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# Bioremediation Potential of *Zinnia elegans* (L.) in Cr enriched Over Burden Soil of Sukinda Chromite Ore Mines, Odisha.

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ABSTRACT

Continues to grow in relation to food safety and sustainable agricultural output. It is a difficult effort to remove Cr from contaminated soils, yet doing so could support agriculture while simultaneously reducing negative environmental effects. Using overburden soil (OBS) of Sukinda, pot investigations were carried out to examine the biochemical and toxicological changes, in an ornamental plant, *Zinnia elegans* (L.). After 30, 45, and 60 days of treatment, the physio-morphological parameters viz total chlorophyll content, proline, protein, reducing sugar content, and antioxidant properties viz. CAT, APX, POX and SOD were examined. Upon $T_4$ treatment, the plants' length, biomass, chlorophyll and protein contents all decreased. However, the application of a larger percentage of OBS boosted the antioxidative enzymes as well as proline levels, suggesting that the plants' adaptive defense system is related to increasing $Cr^{+6}$ induced oxidative stress. This work shows that non-edible ornamentals can be used to efficiently carry out phytoremediation of $Cr^{+6}$ contaminated soils in mining sectors, stopping the ion's impacts in the food chain.



## 1. Introduction

A novel green technology known phytoremediation uses plants that accumulate metal or that can withstand metal to clean up soil that has been contaminated with metal. Any environmental factor that keeps plants from realizing their full genetic capacity to carry out metabolic processes under the best growing conditions is often referred to as stress. Trade-offs between vegetative and reproductive growth occur in plant responses to abiotic stress and these trade-offs might vary based on the kind of plant—annual or perennial. Heavy metal phytotoxicity such as $Cr^{+6}$, disrupts the metabolism of carbohydrates and prevents transpiration and photosynthesis, among other processes. It causes secondary stressors like oxidative stress, dietary stress, lipid peroxidation, hydroxyl radical generation, and $H_2O_2$, OH- and $O_2$ accumulation. The plant is therefore experiencing oxidative stress, which is uncontrollable in the absence of antioxidants and impacts the growth and development of the plant (Kramer and Clemens, 2005; Kochian *et al.*, 2005; Lequeux *et al.*, 2010). Employing phytoremediation technology, attempts have been undertaken to lessen the severe contamination caused by harmful hexavalent chromium (Patra *et al.*, 2018a,b,c, 2019, 2020a).

*Zinnia elegans* L. is an annual blooming plant that is mostly grown for decorative purpose. It can grow in noxious metals and has a rapid growth rate, increased biomass, ease of cultivation, and harvesting (Ehsan *et al.*, 2016). Additionally, using ornamental plants like Zinnia enhances the aesthetic value of the area by improving the air quality in the surrounding area, adding aesthetic value to gardens having the potential to contain alkaloids like anabasin, nor-nicotine, and nicotine. Consequently, *Zinnia elegans* L. is the preferred plant for improved phytoremediation studies when compared to other decorative flowering plants.

Thus, the urge of my current experiment by employing *Zinnia elegans* L. plants are (1) to understand the harmful impacts of hexavalent chromium in soil on biochemical

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changes. (2) Recognize the degree of Cr bioaccumulation. (3) *Zinnia elegans* capacity to hyper-accumulate hexavalent chromium, as well as its absorption and translocation, are assessed by measuring the TI (Tolerance index), Ti (Transportation index) and BCF (Bio-concentration factor).

## 2. Materials and methods

### *2.1. Collection of Plant material and Experimental outline*

Experiments on pot culture were carried out at Utkal University in Bhubaneswar. *Zinnia elegans* L. dry seeds were taken from RPRC, Bhubaneswar, and grown in department of Botany, Utkal University. Following mercuric chloride of 0.1% (w/v) treatment, the seeds were cleaned with distilled water. In cleansed Petri plates covered in moistened cotton cloths, the treated seeds germinated. The seeds germinated within 4–5 days. In every pot containing varying percentages of Over Burden Soil (OBS) enriched with Cr, ($T_0$- 100% garden soil as control, $T_1$- 70% garden soil + 30% OBS, $T_2$- 50% garden soil + 50% OBS, $T_3$- 30% garden soil + 70% OBS, and $T_4$-100% OBS), three seedlings of the same height were planted.

### *2.2. Biochemical evaluation*

The leaf tissues of Zinnia plants that were under treatment and control had a number of biochemical properties analysed.

#### *2.2.1. Photosynthetic pigment*

Using cold alkaline acetone and Arnon's (1949) procedures, chlorophyll was extracted. The result was calculated using a formula and given as mg/g fresh weight (FW).

#### *2.2.2. Reducing sugar and Carbohydrates*

The carbohydrate content present in the samples was evaluated by the protocol given by Yoshida *et al.*, (1972).

Quantitative determination of reducing sugar is widely followed by the procedure prescribed by Nelson (1944) and Somogyi (1945).

#### *2.2.3. Protein and Proline content*

Protein and Proline content were estimated by Lowry *et al.*, (1951) and Bates *et al.*, (1973) methods respectively.

### *2.3. Antioxidant enzymatic activities*

Antioxidant enzymatic properties like superoxide dismutase, peroxidase, ascorbate peroxidase and catalase were measured by Aebi (1984), Marshall and Worsfold (1978), Nakano and Asada (1981) in that order.

### *2.4. Bioaccumulation and uptake of Chromium*

The volume percent of Cr in the shoots and roots of the plants was used to evaluate their capacity for absorption, translocation and phytoextraction. In order to calculate BCF (Bio-concentration factor) and Ti (Transportation index) using the previously used formulas, the metal accumulation in both plants was analysed (Ghosh and Singh, 2005; Zurayk *et al.*, 2002). The methods utilized by Patra *et al.*, (2020b) to assess a plant's ability to grow in the presence of a particular concentration of Cr were applied to determine the tolerance index (TI).

$$\text{BCF} = \frac{\text{Chromium conc. in the plant tissue (mg/ kg)}}{\text{Chromium added in soil (mg/ kg)}}$$

$$\text{Ti} = \frac{\text{Cr conc. of shoot (mg/kg)}}{\text{Cr conc. of root (mg/kg)}} \times 100$$

$$\text{TI} = \frac{\text{Dry wt. of treated plants}}{\text{Dry wt. of control plants}} \times 100$$

## 3. Results and Discussion

### *3.1. Impact of Chromium on biochemical properties*

It was discovered that the total chlorophyll content increased up to $T_2$ and then declined as the percentage of Cr-rich OBS increased. Chromium obstructs the synthesis of δ-aminolevulinicacid (ALA) as the initial step in the biosynthesis of tetrapyrrole, which proceeds to the production of heme (Vajpayee *et al.*, 2000). However, Naito *et al.* (1980) proposed that, in addition to regulating ALA synthesis, δ-aminolevulinic acid dehydratase (ALAD) activity may also control the biosynthesis of chlorophyll. When the amount of $Cr^{+6}$ grew in *Zinnia elegans* plants that were 30, 45, and 60 days old, the total sugar content first increased and then gradually dropped (Fig.2). Throughout the growth stage, there were noticeable changes in the decreasing sugar content. Although the sugar level decreases at $T_3$ and $T_4$, the plants' reduced sugar content increased across all treatments from 30 days to 60 days. One possible explanation is the disintegration or transformation of other sugar derivatives, including polysaccharides, into non-reducing sugar molecules. On the other hand, the carbohydrate concentrations of *Zinnia elegans* were raised to $T_2$ and then progressively lowered. Tiwari *et al.* (2009) reported that a substantial drop in the total carbohydrate content occurred as the concentration of Cr increased. In the current in vivo investigation of *Zinnia elegans*, $T_2$ plants had the highest protein content, followed by $T_1$. As the amount of hexavalent chromium in the soil increased, the protein content dropped. Because the processes of protein manufacture are disrupted, stress by heavy metal limits the enzymatic activities containing sulfhydryl group, which also affects protein's normal concentration (Nagoor, 1999). The primary cause behind the decline in protein content is the increased denaturation of proteins brought on by the harmful effects of ROS (Reactive oxygen species) and the conversion of preexisting proteins into amino acids. Hexavalent chromium boosted the proline levels of *Zinnia elegans* plants that were 30, 45, and 60 days old. $T_4$ has the greatest proline content that was found. Plants that have greater percentages of hexavalent chromium may have accumulated proline as a coping mechanism for the oxidative stress caused by chromium.These outcomes are consistent with our previous research, which looked at *Zinnia elegans* capacity for remediation in $Cr^{+6}$-contaminated soil and reported that the plants could withstand concentrations of $Cr^{+6}$ of up to 10–50 ppm (Panda *et al.*, 2020).

### *3.2. Impact on antioxidant enzymes*

Antioxidant enzymatic properties in 30 days and 60-day-old *Zinnia elegans* were significantly altered by treatment with varying doses of hexavalent chromium rich OBS. As the concentration of hexavalent chromium increased, so did the activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (GPX) and superoxide dismutase (SOD).

Plants under environmental stress may experience oxidative stress, which produces and activates reactive oxygen species (ROS). Lipid peroxidation is brought on by ROS. Consequently, in order to regulate the amount of Reactive oxygen species and defend the cells from oxidative stress, plants have evolved a defensive mechanism to scavenge the ROS (Vranova *et al.,* 2002).

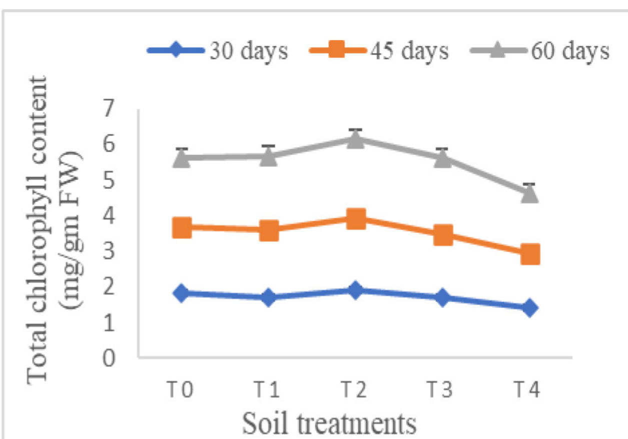


Fig.1. Impact of Cr enriched OBS on total chlorophyll content of 30d, 45d and 60d old *Zinnia elegans* L.

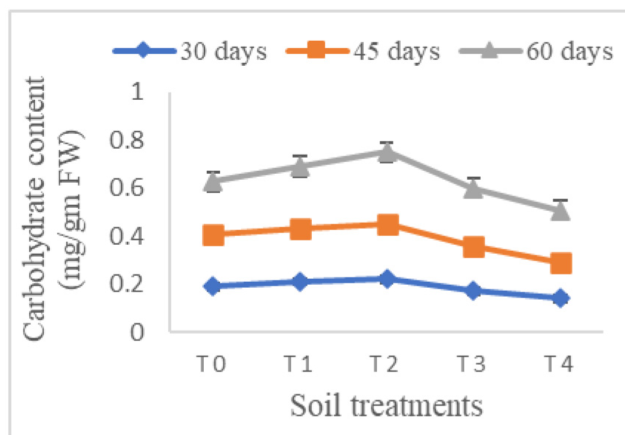


Fig.2. Impact of Cr enriched OBS oncarbohydrate content of 30d, 45d and 60d old *Zinnia elegans* L.

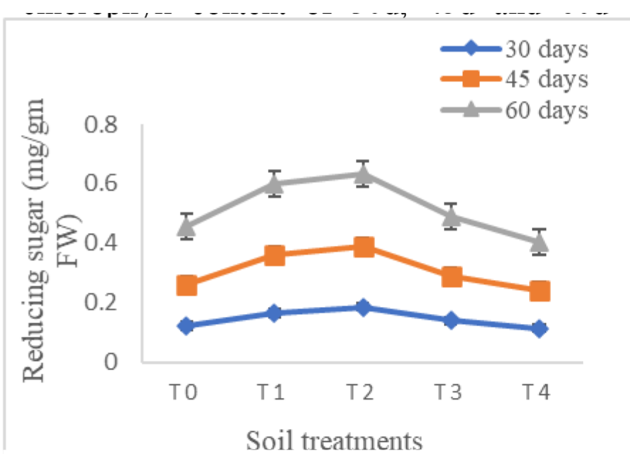


Fig.3. Impact of Cr enriched OBS on reducing sugar content of 30d, 45d and 60d old *Zinnia elegans*L.

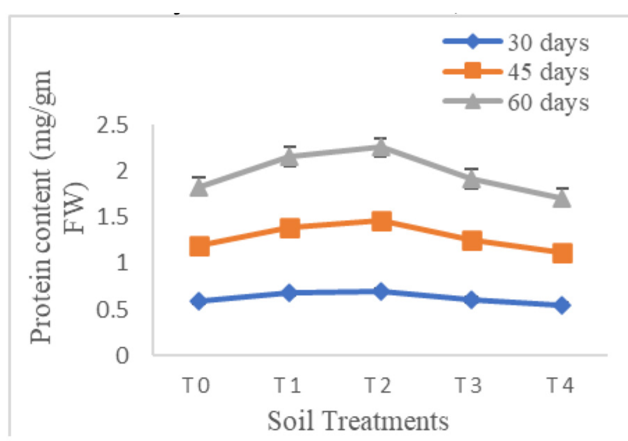


Fig.4. Impact of Cr enriched OBS on protein content of 30d, 45d and 60d old *Zinnia elegans* L.

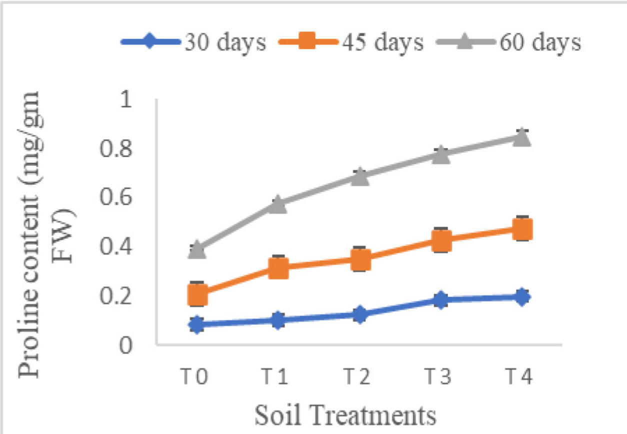


Fig.5. Impact of Cr enriched OBS on proline content of 30d, 45d and 60d old *Zinnia elegans*L.

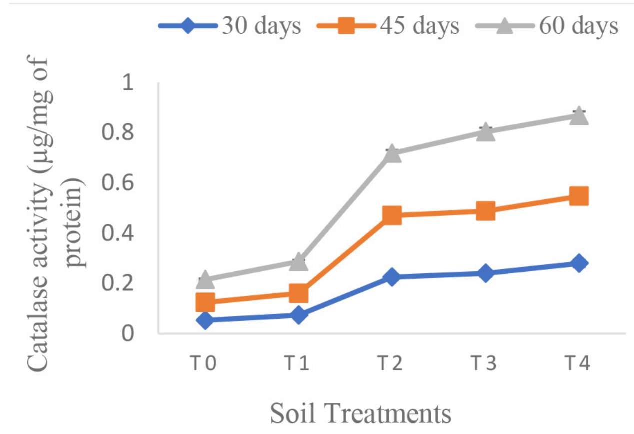


Fig.6. Impact of Cr enriched OBS on catalase activity of 30d, 45d and 60d old *Zinnia elegans*L.

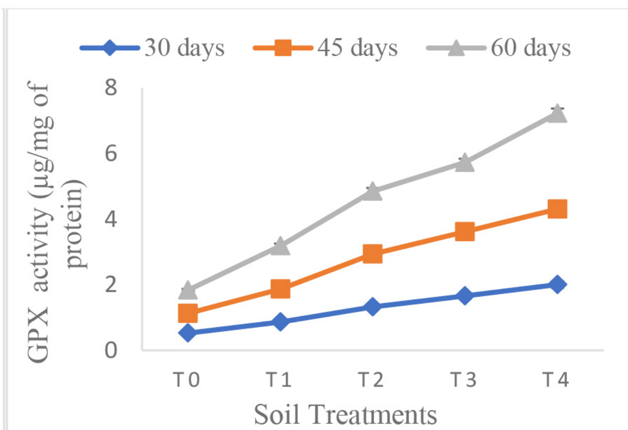


Fig.7. Impact of Cr enriched OBS on peroxidase activity of 30d, 45d and 60d old *Zinnia elegans* L.

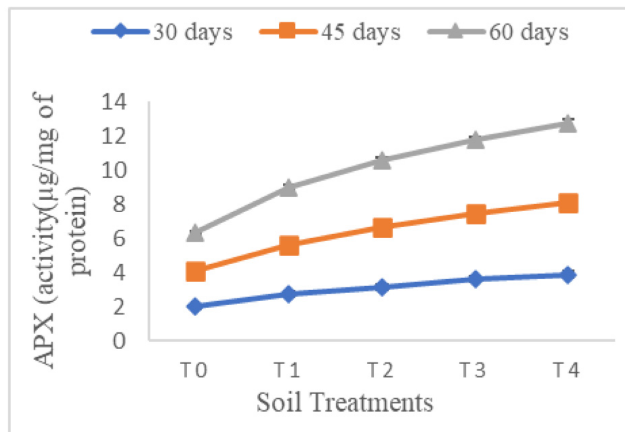


Fig.8. Impact of Cr enriched OBS on APX activity of 30d, 45d and 60d old *Zinnia elegans*L.

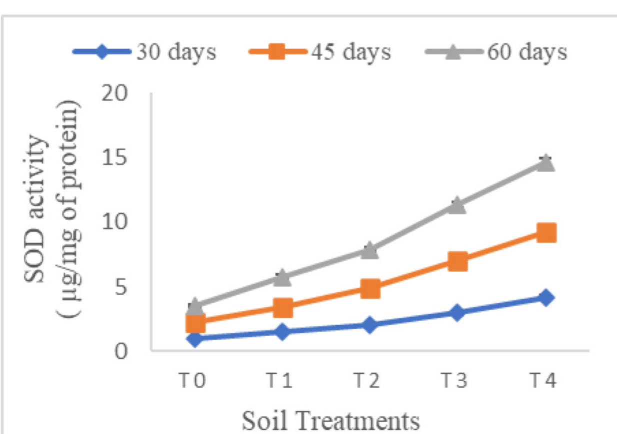


Fig.9. Impact of Cr enriched OBS on SOD activity of 30 d, 45 d and 60 d old *Zinnia elegans* L.

### *3.3. Bioaccumulation of Chromium*

There was a significant difference between the roots and shoots' chromium concentrations. The plants' roots gathered more chromium than their branches. At $T_4$ treatment, roots showed the highest amount of chromium bioaccumulation. According to Ghosh and Singh (2005), the most prevalent heavy metal tolerance trait is a high Cr bioaccumulation in the roots and less transit to the plant shoots. As the concentration of hexavalent chromium increased from $T_1$ to $T_4$, the Ti, TI and BCF all decreased. According to other reports, the plants also generally displayed a tendency for the tolerance index value to decrease as the content of hexavalent chromium increased (Ghosh and Singh, 2005).

Table.1

Effect on bioaccumulation factors of *Zinnia elegans* L.

| Soil Treatments | Cr content in shoot (g/kgdry weight) | | | Cr content in root (g/kgdry weight) | | | BCF (Bio concentration factor) | | | TI (Tolerance index) | | | Ti (Transportation index) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30d | 45d | 60d | 30d | 45d | 60d | 30d | 45d | 60d | 30d | 45d | 60d | 30d | 45d | 60d |
| T1 | 7.023± 0.002 | 7.646± 0.002 | 8.418± 0.002 | 8.965± 0.002 | 9.946± 0.002 | 11.635± 0.002 | 3.648 | 3.869 | 4.002 | 164.69 | 140.84 | 125.16 | 65.03 | 60.87 | 64.09 |
| T2 | 9.201 ±0.002 | 9.601 ±0.002 | 10.001 ±0.003 | 13.427 ±0.003 | 13.988 ±0.003 | 12.468 ±0.003 | 2.431 | 2.462 | 3.930 | 155.25 | 137.93 | 119.73 | 57.21 | 56.85 | 69.72 |
| T3 | 9.86 ±0.002 | 9.826 ±0.002 | 11.69 ±0.003 | 17.291 ±0.004 | 20.812 ±0.005 | 20.991 ±0.005 | 0.984 | 0.992 | 1.003 | 119.40 | 100.73 | 98.16 | 35.89 | 34.27 | 45.17 |
| T4 | 10.671 ±0.003 | 11.32 ±0.003 | 11.93 ±0.003 | 22.37 ±0.006 | 23.705 ±0.007 | 28.523 ±0.009 | 0.868 | 0.986 | 0.986 | 67.58 | 65.14 | 65.19 | 34.21 | 34.89 | 29.48 |

## 4. Conclusion

Chlorophyll, carbohydrate, protein, proline concentration and antioxidant defense system were all impacted by the amount of $Cr^{+6}$ that was present in the soil, demonstrating the plant's capacity to withstand chromium stress. The plants alter a number of metabolic processes to protect themselves against oxidative stress. Furthermore, plants can store Cr in their roots and show a high resistance to Cr in polluted soil. They also develop quickly, have a short life cycle and produce significant biomass. This ornamental plant has little potential of causing heavy metal poisoning to enter the food chain because it is nonedible. Furthermore, *Zinnia* is a beneficial species of plant when used in conjunction with other plants that are necessary to complete the biosystem during mine reclamation once the topsoil at the excavation sites has been restored, such as successional varieties of hardy native perennial grasses and other plants. The current zinnia plant experiment will yield valuable information that may be used to advocate emerging phytoremediation technique for minimizing toxicity of chromium and its practical application in real-world settings.

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**References**

Aebi, H. (1984). Catalase in vitro. Methods in Enzymology. 105: 121–126.

Arnon, D.I. (1949). Copper enzymes in chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15.

Bates, L., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205–207.

Ehsan, N., Nawaz, R., Ahmad, S., Arshad, M., Umair, M. and Sarmad, M. (2016). Remediation of heavy metal-contaminated soil by ornamental plant zinnia (*Zinnia elegance* L.). Asian J. Chem. 28: 1338–1342.

Ghosh, M. and Singh, S.P. (2005). A comparative study of cadmium phytoextraction by Accumulator and weed species. Environ. Pollut. 133: 365–371.

Kochian, L.V., Pineros, M.A. and Hoekenga, O.A. (2005). The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil. 274: 175–195.

Kramer, U., Clemens, S. (2005). Functions and homeostasis of zinc, copper and nickel in plants. In: Tamás, M., Martinoia, E. (Eds.), Topics in Current Genetics, Vol. 14. 215–271.

Lequeux, H., Hermans, C., Lutts, S. and Verbruggen, N. (2010). Response to copper excess in *Arabidopsis thaliana:* Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48: 673–682.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Marshall, M.J. and Worsfold, M. (1978). Superoxide dismutase: A direct continuous linear assay using the oxygen electrode. Anal. Biochem. 86: 561–573.

Nagoor, S. (1999). Physiological and biochemical responses of cereal seedling to graded levels of heavy metals, II. Effect on protein metabolism in maize seedlings. Adva 12: 425–433.

Naito, K., Ebato, T., Endo, Y. and Shimizu, S. (1980). Effect of benzyladenineond-aminolevulinic acid synthetic ability and ä-aminolevulinic acid dehydratase: diferential responses to benzyl adenine according to leaf age. Z. Pfanzenphysiol. 96: 95–102.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867–880.

Nelson, N. (1944). Estimation reducing sugar. J. Biol. Chem. 163: 375.

Panda, A., Patra, D.K., Acharya, S., Pradhan, C. and Patra, H.K. (2020). Assessment of the phytoremediation potential of *Zinnia elegans* L. plant species for hexavalent chromium through pot experiment. Environmental Technology & Innovation. 20, 101042.

Patra, D.K., Pradhan, C. and Patra, H.K. (2018a). An *in situ* study of growth of Lemongrass: *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson on varying concentration of Chromium ($Cr^{+6}$) on soil and its bioaccumulation: Perspectives on phytoremediation potential and phytostabilisation of chromium toxicity. Chemosphere. 193: 793–799.

Patra, D.K., Pradhan, C. and Patra, H.K. (2018b). Chromium stress impact on lemongrass grown in over burden soil of Sukinda Chromite Ore Mine (Odisha), India. Ann. Plant Sci. 7: 2394–2397.

Patra, D.K., Pradhan, C. and Patra, H.K. (2018c). Chelate based phytoremediation study for attenuation of chromium toxicity stress using Lemongrass: *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson. Int J Phytoremediat. 20: 1324–1329.

Patra, D.K., Pradhan, C. and Patra, H.K. (2019). Chromium bioaccumulation, oxidative stress metabolism and oil content in lemon grass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson grown in chromium rich over burden soil of Sukinda chromite mine, India. Chemosphere. 218: 1082–1088.

Patra, D.K., Pradhan, C. and Patra, H.K. (2020a). Toxic metal decontamination by phytoremediation approach: Concept, challenges, opportunities and future perspectives. Environ. Technol. Innov. 18: 100672.

Patra, D.K., Pradhan, C. and Patra, H.K. (2020b). Assessment of chromium phytotoxicity, phytoremediation and tolerance potential of *Sesbania sesban* and *Brachiaria mutica* grown on chromite mine overburden dumps and garden soil. Chemosphere. 252: 126553.

Tiwari, K.K., Dwivedi, S., Singh, N.K., Rai, U.N. and Tripathi, R.D. (2009). Chromium (VI) induced phytotoxicity and oxidative stress in pea (*Pisum sativum* l.): biochemical changes and translocation of essential nutrients. J. Environ. Biol. 30: 389–394.

Vajpayee, P., Tripathi, R.D., Rai, U.N., Ali, M.B. and Singh, S.N. (2000). Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. Chemosphere 41: 1075–1082.

Vranova, E., Inze, D. and Breusegem, F. (2002). Signal transduction during oxidative stress. J. Exp. Bot. 53: 1227–1236.

Yoshida, S., Forno, D.A. and Cock, J.H. (1976). Laboratory Manual for Physiological Studies of Rice, second ed. International Rice Research Institute. 23: 61-66.

Zurayk, R., Sukkariyah, B., Balbaki, R. and Ghanem, D.A. (2002). Ni phytoaccumulation in *Mentha aquatic* L. and *Mentha sylvestris* L. Water Air Soil pollut. 139: 355–364.