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Mallikarjuna Kunjam
Lecturer in Botany, Department of Botany, D.N.R College, Bhimavaram-534202.

Hephzibah Govada
Lecturer in Chemistry, Department of chemistry, P.B. Siddhartha College of Arts & Science, Vijayawada- 522010.

Nataraj Mididoddi
Assistant Professor, Department of Chemistry, AmritaSai Institute of Science and Technology, Paritala, Kanchikacherla- 521180.

Ravi SLN Kota
Lecturer in Biotechnology, Department of Biotechnology, Teegala Krishna Reddy College of Engineering and Technology, Medbowli, Hyderabad, India---500079.

Correspondence:
Mallikarjuna Kunjam
Lecturer in Botany, Department of Botany, D.N.R College, Bhimavaram-534202.

Studies on selected heavy metals on seed germination and plant growth in pea plant (*Pisum sativum*) grown in solid medium

Mallikarjuna Kunjam, Hephzibah Govada, Nataraj Mididoddi, Ravi SLN Kota

Abstract
The present studies have shown that Pea plant (*Pisum sativum*) can grow in some heavy metal-contaminated soils. Based on that, we studied the individual effects of several doses of Zn, Ni, Cu, Cr and Cd on the growth of live Pea plants using solid growth media. The doses used in the present study were 0, 20, 40, 60, 80ppm. The seed germination and plant growth were significantly affected by Cd and Cr at60 ppm, as well as by Cu and Ni at 80 ppm and higher concentrations ($P<1\%$). Zn did not affect seed germination. The roots of the plants exposed to 20-40 ppm-dose of Zn, Ni, Cu, Cr, Cd grew more than the roots of control treatment by more than 30%. Exposure of 40 ppm of Cd reduced the shoot size by 40% as compared to the control. While Zn, Ni, Cu increased the shoot size by37.5%, 52.5% and 27.5% respectively; only Zn promoted the shoot growth at the dose of 40 and 60 ppm.

Keywords: Seed growth, *Pisum sativum,* heavy metals, Solid media.

1. Introduction

Heavy metal contamination of agricultural soil has become a crucial ecological concern due to their potential undesirable ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence, and their acute and chronic toxic effect on plants grown on such soils. Metal concentration in soil range from less than 1mg/Kg (ppm) to high as 100,000 mg/Kg, whether due to the geological origin of the soil or as a result of human activities[1,2]. Excess concentrations of some heavy metals in soils such as Zn, Ni, Cu, Cr and Cd have caused the disruption of plant, natural aquatic and soil microflora [3,4].Since the beginning of the industrial revolution, pollution of the biosphere with toxic metals has accelerated significantly[5]. However, assimilation over the life history of plants growing on contaminated soil can result in a very high concentration of these heavy metals. Currently, cleanup processes of heavy metals pollution are expensive and environmentally destructive [6]. Recently, scientists have started to generate cost-effective technologies that include the use of microorganisms, biomass and live plants in the cleaning process of pollutants [7,8].Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they many cause metabolic alterations and inhibits the growth in many plant species[9, 10, 11]. Researchers have reported that some plant species are endemic to metalliferous soils and can tolerate a higher concentration of heavy metals [12]. Several studies have been conducted in order to calculate the effects of different heavy metals concentrations of live plants [13, 14]. Most of these studies have been conducted using seedlings and adult plants [15, 16]. In a few studies, the seeds have been exposed to contaminates [17]. The present study reports the data regarding the ability of Pea (*Pisum sativum*) seeds to germinate and grow on media containing Zn, Ni, Cu, Cr and Cd ions.

2. Materials and Methods

Fresh Pea seeds were collected from local market of Vijayawada. The seeds of pea plant (*Pisum sativum*) were sterilized in 10% hydrogen peroxide for 20 min. The seeds were then thoroughly washed with distilled water and germinated on moistened filter paper at 25 °C in the dark. The uniform seedlings were then transferred to continuously aerated nutrient solutions containing KH_2PO_4, 0.5 mM; $Ca(NO_3)_2$, 1.25 mM; KNO_3, 1 mM; $MgSO_4$, 0.5 mM; Fe-KEDTA, 50 µM; $MnSO_44H_2O$, 5 µM; $ZnSO_47H2O$, 1 µM; $CuSO_45H_2O$, 1 µM; H_3BO_3, 30 µM, and $(NH_4)_6$ $MO_7O_{24}4H_2O$, 1 µM and Agar-agar, 1%w/v. The heavy metals: Zn (as $Zn(NO_3)_2$ $6H_20$), Ni (as $Ni(NO_3)_2$), Cu (as $CuSO_4$ $5H_2O$),Cr (as $K_2Cr_2O_7$) and Cd (as Cd (as Cd $(NO_3)_2$ $4H_2O$) were used at the concentrations of 0,10,20,30 and 40 ppm.

Plants were grown and treated in a growth chamber (26 °C/ 70% relative humidity during the day, 20 °C/90% during the night). A 16-h (daily) photoperiod was used with a light irradiance of 150 μmol·m–2·s–1 at the canopy level. For each treatment the pH was adjusted to 6.2. Each treatment was replicated three times for statistical purpose. The seeds were set under a photoperiod of 12 hr, and 25/20 ºC day/night temperature. The seedlings were harvested after four weeks and the germination rates, root and shoot length were recorded. The data were analysed through one-way analysis of variance (ANOVA) to determine the effect of treatment and least significant difference (LSD) tests was performed to determine that statistical significance of the differences between means of treatment.

3. Results and Discussion

3.1 Effect of Heavy Metals on seed germination

Fig 1 shows that effect of the concentration of Zn, Ni, Cu, Cr and Cd on seed germination of Pea plant grown in solid medium (agar).In general; there was a reduction in seed germination as metal concentration in the growing media increased. The 40 ppm dose of Cd and Cr and the 60 ppm dose of Cu and Ni significantly reduced the seed germination (P<1%). At concentration of 80 ppm, Ni, Cu, Cr and Cd inhibited seed germination by 30, 50, 62 and 57% respectively. The results are in agreement with [18, 19]. However, in this study Zn was the only metal that has not significantly reduced the seed germination, even at the concentration of 80 ppm.

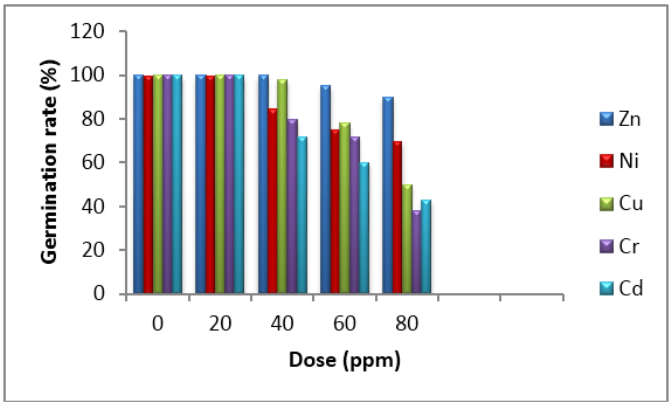

Fig 1: Seed germination of Pea Plant (*Pisum sativum*) after three weeks of heavy metals of exposure.

3.2 Effect of Heavy Metals on Root Growth

The data corresponding to the root growth of Pea plant vs. the dose of the heavy metal reported in this paper is shown in **Fig 2**. The dose of 20 ppm of Zn, Ni, Cu, Cr and Cd promoted the root growth by 189,154,230,269 and 115 % respectively as compared to the root growth of the control plant. The heavy metals Zn, Ni, Cu, Cr at40 ppm concentrations still increased the root growth over the control root size. However, at the 60ppm dose Cd reduced the root size by nearly 30% as compared to the control root elongation. Cr and Cu and Ni demonstrated a concentration dependent inhibition of the root growth at the dose of 40-80 ppm. Oncel *et al*., 2000 [20] found similar effects using cadmium in wheat seedling. All Zn concentrations increased the root length by more than 100% when compared to the control.

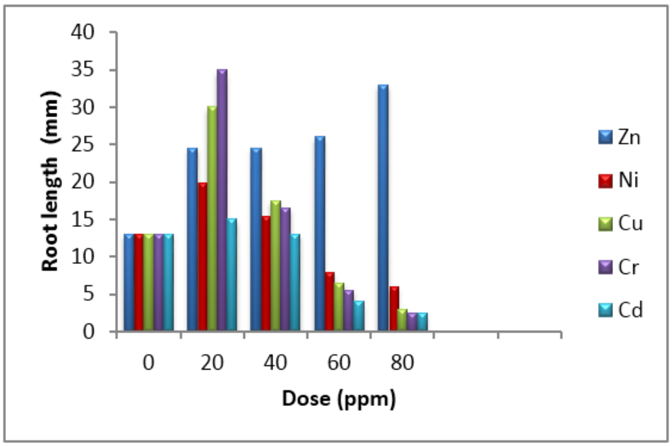

Fig 2: Root length of Pea (*Pisum sativum*) after three week of heavy metal exposure.

3.3 Effect of Heavy Metal on Shoot Growth

The effects of the heavy metals over the shoot growth were different as compared to the effect on root growth **(Fig 3)**. At 20 ppm dose, Cd reduced the shoot size by about 15% as compared with the shoot size of the control group. On the other hand, a dose of 20 ppm of Zn, Ni, Cu and Cr increased the shoot length in 12.5,35, 65 and 17.5% respectively related to the growth of the control treatment. However, Cd and Cr, at 40 ppm dose, significantly reduced the shoot growth as shown in the control plants. When the concentration of these two heavy metals was increased to 60 ppm, the shoot size diminished by32.5% and 50% respectively. However, these heavy metals at 80 ppm showed a lethal effect over the Pea plant. These data are in correlation with the results of [16, 19]. The heavy metal Zn showed positive effect at the concentration of 80 ppm. The results indicate that low concentration of Cr and Cu and Ni have micronutrient like effect on the Pea plant. In case of Zn, the observations indicate that some heavy metals have positive effects on the growth of the Pea plants, even at moderately higher concentrations.

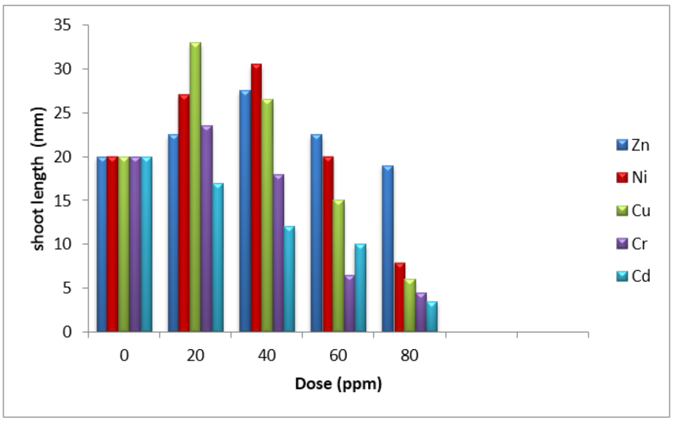

Fig 3: Shoot length of Pea (*Pisum sativum*) after three weeks of heavy metals exposure.

4. Conclusion

Based on the results, we concluded that the seed germination of the Pea plant (*Pisum sativum*) is seriously affected by a concentration of 60 ppm of Cd, Cr, and 80 ppm of Cu and Ni. The root and shoot growth of the plant is stimulated by the concentrations of 20 ppm of Cr, Cu and Ni and Zn. Pea plant did not showed any capabilities of germinate and grow in a medium containing 60 ppm of Cd and Cr , and 80 ppm of Cu and Ni . However, Pea plant was able to germinate and grow efficiently at any Zn concentrations evaluated in this investigation. This study indicated that the Pea plant may be grown directly in soils individually contaminated with moderate amounts of Ni, Cu, Cr and Cd. Further studies need to be performed in order to establish the maximum amount of Zn that the plant may tolerate and the ability of the Pea plants to germinate and grow on media containing mixtures of these heavy metals.

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