An *In vivo* Study on Toxicological Alterations in *Sesamum indicum* L. Under Hexavalent Chromium Stress

Monalisa Mohanty[1], Hemanta Kumar Patra[2]

[1, 2] Laboratory of Environmental Sciences, Post Graduate Department of Botany, Utkal University, Bhubaneswar-751004, India

Abstract: ***The present in vivo pot culture experiments highlight various phytotoxic impacts of hexavalent chromium (Cr^{+6}) in Sesamum indicum L. (Var-Amrit) (Oriya name: Rasi) commonly called Sesame which is an important oil yielding plant grown in.. The periodic investigation of toxicological changes in response to growth parameters, physiological and biochemical alterations in plant were carried out at 15 days (pre flowering), 30 days (flowering) and 45 days (pod forming) exposure to varying concentrations of Cr^{+6} treatment. Stimulatory effect of Cr on plant height and shoot biomass was noticed for 45 days grown seedlings at 10 mg Cr /kg dry soil treatment where as plant dry biomass was increased at 50 mg Cr /kg dry soil treatment. Total chlorophyll and carotenoid content were adversely affected with increasing Cr concentration at 15 and 30 days growth periods but the reverse trend was observed at maturity of the plants after 45 days treatment. Maximum proline synthesis was noticed at 200 mg Cr /kg dry soil treatment for 30 days growth period of plants. The above study emphasizing the interrelationship between the different metabolic effects induced by chromium in a oil yielding plant like Sesamum indicum warrants intensive future research on the its bioaccumulation potential which enable the plant as a green tool for phytoremediation of heavy metal contaminated soil.***

Keywords: Chromium; Photosyhtetic pigments, Proline, Phytoremediation, Sesame**.**

1. Introduction

Heavy metal contamination is now a global concern, caused by various industrial, mining and anthropogenic activities. Metals tend to bio-accumulate in food chain as large areas of agricultural land have been contaminated with heavy metals. India has fairly large resources of chromium mostly concentrated in the state of Orissa which accounts for 98% of the total chromium deposits of the country [1,2,3,4]. Chromium is one of modern industry's essential elements whose strategic importance is attributed to critical application in defense, aero-space, aviation, paints, dyes, chrome plating industries etc. Widespread use of chromium in several industrial and mining activities leads to the release of toxic hexavalent chromium to environment. Hexavalent chromium (Cr^{6+}) stress is one of the major problems in chromite mining area of Orissa (India) [4,5,6,7].

Chromium (Cr), the seventh most abundant element occurs in several oxidation states (from -2 to +6) and among them Cr^{6+} is a potent, extremely toxic, carcinogen causing a lot of mutagenic, toxic and carcinogenic effects in biological systems as evidenced by several researchers [7,8,9]. Compounding this problem it is a fact that traditional physiochemical methods of clean- up are often expensive, difficult and inefficient, detrimental to soil structure and fertility.

Current phytotechnological approach by phytoremediation technique emphasizes reclamation, in environmentally friendly manner. Current research in biotechnology includes investigation that utilizes plants to facilitate reclamation, the technique called phytoremediation (Mohanty et al., 2015). Phytoremediation of heavy metal–contaminated soils has received increasing attention in the past two decades because it is an environmentally friendly and relatively affordable technique in comparison with other traditional methods. Ideal plants for phytoremediation possess high biomass production, deep penetration of roots, fast growing, ability to tolerate and accumulate high amount of Cr in their aerial and harvestable parts [3].

Sesamum indicum Linn. is an important crop plant species for yielding oil. It is an annual plant that reaches 50 to 100 cm tall. *S. indicum* is widely cultivated and naturalized in dry habitats of tropical and subtropical regions. It is cultivated throughout India up to an altitude of 1200 m. grows on a light well-drained soil, which is capable of retaining adequate moisture. It thrives best on typical sandy loams. Considering the above positive characters, phytoreclamation of mine waste soil could be possible. This demands extensive investigations on phytotoxic impacts of varying doses of Cr^{6+} along with its concentration in different plant tissues. There is a huge dearth of information on the toxicological responses in sesame plant under Cr stress. The present study aimed to assess the phytotoxic impacts of Cr^{6+} which include growth impairment studies, physiological, biochemical and toxicological changes in sesban seedlings after 15, 30 and 45 days treatment with varying concentrations of hexavalent chromium. It is essential to ascertain the extent of phytotoxicity induced by Cr^{6+} bioaccumulation in different parts of the sesame (*Sesamum indicum* Linn.). The test plant will be assessed in the pot culture experiments for its toxicological alterations induced by hexavalent chromium.

2. Materials and Methods

2.1. Experimental design and Plant material

Pot culture experiments were conducted in completely randomised design. Dry seeds of sesame (*Sesamum indicum*

Linn.) were collected from Orissa University Of Agriculture & Technology, Bhubaneswar and were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes [4].

2.2 Germination Study

The pretreated uniform healthy sesame seeds were germinated in petriplates over saturated cotton pads supplemented with different concentrations of Cr^{6+} (source: $K_2Cr_2O_7$) viz. 5 mg L^{-1}, 10 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}, 300 mg L^{-1} and 500 mg L^{-1} along with a control for two days inside BOD incubator at 25 ± 2 ^{0}C. After 48 hours (two days) the number of germinated seeds under each treatment of Cr^{6+} was counted. The germination percentage and germination index (IG %) of seeds were calculated.

2.3 Growth of Sesame Seedlings

The seeds were germinated in earthen pots (size: height 30cm and diameter 15cm) containing 5 kg garden soil (control: Cr^{6+}-0 mg L^{-1}) and after 7 days of plant growth in uncontaminated control garden soil, the pots were supplemented with selected concentrations of Cr^{6+} (10 mg Kg^{-1}, 100 mg Kg^{-1}, 200 mg Kg^{-1} and 300 mg Kg^{-1}) and were grown at the nursery site of Department of Botany, Utkal University for 15, 30 and 45 days. Pots supplemented with half strength Hoagland nutrient solution [10] were taken as control treatment.

2.4 Analysis of Biochemical Parameters

Analysis of seedling growth, pigment content (Chlorophyll and carotenoid), praline content was conducted using 15, 30 and 45 days grown sesame seedlings. The extraction of chlorophyll was made using cold alkaline acetone (80% v/v) and calculated as per the methods of Arnon [11] with a little modification [12]. proline content of seedlings at different growth periods and exposed to varying concentrations of Cr^{6+} were also calculated [13].

2.5 Statistical Analysis

The experiments were conducted in triplicates for each treatment and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates.

3. Results and Discussion

3.1 Effects on Seed Germination

The seed germination was found to be inhibited with excess toxic concentration of chromium (5ppm). 95% seed germination was noted when the seeds were treated with 500ppm (Fig 1).

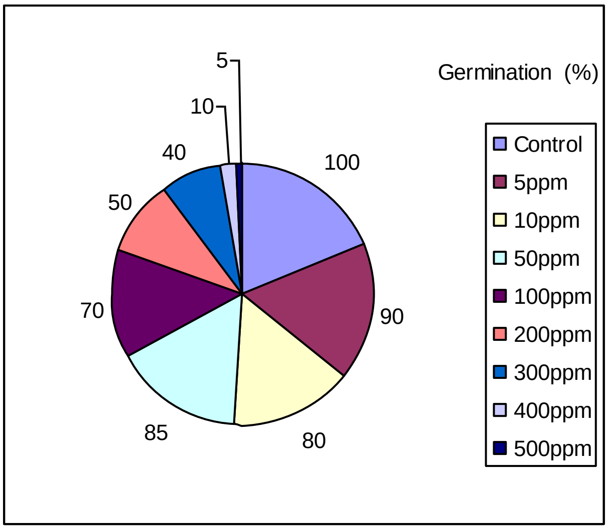

Figure 1: Effect of various treatment concentrations of Cr^{6+} on Germination of sesame seeds

A stiff decline in germination was observed at 50 ppm Cr treatment. Germination of sesame seeds were significantly decreased beyond 100 pm of Cr treatment.

Table 1: Effect of different treatment concentrations of Cr^{6+} on germination and seedling survivability after 45 days of sowing the seed

Treatments of Cr^{+6} mg Kg^{-1} dry soil	*Plant survivability (%) after 45 days treatment*
Control (0)	51
Cr^{+6} (10)	47
Cr^{+6} (50)	33
Cr^{+6} (100)	47
Cr^{+6} (200)	24

Seedling survival showed declining trend after 45 days treatment with varying doses of Cr (Table 1). It was reduced to 24% at Cr^{6+} treatments of 200mg Kg^{-1} dry soil.

The reduction in germination of sunflower seeds may be attributed to increased protease activity under chromium stress and depressive effect of Cr on the subsequent transport of sugars to the embryo axis [15, 16]. Similar results were reported by several researchers [9, 17] in other plants

3.2 Toxicological effects on plant growth

Changes in the shoot length of Sesame seedlings grown under different treatments of Cr^{6+} showed varying trend with plant age and maturity.

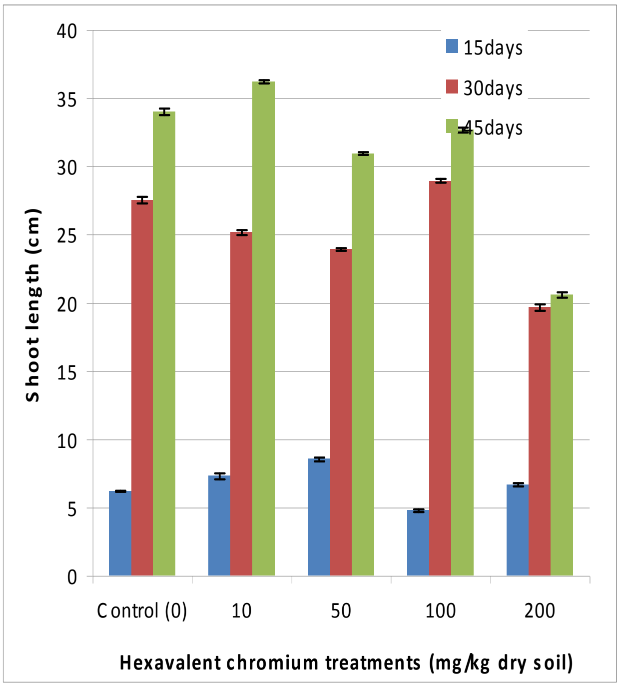

Figure 2: Effect of various treatment concentrations of Cr^{6+} (mg Kg^{-1}) on shoot length of 15, 30 and 45 days treated sesame seedlings

The shoot length treated with Cr^{6+} (50ppm) was higher in 15days, whereas it was maximum in Cr^{6+} (100ppm) and Cr^{6+} (10ppm) in 30days & 45days treated seedlings respectively (Fig 2.). The order of decrease in shoot length under different treatments of Cr^{+6} after 15, 30 and 45 days of treatment with Cr^{6+} was as follows-

15 days: Cr^{6+} (50)> Cr^{6+} (10)> Cr^{6+} (200) > Control > Cr^{6+} (100)
30days: Cr^{6+} (100)> Control > Cr^{6+} (10) > Cr^{6+} (50) > Cr^{6+} (200)
45 days: Cr^{6+} (10) > Control > Cr^{6+} (100) > Cr^{6+} 50) > Cr^{6+} (200).

The fresh shoot weight gradually decreased with increase in Cr^{+6} concentrations. Among the seedlings treated with 200mg Cr Kg^{-1} dry soil had minimum value after 30 and45 days treatment (Fig. 3).

The order of decrease in fresh root matters was as follows-

15 days: Cr^{6+} (10)> Control> Cr^{6+} (200) > Cr^{6+} (50) > Cr^{6+} (100)
30 days: Cr^{6+} (100)> Cr^{6+} (10) >Control> Cr^{6+} (50) > Cr^{6+} (200)
45 days: Cr^{6+} (10) > Control > Cr^{6+} (50) > Cr^{6+} (100) > Cr^{6+} (200)

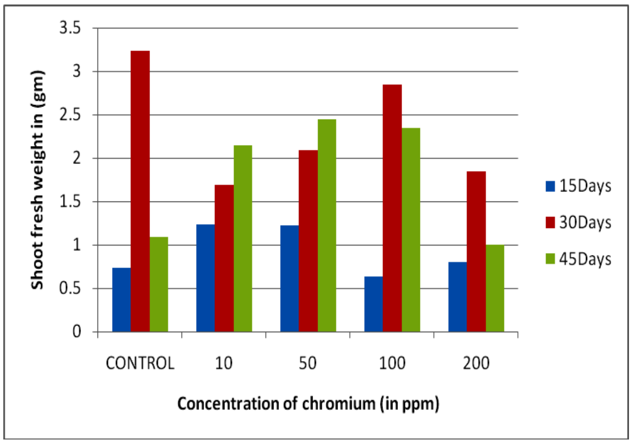

Figure 3: Effect of various treatment concentrations of Cr^{6+} (mg Kg^{-1}) on shoot fresh weight of 15, 30 and 45 days treated sesame seedlings

The ratio of root and shoot dry weight showed declining trend with increased Cr concentration at both the periods of plant growth during pre flowering and pod forming stages of plant growth (Fig. 4).

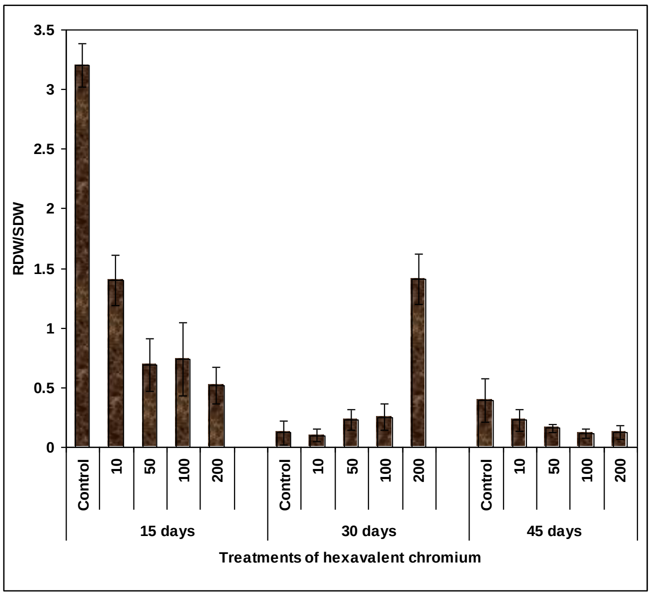

Figure 4: Effect of various treatment concentrations of Cr^{6+} (mg Kg^{-1}) on ratio of Root and shoot dry weight of 15, 30 and 45 days treated sesame seedlings

Hexavalent chromium affects plant growth and metabolism by decreasing nutrient uptake and photosynthetic abilities [19]

3.3 Cr induced alterations in photosynthetic pigments

Deterioration in total chlorophyll content of sesame leaves were observed with increasing supply of Cr^{6+} when grown for 30 days (Fig. 5). Carotenoid content does not show any significant variation among different treatments which showed its tolerance potential. The increased Cr stress causing reduction in chlorophyll content was attributed to ultrastructural damage [19]. Similar reports on the impacts of metal toxicity are available in different plants [20]

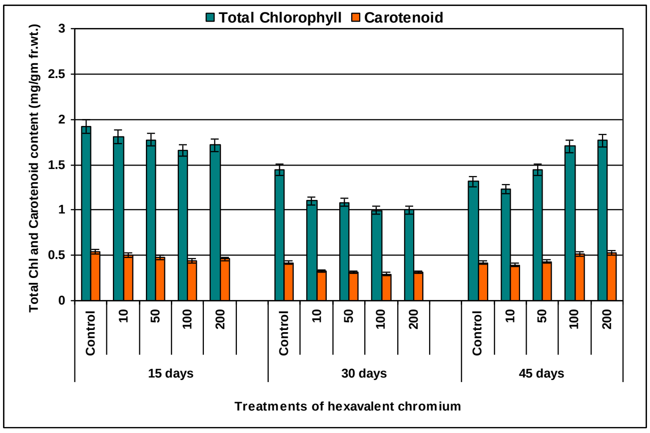

Figure 5: Changes in total chlorophyll and carotenoid contents of sesame seedlings after 15, 30 and 45 days of Cr^{6+} treatments (mg Kg^{-1}).

3.4 Proline accumulation in response to Cr^{6+} stress

Proline content after 15, 30, 45 days of seedling growth is high in 10, 100, 50 mg kg^{-1} Cr treatments respectively (Fig. 6).

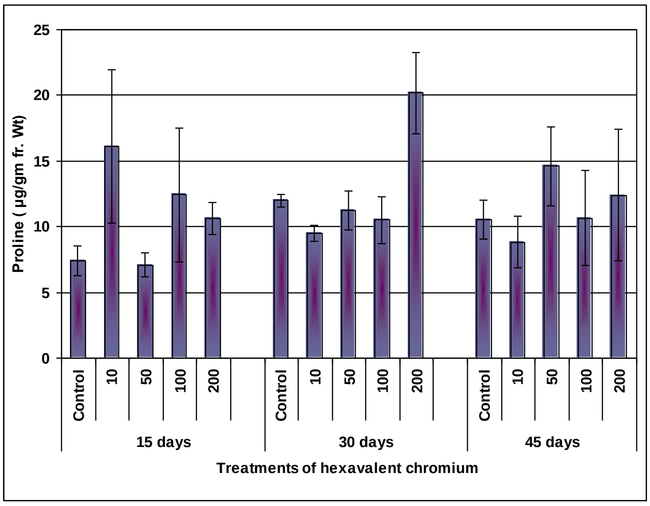

Figure 6: Bar graph showing the comparative values of proline content in sesame seedlings after 15, 30 and 45 days of Cr^{6+} treatments (mg Kg^{-1}).

The proline content treated with different chromium concentration was as follows :

15 days: Cr^{6+} (10) > Cr^{6+} (100) > Cr^{6+} (200) >Control> Cr^{6+} (50).

30 days: Cr^{6+} (100)> Control> Cr^{6+} (50) > Cr^{6+} (100) > Cr^{6+} (10).

45 days: Cr^{6+} (50) > Cr^{6+} (200) > Cr^{6+} (100) > Control> Cr^{6+} (10)

Increasing concentrations of Cr+6 shows linear increase in proline accumulation (Fig. 6). Proline content increases with the enhancing concentration of Cr^{6+} (Fig. 6) because the proline is the only amino acid that accumulates to a greater extent in the leaves of many plants under stress [19]. Proline accumulation may also help in nonenzymic free radical detoxifications [7]. Increasing proline level in sesame seedlings after 30 days treatment is considered to help the cells in osmoprotection as well as in regulating the redox potential, scavenging hydroxyl radicals and gives the protection against denaturation of various macromolecules[21].

4. Conclusion

The experimental results clearly showed that Cr is found relatively less non toxic at different degrees at different stages of plant growth and development even at high concentration which suggests further implication of the plant as a green tool of phytoremediation technology for Cr phytoextraction purpose. This preliminary report contributes in exploring and finding the tolerance limit of sesame at different concentration of chromium. However, further research is needed to determine the effect of different level of other metals in the environment and various parts of the plant through an *in situ* approach. A field study is also recommended to find effect of natural variables (temperature, pH, light, soil quality, etc.) on the above laboratory based results.

5. Acknowledgement

The authors acknowledge the financial support provided by CSIR to Dr. H .K. Patra (CSIR-ES) under CSIR-Emeritus Scientist Scheme, Post Graduate Department of Botany, Utkal University.

References

[1] M. Mohanty, HK. Patra, "Chelate assisted phytoextraction of chromium"– an *in vitro* study in rice. In: Environmental stress impacts on plants (Patra H.K., Eds.). Proc. Nat. Sem., UGC-DRS (SAP-II). Utkal University, India. pp. 85-95. 2010

[2] M. Mohanty, MM. Pattanaik, AK. Misra, HK. Patra, "Chromium Bioaccumulation in Rice grown in Contaminated Soil and Irrigated Mine Waste Water - A Case Study At South Kaliapani Chromite Mine Area, Orissa, India", Int. J. Phytoremediat., (13) pp.397-409, 2011

[3] M. Mohanty, HK. Patra, "Attenuation of chromium toxicity by bioremediation technology", Rev. Env. Cont. Toxicol., (210), pp.1-34, 2011a.

[4] M. Mohanty, HK. Patra, "Effect of Cr^{+6} and chelating agents on growth, pigment status, proline content and chromium bioavailability in rice seedlings", Int. J. Biotech. Appl., (3), pp. 91-96, 2011b.

[5] AM .Zayed, N. Terry, "Chromium in the Environment: Factor affecting biological remediation", Plant Soil., (249), pp.139–156, 2003.

[6] AK. Shanker, C. Cervantes, H.Loza-Tavera, S. Avudainayagam, "Chromium toxicity in plants", Environ Int, (31) pp.739–753, 2005a.

[7] M. Mohanty, C. Pradhan, HK. Patra, "Chromium translocation, concentration and its phytotoxic impacts in *in vivo* grown seedlings of *Sesbania sesban* L. Merrill", Acta Biol. Hungarica, 1 (66), pp.80-92, 2015

[8] M. Mohanty, HK. Patra, "Hexavalent Chromium induced toxicological, physiological and biochemical alterations in *Sesbania sesban* L. Seedlings", J Plant

Physiol Pathol, (2.), pp.1-6, 2014. http://dx.doi.org/10.4172/2329-955X.1000129
[9] F. Andaleeb, M. Anjum Zia, M. Ashraf, Z. Mahmood Khalid, "Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.)", J. Env. Sci., (20), pp. 1475–1480, 2008
[10] DR. Hoagland, DI. Arnon, "The water culture method for growing plants without soil", Calif. Agric. Exp. Station Cir. (347), pp. 1-39, 1938.
[11] DI. Arnon, "Copper enzymes in chloroplast polyphenol oxidase in Beta vulgaris. Plant Physiol. (24), pp. 1-15, 1949.
[12] RJ. Porra, "The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b"., Photosynth. Res., (73) pp. 149 -156, 2002.
[13] LS Bates, RP. Waldren, ID.Teare, "Rapid determination of free proline for water stress studies. Plant Soil, (39), pp. 205–207, 1973.
[14] A. Bonet, C. Poschenrieder, J. Barcelo, "Chromium III-iron interaction in Fe-deficient and Fe-sufficient bean plants, I. Growth and nutrient content". J. Plant Nutr. (14), pp. 403-414, 1991.
[15] M. Mohanty**,** HK. Patra, "Effect of ionic and chelate assisted hexavalent chromium on mung bean seedlings (*Vigna radiata* L. wilczek. var k-851) during seedling growth", J. Stress Physiol. Biochem., (9), pp. 232-241, 2013.
[16] JK. Datta, A. Bandhyopadhyay, A. Banerjee, NK. Mondal, "Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars under laboratory condition". J. Agric. Techy., (7), pp. 395-402, 2011.
[17] H. Amin, BA. Arain, F. Amin, MA. Surhio, "Phytotoxicity of Chromium on Germination, Growth and Biochemical Attributes of *Hibiscus esculentus* L." *Am.J. Plant Sc.*, (4), pp. 2431-2439,2013.
[18] WS. Shu, CY. Lan, ZQ. Zhang, "Analysis of major constraints on plant colonization at Fankou Pb/Zn Mine tailings". Chinese J. App. Ecol., (8), pp. 314-318, 1997.
[19] M. Mohanty**,** HK. Patra, "Effect of Chelate assisted Hexavalent Chromium on Physiological changes, Biochemical alterations and Cr Bioavailability in Crop Plants - An *in vitro* Phytoremediation Approach", Bioremediat. J.**,** (16), pp. 147–155, 2012.
[20] DH. Liu, JH. Zou, M. Wang, WS. Jiang, "Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L.", Biores. Tech., (99), pp. 2628–2636, 2008.
[21] MH. Khan, LBK. Singh, SK. Panda, "Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress" Biol. Plant., (45), pp. 625-627, 2002.

Author Profile

Dr. Monalisa Mohanty is presently working as Lecturer in Botany, Dhenkanal (Autonomus) college, Dhenkanal, Odisha, India. She was working as a CSIR-Research Associate under the scheme at Post Graduate Department of Botany, Utkal University, Bhubaneswar, India.

Prof. (Dr.) Hemanta Kumar Patra, Former Professor in Environmental Science, is presently working as CSIR-Emeritus Scientist under the scheme at Post Graduate Department of Botany, Utkal University, Bhubaneswar, India.