SUMMARY AND CONCLUSIONS
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The present thesis is focused on the use of a tropical plant from Cactaceae family, *Nopalea cochenillifera*, for removal of toxicants viz. textile dyes, a paint preservative (Troysan S89) and hexavalent chromium. *In vitro* cultures have been established in *N. cochenillifera* and the cultures were used for decolorization of textile dyes, detoxification of Troysan S89 and bioaccumulation of Cr(VI). Spectroscopic and chromatographic analysis of metabolites obtained after degradation of dye Red HE7B and assays of enzyme systems involved in phytotransformation of Red HE7B have been completed by using *in vitro* cultures of *N. cochenillifera*. Subsequently, phytotoxicity studies have been carried out in Red HE7B and its degradation metabolites. Detoxification of Troysan S89 by cell cultures of *N. cochenillifera* has been confirmed by using spectroscopic (UV-Vis and FTIR) and toxicity (phyto-, cytogenotoxicity and carcinogenicity) analyses, which has been then compared with detoxification of Troysan S89 by cell cultures of *B. malcolmii*. A successful micropropagation protocol for better multiplication of *N. cochenillifera* plants has been established and these *in vitro* cultures have been used for Cr(VI) accumulation studies.

The callus was induced in ovules of *N. cochenillifera* by using MS medium supplemented with 5 mg/L 2,4-D and 2 mg/L BA. Addition of L-glutamine and freshly obtained coconut milk at concentrations 500 mg/L and 15%, respectively enhanced growth of callus. The best growth of friable callus tissue was obtained in the linear phase between (21 and 91) d of culture followed by stationary phase. However, after (40 to 50) d of culture, white and vigorous calli started to show brown discoloration. 1 g of callus culture could give rise to maximum callus growth of about 17.951 g (FW) and 0.384 g (DW) after 91 d of incubation.

Here, we have reported *in vitro* propagation of *N. cochenillifera* and acclimatization to field conditions by modified method of Brasil et al. (2005). Comparable propagation rate of up to (17 ± 5) individuals for each cycle of multiplication has been obtained. The maximum areole shooting was observed on MS + 2 mg/L BA after 40 d culture incubation. Though, spontaneous rooting of shoots was observed on MS medium; a high number of roots per rooted plantlet was obtained by culturing shoots on MS + 5 mg/L
Phytoremediation approach for removal of environmental pollutants

IBA after 20 d. *In vitro* rooted plantlets showed a 100% survival when sequentially transferred to soil.

UV-Vis spectrophotometric analysis revealed that *N. cochenillifera* (cladode and callus) could degrade selected textile dyes at a concentration 40 mg/L. Malachite green showed the highest decolorization of about 91% after seven days, but a considerable amount (39%) of the dye remained adsorbed on the cell surface. Though greater decolorization was achieved for Green HE4BD than Red HE7B, enzymatic analysis in cells after decolorization did not illustrate promising results. Henceforth, we concentrated our studies on the dye Red HE7B, which was decolorized about 65% without any adsorption onto the surface of cells within seven days. UV–vis spectral scan of culture supernatants removed after seven days indicated decolorization of Red HE7B. Considerable reduction in the percentage decolorization was observed with increase in the dye Red HE7B concentration after 44 d. The HPTLC analysis of dye Red HE7B and its phytotransformation products showed completely different pattern of Rf values and the chromatogram clearly indicated phytotransformation of the dye. The FTIR spectrum of Red HE7B showed the presence of peaks which were confirmed with Red HE7B dye structure. Absence of these peaks and instead existence of new peaks clearly indicated phytotransformation of Red HE7B into simpler metabolites that can be easily consumed. Based on the GC-MS and FTIR analyses of the separated metabolites a possible pathway for the phytotransformation of Red HE7B has been proposed and this notion was further supported by specific enzyme activity studies that demonstrated the induction of activities in biotransformation enzymes involved in dye degradation. Detailed analysis of data obtained from phytotoxicity studies revealed that metabolites extracted after degradation of Red HE7B are very less toxic than that of dye.

The detailed analysis of detoxification of Troysan S89 by *Blumea* and *Nopalea* cells revealed better potentiality of *Blumea* over *Nopalea*. UV-Vis spectral analysis showed greater shift in absorbance of Troysan S89 after degradation by *Nopalea* than that by *Blumea*. The FTIR analysis showed presence of peaks for sulfur containing compounds in degradation metabolites of Troysan S89 by *Nopalea* similar to Troysan S89, while in case of *Blumea* none or minimum number of peaks of degradation metabolites was detected similar to that of peaks for Troysan S89 indicating better phytotransformation of
Phytoremediation approach for removal of environmental pollutants

Troysan S89 by *Blumea*. Cytogenotoxicity studies in *Allium* root cells and phytotoxicity studies in *T. aestivum* and *E. lens* when exposed to Troysan S89, degradation metabolites by *Blumea* and degradation metabolites by *Nopalea* revealed that degradation metabolites by *Blumea* were lesser toxic than degradation metabolites by *Nopalea* when compared to the control. The spectroscopic and toxicity analyses proved better prospective of *Blumea* over *Nopalea*. Though analyses confirmed better capability of *Blumea* over *Nopalea* cell suspensions, considering the characteristics of the plant, *Nopalea* should still be preferred for phytoremediation.

*In vitro* plants of *N. cochenillifera* significantly tolerated up to 100 µM K$_2$Cr$_2$O$_7$. Exposure to ≥ 1000 µM K$_2$Cr$_2$O$_7$ was phytotoxic, causing death in all plants after 40 d of exposure. *Nopalea*’s ability to tolerate up to 100 µM K$_2$Cr$_2$O$_7$ supports its potential utility for phytoremediation of vestiges containing this concentration of Cr. The chlorophyll content of all the treated plants was dose dependent that showed a statistic correlation of decrease in concentration with increase in concentrations of Cr. Both the levels of lipid peroxidation and protein oxidation in root tissues of *N. cochenillifera* increased significantly with increasing concentration of Cr. Exposures of the plant (*N. cochenillifera*) to lower concentrations of K$_2$Cr$_2$O$_7$ (≤ 10 µM) induced CAT and SOD significantly but higher concentrations of K$_2$Cr$_2$O$_7$ (> 100 µM) inhibited the activities. Induction of antioxidative enzymes at lower concentrations of Cr could be due to activations of genes encoding CAT and SOD by Cr-stress; while inhibition of the enzymes at higher toxic concentrations of Cr could be explained on the basis of protein oxidation at respective concentrations.

Conclusively, *in vitro* systems of *N. cochenillifera* have been studied for removal of environmental pollutants such as textile dyes, paint preservative (Troysan S89) and Cr(VI). The plant effectively degraded toxic levels of dyes and Troysan S89 and accumulated Cr(VI). *Nopalea* cell cultures not only degraded organic pollutants but also transformed into less toxic products. Established micropropagation protocol helped enhance faster multiplication of such a slow growing plant from Cactaceae family. The overall study in the present thesis supported *N. cochenillifera* as a better candidate plant for phytoremediation. Society must employ all available tools to meet the challenges of increasing environmental pollution. One such tool can be phytoremediation by relatively
unexploited plant sources such as cacti. Future research goals on cactus must address high and stable yield, multiple disease and pest resistance, improved plant habitat, efficient nutrient uptake, and desirable quality in the plant parts that are utilized. Cactus pear will transcend the ethnical markets only if adequate marketing strategies can be promoted and quality of the plant enhanced and standardized.