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Hyperaccumulation of silver in Indian mustard *Brassica juncea*

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Abstract

Human activities like, landfilling, effluents of industrial waste, ore processing and leaching are few of the major ones contributing towards the discharge of silver into the environment. Phytoremediation is environment friendly and less expensive technique and it is easily applicable on larger contaminated areas. Brassica plants have been used for the purpose of phytoremediation. *Brassica juncea* (Indian mustard) is chosen because of its great tolerance level against high concentration of metal. This study explores the potential of *Brassica juncea* for remediation of silver metal. The pot experiment was conducted and seeds were sown in sand under the natural conditions required for growth. Plants were treated with silver for 2 weeks for accumulation analysis. Silver content in the stem, root and leaves as well as in sand samples was analyzed by Atomic Absorption spectrometer. Maximum accumulation of silver in *Brassica juncea* leaf was 10400 (mg/kg) at 50(mg/L), in stem was 2800 (mg/kg) at 50(mg/L) and in root was 11000(mg/kg) at 100(mg/L). Substantially higher concentration of silver was found in aerial parts and lower quantities were found in roots. Bioaccumulation factor was measured to show the capability of the plant to extract metal from the contaminated medium. The Bioaccumulation factor of *Brassica juncea* was 4.3, 1.92, 3.4, 1.5, 1 with concentration of Silver 25, 50, 100 200 (mg/L). All the values were higher than 1 which shows hyperaccumulation of silver in plant. Hence there is a great scope for utilization of *Brassica juncea* for cleaning the soils polluted by silver metal.

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Introduction

Silver is a naturally occurring metal in our environment. It has a wide usage in photographic, electronic and imaging industry (Reimann and Caritat, 2012). The amount of silver being released to the environment all over the world is more than 13kgs per year. Three-fourth part of this total amount is only due to by tailing and landfill (Eckelman and Graedel, 2007). Therefore, both terrestrial and aquatic ecosystems are affected by the release of silver.

Agricultural soils receive eminent levels of silver primarily due to implementation of sewage sludge and sludge effluents for the purpose of achieving improvement in agriculture. Heavy metals are considered toxic and a major cause of environmental contamination because of their capability to accumulate and their toxic nature (Hesse *et al.,* 2018; Ali *et al.,* 2019). This warrants for the remediation of soils for safeguarding the food chains and restore ecosystem and human health.

The chemical methods that are being used to clean the environment from heavy metal pollution are very costly, produce large amount of sludge and do not perform to the optimal level (Rakhshaee *et al.,* 2009). Constituents of soils are damaged when thermal or chemical methods are used.

These methods can be difficult and costly to implement (Negri and Hinchman, 1996). To solve the issue of heavy metal pollution, a natural technique phytoremediation is used. Hyperaccumulator plants are used in the process to clean up the heavy metal contaminated environment. Hyper accumulators take up the heavy metals from soil and accumulate them in the aerial parts (Sarma, 2011). It is believed that out of all the hyperaccumulators plants, Brassica family contains 25% of the total (Dar *et al.,* 2018). *Brassica juncea* of Brassicaceae family is well known phytoextractor and is used for the purpose of phytoremediation.

Therefore, the present research work was designed to investigate the efficiency of *Brassica juncea* to remediate the silver metal.

Materials and methods

To find out the phyto-extraction ability of *Brassica juncea*, plants were grown in natural conditions in sand. Accumulation of silver was determined. The lab work was accomplished by following work plan;

First, seed collection, plantation and growing *Brassica juncea* in sand medium.

Second, *Brassica juncea* plants treatment with various concentrations of silver.

Third, analysis of silver accumulation in different parts of plant by atomic absorption spectrometry and calculation of bioaccumulation factor.

Research laboratory facility

The entire work was done in the Research Laboratory of Lahore College for Women University (Environmental Science Department).

Plant growth conditions and pot experiment

Seeds of *Brassica juncea* were bought from institute of Agricultural Sciences, University of the Punjab. The pot experiment was conducted from November to February with daily average temperature ranging from 5°C to 15°C. Seeds of *Brassica juncea* were sown in plastic pots containing half Kg of sand.

The pots were placed under sunlight for providing the natural conditions required for growth and moisture content of sand was maintained by providing adequate water. When the seedlings emerged from the sand they were thinned to three per pot. For that analysis all plants were harvested after 15 days of growth under silver treatment.

Silver treatment

Preparation of solution for silver treatment

As 1 Kg equals 1L so the solution were made in mg/L that would be equal to 1 mg/L in sand (Fones *et al.,* 2010). Stock solution of 100mg/L silver was prepared by dissolving 0.396 g of silver nitrate in 500 ml of distilled water. It was then diluted to 2.5, 5, 10 and 20 mg/L with distilled water by applying dilution formula i.e.:

$C_1V_1 = C_2V_2$

Similarly 25, 50, 100 and 200 mg/L of silver was prepared by taking 2.5ml, 5ml, 10ml and 20ml of stock solution in 50ml volumetric flask and diluted up to the mark with distilled water.

Application of silver solution on plants

Two week old plants of *Brassica juncea* were treated with different concentration of silver including 0(Control), 25, 50, 100 and 200mg/L silver the silver was taken from silver nitrate solution ($AgNO_3$). Plants were treated with silver for 2 weeks for accumulation analysis.

Silver analysis

Preparation of samples for atomic absorption spectrometer

The dried plant material including root, stem and leaves of plants treated with different silver concentrations was grinded using mortar and pestle. Samples of sand of each pot treated with silver and control were also dried and grinded.

Subsamples of 0.05g of dried plant material and sand samples were taken in 50ml beaker for acid digestion. 3ml of nitric acid was added in each sample and left overnight in fume hood for the complete digestion. Acid digestion samples were then filtered and were diluted up to 10 ml with distilled water.

Analysis of Silver content in Plant material by AAS

Silver content in the stem, root and leaves as well as in sand samples was analyzed by Atomic Absorption spectrometer. Silver concentration was calculated by using the following formula (Kathal *et al.*, 2016).

$$\text{Silver Concentration (mg/kg)} = \frac{\text{Concentration ppm} \times \text{Solution volume (ml)}}{\text{Sample weight (g)}} \times 100$$

Bioaccumulation factor in Brassica juncea

Bioaccumulation factor was measured to show the capability of the plant to extract metal from the contaminated medium. Bioaccumulation factor was measured using following equation (Zacchini *et al.*, 2009).

$$BAF = \frac{C_{plant}}{C_{sand}}$$

Where,

C_{plant} = is the concentration of silver in plants

C_{sand} = is the concentration of silver in sand.

Results and discussion

Analysis of Silver Accumulation

Accumulation of Silver in leaf of *Brassica juncea* grown in sand decreased with increased concentration of Silver 0 and 25 (mg/L) with 2173.9, 1400 (mg/kg) but increased to 24761 (mg/kg) at 50 (mg/L) and then decreased to 4516.12 and 4285.7 (mg/kg) at 100 and 200 (mg/L).

The accumulation of Silver in leaf was 2173.9, 1400, 24761, 4516.12, 4285.7 (mg/kg) with concentration of Silver 0, 25, 50, 100, 200 (mg/L). The maximum accumulation of silver in leaf observed was 10400 (mg/kg) at 50(mg/L) (Fig. 1).

Table 1. Bioaccumulation Factor in *Brassica juncea* grown in sand.

Ag Treatment (mg/L)	Ag in aerial parts of plant (mg/kg)	Ag in sand (mg/kg)	Bioaccumulation Factor
0	0.26	0.06	4.3
25	0.27	0.14	1.92
50	0.75	0.22	3.4
100	0.75	0.49	1.5
200	0.39	0.48	1

Accumulation of Silver in stem of *Brassica juncea* grown in sand increased with increased concentration of Silver 0, 25, 50 (mg/L) with 1200, 1600, 2800 (mg/kg) and decreased to 1200 and 1600 (mg/kg) at 100 and 200 (mg/L). The accumulation of Silver in stem was 1200, 1600, 2800, 1200, 1600 mg/kg) with concentration of silver 0, 25, 50, 100, 200 (mg/L). The maximum accumulation of Silver in stem observed was 2800 (mg/kg) at 50(mg/L) (Fig. 1).

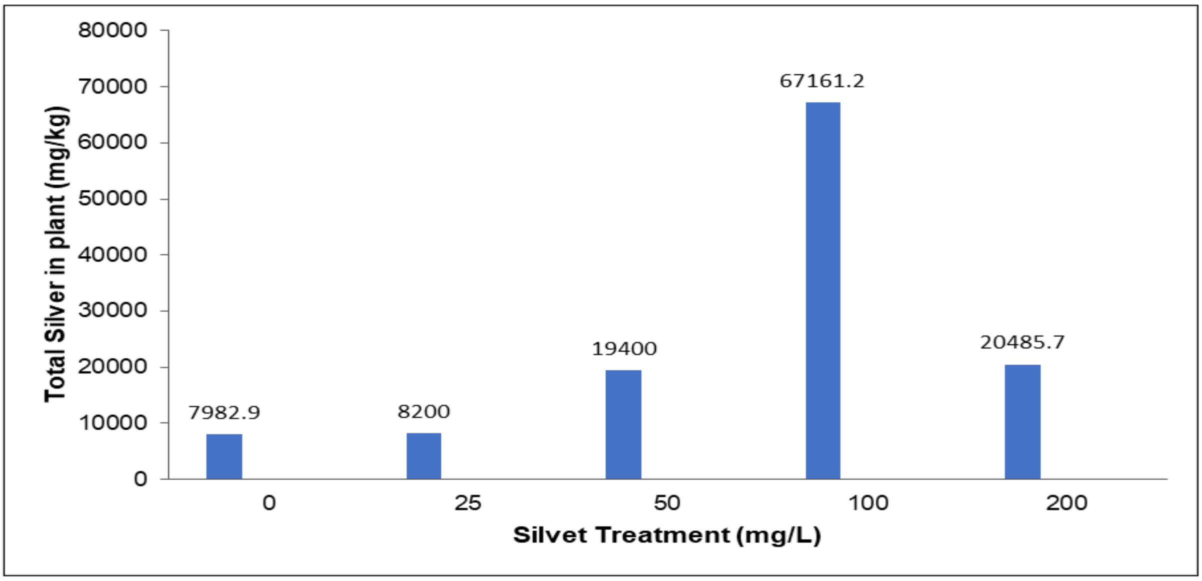

Fig. 1. Total Silver accumulation in *Brassica juncea* grown in various concentrations in sand.

Accumulation of Silver in roots of *Brassica juncea* grown in sand decreased with increased concentration of Silver 0, 25, 50 (mg/L) with 3000, 2400, 1800 (mg/kg) but increased to 11000 (mg/kg) at 100 (mg/L) then again decreased to 5000 (mg/kg) at 200 (mg/L).

The accumulation of silver in root was 3000, 2400, 1800, 1100, 5000 (mg/kg) with concentration of silver 0, 25, 50, 100, 200 (mg/L). The maximum accumulation of silver in root observed was 11000(mg/kg) at 100(mg/L) (Fig. 1).

The various concentration of silver was accumulated in above and ground parts of *Brassica juncea* that were grown in sand. Bioaccumulation factor of *Brassica juncea* grown in sand was maximum at 50mg/L (3.4) and minimum at 200mg/L (1.1). The Bioaccumulation factor of *Brassica juncea* was 4.3, 1.92, 3.4, 1.5, 1 with concentration of Silver 25, 50, 100 200 (mg/L). All the calculated values were higher than 1 which shows hyperaccumulation of silver in plant (Table 1). Silver toxicity in plants has been reported when it exceeds its normal value (Lokeshappa *et al.,* 2012). In the present study, the accumulation of Silver in *Brassica juncea* grown in sand was higher in aerial part (leaf and stem) than in roots as compared to control plants. In a study it was concluded that *Brassica juncea* tends to accumulate far greater concentrations of heavy metals in its aerial parts that of *Brassica napus* (Nouairi *et al.,* 2006). A similar finding was shared where silver was found more concentrated in the stems and leaves and lesser quantities were reported in the underground parts of the plants (Cabrera *et al.,* 2014).

Measure of efficiency of plant species to accumulate a metal from the surrounding environment into its tissues is reflected by plants bioaccumulation factor (Ladislas *et al.,* 2012). Plants exhibiting a bioaccumulation factor value greater than 1 are categorized as hyperaccumulators (Ma *et al.*, 2015).

The result of the present study indicated that bioaccumulation factor for each of the concentration is greater than 1 which shows *Brassica juncea* plant is hyper-accumulator for silver metal because it accumulated more amount of Silver metal. Therefore its potential for use in phytoremediation of silver afflicted soils is enhanced.

Conclusion

Brassica juncea modifies itself according to the contaminated environment and grows well even in the stress of heavy metal. The response of *Brassica juncea* towards heavy metal stressed soil makes it an exceptional model for this purpose. Substantially higher concentration of silver was found in aerial parts and much lower quantities were found in roots of hyperaccumulator, *Brassica juncea*. Hence there is a great scope for utilization of *Brassica juncea* for cleaning the soils polluted by silver metal.

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