

## Abstract

Crop irrigation with untreated wastewater is a routine practice in developing countries that causes multiple human health consequences. A comparative study was performed to evaluate the efficiency of chitosan polymerized silica gel (CP-silica gel), zinc enriched biochar (ZnBc) and three bacterial species such as Trichococcus (B1), Pseudomonas alcaligenes (B2) and Bacillus subtilis (B3) to improve growth and physiology of Spinacia oleracea L. plants grown on soil irrigated with tanneries wastewater. Initially, a biosorption trial was conducted to test the heavy metal removal efficiency of three bacterial species B1, B2 and B3. Among three bacterial isolates, B3 sp. showed maximum Cr<sub>6+</sub> and Pb<sub>2+</sub> removal efficiency. Then, a pot study was conducted with 12 different treatments such as Control; CP-Silica gel (0.2%); ZnBc (1.5%); B1; B2; B3; CP-Silica gel + B1; CP-Silica gel + B2; CP-Silica gel + B3; ZnBc + B1; ZnBc + B2 and ZnBc + B3 having three replicates. The results demonstrated that wastewater application significantly ( $p \leq 0.01$ ) reduced the fresh and dry weights of root, stem and leaves due to high Cr<sub>6+</sub> and Pb<sub>2+</sub> toxicity. Similarly, the concentration of leaf Cr<sub>6+</sub> was significantly decreased (91 and 85 %) with application of ZnBc + B3 and CP-Silica gel + B3, respectively, as compared to control. There was a reduction in stem H<sub>2</sub>O<sub>2</sub> (87%) and MDA (81%) recorded with CP-silica gel + B3 treatment due to enhanced activities of antioxidant enzymes viz. APX (6-folds) and CAT (7-folds) relative to control. Similarly, leaf total phenolics (3-folds) and protein (6-folds) contents were enhanced with CP silica gel+B3 application relative to control. Altogether, it was found that application of CP-Silica gel and ZnBc along with B3 species showed maximum efficiency to combat Cr<sub>6+</sub> and Pb<sub>2+</sub> stress in soil and Spinacia oleracea L. plant. Hence, applications of ZnBc with interaction of microbes proved to be a cost-effective and eco-friendly soil conditioner in developing countries including Pakistan to limit the deleterious effects of tanneries wastewater pollution.