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Article

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Posted Date: May 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1462757/v5>

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COMPARATIVE ANALYSIS OF BIOACCUMULATION OF CADMIUM, LEAD, CHROMIUM, COPPER AND NICKEL IN WHEAT CROP

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Abstract:

In this research, the comparative analysis of heavy metals has been studied. An experiment was conducted to determine heavy metals bioaccumulation in wheat as wheat is the staple cereal crop consumed as food globally. Abiotic stress disturbs the metabolic pathway of plants, and the bioaccumulation of the metals cause enzymatic degradation, reduction in photosynthesis, increased level of the ROS species. Comparative analysis of the Cd, Pb, Cr, Cu, and Ni was analyzed with the help of Inductively Coupled plasma optical emission spectrometry (ICP-OES). The results show that Ni has a maximum value of BCF in shoots and roots, whereas the Cr has maximum TF values. SOD and POD assays were performed to check the ROS level in the plants. The results show that plants increase their anti-oxidant enzymes for the scavenging of ROS produced against the bioaccumulation of heavy metals.

Key words:

POD [Peroxide dismutase], SOD [Superoxide dismutase], TF (Translocation factor), BCF (Bioconcentration factor). ROS (Reacting oxygen species)

Introduction:

Wheat is the major cereal crop and major staple food worldwide. Approximately half of the population consumed wheat crops. It is estimated that around 600 million tons of wheat are harvested annually. Globalization and industrialization are increasing exponentially day by day to overcome the resources and their preservation. On the other hand, it adversely affects our environment directly or indirectly. Due to industrial effluents, many pollutants are leached out in groundwater and the atmosphere and cause contamination. Heavy metal contamination is a serious problem for plants and human beings among these pollutants. However, some metals such as Zn, Fe, Na, Mg and Mn are essential for the plants in minimum concentrations. Bioaccumulation of Cd, Pd, Cr, Cu and Ni in different parts of the plants affects the metabolic pathway of plants. Usually, plants show responses against abiotic stress, increase the production of ROS species, reduce chlorophyll content and cause enzymatic degradation. Under abiotic stress, the plants usually reduce photosynthesis and stunted growth of plants. Plants contaminated with metals are digested by animal digestive tracks and produce serious health problems. Cadmium is involved in renal failure, affects the liver and causes bone demineralization. The research's main objective is to analyze the bioaccumulation of heavy metals in different parts of the plant. Phenotypic and biochemical characterization of the wheat plant is the main goal of the research; the leaf is involved in the development of wheat grain and increases the number of grains per spike. It means the leaf indirectly controls crop yield. Different parameters such as BCF and TF were measured in various plants and rhizosphere soil parts.

SOD and POD analyses were formed to check wheat's ROS production level. Biochemical characterization was performed qualitatively and quantitatively to determine the wheat's proteins, carbohydrates, lipids, cellulose, hemicellulose, and lignin contents. The experiment was conducted in the Government University of Faisalabad [New Camps]. The environment of Faisalabad is suitable for the cultivation of the wheat crop. The temperature fluctuates from 4 to 40⁰c in Faisalabad during the wheat season; Wheat cultivation season starts in December when the temperature is nearly equal to the freezing point.

Materials and Methodology:

1. Wheat cultivation:

We have permission to collect the wheat plant from field at GCUF. Wheat sample collection Galaxy-13 of the wheat variety was sown in December in experimental pots to analyze the other parameters of the wheat plants observed under the abiotic stress, known as heavy Metals stress. There were 144 pots in which different strains of the wheat were cultivated, half of them were taken as control and others were taken as cadmium stress. Approximately 40 ml of Cd, Zn, Pd, Ni, Cr and Cu salts were provided to the abiotic stress pot to assess their effects on the wheat crop. Additional parameters of the soils were measured and recorded, including Cd, Zn, Pd, Ni and Cu concentration, electrical conductivity, and H ion concentration of the earth. The inductometer

measured electrical conductivity, and the digital pH meter determined the H ion concentration. After sowing the wheat, approximately all seeds germinated in different pots; the germination started two days after planting the wheat seeds. After the germination, the first leaf of the wheat emerged five days later. The most important factor in the plant's growth is the plant's watering, adequate and controlled. This first irrigation was provided 21 days after the germination. Tillering of many plants was noticed during the period after germination

2. Sampling of the wheat plant and laboratory processing:

The plants and their rhizosphere were sampled and were taken into the laboratory to assess different phenotypic parameters of the plants. Firstly, the plants were washed to remove suspended soil particles from the soil, and their FW was measured and recorded. Further, roots and the shoots of different plants were separated to measure their diameter and lengths; roots numbers were also counted. Shoot lengths of the other plants were also measured with the help of a meter rod. All the flag leaves of the plants were grided and mixed with methanol and rinsed with 0.01% HCl and deionized water to store plant samples for biochemical analysis and the determination of the heavy metal concentrations in different parts of the plants.

3. Acidic digestion:

Acidic digestion that was performed in the laboratory included nitric acid (HNO_3) and hydrogen peroxide (H_2O_2 or hydrogen fluoride (HF)). Soil and plant tissues were digested in the HotBlock digestion system. The digestion process is also known as nitric acid digestion. Before acidic treatment, all the glassware and vessels were washed with acid and rinsed with water. Plants tissues were weighed with the help of digital balance, approximately 0.5 g of plant tissue and 0.25 g for soil samples were used for the acidic treatment, which proceeded as follows: Each piece was digested with 9ml of 65% nitric in digestion vessels containing plant tissue samples, gently swirled and placed onto a HotBlock system at 95 °C for 30 minutes. Further, 3ml of HF were added and digested at 95°C for another 30 minutes. The temperature was increased to 135°C for 1 hour to evaporate the water for the dryness of samples. Afterward, 3ml of HNO_3 and 40ml of de-ionized water were added and boiled until clear. Subsequently, clear solutions were transferred to 150ml flasks, and the samples were cooled down; deionized water was added to make a final volume. Similarly, 10 ml of 65% HNO_3 and 30% H_2O_2 were added, gently swirled and placed onto the Hot Block digestion system for the roots and shoots samples. After that, the temperature was heated at an altering temperature of 95°C-135°C until a solution became clear. Afterward, the keys in appropriate quantity were transferred to the 100ml volumetric flasks, and de-ionized water was added to make up the final volume.

4. Analysis of heavy metals:

The digested samples were analyzed with the help of injection into Inductively Coupled Optical Emission Spectrometry (ICP-OIES). Metals (Cd, Ni, Pb, Cr and Cu) quantification was performed against the National Institute of Standards and Technology, multi-element Standard Reference

Materials (SRM's); Soil 2709a and Apple leave 1515. Metal analysis of samples collected from the Government College University of Faisalabad (New campus) were analyzed and quantified in ICP-OES. All the experiments were performed in accordance with relevant guidelines and regulations.

Results:

Soil properties:

Soil properties of the soil taken as control and metals treated soil were measured at the Government University of Faisalabad; earth shows a pH of 8.01 ± 0.11 , the electrical conductivity of $4.23 \pm 0.48 \text{ mS/m}$ and $0.52 \pm 0.04\%$ TOC was recorded. There exist no larger difference in pH and electrical conductivity for control and treated plants. Faisalabad's soil is mostly alkaline and enriched in organic matter, iron and clay ingredients.

Heavy metal bioaccumulation:

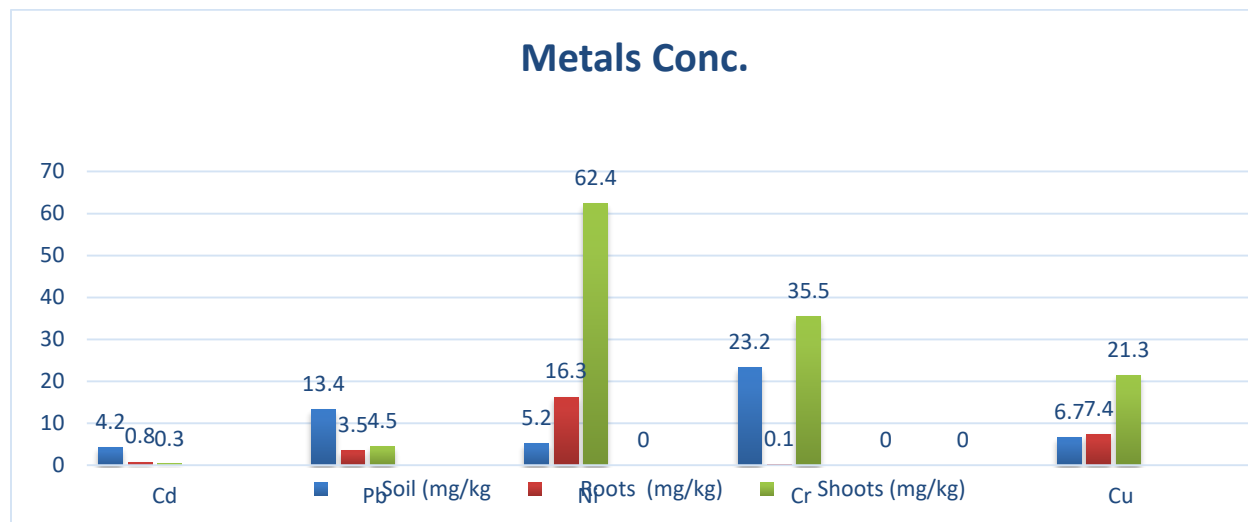
Usually, the plants under abiotic stress show reduced growth due to heavy metals' bioaccumulation that affects the metabolic pathway and reduces photosynthesis and CO_2 assimilation. The concentration of the different metals in the soil was in order $\text{Cr (23.2)} > \text{Pb (13.4)} > \text{Cu (6.7)} > \text{Ni (5.2)} > \text{Cd (4.2)}$ mg/kg, whereas, in plant shoots, bioaccumulation of the metals was noticed in the order of $\text{Ni (62.4)} > \text{Cr (35.5)} > \text{Cu (21.3)} > \text{Pd (4.5)} > \text{Cd (0.3)}$. At the same time, the Bioaccumulation in the root part of the plants was recorded in the order of $\text{Ni (16.3)} > \text{Cu (7.4)} > \text{Pd (3.5)} > \text{Cd (0.8)} > \text{Cr (0.1)}$ mg/kg. It was noticed that Cr and Pd concentrations were much higher than the Cd, Cu, and Ni. However, the Cr (23.2) and Pd (13.4) concentrations were seen to be higher in the soil, while in the shoots part of the plant, maximum bioaccumulation of Ni (62.4) > Cr (35.5) was recorded. But there was more bioaccumulation of Ni (16.3) and Cu (7.4) seen in the plant's roots. The concentrations of the different metals collected after the analysis are given in the (Table:1, Figure:1)

Table:1 BCF for the shoots and roots for Cd, Pb, Ni, Cr and Cu: Ni (12.0) has the highest BCF values for shoots and for roots, Ni (3.13). At the same time, Cr has maximum TF values compared to other metals.

Metals Conc. (mg/kg)	Soil mg/kg	Roots mg/kg	Shoots mg/kg	BCF for Shoots	BCF for roots	TF
Cd	4.2	0.8	0.3	0.071	0.190	0.373
Pb	13.4	3.5	4.5	0.33	0.261	1.264
Ni	5.2	16.3	62.4	12.0	3.13	3.833
Cr	23.2	0.1	35.5	1.530	0.04	38.25

Cu	6.7	7.4	21.3	3.17	1.104	2.871
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Figure:1 Graph represents conc. Of Cd, Pb, Ni, Cr and Cu in soil, roots and shoots. Cr (23.2) conc. In the soil is greater than roots and shoots. Shoots show maximum accumulation of Ni (62.4), and roots show maximum bioaccumulation of Ni (16.3)



Measurements of BCF (Bioconcentration Factor) and TF (Translocation Factor):

The bioconcentration factor is the ratio between the metals bioaccumulated in parts of the plant to the con. Of metals in the soil given in the (Table:1, Figure:2a,2b).

BCF for Shoot = Conc. of [Metal] in shoot / Conc. of [Metal] in soil

BCF for Root = Conc. of [Metal] in root / Conc. of [Metal] in soil

TF = Con. Of [Metal in shoot / Conc. of [Metal] in root

Figure:2a Graphical interpretation of BCF results for shoots and roots. Peaks show Ni has higher values of BCF for shoots (12.0) and for roots (3.19), respectively.

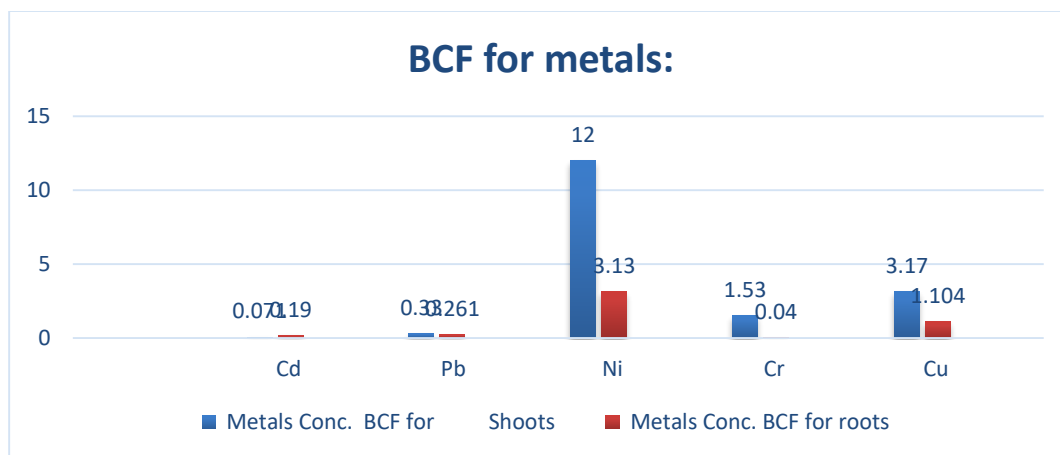
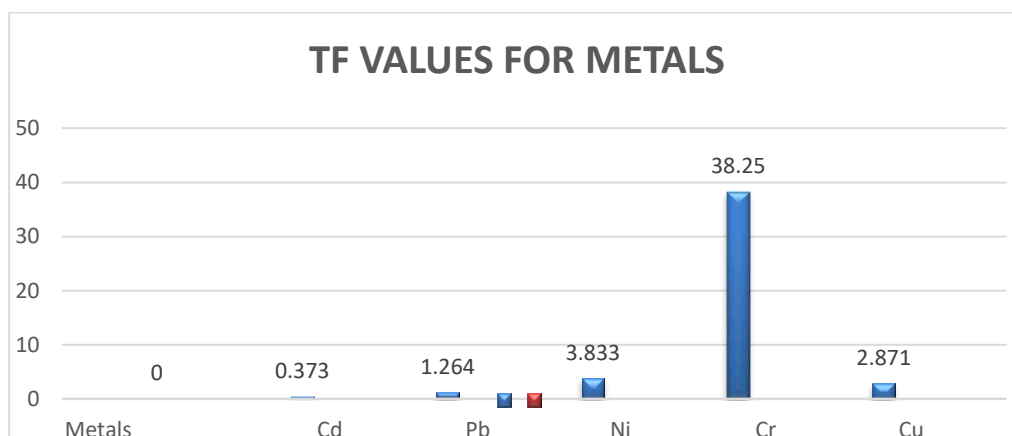


Figure:2b Graph for the TF (Translocation Factor for Cd, Pb, Ni, Cr and Cu values, shows that Cr (38.25) has a maximum value of Translocation factor.



SOD and POD Analysis:

Against the heavy metal stress, plants usually increase their antioxidant enzymes to scavenge the ROS;(Reacting Oxygen Species). In plants, well-organized antioxidative systems, enzymatic and non-enzymatic, exist to cope with environmental biotic and abiotic stress. The anti-oxidants produced against the abiotic stress play a vital role in the scavenging activity of ROS species had due to abiotic stress (Heavy metals) for the maintenance of the physiological redox status of the organism. An increase in SOD (Superoxide dismutase) and POD (Peroxide dismutase) was observed in leaves of the wheat plant due to the bioaccumulation of heavy metals. Increased superoxide production results from the expression of genes encoding the SOD ((Mishra et al., 2006). POD activity imitates the modified mechanical properties of the cell wall and cell membrane integrity of plant leaves under stress conditions.

Discussion:

In this research study, the wheat plants were treated with different heavy metals to analyze the bioaccumulation rate of the heavy metals under the same conditions and concentrations. Galaxy-13 variety of the wheat was selected to assess the concentrations of Cd, Pb, Ni, Cr, and Cu concentrations. Different parameters such as BCF (Bioconcentration factor) and TF (Translocation factor) were measured to determine the concentrations of other metals in the different parts of the plants. SOD and POD assays were performed to measure the ROS (Reacting oxygen species) level in the leaves of the plants. The results show that Ni is more bioaccumulated in shoots and roots. Therefore, the BCF values of the Ni metal in shoot and root are higher than the other metals. But, the TF (Translocation factor) value was higher for the Cr metal than other metals.

Declaration:**Funding's**

No funds

Conflict of interest

All authors have no conflict of interest

Consent for publication

This study is based on research.

Acknowledgment

This research study is self-funded.

Experimental statement

All the experiments were performed in accordance with relevant guidelines and regulations. We have permission to collect the wheat plant from field at GCUF.

Author declaration statement

All authors have participated in this research

AVAILABILITY OF DATA AND MATERIALS:

The data associated with a paper is available, the data available on demand through email contact of co-author.

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