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RESEARCH ARTICLE

Environmental Science

Bioaccumulation and health risks of chromium and cadmium in *Basella alba* with emphasis on Urea, muriate of potash and triple super phosphate mixtures

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Abstract: *Basella alba* is a commonly consumed green leafy vegetable in South Asian countries and it is considered as an economical source of essential vitamins and dietary fibers. Urea, muriate of potash (MOP) and triple super phosphate (TSP) mixture is the most commonly used inexpensive fertilizer mixture in the commercial cultivation of *B. alba*. This study was conducted to assess the effect of using a mixture of Urea-MOP-TSP on the bioaccumulation potential of cadmium (Cd) and chromium (Cr) by the leaves of *B. alba*. Pots treated with the Urea-MOP-TSP mixture and compost were maintained in a greenhouse with controlled light and temperature settings. The concentrations of Cd and Cr in the root zone soil, roots and leaves of *B. alba* were analyzed using an Atomic Absorption Spectrophotometer after acid digestion. The health risk of consuming *B. alba* was evaluated using the daily intake of metals, health risk index and cancer risk index. The results showed that Cd and Cr can bioaccumulate in the leaves of *B. alba*. The bioaccumulation potential of Cr was higher than that of Cd. Although the daily intake of Cd and Cr from *B. alba* were below the maximum values stipulated by the WHO, the cancer risk index indicated potential cancer risks based on Cd intake due to consumption of *B. alba* cultivated using the Urea-MOP-TSP mixture. Furthermore, the health risks associated with the hyper-accumulation of Cd and Cr in the edible parts of *B.alba* cultivated using compost were significantly less compared to that of the plants treated with the Urea-MOP-TSP mixture. Therefore, if *B. alba* is cultivated using chemical fertilizer, it is recommended to regularly monitor the concentrations of Cd and Cr in the cultivation soil, chemical fertilizer and in the edible parts of *B. alba* to prevent the excessive buildup of Cd and Cr along the food chain. In addition, it is recommended to identify the potential microbial assemblages that can be inoculated to the cultivation soil to reduce the bioavailability of Cd and Cr.

Keywords: Bioconcentration, cadmium, cancer, chromium, daily intake of metals, hyperaccumulation

INTRODUCTION

Green leafy vegetables are nutritional foods that contain high concentrations of dietary fibers, vitamins and minerals. The phyto-chemicals found in the edible parts of green leafy vegetables have antioxidative and anti-inflammatory properties that provide health benefits to consumers (Brouwer-Brolsma *et al*. 2020). The nutritional and health benefits of green leafy vegetables have led to an increase in the consumer demand resulting in their cultivation on a commercial basis. Commercial cultivations mainly focus on increasing yield by applying chemical fertilizers in the cultivation plots (Upekshani *et al*. 2018).

Essential plant nutrients are added to the soil in commercial cultivations by applying chemical fertilizer mixtures, which contain nitrate, phosphate, and potassium. However, the fertilizer formulations contain organic and inorganic contaminants such as heavy metals, which have the potential to cause adverse impacts in the receiving environment (AlKhader 2015). Compared to organic fertilizers and soil modifiers, inorganic fertilizers breakdown easily when added to the soil. However, the quick breakdown of inorganic fertilizers can lead to contamination of the soil in agricultural fields. The main nitrogenous and phosphatic fertilizers can modify

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the physical soil properties such as bulk density, hydraulic conductivity and can also alter the soil structure (Rashmi *et al*. 2020, Alengebawy *et al*., 2021)

The root system of green leafy vegetables can absorb heavy metals, which can then accumulate in the edible and non-edible parts of the plant. This uptake of heavy metals can lead to a decrease in the nutritional quality of the vegetables and disrupt metabolic functions. As a result, essential nutrients may not be absorbed properly, chlorophyll synthesis may be inhibited, and enzymatic responses to oxidative stress can be induced (Edelstein *et al*., 2018, Chaturvedi *et al*. 2019, Pavlíková *et al*., 2023). Consumers may experience significant health impairments from consuming green leafy vegetables with accumulated metals, leading to chronic and acute toxic responses with prolonged consumption (Amin *et al*. 2013; Shakya & Khwaounjoo 2013; Wijeyaratne & Kumari 2021).

Basella alba, also known as Malabar spinach, is a very popular green leafy vegetable in South Asian countries due to their high fiber and mineral content (Deshmukh & Gaikwad, 2014). *B. alba* is a creeping vine commonly grown as a pot herb in home gardens in India, Sri Lanka and Indonesia. It can be easily cultivated in a variety of soils and is adaptable to extreme climatic conditions (Gayathree *et al*. 2020). However, several studies have shown that *B. alba* has the capacity to accumulate high concentrations of cadmium (Cd) and chromium (Cr) compared to other commonly consumed green leafy vegetables available in the market.

Furthermore, it has been noted that there are significantly high hazard quotients and health risk index values for Cd and Cr in comparison to As, Ni, Pb, Cu and Zn (Kananke *et al*. 2014; Sultana *et al*. 2019). However, these studies were conducted using samples from local markets in South Asian cities. Therefore there is no information available on the cultivation methods or irrigation water used for cultivation. Some studies conducted on *B. alba* cultivations were irrigated using textile wastewater, which recorded high concentrations of Cd and Cr in the edible parts of the plant and have been identified as potential health risks associated with long-term consumption (Alam *et al*. 2003; Shammi *et al*. 2016). These studies indicate a high risk associated with the accumulation of Cd and Cr in *B. alba*.

The consumer demand for *B alba* in South Asian countries has increased in the past decade as it is a good source of nutrients at an affordable cost. Commercial cultivations of *B. alba* commonly use a fertilizer mixture of urea, muriate of potash (MOP) and triple super phosphate (TSP). This fertilizer mixture provides the essential nutrients required for the growth of the plant and is very effective in producing a greener and higher quality *B. alba* desired by consumers to the market (Rosliza *et al*. 2009). However, the application of Urea, MOP and TSP can affect the mobility of Cd and Cr in the soil solution and can increase the uptake of these heavy metals by plants (Bandara *et al*. 2008; Jayasumana *et al*. 2015). Therefore, the present study focused on assessing the concentrations of Cd and Cr in the edible parts of *B. alba* cultivated using the above fertilizer mixture. Furthermore, the health risks associated with the consumption of leaves of *B. alba* cultivated using a mixture of Urea, MOP and TSP was also assessed. This was done in terms of the bioconcentration factor (BCF), soil to root translocation factor (TF (soil − root)), root to leaf translocation factors (TF (root − leaf)), target hazard quotient (THQ), hazard index (HI) and cancer risk index (CI) for Cd and Cr.

MATERIALS AND METHODS

Soil samples were collected at a depth of 0-25 cm from the Landscape Management Unit of the University of Kelaniya, Sri Lanka. The study was conducted in a greenhouse at the University of Kelaniya, Sri Lanka.

Preparation of the experimental units

Air dried soil samples were sieved through a 4 mm sieve, and used in the seed germination trays (44 x 214 x 52 mm) and in the experimental pots (pot volume: 2 L; pot height: 13 cm; pot diameter - top: 17 cm: pot diameter - bottom: 13 cm). *B. alba* seeds were purchased from the local market and germinated in the seed germination trays. After two weeks, healthy seedlings (6 seedlings per pot) were transplanted into individual prewashed plastic pots which contained 2.5 kg of sieved, air-dried soil.

Two sets of experiments namely Set 1: (chemical fertilizer urea, MOP and TSP mixture applied pots), and Set 2: (organic fertilizer with compost applied pots) were arranged in a randomized block design. Each set consisted of twenty replicate pots. The plants were watered regularly with well water and grown under natural light conditions.

Physical and chemical properties of soil, compost and water

The physical and chemical properties of the soil samples, compost samples and water used for watering the plants were assessed using standard laboratory methods. The pH, and conductivity were measured using a calibrated digital multiparameter water quality meter (YSI Environmental Model-556 MPS). The moisture content of the soil and compost samples was determined by the gravimetric method. The total organic matter content (TOM) and the $CaCO_3$ content were determined by the mass loss on ignition method (550 °C, until a constant weight is attained) using a muffle furnace. The bulk density of the soil was determined using the core method and the bulk density of compost was determined by the method described by Ahn *et al*., (2004). A 10 liter graduated cylinder was filled with compost and slightly compacted to eliminate large void spaces. The bulk density was then calculated by dividing the weight of the compost by the volume of compost in the graduated cylinder (Ahn *et al*. 2004). The percentage of gravel, clay, silt and sand content in the soil was determined using the pipette method as described by Bowman and Hutka (2002).

The soil and compost samples were acid-digested and the water samples acidified by adding concentrated HNO_3 until the pH reached 6.0-6.5. The concentrations of Cd and Cr in the acid digested samples were analyzed using an atomic absorption spectrophotometer (Analytic Jena Model novAA 400p).

Application of fertilizer

Two weeks after transplanting, 25 g of a mixture containing urea, muriate of potash (MOP), and triple super phosphate (TSP) in a ratio of 2:1:1 was added to each experimental pot in Set 1, and 25 g of a commercial compost mixture was added to each experimental pot in Set 2 as recommended by the Department of Agriculture, Sri Lanka (2007).

Sampling of plants and soil

Sampling was conducted on three occasions at 4 wks, 8 wks and 12 wks (at the end of culture cycle) after fertilizer application. At each sampling event, two *B. alba* plants with their root zone soil were sampled from each pot. The roots and leaves of the plant samples were separated, thoroughly washed with distilled water and air-dried. The air-dried root and leaf samples were then oven-dried at 105 ^{0}C until they reached a constant weight. The dried samples were then ground into a powder by using a clean electric grinder. The powdered samples were sieved through a 0.4 mm sieve and kept in a desiccator until they were acid digested.

The air-dried root-zone soil samples were oven-dried at 105 ^{0}C until they reached a constant weight. These samples were ground into fine particles using a pre-acid washed commercial mortar and pestle. The ground soil was sieved through a 2 mm sieve and samples were stored in a desiccator until they were ready for acid digestion.

Acid digestion of plant and soil samples

Acid digestion and metal analysis of the samples were done by following the procedure described in Wijeyaratne and Kumari (2021).

Powdered and homogenized leaf, root and soil samples (0.5 g) were acid digested in the Gerhardt Kjeldatherm digestion system at 270 °C for 2 h using concentrated HNO_3 and concentrated $HClO_4$ in a 1:1 (v/v) ratio as the digestion medium. The digested solutions were allowed to cool and 5 mL of deionized water was added. The dissolved solution was swirled and filtered into a 50 mL volumetric flask through Whatman filter paper number 42. The clear solution was then diluted up to 50 mL with deionized water and analyzed for Cd and Cr by atomic absorption spectrophotometer (Analytic Jena Model novAA 400p) in the graphite furnace mode following the procedure described by the American Public Health Association (APHA, 1999).

The minimum detection limits for Cd and Cr were 0.02 mg/L and 0.001 mg/L, respectively. Sandy loam certified reference material CRM 023 (Sigma-Aldrich, USA) was used as the standard reference material for soil analysis, and white cabbage certified reference material BCR 485 (Sigma-Aldrich, USA) was used as the standard reference material for plant analysis. Continuing control verification was done after every 10 samples to ensure that variability was within 10%.

The accuracy of the analytical procedure was determined by calculating the percentage recovery of Cd and Cr in the spiked sample as described by Addis and Abebaw (2017). Soil and plant samples were spiked in triplicates at a known calibration concentration of Cd and Cr. The same digestion and analysis procedure was followed for both the original samples (non-spiked) and the spiked samples The percentage recoveries of Cd and Cr were then calculated to evaluate the accuracy of the analytical procedure by the formula described by Addis and Abebaw (2017).

Percentage recovery of the metal (%R) = $\frac{S_n - U_n}{A_n} X\ 100$

Where,

S_n – Concentration of metal in the spiked sample
U_n – Concentration of metal in the non-spiked sample
A_n – Spiked concentration

Bioconcentration factor and translocation factors

The heavy metal concentrations were used to calculate the bioconcentration factor, soil-to-root translocation factor, and root-to-leaf translocation factor of Cd and Cr in *B. alba* as described by Sulaiman *et al*. (2016) and Wijeyaratne and Kumari (2021).

The bio-concentration factor (BCF) of *B. alba* at the end of the culture cycle was calculated by the following formula:

$$\mathrm{BCF} = \frac{C_{Plant}}{C_{Soil}}$$

Where, C_{plant} is the metal concentration in the plant part (leaves) and C_{soil} is the metal concentration in the soil (Wijeyaratne & Kumari 2021).

The soil to root translocation factor (TF_{S-R}) at the end of the culture cycle was calculated by the following formula (Wijeyaratne & Kumari 2021):

$$\mathrm{TF}_{S-R} = \frac{C_R}{C_S}$$

Where, C_R is the metal concentration in roots and C_S is the metal concentration in soil.

Root to leaf translocation factor (TF_{R-L}) at the end of the culture cycle was calculated by the following formula (Wijeyaratne & Kumari 2021).

$$\mathrm{TF}_{R-L} = \frac{C_L}{C_R}$$

Where C_L is the metal concentration in leaves and C_R is the metal concentration in roots.

Assessing the health risks associated with consumption of *B. alba*

The health risks associated with the consumption of *B. alba* were calculated based on the daily intake of metals for Cd (DIM_{Cd}) and Cr (DIM_{Cr}), health risk index for Cd (HRI_{Cd}) and Cr (HRI_{Cr}), and cancer risk for Cd (CR_{Cd}) and Cr (CR_{Cr}).

The daily intake of Cd and Cr from *B. alba* was calculated using the following formula described by Gebreyohannes and Gebrekidan (2018):

$$DIM = \frac{C_{Metal}\ X\ C_{Factor}\ X\ D_F}{w}$$

Where,
DIM: Daily Intake for Metal;
C_{Metal}: Concentration of the metal (mg/kg);
C_{Factor}: Conversion factor to convert fresh vegetable weight to dry weight (0.085 for green leafy vegetables (Benson *et al.* 2002));
D_F: Daily average intake of green leafy vegetables (USEPA 2007a, USEPA 2007b, WHO/FAO 2007);
w: Average body weight of a person (70 kg for adults and 30 kg for children (Walpole *et al*. 2012)).

The health risk index of *B. alba* for Cd and Cr was calculated using the following formula described by Gebreyohannes and Gebrekidan (2018):

$$HRI = \frac{DIM}{RoD}$$

Where,
HRI: Health Risk Index for metal;
DIM: Daily Intake for Metal;
RoD: Reference oral Dose for metal (Cd: 0.001; Cr: 1.5 (Benson *et al*. 2002)).
An HRI < 1 is considered safe for the exposed human populations (Gebreyohannes and Gebrekidan 2018; Khan *et al*, 2020; Wijeyaratne and Kumari 2021).

The cancer risk index (CI) of *B. alba* for Cd and Cr was calculated using the following formula described by Gebeyehu and Bayissa (2020):

$$CI = DIM\ X\ CPS_O$$

Where,
CI: Cancer risk for metal;
DIM: Daily intake for metal;
CPS_O: the oral cancer

slope factor in $(\mathrm{mg/kg/day})^{-1}$. CPS_O for Cd: 0.38 and CPS_O for Cr: 0.5 (Zeng *et al*. 2015; Yang *et al*. 2018).

Statistical analysis

The Anderson Darling test was used to test for the normality of data and the non-normalized data were log-transformed.

The Student's t-test was used to compare the concentrations of Cd and Cr in the roots, leaves and root zone soil of *B. alba* across treatments where compost, Urea, MOP and TSP were applied at each sampling event. One-way analysis of variance followed by Tukey's pairwise comparison was used to analyze the variation of Cd and Cr concentrations in the roots, leaves and root zone soil of *B. alba* in pots treated with compost and a mixture of urea, MOP and (TSP) over a culture cycle. Additionally, the variation of health risk assessment indices for Cd and Cr in the different treatments were analysed. Data analysis was conducted using R statistical software.

RESULTS AND DISCUSSION

The physical and chemical properties of the soil, compost and water used in the experiment are given in Table 1. The soil used as the experimental medium had a sandy loam texture with a mean pH of 7.4. The physical and chemical properties of the soil medium and the compost were within favorable ranges for plant growth (Table 1).

Table 1: Physical and chemical properties of the soil, compost and water used in the experiment pots

Physical/Chemical Property	Soil	Compost	Water
pH	7.4 ± 0.2	7.6 ± 0.2	7.2 ± 0.1
Conductivity (µS/cm)	610.2 ± 31.2	410.5 ± 24.3	385.2 ± 25.2
Total organic matter content (%)	2.8 ± 0.6	35.6 ± 1.2	Not Applicable
Bulk density (g/cm^3)	1.55 ± 0.4	0.64 ± 0.2	Not Applicable
Moisture content (%)	22.8 ± 1.3	23.1 ±1.5	Not Applicable
$CaCO_3$ concentration (%)	4.5 ± 0.3	2.2 ± 0.3	Not Applicable
Percentage clay (%)	6.3 ± 1.3	Not Applicable	Not Applicable
Percentage silt (%)	41.0 ± 3.5	Not Applicable	Not Applicable
Percentage sand (%)	42.5 ± 2.6	Not Applicable	Not Applicable
Gravel content (%)	10.2 ± 1.8	Not Applicable	Not Applicable
Cd Concentration	1.39 ± 0.17	0.76 ± 0.23	Not detected
Cr Concentration	15.62 ± 0.5	15.6 ± 0.64	Not Detected

Cadmium and chromium concentrations in the root zone soil of *B. alba* at the sampling events

Mean concentrations of Cd and Cr in the root zone soil of *B. alba* at different sampling events are given in Table 2.

Results are presented as Mean ± SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other. (Student t- test, $p < 0.05$; $n = 40$). MOP: muriate of potash; TSP: triple super phosphate.

The mean concentration of Cd in the root zone of the soil ranged from 0.54 to 1.86 mg/kg and the mean concentration of Cr ranged from 15.59 to 19.67 mg/kg. The highest mean Cd and Cr concentrations were recorded in the root zone of the soil collected from the Urea-MOP-TSP mixture applied samples at the third sampling event (Table 2).

The mean concentrations of Cr were higher than the mean concentrations of Cd in both treatments at all sampling events. The concentrations of Cd and Cr in the root zone of the soil of *B. alba* collected from pots where Urea-MOP-TSP mixture was applied were significantly higher than those in pots where compost was applied at the second and third sampling events (Student's t-test, $p < 0.05$; see Table 2). However, there was no significant difference in the concentrations of Cd and Cr between the pots that were applied with a Urea-MOP-TSP mixture and those with compost in the first sampling event (Student's t-test, $p > 0.05$; see Table 2). The concentrations of Cd and Cr in the soil increased significantly after 8 and 12 weeks following the application of chemical fertilizer. The soil Cd concentration increased due to the application of phosphate fertilizer, which contain Cd based additives. If the amount of Cd added by the fertilizer exceeds the removal of Cd through leaching, bioturbation or erosion, prolonged application of phosphate fertilizer can significantly increase the Cd concentrations in agricultural soil (Grant & Sheppard 2008; AlKhader 2015).

Table 2: Mean concentrations of Cd and Cr in the root zone soil of *B. alba* collected from pots applied with urea, muriate of potash (MOP) and triple super phosphate (TSP) mixture and compost.

Metal	Concentration (mg/kg)	
	Urea, MOP and TSP mixture	Compost
Sampling Event 1: Four weeks after fertilizer application		
Cd	1.393 ± 0.17 [a]	0.758 ± 0.23 [a]
Cr	15.62 ± 0.53 [a]	15.59 ± 0.64 [a]
Sampling Event 2: Eight weeks after fertilizer application		
Cd	1.803 ± 0.10 [a]	0.542 ± 0.24 [b]
Cr	19.50 ± 0.49 [a]	16.44 ± 0.53 [b]
Sampling Event 3: Twelve weeks after fertilizer application / End of culture cycle		
Cd	1.864 ± 0.15 [a]	1.314 ± 0.21 [a]
Cr	19.67 ± 0.49 [a]	16.352 ± 0.36 [b]

In the compost applied treatments, the concentration of Cd in the root zone of the soil at the 12th week after fertilizer application (at the end of the culture cycle/ Sampling Event 3) showed a significant increase, while the concentration of Cr in the root zone of the soil did not show a significant increasing trend throughout the culture cycle. Organic substances in the compost can enhance formation of metal-humic complexes, which can increase solubility and bioavailability of Cd in the compost applied soil (Hanc *et al* 2009; Chaab *et al*. 2016). This could be a reason for the increase in Cd concentration in the root zone of the soil in the compost applied pots. The concentrations of Cd and Cr in the root zone of the soil recorded, in this study, were lower than the maximum permissible limits for heavy metals in vegetable growing soils stipulated by the European Union (180 mg/kg for Cr and 3.0 mg/kg for Cd).

Table 3: Mean concentrations of Cd and Cr in the roots of *B. alba* collected from pots applied with urea, muriate of potash (MOP) and triple super phosphate (TSP) mixture and pots applied with compost.

Metal	Concentration (mg/kg)	
	Urea, MOP and TSP mixture	Compost
Sampling Event 1: Four weeks after fertilizer application		
Cd	2.12 ± 0.06 [a]	1.94 ± 0.34 [a]
Cr	13.9 ± 0.49 [a]	13.36 ± 0.62 [a]
Sampling Event 2: Eight weeks after fertilizer application		
Cd	2.30 ± 0.19 [a]	1.54 ± 0.21 [b]
Cr	20.07 ± 0.59 [a]	13.42 ± 0.31[b]
Sampling Event 3: Twelve weeks after fertilizer application / End of culture cycle		
Cd	2.782 ± 0.19 [a]	1.714 ± 0.18 [b]
Cr	22.39 ± 0.66 [a]	16.215 ± 0.26 [b]

Concentrations of cadmium and chromium in the roots of *B. alba* at the sampling events

Mean concentrations of Cd and Cr in the roots of *B. alba* at different sampling events are given in Table 3. The mean concentration of Cd in the roots of *B. alba* ranged from 1.54 to 2.78 mg/kg, and the mean concentration of Cr ranged from 13.36 to 22.39 mg/kg. The highest mean concentrations of Cd and Cr were found in the roots of *B. alba* collected from the pots treated with the Urea, MOP, TSP mixture at the end of the culture cycle (Table 3).

Results are presented as mean ± SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (Student t- test, $p < 0.05$; $n = 40$). MOP: muriate of potash; TSP: triple super phosphate.

There was no significant difference in the concentrations of Cd and Cr in the roots of *B. alba* between the samples applied with urea, MOP, TSP and compost in the first sampling event (Student's t- test, $p > 0.05$, Table 2). However, in the second and third sampling events, the concentrations of Cd and Cr were significantly high in the roots of *B. alba* in the pots applied with urea, MOP and TSP compared to the pots applied with compost ($p < 0.05$, Table 3). Several studies have documented that, the mobility and bioavailability of Cd and Cr are influenced by the soil structure, availability of organic matter and soil pH. Furthermore, it has been reported that chemical fertilizers containing NH_4^+ can reduce the pH of the soil within 8-10 weeks, increasing the mobility of Cd and Cr (Liao *et al*., 2019; Senila and Kovacs 2024). This may have resulted in increasing the bioavailability of Cd and Cr, in the present study, to be taken up by the roots of *B. alba.*

Cadmium and chromium concentrations in the leaves of *B. alba* at the sampling events

Mean concentrations of Cd and Cr in the leaves of *B. alba* at different sampling events are given in Table 4. The mean concentration of Cd in leaves of *B. alba* ranged from 0.69 to 3.38 mg/kg while the mean Cr concentration ranged from 13.48 to 23.35 mg/kg. The mean concentration of Cr in the leaves of the *B. alba* was significantly higher than that of Cd in all sampling events in both treatments. Trends in the variation of Cd and Cr concentration in the leaves of *B. alba* were similar to that of the roots.

In the first sampling event, there was no significant difference in the concentrations of Cd and Cr in the leaves of *B. alba* harvested from the two treatments. However, in the subsequent sampling events, the concentrations of Cr and Cd in the leaves of *B. alba* harvested from the pots applied with the Urea-MOP-TSP mixture were significantly higher than those harvested from the pots applied with compost (Student t test, $p < 0.05$, Table 4). The recorded concentrations of Cr and Cd in leaves of *B. alba* harvested from both the treatments, exceeded the safe levels recommended by the WHO/FAO (2.3mg/kg for Cr and 0.2 mg/kg for Cd) (Tajik *et al*., 2021).

Results are presented as Mean ± SD. In each row at a particular sampling event, the mean values with different superscript letters are significantly different from each other (Student t- test, $p < 0.05$; $n = 40$).

Table 4: Mean concentrations of Cd and Cr in the leaves of *B. alba* collected from pots applied with (i) urea, muriate of potash (MOP) and triple super phosphate mixture (TSP) mixture, and (ii) Compost.

Metal	Concentration (mg/kg)	
	Urea, MOP and TSP mixture	Compost
Sampling Event 1: Four weeks after fertilizer application		
Cd	1.76 ± 0.20^{a}	0.91 ± 0.38^{a}
Cr	13.80 ± 0.53^{a}	13.48 ± 0.61^{a}
Sampling Event 2: Eight weeks after fertilizer application		
Cd	2.83 ± 0.14^{a}	0.69 ± 0.40^{b}
Cr	19.75 ± 0.50^{a}	18.08 ± 0.32^{b}
Sampling Event 3: Twelve weeks after fertilizer application / End of culture cycle		
Cd	3.38 ± 0.18^{a}	2.33 ± 0.092^{b}
Cr	23.35 ± 0.89^{a}	17.85 ± 0.50^{b}

Cadmium and chromium concentrations in roots, leaves and the root zone soil of *B. alba* in the pots applied with Urea-MOP-TSP mixture and compost, over a culture cycle

There was a significant increase in the concentration of Cr in roots, leaves, and in the rootzone soil in the pots applied with Urea-MOP-TSP mixture in the successive sampling events towards the end of the culture cycle (ANOVA, Tukey's pairwise comparison, $p<0.00$, Table 2, Table 3, Table 4).

The concentration of Cd in the roots of *B. alba* in the pots treated with compost was significantly higher than that in the leaves and root zone soil during the first and second sampling events. However, the concentration of Cd was significantly high in the leaves of *B. alba* harvested from the pots treated with compost at the end of the culture cycle (ANOVA, Tukey's pairwise comparison, $p<0.00$, Figure 2). Similarly, an increasing trend and significantly high concentrations of Cr were recorded in the leaves of *B. alba* in the pots treated with compost and harvested during the second and third sampling events (ANOVA, Tukey's pairwise comparison, $p<0.00$, Table 2, Table 3, Table 4). Therefore, the results of this study indicate that there is a possibility of the bioaccumulation of Cd and Cr in the leaves of *B. alba* towards the end of the culture cycle.

Similar results were reported by Noor *et al* (2023) who found high concentrations of Cd and Cr in the edible parts of spinach (*Spinacia oleracea*) in rural areas of Pakistan. Another study conducted using a pot experiment, with contaminated and non-contaminated soils, to assess the accumulation of heavy metals and major nutrients in leaves of spinach, revealed high levels of Cr (Zakir *et al*., 2018).

Bioconcentration factor

The bioconcentration factors of *B. alba* harvested from pots applied with a Urea-MOP-TSP mixture and compost are given in Figure 1.

The bioconcentration factor of Cd for *B alba* in pots applied with the Urea-MOP-TSP mixture ranged from 1.4 to 2.5 and for compost from 1.2 to 2.2 (Figure 1). The bioconcentration factor of Cr for *B alba* in pots applied with the Urea-MOP-TSP mixture ranged from 0.7 to 1.7 and for compost from 0.75 to 1.5. (Figure 1). The mean bioconcentration factors for Cd and Cr in *B alba* from pots applied with the Urea-MOP-TSP mixture were not significantly different from those in the pots applied with compost (ANOVA, $p>0.05$, Figure 1). The bioconcentration factor is a measure of the uptake, mobilization, and efficiency of heavy metal accumulation in plant tissues. A bio-concentration factor higher than 1 indicates a potential for hyperaccumulation (Raj *et al*., 2020). In this study, it is evident from the results that *B.alba* is a potential hyperaccumulator for both Cd and Cr.

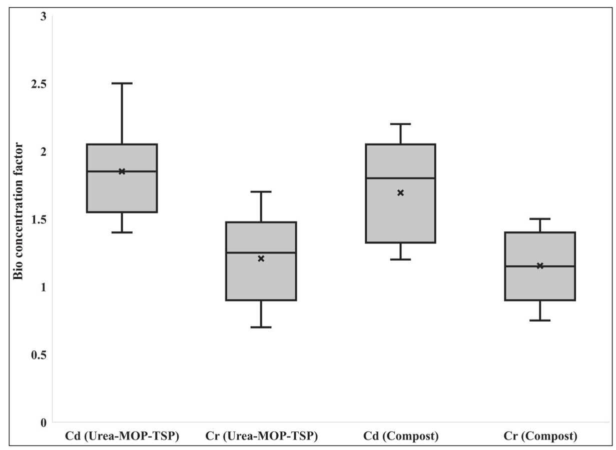

Figure 1: Bioconcentration factors for Cd and Cr of *B. alba* harvested at the end of the culture cycle from the pots applied with the Urea-MOP-TSP mixture and compost.

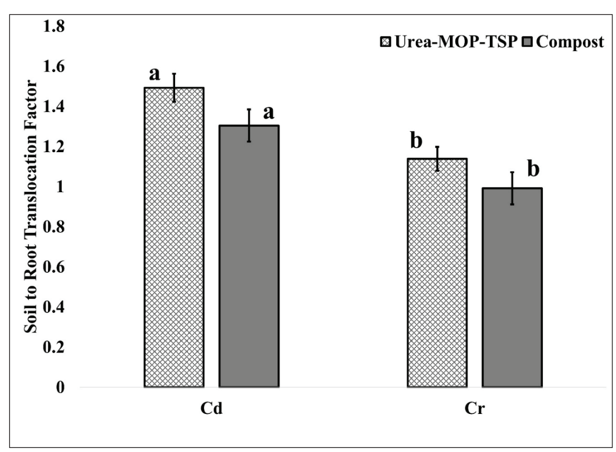

Figure 2: The soil to root translocation factors (TF_{S-R}) for Cd and Cr of *B. alba* harvested from pots applied with the Urea-MOP-TSP mixture and compost at the end of the culture cycle.

Translocation factor

The soil to root translocation factor (TF $_{S\text{-}R}$) and root to leaf translocation factors (TF $_{R\text{-}L}$) for Cd and Cr at the end of the culture cycle of *B. alba*, harvested from pots applied with the Urea-MOP-TSP mixture and compost are given in Figure 2 and Figure 3, respectively.

The TF $_{S\text{-}R}$ of Cd ranged from 1.1 to 1.6 and for Cr it ranged from 0.8 to 1.1 (Figure 2). The TF $_{R\text{-}L}$ for Cd ranged from 0.9 to 1.4 and for Cr from 0.9 to 1.3 (Figure 3).

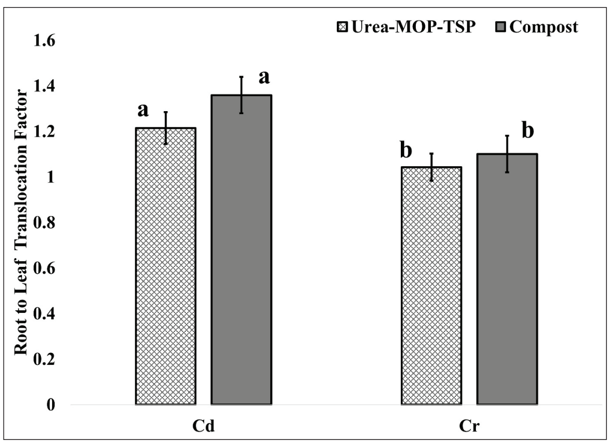

Figure 3: The root to soil translocation factors (TF $_{R\text{-}S}$) for Cadmium and Chromium of *B. alba* harvested from pots applied with the Urea-MOP-TSP mixture and at the end of the culture cycle.

Mean TF $_{S\text{-}R}$ and TF $_{R\text{-}L}$ for Cd and Cr of *B. alba* in the pots applied with Urea-MOP-TSP mixture and compost were not significantly different from each other (ANOVA, $p>0.05$, see Figure 2, Figure 3). The translocation factor is a measure of the movement of heavy metals from the soil to the roots and from roots to the leaves of a plant, and another factor that can be used to assess the hyperaccumulator ability of plants (Wijeyaratne & Kumari 2021). The TF $_{R\text{-}L}$ for both Cd and Cr is higher than that of the TF $_{S\text{-}R}$ in both the pots treated with Urea-MOP-TSP and compost (Figure 2, Figure 3). This is consistent with the conclusion of the BCF indicating that leaves of *B. alba* are hyperaccumulators of Cd and Cr irrespective of the use of chemical fertilizer or organic amendments.

It has been reported that Cr and Cd have a tendency to accumulate in the roots rather than in the shoots, which decreases the bioaccumulation potential of these heavy metals in plants (Chaab *et al*. 2016). However, the results of this study do not agree with Chaab *et al*. (2016) since the bioconcentration factor, the soil to root translocation factor, and the root to leaf translocation factor for Cr and Cd in *B. alba* were greater than 1. This indicates a bioaccumulation potential for these heavy metals in the edible parts of *B. alba*. Furthermore, the bioconcentration factor, the soil to root translocation factor, and the root to leaf translocation factor for Cd in *B. alba* were higher than those of Cr indicating a higher bioaccumulating capacity for Cd in the edible parts of *B. alba* compared to Cr. The decreased bioaccumulation of Cr in the leaves of *B alba* may be due to the ability of Cd to inhibit the translocation of Cr within the plant as seen in *Spinacea oleracea* L. (Çelik and Kunene 2021).

Additionally, studies on microbial interactions in the composting process have revealed that, inoculation of nitrogen retaining microbial agents into the compost helps to reduce the bioavailability of Cd and Cr (Guo *et al*., 2022; Wang *et al*. 2023). Therefore, further studies should focus on the identification and propagation of such natural assemblages of nitrogen retaining microbial communities in the commercial cultivation of *B. alba* to reduce the risks associated with the hyperaccumulation of these heavy metals in the edible parts of *B. alba*.

Assessing the health risks associated with consumption of *B. alba*

The daily metal intake values, health risk index and cancer index for adults and children based on concentrations of Cd and Cr in *B. alba* harvested from pots applied with urea, MOP, TSP and compost are given in Table 5.

The daily intake of Cr from *B. alba* by adults and children was significantly higher than that of Cd in both the urea, MOP, TSP mixture and compost treatments. However, the health risk index values for Cd in *B. alba* harvested from the Urea-MOP-TSP mixture treatment were significantly higher than that of the compost treated sites, for both adults and children, based on the recommended daily intake values by the FAO (Table 5). Further, the Cd based cancer risk index of *B. alba* harvested from the pots applied with Urea-MOP-TSP was significantly higher compared to that of the compost treatment for both adults and children. The Cr based cancer risk index for *B. alba* did not show significant variations among treatments for both adult and child populations. (Table 5).

Table 5: Daily metal intake (mg/day), health risk index and cancer risk index for adults and children based on the concentrations Cd and Cr in *B. alba*.

	Cd		Cr	
	Urea, MOP and TSP mixture	Compost	Urea, MOP and TSP mixture	Compost
Daily metal intake (Adult) (mg/day)	0.014 ± 0.0[a]	0.007 ± 0.0[c]	0.07 ± 0.0[b]	0.07 ± 0.0[b]
Daily metal intake (Children) (mg/day)	0.007 ± 0.0[a]	0.0004 ± 0.0[c]	0.004 ± 0.0[b]	0.0004 ± 0.0[b]
Health Risk Index (Adult)	1.4 ± 0.01[a]	0.7 ± 0.03[c]	0.05 ± 0.0[b]	0.05 ± 0.0[b]
Health Risk Index (Children)	0.7 ± 0.001[a]	0.04 ± 0.001[c]	0.003 ± 0.0[b]	0.003 ± 0.00[b]
Cancer Risk (Adult)	0.005 ± 0.001[a]	0.00003 ± 0.0[c]	0.0035 ± 0.0[b]	0.0035 ± 0.0[b]
Cancer risk (Children)	0.003 ± 0.001[a]	0.00001 ± 0.0[c]	0.002 ± 0.0[b]	0.002 ± 0.0[b]

Results are presented as Mean ± SD. The mean values with different superscript letters in each row are significantly different from each other (ANOVA, Tukey's pairwise comparison, $p < 0.05$; $n = 40$). MOP: muriate of potash; TSP: triple super phosphate.

Even though heavy metals bioaccumulate in the edible parts of green leafy vegetables, their toxic effects on consumers are controlled by the daily intake of metals. The maximum tolerable daily intake for Cd ranges between 0.02-0.07 mg/day, and for Cr between 0.035–0.2 mg/ day (Kamunda *et al*. 2016; Shaheen *et al*. 2016). According to this study, the daily intake values of Cd and Cr from consuming *B.alba* by both adults and children did not exceed the maximum tolerable daily intake values. The health risk index is also important for assessing the health risks in dietary items. A health risk index greater than 1 indicates the possibility of significant health risks for consumers (Wijeyaratne & Kumari 2021). In this study, for Cd, the health risk index for adults and children by consuming *B.alba* cultivated using a mixture of Urea-MoP-TSP and compost was 1.4 and 0.7, respectively (Table 5). Therefore. there is a potential for Cd- associated health impairments in adults consuming *B. alba* cultivated using the mixture Urea-MoP-TSP.

Further, the cancer risk index for Cd and Cr for *B.alba* for both treatments exceeded the acceptable upper limit of $1 \times 10^{-4,}$ (Gebreyohannes & Gebrekidan 2018) for the risk of developing cancer due to long-term consumption. Similar results were also recorded in studies conducted in Ethiopia (Gebreyohannes & Gebrekidan 2018) and Bangladesh (Sultana *et al*., 2019) on the health risks associated with the consumption of vegetables.

CONCLUSION

It is possible for *B. alba* leaves to hyperaccumulate Cd and Cr, irrrespective of the type of fertilizer applied. The long term dieteary intake of *B. alba* grown with chemical fertilizer is associated with a higher risk of health issues related to Cr and Cd. Therefore, reducing of chemical fertilizer use, adopting organic farming practices and introducing nitrogen-retaining microbial communities into compost are recommended to mitigate the health risks associated with the dieteary intake of *B.alba*.

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