

# Impact of two Pesticides on soil Physicochemical Characteristics and the Biological Activity

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## ABSTRACT

Easy to access and use, synthetic pesticides have been very effective in a significant number of cases. They have widely contributed to the increase and regularity of production. However, in recent years, the systematic use of these products is in question following the disturbances generated for all components of the environment. During the spraying, no more than 90% of the quantities reach their targets, but they are diffused at ground level causing the modification of its physicochemical properties and its biological activity. For that purpose, our work aimed at verifying the impact of two pesticides used massively at the Algerian Northeast level. To do it, some characteristics of the ground were determined. The microbial flora and the enzymes of fertilities were also estimated from the fractions of ground. The obtained results show that the values of certain parameters exceed the prescribed standards and in particular those of the electric conductivity and the total carbon. The rates of the analyzed enzymes are lower than those of the witnesses. The isolated micro-flora is established by 60% of bacteria with an ascendancy of *Micrococcus* and by 40% of mushrooms the most plentiful of which are *Aspergillus*.

**KEY WORDS:** Pesticides, Ground, Physicochemical Settings, Acid Phosphatase, Dehydrogenase.

## 1. INTRODUCTION

In the last century, the population has been exposed to an increase in the use of crop protection products. The progress in the protection of plants and the development of synthesis chemicals, such as pesticides, widely contributed to the regularity of the production. However, today the systematic use of these products is questioned, with the increasing awareness of the risks, which they can generate for all the components of the environment, even for man health. Toxicological consequences due to the exhibition in these thousand chemical components by means of the food, water and ground are alarming.

Most of the active substances of these phytosanitary products can form residues bound at the level of the various compartments of the ground by modifying its fertility and its biological activity. The notion of chemical and biological quality of a polluted ground subjected to the effects of the organic pollution by pesticides gave rise to numerous interpretations. According to Morel (1989), the quality of a ground depends of a set of factors of the vegetable growth such as the chemical elements necessary for the plant and the organic substances of growth. The production of these nourishing elements by the microbial actions is translated by the supply of the nitrogen, the carbon, the phosphor and so on. However the presence of pesticides in the ground causes the inhibition of all these elements. Besides, the toxic action of the pesticide can cause a slowing down of the microbial activity of the ground and the selection of populations the best endowed to resist the pollutant (Barriuso, 1994) and the decrease of the diversity of microorganisms (Rouard, 1996; Soulas, 1996; Chaussod and Nicolardot, 1982).

In addition, the presence repeated of several pesticides molecules to exaggerated doses in the ground as well as the interactions between pollutants and microorganisms of the ground can lead to an inhibition of the enzymatic activities or to their stimulation depending at the same time on the nature of the pollutant, on the concentration of the pollutant in the ground, and on the type of enzyme (Ascoli, 2007; Dick and Tabatabai, 1983; Fotio, 2006). Enzymes, which can be affected, are of type respiratory type, and the most common are: dehydrogenases, phosphatases, oxydo-reductases and hydrolases. Among developing countries using the biggest quantities of pesticides, Algeria appears in the first rows with other African countries. Thereby thousands of phytosanitary products are approved every year in Algeria (Bouziani, 2007). This situation is all the more worrisome as the use of pesticides must be periodically repeated and to random doses. This can generate serious consequences in a definitive way in particular on the fertility of grounds.

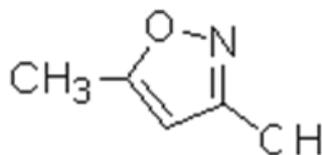
For all these reasons, we tried to study the effect of the repeated employment of two pesticides molecules (hymexazole and prometryne) on the physicochemical quality and on the biological activity of the soil.

## 2. MATERIAL AND METHODS

**Driving Test:** The test was realized in jars containing 1kg of a mixture of several samples of ground taken from a not polluted zone. Prepared jars are thus beforehand irrigated in the sterile distilled water. After 48 hours, jars containing the sterile ground are then treated every 15 days during three months by means of three doses of pesticide. Witnesses pots undergo the same treatment but with some sterile water.

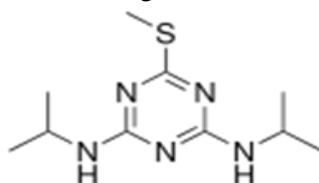
**Preparation of pesticide solutions:** The used molecules of pesticides we were supplied in liquid form by the Plant Protection Station of Ben M'Hidi. They are compounds massively used for the truck and cereal farming's at the level of the region of Annaba. It is about a weed-killer the prometryne and of a fungicide, the hymexazole.

**Fungicide:** The hymexazole is the active substance of a systematic fungicide of seeds resulting from Golden union agrochemical, marketed in Algeria under the name of tachigazol. It belongs to the chemical class of triazines, of crude chemical formula: C<sub>4</sub>H<sub>5</sub>NO<sub>2</sub> of formula 5-methyl-3 (2H)-isoxazolone and whose chemical structure is shown in the following figure (see Fig.1).



**Figure.1. Chemical structure of hymexazole**

**Herbicide/ Weed-killer:** The herbicide is a pre-emergence molecule of certain grasses and broadleaf weeds, marketed under the name Gesagard. Its active substance is prometryne belonging to the family of triazines, manufactured by Syngenta, gross chemical formula and formula C<sub>10</sub>H<sub>19</sub>SN<sub>5</sub> bis (isopropylamino) -2.4 methylthio-1, 3, 5-triazine 6. Its chemical structure is as follows (Fig.2).



**Figure.2. Chemical structure of prometryne**

**Spraying of Pesticides:** Pesticides are solubilized in sterile distilled water and 80% methanol (20v/80v). The concentrations are used on the dose of fields (DC1: 45µl/cm<sup>2</sup>) (D1); the dose of fields multiplied by 10 (DC2X10: 450µl/cm<sup>2</sup>) (D2); and the dose of fields divided by 5 (DC3: 9µl/cm<sup>2</sup>) (D3). The prepared suspensions are mixed well, and then sprayed (pulverized) every 15 days, during three months. Witnesses (Batons) (t) are sprayed (pulverized) with some sterile water. After 90 days, soils samples are taken from each pot to a depth of 0-10cm. Soil fractions obtained from the samples are then used for analysis.

**Analysis of physicochemical soil parameters:** Analyses focused on the following parameters (Table.1): Electrical conductivity, total carbon, phosphorus and nitrogen. All parameters were analyzed in an automatic way through electronic analyzers and compared with soil scales reported by Soltner (2005), Gauchers and Soltser (1981), Durand and Dutil (1972).

**Table.1. Physicochemical parameters of grounds treated by the pesticide**

Parameters	Methods	Units
Electrical conductivity	Multi-parameter typifies WTW197	ms/m
Total carbon	Automatic analyzer	g/kg
Phosphorus	Automatic analyzer	mg/kg
Nitrogen	Automatic analyzer (Dumas method)	g/kg

**Quantitative evaluation of the total micro-flora:** The determination of the microbial biomass was determined by the dilution suspension technique. A calibrated drop of the soil suspension is diluted several times 10<sup>-1</sup> to 10<sup>-6</sup> and then plated on petri dishes containing sterile agar medium. Witnesses seeded with a drop of sterile distilled water are included in the trials. The boxes in triplicate for each case are incubated at a temperature of 30°C for 5 to 7 days. The isolated microorganisms are first purified by successive subcultures of monospore or single colony on specific culture media. The determination of microbial biomass is made by counting of colonies (summer camps) and the results are expressed in UFC (number of Units Forming)/g of ground according to Dutruc-Rosset (2003).

After purification, the bacterial isolates are characterized after gram staining as well as the oxidase test and catalase and biochemical tests of the API 20 E galleries and API 20 NE. Identification is based on the reading table provided in the kit with software (Web API database). Regarding fungal strains are determined using identification keys of Botton (1990).

**Quantitative evaluation of enzyme activities:** In order to achieve a quick estimate of the overall activity of the ground, the contents, dehydrogenase and acid phosphatase, were evaluated from soil fractions based on methods (Eivazi and Tabatabai, 1977; Domsch and Gams, 1980).

The analyzed parameters (physicochemical and enzymatic parameters) were validated by the statistical tests: Anova, Tukey and Dunnett (Dagnelie, 2009).

3. RESULTS AND DISCUSSION

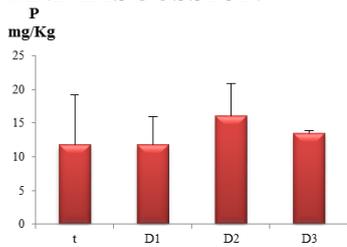


Figure.3. Rate of phosphorus in presence of three doses of the herbicide (prometryne)

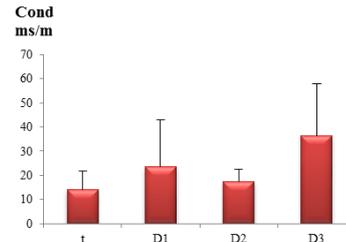


Figure.4. Electrical conductivity in presence of three doses of herbicides (prometryne)

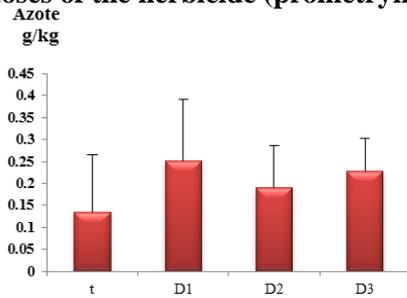


Figure.5. Nitrogen levels in presece of three doses of the herbicide (prometryn)

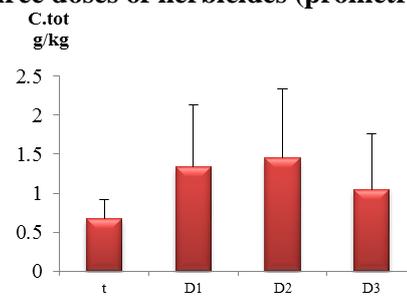


Figure.6. Total carbon levels in presence of three doses of herbicides (prometryne)

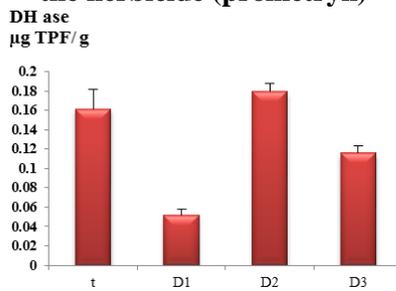


Figure.7. Content of the dehydrogenase in the presece of three doses of herbicides (prometryne)

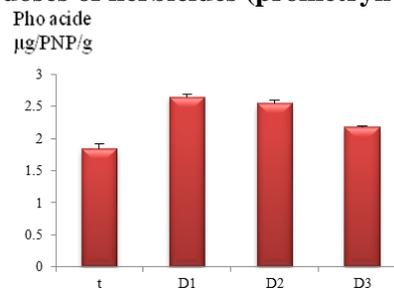


Figure.8. Content of phosphatase acid in the presence of three doses of herbicide (prometryne)

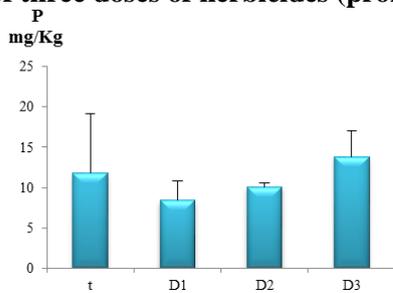


Figure.9. Rates of phosphate in the presence of the three fungicide dose (Hemyxazole)

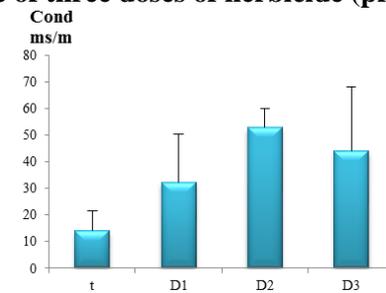


Figure.10. Electrical conductivity in the presence of three fungicide doses (Hemyxazole)

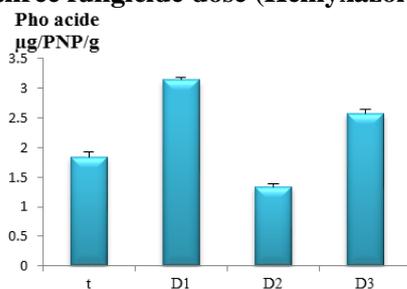


Figure.11. Phosphatase acid content in the presence of the three fungicide doses (Hemyxazole)

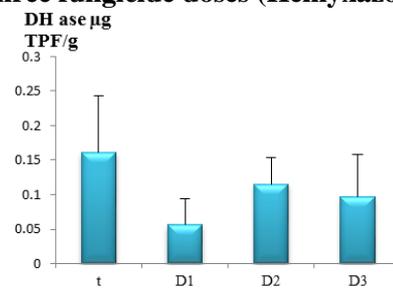
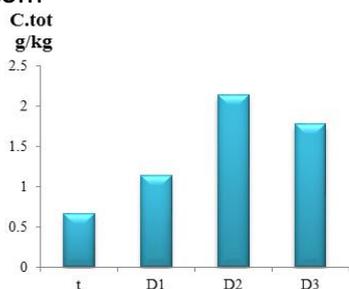
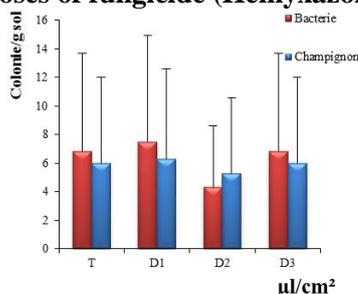


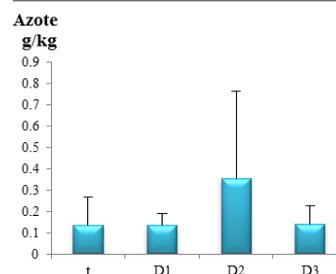
Figure.12. Dehydrogenase content with the three fungicide doses (Hemyxazole)



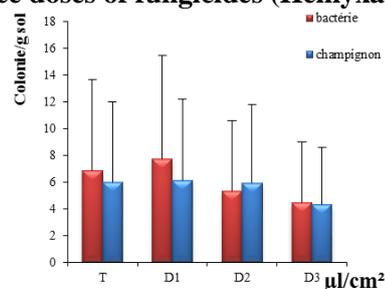
**Figure.13. Rate of carbon in the presence of three doses of fungicide (Hemyxazole)**



**Figure.15. Microbial biomass in the presence of three doses of fungicide (Hymexazole)**



**Figure.14. Rate of the nitrogen in the presence of three doses of fungicides (Hemyxazole)**



**Figure.16. Microbial biomass in the presence of three doses of the herbicide (prometryn)**

The analyzed physico-chemical parameters show a big variability in the results (see Fig.3. to Fig.6. and see Fig.9. to Fig.12). The values vary according to the nature of the pesticide and the used doses. Most of the tested parameters are higher in the treated soils with the three used doses of pesticide. The total carbon and the nitrogen are of a big utility for microorganisms and establish a source of energy, which is transformed into new products of the metabolism, and, also, play a role in the solubility of pesticides. The adsorption of pesticides by the carbon of the organic matter increases their solubility and their stability in the profiles of ground (Chiou, 1986). Although there is a difference in the values of all the physical and chemical parameters between the ground witness and the treated grounds, assessing the degree of soil fertility and quality remains difficult and often imprecise given, uncertainties and lack of data on standards of soil quality. The microbial biomass is more and more considered as an ecological marker (Franco, 2004) and a useful indicator of the improvement or the degradation of grounds (Gil-Sotres, 2005). So, the enumeration realized from the samples of ground highlighted a micro-flora varied as well by the point of view quantitative as qualitative with a total of 1152 bacterial isolates and 1002 fungal isolates. However, the number of microorganisms including the bacteria of the ground witness (baton) is by far the most dominant with  $112 \times 10^5$  colonies/g of soil. If we refer to the analyzed chemical parameters, we notice that there is a perfect correlation between the abundance of the total micro-flora and the contents in total carbon. Indeed, it was indicated that the abundance of the microbial biomass is bound (connected) to high rates of organic matter and carbon necessary for the life of the microorganisms of the ground in particular during the first stages of the decomposition (Bordjiba, 2001). Bacterial and fungal populations appear to be inhibited by the pesticide and, with all doses and in particular the D2 and D3 doses (Fig.15 and Fig.16). The toxic action of pesticides on microbial biomass resulting in a slowdown in growth and a selection of resistant strains was reported by Barriuso (1994).

The quantification of the bio-indicator parameters of the enzymatic activity was realized with the aim of characterizing the quality of ground in a context of organic pollution by molecules of fungicide and herbicide. This enzymatic dosage allowed us to notice that the contents of the dehydrogenase are low for the three doses tested some fungicide as compared to witnesses (Fig.7 and Fig.8). Contents lower than those of the witnesses were also noted for both doses D3 and D1 of herbicide. As for the contents of the phosphatase acid, they seem superior in the treated ground by the pesticide (Fig.13 and Fig.14). Indeed, the enzymatic activities in grounds are sensitive to the environmental modifications (Mbonigaba, 2009) and are therefore considered indicators of natural and anthropogenic disturbances (Schloter, 2003).

The results demonstrate the heterogeneity of the responses of enzyme activities obtained only from dehydrogenase and phosphatase acid in particular according to the type (chap) of the physicochemical characteristics of the ground. The biological activity of the soil cannot be assessed through the study of one or two types of enzyme (Fauvel and Rocquerol, 1970). Other authors faced difficulties in connecting the microbial biomass and the enzymatic activities, even in favorable experimental conditions (Ladd and Paul, 1970). According to Cortez (1972), even if we try to quantify several, the interpretation (performance) is not facilitated because their variations are sometimes contradictory.

**4. CONCLUSION**

The multiple interactions between the nature of the product, the physical and chemical ground characteristics and environmental factors make the estimation of the chemical and biological quality of the soil subjected to the effect of pollution by two and triazines hymexazole prometryne difficult because the values are often contradictory. Likewise, the quantification of the enzymatic activity of a soil with respect to pesticides seems difficult to interpret. However, some enzymes, such as dehydrogenase appear to be sensitive to the presence of the fungicide. Among the parameters studied in the present research, the total number of microflora and enzymatic activity of dehydrogenase turn out reliable criteria in the evaluation of the biological quality of the ground when it undergoes the toxic action of the pesticide or the fungicide and its microbial activity is inhibited. An interesting continuation in this work would, thus, be to measure other enzymes perceptible to pesticides, and to study the density and the functional diversity of the bacterial and fungal populations.

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