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ASSESSMENT OF HEAVY METAL TOXICITY CAUSED BY CO AND PB ON *PORTULACA GRANDIFLORA*

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Abstract

In this research study, the application and accumulation of Metal Pollutants in various parts of *Portulaca grandiflora* were studied. Objectives of this research are to study the growth parameters such as plant height, leaf area, biomass and the biochemical parameters such as Pigment Composition (Chlorophyll) and Proline Estimation and to evaluate the presence and accumulation of different concentrations of metal pollutants (Cobalt and Lead) in the plant parts. Purslane shows negligible effect while treating with different concentrations of Co and more loss while treating with different concentrations of Pb (50 µM, 100 µM, 150 µM). Leaf area and plant height showed less reduction due to application of lowest concentration of Co (50 µM) and highly reduced due to application of highest concentration of Pb (150 µM). Pb shows more negative effect than Co on leaf area and plant height. Primary roots were less affected but secondary and tertiary roots were slightly affected due to application of Co and Pb which, indicated partially reduced root system. In the comparison of control plant, the test plant shows an increase in biomass but in comparison of both the metal pollutant (Co and Pb) Pb shows negative effect as the metal concentration increases. Total Chlorophyll was also affected due to application of metal pollutants. Proline plays a therapeutic role in plants, which increase in plants under stress condition. As the Co concentration increased the total proline content decreased. At low concentration of metal pollutants, less amount of proline was increased. At high concentration of Pb, the test plant shows increased proline content, which shows stressful condition for the plant.

Keywords: Chlorophyll, Proline, Metal Pollutants, *Portulaca grandiflora*, Growth parameters

Introduction: -

Heavy metal toxicity means excess or undesired content naturally produced on the earth and concentrated as a consequence of human activity, entering plant, animal and human tissue through inhalation, food intake and manual handling and may bind and interfere with the functioning of vital cellular functions. An external factor which exerts a disadvantageous effect on the organism. In contrast, any changes in environmental conditions which adversely mutate the plant growth or development. In developing countries. A major problem is open dumping of solid waste, which causes environmental pollutions as water pollution, land pollution, air pollution and except these problems it causes unpleasant odour, health issues and vegetation loss (Sharma ***et al.,*** 2019).

In the environment, the abundance of heavy metals raised significant concerns throughout the world with the development of urbanization and industrialization (Suman ***et al.,*** 2018; Ashraf ***et al.,*** 2019).

Due to different types of anthropogenic activities and many natural processes, the accumulation of heavy metals has been increased rapidly in soil. Heavy metals are persisted in throughout the environment and also are non-biodegradable. Heavy metals have the potential for entering the food chain and accumulating in the human body. Heavy metals have toxic nature which cause serious threat to health of human, animals and ecosystem (Yan ***et al.,*** 2020).

Heavy metals are naturally present in the soil, and geologic and anthropogenic activities increase the concentration of these elements to amounts that are harmful to plants. Growth reduction as a result of changes in physiological and biochemical processes in plants growing on heavy metal-polluted soils has been recorded, along with disturbance of nutrient uptake and translocation, photosynthetic reduction (decrease in photosynthetic pigments, inhibition of electron transport, decrease in CO_2 fixation, chloroplast disorganization, photo-oxidative damage, the generation of reactive oxygen species (ROS), the inhibition of anti-oxidative enzymes, cellular redox imbalance, DNA damage, and protein oxidation (Sperdouli, 2022).

Cobalt: -

Cobalt is a chemical element. Symbol is Co and atomic number is 27. It is found in the form of combination in crust of earth. It is found in alloys of natural meteoric iron. It is produced by reductive smelting and hard, lustrous, silver-grey metal. It is present in period 4 group 9 of d block in periodic table. It is transitional metal having $3d^7$, $4s^2$ electronic configuration and 2, 8, 15, 2 electrons per shell. Cobalt is firstly discovered and isolated by George Brandtin 1735. It is metallic grey in colour and solid in nature having 1768 K (1495ºC, 2723ºF) melting point and its boiling point is 3200 K (2927ºC, 5301ºF) and its density is 8.90 g/cm^3.

Tolerable limit of Co in soil for the growth of plants was found to be 0.2–0.5 ppm. Some plant species are able to grow in soil possessing high concentrations of Co up to 4000–10,000 ppm. Toxic concentrations of Co inhibit active transport in plants. Relatively higher concentrations of Co have toxic effects, including leaf fall, inhibition of greening, discoloured veins, premature leaf closure and reduced shoot weight (Sonia ***et al***., 2014). High concentration of Co leads to numerous dysfunctions in the plant system. These mainly include production of reactive oxygen species (ROS), hydroxyl radical ($^{\cdot}OH$) generation, formation of hydrogen peroxide (H_2O_2) radicals, increased MDA, proline content and alteration of antioxidant enzyme activities (Azarakhsh ***et al***., 2015). ROS generation under Co stress in plants generally causes disturbance in photosystem II and stimulates disruption in electron transport chain (Begovic ***et al***., 2016).

Lead: -

Lead, a chemical element, symbol is Pb, having 82 atomic number. $_{82}Pb$ is a heavy metal, which is soft and malleable, having low melting point. It is bluish-white in colour, when it cuts freshly and when it exposed to air, it turns to dull grey colour. Pb is stable element: because of having highest atomic number. Lead is present in 14^{th} group and 6^{th} period of p-block in periodic table having $4f^{14}$, $5d^{th}$, $6s^2$, $6p^2$ electronic configuration. Lead is post- transitional metal; solid in nature with 600.61 K (327.46ºC, 621.43ºF) melting point and boiling point is 2022 K (1749ºC, 3180ºF). Its density is 11.34 g/cm^3.

Lead is highly toxic and non-biodegradable heavy metal which remains throughout the environment for the long period. It is neurotoxic for living beings and exerts harmful effects. Due to dumping of lead in a huge amount, it leads to numerous harmful effects on soil and living beings. Deposition of Pb in the tissues of plants lead to oxidative stress, cellular damage and reduction in biomass production (Gupta ***et al***., 2024).

***Portulaca grandiflora*: -**

Portulaca grandiflora, also originating from the Portulacaceae family, is succulent ornamental plant with a creeping habit. *P. grandiflora* is native to South America, Southern Brazil, and Argentina. As time goes by, this plant has come across in various countries. The morphological characteristics of *P. grandiflora* include reddish-green leaf colour, short round leaf shape with a leaf length ranging from 2-4 cm, flower diameter ranging from 3-5 cm, and plant length varying from 7-15 cm (Setiawan ***et al***., 2016). Purslane is highly nutritious and has all essential minerals, vitamins, and proteins. It contains dietary minerals like potassium, magnesium, calcium, phosphorus, iron, etc. Propagation of *P. grandiflora* can be performed using seeds (generatively) and shoot cuttings (vegetatively). However, vegetative propagation is preferable for its feasibility and efficiency (Cruz ***et al***., 2019). *P. grandiflora* is widely known as a medicinal plant (Lim ***et al***., 2014; Devi ***et al***., 2019).

Saponin detected in purslane herb *P. grandiflora* with a magenta-colored flower and *P. grandiflora* with pink-colored flower varieties is a secondary metabolite with antibacterial activity. This activity is related to its ability to increase bacteria cell membrane permeability, leading to hemolysis (Wei ***et al***., 2021). The antibacterial activity of saponins of the herb purslane is associated with the ability to increase the permeability of bacterial cell membranes, leading to hemolysis. In addition, saponins are able to increase

collagen production and accelerate the epithelization of cells in the wound. Tannins can also play an important role in the wound healing process by repairing damaged tissues (Ghorani ***et al.***, 2023).

P. grandiflora flowers produce and accumulate betalains, which are divided into yellow orange betaxanthins and red-violet betacyanins these pigments have attracted much attention because of their bioactivities, which range from an antioxidant capacity to the chemoprevention of cancer (Spórna-Kucab ***et al.***, 2020). The effectiveness of *P. grandiflora* on a surface antigen of the hepatitis B virus has been documented. This plant has an anti-mutagenic impact on the mutations caused by cyclophosphamide and aflatoxin B1, two human carcinogens, in mice (Salahuddin ***et al.***, 2024).

Material and Methods: -

Cultivation of Plant: -

- Seeds of test plant were sown in plastic pots of 30×26 cm with 5 kg of test soil, contains $CoCl_2$ and $PbCl_2$ respectively.
- 3 replications of each concentration of heavy metal were taken so, as to get the precise and accurate result.
- Cultivation was carried out in a normal native plant condition at 32°C for 120 days.

Experimental Design: -

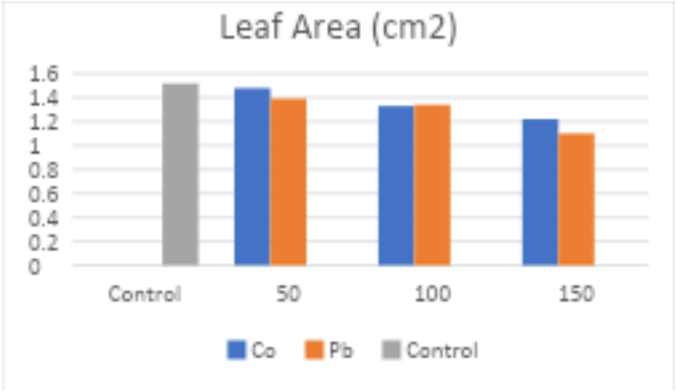
Physiological parameters such as chlorophyll and proline and growth parameters such as leaf area, height and Biomass was investigated in different experiment.

Statistical analysis: -

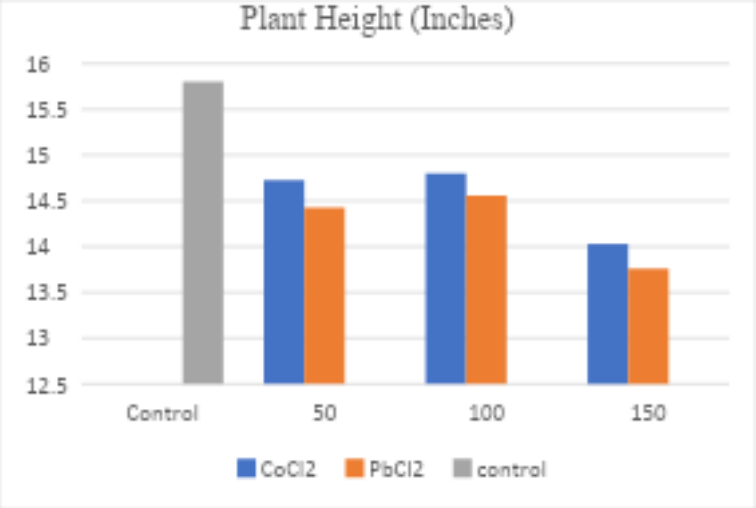
Data were expressed as mean and standard deviation.

Result and Discussion: -

Biomass of control plant was compared with $CoCl_2$ and $PbCl_2$ treated sample of plants and the result of experiment revealed that accumulation of heavy metal in *Portulaca grandiflora* (purslane) had significant impacts on height and biomass of the plant. During the phase of cultivation, the height of Pb-treated and Co-treated plants was observed to be decreased as compared to control and had no significant impact as compared to both the metal pollutants (Co and Pb). The leaf area of Co-treated and Pb-treated plants was observed to be decreased at all concentration in comparison of control plant and both the metal pollutants as well. Pb-treated plants show reduction in leaf area as the concentration went high (150 µM) respectively. While comparing the height and leaf area of Co and Pb-treated plants with control, study suggested that leaf area of Co-treated plants was greater than followed by Pb and height of both the test plants shows negligible effect as compared to each other but lower than followed by control.

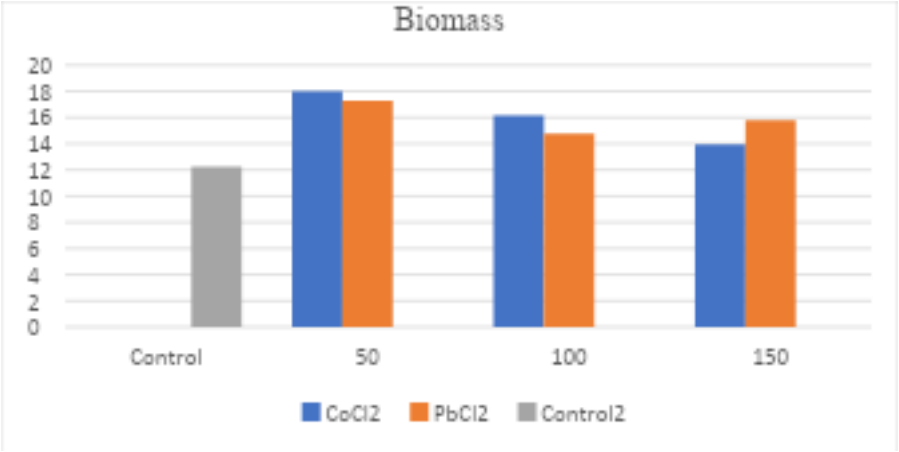


graph 1: leaf area of *portulaca grandiflora* at different concentration of co and pb



graph 2: height of *portulaca grandiflora* at different concentration of co and pb

Total biomass of *Portulaca grandiflora* using Co and Pb treatment was slightly increased as a result of increasing level of heavy metal in plants. However in some cases it was found that Co and Pb treated samples had no or negligeble increament in plant biomass.



graph 3: biomass of *portulaca grandiflora* at different concentration of co and pb

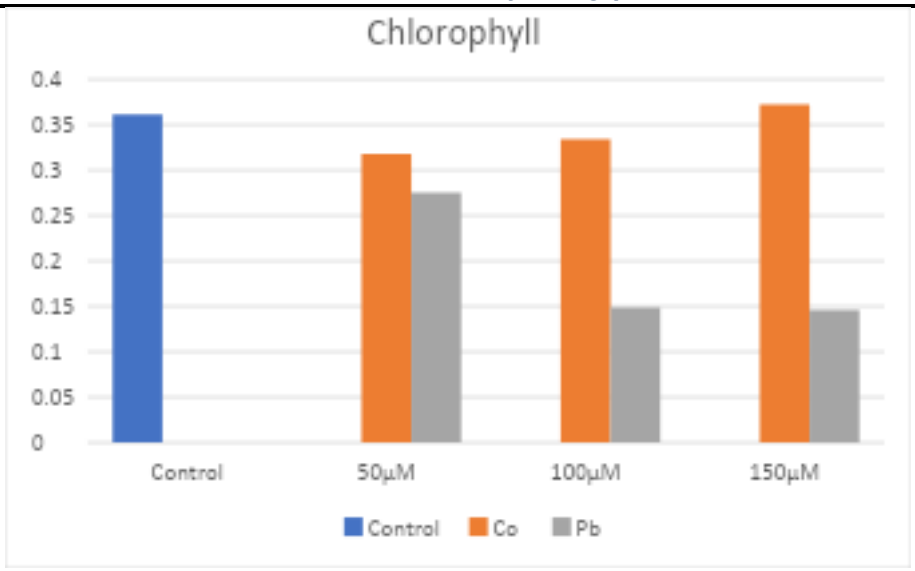
Thus, the data revealed that increasing concentration of heavy metal had negligible effect on plant's growth parameters. *Portulaca grandiflora* has some tendency to with stand against heavy metal stress.

Estimation of Chlorophyll and Proline: -

Research data revealed that chlorophyll content of *Portulaca grandiflora* among different heavy metal concentration as follows: -

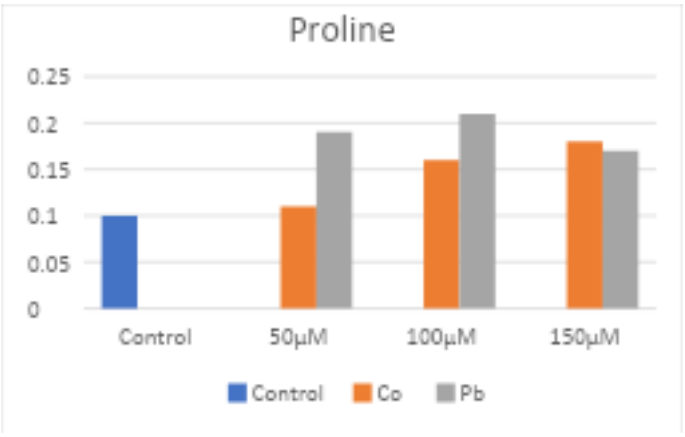
At lower concentration of Pb 50 µM, Chlorophyll was 0.207- 0.375 mg g^{-1}, at Pb 100 µM, Chlorophyll content was 0.185-0.308 mg g^{-1} and at Pb 150 µM, concentration of Chlorophyll was 0.177-0.252 mg g^{-1}. This clearly stated that with an increase in heavy metal concentration the level of chlorophyll decreases but it was lower than that of control which was 0.308- 0.328 mg g^{-1}.

Co had 0.630-0.831 mg g^{-1}, 0.615-0.817 mg g^{-1}, 0.603-0.713 mg g^{-1} of chlorophyll content at Co 50 µM, Co 100 µM and Co 150 µM respectively. This data depicts that increasing in level of heavy metal concentrations *Portulaca grandiflora* shows an increament in chlorophyll content, but the data of control such as 0.308-0.328 mg g^{-1}, suggested that it had lower chlorophyll content than heavy metal treated plant samples.



graph 4: chlorophyll content in *portulaca grandiflora* at different concentration of co and pb

Proline content is stress indicator in plants so it is directly proportional to stress level generated in plants through heavy metals. That means when heavy metal stress induced in plants then proline level will also be increased. Likewise in control plant, proline content was 0.10 mg g^{-1} and at Co 50 µM, 100 µM and 150 µM concentrations, proline content was 0.11 mg g^{-1}, 0.16 mg g^{-1} and 0.18 mg g^{-1} respectively. At Pb 50 µM, 100 µM and 150 µM concentrations, the proline content was 0.19 mg g^{-1}, 0.21 mg g^{-1} and 0.17 mg g^{-1}. It simply suggested that Pb showed high heavy metal stress followed by Co and Control. So Pb has higher proline content than Co and Control.



graph 5: proline content in *portulaca grandiflora* at different concentration of co and pb

Conclusion: -

The research was conducted to determine the effects of different concentrations of Co and Pb on different physiological parameters such as leaf area, height, biomass and biochemical parameters i.e., chlorophyll and proline in *Portulaca grandiflora*. The study revealed that imposition of both heavy metals Co and Pb showed negligible effect in leaf area but showed partial reduction in plant height and partial increment in biomass of *Portulaca grandiflora*. The study was stated that with increasing concentration of Pb the level of chlorophyll decreases as compare to control. But this study depicts that increasing level of Co had negligible effect as compared to control..This research also revealed that Pb showed high heavy metal stress followed by Co and Control. So Pb has higher proline content than Co and Control.

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