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Impact of different concentrations of Pb with or without salinity on the growth and nutrient uptakes of date palm *Phoenix dactylifera* L. seedling

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ABSTRACT

Date palm seedlings were exposed to Pb (100, 300 and 600 mg.kg) alone or in combination with salinity (200 mM NaCl solution). The influences of Pb stress individually or combined with salinity stress on growth was examined based on number and length of the roots and leaves of date palm seedlings. Plant appearance was also observed. H_2O_2, Malondialdehyde (MDA) and Electrolyte leakage (EL) levels were estimated as oxidative stress markers. Macronutrients (NPK) and micronutrients (Zn, Fe and Cu) as well as Pb accumulation in the root and leaves were estimated. Results showed that 300 and 600 $mg.kg^{-1}$ Pb inhibited the growth of date palm seedlings by reducing the length of the roots and leaves. A higher degree of reduction was detected in the treatment combining Pb and salinity. The interaction of Pb with salinity increased the contents of H_2O_2, MDA, and electrolyte leakage, which are oxidative stress markers in the root and leaves. The increase was more pronounced when 600 $mg.kg^{-1}$ Pb was applied with salinity. The N and K contents in the treated date palm seedlings decreased with increasing Pb concentration, especially when the treatment was combined with salinity. By contrast, the P content increased. The contents of Fe and Cu decreased when the seedlings exposed to Pb. This effect was more evident when Pb stress was interacted with salinity. An opposite trend was observed for Zn content. Compared with the control, 600 $mg.kg^{-1}$ Pb increased the Pb accumulation in the roots by 6.43 times and by 7.31 times when combined with salinity. Meanwhile, the Pb accumulation in the leaves increased by 9.91 when Pb stress was applied alone and by 10.88 when Pb was combined with salinity. The main finding of the work: The high level of Pb inhibited the growth of date palm seedlings by reducing the length of the roots and leaves. A higher degree of reduction was detected in the treatment combining Pb and salinity.

***Key words**: Abiotic stress, Lead symptoms, Macronutrients, MDA, Micronutrients, Oxidative stress*

Introduction

Heavy metals pollution and soil salinization are important environmental stresses that threaten traditional agricultural production in arid and semi-arid regions around the world (Li *et al.*, 2010). Lead (Pb) is a hazardous heavy metal, but its biological function remains unknown (Maestri *et al.*, 2010). After arsenic, Pb is a hazardous substance, (ATSDR, 2013). The common phytotoxic effects of Pb are diminished growth, change cellular structures, ion homeostasis, decline of chlorophyll biosynthesis,

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hormonal imbalance and induced overproduction of reactive oxygen species (ROS) (Pourrut *et al.,* 2011; Shahid *et al*., 2011; Abass *et al*., 2016). The two major threats of salinity to plant growth are as follows: the first is in terms of osmotic and ionic stresses, and the second is manifested an oxidative stress (Parida and Das, 2005; Flower and Colmer, 2015). Salinity exerts deleterious effects on different physiological and metabolic processes of plants (Rahneshan *et al.,* 2018). Total concentration of metal is a poor indicator of potential effects and toxicities (Finzgar *et al.,* 2007). Alternatively, indicators of bioavailability and food chain transfer should be given more concern. The mobility and bioavailability of metals could be affected by soil properties such as pH, clay content and total soil metal content (McLean and Bledsoe, 1992). In saline and nonsaline soil environments, the mobility and bioavailability of metals may be altered (Khodavirdilo and Taghlidabad, 2014). In saline soils, the presence of high concentration of Cl ion increases metal mobility due to highly mobile inorganic ligand formation (McLean and Bledsoe, 1992). Some previous works investigated the effect of salinity on Pb uptake behaviour; the results showed that the mobility and bioavailability of Pb increased with increasing salinity. Acosta *et al.* (2011) found that the increase in the salinity led to an increase in the mobility of Pb. Nawaz *et al.* (2016) reported that salinity (applied as 200 mM NaCl) combined with 20 $mg.kg^{-1}$ Pb enhanced the accumulation of Pb in the root and leaves of *Eucalyptus camaldulensis* Dehnh.

The environment in Basrah governorate (southern Iraq) suffers from a rapid increase in Pb accumulation, as evident by the increased Pb concentration in the soil from 115 $mg.kg^{-1}$ in 2015 to 176 $mg.kg^{-1}$ in 2016 (Abass *et al.,* 2015; Al-Jabary *et al.,* 2016); the salinity problem also affects agricultural soils and water used in irrigation. Jabbar *et al.* (2018) reported that saline soil in Basrah city increased from 13.3% in 2000 to 19.4% in 2015. However, no studies have been conducted on the effect of salinity on the bioavailability of Pb to date palm *Phoenix dactylifera* L. trees, which are the most abundant fruit trees in this city.

This study aimed to examine the behaviour of Pb added at different concentrations to soil with or without salinity stress with regard to the accumulation of this metal in the roots and leaves of date palm. The effects on the growth, biochemical response and absorption of macronutrients (NPK) and some micronutrients (Zn, Fe and Cu) were also investigated.

Materials and Methods

For this experiment, 21 date palm seedlings (20 cm high) cultured in plastic pots (18 cm diameter and 15 cm depth) have been selected. The properties of culture soil were as follow: pH= 7.54; Ec=2.31 $ds.m^{-1}$; content of organic matter 5.48%; Exchange Cationic Capacity (CEC) = 21.51 $Cmol.kg^{-1}$, as well as the soil texture was silty clay, which the particle size distribution were: Sand =8.12%, clay= 49.32% and 42.56%.

Three concentrations of lead were prepared (100, 300 and 600 mg.kg) as an aqueous solution of $Pb(NO_3)_2$ (Sigma Aldrich, USA). Chemically pure NaCl (Himedia, India) salts were used to prepare salt solution at 200 mM (Depending on the salinity of Shatt al-Arab water, the largest source of irrigation water used to irrigate date palm orchards in Basra governorate). Seven treatments were created and applied as follow:

1: control treatment, the seedling irrigated with Pb and NaCl – free water.
2: Pb at 100 $mg.kg^{-1}$
3: Pb at 300 $mg.kg^{-1}$
4: Pb at 600 $mg.kg^{-1}$
5: Pb at 100 $mg.kg^{-1}$ +salinity
6: Pb at 300 $mg.kg^{-1}$ +salinity
7: Pb at 600 $mg.kg^{-1}$ +salinity.

The seedlings were irrigated as field capacity.

Growth parameters

The date palm seedlings were exposed to treatments for four months, after which the seedlings were removed from the soil, the growth measurements were taken, as the number of leaves and roots and then lengths the roots and leaves were measured using the measurement tape.

Estimation of oxidative stress markers

H_2O_2 content: Samples of the root and leaf (0.5 g) were homogenized with 5 ml of 0.1% (w/v) Trichloroacetic acid (TCA). The homogenate was centrifuged at 13000 rpm for 15 min. the reaction mixture consisted of 1 ml of supernatant, 0.5 ml of potassium phosphate buffer (10 mM; pH=7.0) and 1 ml of KI (1 M). The absorbance of mixture was read at 390 nm and H_2O_2 content was calculated from H_2O_2 solutions standard curve, the concentration expressed as µmole.g^{-1} fresh weight according to sergiev *et al.* (1997).

Malondialdehyde (MDA) content: procedure of Heath and Packer (1968) was used to determine the content of MDA; 0.5 g of fresh root and leaf tissue samples was homogenized in 5 mL TCA (0.1%, w/v), then centrifuged at 10000 rpm for 5 min; 1 ml of supernatant was added to 4 ml of Thiobaributric acid (TBA; 0.5% w/v) prepared in 20% TCA, the mixture was boiled, after 30 Min. the mixture was placed on ice to terminate the reaction, then centrifuged at 10000 for 15 Min. Absorbance at 532 and 600 nm was done, the MDA content was calculated by followed equation:

$$MDA\ content\ (nmole/g) = \frac{(A532 - A600)1000}{155}$$

Which 155= Extinction Coefficient of MDA.

Electrolyte leakage (EL %)

Dionisio-Sese and Tobita (1999) Method was used to determine Electrolyte leakage. Small part of leaf (5 mm) and segment of root was placed in test tube containing 8 ml distilled water. First electrical conductivity (EC1) was read after two hours from placing the tubes in water bath at 32 ^{0}C, while the second read (EC2) was done after autoclaved the samples at 121 ^{0}C for 20 Min. Electrolyte leakage (LE%) was calculated according to following equation:

$$EL\ (\%) = (EC1/EC2)\times 100$$

Nutrients and Pb uptake

Content of Pb, K, Zn, Fe and Cu in the roots and leaves of date palm seedling was measured by using Flame Atomic Spectrophotometer (Perkin Elmer AAS Analysis 300, US), after digesting 0.5 g of root and leaf sample as wet acid digestion according to Jones (1984). Briefly, a mixture of 5 ml of nitric acid (70%) and 1.5 ml of Perchloric acid (60%) were added to sample, then the solution was heated until the disappearance of the brown fume, after solution cooled, the sample was diluted with 5 ml Hydrochloric acid (1:1), subsequently, mixture was diluted to 25 ml with distilled water as final volume. Kjeldhal method was followed to determined N content and the chlorostannous molybdophosphoric blue color method was used to determined P content (Piper, 1944).

Statistical analysis

The complete randomized design was used to design this experiment, the obtained data was subjected to one-way analysis of variance (ANOVA), then the significant between the treatments was evaluated by the least significant difference (LSD) test at 5% probability level ($P \leq 0.05$). All statistical analysis was carried out on SPSS software version 22.

Results

Morphological description

Plate 1(a-g) shows the growth of date palm seedlings under Pb stress alone at different concentrations (100, 300 and 600 mg.kg^{-1}) or combined with salinity (200 mM). Untreated seedlings and those treated with 100 mg.kg^{-1} Pb individually or combined with salinity were in good condition. The leaves and roots grew well, and secondary root formation was detected. No blackening was observed in the roots, except in the root cap of seedling roots treated with 100 mg.kg^{-1} Pb combined with salinity. The addition of 300 mg.kg^{-1} Pb caused weak growth, slight blackening in the roots and slight dryness in the leaves. When Pb was added at 300 mg.kg^{-1} with salinity, root growth was suppressed, no secondary roots were formed, most of the roots died and the growth apex blackened. When the seedlings were treated with 600 mg.kg^{-1} Pb, blackening was observed in the root cap and growth apex, and most of the leaves were dried. In the same Pb treatment but with salinity stress added, secondary root formation was inhibited, blackening of most root tissues was detected, the growth apex was weak and black and most leaves were wilted.

Table 1 reveals the effect of Pb with or without salinity on the number and length of the roots and leaves of date palm seedlings. No significant effect of Pb on the number of roots and leaves was observed when the metal was added alone or in combination with salinity. The effect of Pb was obvious on the length of the roots and leaves. The longest root and leaf were recorded in the control, with values of 16.41 and 36.95 cm, respectively. No significant difference was observed between control and the treatment with 100 mg.kg^{-1} Pb with or without salinity. The shortest root length (9.66 cm) was found in the treatment with 600 mg.kg^{-1} Pb, and the

Plate (1). Date palm seedling treated with different concentrations of lead with or without salinity.
a: Contrl b: Pb at 100 mg.kg^{-1} c: Pb at 300 mg.kg^{-1} d: Pb at 600 mg.kg^{-1} e: Pb at 100 mg.kg^{-1} with salinity at 200 mM (f) : Pb at 300 mg.kg^{-1} with salinity at 200 mM. g: Pb at 600 mg.kg^{-1} with salinity at 200 mM

shortest leaf length (27.50 cm) was detected in the treatment with 600 mg.kg^{-1} Pb with salinity. The treatment with 600 mg.kg^{-1} Pb either alone or in combination with salinity exerted no significant effect on the length of roots and leaves.

Oxidative stress markers

Table 2 shows the effect of treatment with Pb at three concentrations alone or in combination with salinity on oxidative stress markers including H_2O_2 and MDA content as well as electrolyte leakage index in the roots and leaves of date palm seedlings. No significant effect was observed when 100 mg.kg^{-1} Pb was applied alone, but the effect was significant when the treatment was combined with salinity. Significant effects were observed in the treatments with 300 and 600 mg.kg^{-1} Pb, and such effects were enhanced when the treatments were combined with salinity. In the control, the H_2O_2 contents were 0.68 µmol.g^{-1} in the root and 0.74 µmol.g^{-1} in the leaf. The content of H_2O_2 increased significantly to 2.86 and 2.42 µmol.g^{-1} in the root and leaf, respectively, when the seedlings were exposed to 600 mg.kg Pb combined with salinity.

Table 1. Effect of different concentrations of Pb applied alone or combined with salinity on the growth of date palm seedlings

Treatments	Roots number	Roots length (cm)	Leaves number	Leaf length (cm)
Control	5.66 ± 0.57*	16.41 ± 1.04a	5.33 ± 0.57	36.95 ± 0.38a
Pb 100 mg.kg^{-1}	4.66 ± 0.57	16.16 ± 0.38a	4.33 ± 0.57	36.75 ± 0.75a
Pb 100 mg.kg-1+ salinity	5.00 ± 1.00	15.71 ± 0.62a	5.33 ± 0.57	35.58 ± 0.62a
Pb 300 mg.kg^{-1}	4.66 ± 0.52	13.16 ± 1.25b	4.33 ± 1.52	33.16 ± 0.76b
Pb 300 mg.kg-1+ salinity	4.33 ± 0.57	12.83 ± 0.28b	3.66 ± 0.57	30.00 ± 0.90c
Pb 600 mg.kg^{-1}	4.33 ± 0.57	9.66 ± 0.28c	4.00 ± 1.00	29.33 ± 1.25d
Pb 600 mg.kg-1+ salinity	4.66 ± 1.15	10.08 ± 0.38c	4.66 ± 0.52	27.50 ± 1.50e
LSD	NS	1.24	NS	1.66

*Values are mean ±SD; Different letters indicate significant differences between means at ($P<0.05$)

The MDA content increased significantly in the root (4.92 nmol.g^{-1}) and leaf (4.59 nmol.g^{-1}) under Pb stress at 600 mg.kg^{-1} combined with salinity compared with all treatments. The MDA content in the control was 1.22 nmol.g^{-1} in the root and 1.15 nmol.g^{-1} in the leaf.

The electrolyte leakage (EL%) increased under applied treatments. This parameter significantly increased from 22.14% and 15.16% in the root and leaf of the control seedlings, respectively, and reached the highest average under treatment with 600 mg.kg^{-1} Pb combined with salinity (59.34% in the root and 51.23% in the leaf).

Effect of Pb with or without salinity on NPK uptake

The N, P and K contents in the root and leaf of date palm seedlings exposed to Pb alone or in combination with salinity are presented in Figs. 1, 2 and 3. In general, the N and P contents were higher in the leaf than in the root. An opposite trend was observed in the K content, which was higher in the root than in the leaf. Treatment with Pb at all concentrations whether alone or combined with salinity led to a significant reduction in the N content in the leaf and root compared with the control seedlings (Fig. 1). The N content in the leaves of date palm seedlings was 4.26 mg.kg^{-1}, with an average of 1.24 mg.kg^{-1}, when the seedlings were exposed to 600 mg.kg^{-1} Pb with salinity. The content was not significantly different than that (1.27 mg.kg^{-1}) obtained in the treatment with 600 mg.kg^{-1} Pb without salinity. The same trends were detected in the N content in the root, which had the highest average of 2.61 mg.kg^{-1} in the control and the lowest value of 1.03 mg.kg^{-1} in the treatment with 600 mg.kg^{-1} Pb with salinity. Similarly, the N content in the root seedlings exposed to Pb alone at 600 mg.kg^{-1} was 1.04 mg.kg^{-1}.

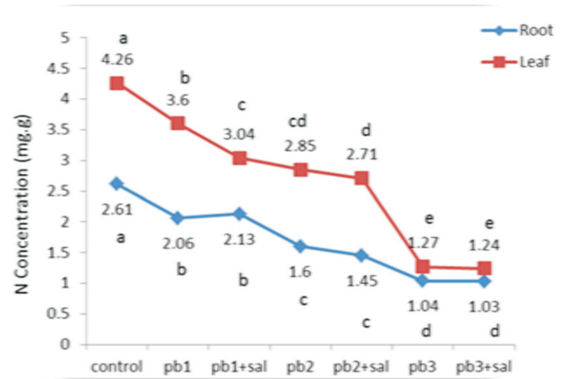

Fig. 1. Effect of different concentration of Pb applied alone or combined with salinity on N content in the root and leaves of date palm seedlings

Fig. 2 shows that treatments with Pb of different concentration whether applied alone or in combination with salinity significantly increased the P content in date palm seedlings compared with the con-

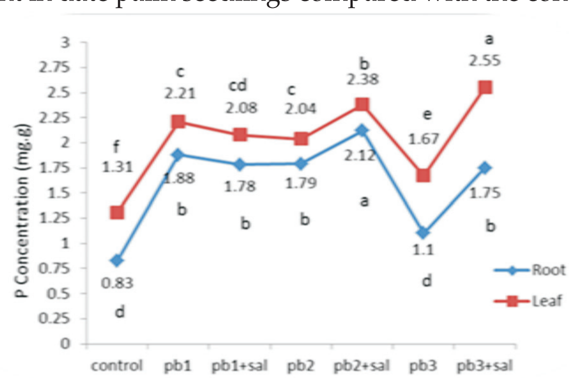

Fig. 2. Effect of different concentration of Pb applied alone or combined with salinity on P content in the root and leaves of date palm seedlings.

Table 2. Effect of different concentrations of Pb applied alone or combined with salinity on oxidative stress markers (H_2O_2, MDA and Electrolyte Leakage) in the root and leaves of date palm seedlings.

Treatments	H_2O_2 (µmole.g^{-1})		MDA (nmole.g^{-1})		EL (%)	
	Root	Leaf	Root	Leaf	Root	Leaf
Control	0.68 ± 0.08e*	0.74 ± 0.21e	1.22 ± 0.05f	1.15 ± 0.08e	22.14 ± 0.09f	15.16± 0.39f
Pb 100 mg.kg^{-1}	0.85 ± 0.95e	0.79 ± 0.09e	1.36 ± 0.09ef	1.18 ± 0.12e	24.19 ± 0.95f	16.21 ±0.96f
Pb 100 mg.kg^{-1}+ salinity	1.18 ± 0.26d	0.92 ± 0.19e	1.77 ± 0.13e	1.22 ± 0.43e	29.66 ±0.46e	21.31 ±0.99e
Pb 300 mg.kg^{-1}	1.32 ± 0.92d	1.26 ± 0.15d	3.12 ± 0.39d	2.65 ± 0.85d	42.73±1.02d	26.23 ± 0.88d
Pb 300 mg.kg^{-1}+ salinity	1.72 ± 1.02c	1.58 ± 0.28c	3.79 ± 0.21c	3.22 ± 0.76c	48.17±0.25c	32.25± 0.31c
Pb 600 mg.kg^{-1}	2.18 ± 0.33b	1.93± 0.55b	4.26 ± 0.66b	3.95 ± 0.22b	52.42 ±0.63b	45.12 ± 0.64b
Pb 600 mg.kg^{-1}+ salinity	2.86 ± 0.45a	2.42 ± 0.12a	4.92 ± 0.96a	4.59 ± 0.64a	59.34 ±0.29a	51.23 ± 0.77a
LSD	0.23	0.31	0.45	0.39	2.82	3.21

*Values are mean± SD; Different letters indicate significant differences between means at ($P<0.05$)

posed to 600 mg.kg^{-1} Pb with salinity. This combined treatment was significantly superior over other treatments. The lowest P content (1.31 mg/kg^{-1}) was detected in control plants. The lowest P content in the root was found in control seedlings (0.83 mg.kg^{-1}) and seedlings exposed to 600 mg.kg^{-1} Pb (1.1 mg.kg^{-1}). The difference between the two treatments was not significant. The highest P content (2.12 mg.kg^{-1}) was found in the root of seedlings exposed to 300 mg.kg^{-1} Pb combined with salinity, and this combined treatment was statistically superior to other treatments. Fig. 3 shows the K content in the root and leaves of date palm seedlings grown under Pb stress at different concentrations with or without salinity. All examined treatments significantly decreased the K content in the roots and leaves compared with the control. The K contents were 3.55 and 3.22 mg.kg^{-1} in the roots and leaves of the untreated seedlings, respectively, and the lowest content was observed in the treatment with 600 mg.kg^{-1} Pb combined with salinity (1.17 and 1.05 mg.kg^{-1} in the roots and leaves, respectively).

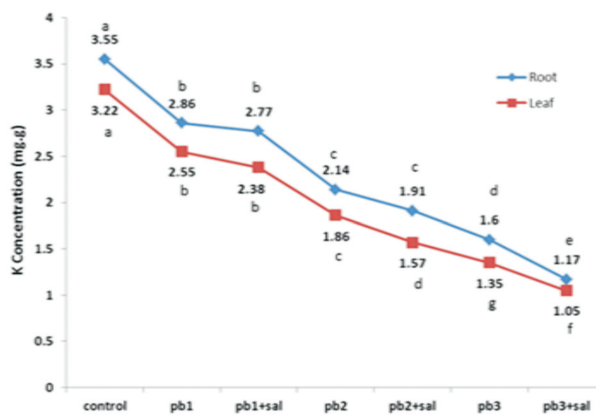

Fig. 3. Effect of different concentration of Pb applied alone or combined with salinity on K content in the root and leaves of date palm seedlings.

Effect of Pb with or without salinity on Zn, Fe and Cu uptake

The concentrations of Zn in the root and leaves of date palm seedlings exposed to different concentration of Pb (100, 300 and 600 mg.kg^{-1}) applied alone or combined with salinity water (200 mM) are shown in Fig. 4. The concentration of Zn in the root was higher than in the leaves. The highest concentration (34.78 mg.kg^{-1}) in the root was found when the seedlings were exposed to 600 mg.kg^{-1} Pb with salinity, and this value was significantly different from that obtained in the other treatments. The lowest concentration (14.93 mg.kg^{-1}) was found in the control and was significantly different from the contents in the other treatments except in the treatment with 100 mg.kg^{-1} Pb alone. The same trend was observed for Zn concentration in the leaves. The highest Zn concentration (14.18 mg.kg^{-1}) was detected in the treatment with 600 mg.kg^{-1} Pb with salinity and was significantly different from the other treatments. The lowest Zn concentration was found in the control, with a value of 2.58 mg.kg^{-1}, which was significantly different from those obtained in the other treatments.

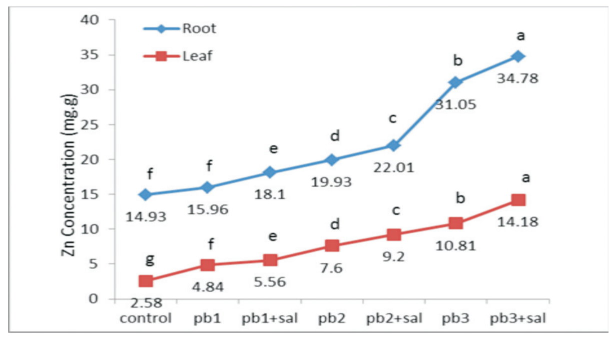

Fig. 4. Effect of different concentration of Pb applied alone or combined with salinity on Zn content in the root and leaves of date palm seedlings.

In contrast to the Zn results, the Fe concentration decreased in the seedlings exposed to Pb either alone or in combination with salinity (Fig. 5). The Fe concentration in the roots was reduced from 339.82 mg.g^{-1} in the control to 141.57 mg.g^{-1} in the treatment with 600 ppm Pb combined with salinity. No significant difference was found when Pb was applied at 600 mg.kg^{-1} either alone or in combination with sa-

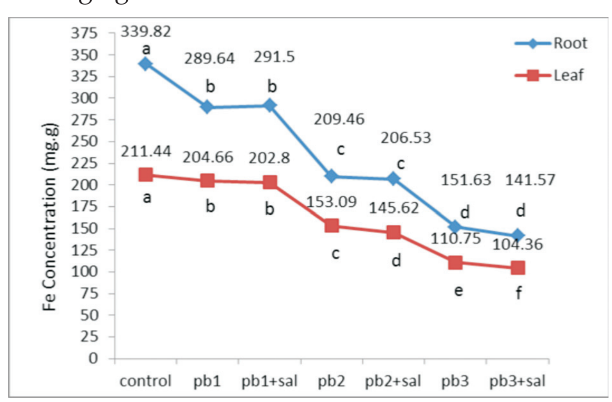

Fig. 5. Effect of different concentration of Pb applied alone or combined with salinity on Fe content in the root and leaves of date palm seedlings.

linity. The lowest Fe concentration in the leaves was found in the seedlings treated by 600 mg.kg^{-1} Pb combined with salinity, and the obtained value of 104.36 mg.g^{-1} was significantly different from those in the other treatments. The highest Fe concentration was found in the control (211.44 mg.kg^{-1}) and was statistically superior to the other treatments.

Fig. 6 Illustrates the Cu concentration in the root and leaves of date palm seedlings treated with Pb at different concentrations alone or in combination with salinity. The highest concentrations of Cu were 11.89 and 8.89 mg.kg^{-1} in the root and leaves of the control, respectively, and these values were reduced significantly to 2.41 and 1.24 mg.kg^{-1}, respectively, when the date palm seedlings were exposed to 600 mg.kg^{-1} Pb and salinity. No significant difference was recorded when Pb was applied at 600 mg.kg^{-1} either alone or in combination with salinity in the roots and leaves.

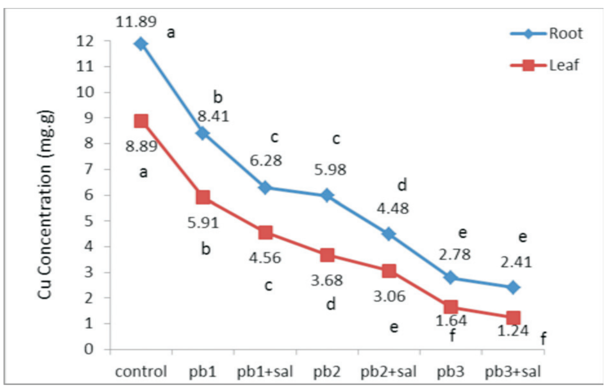

Fig. 6. Effect of different concentration of Pb applied alone or combined with salinity on Cu content in the root and leaves of date palm seedlings.

Effect of Pb with or without salinity on Pb accumulation

The accumulation of Pb in the roots and leaves of date palm seedlings treated with different concentrations of Pb alone or in combination with salinity is presented in Fig. 7. The accumulation of Pb increased in the roots and leaves but was higher in the former than in the latter. The increment was compatible with the increase in the Pb concentration. Moreover, salinity enhanced Pb accumulation. The concentrations of Pb were 42.49 mg.g^{-1} in the roots and 32.35 mg.g^{-1} in the leaves of the seedlings treated with 600 mg.kg^{-1} Pb combined with salinity. This combined treatment was statistically superior to the other treatments. The lowest Pb accumulation

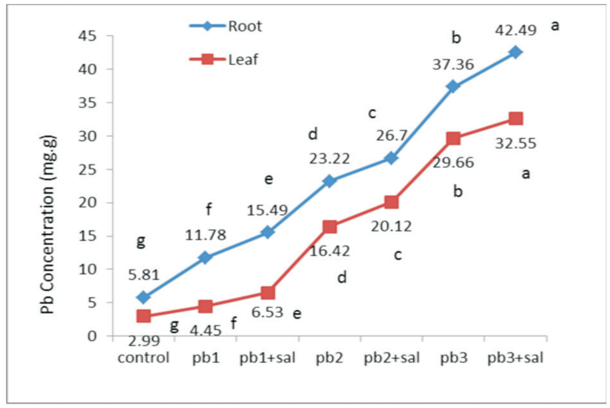

Fig (7) Effect of different concentration of Pb applied alone or combined with salinity on Pb accumulation in the root and leaves of date palm seedlings

with statistical difference was recorded in the control, with values of 5.81 mg.g^{-1} in the roots and 2.99 mg.g^{-1} in the leaves.

Discussion

In general, Pb phytotoxicity inhibits root growth, leads to stunted plant growth and affects several physiological processes, such as nutrient metabolic disturbance, disturbed photosynthesis, hormonal status and water imbalance (Islam *et al.*, 2007; Gopal and Rizvi 2008; Islam *et al.*, 2008). In our experiment conditions, blackening of the roots was less severe when date palm seedlings were exposed to 300 mg.kg^{-1} and higher when the seedlings were treated with 600 mg.kg^{-1} Pb applied alone or in combination with salinity. No blackening of the roots was observed when date palm seedlings were treated with 100 mg.kg^{-1} Pb either with or without salinity. Blackening of the root is one of the visual symptoms when plants are exposed to high concentrations of Pb (Sharma and Dubey, 2005; Nas *et al.*, 2018). Furthermore, the combination of 300 or 600 mg.kg^{-1} Pb with salinity suppressed secondary root formation. The addition of 600 mg.kg^{-1} Pb significantly reduced the root length up to 20% and the leaf length up to 16%. Under the same concentration of Pb but combined with salinity, the length of the roots was reduced up to 38% and that of the leaves up to 20% compared with the control. Previous studies reported that the high concentration of NaCl significantly reduced the growth of date palm, particularly its root (Al-Mansoori and Eldeen, 2007; Alturki, 2018). Changes in the root volume and diameter together with the production or inhibition of secondary roots are plant responses to Pb exposure (Fahr *et al*., 2013). The root growth inhibition could be attributed to the inhibition of cell division in the root tip as a result of Pb presence (Eun *et al*., 2000). This finding might also be associated with disorder of metabolic processes due to Pb and salinity stresses (Isayenkov and Maathuis, 2019).

Compared with the control, the combination of 600 $mg.kg^{-1}$ Pb with salinity (200 mM) increased the level of H_2O_2 by about fourfold in the root and threefold in the leaves; the MDA content was increased by about fourfold in both roots and leaves, and the electrolyte leakage increased by about 2.5-fold in the root and threefold in the leaves. Pb is one of heavy metals that can generate ROS, which could interfere with electron transport activities (Rico *et al*., 2009). Salinity also induce ROS production (Shahid *et al*., 2014). The combination of 600 $mg.kg^{-1}$ Pb and salinity (200 mM) increased the H_2O_2 content to 23.77%, the MDA level to 13.41 and the EL to 11.79 in the roots compared with Pb stress alone (600 $mg.kg^{-1}$). In the leaves, the increase reached 20.24%, 13.94% and 11.92% for H_2O_2, MDA and EL, respectively. Oxidative stress is an outcome of excessive ROS levels (Newsholme *et al*., 2016), thus plants suffer from severe toxic effects, including lipid peroxidation and disruption of membrane integrity (Cuypers *et al.,* 2011). Membrane damage measured indirectly by MDA content and electrolyte leakage may also be attributed to increased ROS levels. The results demonstrate that the ROS levels increased by increasing the accumulation of H_2O_2 in the roots and leaves. Membrane damage and increased H_2O_2 accumulation under heavy metals and salinity stress were reported in date palm in previous works (Abass *et al.,* 2016; Zouari *et al*., 2016; Suhim *et al.,* 2017)

The N and K uptakes decreased when date palm seedlings were exposed to Pb and Pb+salinity. The highest reduction in the N and K uptake (71%) in the roots was found in the treatment combining 600 $mg.kg^{-1}$ Pb with salinity; the reduction was about 70% when Pb at the same concentration was applied alone. In the leaves, the N uptake was reduced to 61% and 60% when the seedlings were treated with 600 $mg.kg^{-1}$ Pb with and without salinity, respectively. The reduction in the K uptake (67%) was similar between the roots and leaves when the seedlings were treated with 600 $mg.kg^{-1}$ Pb with salinity. When only Pb at the same concentration was applied, the reduction percentages were 55% in the root and 58% in the leaves. By contrast, the P uptake increased when date palm seedlings were treated with Pb and salinity. The P accumulation increased to 61% in the root under treatment with 300 $mg.kg^{-1}$ Pb combined with salinity and increased to 47% in the leaves under treatment with 600 $mg.kg^{-1}$ Pb with salinity. In general, abiotic stresses, including salinity and heavy metals, can effect on the nutrients concentration in the plant (Duman, 2012). According to Pourrut *et al.* (2011), Pb can decrease the uptake of nutrients either through competition or by altering some physiological plant activities. Pb is a heavy metal that reduces the absorption of nitrogen indirectly by inhibiting the activity of N assimilation-related enzymes, such as nitrate reductase and glutamine synthetase (Mallick and Rai, 1994). The reduction of K absorption can be attributed to competition with Pb. Sharma and Dubey (2005) reported that the similar radii led to strong interaction of K^+ ions with lead. Some reports indicated the increase in the P concentration in some plant tissues under salinity stress (Navarro *et al*., 2001; Demiral, 2017, Tang *et al*., 2019).

Applying Pb alone or in combination with salinity decreased the concentrations of Fe and Cu and increased the concentration of Zn in the roots and leaves of date palm seedlings. Significant reductions were observed in the Cu content, which were 80% in the root and 86% in the leaves. The reductions in the Fe content were 58% in the roots and 51% in the leaves. Meanwhile, the Zn content increased to 57% in the roots and 82% in the leaves. Alteration of micronutrient uptake due to Pb stress could be either through physical mechanism, which relies on the size of metal radii; chemical mechanism, via induced disorder of cell metabolism (Sharma and Dubey, 2005). Plants tend to accumulate Zn in their tissues under heavy metals stress because of the important role of this element in alleviating heavy metal phytotoxicity (Aravind *et al.,* 2009). According to Pourrut *et al.* (2011), Pb exposure decreases the concentrations of divalent cations (Zn, Fe and Cu). This finding is not consistent with the results of Zn in the present study but consistent with the results of Fe and Cu.

Compared with the control, Pb accumulation in the root and leaves of date palm seedlings increased when Pb was applied at all examined concentrations; in addition, the presence of NaCl led to enhanced accumulation of Pb in plant tissues. The accumulation of Pb in the roots increased to 51%, 75% and 84% when Pb was applied alone at 100, 300 and 600 $mg.kg^{-1}$, respectively. The increments were 62%, 78% and 86% when Pb was applied at the same concentration and combined with salinity. The accumulation of Pb in the leaves increased to 34%, 82% and 90% when Pb was applied without salinity at 100, 300 and 600 $mg.kg^{-1}$, respectively, whereas the accumulation of Pb increased to 54%, 85% and 91% when Pb was applied with salinity at 100, 300 and 600 $mg.kg^{-1}$, respectively. Fig. 7 shows that the concentration of Pb in the root was higher than that in the leaves. This finding could be due to the fact that the roots act as barrier to restrain the translocation of Pb to aerial plant organs and can absorb significant quantities of Pb (Pinho and Ledeiro, 2012). Acosta *et al*. (2011) revealed that salinity increased the mobility of Pb in nutrient solution, thereby increasing Pb absorption; the presence of Cl at high concentrations can form stable compounds, such as PbCl, $PbCl_2$ and $PbCl_3$, which could also increase mobility of Pb (Chu *et al.,* 2015). Nawaz *et al*. (2016) found that the uptake Pb by *E. camaldulensis Dehnh* was higher (49.3$mg.kg^{-1}$) when treated with 20 $mg.kg^{-1}$ Pb combined with salinity (200 mM) compared with the control.

Conclusion

Salinity (at least in the examined concentration) increased the accumulation of Pb in date palm seedling tissues. Salinity affects the bioavailability of Pb in soil as well as its translocation from the roots to the leaves. Date palm seedlings can grow in Pb-contaminated soils (100 $mg.kg^{-1}$) even if combined with salinity. No visual symptoms (e.g. blackening of roots, absence of secondary roots and reduction of root length and leaves) were observed when the seedlings were exposed to 100 $mg.kg^{-1}$ Pb whether alone or in combination with salinity. However, these symptoms appeared when the seedlings were exposed to 300 and 600 $mg.kg^{-1}$ Pb whether alone or in combination with salinity. Pb exerted varied effects, whether positive or negative, on the concentration of macronutrients and some micronutrients; such effects were enhanced by the presence of salinity.

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