TAPPING PHYTOREMEDIATION POTENTIAL FOR SOILS CO-CONTAMINATED WITH HEAVY METAL AND PESTICIDE

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The overall aim of this thesis entitled, "Tapping phytoremediation potential for soils co-contaminated with heavy metal and pesticide" was to determine whether application of phytoremediation could be carried out for co-contaminated soils. Cd/Hg and BC were used as mixed contaminants. The overall aim was initiated by selecting suitable heavy metals and herbicide through survey of agro-industrial lands and laboratory analysis of soil samples, followed by studying the effects of co-contaminants on germination of seeds, biochemical parameters of plants, the role of soil amendments (chelating agents) on biochemical parameters, the phytoextraction potential of *C. roseus* plants under individual (Cd/Hg/BC) and joint contamination (Cd/Hg and BC) and the effect of chelate assisted phytoremediation of heavy metal and herbicide co-contaminated soils in six distinct but complementary chapters, as mentioned below:

- Chapter 4 Soil analysis of agricultural lands in and around Lucknow to ascertain the status of heavy metals & herbicides in contaminated soils.
- Chapter 5 Seed germination rate and early seedling growth of *C. roseus* in presence of single (heavy metal or herbicide) and mixed contaminants (heavy metal and herbicide).
- Chapter 6 Screening of phytoremediation potential of *C. roseus* under Cd/Hg and Butachlor co-contamination using biochemical parameters and SEM-EDX analysis.
- Chapter 7 Effect of chelate addition on biochemical parameters of *C. roseus* under single and joint toxicities of heavy metals along with Butachlor and SEM-EDX analysis.
- Chapter 8 Phytoremediation of Cd/Hg and Butachlor contaminated soils by *C. roseus* under individual treatment and joint contamination.
- Chapter 9 Chelate-assisted phytoremediation of Cd/Hg and Butachlor co-contaminated soils using *C. roseus*

This study was carried out on agricultural industrial soil samples from rural areas situated in and around Lucknow city. The physico-chemical and microbial properties of soil co-contaminated with inorganic and organic compounds at different sites was carried out. The results of correlation matrix among different physico chemical parameters, revealed positive correlation with organic matter while negative

correlation with clay particles and no correlation with rest of the parameters. Whereas, in terms of soil bacterial count the highest was revealed in control soil while the lowest was observed in Diwanganj soil. Hence it can be said that high concentration of heavy metal and herbicide in soil sample significantly affected the diversity of microbes. An analysis of the heavy metal (Pb, Hg, Cu, Cr, Ni, As, Cd, Mn, Fe) content in the soil samples revealed Hg and Cd to be more prevalent and above the tolerable limits in soil sample collected from Kalli West followed by Bijnor and Bhawaniganj and finally from Mau (For Hg), while in case of Cd, Semarou was found to have highest concentration followed by Mohanlalganj and Kalli West. Depending upon the findings, the two heavy metals Hg and Cd were selected for the proposed research work. In addition, the result of cluster analysis showed that any soil having high concentration of Cr might have low amount of Hg. Based upon the laboratory analysis of the soil sample through HPLC, a peak of BC was detected in most of the soil samples. Hence, on the basis of survey and laboratory analysis Butachlor herbicide was chosen along with Hg and Cd for co-contamination study.

Different plant species were checked for their phytoremediation potential, out of which *Catharanthus roseus* revealed high phytoremediation potential for co-contamination study based upon preliminary studies on seed germination and growth. The results for seed germination in the presence of heavy metal (Cd and Hg) and herbicide (BC) alone and in combination affected both, the percentage of germination and early seedling growth of *C. roseus*, differently. In specific, the joint effect of Cd and BC was found to be more toxic as compared with the individual toxicities. Based on EC 50 values for the effect of Hg and BC, the results indicated that the joint effect of Hg and BC was more toxic followed by individual treatments of BC and Hg. Although, morphologically root was more sensitive to shoot both under single and/or joint toxicities, however, the SEM micrographs revealed more structural deformation in shoot as compared to roots.

The effect of Cd, Hg and BC on *C. roseus* on different biochemical parameters was observed differently. For example the effect of metals and herbicide on photosynthetic pigment of plant revealed that Cd in presence of BC induced a decrease in *Chl a* and *Chl b*, carotenoid and total chlorophyll content with increase in concentration. While that of Hg (20-120 $mgkg^{-1}$) and BC (4 $mgkg^{-1}$) revealed an increase in chlorophyll content upto 40Hg+4BC (12.5% to 4.63%) and then there was

a sharp decline observed upto highest concentration 120 Hg+4BC (60.24%). Similarly, co-contamination was found to pose more stress to the plants which may be the cause of lower phenolic content in joint treatments as compared to the alone treatment. Data obtained in this study established the fact that the activities of all antioxidant enzymes inclined to escalate with the Cd/Hg in soil. The SEM images clearly defined that the distinct feature of xylem and phloem was gradually distorted with increasing concentration from 25-200 $mgkg^{-1}$Cd; 20-120 $mgkg^{-1}$ Hg and 0.5-8.0 $mgkg^{-1}$ BC. But more deformation was observed under joint toxicity of heavy metals and BC as compared to alone treatments.

In addition, the effect of heavy metals with and without BC was also observed after adding chelating agent (CA and EDTA) to the soil, to induce heavy metal and herbicide uptake. The results revealed that addition of chelating agents increased the photosynthetic pigment value, protein content and also enhanced antioxidant enzymes, which decreased the production of MDA under co contamination. Results for proline content showed a decline in the presence of chelating agents, which might be due to the reason that a chelator forms complex compounds which tends to reduce the toxicity in co-contaminated soil. For mitigation of ROS-induced in the present study *C. roseus* plant stimulates protective mechanism in the form of enhancement of SOD, APX, CAT, GR and POD activity for all the treatments. The SEM micrograph of treated root, shoot and leave showed a structural deformation in the epidermal layer, with respect to control.

The effect of heavy metal and/or herbicide on plant growth and their anatomical structure was studied. Individual and joint toxicity not only affects heavy metal accumulation but BC dissipation as well. Our study on the plant *C. roseus* revealed that Cd was more effective for decreasing plant biomass of the growing plants as compared to Hg. Results revealed that there was a significant decline in root (44.89%) and shoot (55.52%) length as the toxicity of herbicide (BC) increases. *C. roseus* showed better growth under BC treatment alone in comparison to co-contamination of Cd, Hg and/or BC. In presence of herbicide a higher uptake of metals was observed which results in an increase in concentration of metals in *C. roseus* root and shoot as compared to individual Cd and Hg treatment. The BCF values for shoot and root of *C. roseus* showed higher values under co-contaminated treatments (Cd/Hg and BC) than alone treatments of Cd/Hg. The results of present

study for BC dissipation revealed higher degradation of BC at higher concentration of heavy metal both Cd and Hg.

Chelate-assisted phytoremediation of Cd/Hg and BC co-contaminated soils using plant *C. roseus* was carried out. The biomass (roots and shoots) of the plant *C. roseus* was found to decline in the presence of chelating agents EDTA and CA, in comparison to control. The overall results for BCF values for roots and shoots further revealed that the total metal uptake by *C. roseus* was significantly ($p<0.001$) increased due to application of chelating agents EDTA and CA alone as well as in combined application with respect to metal treatments without chelates. Results also revealed that chelating agents enhanced the dissipation of PAH compound such as BC along with heavy metal in co-contaminated soils. Chelating agents have the ability to desorb the PAH compounds and make them bioavailable for degradation by microbes.

This investigation on the phytoremediation potential of *C. roseus* on co-contaminated soils with different concentrations of heavy metal and herbicide along with the presence of organic and inorganic chelates in this study represented an exhaustive assessment of this area of research. Moreover, *C. roseus* emerged as a good and a potential candidate for phytoremediation more significantly when it comes under co-contamination.