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Effects of Three Pesticides (Atrazine, Deltamethrin and Acetamiprid + Cypermethrin), Two Plant Species (*Vetiveria nigritana* Benth. and *Oxythenantera abyssinica* Linn.) and Their Interactions on the Biological Activity of Lixisols and Vertisols of Cotton Agrosystems of Burkina Faso (West Africa)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IS and ANS designed the study and wrote the protocol. Author IS conducted the field study, data collection and wrote the draft of manuscript. Authors IS and JO managed the analyses of the study, performed the statistical analysis of the data. Author HBN framed, oriented and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The application of pesticides can have adverse effects on the biology of agricultural soils. This study had 3 objectives: (i) evaluate the impact of the three pesticides on soils, (ii) evaluate the impact of the two plant species on soils and (iii) also evaluate the interaction of pesticides and plant species on soils.

Methodology: In order to achieve this aim, an experimental test was conducted in a vegetal vase with 7 treatments. The experimental device used is a split-plot device with four repetitions for each soil type. The main treatments are plant species and secondary treatments consist of pesticides. The main factor had three modalities namely: absence of plant, *Oxytenanthera abyssinica* and *Vetiveria nigritana*. As for the secondary factor, it comprises four modalities which are: control, Atrazine, Deltamethrin and Acetamiprid + Cypermethrin. Deltamethrin and acetamiprid + cypermethrin are not a mixture. They constitute 2 different pesticides. It is rather acetamiprid + cypermethrin which is a mixture of acetamiprid and cypermethrin.

Results: Physicochemical characteristics are generally lower in lixisols compared to vertisols, except P and K. Microbial biomass is lower in the presence of plant species regardless of soil type. Microbial biomass has declined regardless of the type of pesticide. This decrease was significant ($P = 0.045$) in lixisols. Pesticide interaction and plant species significantly increased ($P = 0.01$) microbial biomass in the presence of *Vetiveria nigritana* and respiratory activeness for both species. The highest cumulative release values are found in soils without plants. Analysis of variance revealed highly significant differences ($P < 0.001$) in lixisols. The application of Atrazine and Cypermethrin + Acetamiprid increased respiratory activity regardless of soil type. Cumulative values are higher in lixisols than in vertisols.

Conclusion: The pesticides used induce a decrease in the microbial biomass of the soil and promote a stimulation of the respiratory activity. The plant species tested induce a decrease in microbial biomass as well as the mineralization that expresses the release of CO_2 in lixisols and vertisols. The interaction between plant species and pesticides increases microbial biomass and respiratory activity in both soil types.

Keywords: Respiratory activity; microbial biomass; lixisols; vertisols; pesticides; plant species.

1. INTRODUCTION

Pesticides contribute significantly to improved yields; however, they raise concerns about possible negative effects on soil fertility [1]. After their application, a large quantity of pesticides is found in the soil and this can be harmful to living organisms in the soil [1,2]. However, the biological activity of a soil is, in the same way as its physical and chemical properties, determining for its productivity [1].

Numerous studies have thus highlighted the impact of pesticides on soil microorganisms, particularly in Burkina Faso [3,4,5]. These analyzes emphasize sometimes an inhibition and sometimes a stimulation of the microbial activity. Thus, it is difficult to predict the impact of a pesticide on soil biology.

More over, the risk that arises is the pollution of water and soil. For example, overdose and repeated use of some pesticides that are more or less persistent and even sometimes prohibited can lead to an accumulation of residues in the soil. [2,6,7] have shown that the waters and soils of Boucle du Mouhoun (Burkina Faso) were contaminated by pesticides used in cotton growing. Health problems result in various diseases caused by contact with pesticides, the consumption of contaminated food products (water, fish, meat, vegetables, fruits, etc.). [7,8,9, 10,11] after analysis of various food samples (vegetables and cereals), obtained high concentrations of Cypermethrin and Deltamethrin.

Although many studies on the impact of pesticides on soil microbial activity exist, very few studies have examined the impact of plant species and their interactions on soil microbial activity. This study had 3 objectives: (i) evaluate the impact of the three pesticides on soils, (ii) evaluate the impact of the two plant species on soils and (iii) also evaluate the interaction of pesticides and plant species on soils.

2. MATERIALS AND METHODS

2.1 Study and Soil Sampling Sites

Soils were collected at Boni sites (11 ° 35'N - 3 ° 26W) and Dossi (11 ° 26N - 3 ° 24W). These sites are located in areas of intense cotton growing in Burkina Faso. The vegetation in the sites is a wooded savannah. The experiment was conducted in the forest park of the Nazi Boni University located in Bobo-Dioulasso (4 ° 10'-4 ° 30'West, 11 ° -12 North) located in the Sudanian zone of Burkina Faso. Fig. 1 show the location of the study sites.

2.2 Sampling and Treatments of Soil Samples

Soil samples were taken from fallows (never cultivated) over the 0-20 cm horizon. To take into account the heterogeneity of the medium, a composite sample of each soil type is constituted by mixing the same quantity of soil taken from the different media for chemical analyzes (Table 1).

2.3 Plant Material

Vetiveria nigritana (Benth.) (vetiver) and *Oxythenantera abyssinica* Linn. (bamboo) are perennial grasses. There are 12 species of *Vetiveria nigritana* growing in the tropics, but the species *Vetiveria nigritana,* of southern Africa is the most cultivated. There are over 1200 species of *Oxytenanthera abyssinica*. Whole plants were used. They come from a nursery of 4 weeks.

2.4 Pesticides Used

The trade names of the pesticides used were: Atravic, Deltacal and Conquest. The active ingredients are respectively Atrazine, Deltamethrin and the combination Acetamiprid + Cypermethrin (Table 2). Acetamiprid and Cypermethrin are premixed before being purchased. Acetamiprid + Cypermethrin is composed of 32g/ l of acetamiprid and 144g/ l of Cypermethrin. These pesticides are heavily used in cotton, grain and market gardening in Burkina Faso. Moreover, they are available in the formal and informal markets.

2.5 Experimental Design

The experimental device used is a split-plot device with four repetitions for each soil type. The primary treatments are plant species, and secondary treatments consist of pesticides. The main factor had three modalities namely: absence of plant, *Oxytenanthera abyssinica* and *Vetiveria nigritana*. As for the secondary factor, it comprises four modalities which are: control, Atrazine, Deltamethrin and Acetamiprid + Cypermethrin.

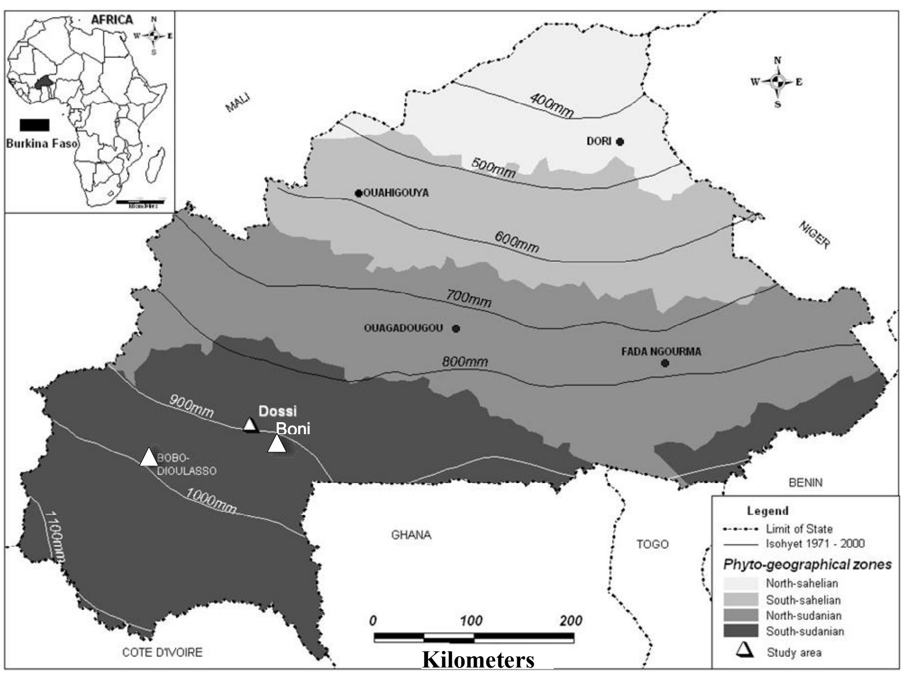


Fig. 1. Location of the study sites

Table 1. Chemical properties of soils

Soil type	pHeau	pHkcl	C (%)	M.O (%)	N (%)	C/N	P-total (mg/kg)	P-ass (mg/kg)	K-total (mg/kg)	CEC (méq/100 g)
Lixisols	6.17	4.98	0.88	1.92	0.062	14.4	140.43	1.81	1972	5.02
Vertisols	6.91	5.45	1.01	1.34	0.073	13.9	114.86	0.27	685	24.56

Table 2. Characteristics of the pesticides used

Trade name of pesticides	Active ingredient	Chemical formula	Chemical family	Use
Atravic 500	Atrazine (500 g/l)	$C_{18}H_{14}ClN_5$	Triazine	Herbicide
Deltacal 12,5 EC	Deltamethrin (12,5 g/l)	$C_{22}H_{19}Br_2NO_3$	Pyrethroids	Insecticide
Conquest C 176 EC	Acetamiprid (32g/l) et Cypermethrin (144g/l)	$C_{10}H_{11}ClN_4$ $C_{22}H_{19}NO_3Cl_2$	Neonicotinoids Pyrethroids	Insecticide Insecticide

A sample is taken before the application of pesticides. One (01) plant of each species is transplanted into a plastic bucket containing 12 kg of soil. Pesticides are applied 20 days after transplanting. The dose considered is that of twice the dose used in a peasant environment. This quantity is estimated at 28 ml per pot for Atrazine, 8 ml per pot for Deltamethrin and 2 ml per pot for Acetamiprid + Cypermethrin. These different amounts are diluted respectively in 0.48 l; 3.2 l and 0.08 l of water. The mixture is homogenized and applied directly into the bucket.

2.6 Soil Analysis

➢ **Chemical analyzes**

- The chemical analyzes of the soil samples were carried out at the Sol-Eau-Plante laboratory of the Natural Resources Management and Production Systems Department (GRN/ SP) of the Institute of Environment and Agricultural Research (INERA) ;
- The methodology of the extraction of the elements studied is as follows:
- The determination of organic carbon is carried out by the method of [12];
- Total nitrogen, phosphorus and potassium were measured by the Macro Kjedahl method using a self-analyzer using calorimetry ;
- pHe and pHkcl by the glass electrode pH meter method ;
- Cation Exchange Capacity (CEC) by the procedure of extraction of the cations with Thioure Silver (Agtu) at 0.01 M ;
- Phosphorus assimilated by the method [13].

➢ **Biological analyzes**

The biological analyzes were carried out at the laboratory of the Institute of the Environment and Agricultural Research (INERA) of Kamboincé (Burkina Faso). Institute of Environmental and Agricultural Research (INERA) is located in Ouagadougou, Burkina Faso. Soil samples were taken 15 days after pesticide application. Microbial soil biomass was measured using the fumigation-incubation method proposed by [14]. Organic carbon was measured according to the method proposed by [15].

The microbial biomass is obtained from the following formula:

BM = (F0-7- F8-14) / 0.14

F0-7 = amount of CO_2 released between 0 and 7 days of incubation.

F8-14 = amount of CO_2 released between 8 and 14 days of incubation.

0.14 is the coefficient of proportionality representing the mineralizable fraction of carbon into CO_2.

As for the determination of the quantity (Q) of CO_2 released, it is given by the formula:

Q = [VHcl (white) - VHcl (sample)] × 2.2

VHCl (white) = volume of hydrochloric acid used for the determination of the control.

VHcl (sample) = volume of hydrochloric acid used for the determination of the sample.

The coefficient 2.2 means that 2.2 mg of CO_2 corresponds to 1 ml of 0.1 N hydrochloric acid [16].

2.7 Statistical Analysis

The data were subjected to an ANOVA analysis of variance. Data processing was performed using Genstat software version 4.10.3. The separation of the means was performed by the Student-Newman-Keuls test at the 5% threshold.

3. RESULTS

3.1 Effects of Plant Species on Soil Microbial Biomass

The impact of plant species on soil microbial biomass is shown in Tables 3 and 4 respectively in the presence of *Oxytenanthera abyssinica* and *Vetiveria nigritana*.

Whatever the type of soil, the presence of species has decreased the amount of microbial biomass in the soil. However, this decline was significant only in vertisols.

Table 3. Microbial soil biomass (mg.100 g-1 soil) in the presence of *Oxytenanthera abyssinica*

Treatments	Soil without pesticides	
	Lixisols	Vertisols
Without plant	40.06a	100.16ab
Oxytenanthera abyssinica	36.49a	89.43a
Probability (5%)	0.91	0.03
Significant	NS	S

The averages in the same column followed by the same letter are not statistically different.
S: Significant; NS: Not significant

Table 4. Biomasse Microbial soil biomass (mg.100 g-1 soil) in the presence of *Vetiveria nigritana*

Treatments	Soil without pesticides	
	Lixisols	Vertisols
Without plant	40.06a	100.16ab
Vetiveria nigritana	39.92a	75.84a
Probability (5%)	0.91	0.03
Significant	NS	S

The averages in the same column followed by the same letter are not statistically different.
S: Significant; NS: Not significant

3.2 Effects of Pesticide Type on Soil Microbial Biomass

The impact of different pesticides on soil microbial biomass is given in Table 5. For both soil types and whatever the pesticide, microbial biomass has decreased. For lixisols, variance analysis revealed significant differences between treatments (P = 0.045). The observed microbial biomass is significantly greater with Atrazine and Deltamethrin.

Table 5. Microbial soil biomass (mg.100 g-1 soil) in the presence of pesticides

Treatment	Lixisols	Vertisols
Control	40.1b	100.2a
Atrazine	37.9ab	98.9a
Deltamethrin	17.9a	95.9a
Acetamiprid + Cypermethrin	39.9b	98.7a
Probability (5%)	0.045	0.75
Significant	S	NS

The averages in the same column followed by the same letter are not statistically different.
S: Significant; NS: Not significant

3.3 Effects of the Interaction between Pesticides and Plant Species on Soil Microbial Biomass

The results of the interaction between different pesticides and plant species on soil microbial biomass are presented in Tables 6a and 6b.

In lixisols, after application of pesticides, there is a non-significant reduction in microbial biomass regardless of the plant species. However, the application of Deltamethrin in the presence of *Oxytenanthera abyssinica* resulted in a slight but not significant increase. In vertisols, there is generally a slight increase in microbial biomass in the presence of both plant species. This increase was only significant (P = 0.01) in the presence of *Vetiveria nigritana*.

Table 6. Microbial soil biomass (mg.100g-1 soil) in the presence of pesticides and plant species

a) In lixisols

Pesticide	*Oxytenanthera abyssinica*	*Vetiveria nigritana*
Control	36.5a	39.92a
Atrazine	28.6a	36.5a
Deltamethrin	42.2a	30.8a
Acetamiprid + Cypermethrin	36.5a	30.1a
Probability (5%)	0.82	0.75
Significant	NS	NS

The averages in the same column followed by the same letter are not statistically different.
S: Significant; NS: Not significant

b) In vertisols

Pesticide	*Oxytenanthera abyssinica*	*Vetiveria nigritana*
Control	89.4a	75.8a
Atrazine	87.3a	111.6b
Deltamethrin	92.3a	81.6a
Acetamiprid + Cyperméthrin	91.6a	92.3ab
Probability (5%)	0.68	0.01
Significant	NS	S

The averages in the same column followed by the same letter are not statistically different.
S: Significant; NS: Not significant

3.4 Effects of Plant Species on Soil Respiration

Figs. 2a and 2b show the cumulative evolution of C-CO_2 in the presence of plant species respectively in lixisols and vertisols. Daily CO_2 releases have daily mineralization rates for 21 days of incubation. The highest values are found at the level of soils without plant resulting in low biological activity in the media with plants. The analysis of the variance of CO_2 accumulation in lixisols revealed highly significant differences (P <0.001). The cumulative release of CO_2 is more intense in lixisols than in vertisols regardless of the plant species.

3.5 Effects of Pesticide Type on Soil Respiration

The cumulative amounts of CO_2 in soils polluted by pesticides are shown in Figs. 3a and 3b. The highest values are obtained in the presence of Atrazine and the combination Cypermethrin + Acetamiprid in both soil types compared to unpolluted soils. Significant differences P <0.001

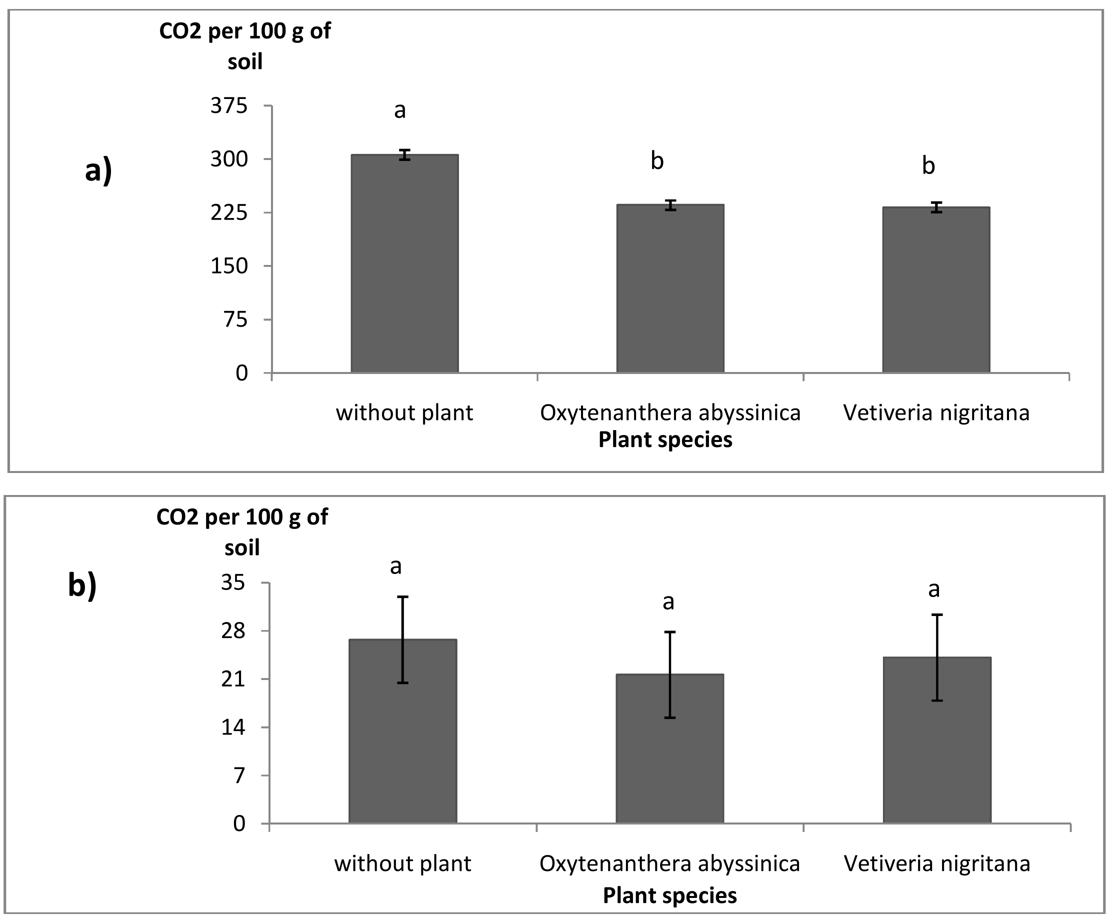


Fig. 2. Cumulative CO_2 production (mg.100 g-1 dry soil) in the presence of plant species; a) In lixisols; b) In vertisols

and P <0.003 between treatments, respectively in lixisols and vertisols, are also noted. The cumulative values reached 291.5 and 31.79 mg of C-CO_2 / 100 g of soil at the end of incubation respectively in the lixisols and the vertisols (Figs. 3a and 3b). Cumulative values are higher in lixisols than in vertisols.

3.6 Effects of Interaction between Plant Species and Pollutant Type on Soil Respiration

Figs. 4a and 4b show the effect of the interaction between plant species and the pollutant respectively in lixisols and in vertisols.

In both lixisols and vertisols, the intensity of cumulative releases increases with the application of pesticides relative to the soil without pesticides, regardless of the plant species and the type of pollutant. The highest values are observed in the presence of *Oxytenanthera abyssinica* in lixisols and in the presence of *Vetiveria nigritana* in vertisols. Cumulative values are generally higher in lixisols than in vertisols (Figs. 4a and 4b).

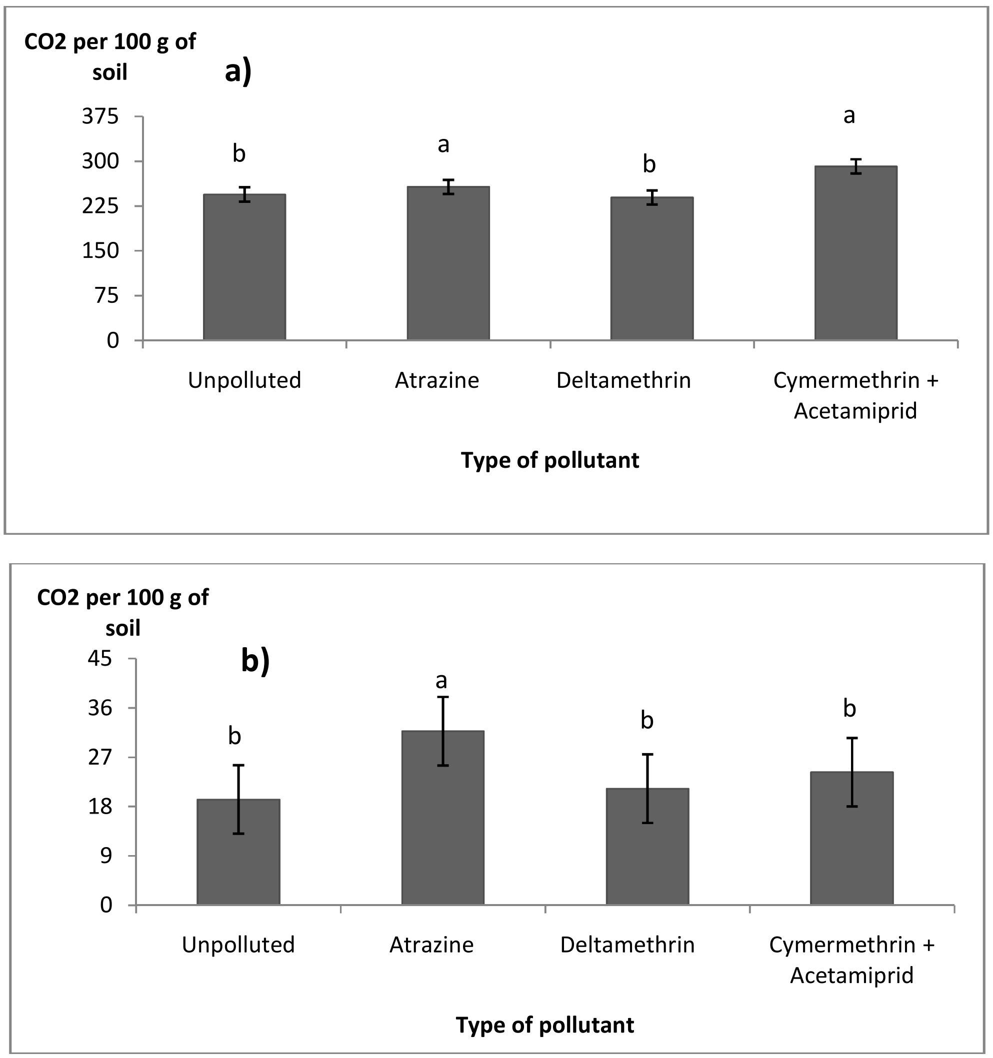
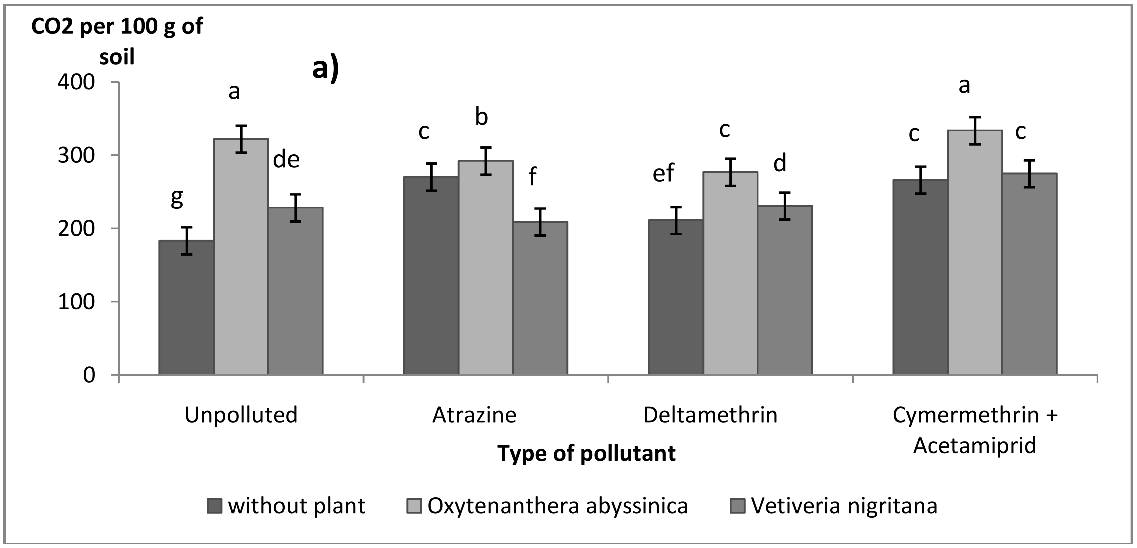


Fig. 3. Cumulative CO_2 production (mg.100 g-1 dry soil) in the presence of pesticides; a) In lixisols; b) In vertisols



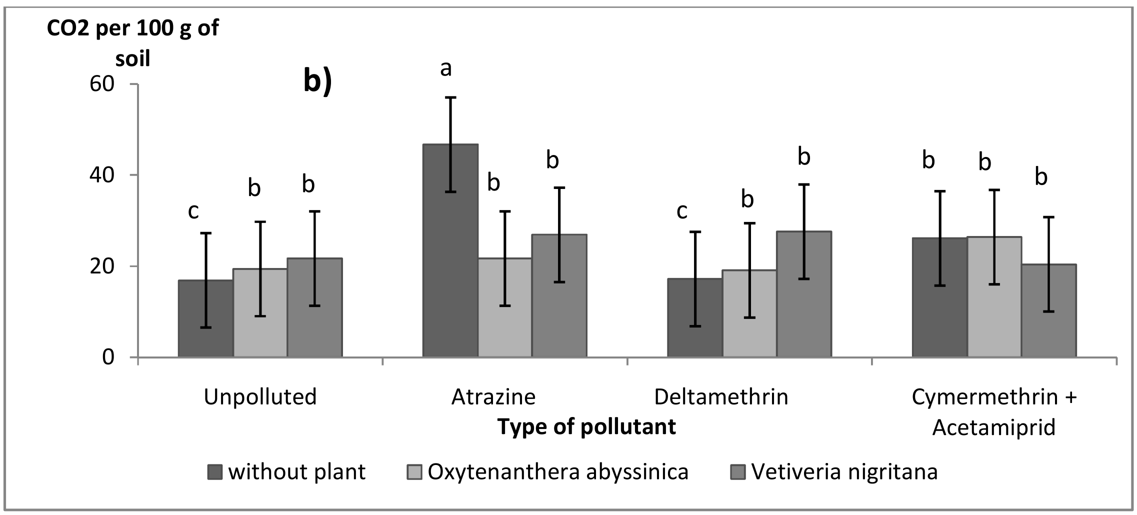


Fig. 4. Cumulative CO_2 production (mg.100g-1 dry soil) in the presence of pesticide and plant species; a) In lixisols; b) In vertisols

4. DISCUSSION

Total microbial biomass in the presence of plant species indicates a decrease in the microbial population regardless of plant species and soil type. In the presence of *Oxytenanthera abyssinica*, microbial biomass decreased by 9.8% and 12%, respectively, in lixisols and vertisols compared to the control without plant. In the presence of *Vetiveria nigritana*, they are 0.3% and 32% respectively in lixisols and vertisols compared to the control. This decrease is significant in vertisols for both plants. The presence of *Oxytenanthera abyssinica* and *Vetiveria nigritana* decreases microbial biomass. Plant species showed some toxicity to microorganisms which allowed reducing the quantities of soil microbial biomass.

Compared to the absolute control (no plant or pesticide), the presence of pesticides induces a decrease regardless of the type of soil and pesticide. The microbial biomasses of lixisols decreased by 5.8%, 124% and 0.5% respectively in the presence of Atrazine, Deltamethrin and Acetamiprid + Cypermethrin compared with the control. In vertisols, decreases were 13%, 4.5% and 1.5% respectively in the presence of Atrazine, Deltamthrin and Acetamiprid + Cypermethrin. This decrease was significant only in the presence of Deltamethrin in lixisols. The pesticides used in this study decrease total microbial biomass in lixisols and vertisols. This decrease effect, related to the presence of these three pesticides consistent with [1], revealed that pesticides such as dinoseb and glufosinate induce a reduction of soil microbial biomass by 20 to 50% 3 weeks after their release. This result is also consistent with [17], which highlights that ammonium glufosinate inhibits soil bacteria. Pesticides can be toxic to soil microorganisms. In this case, the microbial activity is slowed down and there is a selection of microorganisms that are resistant to pesticides or that can be used as a source of carbon. These results in microbial readjustments that may be associated with changes in the physiological characteristics of the soil microflora and may also be associated with a decrease in the diversity of microorganisms [2].

An impact study of the prolonged use of pesticides in a cotton farming system, conducted by [18], revealed that endosulfan, profenofos + alphametrine and methamidophos decreased the bacterial population. The same observation was made by [1] on soil cultivated with potato treated with dinoseb and glufosinate. Concerning the total mushroom population, dimethoate resulted in a decrease in the mushroom population while endosulfan, monocrotophos, profenofos and methamidophos stimulated it. [19] investigated the effects of profenofos on soil microorganisms of an experimental cotton field and peasant fields. The results showed that profenofos has a minimal impact on populations of bacteria and fungi in cotton fields.

The interaction of pesticides with plant species induced a significant increase in microbial biomass with Atrazine and the combination Acetamiprid + Cypermethrin in Vertisols in the presence of *Vetiveria nigritana*. This increase is 32% for Atrazine and 21.76% for Acetamiprid + Cypermethrin. No significant difference in lixisols regardless of the type of pesticide. However, there are slight increases in the presence of *Vetiveria nigritana* for the three pesticides. The interaction of pesticides and plant species to promote the activation of the total biomass of the soil. This would mean that the combined effect of plant species and pesticides had a positive effect on soil microorganisms which could increase the soil's respiratory activity.

The evolution of accumulated CO_2 from the soil without the plant is the largest, indicating a decrease in the soil's respiratory activity in the presence of plant species. In the presence of *Oxytenanthera abyssinica*, the respiratory activity decreased by 30% and 23.46% respectively in the lixisols and vertisols compared to the control without plant. In the presence of *Vetiveria nigritana*, they are 31.6% and 10.72% respectively in lixisols and vertisols compared to the control. This would mean that the presence of plant species had an inhibitory effect on the respiratory activity whatever the type of soil.

In the presence of pesticides, the cumulative amount of CO_2 indicates an intensification of the soil's respiratory activity in both soil types. After 21 days of incubation, the respiratory intensities of pesticide-treated soils are higher than those of the control treatment. Cumulative amounts of CO_2 increased by 5.2% and 16.2% relative to the control in lixisol soils. In the violets, they are 39.22%, 9.3% and 20.46%. This increase was significant in the presence of Deltacal in lixisols. Similar results observed by [20] revealed that Zoomer's herbicide based on glyphosate and oxyflorfen and Cottonex PG based on fluometuron, prometryne and glyphosate stimulated soil respiration activity at 15 days after application. The work [3] showed that endosulfan stimulated the respiratory activity of soils at a concentration of 6 ppm.

The more intense CO_2 release in lixisols compared to vertisols seems to be related to their organic matter content in the soil. This result is reminiscent of those [3,21,22] who observed higher respiratory activity in soils with higher organic matter content. Similar results were observed [23,24] also observed a higher respiratory activity on soil samples richer in organic matter. [25] in a study of the comparative and interactive effects of pesticides and physical factors (freezing, heating, drying) on the mineralization of carbon substrates in the soil, noted that mineralization of substrates was primarily dependent on the concentration of substrate and not the physical or chemical nature of the stress. [24] found a linear correlation ($r = 0.71$) between soil respiration and soil organic matter.

However, contrary results were observed by [26, 19]. The work [26] has shown that 18% reduces the respiratory activity of a soil that has received atrazine and carbofuran from baseline. According to [24], pesticides can be toxic to soil microorganisms. In this case, microbial activity is slowed down and there is a selection of the microorganisms best adapted to pesticides or able to use it as a source of carbon. This results in microbial readjustments that may be associated with changes in the physiological characteristics of the soil microflora and may lead to a decrease in the diversity of microorganisms.

The pesticides interaction and plant species generally induced an increase in the respiratory activity relative to the soil without pesticides and plants, regardless of the plant species and the type of pollutant. The presence of plant species did not reduce the amount of cumulative CO_2 in the soil as in the case of soils without pesticides. This would mean that pesticides had more effect on the soil's respiratory activity compared to the plant species tested.

5. CONCLUSION

Pesticides are mostly used in agriculture but can also be used in other professional sectors or for domestic or therapeutic use. The application of synthetic chemical pesticides in agriculture has adverse consequences on soil biology through its overall microbial activity. Thus, it is more than necessary to understand the effect of these molecules on the microbial activity of the soil under the conditions specific to different soils. The present study has shown that the presence of the tested plant species induces a decrease of the microbial biomass as well as the mineralisation which expresses the release of CO_2 in the lixisols and the vertisols. In the same way, this study revealed that the pesticides induce a decrease of the microbial biomass of the grounds but to favour a stimulation of the respiratory activity. The interaction between plant species and pesticides increases microbial biomass and respiratory activity in both soil types.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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