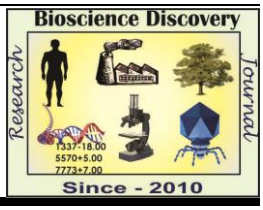
Bioscience Discovery, 8(3): 382-387, July - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article



Heavy metal induced toxic effect on growth and biochemical activities of turnip (*Brassica Rapa*) seedlings

Amrit Kaur, Gagandeep Kaur and Avneet Kaur*

GGDSD College, Sector-32C, Chandigarh (UT) India -160030
*Corresponding Author
avbawa@yahoo.co.in

Article Info

Received: 19-05-2017,
Revised: 22-06-2017,
Accepted: 26-06-2017

Keywords:
Antioxidant, Biochemical, *Brassica Rapa*, Heavy metals, Toxicity

Abstract

The present study was performed in order to evaluate turnip *Brassica Rapa* response to heavy metal (Fe, Mg, Cu, Zn, and Cd) stress. The effect of heavy metals on the seedling growth and biochemical parameters like protein content, antioxidant content, flavonoid content and total phenolic content was evaluated under *in vitro* laboratory conditions. The sterilized turnip seeds were placed in Petri dishes containing Whatman filter papers having different concentration (10, 20, 30, 40 and 50mM conc.) of heavy metals. There was significant decrease in seedling growth and root/shoot length, protein content, antioxidant content, flavonoids content and total phenolic content except at some lower conc. (10mM, 20Mm) of heavy metals whereas there was significant increase in their values which is due to plant tolerance mechanism for cellular detoxification.

INTRODUCTION

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag) and the platinum group elements.

The exposure of plants to toxic levels of heavy metals triggers a wide range of physiological and metabolic alterations resulting in reduction in plant growth (Sharma and Dubey, 2007). It include leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a crippled photosynthetic apparatus, often correlated with progressing senescence processes leading to plant death (Dalcarso *et al.*, 2010).

Heavy metals can be divided into two groups: redox active (Fe, Cu, Cr, Co) and redox inactive (Cd, Zn, Ni, Al, etc.). Heavy metal toxicity could be due to stimulation of ROS and MG production by auto-oxidation and the fenton reaction or by modification of the antioxidant defense system and the glyoxalase system (Emamverdian *et al.*, 2015). Due to direct interaction with proteins due to their affinities for thioyl-, histidyl-, and carboxyl-groups heavy metals target structural, catalytic, and transport sites of the cell because of which there is displacement of essential metal ions from specific binding sites, causing function to collapse (Clemens and Ma, 2016).

Metal toxicity has high impact and relevance to plants and consequently it affects the ecosystem, where the plants form an integral component. Plants growing in metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass production and metal accumulation (Bapurao and Popatrao, 2017). Various physiological and biochemical processes in plants are affected by metals.

The contemporary investigations into toxicity and tolerance in metal-stressed plants are prompted by the growing metal pollution in the environment (Delbari and Kulkarni, 2011)

Keeping in mind the rising concern of metal contamination of soil, we focused our attention to study the effects of heavy metal stress on various growth parameters like seed germination, root/shoot length and various biochemical assays like protein estimation, antioxidant activity, phenolic content, flavonoids etc in *Brassica Rapa*.

MATERIAL AND METHODS

The present study was carried at Dept. of biotechnology at GGDSD College, Chandigarh in November, 2016

Seed preparation: The seeds of turnip were sterilized and were placed in plastic dishes (in triplicate). Fifteen seeds were equally placed into each dish on the surface of filter paper, and 5 mL tested aqueous solution with heavy metal was added The stock solution [0.5M] of $FeSO_4$, $CuSO_4$, $MgSO_4$, $ZnSO_4$, and $CdCl_2$ were prepared in autoclaved distilled water. Then these stock solutions were diluted to 10mM, 20mM, 30mM, 40mM and 50mM with the help of distilled water.

Preparation of Homogenate: The germinated seeds were homogenized in distilled water. The supernatant was collected as a sample for testing of various parameters and debris was discarded.

Statistical analysis: Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of heavy metal treatment were considered statistically significant when $P < 0.05$.

Protein Estimation: The protein concentration determination was done by the Lowry protein assay method (Lowry *et al*., 1951).

Estimation of Total Phenolics by modified Folins-Ciocalteau method: The hydroxyl (-OH) group of phenolic compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of an alkaline medium (present in Folin's reagent). The blue coloured complex was then spectrophotometrically measured at wavelength 760nm **(Li *et al*., 2007)**.

Estimation of Antioxidant activity by FRAP assay: The antioxidants present in the sample reduce $Fe3^+$ to $Fe^{2+.}$ This ion conjugated with the ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at wavelength 700nm (Benzie and Strain, 1996) with some modifications.

Estimation of Flavonoids by Aluminium Chloride method: Flavonoids present in the extract formed a charge transfer complex with several heavy metals to give a pink coloured complex that was spectrophotometrically measured at wavelength 510nm (Almaraz-Abarca *et al*., 2007).

RESULTS AND DISCUSSION

Metal toxicity is an important factor governing germination and growth of plants. The effects of toxic substances on plants are dependent on the amount of toxic substance taken up from the given environment. Seedling growth is considered as an indicator of metal stress on plant ability to survive. The toxicity of some of the metals may be large enough that, plant grown is retarded before large quantities of the element can be transferred.

The significant decrease in seed germination in seeds treated with 30mM, 40mM and 50mM conc. of cadmium and copper implies that uptake and accumulation of Cd and Cu effects plant development. However, in case of iron the germination was inhibited at higher conc. (50mM conc.). Magnesium had no effect on the inhibition of germination of seeds and could accumulate in the seeds without any metabolic disturbance. Reduction in seed germination of turnip could be due to accumulation of metal in their cell sap, failure of sub-cellular organelles to adjust to high metal concentration, accelerated breakdown of stored food materials in seed by the application of heavy metal mixture or due to alterations of selective permeability properties of cell membrane.

The high conc. of metals (30, 40 and 50mM) Fe, Cu, and Cd caused significant decrease in root/shoot length. However, lower conc. of metals (10mM, 20mM) had no affect on root/shoot length except in cadmium ($p \leq 0.05$). Cd toxicity even at low conc. could be due to the inhibition of root cell division/root elongation or due to the extension of cell cycle in the roots, inducement of chromosomal aberrations and abnormal mitosis (Nazar *et al*., 2012). The reduction in root/shoot length due to metals like Fe could be due to its toxic effect on photosynthesis, respiration and protein synthesis (Mittal *et al.*, 2017). There was significant decrease in protein conc. when the seeds were treated with varying conc. such as 10, 20, 30, 40 and 50mM conc. of Cd, Zn, Fe and Mg ($p \leq 0.05$) (Fig.1) which could be due to enhanced protein degradation as a result of increased protease activity (Palma *et al*., 2002).

Cd resulted in a significant inhibition of protein level in Brassica juncea L. and root tips of barley seedlings (Singh and Tewari, 2003). Metal toxicity induced lipid peroxidation and fragmentation of proteins could be due to generation of reactive oxygen species. At lower conc. i.e.10 and 20mM there was significant increase in proteins in Cu treated seeds as compared to control ($p \leq 0.05$). Increased synthesis of stress proteins in Cu treated seeds could be a plant tolerance mechanism for cellular detoxification (Sabatini *et al*., 2009).

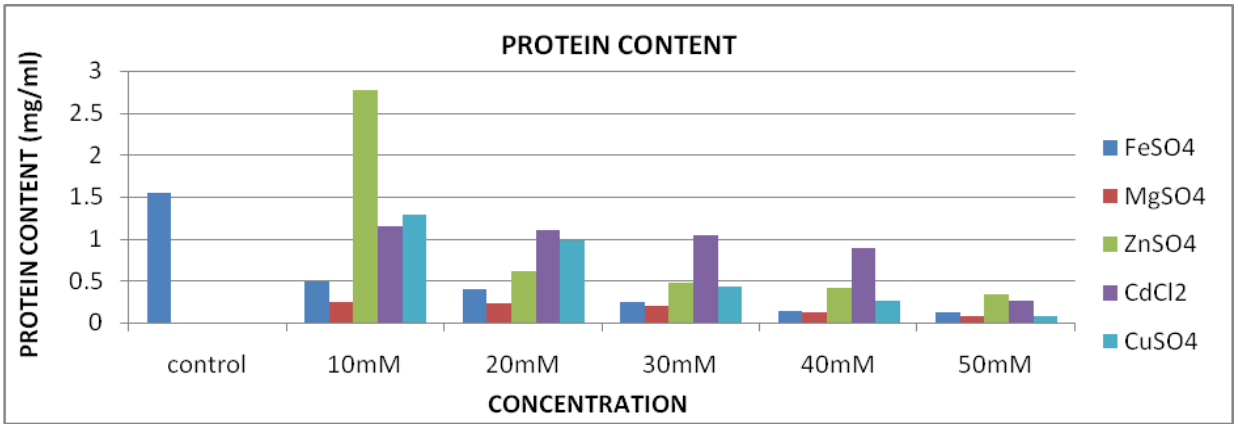


Fig. 1: Effect of varying conc. of metals on Protein content.

There was overall reduction in antioxidant activity between the groups when the seeds were treated with varying conc. such as 10, 20, 30, 40 and 50mM conc. of Cu, Cd, Zn, Fe and Mg (Fig.2) suggesting that plant uses its antioxidants to combat oxidative stress by neutralizing free radicals . However, there was significant decrease in antioxidant activity at 10 and 20mM conc. of Fe and Mg ($p \leq 0.05$). In seeds treated with Cd and Cu there was increase in antioxidant activity at 10 and 20mM conc. of metal as reported by Gulcin *et al*., 2004. Antioxidants like cysteine, proline, ascorbic acid and non-protein thiols (sulfhydryl) play an important role in detoxification of toxic metal ions by inducing resistance to metals by protecting labile macromolecules (Singh and Sinha, 2005)

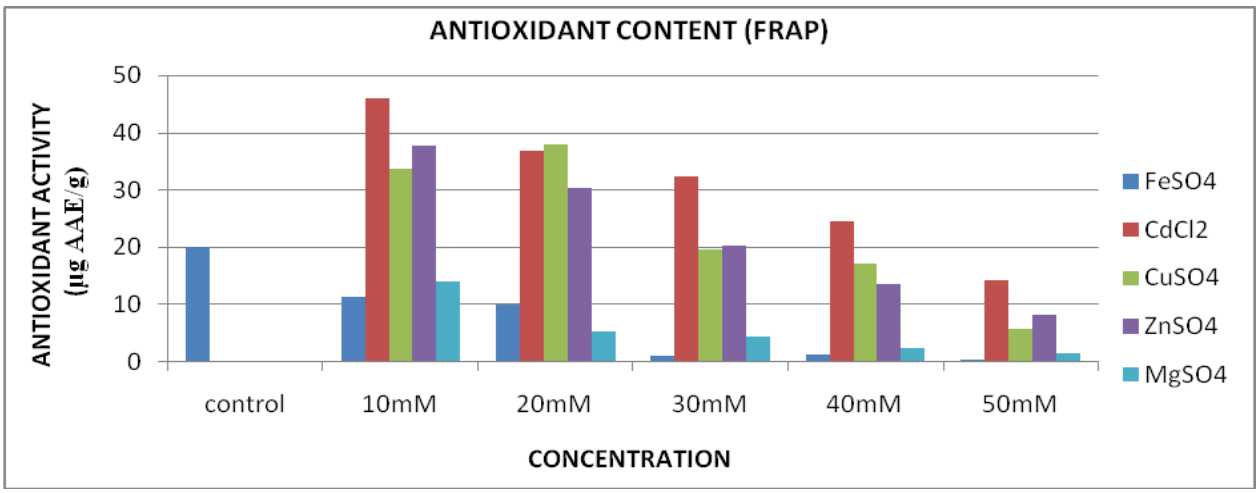


Fig. 2: Effect of varying conc. of metals on Antioxidant Activity.

There was significant decrease in flavonoids content between the groups when the seeds were treated with varying conc. such as 10, 20, 30, 40 and 50mM conc. of Cu, Cd, Zn, Fe and Mg ($p \leq 0.05$) (Fig.3). Flavonoids have multiple protective functions such as antioxidative activity and act as protectants against heavy metal stress However, there was increase in flavonoids content

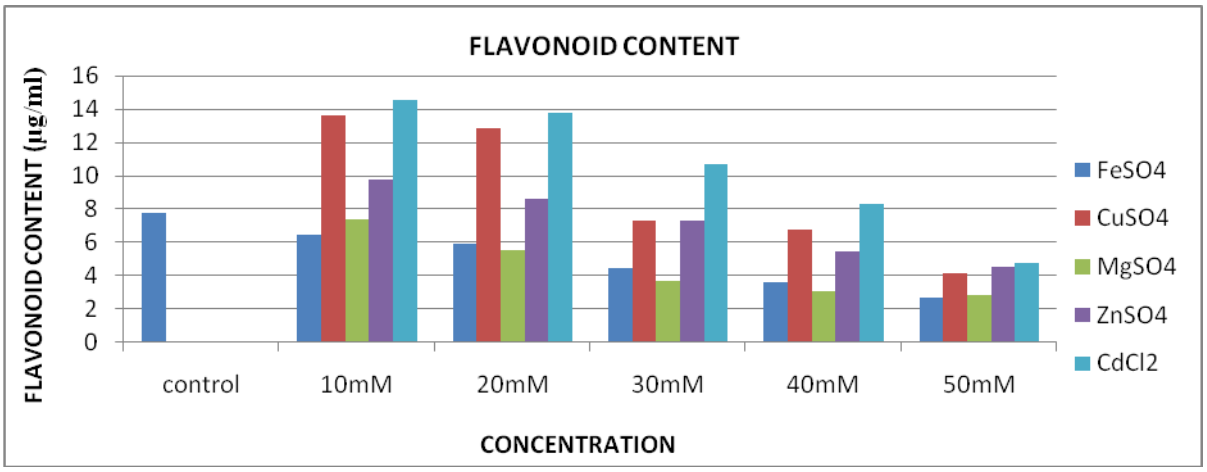


Fig. 3: Effect of varying conc. of metals on Flavonoids.

when seeds were treated with 10mM conc. of Cu as reported by Mohsenpour (2015). During metal stress, phenolic compounds functions as intermediates in lignin biosynthesis and builds physical barrier to preserving the plant cells (Diaz *et al.*, 2001). There was overall increase in total phenolic content between the groups when the seeds were treated with varying conc. such as 10, 20, 30, 40 and 50mM conc. of Cu, Cd, Zn, Fe and Mg (Fig.4) which proves the antioxidative properties of phenolic compounds in plant response to oxidative stress There was significant increase in phenolic content ($p \leq 0.05$) at 20, 30, 40 and 50mM conc. of Cd as reported by Rastgoo and Alemzadeh (2014). During metal toxicity, phenolics act as metal chelators and scavenge molecular species of active oxidant (Kovacik *et al.*, 2009) increase activity of enzymes involved in phenolics synthesis, decrease the ROS synthesis by the prevention of reactions superoxide production (Fenton reactions), inhibit the lipid peroxidation, membrane stabilization, and prevent the ROS transportation (Michalak, 2006).

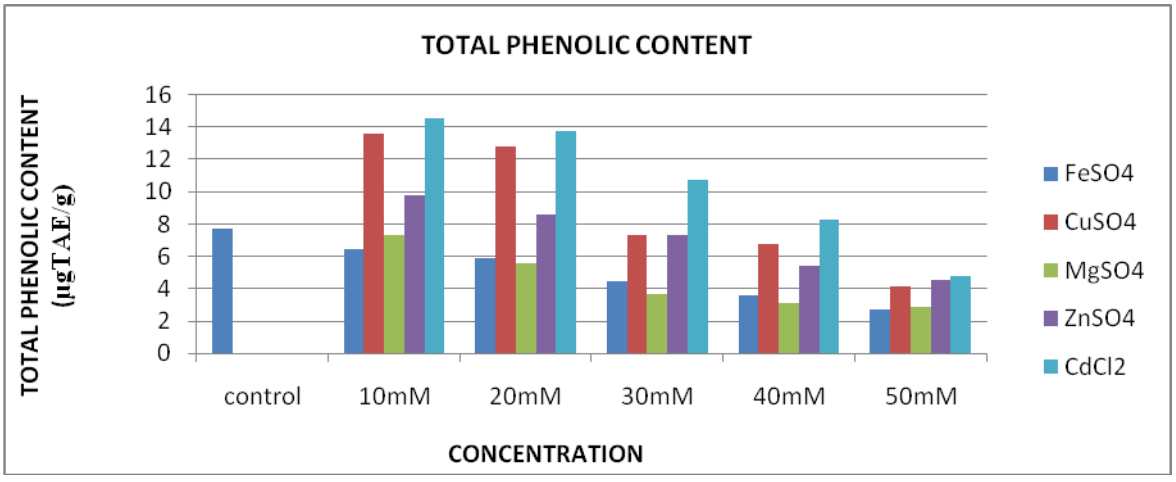


Fig. 4: Effect of varying conc. of metals on Total phenolic content.

The present study gives a understanding of effect of varying conc. of various metals on oxidative stress and possible induction of defence mechanism in *Brassica rapa* showing that higher concentration of metal ions causes toxic effects on turnip seeds like growth inhibition, reduction in root length and shoots length also. The results indicated that there was significant reduction in protein content, antioxidant activity and flavonoids between the different metal groups and increase in total phenolic content. In lower concentration of metal ions (like 10mM and 20mM) there was no significant reduction in seed germination and on antioxidant levels.

The plant counteracts the higher concentration of ROS produced under metal toxicity by a coordinated increase of phenolics or by increased activities of various antioxidant enzymes.

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How to Cite this Article:

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