International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Morphological and Biochemical Responses of *Helianthus annuus* L. to Chromium Contaminated Soil from Sukinda Mining Area (Odisha, India)

Debasis Dash[1], Sujata Hota[2], Bibhu Prasad Rath[3], Tapasi Upadhaya[4], Prabhu Kaibalya Das[5]

[1, 2, 3]P.G. Dept. of Botany, Orissa University of Agriculture and Technology, Bhubaneswar

[4, 5]P.G. Dept. of Zoology, Khallikote University, Berhampur

Abstract: ***Helianthus annuusL.is a crop of interest because of its shorter growing season. However the yield potential is affected by chromium toxicity. The chromium toxicity also affects its morphological and biochemical parameters. Sunflower is highly chromium sensitive plant and acts as chromium toxicity indicator. In our study an attempt has been taken to investigate the effect of chromium contaminated soil of Sukinda mining area of Odisha, on morphological and biochemical responses of Helianthus annuusL. Different concentration of contaminated soil along with control soil were taken as a measure of chromium toxicity on plants. Among the parameters taken, it was showed that the accumulation of chromium was more in roots than shoots, because chromium translocated into the shoots from the roots. May be it was the main cause for poor results obtained in physical and biochemical parameters. In almost all treatments and days of respective observations taken, the physical, biochemical parameters are declining with the increase in chromium contamination (i.e. from control to 25%, 50% and pure contaminated soil).***

Keywords: *Helianthus annuusL.*, Chromium contamination, Physical parameters, Biochemical parameters

1. Introduction

Pollution is a major threat to the world. Nowadays soil pollution is getting considerable public attention due to its serious effects on crop productivity. One of the major causes of soil pollution is heavy metal toxicity. Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity to the biota [1]. Heavy metals tend to adsorb very firmly to the soil matrix, and once released to the environment, they are not degraded like organics by microbial activity or through chemical oxidation [2]. India is the 2nd largest producer of chromite in the world. Orissa accounts for about 98% of total chromium ores (chromite) reserves of the country, of which about 97% occurs in the Sukinda valley, over an area covering 200 sq. km (approximately) in Jajpur district. It contributes nearly 95% of total chromite of the country [3]. Among the chromite reserves of Orissa, South Kaliapani chromite reserve has the extensive chromite mining activity.

2. Materials and methods

2.1 Study area

The study area was in Kaliapani, Sukinda, Odisha. Kaliapani is a small place in Sukinda according to its size, but it is highly economically important place of India. Sukinda is a town in Jajpur District, Odisha, India. Sukinda is listed as one of the 10 most polluted places in the world [3]. As per the statistics Odisha accounts for about 98% of the total proved chromite (chromium ore) reserves of the country, of which about 97% occur in the Sukinda Valley. Sukinda is situated in between coordinates 20º58´0´´N 85º55´0´´E.

Figure 1: Map showing Sukinda in Odisha, India

Figure 2: Map showing Kaliapani mines area in Sukinda

2.2. Analysis of Waste Soil Samples

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

2.2.1 Available Chromium (Cr)

The available chromium of the soil samples after combination was determined by DTPA extraction method. Reading was taken using Atomic Absorption Spectroscopy (AAS).The unit of available Chromium was taken in mg/kg.

2.2.2 Available Nitrogen (N)

The available Nitrogen of the soil samples after combination was determined by alkaline potassium permanganate method in Kel Plus automated machine [4].

2.2.3 Available Potassium (K)

The available potassium of the soil samples after combination was determined by Flame Photometer method. The unit of available potassium was taken in kg/ha.

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

2.3 Preparation of culture pots

The dried and powdered waste soil was used for preparation of culture pots with garden soil in order to study the effect of the waste soil combination on *Helianthus annuus*L. and also to compare the effect with control soil taken from garden on *Helianthus annuus*L. Three different concentrations of soil sample were taken in triplicate with comparison to control soil from garden. The pots were prepared according to the concentration of the waste soil sample taken. Pot 1 was having 2kg of 100% waste soil, pot 2 was having 1kg of waste soil and 1kg of control soil (total 2kg), pot 3 was having 500gm of waste soil and 1.5kg of control garden soil (total 2kg) and pot 4 was having 2kg control soil.The pots were kept on roof and were watered equally. The soil water mixture was allowed to settle for 2days. Seeds of *Helianthus annus*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 and 30th dayfrom seed germination.

2.4 Seed Material Used

Seed material used for the experiment was of Arjuna variety of *Helianthus annus*L.

2.5 Shoot Length (cm)

On the day of final count of the germination test i.e. 15th and 30th days, 5 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.

2.6 Root length (cm)

5 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.

2.7 Seedling dry weight

The same 5 seedlings were used for shoot and root length measurement, were put in the butter paper pockets and kept in an oven maintained at 100 ± 2º C for 24 hours. After drying, the seedlings were kept in a desiccator for cooling. The weight of dried seedlings was recorded and mean dry weight was expressed in mg. [5].

2.8 Biochemical Analysis:

2.8.1 Extraction and Estimation of Chlorophyll

The chlorophyll content was calculated as described by Arnon, 1949 [6].

2.8.2 Extraction and Estimation of Protein

Extracted leaf samples was precipitated with 50% TCA and then centrifuged at 10,000 rpm for 15 minutes. Residue was dissolved in 1N NaOH ,to it 5 ml of reagent mix (2%Na2CO3 in 0.1 NaOH ,0.5%CuSO4 in 1% Rochelle's salt), 0.5 ml of Follin's reagent added. Incubation was done for 30 min and then OD was taken at 650nm [7].

2.8.3 Starch Estimation

The leaves were analyzed for starch content by homogenizing 500mg of leaves in 80% ethanol and the extract was centrifuged and the residue was boiled in 4.6N Perchloric acid for 15minutes. Then again centrifuged and supernatant was collected. 5ml of Anthrone reagent was added to it and boiled. Then analyzed for starch content in a spectrophotometer at wavelength 630nm for starch with 80% ethanol as blank.

3. Results and Discussion

Chromium interferes with several metabolic processes causing toxicity to plants as exhibited by reduced root growth and biomass, chlorosis, photosynthetic impairing and finally plant death [8].

Table 2: Observation on the 15th day of germination

Pot	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	Total chlorophyll (mg/gfw)	Starch (mg/dl)	Protein (mg/100gfw)
Control soil	16.4	7.3	3.6	1.9	0.580	381.6	69.43
25% waste soil	14.0	5.3	2.4	0.8	0.553	356.6	66.72
50% waste soil	10.2	5.3	0.9	0.6	0.510	277	64.97
100% waste soil	7.8	5.3	0.4	0.2	0.324	225.6	42.88

Table 3: Observation on the 30th day of germination

Pot	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	Total chlorophyll (mg/gfw)	Starch (mg/dl)	Protein (mg/100gfw)
Control soil	33.3	10.0	7.7	2.4	0.696	832.33	111.85
25% waste soil	26.5	9.3	6.3	1.5	0.686	822	98.18
50% waste soil	21.4	8.9	4.2	1.0	0.642	680	82.25
100% waste soil	12.2	7.9	1.9	0.9	0.343	511	58.70

The data represent the effect of quality parameters in the table stated that, the root length (as per 15th day of germination) was highest in the control treatment followed by 25%, 50% contaminated soil. Further lowest was recorded in the 100% contaminated soil.

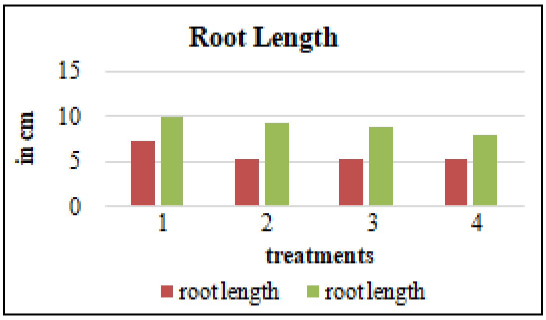

Figure 3: Graph showing root length

In case of shoot length the results were similar. So it was found to be less in waste soil than that of control soil.

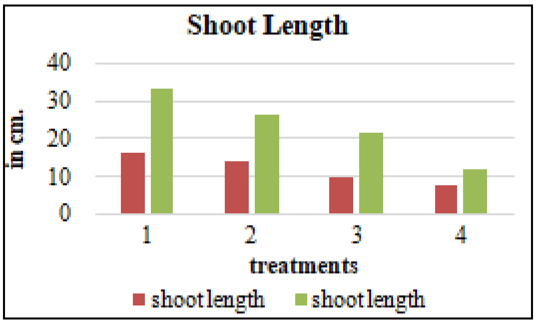

Figure 4: Graph showing shoot length

Symptoms of chromium phytotoxicity induces depressed biomass [9]. Decrease in fresh weight of low water uptake may be due to subsequent membrane damage since plant cell membrane generally considered as the primary sites of metal injury [10]. The data presented in the above table, was recorded that the fresh weight was found highest in treatment of control soil followed by 25%, 50% contaminated soil. Further lowest fresh weight was recorded in 100% contaminated soil.

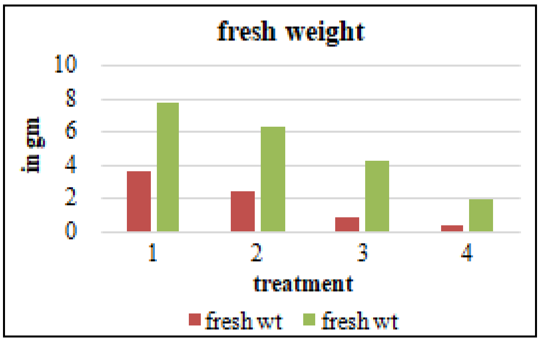

Figure 5: Graph showing fresh weight

According to the data of dry weight it is clear that the effect of chromium contaminated soil on biomass of sunflower was more in control soil whereas it was poor at a concentration of 100% contaminated soil. So it was found to be less in waste soil than that of control soil.

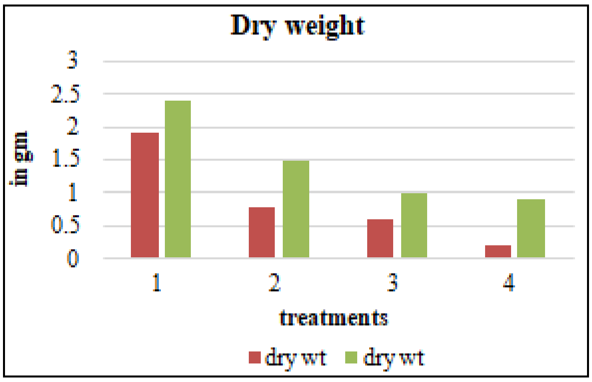

Figure 6: Graph showing Dry weight of the seedlings

The reduction in chlorophyll content of crops may be due to the interaction of enzymes involved in chlorophyll biosynthetic pathways could also contribute to the general reduction in the chlorophyll content in most plants under chromium stress [11]. The tables represent the effect of Chromium contaminated soil on chlorophyll content of Sunflower. The tables showed that the concentration of chlorophyll was found highest in control and followed by 25%, 50% contaminated soil. The concentration was lowest in 100% contaminated soil. So it was found to be less in waste soil than that of control soil.

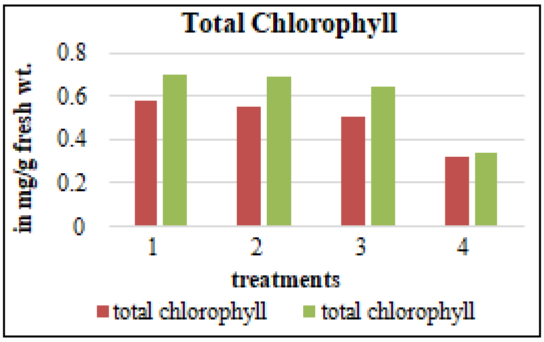

Figure 7: Graph showing Total Chlorophyll contents of leaves of the seedlings

The reduction in germination percentage of plants at higher chromium concentrations may be attributed to the interference of metal ions which may inhibit seed germinations by exerting unfavorable effect in the mobilization of major seed reservoirs like starch [12]. The table represents the effect of Chromium contaminated soil on starch content of Sunflower. The table showed that the concentration of starch was found highest in control and followed by 25%, 50% contaminated soil. The concentration was lowest in 100% contaminated soil. So it was found to be less in waste soil than that of control soil.

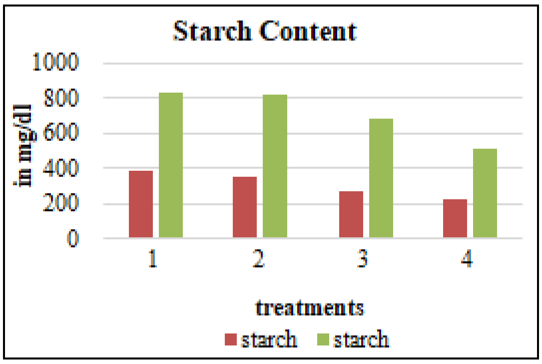

Figure 8: Graph showing starch contents of the leaves

The soluble protein in agricultural crops was reduced by heavy metals like chromium [13]. The table represents the effect of Chromium contaminated soil on protein content of Sunflower. The table showed that the concentration of protein was found highest in control and followed by 25%, 50% contaminated soil. The concentration was lowest in 100% contaminated soil. So it was found to be less in waste soil than that of control soil.

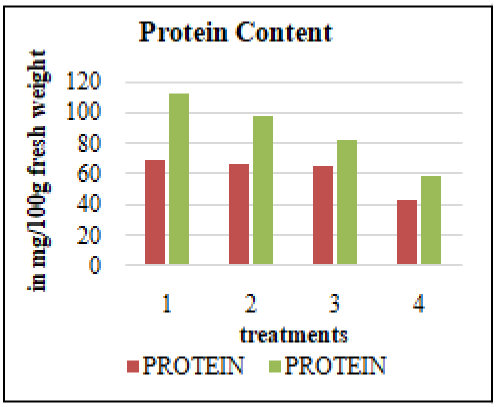

Figure 9: Graph showing protein content of the leaves

4. Conclusion

Plants treated with control soil and 25% of waste soil showed good results in the chemical parameters like photosynthetic pigments, starch contents, protein content and physical parameters like good quality fresh weight. But if we compare control soil and 25% waste soil then also control soil showed good results than 25% waste soil.

But plants treated with pure contaminated soil and 50% contaminated soil showed poor quality results in the chemical parameters like photosynthetic pigments, starch contents, protein content and physical parameters like poor quality fresh weight.

If we see in 15th day and 30th day of germination, then chromium accumulation in plants is also one of the fact which is responsible for the cause of poor results in plants. Though accumulation was found to be higher in root than shoot but it affected the physical and chemical parameters as everything that are translocated to shoots are from the roots.

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Author Profile

Dr. Debasis Dash is currently working as an Assistant Professor, in the Department of Botany, College of Basic Science and Humanities, Orissa University of Agriculture and Technology.

SujataHotareceived the B.Sc. degree in Botany from SailabalaWomens' College in 2015 and M.Sc. degree in Botany from Orissa University of Agriculture and Technology in 2017.

Bibhu Prasad Rath received his B.Sc. degree in Botany from Nimapara Autonomous College in 2015 and M.Sc. degree in Botany from Orissa University of Agriculture and Technology in 2017.

TapasiUpadhayareceived the B.Sc. degree in Zoology from M.P.C. Autonomous College in 2015 and M.Sc. degree in Zoology from Khallikote University in 2017.

PrabhuKaibalya Das received his B.Sc. degree in Zoology from Nimapara Autonomous College in 2015 and M.Sc. degree in Zoology from Khallikote University in 2017.