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Effect of Textile Industry Effluent on Growth and Biochemical Parameters of *Tagetes Erecta*

Aarti, Richa Gupta and Amita Mittal*

Department of Biotechnology, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra- 136119, Haryana, India; amitakuk@gmail.com

Abstract

Tagetes erecta plants were exposed to different concentrations of textile mill effluent. Various physio-biochemical parameters (plant height, number of branches and leaves per plant, chlorophyll content, soluble protein, soluble sugars, proline content and malondialdehyde) were studied at different intervals of time. At lower concentrations, an increase in chlorophyll content, proline, protein and sugar content was observed but on increasing the effluent concentration, a decrease was observed for all these parameters after a certain period of time. Our results indicate that the exposition of *Tagetes erecta* to diluted concentrations of textile mill effluent for short duration of time results in an increase in growth and other parameters which ultimately result in better productivity. The study was investigated in relation to both concentration of effluent and time intervals of supply of . It is suggested that wastewater should be diluted before it is used for irrigation.

Keywords: Chlorophyll, Effluent, Malondialdehyde, Proline, Tagetes Erecta, Textile

1. Introduction

Disposal of wastewater is a worldwide problem faced by industries[1,2]. These wastes (effluents) are released in the environment after treatment (developed countries) or mostly without treatment (developing countries such as India and Pakistan etc). In India also being a cheap source of irrigation farmers are applying this water to their fields. In developing countries, there has not been much emphasis on the installation of effluent treatment plants and all the industrial effluents are generally either released into some water body or directly on to the lands, which are mostly agricultural. Sometimes these effluents are purposely used for irrigation due to scarcity of water, especially for raising vegetables and fodder etc [3]. In India, very few industries are equipped with satisfactory operating treatment facility set up, so there is common trend that industries dispose of untreated effluents via open and covered routes into the water ways which degrade water quality [4]. Effluent, when discharged as such (untreated or partially treated) into the water bodies, adversely affects aquatic ecosystem by reducing the penetration power of sunlight and ultimately reduces the photosynthetic activities and dissolved oxygen content, whereas in agricultural soil it causes inhibition of seed germination by reducing soil alkalinity and manganese availability [5]. These effluents contain heavy metals as well as nutrients, which affect plant and soil in variety of ways[6]. Most of the studies conducted so far are to investigate the effects of different industrial effluents on vegetable crops and cereals. Hence, wastewater requires pre-treatment before its safe disposal into the environment [5,7]. Keeping in view the importance of pollution and lack of knowledge regarding the effects of industrial effluents on plants, present study was carried out.

2. Material And Method

Plants used in the experiment were obtained from Gurukul nursery, Kurukshetra. The experimental plant marigold (*Tagetes erecta*) reaches height of 50-100 cm. To find out the effect of textile industry on the growth of *Tagetes*

* *Author for correspondence*

erecta, a pot experiment was conducted in the natural open weather conditions for 70 days during the plant season. Plants with uniform size and color were chosen for experimental purpose. Plants were grown in pots in untreated soil (control) and in soil to which industrial effluent concentartions (25%, 50% and 75%) had been applied. All the parameters were done in triplicates. Water (100 ml) was given in control as well as treated plants daily except the 9th day in case of treated plants. In treated plants, effluent of varying concentrations was given after every 8 days. Comparison of effluents exposed plants were made with untreated (control) plants. The data pertaining to plant growth, chlorophyll, soluble protein, total soluble sugar, proline content and malondialdehyde content was recorded after 8, 16, 24 and 32 days after treatment.

2.1 Physiochemical Parameters

Analysis of Physicochemical parameters of textile Effluent was done. pH, Temperature and Electrical conductivity were determined.

2.2 Physical Parameters

Physical Parameters were recorded after interval of 8 days in control and treated plants. Plant Height was measured with the help of meter scale. Number of leaves and number of branches per plant were counted. Any visible wilting, whitening or yellowing of leaves or any other plant part was also observed and noted.

2.3 Biochemical Parameters

2.3.1 Determination of chlorophyll

Chlorophyll content was determined[8]. Fresh leaves (100 mg) were kept in 2 ml extraction reagent, dimethylsulphooxide (DMSO). The tubes were kept in the oven at 65^{0}C for 40 min. 1 ml aliquot was mixed with 2 ml DMSO and vortexed. Absorbance was determined photometrically at 645 and 663 nm using DMSO as blank. The amount of chlorophyll was calculated by using the equations as follows[9]:

Chl a (mg/l) = 12.7 A663 – 2.69 A645
Chl b (mg/l) = 22.9 A645 – 4.68 A663
Total Chl (mg/l) = 20.2 A645 + 8.02 A663

2.3.2 Protein Estimation

Protein content was estimated by the method of Bradford[10]. Fresh leaves (300 mg) were homogenized in 1ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5000 rpm for 10 min. Half ml of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000 rpm for 15 min. The supernatant was dissolved in 1ml of 0.1N NaOH and 5 ml of Bradford reagent was added. The colour developed was measured photometrically at 595 nm using bovine serum albumin as a standard.

2.3.3 Estimation of Proline

Proline concentration was estimated[11]. Fresh leaves (200 mg) were homogenized in 10 ml of 3% aqueous sulphosalicylic acid in the pestle and mortar. The homogenate was centrifuged at 9000 rpm for 15 minutes. 2 ml aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin (1.25 g ninhydrin in 30 ml acetic acid and 20 ml 6N phosphoric acid) and kept for 1 hour at 100^{0}C in boiling water bath. After development of color (pinkish red), the tubes were placed on ice bath for termination of reactions. 4 ml of toluene was added to the reaction mixture and extract was vortexed for 20-30 seconds on vortex shaker. Two immiscible layers formed after shaking, were separated using pipette. Upper aqueous pinkish-red layer containing toluene was taken and absorbance determined photometrically at 520 nm using toluene as blank.

2.3.4 Estimation of Soluble Sugars

Soluble sugars were estimated[12]. Fresh leaves (300 mg) were kept in 10 ml of alcohol for 1 hour at 60^{0}C in incubator. The extract was then decanted into a 25 ml volumetric flask and the residue re-extracted with another 10 ml volume of 90% ethanol. Final volume was made up to 25 ml by adding 90% alcohol. 1 ml of alcoholic extract was taken in a test tube and 1.0 ml of 5% phenol was added. 5 ml of conc. sulphuric acid was added. The contents in the tube were shaken carefully. Reaction mixture was allowed to stand for 30 min. at room temperature. Yellow-orange color developed was measured photometrically at 485 nm using distilled water as blank and total sugar content was estimated by using a standard curve of D-glucose.

2.3.5 Malondialdehyde (MDA)

Malondialdehyde was estimated[13]. Freshly harvested plant leaves (300 mg) were homogenized thoroughly in 5% trichloro acetic acid (TCA). The homogenate was

centrifuged at 12,000 rpm for 15 min at 25^0 C. Supernatant was used for the estimation of malondialdehyde. The TBA reactivity (TBAR) was determined by mixing 2ml of the supernatant obtained (as mentioned above) to an equal aliquot of 0.5% TBA in 20% tri-chloro acetic acid. The mixture was heated at 95^{0}C for 25 min to obtain orange colour and then centrifuge for 5 min. to get a clear supernatant. The absorbance of the supernatant was measured photometrically at 532 nm. The amount of malondialdehyde (MDA) was calculated by using the formula:

MDA equivalents (nmol.cm^{-1}) = 1000[(Abs 532- Abs 600 nm)/155].

3 Results and Discussion

3.1 Physicochemical Parameters

The results obtained on the analysis of the physico-chemical parameters of textile industry effluents were summarized and discussed as shown in "Table 3.1".

Table 3.1 Physico-chemical parameters of textile effluents

No.	Physico-chemical parameters	Textile industry effluent
1.	pH	8.02
2.	Electrical conductivity (dSm-1)	5.96
3.	Temperature	280 C
4.	Colour	Brick red
5.	Texture	Clay loam
6.	Odour	Odourless

3.2 Physical Parameters

Effect of different concentrations of textile industry effluent on the plant height (cm), number of leaves and number of branches of *Tagetes erecta* are shown in "Table 3.2, 3.3, 3.4", respectively. Plant height, number of leaves and number of branches increases with increase in concentrations of textile effluent but with continuous use of effluents at high concentrations i.e. 50% and 75%, the decrease of physical parameters were observed after 24 and 32 days. Whitening and wilting of leaves were also observed at higher concentration of effluent after 24 days.

Figure 3.1 Effect of industrial effluents on physical parameters of plants after 24 days at 75% concentration.

The present findings show similarities with those reported earlier, who stated that irrigation with a mix of municipal and textile effluents improved the growth, biomass, and nutritional status of *Eucalyptus camaldulensis* seedlings, and 50% diluted textile effluent increased the seed germination, total sugars, starch, reducing sugars, and chlorophyll compared to distilled water (control) in *Arachis hypogea* seedlings, respectively[14]. Meanwhile, other researcher proposed that the effect of textile effluent

Table 3.2 Effect of different concentrations of textile industry effluent on the plant height (cm) of *Tagetes erecta*. Values are mean ± SE (n=3)

Textile industry effluent (Conc.)	Number of days			
	8	16	24	32
Control	8.75 ± 0.18	13.5 ± 0.34	17.5 ± 0.35	19.75 ± 0.53
25%	12.83 ± 0.89	14.5 ± 0.24	20.5 ± 0.24	24.16 ± 0.59
50%	13.83 ± 1.34	18.33 ± 2.73	23.83 ± 3.20	25.66 ± 2.72
75%	14.16 ± 1.98	18.16 ± 1.11	23.16 ± 2.79	25 ± 2.46

Table 3.3 Effect of different concentrations of textile industry effluent on the number of leaves per plant of *Tagetes erecta*. Values are mean ± SE (n=3)

Textile industry effluent (Conc.)	Number of days			
	8	16	24	32
Control	55 ± 0.71	68.5 ± 1.06	103 ± 1.63	125 ± 2.36
25%	72 ± 8.55	109.66 ± 4.95	144 ± 3.74	166 ± 6.94
50%	84 ± 3.03	131 ± 2.49	155.33 ± 4.77	169 ± 4.78
75%	87 ± 0.94	141.33 ± 1.91	153.33 ± 8.50	165.66 ± 4.74

Table 3.4 Effect of different concentrations of textile industry effluent on the number of branches per plant of *Tagetes erecta.* Values are mean ± SE (n=3)

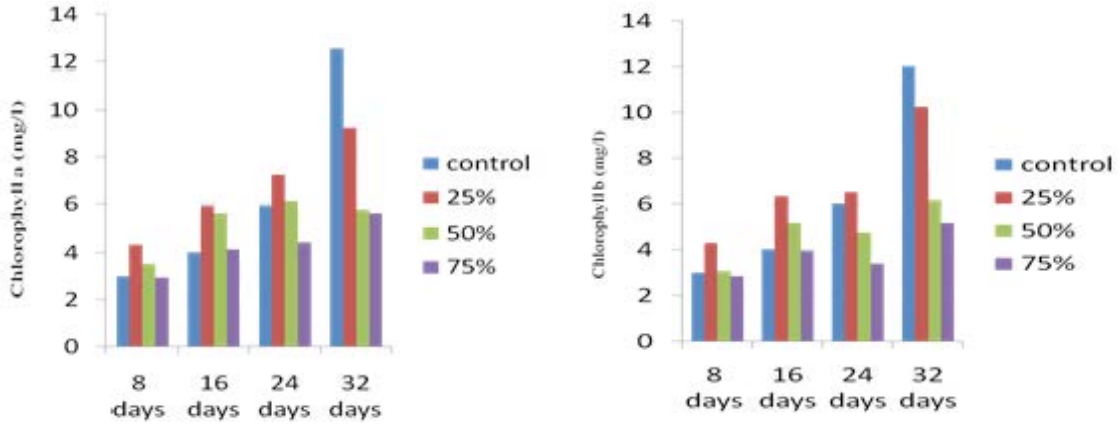
Textile industry effluent (Conc.)	Number of days			
	8	16	24	32
Control	6.33 ± 0.27	7.33 ± 0.27	8.66 ± 0.27	12 ± 0.47
25%	8.33 ± 0.27	9.33 ± 0.72	13 ± 0.47	15.66 ± 0.27
50%	9.33 ± 0.54	9.66 ± 1.44	13.66 ± 1.09	16.66 ± 0.27
75%	10.66 ± 0.27	11.33 ± 0.27	11.66 ± 0.27	14.66 ± 0.27

is cultivar specific, and care should be taken before using textile effluent for irrigation purposes. Generally, while low concentrations promote plant growth, more elevated amounts have negative effects[15].

3.3 Biochemical Parameters

3.3.1 Determination of chlorophyll

It was observed that 25% concentration of textile industry effluent increased the chlorophyll content. The prolonged exposure of industrial effluents reduced the chlorophyll content in plants. Chlorophyll a was decreased at high concentration (50% and 75%) of textile industry effluent after 8, 16, 24 and 32 days "Figure 3.1". Chlorophyll b was decreased at 75% concentration after 16, 24 and 32 days. It was observed that 50% and 75% concentration of textile effluent decreases chlorophyll b content "Figure 3.1". Total chlorophyll content decreased at high concentrations (50% and 75%) of textile industry effluent observed after



A B

Figure 3.1 Effect of different concentrations of textile industry effluent on A. chlorophyll a, B. chlorophyll b (mg/l) of *Tagetes erecta.* Values are mean (n=3).

8, 16, 24 and 32 days "Figure 3.2". It was observed that at low concentration of textile industry effluents the chlorophyll content (chl a, chl b and total chlorophyll) increased marginally. The prolonged exposure to high concentration of industrial effluents reduced chl a, chl b, total chlorophyll content in plants. It may be due to the breakdown of chlorophyll during stress or due to inhibition of chlorophyll biosynthesis[16]. Various abiotic stresses decrease the chlorophyll content in plants[17]. Similarly, decrease in chlorophyll content was observed in *Cyamopsis tetragonoloba* when treated with plate making industry effluent[18].

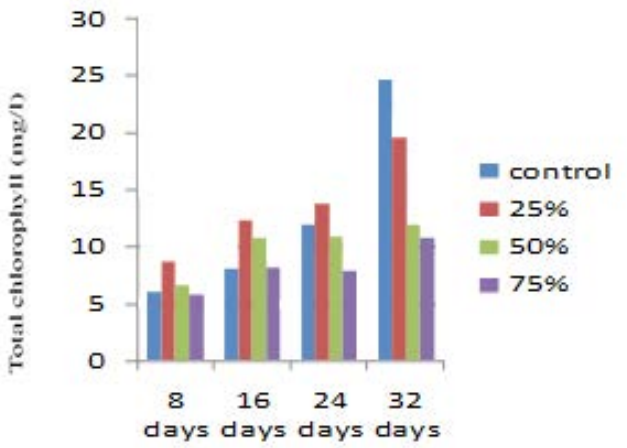


Figure 3.2 Effect of different concentrations of textile industry effluent on total chlorophyll (mg/l) of *Tagetes erecta.* Values are mean (n=3).

3.3.2 Estimation of Protein

Protein concentration increased at lower concentrations (25% and 50%) of textile industry effluents; however, at 75% effluent concentration, the soluble protein concentration declined in *Tagetes erecta* after 16, 24 and 32 days as shown in "Figure 3.3". Protein content was the most sensitive macromolecule affected by effluents. In the present study, it was observed that with high concentration of textile industry effluents treatment the soluble protein content in *Tagetes erecta* declined. Abiotic stress may inhibit synthesis of some proteins[19]. The decrease in protein content in *L. polyrrhiza* may be caused by enhanced protein degradation process as a result of increased protease activity under stress conditions[20].

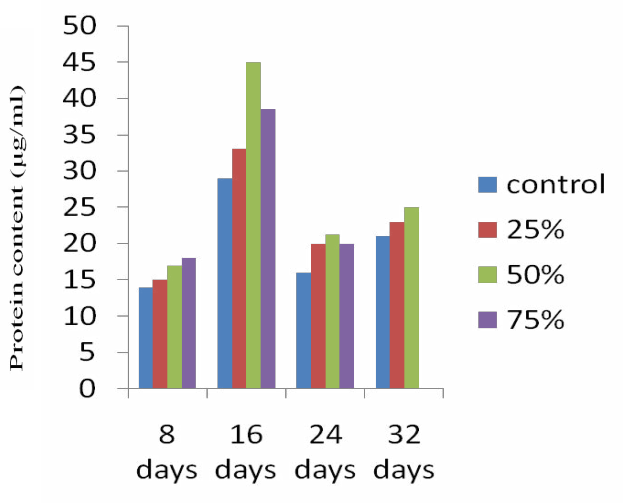


Figure 3.3 Effect of different concentration of textile industry effluent on protein content (µg/ml) of *Tagetes erecta.* Values are mean (n=3).

3.3.3 Estimation of Proline

It was observed that proline content increased at 25% concentration of textile industry effluents, however, at 50% and 75% concentrations, proline content declined after 24 and 32 days as shown in "Figure 3.4". Proline, an amino acid, is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress[17]. Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to cadmium toxicity was demonstrated but was found that proline accumulation decreased with the exposure to cadmium in hydrophytes (*Ceratophyllum, Wolffia*, and *Hydrilla*)[21].

3.3.4 Estimation of Soluble Sugars

It was observed that lower concentration (25%) of textile industry effluents increased the soluble sugar content; however, 50% and 75% concentrations of effluent showed a decrease in soluble sugars after 32 days as shown in "Figure 3.5".

3.3.5 Malondialdehyde (MDA)

It was observed that malondialdehyde content increased at all the concentration of textile industry effluent up to

24 days after treatment however, after 32 days at 75% concentration of textile effluent, MDA content was decreased as shown in "Figure 3.6". Malondialdehyde content was higher in test plants as compared to control plants. Our results correlated with the findings of cassava processing effluent that have reported increased MDA levels in *Allium cepa*[22] and increased MDA content in Maize plants treated with sugar mill effluent[23]. Effluent induced increase in MDA has been reported in *Pisum sativum*[24] and soybean[25].

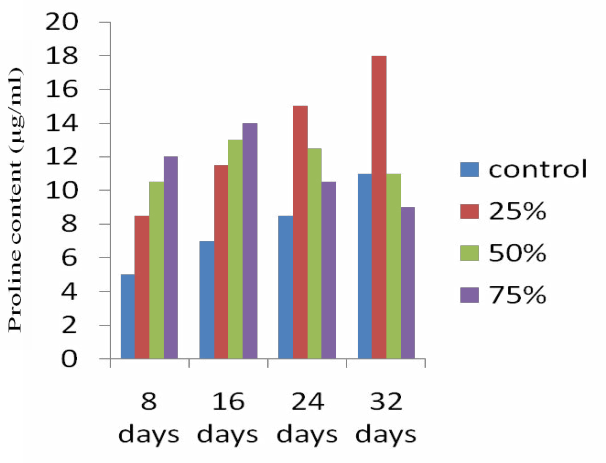


Figure 3.4 Effect of different concentration of textile industry effluent on proline content (µg/ml) of *Tagetes erecta.* Values are mean (n=3).

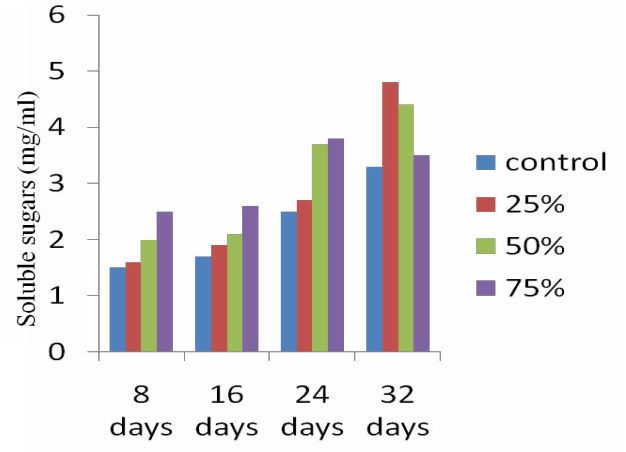


Figure 3.5 Effect of different concentration of textile industry effluent on soluble sugars (mg/ml) of Tagetes erecta. Values are mean (n=3).

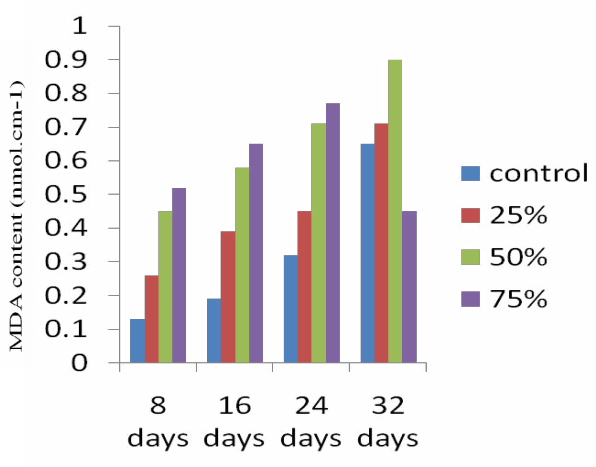


Figure 3.6 Effect of different concentration of textile industry on malondialdehyde content (nmol.cm-1) of *Tagetes erecta.* Values are mean (n=3).

Our results indicate that the exposition of *Tagetes erecta* to different concentrations of textile industry effluents results in an increase in growth, proline content, proteins, soluble sugar content and malondialdehyde at low concentration upto a certain period of time, but with continuous use of effluents at high concentrations, growth and biochemical parameters were decreased. The effluents nominally decreased the photosynthetic pigments in *Tagetes erecta*. The effects were investigated in relation to both concentration of effluent and time of exposure to the effluent. It is suggested that wastewater should be diluted before it has to be used for irrigation. At low concentration (25%), effluents served as a liquid fertilizer and enhanced growth of *Tagetes erecta*. The results suggested that diluted industrial effluents (≤ 25%) could be used for irrigation. Phytoremediation may contribute in the treatment of various sites contaminated with industrial effluents. Therefore, application of industrial effluent to agricultural soils may be sustainable and economical due to nutrient cycling and disposal of waste water.

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5. References

1. Malaviya P,Rathore VS. A correlation study on some physicochemical quality parameters of pulp and paper mill effluent. Poll. Res.2001; 20: 465–70.
2. Kohli R Malaviya P. Impact of tannery effluent on germination of various varieties of wheat (Triticum aestivum L.). J. Appl. Nat. Sci.2013; 5:302–5.
3. Ghafoor A, Rauf A, Arif M, Muzaffar W. Chemical composition of effluents from different industries of the Faisalabad city. Pak. J. Agri. Sci.1994; 31: 367–69.
4. Farid S. Heavy metal ion concentration in wheat plant (Triticum aestivum) irrigated with city effluent. Pak. J. Sci. Ind. Res.2003; 46: 395–98.
5. Dhevagi P,Oblisami G. Effect of paper mill effluent on germination of agricultural crops. J. Ecobiol.2002; 4: 243–49.
6. Kumar P,Chandra R. Decolourisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures of Bacillus sp. Biores. Technol.2006; 7: 2096–102. Crossref.
7. Mohana S, Acharya KB,Madamwar D. Distillery spent wash: treatment technologies and potential applications. J. Haz. Mater2009; 163:12–25. Crossref.
8. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian journal of botany.1979; 57: 1332–34. Crossref.
9. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in Beta vulgaris. Plant physiology.1949; 24: 1–15. Crossref.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem.1976; 72: 248–54. Crossref.
11. Bates L, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil.1973; 39: 205–7. Crossref.
12. Dey PM. Methods in plant biochemistry. Vol-II. Carbohydrates. (Publ.) London: Acad. Press;1990. PMCid:PMC54196
13. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives in Biochemistry and Biophysics.1968; 125: 189–98. Crossref.
14. Bhati M, Singh G. Growth and mineral accumulation in Eucalyptus camaldulensis seedlings irrigated with mixed industrial effluents. Bioresource Technology.2003; 88: 221–8. Crossref.
15. Kaushik P, Garg VK,Singh B. Effect of textile effluents on growth performance of wheat cultivars. Bioresource Technology.2005; 96:1189–93. Crossref.
16. Kumar PS, Kumar SS, Anuradha K, Sudha B, Shahbaj A. Phytoremediation as an alternative for treatment of paper industry effluents by using water hyacinth (Eichhornia crassipes). Int. J. of Research and Environment.2012; 2: 95–9.
17. Ahmad P, Sharma S, Srivastava PS. Differential physio-biochemical responses of high yielding varieties of Mulberry (Morusalba) under alkalinity (Na2CO3) stress in vitro. Physiol. Mol. Biol. Plants.2006; 12: 59–66.
18. Selvaraj K, Sevugaperumal R, Ramasubramanian V. Nullifying the toxicity of plate making industry effluent using seaweed Ulva lactuca. Indian J. of Fundamental and Appl. Life Sciences.2012;, 2(2):79–86.
19. Ericson MC,Alfinito AE. Proteins produced during salt stress in tobacco cell cultures. Plant Physiol.1984; 74: 506–9. Crossref.
20. Palma JM, Sandalio LM, Javier CF, Romero PMC, McCarthy I, DelRio LA. Plant proteases protein degradation and oxidative stress: role of peroxisomes. Plant Physiol. Biochem.2002; 40:521–30. Crossref.
21. Dhir B, Sharmila P, Saradhi PP. Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. Aquat. Toxicol.2004; 66: 141–7. Crossref.
22. Olorunfemi DI, Lolodi O. Effect of cassava processing effluents on antioxidant enzyme activities in Allium cepa L. Biokemistri.2011; 23(2):49–61.
23. Hussain I, Iqbal M, Nawaz M, Rasheed R, Perveen A, Mahmood S, Yasmeen A, Wahid A. Effect of sugar mill effluent on growth and antioxidative potential of maize seedling. Int. J. Agric. Biol.2013; 15: 1227–35.
24. Ahmada PR, Johnb M, Sarwatc, Umard S. Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of Pisumsativum L. under salt stress. Int. J. Plant Prod.2008; 2: 1735–8043.
25. Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Golezani KG. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (Glycine max L.). Plant Omics J.2012; 5:60–7.