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Role of Lead on Chlorophylls and Soluble Proteins in Some Cultivars of *Triticum aestivum* L. under Osmotic Potential

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Abstract

The changes of chlorophylls and main metabolites in three cultivars of *Triticum aestivum* L. under the effects of osmotic and lead stresses, as well as their interaction, were investigated. The results indicated that, the decreased Ψ_s and Pb concentration caused an increase in the chlorophyll content in all plants. Conversely, the chl. *a*/*b* ratio was decreased. Apparently, the total soluble proteins were increased in response to low Ψ_s and Pb, except in roots of cv. Giza168 which were increased under high Pb concentrations. Statically, the Ψ_s had the predominant role on chlorophyll content, chl. *a*/*b* ratio and total soluble proteins in all investigated plants. The significant correlations between chlorophyll content and soluble proteins were negative.

Keywords

Osmotic Potential, Lead, Chlorophyll, Soluble Proteins, *Triticum aestivum* L., Interaction, Correlation

Subject Areas: **Plant Science, Soil Science**

1. Introduction

Photosynthesis is adversely affected by Pb, which could be due to metal-induced reductions in the levels of photosynthetic pigments [1], inhibition in the electron transport system [2], changes in the fine structure of chloroplasts [3]. Song *et al*. [4] found that, all genotypes exhibited gradually decreasing of chlorophyll content with the increase of Pb^{2+} concentration, indicating that greater concentrations of Pb^{2+} exerted relatively greater adverse effects on chlorophyll generation in seedling cells. Nevertheless, Pb is known to inhibit chlorophyll synthesis either due to impaired uptake of Mg^{2+} and Fe^{2+} by plants [5] or because of increased chlorophyllase

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activity [6]. Also, the severe reduction in the Chl. *a*, Chl. *b* and total chlorophyll content and increase in the Chl. *a*/*b* ratio under salinity stress may be due to an increase in chlorophyll degradation or to a decrease in chlorophyll synthesis [7].

Exposure of plants to excess salt causes ion imbalance and ion toxicity-induced imbalances in metabolism. These effects are more marked in the roots, which are likely the organs exposed to the highest lead concentrations [8]. Therefore, salt stress reduces protein synthesis, increases protein hydrolytic enzyme activity, decreases amino acid synthesis and interferes with tertiary and quaternary enzyme structures leading to decreases in soluble protein content [9]. It is found that, abiotic stresses including heavy metal can exert its effects on plants by resulting in changes of soluble protein content. A number of reports have showed negative effects of different heavy metals on the amount of soluble protein in plant seedlings [10]. Lamhamdi *et al*. [11] reported that, the increase of proteins under lead stress was possibly a result of the induction of stress proteins, which may comprise various antioxidant enzymes. This indicated that, this rise in the protein concentration could be also explained by the production of phytochelatins aimed to detoxify the high lead concentrations [12]. It alleviates metal toxicity by acting as a metal chelator and as a protein stabilizer [13].

The aim of this article is to understand the protective mechanisms of chlorophylls, and soluble proteins of *Triticum aestivum* L. cultivars (Sakha93, Jizan baladi, Giza168) under the osmotic potential (salinity), lead and their interaction stresses, and to know the correlations between the investigated parameters in response to the intrinsic conditions.

2. Materials and Methods

2.1. Preparation of Plants for Experimentation

For experimentation, three of *Triticum aestivum* L. of different osmotic stress tolerance were grown in wooden trays containing sawdust suitable for germination of seeds. Two cultivars (Sakha93 and Giza168) of experimental seeds were supplied by Crop Science Department of Agricultural Research Center, Dokki, Giza, Egypt and third cultivar (Jizan baladi) was supplied by the Ministry of Agricultural of Saudi Arabia.

Preliminary germination tests performed before experimentation indicated a high germination percentage, reaching about 100% in these seeds. In the preliminary germination test, a control experiment of the untreated seeds was carried out for comparing both the rate and the amount of germination. Glass Petri-dishes (11 cm diameter) were used for germination tests. Each dish contained ten seeds conveniently spaced over chemically pure filter paper. The filter paper which served as an embedding medium for germinating seeds was kept visibly moist during the test and addition of 15 mL of distilled water was enough to keep the filter paper visibly moist during the test. Ten days after seed germination, healthy seedlings were transferred to grow at optimum germination conditions (at 25˚C) in full strength hydroponic cultures, prepared according to Hoagland and Arnon [14], contained in plastic pots (three individuals/pot). The cultures were kept covered during the experimental periods to prevent direct evaporation in incubator with air circulation under light condition (supplied by 60 watt incandescent bulbs, yielding 1500 - 2000 lux at culture level just about the compensation point). The cultures were constantly aerated with humid air introduced pumped through fine capillary tubes. The culture solution was periodically replaced by draining through siphoning tubes kept in place throughout the experimental period.

2.2. Adjustment of Salinity Levels (Osmotic Potential, Ψ_s) and Lead (Pb) in the Culture Solution

Thirty-day-old plants were transferred into pure distilled water culture (expressing deficiency in macro-and micro-nutrient elements). The water content of each pot was treated with solutions of (NaCl + $CaCl_2$) in concentrations that yield different osmotic potentials (Ψ_s) and Pb in the culture solution: Ψ_s levels were chosen at 0 (control −0.3, −0.7 and −1.0 MPa). The sodium adsorption ratio (SAR) for each Ψ_s level was adjusted at level 12.5% of NaCl and $CaCl_2$ concentrations in solutions according to the calculations explained by El-Sharkawi [15].

Solutions having different osmotic potentials with Pb element (as $Pb(NO_3)_2$), were prepared by dissolving certain amounts of NaCl + $CaCl_2$ in Pb solution. The treatment solutions prepared thus are of certain levels of treatment combinations. At the same levels of osmotic potential. (Ψ_s + Pb) another series of Pb solutions (0, 2, 5 and 10 ppm) for each cultivar were prepared.

2.3. Preparation of Plant Extracts for Analysis

At the end of experimentation (37-day-old plants), average fresh weights of roots and shoots were immediately recorded. For extraction, it is important to freeze the tissues in liquid nitrogen immediately after detaching the tissues from the plants and grind the tissues into powder with a mortar. A known weight of powder sample was rapidly blended with 10 cm^3 of ice cold distilled water. The suspension was quantitatively transferred to centrifuge tubes and decanting the residue with distilled water. Centrifugation at 7000 rpm was carried out for 15 min. After the centrifugation, the supernatant was then transferred to 25 mL Erlenmeyer flask and the supernatant was kept in deep freeze until analysis.

2.4. Determination of Chlorophyll Content

At the same time, chlorophyll *a* and chlorophyll *b* were extracted from the leaves of *Triticum* cultivars by using 85% acetone. The calculation of chlorophyll content ($mg \cdot g^{-1}$ fresh weight) and chlorophyll *a/b* ratio were carried according to Lichtenthaler [16].

2.5. Determination of Water Soluble Proteins

The content of each metabolite in the extract of different cultivar organs of experimental plants is expressed in $mg \cdot g^{-1}$ dry weight by using the spectrophotometer *UNICAM* model *UV-Vis* spectrometry (made in England). Total soluble proteins were determined by using Lowry solutions with Folin reagent. The concentration was calculated using a calibration curve made with bovine serum albumin and measured at 750 nm according to Lowry *et al.* [17].

2.6. Statistical Analysis of Data

Statistical inference necessary to evaluate the significance of effects and the relative roles of the single factors: lead, Ψ_s and their interaction in the total response to different treatment combinations included analyses of variance (F values) and coefficient of determination (η^2), as well as simple linear correlation coefficient (r) [18]. The latter (η^2) is a statistic used to evaluate the relative role (share) of single factors, as well as their mutual interactions in contributing to the total effect of treatment (combination) usually expressed as a percentage or fraction [19]. All these analyses were computerized by using the SPSS program.

3. Results

1) Effect of osmotic potential (Ψ_s) and Pb on the chlorophyll characteristics

In the studied plants the changes of chlorophyll *a*, chlorophyll *b* and chlorophyll *a*/*b* ratio under the effect of Ψ_s and Pb were shown (**Figures 1-4**).

a) Chlorophyll (Chl.) content

Apparently, the Chl. *a* consent in all plants was produced a higher values than the Chl. *b* content. The maximum values (3.5, 3.4 & 2.4 $mg \cdot g^{-1}$ fresh weight) were recorded in Giza168, Sakha93 and Jizan baladi, respectively at lowest Ψ_s (−1.0 MPa) in the absence of Pb (**Figure 1**). Whereas, the minimum Chl. *a* contents (0.9, 1.4 and 0.7 $mg \cdot g^{-1}$ fresh weight, respectively) were existed in unstressed plants at low Pb concentration (2 ppm). At the same Pb concentrations, the Chl. *a* content of Sakha93 cultivar was increased at Ψ_s = −0.3 MPa. Commonly, the presence of Pb were induced the Chl. *a* content in plants under high water potential and *vice versa*.

The behavior of Chl. *b* content in plants was similar to Chl. *a* (**Figure 2**). In both Giza168 and Jizan cultivars, a high Chl. *b* contents (2.1 & 1.2 $mg \cdot g^{-1}$ fresh weight) were recorded at Ψ_s = −1.0 MPa in the absence of Pb. The same was true in case of Sakha93 plants. Whereas, the treated plants of Sakha93 with Pb 2 ppm and Ψ_s = −0.3 MPa increased the Chl. *b* content. Meanwhile, the Chl. *b* in Giza168 and Jizan cultivars was the lowest under Pb = 2 ppm and Ψ_s = 0 MPa, the absence of Pb at the same Ψ_s level produced a low Chl. *b* content in Sakha93, as well as the same was existed at Ψ_s = −0.7 MPa. The total chlorophyll (Chl. *a* + Chl. *b*) in the three cultivars exhibited a high values under lowest Ψ_s level (−1.0 MPa) in the absence of Pb, likewise at Pb = 2 ppm with Ψ_s = −0.3 MPa in case of Sakha93. The minimum values (1.4; 2.2 & 1.0 $mg \cdot g^{-1}$ fresh weight) of total Chl. In Giza168, Sakha93 and Jizan baladi, respectively was found under low Pb (2 ppm) concentration with non-stressed plants (**Figure 3**).

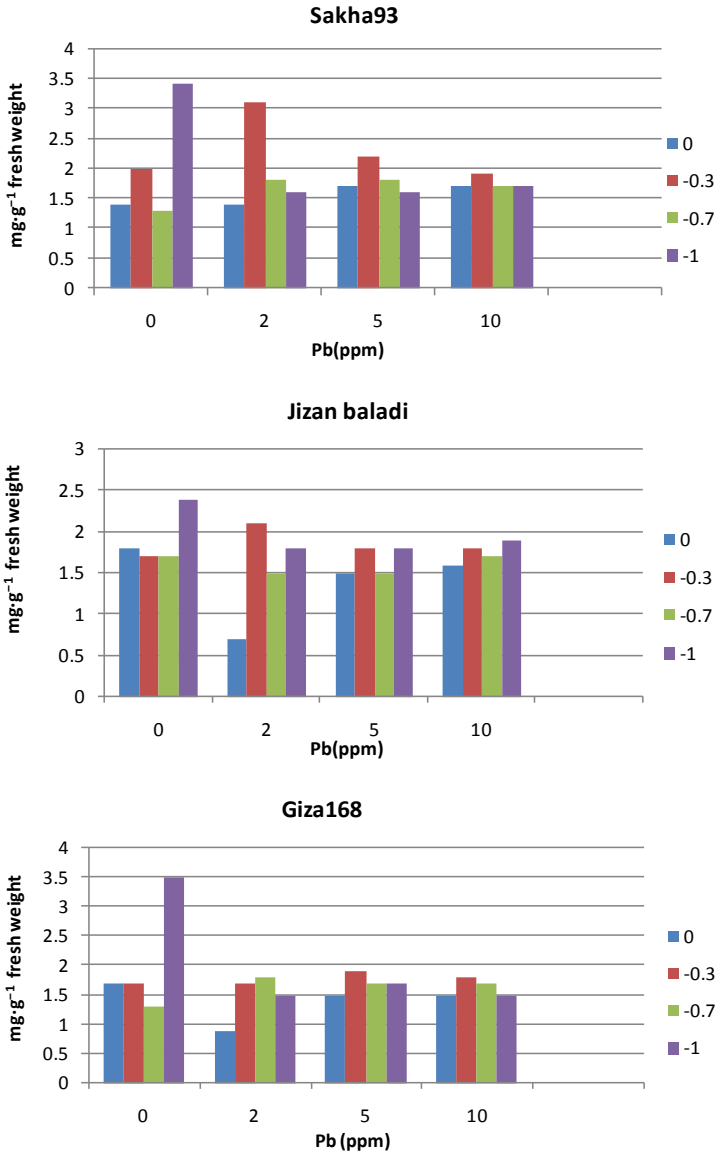

Figure 1. Chlorophyll *a* content in cultivars of *Triticum aestivum* L. at different Pb conc. and osmotic potential Ψ_s levels.

b) Chlorophyll *a/b* ratio

The chlorophyll *a/b* ratio in leaves of tested cultivars were varied (**Figure 4**). The Chl. *a/b* of fresh leaves ranges from 1.66 to 2.23 in Giza168; from 1.47 to 2.16 in Sakha93 and from 1.89 to 2.38 in Jizan cultivars. The proportion of Chl. *a* to Chl. *b* greatly depends on the Chl. content of the cultivar under different Ψ_s and Pb levels. Therefore, the decreased Chl. *a/b* ratio corresponding to the relatively high Chl. *b* content at −0.3 MPa and Pb 2 ppm in Sakha93 and Jizan plants and shifted to low Ψ_s (−1.0 MPa) in the absence of Pb. Whereas the high Chl. *a/b* ratio was detected in unstressed Giza168 plants at Pb = 0 ppm, this high ratio in Jizan plant was shifted to lowest Ψ_s at different Pb levels. This reflect the interactive effects of both Ψ_s and Pb on the Chl. *a* and Chl. *b* formation in the studied *Triticum* cultivars.

The effects of Ψ_s, Pb and the interaction (Pb × Ψ_s) on the Chl. *a*, Chl. *b* and Chl. *a/b* ratio in Sakha93 were significant except Pb on the Chl. *a*. In both Jizan and Giza168, the effect of Ψ_s was significant on Chl. *a*, Chl. *b* and Chl. *a/b* ratio, as well as the effect of the (Pb × Ψ_s) interaction on the Chl. *a/b* ratio. In Jizan only Pb had a significant effect on Chl. *a* (**Table 1**). The effect of Ψ_s was dominant on Chl. *a*, *b* contents and *a/b* ratio (η^2 =

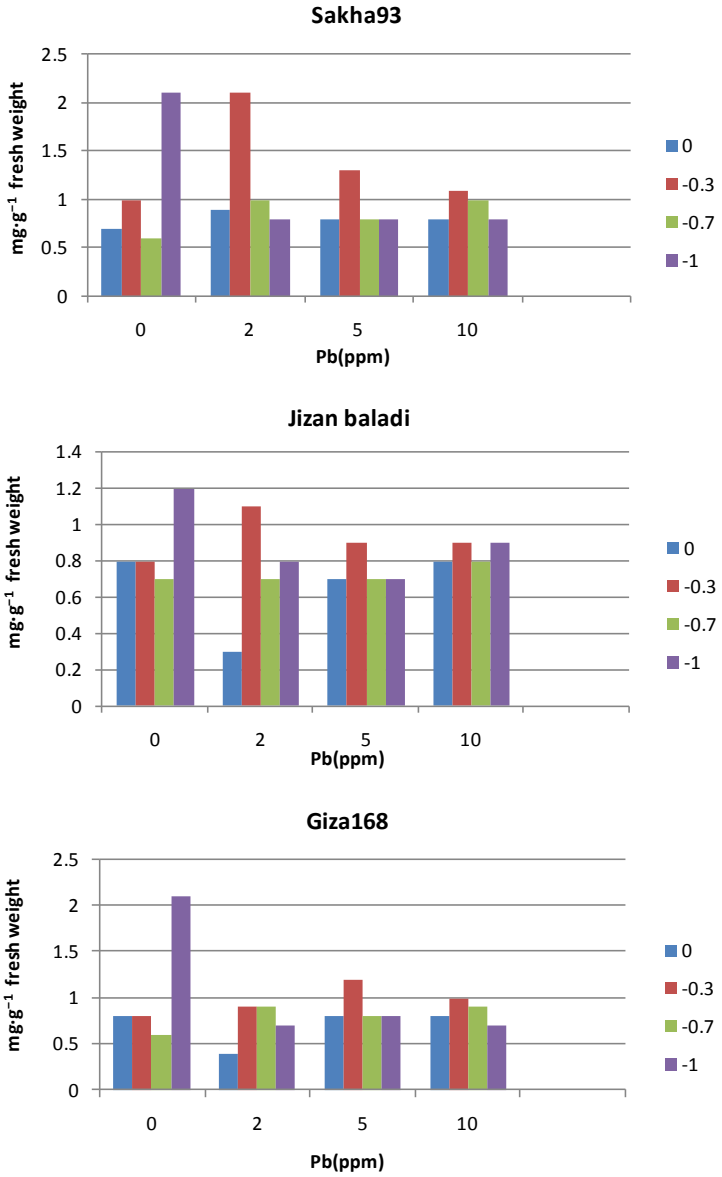

Figure 2. Chlorophyll *b* content in cultivars of *Triticum aestivum* L. at different levels of Pb conc. and osmotic potential Ψ_s levels.

0.69, 0.64 and 0.52 for Sakha93, respectively), (η^2 = 0.75, 0.76 and 0.60 for Jizan baladi, respectively) and (η^2 = 0.80, 0.74 and 0.60 for Giza168 respectively). Meanwhile, Pb had a secondary role on Chl. *a* in Jizan, The (Pb × Ψ_s) interaction had the same role on the chlorophyll contents and ratio in Sakha93. This was true in the role of the interaction factor on the Chl. *a/b* ratios in both Jizan and Giza168 cultivars.

2) Water soluble proteins (S.P.)

Changes in soluble proteins in studied plants under different Pb and Ψ_s levels were illustrated in (**Figure 5** & **Figure 6**). Obviously, the S.P. content in Sakha93 roots exerted a relatively higher value than that in both Giza168 and/or Jizan cultivars. Also, differences among plants and organs were quite clear, particularly concerning the effect of Pb on S.P. content. In Giza168 roots, S.P. contents exhibited a low values in the absence of Pb, whereas in the presence of Pb the S.P. contents were increased, especially under high Ψ_s levels. This is true in case Jizan and Sakha93 roots, in the presence of Pb at moderate (Ψ_s = −0.7 MPa). Meanwhile, the (S.P.) contents were increased in the Pb deficiency under high Ψ_s levels.

In shoots, the total soluble proteins were variable among the investigated cultivars. In Giza168, the S.P.

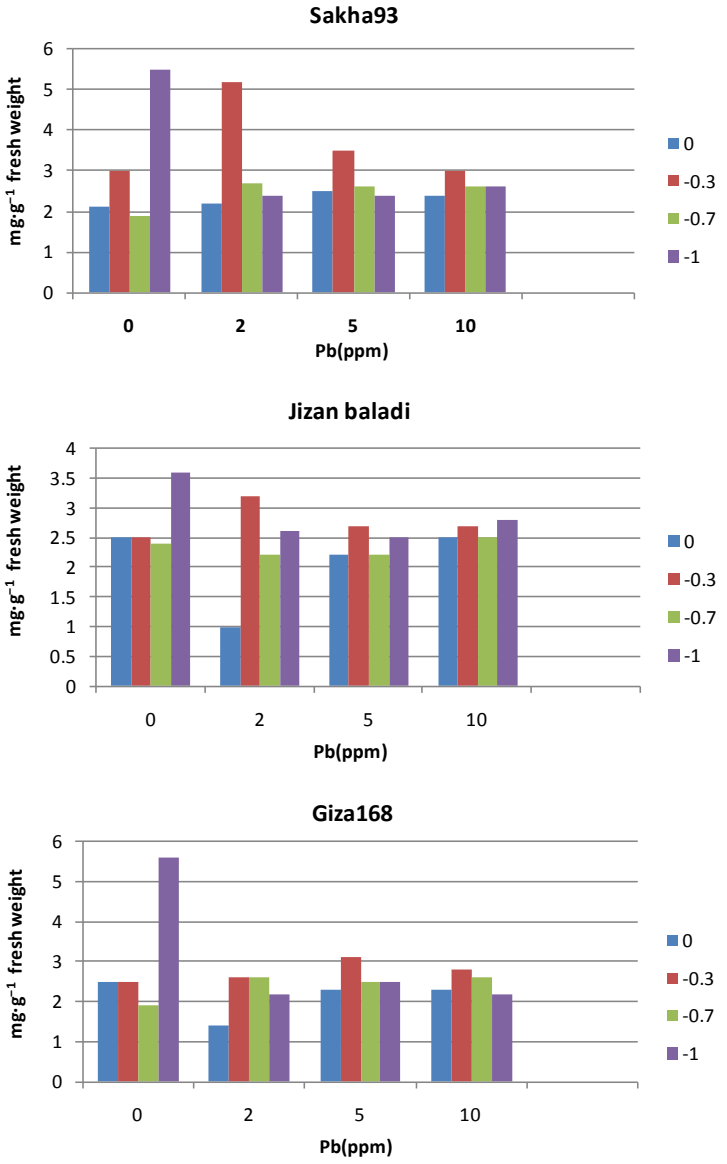

Figure 3. Chlorophyll *a* + *b* content in cultivars of *Triticum aestivum* L. at different Pb conc. and osmotic potential Ψ_s levels.

content in shoots gradually increased with lowering Ψ_s. Also, in the water stressed plants, the total soluble proteins exhibited a high values, particularly with the elevation of Pb concentration (5 - 10 ppm). In unstressed plants of Giza168, shoots produced a low soluble protein content (4.7 mg·g^{-1} dry weight) under Pb treatments, which was gradually increased with increasing Pb concentrations. In both Jizan and Sakha93, the accumulation of S.P. was observed at relatively high Ψ_s levels in the presence Pb element. This high accumulation of S.P. was shifted to relatively lower Ψ_s levels in the absence of Pb. The lowest value of S.P. (0.9 mg·g^{-1} dry weight) was detected in Jizan plants at Ψ_s = −1.0 MPa and Pb = 10 ppm whereas, this was true in case of unstressed plants (Ψ_s = 0 MPa and Pb = 0 ppm) of both Giza168 and Sakha93 cultivars.

The ANOVA test (**Table 2**) indicated that, the Pb had significant effect on soluble proteins of Sakha93 and Jizan roots. Ψ_s had a highly significant effects on the S.P. content of roots & shoots in both cultivars. The same effect of Ψ_s on S.P. was in Giza168 shoots. The Ψ_s and Pb singly had a dominating and sub-dominating (for roots η^2 = 0.71; 0.69 & 0.84, for shoot η^2 = 0.84; 0.84 & 0.56 in Sakha93, Jizan & Giza168, respectively) effects over the interaction effect on S.P. of different organs in tested plants.

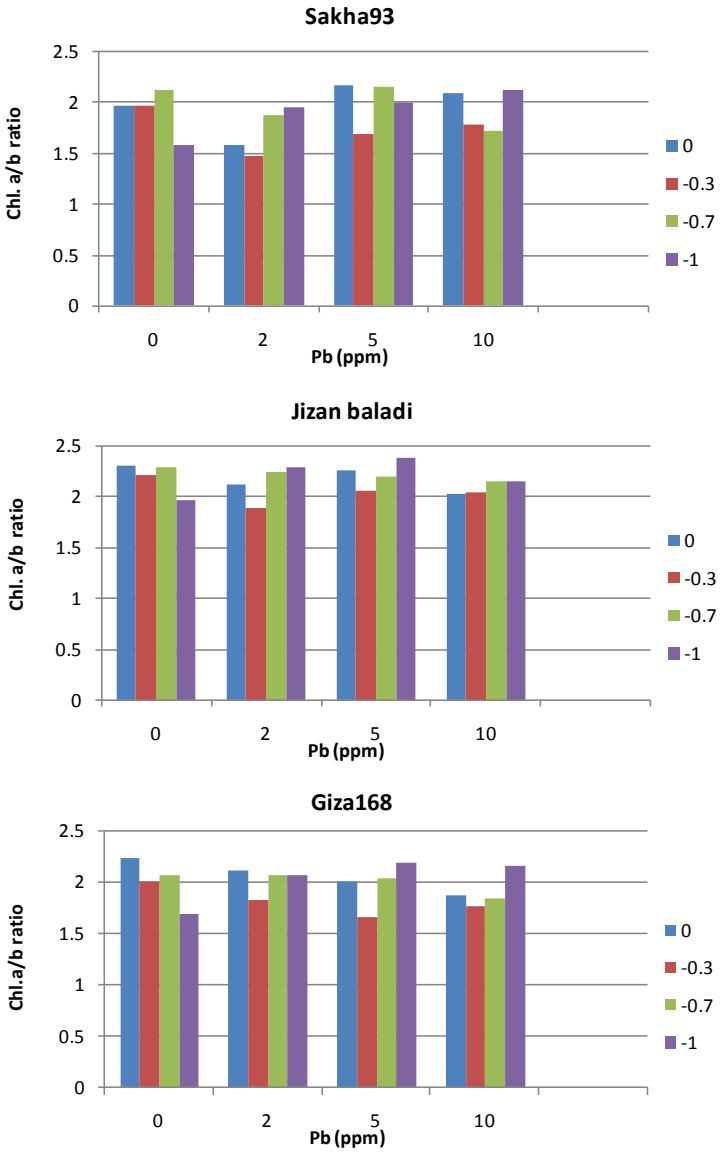

Figure 4. Chlorophyll *a/b* ratio in different cultivars of *Triticum aestivum* L. at different Pb conc. and osmotic potential Ψ_s levels.

Table 1. ANOVA test showed the effect of osmotic potential, lead and their interaction on the, chlorophyll content and chlorophyll *a/b* ratio of investigated *Triticum aestivum* L. cultivars.

Variety		Sakha93		Jizan baladi		Giza168	
Content	**Source of variance**	F	η^2	F	η^2	F	η^2
Chl. *a*	**Pb**	2.853	0.142	3.970*	0.24	1.739	0.120
	Ψ_s	5.622**	0.693	4.255*	0.747	5.364**	0.798
	Pb × Ψ_s	3.045*	0.164	0.150	0.013	1.172	0.082
Chl. *b*	**Pb**	3.212*	0.135	2.435	0.16	2.593	0.143
	Ψ_s	14.842**	0.642	3.748*	0.76	8.271**	0.740
	Pb × Ψ_s	6.354**	0.223	1.104	0.08	2.050	0.117
Chl. *a/b*	**Pb**	4.687*	0.153	0.983	0.061	2.065	0.095
	Ψ_s	10.505**	0.525	4.999**	0.603	7.849**	0.601
	Pb × Ψ_s	15.242**	0.322	7.699**	0.336	9.692**	0.304
D.F		Pb = 3		Salinity = 3		Pb × salinty = 15	

*Significant at P < 0.05 level, **Significant at P < 0.01 level.

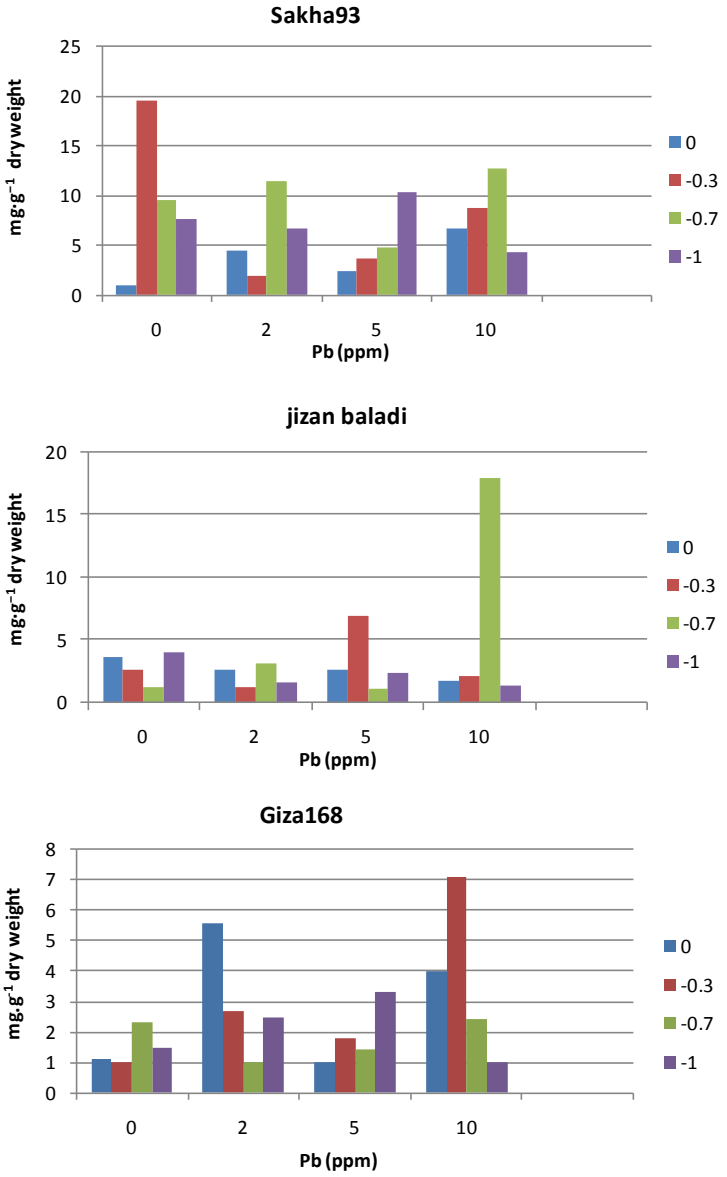

Figure 5. Total soluble protein contents in roots of *Triticum aestivum* L. cultivars at different Pb conc. and Ψ_s levels.

Table 2. ANOVA test showed the effect of osmotic potential, lead and their interaction on the total soluble protein content of both root and shoot of investigated *Triticum aestivum* L. cultivars.

Variety		Sakha93		Jizan baladi		Giza168	
Organ	**Source of variance**	F	η^2	F	η^2	F	η^2
Root	**Pb**	4.612*	0.215	3.802*	0.191	1.495	0.091
	Ψ_s	8.107**	0.712	6.271**	0.691	12.113**	0.835
	Pb × Ψ_s	1.299	0.073	2.116	0.118	1.196	0.074
Shoot	**Pb**	1.074	0.064	0.910	0.059	2.931	0.105
	Ψ_s	23.153**	0.838	9.890**	0.838	16.950**	0.559
	Pb × Ψ_s	1.732	0.099	1.636	0.102	16.787**	0.336
D.F		Pb = 3		Salinity = 3		Pb × salinty = 15	

*Significant at P < 0.05 level, **Significant at P < 0.01 level.

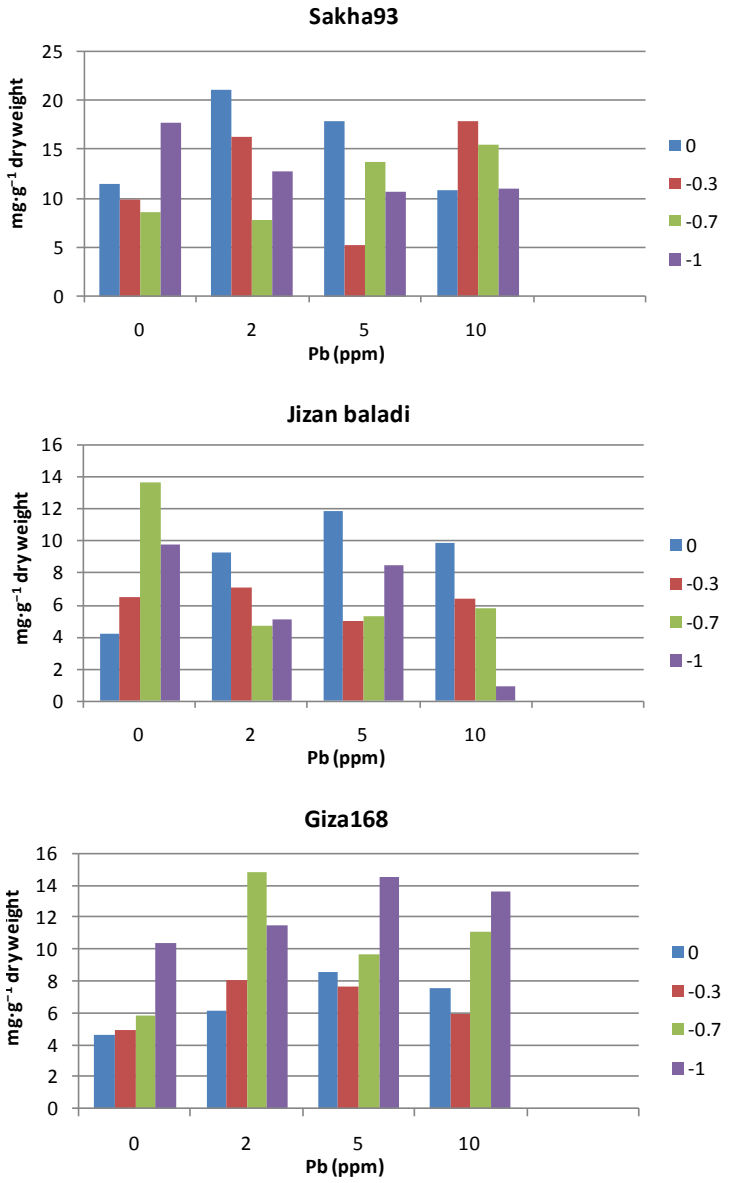

Figure 6. Total soluble protein contents in shoots of *Triticum aestivum* L. cultivars at different Pb conc. and osmotic potential Ψ_s levels.

The most significant correlations between chlorophyll content (*a* & *b*) and soluble proteins were negative in Giza168 and Jizan baladi under Pb and salinity stresses, respectively (**Table 3**).

4. Discussion

The plant growth is affected by the availability of photosynthesis which mainly depending on the photosynthetic pigments. Therefore, the reduction of photosynthetic pigments (Chl. *a* and Chl. *b*) in plants may be attributed to toxic action of salinity and lead on the biosynthesis of pigments [20] [21]. In the investigated *Triticum aestivum* L. cultivars, the photosynthetic pigments tended to a maximum values under low Ψ_s levels in the absence of Pb. This indicates that, the photosynthetic apparatus of plants, hitherto is capable of adapting to the higher salinity [22], whereas, the low Pb concentration encourage the Chl. content (*a* and *b*) in both Sakha93 and Jizan baladi plants at high Ψ_s levels. Conversely, the presence of relatively high Pb concentration induced the Chl. *a* and Chl. *b* content in the absence of osmotic stress. This agrees with John *et al.*, [2] who concluded that, no significant

Table 3. Correlation coefficient (r) values between chlorophyll content (*a* & *b*) and soluble proteins in shoots of different studied cultivars of *Triticum aestivum* L. under osmotic water potential, lead stress and their interaction.

Variety	Source of variance / Contents	Chl. *a* & soluble proteins	Chl. *b* & oluble proteins	D.F
Sakha93	**Lead**	−0.123	0.381	**15**
	Ψ_s	−0.385	−0.362	**3**
	Pb × Ψ_s	0.219	0.292	**3**
Giza 168	**Lead**	**−0.926***	**−0.878***	**15**
	Ψ_s	0.692	0.521	**3**
	Pb × Ψ_s	0.185	0.124	**3**
Jizan baladi	**Lead**	0.493	0.197	**15**
	Ψ_s	**−0.981****	**−0.959****	**3**
	Pb × Ψ_s	−0.150	−0.186	**3**

*Significant at P < 0.05 level, **Significant at P < 0.01 level.

changes in total chlorophyll under low Pb concentration, whereas higher lead concentration led to significant decrease in chlorophyll content. In this respect, the wheat cultivars may have a better defense system against limited Pb concentrations.

The Chl. *a*/*b* ratio in both Giza168 and Jizan baladi yielded a high values in the absence of salinity and Pb stresses, as well as, under moderate Pb concentration and low Ψ_s level. In Sakha93, the same was true at moderate Ψ_s levels and in control plants. Hence, the converted of Chl. *a* to Chl. *b* may explain the increase in the Chl. *a*/*b* ratio at salinity levels [22] and Pb exposure. Therefore, Ψ_s had a predominant role on the Chl. *a* and Chl. *b* and Chl. *a/b* ratio in all tested plants, while the (Ψ_s × Pb) interaction had the secondary role in Chl. *a*/*b* ratio. The same role was released on Chl. *a* and Chl. *b* in Sakha93. Pb had a subdominant role on Chl. *a* content of Jizan baladi. Obviously, in salt tolerant cultivars chlorophyll content increased, while in salt sensitive varieties it was decreased. Furthermore, photosynthesis is adversely affected by Pb which could be due to metal induced reductions in the levels of photosynthetic pigments [1].

A plant tolerance to salt and lead stresses depends on a complex of interrelated systems ensuring plant adaption on metabolic and gene levels [23]. It becomes clear that, marked differences among investigated cultivars were quite evident in their response to either Ψ_s or lead concentration. Therefore, maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under salinity stress [24]. In roots, although the salinity was induced the proteins content in Giza168 plants, the protein content was increased in both Sakha93 and Jizan. Conversely, in shoots the total soluble proteins were higher in Sakha93 and Jizan baladi under high Ψ_s levels and increased in Giza168 shoots with decreased Ψ_s levels. Hence, abiotic stress may inhabit a synthesis of some proteins and promote others with a general trend of decline in the overall content [25]. Thus, the relatively high Pb concentration encourage the protein accumulation in roots of Giza168 and Jizan baladi, while this was took place in Sakha93 root (as a sensitive Pb organ) in the absence of lead treatment. In general, the shoots of investigated plants gained a high amount of soluble proteins under the lower or in the absence of Pb exposure. However, the increase of soluble protein under lead stress is possibly a result of the induction of stress proteins, which may comprise various antioxidant enzymes [11]. The effect of Ψ_s was significant and played the main role on the soluble proteins of both roots & shoots in different cultivars. Whereas, the effect of Pb was sub-dominant on the roots of Sakha93 and Jizan B. plants, the (Pb × Ψ_s) interaction was played the same role on soluble proteins of shoots in Giza168.

5. Conclusion

In general, the osmotic potential had a predominant role on the content and *a/b* ratio of chlorophyll and total soluble proteins; whereas the Pb or its interaction with Ψ_s had the secondary role. Likewise, Ψ_s and Pb were affected on the correlations between chlorophyll content and total soluble proteins. These correlations are negative, which means that the chlorophyll pigment is probably attached to a protein, which gives protection, particularly in the presence of toxic ions such as Pb^{2+} and over the physiological range of Na^{+}.

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