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Phytotoxic effect of chrome liquor on growth and chlorophyll content of *Spirodela polyrrhiza* L. Schleid

Asha Singh and Piyush Malaviya*

Department of Environmental Science, University of Jammu, Jammu-180006 (J&K), INDIA
*Corresponding author. E-mail: piyushmalaviya@rediffmail.com

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Abstract: The present study assessed the tolerance of *Spirodela polyrrhiza* (L.) Schleid (giant duckweed) exposed to different concentrations (5% -100%) of chrome liquor for 7 days. Physiological conditions of *S.polyrrhiza*plants were monitored daily by measuring fresh weight and chlorophyll-a and chlorophyll-b. Fresh biomass of *Spirodela* plant showed concentration and duration dependent reduction with minimum value (5.71 g) reported on 7 d at 100% tannery effluent concentration. Likewise, significant reduction in chlorophyll-a and chlorophyll-b content was observed in concentration-duration dependent manner with maximum reduction reported to be 99.8% and 99.6% respectively on 7 d at 100% effluent concentration in comparison to control.

Keywords: Chlorophyll, Chrome liquor, *Spirodela,* Tannery, Toxicity

INTRODUCTION

The tanning industry is one of the major consumers of water and chrome tanning is the most common type of tanning where a large amount of basic chromium sulphate is used. Tannery wastewater contains very high values of BOD, COD, total solids, chlorides, sulphates and chromium (Bajza and Vrcek, 2001). Chromium (Cr), in the spent chromium liquor from the tannery industry practicing classical chromium tanning method, may be as high as 1300–2500 mg L^{-1} (Hafez and El-Manharawy, 2004; Malaviya and Singh, 2011). Exposure of Cr(VI) to humans may result in chrome ulcers, corrosive reaction at nasal septum, acute irritative dermatitis and allergic eczematous dermatitis (Tahir and Naseem, 2007).While evaluating the environmental impact of complex industrial wastewaters, in addition to physico-chemical analysis,toxicity testing helps to assess pollutant bioavailability, as well as in comprehensive understanding of synergistic toxic effects of different pollutants present in the wastewater. Integration of both chemical and biological approaches is therefore crucial to corroborate ecotoxicity testing (Kuczynska*et al*., 2005; Mwinyihija*et al*., 2006; Malaviya and Sharma, 2011).

Most studies with Cr toxicity have focused on the effects of Cr solution on plant tolerance, growth and survival with only a few studies (Vajpayee *et al*., 2001; Sinha*et al*., 2002; Calheiros*et al*., 2008) conducted on toxicity of tannery effluent. In the present study,an attempt has been made to assess the toxic effects of different concentrations of chrome liquor on growth and chlorophyll content of *Spirodela polyrrhiza* (L.) Schleid as a direct quantitative measure of actual environmental toxicity of tannery effluent.

MATERIALS AND METHODS

Sample collection : For toxicological tests young *Spirodela* plants were collected from a local natural shallow pond. After collection, macrophytes were thoroughly cleaned under running water in order to eliminate any remains of sediment and particles and then acclimatized in large troughs for two days. The spent chrome tan liquor samples used in this study were collected in acid-rinsed polyethylene containers from the wet blue section of a tannery located in Central Leather Research Institute (CLRI) complex, Kapurthala Road, Jalandhar, India. The collected samples were brought to the laboratory and stored in a refrigerator at 4°C to be used in further studies.

Experimental set-up: Plastic troughs of capacity 1350 ml with surface area of 337 cm^2 were used for the experiment. Seven similar sets, each containing 1 L of different tannery effluent concentrations i.e., 5%, 10%, 15%, 20%, 40%, 60%, 80%, and 100% (prepared using tap water) and a control (plants in tap water) were placed. Healthy acclimatized plants with a uniform size and weight (~14 g) were selected and inoculated to different concentrations of tannery effluent. Harvesting of samples of *S. polyrrhiza* was done after every 24 h for analyzingphytotoxicity of different concentrations of tannery effluent.

Plant analysis: All plants, harvested at the end of each exposure period were washed with tap water, then, rewashed in two lots of distilled water to remove any

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adhering remains of effluent. Further, they were blotted dry under uniform conditions and fresh weight of plant samples was measured using Digital Analytical Balance (ML204, Mettler Toledo). Chlorophyll-a and chlorophyll-bmeasurements were made from chilled 80% acetone extracts of fresh *Spirodela* plants following the methods of Arnon (1949). OD of the pigment extracts was measured at different wavelengths using UV-VIS Spectrophotometer (UV-1800, Shimadzu).

RESULTS AND DISCUSSION

The analysis of results of fresh weight of *S. polyrrhiza* plants after chrome liquor exposure showed an overall gradual decrease in a concentration-duration dependent manner (Table 1). Decrease in the fresh weight of the *Spirodela* plants with increasing effluent concentration and exposure duration could be explained by increase in the percentage of moisture loss due to increase in the values of osmotic potential owing to the presence of excess salts and is also supported by the observations of Lai *et al.* (2007); and Neocleous and Minas (2007). Decrease in fresh weight of *Spirodela* with increasing effluent concentration could also be attributed to degradation of frond and root components e.g. proteins and pigments (Keskitalo *et al.*, 2005) and breaking and decaying of fronds and roots.

The effect of chrome liquor on chl-a, and chl-b present in *S. polyrrhiza* showed an overall gradual decrease in chl-a (Table 2), chl-b (Table 3) content in a concentration-duration dependent manner. Such decline in chlorophyll concentration of aquatic macrophytes on exposure to different concentrations of tannery effluent is also supported by the observations of Vajpayee *et al.* (2001) and Sinha *et al.* (2002).Slight increase in chl-a and chl-b concentration upto 3 d of exposure at 5% effluent concentration in *S. polyrrhiza* could be explained by promotion of photosystem I (PS I) and photosystem II (PSII) activity by low concentrations of Cr in 5% concentration of chrome liquor. Such enhancement of activity in plants exposed to Cr-rich wastewater can be attributed to higher flow of electrons to photosystem II required to maintain the physiological status under stress (Dhir*et al.*, 2009).Similar increase in chlorophyll content of *Salvinia* sp. on exposure to low concentrations of chromium has also been reported by Dhir *et al.* (2009) and Prado *et al.* (2010).

Thereafter, the depletion in chlorophyll contents in the plants exposed to different concentrations of chrome liquor might be attributed to both altered chlorophyll biosynthesis due to the disruption of chloroplast phosphorylation (Chandra and Kulshreshtha, 2004) and replacement of Mg ions by

Table 1. Changes in fresh biomass (g) of *S. polyrrhiza* on exposure to different concentrations of chrome liquor.

Exposure period (days)	Effluent concentrations Fresh biomass (g)								
	0%	5%	10%	15%	20%	40%	60%	80%	100%
1d	14.46±0.43	13.81±0.39	12.86±0.56	12.39±0.49	10.39±0.48	9.42±0.56	9.35±0.34	8.84±0.28	8.60±0.38
2d	14.89±0.27	13.48±0.42	12.38±0.57	11.94±0.34	10.15±0.78	8.85±0.30	8.23±0.30	8.09±0.34	7.78±0.64
3d	15.46±0.46	13.14±0.31	11.77±0.34	11.37±0.46	10.04±0.12	8.41±0.24	7.79±0.24	7.54±0.44	7.41±0.39
4d	15.96±0.23	12.79±0.28	11.42±0.54	10.76±0.16	8.93±0.20	7.88±0.26	7.45±0.53	7.21±0.44	7.09±0.36
5d	16.39±0.45	12.42±0.61	10.86±0.61	10.41±0.37	8.25±0.42	7.45±0.47	7.15±0.40	6.86±0.28	6.64±0.40
6d	16.53±0.44	11.97±0.32	10.57±0.48	10.17±0.67	7.92±0.29	7.00±0.26	6.84±0.37	6.60±0.38	6.29±0.56
7d	17.22±0.40	11.82±0.35	9.89±0.45	9.51±0.49	7.56±0.44	6.59±0.50	6.49±0.47	6.08±0.26	5.71±0.47

All the values are mean ± SD (n=3)

Table 2. Variation in chlorophyll-a content (mg g^{-1} fw) of *S. polyrrhiza* on exposure to different concentrations of chrome liquor.

Exposure period (days)	Effluent concentrations Chlorophyll-a content (mg g^{-1}fw)								
	0%	5%	10%	15%	20%	40%	60%	80%	100%
1d	0.2942 ±0.0330	0.3204 ±0.0236 (-8.9%)	0.2472 ±0.0369 (16.0%)	0.2014 ±0.0199 (31.5%)	0.1815 ±0.0274 (38.3%)	0.1501 ±0.0287 (49.0%)	0.1255 ±0.0283 (57.3%)	0.1004 ±0.0234 (65.9%)	0.06654 ±0.0109 (77.4%)
2d	0.3343 ±0.0365	0.3947 ±0.0263 (-18.1%)	0.1702 ±0.0286 (49.09%)	0.1254 ±0.0259 (62.5%)	0.0735 ±0.0171 (78.0%)	0.0685 ±0.0106 (79.5%)	0.0507 ±0.0142 (84.8%)	0.0368 ±0.0162 (89.0%)	0.0319 ±0.0035 (90.5%)
3d	0.3790 ±0.0106	0.4047 ±0.0146 (-6.8%)	0.1017 ±0.0063 (73.2%)	0.0642 ±0.0066 (83.1%)	0.0384 ±0.0089 (89.9%)	0.0301 ±0.0045 (92.1%)	0.0253 ±0.0045 (93.3%)	0.0233 ±0.0019 (93.9%)	0.0211 ±0.0026 (94.4%)
4d	0.4187 ±0.0067	0.2868 ±0.0029 (31.5%)	0.0622 ±0.0034 (85.14%)	0.0184 ±0.0053 (95.6%)	0.0133 ±0.0035 (96.8%)	0.0099 ±0.0015 (97.6%)	0.0077 ±0.0016 (98.2%)	0.0063 ±0.0010 (98.5%)	0.0039 ±0.0009 (99.1%)
5d	0.4668 ±0.0062	0.2448 ±0.0026 (47.6%)	0.0456 ±0.0033 (90.2%)	0.0099 ±0.0022 (95.6%)	0.0078 ±0.0017 (98.3%)	0.0057 ±0.0010 (98.8%)	0.0056 ±0.0010 (98.8%)	0.0048 ±0.0009 (99.0%)	0.0032 ±0.0011 (99.3%)
6d	0.5172 ±0.0044	0.1879 ±0.0018 (63.7%)	0.0238 ±0.0021 (95.4%)	0.0067 ±0.0012 (96.4%)	0.0049 ±0.0011 (99.1%)	0.0041 ±0.0008 (99.2%)	0.0026 ±0.0006 (99.5%)	0.0022 ±0.0009 (99.6%)	0.0016 ±0.0006 (99.7%)
7d	0.5023 ±0.0012	0.1522 ±0.0014 (69.7%)	0.0119 ±0.0009 (92.2%)	0.0045 ±0.0005 (99.1%)	0.0027 ± 0.0008 (99.5%)	0.0018 ±0.0006 (99.7%)	0.0016 ±0.00052 (99.7%)	0.0014 ±0.0007 (99.7%)	0.0007 ±0.0004 (99.8%)

All the values are mean ± SD (n=3), Values in the parenthesis are the percentage reduction values of chlorophyll-a in comparison to the control

Table 3. Variation in chlorophyll-b content (mg g^{-1} fw) of ***S.polyrrhiza***on exposure to different concentrations of chrome liquor

Exposure period (days)	Effluent concentrations Chlorophyll-b content (mg g^{-1}fw)								
	0%	**5%**	**10%**	**15%**	**20%**	**40%**	**60%**	**80%**	**100%**
1d	0.1589 ±0.0042	0.1477 ±0.0024 (7.1%)	0.1442 ±0.0070 (9.3%)	0.1387 ±0.0047 (12.7%)	0.1246 ±0.0065 (21.6%)	0.1032 ±0.0066 (35.1%)	0.0907 ±0.0032 (42.9%)	0.0732 ±0.0050 (53.9%)	0.0511 ±0.0039 (67.8%)
2d	0.1865 ±0.0046	0.1882 ±0.0054 (-0.9%)	0.1056 ±0.0056 (43.4%)	0.0872 ±0.0055 (53.2%)	0.0515 ±0.0031 (72.4%)	0.0485 ±0.0048 (74.0%)	0.0394 ±0.0026 (78.9%)	0.0292 ±0.0007 (84.3%)	0.0265 ±0.0043 (85.8%)
3d	0.2084 ±0.0075	0.1963 ±0.0030 (5.8%)	0.0646 ±0.0053 (69.0%)	0.0455 ±0.0049 (78.2%)	0.0275 ±0.0012 (86.8%)	0.0219 ±0.0031 (89.5%)	0.0200 ±0.0063 (90.4%)	0.0192 ±0.0036 (90.8%)	0.0178 ±0.0042 (91.5%)
4d	0.2110 ±0.0040	0.1458 ±0.0034 (30.9%)	0.0412 ±0.0037 (80.5%)	0.0132 ±0.0036 (93.7%)	0.0098 ±0.0022 (95.4%)	0.0077 ±0.0003 (96.4%)	0.0064 ±0.0023 (97.0%)	0.0055 ±0.0016 (97.4%)	0.0036 ±0.0016 (98.3%)
5d	0.2156 ±0.0044	0.1260 ±0.0043 (41.6%)	0.0338 ±0.0029 (84.3%)	0.0079 ±0.0028 (96.3%)	0.0063 ±0.0028 (97.1%)	0.0048 ±0.0014 (97.8%)	0.0047 ±0.0018 (97.8%)	0.0044 ±0.0011 (98.0)	0.0032 ±0.0011 (98.5%)
6d	0.2274 ±0.0033	0.0977 ±0.0031 (57.0%)	0.0196 ±0.0004 (91.4%)	0.0055 ±0.0012 (97.6%)	0.0042 ±0.0009 (98.2%)	0.0036 ±0.0010 (98.4%)	0.0025 ±0.0012 (98.9%)	0.0021 ±0.0002 (99.1%)	0.0019 ±0.0008 (99.2%)
7d	0.2195 ±0.0031	0.0799 ±0.0045 (63.6%)	0.0099 ±0.0022 (95.5%)	0.0039 ±0.0006 (98.2%)	0.0024 ± 0.0006 (98.9%)	0.0016 ±0.0007 (99.3%)	0.0016 ±0.0003 (99.3%)	0.0014 ±0.0003 (99.4%)	0.0009 ±0.0005 (99.6%)

All the values are mean ± SD (n=3), Values in the parenthesis are the percentage reduction values of chlorophyll-b in comparison to the control

Cr ions (Vajpayee *et al*., 2001). δ-aminolevulinic acid dehydratase (ALAD) is a metalloenzyme (Chen and Nelands, 1973) and its activity in plants is dependent on availability of Mg. It has been reported that Cr causes toxicity to ALAD (an enzyme involved in chlorophyll biosynthesis) by impairing δ-aminolevulinic acid (ALA) utilization (Vajpayee *et al*., 2000). Additionally, exposure to Cr(VI) normally leads to oxidative damage and increase in succinic dehydrogenase activity (Satyakala andJamil, 1992). It may also change the metalloenzymes of the plant by displacement or replacement of metal ions by its ability to generate reactive oxygen species such as $HO^{\cdot}$ and H_2O_2 which in turn may cause oxidative stress (Shanker *et al*., 2005). Further, it has also been reported that Cr inhibits chlorophyll biosynthesis by causing Fe deficiency and creating nutrient imbalances.

Analysis of the percentage reduction of chl-a (Table 2) and chl-b (Table 3) as compared to the respective control sets revealed greater decline of chl-a than chl-b on exposure to different concentrations of the chrome liquor which could be explained by higher sensitivity of chl-a as compared to chl-b and is also supported by observations of Vajpayee *et al.* (2000).

Conclusion

Exposure of *S. polyrrhiza* (L.) Schleid.to different concentrations of chrome liquor (5%-100%) showed significant toxic effects on it in a concentration and duration dependent manner, as revealed by reduction in fresh weight, chlorophyll-a and chlorophyll-b in comparison to the control. However, at very low concentration of chrome liquor (5%) chl-a and chl-b were found to slightly increase up to 3 d of exposure. In addition, assessment of percentage reduction of chl-a and chl-b in comparison to control demonstrated more sensitive nature of chl-a towards chrome liquor toxicity in comparison to chl-b.

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