

INTRODUCTION

Environmental contamination has been defined as the release of unwanted substances into the environment by man in quantities that damage the health and the resources (Thiripurasundari *et al.*, 2013). Majority of water available on the earth is saline in nature, with only a small quantity as fresh water. Due to over exploitation and pollution, freshwater has become a scare commodity (Gupta and Shukle, 2006). Water contamination caused by industrial effluent discharges with enhanced concentration of nutrients, sediments and toxic substances has become an alarming trend worldwide. It has become a question of considerable public and scientific concern in the light of evidence of their acute toxicity to human health, to biological ecosystems (Hassan *et al.*, 2013).

Effluent discharged into the environment may have a severe negative impact on the quality and the life forms of the receiving water body when discharged untreated or partially treated. Water contaminated by effluent from various sources is associated with heavy disease burden and this could influence the shorter life expectancy in developing countries compared with the developed nations (Okoh, 2007).

The textile industry accounts for two-third of the total dyestuff market. More than 10,000 different textile dyes with a predictable annual production of 7×10^5 metric tons are commercially available worldwide (Aksu, 2005). Synthetic textile dyes used each year are lost during manufacture and processing operation and 20% of these dyes enter the environment through effluents that result from the treatment of residual industrial water. Wastewater from printing and dyeing units is often rich in color, containing residues of dyes and chemicals and needs proper treatment before being released into the environment. Without adequate treatment, these dyes can remain in the environment for a longer period of time. Approximately 10-15% dyes are released into the environment during process of dyeing, making the effluent highly colored and aesthetically unpleasant. The effluent from textile industries thus carries a large number of dyes and other additives which are added during the process of coloring. This residual amount is responsible for the coloration of the effluents and cannot be recycled. The dyed organic substances, which are non recyclable

and responsible for the high biological oxygen demand (BOD) and chemical oxygen demand (COD) of the effluents, textile fibers, electrolytes, essentially sodium chloride and sodium carbonate are accountable for the very high saline content of the wastewater (Allegre *et al.*, 2006).

The silk industry involves water intensive processes that need large volume of good quality of water. The silk industry uses a lot of dyes generating about 3773 metric tons per annum (MTA) of hazardous waste of which only 3.25% is recyclable. Process of dyeing emits volatile organic compounds (VOCs). Some of the small scale dyeing units are having storage facilities for hazardous waste but suitable disposal facilities are not available, which contaminate water sources as they are also released into drains and cause grave risk to the health of local communities, in addition to polluting the drinking water (Nupur, 2009).

The color in watercourses is accepted as an aesthetic problem rather than an eco-toxic hazard. Therefore, the public seems to accept blue, green or brown colour of rivers but the 'non-natural' colors such as red and purple usually cause most concern. The polluting effects of dyes against the aquatic environment can also be the outcome of the toxic effects due to their long time presence in the environment (i.e. half-life time of several years). The accumulation in sediments especially in fish or other aquatic life forms and decomposition of pollutants are carcinogenic or mutagenic compounds but they are also of low aerobic biodegradability. Due to their synthetic nature and structure mainly aromatic, most of the dyes are non-biodegradable, having carcinogenic action or causing allergies, dermatitis, skin irritation or different changes in tissues (Bornick and Schmidt, 2006). In addition to being toxic, the dye effluents also include chemicals that are carcinogenic, mutagenic or teratogenic to various organisms (Mathur and Bhatnagar, 2007).

The environmental authorities have begun to target the textile industry to clean up the wastewater that is discharged. The environmental issues related with the residual dye content or the residual color in treated textile effluents are always a concern for each textile operator that directly discharges the effluents into the atmosphere (Zaharia *et al.*, 2011).

The effluent discharge from the silk dyeing industries is common in Salem district. The major issue that has caused failure to small units is the high cost of running a plant, lack of electricity, non-payment of user charges of reagent for the treatment of effluent.

Also the current effluent treatment plants fail when the operation does not involve chemicals at the right time and in the right quantity. The objective is to provide eco friendly environment of dyeing unit effluents that positively affect the lives of the poor artisans as well as their communities who are unable to establish treatment plants in Salem district.

Silk dyeing industries' raw material, the silk filament, is obtained from the silkworm which is spun by two fibroin filaments held together by a cementing layer sericin. Fibrion and sericin account about 75% and 25% weight of raw silk respectively. Initially the silk filament is subjected to degumming process which includes removing the sericin layer. The degumming process of silk has traditionally been carried out near alkaline condition at a pH of 10-11 near boil. At present, two different types of high temperature, high pressure degumming and enzyme degumming are involved. By ultra filtration, sericin is recovered and reused. The dyebath is added to one-third dye solution with a half part of calsolene and one part of citric acid which is boiled in a waterbath. The undyed part of the skein silk is soaked and left for 30 minutes to exhaust. Then milsoft is added, wrung out tightly and partially dried (Gulrajani, 2008).

The silk fibroins are given different colors by colored substance 'dye' that have affinity to substrate fibroins which need the mordant to improve the fastness of the dye on the fiber. The archaeological evidence shows that particular dyeing has been carried out for over 5000 years. Many natural dyes have been known for a long time. They were obtained from animal and vegetable sources. Organic compounds have an ability to impart their color to the material to be dyed, in an aqueous medium (Pallavi *et al.*, 2010). By far, the greatest source of dyes has been from the plant kingdom but only a few have ever been used on a commercial scale.

A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fiber. Usually a substrate is a textile fiber, but it may be paper, leather, hair, fur, plastic material, wax, a cosmetic base or a foodstuff. Dyes are extensively used in the textile, rubber product, paper, printing, color photography, pharmaceuticals, cosmetics and many other industries (Thiripurasundari *et al.*, 2013). Thousands of synthetic dyes have been prepared. They are

quickly replaced by the natural dyes. They cost less and they offer a vast range of new colors.

According to Kiernan (2001), dyes may be classified in several ways, according to their chemical constitutions, application class or end-use. The primary classification of dyes is based on the fibers to which they can be applied and the chemical nature of the dye. They are acid dyes, basic dyes, direct / substantive dyes, mordant dyes, reactive dyes, disperse dyes, azo dyes, pigment dyes, vat dyes, ingrain dyes, solvent dyes and sulfur dyes.

The most important and the largest class of synthetic dyes used in commercial application are the direct dyes. Direct or substantive dyeing is generally carried out in a neutral or slightly alkaline dyebath, at or near boiling point, with the addition of either sodium chloride (NaCl) or sodium sulfate (Na₂SO₄). Direct dyes are used on cotton, paper, leather, wool, silk and nylon (Rajendra *et al.*, 2012).

Amount of dyes in water (less than 1 ppm for some dyes) was extremely visible and affects the aesthetic merits of water transparency and gas solubility in lakes, rivers and other water bodies. The strong color of textile effluent even at below 1 ppm creates a huge impact on the aquatic environment due to its turbidity, high pollution strength and dye concentrations ranging from 10-200 mg/l. About 10-20% of the dye is present in effluent along with organic and inorganic accessory chemicals involved in the process of dyeing (Rajendren and Gunasekaran, 2006).

At present, the common effluent treatment plant (CETP) established in major places involves some physico-chemical methods such as filtration, specific coagulation, absorption by activated carbon, chemical flocculation, precipitation, ozone ionizing radiation, ultra filtration etc. The wastewater disposal on agricultural land is the potentially phytotoxic nature of organic wastes, mainly as an outcome of combination of factors such as high salinity or excess of ammonium ions and organic compounds (Lazarova and Bahri, 2005). These methods are costly, less efficient and have limited application and generate wastes of concentrated sludge that creates secondary disposal problems which is difficult to dispose off. The removal of dyes from effluent wastewater faces many actual problems such as large volume produced, nature of synthetic dyes and control of biomass. In India, most of the large scale industries have effluent treatment plant facilities, but a number of small and

medium sized industries do not have adequate effluent treatment facilities. Such industries cannot afford enormous investments in pollution control equipments as their profit margin is very less (Sagar *et al.*, 2012). Hence the small scale industries due to the lack of financial support and lack of knowledge of the technologies to clean up the effluents, discharge the effluents into the ditches exposing the environment streams with intense color which alters the pH and increase in BOD and COD.

As per the information from the Central Pollution Control Board (CPCB) for central and state governments, the common effluent treatment plant (CETP) financial assistance scheme provides project capital cost of 25% share. As a result, CETPs having a total capacity of 570 million litres per day (MLD) have been setup throughout India covering over 10,000 polluting industries. In spite of the state pollution control board having a watch on this close operation, inspections to a large number of CETPs by CPCB revealed that in general, CETPs are not performing satisfactorily due to improper operation and maintenance. Hence, use of these water resources becomes limited and the ecosystem is affected. Numerous environmental directives, regulations and legislations have been issued in order to define quality standards for water. The high chemical oxygen demand, suspended solids, conductivity, salinity and total dissolved solids still pose an economical problem for the industries since these have been engaged as major parameters for effluent discharge (Rodrigues *et al.*, 2007).

The risk factor in the silk industry is associated with the dye process such as soaking, degumming and process of dyeing where the chemicals are used. These chemicals can cause damage, if not properly treated before discharging into the environment. The presence of these dyes in the aqueous ecosystem leads to severe environmental and health concerns (Thanushree *et al.*, 2013).

The dire need for the small scale industries is a low-cost, efficient method for biotransformation of toxic compounds which should also be environmentally friendlier. The ubiquitous nature of bacteria makes invaluable tools in the effluent biotreatment. Bacteria are very important to the breakdown of the toxic component of the effluent. Microbial decolorization and degradation is an environmental friendly and cost competitive alternative to chemical decomposition process (Watson and Cichra, 2006). Recent research work has

revealed the existence of wide variety of microorganisms capable of decolorizing broad range of dyes. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. This is relatively an inexpensive process, running costs are low and the end products are completely mineralized with no toxicity. Although dye decolorization of industrial effluents has been achieved by degradation using bacteria, there is an increasing demand for effective and economical technologies for color removal (Mohana *et al.*, 2008).

The problem of generation of secondary concentrated sludge disposal is not possible due to the decoloration and degradation can also detoxify the effluent competently without leaving any residue. In this context, the combined or the uncombined anaerobic /aerobic biological treatment of textile dye effluents using single adaptable microbe or a consortia initiates the removal of color which is the first sign of decontamination recognized are in common in literatures (Lodato *et al.*, 2007). This decontamination of the concentration of these pollutants by the microorganism is named as Biodegradation. The efficiency of biodegradation depends on the capacity of the microorganism for producing enzymes, temperature, pH and nutrient status as important moderators. The biodegradation process is a well-established and powerful technique for treating domestic and industrial effluents. Several studies indicate that the biodegradation involving microorganisms is a suitable process for industrial wastewater treatment (Noorjahan *et al.*, 2005).

Biofertilizer is a product that contains living microorganisms, which exert direct or indirect beneficial effects on plant growth and crop yield through different mechanisms. The term biofertilizer as used here could include products containing bacteria to control plant pathogens, but are frequently referred to as biopesticides (Vessey, 2003). Bacteria from genus *Pseudomonas*, *Azospirillum* and *Rhizobium* are the microorganisms which decompose organic pollutants through cometabolism in natural water and soil environment. These microbes are having the capacity of biodegradation of complex chemical compounds and also they act as PGPR (Plant growth promoting rhizobacteria) which produce antibiotics as well as secondary metabolites such as siderophore, phytohormones, volatile compounds and hydrogen cyanide (HCN). The *Azospirillum sp.*, and *Pseudomonas sp.*, belong to the plant growth promoting rhizobacteria that are the bacteria capable of promoting plant growth by colonizing the plant's root (Prakash and Karthikeyan, 2013).

Furthermore, the plant roots seem to be an ideal environment for the degradation of organic compound as a result of numerous mechanisms. The plant root system allows the rapid movement of water and gases through soil structure. It also provides a biologically active soil region (i.e) Rhizosphere enhances the microbial activity and the contaminant bioavailability which is the promising green technology for remediation of contaminated soil (Joner *et al.*, 2007).

Hence a study has been designed with the following objectives

1. To assess the biometric, the biochemical, the antioxidant and the pigment constituents of the selected green leafy vegetables (GLVs) grown in fresh water.
2. To characterize the physico-chemical parameters in the silk dyeing effluent and its effect on the biometric, the biochemical, the antioxidant and the pigment levels of the GLVs.
3. To decolorize the silk dyeing effluent with the selected biofertilizers.
4. To assess the biometric, the biochemical, the antioxidant and the pigment constituents of the selected GLVs grown in biotreated effluent.
5. To identify the functional groups and the compounds, using spectroscopic and chromatographic techniques in the selected GLVs grown in different treatments.

The Null hypothesis (H_0) proposed is “*Pseudomonas fluorescens* do not decolorize the silk dyeing effluent”.

Hence the present study compares the biometric, the biochemical, the antioxidant and the pigment constituents of the selected GLVs grown in normal, effluent exposed and effluent biotreated conditions.