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Bi-Factorial Interactive Effects of Osmotic Potential and Lead on the Content of Na^+, K^+ and Pb^{2+} Ions in Three Cultivars of *Triticumaestivum* L.

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Abstract

This investigation was carried out on three cultivars of *Triticumaestivum* L. namely: Sakha93, Giza168 and Jizan baladi. The Plants were grown in hydroponic solution and treated by ion-deficient cultures under different osmotic water potential levels and Pb concentrations. The results indicated that, the Na^+ content was low in roots of Sakha93 and Giza168 shoot, while the high Na^+ content was observed in Giza168 root and Jizan baladi shoot. The K^+ content was higher in Giza168 plants than in the rest plants. The lead content in roots was gradually increased with increasing Pb concentrations of deficient salinity cultures. The Pb content in roots was markedly decreased under different Ψ_s levels. The interaction ($\Psi_s \times$ Pb) had a significant effect on the content of Na^+, K^+ and Pb in roots and shoots except in case of Giza168. The Pb content was affected by Ψ_s singly. The significant correlations between K^+ and Na^+ in shoots and roots of tested plants under the Pb, Ψ_s or their interaction were positive and between Na^+ or K^+ and Pb were negative.

Keywords

Lead, Osmotic Potential, *Triticumaestivum*, Interaction, Sodium, Potassium, Correlation

Subject Areas: **Agricultural Science, Plant Science**

1. Introduction

Salinity is one of the most severe environmental factors that may impair crop productivity. The ability of plants to survive under high salt conditions is important for the ecological distribution of plant species and agriculture in

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semi-arid, arid, and salinized regions. Hence, plant tolerance to salt stress depends on a complex of interrelated systems ensuring plant adaptation to metabolic and gene levels including ionic homeostasis, synthesis of osmolytes, compartmentation of toxic ions, and structures preventing the generation of reactive oxygen species (ROS) [1]. Likewise, the toxic effects of heavy metals have caused a considerable reduction in crop yield. Among the heavy metals, lead is the most common heavy metal causing environmental pollution and toxicities on soil fertility.

Accumulation of mineral ions such as K^+, Na^+, Cl^-, and SO_4^{2-} facilitate osmotic adjustment of plants under atmospheric aridity and soil dryness. Externally supplied Ca^{2+} reduces the toxic effects of NaCl, apparently by facilitating a higher K^+ to Na^+ selectivity [2] [3]. But, in plants exposed to relatively low Na^+ concentrations, Na^+ is a beneficial element which can promote growth of many plants, in particular when K^+ concentrations in the growth medium are limited [4]. It was found that, calcium concentration was not significantly affected by the salinity, averaged over genotypes, but Mg^{+2} and K^+ decreased and Na^+ increased in proportion to the concentration of NaCl [5]. Otherwise, the high lead concentrations in culture media caused a reduction of most macro- and micro-elements, also reduced in growing plants [6]. Thus, the inhibition of mineral ion uptake appears to be a general consequence of lead exposure [7].

The aim of this research study is to understand the comparative strategies of *Triticumaestivum* L. cultivars for adaptation to salinity and lead stress combination through the accumulation of the specific ions K^+, Na^+ and avoidance of Pb^{2+} accumulation.

2. Materials and Methods

The investigated plants included three cultivars of *Triticumaestivum* 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 (Jizan baladi) was supplied by the Ministry of Agricultural of Saudi Arabia.

2.1. Preparation of Plants for Experimentation

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 in growth chamber at optimum germination conditions (at 25˚C) in full strength hydroponic cultures [8] which were contained in three replicates of plastic pots (3 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 (replicate) 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 concentrations of NaCl and $CaCl_2$ in solutions prepared are based on the calculations explained by El-Sharkawi [9].

Solutions having different water 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. For each cultivar, another series of Pb solutions (0, 2, 5 and 10 ppm) at the same different levels of osmotic water potential (Ψ_s + Pb) were prepared (*i.e.* each treatment had three replicates).

2.3. Preparation of Plant Extracts for Analysis

At the end of experimentation (37-day-old plants), average lengths of both root and shoot were measured, and 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 r.p.m. 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.

The plant extracts were analyzed for the cations sodium, potassium and lead by specific methods and techniques in the Central Lab. of Soil Department, Faculty of Agriculture, Assiut University, Egypt as follow:

1) Sodium, potassium and magnesium were determined by the flame emission technique which is a rapid and sensitive method for the determination of sodium and potassium. The flame photometer method [10] using Carl Zeiss flame photometer.

2) Lead cations (Pb^{2+}) of the plant root extract were determined by atomic absorption spectrophotometry using *ICAP*6200 (*ICP*-OES).

2.4. Statistical Treatments 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 combination included analyses of variance (F. values) coefficient of determination η^2, and a simple linear correlation coefficient (r.) respectively (Ostle, 1963). 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 (El-Sharkawi and Springuel, 1977). These analyses were computerized by using the SPSS program.

3. Results

3.1. Content of Ions

3.1.1. Na^+ Ions in the Roots and Shoot

Decreased Ψ_s, in general caused an increase in Na^+ content in all organs of cultivars studied (**Figure 1** & **Figure 2**). It was quite clear that, the shoots in the majority of plants had more Na^+ content than roots. Apparently, the effect magnitude of the Pb and Ψ_s levels differed according to organ and cultivar. **Figure 1** showed the sodium (Na^+) content in the roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels. In Jizan baladi roots, the Na^+ content was decreased at low Pb conc. and high water potential (Ψ_s = 0 MPa) The Na^+ amount was increased (0.23 $mg \cdot g^{-1}$ dry weight) under low Ψ_s levels especially at high Pb concentrations. The same was true in case of Sakha93 roots, particularly at moderate Ψ_s level (−0.7 MPa), whereas the low amounts of sodium was observed at relatively high Ψ_s and moderate Pb concentration. However, the roots of Giza168 cultivar accumulate Na^+ (0.27 $mg \cdot g^{-1}$ dry weight) at Pb range from 0 - 5 ppm at different Ψ_s levels with some exceptions.

Figure 2 showed the sodium (Na^+) content in the shoots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels. In shoots, the response of Na^+ content to changes in Ψ_s and Pb was depending on the *Triticum* cultivar. It is noticed that, the absence of Pb or salinity caused on increased in Na^+ content of Jizan B. shoots. In both Giza168 & Sakha93 the high Na^+ contents were existed at different Pb concentrations with lower Ψ_s levels, as a reflection of increased Na^+ concentration in the root medium. Generally, the Na^+ amount in both cultivar shoots gradually increased with decreasing osmotic water potential (Ψ_s).

Table 1 showed the effect of osmotic potential, lead and their interaction on the sodium content of both root and shoot of investigated *Triticumaestivum* L. cultivars. The (Ψ_s × Pb) interaction had a highly significant effect on Na^+ content in both roots and shoots of Sakha93. Likewise, this effect of (Ψ_s × Pb) interaction extended to Na^+ content of Jizan B. roots and Giza168 shoots. Whereas, the single factors effect was non-significant on the Na^+ content of different organs.

Table 1. ANOVA test showed the effect of osmotic potential, lead and their interaction on the sodium content of both root and shoot of investigated *Triticumaestivum* L. cultivars.

Cultivar		**Sakha93**		**Jizan baladi**		**Giza168**	
Organ	**Source of variance**	F	η^2	F	η^2	F	η^2
Root	**Pb**	2.129	0.141	0.237	0.028	1.322	0.016
	Ψ_s	2.396	0.589	1.439	0.706	2.132	0.981
	Pb × Ψ_s	4.682**	0.270	2.634*	0.267	0.186	0.0025
Shoot	**Pb**	0.661	0.058	0.889	0.138	1.078	0.090
	Ψ_s	1.411	0.539	1.052	0.797	1.650	0.571
	Pb × Ψ_s	6.202**	0.402	0.407	0.065	5.143**	0.340
D.F		Pb = 3		Salinity = 3		Pb × Salinty = 15	

* Significant at $P < 0.05$ level; ** Significant at $P < 0.01$ level.

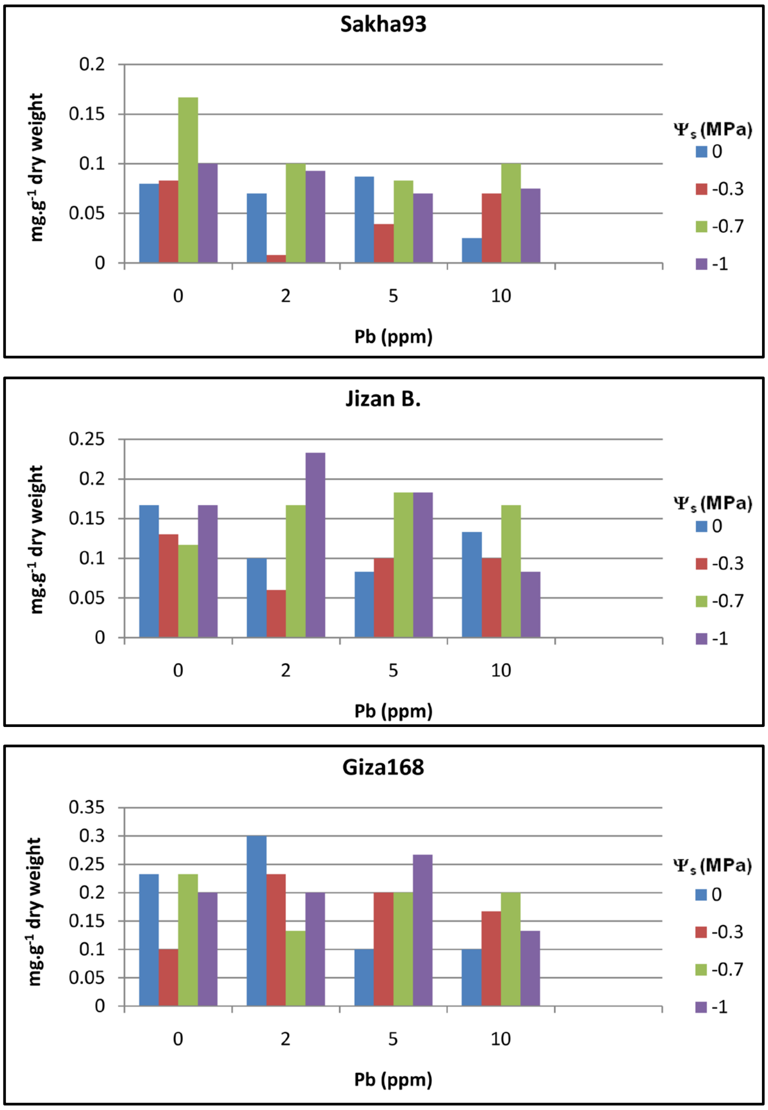

Figure 1. Sodium (Na^+) content in the roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels.

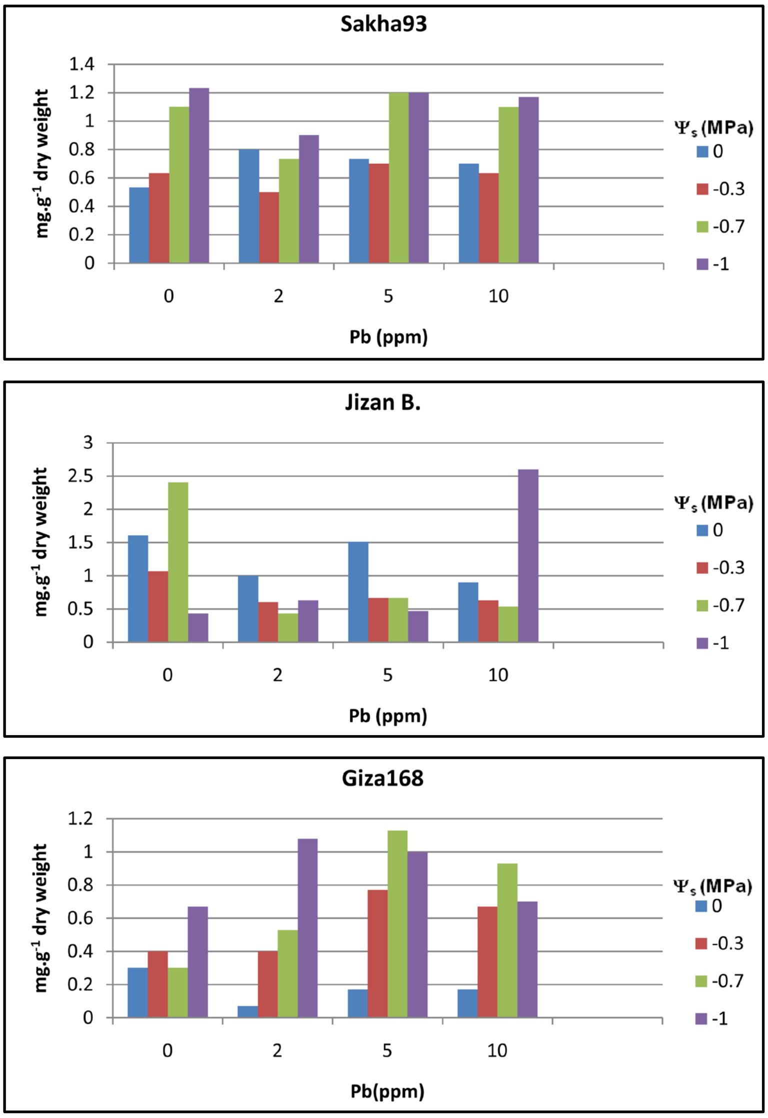

Figure 2. Sodium (Na^+) content in the shoots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels.

3.1.2. Potassium Ions in the Roots and Shoots

The accumulation of K^+ in different plant organs was higher than that of Na^+. The effects of both Ψ_s and Pb levels on the K^+ contents in root and shoot organs of experimental plants were showed in (**Figure 3** & **Figure 4**). **Figure 3** showed the potassium (K^+) content in roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels. In the roots, the K^+ content was low in un-water stressed plants at different Pb concentrations. Whereas, the decreased Ψ_s enhance the accumulation of K^+ ions particularly under low Pb concentration. Therefore, a high K^+ contents in all tested roots were observed at moderate Ψ_s levels at different concentrations of Pb.

Figure 4 showed the potassium (K^+) content in shoot of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels. The K^+ content in the shoots of various plants was higher than that in the roots, which was differed among studied cultivars. In Sakha93, the K^+ content was induced at relatively high Ψ_s levels, in the Pb range 0 - 5 ppm, while low Ψ_s stimulate the K^+ content. Also, this was showed in the absence of Pb element. This stimulation of K^+ content was found in Giza168 shoots at the same Ψ_s levels in the presence of low Pb concentration (2 ppm). The high K^+ accumulation was also observed at high Ψ_s level at Pb = 10 ppm, which was induced by Pb 5 ppm with relatively high Ψ_s levels. Conversely, in Jizan B. shoots, the K^+ content

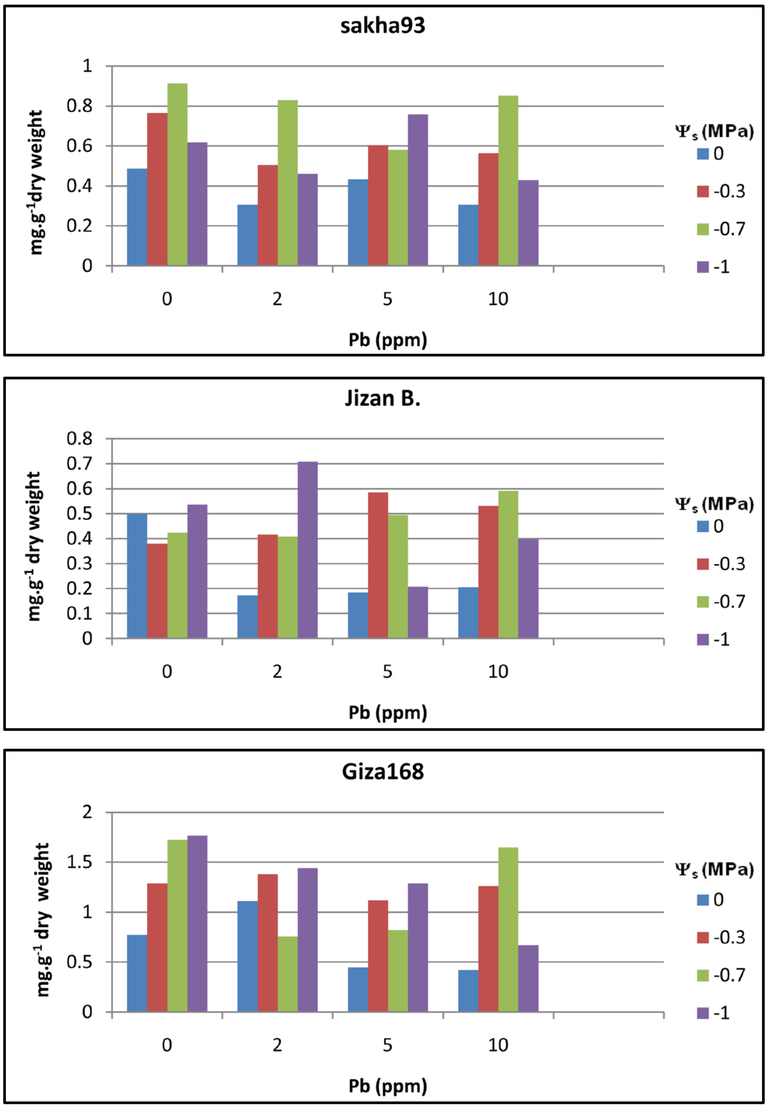

Figure 3. Potassium (K^+) content in roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels.

was induced by low Ψ_s levels at Pb 2 ppm. The high K^+ content was detected at Pb 5 ppm at ($\Psi_s = 0$ MPa) and shifted to Pb 10 ppm at $\Psi_s = -1.0$ MPa. In the absence of Pb, this K^+ stimulation was observed at $\Psi_s = -0.7$ MPa.

The effect of osmotic potential, lead and their interaction on the potassium content of both root and shoot of investigated *Triticumaestivum* L. cultivars was showed in Table 2. As in case of Na^+, the effects of ($\Psi_s \times$ Pb) interaction was significant on K^+ content of roots in different cultivars, which was played the main role. In case of shoots, neither single factors nor their interaction had no significant effect on the K^+ content.

Table 3 presented the mean values of K^+/Na^+ ratio in both roots and shoots of different *Triticumaestivum* L. cultivars at different Ψ_s levels & Pb concentrations. Apparently, the K^+/Na^+ ratio was higher in root than in shoot of Sakha93 plants, whereas in both Jizan B. and Giza168 the shoot had a higher ratio than root. Generally, Giza168 shoot had a higher ratio than that of the rest cultivars.

3.1.3. Lead (Pb) Ions in the Roots

The Lead content in roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels was showed in Figure 5. It is quite clear that, the Pb elements in the roots of investigated plants had a

Table 2. ANOVA test showed the effect of osmotic potential, lead and their interaction on the potassium content of both root and shoot of investigated *Triticumaestivum* L. cultivars.

Cultivar		Sakha93		Jizan baladi		Giza168	
Organ	**Source of variance**	F	η^2	F	η^2	F	η^2
Root	**Pb**	0.876	0.076	0.335	0.034	1.743	0.120
	Ψ_s	1.532	0.565	1.983	0.722	2.854	0.648
	Pb × Ψ_s	5.306**	0.359	2.852*	0.244	3.772**	0.232
Shoot	**Pb**	0.483	0.074	1.078	0.100	0.404	0.072
	Ψ_s	0.952	0.716	1.850	0.891	1.314	0.901
	Pb × Ψ_s	1.458	0.210	0.097	0.0096	0.144	0.026
D.F		Pb = 3		Salinity = 3		Pb × Salinty = 15	

*Significant at $P < 0.05$ level; **Significant at $P < 0.01$ level.

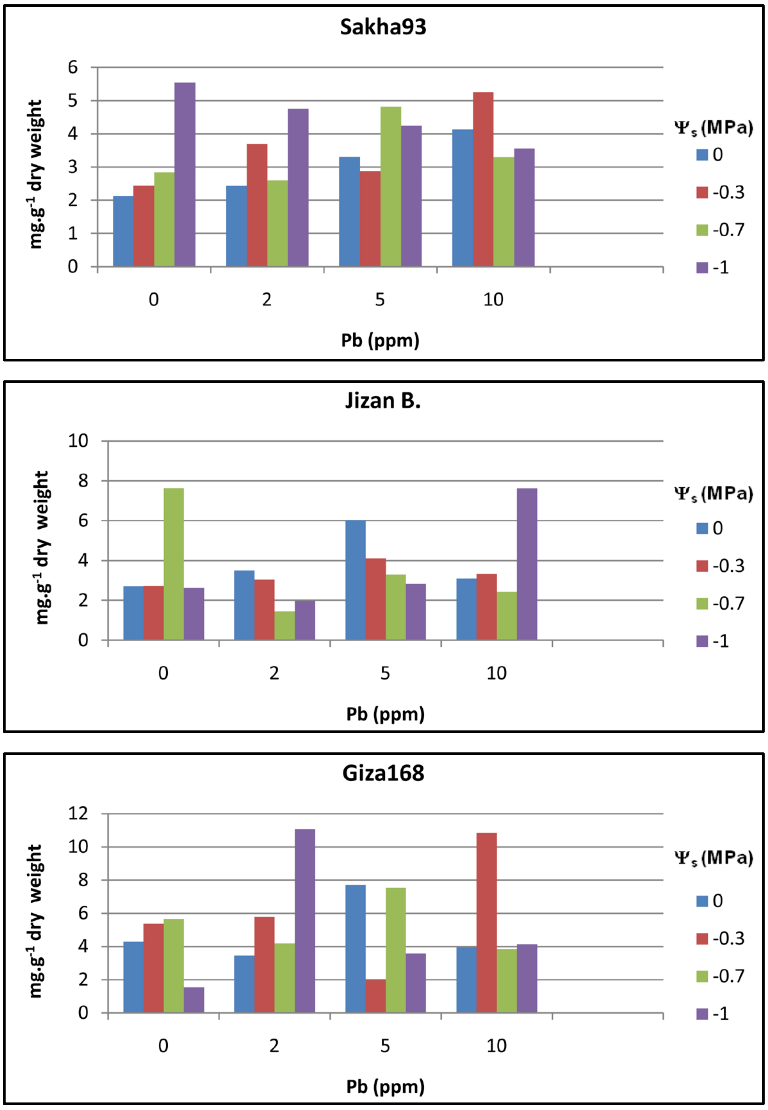

Figure 4. Potassium (K^+) content in shoot of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels.

Table 3. The mean values of K^+/Na^+ ratio in both roots and shoots of different *Triticumaestivum* L. cultivars at different Ψ_s levels & Pb concentrations.

	cultivar	**Sakha93**		**Giza168**		**Jizan baladi**	
Stress		**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
Osmotic water potential (MPa)	**0**	6.93	4.28	3.93	33.2	2.13	3.15
	−0.3	9.75	5.9	8	11.7	5.23	4.75
	−0.7	7.35	3.3	6.35	9.35	3.05	4.03
	−1.0	6.93	4.08	6.45	5.53	3.03	4.55
Lead concentration (ppm)	**0**	6.75	3.73	8.1	12.25	3.18	3.33
	2 ppm	6	4.83	5.53	20.63	3.5	3.78
	5 ppm	8.85	4.03	4.75	14.58	3.95	5.28
	10 ppm	8.78	5.05	6.25	12.4	3.73	4.05

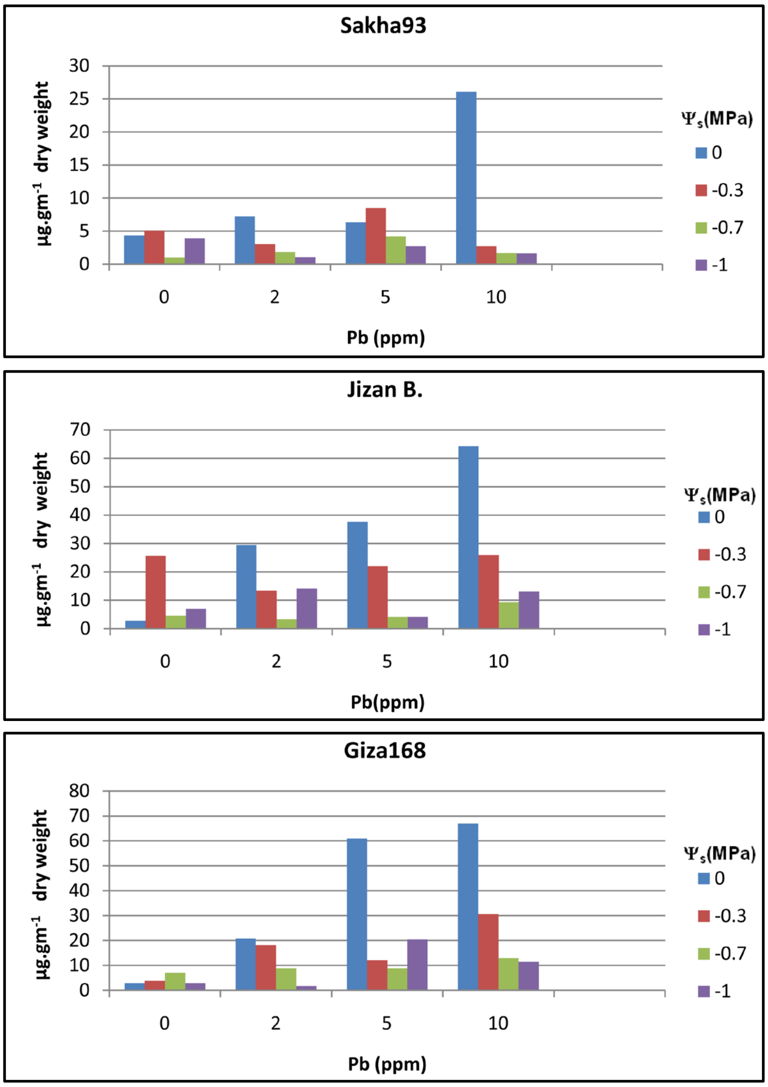

Figure 5. Lead content in roots of *Triticumaestivum* L. cultivars at different Pb conc. and osmotic water potential Ψ_s levels.

tendency to accumulate under the high Ψ_s levels (0 to −0.3 MPa), particularly at Pb 10 ppm. The roots of Sakha93 were accumulated less Pb than that of Jizan baladi and Giza168. The maximum Pb contents was 67.2; 64.3 & 26.1 mg·g^{-1} dry weight in Giza168, Jizan B. and Sakha93, respectively at $\Psi_s = 0$ MPa and Pb = 10 ppm. Also, the decreased Ψ_s accelerated the roots of different plants to minimize the Pb content, particularly at the lowest Ψ_s levels.

Table 4 presented the effect of osmotic potential, lead and their interaction on the lead content of root of investigated *Triticumaestivum* L. cultivars. The Pb content of Sakha93 & Jizan plants roots was greatly affected by the interaction ($\Psi_s \times$ Pb) factor, while Ψ_s effect was significant on the Pb content of Giza168 roots.

Table 5 showed the correlation coefficient (r.) values between Na$^+$, K$^+$ and Pb in both root and shoot of different studied cultivars of *Triticumaestivum* L. under osmotic potential, lead stress and their interaction. The significant correlation between Na$^+$ and K$^+$ in root and shoot of different cultivars was positive, whereas, yielded a negative correlation in case of Pb with both Na$^+$ and K$^+$ under salinity, lead stress or/and their interaction.

4. Discussion

The use of ions for osmotic adjustment may be energetically more favorable than biosynthesis of organic osmolytes [11]. In the present work, the K$^+$ accumulation in the experimented roots and shoots was detected under low Ψ_s levels, particularly in Giza168 plants. This accumulation of K$^+$ was decreased with increasing Pb concentration. The same was true in case of Na$^+$ in shoots of different cultivars, except in Jizan baladi where more Na$^+$ accumulation was detected in the absence of Pb. This means that, the inhibition of mineral ion flux appeared to be a general consequence of lead exposure. Also, a high salinity of the surrounding solution gives rise to a

Table 4. ANOVA test showed the effect of osmotic potential, lead and their interaction on the lead content of root of investigated *Triticumaestivum* L. cultivars.

Cultivar		Sakha93		Jizan baladi		Giza168	
Organ	Source of variance	F	η^2	F	η^2	F	η^2
Root	Pb	0.648	0.067	1.647	0.105	2.098	0.143
	Ψ_s	1.631	0.682	2.953	0.605	3.468*	0.709
	Pb × Ψ_s	2.791*	0.252	5.652**	0.290	2.168	0.148
D.F		Pb = 3		Salinity = 3		Pb × Salinty = 15	

*Significant at $P < 0.05$ level; **Significant at $P < 0.01$ level.

Table 5. Correlation coefficient (r.) values between Na$^+$, K$^+$ and Pb in both root and shoot of different studied cultivars of *Triticumaestivum* L. under osmotic water potential, lead stress and their interaction.

Cultivar	Contents Source of variance	K$^+$ & Na$^+$ Root	K$^+$ & Na$^+$ Shoot	Na$^+$ & Pb Root	K$^+$ & Pb Root	D.F
Sakha93	Pb	**0.940***	0.571	−0.463	−0.381	3
	Ψ_s	0.656	0.652	−0.555	−0.840	3
	Pb × Ψ_s	**0.589***	0.415	**−0.515***	**−0.511***	15
Giza168	Pb	0.363	0.332	−0.656	**−0.935***	3
	Ψ_s	0.287	0.238	−0.473	**−0.969****	3
	Pb × Ψ_s	**0.503***	0.206	−0.422	**−0.579***	15
Jizan baladi	Pb	0.128	0.664	**−0.997***	−0.187	3
	Ψ_s	0.274	**0.938***	−0.745	−0.829	3
	Pb × Ψ_s	0.407	**0.867***	−0.380	**−0.486***	15

*Significant at $P < 0.05$ level; **Significant at $P < 0.01$ level.

change of water and ionic status in the plant cells [12]. Commonly, the shoots had higher contents of both K^+ and Na^+ than the roots. A high K^+/Na^+ ratio in cytosol is essential for normal cellular functions of the plants [13]. Hence, regulation of K^+ uptake, prevention of Na^+ influx, promotion of Na^+ efflux from the cell and utilization of Na^+ for osmotic adjustment are the strategies commonly used by plants to maintain desirable K^+/Na^+ ratio in cytosol [14]. It was found that, the content of K^+ in experimented plants was higher than that of Na^+, ultimately high K^+/Na^+ ratio. This means that, the maintenance of high K^+ content may act as the major cationic osmoticum under salinity stress [15]. Also, the ion partitioning may be contributing to the improved salt tolerance of genotypes [16]. In this respect, the K^+/Na^+ selectivity of potassium channels and the existence of an apoplastic barrier, the Casparian bands of the endodermis, lead to the lateral gradient of K^+ and Na^+ across root tissue, resulting not only in high levels of $[K^+]$ in the shoot, but also a large $[Na^+]$ gradient between the root and the shoot [17]. This phenomenon well detected in case of Giza168 and Sakha93 shoot and root, respectively under Ψ_s exposure. The ANOVA values indicated that, the ($\Psi_s \times$ Pb) interaction had significant effect on the K^+ content of all plant roots, as well as on the Na^+ content in roots of Sakha93 and Jizan baladi plants. Similarly, this interaction had the same effect on Na^+ content in shoots of both Sakha93 and Giza168. Apparently, the applied salinity interacted with Pb in order to minimizing the detrimental effects of toxic ions on crop cultivars of *Triticumaestivum* L.

On the other hand, the largest proportion of Pb^{2+} is accumulated within roots in an insoluble form [18] because of the blockage by Casparian strips within the endodermis and usually limited in shoots. In the present work, the Pb content in Sakha93 roots had lowest values among the studied plants due to its sensitivity to Pbions. Obviously, the absence of salinity accelerated the Pb content in different plant roots, while the Pb accumulation was decreased with increasing salinity, whereas, the content of Pb^{2+} gradually increased with increasing Pb concentration in the root medium. This means that the lead content depends on the concentration, salinity and wheat cultivars. Accordingly, the ($\Psi_s \times$ Pb) interaction had a significant effect on the Pb content in the roots of both Sakha93 and Jizan baladi, while mean Ψ_s effect was significant in case of Giza168 roots. Apparently, the plants absorb Pb from solution in the soil through their roots and, subsequently, the largest proportion of Pb^{2+} is accumulated within roots in an insoluble form [18]. Thus, the inhibition of mineral ion uptake appears to be a general consequence of lead exposure. Conversely, increased provision of certain inorganic salts can antagonize lead effects to some extent [7].

5. Conclusion

It can be concluded that, Sakha93 cultivar was more adapted to decreased osmotic potential and had a capability to avoid Pb accumulation. Additionally, the presence of Ca-salts in the root medium counteracted the deleterious effects of Pb during cultivation to improve any cropping introduced in rural areas when the soil was polluted by automotive exhaust and in fields contaminated with fertilizers containing heavy metal impurities.

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