Summary and Conclusions

The present study concludes that the various antineoplastic compounds are being consumed during chemotherapy in hospitals and households by out-patients for the treatment of continuously increasing cancer cases. Antineoplastic compounds are contaminating aquatic environment and possess mutagenic, cytostatic and eco-toxicological effects on aquatic life and human health. These anti-cancer agents enter the water bodies in their original form or as metabolites via urine and faeces of the out-patients or the patients admitted in hospitals. Due to its high lipid solubility, the antineoplastic drugs accumulate in the fatty tissues of the organisms. These drugs enter through the food chain and cause health effects in humans due to their cytotoxic and genotoxic properties. Many advanced treatments such as membrane filtration, catalytic degradation etc. for the removal of these pollutants were employed but they are exhibiting certain limitations. Here, on the basis of limitations in prior said treatment technologies, the biological approach has been used for the biodegradation of some antineoplastic compounds by using white rot fungi. This approach overcome to limitation exist in physical, physio-chemical and chemical treatments.

Here, we have developed a simple, accurate, fast and reliable HPLC method for detection of the three antineoplastic compounds in environmental samples and validated the same. The developed HPLC methods have several advantages including cost effectiveness, minimal analysis time is required, and no extensive sample preparation is required. Further, use of simple solvent such as acetonitrile and water as mobile phase would not have serious environmental damage**.** Etoposide and paclitaxel were eluted best at the same developed, but they provided different retention *i.e.* 2:40.1 and 3:49.3 min. for etoposide and paclitaxel respectively. Cyclophosphamide provided best elution peak in similar solvent but retention time, mobile phase ratio and wavelength was different. The conditions of developed methods provided better resolution for all the analytes that were tested *viz* cyclophosphamide, paclitaxel and etoposide. The feasibility and reliability of methods including linearity, accuracy and

precision and robustness are good and acceptable for analysis of etoposide, paclitaxel and cyclophosphamide. Thus, we strongly propose to use the method presented here to detect cyclophosphamide, paclitaxel and etoposide in environmental water or wastewater samples.

In biodegradation study of WRFs found that, only *G. lucidum* fungus have the better potential for the removal or degradation of cyclophosphamide, etoposide and paclitaxel. Among three antineoplastic compounds treatment with white rot fungi strains, the highest biodegradation was achieved for etoposide. Etoposide was the only compounds that was removed by all three fungi. Cyclophosphamide showed degradation with only *G. lucidum* and highest biosorption efficiency with *P. chrysosporium*. The removal of paclitaxel was analysed very less in *G. lucidum* and almost negligible in *P. chrysosporium* and *T. versicolor*. This study find that the degradation carried out for these compounds in *G. lucidum* and *T. versicolor* was directly proportional to the biomass and extracellular enzyme production, while in *P. chrysosporium* it was different. The degradation of these compounds followed the pseudo first-order-kinetics study with these fungi. The maximum rate constant was calculated in degradation of etoposide with *G. lucidum*. The change in pH value and glucose utilization for each fungal culture during biodegradation were estimated. So, every WRFs does not have potential for the removal of all antineoplastic compounds and their degradation does not depend only on the biomass and enzyme activity presence, while some other factors may responsible also for the biodegradation and degradation rate.

Moreover, the study focused on toxic effect analysis of cyclophosphamide, etoposide, and paclitaxel and their TPs generated during degradation with WRFs on Raw 264.7 cell line (monocyte macrophage). MTT assay was performed to evaluate the *in-vitro* cytotoxicity and the result indicates that these compounds put an adverse effect on mouse macrophage cell line. According to the investigation all three drugs exhibiting cytotoxic effect on Raw 264.7 cells but etoposide will be classified as more toxic than paclitaxel and cyclophosphamide if present

in their native form. It indicates towards a strong effort should be made to remove or degrade these compounds at source before reaching to the aquatic environment as they are harmful for the immune system of aquatic animal diversity. While, after the degradation with selected white rot fungi, the toxicity level of these compounds reduced in the form of TPs but their toxicity was not completely eliminated still after the degradation. Further studies such as mutagenic, carcinogenic effects are required to help more understanding of the exposure and hazardous effect of these compounds on cell lines and aquatic organisms.

Following are the major conclusions in this study:

- The isocratic mode of HPLC provides the best elution for cyclophosphamide, etoposide and paclitaxel.
- The developed condition for detection and quantification of etoposide and paclitaxel were same but their retention time and peak areas were different.
- The retention time of cyclophosphamide, etoposide and paclitaxel was 3:32.3, 2:40.1, 3:49.3 min. respectively.
- The detection limit of the selected compounds in developed methods was 10 $\mu g.ml^{-1}$.
- *Ganoderma lucidum* showed the highest degradation for etoposide (>99 %), while *P. chrysosporium* showed highest biosorption efficiency for cyclophosphamide (>23 %).
- Maximum degradation rate was achieved for etoposide (k = 0.54 day^{-1}).
- *P. chrysosporium* does not showed biodegradation for cyclophosphamide, while *T. versicolor* showed only 1 % of the initial concentration of cyclophosphamide.
- The co-relation of glucose utilization and rate of degradation was not confirmed due to complete utilization of glucose during initial days of cultivation.

- The initial set pH value was changed in each case of fungal culture during the degradation of antineoplastic compounds.
- Each antineoplastic having potential to cause cytotoxic effect on Raw 264.7 cells but their toxicity limit varying.
- After the biodegradation with white rot fungi, the cytotoxicity was reduced.
- The cytotoxicity of by-products or degraded products of etoposide in treatment with *P. chrysosporium* was higher to the toxicity of by-products of etoposide with *G. lucidum* and *T. versicolor*.

Future scope of the work

In case of WRF, it should require to optimize the condition for each compounds individually with enzyme for the achievement of better degradation efficiency. Present study indicated that, the rate of degradation is not depending on the amount of biomass and enzyme activity of growing culture. So, the separate optimization should be done for the degradation of each compound by each enzyme to identify the individual effect of extracellular and intracellular enzymes on rate of degradation. In concern of toxicity, WRFs cannot eliminate the complete toxic effect of these compounds from the source even after the efficient degradation, so instead of degradation by fungi, their complete removal from water bodies should be done with no generation of toxic by-product.