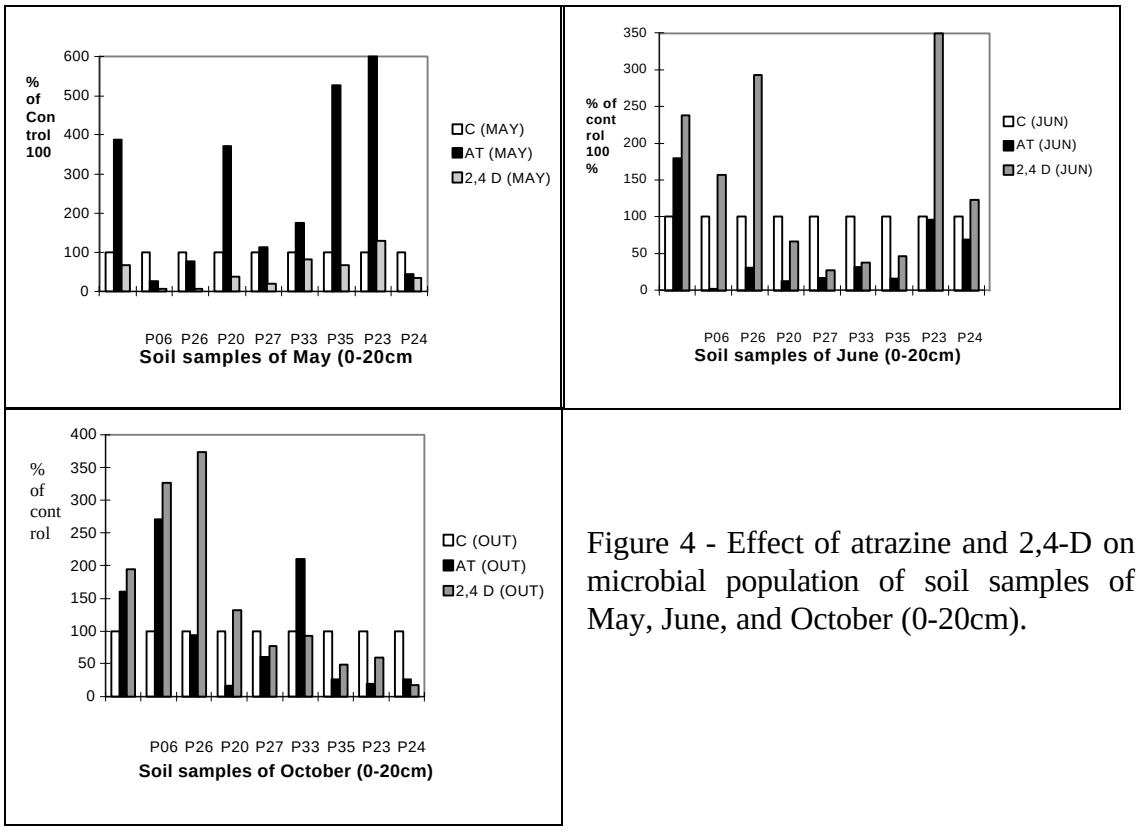
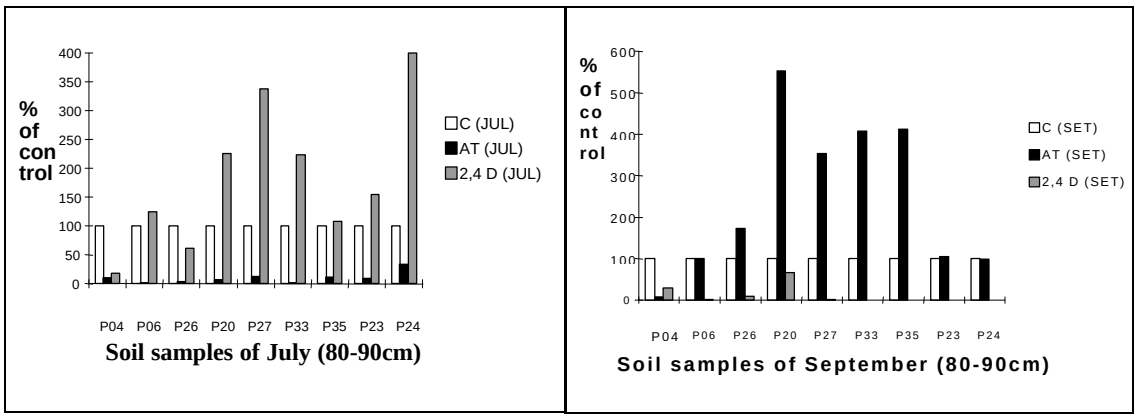


Figure 4 - Effect of atrazine and 2,4-D on microbial population of soil samples of May, June, and October (0-20cm).

samples of July, and November, while 2,4-D inhibited during February to May (Figure 2), and July in 0-20cm samples and from September (Figure 5) to November in 80-90cm samples, considering similar responses of at least 7 out of 9 samples for this conclusion.

Figure 5 - Effect of atrazine and 2,4-D on microbial population of soil samples of July



and September (80-90cm).

With this work we have shown the response of a specific herbicide can change from high inhibition to high stimulation, even when selecting the same species. So, the effects of the herbicides were influenced by the seasonal changing characteristics

of soils probably submitted to environmental conditions or handling by agricultural practice. Observing the determinations of pH, organic matter and humidity (ROCHA et al, 1997, CERDEIRA et al, 1998), it was noticed the coincidence of these parameters variations concomitant to changes in response of microbial population of soil samples from May to August, mainly.

Usually atrazine has selected bacterial and some yeast, while 2,4-D during its highest inhibition period has selected several fungi, instead of a typical yellow bacterial colony present in most of the 2,4-D incubated soil samples.

We have noticed from the colonies observation during the period of this study the high biodiversity of controls, although reduced, probably imposed by the culture conditions, when compared to atrazine and 2,4-D treated samples which selected only one or few distinct populations after the incubation. Some strains appeared in both cultures, while others could be considered typical for atrazine or 2,4-D, showing the effectiveness of the methodology for selection of specific strains, mainly bacteria. Two yeasts isolated from these experiments were already identified and several bacteria are under identification by Fundação Tropical André Tosello, (Campinas, Brazil).

The results presented in this work, although obtained by incubation of soil suspension with herbicides and defined growth medium, could probably explain the controversial results described in the literature (GROSSBARD,1976) and the once believed statement that a characteristic of this field of research is that a herbicide shown to be inactive or inhibitor in one report was shown to exert a stimulating action in another.

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Mots clés : microflore, herbicides, atrazine, 2,4-D

Table 1- Microbial population (10^7 CFU/g soil) of fresh soil samples (0-20 and 80-90cm) from 9 points of Espraiado Basin determined by plate dilution count in YPG medium.

Month	May	May	June	June	July	July	Sept	Sept	Oct	Oct	Nov	Nov	Jan	Jan	Feb	Feb
Depth/ Point	0-20	80-90	0-20	80-90	0-20	80-90	0-20	80-90	0-20	80-90	0-20	80-90	0-20	80-90	0-20	80-90
P04	3.20	0.52	2.20	0.13	0.43	0.09	1.20	0.15	0.75	0.08	0.39	0.02	1.08	0.41	0.20	0.14
P06	0.63	0.12	0.87	0.07	0.34	0.05	1.05	0.05	0.61	0.05	0.80	0.29	0.60	0.27	0.71	0.47
P26	2.35	0.09	0.87	0.07	2.20	0.02	1.43	0.13	0.80	0.07	1.40	0.12	2.12	0.39	2.57	0.23
P20	2.45	0.20	1.40	0.04	0.64	0.07	1.45	0.13	1.95	0.25	2.60	0.14	1.25	0.28	2.12	0.18
P27	1.50	0.68	2.00	0.34	0.40	0.12	1.20	5.00	0.68	0.47	0.49	0.27	1.42	0.09	0.72	0.13
P33	0.48	0.12	0.90	0.09	0.69	0.03	0.60	0.40	0.75	0.34	2.22	0.15	1.38	0.23	0.11	0.60
P35	0.58	0.56	1.10	0.14	0.80	1.00	0.50	0.60	0.80	0.44	0.61	0.46	0.65	0.54	1.68	0.05
P23	1.02	0.16	1.08	0.14	0.93	0.13	0.33	1.70	0.87	0.22	1.08	0.29	1.32	0.12	0.83	0.09
P24	0.37	0.15	1.40	0.05	0.50	0.02	0.80	1.70	0.63	0.73	0.52	0.10	0.98	0.24	1.02	0.16

Values expressed in 10^7 CFU/g soil.