Nutrient Analysis of Two Chickpea Cultivars Grown in Local Soil and Treated with Plant Growth Promoting Bacteria from Bathinda Region

Project submitted

For the award of

Master of Science

In

Life science (Biochemistry)

By

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Supervisor

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May, 2018

DECLARATION

I declare that the project report entitled **" Nutrient Analysis of Two Chickpea Cultivars Grown in Local Soil and Treated with Plant Growth Promoting Bacteria from Bathinda Region"** has been prepared by me under the guidance of Dr. Ramakrishna Wusirika, HOD, Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this Project has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

I certify that Nitin Dogra has prepared his project report entitled **“Nutrient Analysis of Two Chickpea Cultivars Grown in Local Soil and Treated with Plant Growth Promoting Bacteria from Bathinda Region"**, for the award of M.Sc. degree of the Central University of Punjab, under my guidance. He has carried out this work at the Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab.

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Date:

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Although only one name appeared on the cover of this M.Sc. Project but there are many hidden names that helped me to make contents in between the covers. I take this opportunity to express my thankfulness to all of them.

Although I did not find words that could express my thanks to a comparable extent for the help and support that he provided me but still trying to thank my guide Dr. Ramakrishna Wusirika, who guided my M.Sc. and provided all facilities to complete my Project. I have no words to express my gratitude for him. He has motivated me very much and this M.Sc. work would not have been completed without his help. I thank you sir, for all the help and advice you have given me at right time and in right direction.

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ABSTRACT

Nutrient Analysis of Two Chickpea Cultivars Grown in Local Soil and Treated with Plant Growth Promoting Bacteria from Bathinda Region

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Key Words: Plant Growth Promoting Bacteria (PGPB), Shoot and Root length, Plant growth promoting activity, ICP-MS, Biomass quantification.

The bacteria found near plant roots are known to affect plant and soil health. Several important characteristics, such as biological nitrogen fixation, phosphate solubilization and production of siderophores and phytohormones are together called plant growth promoting (PGP) traits. In the current study, the plant growth promoting activity of five bacterial strains, *Pseudomonas citronellis* (PC), *Pseudomonas* sp. RA6, S2, *Serratia marcescens* CDP-13 and Symbion-K (*Frateuria aurantia*) were observed on chickpea varieties PBG1 and PBG5, grown in pots containing soil collected from Central University of Punjab city campus. The plants were harvested after 30 days and shoot length, root length, plant biomass and nutrient content of shoots were evaluated.

Nitin Dogra Dr. Ramakrishna Wusirika

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LIST OF ABBREVIATIONS

Sr. No.	Full Form	Abbreviations
1.	Aluminium	Al
2.	Barium	Ba
3.	Boron	B
4.	Cadmium	Cd
5.	Calcium	Ca
6.	*Capparis decidua* plant	CDP
7.	Chromium	Cr
8.	Cobalt	Co
9.	Copper	Cu
10.	Gibberellic Acid	GA
11.	Indole acetic acid	IAA
12.	Inductively Coupled Plasma–Mass Spectrometry	ICP-MS
13.	Iron	Fe
14.	Lead	Pb
15.	Lithium	Li
16.	Luria-Bertani	LB
17.	Magnesium	Mg
18.	Manganese	Mn
19.	Nickel	Ni
20.	Nitrogen, phosphate, potassium	NPK
21.	Optical Density	OD
22.	Plant growth promoting bacteria	PGPB
23.	*Pseudomonas citronellis*	PC
24.	*Pseudomonas sp. RA6*	RA6
25.	*Serratia marcescens* CDP-13	CDP-13
26.	Sodium	Na
27.	Strontium	Sr
28.	Ultra Violet	UV
29.	Zinc	Zn

1. Introduction

In the last 50-60 years, the use of pesticides, herbicides, and other chemicals to increase the productivity of crops has greatly increased the quantity of food to feed the growing population of the world. However, the use of these chemicals on the land has made the quality of soil worst in terms of heavy metal deposition, fertility, water absorption, etc. It has affected the health of not only humans but also other animals including birds and fishes by biomagnification (Marrugo-Negrete *et al.*, 2017)

The soil is extensively contaminated with heavy metal deposition (Cd, Cu, Pb, Cr, Hg, U). It is important to get rid of these heavy metals as they are non-biodegradable and very harmful to human health as well as the microbial population in soil (Marrugo-Negrete *et al.*, 2017). To deal with this problem phytoremediation technique is employed in which the metal resistant plants are allowed-to precipitate metal ions on or into the roots. This adaptation of plants in the contaminated area is facilitated by microorganisms colonizing both the rhizosphere and phyllosphere, so that they can provide nutrients to plants and can reduce toxic effects of contaminants. Such bacteria associated with plant growth are called plant growth promoting bacteria (PGPB) and contribute to metal tolerance (Navarro-Torre *et al.*, 2016).

1.1 Knowledge Gap

Majority of farmers in Punjab use chemical NPK fertilizers rich in nitrogen, phosphorus, and potassium. Due to the changing soil health of these regions, ways to restore soil health along with production of quality crops with reduced heavy metal contamination are required, this can be achieved by employing local PGPB as biofertilizers for chickpea.

1.2 Hypothesis

Naturally occurring PGPB can be used to promote chickpea growth and nutrient content thereby reducing the reliability on chemical NPK fertilizers and improving soil.

1.3 Objective

1. To study the effect of isolated plant growth promoting bacteria on the growth of chickpea cultivars**.**
2. Determining the nutrient content in plants inoculated with PGPB and in soil used to grow these plants using, inductively coupled plasma–mass spectrometry (ICP-MS).

Chapter 2

Review of Literature

Review of Literature

2.1 Plant growth promoting activities of PGPB

PGPB can stimulate plant growth by either direct or indirect way. In direct way these bacteria produce plant growth regulating hormones and maintain nutritional balance. On the other hand, they induce resistance against plant pathogens and solubilize nutrients for easy uptake by plants to boost their growth.

How PGPB promote plant growth?

- Production of Growth Regulators

  Some bacteria can produce or alter the concentration of growth regulators such as indole acetic acid, gibberellin, cytokinin, and ethylene (Vejan *et al.*, 2016). These growth regulators, also known as phytohormones are found in extremely low amounts and they exert influence on the biochemical, physiological, and morphological processes in plants such as primary root elongation (auxin), seed germination, floral induction, floral and fruit development, stem and leaf growth, cell division, vascular cambium sensitivity, root hair proliferation (cytokinin), ripening of fruits and the abscission of leaves (ethylene) (Lugtenberg *et al.*, 2002).

- Nutrient Fixation and Phosphate Solubilization.

  PGPB can fix the nutrients present in the soil for plants, thereby preventing leaching out of nutrients (Mantelin & Touraine, 2004). Nitrogen required for the synthesis of proteins, amino acids, and nucleic. Plants take the required nitrate and ammonia produced by nitrifying bacteria from atmospheric nitrogen and the rest is converted back to nitrogen dioxide by denitrifying bacteria and liberated back into the atmosphere (Butterbach-Bahl *et al.*, 2013).

  Most PGPB are present in the soil are known for their ability to solubilize phosphate, which is readily taken up by plants (Yadav *et al.*, 2014)

- Production of Secondary Metabolites

  PGPB produce several enzymes which have antibiosis and anti-fungal properties used for the defense purpose from harmful pathogenic microbes present in the soil. These enzymes are hydrolytic in nature e.g. chitinase and glucanase (produce by *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 respectively). They act by degrading the cell wall of the pathogens (Arora *et al.*, 2008)

  Other ways by which PGBP promote plant growth are production of siderophores and volatile organic compounds (Flores-Félix *et al.*, 2015).

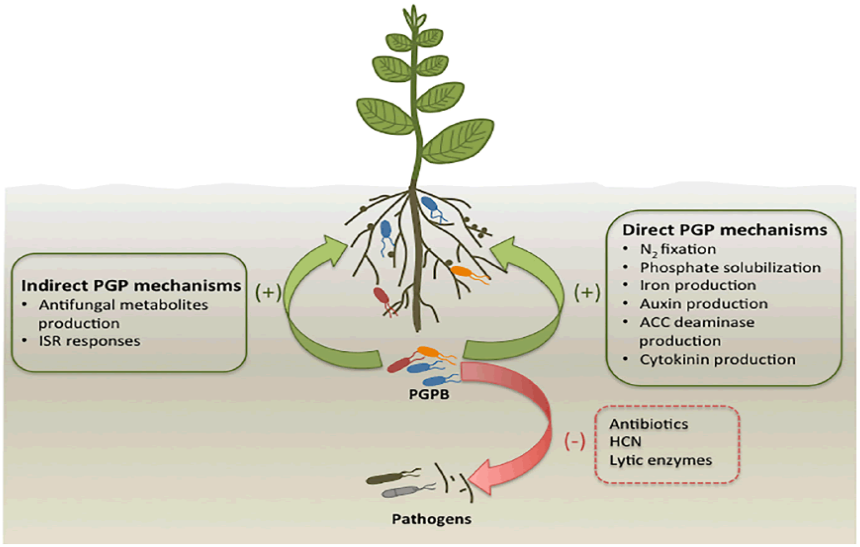

Figure 2.1 Direct and indirect mechanisms employed by plant growth promoting rhizobacteria (Premachandra *et al.*, 2016).

2.2 Use of Nanotechnology and PGPB as Biofertilizers in Agriculture

As the human population is increasing with a blast it is very important to meet the growing demand for food. Seven decades ago Green Revolution saved the lives of billions from undernourishment and starvation by introducing NPK fertilizers (Tarafdar *et al.*, 2013) to enhance the productivity but on the other hand it has resulted in the worsening of the soil health. There is a need to devise a new approach to enhance the crop productivity and restore soil health without affecting the ecosystem. This goal can be achieved combining nanotechnology and agriculture, nano agriculture, focuses on the use of nano sized particles such as nano fertilizers for the efficient uptake of nutrients by plants. This would make it easier to, monitor plant growth, enhance food quality and production and reduce waste. A drawback of using PGPB directly is that 90% of these are lost during incorporation, as they are intolerant to U.V. and heat radiation, run-off etc. Therefore, nanotechnology can be used here for encapsulation (nanoencapsulation technology) of PGPB for their protection enhancement of service life and dispersion in fertilizer formulation for controlled release (Prasad *et al.*, 2010).

2.3 Biofortification

PGPB can be used in biofortification of crops, which is a process to improve the nutritional quality of food crops. This can be done by employing agronomic practices, conventional plant breeding, or modern biotechnology (Khush *et al.*, 2012). Biofortification is one such strategy which can be used to remove the persistent burden of micronutrient malnutrition (Garcia-Casal *et al.*, 2016). Micronutrient malnutrition has an important impact on individuals, health systems as well as societies, resulting in poor health, lower educational attainment among children, and decreased work capacity and earning potential in adults (Bailey *et al.*, 2015).

Chapter 3

Materials and Methods

Material and Methods

3.1. Sample Collection

Five bacterial strains were tested for their ability to promote growth in two chickpea varieties. Two of these bacteria were previously collected from three areas of Bathinda region and characterized as *Pseudomonas sp. RA6* and Pseudomonas *citronellis (*PC), based on biochemical and DNA sequence analysis (Adhikary *et al.*, submitted). The third bacterium, *Serratia marcescens* CDP-13 is a PGPR associated with *Capparis decidua* plant (Singh & Jha, 2016). The fourth bacterial strain designated as S2 was isolated from the soil sample collected from village Ramsara. The fifth one Symbion-K is a commercial bio-fertilizer based on a selective strain of potash solubilizing, beneficial bacteria *Frateuria aurantia* (http://www.tstanes.com/products-symbion-k.html). All these bacterial strains show plant growth promoting traits such as production of the plant hormone, indole acetic acid (IAA) and solubilization of phosphate. Two chickpea varieties (PBG1 and PBG5) procured from Punjab Agricultural University, Ludhiana were used in this study. Plastic pots (diameter 4.5'' and height 4'') were used to grow plants in the soil collected from the Central University of Punjab, Bathinda city campus.

3.2. Preparation of bacterial inoculum

LB media (1 ml) was inoculated with a single colony of the bacterial strains and incubated for 24hrs at 37° C. This culture was inoculated in 250 ml LB and incubated for 24hrs at 37° C. The cell density was adjusted to 10^8 cells/ml based on absorbance at 550nm. The cells were centrifuged for 10 min at 4000rpm at a temperature of 4^0 C (Bashan *et al.*, 2002). The pellet was resuspended in saline buffer. This solution was later used for inoculation of the seeds.

3.3. Inoculation and growth conditions

Seeds were kept in pertriplates having cotton beds and rinsed with distilled water till the seeds started sprouting after which they were immersed in PGPB suspended in saline buffer for 45 minutes. Five seeds were sown in each pot of treatment and control. The pots were kept in the green house and allowed to grow for 30 days.

3.4. Plant growth measurements and nutrient quantification

Shoot lengths of plants were measured after every 15th day and 30th day after germination. At the end of each experiment, root length of each plant was measured and compared with control plants used for each strain. The biomass of plants before drying (fresh weight) and after drying (dry weight) was taken on the 30th day using the weighing balance. Nutrient quantification was performed using the ICP-MS samples were dried and exactly 0.5 gm was taken and mixed with 10 ml digesting solution (8 ml 70% HNO_3 and 2 ml H_2O_2) in tubes designed for microwave digestion (Krachler *et al.*, 2002). The tubes were kept in Microwave Digesting System for 4-5 hours for digestion. After digestion, the samples were filtered using Whatman filter paper followed by filtration using syringe filter. The filtered solution was diluted 10,000 times using sterile water (Becker *et al.*, 2008). These samples were given to the CIL (Central Instrumentation Laboratory) for ICP-MS analysis. The same procedure mentioned above was used for ICP-MS analysis of soil used for growing the plants.

3.5. Statistical analysis

A pair-wise t-test was performed for statistical significance. ($p<0.05$) of each treatment with the control. The parameters taken for this analysis were shoot length, root length and nutrient concentration of each element quantified in the plant samples.

Chapter 4

Results and Discussion

4. Results and Discussion

PBG1 variety plants treated with the strains PC and RA6 showed increased growth but no growth promoting activity was seen with S2, Symbion-K and CDP-13, in the first 15 days (**Figure 4.1**). On the 30th day, the height of the plants treated with PC, Symbion-K and CDP-13 increased compared to the control plants where CDP-13 and PC treatment showed significant growth in the plants. In case of PBG5 variety, the shoot length measured on the 15th day showed that out of all the bacterial strains tested, S2, PC, RA6 and CDP-13 promoted the plant growth by increasing the shoot length but a significant growth was seen only in S2 and CDP-13 treated plants (**Figure 4.2**). On the 30th day, all the treated plants showed higher shoot length compared to the control and this increase was significant in case of S2, PC, RA6 and CDP-13. The root length of treated plants taken on the same day was less than that of the control with no increase in length in both PBG1 (**Figure 4.3**) and PBG5 varieties (**Figure 4.4**).

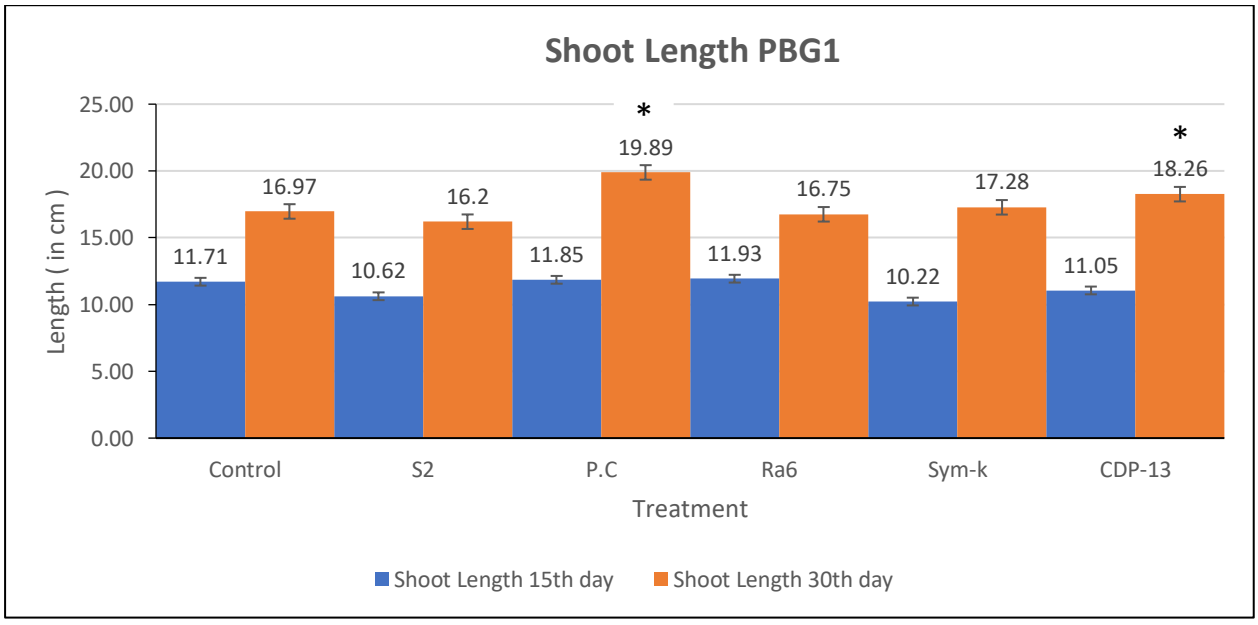

Figure 4.1. Shoot length of chickpea variety PBG1 taken after 15 and 30 days of germination. (* represents p value<0.05 & ** p value<0.01). Error bars show standard error.

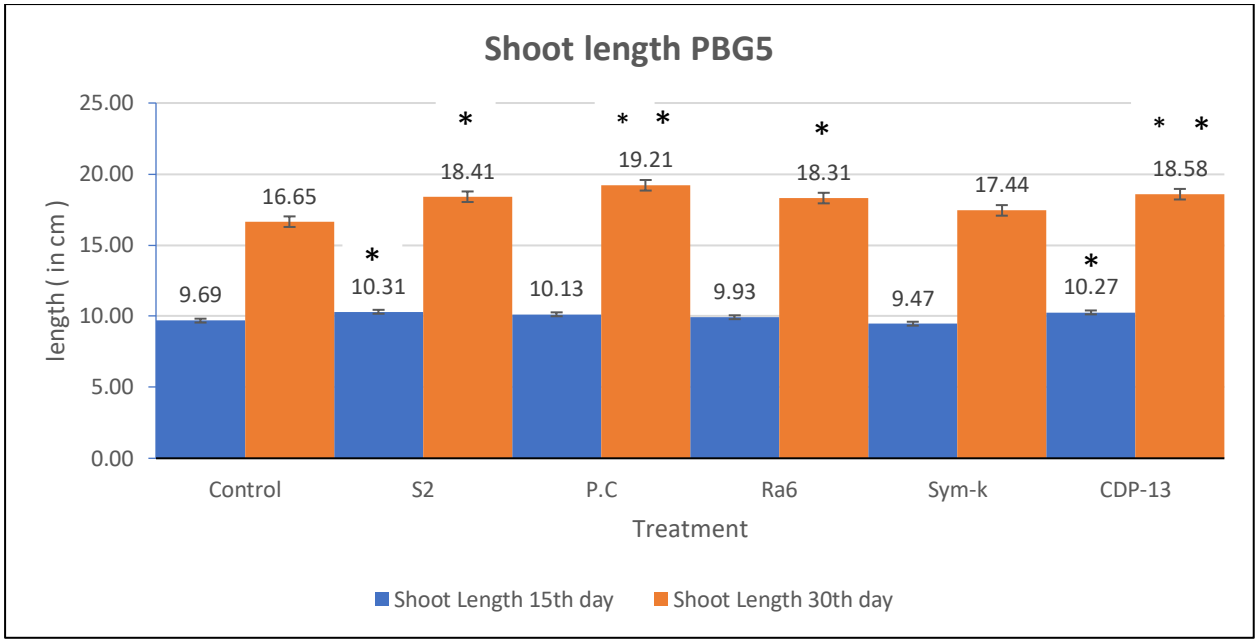

Figure 4.2. Shoot length of chickpea variety PBG5 taken after 15 and 30 days of germination.

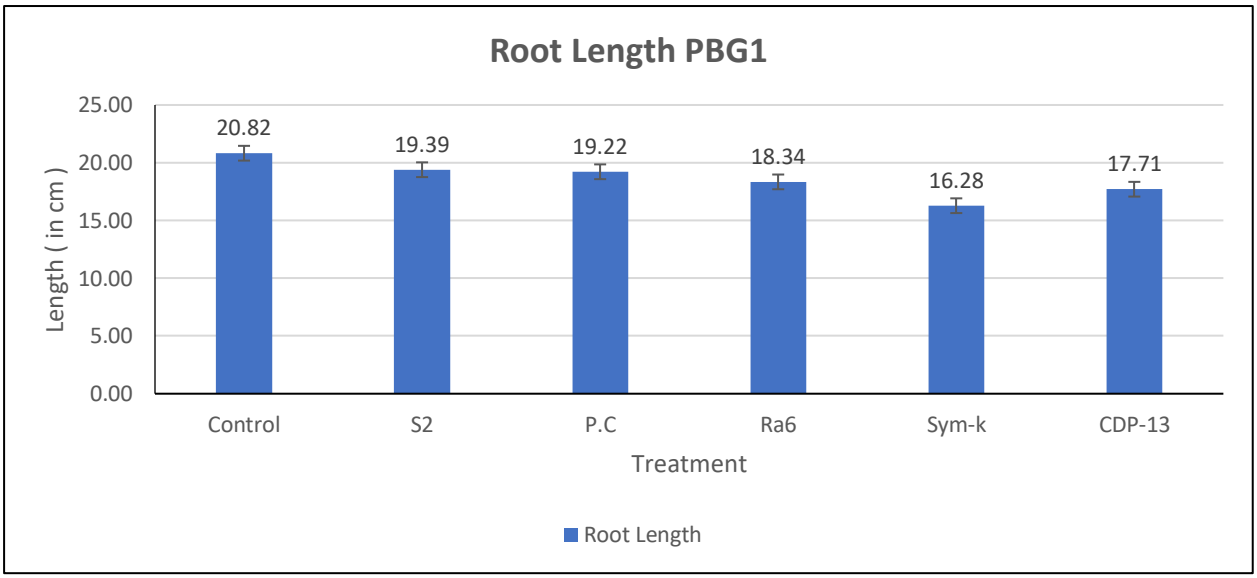

Figure 4.3. Root length of chickpea variety PBG1 taken after 30 days of germination.

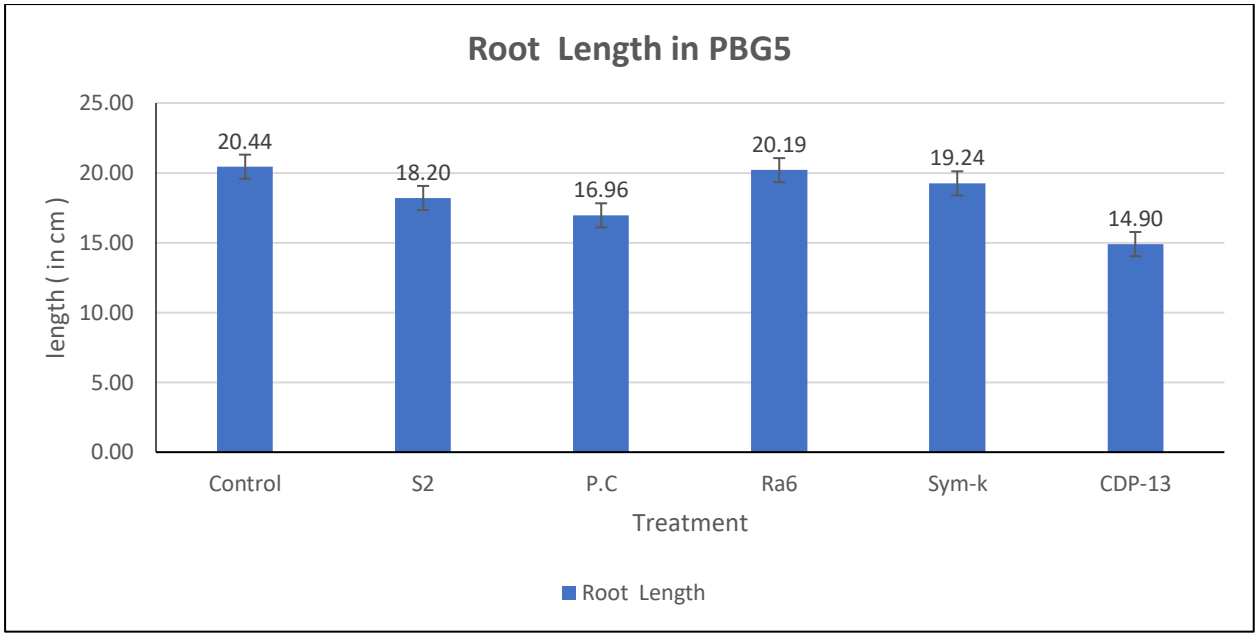

Figure 4.4. Root length of chickpea variety PBG5 taken after 30 days of germination.

The next step was to determine the biomass of the plants harvested. PBG1 variety plants showed higher fresh weight in the CDP-13 and PC treated plants whereas the dry weight measurements showed increased weight only in CDP-13 treated plants (**Figure 4.5**). In case of PBG5 variety, the fresh and dry weight of all the treated plants increased when compared to the control plants (**Figure 4.6**). However, none of the changes were statistically significant ($p<0.05$).

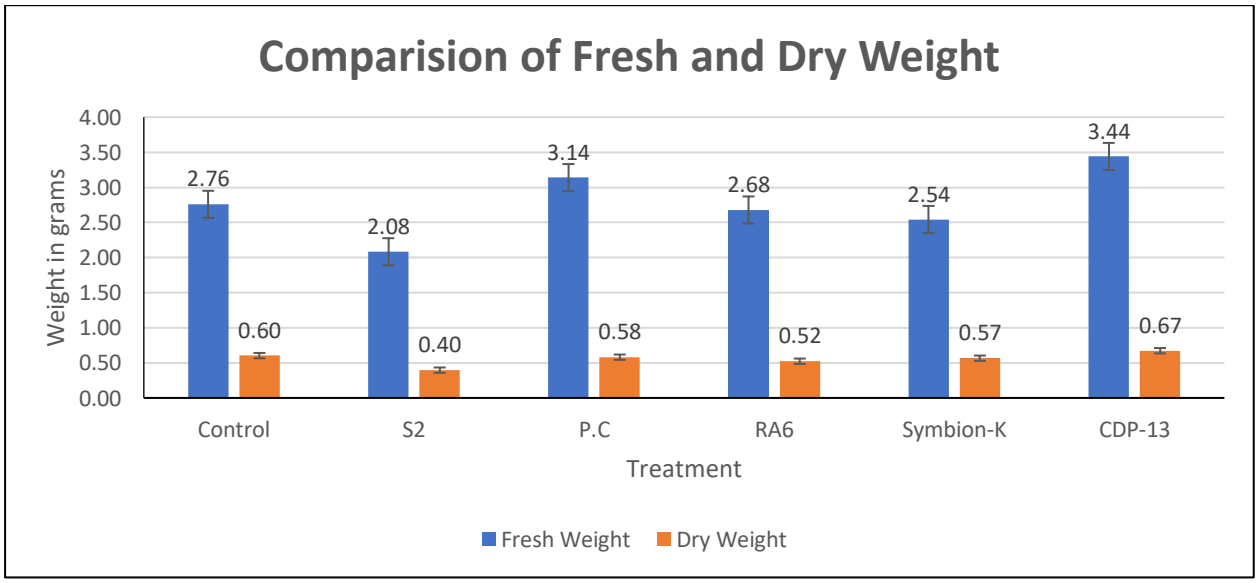

Figure 4.5. Comparison of fresh and dry weight of PBG1 shoots.

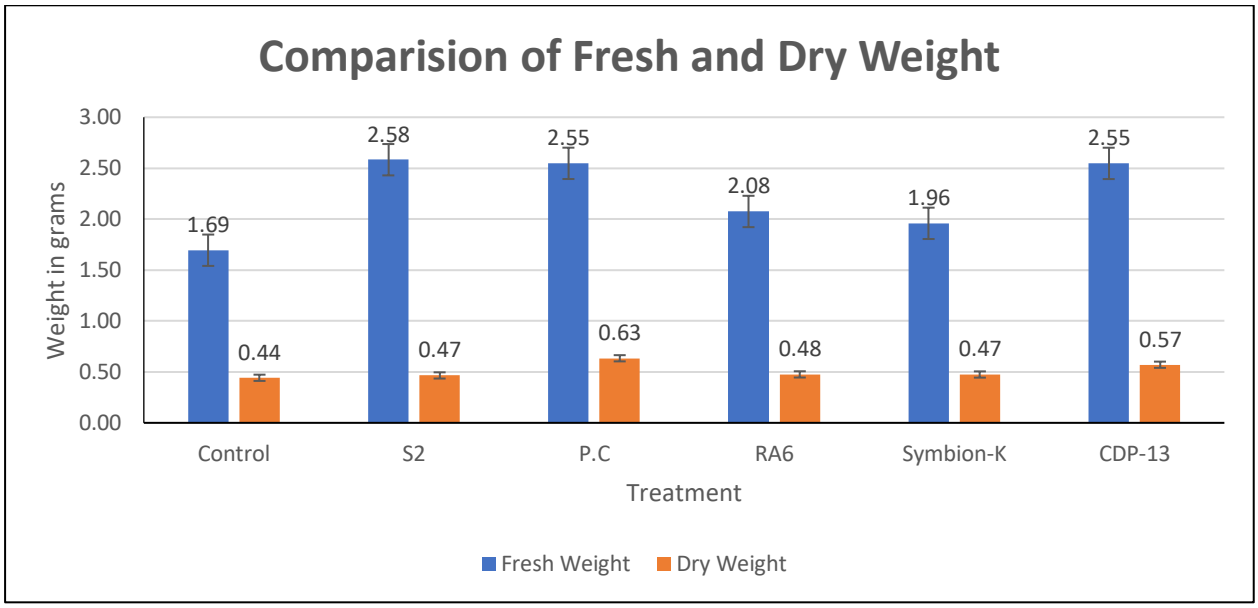

Figure 4.6. Comparison of fresh and dry weight of PBG5 shoots.

Macronutrients (N, K, Ca, Mg, P, and S) are needed in higher quantities and micronutrients (Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni) are needed in lower quantities for optimal plant growth. Most of these nutrient levels and levels of heavy metals were evaluated using ICP-MS. PBG1 variety treated with S2, PC and CDP-13 showed higher concentration of lithium and PBG5 variety showed significant accumulation of lithium in S2, PC, Symbion-K and CDP-13 treated plants (**Figure 4.7**). In PBG1 as well as PBG5 significant concentration of boron was seen in all the treated plants compared to control plants (**Figure 4.8**). In case of sodium, significant accumulation was seen in PC and Symbion-K treated plants whereas in PBG5 variety, significant accumulation was seen in all the treated plants compared to untreated control plants (**Figure 4.9**). Increase in magnesium levels in PBG5 plants treated with CDP-13 and Symbion-K was observed but they were not significant (**Figure 4.10**). In case of PBG1 variety, all treated plants showed less accumulation than the control plants.

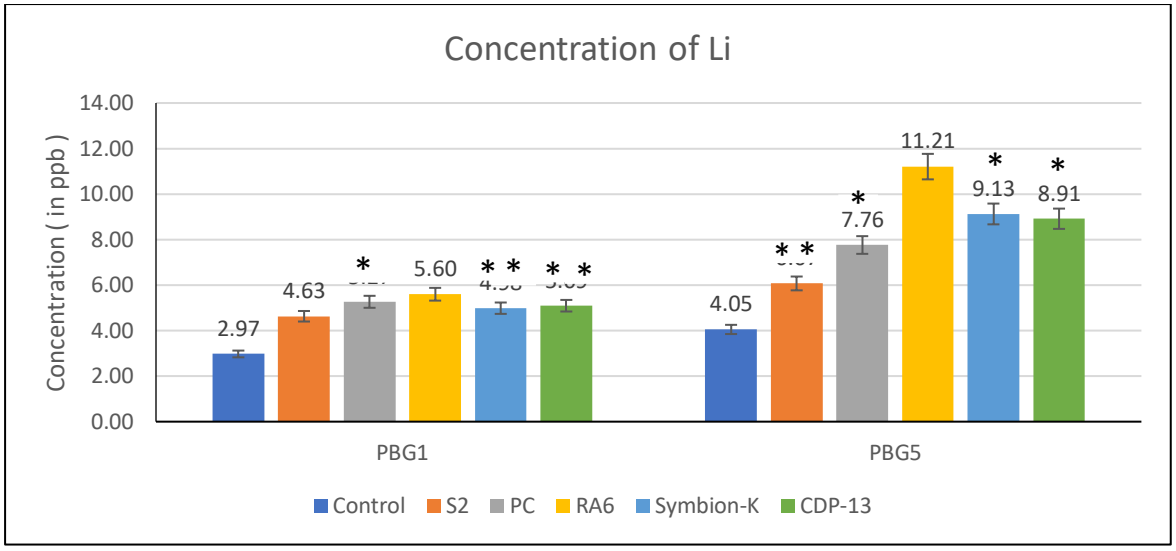

Figure 4.7. Comparison of lithium concentration in shoots of PBG1 and PBG5. (* represents p value<0.05 & ** p value<0.01). Error bars show 5% error.

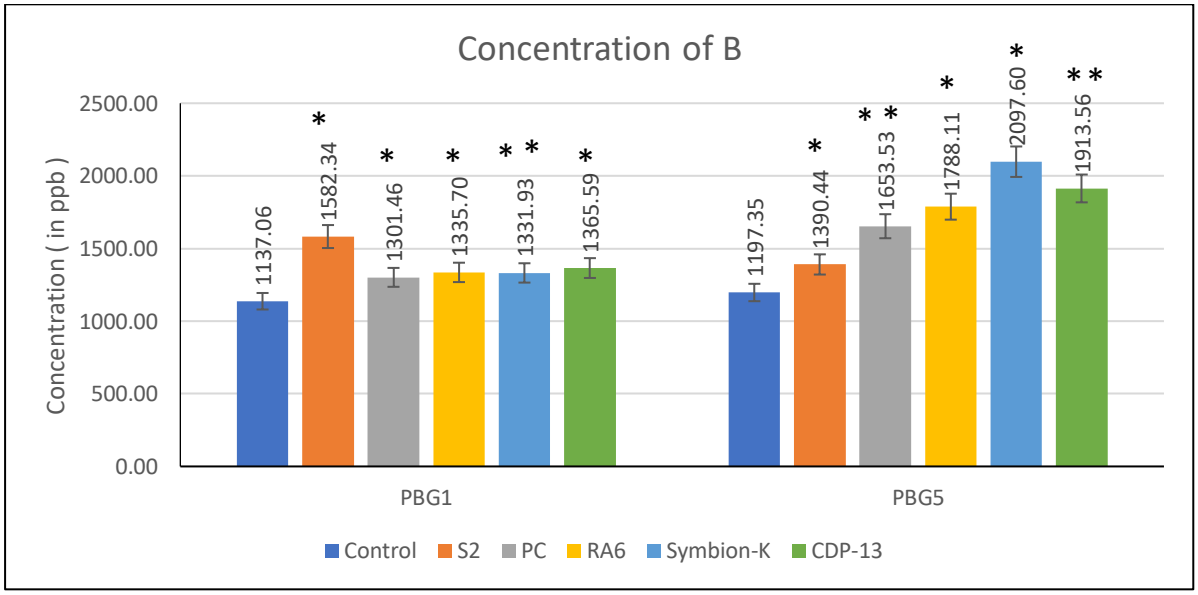

Figure 4.8. Comparison of boron concentration in shoots of PBG1 and PBG5.

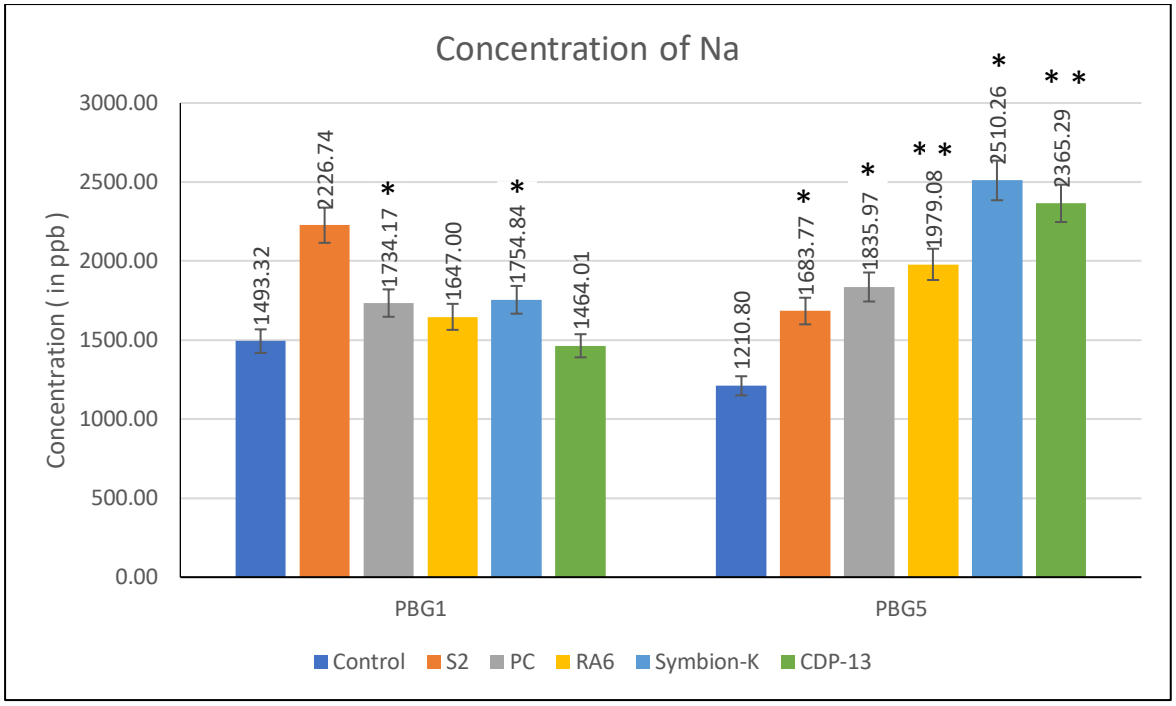

Figure 4.9. Comparison of sodium concentration in shoots of PBG1 and PBG5.

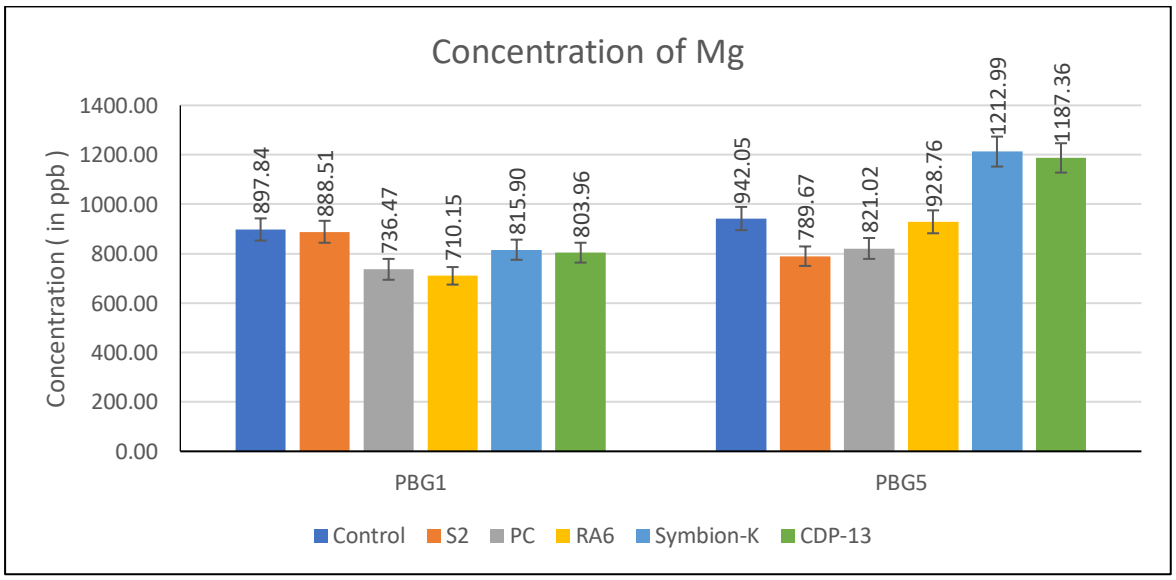

Figure 4.10. Comparison of magnesium concentration in shoots of PBG1 and PBG5.

Aluminium accumulation was higher in the PBG5 variety shoots in all treatments except S2 but PBG1 showed increase in all treatments except RA6 (**Figure 4.11**). However, the increase was not significant. Significant accumulation of calcium was seen in the PBG1 variety treated with CDP-13 and PBG5 variety treated with PC, Symbion-K and CDP-13 (**Figure 4.12**).

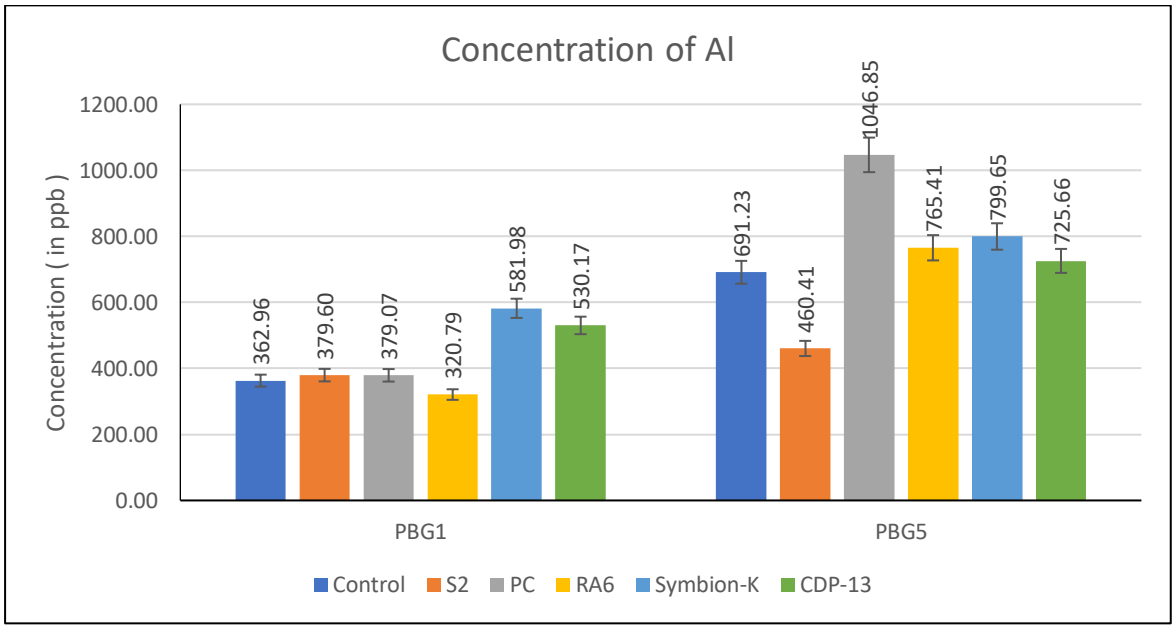

Figure 4.11. Comparison of aluminum concentration in shoots of PBG1 and PBG5.

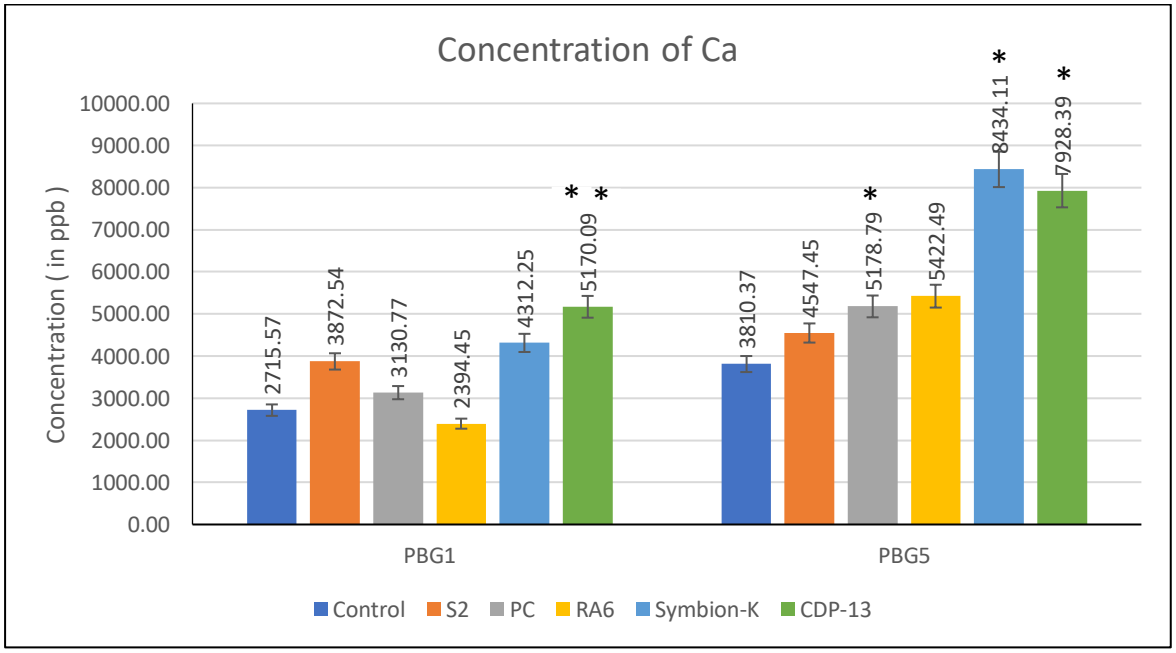

Figure 4.12. Comparison of calcium concentration in shoots of PBG1 and PBG5.

Chromium levels were significantly higher in CDP-13 treated PBG1 shoots and RA6, Symbion-K and CDP-13 treated PBG5 shoots (**Figure 4.13**). In case of manganese, no significant increase was seen in either variety, but higher accumulation was seen in PBG1 treated with S2, Symbion-K and CDP-13 and PBG5 in all treatments compared to control plants (**Figure 4.14**).

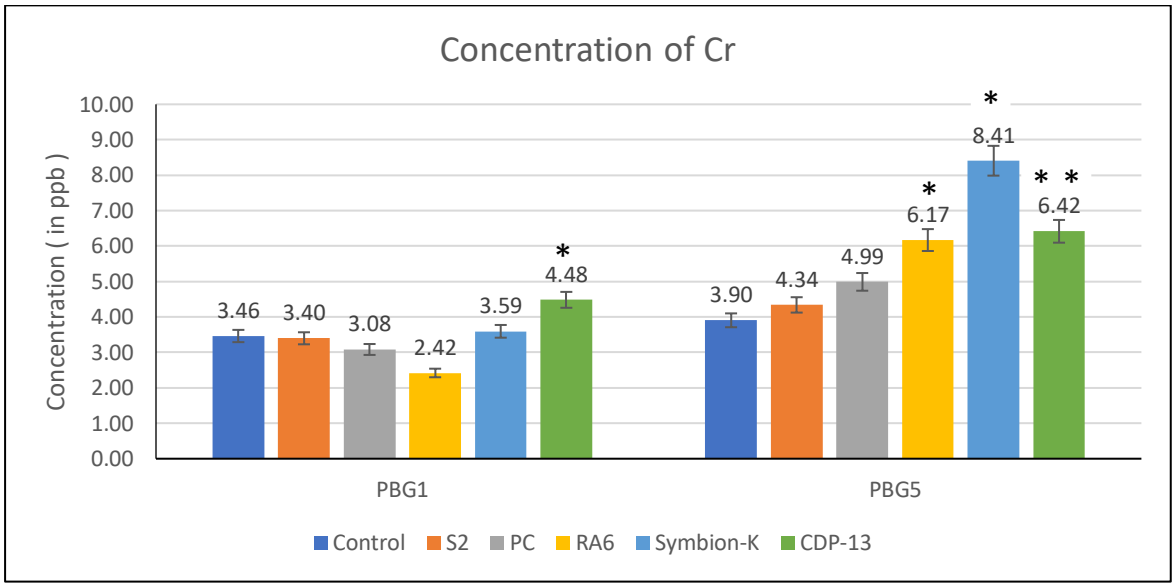

Figure 4.13. Comparison of chromium concentration in shoots of PBG1 and PBG5.

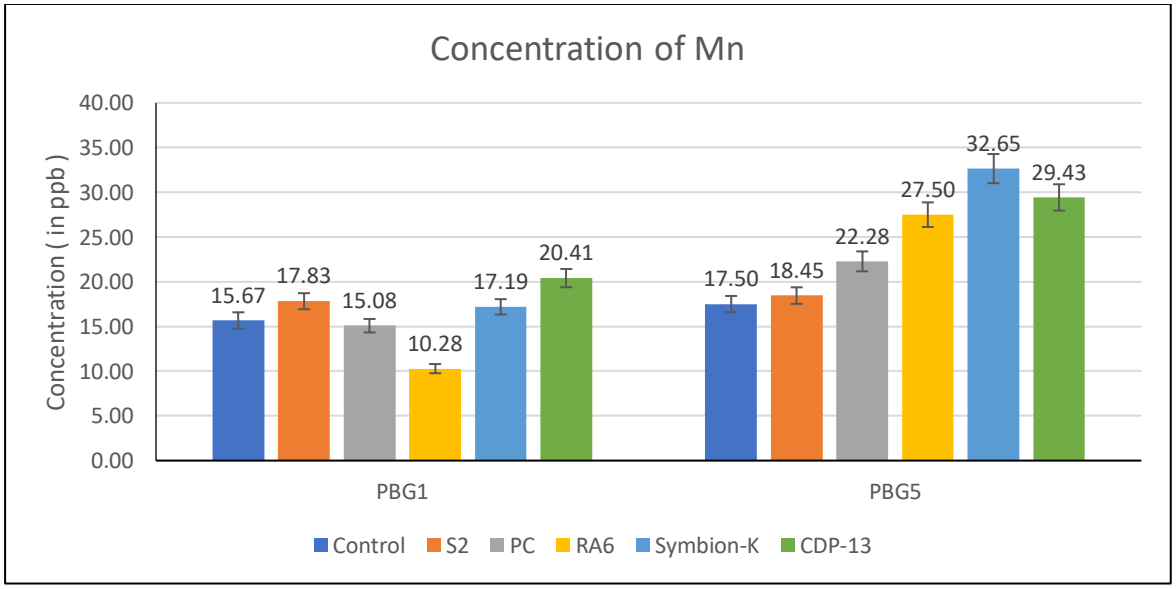

Figure 4.14. Comparison of manganese concentration in shoots of PBG1 and PBG5.

Increase in iron accumulation was observed in PC, RA6 and CDP-13 treated PGB5 shoots but significant accumulation was seen only in CDP-13 treated plants (**Figure 4.15**). Iron levels were higher in all treatments compared to control plants but significant only in case of three treatments: PC, RA6 and CDP-13. Cobalt levels increased in all treatments in PBG5, but significant accumulation was seen only in RA6 and CDP-13 treated plants (**Figure 4.16**).

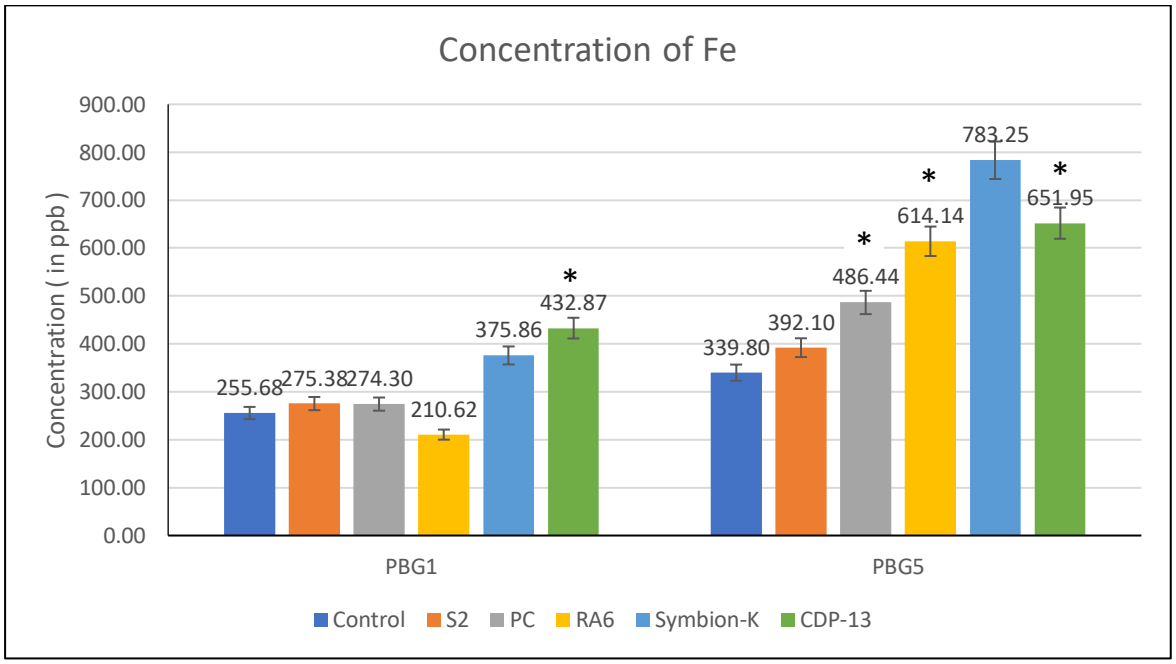

Figure 4.15. Comparison of iron concentration in shoots of PBG1 and PBG5.

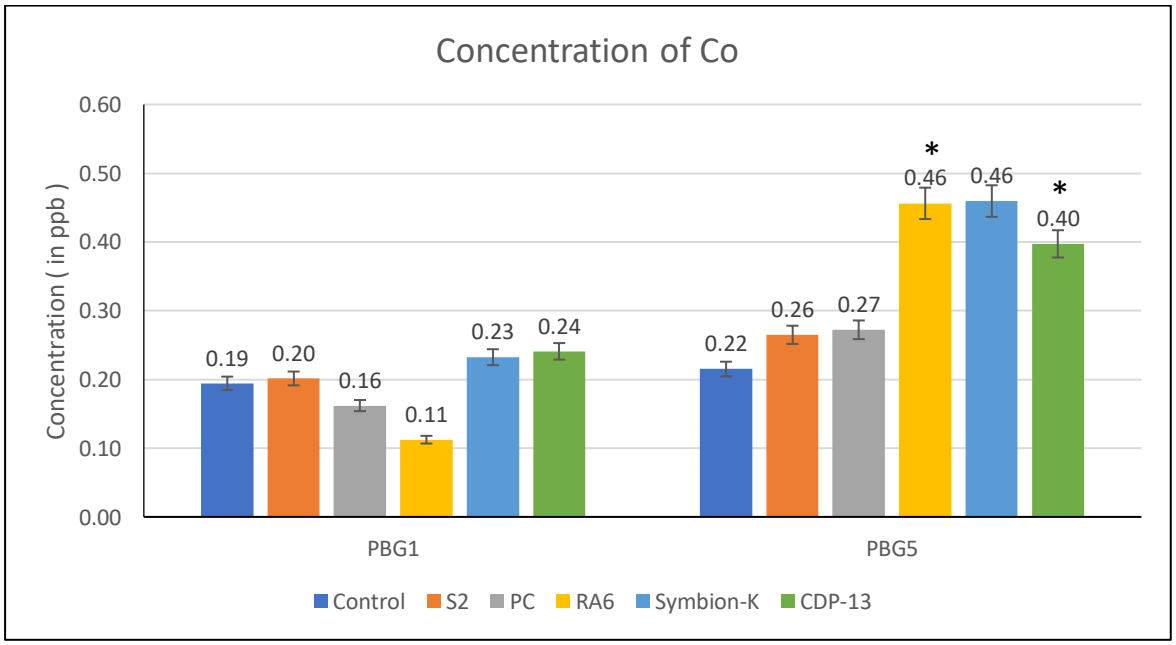

Figure 4.16. Comparison of cobalt concentration in shoots of PBG1 and PBG5.

Nickel levels were significantly higher in RA6, Symbion-K and CDP-13 treated PBG5 plants and Symbion-K and CDP-13 treated PBG1 plants (**Figure 4.17**). Significant accumulation of copper was seen in PBG5 variety shoots only in CDP-13 treated plants compared to control plants (**Figure 4.18**).

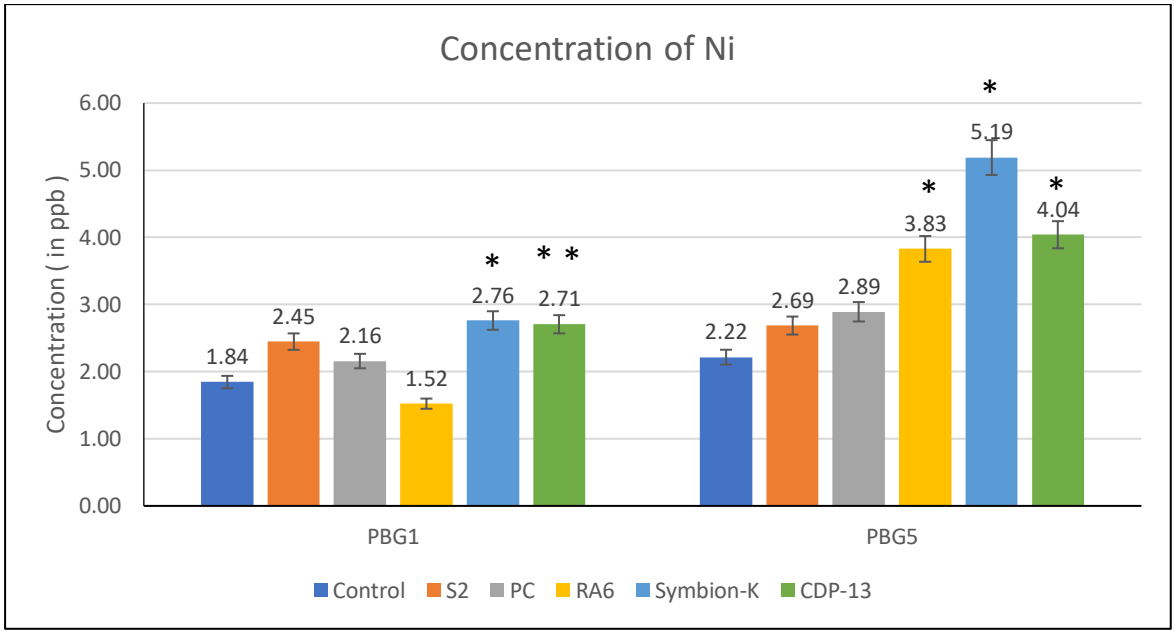

Figure 4.17. Comparison nickel concentration in shoots of PBG1 and PBG5.

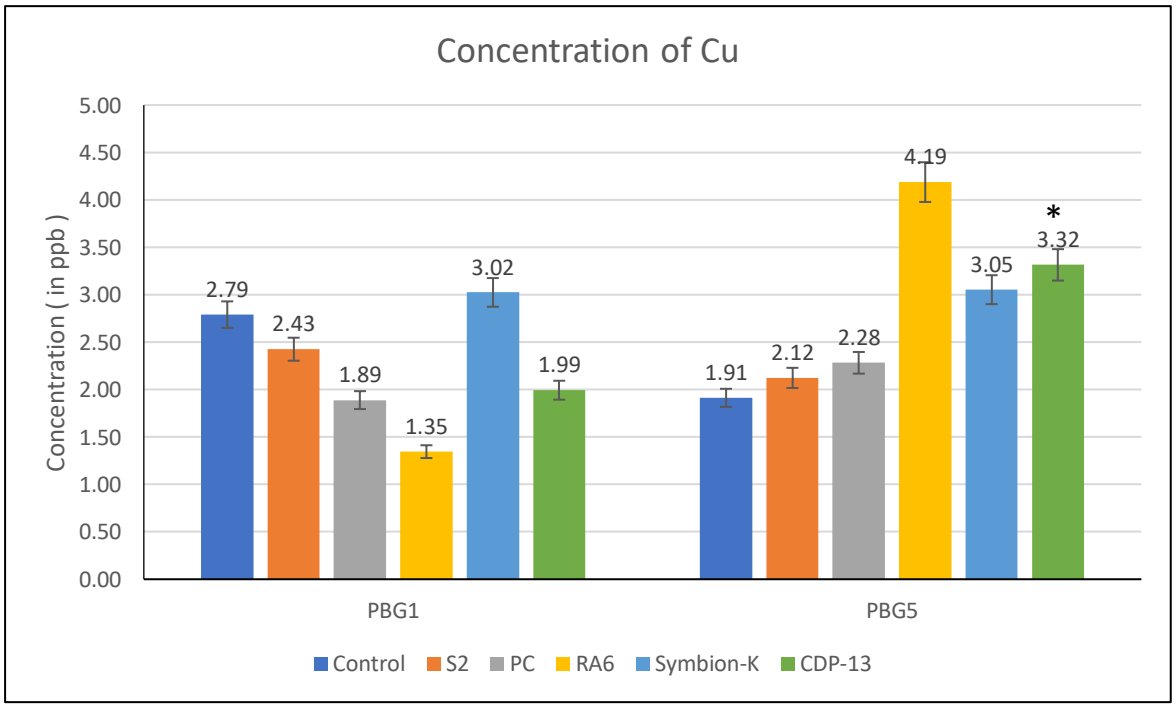

Figure 4.18. Comparison copper concentration in shoots of PBG1 and PBG5.

Significant accumulation of zinc was seen only in shoots of PBG5 variety plants treated with RA6, Symbion-K and CDP-13 (**Figure 4.19**). Strontium showed significant accumulation in PC, Symbion-K and CDP-13 treated PBG5 plants and CDP-13 treated PBG1 plants (**Figure 4.20**).

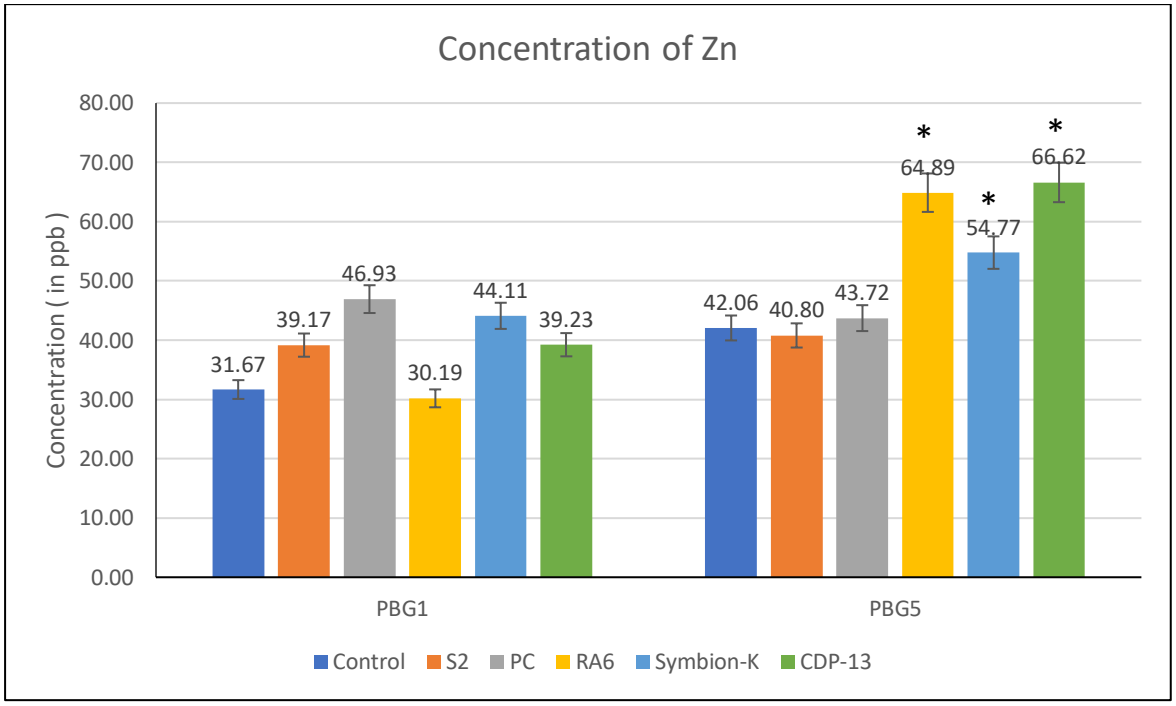

Figure 4.19. Comparison of zinc concentration in shoots of PBG1 and PBG5.

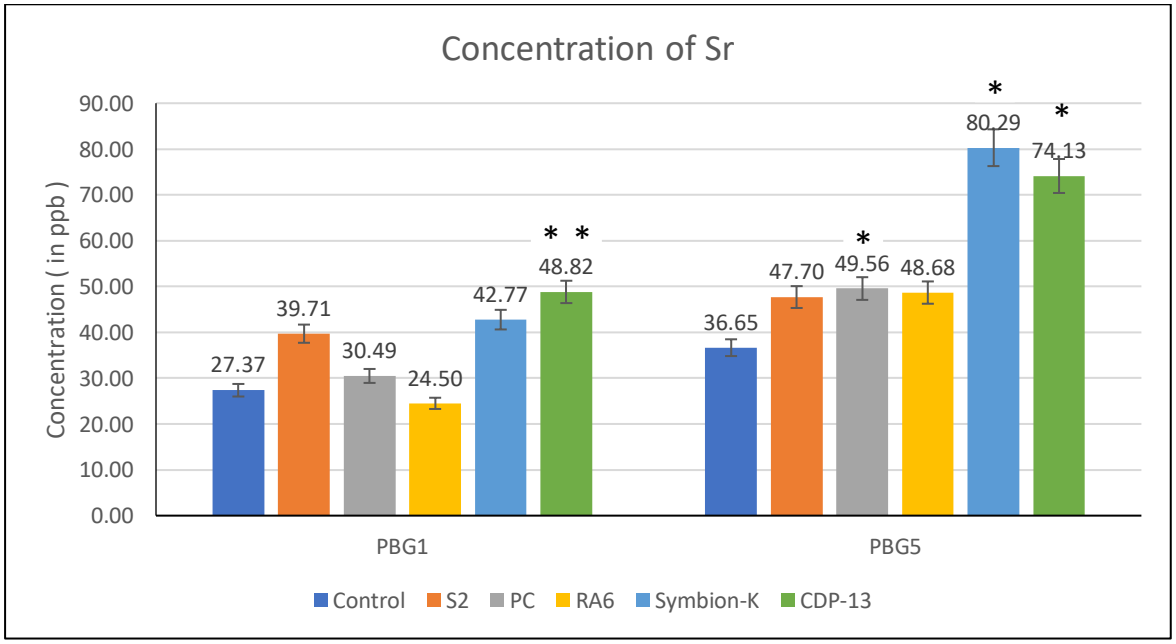

Figure 4.20. Comparison of strontium concentration in shoots of PBG1 and PBG5.

Barium showed significant accumulation in PC, RA6, Symbion-K and CDP-13 treated PBG5 plants and CDP-13 treated PBG1 plants (**Figure 4.21**). Significant accumulation of lead was seen in PBG5 variety treated with RA6, Symbion-K and CDP-13 (**Figure 4.22**).

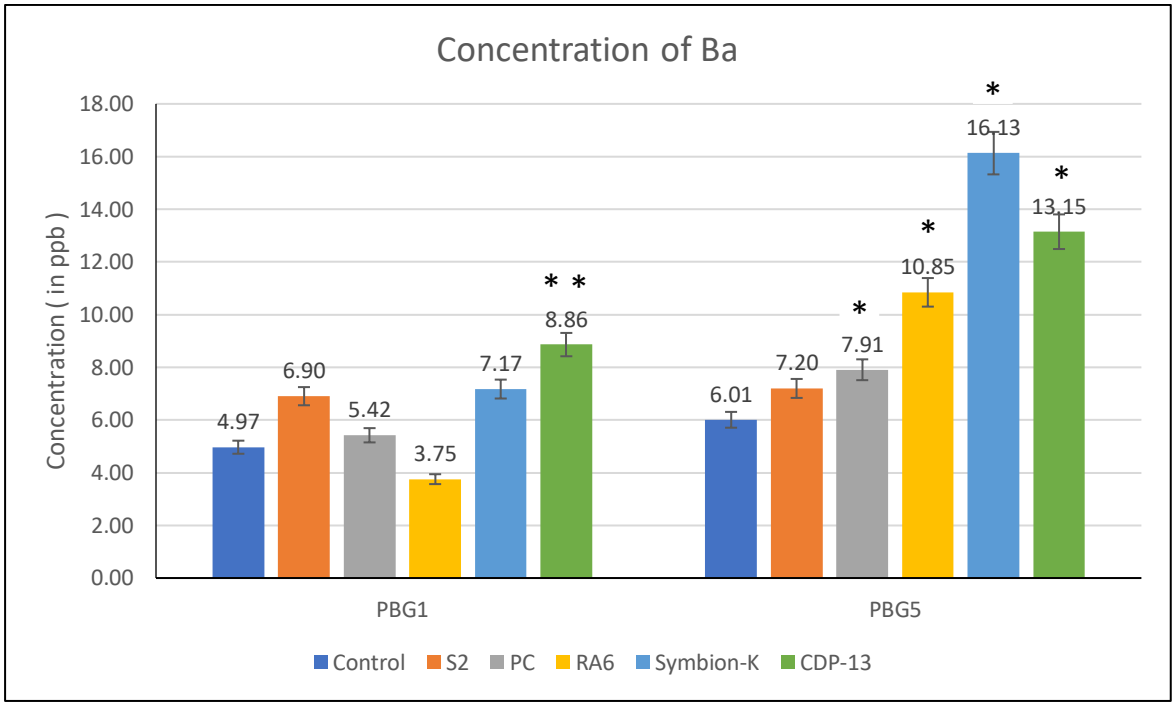

Figure 4.21. Comparison of barium concentration in shoots of PBG1 and PBG5.

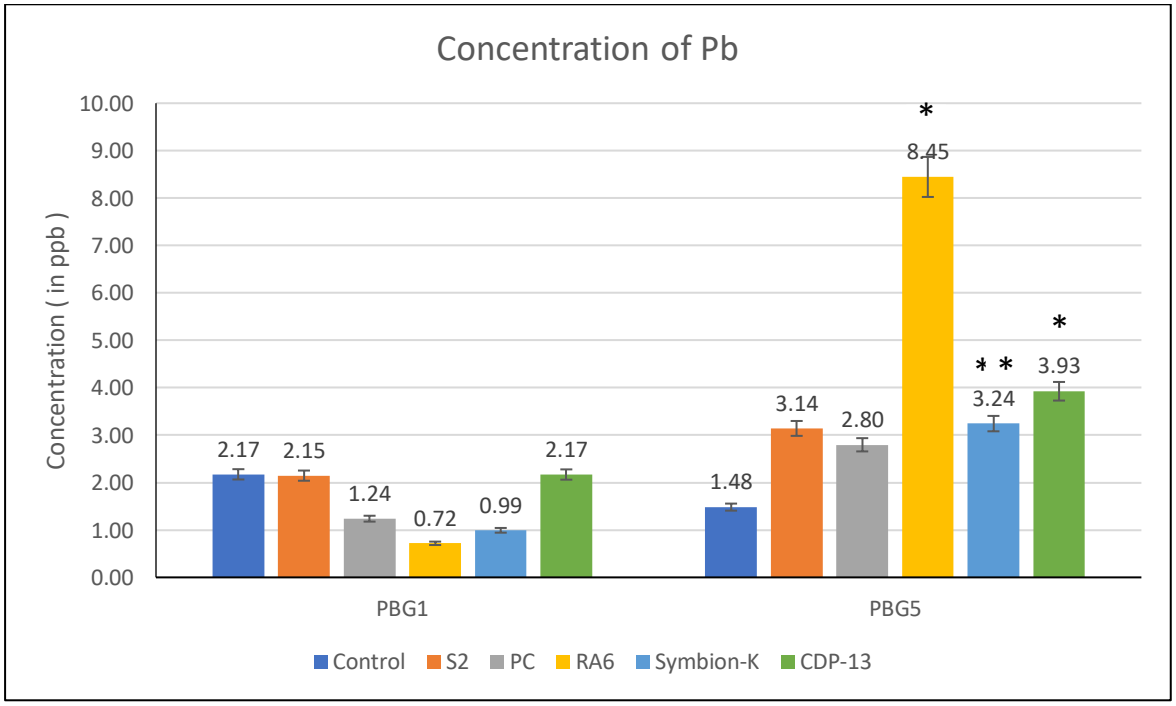

Figure 4.22. Comparison of lead concentration in shoots of PBG1 and PBG5.

From the above-mentioned data, we can conclude that the levels of all the elements except sodium, copper and lead were higher in control plants of PBG5 variety compared to PBG1 variety. PGPB enhanced the macronutrient, calcium in both PBG1 and PBG5, and magnesium only in PBG5. The levels of micronutrients, iron, boron, manganese, zinc and copper were higher in one or more PGPB treated plants in both PBG1 and PBG5. PC and Symbion-K showed higher levels of maximum elements while RA6 showed the least (3) while CDP-13 showed significant enhancement of seven elements compared to control out of a total of 12 elements. In case of PBG5 variety, CDP-13 and Symbion-K showed maximal increase of elements with 15 elements out of 18 and 9 elements out of 18, respectively. RA6 did not show maximum activity in PBG1 variety but in case of PBG5 it showed increase in 11 out of 17 elements. Lead levels did not increase in treated plants as compared to that of control plants in PBG1 variety but in case of PBG5 lead levels were higher than the control plants. However, the higher levels of lead in PBG5 were lower than the toxic level reported in plants. Overall, the ICP-MS data showed an increase in many essential macro and micronutrients in both PBG1 and PBG5. PBG5 is a high yielding variety compared to PBG1 and PBG5 confers better resistance to *Aschochyta* blight and *Fusarium* wilt compared to PBG1 (Sandhu *et al.*, 2004). Therefore, PGPB can be considered as one of the modern-day strategy of biofortification to deal with the malnutrition spread in many areas of the world.

To determine the nutrient content of the soil used for the experiment, three soil samples were collected from the same area and three replicates of 0.5 g were analyzed by ICP-MS (Table 4.1). The concentration of the elements in the soil were much higher compared to plant shoot. The soil ICP-MS data was compared with three other soil samples collected from agricultural fields in Bathinda (unpublished data). The level of potassium is 30-40% higher in field soil samples compared to the soil used in this study, which may be due to the use of fertilizers. The concentrations of magnesium were 40% higher, calcium was 4-fold, strontium and mercury was >2-fold and aluminium 30% lower than the soil from agricultural fields in Bathinda.

Table 4.1. ICP-MS data of soil used for growing PBG1 and PBG5 varieties.

S. No.	Element	Replicate 1	Replicate 2	Replicate 3	Average
1	**Boron**	978.8	1015.7	1109.5	1034.7
2	**Sodium**	1263.4	1637.1	1626.7	1509.1
3	**Magnesium**	3290.4	3326.8	3729.8	3449.0
4	**Aluminium**	3556.6	3570.4	3742.5	3623.2
5	**Potassium**	1091.6	1083.2	1200.9	1125.3
6	**Calcium**	7968.8	8355.7	8612.5	8312.4
7	**Chromium**	11.2	11.7	12.9	12.0
8	**Manganese**	133.7	129.4	135.9	133.0
9	**Iron**	5643.6	5788.7	6224.7	5885.6
10	**Cobalt**	3.1	3.2	3.3	3.2
11	**Nickel**	8.0	8.4	8.7	8.4
12	**Copper**	2.7	2.3	2.7	2.6
13	**Zinc**	21.3	18.3	18.9	19.5
14	**Arsenic**	2.7	2.7	2.9	2.8
15	**Strontium**	69.9	76.7	77.6	74.8
16	**Silver**	0.055	0.044	0.032	0.043
17	**Cadmium**	0.043	0.248	0.051	0.114
18	**Indium**	0.257	0.200	0.163	0.206
19	**Barium**	32.6	31.7	33.2	32.5
20	**Mercury**	6.5	4.4	4.2	5.0
21	**Lead**	2.8	7.3	2.7	4.3
22	**Bismuth**	1.6	1.1	0.861	1.2

Value of elements is in ppb.

To feed the increasing population, it is very important to produce new methods and technology to enhance crop productivity without harming the health of the soil. The currents methods used by most of the countries rely primarily on fertilizers, pesticides, insecticides and other artificially prepared chemicals to enhance the productivity. All these methods increase crop productivity but, they also spoil the soil health by making it contaminated with heavy metals and harmful chemicals. Plant growth promoting bacteria used as part of biofertilizers can enhance plant growth without negatively affecting soil health. Development of local PGPB suitable for a particular region is needed. More studies on these PGPB strains and others can be instrumental for their application thereby reducing the reliance on chemical fertilizers.

References:

Arora, N. K., Khare, E., Oh, J. H., Kang, S. C., & Maheshwari, D. K. (2008). Diverse mechanisms adopted by fluorescent Pseudomonas PGC2 during the inhibition of *Rhizoctonia solani* and *Phytophthora capsici*. *World Journal of Microbiology and Biotechnology,* **24(**4), 581-585.

Bailey, R. L., West Jr, K. P., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism,* **66**(Suppl. 2), 22-33.

Bashan, Y., Hernandez, J.-P., Leyva, L. A., & Bacilio, M. (2002). Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biology and Fertility of Soils,* **35**(5), 359-368.

Becker, J. S., Sela, H., Dobrowolska, J., Zoriy, M., & Becker, J. S. (2008). Recent applications on isotope ratio measurements by ICP-MS and LA-ICP-MS on biological samples and single particles. *International Journal of Mass Spectrometry,* **270**(1-2), 1-7.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Phil. Trans. R. Soc. B,* **368**(1621), 20130122.

Flores-Félix, J. D., Silva, L. R., Rivera, L. P., Marcos-García, M., García-Fraile, P., Martínez-Molina, E.*, et al.* (2015). Plants probiotics as a tool to produce highly functional fruits: the case of Phyllobacterium and vitamin C in strawberries. *PLos One,* **10**(4), e0122281.

Garcia-Casal, M. N., Peña-Rosas, J. P., Pachón, H., De-Regil, L. M., Centeno Tablante, E., & Flores-Urrutia, M. C. (2016). Staple crops biofortified with increased micronutrient content: effects on vitamin and mineral status, as well as health and cognitive function in the general population. *Cochrane Library*, **8**, CD012311.

Khush, G. S., Lee, S., Cho, J.-I., & Jeon, J.-S. (2012). Biofortification of crops for reducing malnutrition. *Plant Biotechnology Reports,* **6**(3), 195-202.

Krachler, M., Mohl, C., Emons, H., & Shotyk, W. (2002). Influence of digestion procedures on the determination of rare earth elements in peat and plant

samples by USN-ICP-MS. *Journal of Analytical Atomic Spectrometry,* **17**(8), 844-851.

Lugtenberg, B. J., Chin-A-Woeng, T. F., & Bloemberg, G. V. (2002). Microbe–plant interactions: principles and mechanisms. *Antonie Van Leeuwenhoek,* **81**(1-4), 373-383.

Mantelin, S., & Touraine, B. (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *Journal of Experimental Botany,* **55**(394), 27-34.

Marrugo-Negrete, J., Pinedo-Hernández, J., & Díez, S. (2017). Assessment of heavy metal pollution, spatial distribution and origin in agricultural soils along the Sinú River Basin, Colombia. *Environmental Research,* **154**, 380-388.

Navarro-Torre, S., Mateos-Naranjo, E., Caviedes, M., Pajuelo, E., & Rodríguez-Llorente, I. (2016). Isolation of plant-growth-promoting and metal-resistant cultivable bacteria from Arthrocnemum macrostachyum in the Odiel marshes with potential use in phytoremediation. *Marine Pollution Bulletin,* **110**(1), 133-142.

Prasad, R., Jain, V., & Varma, A. (2010). Role of nanomaterials in symbiotic fungus growth enhancement. *Current Science,* **99**(9), 1189-1191.

Premachandra, D., Hudek, L., & Brau, L. (2016). Bacterial modes of action for enhancing of plant growth. *Journal of Biotechnology & Biomaterials,* **6**(3), 1-8.

Singh, S., Bains, I., Sharma, T.S., Sidhu, R., & Singh, S. (2004). PBG 5: a new multiple disease resistant desi chickpea variety for Punjab, India. International Chickpea and Pigeonpea Newsletter, pp. 18-20.

Singh, R. P., & Jha, P. N. (2016). The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). *PLos One,* **11**(6), e0155026.

Tarafdar, A., Raliya, R., Wang, W.-N., Biswas, P., & Tarafdar, J. (2013). Green synthesis of TiO2 nanoparticle using *Aspergillus tubingensis*. *Advanced Science, Engineering and Medicine,* **5**(9), 943-949.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules,* **21**(5), 573.

Yadav, J., Verma, J. P., Jaiswal, D. K., & Kumar, A. (2014). Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). *Ecological Engineering,* **62**, 123-128.