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Determining Germination percentage and Protein Levels in Germinating *Pisum sativum* (Green Pea) Seeds Exposed to Lead Stress using the Biurette Method

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ABSTRACT

Green peas, scientifically known as *Pisum sativum*. A legume crop primarily grown for their edible seeds. This study's objective is to assess the germination percentage and protein content in germinated green pea seeds. The seeds were procured from a local market and underwent surface sterilization with a 0.3% hydrogen peroxide (H_2O_2) solution for twenty minutes. Subsequently, they were placed in petri plates lined with two layers of filter paper. In each petri dish, 10 ml of lead nitrate at various concentrations (0, 100, 500, 1000 ppm) was added. Each treatment, including the control group, was replicated three times. All petri dishes were placed under diffused light at room temperature (28±2 °C). Following immediate germination, a protein estimation test was conducted using the biurette method. The study revealed, reduced germination percentage at increasing concentrations of lead nitrate. The protein content initially raised in 100 ppm compared to control and at 500 and 1000 ppm the protein content is gradually reduced. This investigation demonstrates that an increased concentration of $Pb(NO_3)_2$ has a detrimental impact on green pea plants.

Key words: *Lead nitrate stress, Pisum sativum, Biuret method*

Introduction

Within the realm of ecosystem sustainability, soil pollution stands out as a particularly critical environmental issue. Soil contamination resulting from the release of toxic heavy metals has gained global recognition as a major environmental challenge (FAO, 2018). The adverse impacts of heavy metals on ecosystems are compounded by their escalating toxicity and their remarkable persistence in the ecosystem (Järup, 2003; Singh *et al.*, 2018).

Lead (Pb) exerts significant adverse effects on seed morphology and physiological processes, causing inhibition of germination, root elongation, seedling development, transpiration, chlorophyll production, and protein content. These impacts changes the chloroplast structure, disrupts the electron transport chain, inhibits Calvin cycle enzymes, impairs uptake of essential elements like magnesium (Mg), iron (Fe) and also induces carbon dioxide (CO_2) deficiency which is due to stomatal closure. In Pb-contaminated soils, seedling growth is inhibited due to elevated lipid peroxidation. This triggers the activation of enzymes like superoxide dismutase (SOD), guaiacol peroxidase (POD), and ascorbate peroxidase (APX), as well as the glutathione (GSH)-ascorbate cycle, which collectively participate in hydrogen peroxide (H_2O_2) removal. Additionally, Pb exposure leads to the up-regulation of heat shock protein 70 (HSP70), and both lipid peroxidation and HSP70 serve as markers for Pb-induced stress in soil ecosystems.

A multitude of studies have extensively documented the adverse impacts of lead (Pb) on various aspects of plant development. These include negative effects on seed germination (Zhang *et al.,* 2018), seedling development (Kedarini *et al*., 2009), root growth (Sharma and Dubey, 2005), and other plant growth-related characteristics. Once absorbed by plant roots and subsequently translocated to aerial plant parts, these metals can enter the food chain (Dey *et al.,* 2007). The presence of lead in the food chain poses significant risks to human health, potentially causing damage to the nervous system, liver, kidneys, and disrupting various other related biochemical functions in humans (Fu and Wang, 2011).The primary objective of the study is to determine protein levels in germinating *pisum sativum* (green pea) seeds exposed to lead stress using the biurette method.

Materials and Methods

Lead nitrate stock solution was prepared using the stock varied ppms of lead nitrate solutions were made (0 ppm, 100 ppm, 500 ppm, 1000 ppm) and added in different petri dishes which was priorly layered with double filter paper. Added green peas to petridish and incubated for 2-3 hours, keeping it overnight for germination. Once the germination rate is constant, weight of the germinated seeds in grams were measured from each treatment and germination percentage was calculated. Germinated seeds from each treatment were collected separetly and crushed in to paste using mortar and pestle by adding ethanol 10ml and centrifuged it for 10 mins. 0.4 ml of prepared solutions were dispensed into test tubes, followed by the addition of 2.6 ml of distilled water, and 4 ml of biurette reagent in each test tube. The same done to control except adding protein solution and allowed it to react for half an hour. Optical densities of each concentration were measured.

Results and Discussion

Germination Percentage

Each petri plate contained five green pea seeds. Once a constant germination rate was established, the germination percentage was measured. The highest germination percentage, reaching 100%, was observed in the control group. However, as the concentration of $Pb(NO_3)_2$ progressively increased, a gradual decrease in germination percentage was noted. The lowest germination percentages were observed at concentrations of 100 and 500 ppm of Pb, with an even further decrease to very low percentages at 1000 ppm (Fig. 1). This decline in seed germination can be attributed to either the breakdown of stored food material in the seeds or the toxic impact of Pb, as suggested by (Kedarini *et al.,* 2019; Kalimuthu and Siva in 1990).

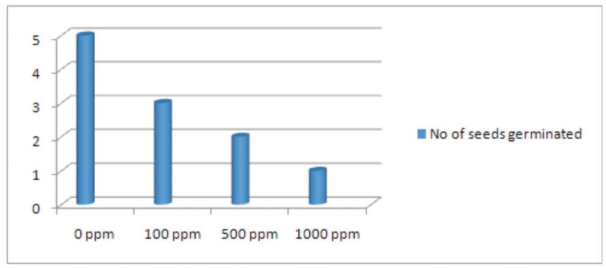

Fig. 1. Impact of varying concentrations of lead nitrite ($Pb\ (NO_3)_2$) on the germination percentage of green pea seeds

Protein test

0.4 ml of prepared solutions were dispensed into test tubes, followed by the addition of 2.6 ml of distilled water, and 4 ml of biurette reagent in each test tube. These test tubes were then incubated for 30 minutes. A similar procedure was carried out for the control group, but no protein or prepared solution was added. After the half-hour incubation, the optical densities of each concentration were measured. It was observed that the protein concentration was slightly high at 100 ppm and at 500 and 1000 ppm protein concentration was gradually declined compared to 100 ppm, as it showing threshold point (Fig. 2). Protease activities exhibit variability, with occasional increases or decreases, leading to alterations in intracellular proteolysis. The observed rise in proteolytic activity, coupled with the concurrent depletion of reserve proteins, aligns with earlier findings in different legume seeds, such as *Vicia sativa* (Schlereth *et al*., 2001), and *Macrotyloma uniflorum* (Rajeshwari and Ramakrishna Rao, 2002). According to studies once the threshold level reached the protein concentration decreases.

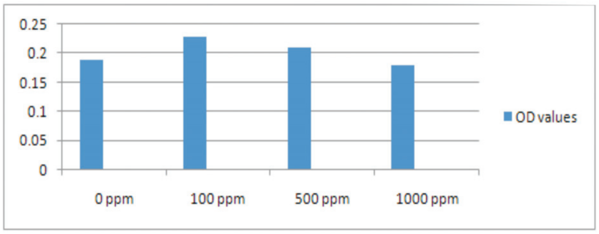

Fig. 2. Impact of varying concentrations of lead nitrite (Pb $(NO_3)_2$) on the protein concentration of green peas.

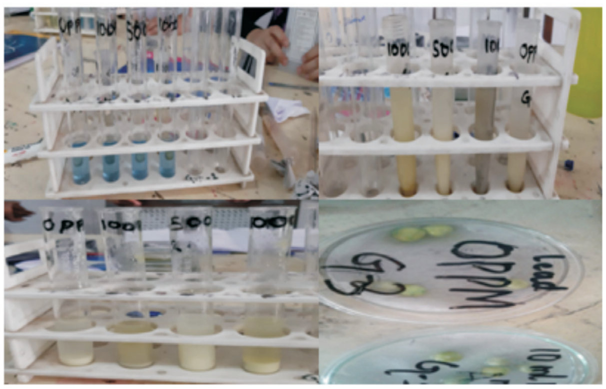

Fig. 2. Prepared samples and germinated seeds

Conclusion

The present study revealed that reduced germination percentage at increasing concentrations of lead nitrate solutions. The protein content initially raised in 100 ppm compared to control and at 500 and 1000 ppm the protein content is gradually reduced due to high levels of lead stress. This study demonstrates that an increased concentration of $Pb(NO_3)_2$ has a detrimental impact on green pea plants.

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