ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Constitutive Effects of Distinct Heavy Metals (Cd, Pb and As) on Seed Germination and Physiological Characters of Groundnut (*Arachis hypogaea* L.)

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Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 11 July 2015
Published Online: August 2015

Key words:

Groundnut, Heavy metals, Bio mass, Tolerance index, phyto toxicity and Photo synthesis

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Abstract

Heavy metals are carcinogenic in nature, damage DNA, proteins and lipids that adversely affects plant growth and yield. The deterrent heavy metals cadmium, lead and arsenic results in root growth inhibition by accumulation of reactive oxygen species. These cellular derailment results change in antioxidant activity, inhibition of transpiration and photosynthesis, disturbs carbohydrate metabolism, lipid peroxidation leading to secondary stresses like nutrition stress. Groundnut is the king of oil seed crops and is very sensitive to low concentration of heavy metals. The present study deals with the effect of different concentrations of heavy metals on groundnut when applied individually and in combination, the germination rate, root length, shoot length, biomass, seedling vigour index, tolerance index, phyto toxicity and seedling inhibition rate was decreased. Shoots become thick and thin, length and surface area of leaves are reduced and leaf plasticity occurred. Interestingly cd shows different results like increased germination percentage, increased root length, shoot length especially in combination forms at particular concentration levels. The results indicates that heavy metals like Cd, Pb , As, Cd + Pb, Cd + AS, Pb + As and Cd + Pb + As will cause phenotypic changes in groundnut which will leads drastic effects on plant metabolisms which cause crop yield loss and ecological imbalance. Cd alone and in combinational forms shows increased germination rate and root length elongation. This study shows the Cd ability in restoring the plant defects caused by Pb and As.

INTRODUCTION

1. Background:

Abiotic stresses like heat, cold, drought, salinity, nutrient and metal stress have huge impact on world agriculture and reduce up to 50% of yield in crop plants (Wang et al., 2003). Heavy metal accumulation in soil and plants is alarming and it adversely affects human health. Metals in low concentrations are essential for growth and at high concentrations they are toxic in nature. Soil pollution by heavy metal accumulation leads to damage ecological and environmental balance and subsequent toxicological problems (Raskin et al., 2000) especially in arid zones with saline soils. Various operations like application of pesticides, phosphatic fertilizers, sewage sludge application, dust from smelters, industrial waste and bad watering practices in agriculture, metallurgical industries, mining operations, discharge of untreated industrial waste, unmanaged human activities such as livestock grazing, logging, intentional fire, burning of fossil fuels and eutrophication lead to the release of various heavy metals such as Fe, Cu, Cd, Cr, Zn, Ni, Co, Cr, Pb and Hg into the environment (Bell et al., 2001, Passariello et al., 2002; Chandra et al., 2009 and Gupta et al., 2010) results in environmental degradation. These adversely affects germination, growth and biodiversity of plants and leads to DNA damage, yield losses and their carcinogenic effects in animals and humans are most probably caused by their mutagenic ability. Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for the production of food affects crop growth, interfere with metabolic functions including physiological and biochemical processes, inhibition of photosynthesis, respiration and degeneration cell organelles (Garbisu and Alkorta, 2001; Schmidt, 2003; Schwartz et al., 2003 Wang et al., 2003 and An et al., 2004; Gratao et al., 2005; Clemens, 2006; Hong-Bo et al., 2010). For example, the diseases known as *itai-itai* and *minamata* were caused by the production of paddy rice grown on soil contaminated with Cd and Hg in Japan (Asami, 1981).

Based on genotoxicity, metals are categorized into: essential and relatively non toxic (e.g. zinc), essential and extremely toxic at elevated concentrations (e.g. copper) and nonessential but highly cytotoxic (e.g. cadmium). Metals like zinc, iron, manganese, nickel and copper are essential micronutrients required for a wide range of physiological processes in plant. They are needed for metal-dependent enzymes, proteins and participate in metabolism like photosynthesis, enzyme metabolism, growth and development (Millalea et al., 2010). However, at elevated levels they are toxic and, result in root growth inhibition, accumulation of reactive oxygen species (ROS) leading to damage of lipids, proteins and DNA. The cellular damage brings change in the activities of antioxidant enzymes such as SOD (superoxide dismutase), catalase (CAT) and guaiacol peroxidase (POD) (Demirevska-Kepova et al., 2004; Maleva et al., 2009). Metals like arsenic, mercury, cadmium and lead are non essential and potentially highly toxic. Phytotoxicity of heavy metals inhibits transpiration and photosynthesis, disturbs carbohydrate metabolism, and drives the secondary stresses like nutrition stress and oxidative stress, initiate hydroxyl radical production, lipid peroxidation, H_2O_2 accumulation, and an oxidative burst which cannot be controlled by antioxidants, by which collectively affect the plant development and growth especially root growth inhibition, with the root apex being the most sensitive part of the root (Kramer and Clemens, 2005 &; Kochian et al., 2005; Rellan-Alvarez et al., 2006; Lequeux et al., 2010).

In *Triticum aestivum* L. reduction of lamina, mesophyll thickness and epidermal cell size was observed due to excess heavy metals like Cd, Pb and Ni (Kovacevic et al., 1999). In fruit vegetables the problem was currently not wide spread, some reports shows that in Japan (Arao et al., 2008) approximately 7% of eggplant (*Solanum melongena* L.) fruit contain cadmium at concentrations exceeding the internationally acceptable limit for fruiting vegetables. In addition to its adverse impact on human health, Cd adversely show effect on plants, including water transport, nitrogen metabolism, oxidative phosphorylation in mitochondria, photosynthesis, chlorophyll content and also shows visible symptoms of chlorosis, growth inhibition, browning of root tips, and finally death (Burzynski and Klobus et al., 2004; Wojcik and Tukiendorf et al,. 2004; Djebali et al., 2005; Mohanpuria et al., 2007 and Feng et al., 2010). Cadmium, do not undergo detoxification immediately, it may trigger ROS and damages the redox control of the cell, leading to growth inhibition, stimulation of secondary metabolism, lignification and finally cell death leads to unspecific necrosis.

Lead (Pb) is one of the ubiquitously and abundantly distributed toxic elements in the soil. It exerts adverse effects on morphology, growth and photosynthetic processes of plants. Elevated level of Pb causes inhibition of enzyme activities at cellular level by reacting with their sulfohydril groups, water imbalance, alterations in membrane permeability and disturbs mineral nutrition (Sharma and Dubey et al., 2005). It induces oxidative stress by increasing the production of ROS in plants (Reddy et al., 2005). Arsenate (As) is an analog of phosphate (P) and competes for the same uptake carriers in the root plasma lemma of plants (Meharg and Macnair et al., 1992) that can disrupts at least some phosphate-dependent aspects of metabolism. It can be translocated across cellular membranes by phosphate transport proteins, leading to imbalances in phosphate supply. It can compete with phosphate during phosphorylation reactions, leading to the formation of As adducts that are often unstable and short-lived. As an example, the formation and rapid auto hydrolysis of As V-ADP sets in place a futile cycle that uncouples photo phosphorylation and oxidative phosphorylation, decreasing the ability of cells to produce ATP and carry out normal metabolism. Arsenic exposure induces ROS production, that can leads to the production of antioxidant metabolites and numerous enzymes involved in antioxidant defense mechanism. It also results change in oxidative carbon metabolism, amino acid and protein relationship, nitrogen and sulfur assimilation pathways (Patrick M. Finnegan and Weihua Chen et al., 2012). All these metals cause a transient depletion of GSH and an inhibition of anti oxidative enzymes, especially glutathione reductase.

Groundnut (*Arachis hypogaea* L.) ranks 4th as source of edible oil, 3rd as most important source of vegetable protein and 13th most important food crop in the world, hence considered as king of oil seeds (Sudhir et al., 2010). Groundnut is an important oil seed crop in India, Andhra Pradesh is the largest edible oil producer followed by Tamilnadu, Karnataka and Gujarat. Peanut (*Arachis hypogaea* L.), which is a principal agricultural crops in the world it has been demonstrated to be highly sensitive even at very low cadmium concentrations (Dinakar et al., 2008). ICGV 87846 semi-erect type was significantly superior to all other genotypes maturing in 125-135 days and has recorded up to 2,400 kg / ha under severe drought and is also tolerant to foliar diseases *viz*., rust and late leaf spot and there by yields higher haulms which are good cattle feed. The following questions were posed: how do peanut plants respond to distinct heavy metal in terms of morphology, anatomy and physiology like germination rate, shoot length, root length, fresh weight and dry weight. Because some studies demonstrate that peanut plants very sensitive to low concentrations of heavy metals. One of the most important stage in life cycle of plant is germination and seedling establishment (Vange v et al., 2004). The present investigation was carried out to observe phenotypic variations of peanut seedlings against different concentrations of three heavy metals stress treatments when applied individually and in combination (Cd, Pb and As) because germination and seedling growth are important aspects in present environmental situation to tolerate and establishment themselves (Welbaum G E et al., 1998).

2. MATERIALS AND METHODS:

Groundnut seeds ICGV 87846 procured from the ICRISAT, were used. Heavy metals like cadmium acetate [Cd $(CH_3COO)_2$], lead acetate [Pb $(C_2H_3O_2)_2$] and arsenic penta oxide [As_2O_5] (sigma Inc.) were used. Different concentrations of metal solutions were prepared like 100, 200, 400, 800, 1600, 2400 and 3200 ppm (µmol/L). The plants are treated with these metals individually and in combination (Cd, Pb, As, Cd + Pb, Cd + As, Pb + As and Cd + Pb + As). The seeds were washed under running tap water for 2-3 times, then washed with distilled water, treated with sodium-hypo chloride / mercuric chloride for five minutes to avoid fungal contamination. After the treatment with sodium-hypo chloride / mercuric chloride the seeds were washed with distilled water several times. After washing, the seeds were sown three seeds per pot and labelled according to stress treatments and irrigated with 5 ml of prepared metal solutions according to labeling. This will be continued about 14 days, controls were maintained by irrigating with normal water. This entire program was maintained, at temperature 28±2 °C temperature under a photoperiod of 12 hr in glass house. The seedlings were harvested after 14 days and the germination percentage, root and shoot length and bio mass were recorded.

Germination percentage was calculated by using number of seeds germinated/ number of seeds planted × 100.
Root and shoot length were measured by using scale and seedling fresh bio mass were measured by electronic balance and dry biomass was determined by placing the seedling in oven at 80°C for 24 hours, then weigh the dried seedling by using electronic balance.

Moisture percentage was calculated by formula:

$$\textbf{Moisture \%} = \frac{\text{fresh wt of seedlings- dry wt of seedlings}}{\text{fresh wt of seedlings}} \times 100$$

Seedling vigour index was calculated according to the formula (Abdul-Bakiand and Anderson et al., 1973)

Vigour index = germination percentage X length of seedlings

Tolerance index was calculated according to the equation (Turner and Marshal et al., 1972).
Tolerance index = length of longest root in treatment/ length of longest root in control

$$\textbf{Percent Phytotoxicity} = \frac{\text{radicle length of control - radicle length of test}}{\text{Radicle length of control}} \times 100$$

% inhibition of seedling growth was calculated according to
% reduction = value in control – value in treatment / value in control X 100

3. Results and Discussion:

3.1 Effect of heavy metals on groundnut seed germination:

Heavy metals exposure results in reduction in germination rate, shoot length, root length, biomass and tolerance index. Excess concentrations of heavy metals affected the plant growth and metabolism (Foy et al., 1978). Copper at low concentration is important for certain metabolisms during plant development like leaf and reproductive organs, and is toxic at elevated leading to chlorosis, necrosis, stunting, leaf discoloration and inhibition of root growth (van Assche and Clijsters, 1990; Marschner, 1995). Cd was the most toxic heavy metal which inhibits seed germination and also it shows more inhibition in germination and seedling growth of soybean rather than Cu, Ni and Zn (Dubey and Dwivedi et al., 1987). The reduced germination percentage and growth of plants observed during Cd stress, which alters the biochemical, physiological and molecular functions of plants and shows stunted growth, leaf chlorosis and damage the various metabolic pathways by altering the activity of many key enzymes (Bharti and Singh, 1994; Di Toppi and Gabbrielli, 1999; Chaudhary and Singh, 2000; Sharma et al., 2010). In general, while increasing the concentration of metals, there is a reduction in seed germination rate. Germination rate was studied after 7 days of sowing. (**Fig 1**). It is observed in Pb and As treatments, but in the case of cadmium it is reverse, the germination rate increases with increase in concentration. In combination of stress treatments Cd along with Pb and As at 800 ppm concentration shows maximum germination compare to AS + Pb treatment. Cd shows only 33% seed germination inhibition at 2400 and 3200 ppm but Pb and As shows complete germination inhibition, along with Cd in combinational form at these stress treatments they show 33% germination frequency (**Fig 1**). Hg and Mn inhibit the barley seedling germination and growth while the concentrations increases (Pathak et al., 1987). In *Spartina alterniflora* germination percentage and speed of germination was reduced by Hg and Cd (Mrozek, 1980). Germination percentage of *spartina alterniflora* was inhibited by Pb and stimulated by Zn and Cd (Mrozek and Funicelli, 1982). In this experiment Pb and As inhibits the seed germination, growth and biomass and which was stimulated by Cd. The seed germination in the presence of high concentrations of heavy metals occurs due to the accelerated breakdown of stored food material and due to changes of selection permeability of cell membrane (Heidari et al., 2011 and Shafiget al., 2008). The embryos against heavy metals are protected by tissue covering, which have selected penetration but the following seed growth was virtually effected by lesser concentrations (Weiqiang et al., 2005).

3.2 Shoot and Root length of Groundnut seedlings:

Heavy metals inhibit the growth of *Phaseolus aureus* (Sharma S.S, 1982), reduction in meristematic cells and some enzymes present in cotyledon and endosperm which are participated in different metabolisms cause reduced shoot length. The maximum shoot length of control plant was 14.8 cm. Pb and As at 100 ppm stress treatment shows 11% increase compare to control shoot, while cd shows 27% decrease. While increasing Cd concentration at 200 ppm 13%, at 400 ppm 12% at 800 ppm 8% enhanced shoot size observed as compared with the control group. But Pb and As shows decreased shoot length. At 200 ppm Cd+Pb shows 20% increase compare to Pb at 200 ppm concentration. Cd+ As shows 36% shoot length increase compare to As at 200 ppm (**Fig 2**). While increasing concentrations of Pb and As shows decreased shoot length compare to Cd and controls but in combinational forms like Cd + Pb, Cd + Pb + As shows enhancing shoot lengths compare to individual stress treatments. The heavy metals adversely affect on roots and shoot growth, but roots are less affected compared to shoots, because root translocates the heavy metals in to shoot and cause reduction in leaf pigment and also in fresh weight of the plant.

High concentration of heavy metals leads root hair browning (Punz and Sieghardt et al., 1993) is the result of suberin deposits. Reduction in mitotic cells in meristematic zone of root is the result of decreased root length due to metal stress (Lerda, D et al., 1992). The maximum root length of control Groundnut seedlings was 11 cm. The stress treatment of Cd promoted the root growth by 22% at 200 ppm, 17% at 400 ppm, 14.5% at 800 ppm and 11% at 3200 ppm concentrations compare to controls. The heavy metals Pb and As at 100 ppm concentrations increased the root length 11% and 13% compare to controls. However at the same dose Cd reduce the root growth 6.6% as compared to controls. Pb and As shows a concentration dependant suppression of root growth, while comparing Cd increase at certain concentrations. Pb along with combination of Cd promoted the root growth 14 % at 200, 400, 800 and 1600 ppm concentrations compare to control root growth. As in the presence of Pb shows 19% root enhancing compare to controls and 14% increased root length compared to AS individually at 100 ppm (**Fig 3**). While increasing concentration Pb and As suppresses root length, root hair development and growth due to decreased photo synthetic rate and stomatal closure (Sarkar et al., 1986).

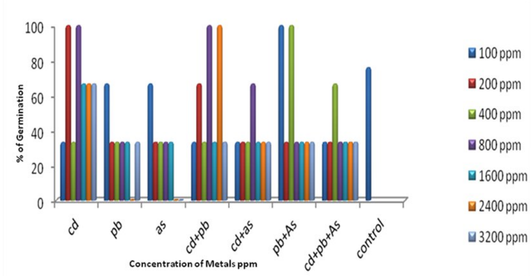

Fig 1 Germination Percentage of Groundnut seedlings

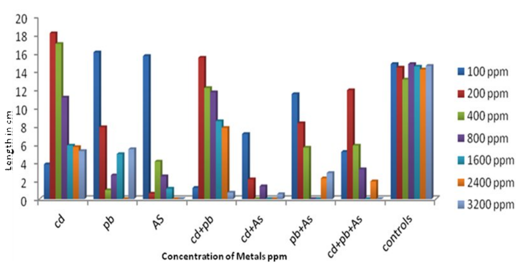

Fig 2 Shoot lengths of Groundnut seedlings upon exposure to Cd, Pb and As

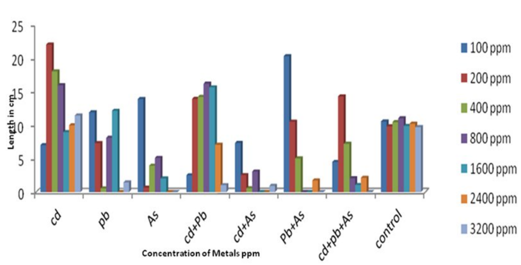

Fig 3 Cd, Pb & As effect on Root length of Ground seedlings

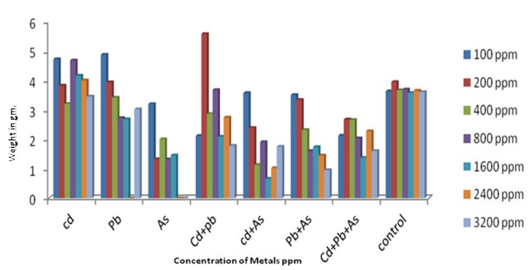

Fig 4 Cd, Pb and As effect on Fresh Weights of Groundnut seedlings

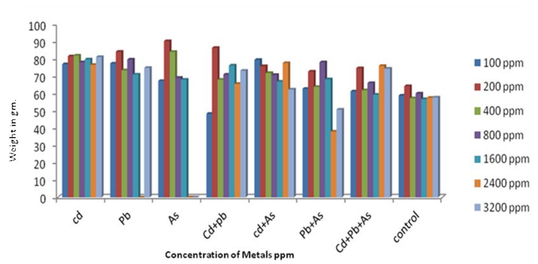

Fig 5 Effect of Cd, Pb & As on Dry Weight of Groundnut seedlings

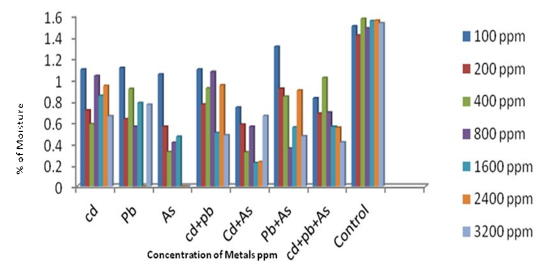

Fig 6 Groundnut Seedlings Percentage of Moisture alters by Cd, Pb & As

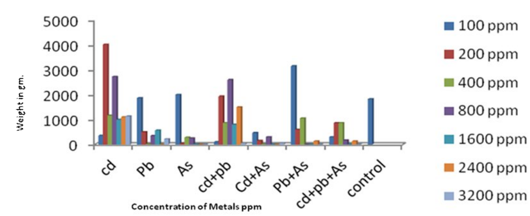

Fig 7 Groundnut Seedling Vigour Index altered by Cd, Pb & As

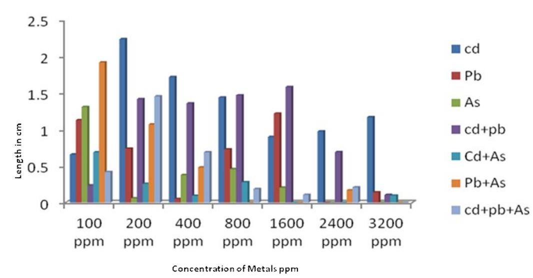

Fig 8 Illustrates Groundnut Seedling Tolerance Index after exposing to Cd, Pb & As

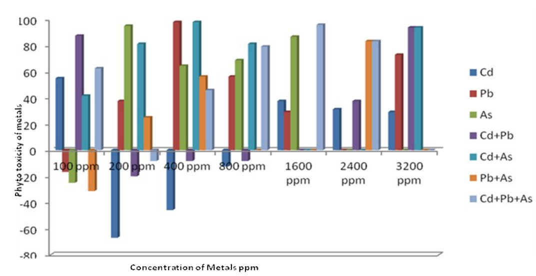

Fig 9 Phyto toxicity of Cd, Pb & As on Groundnut Seedlings

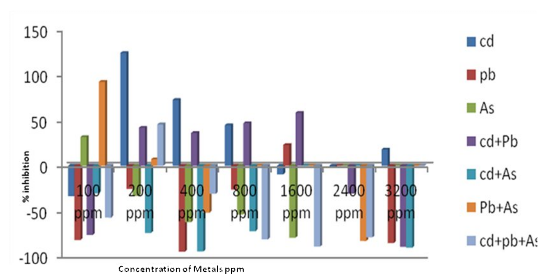

Fig 10 Inhibition of Growth of Groundnut Seedlings exposure to Cd, Pb & As

Fig. 1 – 10 Effects of Heavy metals Cd, Pb and As on Groundnut seedlings

3.3 Bio mass:

The reduced bio mass indicates the plants sensitivity to various stress viz heavy metal stress that damage physiological functions (Haghiri et al., 1973). By increasing the concentrations of metals decreasing the fresh weight was observed. While applying Cd individually and in combinational forms like Cd + Pb and Cd + Pb + As enhancing fresh weights observed at certain concentrations like 800 and 1600 ppm compare to controls. Cd and Pb alone promoted 13% more fresh weight compared to control fresh weight (**Fig 4**). While increasing the concentrations of stress treatments the dry weight was decreased compare with control dry weights. Cd along with Pb show enhanced dry weight compare to individual treatments at 400 and 800 ppm concentrations. As in the presence of Pb shows increased dry weight compare to alone (**Fig 5**). Reduced biomass results as application of Cd to plant rhizosphere in many plants (Bharti and Singh, 1994; Bharti et al., 1996; Chaudhary and Singh, 2000; Correa et al., 2006; Simnova et al., 2007), due to formation of metal thiolate bond and alteration of cell wall and membrane permeability by binding of nucleophilic groups (Stohs et al., 2000; Schutzendubel et al., 2003) causes damages root tip, reduce nutrient and water uptake, impairs photosynthesis and inhibits growth of the plants (Chaudhary and Singh, 2000; Jihen et al., 2010; Lag et al., 2010). Increased concentration of Cadmium reduced percent moisture content in 7 day old Indian mustard seedlings (Rana P. Singh, 2011). In the present investigation increased concentration of Pb and As shows reduced percent moisture level but Cd individually and in combinational forms shows increased percent moisture levels compare to controls, but in the case of Pb and As combinational form it dicreases the percent moisture. (**Fig 6**).

3.4 Groundnut Seedling vigour index, Tolerance index and Phyto toxicity:

Seedling vigour index decreasing while increasing concentration of heavy metals. Cd shows the maximum vigour index at 200 ppm 4017, 2723 at Cd 800 ppm and 2600 at combined treatment of Cd + Pb at 800 ppm. Cd along with Pb and As shows increased vigour 866 at 200 and 400 ppm concentrations and later it decreases. The control shows 1824 vigour index and the minimum observed in Pb and As stress treatments 0 at Pb 2400, As 2400 and 3200, Cd + As 1600 and 2400, Pb + As 800, 1600 and 3200 and cd+pb+As 1600 and 3200 ppm concentrations (**Fig 7**). The maximum tolerance index 2.24 observed in Cd 200 ppm concentration, 1.72 observed at Cd 400, 1.42 at Cd 800 and 1.17 at Cd 3200 ppm. The tolerance index decreases with increasing stress treatment concentrations. In case of Cd along with Pb combination it gradually increases at particular concentration level and again it decreases, the maximum tolerance index 1.584 at 1600 ppm observed in increasing concentration and minimum 0.106 at 3200 ppm was observed (**Fig 8**). While increasing the concentration of metals the phyto toxicity was decreased. By increasing Cd concentration in rhizosphere increased phyto toxicity and decreased tolerance index observed in *Cicer arientinum* and *Phaseolus mungo* (Kumar et al., 2009). But in the case of Cd at 200 ppm shows 124.48 phyto toxicity and 2.24 tolerance index which is maximum. Cd in combinational forms along with Pb and As shows increased phyto toxicity and tolerance index (**Fig 8 & 9**). The metal tolerance index was found in contrast to the phytotoxicity for all the cultivars. The metal tolerance was highest in the cultivar Pusa Jai Kisan whereas, it was lowest in the cultivar Gangotri (Rana P. Singh, 2011).

3.5 Inhibition of seedling growth:

Inhibition of mitosis, reduction in synthesis of cell wall components, damage to the Golgi apparatus and changes in the polysaccharide metabolism results seedling growth inhibition (Heidari, M. and S. Sarani et al., 2011). Reduction in meristematic cells, inhibition of biochemical and some enzyme activity leads to inhibition of seedling growth under heavy metal stress (Malhotra S.S., 1981 and Kabir M., 2008). The inhibition of seedling growth of groundnut was increased with increase in the concentration of heavy metals. The Cd shows the seedling growth inhibition of 55% at 100 ppm, 37.5% at Cd 1600 ppm, 31.25% at Cd 2400 ppm, 29.16% at Cd 3200 ppm. Cd 200 ppm shows 67%, 400 ppm shows 45.8% and 800 ppm shows 12.5% more survivalance rate. Pb 100 ppm and As 100 ppm shows 16.6% and 25% more surviaval rate. Cd along with Pb shows more survival rate 8.3 % at particular concentrations like 800 ppm. Pb and As at high concentrations like 2400 and 3200 ppm individually and in combined forms shows complete seedling growth inhibition (**Fig 10**). However in the present investigation it was found that Cd shows less inhibitive effect compare to Pb and As individually and in combined treatments.

4.Conclusion:

The experiment conclude that heavy metals concentration on germination shows lesser effect but later even minute concentrations also repress the growth of seedlings. Previous reports shows that the effect of Cd on germination rate and root growth is more when compare to other heavy metals like Pb, Hg, As and Ni. But surprisingly in this experiment it promotes the germination and root length individually and in combination forms along with Pb and As, which will support the restoration capacity of Cd. ICGV 87846 could germinate and grow at high concentrations of heavy metals, suggesting that it confers tolerance to metals which will useful to studying molecular mechanism of heavy metal tolerance, role of metal transporters and gene networks involved in conferring tolerance.

Abbreviations

Cd: Cadmium acetate [Cd $(CH_3COO)_2$], Pb: lead acetate [Pb $(C_2H_3O_2)_2$], As: Arsenic penta oxide [As_2O_5], ppm: parts per million

Competing interest

The authors declare that they have no competing interests.

Author's contributions

M-NR carried out the germination and physiological studies. D-MR was an adviser to carry out the germination and physiological studies. A-S, M-NR and D-MR drafted the manuscript and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

M- NR is thankful to the UGC, New Delhi, for providing fellowship. D- MR is thankful to the UGC, New Delhi, for financial support through UGC-MRP.

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