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Research Article

POLYPHENOLS AND ANTIBACTERIAL ACTIVITY OF *XANTHORIA PARIETINA* (L.) Th. Fr. METHANOL EXTRACTS UNDER LEAD STRESS

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ABSTRACT. The main objective of this study was to investigate the variations in the content of polyphenols and flavonoids in lead-stressed *X. parietina* (L.) Th. Fr. lichen and to study the antibacterial activity of its methanol extract, Lichen thalli have been incubated at lead concentrations of 0, 0.5, 1.0, 5.0 and 10.0 mM for 96 hours. The antibacterial activity of methanol extract was evaluated against three Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*) and five Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) The analysis of the obtained results data showed that *X. parietina* is able to accumulate lead correlating with $Pb(NO_3)_2$ availability in the substrate, it also highlight that lead-induced stress causes significant increase in polyphenol and flavonoid contents with increasing $Pb(NO_3)_2$ concentrations, but with high concentrations, polyphenol and flavonoid contents decrease . Furthermore, results show a positive correlation between the polyphenol, flavonoid contents and the variations of the size of the inhibition zone diameter. Based on these results, Gram-negative bacteria were shown to be more resistant to the extracts than Gram-positive- bacteria.

Keywords: *Antibacterial activity, flavonoids, lead, lichen, polyphenols.*

INTRODUCTION

Lichens are symbiotic organisms associating a mushroom called mycobiont, that derive fixed carbon from green algae and/or cyanobacteria called photobionts [1, 2, 3, 4]. They have been used for several decades to assess the quality of the air [5, 6, 7, 8, 9, 10, 11, 12, 13]. Lichens can also considered as a source of original compounds, and more particularly bioactive secondary metabolites [14], where, they can be used for their antimicrobial [15, 16, 17] and antioxidant activity [18, 19, 20]. Among them, polyphenols are known to be a large group of pharmacologically active compounds [21]. In plants, polyphenols are essential for growth, nutrition, survival, and defenses [22]. As they participate very effectively in the tolerance of plants to different stresses [23, 24, 25]. Currently, in addition to its uses, lichens are used in the treatment of various human pathologies particularly, as an anti-cancer activity [26, 27, 28, 29].

Polyphenols, like all other plant elements such as chlorophyll, react to contaminants in the atmosphere in varying degrees. Plants employ phenolic chemicals to protect themselves from oxidative stress caused by numerous contaminants in the air [30]. Plant polyphenols have the characteristic of having a natural antioxidant activity and being weakly toxic. Furthermore, due

to their particular chemical molecular structure, they can chelate lead and hence resist lead toxicity [31].

The aim of this research is to investigate the effects of lead on polyphenol content in the lichen *Xanthoria parietina*, as well as to test the antibacterial activity of its methanol extracts in vitro.

MATERIALS AND METHODS

Lichen material

Lichen thalli samples of *X. parietina* were gathered in the Beni Metrane region, south of Jijel (Algeria) in February-March 2019. After collection, samples were transferred to the laboratory in clean closed boxes, impurities were removed, and samples were washed three times for five seconds with distilled water to remove superficial dust and adherent particles. In each experimental vessel, fresh weights of thalli were isolated and acclimatized to laboratory conditions until analysis.

Lead treatments

In comparison to distilled water, the lichen thalli of *X. parietina* were incubated in 100 ml of lead nitrates (0.5, 1, 5, and 10 mM) at room temperature. H_2SO_4 or HNO_3 were added to the solutions immediatly before treatment to adjust pH to 3.5. A 96-hour lead stress was applied. The samples were rinsed three times for five seconds with distilled water after treatment and before each assay and dried at room temperature [32].

Lead analysis

Flame atomic absorption spectrometry was used to determine lead levels. Lichen thalli were dried at 90 °C for 24 hours, then digested in 3 ml of concentrated HNO_3 and H_2O_2 (2:1, v/v) for 48 hours [33]. Residues were filtered through Whatman Filter paper no. 42, then deionized water was added to bring the content to 10 ml. The Flame Atomic Absorption Spectrophotometer 6200 (SHIMADZU) was used to test lead. Deionized water was used to make the working standard from the internal stock. Results were expressed in μg g-1 (dry weight).

Phenolic compounds extraction

Several organic solvents can be used for the extraction of phenolic compounds such as acetone, ethanol, and methanol [34]. Methanol is the best solvent because it provides better extraction efficiency and has the advantage of being easier to remove [35] and when mixed with water (80%), it promotes good extraction of polyphenols [36, 37].

The total extract is obtained by adding approximately 15 g of the ground lichen to 60 ml of methanol (80%). The mixture obtained is then subjected to continuous stirring in the absence of light in order to avoid oxidation phenomena for 48 hours at room temperature. After maceration, the solutions were filtered with filter paper.

The methanol phases of each sample were evaporated using a Heidolph type rotavapor and then dried at 40 °C until the methanol had completely evaporated. The mass of the residue obtained after evaporation of the solvent is extracted with crude methanol. The extracts obtained are stored in the freezer at -20 °C until they have been used.

Determination of total soluble phenolic contents

Total soluble phenolic contents in extracts of *X. parietina* were determined according to the method of Slinkard and Singleton [38] using gallique acid as standard. To 1 ml of the methanol extract, 1 ml of the Folin-Ciocâlteu reagent was added, after 5 min, 1 ml of Na_2CO_3 (20 g/l) was added, the mixture was then incubated at room temperature for 2 hours, the absorbance was measured at 760 nm. The results are thus expressed in mg gallic acid equivalent per 1 g dry weight (mg GAEPGDW) of the extract using the equation obtained from the calibration curve established by gallic acid (y=6,574 x, R^2=0.99).

Determination of total flavonoid contents

Flavonoids were determined using the method of Meda et al. [39]. 2 ml of the extract was mixed with 2 ml of aluminum trichloride ($AlCl_3$), the mixture was incubated for 10 min at room temperature, and absorbance was measured at 415 nm against a blank sample using a spectrophotometer. The concentration of the total flavonoids was expressed as Quercetin equivalents per g dry weight (QEPGDW) of extract using the equation obtained from reference to standard curve (y=31.68 x, R^2=0.99).

Antibacterial activity of methanol extracts

Strains tested

The American Type Culture Collection (ATCC) provided all of the microorganism strains. The strains tested are part of two bacterial groups: positive Gram represented by *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 15313, and negative Gram represented by *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhimurium* ATCC 25842, *Pseudomonas aeruginosa* ATCC 27853, and *Enterobacter spp.* ATCC 25639. These bacteria are first incubated in a nutrient broth at 37 ° C for 18 h. They are then seeded by the quadrant method on nutrient agar so as to obtain well-isolated colonies [40].

Inoculum preparation

Each bacterial suspension was prepared in 9 ml of saline water (NaCl 0.9%) by diluting a colony of the concerned strain from a fresh culture. The opacity of the suspension should be equivalent to 0.5 according to the standard McFarland method with around 10^8 CFU/ml (CFU=colony-forming unit), the inoculum should be used within 15 minutes of its preparation [41].

Antibacterial activity test

To test in vitro the antibacterial activity of methanol extracts of lichen *X. parietina* treated with increasing concentrations of lead (MEXTL), an adapted disc diffusion method on Mueller Hinton agar was used [41]. The supercooled culture medium is poured into Petri dishes with a diameter of 90 mm and a thickness of 4 mm. After solidification, the inoculation of each inoculum is carried out under rigorous aseptic conditions by rubbing the surface of the medium with sterile swabs soaked in the bacterial suspensions. The whole of the agar surface must be seeded from bottom to top, in tight streaks, we repeated the operation three times turning the box of 60 ° each time. Sterile disks of Whatman No. 1 paper were then deposited on the surface of the Mueller Hinton agar in each seeded box, each disk was then filled with a volume of 20 µl of the methanol extract from each concentration [42].

The negative control was filled with methanol, distilled water and different concentrations of the Pb $(NO_3)_2$ solution. Each test is repeated three times to obtain an average and to calculate a standard deviation. The Petri dishes are then closed and remain placed next to the Bunsen spout for 30 min for the diffusion of the methanol extracts before the bacterial growth, then placed in an oven at 37 °C for 18 to 24 hours [43].

At the exit of the oven, the absence of the bacterial growth results in a translucent halo (zone of inhibition) around each disc, identical to that of the sterile agar. Results were expressed as the size (mm) of the inhibition zone diameter (IZs).

Statistical analysis

Three repetitions were performed at each concentration, so we can calculate the standard deviation. The statistical study was performed using the ORIGIN 6.0 system using the test univariate variance (one way ANOVA). For the 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 $0.01<p<0.05$ (*), haut significant when $0.001<p<0.01$ (**) and very haut significant when $p<0.001$ (***).

Correlation matrices between the different parameters were analyzed by STATISTICA Version 10 software.

RESULTS AND DISCUSSION

Lead accumulation

Treatment with increasing concentrations of $Pb(NO_3)_2$ caused a general increasing accumulation of lead in *X. parietina* (Fig. 1), correlation matrices between lead accumulated and $Pb(NO_3)_2$ solutions are presented in Fig. 2.

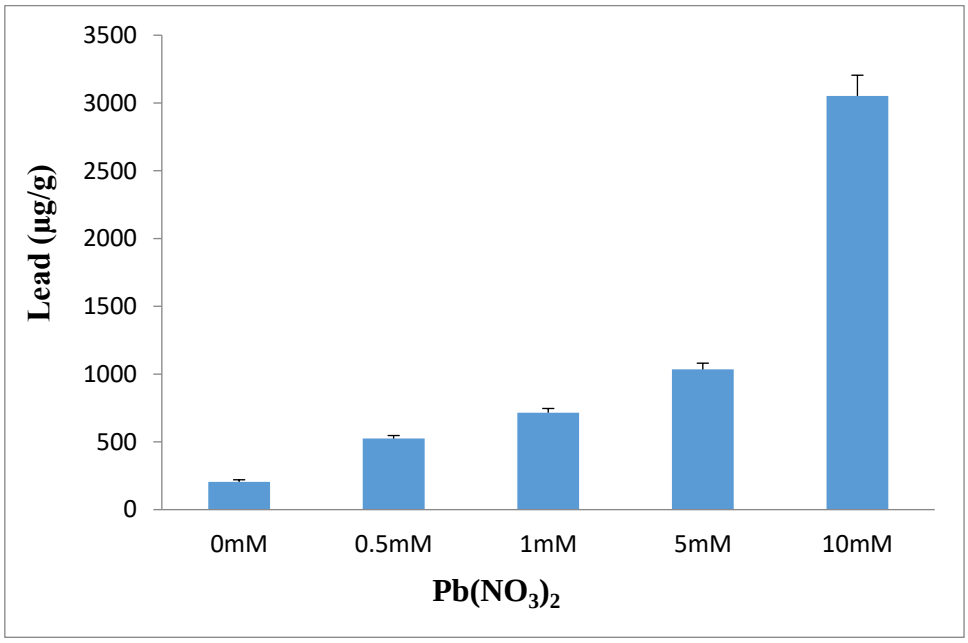

Fig. 1. *Lead accumulation in the lichen X. parietina after incubation of thalli for 96h in the presence of 0, 0.5, 1, 5 and 10 mM of Pb $(NO_3)_2$.*

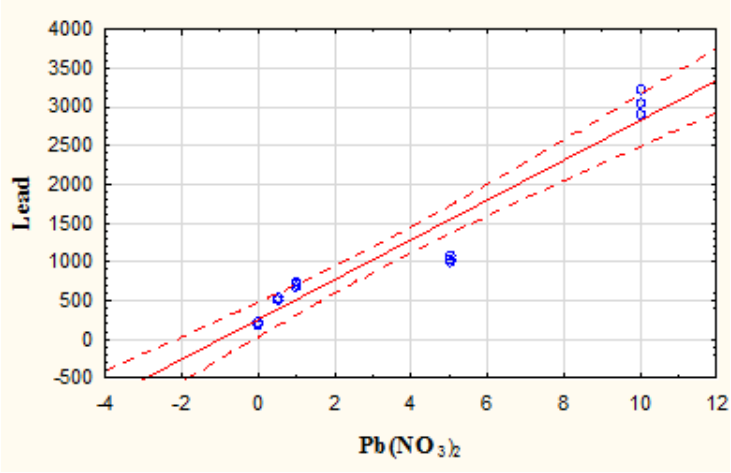

Fig.2. *Correlation matrices between accumulated lead in X. parietina and Pb $(NO_3)_2$, r=0.96122, p<0.001, Significance: ****

According to Fig. 1, the results show that *X. parietina* is able to accumulate lead from $Pb(NO_3)_2$ solutions, where there was a significant increase in accumulated lead with increasing $Pb(NO_3)_2$ concentrations (p=0.018*) with a maximum value of 3052 µg/g in thalli treated with 10 mM $Pb(NO_3)_2$ against 205.21 µg/g recorded in the control test. Fig. 2 shows a positive correlation between accumulated lead in *X. parietina* and $Pb(NO_3)_2$ solutions.

Total phenolic and flavonoid compounds

Results of the total phenolic and flavonoid compounds in the thalli of *X. parietina* treated by different concentrations of $Pb(NO_3)_2$ during 96 hours of treatment are presented in Fig. 3. Polyphenols/lead and flavonoids/lead correlation matrices are presented in Fig. 4 and Fig. 5 respectively.

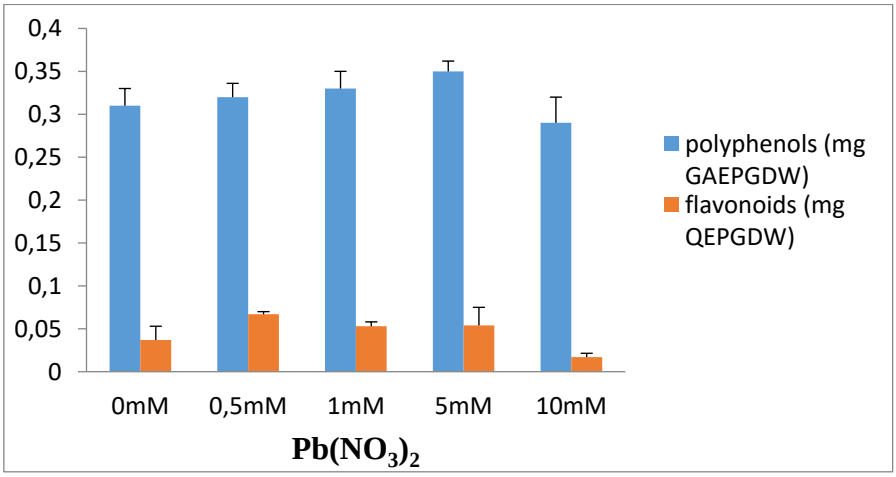

Fig. 3. *Total phenolic and flavonoid compounds of methanol extract in X. parietina after incubation of thalli for 96h in the presence of 0, 0.5, 1, 5 and 10 mM of Pb $(NO_3)_2$,*

mg GAEPGDW: gallic acid equivalent per g dry weight, mg QEPGDW: quercetin equivalent per g dry weight.

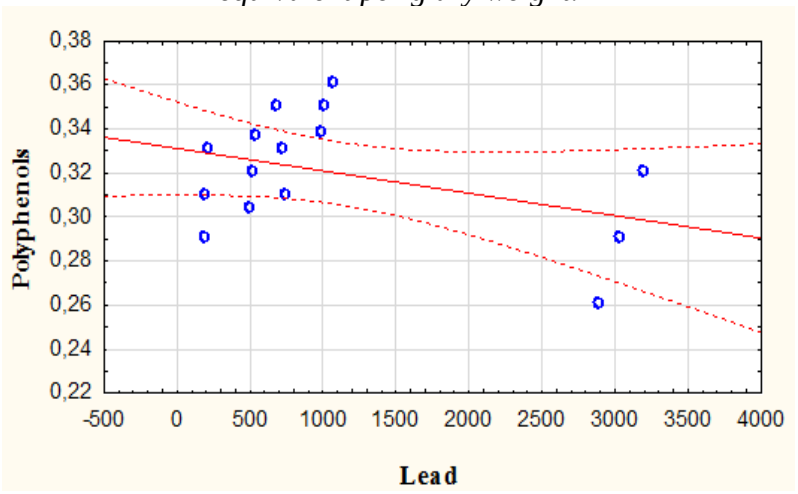

Fig.4. *Correlation matrices between total phenolic compounds and accumulated lead in X. parietina, r=- 0.39744, p=0.142374, Significance:* ^{NS}

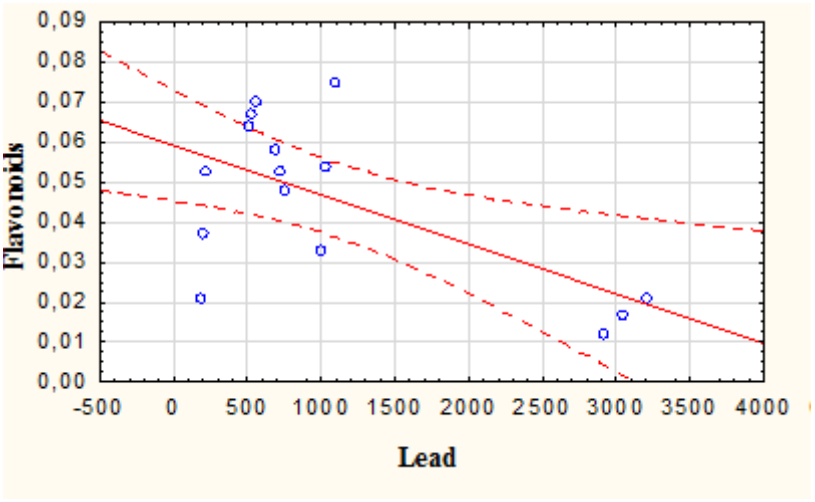

Fig.5. *Correlation matrices between flavonoids and accumulated lead in X. parietina, r =- 0.62677, p=0 .012402, Significance: **

From Fig. 3, the results show that the total phenolic compounds in *X. parietina* was 0.31 mg, this value is calculated in the control thalli treated with distilled water at T 0 (0h), in contrast, this value varies after 96 h in the thalli treated with different concentrations of $Pb(NO_3)_2$, where a significant increase was noted in the thalli treated with the concentrations 0.5, 1 and 5 mM of $Pb(NO_3)_2$, (p=0.04081*), beyond the concentration 5 mM and with the concentration 10 mM, the decrease of the polyphenol contents was not significant ($p>0.05^{NS}$).

A significant variation (p=0.00329**) in content of flavonoids was noted in thalli treated with various concentrations of Pb $(NO_3)_2$, the results show a remarkable increase

in thalli treated with the 0.5 mM of Pb $(NO_3)_2$. However, a significant decrease (p=0.0018**) in flavonoid contents was noted in the thalli treated with the other concentrations of Pb $(NO_3)_2$ (1, 5 and 10 mM). These results are confirmed by data from the statistical analyzes presented in Fig. 4 and Fig. 5, where a negative correlation was noted between polyphenol, flavonoid contents and accumulated lead in *X. parietina respectively.* Similarly, the results show that polyphenols/lead correlation was not significant, however flavonoids/lead correlation was significant.

Results of antibacterial activity

Results showing the effects of methanol extract of *X. parietina* treated with lead (MEXTL) on *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Enterobacter* spp. ATCC25639, *Klebsiella pneumoniae* ATCC700603, *Salmonella typhimurium* ATCC25842, *Bacillus cereus* ATCC 10876, *Listeria monocytogenes* ATCC15313 *and Staphylococcus aureus* ATCC25923 are shown in Fig. 6.

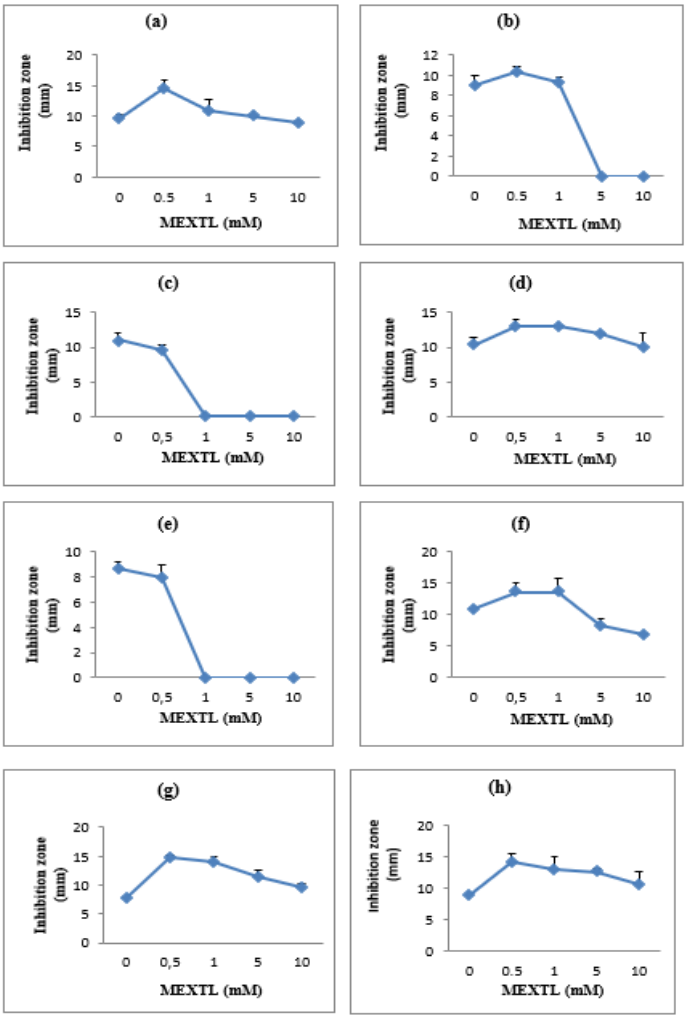

Fig. 6: *Antibacterial activity of methanol extracts of lichen X. parietina stressed by lead against different bacteria: (a) E. coli, (b) P. aeruginosa, (c) Enterobacter spp. (d) K. pneumoniae, (e) S. typhimurium, (f) B. cereus, (g) L. monocytogenes, (h) S. aureus. MEXTL: methanol extract of X. parietina treated with lead.*

According to the results given in Fig. 6, the same antibacterial effect of the extract (MEXTL) against *E. coli, B. cereus, L. monocytogenes* and *S. aureus* bacteria was noted. Against *P. aeruginosa*, only MEXTL 5 mM and 10 mM do not cause any antibacterial effect. Whereas, against *Enterobacter spp.* and *S. typhimurium* bacteria, a similar result was noted, only the MEXTL 0.5 mM has identical antibacterial activity to that obtained by the control. Against *K. pneumoniae* bacteria, variations in the antibacterial activity of MEXTL are negligible.

Against *E. coli* bacteria, it can be seen from Fig. 3(a) that the MEXTL 0.5 mM has a remarkable effect, with a highly significant increase (p=0.003**) of the size of the inhibition zone diameter (IZs=14.67 mm), however, the antibacterial activity decreases significantly (p=0.019*) in the thalli treated with MEXTL 1, 5 and 10 mM. Fig. 3(b) shows that the antibacterial activity of MEXTL against *P. aeruginosa* is similar between concentrations 0 and 0.5 mM, while, a significant decrease (p=0.02791*) in the antibacterial activity from 0.5 mM concentration was noted. From the data of Fig. 3(c) it has been noted that the decrease of the antibacterial activity against *Enterobacter spp.* is significant (p=0.00211**) with increasing concentrations of MEXTL, no activity was noted with MEXTL 1 mM, 5 mM and 10 mM.

Compared to the control (IZs = 10.33 mm), Fig. 3(d) shows a significant increase (p = 0.0007***) in antibacterial activity of MEXTL 0.5 mM against *K. pneumoniae* (IZs=13 mm), the same antibacterial effect was obtained to by MEXTL 1 mM with a slight reduction by MEXTL 5 mM, a significant decrease (p=0.01566*) of antibacterial activity was noted with MEXTL 10 mM.

Against *S. typhimurium* bacteria, Fig.3 (e) shows a significant decrease of antibacterial activity with increasing MEXTL concentrations (p=0.00251**). A total resistant of *S. typhimurium* was registered against MEXTL 1 mM, 5 mM and 10 mM. Fig. 3(f) shows that the antibacterial activity of MEXTL against *B. cereus* tends to increase significantly (p=0.00001***) to reach the maximum with MEXTL 0.5 mM and 1 mM. A significant decrease (p=0.02144*) should be observed with MEXTL 5 mM and 10 mM.

According to Fig. 3 (g, h), the same result was observed in *L. monocytogenes* and *S. aureus,* with a significant increase in the antibacterial activity of MEXTL 0.5 mM in comparison with the control (p=0.02* and p=0.004** respectively**)**. A significant decrease (p = 0.007** and p=0.0089**) in antibacterial activity against *L. monocytogenes* and *S. aureus* was noted by the other concentrations of the extract (MEXTL 1 mM, MEXTL 5 mM and MEXTL 10 mM) respectively.

The negative control (methanol, distilled water, various concentrations of Pb ($[NO_3]_2$) did not have any inhibitory activity on any bacteria (IZs=0 mm).

Polyphenols/antibacterial activity and flavonoids/antibacterial activity correlation matrices are presented in Tables 1 and 2 respectively.

From the data presented in Table 1, the statistical analysis results showed a positive correlation between the polyphenol contents and the antibacterial activity against *E. coli, P. aeruginosa, K. pneumoniae, B. cereus, L. monocytogenes* and *S. aureus*, however, a negative correlation was noted against *Enterobacter spp.* and *S. typhimurium*. Only, a significant correlation was noted between polyphenols and antibacterial activity against *S. aureus*.

The statistical analysis results presented in Table 2 showed a positive correlation between the flavonoid contents and the antibacterial activity against all studied strains except for the negative correlation obtained with *S. typhimurium.* The results also show a

significant correlation between flavonoids and antibacterial activity against *E. coli, B. cereus, L. monocytogenes* and *S. aureus.*

Table 1. *Correlation matrices between antibacterial activity and total phenolic contents in X. parietina.*

Correlation matrices	*r*	*p*	Significance
Polyphenols / *E. coli* IZs	0.31132	0.25868	NS
Polyphenols / *P. aeruginosa* IZs	0,01132	0.96805	NS
Polyphenols / *Enterobacter spp.* IZs	- 0,13514	0.63108	NS
Polyphenols *K. pneumoniae* IZs	0,36287	0.18373	NS
Polyphenols / *S. typhimurium* IZs	- 0,16468	0.55753	NS
Polyphenols / *B. cereus* IZs	0.33484	0 .22248	NS
Polyphenols / *L. monocytogenes* IZs	0.41141	0.12761	NS
Polyphenols / *S. aureus* IZs	0.65596	0.00792	**

Table 2. *Correlation matrices between antibacterial activity and flavonoid contents in X. parietina.*

Correlation	*r*	*p*	Significance
Flavonoids / *E. coli* IZs	0.71298	0.00284	**
Flavonoids / *P. aeruginosa* IZs	0.41610	0.12290	NS
Flavonoids / *Enterobacter spp.* IZs	0.25209	0.36472	NS
Flavonoids *K. pneumoniae* IZs	0.51179	0.05115	NS
Flavonoids / *S. typhimurium* IZs	0.24803	0.37274	NS
Flavonoids / *B. cereus* IZs	0.69038	0.00438	**
Flavonoids / *L. monocytogenes* IZs	0.69477	0.004	**
Flavonoids / *S. aureus* IZs	0.67523	0.00574	**

Our results reveal that *X. parietina* may accumulate lead correlating with $Pb(NO_3)_2$ availability in the substrate (r=0.96122, p <0.001***). The same results was obtained by Caggiano et al. [44], Darnajoux et al. [45], Belguidoum et al. [46], Sujetovienė and Česynaitė [47] who indicate that lichens accumulate metals from their environment.

Lichen extract is usually attributed to the presence of phenolic compounds. We confirmed the presence of these compounds in these extracts. The total content of phenolic compounds was estimated by 0.31 mg/g of dry weight of extract, expressed in equivalents of gallic acid.

Our results show that lead-induced stress causes significant increase in polyphenol and flavonoid contents with increasing Pb $(NO_3)_2$ concentrations, these results are in agreement with results obtained by Khedim et al. [48] which showed an increase in the total polyphenols and flavonoids in *Atriplex canescens*, depending on the increasing concentration of heavy metals (zinc, lead and cadmium). The same results was also obtained by Ren et al. [49] and Kiani et al. [23] who show that polyphenols are accumulated by plants as a defense mechanism against stress. In a study carried out by Kısa et al. [50], it was discovered that applying Cd, Cu, and Pb to *Zea mays* increased total phenolics in all treatments when compared to control groups. Likewise, Harangozo et al. [51] show that higher levels of polyphenol compounds in flax seeds due to increased

doses of lead. Benhabiles et al. [52] show also that cadmium induced an increase in total phenolic contents. Another study carried out by Mamat et al. [53] showed that high concentrations of copper increase the activity of cytochrome c oxidase in cells which produces more phenolic compounds. Lichens increase the synthesis of polyphenols and flavonoids for use in the phenomenon of detoxification and biodegradation of xenobiotics [54].

According to our results, the polyphenol content of thalli stressed by high concentrations of $Pb(NO_3)_2$ (10 mM) did not significantly decrease ($p > 0.05^{NS}$), while all concentrations of $Pb(NO_3)_2$ except 0.5 mM, cause a significant decrease in the content of flavonoids, these results are justified by the statistical data presented in Fig. 4 and Fig. 5; lead-induced stress causes not significant decrease in polyphenol contents and significant decrease in flavonoid contents correlating with $Pb(NO_3)_2$ concentrations (r =-0.39744, p=0 .14237^{NS}; r =-0.62677, p = 0 .0124* respectively). In the biological system, oxidative stress results from an imbalance between the formation of reactive oxygen species (ROS) and the decrease in polyphenol and flavonoid levels in stressed lichens. Because of the disruption of secondary metabolism and the destruction of the antioxidant defense system, these quantities decrease, according to Sharma et al. [55] and Pizzino et al. [56]. Heavy metals contribute to a significant increase in the generation of ROS, resulting in an imbalance that leads to cell and tissue damage. According to Kısa [57], the excessive accumulation of heavy metals in tomato cultivated under heavy metal-induced stress induces a reduction in (ascorbate peroxidase) APX, (peroxidase) POD, and (superoxide dismutase) SOD activities. The decrease caused by high lead concentrations in polyphenol contents in *X. parietina* can be explained by the result of lead-induced the over production of free radical.

Our results imply that *X. parietina* could be exploited as a natural antibacterial agent source, this result is in agreement with those of Basile et al. [58] and Alqahtani et al. [59], the same results was obtained by Ranković et al. [60] with *Hypogymnia physodes* lichen. From the results obtained, it has been found that the methanol extracts of lead-treated lichen cause an increase of the size of the inhibition zone diameter of the studied bacteria; this increase can be explained by the increase of the polyphenol and flavonoid levels. Our results are in agreement with those of Coppo and Marchese [61] which show that polyphenols represent a possible source of antimicrobial agents and those of Akpinar et al. [62], Devi et al. [43], Alqahtani et al. [59] and Popovici et al. [63] which indicate that methanol extracts of lichens have antibacterial activity on several bacteria.

According to the results obtained it was noticed that the methanol extracts of the lichen treated by the high concentrations of lead cause the decrease of the size of the inhibition zone diameter in the studied bacteria. This decrease can be explained by the decrease in polyphenol and flavonoid levels under lead stress, where we found a positive correlation between the size of inhibition zone diameter and polyphenol, flavonoid contents.

From the statistical analysis results presented in Tables 1 and 2, in the majority of cases the increase and the decrease in the size of the inhibition zone diameter are positively correlated with polyphenol and flavonoid contents, we found a significant correlation between polyphenols/*S. aureus* IZs (r = 0.65596, p = 0.00792**), flavonoids/*E.coli* IZs (r=0.71298, p=0.00284**), flavonoids/*B. cereus* IZs (r = 0.69038, p = 0.00438**), flavonoids/*L. monocytogene*s IZs (r=0.69477, p=0.004**) and flavonoids/*S. aureus* IZs (r=0.67523, p=0.00574**).

Based on our results, the extracts were found to be more active against Gram-positive bacteria (*B. cereus, L. monocytogenes, S. aureus*) than the other Gram-negative bacteria

(*S. typhimurium, P. aeruginosa, and Enterobacter spp.*) whose most sensitive strains are *B. cereus* (IZs=10.83–13.67 mm), *L. monocytogenes* (IZs =7.67–14.67 mm) and *S. aureus* (IZs=9 –14.33 mm), while the most resistant strains are *S. typhimurium* and *Enterobacter spp.* (IZs=0 mm) against MEXTL 1, 5 and 10 mM. When Gram-positive bacteria are compared to Gram-negative bacteria, our results show that methanol extract has a significantly higher antibacterial action against Gram-positive bacteria (p=0.001***). The same result was obtained by Alghazeer et al. [64] who indicate that the methanol extracts of marine green showed a significant antibacterial activity against Gram-positive (*S. aureus, B. subtilis, Bacillus spp*., and *S. epidermidis*) as well as Gram negative bacteria (*E. coli, S. typhi, Klebsiella spp*., and *P. aeruginosa*), and by Chen et al. [65] who studied the antibacterial effect of polyphenol extract from fresh sweet sorghum stems against *S. aureus, E. coli, Listeria spp.* and *Salmonella spp.* and who concluded that its effect on Gram-positive bacteria is significantly higher than the effect on Gram-negative bacteria. The reason of this difference may be the presence of the outer membrane in Gram-negative bacteria which is totally absent in Gram-positive bacteria [66].

Among Gram-negative bacteria, our results show that *E. coli* and *K. pneumoniae* offer significant sensitivity to MEXTL 0.5 mM (IZs=9.67 mm–14.67 mm and IZs=10.33 mm – 13 mm respectively). The same result was obtained by Aghraz et al. [67], who show that the polyphenol extracts from *Cladanthus arabicus* and *Bubonium imbricatum* exert strong antibacterial activity in vitro, especially against *E. coli*.This result allowed us to put forward the hypothesis: in plants, inducing stress, increasing polyphenol contents, decreasing Gram-negative bacteria resistance.

CONCLUSION

The results of the present study showed that experimental exposure of *X. parietina* thalli to Pb $(NO_3)_2$ solutions caused lead accumulation which affects several parameters. Exposing thalli of the lichen *X. parietina* to Pb $(NO_3)_2$ solutions caused an increase in polyphenol and flavonoid contents but the high concentration of lead caused an imbalance of detoxification system which explains the decrease of polyphenol and flavonoid contents. Our results show also that methanol extracts of lead-stressed *X. parietina* lichen were found to be more active against Gram-positive bacteria (*B. cereus, L. monocytogenes* and *S. aureus*) than compared to other Gram-negative bacteria (*S. typhimurium, P. aeruginosa*, and *Enterobacter spp.*). Among Gram-negative bacteria, our results show that the most resistant strains are *S. typhimurium* and *Enterobacter spp.* however, *E. coli* and *K. pneumoniae* offer significant sensitivity to MEXTL 0.5 mM (IZs=9.67 mm–14.67 mm and IZs=10.33 mm–13 mm respectively).

In perspective, further work will be very important to study the accumulation of polyphenols in lichens under heavy metals stress and to specify their optimal concentrations at which the polyphenol content is the most important, and therefore the exploitation of polyphenols as antibacterial agents.

Conflict of Interest. The authors declared that there is no conflict of interest.

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