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# Cadmium uptake potential of *Brassica napus* cocropped with *Brassica parachinensis* and *Zea mays*

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ABSTRACT

Cadmium uptake potential of *Brassica napus* cocropped with *B. parachinensis* or *Zea mays* plants in split pot (allow the solutes to pass but prevent the interaction of roots between compartments) experiments was evaluated. Plants were grown in split pots filled with soil spiked at 0, 3, 6, 12, 25 and 50 mg Cd/kg soil. Biomass and Cd uptake were detemined after 6 weeks, and rhizospheric soil solutions, extracted using soil probes, were analyzed for pH and water soluble Cd at weekly intervals. Cadmium treatments affected the biomass. Cadmium concentration in the shoots of *B. napus* was higher when cocropped with *B. parachinensis* and significantly higher with *Z. mays*; however, the biomass was negatively affected implying the higher nutrient apportionment to the crop plants than *B. napus*. Concentration of Cd in *B. napus* was higher in shoots than in roots as revealed by shoot/root Cd quotient and was always >1; the quotient for *B. parachinensis* was ~1 and that of *Z. mays* was <1, indicating the potential of Brassicaceae members to translocate the Cd to aboveground tissue. Results indicate the feasibility of cocropping method to clean the Cd contaminated soils.



## 1. Introduction

Mining, manufacturing and the use of synthetic products, and land application of industrial or domestic sludge can result in cadmium (Cd) contamination of urban and agricultural soils [1]. In China, the average content of Cd in soil is 0.097 mg/kg and in soils of a wastewater irrigation zone, the content of Cd even reached 3.16 mg/kg [2]. Further, reports suggest that more than 10,000 ha of arable lands in China are contaminated with Cd [3,4]. Remediation of these agricultural fields is essential to prevent the movement of Cd through the food chain to human. Conventional soil and crop management methods such as increasing the soil pH, draining wet soils and applying phosphate can help prevent the uptake of heavy metals by plants, leaving them in the soil and the soil becomes the sink of these toxic metals in due course of time. Phytoextraction using hyperaccumulator plants has been proposed as a promising, environmental friendly, low-cost technology for decreasing the heavy-metal contents of contaminated soils and has emerged as an alternative to the engineering-based methods [5,6].

Metal hyperaccumulator plants can grow in soils containing high concentration of metals and can accumulate heavy metals at high concentrations in their shoots [5]. For a Cd hyperaccumulator, the threshold foliar concentration of Cd has been defined in the literature as 0.01% [7]. Unfortunately, most hyperaccumulators are poor yielding, slow growing and rare. For these reasons, research is focusing on heavy metal tolerant, high-biomass and fast-growing plants. Many cultivated *Brassica* species are potentially useful candidates for phytoremediation [8,9]. Earlier reports suggests that *Brassica napus* can be a useful candidate for phytoextraction of Cd due to its high above ground biomass, faster growth and high Cd uptake [10–13].

Stopping the regular crop and entering into the phytoremediation program would affect the economy and will not be welcomed by the farmers. In that case, the planting of a hyperaccumulator along with the regular crop (cocropping) will be an alternate option. Earlier, it has been shown that cocropping a hyperaccumulating *Thlaspi caerulescens* effectively depleted the plant available Zn from the soil and increased the growth and decreased the Zn uptake of a Zn-sensitive *Thlaspi arvense* [14]. It is interesting to note that such an enhancement in biomass was not observed when their roots were not allowed to mingle. This indicates that the changes in rhizosphere of hyperaccumulator plant facilitated the growth of sensitive species. The obvious change might be the depletion of available Zn by the hyperaccumulator and making them unavailable to the sensitive plant. The efficient removal of bioavailable and phytotoxic metals from soil solution by a hyperaccumulator might aid the establishment of other co-planted less tolerant species. This might enhance the efficiency and revegetation of contaminated soils with less tolerant species, referred by Whiting et al. [14] as 'phytoprotection'.

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**Table 1**
Selected physico-chemical properties of soil used in the study.

| Parameter | Value |
|---|---|
| pH | 5.201 ± 0.043[a] |
| Total organic C (%) | 0.643 ± 0.021 |
| Total N (%) | 0.009 ± 0.001 |
| Total P (%) | 0.037 ± 0.004 |
| Cd (mg/kg) | 0.343 ± 0.004 |
| Cu (mg/kg) | 3.392 ± 0.140 |
| Zn (mg/kg) | 4.778 ± 0.116 |
| Ni (mg/kg) | 0.546 ± 0.063 |

[a] mean ± S.E. ($n$ = 3).

Understanding the possible interactions between the cocropped plants will improve the application of this technique to major agricultural crops. Hence it is essential to characterize the cocropping system from the perspective of phytoextraction of metals. Since reports on cocropping are very scarce [14–17], information from monoculture experiments can be applied and tested in a cocropping system. The availability of heavy metals to plants and, thus their toxicity depends on complex rhizospheric reactions involving not only exchange processes between soil and plants but also microbial activities. Hence the processes occur in the rhizosphere of the plants, especially in a cocropping system with a hyperaccumulator and a crop plant, deserve to be elucidated.

In the present study, the cocropping of the Cd-hyperaccumulator *Brassica napus* (rapeseed plant) [18] with *Brassica parachinensis* (false pak choi) or *Zea mays* (maize), was investigated. It is designed to test whether the cocropping of a hyperaccumulator with a crop plant increases the uptake of Cd in the hyperaccumulator plant. Cocropped crop plants were selected based on the commercial value. *Brassica parachinensis*, also belong to the crucifer family, is one of the important leafy vegetables in the South China. *Zea mays* is one of the most important agricultural crops worldwide and it is also a very interesting species due to its potential usefulness in phytoremediation of the areas contaminated with heavy metals, especially in one of the phytoremediation technologies—induced hyperaccumulation [19].

## 2. Materials and methods

### 2.1. Soil

A fine loamy soil from the Experimental Farm of Agriculture, Fisheries and Conservation Department was sampled to a depth of 15 cm, air dried and sieved to <2 mm using a stainless steel sieve. Selected soil characteristics are presented in Table 1. The soil was spiked with $Cd(NO_3)_2.4H_2O$ solution to obtain 3, 6, 12, 25 and 50 mg/kg levels of Cd and incubated at approximately 60% water-holding capacity (WHC) for 1 week until potting. After incubation, soils were filled in 14 cm × 12.5 cm × 12.5 cm size pots made of Pyrex glass. The pots were divided into two parts using 35 μm nylon mesh to prevent the roots moving to the adjacent section. Soil solution probes were inserted into pots during soil filling. The spacing between plants and soil solution extraction probes are illustrated in Fig. 1.

### 2.2. Plant materials and growth conditions

Two cocropping systems, BN–BP (*B. napus* and *B. parachinensis*) and BN–ZM (*B. napus* and *Z. mays*) were established. In each system, plants were grown in the following treatments: control soil, 3, 6, 12, 25 and 50 mg Cd/kg soil. To evaluate the potential of cocropping and for comparison two additional treatments with 6 mg Cd/kg soil were setup. The first one was a monocropping control, with both sides of a divided pot being sown with *B. napus* (hereafter mentioned as monocropping system). The second one was the cocropping system, in which *B. napus* and *B. Parachinensis*/*Z. mays* were gorwn in a pot without compartmentation, i.e., without any nylon barrier so as to allow the root interaction. For each treatment, five seeds each of *B. napus* and *B. parachinensis* or *Z. mays* were sown in each pot and thinned to one plant after 1 week (Fig. 1). Pots, three replicates each for a treatment, were placed on greenhouse bench top in a randomized block design with a temperature range of 25–35 °C. The water content of the soil was maintained at an average of 60% WHC by watering to weigh daily with deionised water. Nutrients were provided to plants after 14 and 28 days of planting as described by Wong et al. [20].

Soil solution was extracted from the soil at weekly intervals by applying a gentle suction for 16 h using an acid-washed plastic syringe attached to the probes. Six weeks after sowing, soil and plant samples were collected. The plants were rinsed in deionised water, separated into root and shoot and oven dried at 80 °C. The dry weights were recorded and the plants were ground in a mechanical pulverizer and analyzed for Cd. Soil samples were dried at 105 °C and analyzed for pH and DTPA extractable Cd.

### 2.3. Chemical analyses

The pH of the soil was measured in 1:10 water extracts. Total organic content of the soil was determined by Walkey–Black method. The total N and P contents of the soil were extracted by a Kjeldhal digestion method and analyzed using Indophenol Blue and Molybdenum Blue methods, respectively [21]. For bioavailable Cd, soils were extracted with 1:5 (sample:extractant, w/v) diethylene triaminepentaacetic acid–triethanolamine (DTPA–TEA) [22], shaken at 200 rpm for 2 h and centrifuged at 8000 × $g$ for 5 min. After filtration, the supernatants were stored in polyethylene bottles until analysis. For total Cd analysis in plant materials, and Cd, Cu, Ni and Zn in soils, samples were subjected to mixed acid digestion (conc. $HNO_3$ and conc. $HClO_4$) and analyzed using atomic absorption spectrophotometer (Varian Techtron Model AA-10) and graphite furnace atomic absorption spectrophotometer (GFAAS) with deuterium background correction. Certified reference soil or orchard leaves were included in each batch for quality control. The pH of the soil solution extracted using soil probes were measured immediately and the solutions were stored at 4 °C until Cd analysis. The Cd concentrations in the soil solutions were determined using GFAAS.

### 2.4. Statistical analyses

Analyses were performed in triplicate samples and the mean values with standard error were presented. The data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS, ver.11.5 software.

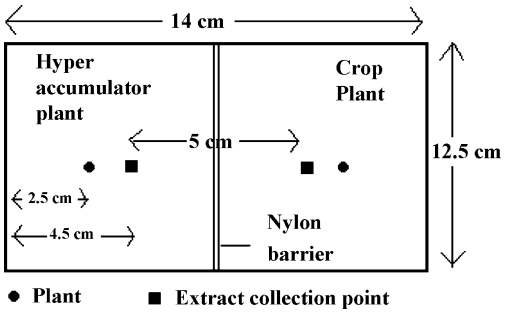


**Fig. 1.** Design of the pots and spacing between plants. Hyperaccumulator plant is *B. napus* and the crop plant is either *B. parachinensis* or *Z. mays*.

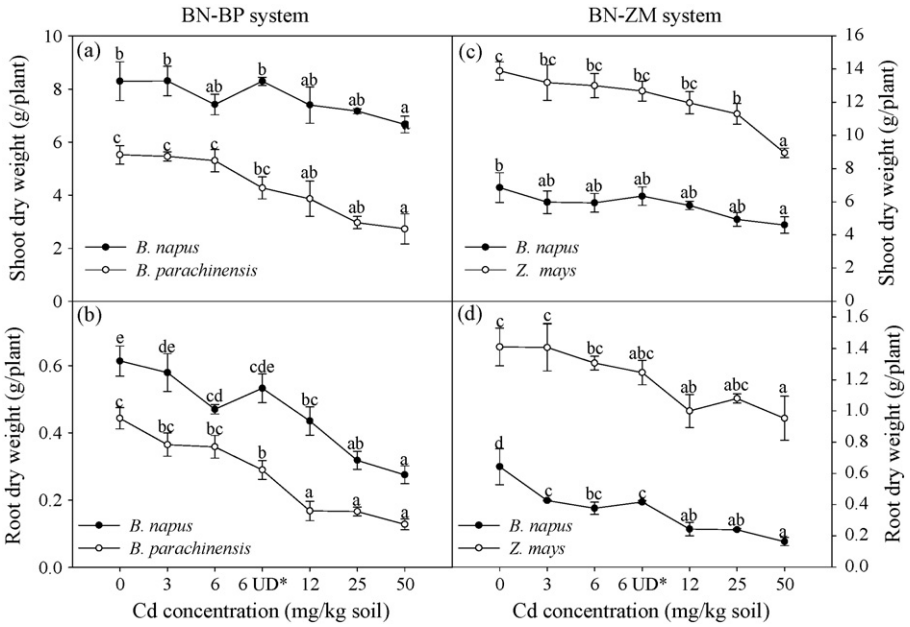


**Fig. 2.** Shoot and root dry weights of cocropped plants: (a) shoot dry weight; (b) root dry weight of BN–BP (*B. napus–B. parachinensis*) cocropping system; (c) shoot dry weight; (d) root dry weight of BN–ZM (*B. napus–Z. mays*) cocropping system. UD* pots were not divided with nylon barrier. Means sharing the common lower case alphabets within a group are not significant at 0.05% level according to DMRT. Error bars are standard errors ($n = 3$).

## 3. Results

### 3.1. Plant biomass

Visible symptoms of Cd toxicity were not evident in all the experimental plants even at 50 mg Cd/kg soil level. In *B. napus–B. parachinensis* (BN–BP) cocropping system, *B. napus* plants showed higher shoot and dry weight than *B. parachinensis* plants (Fig. 2). The shoot and root biomass decreased with an increase in Cd concentrations. The differences in shoot dry weight of *B. napus* plants were not statistically significant at 0.05% level, except at 50 mg Cd/kg soil. But in *B. parachinensis* plants, the reduction in shoot dry weight was significant beyond 12 mg Cd/kg soil treatment (Fig. 2a). For both plant species, significant ($p < 0.05$) reduction in root dry weight was observed (Fig. 2b). In *B. napus–Z. mays* (BN–ZM) cocropping system, higher shoot and root dry weights were recorded for *Z. mays* plants than *B. napus* plants. Reduction in shoot dry weight was significant ($p < 0.05$) only at 50 mg Cd/kg soil treatment for *B. napus* and at 25 and 50 mg Cd/kg soil level for *Z. mays* (Fig. 2c) when compared to controls. However, the root dry weight decreased significantly in *B. napus* plants (Fig. 2d). In both the plant systems, when the plant roots were allowed to mingle at 6 mg Cd/kg soil, although statistically not significant, the dry weight of *B. napus* plants was higher than the pots with nylon divider in the same concentration. But in *B. parachinensis* and *Z. mays* plants, a marginal decrease was noticed. Shoot and root dry weights of *B. napus* plants were lower in BN–ZM system when compared with BN–BP cocropping system.

### 3.2. Effect of plant growth on the soil solution pH and Cd

Soil solution was extracted from the rhizospheric soil of plants using soil probes at weekly intervals and analyzed for pH and Cd concentration. Generally, the pH continues to increase up to 5 weeks of plant growth and then stabilized, and ranged between 6.1 and 6.8 in BN–BP system and between 6.2 and 6.9 in BN–ZM

**Table 2**
pH of soil solution extracted from the rhizosphere of *B. napus–B. parachinensis* cocropping system.

| Soil Cd concentration (mg/kg soil) | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|
| pH of the soil solution extracted from *Brassica napus* grown soil | | | | | | |
| 0 | 5.27 ± 0.06 aA[b] | 5.44 ± 0.18 aA | 5.57 ± 0.27 aAB | 6.01 ± 0.11 abBC | 6.32 ± 0.04 aC | 6.42 ± 0.05 aC |
| 3 | 5.34 ± 0.05 aA | 5.58 ± 0.10 aAB | 5.80 ± 0.05 abB | 6.45 ± 0.13 cC | 6.61 ± 0.08 abC | 6.51 ± 0.16 aC |
| 6 | 5.40 ± 0.01 aA | 5.63 ± 0.08 aAB | 5.65 ± 0.03 aAB | 6.00 ± 0.17 abB | 6.68 ± 0.09 abC | 6.55 ± 0.19 aC |
| 6 UD[a] | 5.75 ± 0.25 bA | 6.08 ± 0.24 bA | 6.04 ± 0.18 abA | 6.25 ± 0.02 bcAB | 6.85 ± 0.07 bC | 6.77 ± 0.13 aBC |
| 12 | 5.21 ± 0.05 aA | 5.56 ± 0.01 aAB | 5.62 ± 0.05 aAB | 5.87 ± 0.04 aB | 6.40 ± 0.28 aC | 6.55 ± 0.16 aC |
| 25 | 5.99 ± 0.07 bA | 6.05 ± 0.09 bA | 5.98 ± 0.12 abA | 6.35 ± 0.03 bcB | 6.51 ± 0.09 abB | 6.53 ± 0.11 aB |
| 50 | 5.99 ± 0.02 bA | 6.38 ± 0.07 bA | 6.12 ± 0.24 bA | 6.23 ± 0.10 bcA | 6.36 ± 0.09 aA | 6.09 ± 0.47 aA |
| pH of the soil solution extracted from *Brassica parachinensis* grown soil | | | | | | |
| 0 | 5.48 ± 0.08 abA | 5.63 ± 0.18 aA | 5.62 ± 0.11 aA | 6.35 ± 0.18 bB | 6.47 ± 0.05 aB | 6.35 ± 0.05 aB |
| 3 | 5.54 ± 0.18 bA | 5.60 ± 0.11 aA | 5.70 ± 0.12 aA | 6.17 ± 0.11 abB | 6.61 ± 0.05 aC | 6.56 ± 0.10 abC |
| 6 | 5.38 ± 0.01 abA | 5.68 ± 0.05 aA | 5.72 ± 0.03 aA | 6.11 ± 0.29 abB | 6.78 ± 0.03 aC | 6.79 ± 0.02 bC |
| 6 UD[a] | 5.39 ± 0.09 abA | 5.91 ± 0.08 abB | 6.00 ± 0.05 abB | 6.12 ± 0.10 abB | 6.79 ± 0.05 aC | 6.77 ± 0.08 bC |
| 12 | 5.22 ± 0.03 aA | 5.60 ± 0.04 aB | 5.60 ± 0.08 aB | 5.77 ± 0.05 aB | 6.48 ± 0.12 aC | 6.59 ± 0.09 abC |
| 25 | 5.94 ± 0.06 cAB | 6.29 ± 0.20 bABC | 5.85 ± 0.08 abA | 6.29 ± 0.12 abABC | 6.57 ± 0.25 aC | 6.41 ± 0.04 aBC |
| 50 | 6.08 ± 0.01 cA | 6.22 ± 0.15 bA | 6.30 ± 0.33 bA | 6.38 ± 0.15 aA | 6.53 ± 0.22 aA | 6.59 ± 0.22 abA |

[a] UD—pots were not divided by nylon barrier.

[b] Mean ± standard error ($n = 3$). Means sharing the common lowercase alphabets within a column for a plant and means sharing the common uppercase alphabets within a row are not significant at 0.05% level according to DMRT.

**Table 3**
pH of soil solution extracted from the rhizosphere of *B. napus–Z. mays* cocropping system.

| Soil Cd concentration (mg/kg soil) | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|
| pH of the soil solution extracted from *Brassica napus* grown soil | | | | | | |
| 0 | 5.19 ± 0.09 aA[b] | 5.79 ± 0.08 aC | 5.52 ± 0.04 aB | 5.58 ± 0.07 aBC | 6.52 ± 0.11 abD | 6.66 ± 0.05 bcdD |
| 3 | 5.38 ± 0.07 aA | 5.82 ± 0.13 aB | 5.69 ± 0.07 abB | 5.79 ± 0.06 abB | 6.54 ± 0.17 abC | 6.83 ± 0.04 dC |
| 6 | 5.42 ± 0.05 abA | 5.83 ± 0.10 aB | 5.98 ± 0.07 bB | 5.89 ± 0.25 abB | 6.69 ± 0.08 abC | 6.77 ± 0.07 cdC |
| 6 UD[a] | 5.68 ± 0.17 bA | 5.76 ± 0.16 aA | 5.72 ± 0.23 abA | 5.58 ± 0.26 aA | 6.78 ± 0.03 bB | 6.80 ± 0.09 dB |
| 12 | 5.26 ± 0.01 aA | 5.73 ± 0.01 aB | 5.68 ± 0.05 abB | 5.83 ± 0.03 abB | 6.69 ± 0.20 abC | 6.90 ± 0.09 dC |
| 25 | 5.99 ± 0.11 cA | 6.04 ± 0.21 aA | 6.01 ± 0.08 bA | 6.20 ± 0.07 bcA | 6.35 ± 0.14 aA | 6.16 ± 0.14 aA |
| 50 | 6.14 ± 0.04 cA | 6.43 ± 0.04 bB | 6.47 ± 0.12 cB | 6.39 ± 0.08 cAB | 6.42 ± 0.05 abAB | 6.35 ± 0.12 abcAB |
| pH of the soil solution extracted from *Zea mays* grown soil | | | | | | |
| 0 | 5.16 ± 0.08 aA | 5.59 ± 0.12 aAB | 5.58 ± 0.14 aAB | 5.97 ± 0.28 abB | 6.72 ± 0.06 aC | 6.64 ± 0.05 bcC |
| 3 | 5.43 ± 0.14 abA | 5.79 ± 0.34 aAB | 5.78 ± 0.40 aAB | 5.94 ± 0.40 abABC | 6.73 ± 0.20 aBC | 6.82 ± 0.13 cC |
| 6 | 5.40 ± 0.06 abA | 5.80 ± 0.14 aAB | 5.90 ± 0.20 abB | 6.08 ± 0.21 abB | 6.76 ± 0.05 aC | 6.83 ± 0.10 cC |
| 6 UD* | 5.53 ± 0.09 bA | 5.76 ± 0.20 aA | 5.50 ± 0.21 aA | 5.42 ± 0.21 aA | 6.60 ± 0.03 aB | 6.36 ± 0.01 abB |
| 12 | 5.28 ± 0.03 abA | 5.55 ± 0.04 aA | 5.57 ± 0.15 aA | 5.76 ± 0.22 abA | 6.78 ± 0.24 aB | 6.70 ± 0.16 cB |
| 25 | 5.92 ± 0.18 cA | 6.21 ± 0.40 abA | 5.93 ± 0.13 abA | 6.16 ± 0.05 abA | 6.32 ± 0.05 aA | 6.16 ± 0.05 aA |
| 50 | 6.20 ± 0.12 cA | 6.61 ± 0.07 bB | 6.51 ± 0.08 bAB | 6.50 ± 0.13 bAB | 6.54 ± 0.16 aAB | 6.32 ± 0.10 aAB |

[a] UD—pots were not divided by nylon barrier.
[b] Mean ± standard error ($n = 3$). Means sharing the common lowercase alphabets within a column for a plant and means sharing the common uppercase alphabets within a row are not significant at 0.05% level according to DMRT.

cocropping system (Tables 2 and 3). The increases in pH were progressive and significant at concentrations below 25 mg $kg^{-1}$ soil. In higher concentrations, the pH markedly increased in the 1st week and increased slowly up to 6 weeks. The pH of the Cd amended soils, especially at 50 mg Cd/kg soil, were significantly ($p < 0.05\%$) higher than the control plants up to 3 weeks of plant growth. Another interesting observation is that in BN–BP cocropping system, although statistically not significant, the pH of soils from undivided pots at 6 mg Cd/kg soil were higher than the pH of the soils from pots divided with nylon barrier. This tendency extended till the end of the experiment (6 weeks) in *B. napus* plant rhizosphere. However, from *B. parachinensis* rhizospheric soil, the difference was not evident after 3 weeks (Table 2). In contrast to BN–BP system, in BN–ZM system, the rhizospheric pH was higher in nylon divided pots than the undivided pots.

Cadmium in soil solution increased significantly with increasing Cd amendment (Tables 4 and 5). However, after 4 weeks, the differences in solution Cd are significant only at high concentrations. In both the cocropping systems, water soluble Cd increased up to 4 weeks and decreased thereafter; a sharp decrease observed in treatments with >12 mg/kg soil especially from the solution collected from *Z. mays* plants. At 50 mg Cd/kg soil level, the solution collected from *B. napus* plants contained more Cd than *B. parachinensis* or *Z. mays*.

### 3.3. Cadmium uptake in plant tissue

After 6 weeks of growth, the Cd concentration in shoots and roots were analyzed and presented in Fig. 3. Since the biomass of the tested plants were different, the actual Cd uptake/plant is pre-

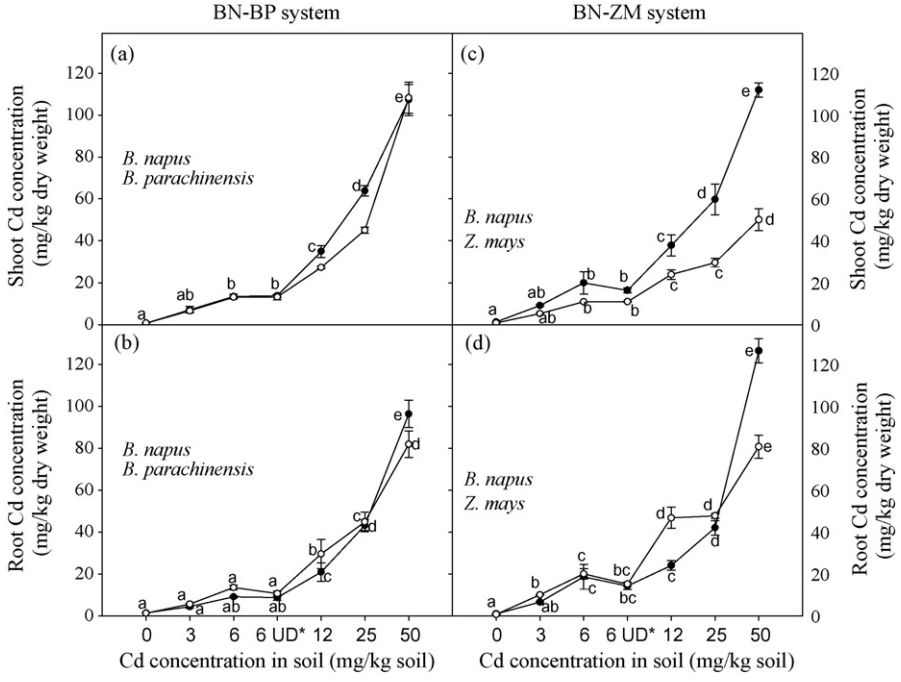


**Fig. 3.** Cadmium concentration in the plant tissue of cocropped plants grown for 6 weeks in different concentrations of soil Cd: (a) Cd concentration in shoot; (b) Cd concentration in root of BN–BP (*B. napus–B. parachinensis*) cocropping system; (c) Cd concentration in shoot; (d) Cd concentration in root of BN–ZM (*B. napus–Z. mays*) cocropping system; UD* pots were not divided with nylon barrier. Means sharing the common lower case alphabets within a group are not significant at 0.05% level according to DMRT. Error bars are standard errors ($n = 3$).

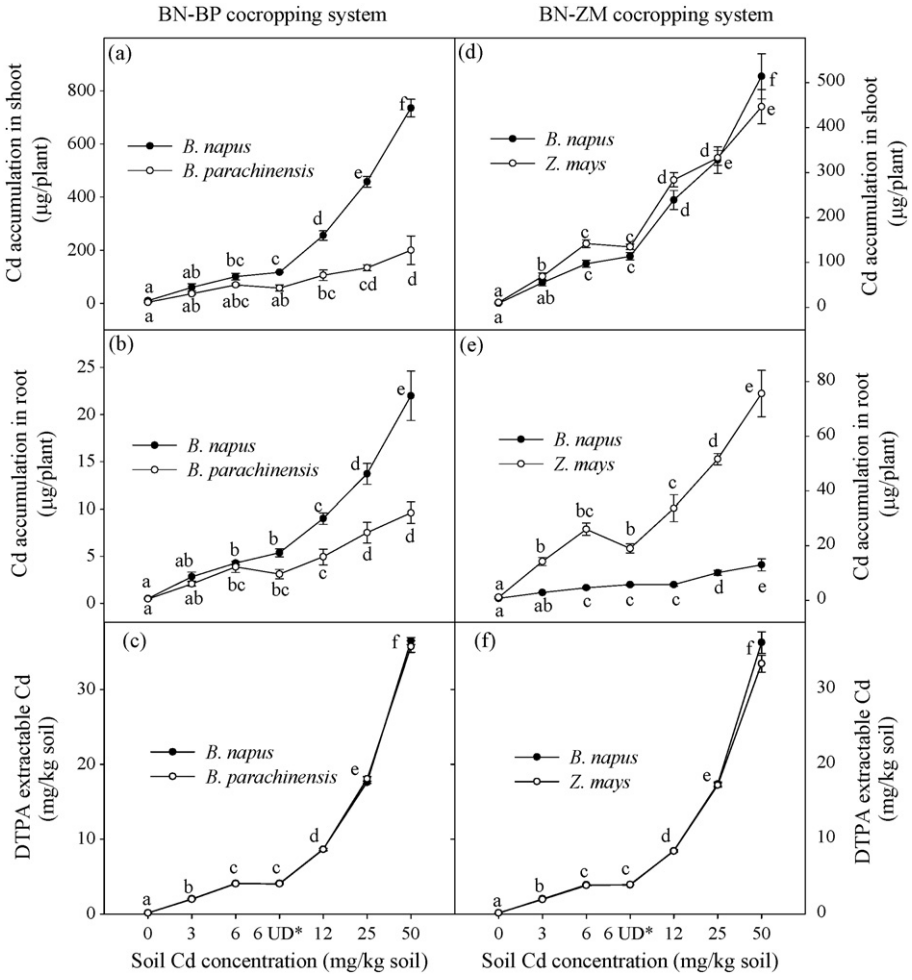


**Fig. 4.** Cadmium accumulated in the plant tissue and DTPA extractable Cd in the soils of cocropped plants: (a) Cd accumulation in shoot; (b) Cd accumulation in root; (c) DTPA extractable Cd contents in soils after 6 weeks of BN–BP (*B. napus–B. parachinensis*) cocropping system; (d) Cd accumulation in shoot; (e) Cd accumulation in root; (f) DTPA extractable Cd contents in soils after 6 weeks of BN–ZM (*B. napus–Z. mays*) cocropping system; UD* pots were not divided with nylon barrier. Means sharing the common lower case alphabets within a group are not significant at 0.05% level according to DMRT. Error bars are standard errors ($n = 3$). (c and f) Level of significance is same for both the plants.

sented in Fig. 4. In both the cocropping systems, Cd accumulation increased significantly with increasing soil Cd. In BN–BP system, Cd accumulation was higher in *B. napus* plants than *B. parachinensis*; and the differences were obvious and significant after 12 mg/kg soil Cd level. Cd concentrations and contents were higher in shoots than roots. Above 12 mg Cd/kg soil treatment, the Cd concentration was high in *B. napus* than *B. parachinensis* plant (Fig. 3), which resulted in significant difference in Cd content/plant between these two plants (Fig. 4a and b) as the biomass of the *B. napus* was higher than *B. parachinensis*. Similar trend was observed both shoots and roots. Cd contents were almost similar when the roots of *B. napus* and *B. parachinensis* plants were allowed to mingle at 6 mg Cd/kg soil.

In BN–ZM cocropping system, Cd concentrations in *B. napus* shoots were significantly higher than *Z. mays* (Fig. 3). However, Cd content/plant was almost similar in shoot between *B. napus* and *Z. mays* due to the higher biomass of *Z. mays* (Fig. 4d and e). The Cd concentrations in the roots of *B. napus* was significantly higher than the *Z. mays* plants only at 50 mg Cd/kg soil level. However, as the root biomass of the *Z. mays* was 3–5-fold higher than the *B. napus*, the Cd content/plant in the roots was significantly higher in *Z. mays*. In *Z. mays* plants, shoot Cd concentration was lower than root Cd concentration may be due to the higher shoot biomass. When the roots are allowed to mingle between cocropped plants, at 6 mg Cd/kg soil level, shoot and root Cd concentrations of *B. napus* plants ($18.03 \pm 0.90$ mg/kg DW and $13.67 \pm 0.58$ mg/kg DW, respectively) were slightly higher than plants grown with nylon barrier between them($16.60 \pm 1.96$ mg/kg DW and $12.39 \pm 0.56$ mg/kg DW, respectively), however, statistically not significant.

### 3.4. DTPA extractable Cd in soil

After 6 weeks of plant growth, the plants were harvested and the soil was analyzed for the DTPA extractable Cd. DTPA extractable Cd in soils of different treatments was presented in Fig. 4c and f. In both the systems and plants, the residual DTPA extractable Cd was same and was accounted for about 65–70% of the spiked Cd. The percentage of residual DTPA extractable Cd increased with increasing Cd concentration in the soil.

### 3.5. Accumulation factor and shoot/root Cd quotient

Accumulation factor (mean shoot Cd concentration/mean soil Cd concentration) was higher at 12 mg Cd/kg soil treatment, when compared with other Cd treatment levels (Fig. 5). In BN–BP system, the differences are significant at 50 mg Cd/kg treatment when compared to the control (Fig. 5a). However, in BN–ZM system, higher Cd treatments (25 and 50 mg) showed significant differences when compared with other Cd treatments (Fig. 5c). In both the plant systems, accumulation factor slowly increased up to 12 mg Cd/kg

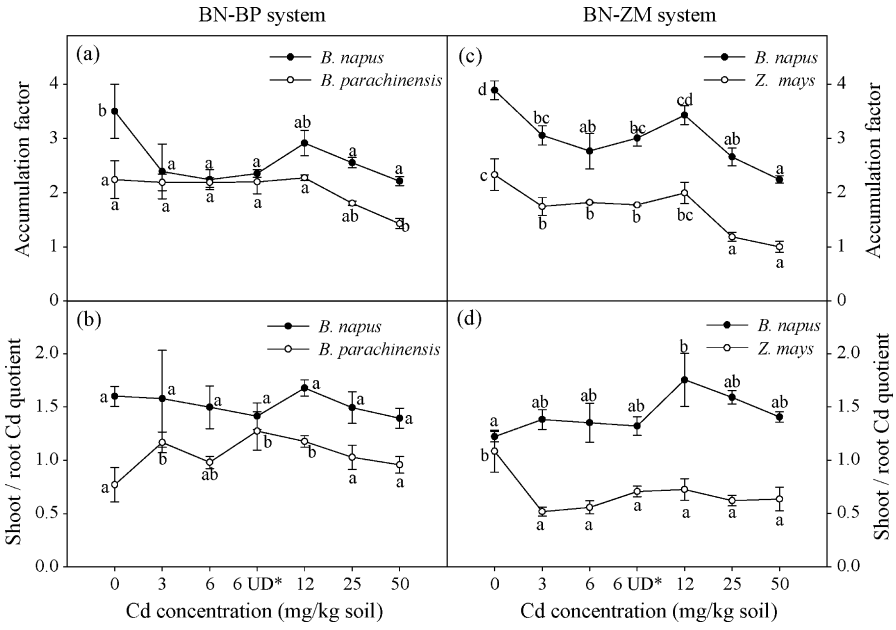


**Fig. 5.** Accumulation factor (shoot Cd concentration/soil Cd concentration) and shoot/root Cd quotient of cocropped plants. BN–BP, *B. napus–B. parachinensis* cocropping system; BN–ZM, *B. napus–Z. mays* cocropping system; UD* pots were not divided with nylon barrier: (a) accumulation factor; (b) shoot/root Cd quotient of BN–BP cocropping system; (c) accumulation factor; (d) shoot/root Cd quotient of BN–ZM cocropping system. Means sharing the common lower case alphabets within a group are not significant at 0.05% level according to DMRT. Error bars are standard errors ($n = 3$).

soil level and gradually decreased thereafter. In all the cases, the accumulation factor was ≥1. In both the cocropping system, the accumulation factor of *B. napus* plants was >2 and higher than the *B. parachinensis* or *Z. mays* plants. The order of accumulation factor was *B. napus* > *B. parachinensis* > *Z. mays*. The accumulation factor for the control soil was higher when compared with Cd treatments. When the roots of cocropped plants allowed to interact at 6 mg Cd/kg soil, the accumulation factor was higher; however, is not statistically significant.

Shoot/root (S/R) Cd quotient was also slowly increasing up to 12 mg Cd/kg soil treatment and decreased thereafter in *B. napus* plants of both cocropping system (Fig. 5b and c). However, the trend was not clear as observed in accumulation factor. In all the treatments, S/R Cd quotient was higher for *B. napus* than the other cocropped plants. The order of S/R Cd quotient for the tested plants was *B. napus* > *B. parachinensis* > *Z. mays*. Both *Brassica* species have S/R Cd quotient of >1 and the quotient for *Z. mays* ranged between 0.62 and 0.73 for Cd treatments. However, for control *Z. mays* plants S/R Cd quotient was 1.08 and significantly higher than Cd treatments.

## 4. Discussion

Earlier reports suggest that the *B. napus* can be useful as a Cd hyperaccumulator [10–12]. In our study also, Cd accumulation of more than 100 mg/kg dry weight indicates the potential of this species in Cd phytoextraction. Generally, Cd in plants causes chlorosis and reduces both shoot and root growth [1] by affecting the photosynthetic apparatus [23] and water balance [24,25]. Larsson et al. [26] reported that the Cd affected chlorophyll and carotenoid contents, and increased the non-photochemical quenching in *B. napus*. To evaluate the potential of cocropping, a monocrop, i.e., pots with *B. napus* on both sides of a divided pot with 6 mg Cd/kg soil was conducted and the results are compared with BN–BP and BN–ZM

**Table 4**
Cadmium concentration of soil solution extracted from the rhizosphere of *B. napus–B. parachinensis* cocropping system.

| Soil Cd concentration (mg/kg soil) | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|
| Cd content in the soil solution extracted from *Brassica napus* grown soil (μg/L) | | | | | | |
| 0 | 3.6 ± 0.3 aD[b] | 2.4 ± 0.7 aC | 1.8 ± 0.5 aBC | 2.6 ± 0.2 aCD | 0.3 ± 0.1 aA | 1.1 ± 0.1 aAB |
| 3 | 33.3 ± 1.5 bD | 25.3 ± 2.4 bC | 26.0 ± 1.5 abC | 14.9 ± 1.3 abB | 2.4 ± 0.5 aA | 2.8 ± 0.4 aA |
| 6 | 51.7 ± 3.4 cC | 44.0 ± 4.0 cBC | 38.7 ± 2.7 bB | 46.5 ± 2.2 cBC | 3.4 ± 0.6 aA | 2.8 ± 0.2 aA |
| 6 UD[a] | 51.7 ± 2.2 cD | 38.7 ± 0.3 bcC | 31.0 ± 1.5 bB | 37.4 ± 4.8 bcBC | 4.8 ± 0.7 aA | 3.7 ± 0.3 aA |
| 12 | 70.7 ± 1.8 dB | 121.3 ± 6.9 dC | 68.7 ± 7.7 cB | 139.5 ± 8.1 dD | 38.0 ± 4.0 bA | 27.0 ± 4.1 bA |
| 25 | 119.3 ± 7.5 eB | 134.7 ± 4.8 dB | 166.0 ± 10.8 dC | 160.0 ± 7.0 dC | 52.0 ± 6.2 bA | 58.0 ± 4.5 cA |
| 50 | 171.3 ± 7.7 fB | 190.0 ± 10.3eB | 361.3 ± 18.7 eC | 326.3 ± 20.7 eC | 143.0 ± 14.7 cAB | 110.3 ± 13.6 dA |
| Cd content in the soil solution extracted from *Brassica parachinensis* grown soil (μg/L) | | | | | | |
| 0 | 2.5 ± 0.2 aB | 2.3 ± 0.4 aB | 2.3 ± 0.4 aB | 0.6 ± 0.1 aA | 0.3 ± 0.03 aA | 0.9 ± 0.1 aA |
| 3 | 37.0 ± 1.5 bD | 23.7 ± 1.2 bC | 25.3 ± 1.3 abC | 19.3 ± 0.9 bB | 1.8 ± 0.2 aA | 1.9 ± 0.2 aA |
| 6 | 62.0 ± 2.5 cD | 48.7 ± 4.7 cC | 36.0 ± 0.6 bB | 53.2 ± 5.1 cCD | 2.9 ± 0.2 aA | 1.7 ± 0.1 aA |
| 6 UD | 45.3 ± 1.8 bcC | 36.3 ± 3.3 bcBC | 32.7 ± 0.7 abB | 42.1 ± 7.5 cBC | 2.8 ± 0.4 aA | 2.8 ± 0.6 aA |
| 12 | 83.3 ± 8.1 dB | 117.0 ± 7.4 dC | 73.7 ± 7.0 cB | 101.3 ± 3.5 dC | 18.3 ± 2.9 bA | 10.0 ± 1.7 aA |
| 25 | 125.7 ± 12.2 eB | 149.7 ± 10.2 eBC | 173.7 ± 9.2 dC | 164.7 ± 3.2 eC | 44.3 ± 4.9 cA | 52.0 ± 4.6 bA |
| 50 | 146.3 ± 7.9 fB | 144.0 ± 11.3 fB | 250.7 ± 24.5 eC | 226.7 ± 10.1 fC | 112.3 ± 7.8 dAB | 88.0 ± 8.7 cA |

[a] UD—pots were not divided by nylon barrier.

[b] Mean ± standard error ($n = 3$). Means sharing the common lowercase alphabets within a column for a plant and means sharing the common uppercase alphabets within a row are not significant at 0.05% level according to DMRT.

**Table 5**
Cadmium concentration of soil solution extracted from the rhizosphere of *B. napus–Z. mays* cocropping system.

| Soil Cd concentration (mg/kg soil) | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|
| Cd content in the soil solution extracted from *Brassica napus* grown soil (μg/L) | | | | | | |
| 0 | 1.3 ± 0.1 aB[b] | 1.8 ± 0.1 aC | 1.9 ± 0.2 aC | 1.6 ± 0.2 aBC | 0.3 ± 0.1 aA | 0.2 ± 0.06 aA |
| 3 | 32.0 ± 1.2 bC | 28.7 ± 0.3 bC | 14.3 ± 1.8 abB | 18.3 ± 4.1 abB | 2.2 ± 0.2 aA | 1.5 ± 0.2 aA |
| 6 | 49.3 ± 0.7 cBC | 50.7 ± 3.3 bC | 38.7 ± 3.8 bcBC | 37.9 ± 7.6 bB | 4.0 ± 0.6 aA | 3.1 ± 0.3 aA |
| 6 UD[a] | 42.0 ± 3.5 bcB | 47.7 ± 2.7 bB | 50.7 ± 7.7 cB | 76.5 ± 6.0 cC | 3.0 ± 0.2 aA | 7.6 ± 0.5 aA |
| 12 | 90.0 ± 3.8 dB | 115.3 ± 10.7 cC | 145.7 ± 6.5 dC | 170.1 ± 11.1 dD | 25.1 ± 2.2 bA | 9.3 ± 1.3 aA |
| 25 | 112.3 ± 5.7 eBC | 126.7 ± 14.8 cC | 161.7 ± 9.3 dD | 199.0 ± 15.0 dE | 36.0 ± 4.0 bA | 82.0 ± 8.3 bB |
| 50 | 158.3 ± 8.4 fB | 130.0 ± 5.9 cB | 205.3 ± 16.7 eC | 272.0 ± 18.0 eD | 121.7 ± 14.3 cAB | 101.7 ± 3.5 cA |
| Cd content in the soil solution extracted from *Zea mays* grown soil (μg/L) | | | | | | |
| 0 | 4.1 ± 0.1 aE | 1.9 ± 0.1 aD | 1.8 ± 0.1 aD | 0.9 ± 0.02 aC | 0.4 ± 0.1 aB | 0.2 ± 0.03 aA |
| 3 | 29.7 ± 2.4 bD | 26.7 ± 0.9 bD | 10.3 ± 0.7 aC | 6.7 ± 0.6 abB | 1.1 ± 0.3 aA | 1.0 ± 0.3 aA |
| 6 | 54.7 ± 2.8 cE | 43.7 ± 4.6 cD | 29.7 ± 2.2 bC | 17.2 ± 2.5 abB | 2.4 ± 0.6 aA | 3.9 ± 0.5 aA |
| 6 UD | 47.0 ± 2.1 cC | 46.3 ± 3.5 cC | 43.0 ± 3.2 bC | 30.6 ± 2.1 bB | 2.6 ± 0.1 aA | 6.5 ± 0.8 abA |
| 12 | 105.3 ± 6.7 dB | 127.3 ± 4.8 dC | 135.0 ± 5.0 cC | 100.0 ± 10.1 cB | 15.6 ± 3.3 aA | 18.1 ± 0.8 bA |
| 25 | 128.7 ± 4.1 eBC | 132.0 ± 8.5 dC | 155.7 ± 6.6 dD | 106.8 ± 11.2 cB | 34.3 ± 4.1 bA | 39.7 ± 3.2 cA |
| 50 | 198.0 ± 2.0 fC | 132.3 ± 4.5 dB | 232.7 ± 11.6 eD | 265.3 ± 17.1 dD | 168.0 ± 12.1 cC | 73.3 ± 9.7 dA |

[a] UD—pots were not divided by nylon barrier.
[b] Mean ± standard error ($n = 3$). Means sharing the common lowercase alphabets within a column for a plant and means sharing the common uppercase alphabets within a row are not significant at 0.05% level according to DMRT.

cocropping systems at the same soil Cd concentration (Table 6). In the present study, all the tested plants did not show any toxicity symptoms up to 50 mg Cd/kg soil, indicating their potential to tolerate the Cd treatment. Higher dry weights of *B. napus* in BN–BP system and *Z. mays* in BN–ZM system may be attributed to their growth habit. Similar to earlier reports [13,27], increase in soil Cd concentration reduced both root and shoot biomass. But the differences in shoot dry weights were significant only at 50 mg Cd/kg soil treatment for *B. napus*. But *B. parachinensis* or *Z. mays* plants showed significant shoot biomass reduction after 12 and 25 mg Cd/kg, respectively, indicating the higher tolerance of *B. napus* than the cocropped plants. However, in root dry weights, the reduction was significant for both *B. napus* and *B. parachinensis* plants after 6 mg Cd/kg soil treatment in BN–BP cocropping system. Galli et al. [28] reported a strong reduction in root dry weight of *Z. mays* plants exposed to Cd. But in BN–ZM plant system, such a significant reduction was absent, which may indicate that either the *Z. mays* used in the study may be Cd tolerant or the rhizospheric effects of *B. napus* plants influenced the *Z. mays* rhizosphere. Further, the shoot and root dry weights of *B. napus* plants cocropped with *Z. mays* was lower than the *B. napus* plants cocropped with *B. parachinensis*. The root system of *Z. mays* developed well alongside the partition and abundant than *B. napus* plants, implying the dominance of *Z. mays* plants over *B. napus* plants for the available nutrients. Further, in comparison with *B. napus* monocropping experiment, *Z. mays* plants negatively influenced the shoot and root biomass of *B. napus* significantly (Table 6). In both the plant system, when the plant roots were allowed to mingle at 6 mg Cd/kg soil, the dry weight was higher in *B. napus* when compared to the pots with nylon divider in the same concentration. Such variations were not observed in *B. parachinensis* and *Z. mays* cocropped with *B. napus* but a marginal decrease was noticed.

Soil pH is considered to be one of the most important chemical factors controlling the availability of heavy metals in soil. Some plants increase their uptake of nutrients through the acidification of the rhizosphere via proton release [29] and the rape plants

**Table 6**
Comparison of *B. napus* growth, and elemental accumulation when is grown with *B. napus* (monocropping), *B. parachinensis* (BN–BP system) or *Z. mays* (BN–ZM system). Plants were grown at 6 mg/kg Cd levels and the different parameters after 6 weeks of growth are presented.

| Parameter | Brassica napus cocropped with | | |
|---|---|---|---|
| | *B. npaus* (monocropping) ($n = 6$) | *B. parachinensis* (BN–BP system) ($n = 3$) | *Z. mays* (BN–ZM system) ($n = 3$) |
| Shoot dry weight (g/plant) | 8.39 ± 0.39 b[a] | 7.42 ± 0.39 ab | 5.93 ± 0.56 a |
| Root dry weight (g/plant) | 0.70 ± 0.03 b | 0.47 ± 0.01 a | 0.38 ± 0.04 a |
| Shoot Cd concentration (mg/kg) | 12.35 ± 0.72 a | 13.43 ± 1.10 ab | 16.60 ± 1.96 b |
| Shoot Cd (μg/plant) | 104.07 ± 9.28 a | 100.47 ± 13.14 a | 97.03 ± 7.78 a |
| Root Cd concentration (mg/kg) | 11.85 ± 0.14 b | 9.11 ± 0.48 a | 12.39 ± 0.56 b |
| Root Cd (μg/plant) | 8.27 ± 0.46 b | 4.27 ± 0.17 a | 4.60 ± 0.30 a |
| DTPA extractable Cd (mg/kg) | 4.28 ± 0.33 a | 4.08 ± 0.05 a | 3.90 ± 0.08 a |
| pH of soil solution[b] | | | |
| Week 3 | 5.85 ± 0.05 b | 5.65 ± 0.03 a | 5.98 ± 0.07 b |
| Week 4 | 5.83 ± 0.10 a | 6.00 ± 0.17 a | 5.89 ± 0.25 a |
| Week 5 | 6.52 ± 0.08 a | 6.68 ± 0.09 a | 6.69 ± 0.08 a |
| Week 6 | 6.43 ± 0.17 a | 6.55 ± 0.19 a | 6.77 ± 0.07 a |
| Cd in soil solution (μg/l)[b] | | | |
| Week 3 | 24.2 ± 1.8 a | 38.7 ± 2.7 b | 38.7 ± 3.8 b |
| Week 4 | 19.1 ± 1.0 a | 46.5 ± 2.2 b | 37.9 ± 7.6 b |
| Week 5 | 13.8 ± 0.8 b | 3.4 ± 0.6 a | 4.0 ± 0.6 a |
| Week 6 | 13.8 ± 0.8 b | 2.8 ± 0.2 a | 3.1 ± 0.3 a |
| Accumulation factor | 2.06 ± 0.12 a | 2.24 ± 0.19 ab | 2.77 ± 0.33 b |
| Shoot/root Cd quotient | 1.04 ± 0.05 a | 1.50 ± 0.20 b | 1.35 ± 0.18 ab |

[a] Means ± S.E. Means sharing a common lowercase alphabet within a row are not significant at 0.05% level according to DMRT.
[b] pH and Cd content of the soil solution were not determined during 1st and 2nd weeks and hence comparison was not presented.

are reported to intensively acidify the rhizosphere in response to the low P status. Hedley et al. [30] reported that the changes in the rhizosphere pH of rape plants (*Brassica napus* var. Emerald), grown at high root densities (>90 cm $cm^{-3}$) in a soil of low P status, were not associated with any detectable increase in the amount of extractable organic acids or their anions, however, the rhizosphere acidification led to the efficient P uptake [31]. Although root exudation of organic acids may alter rhizosphere pH in some instances [32], most studies have identified differences in anion/cation uptake as the cause of the pH change [30,33]. In the present study, in both the cocropping systems, the pH continues to increase up to 5 weeks of plant growth and then stabilized and ranged between 6.1 and 6.9 after 6 weeks. Hinsinger and Gilkes [34] reported that the rhizosphere pH increased by three units when rape plants were grown with rock phosphate as the sources for Ca and P, while little or no change in pH occurred for ryegrass. Further, in our study, pH might be influenced by the addition of nutrient solution (pH 6.0) after 14 and 28 days. However, increase in pH during the initial stages implies the role of other factors. Wu et al. [35] reported that addition of Cd salt to the soil decreased the buffering capacity. However, the changes in pH may not be related with the heavy metal uptake. Previous studies using *Thlaspi caerulescens* have ruled out the role of rhizosphere acidification in metal accumulation [36–38]. Similarly, no change in rhizosphere pH was recorded in a Ni hyperaccumulator *Alyssum murale* [29,39]. In contrast, Mench and Martin [40] found that extraction of Cd from soil, using root exudates isolated in hydroponic culture, followed the same order as Cd bioavailability for three plants: *Nicotiana tabacum* > *Nicotiana rustica* > *Zea mays.* These authors suggest that root exudates of the *Nicotiana* spp. may play an important role in Cd accumulation. Similarly, Robinson et al. [41] found that Cd concentration of *Thlaspi caerulescens* was negatively correlated with pH. However, the role of root exudates in metal hyperaccumulation has been little researched.

Generally, the concentration of water soluble Cd increased up to 4 weeks, and sharply decreased thereafter. This may be correlated with the increasing pH, especially after 4 weeks. Cadmium is more available than other heavy metals to migrate to deeper soil layers or to underground water by leaching [42]. Wu et al. [35] reported that addition of Cd salt to the soil substantially enriched the soil solution with Cd. However, the increasing pH might have reduced the water soluble Cd. From the results we can suggest that adequate Cd was available for the plant for uptake and it was not the limiting factor. Further, after 6 weeks of plant growth, the DTPA extractable Cd in soils accounted for about 65–70% of the spiked Cd, which indicates the limitation of the plant species to extract the available Cd rapidly. As the DTPA extractable heavy metal gives a measure of plant available metals [22], most of the spiked Cd were in available form after 6 weeks of growth. There is no significant difference between with and without root barrier at 6 mg/kg Cd with respect to the water soluble and DTPA extractable Cd concentrations. These results indicate that the *B. napus* do not voraciously take up Cd but take up if available and accumulate without affecting its physiological functions as evidenced from the lack of typical Cd toxicity symptoms. Hence we suggest that the *B. napus* used in our study may be a moderate Cd acuumulator.

After 6 weeks of growth, the Cd concentration in plant tissue increased linearly with Cd concentrations in the soil. In both the cocropping systems, the shoot Cd concentration of *B. napus* plants exceeded 100 mg/kg dry weight, a limit defined for a Cd hyperaccumulator [7]. The accumulation factor for *B. napus* plants was >2 in both the cocropping systems, which is higher than both *B. parachinensis* and *Z. mays*. In both cocropping systems, the shoot Cd concentrations were different between cocropped plants but the root Cd concentration remained similar, indicating the efficiency of *B. napus* to translocate the Cd to the shoot, an important trait for a hyperaccumulator. Further, *B. napus* plants consistently exhibited S/R Cd quotient of >1, typical of an accumulator plant as suggested by Baker [43]. Baryla et al. [10] reported 2.5 times higher Cd concentration in shoot than that of roots in *B. napus* plants grown at 25 mg Cd/kg soil for 47 days, however, at 50 mg Cd/kg concentration, the Cd concentration was about 2 times higher in shoot than the root. Rossi et al. [12] also reported 1.4 times higher Cd concentration in the shoots than that of roots in *B. napus* plants grown at 50 mg Cd/kg soil for 5 weeks. However, the concentration of Cd in the shoot (37 mg/kg DW) and root (27 mg/kg DW) reported by Rossi et al. [12] was very low when compared to the reports of Baryla et al. [10] and the present study.

In BN–BP system, the Cd concentration was higher in shoots than roots in both the plants. Since the biomass of *B. napus* plant was higher than the *B. parachinensis* plants, the quantity of Cd extracted (Cd content/plant) was higher for *B. napus*. *Brassica parachinensis* plants also showed S/R quotient ~1, a possible indication of human health risk when the leaves of the *B. parachinensis* are consumed if the soil is contaminated with Cd, since it is grown as a leafy vegetable. However, in BN–ZM system, the root Cd concentration of *Z. mays* was higher than the Shoot Cd concentration. Our results are in agreement with a number of reports which indicate that Cd accumulates more in roots than in maize shoots [44–48]. Higher root Cd concentration was also revealed by the S/R quotient of <1 in all Cd treatments; however, the quantity of Cd accumulated in the shoots was about 6 times of Cd accumulated in the roots due to the high shoot biomass compared to the root biomass. Concentration and contents of Cd was the same when the roots of *B. napus* and *B. parachinensis* or *Z. mays* plants were allowed to mingle at 6 mg Cd/kg soil. Interestingly, the shoot Cd concentrations of *B. napus* from BN–ZM cocropping was significantly higher than the *B. napus* from monocropping system at 6 mg Cd treatment, and the differences in root Cd, although higher, was not significant. In contrast, *B. napus* from BN–BP system, exhibit higher but insignificant shoot Cd and significantly lower root Cd. The accumulation factor and S/R quotient also follow the same trend. Cocropping with *B. parachinensis* or *Z. mays* negatively affected the biomass of *B. napus*. Although the Cd concentration in shoot of *B. napus* was higher than in *B. napus* of monocropping, the Cd accumulation (Cd content/plant) was less due to the reduction in the biomass. Alternatively, cocropping might resulted in growth enhancement of *B. parachinensis* and *Z. mays* due to the higher nutrient apportionment to the crop plants than *B. napus*. Although, the cocropping negatively affected the biomass of *B. napus* at 6 mg/kg soil concentration, they are expected to grow better at higher concentration than the crop plant and extract more cadmium, thus providing a less toxic environment to the crop plant. Monocropping controls at higher Cd concentrations (i.e., >12 mg/kg soil) would give more information. However, the lack of symptoms in *B. napus* up to 50 mg/kg soil suggests that they can thrive better at high Cd concentrations also. Further, both the crop plants seem to be Cd tolerant, especially *B. parachinensis* accumulated >100 mg/kg dry weight. More information could be obtained if these crop plants were sensitive to Cd. However, the overall results indicate that, when the *B. napus* plants cocropped with *B. parachinensis* or *Z. mays*, they take up more Cd and the cocropping with *Z. mays* is more effective than with *B. parachinensis*.

## 5. Conclusions

High aboveground biomass and the Cd accumulation in the shoot of *B. napus* offer potential opportunity for the phytoextraction of Cd as the concentration exceeds the limit of a hyperaccumulator. Since, the *B. napus* used in this study did not voraciously take up the Cd, we suggest that it may be a moderate accumulator of Cd. When *B. napus* was cocropped with *B. parachinensis* or *Z. mays*, the Cd concentration and accumulation in the shoot was significantly

($p < 0.05\%$) higher indicating the potential of cocropping method to remediate the Cd contaminated soils. However, the cocropping of *B. napus* with another Brassicaeae member was not much useful. Further, consumption of *B. parachinensis* from Cd contaminated soil might pose health risk.

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