International Journal of Ecotoxicology and Ecobiology
2016; 1(2): 20-27
http://www.sciencepublishinggroup.com/j/ijee
doi: 10.11648/j.ijee.20160102.11

Impact of Lead on Water Soluble Metabolites in Some Cultivars of *Triticum aestivum* L. Grown under Osmotic Water Potential

Kotb Amer Farghali[1, *], Afnan Samy A. Quronfulah[2]

[1]Botany Department, Faculty of Science, Assiut University, Assiut, Egypt
[2]Biology Department, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia

Email address:

farghali52@yahoo.com (K. A. Farghali)
[*]Corresponding author

To cite this article:

Kotb Amer Farghali, Afnan Samy A. Quronfulah. Impact of Lead on Water Soluble Metabolites in Some Cultivars of Triticum aestivum L. Grown under Osmotic Water Potential. *International Journal of Ecotoxicology and Ecobiology*. Vol. 1, No. 2, 2016, pp. 20-27.
doi: 10.11648/j.ijee.20160102.11

Received: May 24, 2016; **Accepted**: June 3, 2016; **Published**: June 20, 2016

Abstract: The changes of main metabolites in three cultivars of *Triticumaestivum* L. under the effects of lead and osmotic stresses were investigated. The results indicated that, the amount of soluble sugars in different plant organs were increased under low Ψ_s and in the presence of Pb element. Also, the decreased Ψ_s and Pb concentration led to an increase in the free amino acids of roots. The content of soluble proteins was variable among the cultivars. Apparently, the soluble proteins were increased in response to low Ψ_s and Pb, except in roots of cv.Giza168which were increased under high Pb concentrations. Statically, the Ψ_s had the predominant role on the soluble sugars, free amino acids and total soluble proteins in all investigated plants. The significant correlations among the main metabolites were positive under the effect of lead, Ψ_s and their interaction with few exceptions.

Keywords: Osmotic potential, Lead, Metabolites, Soluble Sugars, Free Amino Acids, Soluble Proteins, *Triticumaestivum* L., Interaction, Correlation

1. Introduction

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 [1]. It is believed that under heavy metal stress accumulation of sugars along with other compatible solutes contribute to osmotic adjustment and/ or stabilization carbohydrates (such as glucose fructose, and fructans) and starch accumulation under salt stress [2]. Hence, abiotic stress like salinity and heavy metals increased reducing sugars and sucrose in plants [3]. Whereas, the reducing sugar of the wheat plant organs grown under salinity stress showed significant decreases. It is known that drought tolerance can be partly attributed to the accumulation of soluble sugars [4] as they are able to protect the structural integrity of membranes during dehydration by preventing membrane fusion, phase transition and phase separation [5].

Abiotic stresses including heavy metal can exert its effects on plants by resulting in changes of soluble proteins content. A number of reports have showed then negative effects of different heavy metals on the amount of soluble protein in plant seedlings [6]. Although salinity causes decreasing in protein synthesis and increases in proteolysis in different plants, in many plants, the protein content increases under saline conditions. Meanwhile, soluble proteins increase the surface exposed to water binding, the free amino acids is beneficial in maintaining viscous properties and contribute to increasing osmolality of the cytoplasm [7,8]. On the other hand, changes in amino acids content, whether resulting from degradation of proteins under unsuitable conditions for metabolism (catabolism) or as a product of active metabolism (anabolism or de novo synthesis, especially in roots), are quite indicative of suffering or normal metabolism in response to prevailing factors [9]. 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 proteins content [10]. The increase of protein under lead stress is possibly a result of the induction of stress proteins, which may comprise various antioxidant enzymes [11]. Also, proline is one component of the non-specific defense systems towards lead toxicity. It alleviates metal toxicity by acting as a metal chelator and as a protein stabilizer ([12].

The aim of this article is to understand the protective mechanisms and strategies of soluble sugars, free amino acids and soluble proteins in three cultivars of *Triticum aestivum* L. (Sakha93, Jizanbaladi, Giza168) under the osmotic potential (salinity), lead and their interaction stresses.Also, to know the correlations between different investigated parameters in response to the intrinsic conditions.

2. Materials and Methods

2.1. Preparation of Plants for Experimentation

The investigated plants included three cultivars of*Triticum aestivum* L. 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 (Jizanbaladi) was supplied by the Ministry of Agricultural of Saudi Arabia.

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. Preliminary germination tests performed before experimentation indicated a high germination percentage, reaching about 100% in these seeds.

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[13], 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) Concentrations 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 0ppm (control -0.3, -0.7 and -1.0MPa.). 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[14].

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 solutions. 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 (37day-old plants), average fresh and dry 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. Centrifugation at 7000 r.p.m. was carried out for 15 min. After the centrifugation,. The supernatant was kept in deep freeze until analysis.

2.4. Determination of Water Soluble Metabolites

The content of each metabolite in the extract of different cultivar organs of experimental plants is expressed in mg. g^{-1} dry wt. using the spectrophotometer *UNICAM* model *UV-Vis* spectrometry (Made in England).

2.4.1. Water Soluble Sugars

The soluble sugars was measured at 490 nm and calculated after the realization of a standard curve by using glucose according to Dubois *et al*., [15].

2.4.2. Water Soluble Nitrogen Compounds

The two types of nitrogen compounds (Soluble proteins and total free amino acids) represent the major fractions of active metabolites that can contribute to the bound water (and partially to osmotic adjustment) in the plants.

a-Total free amino acids were determined by using ninhydrin reagent (measured at 570 nm) and calculated after the realization of a standard curve made with glycine according to procedures Lee and Takahanshi[16].

b -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].

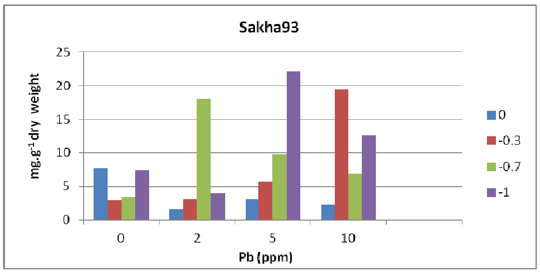
2.5. Statistical Analysisof Data

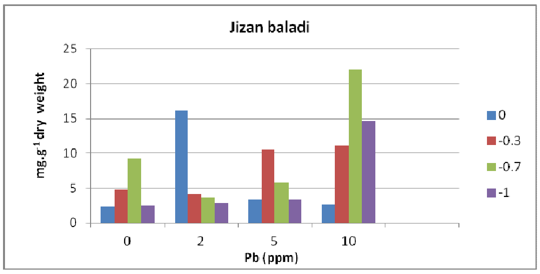
Statistical inference necessary to evaluate the significance of effects and the relative roles of the single factors: lead(Pb), osmotic (Ψ_s)and their interaction(Pb X Ψ_s) in response to different treatment combinations including analysis of variance (F. values) and coefficient of determination (η^2), 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 response of treatment, expressed as a percentage (%). [19]. All these analyses were computerized by using the SPSS program.

3. Results

3.1. Soluble Sugars (S.S)

The changes in soluble sugars in organs of different *Triticum aestivum*L. cultivars were shown in (Figures 1&2). It is quite clear that, the soluble sugars in roots were lower than that in the shoots of investigated plants under different Pb and $\Psi_s$treatments. In Giza168 cultivar, the soluble sugars in both organs (root &shoot) were increased under low $\Psi_s$levels. While mean, the low (S.S) contents were observed at high $\Psi_s$levels, regardless of Pb treatments. In the presence of Pb, the soluble sugar content of roots was increased at moderate Pb (5 ppm under low Ψ_s. This was true in shoots at relatively low (2 ppm) and high (10ppm) Pb concentrations. In general a high amounts of soluble sugars [(20.54 & 19.01 mg.g^{-1} dry weight) in both roots and shoots, respectively were found under lowest Ψ_s in the presence of Pb. In Jizan cultivars, the change in soluble sugars in both organs was varied. Regardless of Pb treatments, a moderate Ψ_s(-0.7MPa) yielded a high amounts of S.S in roots, whereas a low S.S content was found in the absence of Pb. Conversely, a high S.S content in shoots existed in the absence of water stress (Ψ_s= 0MPa) and low S.S was found at low Ψ_s levels. The presence of Pb caused an increased in S.S both Jizan organs, particularly at Pb 10 ppm and Ψ_s= -0.7 MPa in case of roots (22.05 mg.g^{-1} dry weight) and at Pb range from 2-10 ppm at Ψ_s =0 MPa in shoots.





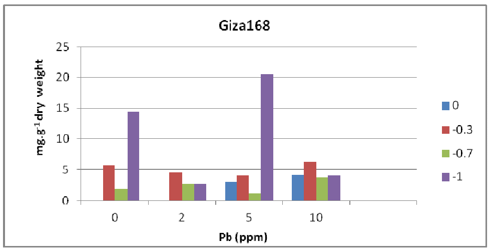
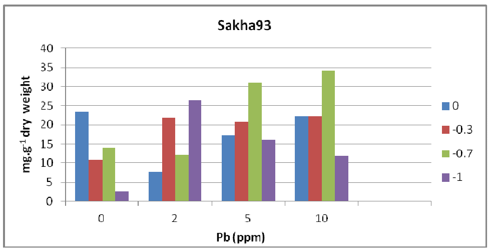
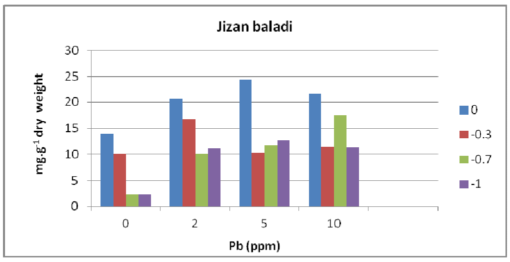


Figure 1. *Soluble sugar contents in roots of Triticumaestivum L. cultivarsat different Pb conc. and osmotic potential Ψs(MPa)levels.*





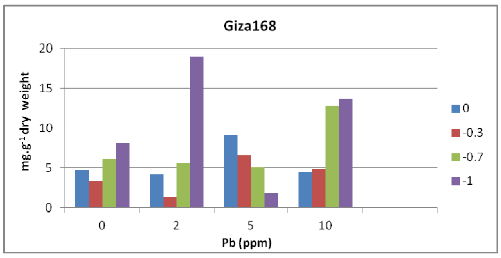


Figure 2. *Soluble sugar contents in shootsof Triticumaestivum L. cultivarsat different Pb conc. and osmotic potential Ψs(MPa)levels.*

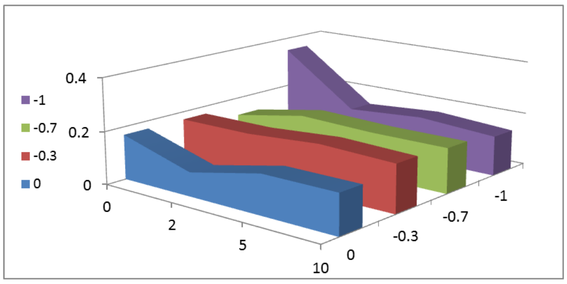
A similar behavior of S.S in Giza168 roots was found in case of Sakha93 roots, where a low S.S content was yielded in the absence of salinity stress. A high amount of S.S in roots (22.08 mg.g^{-1} dry weight) was existed with the lowering of Ψ_s at different Pb doses. In shoots, the Ψ_s = -0.7 MPa produced a high soluble sugar contents, particularly under Pb 5-10 ppm treatments, while a low S.S was observed under low Ψ_s level at different Pb treatments, except at Pb = 2 ppm. The S.S in shoot of Sakha93 tended to a maximum (34.18 mg.g^{-1} dry weight) at Ψ_s = -0.7 MPa and Pb = 10 ppm, while a low (S.S) content (2.52 mg.g^{-1}dry weight) was observed at Ψ_s = 1.0 MPa in the Pb deficiency.

The effects of Ψ_s and its interaction with Pb were highly significant on the soluble sugars of roots and shoots of investigated plants with some exceptions (Table 1). Only, Pb had a highly significant effect on (S.S) in Jizan roots and non-significant on the (S.S) of the rest plant organs.The relative role of factors (η^2) indicated that,Ψ_s had the majority effect on the (S.S) of roots and shoots of different plants (For roots η^2= 0.73 & 0.72 for shoots η^2 =0.86, 0.73 & 0.72 of Sakha93,Jizan and Giza168, respectively). The secondary role was played by Pb or its interaction with Ψ_s factors in case of soluble sugars of roots or shoots.

Table 1. *ANOVA test showed the effect of osmotic potential(Ψ_s), lead (Pb) and their interaction (Pb XΨ_s) on the soluble sugars content of both root and shoot of investigated Triticum aestivum L. cultivars.*

	Variety	Sakha 93		Jizanbaladi		Giza168	
Organ	Source of variance	F	η^2	F	η^2	F	η^2
	Pb	2.682	0.097	4.218*	0.155	1.548	0.082
Root	Ψ_s	16.992**	0.558	437.318**	0.692	48.687**	0.82
	Pb X Ψ_s	17.842**	0.345	4.115**	0.152	1.903	0.098
	Pb	0.34	0.019	1.789	0.09	9.247**	0.231
Shoot	Ψ_s	14.614**	0.744	16.802**	0.733	24.481**	0.552
	Pb X Ψ_s	5.625**	0.236	4.013**	0.177	8.365**	0.218
D.F		Pb =3 Salinity =3 Pb X Salinty =15					

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



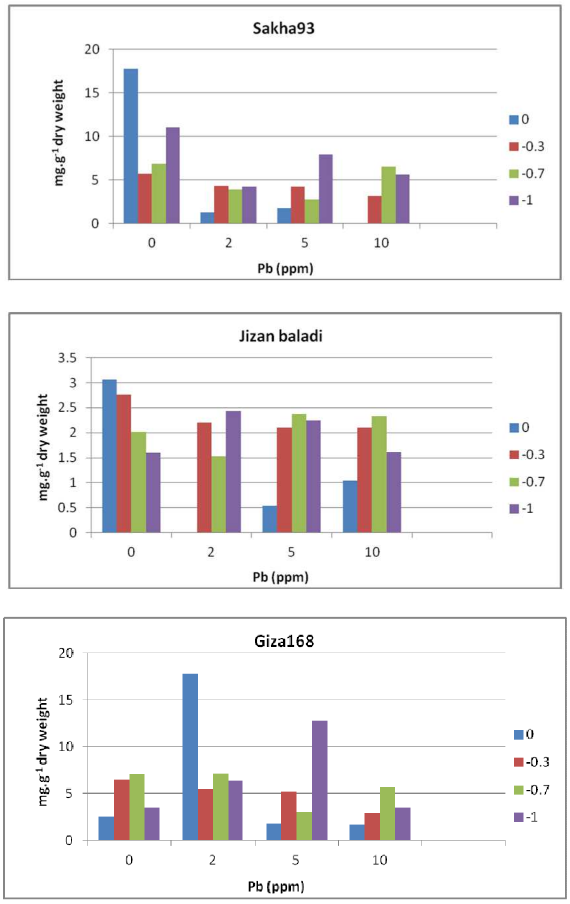


Figure 3. *Total free amino acids contents in roots of Triticumaestivum L. cultivarsat different Pb conc. and osmotic potential Ψs(MPa)levels.*

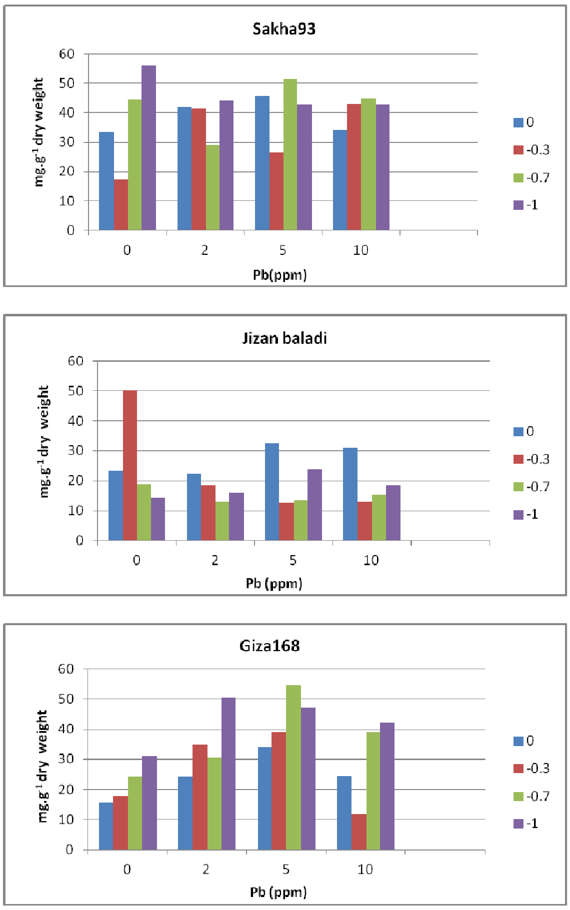


Figure 4. *Total free amino acids contents in shoots of Triticumaestivum L. cultivarsat different Pb conc. and osmotic potential Ψs(MPa)levels.*

3.2. Total Free Amino Acids (TA.A.)

In the investigated plants, the total free amino acids in roots showed a lower contents than that of shoots at different Ψ_s and Pb treatments (Figures 3&4). The free amino acids in Jizanbaladi roots however showed the lowest values among the studied cultivars (Figure 3). Moreover, the T.A.A. content in the same cultivar was slightly changed under different Pb and Ψ_s treatments. Meanwhile, the low A.A. content (0.54 mg.g^{-1} dry weight) was observed under high osmotic water potential (Ψ_s= 0 MPa) at Pb 2-5 ppm, the highest value existed in the absence of Pb. Likewise in Sakha93roots, the control (Ψ_s = 0 MPa and Pb= 0 ppm) yielded a maximum T.A.A. amount (17.76 mg.g^{-1} dry weight). However, the lower T.A.A. contents was found at Pb range 5-10 ppm. Under low Ψ_s levels, the roots of Sakha93 produced a relatively high A.A. particularly at the same Pb treatments which indicates that the presence of Pb enhance the A.A. anabolism. In case of Giza168roots, the total free amino acid content was increased under Ψ_s= 0MPa and Pb = 2ppm. The increasing of A.A. was shifted to Pb 5 ppm treated plants with the moderate Ψ(= -0.7MPa) level. A similar to A.A. in the Sakha93 roots, the A.A. in Giza168 was markedly decreased at Pb range from 5 to 10 ppm.In shoots, the free amino acids of Sakha93 & Giza168 plants tended to maximum contents (50 and 56 mg.g^{-1} dry weight, respectively) under the low Ψ_s levels. Furthermore, the shoots of both cultivars were gained a high A. A. contents compared to that in the shoots of Jizan plants, which had a relatively low contents, especially under water stress (Figure 4). In Giza168 shoots, the free amino acids gradually increased at Pb range 0-5 ppm under high and moderate Ψ_s. In contrast, the lowering Ψ_s decreased the A.A. content of Jizan shoots with Pb treated plants may be due to disturbance in the free amino acid metabolism. Hence, a high A.A. in Jizan shoots was existed at relatively high Ψ_s level (-0.3 MPa) in the absence of Pb.

The single factors and their combination had a significant effects on the free amino acids of both roots and shoots of experimented plants with some exceptions (Table 2). The effect of salinity was predominant (η^2= 0.56, 0.63 & 0.82 for roots and 0.74, 0.73, 0.55 for shoots of Sakha93, Jizan and Giza168, respectively) on the A.A. contents of plants. The effect of interaction was subsidiary on the A.A. of Sakha93 organs and Jizan shoots. Whereas, the effect of Pb was subdominant on A.A. of Giza168 shoots, the same role was equal share between Pb and interaction effect on the A.A. content of Jizan roots.

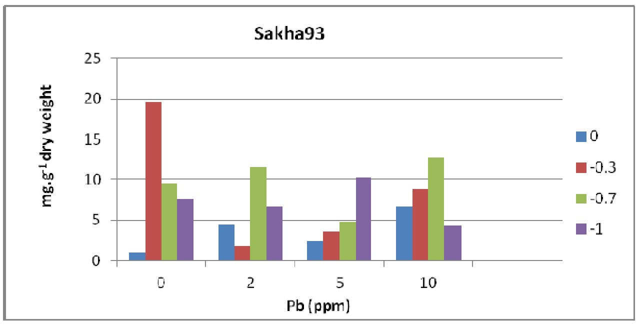
Table 2. *ANOVA test showed the effect of osmotic potential(Ψ_s), lead (Pb) and their interaction(Pb X Ψ_s) on the free amino acid content of both root and shoot of investigated Triticumaestivum L. cultivars.*

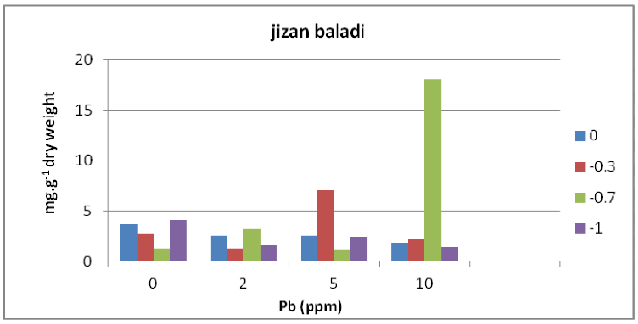
	Variety	Sakha 93		Jizanbaladi		Giza168	
Organ	**Source of variance**	**F**	**η^2**	**F**	**η^2**	**F**	**η^2**
	Pb	2.682	0.097	4.218*	0.155	1.548	0.082
Root	Ψ_s	16.992**	0.558	437.318**	0.692	48.687**	0.82
	Pb X Ψ_s	17.842**	0.345	4.115**	0.152	1.903	0.098
	Pb	0.34	0.019	1.789	0.09	9.247**	0.231
Shoot	Ψ_s	14.614**	0.744	16.802**	0.733	24.481**	0.552
	Pb X Ψ_s	5.625**	0.236	4.013**	0.177	8.365**	0.218
D.F		Pb =3 Salinity =3 Pb X Salinity =15					

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

3.3. Total Soluble Proteins (S.P.)

Changes in soluble proteins in studied plants under different Pb and Ψ_s levels were illustrated in (Figures 5&6).Obviously; the S.P. content in Sakha93 roots exerted a relatively higher values 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. concentrations. 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 was true in case of Jizan and Sakha93 roots,at moderate Pb concentration (Ψ_s= -0.7 MPa).Meanwhile, S.P. contents were increased in the Pb deficiency under high Ψ_s levels.





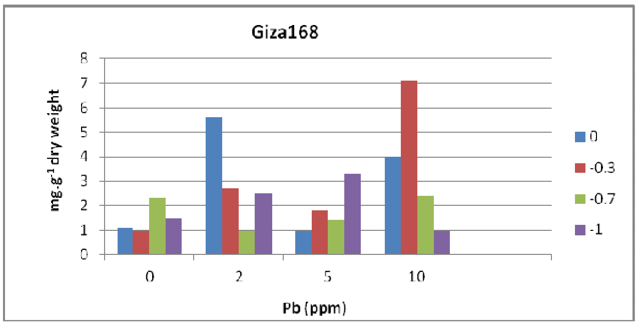


Figure 5. *Soluble proteins contents in roots of TriticumaestivumL.cultivars at different Pb conc. and Ψs(MPa) levels.*

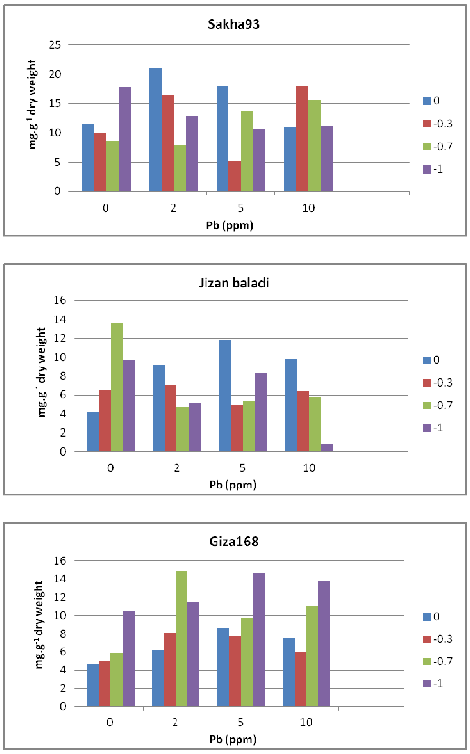


Figure 6. *Soluble protein contents in shoots of TriticumaestivumL. cultivars at different Pb conc. and osmotic potential Ψs(MPa)levels.*

In shoots, soluble proteins were variable among the investigated cultivars. In Giza168, the S.P. content in shoots gradually increased with lowering Ψ_s. Also, in the water stressed plants, soluble proteins exhibited a high values, particularly with the elevation of Pb concentration (5-10ppm). In unstressed plants of Giza168, shoots produced a low soluble proteins content (4.7 $mg.g^{-1}$ dry weight) under Pb treatments, which was gradually increased with increasing Pb concentration. 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 3) indicated that,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. 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. Exceptionally, (Ψ_s×Pb) interaction effect on S.P. of Sakha93 shoot was sub-dominant.

Table 3. *ANOVA test showed the effect of osmotic potential(Ψ_s), lead(Pb) and their interaction(Ψ_s×Pb) on soluble proteins content of both root and shoot of investigated Triticumaestivum L. cultivars.*

	Variety	Sakha 93		Jizanbaladi		Giza168	
Organ	Source of variance	F	η^2	F	η^2	F	η^2
	Pb	4.612*	0.215	3.802*	0.191	1.495	0.091
Root	Ψ_s	8.107**	0.712	6.271**	0.691	12.113**	0.835
	Pb X Ψ_s	1.299	0.073	2.116	0.118	1.196	0.074
	Pb	1.074	0.064	0.91	0.059	2.931	0.105
Shoot	Ψ_s	23.153**	0.838	9.890**	0.838	16.950**	0.559
	Pb X Ψ_s	1.732	0.099	1.636	0.102	16.787**	0.336
D.F		Pb =3 Salinity =3 Pb X Salinty =15					

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

Table 4 showed that, under the effect of the tested factors, the significant correlations between free amino acids, soluble proteins and soluble sugars in root and shoot in all cultivars were positive. Exceptionally, in case of Jizan baladi shoot, the correlation was negative under Pb effect. In the roots, the correlation between free amino acids and soluble proteins or soluble sugars was non-significant.

Table 4. *Correlation coefficient (r.) values between free amino acids, soluble proteins and soluble sugars in both root and shoot of different studied cultivars of Triticum aestivum L. under osmotic water potential(Ψ_s), lead(Pb) stress and their interaction(Ψ_s×Pb).*

Variety	Contents variance	amino acids & soluble proteins	amino acids & soluble proteins	amino acids & soluble sugars	amino acids & soluble sugars	Soluble proteins & soluble sugars	Soluble proteins & soluble sugars	D.F
		Root	Shoot	Root	Shoot	Root	Shoot	
	Lead	0.757	0.038	-0.683	0.969**	-0.466	0.241	
Sakha 93	Ψ_s	-0.16	-0.015	0.557	-0.296	0.726	-0.519	
	PbxΨ_s	-0.064	0.607*	0.076	0.083	0.189	-0.052	
	Lead	-0.023	0.829	-0.541	-0.109	-0.623	0.453	
Giza 168	Ψ_s	-0.58	0.997**	0.499	0.933*	-0.2	0.959**	
	PbxΨ_s	0.351	0.687**	0.198	0.425	0.096	0.368	
	Lead	-0.261	0.82	-0.541	-0.931*	0.887*	-0.808	
Jizanbaladi	Ψ_s	0.347	0.489	0.457	0.878*	0.986**	0.859	
	PbxΨ_s	0.15	0.257	0.106	0.323	0.617**	0.065	

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

4. Discussion

A plant tolerance to salt and lead stresses depends on a complex of interrelated systems ensuring plant adaption on metabolic and gene levels [20]. It becomes clear that, marked differences among investigated cultivars were quite evident in their response to either Ψ_s or lead concentration. Apparently, the soluble sugars in roots were increased with increased salinity. Also, the presence of Pb in the root medium increased the soluble sugars in roots of wheat plants. This implies that, the osmotic adaption to salinity via soluble sugars accumulation was well documented [21]. Likewise, the soluble sugars in shoots were variable among plants. Meanwhile, high soluble sugars conents in Giza168 and Sakha93 were observed in salinized plants, the same was true in unstressed Jizanbaladi plants. This emphasized that, the accumulation of certain carbohydrate fractions may be induced in the stressed plants [2. The F test and η^2 indicated that, the Ψ_s had a significant and predominant role on the soluble sugar content in various organs of all plants. The interaction ($\Psi_s \times$ Pb),however was played the subsidiary role on the soluble sugars in both roots and shoots, except in case of Jizan baladi root where Pb had the same role. This was agrees with Khidr *et al.,* [22] who concluded that the concentration of soluble sugars was significantly increased with increasing NaCl concentration. In contrast, the soluble sugars of shoots in most varieties were increased under Pb doses and low content was existed in the absence of Pb. This increase in soluble sugars was considered as a protective mechanism against protein denaturation [23; 24], or may be able to protect the structural integrity of membranes [5] against deleterious effect of Pb. Accordingly, Parida & Das [25] concluded that, the major functions of sugars are osmo-protection, osmotic adjustment, carbon storage and radical scavenging.

The adaptability of plants to high salt concentrations in the root medium was accompanied by accumulation of osmotic solutes such as soluble sugars and total free amino acids. Accordingly, the accumulation of these solutes allows the plants to minimize sufficient storage reserve to support based metabolism under abiotic conditions [26; 27; 9]. The present data indicated that, the free amino acids in the roots were increased under low Ψ_s levels (high salinity), particularly under relatively moderate Pb concentration and vice versa. In this respect, Youssef [28] concluded that, the osmolytes that support the increase of the cell Ψ_s under salt stress are mainly: glycine, proline and glycinebetaine. In shoots, the trend of amino acids in tested plants was differed.A high free amino acids in both Sakha93 and Giza168 were observed under low Ψ_s levels and relatively high Pb concentration. This accumulation of free amino acids was showed in Jizanbaladi under high Ψ_s in the absence of Pb. This means that, some free amino acids such as proline provide protection for wheat cultivars under Ψ_s& lead stresses [1]. In general, the accumulation of amino acids was increased in shoots more than into the roots under different Ψ_s and Pb levels. Statistical inferences, however indicated that, Ψ_s had a predominant role on the total free amino acids in both root and shoots in all cultivars. The secondary effect on amino acids in both organs of Sakha93 and shoot of Jizanbaladi was played by the interaction ($\Psi_s \times$Pb). Meanwhile, Pb had the same role in case of Giza168 shoots, the Pb or its interaction with Ψ_s had equal share role in case of Jizan roots.

Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under salinity stress [29]. In roots, although the salinity induced the proteins content in Giza168 plants, the protein content was increased in both Sakha93 and Jizanbaladi. Conversely, in shoots the soluble proteins were higher in Sakha93 and Jizanbaladi 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 [30]. Thus, the relatively high Pb concentration encourage proteins accumulation in roots of Giza168 and Jizanbaladi, 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 high amount of soluble proteins under the lower or in the absence of Pb. However, the increase of soluble proteins 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 Jizanbaladi plants, the (Pb $\times \Psi_s$) interaction 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 of soluble sugars, total free amino acids and soluble proteins; whereas the Pb or its interaction with Ψ_s had the secondary role. Likewise, $\Psi_s$and,Pb and/or their interaction affected the correlations between contents of main metabolites to each other which were significantly positive with few exceptions. This means that soluble sugars and free amino acids is probably attached to proteins, which gives a protection, particularly in the presence of toxic ions such as Pb^{+2} and Na^+.

References

[1] Lamhamdi, M., Bakrim, A., Aarab, A., Lafont, R., Sayah, F., 2011. Effects of lead phytotoxicity on wheat (*Triticumaestivum L.*) seed germination and seedling growth. C. R. Biology. 334, 118–126.

[2] Parida, A., Das, A.B., and Das, P., 2002.NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *(Bruguieraparviflora)*, in hydroponic cultures, Journal of Plant Biology. 45: 28-36.

[3] Dubey, R. S and Singh, A. K., 1999. Salinity Induces Accumulation of Soluble Sugars and Alters the Activity of Sugar Metabolising Enzymes in Rice Plants. Biologia Plantarum, 42, (2): 233-239.

[4] Pelah, D., Wang, W., Altman, A., Shoseyov, O., and Bartels, D., 1997. Differential accumulation of water stress related proteins, sucrose synthase and soluble sugars in *Populus*s pecies that differ in their water stress response. Physiologia Plantarum, 99: 153-159.

[5] Crowe, J. H., and Crowe, L.M., 1992. Membrane integrity in an hydrobiotic organisms: Toward a mechanism for stabilizing dry cells.– In Water and Life (Somero, G. N., Osmond, C. B., and Bolis, C.L.,(eds.). Springer- Verlag, Berlin, pp: 87-103.

[6] Bhardwaj P.; Chaturvedi, A. K. and Prasad, P. 2009. Effect of Enhanced Lead and Cadmium in soil on Physiological and Biochemical attributes of *Phaseolus vulgaris* L. Nature andScience, 7(8): 63-75.

[7] El-Sharkawi, H. M.; Salama, F. M. and Ahmed, M. K., 1988.Some aspects of drought resistance in desert plants, I. Metabolic components of osmotic adjustments. Bulletin of Faculty of Science, Assiut University, 17:153-172.

[8] Gengmao Z, Quanmei S, Yu H, Shihui L, Changhai W. 2014. The physiological and biochemical responses of a medicinal plant (Salvia miltiorrhiza L.) to stress caused by various concentrations of NaCl. PLoS ONE 9(2): 1-6.

[9] EL-Sharkawi, H. M., Farghali, K. A. andTammam, S.A., 2012. Interactive Effects of Sodocity and Salinity on the Nitrogenous Metabolites of Three Economic Plants. Journal of Botany, Assiut University, 41(2): 265-280.

[10] Bavei, V., Shiran, B., Arzani, A., 2011.Evaluation of salinity tolerance in sorghum (*Sorghum bicolor* L.) using ion accumulation, proline and peroxidase criteria. Plant Growth Regulation, 64, (3): 275-285.

[11] Lamhamdi, M., Bakrim, A., Aarab, A., Lafont, R., Sayah, F., 2010.Acomparison of lead toxicity using physiological and enzymatic parameters on spinach (*Spinaciaoleracea*) and wheat (*Triticumaestivum*) growth. Moroccan Journal of Biology. 6–7, 64–73.

[12] [12]Sharma, P., and Dubey, R. S., 2005. Lead toxicity in plants. Braz. Journal of Plant Physiology. 17, 35–52.

[13] Hoagland, D.R. and D. I. Arnon., 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347: 1-32.

[14] El-Sharkawi, H. M., 1968.Water relations of some grasses with phreatophytic properties. Ph. D. Thesis Oklahoma State University, U.S.A.

[15] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F., 1956.Colorimetric Method for Determination of Sugars and Related Substances.Anal. Chem., 1956, 28 (3), pp 350–356 DOI: 10.1021/ac60111 a017.

[16] Lee, Y. P., & Takahashi, T. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Analytical Biochemistry. Volume 14, Issue 1, Pages 71–77.

[17] Lowry, O.H., Rosenbrugh, N. J., Farr, A. L., Randall, R. J., 1951.Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 193: pp. 265-275.

[18] Ostle, B. 1963.Statistics in research. Iowa: Iowa State University Press, Ames.

[19] El-Sharkawi, H. M., Springuel, I., 1977. Germination of some crop plant seeds under reduced water potential. Seed Science and Technology5, 677–688.

[20] Flowers, T. J. and Colmer, T. D., 2008. Salinity Tolerance in Halophytes, New Phytol., vol. 179, pp. 945–963.

[21] Patakas, A., Nikolaou, N., Zioziou, E., Radoglou, K., and Niotsakis, B., 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought stressed Grapevines. Plant Science, 163: 361-367.

[22] Khidr, Z. A., Abo-Kassem, E. M., Tahoon, S. K., Sabal, A. E., 2010.Stress Evokes Changes in Response to Sakha-69 Wheat According to Sodium and Calcium Anions. Australian Journal of Basic and Applied Sciences, 4(12): 6140-6153.

[23] Todd, G.W., 1972. Water deficits and enzymatic activity. In:Kozlowski TT (eds.) Water deficits and plant growth Vol III. Academic Press, London New York, pp 177–216.

[24] Naderi, N., Mirzamasoumzadeh, B. & Aghaei, A. 2013.Effects of different levels of Lead (Pb) on physiological characteristics of sugar beet.International Journal of Agriculture and Crop Sciences. Vol., 5 (10), 1154-1157.

[25] Parida, A. K., and Das, A. B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60: 324–349.

[26] Smeekens, S., 2000. Sugar-induced signal transduction in plants. Annu. Rev. Plant Physiol. Plant Molecular Biology. 51, 49–81.

[27] Jaleel, C. A., Kishorekumar, P., Manivannan, A., Sankar, B., Gomathinayagam, M., and Panneerselvam, R., 2008. Salt stress mitigation by calcium chloride in *Phyllanthusamarus*. Acta Botanica. Croatica., 67: 53-62.

[28] Youssef, A. M., 2009. Salt Tolerance Mechanisms in Some Halophytes from Saudi Arabia and Egypt. Research Journal of Agriculture and Biological Sciences, 5(3): 191-206.

[29] Baby, J., and Jini, D., 2010. Proteomic Analysis of Salinity Stress- responsive Proteins in Plants. Asian Journal of Plant Sciences, 9: 307-313.

[30] John, R., Ahmad, P., Gadgil, K., Sharma, S., 2008.Effect of cadmium and lead on growth, biochemical parameters and uptake in (*Lemnapolyrrhiza L.*) Plant Soil Environ. 54:262–270.