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Full Length Research Paper

Effect of different doses of urea on the uptake of cadmium from soil by *Brassica napus*: A case study in Sargodha, Pakistan

Zafar Iqbal Khan[1]*, Kafeel Ahmad[1], Shehnaz Yunas[1], Nudrat Aisha Akram[2], Yasir Rizwa[1], Muneeba Shaheen[1], Mian Jehan Zaib[1] and Munawar Aziz[1]

[1]Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan.
[2]Department of Botany, G.C. University, Faisalabad, Pakistan.

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The present investigation was conducted to study the effect of different doses of urea on cadmium uptake by canola (*Brassica napus* L.) applied in full and split doses. Nine different treatments of urea used were: 0 (control), 60, 90, 120 and 150 kg/ha applied to soil as full doses before sowing, and 60, 90, 120, and 150 kg/ha applied as two equal splits; the first was before sowing, while the second was before flower initiation. Samples of soil were taken just after mixing the appropriate dose of urea, whereas those of stems and leaves were taken at the vegetative stage. Cadmium (Cd) concentrations in soil, stems and leaves samples were determined unconnectedly. Based on the data recorded, it was concluded that all the treatments had significant effect on Cd concentrations in soil, leaves, stems, roots and pods. The mean Cd value in soil, leaves and stem ranged from 0.84 to 1.33, 0.32 to 1.07 and 1.27 to 1.69 mg kg^{-1} dry weight, respectively. While in roots and pods, Cd concentration varied from 1.31 to 1.77 and 1.05 to 1.36 mg kg^{-1}, respectively. The mean Cd level in soil and plant body was much lower than the critical level reported by different researchers, thus an additional supplementation of Cd is required to meet the needs of plants. Mineral supplements with rational amount of Cd sources are suggested to avoid complications caused by Cd deficiency as well as to achieve an optimal animal production of ruminants being reared on the pasture.

Key words: Brassica, cadmium, forage, soil, Pakistan.

INTRODUCTION

The Brassicaceae, which currently includes 3709 species and 338 genera, is one of the 10 most economically important plant families (Rich, 1991; Warwick and Al-Shehbaz, 2006). Canola, one of the important members of *Brassica* family, has long been cultivated by ancient civilizations in Asia and the Mediterranean for its use as oil for lamps (Colton and Sykes, 1992). Brassica is important due to its high nutritional value as well as feed value particularly during winter. A research on canola in aqua feeds has been carried out in Western Australia, and it was estimated that approximately 60% diet can be made up of canola meal. In addition, canola oil can be used in diets for marine fish (Glencross, 2003). Due to the extensive utilization of canola oil, its production ranks second among all oil-seed crops (Chopra and Prakash, 1991). Meanwhile, Pakistan is deficient in edible oil. Local production stood at 0.606 million tons during previous crop year, which was sufficient to meet 29% of the demand, while the rest of the demand (71%) was met through import (Anonymous, 2003, 2005, 2006).

Application of nitrogen containing supplements is very common due to relatively high N requirement (Grant and Bailey, 1993). Nitrogen is a major nutrient element which provides lush green color to the plant due to increase in chlorophyll. Nitrogen plays a key role in plant growth and protein synthesis, protoplasm, cell size and photosynthetic activity and thus provides a huge frame on

*Corresponding author. E-mail: zikhan11@gmail.com. Tel: +92489230728, +923007506618.

Table 1. Different treatments of urea applied to soil before and after sowing the forage crop.

Treatment	Applied dose (kg h^{-1})
Full dose applied before sowing	
1	0 (Control)
2	60
3	90
4	120
5	150
Dose in two equal splits (I[st] split applied before sowing and the 2[nd] before flowering)	
6	60
7	90
8	120
9	150

which more flowers and pods are produced (Yasari and Patwardhan, 2006). Urea is the most common form of combined nitrogen fertilizer used in today's agricultural practices worldwide (Vavrina and Obreza, 1993). The utilization of urea has significantly improved from the last 20 years and nowadays it is considered as a chief fertilizer in farming (Engelstad and Hauck, 1974; Voss, 1984). However, the use of urea has harmful outcomes on germination of seed and plant seedling growth entirely because of ammonia production during hydrolysis of the urea fertilizer in the presence of an enzyme urease in the soil. It causes a considerable loss of nitrogen from urea in the form of ammonia (Catchpoole, 1975; Terman, 1979).

Generally, metal concentration in the soil which ranges from 1 to 100, 000 mg kg^{-1} may be due to human activities or geological factors (Blaylock and Huang, 2000). A high concentration of heavy metals such as zinc (Zn), chromium (Cr), cadmium (Cd), nickel (Ni) and copper (Cu) in the soil induces disruption of natural terrestrial and aquatic ecosystems (Gardea-Torresdey et al., 1996; Meagher, 2000). It has been reported that Cd inhibit significantly seed germination by 45% at concentration of 40 mg L^{-1}. Also, the number of stomata in oilseed rape plants reduces at the rate of 40% on exposure to Cd (Claire et al., 1991). Cd at the level of 100 mg/kg severely affect the leaves and result in chlorosis.

In addition, a substantial reduction in chloroplast density, number of chloroplast and in cell size has been observed (Aurore et al., 2001). Considering the importance of Cd for growth, the present investigation was conducted and effects of different concentrations of urea were studied.

MATERIALS AND METHODS

The investigation was done to assess the level of Cd in the soil and the forage that were subjected to varying doses of urea as a nitrogen source. This experimental work was conducted during December 2008 to April 2009, at the University of Sargodha, Pakistan, which falls under semi-arid climatic conditions. The seeds of *Brassica napus* (Dunkeld variety) were sown in the first week of December 2008 in pots filled with loamy soil at a rate of 10 seeds per pot. The climatic conditions during the experiment were: 18 to 25/10 to 17°C day/night temperature, 55 to 60% RH and 12-h photoperiod. Urea was added to the soil contained in the pots before sowing and/or before flower initiation. Split doses were applied twice; 1[st] just after sowing and 2[nd] at an interval of one month before flowering; while full dose was applied at once before sowing.

The detail of varying urea treatments is presented in Table 1. The complete randomized design (CRD) was used in this study. Polythene pots were used for sowing the seeds of plant and each plastic pot was lined with polyethylene bag. Seven kilogram soil was taken in each plastic pot that was lined with polyethylene bag. Different parts of plants were harvested at maturity. Five replicates of plants from each dose were taken. All protective measures were adapted to ensure healthy crop. All the pots were irrigated with tap water throughout the experimental period.

Soil and plants

Samples of each soil and plants were taken randomly from pots that were given different doses of urea. The samples of soil were obtained after mixing the urea with soil in each pot before sowing. The harvest of different parts (root, stem, leaves and pods) of plants was taken after 90 days of sowing after grain filling. All plant samples were washed well with distilled water. These samples were then air-dried, stored in labeled sealed paper bags and placed in an oven for drying for three days at 70°C.

Wet digestion and analysis

One gram air- and oven-dried soil and plant samples were transferred to digestion tubes and 5 ml of H_2SO_4 were added to each tube. All tubes were then incubated overnight at room temperature. Next, H_2O_2 (25 ml) was poured down through the sides of the digestion tubes and were placed on a hot plate to heat until there was a complete digestion of the material. The volume of the extract was made up to 50 ml with distilled water. After filtering the extract, this was used for the analysis of Cd concentration. The contents of cadmium in soil and plant parts were determined using an atomic absorption spectrophotometer (Model #AA-6300, Shimadzu, Japan).

Statistical analysis

The data obtained from all analyses was tested for significance at 0.05, 0.01 and 0.001 by using the software SPSS (Steel and Torrie, 1986). Standard error values were calculated to compare the mean values of each attribute.

RESULTS AND DISCUSSION

Based on the data recorded, it was concluded that all the treatments had significant effect ($P \leq 0.001$) on Cd concentration in soil, leaves, stems, roots and pods (Figures 1 to 5). The mean Cd value in the soil varied from 0.84 to 1.33 mg kg^{-1}. The highest value was observed during the 4[th] treatment, while the least was during 1[st] treatment. Moreover, there was an abrupt

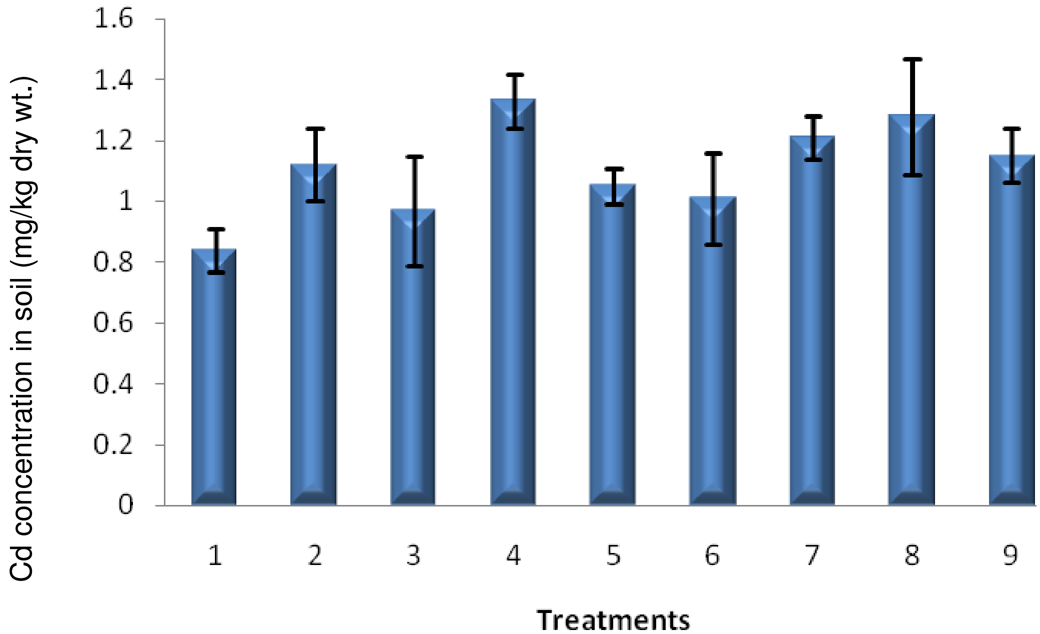

Figure 1. Cd level fluctuations in the soil at nine different treatments (see Table 1).

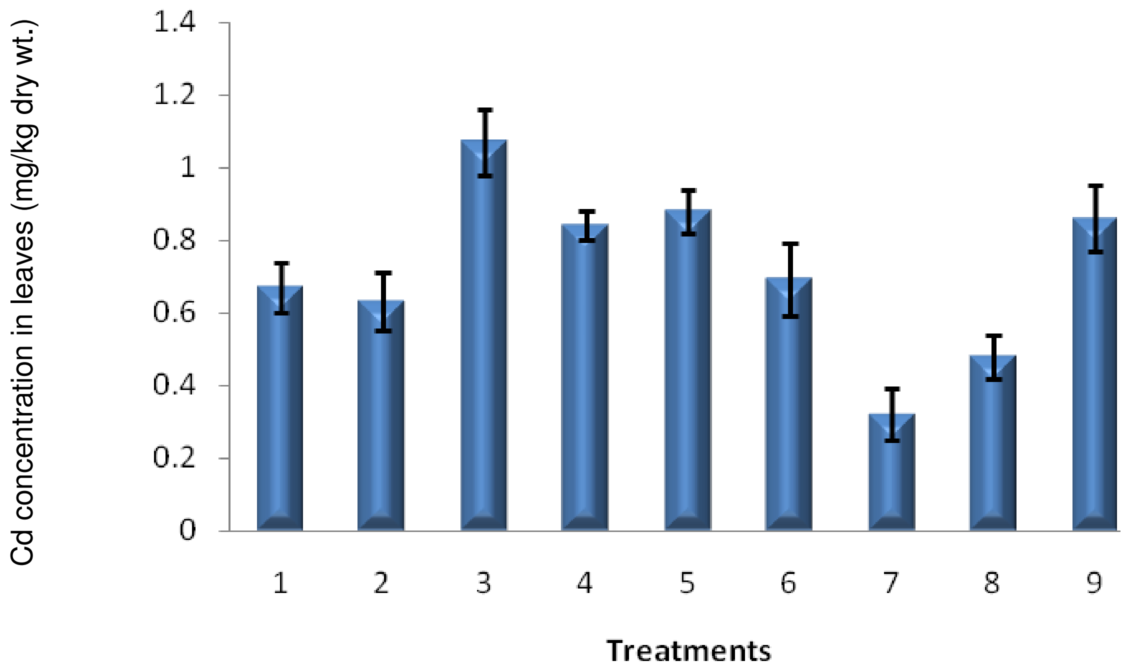

Figure 2. Cd level fluctuations in leaves at nine different treatments (see Table 1).

decrease and increase in values while in all the treatments; the Cd level was higher than control. In the present investigation, the mean Cd levels were lower than critical level of 3 mg kg^{-1} reported by Kloke (1980) and also lower than toxic level reported by Ross (1994). These values are also lower than values reported by Oncel et al. (2000). Hence according to the value of toxicity described above, the level of Cd in soil was below the toxic level and was similar to the level reported by Oluokun et al. (2007).

Furthermore, the mean Cd concentration in the leaves and stem varied from 0.32 to 1.07 and 1.27 to 1.69 mg kg^{-1}, respectively. Cd concentration in leaves and stem was not toxic and was lower than the critical and toxic

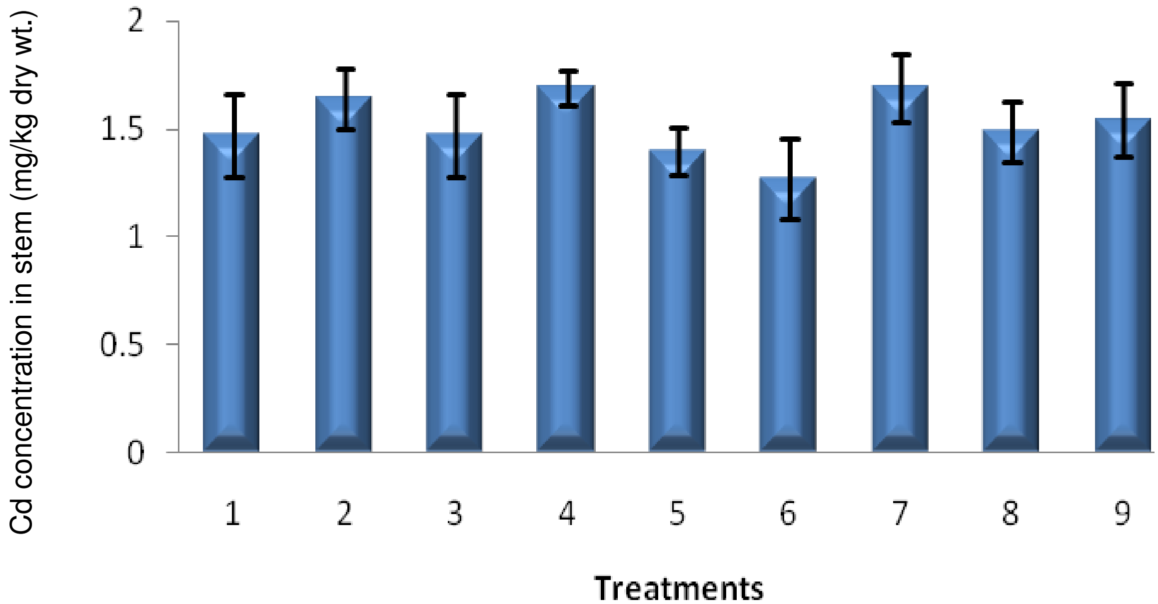

Figure 3. Cd level fluctuations in stem at nine different treatments (see Table 1).

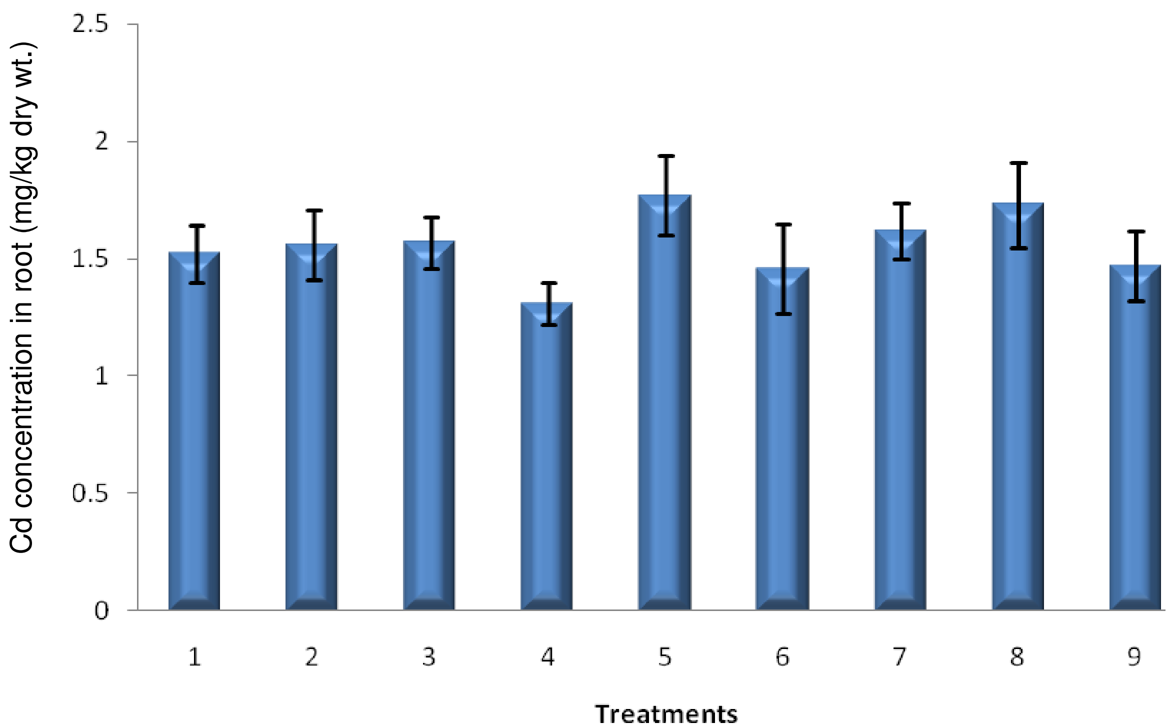

Figure 4. Cd level fluctuations in roots at nine different treatments (see Table 1).

level of 3 to 8 mg kg^{-1} (Ross, 1994; Oncel et al., 2000), but similar to those reported by Aksoy et al. (1999). While in roots and pods, Cd concentrations ranged from 1.31 to 1.77 and 1.05 to 1.36 mg kg^{-1}, respectively. Mean Cd level in soil as well as plant material was much lower than the critical level reported by different researchers, thus additional supplementation of Cd is required to meet the needs of plants. Whereas the forage content of well-fertilized meadow is normally less, the higher level of forage Cd for meadow sites indicated that the presence

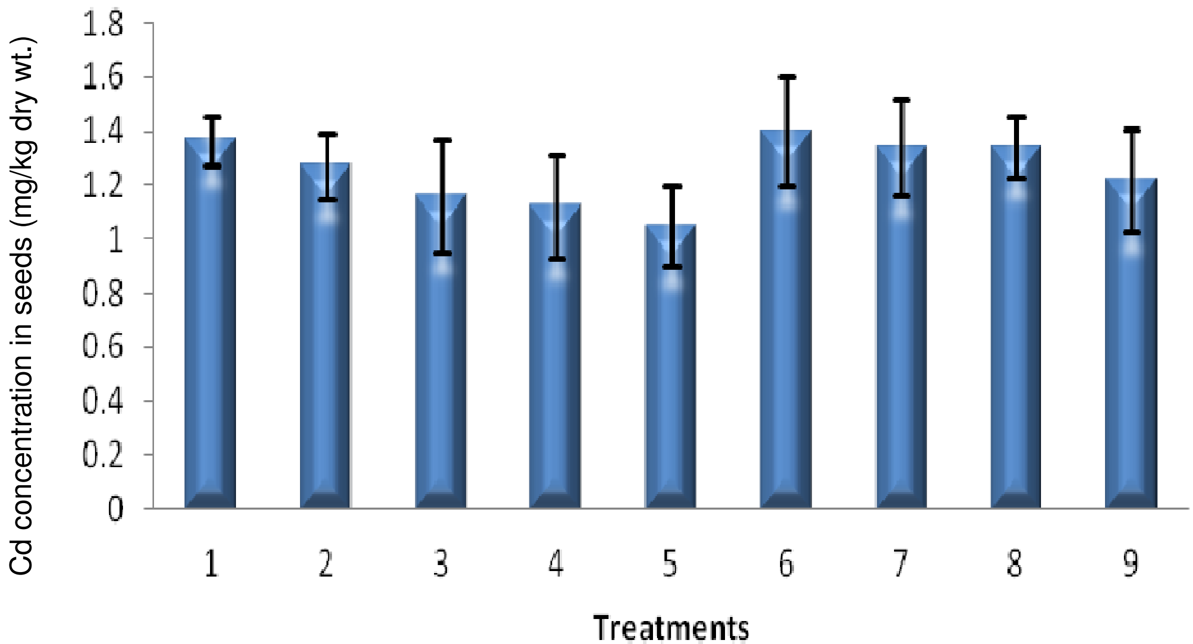

Figure 5. Cd level fluctuations in seeds at nine different treatments (see Table 1).

of forage species in the field should be actively depressed. This may be attained by suitable grazing management practices and fertilizer policies which will support dynamic grass and legume pastures hence suppressing forage establishment and growth having high accumulation potential for heavy metals.

Often, the higher Cd concentration in rural soil was revealed in the Cd contents of wild species, to a very low amount for grasses, and not for legumes.

Commonly, adult animals had elevated level of Cd in kidney because of longer exposure to Cd in their environment and therefore more chances of utilizing and preserving Cd.

Undomesticated deer and sheep segregated from human interference, also demonstrated the age-related preservation of Cd in kidney tissue as a result of exposure to naturally occurring Cd in their environment. However, based on our findings, there seems to be no potential threat of cadmium accumulation for grazing animals being reared on the pasture containing forage species of canola

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