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Consequence of chromium-tainted soil on physical and biochemical responses of *Vigna radiata* L.

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ABSTRACT

Mung bean (*Vigna radiata* L.) is one of the important leguminous plants of India, with shorter growing season. In this work, the intention was to correlate the effects of chromium on physical and biochemical responses of *V. radiata* L. Mung bean seeds were germinated and grown under controlled treatment, with different concentrations of waste soil mixed with garden soil. Physical parameters such as shoot length, root length, and fresh weight were found highest in control soil and 25% of contaminated soil, whereas 50% and pure contaminated soil showed poor growth stage and poor quality physical parameters. Biochemical parameters such as total chlorophyll content, protein content, and starch content were found highest in control soil and 25% contaminated soil, but these parameters were found to be less in 50% and pure contaminated soil. It was observed that physical and chemical parameters were declining with increasing chromium contamination.

1. INTRODUCTION

Heavy metals are major pollutants as they influence multiple organ damages even at low levels of exposure. Heavy metal pollution is the result of anthropogenic activities such as mining, energy and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluent dumping, and military operations [1,2]. Metals such as aluminum, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium, and zinc have been considered as the major environmental pollutants, and their phytotoxicity has already been studied [1]. Raised concentrations of both essential and non-essential heavy metals in the soil and water can lead to toxicity symptoms and growth inhibition in most plants [3-5]. Absorption, translocation, and accumulation of heavy metal ions of Hg, Pb, Cr, and Cd by plants, reduce productivity of the species and cause severe health hazards through the food chain to other life forms [6-8].

1.1. Effect of Chromium Toxicity in Plants

Cr phytotoxicity can result in reticence of seed germination, degrade pigment status, nutrient balance, antioxidant enzymes, and prompt oxidative stress in plants [9]. Cr can change chloroplast and membrane, ultrastructure [10,11]. In higher plants, heavy metals induce oxidative stress by generation of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet of oxygen (O_2) collectively termed as reactive oxygen species (ROS). ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, and amino acids, leading to irreparable metabolic dysfunction and cell death. ROS produced under stress is unfavorable to growth because these molecules cause steady lipid peroxidation, inactivation of antioxidant enzymes, and oxidative dehydroascorbic acid damage [12,13]. Therefore, induction of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) is an important protective mechanism to minimize oxidative damage in plants. SOD is ubiquitous enzyme which plays a key role in cellular defense mechanism against ROS [14]. Its activity modifies the relative amount of O_2^- and H_2O_2 by Haber–Weiss reactions and reduces the risk of OH radical formation which is highly reactive and may cause severe damage to membrane proteins and DNA [5,15]. CAT is less effective in eradicating H_2O_2 due to its low substrate affinity while the main response of tolerant plants to heavy metal is increase in SOD and POD activities [16,17]. Cr (III) is toxic to plants even at low concentration and reported to causes severe oxidative damage to plant cell. It affects growth, water balance, and pigment content and initiates lipid peroxidation causing oxidative damage to plants [18,19]. Cr (VI), on the other hand, is more phytotoxic than Cr (III) and retards growth, reduces the number of palisade and spongy parenchyma cells in leaves, and increases the number of vacuoles and electron dense material along the walls of xylem and phloem [20].

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2. MATERIALS AND METHODS

2.1. Study Area

As per the statistics, Odisha accounts for about 98% of the total chromite (chromium ore) reserves of the country, of which about 97% occur in the Sukinda Valley, Sukinda is a town in Jaipur district, Odisha, India. Sukinda is situated in between longitude 20°58′0″N and latitude 85°55′0″E. The area of study was Kaliapani, Sukinda, Odisha. Kaliapani is a small place in Sukinda according to its size, but it is a highly economically important place of India.

2.2. Collection of Soil Sample

The waste soil of the overburden was collected from Saruabil Chromite Mines, Kaliapani, Sukinda, Odisha. The waste soil was collected in gunny bags and kept under air in field condition for 48 h to decrease its moisture level. It was then powdered, sieved, and subjected to analysis for various important constituents.

The pH of the dark-colored soil of the study area was measured to be 7.10 ± 0.03 having electrical conductivity 46.33 µs/ppm. It contains 5.433 mg of chromium per kg of the waste soil as analyzed by atomic absorption technique. Potassium contents of the waste soil were 0.224 kg/ha, respectively.

2.3. Preparation of Culture Pots

The dried and powdered waste soil was used for the preparation of culture pots with garden soil to study the effect of the waste soil combination on *Vigna radiata* L. and also to compare the effect with control soil taken from garden on *V. radiata* L. Three different concentrations of soil sample were taken in triplicate with comparison to control garden soil (pH 6.88 ± 0.03) [Table 1]. The concentrations of waste soil in pots were as follows:

- Pot 1 - 100% waste soil (pH 7.10 ± 0.03, EC 46.33 µs/ppm)
- Pot 2 - 50% waste soil with 50% control soil (pH 6.85 ± 0.03, EC 44.16 µs/ppm)
- Pot 3 - 25% waste soil with 75% control soil (pH 6.70 ± 0.03, EC 43.50 µs/ppm)
- Pot 4 - Control soil (100% normal garden soil) (pH 6.88 ± 0.03, EC 46.20 µs/ppm).

In each pot soil, sample was taken in a constant weight of 2 kg, and combinations were prepared according to the concentration of the waste soil sample taken. Pot 1 was having 2 kg of 100% waste soil, pot 2 was having 1 kg of waste soil and 1 kg of control soil (total 2 kg), pot 3 was having 500 g of waste soil and 1.5 kg of control garden soil (total 2 kg), and pot 4 was having 2 kg control soil.

The pots were kept on roof and were watered equally. The soil water mixture was allowed to settle for 2 days. Seeds of *V. radiata* L. were sown in the pots containing varying contaminated waste soil combinations. The pots were arranged in triplicate and various parameters were observed on the 15th day from seed germination and on the 30th day from germination.

2.4. Available Chromium (Cr)

The available chromium of the soil samples after combination was determined by DTPA (Pentetic acid or diethylene triamine penta-acetic acid) extraction method. 10 g of each soil sample was dissolved in 20 ml of DTPA solution. Then, it was shaken for 2 h and filtered using 42 number filter paper. Reading was taken using atomic absorption spectroscopy. The unit of available chromium was taken in mg/kg. The waste soil had 5.433 mg/kg of available chromium, whereas the combinations showed decrease in concentration of available chromium content varying from 2.016 mg/kg (50% waste soil + 50% control soil) and 1.725 mg/kg (25% waste soil + 75% control soil), respectively.

2.5. Available Nitrogen (N)

The available nitrogen of the soil samples after combination was determined by alkaline potassium permanganate method in Kel Plus automatic machine [21]. The waste soil had 25% of available nitrogen, whereas the combinations showed increase in the concentration of available nitrogen content varying from 75% (pot 2), 87.5% (pot 3), and control was having 112.5% of nitrogen, respectively.

2.6. Available Potassium (K)

The available potassium of the soil samples after combination was determined by flame photometry. 7.709 g of ammonium acetate dissolved in 100 ml of distilled water. Then, 5 g of each soil sample was dissolved in 25 ml of ammonium acetate solution. Then, it was shaken and pH was maintained up to 7.0 and filtered. Reading was taken using flame photometer. The unit of available potassium was taken in kg/ha. The waste soil had 0.224 kg/ha of available potassium, whereas the combinations showed increase in the concentration of available potassium content varying from 18.37 kg/ha (pot 2), 40.21 kg/ha (pot 3), and control was having 61.04 kg/ha.

2.7. Seed Sowing

Seeds are rinsed with water for 3–4 times. The seeds were sown in the pots at a rate of 6 (*V. radiata* L.) seeds per pot.

2.8. Shoot Length (cm) and Root Length (cm)

On the day of final count of the germination test, i.e., 15th and 30th days, 5/6 normal seedlings were collected from each treatment and in each replication. The shoot length was measured from the base of primary leaf to the base of hypocotyl, and mean shoot length was expressed in centimeters.

5/6 seedlings used for shoot length measurement were also used for the root length measurement. It was measured from the tip of primary root to the base of hypocotyl, and mean root length was expressed in centimeters.

Table 1: Different soil parameters

Treatment	pH	EC (µs/ppm)	Available Cr. (mg/kg)	Available n (%)	Available K (kg/ha)
100% waste soil - pot 1	7.10±0.03	46.33	5.433	25	0.224
50% waste soil+50% control soil - pot 2	6.85±0.03	44.16	2.016	75	18.370
25% waste soil+75% control soil - pot 3	6.70±0.03	43.50	1.725	87.5	40.210
100% control (normal garden soil) - pot 4	6.88±0.03	46.20	Not detected	112.5	61.040

2.9. Sampling Procedure

2.9.1. Extraction and estimation of chlorophyll

The leaves were analyzed for total chlorophyll content by homogenizing 500 mg of leaves in 80% acetone, and the extract was centrifuged, and the supernatant was analyzed for chlorophyll content in a spectrophotometer. The chlorophyll content was calculated as described by Arnon's method [22].

2.9.2. Extraction and estimation of protein

Extracted leaf samples were precipitated with 50% trichloroacetic acid and then centrifuged at 10,000 rpm for 15 min. Residue was dissolved in 1 N NaOH to it 5 ml of reagent mix (2% Na_2CO_3 in 0.1 NaOH, 0.5% $CuSO_4$ in 1% Rochelle's salt), 0.5 ml of Folin's reagent added. Incubation was done for 30 min and then OD was taken at 650 nm [23].

2.9.3. Starch estimation

The leaves were analyzed for starch content by Anthrone reagent method as described by Clegg. Then, it was analyzed for starch content in a spectrophotometer [24].

3. RESULTS AND DISCUSSION

In heavy metal contamination, heavy metals are dispersed with soil particles from early contaminated region by air and water. When these particles are settled, heavy metals may scatter into the adjoining areas.

3.1. Effect of Chromium-contaminated Soil on Quality Parameters of *V. radiata* L.

In Table 2, it was observed that the shoot length [Figure 1] and root length [Figure 2] (as per the 15th day of germination) were highest in the control treatment followed by 25% and 50% contaminated soil. Further, lowest was recorded in the 100% contaminated soil.

However, the results were changed in the 30th day of germination. The shoot length [Figure 1] and root length [Figure 2] were highest recorded in 25% contaminated soil than the control soil. Then, it was followed by 50%, and finally, the lowest was recorded in 100% contaminated soil [Table 3].

After the 15th day of germination, the fresh weight [Figure 3] was found highest in the treatment of control soil followed by 25% contaminated soil, followed by 50% contaminated soil. Further, lowest fresh weight was recorded in 100% contaminated soil [Table 2].

However, after the 30th day of germination, the fresh weight [Figure 3] was found highest in 25% contaminated soil, followed by control soil, 50% contaminated soil and was lowest in 100% waste soil [Table 3]. Decreased root growth and seedling development are persuaded by chromium phytotoxicity [25].

3.2. Effect of Chromium-contaminated Soil on Total Chlorophyll Content of *V. radiata* L [Figure 4]

Table 2 summarizes the effect of chromium-contaminated soil on total chlorophyll content of *V. radiata* L. After the 15th day of

Table 2: Observation on the 15th day of germination

Pot	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Total chlorophyll (mg/g fresh weight)	Starch (mg/dl)	Protein (mg/100 g fresh weight)
Control soil	12.1	4.5	0.62	0.504	150	29.789
25% waste soil	11.6	4.4	0.60	0.553	148	29.022
50% waste soil	8.9	3.5	0.55	0.460	135	23.628
100% waste soil	8.1	3.2	0.40	0.357	115	18.070

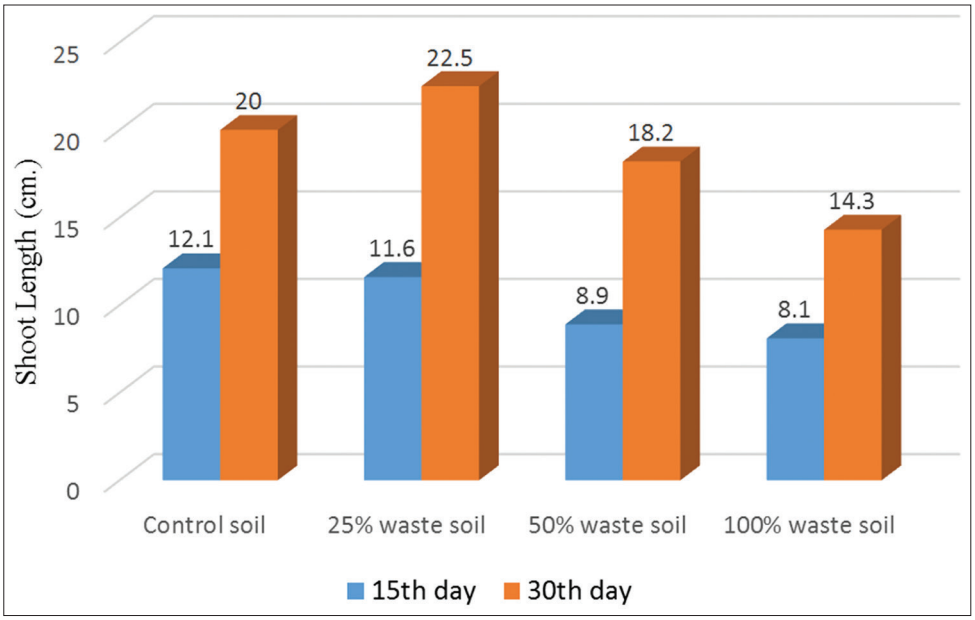

Figure 1: Comparative shoot length of *Vigna radiata L*

Table 3: Observation on the 30th day of germination

Pot	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Total chlorophyll (mg/g fresh weight)	Starch (mg/dl)	Protein (mg/100 g fresh weight)
Control soil	20.0	7.2	2.21	0.579	227	51.06
25% waste soil	22.5	7.9	2.39	0.629	236	65.90
50% waste soil	18.2	6.3	2.09	0.500	215	38.57
100% waste soil	14.3	5.2	1.97	0.397	153	33.07

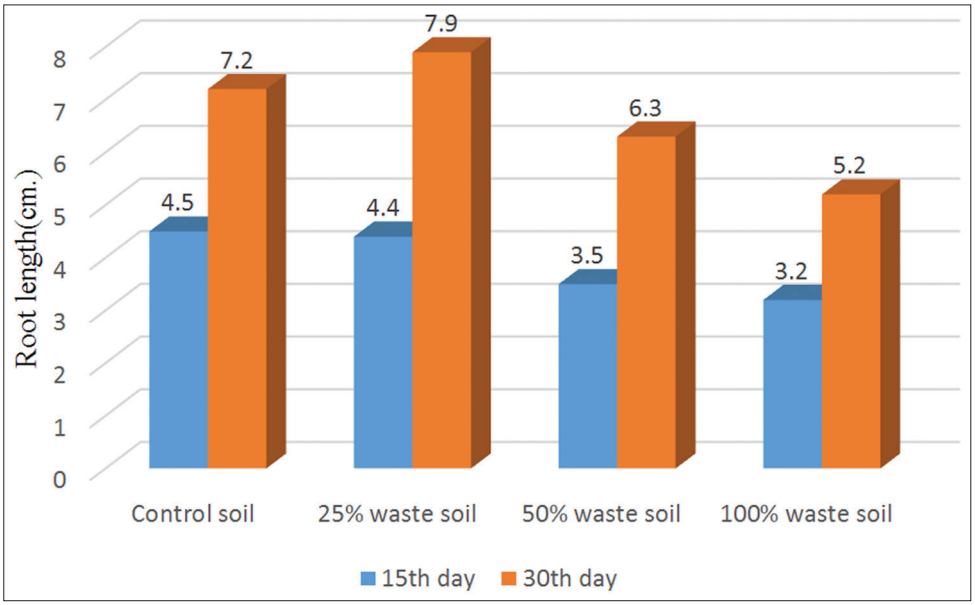

Figure 2: Comparative root length of *Vigna radiata* L

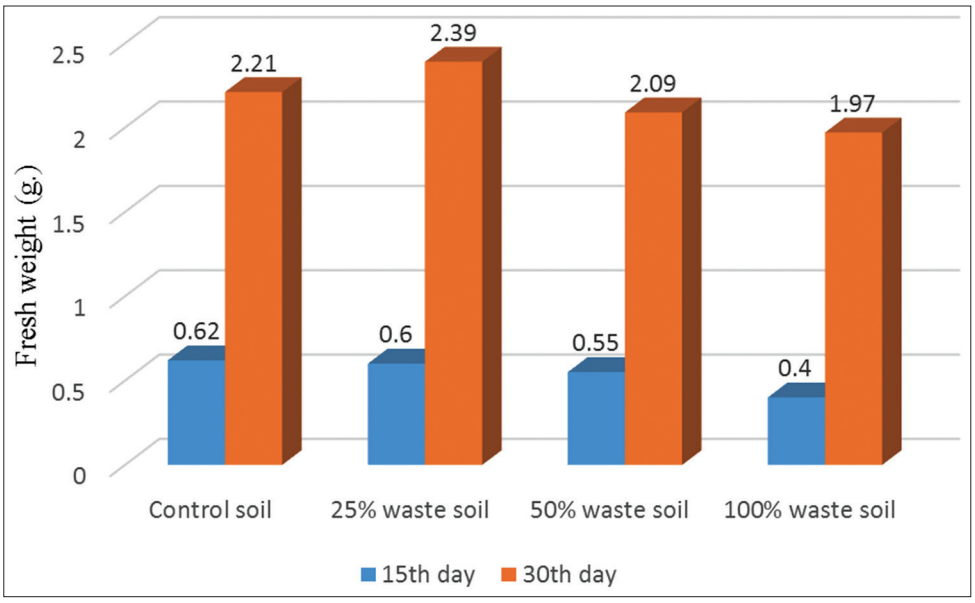

Figure 3: Comparative fresh weight of *Vigna radiata* L

germination, the table showed that the concentration of chlorophyll was found highest in 25% contaminated soil and followed by control, 50% contaminated soil. The concentration was lowest in 100% contaminated soil. After the 30th day of germination [Table 3], the chlorophyll content was also recorded highest in 25% contaminated soil, followed by control, the 50% contaminated soil. The lowest was also recorded in 100% contaminated soil after the 30th day of germination.

Reduced chlorophyll content of crops may be due to the interaction of enzymes involved in chlorophyll biosynthetic pathways in most plants under chromium stress [26].

3.3. Effect of Chromium-contaminated Soil on Starch Content of *V. radiata* L [Figure 5]

Table 2 summarizes the effect of chromium-contaminated soil on starch content of *V. radiata* L. After the 15th day of germination, the table showed that the concentration of starch was found highest in control and followed by 25% and 50% contaminated soil. The concentration was lowest in 100% contaminated soil.

After the 30th day of germination [Table 3], the concentration of starch was found to be highest in 25% contaminated soil and followed by control, 50% contaminated soil. The concentration was lowest in 100% contaminated soil.

Reduced germination percentage of plants at higher chromium concentrations may be attributed to the interference of metal ions which may obstruct seed germinations by exerting unfavorable effect in the utilization of major seed reservoirs like starch [27].

3.4. Effect of Chromium-contaminated Soil on Protein Content of *V. radiata* L [Figure 6]

Table 2 summarizes the effect of chromium-contaminated soil on protein content of *V. radiata L.* After the 15th day of germination, the table showed that the concentration of protein was found highest in control and followed by 25% and 50% contaminated soil. The concentration was lowest in 100% contaminated soil.

After the 30th day of germination [Table 3], the concentration of protein was found to be highest in 25% contaminated soil and followed by control, 50% contaminated soil. The concentration was lowest in 100% contaminated soil. Chromium reduces soluble protein in agricultural crops [28].

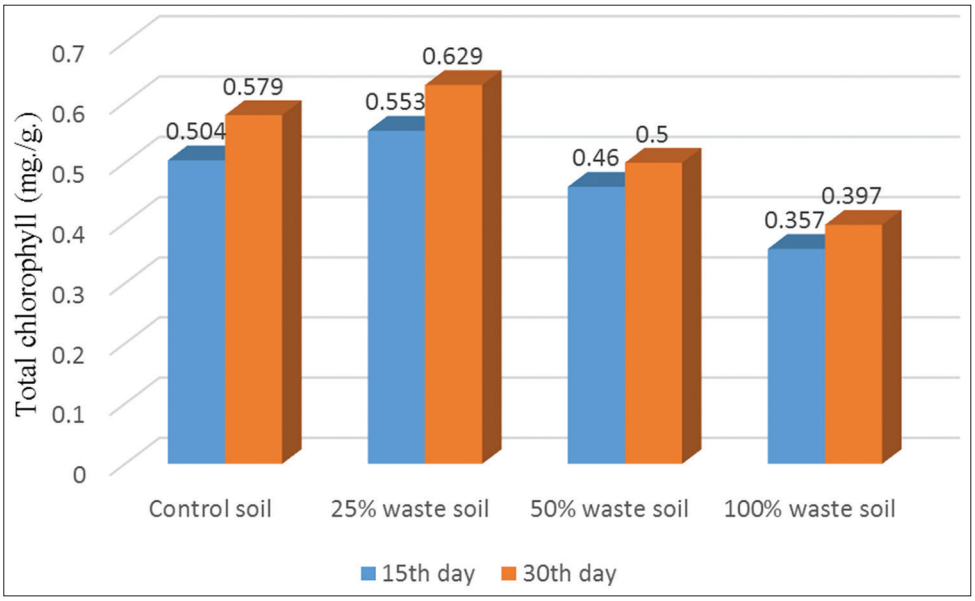

Figure 4: Comparative total chlorophyll content in leaves of *Vigna radiata* L

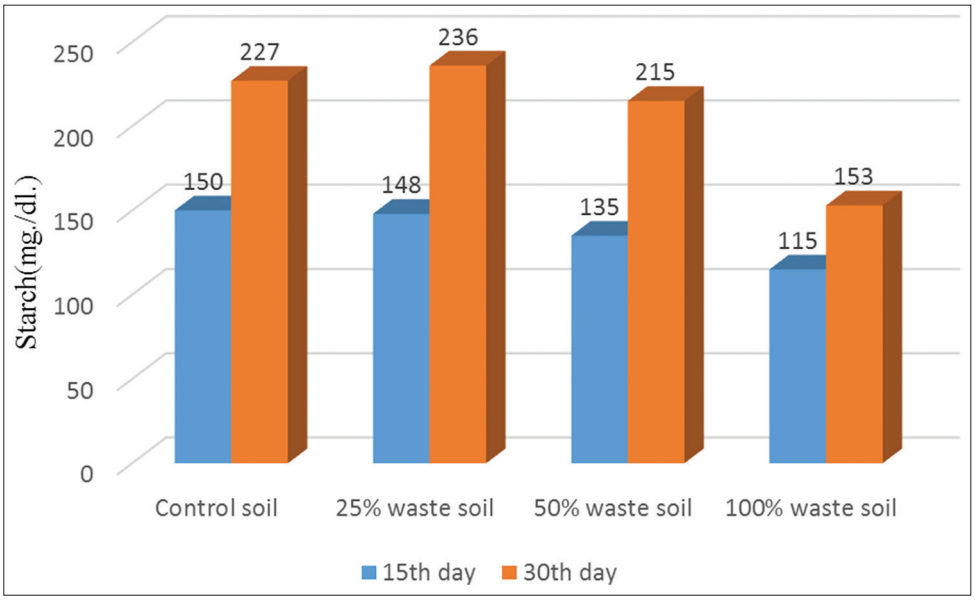

Figure 5: Comparative starch content in leaves of *Vigna radiata* L

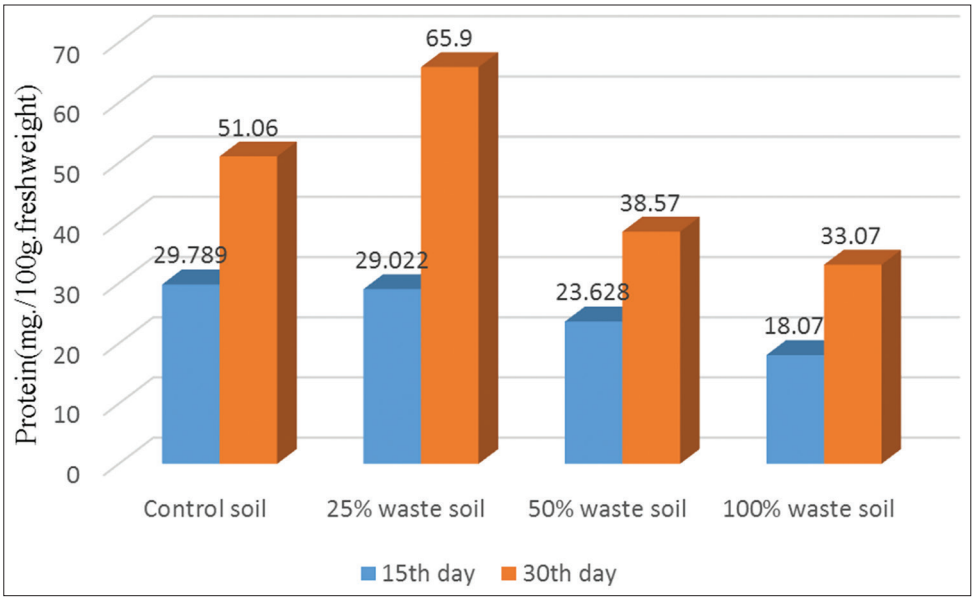

Figure 6: Comparative protein content in leaves of *Vigna radiata* L

4. CONCLUSION

From the above discussion, it is concluded that harmful effects on physiological and biochemical parameters of *V. radiata* L. are increasing with increasing chromium toxicity. However, the parameters are normal in control soil. Hence, the heavy metal contamination should be limited to get better response. Remediation techniques such as excavation, stabilization of metals in soil, and use of growing plants from increasing contamination or to evacuate metals by phytoremediation should be implemented to get heavy metal contamination under control.

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