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Effects of Some Heavy Metals on Seed Germination Characteristics of Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and Safflower (*Carthamus tinctorious*) to Evaluate Phytoremediation Potential of These Crops

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Abstract

Heavy metal pollutants are the main concern of new agricultural productions. Industrial products and using synthetic materials leads to drastically increase in concentration of different heavy metals in the environment. Lead and Cadmium are two famous heavy metals which are largely used in electronic industries thus the waste water of factories could pollute agricultural lands. Different heavy metal solutions were investigated for their effects on seed germination characteristics and phytoremediation potential of two oil crops (canola, *Brassica napus*andsafflower, *Carthamustinctorious*) and a cereal crop (wheat, *Triticumaestivum*). The Canola, Safflower and Wheat seeds were germinated after treatments in solutions containing varying concentrations of heavy metals. Five different concentrations of heavy metals including (BiNO3, BiNO3, CdNO3, Sr (NO3)2, ZnNO3) at 50, 200, 350, 500, 1000 ppm and distilled water considered as control treatment. Results showed that in all treatments the percentage of seed germination, root and shoot length decreased as concentrations of solution increased. No germination was observed at 1000 ppm of cadmium level. Root and seedling vigor increased by application of 200 ppm of BiNO3. There were no seedling growth at 350 and 500 ppm of cadmium and lead concentration.

Keywords: heavy metal, phytoremediation, seed germination, root length, vigor

1. Introduction

Earth crust naturally contains different heavy metals. Heavy metals could get into our bodies by different ways such as respiration, eating food and drinking water. Some heavy metals are trace elements and needed to maintain good metabolism of plants or human body (provide source(s)). Higher concentrations or hyper accumulation of heavy metals can lead to toxic effects in human, animal, plant and other microorganisms (Nodelkoska & Doran, 2000; Donderski & Brzezinska, 2005; Tkaczuk, 2005; Topolska et al., 2004; Nasiadek et al., 2005; Rydzewska, 2001). Environmental contamination of heavy metals has been increased in last decades. Heavy metals have recently received more attention of researchers all over the world, and this is due to their perniciouseffects on living organism such as plants, animals. The toxic and side effects of different metals have also been intensively studied at the level of biochemical and physiological process such as photosynthesis (Kuper et al., 2002), transpiration (Pandy et al., 2002), enzyme activity (Astolfi, 2005) or metal accumulation in tissue (Palmieri, 2005). Concentrations of heavy metals in different soils vary from less than 1 mg/kg (ppm) to high as 100,000 mg/kg, which could because of their geological origin in the soil or as a result of pollution by human and industrial activities (Blaylock & Huang, 2000). There is some reports that excess concentrations or hyper accumulation of some heavy metals in soils such as Cd(II), Cr(VI), CuII), Ni(II), and Zn(II) have caused the disturbanceof both natural aquatic and terrestrial ecosystems (GardeaTorresdey et al., 1996; Meagher, 2000). There are some reports which show that some plants species are well adapted to metalliferous soils and can tolerate high concentration of heavy metals and other toxic substances (Banuelos et al., 1997; Blaylock & Huang, 2000; Raskin & Ensley, 2000; Dahmani Muller et al., 2000). More over different studies have been conducted in order to evaluate the effects of different heavy metal concentrations on herbal organisms (Thompson et al., 1997; Reeves & Baker, 2000; Raskin & Ensley, 2000) but in contrast there are small number of studies on seeds which have been exposed to the pollutants (Claire et al., 1991; Vojtechova & Leblova, 1991; Xiong, 1998). Judia and Fulekar (2008) reported that inhibitory effect of heavy metal on seed gerniantion sis concentration depedent process in alfalfa. They also indicate that cadmium and lead are very toxic heavumetls in 50ppm concentration. Cadmium and Nikel are both inhibitor of seed germination in sunflower (Khan & Moheman, 2006). Approach of phytoextraction is an environmentally friendly, low-cost method which could be very applicable for removing pollution of heavy metals from polluted soils and has been introduced as a favorablealternative to conventionalengineering-based methods (Baker et al., 2000; Dickinson et al., 2009). Seed germination is the first step of plant life and so this stage is very sensitive to environmental factors such as biotic and abiotic stress.

It is reported that during in the process of rhizofiltration, that young plant seedlings grown in ventilated water are more efficient than roots in elimination of heavy metals from water (Salt, 1997). After initiation of seed germination dramatically increase in surface to volume ratio is happen and in young seedlings also absorb large amount of toxic metal ions. This characteristics of young plants makes seedlings exceptionally suitable for remediation process. Phytofilteration using cultured seedlings in light or in darkness can be practical, and required constituent are only seeds, water and air. (Raskin, 1997).

The present study was performed in order to investigate the effects of some heavy metals (Cd (II), Sr (VI), Pb (II), Bi (II), and Zn (II)) on canola, safflower and wheat seed germination and seedling characteristics and to evaluate of phytoremediation potential of these crops at their first life stage.

2. Materials and Methods

Heavy metal solutions were prepared from five different concentration of heavy metals (BiNO3, BiNO3, CdNO3, Sr (NO3)2, ZnNO3) at 50, 200, 350, 500, 1000 ppm and distilled water as control. Seed were subjected to standard germination test using four replications of 25 seeds (ISTA, 1997). The selected seeds were placed on two sheets of filter paper (Whatman No. 1) contained in petri dishes (9 cm diameter). Solutions of heavy metals (5 mL) were subsequently added to the seed-containing Petri dishes to wet the filter paper and contaminate the samples Petri dishes and seeds were put into sealed plastic bags to avoid moisture loss. During germination test, no extra solution was added to petri dishes. Seeds were subjected to germinate at temperature of 20±1°C for wheat and canola and 25±1°C for safflower under dark condition for 14 days. Seeds were stored at refrigerator with temperature of 4 ^{o}C to avoid any deterioration before performing a test. Germination was considered to have occurred when radicles were 2mm long. Germination percentage was recorded every 24 h for 14 days. Day 14th of experiment was considered as the final day because no more germination was observed and root and shoot length was measurable for all petri dishes. Mean germination time and rate of germination was calculated to assess the speed of germination (Ellis and Roberts, 1980). Vigor index was measure using following formula:

Vigor index=seedling length (cm) * germination percentage (moradi et al, 2008).

In order to investigate the effect of different heavy metal consentration on three agronomic crops a factorial experiment was conducted base on complete randomized design in four replications. Mean comparision was performed by using Tukey method. Minitab v16 and Microsoft Excel 2010 were used for statistical analysis and drawing graphs respectively.

3. Results and Discussion

Analysis of variance showed that there is significant difference for interaction effects between heavy metal concentrations and plant species ($p<0.01$) for all investigated traits.

3.1 Effects of Heavy Metals on Seed Germination

Analysis of variance showed that heavy metals had significant effects on seed germination characteristics. Increasing the concentration of different heavy metals resulted dramatically in reduction in germination percentage. No seed germination was observed at 1000 ppm of CdNO3 (Figure 1). Among all the heavy metals, side effects of Cadmium (CdNO3) were more obvious than other treatments. Wheat seeds showed the highest germination percentage. Seed which were unable to germination under high cadmium concentration were counted to measuring germination percentage.

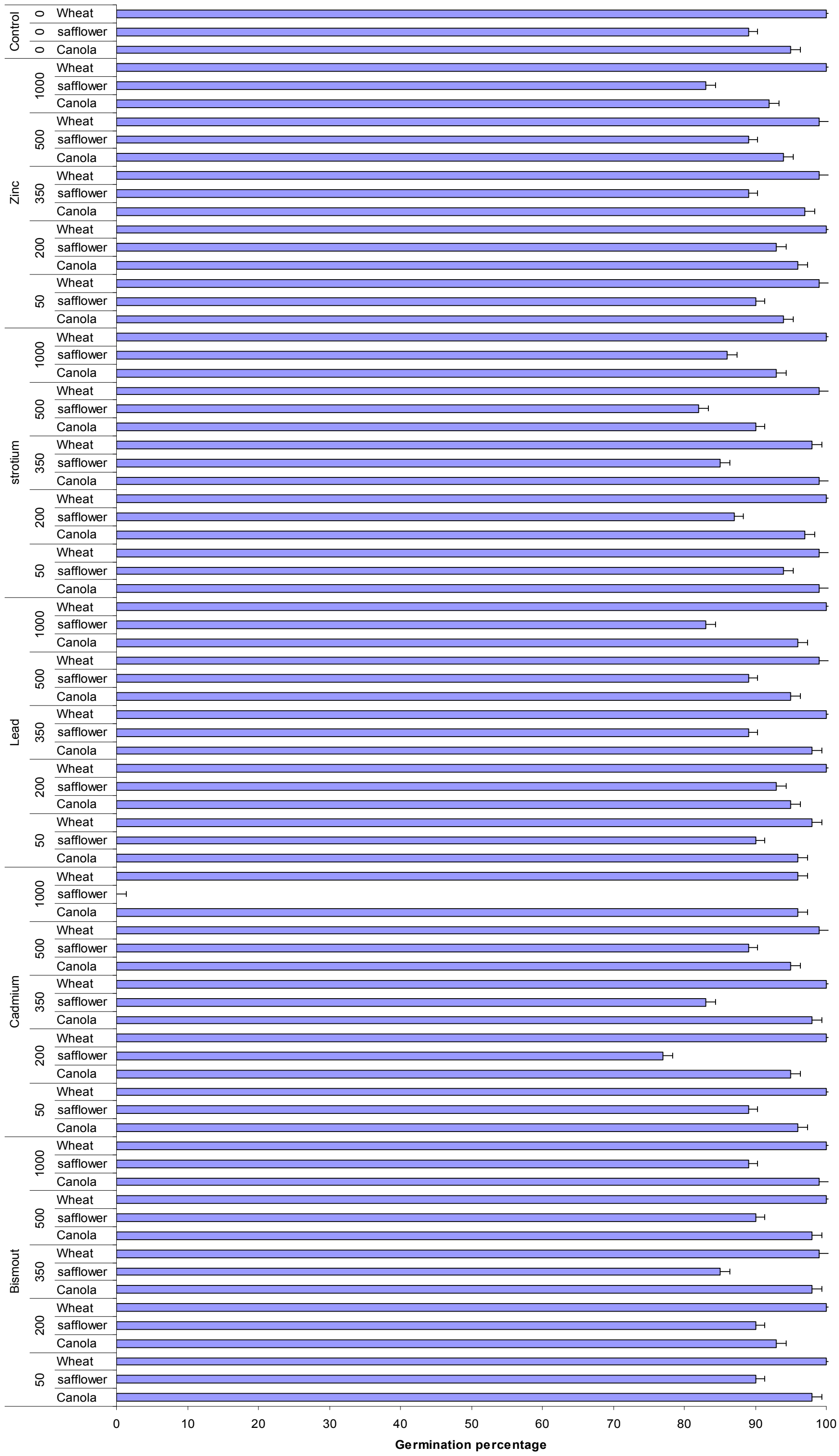

Figure 1. Effect of different heavy metals on seed germination

In general, there was a reduction in seed germination as metal concentrations in the growing media increased.

3.2 Effects of Heavy Metals on Germination Rate (GR)

Results showed significant differences for these trait. Germination rate was decreased with increasing heavy metal concentrations (Figure 2). Germination rate of Cadmium treatments is due to lower germinated seeds at the time of germination test and so according to germination rate formula (Elis and Roberts), it showed faster germination rate for 500 ppm cadmium.The decrease in percentage of germination may be due to loss of viability because of decreased energy generation by the embryo. Energy generation is very important for seed germination and its blockage could affect protein, RNA and DNA synthesis as well as mitosis, since energy is required for these processes to occur (John & van-Laerhoven, 1976).

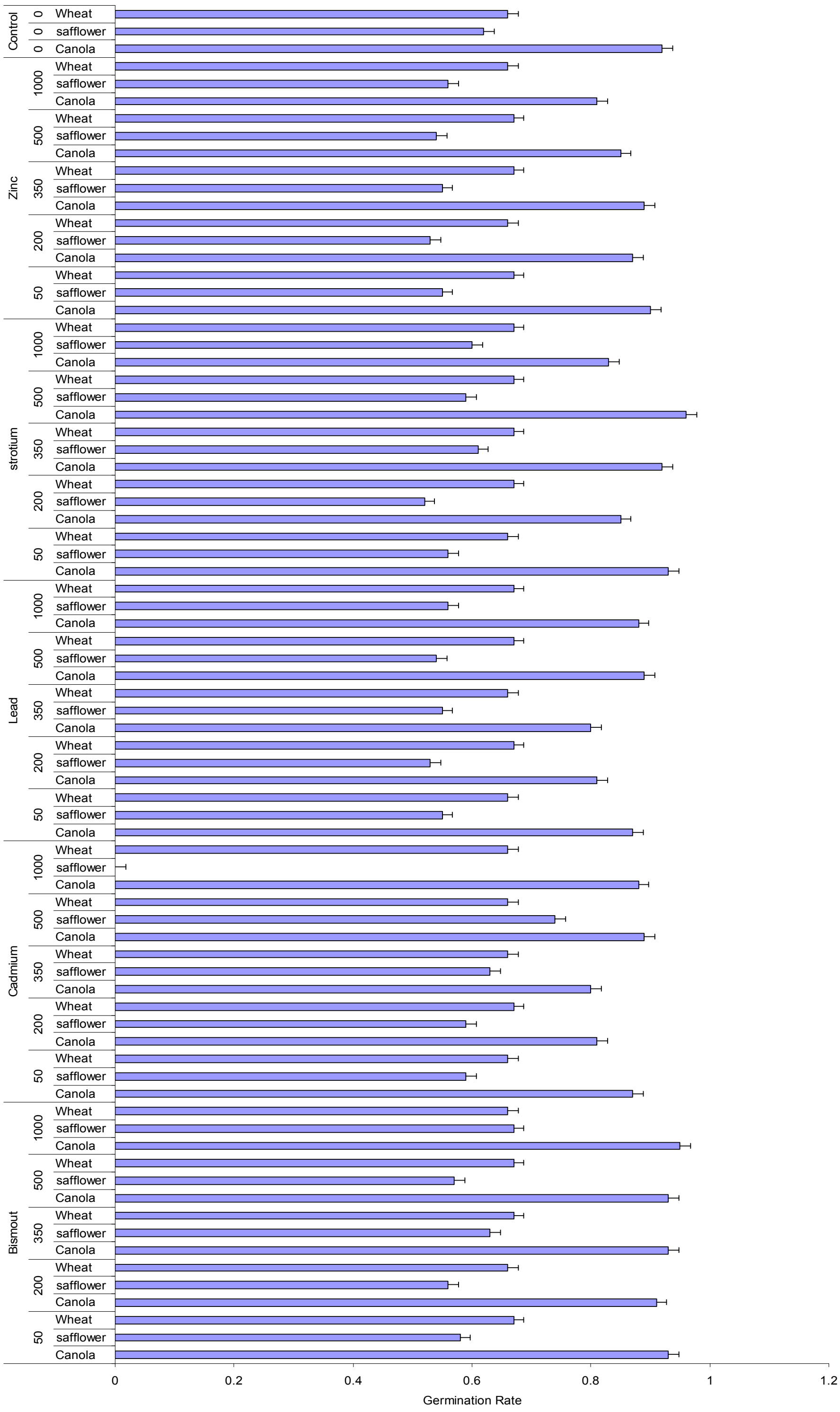

Figure 2. Effect of different heavy metals on germination rate

Our results showed that different concentration of heavy metals did not seriously affect germination percentage and germination rate except of Cadmium.

3.3 Effects of Heavy Metals on Root Length

Heavy metals significantly affected root length. Cadmium had the highest side effect on seedling root than the others and BiNO3 and SrNO3 exhibited higher root length comparing the control (Figure 3). The inhibitory effect of cadmium on cell expansion and division might due to inhibition of growth promoters primarilythrough blocking enzyme activation and lowering or direct blocking of cell division by intrusivewith the cell membrane integrity. Inhibition in cell division process through mitosis may be happen due to the interference with the formation of mitotic apparatus (Brachet & Mirsky, 1961). Repressionof protein synthesis, DNA replication and could also block cell division. It is suggested that inhibition of mitosis by Cadmium probably is due to it's binding to the –SH groups of protein molecules which are part of the tiny fibers that are seen in cell divisionor mitotic apparatus. There is some reports that inhibition of –SH groups of such proteins could be happen during meta-phase of mitosis with other inhibitors such as fluoride, iodoacetic acid, chloroacetophenone and this inhibition might be due to prevention of spindle formation (Brachet & Mirsky 1961). Cadmium could also affect the plasticity and fluidity necessary for division of meristematic cells. Cadmium ion, Cd2+, like other divalent ions like Ca2+, could reduce cell memberane permeability and fluidity which is necessary for cell division.

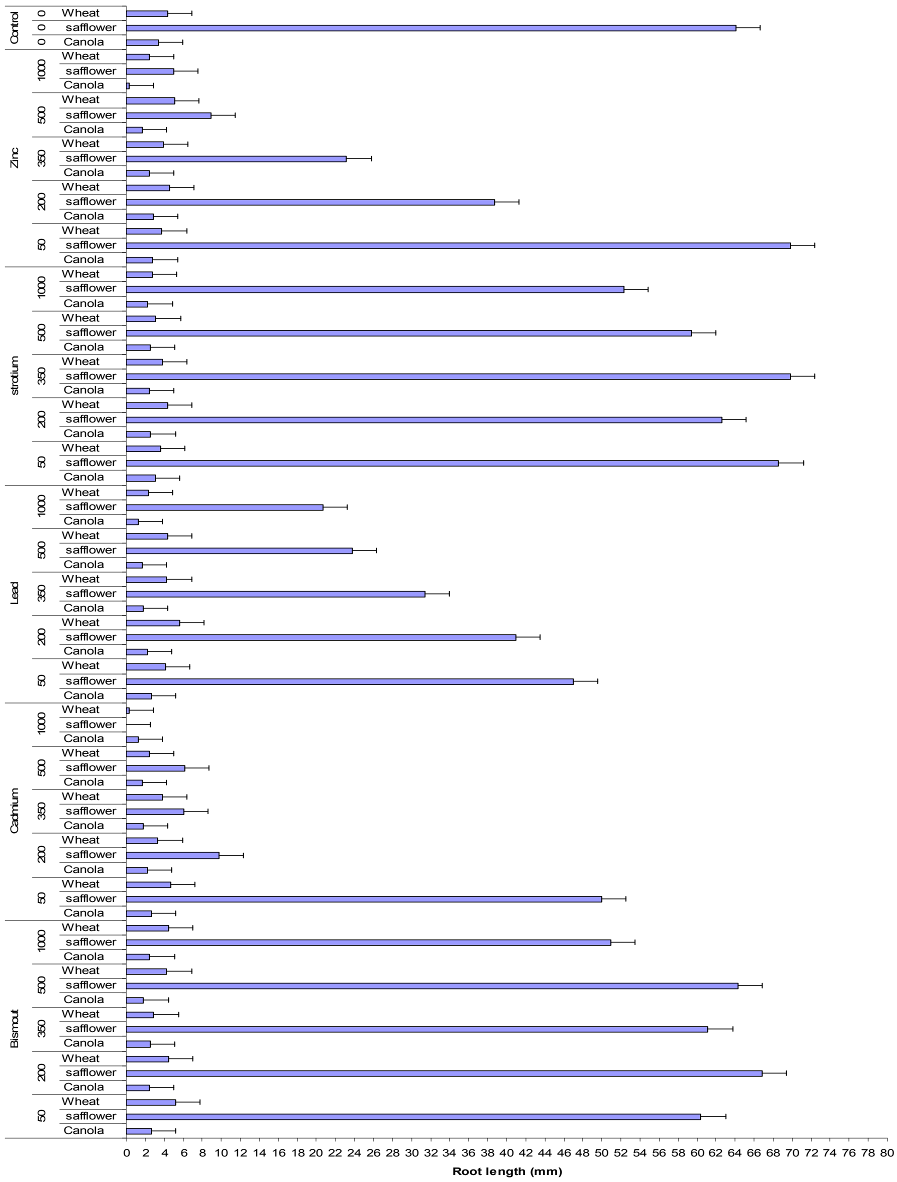

Figure 3. Effect of different heavy metals on root length

3.4 Effects of Heavy Metals on Shoot Length

Heavy metals significantly affected shoot length. Cadmium had the highest side effect on seedling root than the others and BiNO3 and ZnNO3 exhibited higher root length comparing the others (Figure 4). Root growth in all species was reduced by increasing the tested Zn concentrations. No differences in species sensitivity to Zn regarding root growth were observed. In contrast to our results, in some forest tree species seed germination enhanced at low levels of Cadmium (Scherbatskoy et al., 1987) but reason of this phenomenon was unknown. In the present study, results showed that tested levels of Bi and Zn significantly enhanced seed germination. Some studies reviled that root growth was more easily affected by the tested heavy metals comparing to seed germination. Roots were the first plant organs that impose and get into contact with heavy metal(s) contaminated environments. Potential of root growth has been proven to be an index of metal tolerance in plants (Wilkins, 1978). According to results obtained form this study, it is suggested that saffolwer could be used for phytoremediation of soil contaminated with Cadmium or lead.

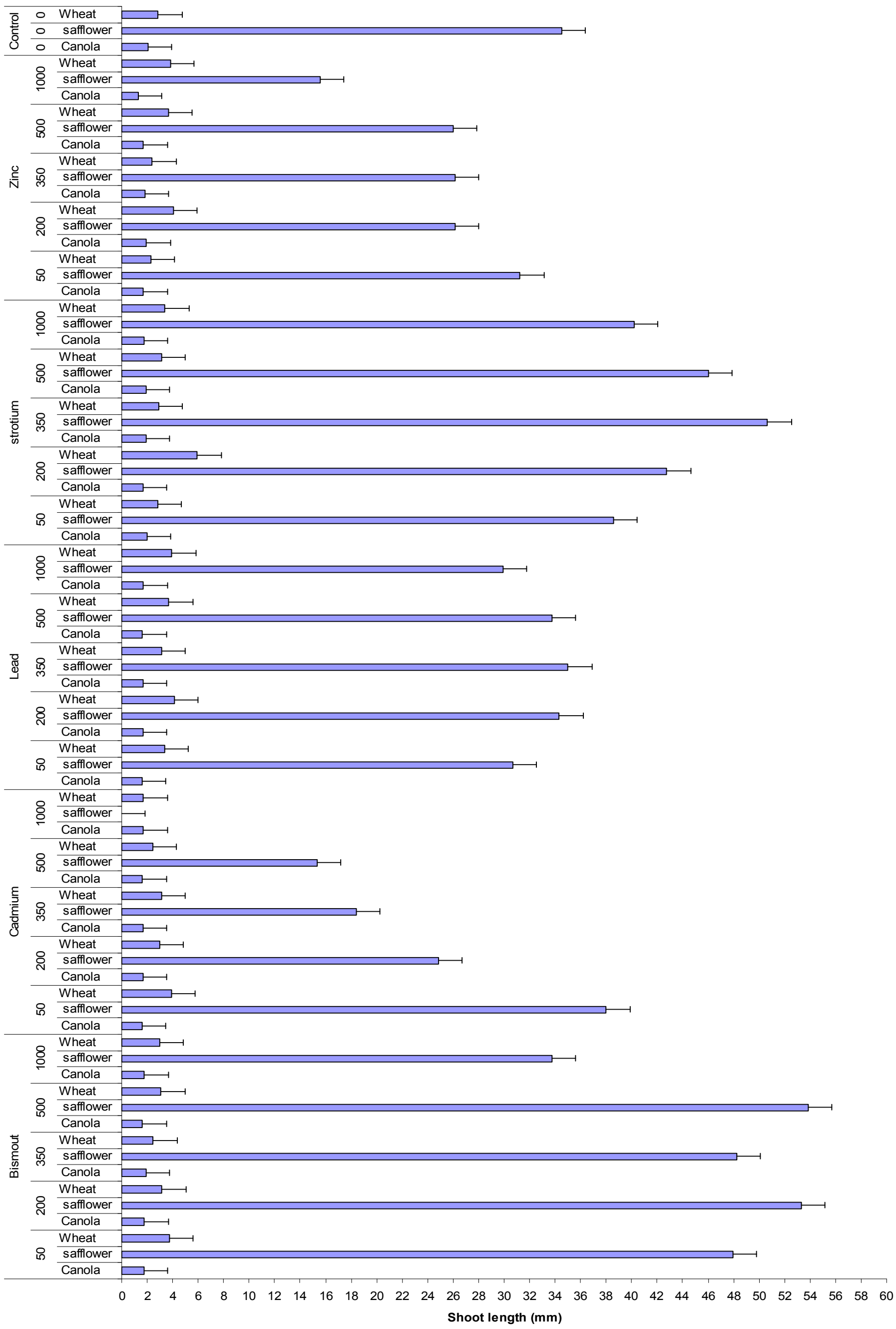

Figure 4. Effect of different heavy metals on shoot length

3.5 Effects of Heavy Metals on Vigor index

With increase in the concentration of solution, seedling vigor started to decrease and this was clearer in the Cadmium and lead solutions. Treatments of BiNO3 and SrNO3 at concentration of 200 ppm significantly increase seedling vigor comparing to control (Figure 5). Inhibition in root growth due to high concentrations of heavy metals especially leads and cadmium may be a main reason of seedling vigor reduction. Heavy metals at toxic concentration can interrupt cell division and RNA replication could clock DNA repair process and other vital physiological process.

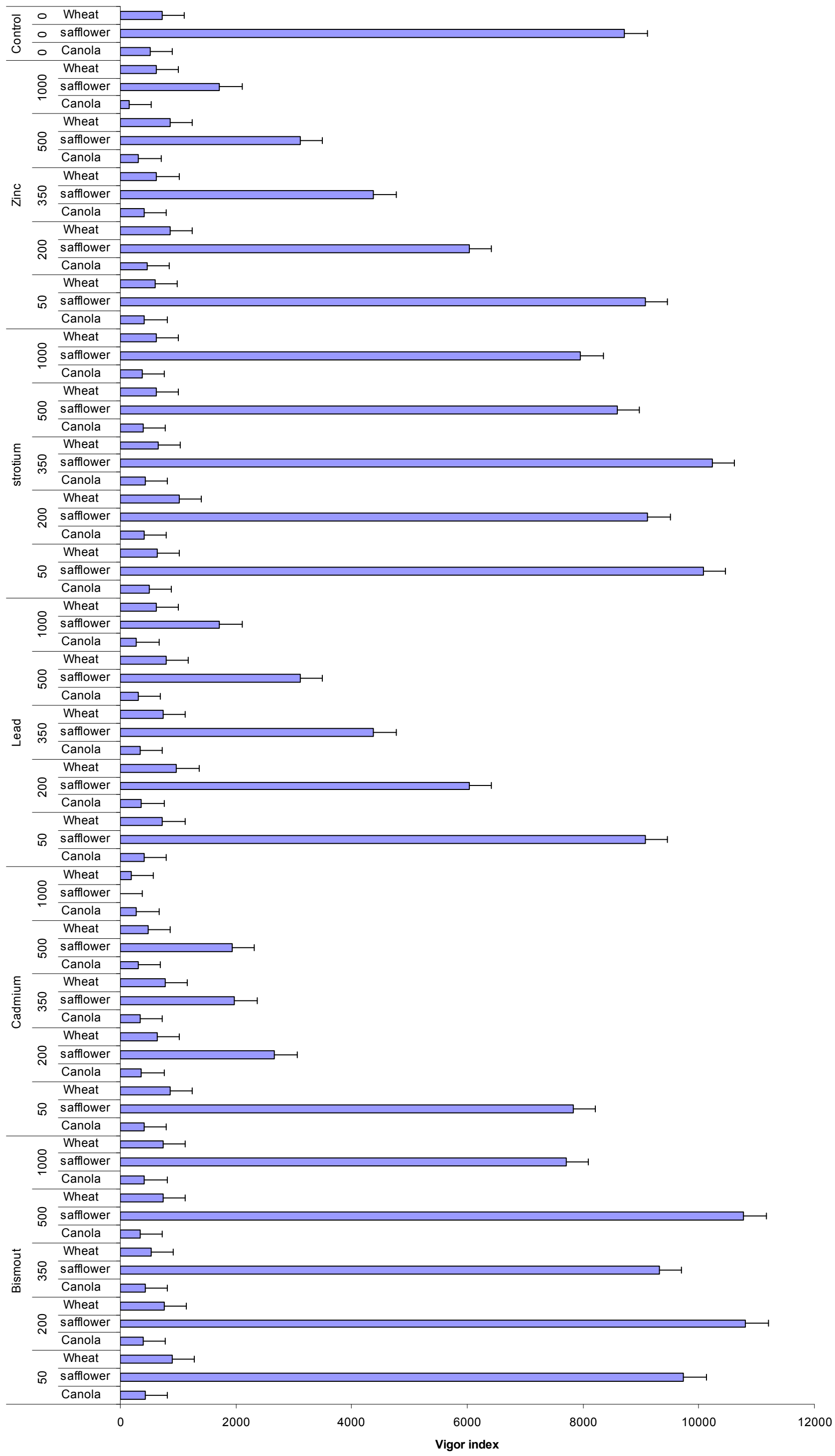

Figure 5. Effect of different heavy metals on seed vigor

Recent studies suggested that heavy metals are taken up by the cell through metal transporters. Some protein transporters have recently been identified (Clemens, 2001).

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