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**THE EFFECT OF HEAVY METAL (Pb) ON SEED GERMINATION  
AND SEEDLING GROWTH ON COCCINIA, MENTHA, AND  
TRIGONELLA.**

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**ABSTRACT**

The current study examines how soil polluted with nickel and lead affects the growth and seed germination of *Coccinia*, *Mentha*, and *Trigonella* plant species. This was done in a lab setting using a general phytotoxicity test and an evaluation of growth inhibition. The ability of three plant species—*Coccinia* (*Coccinia indica*, Cucurbitaceae), *Mentha* (*Mentha viridis*, Lamiaceae), and *Trigonella* (*Trigonella foenum-graecum*, Fabaceae)—to germinate and survive in a gradient of soil contaminated with heavy metals like lead (Pb) was examined. The technique makes advantage of these plants' seed germination and early seedling growth in the presence of different concentrations of Pb (1 mg, 50 mg, and 100 mg). The concentration of these metals, the metal itself, and the type of plant all affected the inhibition they caused. As metal concentrations increased, the species' tolerance index, percentphyto-toxicity, root and shoot lengths, and seed germination all decreased.

**INTRODUCTION**

Metals are found in the environment naturally and are parts of the Earth's. The term "heavy metals" is usually used to refer to metals and semi-metals associated with pollution and toxicity, while some elements that are vital nutrition for cells in small amounts are also referred to as well. They tend to accumulate and persist in ecosystems due to their stability, mainly because they cannot be eliminated or degraded. Toxic metals have been introduced to agricultural soils as a result of growing reliance on chemical fertilizers, irrigation with sewage waste water, and fast industrialization, which has a negative impact on the soil-plant

environment system. The environment is severely harmed by these components. Even in small quantities, these non-essential trace elements are extremely toxic and detrimental to all living things, including humans, animals, and plants.(Gang et al., 2013).

Plants can be dangerous to people and animals since they are part of food chains and can contaminate food sources (Zhou et al. 2014). At low quantities, many of these elements—like Cu and Zn—are necessary for plant growth and development, but at greater concentrations, they become toxic). Higher concentrations of metals interfere with plant germination, growth, and productivity, it may interfere with many physiological and biochemical processes such as cell membrane permeability, nitrogen assimilation, photosynthesis, enzymatic activities, growth regulation and cell reproduction. When their concentrations beyond a threshold point, poisonous heavy metals can become extremely hazardous and serve no purpose for organisms.

Research on seed germination explores the effects of environmental conditions on seed quality and expands our understanding of the physiology and morphology of the embryo and seedlings. Heavy metals, on the other hand, are abiotic elements that could hinder seed germination. In an effort to increase crop production, scientists have become more interested in creating new methods to encourage seed germination and plant. Seed germination is a fundamental trait and it is considered as a determining factor in the success of crop production especially in arid and semi-arid regions. According to Swapna and Rama Gopal (2014), germination is an important criterion for the evaluation of germplasm tolerance to various stresses including heavy metals. Therefore, it is necessary to understand seed germination process under adverse environmental conditions as well as heavy metal stress. The aim of the current study was to improve our knowledge of how plants respond to various types and levels of metal exposure as well as their capacity to grow in the presence of metallic stress. A variety of aqueous treatments of the three Lead was used in the experiment to examine their phytotoxic effects on the germination of seeds and the growth of seedlings Coccinia, Mentha, and Trigonella.

## **MATERIAL AND METHODS**

Seeds of Coccinia, Mentha, and Trigonella were collected from nearby nursery, Yeleswaram, East Godavari district were used as experimental plants in this study. Experiments were carried out in two sets during January to February 2025 at the Department of Botany, Adikavi Nannaya University, Rajamahendravaram.

First set of experiment was conducted in petridishes for germination test. For second set of the experiments soil culture medium was used for the seedling growth under metal exposure. Metal stress was created in both sets of experiments by applying lead (Pb) in the form of Pb(NO<sub>3</sub>). Three concentrations (1 mg, 50 mg, and 100 mg) of metal solutions were prepared with distilled water.

These solutions are used for watering the seeds in experimental trays 1, 2, 3 respectively. Effect of different concentrations of metal on seedling growth *Coccinia*, *Mentha*, and *Trigonella* was carried out. The seeds were allowed to germinate for 4 weeks in trays under different concentrations of metal solution. The different concentrations of solution were used to create stressful conditions to the plants. The seeds grown under these conditions are termed as treatments and the seeds with normal conditions are termed as controls.

#### **Germination test (GT):**

GT was performed in sterilized petridishes using Whatman No.1 filter paper. Petriplates were tagged for the treatments and made ready by placing filter paper on it. The papers were moistened with respective concentration of metal solutions. One treatment was kept as control using distilled water. Washed seeds were dipped in 2% sodium hypochlorite solution for 30 minutes to make it disinfected and washed it repeatedly to remove the sodium hypochlorite traces.

About 100 sterilized seeds were placed in each petridishes and the filter papers were kept moisten with respective treatment solutions throughout the experimental period by adding the solutions periodically. Each treatment was repeated for four times.

#### **Germination parameters:**

The number of seeds germinated in each day was recorded and length of plumule and radical were noted at 7<sup>th</sup> day of the germination test. Percentage germination, Germination Index, Percent inhibition of germination performance under the applied treatments following (Sarma et al 2014)

#### **Percentage germination (PG):**

$$PG = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds taken}} \times 100$$

Seed was considered as germinated when its root length was longer than 2 mm. After seven days, the seedlings of each petridishes were sampled randomly and shoot and root length measured using a ruler (against black background). A fresh weight was also evolved by weighing of these ten seedlings.

## STATISTICAL ANALYSIS

The experiments were conducted in randomized block design in triplicate. The data was analyzed using one-way analysis of variance (ANOVA) to determine the effect of heavy metal treatments and least significant difference (LSD at  $P=0.05$ ) tests were performed to determine the statistical significance of the differences between means of treatments

## RESULTS AND DISCUSSION

In the present study, seed germination studies were performed to study the behaviour of *Coccinia*, *Mentha*, and *Trigonella* under heavy metal stress. Generally, increased concentrations of lead solution significantly reduced the strength of seed germination. Higher seed germination (92%) was observed in control seedlings (Table 1). We made observations up to 4 weeks.

**Table 1: Seed Germination Percentage of Exposure to Heavy Metal.**

| Concentration (mg/L) | <i>Coccinia</i> | <i>Mentha</i> | <i>Trigonella</i> |
|----------------------|-----------------|---------------|-------------------|
| Control              | 92%             | 92%           | 92%               |
| 1 mg/L               | 82%             | 54%           | 54%               |
| 2 mg/L               | 62%             | 40%           | 40%               |
| 3 mg/L               | 45%             | 22%           | 22%               |

In *Coccinia*, 1mg of concentration of lead the seed germination was significantly 82% whereas in *Mentha and Trigonella* the seed germination was around 54%. In 2mg concentration of concentration of lead the seed germination was significantly 62% in *Coccinia* whereas in *Mentha and Trigonella* it was 40%. In 3 mg concentration of lead, the seed germination was around 45% in *Coccinia* whereas in *Mentha and Trigonella* it was around 22% and it was observed that the effect of lead exhibited gradual decrease in the germination percentage. Compared to control seedlings exposed to high concentration of lead exhibited substantial growth reduction while the plant growth decreased progressively with increasing metal concentration. Metals are essential for growth and development of plants at low concentrations (Wintz et al., 2002). However, plants are highly sensitive to either deficiency or excess of metals and it could be leads to inhibition of seed germination or death

of germinated seedlings. This is due to impairing root system (Singh and Thakur, 2014), causing oxidative stress by producing free radicals (Shah *et al.*, 2010), lowering water uptake and transport, damaging embryogenic sac and by replacing the nutrients and essential elements required for plant growth and development.

In this study of heavy metal, when compared to three concentrations the lesser the concentration the more is the germination seen in both the plants. At higher concentration, seed germination at initial stage is little less when compared to the others. Later due to prolonged watering of the concentrated solution the seedlings shows stunted growth which after few days resulted in wilting and death of the plant as per the increased concentrations.

Accumulation of heavy metals in plant tissues by a complex toxicity process (Silva *et al.*, 2015). After mobilization of the soil for root cells, they tend to accumulate in the vacuole cells or they are transported to the aerial parts through the xylem.

When the plant accumulate metals, such as lead, at phytotoxic levels, biochemical and molecular disturbances may occur that cause oxidative stress in cells and, consequently, such effects as cell membrane changes, DNA damage, genetic mutation, rust protein, lipid peroxidation and growth inhibition. And these findings were in concordance with the results obtained by Gang *et al.*, 2013 and Habtamu *et al.* 2013, they reported that metal toxicity on roots decreased at lower concentrations and increased at higher concentrations.

**Table 2: Shoot growth of *Coccinia*, *Mentha* and *Trigonella* under controlled conditions.**

| No. of weeks         | <i>Coccinia</i> | <i>Mentha</i> | <i>Trigonella</i> |
|----------------------|-----------------|---------------|-------------------|
| 1 <sup>st</sup> week | 3 cm            | 2 cm          | 2 cm              |
| 2 <sup>nd</sup> week | 3.6 cm          | 3.1 cm        | 3.2 cm            |
| 3 <sup>rd</sup> week | 5.8 cm          | 5.4 cm        | 5.4 cm            |
| 4 <sup>th</sup> week | 9.4 cm          | 8.5 cm        | 8.3 cm            |

It was observed that, as lead concentration increased, the shoot length decreased (Table 2). The highest decrease in shoot length of both *Mentha* and *Trigonella* and *Coccinia* was found at the highest concentration of lead that is 3g. The shoot length of *Coccinia* was 3cm, 3.6cm, 5.8cm, 9.4cm whereas in *Mentha* and *Trigonella* it was 2cm, 3.1cm, 5.4cm, 8.5cms, in respective weeks of the control (Table 2). The reduced shoot length is due to the reduction in meristematic tissue and cells in this tissue are metabolically active and involved in rapid cell division (Naseer *et al.*, 2001). Also, some hydrolytic enzymes present in the cotyledon and endosperm cells become inactive and unable to digest the stored food into soluble form and transported to the embryogenic axis. Heavy metals can inactivate hydrolytic enzymes in

endosperm and aleuron layer by binding to cysteine residues and block the conversion of insoluble starch into simple sugars. Heavy metal can also displace the essential metal ions in bimolecular. Many enzymes contain metals in positions important for their activity. Therefore, the displacement of one metal by another will normally also lead to inhibition or loss of enzyme activities. Broadley et al., (2007) reported that lead may interact metabolically with nutritionally essential metals and replace zinc on heme enzymes.

Hydrolyzing enzymes play a major role in seed germination. Enzymes such as acid phosphatases, proteases and  $\alpha$ -amylases are known to facilitate both seed germination and seedling growth via mobilizing nutrients in the endosperm. In the presence of heavy metals, starch is immobilized and nutrient sources become limited. Moreover, a reduction in proteolytic enzyme activity and an increase in protein and amino acid content can be observed under heavy metal stress. Proline, is an amino acid which is essential for cellular metabolism. Numerous studies have shown an increase in proline content under oxidative stress in higher plants. Furthermore, heat shock protein production has also been observed under heavy metal stress. The chloroplast small heat shock proteins (Hsp) reduce photosynthesis damage, rather than repair or help to recover from heavy metal-induced damage. Heavy metals are destructive substances for photosynthesis. They are involved in destabilizing enzymes, oxidizing photosystem II (PS II) and disrupting the electron transport chain and mineral metabolism

*Mentha and Trigonella* exhibit different susceptibilities to lead treatment when compared to *Coccinia*. In the present study, *Coccinia* can be said as a good halophyte. They could be sustainably cultivated in different concentrations of lead and appears to be more resistant. Conversely seedling growth was positively stimulated at moderate levels and adversely affected at varying rates. *Coccinia* is able to adapt to adverse environments. Recent studies have documented that via inhibition of storage food mobilization, reduction in radical formation, disruption of cellular osmoregulation and the degradation of proteolytic activities, heavy metals cause inhibition of seed germination and seedling development (Adrees et al. 2015)

Seed germination inhibition by toxic metals depends on the concentration used, the metal itself, and the plant species. The effect of toxic metals can also be influenced by the ability of a metal to interact with the tegument and embryo tissues of the seeds of different species and depends the physical-chemical properties of these elements. Different plant species have varying types of seed teguments, structure and anatomy; therefore, among species, the same concentration of an element can have different toxic effects

## CONCLUSIONS

The current study concludes that, in comparison to the control, lead and nickel treatments had harmful effects on the germination and growth of *Coccinia* (*Coccinia indica* – Cucurbitaceae), *Mentha* (*Mentha viridis* – Lamiaceae), and *Trigonella* (*Trigonella foenum-graecum* – Fabaceae) plants. The majority of plant growth metrics changed when the concentration of both metals in the medium increased. As a result, regulations that aid in lowering metal levels from a variety of sources, including power plants and the metal processing sectors, must be put into place.

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