5

RESULTS AND DISCUSSION

Industrialization is the record of modernization which prompts modification in the physical, chemical and biological properties of the environment. The historical background of human progress uncovers that water source and development runs parallel. The period of boundless freshwater supply is would be coming to an end because of contamination of water sources inferable from the expanding release of huge volume of waste water and toxic nature of wastes (Mohapatra and Singh, 1998).

The textile industry is playing a key role in the economy of many countries. Textile industries are the major sources of pollution due to the nature of their operations which require huge volumes of water that eventually result in higher waste water generation (Nemerow, 1978). The effluents from the dyestuff manufacturing and textile industries, in particular, are highly colored with a large amount of suspended organic solids and considered as important sources of water pollution (Hebeish *et al.*, 2011). Discharging of unfixed dyes into the receiving water may cause a negative impact on the ecosystem, such as reduction of photosynthetic activity, causing of aesthetic problems, and toxicity to life (Daneshvar *et al.* 2012). Therefore, it is necessary to remove these recalcitrant compounds from wastewater, prior to discharge into environment.

Adsorption is an attractive, cheap, and effective method for the treatment of dye-bearing effluents. Several researchers try to optimize adsorption process and develop novel alternative adsorbents with high adsorptive capacity and low cost (Prola *et al.* 2013; Reddy and Lee, 2013). In the current study, textile dye adsorption on *Sargassum wightii* was studied. The results of the present investigation entitled "Studies on removal of brilliant green dye using marine brown macroalgal biomass *Sargassum wightii*" are discussed below.

5.1. Phase - I

5.1. Identification of alga

The collected alga was identified as *Sargassum wightii* (Greville, 1848) (Fig. 9). The alga was dark-brown, 20-30 cm in height with a well marked holdfast, upper portion richly branched, axes cylindrical, glabrous, leaves 5-8 cm long and 2-9 mm broad, leaves tapering at the base and apex, midrib inconspicuous vesicles large, spherical or ellipsoidal being 5-8mm long and 3-4 mm broad, stripe of the vesicle 5-7 mm long seldom ending into a long tip, receptacles in clusters and repeatedly branched.

Fig. 9 Dried *Sargassum wightii*

5.2. Phase - II

5.2. Biosorption optimization study (decolourization)

5.2.1. Effect of adsorbent dosage

The effect of biosorbent dose (0.05-0.6g) on BG dye removal by *S. wightii* is shown in Fig. 10. The maximum dye uptake capacity was observed at 0.1g (25.1579mg/g) adsorbent dose and minimum was observed at 0.05g (19.1349mg/g). When the adsorbent dose was further increased from 0.2 to 0.6 there was decrease in the sorption capacity of the adsorbent. At the adsorption dose of 0.6g, the dye uptake capacity was only 3.77193mg/g.

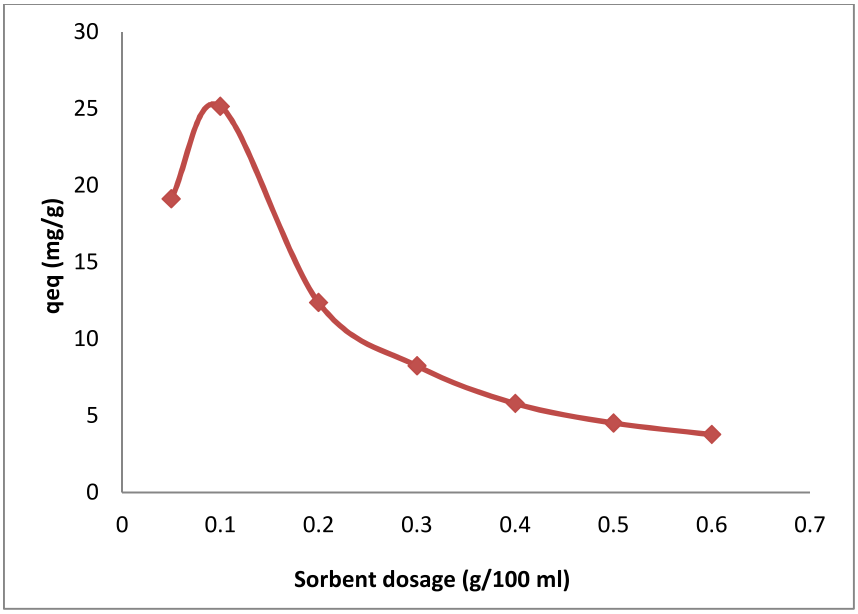


Fig. 10 Effect of biomass dosage on BG dye removal by *S. wightii*

The results also revealed that dye uptake capacity tremendously increased at lower adsorbent dosage and in contrast decreased at higher doses. This is because the active sites in the biosorbents could be efficiently utilized when the dosage is low. When the biosorbent dosages are higher, it is more likely that a significant portion of the available active sites remain uncovered, leading to lower specific uptake. Thus, adsorption efficiency decreased with an increase in the biosorbent dose. The increase in adsorption might be due to increasing number of adsorption sites caused by breaking of some of the internal bonds near the edge of the active surface sites of the adsorbents (Kanawade and Gaikwad, 2011; Kousha *et al.*, 2012).

Present results also concur with Tan *et al.* (2009) who studied the biosorption of Basic yellow 11 using *Sargassam binderi* and stated that the uptake capacity limit was found at 0.1g adsorbent dose. The adsorption of brilliant green dye using guava leaves and potato peels showed an increasing trend with increasing adsorbent dosage initially which then decreases and observed adsorbent dosage of 0.6 g of guava leaves and 0.8g of potato peels (Rehman *et al.*, 2015). Zazouli and Moradi (2015) studied the biosorption of Acid Red18 dye using *Sargassum glaucescens* biomass varied from 10 to 20 mg. They reported that the maximum adsorption rate was achieved with 20 mg/L of adsorbent dose.

5.2.2. Effect of pH

The effect of pH on BG dye removal by *S. wightii* is shown in Fig. 11. Biological treatment process using marine macroalgae are highly pH dependent. The effect of pH was studied by varying the pH from 2 to 10. At pH 2 the minimum adsorption (uptake capacity) of 4.63mg/g was observed. With further increase in pH, a gradual increase was observed and maximum of 14.73mg/g adsorption rate at pH 7.00. Above pH 7 the adsorption was gradually decreased.

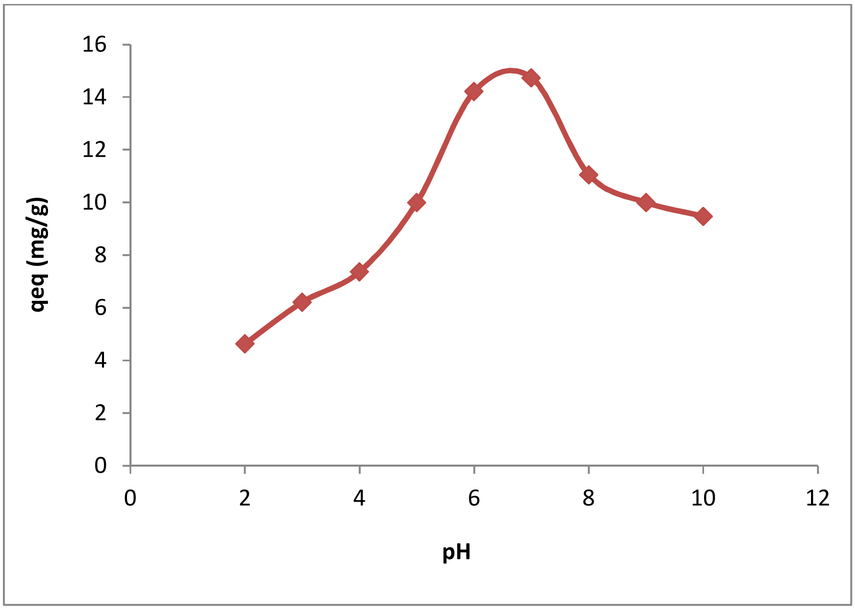


Fig. 11 Effect of pH on BG dye removal by *S. wightii*

The above results indicated that the dye adsorption considerably increased when pH was raised from 3.00 to 6.00. At pH 2.00 uptake capacity was very low which increased as pH was raised to 7.00. pH 7 (neutral) was optimum and favours dye adsorption. Similar observations were reported by Tahir *et al.* (2008) and they stated that optimum pH was 7 for methylene blue dye removal using *U. lactula* and *Sargassum* sp.

The optimum pH for malachite green dye removal by *Ulva intestinalis* and *Ulva rigida* was 7 and showed the highest removal ability (Pansamrit and Ruangsomboon, 2010). The pH of the solution might influence biosorption performance through different mechanisms and also biosorbent surface properties (Daneshvar *et al.*, 2007). The pH of the solution affects surface charge of the adsorbent, the degree of ionization of adsorbate molecule and extent of dissociation of functional groups on the active sites of the adsorbent (Nandi *et al.*, 2009). The carbon atom of the BG dye acts as an electrophilic center and the OH^- group favoured in adsorption, thus the uptake of the colour decreased with the increase in pH (Rao *et al.*, 2016). pH 7 was considered as an optimum pH due to high sorption of Brilliant Green dye using red clay, as the surface of the adsorbent was negatively charged and the dye cations are positively charged (Rehman *et al.*, 2013).

In addition, the competition of H^+ ion with the cationic dye molecules also decreased the adsorption. On the contrary, the functional groups such as hydroxyl and carbonyl groups could act as biosorbing agents or sites with negative charges. The surface of the adsorbent was negatively charged at higher pH, which favoured for adsorption of the positively charged dye cations through electrostatic force of attraction. The adsorption of malachite green to wood fiber consequently increased with increasing of pH values (Pan and Zhang, 2009).

5.2.3. Effect of initial dye concentration and contact time

The effect of initial dye concentration on BG dye removal by *S. wightii* was investigated by changing concentrations from 10 to 110 mg/L. Fig. 12 shows the equilibrium sorption capacity of BG dye at various concentrations. The amount of BG dye achieved 94% colour removal in a very short time (10mg/L; 43.7mg/L in 180 min). For the first 30 minutes, the dye uptake capacity was rapid and thereafter it proceeded at a slower rate and finally attained saturation at different contact time for different initial concentrations of dye. The higher concentration solution of dyes employed, the longer equilibrium time was needed. Thus the decolourization of dye was very rapid initially, followed by gradual decolourization until equilibrium. The plot also reveals that the uptake of dye adsorbed increases with increase in initial dye concentration. The curve on contact time was found to be smooth and continuous, leading to saturation indicating monolayer coverage of dye on adsorbent surface. It reveals that the rate of uptake capacity increased depending on the contact time.

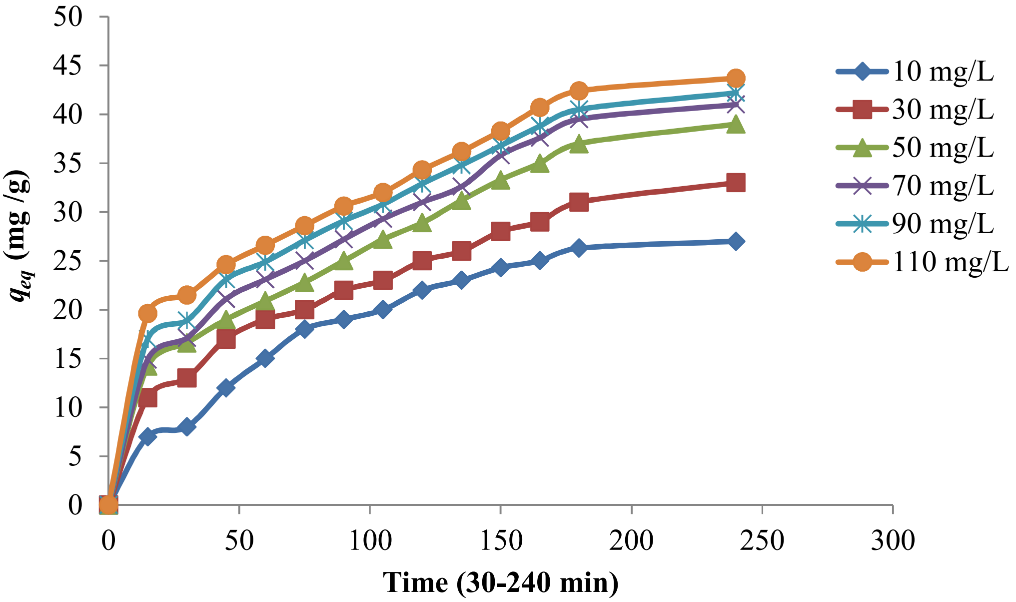


Fig. 12 Effect of initial concentration and contact time on BG dye removal by *S. wightii*

Similar results have been reported in literature, the effect of initial dye concentration on the adsorption efficiency of biomass and their effects increased with increasing the initial dye concentration. Adsorption of brilliant green dye using neem leaf powder showed that the adsorption rate was rapid during the first 5-15min followed a gradual increase during 15-240min with further increasing time there was no improvement in adsorption (Bhattacharyya and Sarma, 2003). The equilibrium time reported for the adsorption of Acid green 3 dye using dried *Azolla* was rapid for first 60-90min and reached after 1.5h (Balarak *et al.*, 2016). Saturation of biomass occurred with 10 mg/L concentration of Acid Black 1 dye using *N. zanardini* and *S. glaucescens* and 50 mg/L dye concentration for *S. marginatum* (Kousha *et al.*, 2012). The maximum percentage and q_{eq} were observed at 100ppm for Methylene blue dye using *Ulva lactuca, Caulerpa taxifolia, Chaetomorpha media and Enteromorpha intestinalis* (Deokar and Sabale, 2014).

The results were in accordance with Zazouli and Moradi (2015) who reported that the adsorption increases with the increase of the contact time and reaches the equilibrium after 240min. The removal was obtained in 60 min. The effect of the initial dye concentration on the adsorption efficiency was showed that increasing the initial dye concentration; the adsorption efficiency has a perceptible increase. The most adsorption was obtained in 15mg/L of the dye concentration.

5.2.4. Effect of temperature

The effect of temperature was studied in the range from 20°C to 60°C (Fig. 13). The dye uptake capacity was increased from 9.47 to 9.77mg/L for the rise in temperature from 20°C to 35°C. Further increase in temperature from 40°C to 60°C could not increase dye removal, rather a decrease was observed from 9.55 to 8.78mg/L. Therefore the maximum adsorption (uptake capacity) of BG dye from aqueous solution was achieved at 35°C.

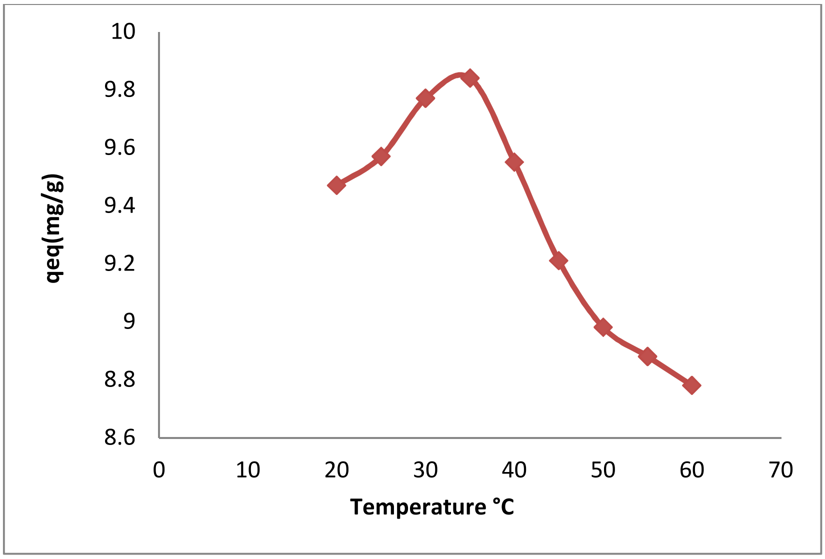


Fig. 13 Effect of temperature on BG dye removal by *S. wightii*

This was achieved because as temperature increased from 20°C to 35°C, a slight increase in surface area of the adsorbent could be possible but further increase in temperature could result in the loss of active surface area resulting from prolonged exposure to high temperatures. Hence, the adsorption was slow at higher temperature. The optimum temperature was 35°C at which the adsorption was very effective. The decrease of sorption capacity with increasing temperature suggested that the adsorption process was exothermic and the mechanism was mainly physical adsorption, dominant at lower temperature (Deniz and Saygideger, 2010; Abbas *et al.*, 2012). Hence the present study considered as exothermic nature of adsorption. An elevation in the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores with temperature owing to the decrease in the viscosity of the solution (Durairaj and Durairaj, 2012).

An increased number of molecules might acquire sufficient energy to undergo an interaction with active sites on the surface. At high temperature, the decrease in biosorption of dyes might be due to the weakening of adsorptive forces responsible for the adsorption of dye molecules. This could be due to the deactivation of biosorbent active sites leads to decreased biosorption (Asgher and Bhatti, 2012; Vigneshpriya and Shanthi, 2016). The results of the present study were supported by Boudechiche *et al.* (2015) reported that the biosorption of methylene Blue on *Luffa cylindrica* fiber over the range (20–60^{0}C). A slight decrease in the dye biosorption with raising temperature and suggesting the study was an exothermic process. Adsorption is normally exothermic in nature and the rate of adsorption in most cases decreases with increase in temperature of the system. Some of the adsorption studies show increased adsorption with increasing temperature and found to be an example of endothermic adsorption.

Under above optimised conditions, the maximum adsorption capacity of BG dye from an aqueous solution by *S. wightii* was achieved and depicted in Table 5.

Table. 5 Optimum conditions for maximum BG dye removal by *S. wightii*

Parameters	Adsorption condition	Uptake capacity (q_e)
Adsorbent dose	0.1g/ 100mL	25.1579
pH	7	14.73
Initial dye concentration and contact time	10g/L and 180min	43.7
Temperature	35°C	9.77

5.2.5 Dye desorption and regeneration study

Desorption study has been carried out to recover the biosorbent for reuse and regeneration in order to reduce the process costs and to determine the desirability of the biosorbent material to desorb the sorbed dye for another cycle. The study was conducted with different desorbing agent's like 0.1M HCl, 10% CH_3COOH, 0.1M NaOH and 0.1M HNO_3. Prior to each experiment, seaweed after adsorption was washed repeatedly with tap water followed by distilled water to remove the unbound dye particles. For the desorption study, dye loaded seaweed was filtered from results obtained in adsorption study under optimized conditions of 7 of pH at 35 °C was mixed with 100 mL of the desorbing medium and it was agitated at 150 rpm at 30 ºC for 30min.

Table. 6 Percentage of dye desorption by various solvents

Time	Desorption (%)		
(min)	HCl (0.1M)	CH_3COOH (10%)	HNO_3 (0.1M)
0	0.00	0.00	0.00
10	16.77	13.63	12.53
20	28.63	21.77	23.67
40	35.77	27.16	28.34
60	43.78	33.53	35.45
80	51.42	42.06	41.07
100	59.23	49.42	47.78
120	64.42	43.34	45.03
140	71.34	41.12	43.21
160	77.27	39.11	41.45
180	82.92	37.34	38.97
200	79.15	34.12	35.73

From the results (Table 6), HCl was found as the most powerful desorbing agent. Although HNO_3 and CH_3COOH showed minimum rate in desorption compared to HCl, they too performed as powerful desorbing agents. NaOH is a strong base that has been widely used as a desorbing agent in desorption process but it might have effective as precipitated (Fig. 14).

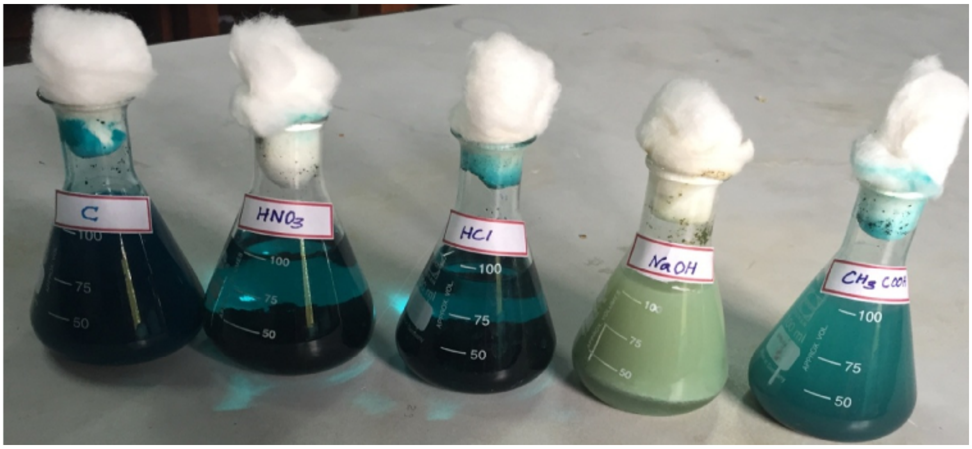


Fig.14 Desorption study under different desorbing medium

Table. 7 Percentage of reuse efficiency (adsorption cycle 1)

Time (min)	Decolourisation (%)
0	9
10	21
20	27
40	33
60	39
80	43
100	49
120	51
140	55
160	58
180	61
200	57

After desorption process, regeneration step (adsorption) (Table. 7) was applied (HCl washed biosorbent in 100 ml of dye) to prevent biomass deterioration or loss of biosorption capacity. The selection of desorbing (regenerating) agent depends on the type of biosorbent used and also the materials being adsorbed, as it determines the type of ion interaction with the biosorbent material (Mata *et al.*, 2010). If a protonated biomass like *Sargassum* alga was used, desorption with acid could be more useful, provided that the release of dye and regeneration process also could be achieved and also reducing the process cost (Lodeiro *et al.*, 2006). From the study, we observed that biosorbent like

Sargassum wightii could be regenerated by a simple acidic wash that would quickly release the loaded dye. The main purpose of regeneration is to have a better overall process economy and also to recover the materials. Thus recovery and recycle of adsorbent in adsorbate might be possible.

5.2.6 Biosorption isotherm

Two isotherm models were used to analyze the equilibrium curve at different initial dye concentration. The linear correlation coefficient of determination (R^2) was used as an error function to evaluate the fitness of each isotherm equation obtained for the two isotherm models and their respective values are listed in Table 8.

Table. 8 R_L values from Langmuir isotherm model from various initial dye concentrations in removal of BG dye by *S. wightii*

Initial Dye Concentrations	R_L (10^{-2})
(mg/L)	BG dye
10	3.86
30	1.97
50	0.80
70	0.40
90	0.20
110	0.13
130	0.08

5.2.6.1 Linearised Langmuir isotherm model

Linearised Langmuir isotherm model was expressed in eq. (4) and separation factor was shown in eq. (5)

$$1/q_{eq} = 1/Q^{\circ} + 1/Q^{\circ} b\, C_{eq} \qquad (4)$$

Where q_{eq} is the amount of dye adsorbed at equilibrium over unit mass of sorbent (mg/g), C_e is the equilibrium concentration (mg/L), Q^o the maximum sorption capacity (mg/g) and b is the Langmuir constant (L/mg). Linear plot of $1/q_{eq}$ vs $1/C_e$ was plotted in order to determine the best fit isotherm data for the adsorption system.

Crucial characteristic of Langmuir adsorption model was expressed as dimensionless separation factor, R_L which can be derived from eq. (5) (Table 8)

$$R_{L}=\frac{1}{1+bC_{L}} \tag{5}$$

b is the Langmuir adsorption constant (L/mg) and $C_L$is the highest concentration of solution. R_L value indicates the favourable condition of adsorption process.

5.2.6.2. Linearised Freundlich isotherm model

Freundlich isotherm was expressed as in eq. (6), and linearised equation was shown in eq. (7).

$$q_{eq} = k_f(C_{eq})1/n \tag{6}$$

$$\ln(q_{eq})= \ln (k_f)+ (1/n) \ln(C_{eq}) \tag{7}$$

K_f(mg/g(L/mg)1/*n*) and *n* were Freundlich constants derived from the linear slope of lnq_{eq} versus lnC_e.

Table 9 also revealed that R^2 value of Langmuir (Fig. 15) and Freundlich isotherm (Fig. 16) are 0.996 and 0.959 respectively. Based on the correlation coefficients, the applicability of the isotherms was compared and it might assume that Langmuir model was best fit to the adsorption data of BG dye than Freundlich model. In addition, the R_L of Langmuir isotherm was obtained from 3.86 (10mg/L) to 0.08 (130mg/L) for biosorption of BG dye (Table 8) indicating that biosorption process was favourable. From Table 4, Langmuir constant *b* and q_m were 2.00 and 43.48 mg/g, respectively, while Freundlich constant K_f and 1/*n* were 2.641 mg/g and 1.41 respectively. Both two sets of the isotherms constants are valid and both supported the applicability of the isotherms. Similar observation was reported for removal of malachite green dye from aqueous solution with adsorption technique using *Limonia acidisimma* (wood apple) shell (Sartape *et al.*, 2013). These experimental results and values of R^2 indicated that the sorption of BG dye onto *S. wightii* followed Langmuir model. The sorption process was described by Esan *et al.* 2014 who observed that the Langmuir isotherm with maximum monolayer adsorption capacity of brilliant green dye onto Luffa cylindrical sponge.

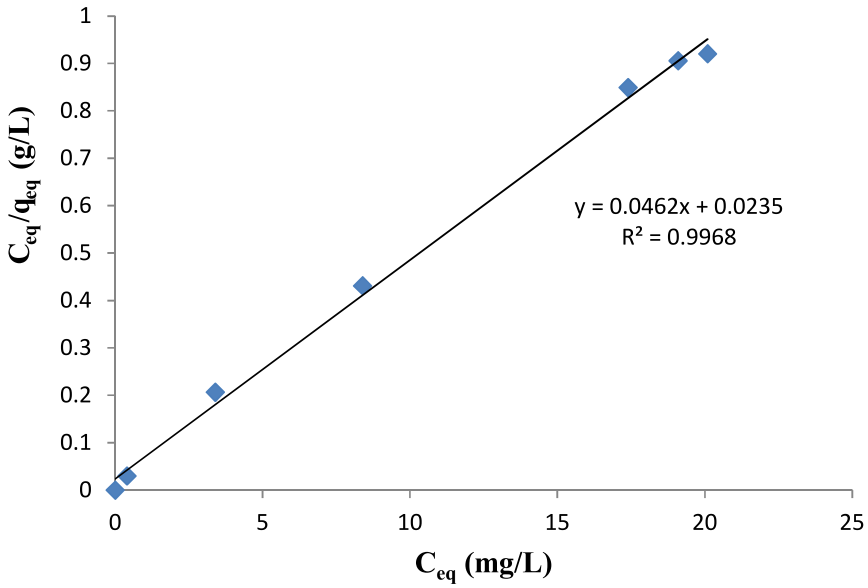


Fig. 15 Linearised Langmuir isotherm curve for BG dye removal by *S. wightii*

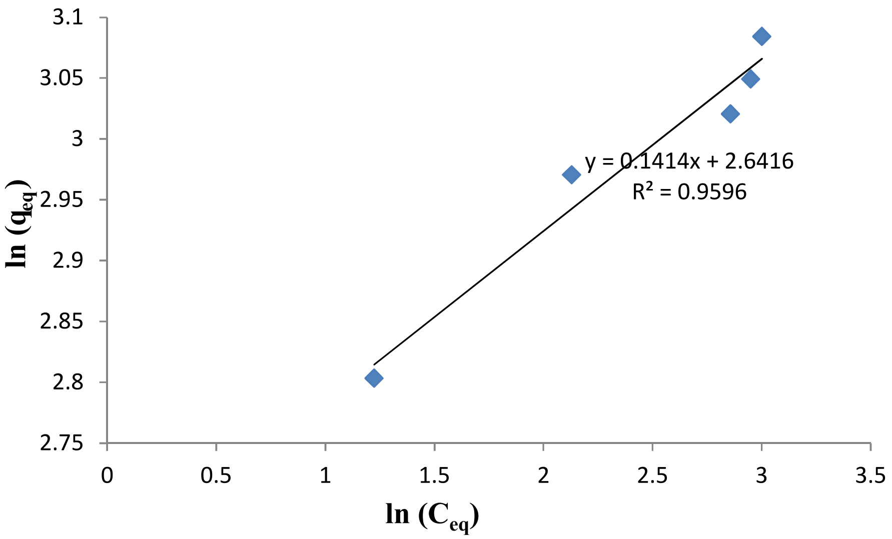


Fig. 16 Linearised Freundlich isotherm curve of BG dye removal by *S. wightii*

Table. 9 Comparison of Langmuir and Freundlich isotherm constants for removal of BG dye by *S. wightii*

Isotherms	Parameters	Values
Langmuir	q_m (mg/g)	43.48
	b (L/mg)	2.00
	R^2	0.996
Freundlich	1/n	1.41
	K_f (mg/g)	2.641
	R^2	0.959

From the result, the fitness of isotherm models describing the type of adsorption based on high correlation coefficient value (R^2) and langmuir constant, accordingly the langmuir isotherm model considered favourable towards adsorption process and described the characteristic of biosorption of BG dye onto *S. wightii*. The Langmuir model assumes monolayer coverage and constant adsorption energy while the Freundlich equation deals with heterogeneous surface adsorption. The applicability of Langmuir isotherm to the studied system implies the monolayer sorption and homogeneous surface conditions exist under the used experimental conditions.

5.2.7 Biosorption kinetics

5.2.7.1. Pseudo-first-order kinetic model

Pseudo-first-order kinetic model (Fig. 17) was theorized by Lagergran (1898) as shown in eq. (8) which was further integrated as linearized form eq. (9).

$$\frac{dq_t}{d_t} = k_{1(q_{eq-q_t})} \qquad (8)$$

$$\log(q_{eq}\text{-}q_t)=\log q_{eq}\text{-}(k_1ad/2.303)*t \qquad (9)$$

q_t indicates the sorption capacity at time t (mg/g), q_{eq} is the sorption capacity at equilibrium (mg/g), and k_1 is the constant. Linear plot of log ($q_{eq} - q_t$) versus t was used in determination of k_1 constant (min^{-1}) and R^2 values. The values of pseudo-first-order kinetic model are tabulated in Table 10.

5.2.7.2 Pseudo-second-order kinetic model

Pseudo-second-order kinetic model (Table. 10 and Fig. 18) was expressed (Mckay and Ho, 1999) in eq. (10).

$$t/q_t = (1/k_2ad\ q_{eq}^2)+(1/q_{eq})t \qquad (10)$$

k_2 (g/mg.min) indicates pseudo-second-order kinetic constant which was derived from linear plot of *t/qt* versus *t.*

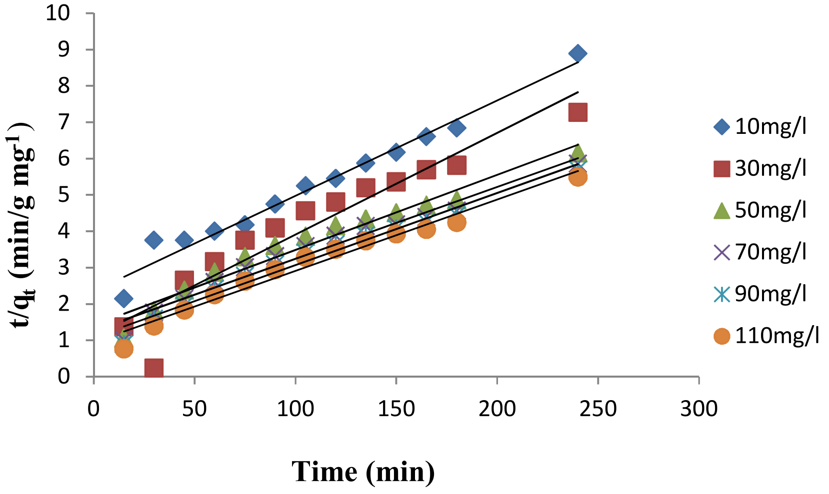


Fig. 17 Pseudo-first-order kinetic model at various initial dye concentrations for the removal of BG dye biosorption by *S. wightii*

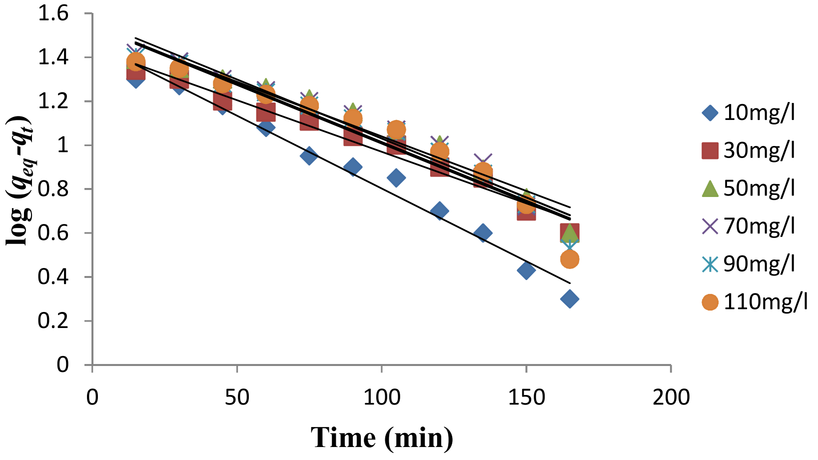


Fig. 18 Pseudo-second-order kinetic model at various initial dye concentrations for the removal of BG dye removal by *S. wightii*

Table. 10 Biosorption kinetic model constants at various initial dye concentrations for the removal of BG dye by *S. wightii*

Initial dye concentration	Pseudo-first-order				Pseudo-second-order		
(mg/l)	q_{eq}exp (mg/g)	K_1ad (1/min)	q_{eq}cal (mg/g)	R^2	K_2ad (g/mg/mi	q_{eq}cal (mg/g)	R^2
10	27	-0.0015	29.3	0.98	38.17	101.69	0.99
30	33	-0.0108	27.57	0.96	35.71	216.22	0.98
50	39	-0.0115	34.39	0.95	48.31	169.33	0.96
70	41	-0.0124	36.96	0.93	50.5	188.74	0.96
90	42.2	-0.0122	35.33	0.95	50.25	221.28	0.97
110	43.7	-0.0122	34.79	0.92	51.02	252.39	0.97

From Table 10, it could be observed that pseudo-second-order kinetic model was best fit rather than pseudo-first-order. The linearity of the plot showed the applicability of the pseudo-second-order kinetic model, which has regression coefficients (R^2) in the range from 0.96 to 0.99. These values are very close to 1 and thus it can be suggested that the present adsorption system are more favorably controlled by the pseudo-second-order kinetic model while that of pseudo-first-order varied from 0.92 to 0.98 randomly. The calculated $q_{eq}(cal)$ based on pseudo-first-order model increased from 29.3 to 34.79 (10- 90 mg/g) and with increase of initial concentration (90-110 mg/L) decreased in $q_{eq}(cal)$ was observed while that of pseudo-second-order model increased from 101.69 to 252.39 mg/g with the increase of initial dye concentration from 10 mg/L to 110 mg/L. Also, the theoretical values of calculated q_{eq} for pseudo-second order kinetic model agreed with the experimental data. In contrast, q_{eq} *(cal)* values of pseudo-first-order kinetic model do not match the experimental values. This expresses the chemisorptive behaviour of the biosorption process (Mittal *et al.*, 2008). It thus showed that the system of study was more appropriately described by the pseudo-second-order model and were in good agreement with Esan *et al.* (2014) and Naveen *et al.* (2011).

5.2.8 Biosorption thermodynamics

The Gibbs free energy change (Gº), enthalpy change (H°) and entropy change (Sº) for the biosorption of dye from aqueous solution using *Sargassum wightii* were determined.

Thermodynamics analysis was conducted by using different temperature (25- 60° C) (Table. 11). Important properties such as Gibbs free energy (*G*º), enthalpy change (*H*°) and entropy change(*S*º) were determined and the values were shown in Table 11. The Van't Hoff plots were obtained by plotting 1/T vs In Ke. The Langmuir constant, b (l/mg), was used to calculate the change in the standard Gibbs free energy (*G*º) according equations 11-13.

$$\Delta G = -RT \ln b \qquad \text{Eq. 11}$$

$$ln\,\frac{b_2}{b_1} = \frac{-\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad \text{Eq. 12}$$

$$\Delta G = \Delta H - T\Delta S \qquad \text{Eq. 13}$$

From table 11, a negative ΔG^o value at all the temperatures indicated that the process was feasible and spontaneous. The negative values of free energy suggested that the feasibility of malachite green dye and confirms the affinity of the biosorbent towards the sorbate (Vijayaragavan and Yun, 2008). The negative value of change in enthalpy (ΔH^o) showed the adsorption was exothermic. Positive values of ΔS suggested good affinity of the dye towards the adsorbent. Thus thermodynamic analysis clearly indicated the spontaneity of adsorption process and exothermic in nature.

Table 11 Thermodynamic parameters for the removal of BG dye by *S. wightii*

Temp (°C)	Kelvin (K)	1/T	qe (mg/g)	Ce (mg/g)	Kc	ln Kc	ΔG kJmol^{-1}	ΔH° (kJ mol^{-1})	ΔS°(J mol^{-1} K^{-1})
25	298	0.00469	9.9	0.17	58.23	1.7651	-14.67	-26.784	94.37
30	303	0.00449	9.9	0.11	90.01	0.2909	-2.41		
35	308	0.00379	9.9	1.66	3.61	0.5575	-4.63		
40	313	0.00359	5.5	2.98	1.84	0.2648	-2.2		
45	318	0.00330	4	4.77	0.83	0.0809	-1.67		
50	323	0.00329	4	4.11	0.77	0.0789	-1.27		
55	328	0.00319	3.3	3.99	0.67	0.0509	-0.69		
60	333	0.00309	3.1	3.11	0.49	0.0439	-0.39		

5.3. Phase - III

5.3.1 Physico-chemical analyses of untreated and *S. wightii* treated dye

The physical characteristics of untreated and *S. wightii* treated BG dye were presented in table 12. Physico-chemical characterization of the treated dye was pale green in colour with odourless and showed a pH of 7.6 at 30º C. The electrical conductivity recorded was 240 µmho/cm. Dissolved oxygen content (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS) and total solids (TS) were reduced to the level of 2.0, 21,240, 90, 2000, and 2090 mg/L, respectively. The amount of chlorides, sulphates, phosphates and nitrates were 640, 510, 1.21 and 40 mg/l respectively. Whereas untreated dye was dark green in colour with bad odour. The electrical conductivity recorded was 350µmho/cm. It showed a pH of 9.0 at temperature of 40ºC. Dissolved oxygen content (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS) and total solids (TS) were found to be 0.5, 90,471, 240, 5000, and 5240 mg/L, respectively. The amount of chlorides, sulphates, phosphates and nitrates were 1190, 918, 2.18 and 80 mg/l respectively.

The results of the physicochemical analyses revealed that the untreated BG dye is characterized by the presence of colour with bad odour, high electrical conductivity, high TSS, TDS values, alkaline pH, high BOD and COD, low DO, high amount of chlorides, sulphates and nitrates (exceeds the BIS limits). Whereas treated BG dye showed pale colour with objectionable odour, low electrical conductivity, low TSS, TDS values, neutral pH, low BOD and COD, high DO, lower amounts of chlorides, sulphates and nitrates (within the BIS limits).

Colour present in dye effluent is the indication of water being polluted and can damage the receiving water bodies. The industrial effluents are coloured, turbid and they are highly resistant to biological activities. The presence of high amount of colour in water causes depletion in DO and BOD which affects aquatic life for respiration and photosynthesis (Manikandan *et al.,* 2015).

Unpleasant odour may be due to the presence of volatile compounds. Similar results regarding the pungent odour in the textile dyeing effluent was reported by Arul *et al.* (2011). The dye sample was more alkaline pH might be due to the excess use of chemicals during bleaching process.

Electrical conductivity is an important parameter that indicates salinity of water. High Electric conductivity (EC) is due to the presence of high amount of dissolved salts (Dahaan et al., 2016). Ahmed (2012) recorded highest value of electrical conductivity (2515µs cm^{-1}) in untreated textile dyeing effluent which is supportive for the present study.

The presence of high amount of TDS in effluent affects the growth of the plant directly, soil structure, permeability and aeration (Varsha *et al.,* 2013).

Total hardness and the amount of calcium and magnesium recorded were low. Their low concentration in the wastewater may be due to the use of softwater in the dyeing and printing process to avoid coagulation of dyes (Hussain and Hussain, 2012).

Oxygen demand of the effluent was quite high and the DO content was very low. This indicates that the effluent is high in recalcitrants and hardly degradable compounds which may not undergo more than 50% substrate biodegradation. It is known that organic matter with 50 – 90% substrate biodegradation has a COD: BOD ratio between 2 and 3.5 .

Most of the industrial effluents have low DO values, below 3 mg/L. The DO level of effluents increases with distance, which may be due to long contact time and large surface area for exposure for the adsorption of atmospheric oxygen.

Siyanbola *et al.* (2011) reported that the presence of very small amounts of dyes in water is very visible and affects the aesthetic merit, water transparency and gas solubility in water bodies and can be toxic to aquatic flora and fauna and cause severe environmental problems worldwide. The removal of colour from wastewaters is often more important than the removal of the soluble colourless organic substances, which usually contribute the major fractions of the biological oxygen demand.

High alkalinity may indicate the presence of bases which are used during the textile process (Mohabansi *et al.,* 2011). The high value of alkalinity in textile dyeing effluent may lead to metabolic alkalosis by affecting the mucous membrane of grazing animals (Singh *et al.*, 1998).

Hardness in industrial wastewater was due to the presence of dissolved salts of multivalent metallic ions such as calcium, magnesium and other mineral salts. The level of total hardness in textile effluent was high which is coherence with Ohioma *et al.* (2009) who reported that the amount of total hardness in textile processing industries was 1050 mg/l.

High chloride content might be due to water softening process or sodium chloride is used to recharge softterns and may harm the agricultural crops, microorganisms which are important in some food chains of aquatic life (Nosheen *et al.,* 2000). Similarly sulphates in wastewater may be due to use of sulphuric acid in various steps of dyeing and printing process.

Table.12 Physico-chemical analyses of untreated and *S. wightii* treated BG dye compared with BIS permissible limits

S. No.	Parameters	Untreated dye	Treated dye	BIS permissible limits
1.	Colour	Dark green	Pale green	1
2.	Odour	Bad odour	Objectionable odour	2
3.	Electrical conductivity (EC) (μmho/cm)	350	240	600
4.	Temperature (ºC)	40	30	>40
5.	Total suspended solids	240	90	100
6.	Total dissolved solids	5000	2000	2100
7.	Total solids	5240	2090	
8.	pH	9.0	7.6	5.5 - 9.0
9.	Alkalinity	430	350	200-600
10	Total hardness	460	360	600
11	Dissolved oxygen	0.5	2.0	-
12	Biological oxygen demand	90	21	30
13	Chemical oxygen demand	471	240	250
14	Chloride	1190	640	1000
15	Sulphate	918	510	1000
16	Phosphates	2.18	1.21	
17	Nitrate	80	40	50

All the values except pH are in mg/l

5.3.2. Analytical studies (characterization of dye solution)

5.3.2.1. FT-IR Analysis (Fourier Transform Infrared Spectroscopy)

To better understand the nature of the functional groups responsible for the biosorption of BG dye by *S. wightii* before and after adsorption, the FT-IR spectra was obtained within the range of 4000 to 800 cm^{-1} (Fig.19a and b).

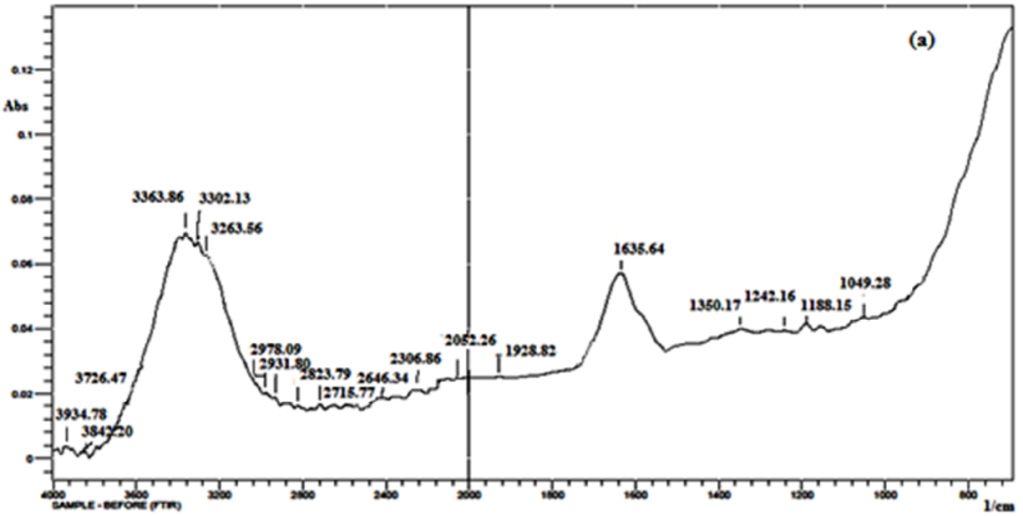


Fig. 19(a) FTIR spectra of the BG dye before adsorption

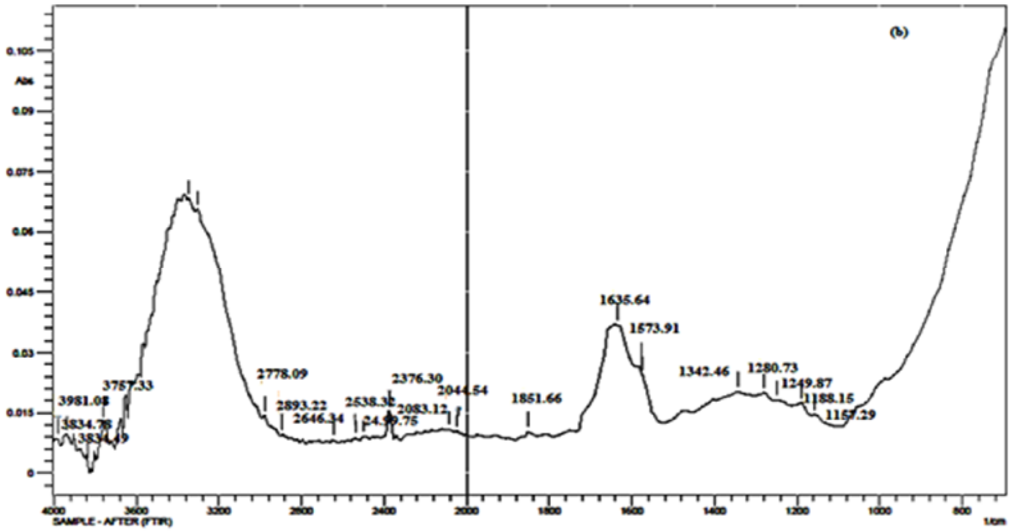


Fig. 19(b) FTIR spectra of the BG dye after adsorption

The major difference after seaweed treated BG dye, a new IR band was observed at 2736 cm^{-1}. However, the following assignments can be done, the band at 1635cm^{-1} may be assigned to C=N bond in dye molecule. The shoulder peak at 1573cm^{-1} is due to the aromatic phenyl groups in BG dye. The band at 2890 and 2978cm^{-1} are due to the symmetric and asymmetric stretching vibration of alkyl groups. The broad band centered at 3360cm^{-1} is due to the moisture content in sample. The broad and strong vibration around 3000–3500 cm^{-1} is an indicative of existence of –OH groups and –NH groups on the surface of *S. wightii*. The peaks at 2823.79cm^{-1} and 2893.22cm^{-1}were assigned to the stretching vibration and bending vibration of C–H of the aliphatic groups. The absorption band at 1242cm^{-1}and 1249 cm^{-1}were attributed to stretching vibration of S=O group. The peak at 1350.17cm^{-1} and 1342.46 cm^{-1}were correspond to –N=O stretching vibrations for the biomass. The bands observed at 1049.28 cm^{-1}and 1157.29cm^{-1} were assigned to C-O stretching vibration of alcohols and carboxylic acids.

These observations indicated that several functional groups (hydroxyl, carboxyl, amine) on the surface of the biosorbents might be responsible for the binding of BG dye. Moreover, each adsorbent has different binding capacity for each dye molecules. Adsorption capacity is not only affected by the textural or porous structure of adsorbents but also strongly influenced by the chemical functionalities at the surface.

5.3.2.2 SEM analysis (Scanning Electron Microscopy)

The surface feature and morphological characteristics of the biosorbent was studied using SEM (Fig. 20). The study was useful in determining the particle shape and porous structure of the seaweed. From the micrograph it was evident that before adsorption (20 B), pores within the seaweed particles are assorted, the surface was rough and irregular in shape which might be indicated that there is an adequate space for dye adsorption. Whereas after BG dye was adsorbed on seaweed, a significant change was observed in surface of the adsorbent (20A), which clearly indicated the porous and fibrous texture of the biosorbent with homogeneity that could contribute to the biosorption of the dye. Also it could be seen that the surface is smooth and linkage. Since they are regular in shape, there is high possibility that the dye might be trapped by adsorbent and causes the higher percentage removal of dye from the aqueous solution.

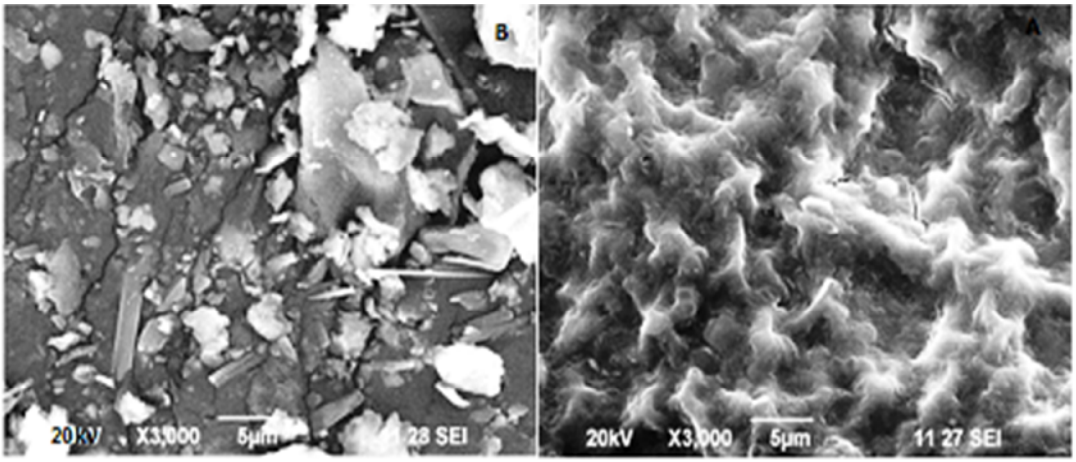


Fig. 20 SEM micrographs before (B) and after adsorption (A)

5.3.2.3 EDX (Energy dispersive X- ray spectroscopy)

In this study various elements were identified from biomass before and after the biosorption by EDX spectrum (Fig. 21). The variation in elemental quantity in brown seaweed was presented in Fig. 19. The amount of K, C, Ca, Cl and O were increased while that of Mg, Na, Si, Al and S were found decreased and Al and Si are found as new after biosorption. It could be due to the chemical interaction mainly employed in the adsorption process. Thus the study determined possible interaction of seaweed in dye adsorption.

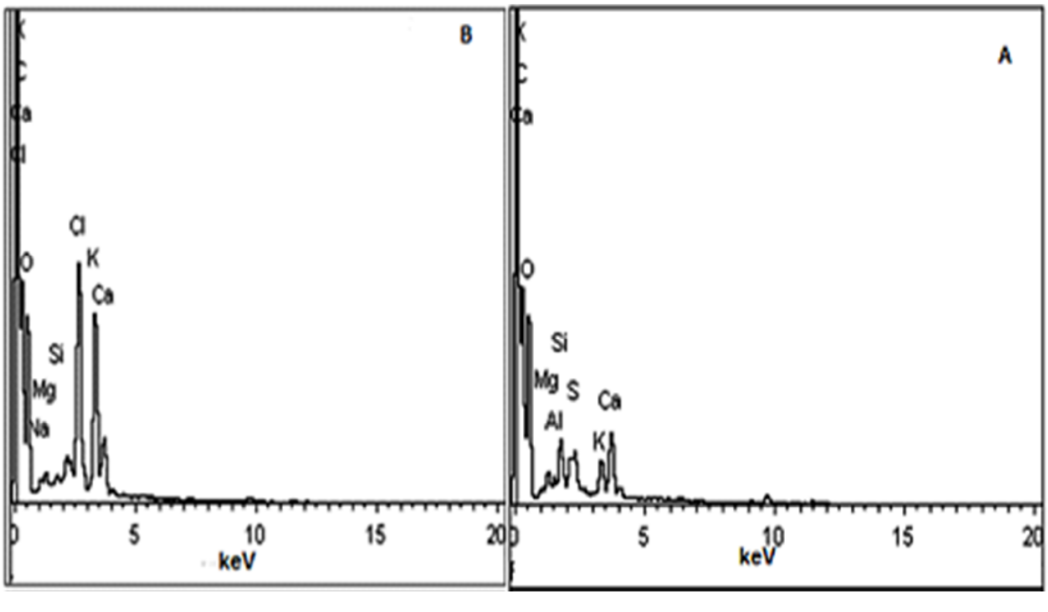


Fig. 21 EDAX spectrum before (B) and after adsorption (A)

5.3.2.4 UV-Vis spectroscopic analysis

The initial and final absorbance values of untreated and treated peaks of BG dye were used to determine the intensity of dye decolourisation (Fig. 22). The spectrum was recorded using at a range of 200-800 nm. The dye UV spectrum before treatment exhibited absorption band at 538 nm. The main absorption features of BG dye, absorption maximum (the alpha band) near 630 nm and a shoulder band (the beta band) near 590 nm are diminished in the sample treated with seaweed. The significant reduction of the absorption peak at 630 nm indicates the significant adsorption of the dye molecule. The appearance of new peak at 250nm indicated the maximum adsorption of dye from aqueous solution. From the result, it could be confirmed that the spectrum displays the shift of the maximum absorbance towards shorter wavelengths upon seaweed treatment which indicates considerable amount of dye was adsorbed on sorbent.

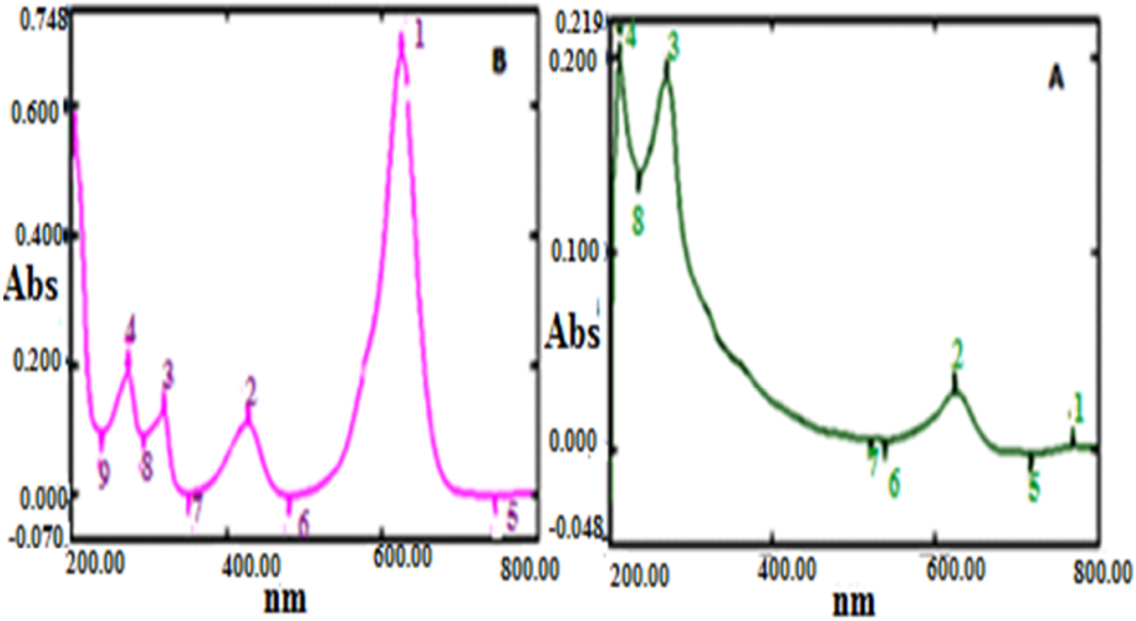


Fig. 22 UV-VIS peak values before (B) and after adsorption (A)

5.4. Phase - IV

5.4.1 Phytotoxicity study of untreated and *S. wightii* treated BG dye on black gram

5.4.1.1 Seventh day of experiment

Treated textile effluent could be used for irrigation and industrial reuse. In this aspect, the reuse of textile effluent for irrigation purpose seems to be the most promising method. Therefore, the treated effluent is a potential water resource for agricultural irrigation. Thus, the present study aimed to assess the treated BG dye used for irrigation of *Vigna mungo* L.

The phytotoxicity study of untreated (T1) and *S. wightii* treated (T2) BG dye solutions compared with control (tap water) were carried out with black gram, *Vigna mungo* L. and the results were tabulated in Table 13. From the result, ANOVA test showed that the germination percentage, seedling growth and their fresh weight, dry weight were maximum in control followed by *S. wightii* treated BG dye and minimum was observed in untreated BG dye. Untreated BG dye influences their toxic effect whereas treated dye after decolourization showed better seed germination and spontaneous growth of plants.

Table. 13 Phytotoxicity study (on 7th day) of control, untreated and *S. wightii* treated BG dye (n=20) and its effect on seeds germination (%), shoot length (cm), root length (cm) weight (g), vigour index and tolerance index.

Germination Seeds	Observations	Control (tap water)	Untreated dye	Treated dye
Black gram	Germination (%)	93	42	80
	Shoot length (cm)	13.43±0.208^{c}	3.10±0.020^{a}	12.60±0.057^{b}
	Root length (cm)	6.43±0.152^{c}	2.26±0.020^{a}	5.40±0.0570^{b}
	Fresh weight (g)	0.27±0.020^{c}	0.10±0.005^{a}	0.24±0.005^{b}
	Dry weight (g)	0.23±0.010^{c}	0.09±0.005^{a}	0.18±0.005^{b}
	Viguor index	558	84	400
	Tolerance index	-	0.333	0.833
	% of phytotoxicity	-	-7.42	-78.85

Results are the mean value of triplicates ± Standard Deviation followed by different superscripts with significant difference at $p<0.05$

5.4.1.2 Sixtieth day of experiment

Irrigated BG dye on black gram yield (Fig. 23-32) varied significantly ($P \leq 0.05$) among treatments as compared with the control. The soil analyses (treated and untreated) were shown in table 15. In the present study, black gram grown in untreated BG dye, the plant height (shoot and root length), plant fresh weight, plant dry weight, seed dry weight, no.of leaves, total leaf area, number of root nodules and number of fruits were significantly low. Whereas plants grown in irrigated treated dye, the plant height (shoot and root length), plant fresh weight, plant dry weight, no. of leaves, total leaf area, number of root nodule and number of fruits were significantly high (Table 14) and the values were close to black gram grown in control. The results revealed that on sixtieth day, plant grown in treated BG dye showed better growth than untreated BG dye.

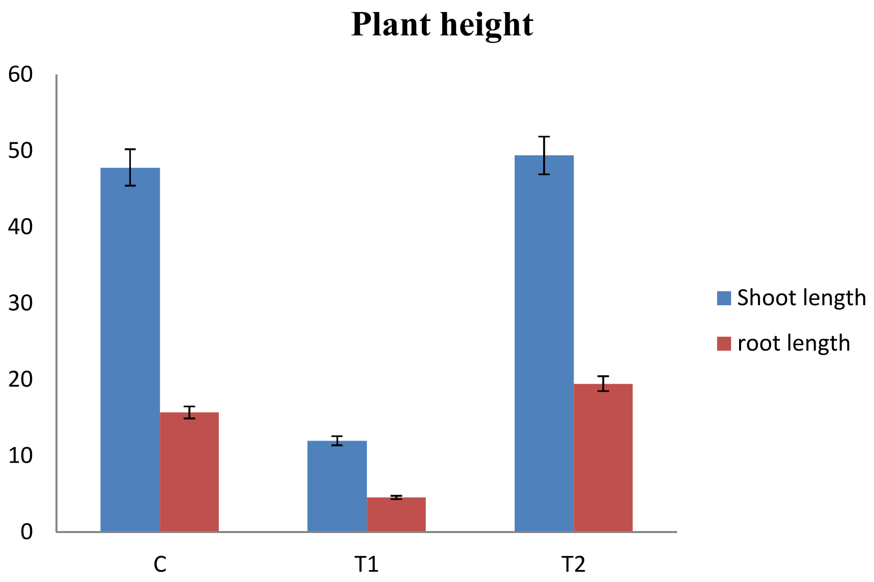


Fig. 23 Shoot and root length of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

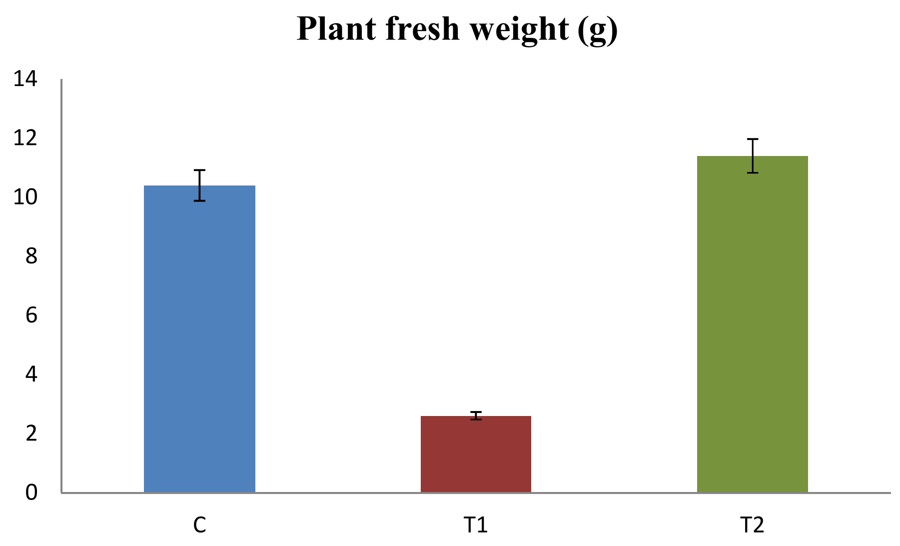


Fig.24 Plant fresh weight of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

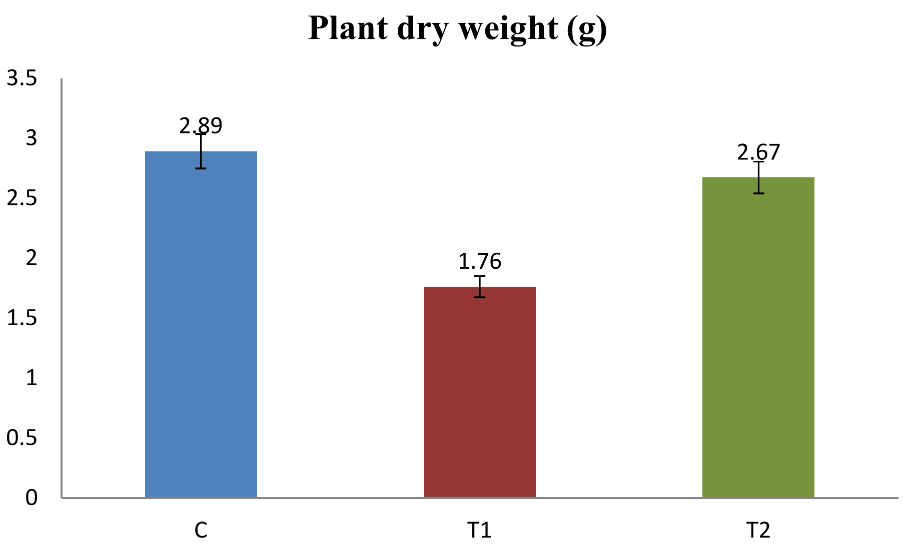


Fig. 25 Plant dry weight of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

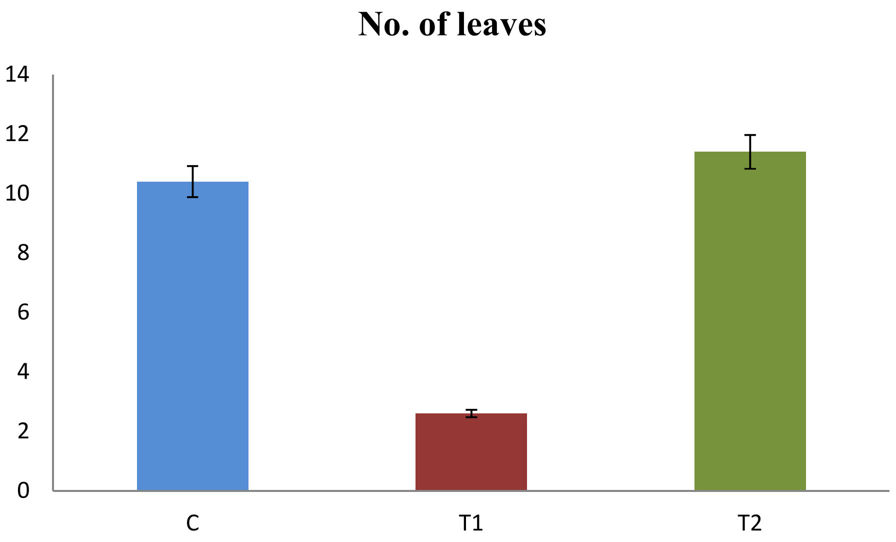


Fig. 26 Number of leaves of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

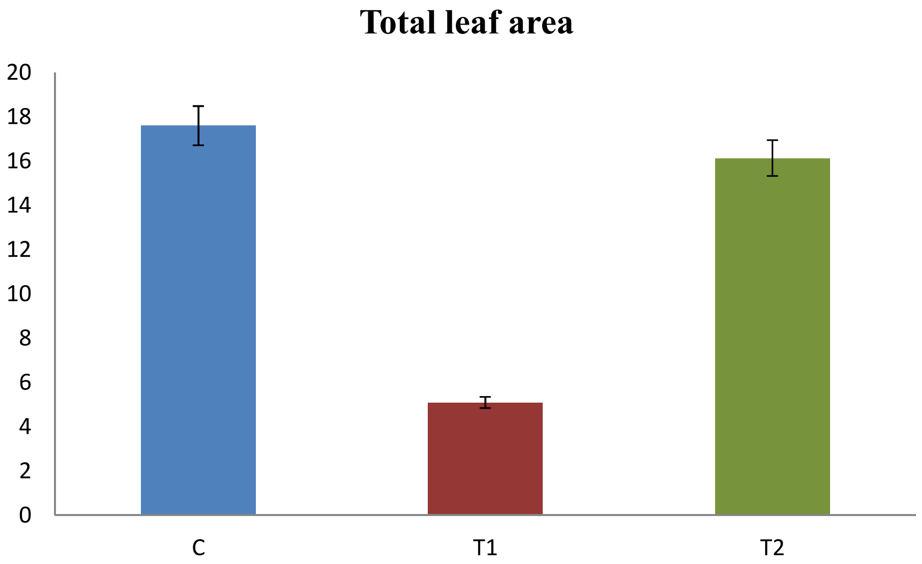


Fig. 27 Total leaf area of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

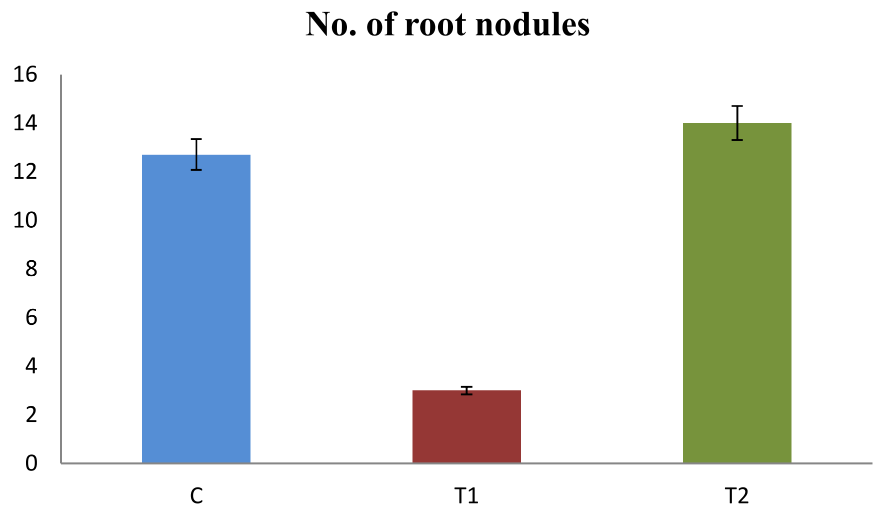


Fig. 28 Number of root nodules of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

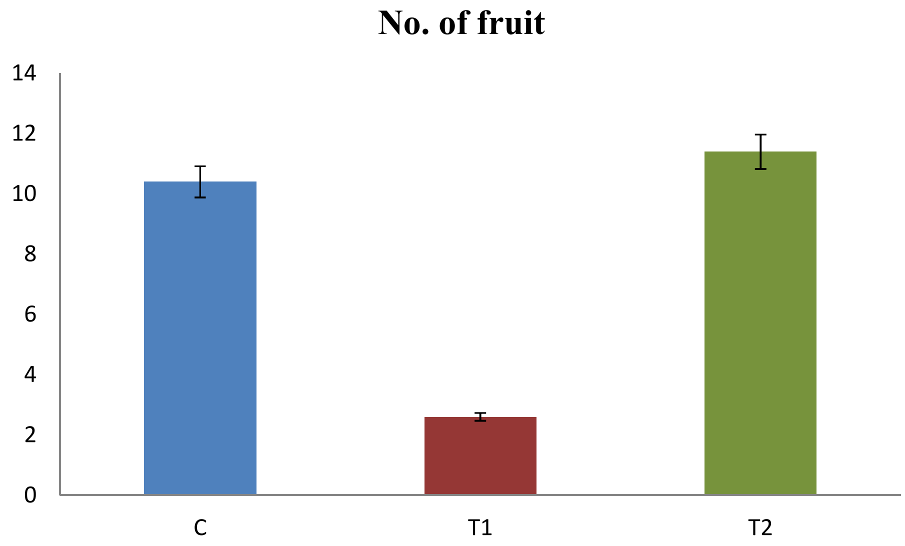


Fig. 29 Number of fruits of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

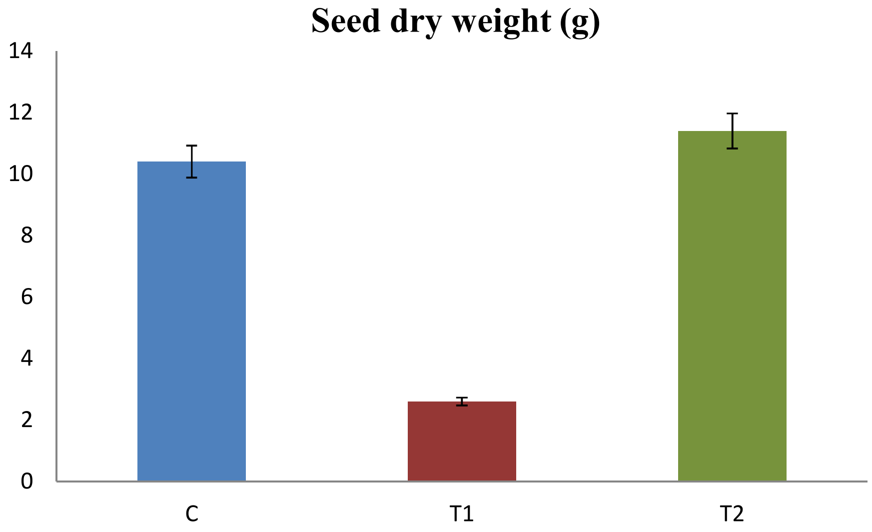


Fig. 30 Seeds dry weight of *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

ANOVA test showed that the value of the yield fruit attributes to the plants were obtained high in treated BG dye when compared with untreated dye. Treated BG dye promoting the number of fruits yield and betterment in growth development whereas untreated BG dye affecting the numbers of fruit yield and inhibiting the growth development of black gram. The results of the present investigation suggested that the significant increasing growth in treated dye solutions might be due to the prevented accumulation of dye in the sample there by reducing its toxicity significantly.

Present results also coincide with the findings of Phugare *et al.* (2010) who reported the toxic and nontoxic effects of untreated and treated effluent on the seed germination of *T. aestivum* and *P. mungo*. Phytotoxicity study showed higher germination rate of *Triticum aestivum* and *Ervum lens* plants grown after decolourization when compared to the plants grown in dye (Shedbalkar and Jadhav, 2011). *Triticum* sp grown in treated dye showed better growth when compared to untreated dye (Brahmbhatt and Jasrai, 2016). The lower concentration of textile dye effluent promoted the growth of cow pea seedlings (Vigneshpriya *et al.*, 2017). Jolly *et al.* (2012) studied the impact of dyeing industry effluent on wheat cultivation who revealed that wheat irrigated with treated effluent shown better performance in growth and yield compared to untreated irrigated wheat. Malaviya *et al.* (2012) studied the impact of dyeing industries effluent at five different concentrations (20%, 40%, 60%, 80% and90%) on growth of pea in pot culture. The plant exposed with 20% diluted effluent shown highest growth (10.78±0.6) and poor growth (3.51±0.4) was found in 90% diluted effluent.

Fig. 31 Phytotoxicity impact of untreated and treated BG dye by *S. wightii* on biometric parameters on 60th old day plant *Vigna mungo*

C T1 T2

Fig. 32 Sixtieth days old *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

Table. 14 Biometric parameters on 60th days old *Vigna mungo* grown in untreated and *S. wightii* treated BG dye

Treatments	Height (Shoot and root length)		Plant fresh weight (g)	Plant dry weight (g)	No. of leaves	Total leaf area	Yield seed dry weight (g)	No. of root nodules	No. of fruit yield
	Shoot length	Root length							
T1	47.8± 0.3	15.7±0.8	4.44± 0.1	2.89± 0.4	25± 0.3	17.6± 0.0	8.89±0.5	12.71± 0.2	10.4±0.5
T2	11.97± 0.4	4.54±0.1	1.69± 0.3	1.76± 0.6	14±0.4	5.1± 0.3	2.92±0.3	3± 0.1	2.6± 0.2
T3	49.4± 0.3	19.45±0.3	3.49± 0.1	2.67± 0.3	27± 0.3	16.14± 1.4	10±0.4	14± 0.4	11.4±0.3

Results are the mean value of triplicates ± Standard Deviation with significant difference at $p<0.05$

Table. 15 Physicochemical analyses of untreated and treated soil

Parameters	Control (T1)	Untreated soil (T2)	Treated soil (T3)
pH (µmhos/cm)	9.64	12.04	9.75
EC (%)	176	730	200
Total Nitrogen (mg/kg)	0.55	2.94	0.53
Total Phosphorus (mg/kg)	2.5	8.93	2.2
Total Potassium (mg/kg)	0.41	3.7	0.33
Sodium (mg/kg)	27	85.1	28.3
Calcium as Ca (mg/kg)	30.0	96.0	22.0
Iron (mg/kg)	0.57	2.81	0.66
Copper (mg/kg)	9.34	12.64	6.76
Zinc (mg/kg)	1.37	4.92	1.34
Chromium (mg/kg)	20.4	56	23.16
Cadmium (mg/kg)	32.1	68.4	47
Nickel (mg/kg)	20.05	52.92	25.83
Manganese (mg/kg)	21.6	44.70	27.41

5.4.2. Microbial toxicity of the untreated and *S. wightii* treated BG dye

5.4.2.1. Bacterial toxicity of the untreated and *S. wightii* treated BG dye

The untreated and treated dye solution was tested for their bacterial toxicity against gram positive as well as gram negative bacteria (Table 16). Untreated dye which has substantial amount of the dye exhibited significant bacterial toxicity. It exhibited vibrant and clear inhibition zone of diameter of about 2.1 cm against *Escherichia coli*, followed by *Salmonella* sp. (2 cm). Marginally high zone of inhibition (1.8 cm) was observed against *Streptococcus epidermis, Staphylococcus aureus* and *Shigella* sp. Next to it, the untreated dye exhibited considerable zone of inhibition (1.7 cm) against *Pseudomonas aeruginosa* and *Proteus vulgaris*. Moderate inhibition zones of diameter 1.5 cm, 1.4 cm and 1.2 cm were observed against *Bacillus cereus, Vibrio cholera* and *Klebsiella* sp. respectively (Fig 33). The clear zone of inhibition exhibited by the untreated dye reflected its extent of microbial toxicity and inferred that the untreated dye had anti-bacterial activity. The index of toxicity was represented by the zone size. No inhibition zones were observed with *S. wightii* treated dye which also confirmed the nontoxic nature of the extracted metabolite.

Potent bacterial toxicity of the untreated dye might be due to the presence of BG dye (one of the triarylmethane dyes), which is used as an effective antiseptic agent. Similar inhibitory results were obtained by Bakker *et al.* (1992) who experimented the activity of brilliant green against *Streptococcus, Proteus* and *Staphylococcus* sp. Parshetti *et al.* (2011) suggested the degradation products were less toxic compared with crystal violet to an exploited microorganism such as *A. radiobacter*, a phosphate-solubilizing bacterium *P. aeruginosa* and nitrogen-fixing bacterium *A. vinelandii*.

Table. 16 Bacterial toxicity of the untreated and *S. wightii* treated BG dye

S. No.	Organisms	Diameter of the zone of inhibition (cm)		
		Control	Untreated BG dye	Treated BG dye
1.	*Klebsiella sp*	NI	1.2±0.11	NI
2.	*Streptococcus epidermis*	NI	1.8±0.51	NI
3.	*Vibrio cholera*	NI	1.4±0.50	NI
4.	*Pseudomonas aeruginosa*	NI	1.7±0.30	NI
5.	*Shigella sp.*	NI	1.8±0.52	NI
6.	*Bacillus cereus*	NI	1.5±0.75	NI
7.	*Proteus vulgaris*	NI	1.7±0.30	NI
8.	*Salmonella enteriditis*	NI	2.0±0.30	NI
9.	*Staphylococcus aureus*	NI	1.8±0.52	NI
10.	*Escherichia coli*	NI	2.1±0.25	NI

Values are the mean of triplicates (Mean±SD); NI- No zone of inhibition

Kahraman and Yalcin (2005) studied the antimicrobial effect of the treated dyes on *P. aeruginosa* which was tested to evaluate the toxicity after decolourization. The untreated dyes used were toxic and their effects increased with the concentration gradually. It was found that the treated dyes were less toxic and also the removal of treated Astrazone black and Astrazone yellow with cotton stalk reduced the toxic effect on *P. aeruginosa.* According to Brahmbhatt and Jasrai (2016), untreated and treated effluents were tested for their effect on the agriculturally important soil bacterial flora, *Azotobacter* sp. and *Rhizobium* sp. From their experiment, no zone of inhibition was obtained for decolorized dye water which indicated that the biodegraded or decolorized product was non toxic to beneficial soil bacteria. The results were also in agreement with Mali *et al.* (2000) who studied the toxic effect of biodegraded products on agriculturally important soil microflora like *Bacillus* sp. (phosphate solubuliser), *Rhizobium* and *Azotobacter* (Nitrogen fixers). They reported from the zone of inhibition that it is non toxic to soil beneficial microflora which is necessary for sustainable agricultural practices.

Microbial toxicity of the textile azo dye (Reactive Red 81) was studied on microorganisms *Azotobacter* sp., *Pseudomonas* sp. and *Rhizobium* sp and their results showed that the wells with decolorized broth showed no zone of inhibition but wells with original dye exhibited a zone of inhibition. This confirmed that the original dye solution (3000µg/ml) was toxic to the bacteria but its degradation products were non toxic according to Shertate and Thorat (2016). Also microbial bioassay of basic fuchsin showed that untreated dye inhibited the growth of *E. coli* (forming a zone of inhibition) when compared to the treated dye (no zone of inhibition) (Rani *et al.*, 2014).

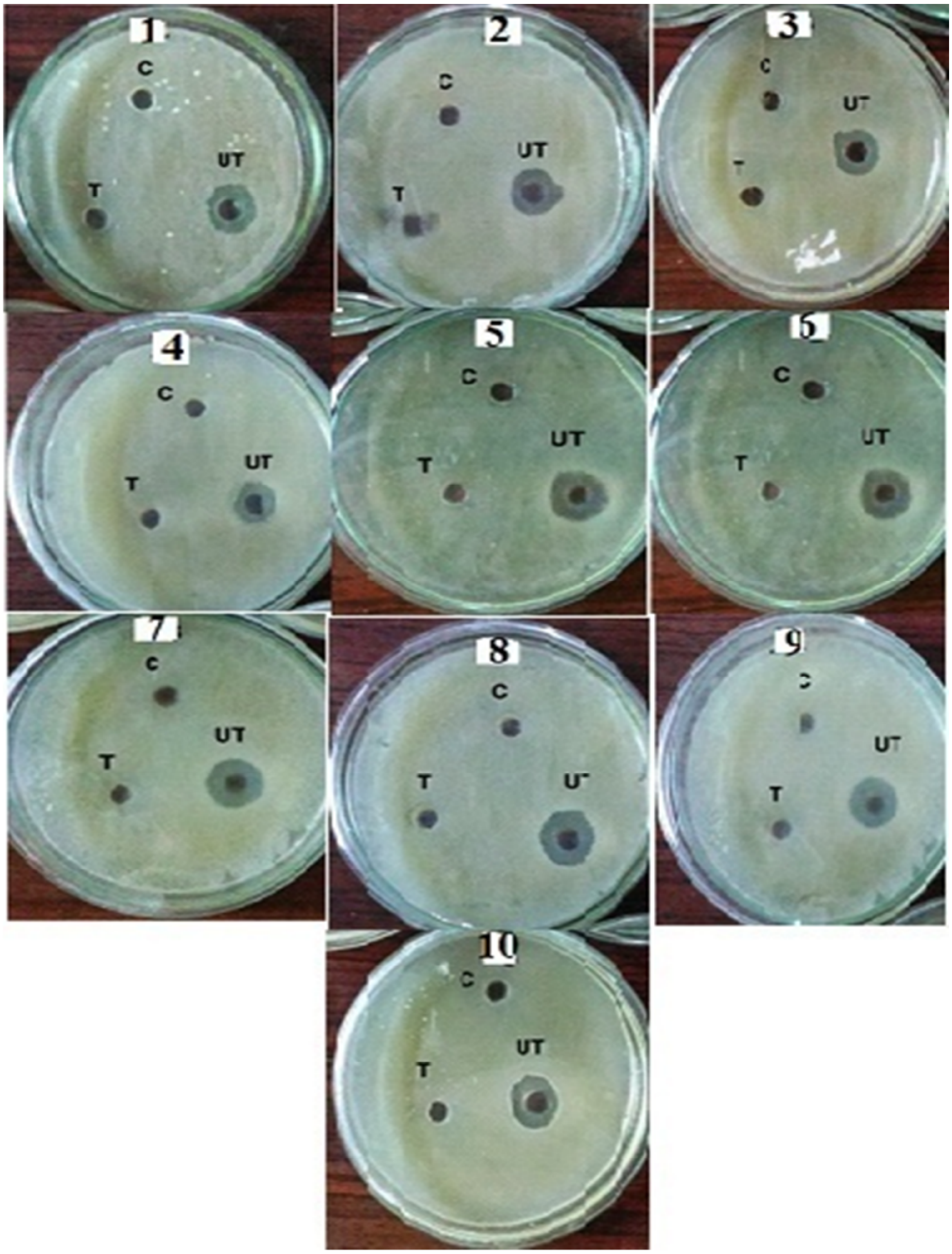


Fig.33 Bacterial toxicity of the untreated and treated dye samples against 1. *Klebsiella* sp. 2. *Streptococcus epidermi* 3. *Vibrio cholera* 4. *Pseudomonasaeruginosa* 5. *Shigella* sp. 6. *Bacillus cereus* 7. *Proteus vulgaris* 8. *Salmonella* sp. 9. *Staphylococcus aureus* and 10. *Escherichia coli*.

5.4.2.2. Fungal toxicity of the untreated and *S. wightii* treated BG dye

The untreated and treated dye solution was also tested for their fungal toxicity against few prominent fungal cultures, *Rhizopus* sp*., Aspergillus flavus, Acremonium* sp*., Trichoderma viride, Aspergillus fumigatus and Aspergillus niger* (Table 17). Eminent zones of inhibition were observed in the untreated dye when compared with the treated dye (no zone of inhibition). Largest zone of inhibition (0.8 cm) was observed against *Rhizopus* sp*., Trichoderma viride, Aspergillus fumigatus* and *Aspergillus niger.* Considerable inhibition zone of diameter 0.6 cm was observed against *Aspergillus flavus* and *Acremonium* sp. (Fig. 34). Treated dye did not exhibit antifungal activity.

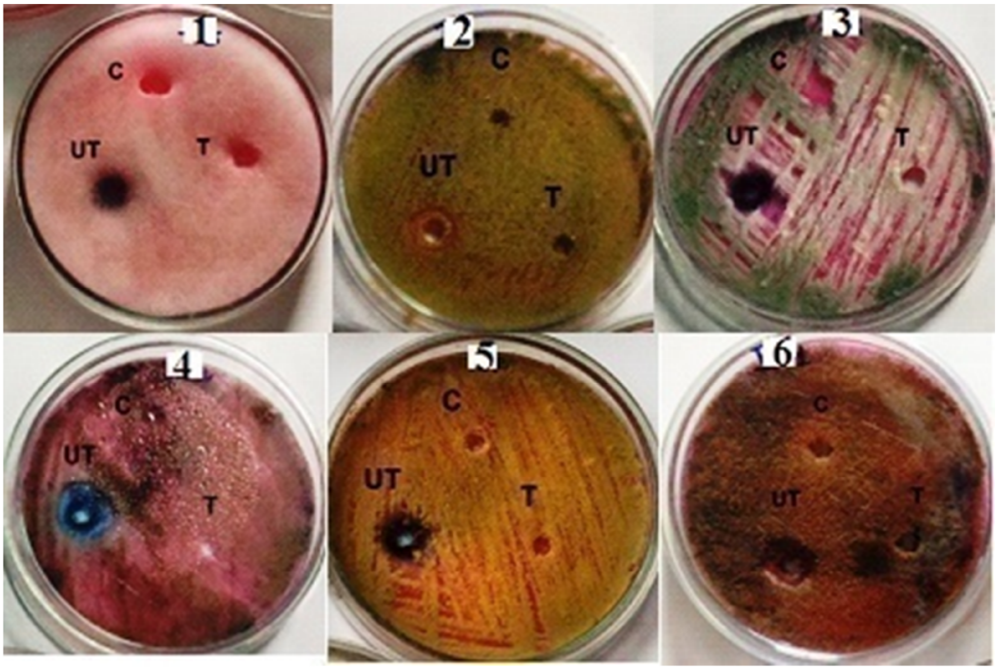


Fig.34 Fungal toxicity of untreated and treated dye samples against 1. *Rhizopus* sp. 2. *Aspergillus flavus* 3. *Acremonium* sp 4. *Trichoderma viride* 5. *Aspergillus fumigates and* 6. *Aspergillus niger.*

BG dye has been used widely as an antifungal agent in fish hatcheries (Allen *et al.*, 1994; Culp *et al.*, 1996; Srivastava *et al.*, 2004). Similar results were obtained by Ilyas and Rehman (2013) reported no zone of inhibition when using *Aspergillus niger* and *Nigrospora* sp. The results of this study (Jadhav *et al.*, 2010) suggested that potentially competent fungal strains can be efficiently used for detoxification and bioremediation of harmful dyes. Thus detoxification ability of seaweed directly proved against bacterial and fungal growth bioassay. Absence of zone of inhibition, in the case of treated dye infers complete removal of the dye by the marine algae (*S. wightii*). Hence, treated dye does not exhibit microbial toxicity due to the complete absence of the dye in it. This indirectly proves the efficiency of marine alga *S. wightii* in complete removal of the dye from the present study.

Table. 17 Fungal toxicity of the untreated and *S. wightii* treated BG dye

S. No	Organisms	Diameter of the zone of inhibition (in cm)		
		Control	Untreated BG dye	Treated BG dye
1.	*Rhizopus* sp.	NI	0.8±0.25	NI
2.	*Aspergillus flavus*	NI	0.6±0.72	NI
3.	*Acremonium* sp.	NI	0.6±0.75	NI
4.	*Trichoderma viride*	NI	0.8±0.30	NI
5.	*Aspergillus fumigates*	NI	0.8±0.41	NI
6.	*Aspergillus niger*	NI	0.8±0.32	NI

Values are the mean of triplicates (Mean±SD); NI- No zone of inhibition

5.4.3 Cytotoxicity of the untreated and *S. wightii* treated BG dye on *Allium cepa*

In this study, treated and untreated BG dye solution was analyzed for their toxicity potential by the *A. cepa*. The macroscopic results clearly showed that toxicity of the dye prompted *A. cepa* root growth inhibition. After 72 hours of exposures, maximum root length was observed in onion bulbs grown in treated dye solution (T3) and control (T1). The minimum root length was observed in bulbs grown in untreated dye solution (T2) (Table 18).

Table 19 describes the effect of control, untreated and *S. wightii* treated BG dye on number of dividing cells, mitotic indices (MI) and mitotic depression (MD). A total of 1000 cells were scored for control and each treatment. The maximum number of dividing cells was observed in control (735.2± 3.9) and *S. wightii* treated BG dye (659.4±2.1) exposed bulbs. The minimum number of dividing cells was observed in onion bulbs grown in untreated BG dye (161.7±0.2). To study the MI (mitotic index) 1000 cells were counted for each treatment group. From table 19, MI of samples exposed to *S. wightii* treated dye (68.9± 2.1) was significantly higher ($P<0.05$) and close to that of control (73.5± 3.9) might be due to the potential of *S. wightii* in the removal of pollutants. Significant reduction ($P<0.05$) was observed in untreated dye sample (16.1± 0.2). The mitotic depression was observed significantly minimum number ($P<0.05$) in treated BG dye (12.9±7.2) was close to that of control (12.5±5.3). In contrast, maximum number of mitotic depression was observed in untreated BG dye (98.2±6.4). Besides treated dye showed less number of alterations compared to the untreated dye includes the abnormal cells and occurrence of different chromosomal aberrations like bridge formation, laagering of chromosome, binuclear cells, polyploidy cells and multinucleated cell etc (Table 20). A similar result was observed by Phugare *et al.* (2010) was observed that cells exposed to treated dye showed minimum alterations because the depletion of pollution loads after treatment.

The similar observation was reported by Tripathy and Patel (2014) who stated that the high concentration of dye reduces the mitotic index and increases the rate of mitotic abnormalities. Stickiness is the indicator and also signifies high toxicity of dye which will lead to irreversibility of chromosomal aberrations and cell death (Alimba *et al.*, 2013). This result similar to the previous report in which onion bulbs exposed to pulp and paper mill effluent induced chromosomal aberrations and nuclear abnormalities compared to the control bulbs (Haq *et al.*, 2016).

This confirmed the interference of the chemicals presence in the dye whereas the bulbs grown in treated dye solution did not showed any inhibition effect on root elongation. Also the roots grew well due to the elevation dye. Untreated dye solution is believed to be toxic as they might exist various harmful effects whereas after treatment dye revealed the nontoxic nature. It also showed that the dye induced chromosomal aberrations at significant levels. The results revealed that the untreated dye was toxic to eukaryotic cells.

Table. 18 Root length of onion bulb, *Allium cepa* grown in control, untreated and *S. wightii* treated BG dye solution

Duration (in hours)	Control			Untreated BG dye			Treated BG dye		
	T1 (a)	T1 (b)	T1 (c)	T2 (a)	T2 (b)	T2 (c)	T3 (a)	T3 (b)	T3 (c)
24	1.13±0.50	1.31±0.40	1.35±0.36	0.67±0.27	0.42±0.30	0.45±0.20	1.26±0.17	1.36±0.30	1.42±0.19
48	1.23±0.28	1.43±0.26	1.41±0.32	0.35±0.13	0.35±0.23	0.43±0.26	1.30±0.30	1.33±0.27	1.45±0.18
72	1.29±0.22	1.35±0.44	1.45±0.17	0.80±0.52	0.58±0.31	0.47±0.19	1.37±0.26	1.37±0.21	1.40±0.26

(Results are the mean value ± Standard Deviation with significant difference at $p<0.05$)

Table.19 Mitotic Index and mitotic depression of onion bulb, *Allium cepa* grown in control, untreated and *S. wightii* treated BG dye solution

Treatments	Cells in division	Mitotic index	Mitotic depression
Control	735.2±3.9	73.5±3.9	12.5±5.3
Untreated	161.7±0.2	16.1±0.2	98.2±6.4
Treated	659.4±2.1	68.9±2.1	12.9±7.2

Represents mean ± Standard Deviation with significant difference at $p<0.05$)

Table. 20 Effect of untreated and *S. wightii* treated BG dye on the induction of chromosomal aberration in onion bulb, *Allium cepa*

Chromosome aberrations	Control	Untreated BG dye	Treated BG dye
Chromosomes with Spindle disturbances	-	14	2
Anaphase chromosome bridge	1	9	20
Normal telophase	45	3	52
Disturbed metaphase	-	27	3
Laggard chromosome	-	11	5
Chromosome displacement at anaphase	-	25	-
Normal prophase	50	40	64
Sticky chained metaphase	-	33	14
Chromosome bridge at telophase	-	97	5
Chromosome fragment	-	78	8
Normal metaphase	35	30	49
Normal spindle fibre separation	-	46	18
Disturbed prophase	-	92	5
Chromosome bridge at late anaphase	-	32	3
Sticky chromosomes	-	46	6
Normal anaphase	36	25	35
Abnormal grouping of chromosomes	-	87	2
Equatorial separation at anaphase	-	63	2
Abnormal telophase	-	89	3
Chromosomes in equatorial plate	27	64	5
Telophase with chromosomal loss	-	93	2

5.4.4. Zootoxicity of untreated and *S. wightii* treated BG dye on fish *Labeo rohita*

5.4.4.1 Mortality percentage

Acute toxicity of untreated and treated BG dye on fish *Labeo rohita* was studied and percent mortality rate was presented in table 21 and figure 35 and 37. Upon exposure to control and two different treatment fishes showed abnormal behavioural changes including jerky movement, restless, fast swimming, excess mucus secretions, loss of equilibrium, sluggish and static condition at the bottom (Ramesh *et al.*, 2014) were removed. The observed behavioural changes were severe and high mortality rate (T_2(I)65%, T_2(II)70% T_2(III)65%) in the fishes exposed to untreated BG dye which shows the toxic content presence in dye. Whereas fishes grown in control and treated BG dye showed less mortality rate (T_1(I)30%, T_1(II)35%, T_1(III)20% and T_3(I)25%, T_3(II)30%, T_3(III)30%)which depicts the less toxic content.

Similar observation was made by Gabriel and Okey (2009) in cat fish exposed to textile industrial effluent. Angelin *et al.* (2015) reported that the *Poecilia reticulata* fish mortality rate was increased with the increase time of exposure to chrome plating effluent. The behavioural changes like erratic swimming, increased activity, inconsistent jumping in fishes were directly proportional to the toxicants present in the sample at different duration of exposure (Bobmanuel *et al.* (2006); Roopadevi and Somashekar (2012)).

Dhanve *et al.* (2014) observed that the mortality percentage was high in fish (*Acantopsis choirorhyncus*) grown in untreated textile effluent and in contrast, the mortality rate was low in fishes grown in treated effluent because the degradation product of the effluent did not induce any hazardous to fishes. The present study was also supported by Sridevi and Kanmani (2010) who reported that the mortality percentage of *Cyprinus carpio* was high in untreated textile dyeing wastewater when compared ozonation treated effluent and control. Francis and Portia (2013) have reported that the more physiological stress to the fish exposed in higher concentration of sago effluent than the lower concentration.

Fig. 35 Experimental setup shows mortality rate of fish *Labeo rohita*

5.4.4.2 Haematological parameters

The haematological parameters of *Labeo rohita* on control and two different treatment groups were observed and presented in table 22 and figure 43-47. During acute toxicity study, red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb), haematocrit (Hct) (Nelson and Morris, 1989), mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH) and mean corpuscular hemoglobin concentration (MCHC) levels were significantly decreased ($p<0.05$) in untreated BG dye exposed fish (1.02 million/cu.mm), 1,00,150 thousand/cu.mm, 2.3g/dl, 5.17%, 117.0 fl, 29.7 pg, 18.1 gm/dl). Whereas there is no change was observed in the level of red blood corpuscles, white blood corpuscles, haemoglobin, haematocrit, MCV, MCH and MCHC level in fishes grown in treated BG dye (1.15 million/cu.mm), 1,96,000 thousand/cu.mm, 6.7g/dl, 16.15%, 191.1 fl, 61.9 pg, 32.0 gm/dl) as compared to control (1.019 million/cu.mm), 2,02, 000 thousand/cu.mm, 7.9g/dl, 17.15%, 190.0 fl, 62.6 pg, 32.5 gm/dl).

The results were in accordance with Kavitha *et al.* (2010) who stated that the significant decrease in RBC and WBC count during acute toxicity might be due to the extent toxic effect in kidney tissue which is the primary site of haematopoiesis provoking immune suppression. The decreased level of haemoglobin and packed cell volume were noticed in *Clarias batrachus* fishes (Tripathi *et al.*, 2003).

RBC level was found to be decreased in fishes subjected to stressful conditions. An anaemic condition is indicated the reduction of RBC count and Hb count when the fishes exposed to environmental pollution (Remyla *et al.,* 2008). In this study, the significant decrease in the level of RBC in untreated dye exposed fish might be due to the anaemic condition or the inhibition of erythropoiesis caused by brilliant green dye.

The significant reduction in MCV and MCHC level which results the swelling of RBC's and also reduce the matured RBC count leads to the formation of sphaerocytosis (Sobecka, 2001). The decrease in haematological parameters might have resulted from impaired oxygen uptake due to gill damage caused by the toxicity (Saravanan *et al.*, 2011). MCV gives an indication of the status or size of RBCs (Alwan, 2009). The MCHC is a good indicator of red blood cell swelling or shrinkage (Wepener *et al.*, 1992). The increase in the MCHC values in the exposed fish is thus probably an indication of shrinking of the red blood cells and/or a decrease in hemoglobin synthesis. The decreased Hb content may also be attributed to decreased hemorrhage or anemia and hemoglobin synthesis which, in turn, explains a decreased MCHC (Southamani *et al.*, 2015). The results were supported by Abdel Moneum *et al.* (2008) who reported that lower concentration of dye waste water promotes an increase in hematological parameters of cat fish *Clarias lazera*.

5.4.4.3 Biochemical parameters

The fish *Labeo rohita* was exposed to control, untreated and *S. wightii* treated BG dye were analyzed for plasma glucose and plasma protein and were presented in table 23 and figure 40 and 41. During acute toxicity, the glucose (35.0 mg/100ml) and protein level (1.13µg/ml) in the plasma of fishes grown in untreated BG dye decreased significantly due to stress by toxic presence in the dye. Whereas fishes exposed to treated BG dye [glucose (76.0 mg/100ml) and protein level (3.62µg/ml)] and control [glucose (77.0 mg/100ml) and protein level (3.80µg/ml)] showed significantly ($P<0.05$) increased level of glucose and protein. It indicated that the elevation of toxicity in treated BG dye. A similar observation was reported in *Cyprinus gariepinus* fish (Nwani *et al.*, 2014) and *Oreochromis niloticus* (Sayed and Moneeb, 2015) treated with toxicants.

The decrease in plasma protein might be due to impaired protein synthesis due to toxicity (Saravana *et al.*, 2015) and liver cirrhosis or nephrosis might be due to modification in enzymatic activity in protein synthesis (Yousef *et al.*, 2008). Glucose plays an important role in all living organisms. The enhance glucose level in the fishes grown in treated BG dye might be due to high mobilization of glucose via glycogenolysis to meet metabolic demands. The plasma glucose has been extensively used as a sensitive bioindicator of environmental stress in fish (Kavitha *et al.*, 2010). It has been reported that the alteration in plasma glucose level indicates a stress response triggered by the stress which might be due to hypotoxic condition and gluconeogenesis. The results of the study was supported by Muley *et al.* (2005) who noticed that the significantly decreased level of total protein in various tissues of *Labeo rohita* exposed to textile mill effluent as compared to control groups.

5.4.4.5 Enzyme analysis

The fish *Labeo rohita* was exposed to acute toxicity studies on control, untreated and treated BG dye and the changes in enzyme activities (Glutamic oxaloacetate transaminase (GOT), Glutamic pyruvate transaminase (GPT), Glutathione (GSH) and Lactate dehydrogenase (LDH)) were recorded and the results were depicted in table 24 and figure 42. The enzymes GOT, GPT and GSH could be used as bioindicators in animals subjected to acute and chronic studies (Nemcsok and Benedeczky, 1981). GOT and GPT are the two key enzymes known for their role in the utilization of proteins and carbohydrates. The enzyme LDH involved in carbohydrate metabolism could be used as a good indicator to chemical exposure and stress in fish (Jos *et al.*, 2003). GSH is a biologically active tri peptide composed of three amino acids doing various physiological functions (Sen *et al.*, 1992).

In the present investigation, the significant decrease ($p<0.05$) level of GOT (107.0 m-units/ml), GPT (20.10 m-units/ml), GSH (14.2 m-units/ml) and LDH (55.11 μM) was observed in untreated BG dye exposed fish which showed the accumulation and toxicity of dye in an organism. A decrease in GOT and GPT during treatment might have results from severe destruction of hepatic cells which resulted in decrease synthesis of the protein. The treated (GOT (182.0 m-units/ml), GPT (77.67 m-units/ml), GSH (48.12 m-units/ml) and LDH (161.23 µM)) and control fishes (GOT (169.0 m-units/ml), GPT (71.36 m-units/ml), GSH (47.6 m-units/ml) and LDH (157.21 µM)) showed significant ($P<0.05$) increase level of observed enzymes.

The same results were obtained by Saravanan *et al.* (2011) who reported that the elevation of GPT activity indicated that the organism tries to mitigate the drug induced stress. Wenjuan *et al.* (2012) have stated that the zebra fish exposed to the textile effluent indicates pro-oxidants presence might be elevate the activities of GSH, GST enzymes. According to Zaki *et al.* (2009) exposure of Nile tilapia to sublethal concentration of lead acetate resulted in a makeable increase in the activities in serum enzyme level. A decrease in the activity of LDH of fishes in untreated BG dye might be due to the declined operation of oxidative metabolism (Shaffi, 2001). The increase in glutathione level in catfish exposed to effluent was based on duration exposure (Ahmad *et al.*, 2000).

5.4.4.6 Histopathology

The fish *Labeo rohita* exposed to control, untreated and treated BG dye was analyzed for its histopathological changes (gill, liver and kidney). The morphological sections were showed in figure 25-27.

5.4.4.6.1 Histopathology of gill

The morphological section of gill of fishes exposed to control group (Fig. 36C) showed the normal structure in which primary and secondary gill lamellae and a prominent branchial simple squamous epithelium composed of non differential cells. The fishes exposed to treated BG dye showed regenerating respiratory epithelium of the gills (Fig. 36T2). Fishes in untreated BG dye showed mucous secretion on gill region, degenerated central axis, erosion of the secondary lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae, degeneration of epithelium, necrosis and curling of secondary lamellae were noticed (Fig. 36T1).

Gill is a vital organ for histological examination to determine the direct effect of a pollutant. Gill alterations such as hyperglycemia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barricade to the entrance of contaminants. The obtained results were in accordance with Jagruti (2015) who reported that the *Catla catla* fingerlings exposed to control group showed normal morphology whereas fingerlings exposed to different concentration of reactive red 120 dye showed makeable changes in morphology and in nuclear material. Sivakumar *et al.* (2015) have reported that the necrosis of the epithelial layer resulted in the erosion of the respiratory epithelium of *Danio rerio* exposed to leather industrial effluent. *Labeo rohita* fish exposed to the textile mill effluent caused severe damage to gills, resulting in shortening of secondary lamellae cells and destruction of mucous cells which supports the present findings (Nikalje *et al.*, 2012).

5.4.4.6.2 Histopathology of liver

Labeo rohita exposed to control group (Fig. 37C) exhibited normal hepatocytes with minimal congestion of the sinusoids. The liver of fishes exposed to untreated BG dye (Fig. 37T1) showed vacuolar degeneration of the hepatocytes and sinusoid congestion. In contrast, the liver of treated BG dye fish also showed normal hepatocytes with mild vacuolar degeneration (Fig. 37T2). These results are in congruence with the findings of Maharajan *et al.* (2016) who observed the significant changes in the liver tissue at the lethal and sublethal concentrations of copper with marked swelling of the hepatocytes in places with areas of diffuse necrosis. Enlarged nuclei, blood congestion in sinusoid, vacuolation of hepatocytes and necrosis in the liver of *Labeo rohita* exposed to heavy metals were observed by Bhatkar (2011).

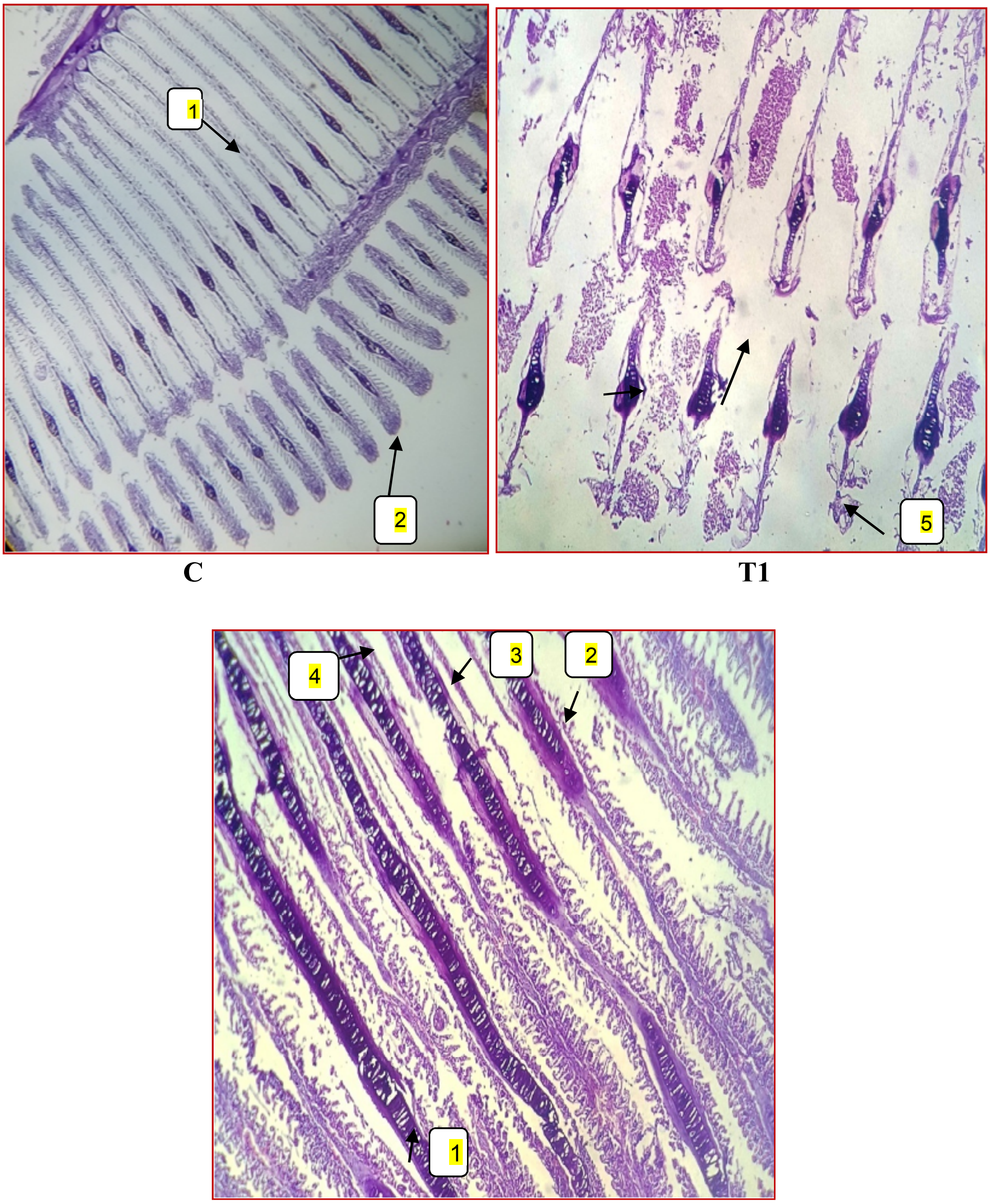
Similar results were observed by Bakhiet (2015) who reported that fishes collected from sewage contaminated Nile river stretch showed histological changes in liver tissues due to bioaccumulation of heavy metal. The result revealed that degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by the elevated concentrations of these compounds, and could subsequently result in structural damage (Brusle and Anandon, 1996).

5.4.4.6.3 Histopathology of kidney

The section of kidney tissue of control (Fig. 38C) and treated BG dye (Fig. 38T2) exposed *Labeo rohita* showed normal pattern appearing glomeruli and tubules. In contrast, fishes exposed to untreated BG dye showed contraction of glomerulus, hypertrophy of epithelial cells, degeneration of the tubules and reduction in lumen (Fig. 38T1). Kidney is the vital organ of the body and its function is to maintain the homeostatis. It is not only involved in the removal of waste from the blood but it also responsible for the selective reabsorption, which helps in maintaining the volume and pH of blood, body fluids and erythropoices (Iqbal *et al.*, 2004).

The degeneration and necrotic changes in tubular luman and haemorrhage were observed in *Labeo rohita* exposed with aflatoxin B_1 (Sahoo *et al.,* 2003). The present results were in accordance with Himri *et al.* (2011) who observed that tatrazine dye toxic irritant substances brought to the kidneys by circulatory blood cause degenerative changes in the kidney tissues.

In the present study, hiostopathological alterations in gill, liver and kidney of *Labeo rohita* exposed to control and treated BG dye showed normal structure and in contrast fishes exposed to untreated BG dye showed degenerative changes were comparatively more in gill, liver and kidney tissues. This might be due to the fact that liver tissues are the main site for detoxification, gills remains in direct contact with the toxicants presence in dye waste water. The kidney tissues generally concerned with excretion and removal of adsorbed pollutants. It is quite evident from the present studies that treated BG dye did not pose any threat to fishes. In contrast fishes grown in untreated BG dye seriously affect the organs like gill, liver and kidney of *Labeo rohita*, ultimately impair the growth and behavior leading to death.



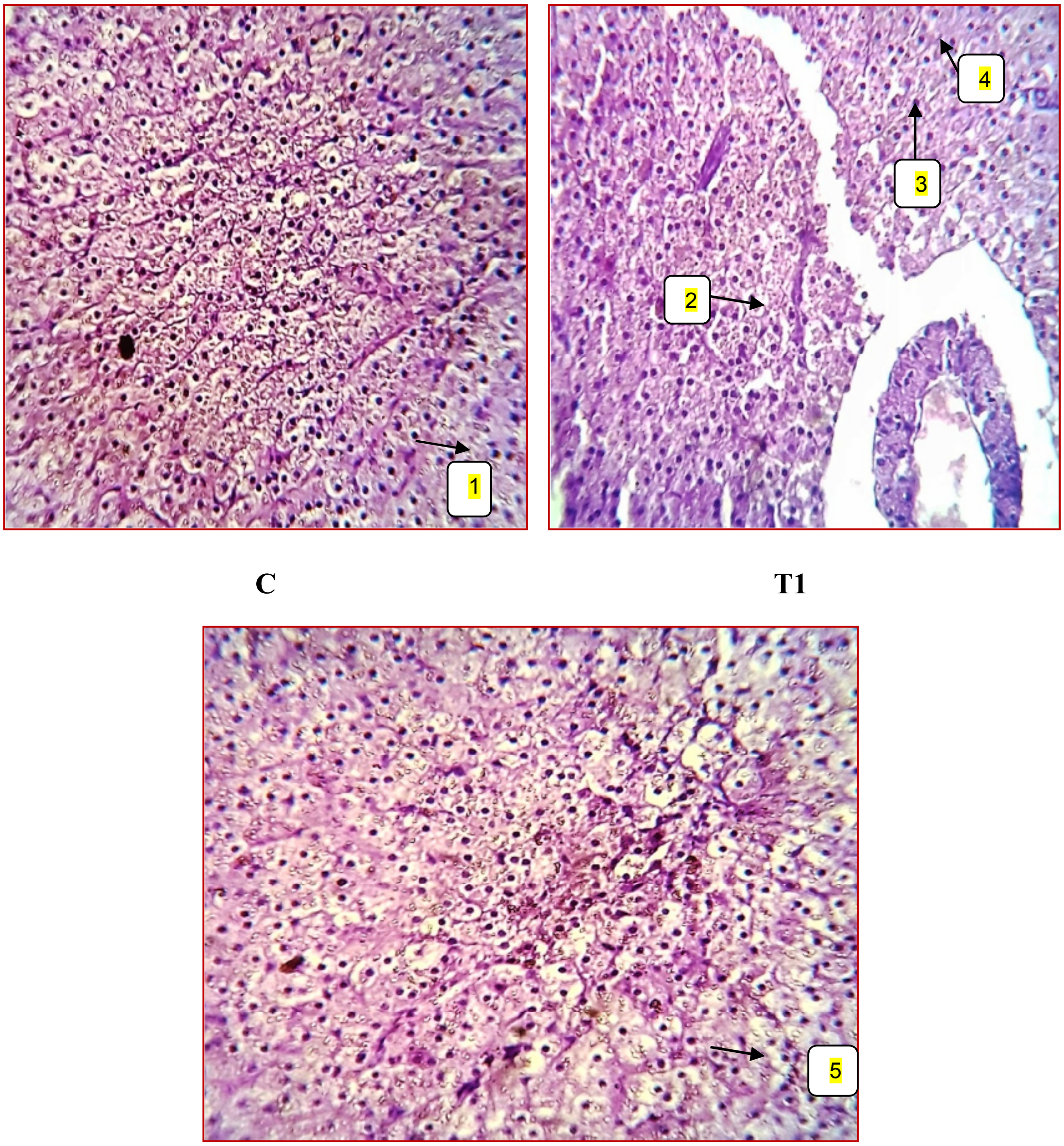
C

T1

T2

1- normal secondary lamelle, 2- normal gill filament, 3- normal Interlamellar epithelium, 4- normal hyaline cartilage, 5- shorting of secondary lamella, degenerative necrotic cells and dissorted capillary channels

Fig. 36 Histological changes in the gill exposed to control (C), untreated (T1) and *S. wightii* treated (T2) BG dye



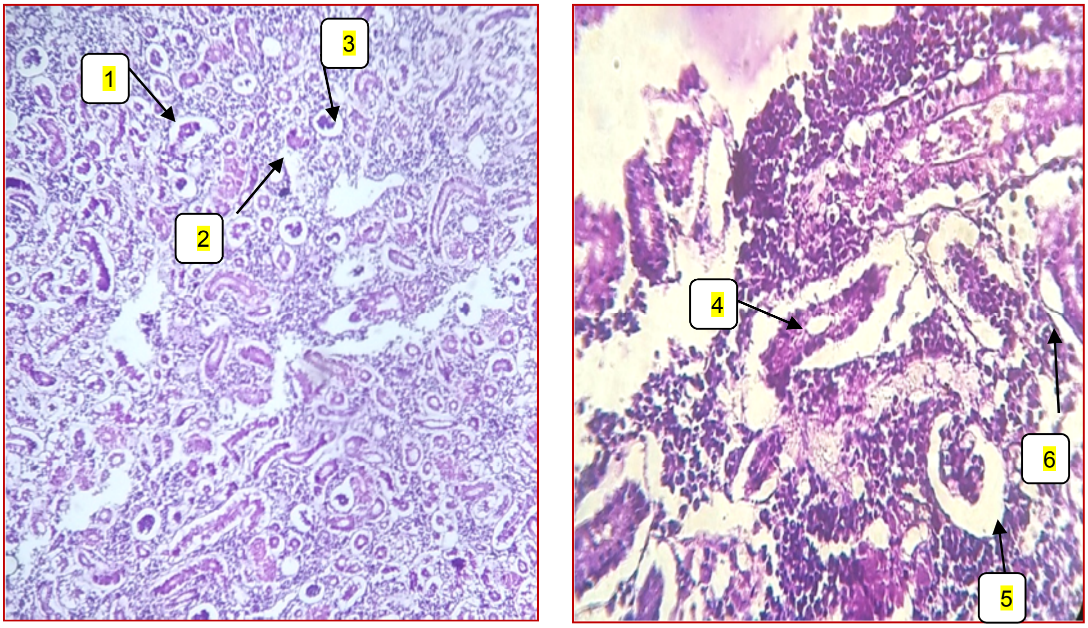
C

T1

T2

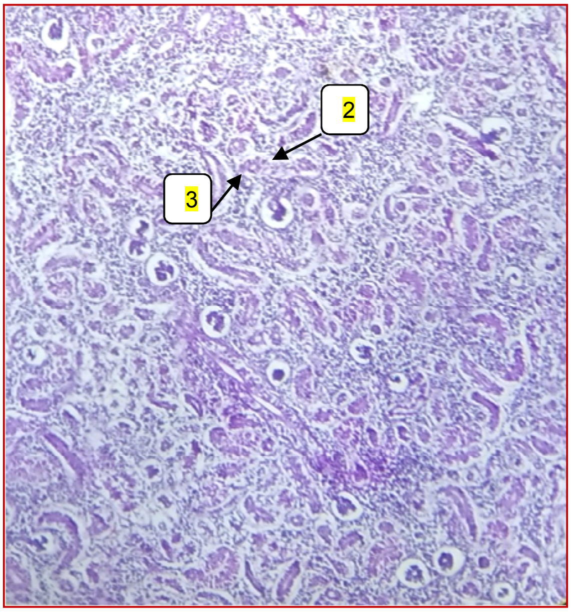
1- normal structure, 2-3- necrotic tissue and congestion of sinusoids, 4- degenerative nuclei 5- normal structure

Fig. 37 Histological changes in the liver exposed to control (C), untreated (T1) and *S. wightii* treated (T2) BG dye



C

T1



T2

1- bowman's capsule, 2- glomerulus, 3- glomerular capillaries, 4-5, shrinkage of renal tubules, 6- renal tubule- vacuolation

Fig. 38 Histological changes in the kidney exposed to control (C), untreated (T1) and *S. wightii* treated (T2) BG dye

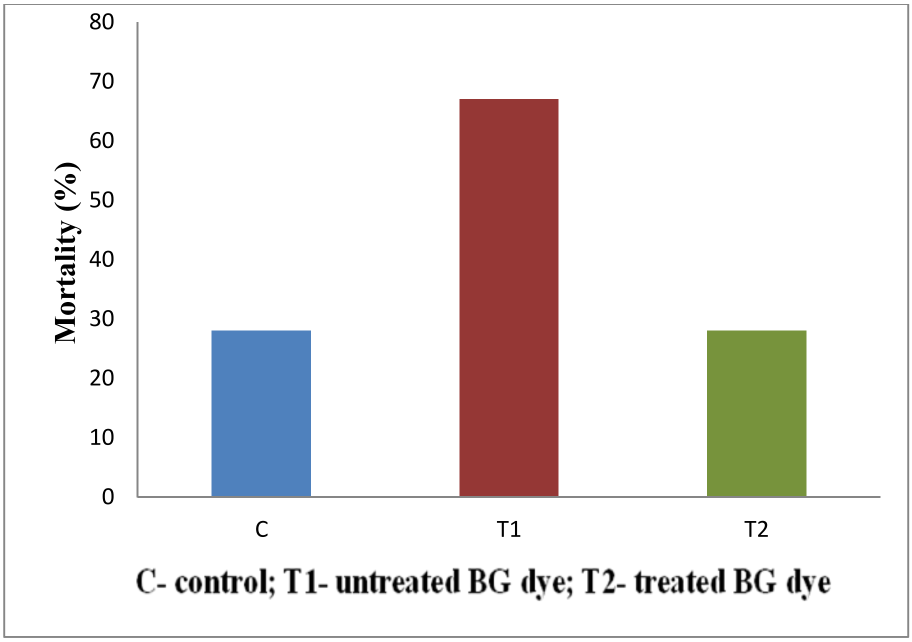


Fig. 39 Mortality rate of fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

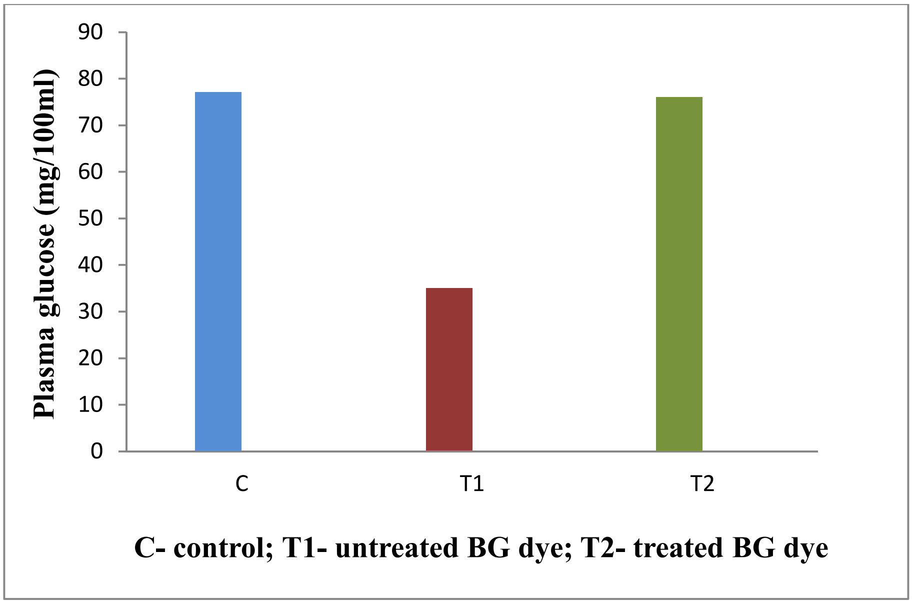


Fig. 40 Level of plasma glucose in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

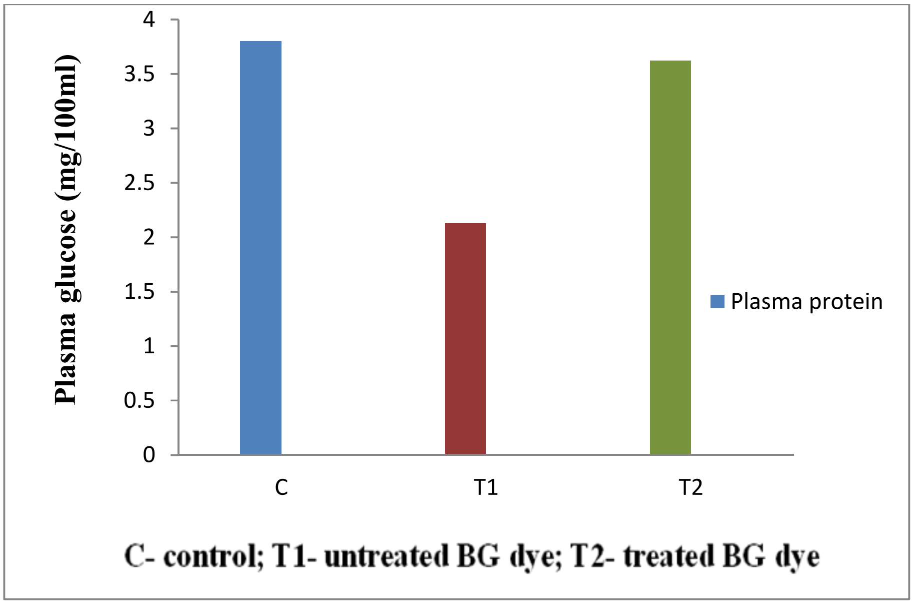


Fig. 41 Level plasma protein in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

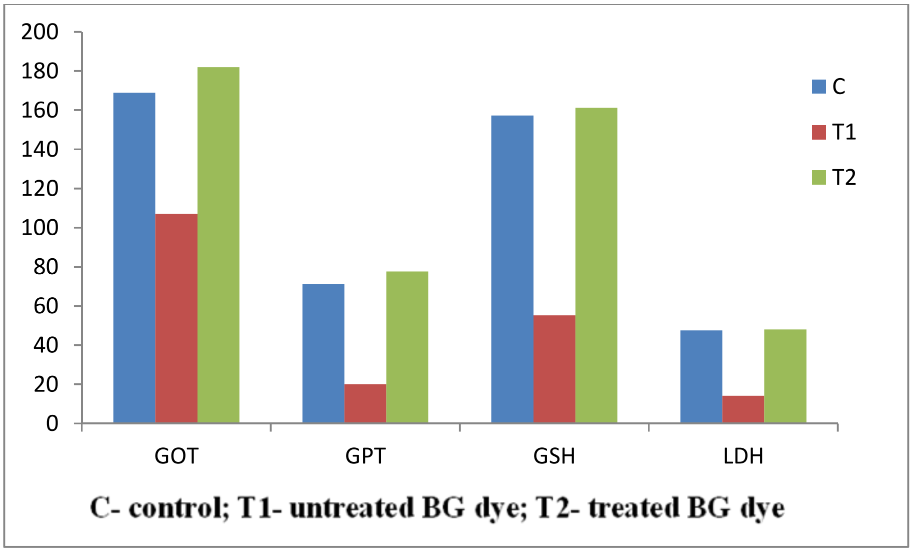


Fig. 42 Level of enzymes in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

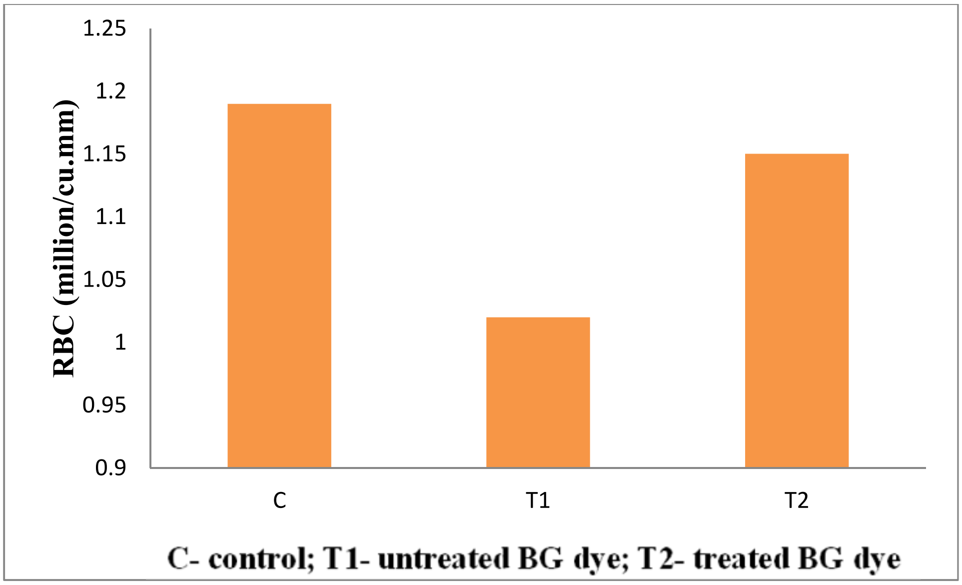


Fig. 43 Level of red blood corpuscles count in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

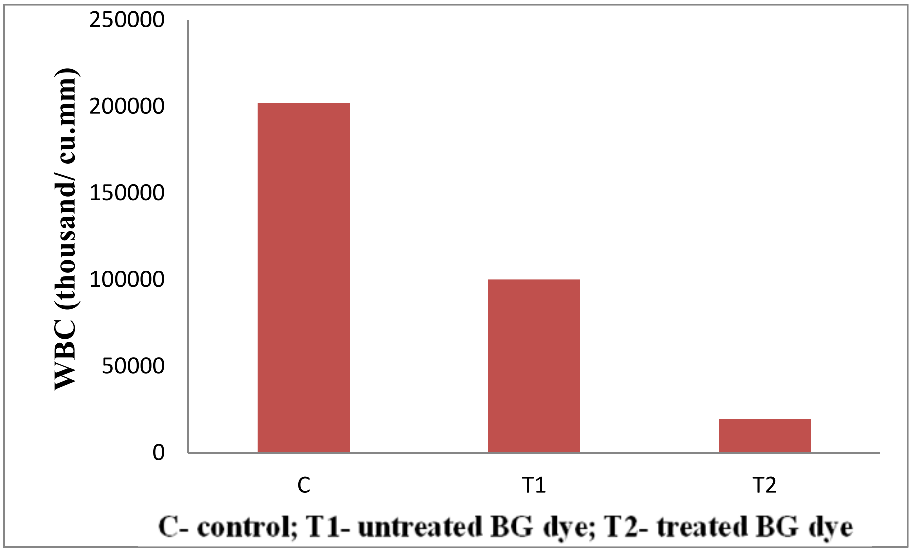


Fig. 44 Level of white blood corpuscles count in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

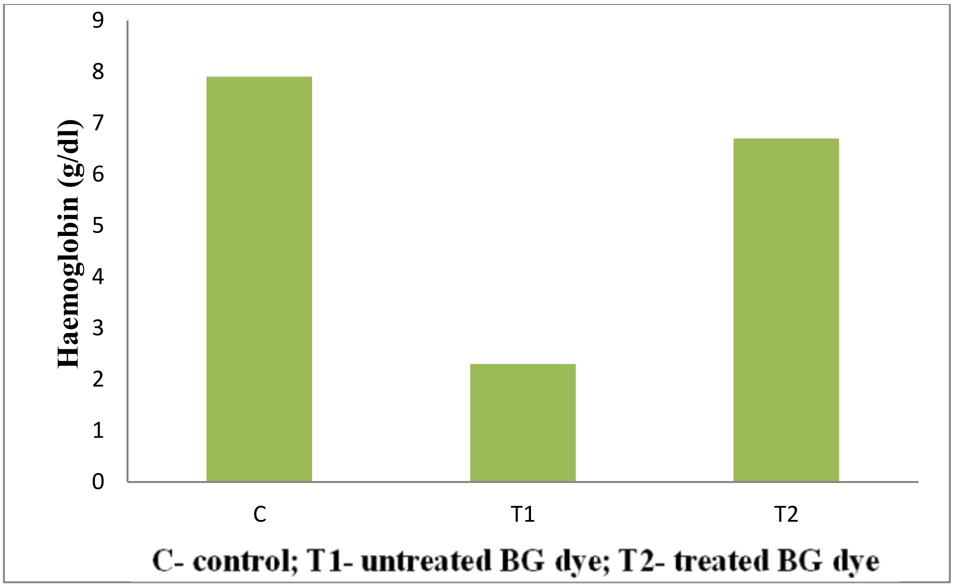


Fig. 45 Level of haemoglobin in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

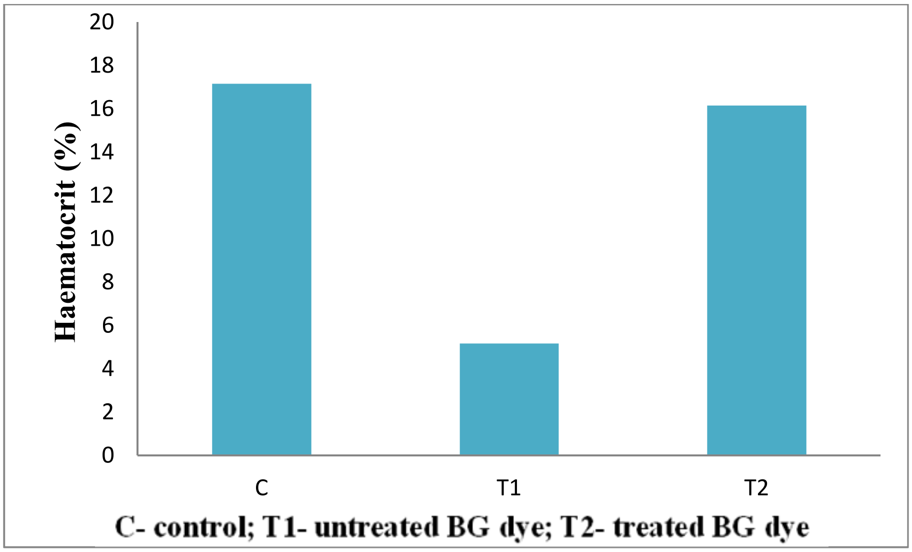
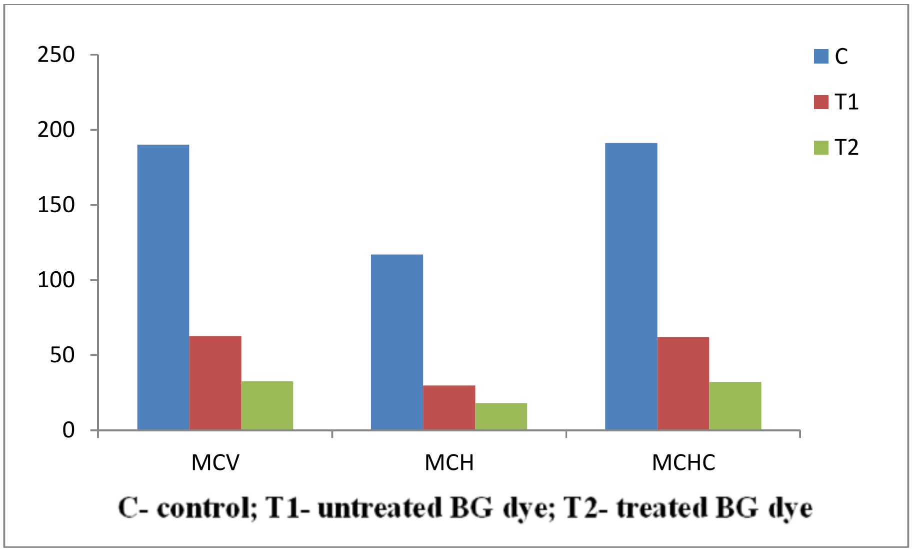


Fig. 46 Level of haematocrit in the fish *Labeo rohita* exposed to control, untreated *S. wightii* and treated BG dye



C- control; T1- untreated BG dye; T2- treated BG dye

Fig. 47 Level of MCV, MCH and MCHC in the fish *Labeo rohita* exposed to control, untreated and *S. wightii* treated BG dye

Table. 21 Mortality rate of *Labeo rohita* fishes grown in control, untreated and *S. wightii* treated BG dye

Treatments	No. of fishes introduced	Experimental Days							No. of fishes dead	Mortality (%)
		I	II	III	IV	V	VI	VII		
$T_1(1)$	20	1	1	3	-	1	-	-	6	30
$T_1(2)$	20	1	1	2	1	2	-	-	7	35
$T_1(3)$	20	-	-	2	2	-	-	-	4	20
$T_2(1)$	20	1	3	2	2	3	1	-	13	65
$T_2(2)$	20	-	1	3	3	4	1	1	14	70
$T_2(3)$	20	-	-	5	5	1	2	-	13	65
$T_3(1)$	20	-	1	1	1	2	-	-	5	25
$T_3(2)$	20	1	1	1	2	1	-	-	6	30
$T_3(3)$	20	-	-	2	3	1	-	-	6	30

Table. 22 Level of haematological parameters of fish *Labeo rohita* fishes grown in control, untreated and *S. wightii* treated BG dye

Parameters	T_1(Control)	T_2 (Untreated BG dye)	T_3 (Treated BG dye)
R.B.C (Million/cu.mm)	1.19 ± 0.45	1.02 ± 0.19	1.15 ±0.45
W.B.C (thousand/cu.mm)	55.78 ±9.97	21 ± 3.31	54.79 ± 9.98
Haemoglobin (gm/dl)	7.9 ±1.34	2.3 ±0.12	6.7 ±1.24
Haematocrit (%)	17.15 ±3.07	5.17 ±1.25	16.15 ±2.57
MCV (fl)	190.0	117.0	191.1
MCH (pg)	62.6	29.7	61.9
MCHC (gm/dl)	32.5	18.1	32.0

Table. 23 Level of biochemical parameters of fish *Labeo rohita* grown in control, untreated and *S. wightii* treated BG dye

Parameters	T_1(Control)	T_2 (Untreated BG dye)	T_3 (Treated BG dye)
Plasma Glucose (mg/100ml)	77.0 ±11.04	35.0 ±7.41	76.0 ±11.01
Plasma protein – Total (µg/ml)	3.80 ±0.63	1.13 ±0.24	3.62 ±0.61

Table. 24 Level of enzymes in the plasma of fiah *Labeo rohita* fishes grown in control, untreated and *S. wightii* treated BG dye

Parameters	T_1(Control)	T_2 (Untreated BG dye)	T_3 (Treated BG dye)
Glutamic oxaloacetate transaminase (GOT) (m-units/ml)	169.0 ±17.72	107.0 ±13.03	182.0± 19.07
Glutamic pyruvate transaminase (GPT) (m- units/ml)	71.36 ±10.09	20.10 ±3.31	77.67 ±11.02
Lactate dehydrogenase (LDH) (U/I)	157.21±15.12	55.11±7.79	161.23±17.01
Glutathione (GSH) (μ M)	47.6±5.41	14.2± 2.12	48.12±5.79

5.5. Phase - V

5.5.1 Physicochemical characterization of untreated and *S. wightii* treated effluent

The physicochemical characteristics of the raw and treated textile effluent sample were depicted in table 25.

5.5.1.1 Untreated textile effluent characterization

The textile effluent sample was found to be dark violet in colour with bad odour. The electrical conductivity recorded was 3100 µmho/cm. The temperature of the sample at the time of collection was 40ºC. The values of total suspended solids (TSS), total dissolved solids (TDS) and total solids (TS) were found to be 2000, 6000 and 8000 mg/l respectively.

The effluent showed a pH of 9.5 and a total alkalinity of 470 mg/l. Total hardness recorded was 895 mg/l. Dissolved oxygen content (DO) was 0.28 mg/l. Biological oxygen demand (BOD) and chemical oxygen demand were 90 mg/l and 571 mg/l respectively.

The amount of chlorides, sulphates, phosphates and nitrates were 1298, 1496, 2.20 and 159 mg/l respectively. The amount of lead, nickel, zinc, chromium and copper recorded the values of 1.3, 2.50, 5.46, 1.83 and 3.21 mg/l respectively. The amount of oil and grease estimated was 14.7 mg/l.

The results of the physicochemical analysis showed that the untreated textile effluent is characterized by the presence of colour with objectionable odour, high electrical conductivity, high TSS, TDS values, alkaline pH, high BOD and COD, low DO, high amounts of chlorides and sulphates, nitrates and also showed the presence of heavy metals, oil and grease.

5.5.1.2 Characterization of *S. wightii* treated effluent

After treatment the effluent became colourless and odourless and recorded a decrease in EC, TSS, TDS and TS. These values were 490 µmho/cm and 200, 2000, 2200 mg/l respectively.

Change in pH from 9.5 to 7.5 was observed. Considerable decrease was observed in total alkalinity (70 mg/l) and total hardness (140 mg/l). Reduction in BOD (21.0 mg/l) and COD (225 mg/l) were recorded. The amount of chlorides, sulphates, phosphates and nitrates recorded a decrease from 1298, 1148, 2.18 and 80 mg/l to 900, 507, 1.31 and 25.58 mg/l respectively. Decrease in heavy metal concentration was also observed. The values were 0.34, 0.74, 2.36, 1.60 and 2.74 for lead, nickel, zinc, chromium and copper respectively. The value for oil and grease decreased from 14.7 to 3.0 mg/l.

The above results showed a significant difference between treated and untreated textile effluent with respect to the physicochemical parameters. In comparison with BIS limits it was found that the parameters exceeding the prescribed permissible limit are TSS, TDS, pH, BOD, COD, chlorides, nitrates, lead, zinc, copper, oil and grease for raw effluent, whereas the treated effluent recorded all these parameters within the permissible limit.

Colour of the effluent depends on the dyes used. Different colour marks are used for dyeing different types of cloth. Both synthetic and natural colours easily disperse in water (Tortman and Tortman, 1964; Cock, 1964). The color value of wastewater is extremely pH dependent and it invariably increases as the pH of the effluent is raised or lowered. The textile wastewater is highly coloured showing the presence of high concentrations of unused dyes. According to Oke *et al.* (2006), the textile effluents show high colour and this may be the combined results of pH, temperature and acidic conditions that do not allow the chromophore group of dye to degrade making effluent highly colored.

The bad odor could be due to unpleasant odor of volatile compounds. Unpleasant or pungent odour for textile effluent was reported (Arul *et al.*, 2011; Ogunlaja and Aemere, 2009).

The electric conductivity (EC) is usually used for indicating the total concentration of the ionized constituents of water and an indirect measure of ions or charge carrying species in the effluents (Sultana *et al.*, 2009).

High TSS and TDS detected could be attributed to the presence of high colour and they may be the major sources of the heavy metals (Yusuff and Sonibare, 2004).

The high pH of effluent indicates the excessive use of dyes. pH value of wastewater has no health implication but many chemical reactions are controlled by pH. Biological activities and some chemical treatment processes are usually restricted by pH. Federal Environmental Protection Agency (FEPA) recommends pH value of 6 – 9 for effluent to be discharged into stream, as either high or low pH will be harmful to man, aquatic animals and will disturb biological activity of stream if discharged untreated.

Total alkalinity has been reported as a major factor which influences pH (Wetzel, 1972; Verma and Deleta, 1975). Alkalinity is the capacity of water to neutralize acid and is characterized by the presence of hydroxyl ions.

The hardness of water is not a chemical parameter but indicates the water quality mainly in terms of Ca^{2+} and Mg^{2+} and expressed as $CaCO_3$. The hardness has no known adverse effect. Their low concentration in the textile wastewater may be due to the use of soft water in the dyeing and printing process to avoid coagulation of dyes (Hussain and Hussain, 2012).

Dissolved oxygen is essential to all forms of aquatic life including those organisms responsible for the self purification processes in natural waters. Like terrestrial animals, fish and other aquatic organisms need oxygen to live. The presence of oxygen in water is a positive sign of a healthy body of water but the absence of oxygen is a signal of severe pollution (Sultana *et al.*, 2009).

High BOD indicates that there could be low oxygen available for living organisms in the waste water. The high BOD may deplete dissolved oxygen, causing death of aerobic organisms and increase anaerobic properties of water (Jody and Dons, 2003). A high level of COD implies toxic conditions and the presence of biologically resistant organic substances in waste water. It determines the oxygen required for the chemical oxidation of organic matter and assesses the quantity of chemically oxidizing matter in water (Sawyer and McCarty, 1978).

Chloride in the waste water might be due to water softening process or when sodium chloride is used to recharge softeners. The high concentration of sulfate in the untreated waste water is due to use of sulfuric acid in various steps of dyeing and printing process (Hussain and Hussain, 2012).

Presence of heavy metals in the waste water may be due to use of some dyes in which these metals are complexed. It have been reported that the major problem associated with textile processing effluents is presence of heavy metals, which arise from materials used in the dyeing process or in a considerably high amount, from metal containing dye (Correia, 1998).

Table. 25 Physico-chemical analyses of untreated and *S. wightii* treated dye compared with BIS permissible limits

Parameters	Untreated effluent	Treated effluent	BIS permissible limits	Appendix
Colour	Dark violet	Pale violet	-	1
Odour	Bad odour	Obnoxious	-	2
pH	9.5	7.5		4
Temperature	40	35	>40	3
EC (µmhos/cm)	3100	490	600	5
TSS	2000	200	100	6
TDS	6000	2000	2100	7
TS	8000	2200	-	8
DO	0.28	3.5	-	11
BOD	90	21	30	12
COD	571	225	250	13
Total hardness	895	140	600	9
Total alkalinity	470	70	200-600	10
Chloride	1298	900	1000	14
Sulphate	1496	507	1000	16
Phosphate	2.20	1.31	5	15
Nitrate	159	25.58	50	17
Lead	1.3	0.34	0.1	18
Nickel	2.50	0.74	3	19
Zinc	5.46	2.36	5	20
Chromium	1.83	1.60	2	21
Copper	3.21	2.74	3	22
Oil and grease	14.7	3	10	23

All the values except pH are in mg/l

5.5.2 *Sargassum wightii* efficiency in the removal of textile effluent- a batch experiment

5.5.2.1 Effect of pH

Table. 26 Effect of pH on textile effluent removal by *S. wightii*

pH	Decolourization (%)
3	25
4	36
5	49
6	**65**
7	62
8	55
9	50
10	45

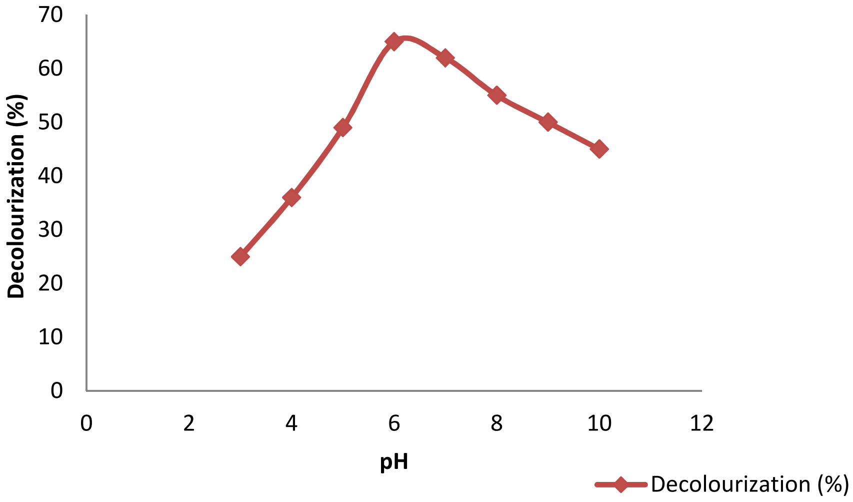


Fig. 48 Effect of pH on textile effluent removal by *S. wightii*

The effect of pH on textile effluent removal by *S. wightii* was presented in table 26 and figure 48. The effect of pH was studied by varying the pH from 3 to 10. At pH 3.0 the minimum adsorption of 25% was observed. With further increase in pH, a gradual increase in adsorption was observed, with 36% at pH 4 and 49% at pH 5.00, which recorded a maximum of 65 at pH 6. pH 7 recorded a slight decrease in adsorption (62). Above pH 7 the adsorption percentage was gradually decreased and recorded 45% at pH 10.

The above results indicate that the dye adsorption considerably increased when pH was raised from 3 to 6. At pH 3, per cent dye removal was very low which increased as pH was raised to 6.00. Slightly acidic pH favours dye adsorption. Similar observation was reported by Nagarani *et al.* (2012) who reported that maximum textile effluent colour removal using *Sargassum wightii* was observed at pH 5 and surface charge of the adsorbent was positive.

Several reasons may be attributed to dye adsorption behaviour of the adsorbent relative to solution pH. The electrostatic attraction as well as the organic properties of the adsorbent and structure of dye molecules could play important roles in dye adsorption. Adsorbent's surface is positively charged at acidic pH. This causes competition between protons and dye cations for adsorption locations. Under acidic condition the dye uptake for acidic dye (anionic dye) is higher as compared to the basic and neutral conditions. When pH is raised a negative charge is present on the surface of the adsorbents causing better dye cation adsorption through electrostatic attraction. Sharmila *et al.* (2016) reported that the maximum reduction of effluent colour was found in activated carbon of seaweed *Gracilaria corticata* at pH 7 of 37° C.

Thus the pH of the solution is an important process controlling parameter in the adsorption of dye. The initial pH values of the dye solutions affect the surface charge of the adsorbent and thus the adsorption of charged dye groups on it. In the present study, slightly acidic condition (pH 6) favours dyes adsorption from the textile effluent. Therefore, it is suggested that the optimum pH for the removal of different dyeing effluent is around 6.

5.5.2.2. Effect of temperature

Table. 27 Effect of temperature on textile effluent removal by *S. wightii*

Temperature (°C)	Decolourization (%)
25	28
30	36
35	**49**
40	47
45	44
50	43
55	42

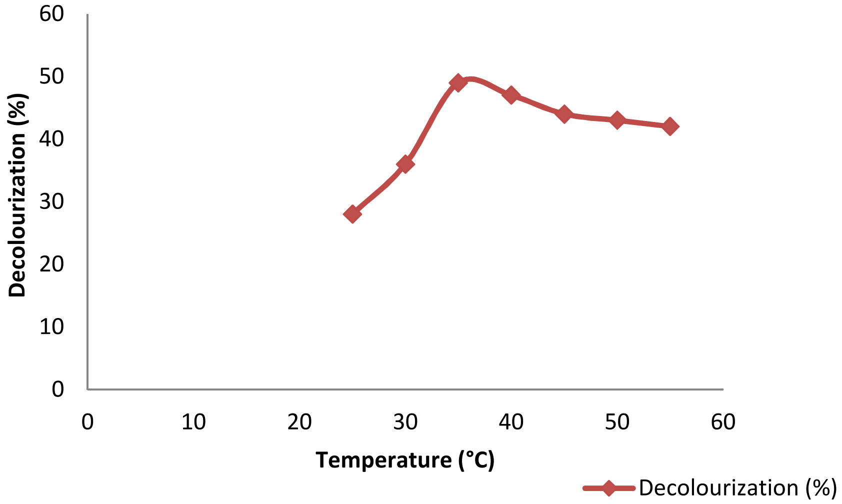


Fig. 49 Effect of temperature on textile effluent removal by *S. wightii*

The effect of temperature on effluent removal by *S. wightii* was presented in table 27 and figure 49. The effect of temperature was studied in the range from 25°C to 45°C. The percentage of dye removal was increased from 28.57 to 48.28 for the rise in temperature from 25°C to 35°C. Further increase in temperature from 35°C to 45°C could not increase dye removal, rather a decrease was observed from 49 % to 44 %. Therefore, the maximum removal of dye from effluent was achieved at 35°C.

This was achieved because as temperature increase from 25°C to 35°C, a slight increase in surface area of the adsorbent could be possible but further increase in temperature could result in the loss of active surface area resulting from prolonged exposure to high temperatures (Amin, 2009). Hence, the adsorption was slow at high temperatures. The optimum temperature was 35°C at which the adsorption was very effective and the present study shows the exothermic nature of adsorption.

5.5.2.3. Effect of contact time

Table. 28 Effect of contact time on textile effluent removal by *S. wightii*

Days	Decolourisation (%)
1	31
2	45
3	60
4	61
5	**62**
6	61
7	61

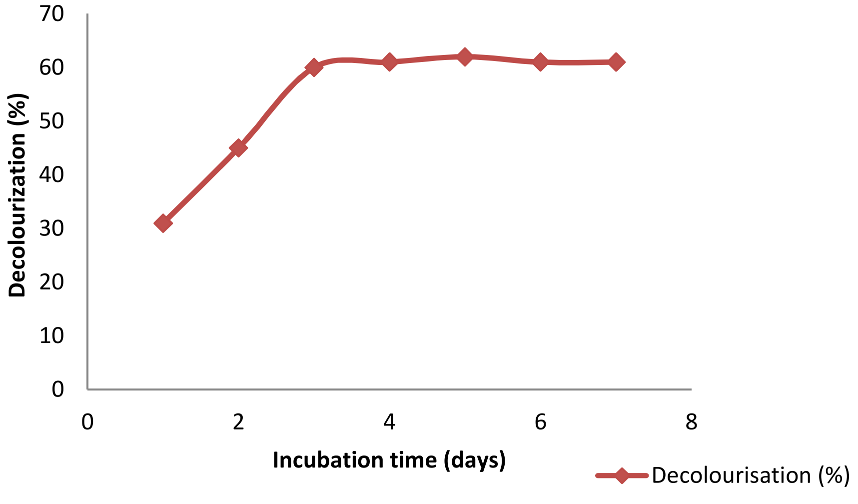


Fig. 50 Effect of contact time on textile effluent removal by *S. wightii*

The effect of contact time on colour removal was observed from 1 to 7 days and the result was presented in table 28 and figure 50. From the figure, the plot reveals that the uptake of dye was rapid in the first 5 days and after 5th day the amount of dye removal was almost constant. The curve on contact time was found to be smooth and continuous leading to saturation indicating monolayer coverage of dye on adsorbent surface. Sorption rapidly occurs and normally controlled by the diffusion process from the bulk to the surface. In the later stage, the sorption is likely an attachment controlled process due to less available sorption sites.

At the beginning the dye is adsorbed by the exterior surface of the adsorbent and the adsorption rate is fast. When the exterior adsorption surface saturate the dye enters into the pores of the adsorbent and is adsorbed by the interior surface of the particles. The first rapid uptake can be rationalized as a rapid attachment of dyes to the biosorbent surface or due to the large number of vacant sites available at the initial stage. After laps of some time, the remaining vacant surface sites are difficult to be occupied due to repulsive forces between the solute molecules adsorbed on the solid surface and the bulk phase (Vijayaraghavan *et al.*, 2008). Thus the contact time between dyes and the adsorbent is of significant importance in the wastewater treatment by adsorption. A rapid uptake of dyes and establishment of equilibrium in short period signifies the efficiency of the adsorbent for its use in wastewater treatment.

5.5.2.4. Effect of adsorbent dose

Table. 29 Effect of adsorbent dose on textile effluent removal by *S. wightii*

Adsorbent dose (g)	Decolourisation (%)
0.1	52
0.2	58
0.3	**69**
0.4	56
0.5	46
0.6	38

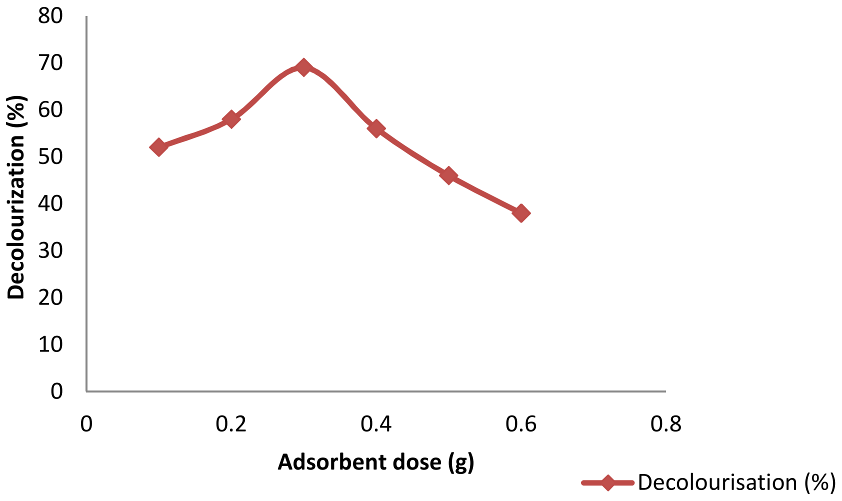


Fig. 51 Effect of adsorbent dose on textile effluent removal by *S. wightii*

The effect of bisorbent dose (0.1-0.6g) on the removal of effluent by *S. wightii* was presented in table 29 and figure 51. The maximum dye removal was observed at 0.3g (69%) biosorbent dose. When the biosorbent dose was further increased from 0.3 to 0.6 there was decrease in the biosorption capacity of biosorbent. At the adsorption dose of 0.6g, the per cent dye removal was 38. Adsorption efficiency decreased with increase in the biosorbent dose.

The same result is in coherence with Aswin, 2015 who studied the impact of sorbent dose (0.1- 0.5g) of *Sargassam longifolium* and stated the greatest balance uptake limit is found to happen for sorbent measurements of 0.1g. The equilibrium uptake limit was found to decrease with expanding adsorbent measurement. This could be clarified by the way that the measure of dye to be adsorbed is part among the expanded dye adsorption sites with increasing sorbent dosage prompting lower particular dye uptake limit.

The results also revealed that the colour removal percentage increase with increase in adsorbent dosage and decrease at higher doses. This is because, the active sites in the bio-adsorbents could not be effectively utilized when the dosage is low and thereafter bio-adsorbents could be effectively utilized. When the bio-adsorbent dosages are higher, it is more likely that a significant portion of the available active sites remain uncovered, leading to lower specific uptake. Biosorbent dose plays a very important role in the process of biosorption. The dye biosorption capacity decreased at higher biosorbent doses due to the aggregation of the biomass which results in the decrease in active sites on the surface of biosorbent available for the attachment of dye molecules.

From the optimization studies it may be concluded that the most efficient operational parameters in the current study were found to be; pH of 6, temperature of 35ºC, contact time of 5th day and adsorbent dose of 0.3g for the treatment of textile effluent of 50% dilution using *Sargassum wightii* as an adsorbent.