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Phytodegradation of Textile Dyes by Water Hyacinth *(Eichhornia Crassipes)* and Composting the Waste by Earthworm

A. Ranjitha
P.G. Student, Department of Biochemistry,
Dharmapuram Gnanambigai Govt. Arts College (w), Mayiladuthurai, Tamil Nadu, India
P. K. M. Anu Geetham
Guest Lecturer, Department of Biochemistry,
Dharmapuram Gnanambigai Govt. Arts College (w), Mayiladuthurai, Tamil Nadu, India
A. Malarvizhi
Assistant Professor & HOD, Department of Biochemistry,
Dharmapuram Gnanambigai Govt. Arts College (w), Mayiladuthurai, Tamil Nadu, India

Abstract:
The potential of water hyacinth to degrade the dye waste water and the tolerant ability was investigated. Further, the used water hyacinath was recycled to vermicomposting with cow dung and garden wastes. The methylene blue and Methyl red was used as dye waste water. The water hyacinath was submerged in the dye waste waters at various concentrations for 144 hours. The methylene blue showed better degradation (85.20%) in 20ppm and lower degradation(53.50%) in 50ppm.The methyl red showed higher degradation (95.5%) in 10ppm and lower degradation in (30%) 50ppm. The physico-chemical characteristic of the vermicompost such as N, P, K, P^H and colour was also investigated. It has been concluded that the water hyacinth was an efficient macrophyte for the treatment of the dye waste water. The study also concludes that water hyacinth was a good vermicompost.

Keywords: *Eichhornia crassipes, Methylene blue, Methyl red, Vermicopost*

1. Introduction

The water hyacinth, *Eichhornia crassipes,* is an invasive plant that is native of the Amazon basin and whose capacity for growth and propagation causes major conservation problems with considerable socioeconomic repercussions. It is a species of great ornamental value used in gardening because of the beauty of its foliage and flowers, but is one of the IUCN's lists of the 100 most dangerous invasive species and the TOP20 of Spain's GEIB (Biological Invasion Specialist Group). Most of the problems associated with *Eichhornia crassipes* are due to its rapid growth rate, its ability to successfully compete with other aquatic plants, and its ease of propagation. These characteristics give rise to enormous amounts of biomass that cover the water surface of a great variety of habitats, often interfering with the use and management of water resources (Trinidad *et al.*, 2008).

The water hyacinth *Eichhornia crassipes* is a floating macrophyte that originated in tropical South America and is now widespread in all tropical climates. It is listed as one of the most productive plants on earth and is considered one of the world's worst aquatic plants. Biological invaders such as water hyacinth have become widespread on a global level. The extreme for worldwide invasions of hyacinth, as hyacinth is often termed a 'perfect invader' (Jason, 2000).

Water hyacinth (*Eichhornia crassipes*) is the most predominant, persistent and troublesome aquatic weed in India. It was first introduced as an ornamental plant in India in 1896 from Brazil (Rao, 1988). In India, water hyacinth has stretched over 2,00,000 ha of water surface in the country (Murugesan *et al.*, 2005) and its exuberance has been highly noticed throughout the course of the river Thamirabarani, a prerennial river in south India (Murugesan *et al.*, 2002; Murugesan, 2001). Because of its beautiful blooms and foliage, water hyacinth has been carried by tourists, plant collectors and botanists to over 80 countries around the world in the last 100 years (Jafari, 2010).

Dyes exhibit considerable structural diversity and thus become difficult to treat them by a single process. It is a fact that due to their visibility, dyes are recognized easily even at the levels as less as 1ppm. Toxicity of dyes to fauna and flora is well documented (Karaca *et al.*, 2008). Colour of textile effluents escalates environmental problem mainly because of its non-biodegradable characteristics. Today industries are the backbone of economy in many developed as well as developing countries. In India, it contributes to about 25% of total export earning and providing employment to almost ¼ of the total labor force (Rouf *et al.*, 2010).

Vermiculture or vermicomposting is the non-thermophilic process by which organic materials are converted by earthworms and microorganisms into rich soil amendments with greatly increased microbial activity and nutrient availability. The term has its origin from *vermis*, the Latin word for worm. The term is also used to refer to the technology of converting raw word for worm. The term is also used to refer to the technology of converting raw organic materials into organic fertilizer, called vermicompost, mainly through microbial action and the use of certain species of earthworm. In addition, the technology is applied in waste management by which organic "wastes" are recycled and made available for plant growth (Ben and Bareja, 2011).

2. Materials and Methods

2.1. Plant Used for the Study

Free floating macrophytes, Water hyacinth (*Eicchornia crassipes)* were used for the study. The plants were collected from the local pond in kaliyakudi, Thiruvarur district.

Figure 1: Free floating water hyacinth(Eichhornia crassipes)

2.2. Experimental Period: 60 Days.

2.3. Dyes Used for the Study

The reactive dyes used as adsorbent for the study were Methyl red and Methylene blue. The structures of dyes are elucidated below:

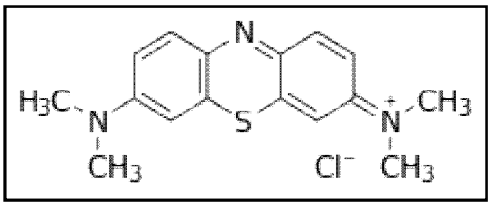

Figure 2: Structure of Methylene blue

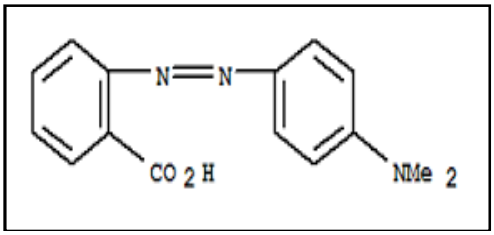

Figure 3: Structure of Methyl red

2.4. Dye Solutions

Dye stock dye solutions of all the dyes were prepared by dissolving 100 mg of dye in 100 ml sterile distilled water to get 1000 ppm dye solution. A suitable aliquot of the sample solution containing dye was transferred into a 100 ml volumetric flask and the solution was made up to the mark with double distilled water. The absorbance was measured at the respective λ max against a blank. A standard graph was plotted for 10 – 100 mg/L of dye.

2.5. Outdoor Cascade Experiments (Set 1 and 2)

The first, second, third and fourth sets of laboratory experiments were performed with five *identical* containers. These containers were operated at 10 L levels of aqueous dye solutions of concentrations ranging from 10, 20, 30, 40 and 50 ppm (each) were prepared for all the sets. The color reductions in the cascade were checked in terms of optical density in all the concentrations at different time intervals namely 24, 48, 72, 96, 120 and 144 hours.

2.6. Set no 1 (Methylene Blue)

The similar set up was also used for Methylene blue aqueous dye solutions. In five containers of Methylene blue aqueous dye solutions, which were set as a cascade, floating *Eichhornia crassipes* plants (12 pieces in each) were introduced and one container each (without plants) for all the five concentrations of aqueous dye solutions was maintained as the controls (Table 1). The color reductions in the cascades were checked in terms of optical density in all the concentrations at different time intervals namely 24, 48, 72, 96, 120 and 144 hours. The λ max for the dye was 520 nm.

2.7. Set no 2 (Methyl Red)

In five containers of Methyl red aqueous dye solutions, which were set as a cascade, floating *Eichhornia crassipes* plants (12 pieces in each) were introduced and one container each (without plants) for all the five concentrations of aqueous dye solutions was maintained as the controls (Table 1).The color reduction in the cascades were checked in terms of optical density in all the concentrations at different time intervals namely 24,48,72,96,120 and 144 hours. The λ max for the dye was 680 nm.

Aqueous reactive Dyes	Concentration of dye(ppm)	Biomass of Eicchornia crassipes	Time
Methylene blue	10-50	500	24 48 72 96 120 144
Methyl red	10-50	500	

Table 1: Outdoors pulsed cascade setup

2.8. Preparation of Compost

The waste plant materials (*Eichhornia* sp.,) obtained after the treatment of the aqueous dye solutions were subjected for the process of composting along with garden waste and cow dung (Table 2). The pre composted compost was further subjected to vermicomposting using the earthworm species.

S. No.	Component	Weight (Kg)	Total weight (Kg)
1.	Cow dung + Garden waste + dye treated *Eichhornia crassipes waste* plant	2 kg+ 1 kg + 3 kg	6

Table 2: Preparation of precompost

2.9. Procedure for vermicomposting: (Nagavallemma et al., 2004)

1. Pits made for vermicomposting were 1 m deep and 1.5 m wide. The length varied as required.
2. Cover the bottom of the cement ring with a layer of tiles or coconut husk or polythene sheet.
3. Spread 15–20 cm layer of organic waste material (precompost prepared in the table:2)on the polythene sheet.Sprinkled rock phosphate powder if available (it helps in improving nutritional quality of compost) on the waste material and then sprinkled cow dung slurry on the top of the soil. Allow the material to decompose for 15 to 20 days.
4. When the heat evolved during the decomposition of the materials had subsided (15–20 days after heaping), released the earthworms through the cracks developed.
5. Cover the ring with wire mesh or gunny bag to prevent birds from picking the earthworms. Sprinkled water every three days to maintain adequate moisture and body temperature of the earthworms.

2.10. Determination of pH

20g air-dry vermicompost and 40 mL distilled water were shaken on a rotary shaker for 10 to 15 minutes. This gave 1:2 soil- water suspensions. The pH of the soil samples was then determined by digital pH-meter.

2.11. Estimation of Total Nitrogen (by Kjeldahl Method)

500 mg vermicompost samples were taken in the digestion tube, 5 ml conc. H_2SO_4 and 1 - 2 g catalyst were added into the tube. The digestion tube was then placed in digestion unit or block and heated to boiling until green heating time was very long and temperatures at that time was very high. After complete digestion, the digestion tubes were allowed to cool for 5 – 10 minutes outside the block and then 20 ml of distilled water were used to dilute the contents. Finally, the volume was made up 50 ml.

2.11.1. Distillation and Titration

Distillation was done in the Kjeldahl apparatus (Micro Kjeldahl apparatus – Borosil). 10 ml digest (aliquot) were placed in steam chamber of Kjeldahl apparatus with 10 ml of 2N NaOH. A 50 ml conical flask containing 10 ml of H3BO3, a few drops of mixed indicator, which was placed under the condenser steam of the distillation apparatus. The liberated NH4 –N, liberated by distillation of the digest with 2N NaOH was absorbed in unstabilization H_3BO_3 in the form of ammonium borate. The contents were titrated against standard 0.01 N HCl by Auto-Titrator (Systronics – 351).

Calculation:

$$\text{Total Nitrogen \%} = \frac{(T-B) \times \text{Molarity of Standard HCl} \times 1.401}{\text{Mass of Soil Sample (g)}}$$

Where,

T = Volume of Standard HCl for titration of the vermicompost sample.

B = Volume of Standard HCl for titration of the blank solution.

The blank solution mentioned here was prepared as described without the vermicompost sample.

2.12. Determination of Potassium by Flame Photometry

2.12.1. Preparation of Stock Solutions

Potassium stock solution was prepared by dissolving 1.909g KCl in 1 liter of distilled water. It contains 1mg potassium per ml (i.e. 1000 ppm). Stock solution was diluted to give four solutions containing 20, 10, 5 and 2 ppm of potassium ions.

2.12.2. Procedure

The flame intensity of the potassium corresponding to the concentration of stock solution was noted using appropriate filters. The results were plotted in a graph. The flame intensity of the sample was noted. The concentration of potassium ions was calculated from the graph.

2.13. Determination of Phosphorus (Dickman and Bray's, 1940)

2.13.1. Extraction

Weighted 5 g vermicompost and transferred it to a 100 ml conical flask. Added 50 ml extract solution to the soil. Shake the contents for 5 minutes, and filtered through Whatman No: 42 filter paper. Prepared a blank in which all the reagents were addeded similarly, except the vermicompost.

2.13.2. Procedure

Pipetted out 5 ml of vermicompost extract into one 25 ml standard flask which was labeled. Pippetted out 5 ml of "Dickman and Bray's Reagent" to the

vermicompost extract. Then, added 5 ml Boric acid to the extract flask. Take the "Standard Phosphorus solution" in a clean burette. From this burette added 1, 2, 3 etc up to 5 ml "Standard Phosphorus solution" in previously labeled 25 ml standard flasks. Pipetted out 5 ml "Dickman and Bray's Reagent" and transferred into 25 ml standard flask containing "Standard Phosphorus solution". To that added 7.5 ml Boric acid. Take a test tube full of distilled water and added through the neck of the flask down to remove the adhering Ammonium Molybdate. Mix thoroughly the content and kept aside. Finally, added 1mL $SnCl_2$ working solution and made upto the mark with distilled water once again, mix the solution thoroughly. Measure the intensity of blue color just after 10 minutes at 690 nm. Plot a graph between absorbance against the concentration in % and determine the concentration of Phosphorus in vermicompost samples from the standard curve.

2.14. Demonstration of Ammonification

2.14.1. Procedure

1. Prepared three flasks of sterile 4% peptone broth (50ml in each) and labeled A and B.
2. Inoculated the flasks with 1g of fine sieved samples A and B.

. A. Control medium with vermicompost sample

B. Control medium without any vermicompost sample.

3. Incubated the flasks at 25^0c for 7 days.

2.14.2. Observation and Results

1. Checked for the release of ammonia on 7^{th}, day of incubation.
2. Added 1ml of culture from each flask to a tube containing 3ml Nessler's reagent.
3. Note the color change and appreciate ammonification.

2.15. Demonstration of Nitrification

1. Prepared three flasks of sterile nitrite broth (50 ml in each) and lableled them A and B.
2. Inoculate the flasks with 1g of fine sieved samples A and B
   a. control medium with vermicompost sample
   b. Control medium without any vermicompost sample.
3. Incubate the flasks at 25˚C for 7 days.

2.15.1. Observation and Results

Mix one drop of diphenylamine and two drops of concentrated Sulphuric acid in a cavity slide. Added one drop of vermicompost culture from nitrite broth. Mix with an applicator stick and observed for color change.

3. Results and Discussion

The maximum color reduction was observed at 144 hours after the introduction of the floating and submerged plants into the 10 ppm Methyl red and 20 ppm Methylene blue aqueous dye solutions. It accounts for 95.5% removal in Methyl red dye and 85.20 % in Methylene blue dye in 10 and 20 ppm aqueous dye solution at 144 hours respectively. In the 50 ppm aqueous dye solutions the color removal was observed after 6 days at the rate of 39% removal in Methyl red dye and 53.50% respectively (Tables 5 and 6) (Figures 3 and 4).

Concentration (µg)	Percentage					
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
10	25	50	52.8	60.72	65	73.6
20	21.0	32.5	41.52	51.66	63.33	85.20
30	13.33	23.33	37.41	43.5	56.7	72.61
40	12.5	20.75	25.3	41.6	52.5	62.7
50	10	15	17.4	27.5	33.6	53.50

Table 3: Effect of time on percent dye (Methylene blue) removal using Eicchornia crassipes

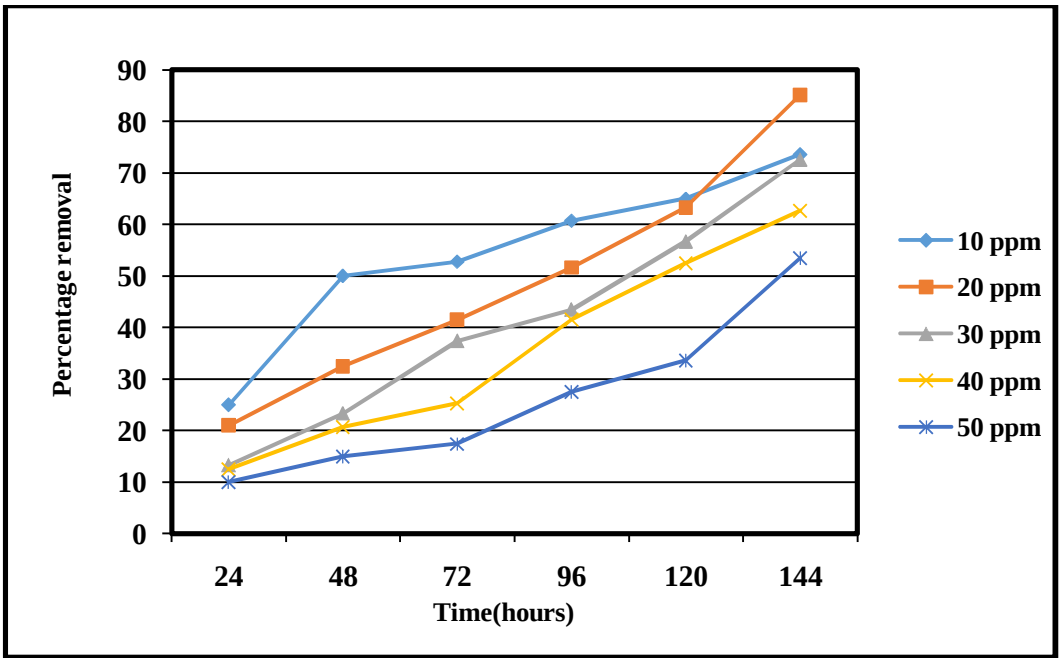

Figure 4: Effect of time on percent dye (Methylene blue) removal using Eichhornia crassipes

Concentration (µg)	Percentage (%)					
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
10	35	55	60	70	85	95.5
20	27.5	42.5	32.5	37.5	45.7	56.9
30	23.33	26.66	28.33	30	41.44	56.7
40	18.75	21.25	16.25	18.75	27.65	39.89
50	17	16	18	19	26	39

Table 4: Effect of time on percent dye (Methyl red) removal using Eicchrnia crassipes

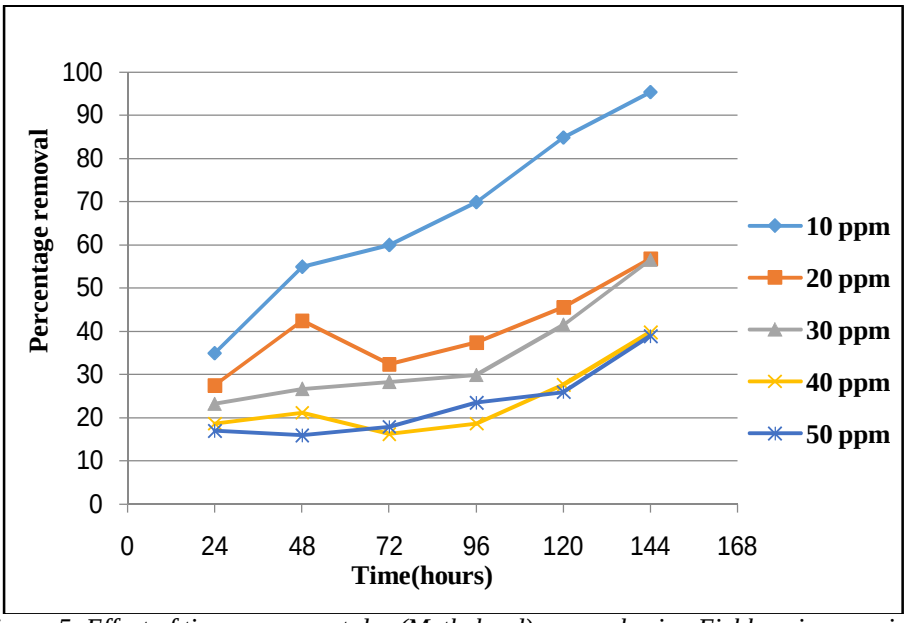

Figure 5: Effect of time on percent dye (Methyl red) removal using Eichhornia crassipes

The removal of the aqueous dyes might be due to Biosorption i.e., the sorption of dye molecules onto the root, shoot and the leaves of the plant. Similar result has been put forth by Vengata Mohan *et al*., (2002). Interestingly, the insight into the speciation and localization of dyes in plant tissues also provides a due rate and extent of uptake by particular plant parts. It was often observed that roots accumulate much higher concentration of pollutants (Anushree Malik, 2007). It has been found that *Eichhornia crassipes* showed maximum performance in 10µg and 20µg dye waste water. This might be due to dilution by tap water. The composition of diluted dye water for the plant uptake become easy than in highly concentrated dye waters.

The results showed that *Eicchornia crassipes* performed well in 10 ppm and 20 ppm dye water. This indicator, dilution enhanced the performance of test plants in dye water. In 50µg dye water, plant was unable to acclimate well. Because dye water contains toxic compounds, (CO_2H, (N=N), =N) that may have affected the test plants to grown in it (figure 7).This similar results has been put forth by Rouf Ahmad shah *et al*.,(2010). The best performance showed by water hyacinth in degradation of dye solutions was due to the plant roots. This permformance was already discussed by Shilpi sharma (2012) on metals had to be adsorbed by plant roots that can be easily removed from a hydroponic system.

The results showed the absorbing capabilities of water hyacinth. This findings was agreed with Suthersan, (1991) who stated that plants that are naturally immobilized, such as attached algae and rooted plants, and those that could be easily separated from suspension, such as filamentous microalgae, macroalgae, and floating plants, have been found to have high adsorption capacities. In a recent study, one blue-green filamentous algae of the genus *Phormidium* and one aquatic rooted plant, water milfoil (*Myriophyllum spicatum*) exhibited high specific adsorption for Cd, Zn, Ph, Ni, and Cu[12].

The result showed that water hyacinth had a capacity to degrade any toxic with varying pH. Wolverton (1989), Brix (1993) and Johnston (1993) explained that reason for turbidity reduction i.e. the root hairs had an electrical charges that attract opposite charges of colloidal particles such as suspended solids and cause them to adhered on the roots where they were slowly digested and assimilated by the plant and microorganisms.

3.1. Performance of free floating macrophytes-water hyacinth (Eichhornia crassipes)

The healthy water hyacinth (*Eichhornia crassipes*) looked bright green in colour (figure 6). Further, water hyacinth plants had bulbous petiole and glossy lamina. On the other hand, water hyacinth plants grown in dyes looked pale green, i.e., the leaves of the species were greenish-yellow (chlorosis) with brown necrotic lesions in the dyes. The plants succumbed to ill effects of dyes, suggesting the photoliabile nature of toxic constituents. Thus, the tolerance of free-floating macrophytes of dyes was noticed (KP Sharma *et al.*, 2005).

Figure 6: Performance of free-floating macrophyte water hyacinth (Eichhornia crassipes) before treatment

Figure 7: Performance of free-floating macrophyte water hyacinth (Eichhornia crassipes) after treatment

Sukha and Srivastava (2008) stated the reason for chlorosis.Here, the lack of chlorophyll due to stresses on the plant, such as lack of nutrients, might results in chlorosis (the yellowing of normally green plant leaves). The dye water contains many toxic compounds in high concentration that may have affected test plants grown in it. The similar effect was observed in the treatment of textile wastewater with water hyacinth effects on the growth of the plant, the small size of which might be due to nutrient imbalance mainly of nitrogen in water (Jabari, 2010).

S. No	Name of the parameters	Values
1	Available Nitrogen	5.25%
2	Available Phosphorus	2.65%
3	Available Potassium	4.36%
4	p^H	7.21
5	Colour	Brown

Table 5: Analysis of vermicompost

The table showed the initial and final values of N, P, K, pH and color of the vermicompost. There was a significant increased of N, P, K in vermicompost. The p^H was 7.2, which was well suited for plant growth. Li *et al*., (2001) have recorded the similar results in the Vermicomposting process.

4. Conclusion

The light of water hyacinth to dye and dye absorption along with good root development.

1. The above mentioned experiment has proved the efficiency of *Eichhornia crassipes* to remove the color and degrade the dye by about 95.5% with Methylene blue and 85.20% with Methyl red.
2. It has been further established by subjecting the *Eicchornia* plants used for treatment for vermicomposting with cow dung and leaves.
3. Vermicompost showed a greater availability of plant nutrient, such as N, P, and K. The p^H was well suited for the plant growth.
4. Also reduces the proportion of water soluble chemicals, which cause possible environmental

5. Acknowledgement
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6. References

i. Anushree Malik (2007), Environmental challenge vis a vis opportunity: The case of water hyacinth. Journal of Environment International, 33, pp122-138.
ii. Ben G, and Bareja (2011), "Basic Information and Procedure in Vermicomposting".
iii. Dickman SR, and Bray RH (1940), Colorimetric determination of phosphate. Indus and Engin: Chem., Analyt. Ed. 12, 665-668.
iv. Jabari N (2004), Ecological and socio-economic utilization of water hyacinth (Eicchornia crassipes Mart solms). Journal application of science and Environmental management, 14(2) 43-49.
v. Jason David Toft (2000), Community Effects of the Non-Indigenous Aquatic Plant Water Hyacinth (Eichhornia crassipes) in the Sacramento/San Joaquin Delta, California. pp 1-86.
vi. Johnston CA (1993), Mechanism of water wetland water quality interaction. In G. A. Moshiri (Ed.), Constructed wetland for water quality improvement. Ann Arbor: Lewis pp 293-299.
vii. Karaca S, Gurses A, Acikyildiz M, and Ejder Korucu M (2008), Adsorption of cationic dye from aqueous solutions by activated carbon. Microporous and Mesoporous Materials, 115: 376- 382.
viii. Murugesan AG (2001), Environmental status of the perennial river Thamirabarani with special reference to domestic and industrial pollution. Proc. Workshop on Enhancing Awareness of Ecological Status of River Basins pp15-21.
ix. Murugesan AG (2002), Integrated biological control of water hyacinth, Eichhornia crassipes in the fresh habitats of India. In Ecology and Ethologyof Aquatic Biota, Daya Publishing House, New Delhi, India, 361-372.
x. Murugesan AG, Ruby J, Paulraj, MG, and Sukumaran, N (2005), different densities and temperature regimes on the feeding behaviour of water hyacinth weevils, Necochetina Bruchi and Neochetina Eichhorniae on Eichhornia crassipes. Asian Jr of Microbiol Biotech Env Sc 7(1): 73-76.
xi. Nagavallemma KP, Wani SP, Stephane Lacroix, Padmaja VV, Vineela C, Babu Rao M and Sahrawat KL (2004), Vermicomposting: Recycling wastes into valuable organic fertilizer. An Open Access Journal published by ICRISAT. (2) pp 1-16.
xii. Rao VS (1988), Principles of weed science. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi (India), 544 pp.
xiii. Rouf Ahmad shah, Kumawat DM, Nihal singh and Khursheed Ahmad wani (2010), Water hyacinth (Eicchornia crassipes) as a remediation tool for dye –effluent pollution. International journal of science and nature. 1(2)172-178.
xiv. Sharma KP, Kamayani Sharma, Suresh kumar, Shwetha, Ruby Grover, Pratima soni, Bhardwaj SM, Chaturvedi RK and Subhasini Sharma (2005), Response of selected aquatic macrophytes towards textile dye wastewater. Indian journal of biotechnology.4:538-545.
xv. Shilpi Sharma*(2012), Bioremediation: Features, Strategies and applications. Asian Journal of Pharmacy and Life Scince. Vol. 2(2), pp 202-213.
xvi. Sukha Ram Vishnoi and Srivastava PN (2008), Phytoremediation- Green for Environmental clean. pp 1016-1021.
xvii. Suthersan **SS,** Phytoremediation.
xviii. Trinidad Ruiz Téllez, Elsa Martín de Rodrigo López, Gloria Lorenzo Granado, Eva Albano Pérez,Ricardo Morán López and Juan Manuel Sánchez Guzmán (2008), The Water Hyacinth, Eichhornia crassipes: an invasive plant in the Guadiana River Basin (Spain). Aquatic Invasions.3, pp 42-53.
xix. Vengata Mohan S, Chandrasekhar RH, and Karthikeyan J, (2002), Adsorptive removal of direct azo dye from aqueous phase onto coal based sorbents: A Kinetic and mechanistic study. Journal of Hazardous Materials, 90, pp189-204.
xx. Wolverton BC (1989), Aquatic plant/microbial filters for treating septic tank effluent in wastewater treatment. In D. A. Hammer (Ed.), Municipal industrial and agricultural waste. Chelsea MI: Lewis.