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Study of Changes in physiological and Enzymatic Metabolism of a Bryophyte "*Rhytidium Rugosum*" Under the Effect of a Herbicide "TREFLAN"

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***Abstract* – The biodiversity was preserved a long time, but the principal components of our environmentwere particularly modified by the addition of molecules resulting from the agricultural and industrial activities.These pollutants disturb the balance and the radiant energy flux of the ecosystem. They can poison the organizations and cause fast harmful changes in the environment. These changes can stress certain species and make them more vulnerable.**

In our work, we determined the disturbance caused by the addition of a xenobiotic and to try to bring one the behavior of mosses with respect to this herbicide. Our choice was made on a vegetal model in fact the moss (*Rhytidium rugosum*) since it is regarded biological indicator. We highlighted in addition to an increase of the chlorophyll (Chla, b, a+b), the disturbance of the macromolecules (glucids, proteins, proline) as well as a disturbance in the enzyme of detoxification (glutathione-SH: GSH).It seems that the specie *Rhytidium rugosum* is very sensitive to xenobiotic TREFLAN (pollutant).

***Keywords* – *Rhytidium Rugosum, TREFLAN*, Xenobiotic, Mosses, Chlorophyll, Proline, Proteins and Glucids Total, GSH**.

I. Introduction

The atmosphere is transparent, colorless and odorless, has long been considered "clean". Past few decades, we see that it contains trace elements which are more or less dangerous to humans.

Today, on the ground, many ecosystems are disturbed because of human activities. Many species of plants and animals are threatened with extinction, these concerns environmental pollution continue to grow in importance. This pollution has adverse effects on the health of humans, ecosystems and climates. These disturbances can touch the heart of the city like the rest of the world and have led to a global awareness of the need to reduce the emission of pollutants (Vasconcelos and Tavares, 1997). Contamination of the environment by pesticides (fertilizers, pesticides, insecticides) is a new component of air pollution, which is still misinformed by the other components of the primary pollution (NOx, SO2, dust ... etc.). This is due, in part, the recent acquisition of this form of pollution. Among these pollutants it will consider in this study mainly on pesticides. While the majority of these products are an undesirable result of human activity, they represent the largest group of organic compounds are intentionally introduced by humans. However, during the application and depending on weather conditions and patterns of applications, the majority of pesticides are not deposited on the treated areas, pesticides can therefore be introduced directly into the atmosphere during application but also after deposit volatilizing or by spreading it by erosion (Viala and Botta,2005).

The atmosphere plays an important role in the dissemination of pesticides at the local, regional and global. Pesticides entering the environment by various processes and can then be transported in the different phases of the latter in areas far from their site of application and contaminate non-target ecosystems. Many pesticides have been banned in recent years, or are being re-evaluated to maintain their authorization on the market due to their persistence in the environment. They have been replaced by new molecules, less stable, but few data are available on the fate of these compounds in our planet (Viala and Botta, 2005).

This memory is designed to gain a better understanding of the fate and effects of pesticides in the environment. It proposes and evaluates new approaches, in order to reduce the use of these substances and / or risks.To learn more about the ways of dispersion, transformation and accumulation of pesticides in the environment. Their transfer and retention in different compartments (water, air, soil) where the molecules are transformed and degraded and, assessment of risks associated with the use of pesticides and take steps to minimize their unintended effects at different levels of biological organization (cells, organisms, populations, communities) and their effects on the interactions between species and impacts on the ecological balance (Ramade,1995).

In our work, we try to highlight the effect of a herbicide that is "TREFLAN" on the biochemical and physiological parameters of a biological model no target considered excellent bio-indicator of pollution. Our choice fell on a bryophyte: *Rhytidium rugosum*.

II. Chemical Material

Our equipment is the chemical "TREFLAN" herbicide into the soil before germinating plants, it contains an active ingredient: Trifluralin ($C_{13}H_{16}F_3N_3O_4$, with molecular weight=333,279±0; 0133 g/mol; density=1, 36 g/cm^3 at 22°C). TREFLAN can control a wide range of herbs, can be selectively used on a wide variety cultivated harvest including garlic, onion, vegetables and fruit trees in all weather conditions at 2.5L/ ha. This product is a yellow granular flow fluid exudes a faint aromatic odor (SAFETY DATA SHEET, 2009).

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Table 1: *TREFLAN* is on the following plants *(SAFETY DATA SHEET, 2009)*

DICOTYLEDONS		GRASSES	
Acalyphaindica	*Papaverrhoeas*	*Agrostis spp.*	*Eleusineindica*
Amaranthus spp.	*Portulacaoleracea*	*Alopercurusagrestis*	*Ischaemumafrum*
Atriplexpatula	*Richardiascabra*	*Apera spica-venti*	*Leptochloa spp.*
Chenopodium spp.	*Salsola kali*	*Avenaspp.*	*Loliummultiflorum*
Fumariaofficinalis	*Stellaria media*	*Bromustectorum*	*Panicumcapillare*
Galiumaparine	*Tribulusterrestris*	*Cenchrus spp.*	*Poaannua*
Kachiascoparia	*Urticaurens*	*Digitariaspp.*	*Setaria spp.*
Lamiumspp.	*Veronica spp.*	*Dinebraretroflexa*	

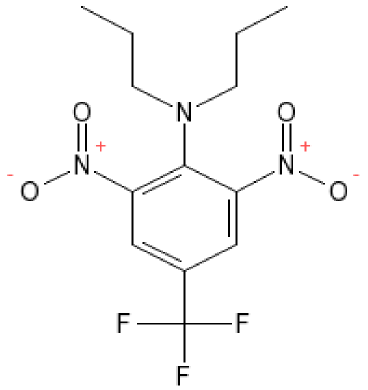

Fig.1. Trifluralin chemical formula

Table 2: Systematic position of *Rhytidiumrugosum*

Systematic Position	
Kingdom	Plantae
Division	Bryophyta
Class	Bryopsida.
Subclass	Bryidae
Superorder	Hypnanae
Order	Hypnales
Family	Rhytidiaceae
Genre	Rhytidium.
Species	***Rhytidiumrugosum***

III. METHODS

- *Determinationof physiological parameters*

The method used for extraction of chlorophyll is the traditional method established by Holden, (1975) which is a maceration of the plant in acetone.For the determination of proline, the technique used is that of Monneveux and Nemmar, (1986).

The proteins are assayed by the Bradfordmethod (1976) using BSA as standard,Soluble sugars were determined by the method of Schields and Burnett (1960) using anthrone in sulfuric acid..

- *Determination of biomarkers*

The glutathione was assayed by the method of Weckberker & Cory (1988), based on measuring the absorbance of the 2-nitro-5 mercapturic resulting from the reduction of the acid 5-5 'thiol-bis-nitrobenzoic acid (DTNB) by the thiol groups (-SH glutathione).

- *Method of growing Foams*

TREFLAN herbicidewas testedbeakers250 ml of mediumimbibitionofFoams withconcentrations : 0.01(C1), 0.02(C2), 0.03(C3) ppm. Approximately, 5 g of thallus of the specieshas been dipped insolutionspreparedfor three days(Khaldi,2009).

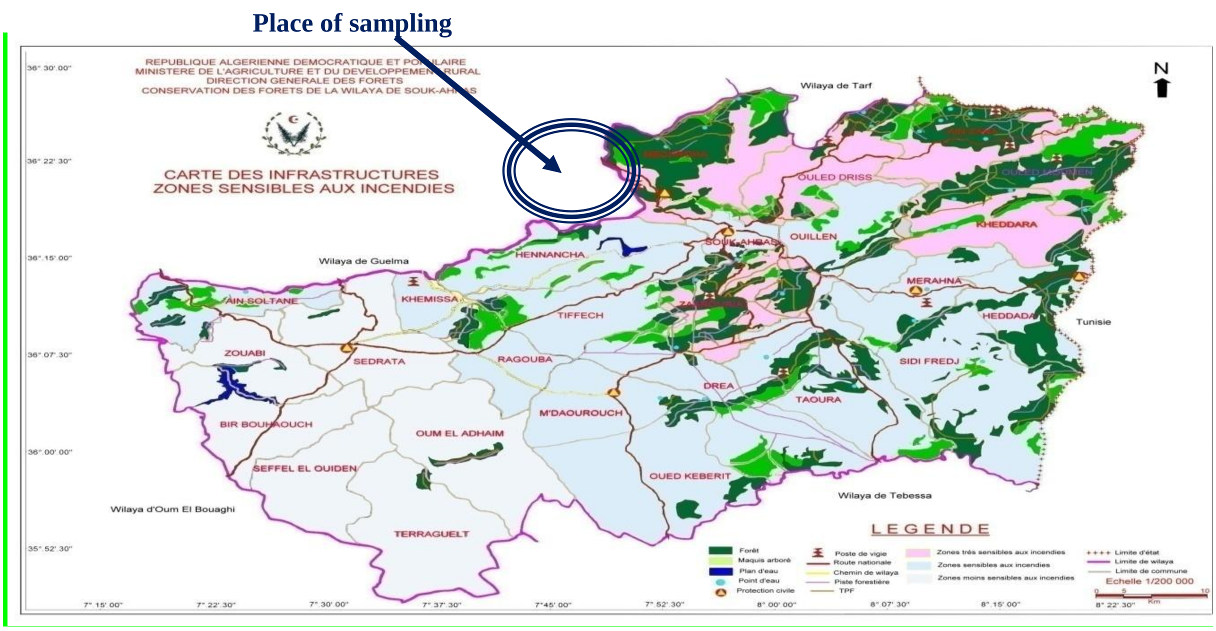

Fig.2. Geographical location of the sampling site "MECHROHA" (Weather Station SoukAhras, Algeria)

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- *Sampling strategy*

Samples of the species Foam *Rhytidium rugosum* were collected during the month of April of 2009. On the ground, we detached thalli of foam that exist on the trunks of trees with a knife. (Cork oak, Oak zene), types of foam carrier are: *Quercussuber, Quercuscaneriensis.* The samples were then stored in tightly closed plastic bags to reduce water loss through evapotranspiration.

- *Choice of sampling site*

Our choice fell on the town of MECHROHA (SoukAhras, Northeast Algerian) more accurately Forest OUZEGOUN more precisely the region EL Table because it is an area considered less polluted, mainly because of its altitudethatallows the existenceseveral speciesofMosses. The climate is sub humid, the altitude of the region EL Table is the order of 750-1045m (Weather Station, SoukAhras, Algeria).

- *Statistical analysis on the ANOVA*

All calculations were performed using the statistical analysis software MINITAB Release 14. This test is designed to test the equality of means (Dagnelie, 1999).

IV. Results

Table 3: Results of the analysis of variance of different parameters

Parameters	P	Signification
Chlorophyll (*a*)	0,000	*The ANOVA shows differences very highly significant (***) between Control, C1, C2, C3.*
Chlorophyll (*b*)	0,000	*Very highly significantdifferences(***)are noted by thestatistical analysis betweenControl,C1, C2, C3.*
Chlorophyll (*a*+*b*)	0,000	*There arevery highly significantdifferences(***) between the four samples(Control,C1,C2,C3).*
Proteins	0,002	*According to the statistical test, there are highly significant differences (**) between Control, C1, C2, C3.*
Proline	0,027	*The effect of different concentrations of the herbiciderate Proline is significant between Control, C1, C2, C3..*
Glucids	0,000	*The ANOVA shows differences very highly significant (***)between Control, C1, C2, C3.*
GSH	0,000	*Statistical analysisbetweenControl,C1, C2, C3 shows that there arevery highly significantdifferences(***)*

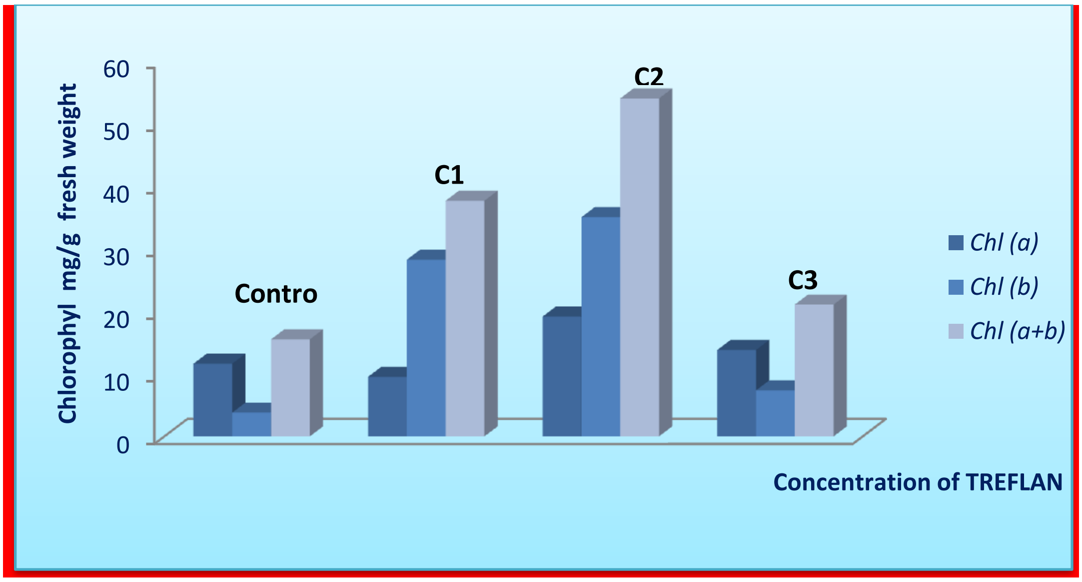

Fig.3. Effect of herbicide on content averages of chlorophyll *(a), (b), (a + b).*

Table 4: *Effect of TREFLAN on content average of Proteins, Proline and Glucids (concentration of proteins,proline and glucids: µg/mg de fresh weight)*(n=5;±: standard error of the mean values.)

Sampling	Control	0.01ppm	0.02ppm	0.03ppm
Proteins	*34.489±0.02*	*34.558±0.1*	*23.524±0.06*	*8.765±0.11*
Proline	*65.921±0.3*	*71.483±0.01*	*89.621±0.2*	*91.855±0.14*
Glucids	*57.770±0.12*	*81.227±0.11*	*101.555±0.07*	*110.886±0.21*

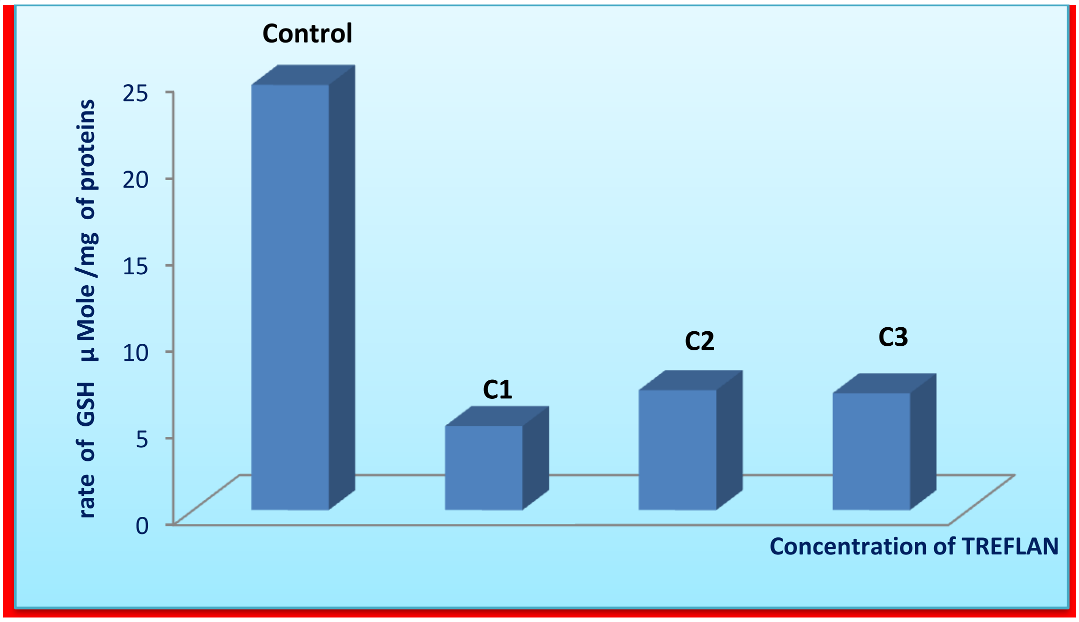

Fig.4. Effect of the herbicide on the rate of GSH.

V. Discussion

The present study aims to characterize the physiological and enzymatic metabolic of bryophyte *"Rhytidium rugosum"* using a herbicide "TREFLAN."Our choice fell on these plants "foam or moss" because they are often used in research on the impact of pollution as much as bioindicators.

The results show that treatment with the herbicide foams substantially affects all parameters studied. Our study focused on the effects of the herbicide "TREFLAN" on average levels of chlorophyll (a) and (b) in the case of foam "*Rhytidiumrugosum*." In general, we note that the rate of chlorophyll seems to be affected by the herbicide and results in an increase in chlorophyll (a), (b) and hence and depending on the concentrations used C1, C2 thereafter, there was a decrease in these rates with the highest concentration C2, which explains the phenomenon of adsorption at this plant. That is to say, after the reaction of bryophyte vis-à-vis the first concentration (C1) and the second concentration (C1), which presents a potential stress explained by higher rates: Chl (a) Chl (b) and Chl (a + b), it will return with the third concentration (C3) at a rate almost equal to that of the control.

These results therefore explain increased photosynthetic intensity foams, which in perfect agreement with the work that focused on the behavior of foams vis-à-vis a pollutant (Asta, 1993, Khalil and Asta, 1998).These studies showed an attenuation of photosynthetic activity by increasing the rate of chlorophyll resulting in a change resulting from a displacement of Mg chlorophyll molecules in the xenobiotic (Duruelle and lallement, 1983).

In order to detect the phenomenon of stress under the effect of different concentrations of the herbicide, we made the determination of proline in these plants. Our results show an increase in proline samples treated with the herbicide compared to the control. According (Lagadicand *al.*, 1997), an increase of proline can be observed if the plants are subjected to oxidative stress created by a pollutant (May and leaver, 1993; Bender and*al.*, 1994).

Concerning the evaluation of the effect of the herbicide on the rate of total protein in the moss species *"Rhytidiumrugosum"* there is any disturbance of the metabolism reflecting toxicity of the pollutant (Khaldi, 2003).To confirm the effect of the herbicide as a pollutant on the bryophytes, we assayed total sugars. The process of concentration of sugars in plants under stress is recognized as a characteristic of adaptation (Deraissac, 1992). Disruption of GSH as a biomarker is observed in foams treated compared to control.

These results confirm the work (Gunnarsson and Rydin, 2000; Dazy and *al.*, 2009; Britton and Fisher, 2007) found that disturbances in levels of glutathione in plants below examples: "moss" and aquatic plants example Macrophyte, under the effect of heavy metals. All these disturbances result in a significant impact (positive or negative) of the herbicide on bryophyte species.

In conclusion, the results for the bioindication highlight the effectiveness of the studied species of mosses time bioindicator of pollution.

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