

Exploring the Toxic Effects of Pb & Ni on Stem Anatomy of *Pisum Sativum* L.

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Abstract - Heavy metals are natural components of Earth's crust which cannot be degraded. They are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Due to this contamination by heavy metals such as Pb, Ni, Cu, Al, Cr etc. cause serious problems. They accumulate in different parts of the food chain. Plants growing in polluted environment often show symptoms of injury, general debility and premature ageing. An investigation was carried out to check the effects of Pb and Ni on seed germination and growth of *Pisum sativum* L. Seeds were placed in petri dishes in triplicates and allowed to grow for 15 days in different concentrations of Pb [as Pb(NO₃)₂] and Ni [as NiCl₂]. Plant samples were collected at regular interval and subjected to morphological and anatomical studies. Our investigation suggests that higher concentrations of Pb exhibit certain anatomical abnormalities like abnormal vascular system, abnormal lignifications in the pith parenchyma and enlarge cortical cells. However, no specific abnormalities were observed with Ni doses.

Keywords – Ageing, anatomical abnormalities, contamination, lignifications, Ni, Pb.

I. INTRODUCTION

Heavy metals are natural component of Earth's crust. They cannot be degraded or destroyed. Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic (5.7), cadmium (8.65), iron (7.9), lead (11.34), and mercury (13.546) (Lide 1992). The term heavy metal is often used to cover a diverse range of elements which constitute an important class of pollutants. Heavy metals are among the most dangerous substances in the environment, because of their high level of durability and harmfulness to live organisms. The increasing pollution of our environment is causing complex changes in the different natural communities. These changes include simplification of the structure and diversity of biological communities, losses of nutrients, and shifts in the proportion of gross production to respiration.

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Heavy metals including Pb, Ni, Cd, Cr and Hg cause toxic effects to plant (Sethy 2013). Fluorides have been found to damage young plants of conifers. A few metals, including Copper, Manganese, Cobalt, Zinc and Chromium are essential to plant metabolism in trace amount. However, when these metals are present in bioavailable forms and at excessive levels, they have potential to become toxic to plants (Nagajyoti 2010).

Field pea (*Pisum sativum* Linn. Fabaceae) is a legume native to Southwest Asia and was one of the first crops cultivated by man. World production of field pea is 5,389×10⁶ ha with the most important producing countries being Canada, China, India, and the Russia. Peas are high in vitamin A, vitamin B, and Lutein.

Therefore the present work is focused towards the toxic effects of two heavy metals lead (Pb) and nickel (Ni) on widely cultivated plants in Gujarat, *Pisum sativum* L (Sweet pea).

A. Lead

Lead (Pb) is one of the prominent examples for anthropogenic environmental metal pollution that originates from various activities including mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal planting and finishing operations, pesticides and from additives in pigments and gasoline (Sharma and Dubey, 2005).

Lead is one of the potentially toxic heavy metal pollutants of the environment with no known biological function and its concentration are rapidly increased in agriculture soil (McGrath et al, 1995). Lead causes drastic morphological and physiological deformities in *Ipomoea lacunose* plant (Kambhampati et al, 2005). Elevated Lead in soils may adversely affect on soil productivity and even a very low concentration can inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption showing toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short root (Patra et al, 2004).

B. Nickel

Nickel is a hard, ductile and silvery-white heavy metal. In general, naturally occurring concentrations of Ni in soil and surface waters are lower than 100 and 0.005 ppm, respectively (McGrath, 1995). Ni is also released into the environment from anthropogenic activities, such as metal mining, smelting, and disposal of hazardous household, municipal and industrial wastes, fertilizer application and organic manures. Increasing

Ni pollution, excess Ni rather than a deficiency is more commonly found in plants. Nickel greatly reduced root meristem mitotic activity and this is probably the main cause of the nickel induced Reduction of root and shoot growth of maize, since cell expansion is not inhibited (Huillier 1996) (Hussain et al. 2013).

II. MATERIALS AND METHODS

Experiments were carried out at department of biosciences, VNSGU, Surat. The seeds of *Pisum sativum* L. were surface sterilized with H_2O_2 to avoid fungal and/or bacterial contamination. The germination of *Pisum sativum* L. was carried out in petri dishes. Different concentrations of Pb and Ni were prepared in double distilled water using $Pb(NO_3)_2$ and $NiCl_2$ i.e. 100 ppm, 200 ppm and 300 ppm. Pure double distilled water was used as control for the study. 10 seeds of *Pisum sativum* L. were placed on cotton in each petri dish and 40 ml of each solution was supplied once for seed germination. Tap water was applied every alternate day after this treatment. Petri dishes were observed daily for contamination. Study was carried out for 15 days and samples were collected after 5th day, 10th day and 15th day. Plants were observed for morphological changes. For anatomical studies stems were harvested and cut into 10-15 cm pieces and preserved in formalin-acetic acid-alcohol (FAA). Manual hand sectioning was done to study anatomy. After sectioning the materials were stained in Safranin stains and then mounted in a drop of glycerin jelly on glass slides. A cover slip was placed over them and observations were made.

A. Photography

Fine sections were observed at 10X and 40X and photographed by Carl Zeiss Axioscope-A1 photomicroscope and processed by Axio Vision software.

III. RESULTS AND DISCUSSIONS

A. Morphological Study

The morphological studies of *Pisum sativum* indicated the effect of Pb and Ni metal toxicity. In normal conditions seeds of *Pisum sativum* show healthy germinated plant. However in both the cases, it was observed increasing concentrations of heavy metal adversely affected the plant growth. Lowest growth was observed at 300 ppm solutions of Pb and Ni, compared to control (Plate 1 A -F).

B. Anatomical Study

The stem anatomy of *Pisum sativum* is very much interesting. In normal condition stem of *Pisum sativum* is more or less square in shape in transactional view. The main anatomical part of stem is epidermis, hypodermis, cortex, endodermis, pericycle and vascular system and pith.

Thin cuticle layer found at the outer wall of epidermis. Epidermis is single layer. Epidermal cells are small and tubular, Hypodermis situated below the epidermis (Plate 2 A). Cells are also smaller in size, the cell wall of cortical cells became wavy somewhere and the cells are larger in size, Crystal is also found in the cortex cell. Zigzag endodermis present below the cortical cells (Plate 2 A). Single layer pericycle located below the endodermis. Large pith

parenchyma cells were found in the centre of vascular cylinder (Plate 2 A). Four vascular bundles in a vascular cylinder at centre four vascular bundles arranged in periphery, whereas two are made up only by xylem. The entire stem anatomy show the six vascular bundles (four lateral exarch and two at each end are endarch), two cortical fibro vascular bundles and two cortical fibers group. Cortical fibrovascular bundles and fiber groups are found in ground tissue of cortex (Plate 2 A).

C. Nickel Treatment

No any specific effect was found after up to lower to higher dose of nickel treatment. Epidermis is single layer, hypodermis situated below the epidermis and cortex are present, fibrous strand is also present, cortical vascular bundle is found in the ground parenchymatous tissue, after 15 days of the growth of plant stem secondary growth in vascular system is also found.(Plate 2 B)

D. Lead Treatment

The stem of *Pisum sativum* treated with lower to higher dose of lead showed few specific effects on stem anatomy. Treated stem anatomy showed presence of damaged epidermis (Plate 2 C).

Due to the effect of Pb, structural abnormality in vascular bundles reported (Plate 2 E), abnormal lignifications found in the pith parenchyma (Plate 2 D) Metaxylem development was found to be developed in pith region (Plate 2 D). Sometime ruptured and enlarge cortical cells development take place (Plate 2 F).

IV. CONCLUSION

Current study indicates that both Lead and Nickel exhibit toxicity to *Pisum sativum*. Lead gives more toxic effects to *Pisum sativum* compare to nickel. Lead shows some lethal anatomical abnormalities which are responsible for abnormal or sometime very slow growth of *Pisum sativum* under lead contaminated conditions. It bring abnormal enlargement of cortical cells as well as also induced abnormal cell division in cortical cells. Due to the toxic effects of this heavy metal ectopic lignifications is also found in pith parenchyma cells. The ultimate effect of this toxic metal was cell death.

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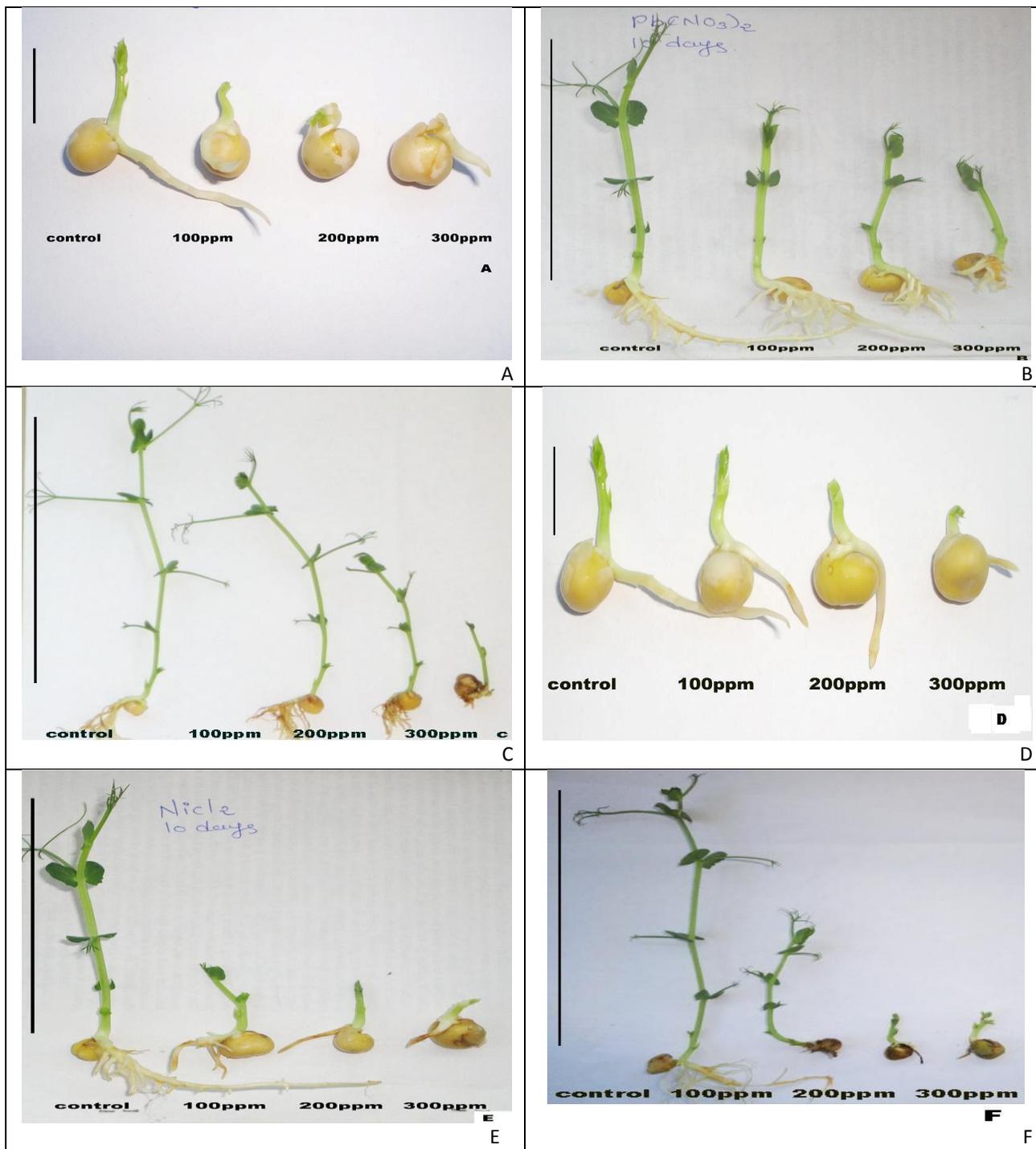


Plate 1: Morphology of *Pisum sativum* seedlings

A - effect of Lead on 5 days plants of *Pisum sativum*; **B** - effect of Lead on 10 days plants of *Pisum sativum*; **C** - effect of Lead on 15 days plants of *Pisum sativum*; **D** - effect of nickel on 5 days plants of *Pisum sativum*; **E** - effect of nickel on 10 days plants of *Pisum sativum*; **F** - effect of nickel on 15 days of *Pisum sativum*.

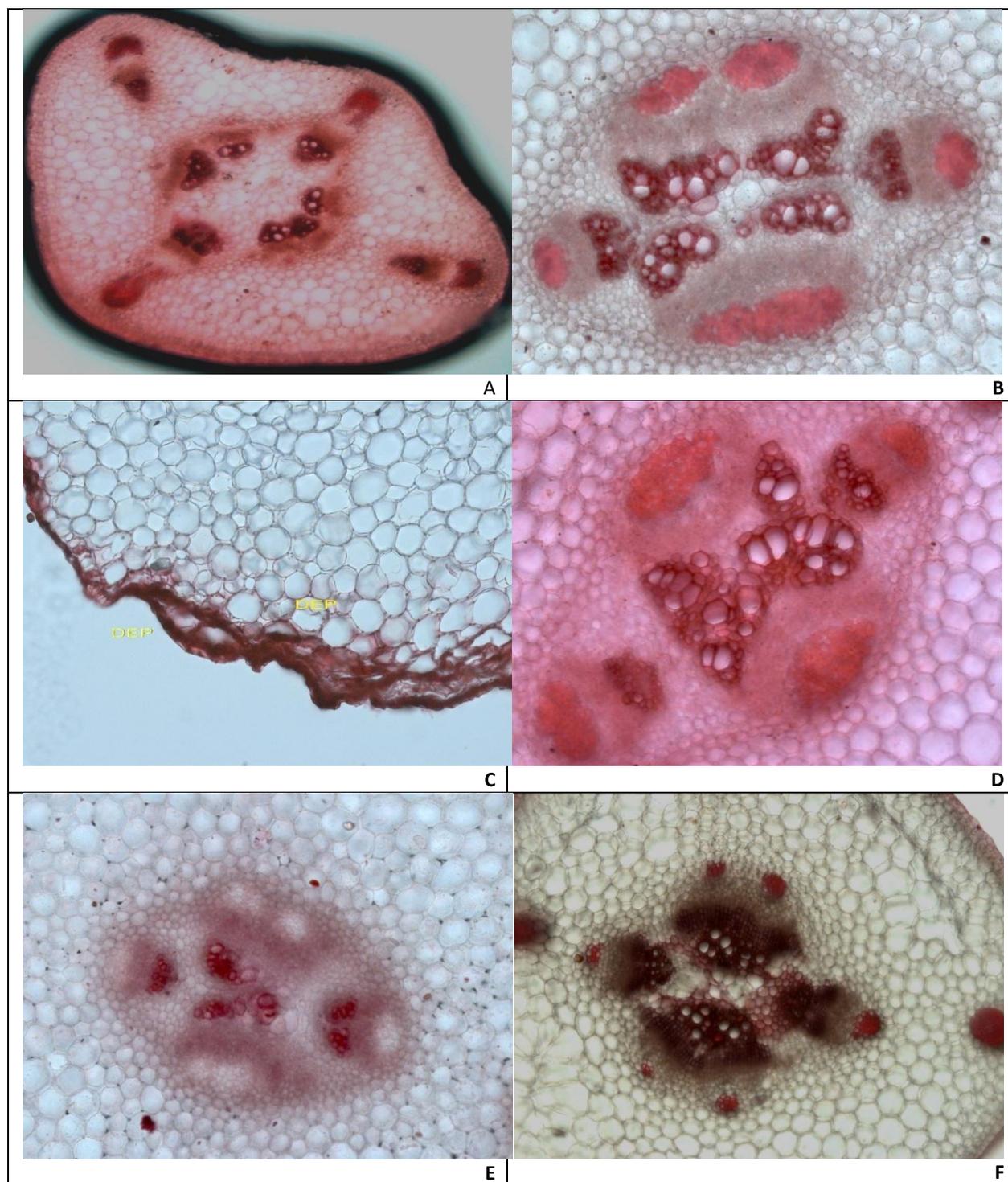


Plate 2: Stem anatomy of *Pisum sativum*

A – T.S of young stem - Normal; B – T.S of stem - Normal; C – Damage epidermis; D – Pith is lignified (Treated by Pb); Meta xylem development in Pith ; E – Structural abnormality of Vascular bundles; F – Ruptured and enlarged cortical cells.
 (A – Control; B – Treated by Ni; C to F – Treated by Pb.)



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