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Absorption capacity of lead by different lichenic species

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Abstract

The use of bioindicator is very important for assessing the pollution intensity by accumulation of pollutants and lead in particular. The measurement of this accumulation in some organisms such as lichens and its effects on the physiological and morphological characteristics was found very effective to answer this problem. It is in this context that we have oriented our research whose objective is to determine the accumulating power of some lichenic species namely: *Flavoparmelia sordians*, *Ramalina farinacea* and *Xanthoria parietina* under the influence of three concentrations of lead nitrate: low, medium and high, respectively (0.207 gPb/l, 2.07 gPb/l and 20.7 gPb/l) in other parts highlight the impact of this accumulation on the Physiology (determination of chlorophyll) and morphology (morphological observation) of these species. The comparison between the three species for the accumulation of lead using the Fisher test revealed significant differences between all our species, while the ANOVA test for a factor showed results ranging from significant (p = 0,349 in *Xanthoria parietina* after treatment 4) to very highly significant (p = 0,000*** in all species after the last treatment) for the measured physiological parameters.

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Introduction

The pollution with lead is a global problem currently subject to a general awareness. Human activity and in particular the use of lead significantly modify the concentration of lead in the atmosphere. Many researchers have been undertaken in United States, Japan and Europe as part of the office organization, including the commission of the European communities (Caplun *et al.,* 1984). This problem is so dramatic that caused this last decade confrontation automakers, environmentalists and many governments on emission standards for vehicles. Unleaded gasoline, poor engine today is translations of political and economic issues of the environment (Georgiades *et al.,* 1988). The various components of the environment react differently to air pollution (as well as lead pollution). Plants and especially lichens often exhibit morphological alterations and physiological changes occur well before the smallest symptoms in animals including humans.

The choice of lichens as a bioindicator is explained by many peculiarities of these plants and in particular by some fundamental differences with the higher plants: continuous photosynthetic activity, slow metabolism, heavy accumulation of heavy metals, especially the lead and the lack of self-defense about the atmosphere (Deruelle, 1992, 1996; Market, 1993).This property of lichens as indicators of pollution has been used many times throughout the world since the Seventies (James, 1973), their reactions to atmospheric pollution have been the subject of much work (Deruelle, 1978; Deruelle and Lallemant, 1983; Semadi and Deruelle, 1993; Rahali, 2003; Serradj Ali Ahmed, 2007; Alioua *et al*., 2008; Serradj Ali Ahmed and *al.,* 2014, 2016).In the region of Annaba (East of Algeria) there is a development of several industrial units in addition to road traffic in constant increase. That is why we considered studying the impact of this metal on three lichenic species and their ability to accumulate this pollutant.

Material and methods

Plant material

Three species were used *Xanthoria parietina* (L.) Th. Fr. (Foliaceous), *Flavoparmelia soredians* (Ny.) Hale (Foliaceous) and *Ramalina farinacea* (Hoffm.) Fürnrohr (Fruticulous).

The identification was made according to the French Association of Lichenology (AFL).

These species were harvested in April 2014 at the level of the Seraidi region exactly below 400 m from the massif of the Edough (mountain range west of Annaba) on *Qercus suber*.

Preparation of solutions

The solutions required for the treatments are made from lead nitrate $Pb(NO_3)_2$.

10^{-3} which correspond to C1 (0.207gPb/l).

10^{-2} which correspond to C2 (2.07gPb/l).

10^{-1} which correspond to C3 (20.7gPb/l).

Treatment of species with prepared solutions

This protocol was carried out from a completely randomized experiment (total randomization).In the laboratory the lichens are placed in boxes of petri dishes (2 g / box), 12 plates of petri dish for each lichenic species which will be divided into 3 series formed each of 4 boxes.

The treatments carried out (Table 1) are used by spraying, in order to study the absorption capacity of lead by lichens; we have increased the amount of solution used as a function of time. The amount of solution sprayed is identical for the three species and for the different concentrations. The control sample was sprayed with distilled water. After the last treatment (T7), we performed: A morphological approach on the thalli of each lichenic species; and different dosages on thalli of the same species.

Determination of chlorophylls

The extraction of chlorophyllsis carried out according to the method proposed by (Rao and Le Blanc, 1965), which consists of a maceration of the plant in acetone. Sample processing is as follows: 1g of the plant leaf cut into small pieces and ground in a mortar with 20 ml of 80% acetone and about 100 mg of calcium bicarbonate ($CaCO_3$). After the total grinding, the solution is then filtered and black boxes in order to avoid oxidation of chlorophyll by light. Reading is done at both wavelengths 645nm and 663nm, after calibration of the instrument with 80% acetone control solution. This dosage is carried out after the T4 and last treatment T7.

Determination of lead

After the last treatment (T7) a lead assay was carried out which consists of mineralization of thalliums which after drying the samples in an oven at 105 ° C., was carefully ground, placed in pillboxes where they were treated with hydrogen peroxide until complete mineralization (Deruelle, 1996).

The hydrogen peroxide used was of the standard RP Norma type at 110 volumes for analysis (stabilized with 0.0005% of sodium stannate). Some authors (Garty and *al.,* 1977; Goyal and Seaward 1981; Seaward and *al.,* 1981) wash the thallus before mineralization, while others (Pilegaarde and *al.,* 1979; Folkeson, 1981) mineralization directly on the harvested samples.

As far as we are concerned, we have opted for the second method. Recent determinations of lead have been performed using the spectrophotometric atomic absorption technique (S.A.A).

The measurements were made from solutions of 20 ml of 2% nitric acid. For the same solution, three measurements (repetitions) were made, the average being considered. Before the determination of lead in the samples, a calibration curve must first be established from solutions of known concentrations of lead.

The results are read directly from the instrument if it is preset according to the manufacturer's instructions or on the calibration curve in micrograms of lead. The apparatus used is a spectrophotometer (Perkin-Elmer model 400).

Statistical analysis

The ANOVA test for a factor and the Fisher test were used to make a comparison for each characteristic as a function of lead nitrate concentrations $Pb(NO3)_2$ on the one hand and between Lichenic species on the other. All calculations were performed by the software stepwise command (Minitab® 15.1.30.0).

The values in parentheses are those of probability p: if ($P \leq \alpha = 0.05$) the correlation is significant *, if ($P \leq \alpha = 0.01$) the correlation is highly significant ** and if ($P \leq \alpha = 0.001$) the correlation is very highly significant ***. If ($P > \alpha = 0.05$), there is no correlation between the characteristics (Dagnelie, 1999).

Results and discussion

Morphological observations

Two days after the first treatment, we find that the *Ramalina farinacea* species has a higher absorption capacity and that there is a deformation and contraction of the thallus compared to the other species. After the second treatment, there was chlorosis in the thallus of the *Ramalina farinacea* species and necrosis in the samples treated with concentrations C2 and C3.

Table 1. Amount of solution used as a function of time.

Treatments (ml) / Species	T1	T2	T6	T3	T4	T5	T7
Xanthoria parietina	5	5	10	8	9	9	10
Flavoparmelia soredians	5	5	10	8	9	9	10
Ramalina farinacea	5	5	10	8	9	9	10
Control Distilled water	5	5	10	8	9	9	10

T1 (06/04/2014), T2 (09/04 /2014), T3 (12 /04/2014), T4 (15/04/2014), T5 (18/04/2014), T6 (21/04/2014 and T7 (24/04/2014).

After the last treatment, we observe the following changes (Table 2). As a result of these observations, deformation and discoloration of the thallus, we can deduce that lead nitrate at concentration of C3 (10^{-1}) is toxic and can cause damage at the cellular level. Biological and physiological alterations have been observed by many authors in living species in environment polluted by lead.

These alterations may be due to a change in the symbiotic exchanges (Deruelle and Petit, 1983) and their importance varies according to the species. Several authors have also reported that heavy metals cause deformations of the thallus (Hellman *et al.*, 2000; Zambrano and Nash III, 2000); that some pollutants cause necrosis at the thallus (Clerc and Roh, 1980) and the lead is fixed at the hyphae (Garty and Theiss, 1990).

Table 2. Color change in the thallus of the different lichen species treated with $Pb(NO_3)_2$.

Concentrations (gPb/l) / Species	Control	C1	C2	C3
Xanthoria parietina	Yellowish green	Yellowish green	Chlorosis	Chlorosis
Flavoparmelia soredians	Green Blue	Green Blue	Light brown	Light brown
Ramalina farinacea	Green	Green	Dark green	Light brown

Determination of chlorophyll

Flavoparmelia soredians

Following the treatment 4, we observed a decrease in chlorophyll with an increase in the concentration of $Pb(NO_3)_2$ (p=0.001)***, and concentrations of chlorophyll in *Flavoparmelia soredians* treated with C1 are clearly close to the control values (Fig.1).

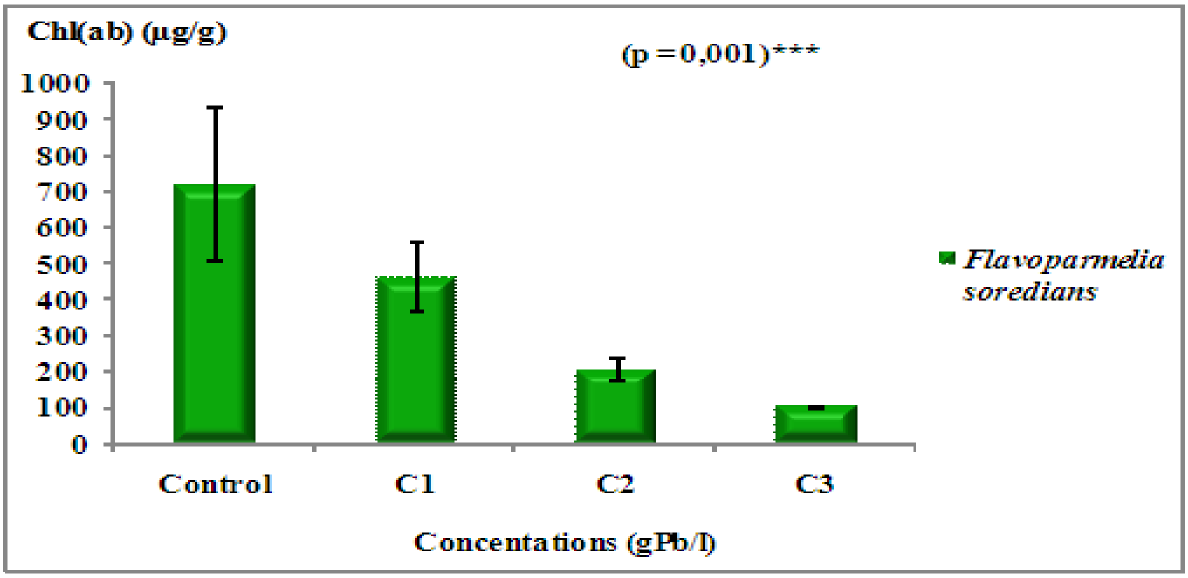

Fig. 1. Mean variation of Chlorophyll (ab) at *Flavoparmelia soredians* in ug/g of dry matter 15/04/2014. Values are expressed as the average (±) standard errors of three determinations.

After the last treatment, there is always a decrease of chlorophyll at a concentration C3 and the contents of chlorophyll recorded at the level of Flavoparmelia treated with the concentrations C1 and C2 are close to that of the control (Fig.2). Several authors have shown that lead affects lichen photosynthesis (Ronen *et al.*, 1984: Kardish *et al.*, 1987; Wietshorke *et al.*, 1990; Fornasiero, 2001). Clijsters and Van Assche (1985) show that inhibition of photosynthetic intensity and transpiration may be visible due to chloroplast alteration. Prasad and Prasad (1987) was more precise on the existence of two enzymes of photosynthesis known for their sensitivity to lead which are:

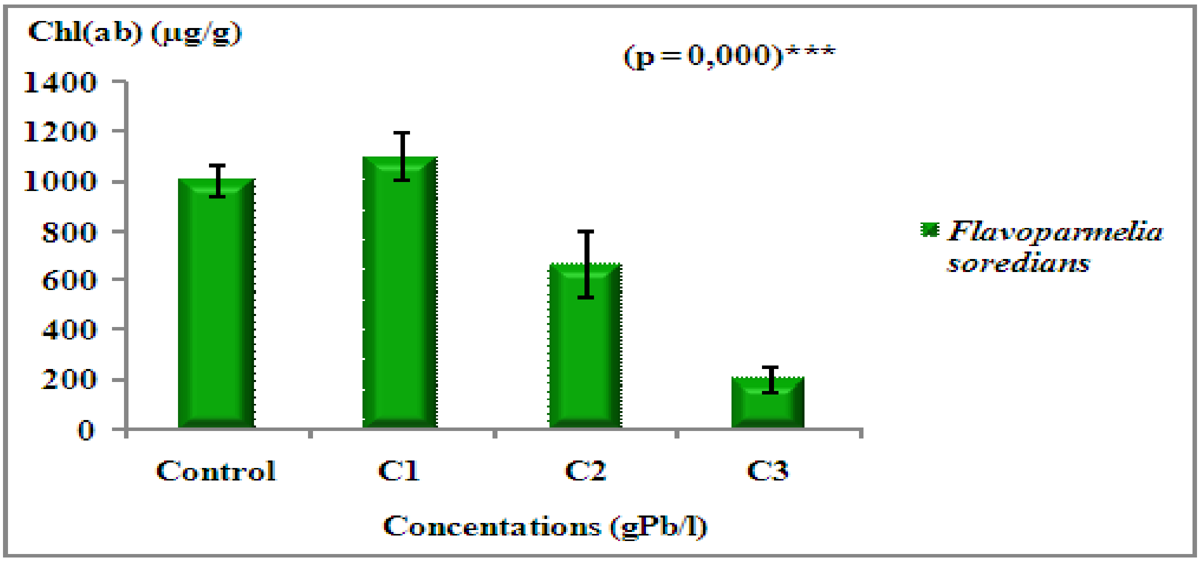

Fig. 2. Mean variation of Chlorophyll (ab) at *Flavoparmelia soredians* in ug/g of dry matter 24/04/2014. Values are expressed as the average (±) standard errors of three determinations.

The α-aminolevulinic acid desyhdratase (A L D). Ribulose 1.5 biphosphate-carboxylase. Indeed, according to our results, we can conclude that lead at a certain concentration (20.7gPb/l) causes to a drop in chlorophyll which is expressed from a cellular point of view by the fact that the metabolism of the lichens and the photosynthesis in particular, is regulated by the degree of hydration of the thallus.

Moreover, the lichens which assimilate by all their surfaces are endowed with a remarkable longevity of several decades and more (Maizi *et al.,* 2010). The analysis of the variance to classification criterion relative to the mean variation of chlorophyll in *Flavoparmelia soredians* in µg/g of dry matter following treatment 7 shows that the latter is very highly Significant (p = 0.000) ***.

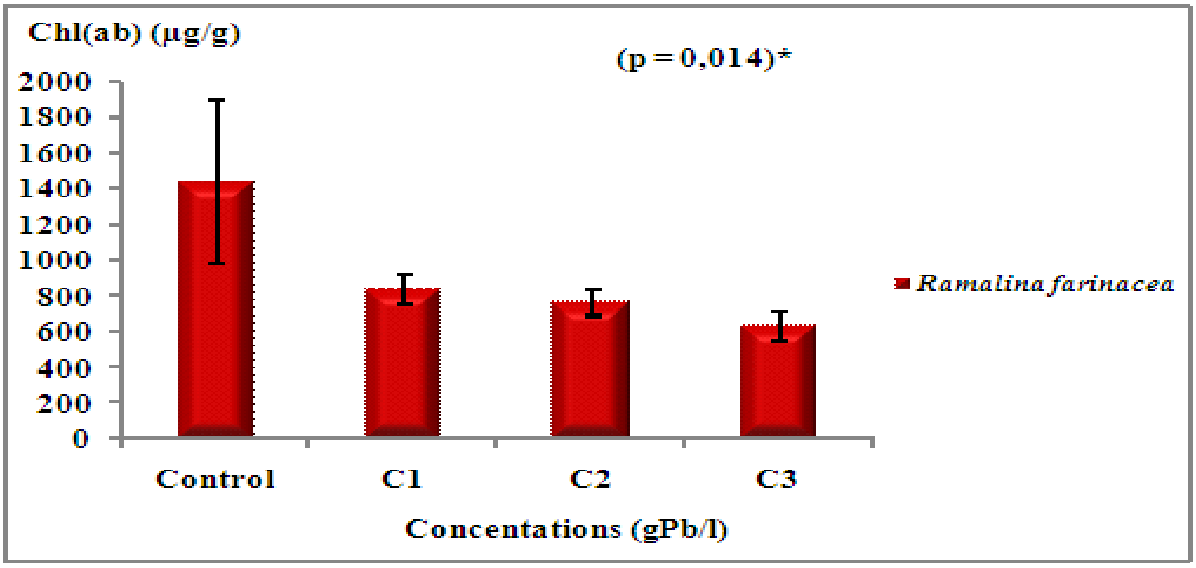

Fig. 3. Mean variation of chlorophyll (ab) at *Ramalina farinacea* in ug/g of dry matter 15/04/2014. Values are expressed as the average (±) standard errors of three determinations.

Ramalina farinacea

The total chlorophyll (ab) varies from 632,53µg/g to 1441, 04µg/g of dry matter (Fig. 3). Comparing these results with the control, we found that chlorophyll decreased as a result of treatment 4. The comparison of the mean chlorophyll content in

Ramalina farinacea shows that this variation is only significant (p = 0.014*). There was also a decrease in chlorophyll as a function of concentration. This decrease in chlorophyll appears clearly following treatment 7 (Fig. 4) (after accumulation of lead in the species *Ramalina farinacea*). Concerning the comparison of the variation of chlorophyll (ab) in *Ramalina farinacea* as a function of the concentrations of lead nitrate, we note that it is very highly significant (p = 0.000 ***).

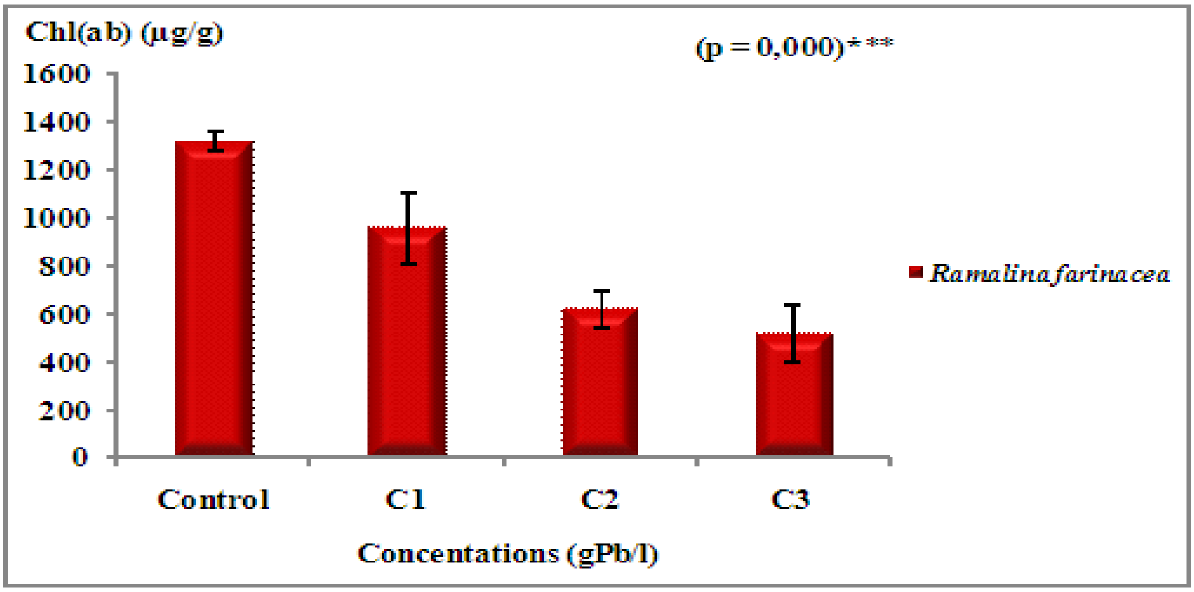

Fig. 4. Mean variation of chlorophyll (ab) at *Ramalina farinacea* in ug/g of dry matter 24/04/2014. Values are expressed as the average (±) standard errors of three determinations.

Xanthoria parietina

The total chlorophyll (ab) varies from 756.21µg /g to 964.54µg /g of dry matter (Fig.5). Following treatment 4, we recorded a slight decrease of chlorophyll (ab) at the concentration C1, C2 and C3 which are always close to the control. The analysis of the variance with a classification criterion confirms these results, since it is noted from the value of (p = 0.349) that this variation is not significant.

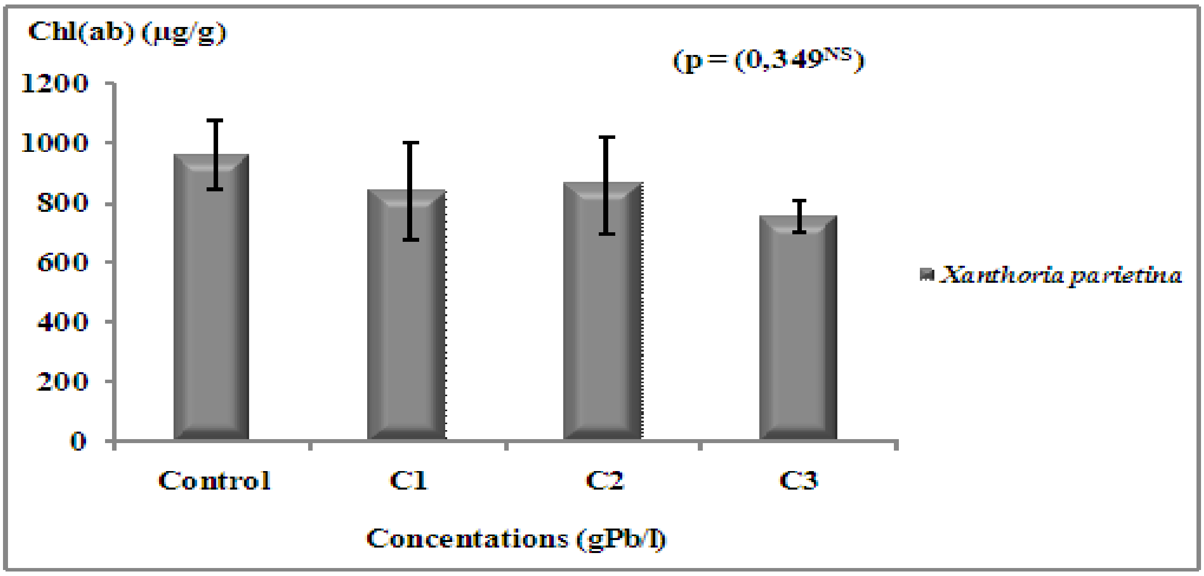

Fig. 5. Mean variation of chlorophyll (ab) at *Xanthoria parietina* in ug/g of dry matter 15/04/2014. Values are expressed as the average (±) standard errors of three determinations.

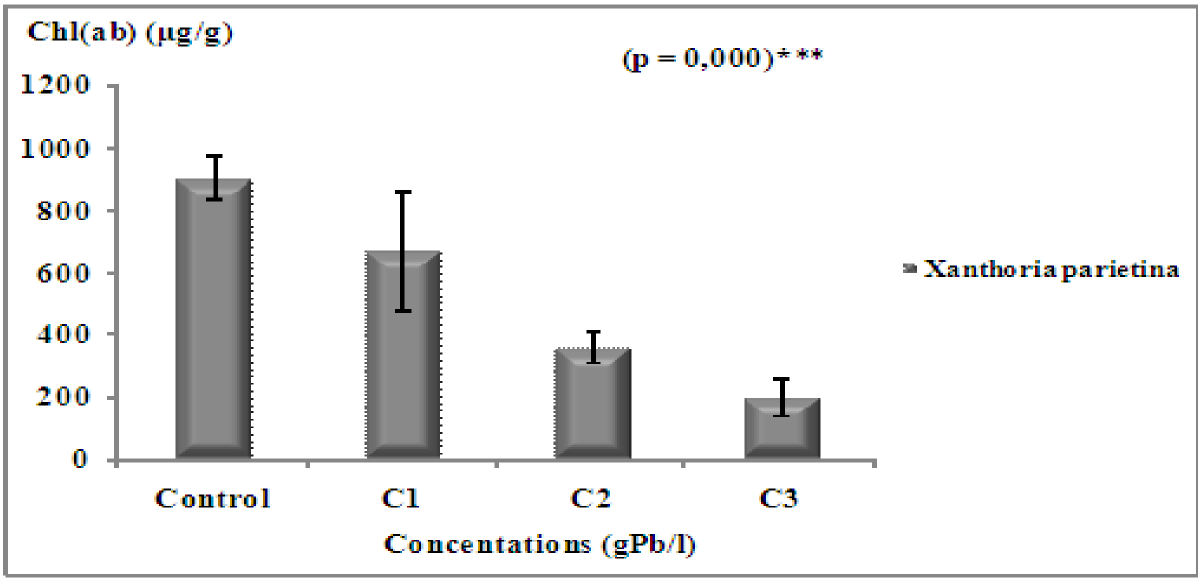

Fig. 6. Mean variation of chlorophyll (ab) at *Xanthoria parietina* in ug/g of dry matter 24/04/2014. Values are expressed as the average (±) standard errors of three determinations.

After the last treatment (T 7), we obtained very highly significant results (p = 0.000***) (Fig. 6). We can explain this slight decrease in chlorophyll by the fact that *Xanthoria parietina* is less sensitive to lead. Furthermore, our results showed that the fruticulous species *Ramalina farinacea* is more sensitive to lead than the species *Xanthoria parietina*. Our results coincide with the work of (Rahali, 2002). The Comparison of our lichen species using the Fisher test for chlorophyll (ab) variation revealed that the differences ranged from non-significant to significant; the difference between *Ramalina farinacea* and *Flavoparmelia soredians* is not significant for C1, whereas it is significant between *Ramalina farinacea* and *Xanthoria parietina* for the same concentration. On the other hand, we deduce a non-significant difference between the three species for C2 and significant difference between *Ramalina farinacea* and *Flavoparmelia soredians* for C3.

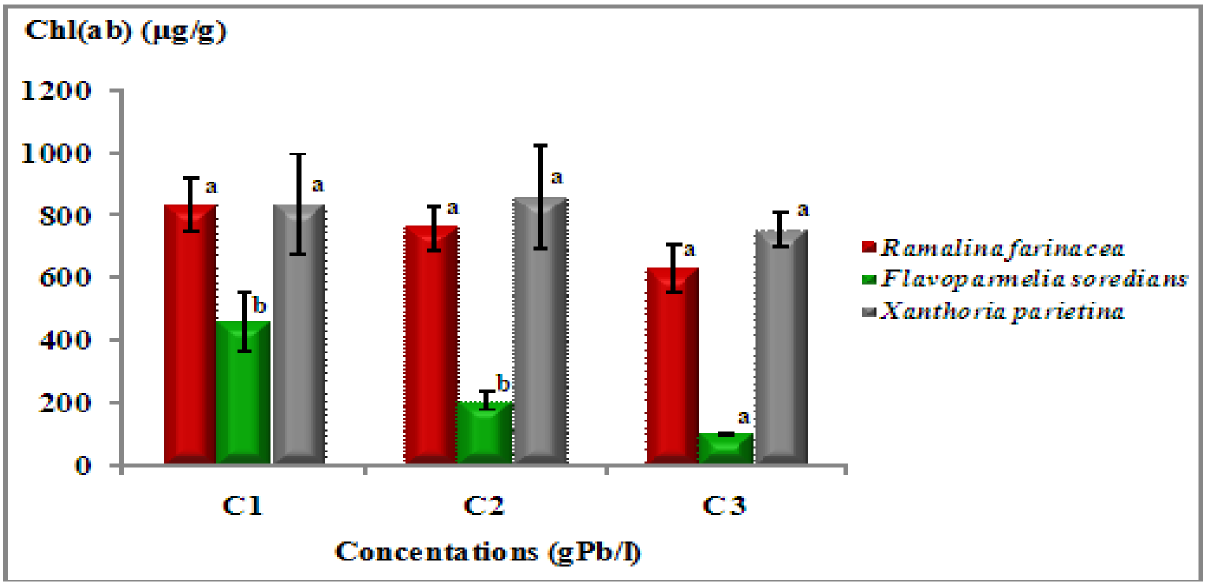

Fig. 7. Mean variation of chlorophyll (ab) in lichenic species: *Ramalina farinacea*, *Flvoparmelia soredians*, *Xanthoria parietina* (15/04/2014). Fractions and concentrations with the same letter indicate a non-significant difference according to the Fisher test ($P \leq \alpha = 0{,}05$).

Determination of lead in the different lichenic species

The (Fig. 9) records results indicating a strong accumulation of lead in the species *Ramalina farinacea* treated with concentration C3. At the cellular level, we can explain this by "negative accumulation", "rejection" or even better "exorption.". This phenomenon was demonstrated and described for the first time by Deruelle at Fontainebleau (1983, 1986) and still observed at the level of the lichenic species transplanted on the ground by Rahali (2002).

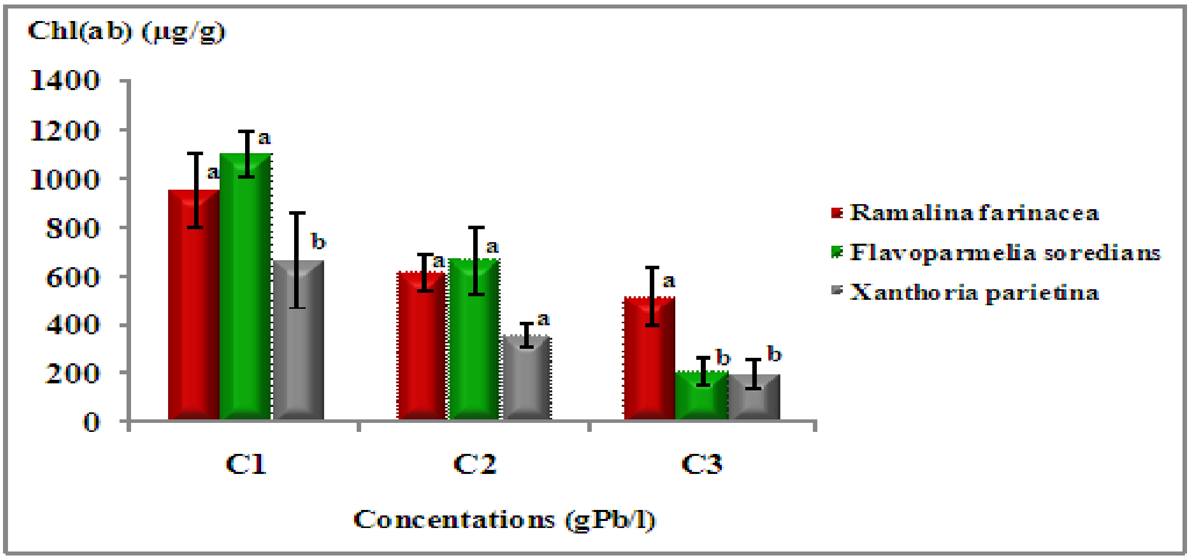

Fig. 8. Mean variation of chlorophyll (ab) in lichenic species: *Ramalina farinacea*, *Flvoparmelia soredians*, *Xanthoria parietina* (24/04/2014). Fractions and concentrations with the same letter indicate a non-significant difference according to the Fisher test ($P \leq \alpha = 0{,}05$).

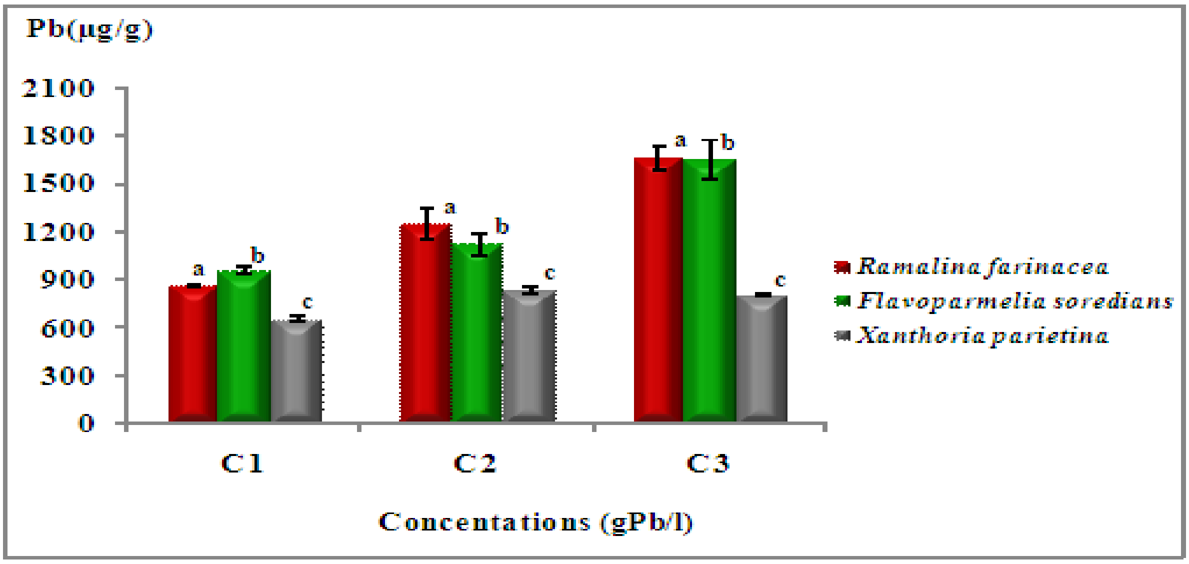

Fig. 9. Mean change of lead accumulated (µg/g) by lichen species: *Ramalina farinacea*, *Flvoparmelia soredians*, *Xanthoria parietina*. Fractions and concentrations with the same letter indicate a non-significant difference according to the Fisher test ($P \leq \alpha = 0{,}05$).

This rejection or exorption is also observed in *Flavoparmelia soredians* and *Xanthoria parietina*. The lichens devoid of roots under the direct dependence of the water loaded with lead is absorbed rapidly and non-selectively by the wall of the hyphae rich in hydrophilic mucilage and reaches the cells directly. The absence of stomata and the numerous accidental or natural cortical fractures, such as pores or pseudocyphellae, allow the water charged with lead to penetrate into the lichen or are trapped in the filament lace.

The variation of lead shows that this element traces has tendency to increase with increasing concentration, and this can be explained according to (Bergamaschi and *al.*, 2007) that these trace elements are likely to be associated with the substances particles deposited on the surface of the lichens. Temporal increases and decreases in element trace concentrations in lichens are generally reported in the literature (Bargagli, 2002). Indeed, the accumulation of metals by lichens occurs according to Brown (1987) either by a deposit of the particles suspended in the atmosphere on the surface of the thallus, by an intracellular accumulation or by accumulation of the metals at the level of the cell wall. Thus, we can deduce on the one hand that there has been a high absorption capacity at high, medium and low concentrations. On the other hand, *Ramalina farinacea* is the species most sensitive to lead (Maizi and *al.,* 2010). Concerning the accumulation of lead in the three lichenic species, the comparison using the Fisher test revealed significant differences between all our species, namely: *Ramalina farinacea*, *Flavoparmelia soredians*, *Xanthoria parietina*.

Conclusion

Finally, the study of the absorption capacity of Lead as a pollutant emitted by various sources of pollution (industries, road traffic and so on.) on some lichen species to different types of thalli (Foliaceous, Fruticulous), showed that this pollutant affects lichens : by deforming the thallus with the appearance of chlorosis and sometimes even necroses. In addition, by affecting the chloroplasts and from there decrease of the photosynthesis with decrease of the concentrations of the total chlorophyll. Our results showed also that some lichen species have a high absorptive capacity and are therefore more sensitive to this pollutant than others, which allowed us to classify them from most to least sensitive: *Ramalina farinacea*, *Flavoparmelia soredians* and *Xanthoria parietina*.

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