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Oxidative stress caused by lead in the lichen *Xanthoria parietina*

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ABSTRACT. The assessment of the air quality is a major concern to the current time. The monitoring and maintenance of air quality necessarily pass by detecting and estimating the overall air pollution. The use of lichens must be an assessmenttool to be studied. In our work we were interested about the toxicity of lead on the various parameters of stress in the lichen *Xanthoria parietina*. For this purpose, lichen thalli have been incubated at lead concentrations of 0, 0.5, 1.0, 5.0 and 10.0 mM, for time scale of 0, 24, 48 and 96 hours. The obtained results showed that lead has an action on the various studied parameters, and the intensity of oxidative stress observed in lichens thalli depends on the concentration, and time of exposure. Lead induced a decrease in chlorophyll and protein contents, and an increase in the contents of catalase, hydrogen peroxide and reduced glutathione. Furthermore, the results also showed that high concentrations of lead caused total destruction of reduced glutathione.

Keywords: lead; lichen; stress; chlorophyll; catalase; proteins; hydrogen peroxide; glutathione.

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Introduction

Lichens present a very important model of symbiotic organisms when associating a mushroom called mycobiont and green algae and/or cyanobacteria called photobionts (Nash III, 2008). They are used in the bio indication of air quality (Bosch-Roig, Barca, Crisci, & Lalli, 2013; Kar, Samal, Maity, & Santra, 2014; Loppi, 2014; Kuldeep & Bhattacharya, 2015; Pescott et al., 2015; Sulaiman, Fuzy, Muis, & Ismail, 2018; Benítez et al., 2019; Mohamed, Mohamed, & Abdelhay, 2020; Quijano-Abril, Ramirez-Ospina, Domínguez-Rave, & Londoño-Valencia, 2021). Lichens are devoid of roots, and mineral nutrition takes place from atmospheric inputs (Garty et al., 2008). Lichens are sensitive to numerous environmental conditions, which might cause changes in some of their components and/or specific parameters. In addition, lichens have the ability to capture mineral nutrients from wet deposits (Gauslaa, Mikulec, & Solhaug, 2021). Such properties allows us to use them as one of the excellent bio-indicators of air pollution.

Lichens have the ability to absorb significant amounts of trace elements from the atmosphere (Caggiano, Trippetta, & Sabia, 2015; Darnajoux, Lutzoni, Miadlikowska, & Bellenger, 2015). Lichens are also used as biomonitors (Kularatne & Freitas, 2013; Ite, Udousoro, & Ibok, 2014; Conti & Tudino, 2016; Demková, Bobul'ská, Árvay, Jezný, & Ducsay, 2017; Abas, 2021; Tarawneh, Salamon, Altarawneh, & Mitra, 2021) and bioaccumulators of heavy metals (Węgrzyn, Wietrzyk, Lisowska, Klimek, & Nicia, 2016; Winkler et al., 2019; Rola, 2020; Vannini et al., 2021). Lichens react with atmospheric pollutants often having physiological, morphological and structural changes (Matos et al., 2015). From physiological changes, lichens react against the stress through reduction of chlorophyll (Sujetovienė & Sliumpaitė, 2013; Balarinová, Barták, Hazdrová, Hájek, & Jílková, 2014).

Heavy metals cause inhibition of chlorophyll synthesis in lichens (Rola, Latkowska, Myśliwa-Kurdziel, & Osyczka, 2019). In green plants, abiotic stress seriously affects photosynthesis in all its stages (Ashraf & Harris, 2013). Lead causes a disturbance of the prooxidant/antioxidant equilibrium, which induces various cell and tissue damage that evolve towards damage at the subcellular level (Nareshkumar et al., 2015). Among the lichen species, *Xanthoria parietina* is the most used in bioindication and biomonitoring programs (Nimis, Andreussi, & Pittao, 2001; Pisani, Munzi, Paoli, Bačkor, & Loppi, 2009). Although various species, such as *Hypogymnia physodes* and *Parmelia sulcata*, are utilized in bioindication, *X. parietina* is capable of

accumulating considerable levels of heavy metals due to its wide surface in contact with contaminants (Cuny, Davranche, Thomas, Kempa, & Haluwyn, 2004).

Our work which relates to the study of the toxic effect induced by lead on *X. parietina,* is the first to be carried out in our region.

The purpose of this study is to investigate the toxic effect of lead which is in the form of $Pb(NO_3)_2$ on the lichen *X. parietina* by assaying different biomarkers of stress on chlorophyll, protein contents, catalase, hydrogen peroxide (H_2O_2) and reduced glutathione (GSH).

Material and methods

Plant material

During February and March 2017, Lichen thalli samples of *X. parietina* were gathered in the Beni Metrane region located in Jijel Northeast of Algeria, in an abandoned orange grove that covered several hectares. The site was chosen because it is in a rural area far from any urban or industrial region. Samples were transported to the laboratory in sterilized closed boxes and rinsed with distilled water to remove adherent particles. The thalli were then isolated from their supports and stored under laboratory conditions until analysis.

Lead treatment

Under a binocular microscope, lichens were thoroughly cleaned. In each experimental vessel, 3 g of fresh thalli were soaked in 100 mL of 0.5, 1, 5, and 10 mM $Pb(NO_3)_2$ solution and compared to control samples soaked in deionized water (Carreras & Pignata, 2007). These solutions were then incubated in the dark for 0, 24, 48, and 96 hours at room temperature. They were then filtered and rinsed three times with deionized water for five seconds each time. Each experiment was carried out three times. On a scale of time of 0, 24, 48, and 96 hours, lichens were stored to perform the following dosages: chlorophyll, protein, catalase, H_2O_2, and GSH dosages.

Lead analysis

Flame atomic absorption spectrometry was used for the determination of lead. After drying at 90°C for 24 hours, 3 mL of concentrated HNO_3 and H_2O_2 (2:1, v/v) was used to digest 100 mg of dry matter for 48 hours. After filtration through a Whatman Filter paper N° 42, the mixture was completed to 10 mL with deionized water. Lead analysis was performed with the Shimadzu AA 6200 Flame Atomic Absorption Spectrophotometer. To quantify the accumulated lead, the internal stock was used to create a calibration curve. Results were expressed in µg g^{-1} (dry weight).

Chlorophyll analysis

To determine the content of chlorophyll a (C_a), chlorophyll b (C_b) and total chlorophyll (C_{a+b}), the method described by Lichtenthaler (1987) was used. The concentration of 80% acetone was used for the maceration of the fresh lichen sample; the maceration extract was then filtered and read by a spectrophotometer at A_{663} and A_{645}. Chlorophyll content was calculated according to the following equation:

$$C_a = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$C_b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$C_{a+b} = 20.2 \times A_{645} - 8.02 \times A_{663}$$

where A_{663}, A_{645} are absorbances at 663 and 645 nm, respectively. Results were expressed in µg g^{-1} dry wt.

Chlorophyll a/b ($c_{a/b}$) has become useful as an indicator for measuring the physiological activity of algal cells.

Proteins assay

The technique of Bradford (1976) was used to test the protein content. Homogenization of 100 mg of fresh weight of lichens in 2 mL of 0.05 M phosphate buffer pH 6.8 and centrifugation of the homogenate at 4°C for 20 min at 12,000 t min^{-1} were carried out. An amount of 2 mL of Bradford's solution was added to 50 µL of the supernatant. After 10 minutes, a reading was taken at 595 nm. The calibration curve was established by the BSA (bovine serum albumin) at different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 and 0.08) mg mL^{-1}. Protein levels (mg g^{-1}) were calculated using equation established by BSA ($y = 28.9\ x$, $R^2 = 0.9911$).

Catalase activity

Using Chance and Maehly's method (1955), the activity of catalase was measured. Homogenization of 50 mg fresh weight of lichens in 2 mL of 0.05M phosphate buffer at pH 7 was made, followed by centrifugation at 15,000 t $min.^{-1}$ at 5°C for 20 minutes. 50 µL of the supernatant was added to 2.95 mL of 0.015 M H_2O_2 in the phosphate buffer. A first reading was performed immediately at 240 nm, then a second one was taken after 3 minutes. The enzymatic activity of catalase was calculated using the following formula:

$k = 2.303 / T \times \log (A_1/A_2)$

of which:
K: the reaction rate constant.
T: Time interval in minutes.
A_1: Absorbance at t = 0.
A_2: Absorbance after 3 min.

H_2O_2 concentration

Method of Sagisaka (1976) was used to determine the concentration of H_2O_2. Approximately 1g of lichen fresh material was homogenized with 2 mL of 5% trichloroacetic acid (TCA). The resulting mixture was centrifuged at 14,000 g for 20 minutes at 0°C. The amount of 1.6 mL of supernatant was added to 0.4 mL of TCA (50%) and 0.4 mL of ferrous ammonium sulfate (1%) and 0.2 mL of potassium thiocyanate (1 %). The optical density at 480 nm was used to determine the amount of H_2O_2 in the supernatant. The H_2O_2 level (mmol g^{-1} (dw)) was determined using an equation established by known concentrations of H_2O_2 standard ($y = 0.1864 x + 0.2281$, $R^2 = 0.09691$).

Determination of GSH

GSH assay was carried out by the colorimetric method described by Ellman (1959). In the presence of 5.5'-dithiobis 2-nitrobenzoic acid (DTNB), GSH is oxidized by releasing thionitro-benzoic acid (TNB) which shows absorbance at 412 nm. Homogenization in 50 mM phosphate buffer (pH 6.5) and centrifugation at 12,000 g for 15 min. at 4°C of lichen thalli were carried out. For this assay, the absorbance at 412 nm of a mixture containing 100 µL of the extract and 1,200 µL of DTNB solution was read. Results were expressed in mmol g^{-1} using an equation established by known concentrations of GSH standard ($y = 0.2012x + 0.3852$, $R^2 = 0.9573$).

Statistical analysis

Three repetitions were performed at each concentration, so we could calculate the standard deviation. The statistical study was carried out using the ORIGIN 6.0 system using the univariate variance test (one way ANOVA). For this study, the results were expressed as mean ± SD (standard deviation). The difference was considered to be not significant when $p > 0.05$(NS), significant when $0.01 < p < 0.05$ (*), very significant when $0.001 < p < 0.01$ (**) and highly significant when $p < 0.001$ (***).

Results

Lead accumulation

The accumulation of lead in treated lichens augmented with increasing exposure time. Treatment with increasing concentrations of $Pb(NO_3)_2$ caused a gradual accumulation of lead in *X. parietina* (Table 1).

Table 1. Lead accumulation in the lichen *Xanthoria parietina* after incubation of thalli for 0, 24, 48 and 96 hours in $Pb(NO_3)_2$ solutions.

	0 mM	0.5 mM	1 mM	5 mM	10 mM
0h	114.52 ± 5.07	114.52 ± 5.07	114.52 ± 5.07	114.52 ± 5.07	114.52 ± 5.07
24h	200.13 ± 3.02	314.49 ± 4.40	594.98 ± 7.99	898.54 ± 20.53	1072 ± 50.99
48h	197.92 ± 2.12	324.50 ± 0.20	779.55 ± 41.38	1128.04 ± 13.56	1287.1 ± 18.22
96h	205.21 ± 15.02	524.52 ± 22.26	715.19 ± 31.21	1034.54 ± 46.22	3052 ± 152.97

Table 1 showed that the accumulation of lead is not significant in treated thalli with different concentrations after 24 and 48 hours of exposure. However, these concentrations increased significantly ($p = 0.018$*) in samples treated by 10 mM of $Pb(NO_3)_2$ after 96 hours of treatment.

Chlorophyll concentration

Values of the C_a, C_b, C_{a+b}, contents and $C_{a/b}$ ration in *X. parietina* thalli treated by $Pb(NO_3)_2$ are shown respectively in Tables 2, 3, 4 and 5.

Lichens incubated in distilled water showed the highest levels of C_a and C_b. The concentration of C_a ($p = 0.04^*$) and C_b ($p = 0.02^*$) decreased significantly after 24 hours of treatment. Such reduction is not significant after 48 and 96 hours of treatment. We have noticed that C_b was more affected than C_a.

Table 4 shows that C_{a+b} decreased significantly ($p = 0.0032^{**}$) in thalli treated with 10 mM of $Pb(NO_3)_2$ after 96 hours of exposure with a clear start-point from 24 hours onward with all concentrations (0.5, 1, 5 and 10 mM).

$C_{a/b}$ ratio was not changed very dramatically and no significant differences were observed in this parameter between different exposure times of lead ($p = 0.5198^{NS}$).

Table 2. C_a concentrations in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

	0 mM	0.5 mM	1 mM	5 mM	10 mM
0h	490.13 ± 41.86	490.13 ± 41.86	490.13 ± 41.86	490.13 ± 41.86	490.13 ± 41.86
24h	480.54 ± 18.73	218.43 ± 10.59	213.5 ± 15.23	163.56 ± 6.18	122.11 ± 17.38
48h	430.19 ± 15.19	207.48 ± 9.04	190.37 ± 20.20	127.37 ± 8.00	89.61 ± 10.9
96h	496.78 ± 25.31	182.99 ± 28.94	139.58 ± 15.10	110.79 ± 6.46	71.09 ± 8.42

Table 3. C_b concentrations in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

	0 mM	0.5 mM	1 mM	5 mM	10 mM
0h	589.13 ± 10.22	589.13 ± 10.22	589.13 ± 10.22	589.13 ± 10.22	589.13 ± 10.22
24h	580.72 ± 21.53	220.81 ± 19.13	164.43 ± 8.41	120.55 ± 7.29	77.12 ± 4.94
48h	398.17 ± 24.10	118.87 ± 13.53	102.78 ± 23.53	114.26 ± 12.79	61.54 ± 6.30
96h	420.15 ± 17.91	112.7 ± 3.25	101.83 ± 8.59	104.04 ± 11.55	66.88 ± 2.58

Table 4. C_{a+b} in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

	0 mM	0.5 mM	1 mM	5 mM	10 mM
0h	1078.2 ± 44.74	1078.2 ± 44.74	1078.2 ± 44.74	1078.2 ± 44.74	1078.2 ± 44.74
24h	1061.26 ± 36.77	439.24 ± 28.52	377.93 ± 19.20	284.11 ± 6.86	199.23 ± 22.22
48h	828.36 ± 38.45	326.55 ± 15.93	293.15 ± 15.63	241.63 ± 19.64	151.15 ± 14.36
96h	889.93 ± 40.78	295.69 ± 30.16	241.41 ± 23.63	214.83 ± 5.77	143.97 ± 11.12

Table 5. $C_{a/b}$ ratio in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

	0 mM	0.5 mM	1 mM	5 mM	10 mM
0h	0.83 ± 0.07	0.83 ± 0.07	0.83 ± 0.07	0.83 ± 0.07	0.83 ± 0.07
24h	0.82 ± 0.02	0.98 ± 0.05	1.29 ± 0.09	1.35 ± 0.10	1.58 ± 0.13
48h	1.08 ± 0.03	1.74 ± 0.20	1.85 ± 0.23	1.11 ± 0.08	1.45 ± 0.11
96h	1.11 ± 0.03	1.62 ± 0.32	1.37 ± 0.04	1.06 ± 0.22	1.06 ± 0.08

Effect of lead on protein, catalase, H_2O_2 and GSH contents

$Pb(NO_3)_2$ treatment caused a decrease in protein contents (Figure 1) and an increase in catalase, H_2O_2 and GSH contents (Figure 2, 3 and 4, respectively). These variations were based on different concentrations of $Pb(NO_3)_2$, and exposure time. However, concerning the content of H_2O_2, statistically significant differences have only emerged on thalli incubated for 96 hours.

Figure 1 shows that protein levels are affected by lead, with significant decrease at all concentrations of lead after 24 hours of exposure ($p = 0.008^{**}$) in comparison with the control. The reduction is also a function of the lead concentration. This decrease is greatest between 24 and 96 hours of exposure but it is not significant ($p > 0.05^{NS}$).

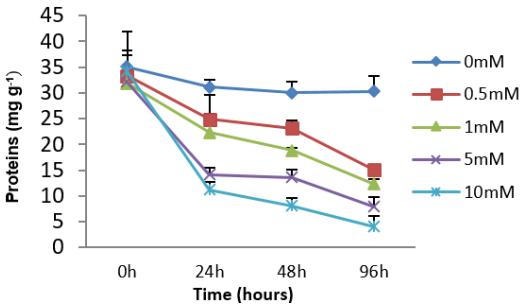

Figure 1. Protein concentrations in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

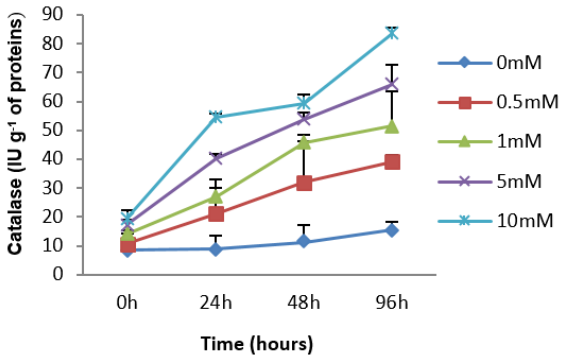

Figure 2. Catalase activities in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

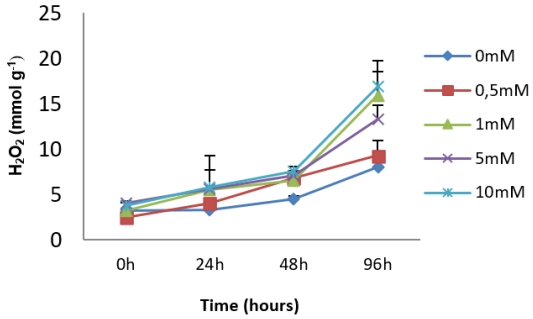

Figure 3. H_2O_2 concentrations in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

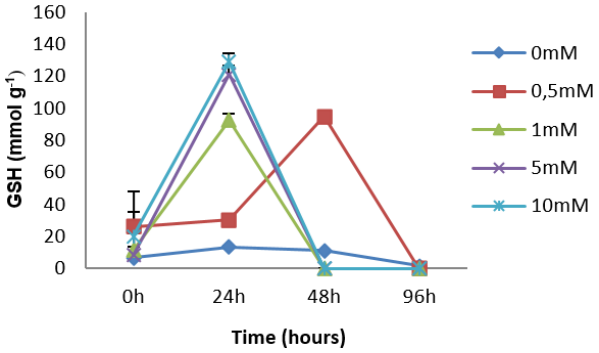

Figure 4. GSH concentrations in the lichen *Xanthoria parietina* after incubation of thalli in $Pb(NO_3)_2$ solutions.

According to Figure 2, it was observed that the expression of catalase in the lichen *X. parietina* was affected after treatment with lead. Thus, it was found that the expression of the catalase was in correlation with the time treatment and concentration of $Pb(NO_3)_2$. When the time of exposure and the concentration of lead augmented, the levels of catalase also increased. Accordingly, a significant increase was observed in function of the time of treatment ($p = 0.036^*$) and in function of lead concentrations ($p = 0.014^*$). Greater amount of catalase was recorded at concentrations of 10 mM of $Pb(NO_3)_2$ after 96 hours of the contact.

In *X. parietina*, the content of H_2O_2 increases in function of the time of exposure, particularly after 96 hours of treatment by 1 mM, 5 mM and 10 mM of $Pb(NO_3)_2$ ($p = 0.0018^{**}$). We noticed that low concentration (0.5 mM) has a negligible effect on the accumulation of H_2O_2, which is similar to the control. The increase in H_2O_2 concentrations was not significant in function of various concentrations of lead.

At low concentration of $Pb(NO_3)_2$ (0.5 mM), Figure 4 shows a significant increase in GSH content after 48 hours of treatment ($p = 0.05^*$), then GSH decreases significantly to zero after 96 hours of treatment ($p = 0.05^*$). Conversely, the other concentrations of $Pb(NO_3)_2$ caused a significant increase ($p = 0.0019^{**}$) of the levels of GSH just after 24 hours of exposure, these concentrations reached the maximum values after 24 hours of treatment and then decreased significantly to zero after 48 hours of exposure ($p = 0.0038^{**}$). Concerning the effect of concentrations, no significant variation of GSH content as a function of different concentrations were noted ($p > 0.05^{NS}$).

Discussion

Accumulation of lead

According to results obtained by Ma et al. (2016), it was found that vegetation is capable to accumulate heavy metals such as lead, and this accumulation induced oxidative stress (Mourato et al., 2015). Our results confirmed the accumulation of lead in *X. parietina* thalli. According to Abas and Awang (2017), lichens are effectively used as powerful biological indicators for biomonitoring. *X. Parietina* is one of the most regularly employed lichens in metal accumulation research. Belguidoum, Lograda, and Ramdani (2021) show that *X. parietina* is able to accumulate large amount of heavy metals in comparison with other fruticose lichen such as *Ramalina farinacea*. Our results show that the accumulation of lead increases with increasing exposure time, the same result was obtained by Douibi et al. (2015), and Sujetovienė and Česynaitė (2021). Lichens have the ability to extract metals from their environment (Subhashini & Suganthi, 2014; Caggiano et al., 2015; Darnajoux et al., 2015). According to results obtained by Carreras and Pignata (2007), Hg, Cu, Cd, Ag, Pb, Zn are considered the most toxic heavy metals. Our results show also that accumulation of lead increases with increasing $Pb(NO_3)_2$ concentrations.

Effect of lead on chlorophyll

According to the results presented in Tables (2, 3, 4 and 5), it was noticed that lead exerts toxicity on lichen by the decrease of C_a, C_b and the total chlorophyll content. It is the same result obtained by Emamverdian and Ding (2017), which indicate that lead is toxic for photosynthetic properties.The obtained results also show that the decrease in the chlorophyll content is important in thalli treated with high concentrations of lead, the same result was obtained by Zhao et al. (2021), who indicates that high concentrations of cadmium inhibit photosynthesis in *Sassafras* seedlings.

Due to their tendency to accumulate large levels of hazardous metals, foliose lichens are frequently used as biomonitors of metal pollution in the environment (Purvis, 2014). Our results indicate that the accumulation of lead in the lichen thalli causes a decrease in the content of C_a, C_b and total chlorophyll. According to Wang et al. (2021), C_a, C_b, and total chlorophyll decrease under lead stress. The decrease in chlorophyll content and $C_{a/b}$ ratio are apparent markers of degradation by senescence or stress (Aboal, Couto, Fernndáez, & Carballeira, 2008). Using treatment with $Pb(NO_3)_2$ concentrations for 24, 48 and 96 hours of *X. parietina* thalli, in addition to control test, quantification of total chlorophyll content was done as a general indicator of lead stress. The decrease in the content of total chlorophyll after 96 hours of treatment of *X. parietina* thalli was taken as a direct indicator of lead intracellular damage in long-term exposure. Abu-Muriefah (2015) shows that the treatment with lead had a considerable impact on the concentration of C_a. The levels of C_a decreased with increasing lead concentrations in solution. The lowest contents of this pigment were recorded in thalli treated with high concentrations of lead, implying that Pb had a negative impact on C_a content (Carreras & Pignata, 2007).

According to a study conducted by Sędzik, Smolik, and Krupa- Małkiewicz (2015), the application of lead nitrate causes a decrease in chlorophyll concentration in seedlings of diverse plant species. The study of Bajpai, Upreti, Nayaka, and Kumari (2010) who were interested in the toxic effect of heavy metals accumulation in lichens and higher plants, showed that heavy metals (Zn, Cd and Cu) even at very low concentrations cause physiological changes and inhibit photosynthesis, while lead causes a decrease in total chlorophyll and $C_{a/b}$ ratio. Our results show that chlorophyll b is more affected than chlorophyll a in thalli treated by $Pb(NO_3)_2$, this is the same result obtained by Purnama, Soedarti, and Purnobasuki (2015), which shows a significant influence of Pb on total chlorophyll in Seagrass ($p = 0.006^{**}$), even if, they indicate that chlorophyll b was more affected than chlorophyll a as result of lead effect.

Effect of lead on proteins

Plants for reconstruction, tolerance, resistance and response to stressful conditions change the protein content (Firuzeh, Khavari-Nejad, Najafi, & Saadatmand, 2015; Amnan, Aizat, Khaidizar, & Tan, 2022). Indeed, the plant changes its protein content to adapt or react against an abiotic stimulus (Nouri, Moumeni, & Komatsu, 2015). Our results show that lead causes the decrease of the soluble protein content, and it is concomitant with the result obtained by Esposito, Sorbo, Conte, and Basile (2012) and Chetia, Gogoi, Gogoi, and Yasmin (2021). This decrease is a function of the increase in lead concentration. In addition, Khan et al. (2021) has indicated that the reduction in the total protein content was observed in cultivated rice in lead contaminated soil.

On the other hand, treatment with 0.3 and 0.6 g L^{-1} lead acetate, resulted in an increase in total protein content in *Triticum durum* leaves and roots (Souahi, Chebout, Akrout, Massaoud, & Gacem, 2021).

Effect of lead on catalase activity

The increased activity of catalase is due to the production of reactive oxygen species (ROS) caused by pollutants. Our results show that lead causes an increase of catalase activity, in agreement with the results obtained by AL-Zurfi, AL-Tabatabai, and Abojassim (2021), which indicate that the plant *Hydrilla verticillata* reacts against cadmium stress by steadily increasing the catalase enzyme content. In addition, several other studies confirm that heavy metals cause oxidative can stress in biomarkers (Taiwo, Henry, Imbufe, & Adetoro, 2014; Batool et al., 2021; Ullah et al., 2021). Plants use catalase to limit and eliminate oxidative harm due to ROS (Lei, Rossi, & Huang, 2022). Emamverdian, Ding, Mokhberdoran, and Xie (2015), report that some antioxidant components such as catalase are likely to act in an integrated manner at excessive levels of heavy metals, and raise plant tolerance to heavy metals stress.

Our results are similar to the results obtained by Abu-Muriefah (2015) and Khan et al. (2021) who indicate that catalase increases significantly in lead stressed plants. Differing from the results obtained by Orabi, Dawood, and Salman (2015), which indicate that the excess of H_2O_2 causes a decrease in the activity of catalase, our results show that the accumulation of H_2O_2 causes an increase in the activity of the catalase.

Effect of lead on hydrogen peroxide

H_2O_2 is one of the ROS produced by plants in response to abiotic stress (Muneer, Kim, Choi, Lee, & Lee, 2014; Qi et al., 2018; Zhang et al., 2022). The hydrogen peroxide performs a crucial role in the transfer of a signal all through abiotic stress in plants (Niu & Liao, 2016). The H_2O_2 is also vital for stressful tolerance conditions in plants (Černý, Habánová, Berka, Luklová, & Brzobohatý, 2018). Our results show that the lichen stressed by lead products H_2O_2, was also obtained by Sofo, Scopa, Nuzzaci, and Vitti (2015) who found that the production of H_2O_2 is considered as a stress marker.

Our results are consistent with those obtained by Panda (2007), who studied the effect of chromium on rice, and who found that this pollutant produced H_2O_2 and this production is proportional to the exposure time and to the pollutant concentration. Our results are also similar to those obtained by Liu et al. (2021), which show that lead causes the increase of H_2O_2 in edible amaranth when lead from soils and atmospheric stresses are present, and Li et al. (2022), who discovered higher levels of H_2O_2 in response to high cadmium concentrations.

Effect of lead on reduced glutathione

The results obtained for glutathione assay are in agreement with those obtained by Sanità di Toppi et al. (2008), who reported that lichens used glutathione in detoxification; the low levels of glutathione are observed in the controls. Our results show that lead causes a gradual decrease in GSH levels in treated lichens with highest concentrations (5 mM and 10 mM). According to Shelly and Lu (2013), the availability of the sulfur amino acid precursor cysteine, as well as the activity of two enzymes glutamate cysteine ligase and GSH synthetase, is the primary determinants of GSH production, so we can say that the decrease in the GSH content is mostly due to an inactivation of enzymes involved in GSH biosynthesis. Our results are also similar to the results obtained by Cao, Ma, and Tu (2004) and Freeman et al. (2004), who demonstrated that the level of GSH decreases with higher tolerance to pollutants at low concentrations.

The opposite was obtained by Cempírková, and Večeřová (2018), which indicate that no light treatment changed the total GSH content with various levels of irradiance for several periods of time in comparison to the control test, whereas, this content varied depending on the species. High concentrations of pollutants affect the detoxification system which explains the total degradation of glutathione. Chaabene et al. (2018) indicate that in plants exposed to copper, antioxidant enzyme activity increases as copper concentrations rise, while, at high concentrations, the enzymatic defense system is disturbed and deregulated, which causes a decrease in enzyme activity. Liu et al. (2021) also indicate that the antioxidant enzyme activity can be significantly reduced by high levels of lead in plants. Our results are similar to those obtained by Sofo et al. (2015) which indicated that elevated and non-metabolized cellular hydrogen peroxide due to stress conditions can cause severe damage to biomolecules such glutathione. They are also concomitant with those found by Li et al. (2015) which reported that increased heavy metal concentrations resulted in a considerable reduction in GSH content in the roots and leaves of *Safflower* plants (*Carthamus tinctorius L.)*.

Conclusion

The results of the current study revealed that treating *X. parietina* thalli with $Pb(NO_3)_2$ solutions generated lead accumulation, which influenced several parameters. Accumulated lead resulted in the reduction of chlorophyll and proteins, as well as an increase in catalase activity, H_2O_2 and glutathione levels. We may deduce that lichen used catalase and GSH as detoxification mechanisms in response to lead stress, but that the high concentration of lead disturbed the detoxification system, resulting in total glutathione decomposition. This lichen can be used as a tool for monitoring environmental pollution. Further studies will be very effective in order to specify the site of action of lead at the cellular, intracellular and molecular level, as well as to look for other molecules developed by this lichen to combat stressful conditions.

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