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Original Article

Halophyte quinoa: a potential hyperaccumulator of heavy metals for phytoremediation

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Abstract

Agricultural soils are becoming contaminated with heavy metals due to industrialization and increase in anthropogenic activities. Phytoremediation of heavy metals is an environment-friendly technique for reclamation contaminated soils. Quinoa is halophyte with excellent nutritional qualities and can also be used for the reclamation of polluted soil. This study was executed to evaluate the phytoextraction potential of heavy metals in older and younger leaves of quinoa. Six genotypes of quinoa were cultivated on artificially heavy metals polluted soil in controlled block with use of sewage wastewater at MNS University of Agriculture Multan, Pakistan. Leaves samples were analyzed to evaluate the concentration of cadmium (Cd), lead (Pb), copper (Cu), and nickel (Ni) by using atomic absorption spectrophotometer. Maximum amount of heavy metals were accumulated in older leaves Q-76, Q50 and Q-82 but Q-76 genotype was higher accumulator. In case of yield, Q-7 and Q-76 were best performers with maximum seed yield, biomass, main panicle length and width. It can be concluded that Q-76 genotype was best among the six for producing higher yield with better extraction of heavy metals (Cd, Pb, Cu and Ni) from polluted soil.

Keywords: Heavy metals, Phytoextraction, Quinoa, Reclamation

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Introduction

Increase in world population, irrigation with sewage water, mining, use of pesticides and industrialization are causing degradation in agricultural lands due to accumulation of organic, inorganic and metal pollutants (Sivarajasekar et al., 2018; Zahra et al., 2021). Primarily food and soils are contaminating due to increase of wastewater irrigation, atmospheric deposition, fertilizers, animal manure, metallo-pesticides or herbicides sludge amendments in soil (Woldetsadik et al., 2017). Pakistan is facing acute shortage of fresh irrigation water and trend of wastewater usage for crops irrigation is enhancing and is approximately 32,500 hectares (Saleem et al., 2005). Sewage wastewater contain different deleterious carcinogenic heavy metals mainly copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and nickel (Ni) and these metals not only deteriorate food chain of agricultural system but also are responsible of many diseases in human (Liu et al., 2005; Yang et al., 2018). Industrial and domestic wastewater contain minute amount of heavy metals but long-term use of wastewater also causes heavy metal pollution (Chaoua et al., 2019). Food chain is becoming contaminated due to soil saturation with heavy metals because these metals have ability to accumulate in food crops and move towards the human body (Wang et al., 2017). These metals become the part of edible portion of crops grown in such soils (Uzma et al., 2016; Raza et al., 2021).

Many studies reveal that heavy metals play toxic role in plants and decrease growth and development, yield, biomass and quality of produce (Ramzani et al., 2016; Haseeb et al., 2022). Such as Cd toxicity in plants results in retardation in photosynthesis due to reduce amount of chlorophyll and dysfunctioning of enzymes compulsory for CO_2 fixation. It also alters many other plant functions such as water potential, leaf chlorosis, minimize nutrients uptake and growth reduction (Nagajyoti et al., 2010), also interrupt the steadiness among antioxidants and reactive oxygen species that have lethal effects on membranal organelles (Raza et al., 2021; Nizar et al., 2022). Metal contamination have dangerous biological consequences in plants and animals, their chemical synchronization and redox properties enable them to cause alteration in cell composition by ionic imbalance, compartmentalization and transport of nutrients (Naushad et al., 2016).

In human, intake of these metals by food results the depletion of many essential minerals which cause many malnutrition issues, mental growth retardation, intestinal cancer and problems of immune system (Dickin et al., 2016). For example, Cd and Pb have carcinogenic effects, when their concentration in vegetables is high kidney, heart, bone and nervous system disorders, cardiovascular complications, lungs and liver problems occur (Zhou et al., 2016). Turkdogan et al. (2003), found that ingestion of lead, copper, chromium and cadmium present in fruit and vegetables increase the chances of stomach cancer in humans. It is extremely evident that pollution of food crops due to Cd have many health-related problems such as postmenopausal breast cancer due to its higher concentration in rice (Yang et al., 2018).

Phytoextraction is a practice which is commonly used for phytoremediation in which many hyperaccumulator and non-hyperaccumulator plant species are used for absorption, accumulation and degradation of toxic chemicals from water, soil or sediments and store them in roots or upper parts of plant (Chandra et al., 2017). Phytoremediation reduces the risk of pollutant dispersal and being environment friendly protect the natural ecotype due to avoiding the polluted sites excavation (Nejad et al., 2018).

Quinoa belongs to family Amaranthaceae and is not a cereal crop, but flour of its seeds can be used as cereals, therefor it is considered as a pseudo-cereal crop (Rashid et al., 2018; Akram et al., 2021). Due to unique nutritional profile and resilience against abiotic stress, FAO designated it as a future smart crop (Xuan and Siddique, 2018; Saddiq et al., 2021). Quinoa has the ability of extraction especially Ni, Cr and Cd heavy metals from the contaminated soil in its leaves tissues (Bhargava et al., 2008). It may also be stated that halophytes have better ability of phytoextraction then glycophytes, because these accumulate more amount of toxic metals (Cl and Na) and have efficient oxidative defense system as compare to glycophytes (Srivastava et al., 2015). It is also documented in previous study that heavy metal tolerant plants and halophytes have many similar traits of tolerance (Oosten and Maggio, 2015). Bhargava et al. (2008) analyzed 40 different species of Chenopodium and found that *Chenopodium quinoa* genotypes have the greater potential to extract heavy metals like Fe, Zn, Cu, Ni, Cr and Cd even at low concentration in soil and store these metals in leaves and other aerial parts. This study was conducted to explore the phytoextraction potential and partitioning of heavy metals (Cd, Pb, Ni and Ni) in leaves. In this study partitioning of heavy metals in leaves (older and younger) of six quinoa genotypes was assessed.

Material and Methods

Geographical location

City of Multan is situated in southern part of Punjab province, Pakistan. Geographically it is situated 30.2^0N latitude and 71.43^0E longitude and 423 ft. above the sea level. It has extreme summer and winter seasons with arid climatic conditions (Fig. 1). Research area was situated at research farm of MNS University of Agriculture Multan which was artificially irrigated with sewerage water in controlled block for this experiment. This study was conducted on this contaminated soil.

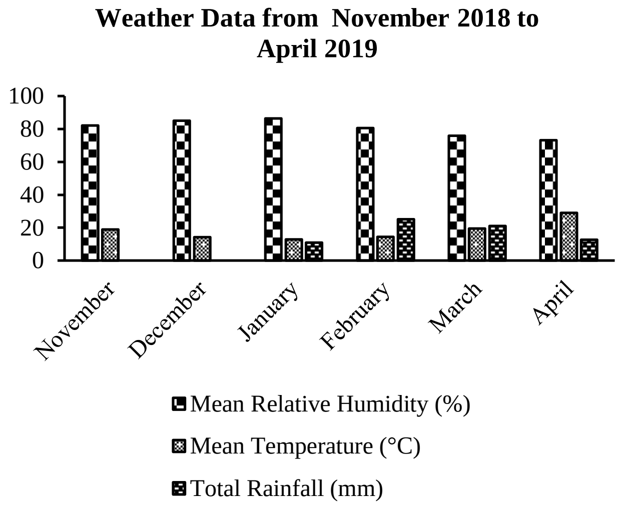

Figure-1: Meteorological data during the quinoa growth season (November 2018 to April 2019)

Experimental setup

After wheat harvesting, the soil was well prepared by ploughing two times followed by planking and then pulverized the soil using rotavator. Ridges were made with the help of ridger with row to row distance of 75 cm. Experimental design used was randomized complete block design (RCBD). Net plot size was kept 4 m × 1.9 m with 0.5 m path between each plot. Recommended dose of fertilizer 75:50:50 N:P:K kg ha^{-1} was applied in the form of Urea, DAP and SOP, in case of nitrogen half dose was applied before preparation of ridges and half dose was applied during flowering stage (Iqbal et al., 2019). In this research six different genotypes of quinoa (Q-6, Q-7, Q-50, Q-51 and Q-76, Q-82) obtained from USDA were used to check the heavy metals extraction potential from contaminated soil. Water was applied before sowing and seeds were sown by hand dibbling method on ridges at depth of 2-3 cm using seed rate of 10 kg ha^{-1} on 21 November, 2018. After germination crop was irrigated 4 times, 1st irrigation 40 DAS, 2nd 75 DAS, 3rd 100 DAS and final 120 DAS was applied (Basra, 2016). Pesticide and herbicide were not applied and weeding was done manually when needed.

To check physical and chemical properties of soil samples were collected from depth of 0-15 cm and analysis was done before sowing of crop. Type of soil was loamy with total nitrogen 0.0039 g kg^{-1}, available phosphorous 4.7 mg kg^{-1}, extractable potassium 300 mg kg^{-1}, 1.84 dS m^{-1} EC and 8.4 pH, while organic matter was 0.77%. Concentration of heavy metals was also recorded in soil samples and amount of Cd, Pb, Cu and Ni was 72.83, 24.06, 109 and 5.9 mg kg^{-1}, respectively.

Materials and apparatus used

To measure the panicle width digital vernier caliper was used and for panicle length centimeter scale was used. Samples collection was done in cleaned yellow paper bags to avoid the spoilage of leaves due to moisture. Drying oven was used to dry the shade dry leaves. The process of digestion was done on hot plate (Model: RTH-340) of Robus Technologies and for weighing samples analytical weight balance (Model: JJ324BC) was used. For safe and accurate handling of chemicals, measuring cylinder, digestion tube and pipette were used in this study. To filter the digested material Whatman No. 1 filter papers were used. Desired heavy metals in leaves samples were analyzed with the help of atomic absorption spectrophotometer (Model: nova ® 400P) fully computer controlled. In this study all chemicals used were of analytical grade. Distill water was used in the whole study for preparation and dilution of all chemical solution preparation, digestion and washing purposes. For digestion HNO_3 and $HClO_4$ were used.

Samples collection

Before attained maturity leaves from upper part (fully expanded young leaf) and lower part (old leaf) of tagged plants were detached and put in the yellow paper bags. After washing with distill water in lab first leaves were air dried in shade and then oven dried for 24 hours at temperature of 70^0C. After drying in oven samples were cut into small pieces with stainless steel scissor and ground manually to obtain fine powder.

Digestion

For digestion of plant material wet digestion method by following the AOAC rules both for young and older leaves. For digestion 1g of fine ground leaf sample was added into 150 ml volumetric flask. Then 7 ml concentrated HNO_3 and 3 ml $HClO_4$ was added (in fume hood). Then these volumetric flasks were put on the hot plate in the fume hood. Temperature of hot plat was set 180-200^0C until fumes disappear or white fumes were evolved and transparent content was left. Then volumetric flasks were lifted out and were cooled to room temperature. Then material was filtered through Whatman No. 1 filter paper and volume was made up to 100 ml (AOAC, 2006). For soil 1 g of soil was analyzed with 7 ml of HNO_3 and 3 ml of $HCLO_4$. Predigested for two hours and then heated on hot plat at temperature 180⁰C to 200⁰C until clear solution obtained.

Parameters study

The heavy metals Cd, Pb, Cu and Ni in µg g^{-1} were estimated in older and younger leaves of six different quinoa genotypes. In yield parameters main panicle length and width (cm), seed yield (Kg ha^{-1}) and biomass (Kg ha^{-1}) were recorded.

Analysis of HMs

To record desired heavy metals in older and younger leaves, samples were analyzed with the help of atomic absorption spectrophotometer (Model: nova ® 400P) fully computer controlled. First of all standards of 0.25, 0.5 ppm, 1 ppm, 2 ppm, 4 ppm, 8 ppm and 10 ppm were run. Samples were examined after running the standards by adjusting the desired wavelengths for heavy metals analysis. To check the Cu in samples of leaves wavelength was kept 217 nm, for Cd 317 nm, Pb 324 nm and for Ni analysis wavelength was kept 224 nm.

Statistical analysis

Statistical analysis of raw data was done by using statistical software statistix 8.1. Graphs were made with the help of Microsoft excel 2013. Analysis of variance (ANOVA) was performed to check the difference between observations for heavy metals accumulations in seeds and leaves of quinoa genotypes and yield related variables (Steel et al., 1997). Difference between mean values of all observations were compared by Tukey's HSD Post Hoc test at probability level of 0.05.

Results

Older leaves

In case of older leaves, statistically significant variations ($P<0.05$) were recorded for heavy metals among quinoa genotypes grown on heavy metal contaminated. There was a clear variation among the concentration of four heavy metals and also genotypes, older leaves of Q-76 genotype accumulate the maximum amount of Cd 59.76 µg g^{-1} (Figure 2A). In case of lead (Pb) in older leaves also Q-76 absorbed highest amount of lead 44.63 µg g^{-1} (Figure 2B).
Maximum amount of copper was stored in older leaves of Q-82 genotype with value of 48.86 µg g^{-1} and amount for Q-76 was 43.49 µg g^{-1} (Figure 2C). Genotype Q-50 showed maximum concentration of Ni in older leaves (2.99 µg g^{-1}) shown in Figure 2D. In overall situation Q-76 appeared most efficient for accumulating heavy metals in its older leaves.

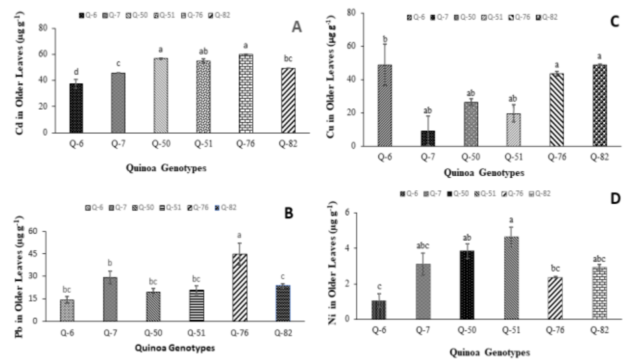

Figure-2: Concentration of heavy metals, Cd (µg g^{-1}) in older leaves (A), Pb (µg g^{-1}) in older leaves (B), Cu (µg g^{-1}) in older leaves (C) and Ni (µg g^{-1}) in older leaves (D) in six quinoa genotypes grown on heavy metal contaminated soil. The same letters on graphs represent statistically similar effect ($P<0.05$) and n=3.

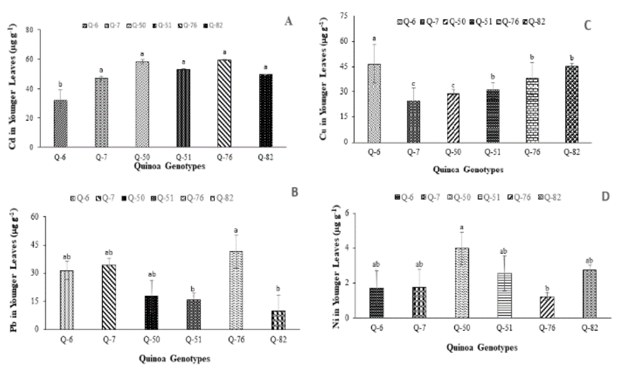

Figure-3: Concentration of heavy metals, Cd (µg g^{-1}) in younger leaves (A), Pb (µg g^{-1}) in younger leaves (B), Cu (µg g^{-1}) in younger leaves (C) and Ni (µg g^{-1}) in younger leaves (D) in six quinoa genotypes grown on heavy metal contaminated soil. The same letters on graphs represent statistically similar effect ($P<0.05$) and n=3.

Yield attributes

Statistical significant difference ($p<0.05$) was observed in case of seed yield attributes. Maximum amount of seed yield and biomass was observed in Q-7 and Q-76 as compare to other genotypes (Q-6, Q-50, Q-51 and Q-82) (Figure 4A). Seed yield of 3438.33 kg ha^{-1} and 10395 kg ha^{-1} biomass in Q-7 was recorded, while in Q-76 yield was 1485 kg ha^{-1} and with 5205 kg ha^{-1} biomass production (Figure 4B). Results regarding main panicle length and main panicle width had also differences. Main panicle length of Q-7 and Q-76 was also maximum (36.35, 14.15 cm and 24.78 and 16.12 cm) while a clear variation was seen in other genotypes (Figure 4C and 4D).

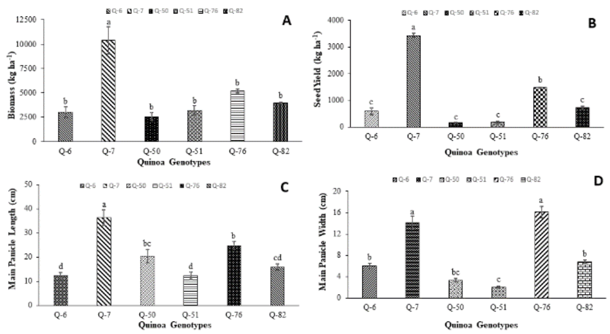

Figure-4. Difference between Biomass in Kg ha^{-1} (A) seed yield in Kg ha^{-1} (B), main panicle length in cm (C) and main panicle width in cm (D) of six quinoa genotypes grown on heavy metal polluted soil. The same letters on graphs represent statistically similar effect ($P<0.05$) and n=3.

Discussion

To check the partitioning of heavy metals, analysis of older and younger leaves of quinoa genotypes was done. Results of this study revealed that uptake and accumulation of heavy metals in plants leaves were different in all genotypes. These results also correlate with research that described that uptake and accumulation of heavy metals varies greatly with plant species and within cultivars within a species (Schneider et al., 1996; Li et al., 1997; Liu et al., 2005). Bhargava et al. (2008) stated that some quinoa genotypes stored maximum amount of Cd in leaves as compared to other parts, in our study same results were observed among six genotypes of quinoa. Genotype Q-76 among all quinoa genotypes accumulated maximum amount of Cd and Pd in older leaves as compare to younger leaves (Fig. 1A and B). In case of Cu Q-82 older leaves extract higher amount and Ni was greater in younger leaves of Q-50 (Fig. 1C and D). Efficiency of genotypes may be written as Q-76<Q-82<Q-50<Q-51<-Q-7<Q-6. Bhargava et al. (2008) stated that the uptake tendency of Ni, Cd, Zn and Cr was considerably higher in leaves of Chenopodium quinoa then other species such as C. giganteum, C. album, C. murale. Our findings are also in accordance with Kim et al. (2003) who concluded that many accessions of Chenopodium quinoa uptake higher amount of Zn and Cu as compare to aquatic plant *Polygonum thunbergii* which is considered more efficient in reclamation of soils contaminated effluents of industry. Ramos et al. (2002) also observed that in lettuce maximum amount of Cd was accumulated in leaves regardless the concentration of Cd in nutrient solution. Another study also conferred our results which stated that maximum Cd stored in older leaves was due to detoxification of metal through immobilization of it by cell wall of plant organ (Xin et al., 2013). Cosio et al. (2006) recorded that localization of Cd was in tips and margins of younger leaves of willow and in older leaves leaf veins were the main point of maximum cadmium storage. Similarly, Xin et al. (2013) described that higher concentration of cadmium in older leaves of water spinach protect younger leaves from toxic effect of Cd. Vacuolar compartmentalization is also an important mechanism involved in storage and detoxification of heavy metals (Cosio et al., 2004).

Sharma and Dubey (2005) documented that Pb even in low concentration is very lethal for plant growth and development. Results of previous study which described that beans accumulate highest concentration of Pb in shoot parts especially leaves also strengthen our findings (Antoniadis et al., 2017). Akinci et al. (2011) reported that lead accumulation in leaves results in chlorophyll destruction by disorganization of grana and thylakoid membrane. Similarly, Gopal and Rizvi (2008) observed that accumulation of lead in older leaves and unwashed seeds caused the reduction in nutrients uptake of plant, so it results in reduction of plant yield attributes, it also strengthens our findings of research. According to Bhargava et al. (2008) copper content in some quinoa accessions was high in leaves as compared to other foliage species. Our study was also correlated with findings of Bhargava et al. (2008) who concluded that Ni and Cd was higher in the older leaves of C. quinoa as compared to other younger leaves. The concentration of Cu in Chenopodium spp. was greater than that reported for flax, cotton and hemp, these crops were considered suitable for cultivation on metal polluted soils (Yanchev et al., 2000; Angelova et al., 2004). Bhargava et al. (2008) concluded that C. quinoa Ames 22156 accumulate the maximum nickel followed by C. quinoa Ames 13719 and C. quinoa PI 614881.

McGrath and Zhao (2003) studied that production of higher biological yield or biomass of a plant species is an indicator of good phytoextraction. According our study Q-7 produced maximum biomass 10395 kg ha^{-1}

followed by Q-76 with 5205 kg ha^{-1}(Figure 3A). Nehnevajova et al. (2005) described that higher biomass in sunflower can be used as an indicator of metal extraction in contaminated soil, this statement also support our results that Q-76 may be used for phytoextraction due to production of high biomass. Q-7 producing maximum biomass might be declared tolerant against heavy metals or less efficient for heavy metals accumulator of heavy metals towards upper parts. Toxicity of lead results in destruction in chlorophyll pigment and oxidative stress which ultimately reduced the biomass and yield of rice crop (Zeng et al., 2007a). Similarly in our study, decrease in biomass of other genotypes (Q-50, Q-51, Q-6 and Q-82) might be due to the accumulation of HMs in plant leaves and disturbance in physiological processes of plant (Figure 3A).

According to Zeng et al. (2007b) lead toxicity is the reason of reduction in yield of sugar beet crop, similarly seed yield of quinoa genotypes other than Q-7 was also reduced due to accumulation of heavy metals in plant. Genotype Q-50 produced minimum yield 163.33 kg ha^{-1} and maximum was observed in Q-7 (3438.33 kg ha^{-1}) (Figure 3B). This reduction was due to heavy metal storage in plant photosynthetic parts, nutrient imbalance and retardation in plant physiological mechanisms (Sharma and Dubey, 2005). Quinoa yield depends upon the no. of sub-panicles, length and width of main panicle. Haseeb et al. (2018) described that no. of panicles were also decreased due to lead toxicity. Those genotypes which absorbed more amount of metals produced minimum main panicles length and also width. In this experiment maximum main panicle length and width was observed in Q-7. Minimum number of sub panicles were seen in genotype Q-51, low panicle length was measured in Q-6 and lowest main panicle width was observed in Q-51 (Figure 3C&D). This reduction in yield and yield attributes might be due to the accumulation of heavy metals in quinoa plant parts.

Conclusion

Quinoa genotypes showed different potential for accumulation of heavy metals (HMs). Older and younger leaves accumulates heavy metals but maximum amount was recorded in older leaves. For accumulation in leaves Q-76, Q-50 and Q-82 performed best for Cd, Pb, Cu and Ni but Q-76 accumulate relatively higher concentration. Trending pattern of HMs in older and younger leaves of Q-76 was Cd>Pd>Cu>Ni. Q-50 and Q-82 younger and older leaves showed the same trending pattern Cd>Cu>Pd>Ni. Hense Quinoa can be used in reclamation of heavy metal contaminated soils.

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Contribution of Authors

Ghous M & Iqbal S: Designed and conducted the experiment, collected & analysed data and prepared the first draft

Bakhtavar MA, Nawaz F, Haq TU & Khan S: Literature review, collected & interpreted data and helped in article write up