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# Uptake of mineral elements by *Brassica juncea* and its effects on biochemical parameters

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**ABSTRACT**

*Plant growth, uptake of mineral elements and biochemical parameters are determined for Brassica juncea. Mineral elements such as Cu, Zn, Mn, Mg, Na and P, protein content, amino acid content and chlorophyll content were estimated. 88.8 ± 0.2 mg/g is the highest protein content in shoot increased by Zn uptake and 0.94 ±0.02 mg/g is the high amino acid concentration in shoot achieved by Na uptake. Cu and Zn uptake gives no effect in total chlorophyll content. Mineral treated Brassica juncea plants showed enhanced uptake of Copper which would be an attractive property for use in phytoextraction, where plants are used to accumulate pollutants followed by harvesting of the plant material.*

**Key words:** Plants; Brassica *juncea*; mineral elements; uptake; biochemical effects.

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## INTRODUCTION

Human evolution has led to immense scientific and technological progress. Global development, however, raises new challenges, especially in the field of environmental Protection and Conservation [1]. Elemental pollution of the environment is a major problem today. It causes health problems in livestock and human beings. Some microelements like zinc, copper, and manganese enter into the environment through air, water and soil and finally reach the food chain through contaminated water, edibles and other food stuffs. Besides, human beings can be directly exposed through occupational and environmental exposures. The productivity of agricultural land and soil quality needs improvement in zinc, iron, copper and potassium [2]. Elemental contaminants are among the most prevalent forms of contamination found at waste sites and their remediation in soils and sediments are among the most technically difficult. The high cost of existing cleanup technologies led to the search for new cleanup strategies that have the potential to be low-cost, low-impact, visually benign and environmentally sound [3.4]. Phytoextraction techniques involve the elimination of the pollutants from the soil, the toxic elements accumulating in the harvestable parts of the plants [5, 6]. The family Brassicaceae consists of 350 genera and about 3500 species and includes several genera [7]. *Brassica juncea*, commonly referred to as Indian mustard is an oilseed *Brassica* crop for which cultivation extends from India through western Egypt and Central Asia to Europe (Kingdom: Plantae, Order: Brassicales, Family: Brassicaceae, Genus: *Brassica,* Species: *B. juncea)* [8]. It known as rai, raya and laha is one of the most important oil seed crops of the country and it's occupies considerably large acreage among the Brassica group of oil seed crops [9]. This plant used to remove metal elements from the soil in hazardous waste sites because it has a higher tolerance for these substances and stores the elements in its cells. It also prevents erosion of soil from

these sites preventing further contamination [10]. To study elemental accumulation in reasonable time, plants with short lifetime are needed, and *Brassica juncea* would be ideal for this purpose.
In this study, we sought to analyze the potential of *B. juncea* to grow and counter the presence of high levels of certain mineral elements some macro and some micro elements. The elements selected for this study are essential for various physiological processes, over accumulation of which can lead to deleterious effects. It is assumed that enhanced supply of cations could lead to increased uptake and accumulation by this plant, as it has been shown to display this propensity. The study was carried out using *B. juncea* grown hydroponically and its response to elevated levels of the elements Mg, K, Na, Cu, Zn and Mn, were monitored in relation to its capacity for hyper accumulation.

## MATERIALS AND METHODS

### 2.1 Growth of *Brassica juncea* and treatment with mineral elements
The seeds of *Brassica juncea* were soaked in water for 24 hr and allowed to germinate for 48 hr on moistened filter paper in a sterile growth chamber maintained at 37ºC and with controlled aeration under a light-dark regimen of 8:16 hours. The germinated seedlings were split into 7 sets. They were the transferred to nylon mesh placed in different plastic trays. The 7 trays were filled with Hoagland's solution and seedlings were grown for 7 days. Hoagland's No.2 Basal Salt Mixture (Sigma Chemical Co, USA) was diluted as 1.6 gm to 1L with deionised water. Seven days later, each tray was amended with one mineral element. The elements added were Na, K, Mg, Mn, Cu and Zn which were added as NaCl, KCl, $MgSO_4.5H_2O$, $MnSO_4.H_2O$, $CuSO_4$ and $ZnSO_4.7H_2O$ respectively at a level of 100 ppm each.

### 2.2 Determination of elements
25 gm of well homogenized sample was taken into a clean silica dish. 25 ml of 20 % aqueous sulphuric acid was added and mix thoroughly. The contents of the dish were dried thoroughly in an oven around 110°C and heated with a soft flame (such as that of Argand burner), until all volatile or readily combustible matter has been removed. Ashing was performed at 500°C for about 6 – 8 hours [11]. The contents of the samples were quantitatively transferred in to 50 ml volumetric flasks, the dish heated with 10 ml of HCl (1+1). The solutions again transferred in to the same volumetric flask and diluted. Appropriately diluted standard solutions was taken and standardized by the AAS. The readings are related to the dry weight of the plant material used for the study. Sodium and potassium in plant material were estimated by the method of A. J. Cavell (1954) [12].

### 2.3 Biochemical estimation
The proteins were determined by the method of Lowry *et al.*, (1951) [13] with bovine serum albumin as standard. Amino acids were determined by the Ninhydrin method of Moore and Stein (1948) [14].Chlorophyll content was determined by the method of Hiscox and Israelstam (1979) [15].

## RESULTS

### 3.1 Uptake of mineral elements by *Brassica juncea*
Short-term uptake and effect of mineral elements in *Brassica juncea* was predicted in table 1 and the results revealed that for all the elements, there was an increase in the intracellular levels of the respective element which was supplemented in the soil, while all the other nutrients remained constant.

**Table1. Short-term uptake and effect of mineral elements in *Brassica juncea***

| Mineral Elements After 18 hours | Treatment in *Brassica juncea* | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control (ppm)** | **Zn (ppm)** | **Cu (ppm)** | **Mn (ppm)** | **Mg (ppm)** | **Na (ppm)** | **P (ppm)** |
| Zn | 0.05 | 0.09 | 0.05 | 0.02 | 0.02 | 0.02 | 0.04 |
| Cu | 0.10 | 0.05 | 0.51 | 0.04 | 0.04 | 0.09 | 0.05 |
| Mn | 0.03 | 0.02 | 0.04 | 0.13 | 0.07 | 0.03 | 0.03 |
| Mg | 0.62 | 0.24 | 0.76 | 0.47 | 0.83 | 0.65 | 0.71 |
| Na | 4.65 | 3.80 | 5.21 | 2.71 | 4.15 | 4.68 | 4.12 |
| P | 2.79 | 3.14 | 4.16 | 3.21 | 3.10 | 2.60 | 2.45 |

*Values are means ±S.E (n = 5)*

For long term uptake, the content of mineral elements was estimated separately in shoots and roots. Long term uptake and effect of mineral elements in *Brassica juncea* was predicted in table 2. The results presented for all the elements, the shoots and roots of the plants showed an increase in the intracellular levels of the respective element which was supplemented in the soil.

**Table 2. Long-term uptake and effect of mineral elements in Brassica juncea**

| Content | Plant Tissue | Treatment in *Brassica juncea* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **C** | **Cu** | **Zn** | **Mn** | **Mg** | **Na** | **P** |
| | | mg/g | | | | | | |
| Zn | Shoot | 0.06 | 0.10 | 0.12 | 0.06 | 0.05 | 0.08 | 0.06 |
| | Root | 0.12 | 0.08 | 0.14 | 0.12 | 0.05 | 0.10 | 0.05 |
| Cu | Shoot | 0.07 | 0.80 | 0.07 | 0.08 | 0.06 | 0.11 | 0.05 |
| | Root | 0.15 | 1.06 | 0.13 | 0.20 | 0.26 | 0.09 | 0.09 |
| Mn | Shoot | 0.02 | 0.03 | 0.03 | 0.04 | 0.02 | 0.03 | 0.02 |
| | Root | 0.32 | 0.31 | 0.21 | 0.37 | 0.23 | 0.33 | 0.30 |
| Mg | Shoot | 0.38 | 0.51 | 0.41 | 0.32 | 0.52 | 0.34 | 0.36 |
| | Root | 0.50 | 0.40 | 0.51 | 0.40 | 0.53 | 0.45 | 0.50 |
| Na | Shoot | 4.46 | 7.04 | 3.74 | 5.06 | 3.41 | 5.23 | 5.02 |
| | Root | 4.44 | 7.18 | 4.44 | 4.46 | 4.35 | 4.76 | 4.40 |
| P | Shoot | 3.91 | 10.86 | 1.50 | 2.86 | 3.11 | 7.40 | 8.03 |
| | Root | 5.72 | 6.31 | 4.74 | 3.29 | 4.14 | 4.85 | 6.81 |

*Values are means ±S.E (n = 5)*

### 3.2 Protein content

The results related to the effect of elements on protein content are depicted in table 3. Among the macro elements, treatment with Mg resulted in an increase in protein content of the shoot by 25 % whereas addition of K resulted in only 1 % increase, while Na addition lead to a decrease in protein by 44 %.

In the roots, there was an increase in protein content by 8 % with Mg and 7 % with K, while Na induced a decrease as high as 51 %. The microelements Cu and Zn induced a reduction in protein content by 7 and 6 % respectively, while Mn induced an increase of 5 % in the shoots. In the roots, all the 3 elements brought a decrease in protein content, being 46 % with Cu, 28 % with Mn and 23 % with Zn.

**Table 3. Protein Estimation**

| Para Meters | Treatment in *Brassica juncea* | | | | | | |
|---|---|---|---|---|---|---|---|
| | **C** | **Cu** | **Zn** | **Mn** | **Mg** | **Na** | **P** |
| Plant Tissue | mg/g | | | | | | |
| Shoot | 56.8 ±0.2 | 44.8 ±0.2 | 88.8 ±0.2 | 80.4 ±0.2 | 82.4 ±0.2 | 76.8 ±0.2 | 88.4 ±0.2 |
| Root | 81.6 ±0.2 | 41.2 ±0.2 | 52.0 ±0.2 | 40.4 ±0.2 | 31.6 ±0.2 | 39.6 ±0.2 | 72.8 ±0.2 |

*Values are means ±S.E (n = 5)*

**Table 4. Amino acid Estimation**

| Para Meters | Treatment in *Brassica juncea* | | | | | | |
|---|---|---|---|---|---|---|---|
| | **C** | **Cu** | **Zn** | **Mn** | **Mg** | **Na** | **P** |
| Plant Tissue | mg/g | | | | | | |
| Shoot | 0.70 ±0.02 | 0.72 ±0.02 | 0.50 ±0.02 | 0.52 ±0.02 | 0.68 ±0.02 | 0.94 ±0.02 | 0.88 ±0.02 |
| Root | 0.46 ±0.02 | 0.40 ±0.02 | 0.26 ±0.02 | 0.44 ±0.02 | 0.58 ±0.02 | 0.38 ±0.02 | 0.32 ±0.02 |

*Values are means ±S.E (n = 5)*

### 3.3 Amino acid content

The results related to the effect of elements on amino acid content are depicted in table 4. Among the macro elements, treatment with Na and K resulted in an increase in amino acid content of the shoot by 34 and 26 % respectively, whereas addition of Mg addition leads to a marginal decrease in amino acid content by 3 %. On the contrary, Mg induced an increase in amino acid content in the roots by 26 %, whereas K and Na induced a decrease by 30 and 17 % respectively.

Among the microelements only Cu induced an increase in amino acid content by a mere 3 %, while Zn and Mn induced a decrease by 29 and 26 % respectively in the shoots. In the roots, all the 3 elements brought a decrease in amino acid content, being 44% with Zn, 13 % with Cu and 4 % with Mn.

### 3.4 Chlorophyll content

The levels of chlorophyll 'a', chlorophyll 'b'; and total chlorophyll were measured separately, and the results are given in table 5. Among the macro elements, only plants treated with Mg showed a 4 % increase in chlorophyll content, while addition of K and Na resulted in 32 and 26 % decrease respectively. The microelement Mn resulted in an increase in chlorophyll content by 7 % Cu caused no change while Zn induced a marginal reduction of 1 % compared to control plants.

**Table 5. Chlorophyll Estimation**

| Parameters | **Treatment in *Brassica juncea*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **C** | **Cu** | **Zn** | **Mn** | **Mg** | **Na** | **P** |
| Plant Tissue | mg/g | | | | | | |
| Chlorophyll a | 17.1±0.2 | No effect | 13.0 ±0.2 | 18.0 ±0.2 | 15.1 ±0.2 | 11.0 ±0.2 | 10.3 ±0.2 |
| Chlorophyll b | 7.2±0.2 | No effect | 11.0 ±0.2 | 8.1 ±0.2 | 10.1 ±0.2 | 7.1 ±0.2 | 6.2 ±0.2 |
| Total Chlorophyll | 24.3±0.2 | No effect | No effect | 26.1 ±0.2 | 25.2 ±0.2 | 18.1 ±0.2 | 16.5 ±0.2 |

*Values are means ± S.E (n = 5)*

## DISCUSSION

This study has analyzed the response on *B. juncea* to the presence of high levels of certain mineral elements - some macro and some micro elements. All those studied herein are essential for various physiological processes. But over accumulation can lead to deleterious effects. In the past, several studies have been conducted using *B. juncea* and shown that it is an ideal candidate for remediation as it can accumulate several elements. Under such a situation, enhanced supply of cations could lead to increased uptake and accumulation [16] tested many fast growing *Brassicas* for their ability to tolerate and accumulate metals, including Indian mustard (*B. juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L). Although all *Brassicas* accumulated metals, *B. juncea* showed a strong ability to accumulate and translocate Cu, Cd, Ni, Pb, and Zn to the shoots.

In this study, treatment with Cu led to accumulation of K within 18 hours. On the $7^{th}$ day, Cu treated plants showed accumulation of K in the shoot and Na in the root. All other minerals are also found in higher levels in Cu treated plants. Therefore, it appears that Cu plays a major role in influencing the elemental balance in *B. juncea.* Walker and Bernal (2005) [17] observed similar increase in uptake of the metals Zn, Cu and Pb when *B. juncea* was grown in a site affected by toxic spillage of acidic and metal-rich waste, although the total uptake of metals was fairly low. Similarly, *B. juncea* grown on tannery waste accumulated the metals Cr, Fe, Zn and Mn with the seeds accumulating the least quantities of all the metals tested [18]. Phytoextraction of the metals, Cd, Cr, Cu, Pb and Zn by mustard plants grown on a multiple contaminated soil in presence of chelates has also been observed [19].

Potassium is an essential macronutrient and the most abundant cation in plants [20]. In this study on *B. juncea*, the K level was found to be marginally lesser in both Na and K treated plants in comparison to control plants. Salt tolerance is generally correlated with the capacity of plants to keep their K level in a defined range [21]. When treated with Na, a decrease in leaf K concentrations is often found in most plants leading to a suboptimal K supply [22]. As noticed in *B. juncea* in the present study, in *Populus euphratica*, the K concentrations of leaves decrease moderately, even under persisting salt stress [23].

Treatment with various elements resulted in slight variation in the protein content of *B. juncea.* These variations in protein content showed an inverse correlation with the amino acid content. This was clearly observed in the case of plants treated with Na, Mn and K. In Mn treated plants, both the protein content and amino acid levels were higher indicating a higher turnover. This is understandable because of the increase in chlorophyll content. In Zn treated plants, the level of protein and amino acids were both lowered than control, probably because of the high level of K accumulated by these plants. In suspension cultured *B. juncea* accumulation of metallothionein in the presence of Zn and reduction in the presence of Cu has been reported [24].

Results obtained in this study showed that the carbohydrate content increased in all the treated plants when compared to control, except in the case of Cu. K treated plants showed very high levels of carbohydrates along with a maximum decrease in chlorophyll content indicating that K plays a large role in the overall metabolism of the plant. An increase in the photosynthetic pigment, protein and carbohydrate has been recorded in *B. juncea* plants grown on diluted sludge [18].

A major factor influencing the efficiency of phytoextraction is the ability of plants to absorb large quantities of metal in a short period of time. Hyper accumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil [25], as long as the metal in question is present. This property is unlike moderate accumulators now being used for phytoextraction where the quantity of absorbed metal is a reflection of the concentration in the soil. Although the total soil metal content may be high, it is the fraction that is readily available in the soil solution that determines the efficiency of metal absorption by plant roots.

In recent years, there has been a considerable interest in use of plant as a scavenger of heavy metals from aqueous solutions because, the methods are simple and environment friendly, cost effective and both living and nonliving biomass are used [26]. Many herbaceous species, including members of the *Brassicaceae,* also accumulate moderate amounts of various metals in their shoots. One of the most promising, and perhaps most studied, non-hyper accumulator plant for the extraction of heavy metals from contaminated sites is Indian Mustard (*B. juncea*) [27]. In this study, *B.juncea* found to be one of the most effective plants for the uptake of mineral elements.

## CONCLUSION

Experiments with seedlings suggest that the mineral transporter can mediate the uptake of Cu, and possibly of Mn, Zn and Mg, since mineral treated *Brassica juncea* accumulated more Cu and to a lesser extend Mg, Zn, Mn compared to the control seedlings. Mature plants are appears with the elemental transporter facilities, that uptake copper into shoots. Constitutive expression of the transporter in mineral treated *Brassica juncea* plant also facilitated uptake of Zn, K, Mg and Na into roots, but not their translocation to the shoot. Seedlings and mature plants were fairly consistent, except that the additional Na, Mg, K and taken up by mineral treated roots was not translocated to the shoot in mature plants. So these are limited by the activity of endogenous transporters.

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