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Full Length Article

Quinoa Response to Lead: Growth and Lead Partitioning

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Abstract

Chenopods are known for salt and metal ion tolerance. *Chenopodium quinoa* is a recent introduction in the country but yet to be tested on metal contaminated soils. A pot experiment was conducted to explore growth and phytoextraction potential of four quinoa lines (A1, A2, A7 and A9) against different lead concentrations (0, 50 and 100 mg kg^{-1}). Required lead concentrations were developed in polythene bags filled with sandy loam soil (5 kg) using lead nitrate salt prior to two month sowing and kept sealed up to sowing. Fifteen seeds of each quinoa lines were sown in each treatment bags with three replications. Five plants were kept in each bag after their complete emergence and allowed to grow till yield production (120 days). Results showed that translocation of lead increased from roots to shoots with increase in soil lead concentration in all lines with the following trend: A1>A9>A7>A2. However, A1 accumulated higher in leaf compared to other lines as depicted by translocation factor of 2.34 and 1.34 when grown at soil having 50 and 100 lead mg/kg, respectively. This trend was similar in case of root (A1>A9>A7>A2). There was 25–66% decline in seed yield at 50 mg kg^{-1} lead level in all lines compared to control. However, growth and yield declined with further increase in lead level. The maximum reduction in yield was observed in A7. More importantly lead concentrations in seed of A2 and A7 quinoa lines were within the permissible value set (0.3 mg/kg DW) by FAO/WHO. It can be concluded that quinoa is suitable for phytoextraction and despite hyper accumulation the concentration in seed remains within safe limit for human consumption.

Keywords: Phytoextraction; Quinoa; Lead; Translocation factor

Introduction

Heavy metals toxicity is an impending issue to agriculture posing serious threat worldwide due to unlimited discharge of industrial effluents to peri-urban areas of developing countries (Wahid *et al*., 2009; Hussain *et al*., 2010; Sabir *et al*., 2011). Heavy metals toxicity wreaks havoc on the ecological balance and food chain. Sewage irrigation, sludge, use of fertilizers having metals and industrial wastes are important sources of metal increment in soil (Abdollahi *et al*., 2011). Since heavy metals cannot be degraded so they can exist in environment for long time as compared to organic contaminants. Heavy metals disturb food chain and cause serious threat to the biota, because the half-life of these lethal elements is more than 20 years (Ruiz *et al*., 2009).

To reduce the hazardous effects of metals and food security, it needs remediation harnessed with such methods which are energy efficient, economically viable and environment friendly. One of there is "phytoremediation" in which target is achieved without losing anything (Paz-Ferreiro *et al*., 2014). Phytoextraction is a long term approach for cleaning the soil from heavy metals (Sas-Nowosielska *et al*., 2008). Phytoextraction is extraction of metals in harvestable parts of plants from contaminated soils (Tangahu *et al*., 2011). Phytoextraction success depends upon different factors like potential of plant to extract metals and quantity of metals present in the soil medium. It has been advised that halophytes have the ability to extract heavy metals so it is healthy option for phytoremediation than glycophtes (Prasad and Freitas, 2003).

Lead (Pb) is toxic, non-essential and also called protoplasmic poison. Sharma and Dubey (2005) documented that Pb in low concentration is very lethal for plant growth and development. Use of pesticides, fertilizers, gasoline and additives in pigments are key Pb sources (Wuana and Okieimen, 2011). Pb application disturbs mineral nutrition and water balance, ceases enzyme activities and inhibits photosynthesis (Sharma and Dubey, 2005). High Pb concentration disrupts the whole plant metabolism, impede the cell division and finally lead to cell death (Singh *et al*., 2015).

Quinoa (*Chenopodium quinoa* Willd.) a pseudo cereal, is a new introduction in Pakistan, also belongs to Chenopodiaceae family. Quinoa being a facultative halophyte has the ability to survive under the problem soils (Matiacevich *et al*., 2006). Due to its high nutritional profile and wide range of adaptability under stress conditions it could be best option for food security. Quinoa can tolerate

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combination of abiotic stresses like high temperature, drought, salinity and frost (Mujica *et al*., 2001).

This study was conducted to expose the morphological and physiological mechanisms of lead tolerance, comparative phytoextraction potential and seed quality of four quinoa lines under lead contaminated soil.

Materials and Methods

A pot experiment was carried out during winter 2014–2015 in a net house, Department of Agronomy, University of Agriculture, Faisalabad Pakistan. Different levels of lead (50 and 100 mg kg^{-1}) were developed in air dried soil by using lead nitrate salt. Lead nitrate salt was used in solution form. Each pot was laminated from inside with a plastic bag to avoid the leaching of lead nitrate salt filled with 5 kg of soil and replicated 3 times. Soil used in this experiment was analyzed according to standard procedures are presented in Table 1. The lead spiked soil was equilibrated for 2 months at 60% water holding capacity as explained by Niazi *et al*. (2011).

Seeds of quinoa lines A1, A2, A7 and A9 were obtained from the Alternate Crops Lab Department of Agronomy, University of Agriculture, Faisalabad (Table 2). Fifteen seeds of four quinoa lines (A1, A2, A7 and A9) in each pot were sown. After complete emergence five plants were maintained in each pot. The pots were irrigated based on the water requirement of plants. The recommended dose of P and K fertilizers (60 kg ha^{-1} each) as diammonium phosphate and sulfate of potash respectively was applied as basal dose however N dose was applied @ 75 kg ha^{-1} using urea half dose at sowing and half at flowering. The plants were harvested after 120 days of sowing.

Growth Parameters

After 80 days of sowing, plants from each replication were uprooted and separated into shoots and roots. Shoot and root length were measured using a scale. Fresh weight (g) of shoot and roots were recorded with analytical balance. Shoot and root samples were oven dried at 70ºC for 72 h in an oven and their dry shoot and root weights were recorded.

Lead Determination

The dried ground material (0.1 g of root, shoot and seeds) was taken in digestion flasks and 5 mL of HNO_3 was added to each flask (Wolf, 1982). All the samples were incubated overnight at room temperature. The flasks were placed on a hot plate and heated up to 250ºC until fumes were produced, after which heating was continued for another 30 min. The digestion flasks were removed from the hot plate, cooled and 1 mL of HNO_3 was slowly added in each sample and placed the flasks back on the hot plate. The above steps were repeated until the digested material became clear and colorless. The volume of the extract was made up to 50 mL with distilled water. The extract was filtered into labeled bottles and used for the determination of lead contents. The lead was determined by atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following the conditions described in AOAC (1990).

Physiological Attributes

Chlorophyll a and b was determined by using the method of Arnon (1949) and carotenoids was determined by the method of Davis (1976). Fresh leaves samples (0.5 g) were taken at panicle emergence stage (after 80 days of sowing) from each pot carefully. Sample material was homogenized with pestle and mortar in 80% acetone in the darkness, filtered and made the volume of filtrate up to 10 mL. Photosynthetic pigments (chlorophyll a and b) were determined from the light absorbance at 663 and 645 nm using an UV-Vis spectrophotometer (Dynamica Co., UK; Halo DB-20/DB 20S) (Metzner *et al*., 1965).

Total soluble phenolics were evaluated using the method of Julkunen-Titto (1985). Fresh plant material (0.5 g) was extracted in 80% acetone; the extract was centrifuged at 12,000 rpm for 5 min. Extract (100 µL) was mixed with 0.5 mL of Folin-Ciocalteu's phenol reagent and 2.5 mL of 20% Na_2CO_3. The volume of the mixture was made up to 5 mL with distilled water and vortexes. Absorbance of the reaction mixture was measured at 750 nm.

Yield Estimation

Harvesting was done from each pot when plants were matured as described by Jacobsen and Stølen (1993). Panicles were dried on filter paper at 25–30°C. After ten days' seeds were threshed manually. Remaining crop portions were tied into bundles and sundried for a week and recorded the dry weight and added to seed weight to calculate total biomass. Later on thousand seeds weights was also recorded using digital balance.

Statistical Analysis

Each treatment was replicated three times, and data were statistically analyzed by a two-way ANOVA analysis under a completely randomized design (CRD) comprising factorial arrangement using statistical software "Statistix" (ver. 8.1, Tallahasse, FL, USA). Lead levels and quinoa lines were taken as factor.

Results

Growth Parameters

Lead phytotoxicity symptoms appeared in all quinoa lines, although relatively less in A2 and A7 lines. In the high lead treatment (100 mg/kg), plant growth was significantly reduced, leaf chlorosis and stunted growth with brown red leaf color was observed. Overall A1 and A9

Table 1: Physical and chemical analysis of soil

Determination	Unit	Value
pH		7.6
EC	dS m^{-1}	1.38
Nitrogen	mg/kg	0.043
Phosphorus	mg/kg	13.11
Potassium	mg/kg	87
Lead	mg/kg	0.07
Organic Matter	%	0.88
Textural Class	-	Sandy loam
Saturation percentage	(%)	32

Table 2: Detail of quinoa lines

Code*	G. Line**	Origin	Plant name
P1 596293	A1	Colorado, USA	Colorado 407D
Ames 13730	A2	New Mexico, USA	IESP
Ames 13737	A7	New Mexico, USA	2WANT
P1 634919	A9	Chile	Pichaman

*as per the germplasm database ** local coding of lines

displayed more lead stress and apparent toxicity symptoms than those of A2 and A7.

A negative effect of lead treatments (50 and 100 mg/kg) was observed on all growth attributes of the studied quinoa lines. Shoot length decreased by 25.3, 29.7, 29.3 and 18.8% in A1, A2, A7 and A9 lines respectively. Quinoa A1 line had undergone 7.4% increment in root length while A2, A7 and A9 lines experienced 5.07, 36.09 and 6.96% less root length in comparison to control. For shoot dry weight A2 and A7 lines displayed (28.3 and 40.9%) at 50 mg/kg lead and (45.8 and 61.6%) less biomass at 100 mg/kg lead application respectively in comparison to control (Fig. 1 and 2).

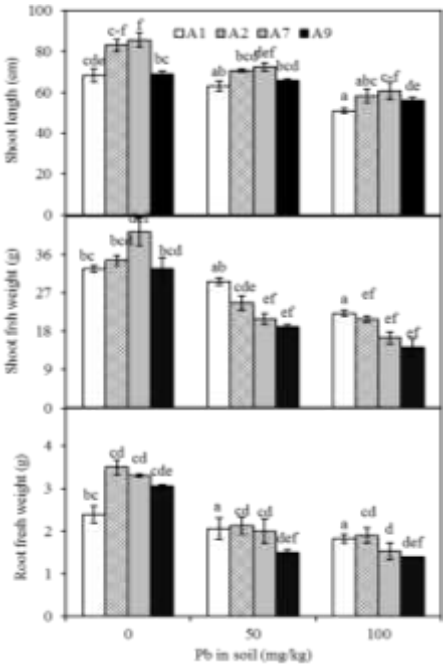

Fig. 1: Plant height, shoot fresh weight and root fresh weight of quinoa lines affected by different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

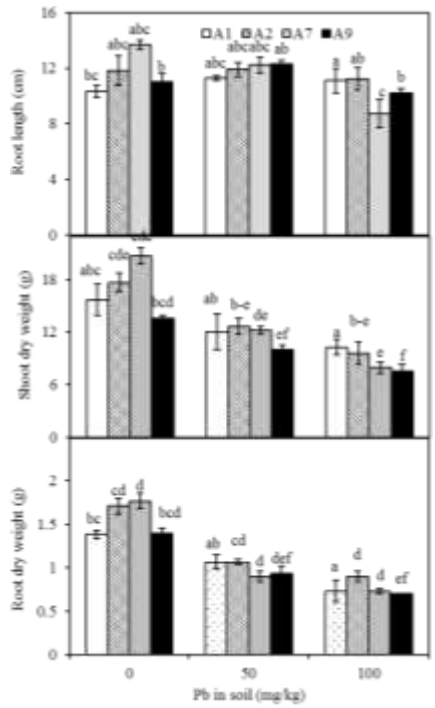

Fig. 2: Root length, shoot dry weight and root dry weight of quinoa lines affected by different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

Lead Effect on Physiological Parameters

The photosynthetic pigments of lead treated lines were significantly ($P<0.001$) decreased. For instance compared to control, the maximum reduction of chl *a* recorded 47.1, 43.5, 42 and 32.07% in A2, A7, A9 and A1 lines respectively and chl *b* content decreased 43.6, 40.2, 34.6 and 27.5% in A9, A7, A2 and A1 lines at 100 mg/kg lead application.

Minor reduction was observed in carotenoid contents in all lines in which the values did not significantly reduced. Maximum carotenoid contents were found in A7 line at control having no lead application (0 mg/kg), while minimum value was found in A9 at 100 mg/kg lead dose. The A9 line showed a higher aptitude to accumulate (52.6%) phenolic at 100 mg/kg lead exposure comparative to other lines (Fig. 3–4).

Lead Accumulation and Translocation

Determination made for lead contents in (leaf, stem, root and seed) indicated significant ($P<0.01$) difference among treatments in quinoa lines grown in lead contaminated pots. Lead concentration increased in quinoa lines with increase in lead dose in pots. The pattern followed by quinoa lines (A1>A9>A7>A2) for leaf lead accumulation. Quinoa lines A1 and A9 experienced maximum lead accumulation in leaf

relative to other lines at 100 mg/kg lead. In quinoa A1, shoot lead uptake varied from 2.9–9.82 mg/kg, whereas it was comparatively lower in A9 (1.84–5.16 mg/kg) for 50 and 100 mg/kg lead respectively.

Data for root lead uptake demonstrated that high lead treatment significantly ($P<0.001$) affected lead uptake in roots of quinoa lines. However, A1 and A9 lines accumulated (1.23–7.3 and 2–5.5 mg/kg) under 50 and 100 mg/kg lead respectively. Contrarily, the lowest root lead concentration was noted in A2 as compared to other lines (Fig. 5)

Bioconcentration factor (BCF) and Translocation factor (TF) used as a tool to access the phytoextraction potential of quinoa lines to remediate the lead contaminated soil. The values of bioconcentration factor showed significant differences ($P<0.05$) among quinoa lines for different Pb levels. The data showed that BCF (0.032) in A2 was significantly higher at 50 mg/kg as compared to 100 mg/kg soil Pb level. On the other hand quinoa lines A1, A7 and A9 exhibited higher BCF in 100 mg/kg Pb as compared to lower dose (50 mg/kg). An increasing trend of TF was noted in all lines with increasing application of lead level in external environment. The difference between four lines was significant at both applied soil Pb levels. The value of TF was recorded maximum in quinoa lines (A1, A2 and A7) except A9. Quinoa lines A1 and A2 got maximum values (2.34–1.34) and (1.94–1.46) at 50 and 100 mg/kg lead respectively at before harvest (Fig. 6). The lead translocation from shoot to seed was observed in all quinoa lines. Maximum seed lead was found in A1 (0.37 mg/kg) with application of 100 mg/kg lead, respectively. The trend of quinoa lines regarding lead storage in seed was A1>A9>A7>A2 (Fig. 7).

Yield Related Parameters

Data in Fig. 1 reveal that main panicle length of all quinoa lines was significantly influenced by Pb concentrations. Applied lead significantly decreased main panicle length (27.2 and 36.8%) in A2 and A7 lines at 100 mg/kg lead respectively in comparison to control. The data indicated that lead application negatively affected panicles number in all lines except A9 at 100 mg/kg lead. Quinoa lines (A1, A2, A7 and A9) showed less biological (5.5, 38.7, 38.2 and 34.07%) and seed yield (30, 59.5, 66.7 and 44.11) in comparison to control under maximum 100 mg/kg lead. Furthermore, A2 and A7 had larger main panicle length, plant biomass and greater yield in comparison to A1 and A9 lines (Fig. 7 and 8).

Discussion

Lead has gained considerable attention as a persistent toxic pollutant, because of the growing anthropogenic pressure on the environment (Pourrut *et al*., 2011).

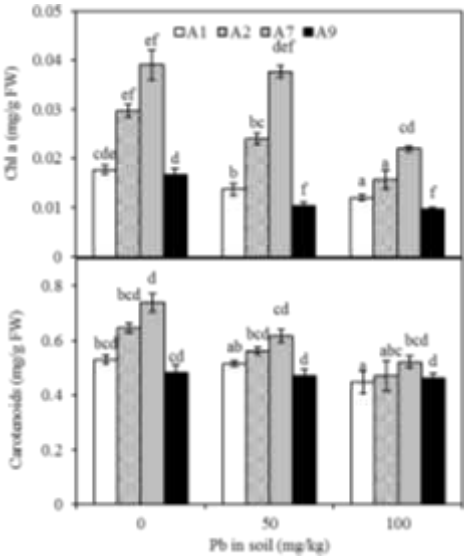

Fig. 3: Chlorophyll a and carotenoids of quinoa lines affected by different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

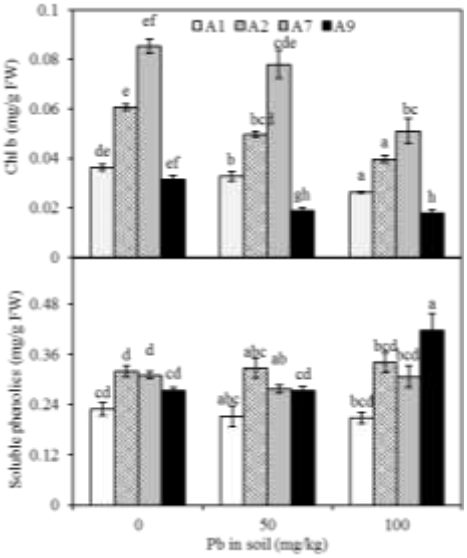

Fig. 4: Chlorophyll b and soluble phenolics of quinoa lines affected by different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

Excessive lead accumulation in plant tissue impairs various morphological, biochemical and physiological functions in plants (Shahid *et al*., 2012).

In present study, high concentration of lead (100 mg/kg) reduced the root length, fresh and dry weight of shoot and root of four quinoa lines in comparison to 50 mg/kg lead treatment. The results showed that high concentrations (100 mg/kg) of lead affected the dry biomass but it did not cease the growth of quinoa lines. At panicle emergence stage (after 80 days of sowing)

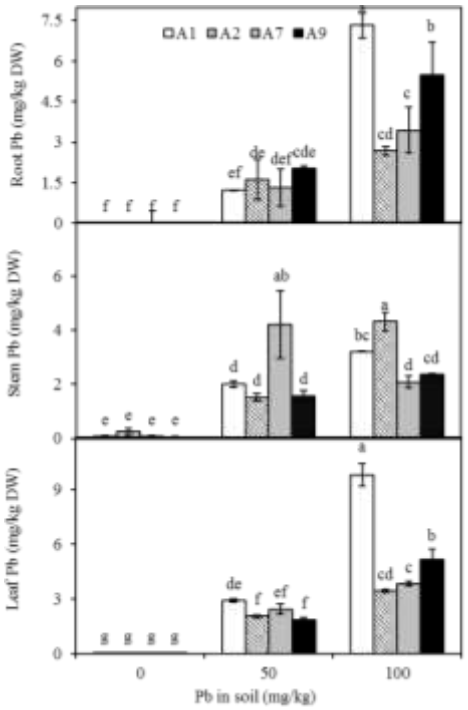

Fig. 5: Pb concentration in root, stem and leaf of quinoa lines in response to different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

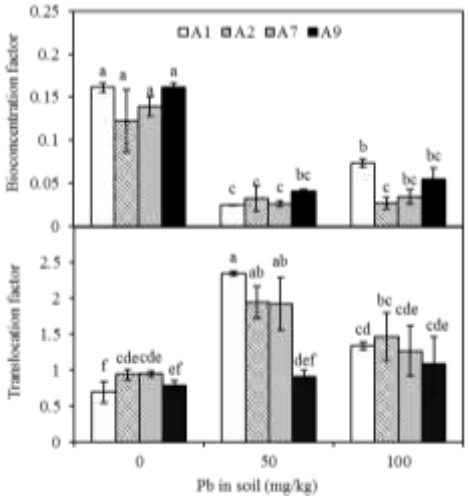

Fig. 6: Bioconcentration factor (BCF) and translocation factor (TF) of quinoa lines in response to different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

lead application (100 mg/kg) resulted substantially less plant height in all quinoa lines. Growth of sunflower was badly affected by high concentration of lead on reproductive stage (Paliwal *et al*., 2014). Growth response of A2 and A7 was incredible as compared to A1 and A9 lines. Slight reduction in growth was found at high Pb dose (100 mg/kg).

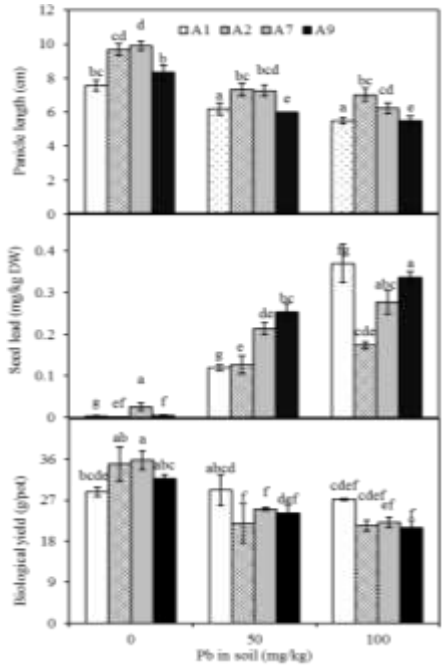

Fig. 7: Panicle length, seed lead concentration and biological yield of quinoa lines in response to different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

Lead accumulation impedes the growth and development of crop plant due to lower uptake of essential minerals (Gopal and Rizvi, 2008). The growth inhibition under Pb stress was similar to that previously described by Jezler *et al*. (2015) in *Mentha arvensis*. Lead concentration decreased the stem girth, root length; fresh and dry weight of 12 weeks old cassava varieties (Ano *et al*., 2013). Similarly, germination of maize seeds in lead contaminated soils significantly reduced the primary root length, mesocotyl root as well as the root mass in *Leucaena leucocephala* (Shafiq *et al*., 2008) and in *Thespesia populnea* L. (Kabir *et al*., 2010).

Buildup of heavy metals in crop plants is of great concern due to the probability of food contamination through the soil-root interface (Mukhtar *et al*., 2010). Plants absorb lead mainly through the roots from soil solution and thereby may enter the food chain. The absorption of lead by roots occurs via the apoplastic pathway or via Ca^{2+}-permeable channels (Sharma and Dubey, 2005). Bioconcentration factor (BCF) and translocation factor are key factors to estimate the phytoextraction potential of any crop plants. As the BCF is the ratio of metal accumulation in harvested plant root tissue as compared to same metal concentration in soil (Zhuang *et al*., 2007). The lower BCF values (less than 1) were observed in all quinoa lines. Translocation factor is the ratio of metal concentration accumulated in shoot to same metal concentration in plant roots

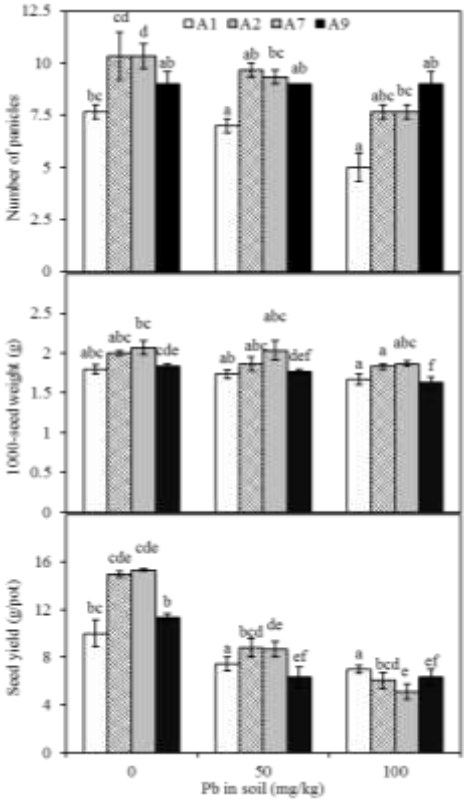

Fig. 8: Number of panicles, 1000-seed weight and seed yield of quinoa lines in response to different Pb concentrations. A1, A2, A7 and A9 indicate the quinoa lines

(Padmavathiamma and Li, 2007). In the present experiment though TF increased with increase in soil Pb concentration at 50 mg/kg, while slight reduction was observed at high Pb dose (100 mg/kg) in all quinoa lines. Garba *et al*. (2012) described that TF more than 1 shows the high metal transfer and accumulation.

Quinoa lines take up lead but it was not translocated at the reproductive stage. This is the unique character found in quinoa while in other crops external amendments are required to hamper the metal sequestration at seedling stage and stop it to transfer to maturity stage as in rice, biochar immobilize lead and reduced its phyto-availability (Li *et al*., 2016).

Lead is accumulated in a dose-dependent manner in plants; therefore, lead at high concentration inhibits the translocation of other essential mineral ions (K, Ca, P, Mg, Fe, Cu and Zn) leading deficiency of other metals in plants. The deficiency of mineral nutrients correlates in a strong decrease in the contents of chlorophylls *a*, *b* and carotenoids. Reduction in chlorophyll contents in wheat (Lamhamdi *et al*., 2013) and mung bean (Dashna and Bufna, 2013) was observed under lead toxicity. Similar results were reported by Akinci *et al*. (2011) who reported that lead accumulation results in chlorophyll destruction by disorganization of grana and thylakoid membrane, alter the chlorophyll structure due to replacement of main nutrients by lead.

In comparison to (50 mg/kg) lead, chlorophyll *a* and chlorophyll *b* of all quinoa lines were reduced at high concentration of lead (100 mg/kg). Reduction in the contents of primary photosynthetic pigments is mainly due to over production of reactive oxygen species (ROS), which disrupts the ultrastructure of chloroplast (Pourrut *et al*., 2011). Lead toxicity resulted into oxidative stress and chlorophyll destruction in rice (Zeng *et al*., 2007). Carotenoids act as photosynthetic pigment as well as antioxidants under abiotic stress conditions (Kasote *et al*., 2015). In present study, the increase in concentration of carotenoids in quinoa lines was more than chlorophyll although it was slightly affected by the lead contents. High carotenoids may have key role in quenching reactive oxygen species produced under lead treatment (Kong *et al*., 2015).

Plants possess several defense strategies to cope with lead toxicity. Such strategies include reduced lead uptake into the cell; sequestration of lead into vacuoles by the formation of complexes; binding of lead by phytochelatins, glutathione and synthesis of osmolytes (Pourrut *et al*., 2011). In addition, activation of various antioxidants to combat increased production of lead induced reactive oxygen species constitutes a secondary defense system. Phenolic compounds stabilize membranes by decreasing membrane fluidity and hinder the diffusion of free radicals. Soluble phenolic bind to the membranes phospholipids by hydrogen bond with the polar head groups of phospholipids (Michalak, 2006). In present study, quinoa lines showed differential behavior i.e. high concentration of lead increased soluble phenolics contents with this pattern (A2>A7>A9) while lead sensitive A1 line had reduced soluble phenolic at higher concentration of lead.

Quinoa yield fluctuations with variation in number and weight of panicles which otherwise positively related with dry biomass of quinoa that significantly affected by lead toxicity. Zeng *et al.* (2007) reported that lead accumulation negatively affected the yield of sugar beet (*Beta vulgaris*). The reduction in yield is mostly due to inactivation of photosynthetic enzymes, disturbs water balance and mineral nutrition which results in poor assimilate production (Sharma and Dubey, 2005).

Conclusion

In conclusion, lead accumulates in different parts of plant and its concentration increased with increase in soil lead concentration. The translocation of lead to stem and leaves affected photosynthetic and defensive processes. The high concentration of lead had inhibitory influence on dry biomass, biological yield and seed yield of quinoa lines. Among four quinoa lines, A2 and A7 was found to be tolerant and low concentration of lead in seeds and greater number of panicles made it economically importance lines as compared to other two quinoa lines.

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