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Assessment of the absorption ability of nitrate and lead by japanese raisin under salt stress conditions

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Abstract. Heavy metals pollution is an important challenge that was cussed by human activity, this stress decreases under salinity. The aim of this study was to investigate the ability of Japanese raisin in the absorption of nitrate (0, 30, and 60 mgL^{-1}) and lead (0, 300, and 600 mgL^{-1}) under salinity stress (0 as control and 3 and 6 dSm^{-1}). Results showed that the studied plant continued to uptake nitrate and potassium under stress conditions of Pb and salinity. Although Na and Cl uptake were observed as a defense mechanism in the plant, the K/Na ratio, and K content increased from 1 to 6 and from 1.8 to 5%, respectively. Also, the most appropriate physiological responses were observed at treatments under contamination level of 300 mg Pb and salinity level of 3 dSm^{-1}, so that the synthesis of malondialdehyde (MDA) and enzymatic activity increased at these levels of HMs and salinity. Based on the results, the studied species were able to uptake moderate concentrations of Pb (34.1-71mg kg^{-1}) under experimental conditions. Hence, its potential for the clean-up of some contaminants in the environment can be considered by researchers for further research.

Novelty statement. This study investigated the cleaning up of some heavy metals and nitrate from the environment and plants' physiological responses under stressful conditions. The plant species (Japanese raisin) characteristics and the results have enough novelty and will be published for the first time. Most hardwood trees such as walnut, oak, beech, poplar, etc. have a slow growth rate. But Japanese raisin tree a new and unknown plant in Iran has very important features. This tree is one of the few trees that in addition to having hardwood, has a very high growth rate. Which can be useful in creating artificial forests, Landscapes, as well as in industrial applications, buildings, and furniture industries. So far, no special research has been done on the phytoremediation characteristics of this plant and the selection of this plant in the present study and the study of the ability of this plant to absorb nitrate and lead under salinity stress is a completely new and innovative topic.

Keywords: Antioxidant enzymes, Heavy metal, Phytoremediation, Proline, Malondialdehyde, Salinity.

1. INTRODUCTION

Metal pollution is harmful for human health and the environment. Human activities have been considered an important factor in the contamination of the soil with heavy metals (HMs) (Akinci and Guven 2018; Motesharezadeh et al, 2016). The presence of HMs reduces soil fertility, crop yield, and soil microbial activity (Pinto et al, 2004; Sumiahadi and Acar, 2018). Lead (Pb), a HM pollutant in industrial ecosystems, is important in plant life because of its easily absorption by the plant roots, which is induced by its high accumulation in the surface area of the soil (Mosaferi et al, 2008; Wang et al, 2019). In addition to natural processes, Pb is also produced through the artificial sources (exhaust fumes from automobiles, factories, battery tanks, and pesticides). After Pb is absorbed by roots, it causes changes in metabolic activities of plants, disrupting their growth and development (Sharma and Dubey, 2005; Oguntade et al, 2018). Presence of Pb, leads to a disruption of membrane carriers' activity of the root cells, depleting nutrients such as magnesium, calcium, and iron. As a result of an experiment, deficiency symptoms of these nutrients were reported in Pb-treated plants (Sharma and Dubey, 2005). Moreover, the overuse of nitrate fertilizers in agriculture fields leads to nitrate pollution of ground and surface waters (Castro-Rodríguez et al, 2016).

Climate change and water deficit is the important challenge in agriculture activities all over the world and soil salinity is the one of most important problem that cause by these challenges (Isayenkov and Maathuis, 2019). There are many studies have reported that salinity stress induced by NaCl restricts agriculture and crop yield (Isayenkov and Maathuis, 2019). Plant resistance to salinity depends on some mechanisms such as antioxidants activity, ion homeostasis, biosynthesis of osmolytes, and gene expression. Phytoremediation is a useful technic based on the living plant's ability to absorb ionic compound by their roots or leaves and clean up soil, air and water contamination (Berti and Cunningham, 2000). There are many reports on phytoremediation, such as phytoremediation of high levels of nitrate with poplar trees (Castro-Rodriguez et al, 2016), zinc (Zn) and Pb nitrates with sunflowers (Adesodun et al, 2010), nitrate with *Salvinia molesta* (Ng and Chan, 2017) and Zn, Cd, and Pb with *Typha angustifolia* and *Eichhornia crassipes* (Sricoth et al, 2018). Generally, stresses such as salinity and heavy metals in which salinity increases the uptake of heavy metals, occur simultaneously in the environment. The results of a study indicated that the presence of Cd along with NaCl in the root environment of four barley cultivars significantly reduced soil Cd concentration and increased its uptake by plants (Huang et al, 2007). Similarly, Abbasi et al. (2013) investigated the effect of irrigation water salinity on the rate of heavy metals uptake in *Potamogeton berchtoldi* and reported that the concentration of heavy metals (Pb and Cd) in plant increased with the increasing salinity up to 4 and 6 dSm^{-1}, respectively. In general, based on the results of the numerous studies, it can be concluded that under conditions of HMs (such as Pb and Cd) and salinity stresses, the plant's nutritional needs for nutrients such as N, P and K will increase (Khoshgoftarmanesh, 2010; Yan et al, 2020). In fact, the recommendation for more application of these nutrients under stress conditions, is a strategic management to prevent reducing plant dry matter. It should be mentioned that nitrate, in principle, increases the resistance to salinity, which has been similarly reported in several studies (Bai et al, 2021).

However, there are limited reports on the phytoremediation ability of Japanese raisin or its ability to grow in the contaminated soils. Based on this background, this study was aimed to assess the potential use of Japanese raisin for the phytoremediation of a nitrate/Pb polluted soil under conditions of salt stress.

2. MATERIAL AND METHODS

2.1. Plant material and growth condition

One-year old seedlings of Japanese raisin (*Hovenia dulcis* L.) were prepared from Hirkania greenhouse (Nowshahr, Mazandaran, Iran). Seedlings were grown in plastic bags (25×30 cm), and fertilized with NPK fertilizer and a Hoagland based solution during the test period (Motesharezadeh et al, 2016), also the average temperature and the humidity were 25 °C and 70%, respectively (Ramesh et al, 2006). The seedlings were kept for five months and then harvested. Due to the stress induced by moving seedlings from a distant place in the north of the country, it was necessary to apply some treatments to plants reinforcement (Table S[1]1). Therefore, to improve the seedlings growth and before the application of experimental treatments, complete fertilizer and Hoagland nutrient solution were used and also leaching was considered.

[1] Supplementary data.

2.2. Experimental design

To execute the experiment, a neutral culture media (a combination of 70% non-enriched cocopeat and 30% perlite) was used. In order to investigate the capability of Japanese raisin for the phytoremediation of a NO_3^- / Pb polluted soil under conditions of NaCl stress, a factorial greenhouse trial was arranged in a completely randomized design with four replications. The treatments consisted of (1) NaCl salinity as the primary factor at three levels of 0 (control), 3, and 6 dSm^{-1}, according to previous reports (Salimi et al, 2012); (2) Nitrate derived from potassium nitrate, as the secondary factor, was applied at three levels (0, 30, and 60 mgL^{-1}) (Gheshlaghi et al, 2015); and (3) Pb form the source of lead nitrate (0, 300, and 600 mgL^{-1}) (Shabani et al, 2015) as the third factor. To have only the effect of nitrate, equivalent of potassium added in the treatments (from the source of potassium nitrate), potassium sulfate was added to other pots. In order to avoid the negative effect of possible stress on plant, potassium nitrate was applied during the holding period every two weeks via irrigation water.

2.3. Biochemical measurements

2.3.a Lead content measurement

Dry ashing method was used to analyze plant samples and measure Pb (Wallis, 1996). During the method, to measure metals, plants organic matter is destroyed with controlled heat. Based on the method, one gram of dried and powdered sample of the plant was poured into a crucible and placed in an electric oven at 550 degrees for 4 hours. After leaving the crucibles out of the furnace and reaching ambient temperature, samples were transferred to small beakers using 10 ml of 2 M hydrochloric acid. Then, the beakers were placed on an electric stove until the first white vapors appeared. Next, after reaching the ambient temperature, the contents of beakers were filtered through a filter paper inside a 100 ml volumetric flask and made up to volume with distilled water. Finally, Pb concentrations were reported in samples extract by use of ICP-OES (Inductively Coupled Plasma Atomic Spectroscopy). Pb uptake was measured and reported by multiplying its concentration by the plant dry matter (Sharma et al, 2012).

2.3.b Total Nitrogen and Nitrate content measurement

Total concentration of nitrogen (N) in plant samples was measured by Kjeldahl method (Horneck and Miller,1998). In this method ground plant material were digested to H_2SO_4 at high temperatures by the help of metal catalyst. Total nitrogen of plant was changed to ammonium (NH_4^+), which is then by titration the concentration of N was quantified. To measure the nitrate of the plant samples, ion-selective electrode method was used (Miller, 1988).

2.3.c Potassium/sodium ratio and chlore content measurements

Sodium and potassium concentrations were determined based on the method of wet digestion with hydrochloric acid using flame photometer ELEA (Ryan et al, 2002).

In order to measure the chlore (Cl) content in the plant material (Liu, 1998), after extraction, the Cl in the filtrate was analyzed using the colorimetric method on the TRAACS 800TM Auto-Analyzer. In this method, the sample is mixed with the color reagent and dialyzed into the color reagent again. The procedure is based on the release of thiocyanate ions from mercuric thiocyanate by Cl ions in the sample. The liberated thiocyanate reacts with ferric iron to form a red color complex of ferric thiocyanate. The color of the resulting solution is stable and directly proportional to the original Cl concentration. The color complex is measured at 480 nm using a 10-mm flow cell. Nitrite (NO_2), sulfide, cyanide, thiocyanate, bromide, and iodine ions cause interferences when present in sufficient amounts.

2.4. Antioxidant enzymes activity measurement

In order to evaluate the effect of salinity, nitrate and lead contamination stresses on plant physiological responses, the changes in enzymatic activity were measured. Among plant enzymes, catalase (CAT) and superoxide dismutase (SOD) are considered as sensitive enzymes indicating plant resistance mechanisms under stress conditions (Khadem Moghadam et al, 2016). Hence, these two enzymes were selected for this goal of the present study. Total protein content was determined following the method described by Bradford (1976). Protein content was determined using spectrophotometry at a wavelength of 595 nm. Also, the modified method of Chance and Maehly (1955) was used to measure the activity of peroxidase (POD) enzyme. The activity of superoxide dismutase, the basis of which is its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), was determined according to the method described by Dhindsa et al. (1981).

2.5. Soluble carbohydrate content measurement

The method of Irigoyen et al. (1992), was used to measure soluble carbohydrates. For this purpose, leave sample were ground in liquid nitrogen, 100 mg of them were blended with 5 mL of 70 % ethanol (wv^{-1}) for 5 min, then centrifuged at 3500 rpm for 10 min at 4 °C. After that 200 mL of the supernatant were added to 1 mL of an anthrone solution, then the absorbance was read by UV/vis spectrophotometer at 625 nm.

2.6. Malondialdehyde level assessment

Malondialdehyde (MDA) concentration was measured by thiobarbituric acid method by spectrophotometry. Its concentration was calculated using the extinction coefficient MDA-TBA for the complex (Dandekar et al., 2002).

2.7. Proline content measurement

To measure proline level, the procedure of Bate et al. (1973) were used. First, 10 ml of acid sulfuric were added to 100 mg of fresh leaf, they were then passed through filter paper. After 2ml ninhydrin and 2ml acid acetic glacial were added to 2 ml of extract and kept in benmary counter for 1 h, then toluene was added to them. After 2 h the supernatant was extracted. The absorbance at 520 nm was recorded.

2.8. Lipid peroxidization measurement

To quantify the amount of this enzyme, the modified Chance and Maehly (1955) method was used. In this method, 1 ml of potassium phosphate buffer (pH = 6.7) was poured into the cuvette and 17.6 µl of hydrogen peroxide and 17.6 µl of leaf extract were added to it. The resulting solution was immediately read in a spectrophotometer at a wavelength of 240 nm for 2 minutes at intervals of 15 seconds to calculate the activity of this enzyme according to the amount of light absorption.

2.9. Statical analysis

The present study was executed based on a factorial trial arranged in a completely randomized design (CRD) with four replications. Data were analyzed using SAS 9.2 and MSTATC software. Differences between treatments were determined following Duncan's Multiple Range Test (DMRT), ($P \leq 0.05$). figures were drawn using Excel 2010 software.

3. RESULTS

3.1. Biochemical content measurements

3.1.a. Plants lead level

According to the variance analysis results, the interactions of salinity, NO_3^-, and Pb significantly affected Pb concentration (Table S2). Based on the means comparison results, salinity at levels of 3 and 6 dSm^{-1} reduced Pb content by 13% and 36%, respectively; nitrate at levels of 30 and 60 mgL^{-1} decreased Pb content by 11% and 12%, respectively; and Pb at levels of 300 and 600 mgL^{-1} increased Pb content by 45% and 53%, respectively. Also, the highest Pb content (69 mg kg^{-1}) belonged to the treatments of S1N0Pb2 and S0N0Pb2, and the lowest one was observed at treatments with no added Pb (Fig. 1).

To better understand the ability of the studied plant how cope with stress and phytoremediation, the uptake rate was calculated for different treatments (Fig. 2). Results showed that in high salt concentration the high uptake of lead was observed in high nitrate concentration. Accordingly, Pb uptake and accumulation can be considered as a reliable indicator for phytoremediation under salinity stress conditions.

3.1.b. Plants total nitrogen and nitrate level

Regarding nitrogen content, it is understandable that there was a significant ($P \leq 0.01$) interaction between salinity, NO_3^-, and Pb. Additionally, NO_3^- significantly affected shoot nitrogen content ($P \leq 0.01$) (Table S2). The application of 30 and 60 mgL^{-1} NO_3^- increased N content by 10% and 18%, respectively. The highest N content (7.3%) was

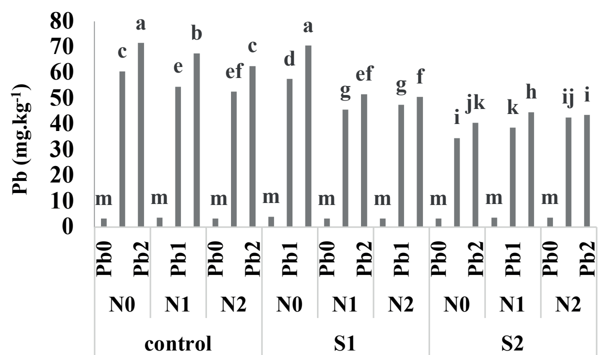

Figure 1. Lead (Pb) content in Japanese raisin in response to salinity (S0: Control, S1: 3 and S2: 6 dSm^{-1}), nitrate (N0: 0, N1: 30 and N2: 60 mgL^{-1}), and Pb (Pb0: 0, Pb1: 300 and Pb2: 600 mgL^{-1}). Values in each group followed by the same letter are not significantly different according to DMRT at $P \leq 0.05$

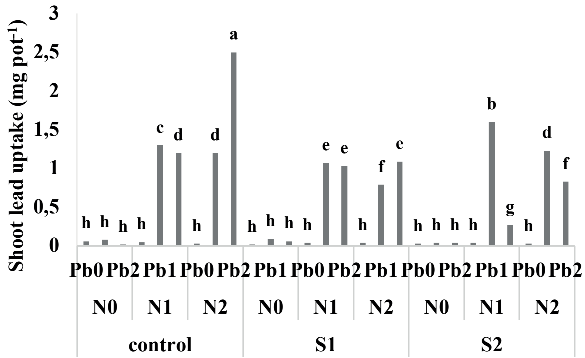

Figure 2. Shoot lead (Pb) uptake in Japanese raisin in response to salinity (S0: Control, S1: 3 and S2: 6 dSm^{-1}), nitrate (N0: 0, N1: 30 and N2: 60 mgL^{-1}), and Pb (Pb0: 0, Pb1: 300 and Pb2: 600 mgL^{-1}). Values in each group followed by the same letter are not significantly different according to DMRT at P≤0.05

observed in S0N2Pb0 and S2N1Pb2 treatments, while the lowest N content (3.3%) was recorded for S1N0Pb2.

Based on the results, the interaction of salinity, NO_3^-, and Pb, significantly affected shoot NO_3^- content (Table S2). NO_3^- content reduced by 11% and 21% with the application of 3 and 6 dSm^{-1} salinity. However, application of 30 and 60 mgL^{-1} of NO_3^- increased NO_3^- content by 24% and 36%, respectively. The interaction between all three treatments showed that the highest NO_3^- content (0.61%) was recorded for the treatment of S0N1Pb0, while the lowest NO_3^- content (0.05%) was observed at the treatment of S2N0Pb2.

3.3.c Plants sodium, potassium and chlore level

In accordance with the obtained results, the interactions of salinity, NO_3^-, and Pb significantly (P≤0.01) affected shoot potassium content (Table S2). The lowest values of shoot K concentration were observed at salinity treatments of 3 and 6 dSm^{-1}, which were reported to be 1.85% and 1.95%, respectively; while NO_3^- application at levels of 30 and 60 mgL^{-1} increased K by 4% and 22%, respectively. The interaction between the treatments indicated that the highest shoot K concentration (5.05%) belonged to the treatment of S0N2Pb0, that was almost 3 times more than the lowest one at treatment of S1N1Pb0, S1N0Pb0 and S2N0Pb2 (1.85%).

As results showed, the interactions of salinity, NO_3^-, and Pb significantly (P≤0.01) affected shoot Na concentration (Table S2). Considering to the data, it can be found that shoot Na concentration increased up to the 39% by salinity application of 3 or 6 dSm^{-1}, while NO_3^- application at levels of 30 and 60 mgL^{-1} decreased shoot Na content by 26% and 25%, respectively. Based on the interactions of studied factors, the highest shoot Na concentration)2.77% (belonged to the treatments of S2N0Pb0, S2N1Pb0 that was fivefold of the S2N2Pb2 treatment as the lowest one.

Considering to the K/Na ratio shown in Table 2, it can be found that the accumulation of K effectively controlled salinity stress. The highest ratio was recorded in the SIN0Pb1treatment that was six times more than S2N0Pb2 treatment as lowest one.

According to variance analysis results it is clear that the interactive effects of salinity, NO_3^-, and Pb, significantly affected Cl concentration (Table S2). Also, the results of means comparison ~~of~~ indicated that salinity at levels of 3 and 6 dSm^{-1} increased Cl content by 17% and 30%, respectively; Nitrate at levels of 30 and 60 mgL^{-1} increased Cl content by 9% and 2%, respectively; and Pb at levels of 300 and 600 mgL^{-1} increased Cl content by 4.9% and 6.8%, respectively. The highest (4.7%) and the lowest (1.4%) values of Cl content were recorded for the treatments of S2N1Pb2 and S0N0Pb0, respectively. High Cl concentration was observed in the treatments with high salinity and nitrate concentration.

3.2. Antioxidant enzymes activity

Results showed salinity significantly affected the activities of antioxidant enzymes (Table S3), so that the levels of 3 and 6 dSm^{-1} increased the activity of POD by 11% and 6%, respectively, while they reduced the activities of SOD by 2 and 15% and CAT by 17 and 63% (Table 1), respectively. Additionally, $NO3^-$ at levels of 30 and 60 mgL^{-1} significantly decreased the activities of POD by 12% and 20%, SOD by 9% and 24%, and CAT by 20% and 15% (Table 1), respectively. Furthermore, Pb at levels of 300 and 600 mgL^{-1} significantly increased the activities of POD by 12% and 11% and CAT by 27% and 34%, respectively, while they reduced the activity of SOD by 39% and 50%, respectively (Table 1). Considering to the results, it can be found that the highest enzymatic activity of SOD, POD and CAT, which are the best indicators of assessing stress conditions, were observed at treatments of 3 dSm^{-1} salinity + 30 mgL^{-1} nitrate (without Pb), 3 dSm^{-1} salinity + 600 mgL^{-1} Pb (without nitrate) and 600 mgL^{-1} Pb + 30 mgL^{-1} nitrate application (without salinity), respectively. (Table 1). In other words, with increasing the studied stresses levels including Pb contamination up to 600 mg, salinity up to 6 dSm^{-1} and nitrate up to 60 mgL^{-1}, the enzymatic activity reduced indicating the reduction of plant defense mechanisms under severe stress conditions.

Generally, the relationships between biochemical traits and nutrients status can provide a clear under-

Table 1. Activities of antioxidant enzyme in response to treatments.

Salinity (dSm^{-1})	Nitrate (mgL^{-1})	Pb (mgL^{-1})	POD	SOD	CAT
Control	0	0	0.52^{d*}	161.9^{c}	5^{kl}
		300	0.29^{lmn}	82.15^{ijk}	56.37^{cd}
		600	0.36^{hij}	87.87^{ij}	21.83^{gh}
	30	0	0.19^{p}	68.63^{lmn}	3.01^{l}
		300	$0.35^{\ h\text{-}k}$	93.02^{i}	54.08^{cd}
		600	0.38^{ghi}	128.4^{ef}	86.26^{a}
	60	0	0.45^{ef}	120.7^{fg}	30.8^{f}
		300	0.56^{cd}	140.1^{d}	58.77^{c}
		600	0.58^{c}	117.5^{g}	67.41^{b}
3	0	0	0.34^{ijk}	67.05^{mn}	72.25^{b}
		300	0.34^{ijk}	132.9^{de}	50.19^{d}
		600	0.8^{a}	175.5^{b}	55.34^{cd}
	30	0	0.54^{cd}	188.5^{a}	43.41^{e}
		300	0.69^{b}	154.3^{c}	11.05^{jk}
		600	0.31^{klm}	59.99^{no}	9.76^{jkl}
	60	0	0.23^{op}	52.44^{o}	53^{cd}
		300	0.27^{mno}	74.59^{klm}	10.55^{jkl}
		600	0.33^{jkl}	86.29^{ij}	14.98^{hij}
6	0	0	0.48^{e}	115.2^{gh}	3.52^{jl}
		300	0.57^{c}	132.3^{de}	25.93^{fg}
		600	0.36^{hij}	105.6^{h}	27.79^{fg}
	30	0	0.42^{fg}	106.3^{h}	14.84^{hij}
		300	0.36^{hij}	79.4^{jkl}	12.66^{ij}
		600	0.38^{ghi}	89.69^{ij}	20.79^{gh}
	60	0	0.23^{op}	49.88^{o}	8^{jkl}
		300	0.39^{gh}	114.4^{gh}	18.7^{hi}
		600	0.26^{no}	58.77^{no}	10.07^{jkl}

* Means within a column followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

standing of positive and negative correlations among whole studied parameters. For this purpose, the correlation between traits were calculated. The results showed the high positive correlation between proline and MDA by R^2=0.879, N and K by R^2= 0.718, soluble carbohydrate and MDA by R^2= 0.612 and lipid peroxidase and MDA by R^2= 0.574 (Table 2).

3.3. Soluble carbohydrates content

To evaluate the biochemical and physiological responses of plant against studied stresses, soluble carbohydrates were measured. Based on the results, the most values of soluble carbohydrates were reported at treatments of S1N0Pb2 and S0N0Pb2, indicating that HMs stress had more effect on this trait in comparison with salinity. The highest content of soluble carbohydrate was recorded in S0N0Pb2 that was 3 time more than S2N2Pb2 as lowest treatment.

3.4. Malondialdehyde level of plants

Also, there was a significant difference in proline concentration among different studied treatments compared to control. Malondialdehyde was measured as an important indicator of plant response to abiotic stresses. Results showed, the most values of this parameter were observed at moderate levels of Pb application (without salinity) and also the synthesis of this biochemical product significantly reduced by the expansion of HMs, salinity and nitrate stress. Lowest value of MDA was recorded in S2N2Pb2 treatment that show this parameter were deceased in high salt, nitrate and lead concentration.

3.5. Proline level of plants

The most values of proline were observed at moderate levels of Pb application (without salinity) and also the synthesis of this biochemical product significantly reduced by the expansion of HMs, salinity and nitrate stress. The highest value of proline was recorded in the S0N0 treatment that was twofold higher than S2N2 treatment as last one.

3.6. Lipid peroxidation

The increasing trend was observed for lipid peroxidation and the most values of this parameter belonged to the treatments with low levels of stress (without salinity and without nitrate). Highest content was observed in the S0N0 treatment, while the treatment with high level of nitrogen and lead ranked last treatment.

4. DISCUSSION

4.1. Biochemical traits affected by different level of nitrate and lead under salinity stress

4.1.a lead uptake and concentration were affected under different nitrate level

Based on the results, Pb pollution caused more salinity (Na) uptake. In other words, salinity can increase HMs stress, which means the intensification of the stress induced by salinity and also has been

Table 2. Pearson correlation coefficients between characteristics.

	N	NO3	K	Na	K/Na	Cl	Pb Con.	Pb Uptake	Sol. Carb	Proline	MDA	Lipid	POX	SOD	CAT
N	1														
$NO3^-$	0.495**	1													
K	0.768**	0.575**	1												
Na	-0.093ns	0.071ns	0.049ns	1											
K/Na	0.468**	0.359**	0.520**	-0.721**	1										
Cl	0.497**	0.409**	0.412**	0.104ns	0.249*	1									
Pb Con.	0.340**	0.158ns	0.344**	-0.331**	0.259*	0.145ns	1								
Pb Uptake	-0.209ns	-0.217ns	-0.198ns	-0.174ns	0.086ns	0.060ns	-0.149ns	1							
Sol. Carb	-0.084ns	0.083ns	-0.065ns	0.215ns	-0.140ns	0.022ns	-0.484**	0.008ns	1						
Proline	-0.006ns	0.179ns	0.189ns	0.366**	-0.180ns	-0.094ns	-0.249*	-0.222*	0.504**	1					
MDA	-0.126ns	0.064ns	-0.054ns	0.009ns	0.016ns	-0.136ns	-0.418**	0.042ns	0.818**	0.612**	1				
Lipid	0.056ns	0.215ns	0.195ns	0.302ns	-0.067ns	-0.071ns	-0.234*	-0.288**	0.475**	0.879**	0.574**	1			
POX	-0.221*	-0.146ns	-0.032ns	0.259*	-0.293**	0.131ns	-0.205ns	0.165ns	0.256*	0.106ns	0.122ns	0.004ns	1		
SOD	-0.113ns	-0.140ns	-0.085ns	0.037ns	0.067ns	-0.285**	0.218ns	0.073ns	0.328**	0.158ns	0.363**	0.206ns	0.386**	1	
CAT	-0.213ns	-0.270*	-0.099ns	-0.299**	0.163ns	-0.240*	-0.050ns	-0.219*	0.202ns	0.302**	0.373**	0.276*	-0.081ns	0.222*	1

** represent significant difference at $P \leq 0.01$, * represent significant difference at $P \leq 0.05$, n.s represent no significant difference

considered by many researchers. Generally, when the stress inhibits plant growth and reduces transpiration, the increase in contaminant uptake will be stopped or reduced. Accordingly, in the present experiment, the salinity stress without HMs contamination, led to reduce contaminant (Pb) uptake. The critical level of Pb contamination in soil, considered by researchers, is 50 mg kg^{-1} soil (Prasad, 2004). Also, the normal range of Pb in plant tissues is between 0.2 to 20 mg kg^{-1}, but its critical and contamination levels in plant is more than 20 mg kg^{-1}, reducing the yield and plants dry matter (Alloway, 1990). Accordingly, high concentrations (34.6-71.6 mg kg^{-1}) of Pb accumulated in treatments of Pb contamination indicating the ability of the studied species for HMs phytoremediation. In fact, the studied plant has an appropriate potential for phytoremediation under conditions of simultaneous stresses.

4.1.b N and Nitrate level improved the plant resistance under salinity stress

The percentage of N and NO_3 and the accumulation of K, Na and Cl represent the intensity of plant response to the studied stresses (HMs and salinity). Results indicated that salinity or Na content reduced by supplying nitrate. It should be mentioned that nitrate increases plants resistance to salinity, which also has been reported in numerous researches (Kafkafi et al, 1992). Some researchers believe that reduction of nitrate concentration is because of the negative interaction between Cl and nitrate and antagonistic effect of Cl on nitrate uptake. While, others attribute it to the plant's response under saline conditions reducing water uptake (Lauter and Munns, 1986). The results of a study showed that phytoremediation of HVs (Co, Cu, Cr, Ni, and Pb) pollution by aquatic hyacinth was only effective at high concentrations of nitrate and by decreasing nitrate concentration the phytoremediation efficiency decreased (Tangahu et al, 2011; Bai et al, 2021). Loska and Wiechula (2003) reported that the presence of any type of contaminants in water and soil resources led to pollutants accumulation in plant organs, changing enzymatic activities. Similarly, the results of the present study are consistent with those of recent studies (Dayani et al, 2009; Husejnovic et al, 2018).

4.1.c High potassium to sodium ratio improve phytoextraction in contract to Chlore

Khoshgoftar et al. (2004) reported that HMs uptake from the soil solution increased with the increase of NaCl level, while no such effect was observed for $NaNO_3$. It is assumed that chloride ion positively affected HMs (Pb and Cd) solubility in soil and their uptake by plants. Generally, chloride ion increases the dynamics and adsorption capacity of the metals. Having high salinity tolerance, is another important strategy of plants for resistance against salinity. For example, grasses and Atriplex/Salicornia are capable to grow at the salinity levels up to 1.2-1.7 dSm^{-1} and 21-28 dSm^{-1}, respectively.

Regarding to the results of the present study, it seems that the Japanese raisin (*Hovenia dulcis*) can be considered as a relatively tolerant species at moderate salinity levels due to its appropriate responses to salinity levels of 3 and 6 dSm^{-1}, the accumulation of K and Na and also the high ratio of K/Na in different stress treatments. In addition to salinity stress, Pb contamination also has a specified critical level based on soil and plant studies. Potassium accumulation is probably a defense mechanism and increasing the ratio of K/Na is a strategic way for resistance to salinity stress (Kibria and Hoque, 2015). Generally, these results indicated the intensity of the effect of stress treatments (HMs and salinity) on the one hand and the plant resistance responses to Pb and salinity. Also, Kibria and Hoque (2015) conducted a field experiment to investigate the effect of the mitigation of soil salinity on rice by application of K and Zn fertilizers. The results demonstrated that K^+/Na^+ ratio in the grains significantly affected by the application of K. Therefore, it may be induced by the fact that the application of higher doses of K and Zn fertilizers could alleviate the adverse effects of salinity in rice via increasing nutrient uptake and maintaining a higher K^+/Na^+ ratio. Boudaghi Malidareh et al. (2014) reported a significant relationship between the amount of K fertilizer and HMs pollution (cadmium concentration) in the soil. Furthermore, the results of a study demonstrated that soil salinity can be improved by the application of nitrogen and potassium fertilizers (Shanker, 2005). Azari et al. (2005) investigated the role of potassium on nitrate and Cd contamination in potato and onion. They found that the concentrations of nitrate and cadmium in potato and onion tubers significantly decreased following the application of potassium and zinc fertilizers, and the highest nitrate and Cd contamination was recorded for the treatment with the unbalanced fertilizer use. Under salinity stress, the concentrations of potassium and phosphorus in the stem significantly decreased, while the concentration of sodium in the leaves increased. The similar results were also reported by Khalilpoor and Jafarinia (2017) and Yousefinia and Ghasemiyan (2016).

4.2. Antioxidant enzymes activity was affected by different levels of HM and salt stress

It seems that the increase in enzymatic activity is one of the main strategies tolerating HMs contamination and salinity stresses (Malar et al, 2016). Accordingly, the changes in activity of SOD enzyme are considered as the appropriate indicators of stress management. The results of the present study showed that the increased SOD activity under stress conditions was due to the plant's survival on the one hand and contaminants purification on the other hand. However, the simultaneous increase in the stresses of HMs contamination, salinity and nitrate, up to the maximum levels, reduced the activity of all three studied enzymes. Malar et al. (2016) reported that plants use different mechanisms to cope with HMs toxicity. Alizadeh (2012) reported that the contamination lead and cadmium disrupted the growth of two poplar species and plant biomass significantly reduced at high levels of pollutants. Furthermore, the reduced vegetative growth caused by HMs stress in plants may be because of the suppressed activities of catalase and superoxide dismutase (Schutzendubel and Polle, 2002). Jabeen and Ahmad (2012) reported that salinity increased the activity of peroxidase but decreased that of catalase. Similar results were also reported on canola (Abili and Zare, 2014) and maize (AbdElgawad et al, 2016). Michalak (2006) has found that antioxidant enzymes can scavenge reactive oxygen species (ROS) when the plant grows under HMs stress. Also, Verma and Dubey (2003) observed that Pb toxicity changed the activity of antioxidant enzymes in rice plants. Barandeh and Kavousi (2017) reported that the activities of antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase significantly increased in lentil seedlings with the increasing in Cd concentration. Similarly, Hendry et al. (1992) illustrated that HMs are also a reason for oxidative stress via the production of free radicals of reactive oxygen which can react with lipids and finally lead to the lipid peroxidation, membrane damage, and enzymatic inactivation (Dixi et al, 2001). Similar results were reported by Verma and Dubey (2003) and AbdElqawad et al. (2016). Generally, it has been reported that stress increases the activities of antioxidant enzymes (Meloni et al, 2003) but at an intolerable intensity obviously reduces their activities (Amiriyan Mojarad et al, 2018). The results of the present study in agreement with previous reports showed the increment of SOD, POD and CAT by increasing the HM and salt stresses level.

4.3. Soluble carbohydrate was affected by different levels of HM and salt stress

Soluble carbohydrate content was affected by different nitrate level and stress condition. The results of current study showed the decreasing trend by increasing salt and lead level. Weisany et al. (2014) reported that at all three growth stages (pre-flowering, post-flowering, and seed filling), salinity stress decreased shoot fresh and dry weights, plant yield, root and leaf also soluble carbohydrate content of these tissue in soybean, but the application of zinc fertilizer alleviated these negative effects. Decreased biomass production induced by HMs stress may be because of a disturbance in uptake and transmission of nutrients and water into the aerial parts of plants (Sudova and Vosatka, 2007).

4.4. Malondialdehyde synthesis were changed under HM and salt stress

The synthesis of biochemical compounds such as malondialdehyde has been considered as another important mechanism to withstand stress conditions. The stress-adapted plants seem to be more capable to synthesis these metabolites. There are numerous studies confirming the increased synthesis of malondialdehyde and some plant biochemical/enzymatic compounds as a response to stress conditions (Juknys et al, 2012; Aljahali and Alhassan, 2020). Based on the results of the present study, there was a significant and negative correlation between shoot Pb concentration with MDA synthesis and also between the activity of POX with the K/Na ratio. The similar results have been reported by Aljahali and Alhassan (2020).

4.5. Proline level affected under stress condition

Proline is a non-enzymatic antioxidant known as bio-marker that showed plants response to the stress (Petrovic et al, 2020). In the water caltrop plant, they suggested proline accumulation as a good biomarker of HMs stress (Petrovic et al, 2020; Bi, *et al.,* 2021; Duan, *et al.,* 2022; Guo, *et al*, 2021; Guo, *et al,* 2022). The results of proline accumulation, showed the decreasing trend by increasing the nitrate concentration under high level of lead. These results indicated the positive effect of nitrate to deceasing the side effect of HMs and salt stress as mentioned before. Similarly, Bai et al, (2021) suggested that phytoremediation of HVs pollution by sweet sorghum was only effective at high concentrations of nitrate and by decreasing nitrate concentration the phytoremediation efficiency decreased.

4.6. Lipid peroxidation were affected under stress condition

Heavy metals pollution, causes changes in some plant processes such as lipid peroxidation (Ashraf et al, 2017). The results of this study showed the high lipid peroxidation under control treatment. Similar results were reported on rice, that by increasing lead level the lipid peroxidation was decreased (Ashraf et al, 2017; Li, *et al*, 2021; Sun, *et al.* 2021; Xu, *et al*, 2021; Zhang, *et al.* 2022).

5. CONCLUSIONS

Based on the obtained results of the present study, it can be concluded that with the increasing in salinity stress from 0 to 3 dSm^{-1}, the content of N (from 5.69% to 5.26%), K (from 4% to 3.21%) and nitrate (from 0.36 to 0.28 mg kg^{-1}) significantly reduced. Also, results showed that with the increasing in salinity stress (from 3 to 6 dSm^{-1}) and nitrate level (from 30 to 60 mgL^{-1}) in the soil, plant Pb concentration significantly decreased. Additionally, under conditions of Pb stress, the uptake of nutrients (especially macronutrients) significantly improved with the increasing in the nitrate level from 30 to 60 mgL^{-1}. It appears that stress conditions increased plant's nitrate requirement, which could be considered as a strategy for the improvement of plant tolerance under HMs stress. On the other hand, the increase in K ranged from 1.8 to 5% and also K/Na ratio ranged from 1 to 6 can be considered as a resistance mechanism of plants under salinity stress. In addition, the synthesis of MDA and other biochemical compounds in Japanese raisin, grown under Pb contamination stress, has been reported for the first-time providing ideas for future studies. It should be mentioned that the studied species absorbed the moderate concentrations of Pb (34.1-71 $mgkg^{-1}$) indicating its potential for HMs phytoremediation. Generally, based on the results of the present study described before, Japanese raisin can be considered as a relatively susceptible to salt stress.

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