# 5

# SUMMARY AND CONCLUSION

Industrial effluents are adverse by-products of economic development and technological advancement. When improperly disposed off, they endanger human health and the environment. The silk dyeing effluent with organic, inorganic and heavy metals are discharged from the small scale dyeing industries toxic to the environmental streams which affect the plants.The dyeing industry provides direct employment to nearly thirty million people. Dyeing industrial establishments have become the cause for the public concern because environmental issues result in the winding up of several small-scale industries. In the present study, silk dyeing effluent was collected and different concentrations of the effluent were treated using the randomly selected biofertilizers to assess the growth of the GLVs and compared with the plants grown in fresh water.

The findings of the four phases were discussed below.

## Phase 1

It involves the growth studies of the selected five green leafy vegetables namely *Brassica juncea*, *Trigonella foenum*, *Amaranthus polygonoides*, *Amaranthus tristis* and *Sesbania grandiflora* grown in fresh water. The plants of the selected GLVs were harvested on the 45$^{th}$ day and subjected to biometric parameters, phytochemical analysis, proximate principles, mineral contents, enzymic, non-enzymic antioxidants, lipid peroxidation, pigment analysis and histological studies. The soil used to grow the GLVs were also analysed for macro and micronutrients.

- The **biometric parameters** such as first cotyledon, percentage seed germination, seedling length, number of leaves, surface area of leaves, root length, shoot length, root shoot ratio, root mass, dry matter, number of seed bunch per plant, distance between the nodes were observed in selected GLVs grown in fresh water. The biometric parameters such as seedling length, number of leaves, shoot length, root shoot ratio, root mass, dry matter and distance between the nodes were found to be maximum in *Sesbania grandiflora.* The mustard plant *Brassica juncea* were found to have large surface area of leaves. The percentage seed germination and the root length were found to be maximum in *Amaranthus polygonoides*.

- In phytochemical analysis, the carbohydrate, protein, cellulose and quinone were present in the leaf, the stem and the seeds of all the selected GLVs except the seeds of *S.grandiflora*. Catechol, saponin, cynogenic glycosides and lignin analysis of the selected GLVs revealed their absence in all the parts of all the extract of the GLVs. The phytochemicals were rich in these GLVs.
- All the selected GLVs were found to be rich in proximate principles, mineral contents, enzymic and non-enzymic antioxidants, lipid peroxidation and pigment.
- The soil analysis revealed that the pH of the soil used in this study was within the optimal range and found to be a sandy loamy type of soil. The electrical conductivity, the level of P, K and the micronutrients (Mn, Cu and Zn) were within the optimal range whereas the calcium and the nitrogen contents of the soil were below the prescribed optimal range.
- The histological sections of the leaf, the root, and the stem tissues of all the selected GLVs showed a fine structural integrity.

**Phase 2**

The physico-chemical properties of the silk dyeing effluent, decolorization in silk dyeing effluent inoculated with *Rhizobium sp*., *Azospirillum sp.* and *Pseudomonas fluorescens* and production of phytohormones were assessed. The GLVs grown in different concentrations (25%, 50%, 75% and 100%) of the silk dyeing effluent were subjected to biometric parameters. The phytochemicals, proximate principles, mineral contents, enzymic and non-enzymic antioxidants, lipid peroxidation, pigment analysis were carried out in the GLVs grown in 75% of effluent. The histological studies of the leaf, the root, and the stem tissues of all the selected GLVs were performed in the highest concentrations of effluent at which they survived. The effluent contaminated soil was also analysed.

- In physico-chemical analysis of the effluent, the appearance and the color of the effluent was dark violet at the time of collection which turned turbid later. The turbidity NT unit of the present study was 18 for the initial untreated effluent. There was no significant odour and foam. The total dissolved solids (TDS), EC, BOD and COD were above the acceptable limits.

Though the GER limits (2010) is 37°C, the collected effluent from the spot area was 45°C which had come down to room temperature (27°C) after sometime. The total hardness as $CaCO_3$, calcium, magnesium, chloride, sulphate, iron, free ammonia, fluoride, nitrite and phosphate levels in the untreated effluent were within the standard limits whereas the level of sodium and nitrate contents was slightly above the standard limits.

The nickel level was above the prescribed limits. The levels of chromium and zinc in the crude silk dyeing effluent were within the set limits and above the prescribed limits respectively. The cadmium of the crude effluent was almost close to the standard limits. Thus the study has revealed that the unsafe silk dyeing effluent from small scale industries is found to have organic, inorganic compounds and heavy metals.

- In bioassay test, the fish allowed to grow in fresh water were survived till 96 hrs of experimental period whereas those in the crude effluent survived only upto 12 hrs.
- The preliminary decolorization of the silk dyeing effluent was carried out with 10% of the diluted effluent by the biofertilizers of *Rhizobium sp*., *Azospirillum sp*. and *P.fluorescens* separately. The percentage decolorization of different concentrations of the crude silk dyeing effluent (25%, 50%, 75% and 100%) with co-substrates glucose (0.002g) was found to be least in *Rhizobium sp*. and the maximum decolorization was observed in *P. fluorescens* followed by *Azospirillum sp*.
- In phytohormone studies, the *P.fluorescens* and *Azospirillum sp.,* in the silk dyeing industrial effluent produced more IAA than $GA_3$.
- The biometric parameters and phytochemicals studied in the selected GLVs were affected by increasing concentrations of the effluent compared to the GLVs grown in fresh water. The proximate principles, mineral contents, enzymic and

non-enzymic antioxidants, lipid peroxidation and pigment levels were found to be reduced in the selected GLVs when compared to the control.

- The pH and the EC noticed in the effluent soil were above the standard limits. All the macronutrients (N, P, K and Ca) and the micronutrients (Fe, Cu and Zn) in the effluent soil were detected to be lower than the optimal range. The results indicate that the effluent reduces the level of both the macronutrients and the micronutrients below the optimal range. The clear view of histological sections was not revealed in the root and the stem of the GLVs affected by the effluent.

**Phase 3**

It involved the assessment of the physico-chemical parameters of biotreated effluent by *Pseudomonas fluorescens*. The post harvested samples of the selected GLVs grown in biotreated soil were subjected to biometric parameters, phytochemical analysis, proximate principles, mineral contents, enzymic and non-enzymic antioxidants, lipid peroxidation, pigment analysis and histological studies. The soil used to grow the GLVs were analysed for macro and micronutrients.

- In physico-chemical analysis of the effluent, the appearance and the color of the effluent after biotreatment I by *Azospirillum sp.* and biotreatment II by *P.fluorescens* were slightly pinkish. Among the two microorganisms used, *P. fluorescens* (biotreatment II) had a higher reduction of turbidity, TDS, BOD, COD and EC when compared to the biotreatment I by *Azospirillum sp.*

  The pH range was maintained between 6 and 9. The biotreated effluent by *Azospirillum sp.* and *P.fluorescens* was basic in nature. Though the GER limits (2010) are 37°C, the collected effluent from the spot area (45°C) was reduced to room temperature 27°C after sometimes. The biotreatment with *Azospirillum sp.* as well as *P. fluorescens* did not change the pH alkalinity as $CaCO_3$ (20mg/l). The levels of total hardness as $CaCO_3$, calcium, magnesium, iron, free ammonia, chloride, fluoride, sulphate and phosphate of the silk dyeing effluent were within the prescribed limits before biotreatment and were reduced maximally after biotreatment with *P.fluorescens.* The manganese level was not detected in both the untreated and the biotreated samples. The sodium, potassium and nitrate of the effluent were highly reduced by biotreatment II. The results revealed that the *P.fluorescens* had efficiently reduced the physico-chemical characteristics of the silk dyeing effluent when compared to the *Azospirillum sp*.

The heavy metals of the silk dyeing effluent such as chromium, zinc, cadmium were within the FMENV limits. The nickel which was above the set limits was highly reduced by biotreatment with *P.fluorescens*. In the bioassay test, the biotreated effluent water (by *P. fluorescens*) supports the survival of the fish showing 0 % mortality in 0 hr - 96 hrs.

- The PGPR biotreated soil had enhanced the growth of the selected GLVs compared to the normally grown and effluent treated plants. The phytochemical analysis results were similar to the selected GLVs grown in fresh water. The proximate principles, mineral contents, enzymic and non-enzymic antioxidants, lipid peroxidation and pigment levels were found to be higher in biotreated effluent, compared to the GLVs grown in untreated and effluent treated water. The biotreated effluent soil has improved the levels of macronutrients and micronutrients when compared to the control and effluent treated soil. The histological sections of the root, the stem and the leaf for all the GLVs grown in biotreated effluent were similar to that of the GLVs grown in fresh water.

**Phase 4**

It involves the identification of functional groups and compounds using spectroscopic and chromatographic techniques in the selected GLV plants.

- The FT-IR spectrum confirms that the alcohol, the alkane and the amine groups were found in all the GLVs irrespective of the treatments, even in crude effluent, the plants managed to form these organic groups. The amide group was completely absent in all the treatments. The phosphine group was detected only in *A.polygonoides* plant grown in crude effluent. The isocyanide group was found only in *B.juncea*, *A.polygonoides* and *S.grandiflora* grown in fresh water, which was unable to synthesize in plants grown in crude effluent and biotreated soil. Irrespective of the treatments, the aromatic groups were synthesized by *B.juncea*, *A.tristis* and *S.grandiflora* as well as *T.foenum* grown in biotreated soil and *A.polygonoides* grown in both the effluent exposed and the biotreated soil. The alkene group was detected in different treatments of *B.juncea*, *T.foenum* and *S.grandiflora* as well as *A.polygonoides* grown in fresh water and *A.tristis* grown in biotreated soil.

- In HPLC analysis, the compounds were identified on comparison of the standards (pigments, alkaloid and monosaccharides) with the HPLC of methanolic extract of the selected GLVs of different treatment with the difference of ±0.5 retention time (tR). Comparison of the retention time (tR) has revealed the presence of the pigments chlorophyll $C_3$ in *A.tristis* of normal, effluent and biotreated treatments, chlorophyll $C_2$ in *A. polygonoides* grown in fresh water, chlorophyll B in *T.foenum* grown in fresh water, carotene in *T.foenum* grown in fresh water and biotreated effluent. The alkaloid caffeine was found in *A.tristis* grown in biotreated effluent. The monosaccharide glucose was present in all the GLVs of all the treatments and fructose present in *A.polygonoides* grown in fresh water and *T.foenum* grown in crude effluent and biotreated effluent. The mannose and the galactose were present only in *T.foenum* grown in fresh water. The HPLC of the silk dyeing industrial effluent with 99.88 % and 0.115 % area whereas the chromatogram of biotreated effluent has shown five peaks with reduced percentage area of 39%, 18.5%, 10.1%, 8% and 24.18% which clearly indicates that the dye in the effluent has been degraded by *Pseudomonas fluorescens*.

Hence, it can be concluded that the null hypothesis ($H_0$) "*Pseudomonas fluorescens* do not degrade the silk dyeing effluent" is rejected and also confirms that the biodegradation technology using the biofertilzer *Pseudomonas fluorescens* will lead to a safer disposal of the silk dyeing effluent. Thus, the ecofriendly technology can be implemented to enhance the growth of the plants in the effluent discharged area. It will also avoid the threat to the lives health wise and also the small scale industrial entrepreneurs can establish their own zero discharge of the effluent. The government can encourage the biofertilizer mediated technology for the betterment of poor entrepreneurs of the silk dyeing units and safeguard many industries from closure.

## Recommendations

The biotreated silk dyeing industrial effluent by *Pseudomonas fluorescens* can be subjected to NMR studies to confirm the degradation i.e dye bond breakage.