

Bioremediation of tannery wastewater by a salt-tolerant strain of *Chlorella vulgaris*

Cindrella Das¹, Naseera K.¹, Anirudh Ram², Ram Murti Meena¹, Nagappa Ramaiah^{1*}

¹CSIR - National Institute of Oceanography, Dona Paula, Goa – 403004, India

²CSIR-National Institute of Oceanography, Regional Centre, Mumbai- 400053

*Corresponding author: E-mail – ramaiah@nio.org, Tel. – (0832)2450515

Abstract

Phycoremediation is the use of algae for removal or reduction of inorganic nutrients and xenobiotics from wastewaters. It is a reliable process for biotransformation and detoxification of a variety of pollutants. This study focused on the potential of a strain of microalga, *Chlorella vulgaris*, to reduce various pollutants in tannery wastewater (TW). The microalga was grown in TW for a culture period of 21 days and the resultant removal/reduction/biotransformation of biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrates (NO₃-N), phosphates (PO₄-P), sulphates (SO₄-S), dissolved solids and chromium (Cr) was monitored. The isolate was efficient in the removal of excess nutrients in wastewater. Most notably, complete removal (100% reduction) of NO₃-N and Cr was observed by the 6th and 12th day of culture period, respectively. Removal of phosphates was as high as 91.73% by day 6 and over 99% by day 21. This strain also reduced sulphate concentrations to 67.4% by day 21. Levels of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in TW were reduced by 94.74% and 95.93%, respectively, after 21 days. Our results are useful to suggest that the isolate, *Chlorella vulgaris* is promising for bioremediating and detoxifying tannery wastewater to improve its quality to meet up recommended effluent discharge limits.

Keywords: *Chlorella*; microalgae; tannery; wastewater; BOD; bioremediation

INTRODUCTION

Globally, an estimated total of more than 52 billion domesticated animals are slaughtered every year for human consumption (FAO 2004) and the hides of larger animals are processed for varied uses. Common commercial hides include leather from cattle and other livestock animals which are used for manufacturing shoes, clothes, upholstery, interior decoration among many others.

The tanning industry is a significant contributor to economy and provides large scale employment opportunities in particular to unskilled and/or semiskilled people who also economically weak in the societies. Unfortunately, it is one of the worst anthropogenic polluters (Khwaja et al. 2001). Tanneries process the foul-smelling, damp, hairy and assorted sized hides employing cheap labour. In spite of the impending deleterious ecological consequences, the developing world still practices traditional, archaic processing technologies (Kennedy 1999). To circumvent the obnoxious impacts of tannery wastewater, several treatment methods have emerged during the early 1990s (Durai and Rajasimman 2011; Saranraj and Sujitha 2013).

Notably, most of the world's tanneries are located in the Indian subcontinent. India alone has about 3,000 tanneries with an annual processing capacity of 700,000 tons of hides and skins. These tanneries produce and discharge an estimated 30 billion litres of effluent annually (Srivastav 2012). Conventional leather tanning technology is highly polluting and produces large amounts of organic and chemical pollutants (Kabdasli et al. 2002). These pollutants, in the discharged effluent, pose serious threats to the environment.

Tannery wastewaters are characterized mainly by high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), total dissolved solids (TDS), chromium and sulfides (Leta et al. 2004). Conventional wastewater treatment schemes typically include primary and secondary treatment. Primary treatment consists of sulfide oxidation, solids separation and chromium precipitation. Secondary treatment is usually accomplished by activated sludge systems (Dotro 2003). The large amounts of sludge generated by wastewater treatment processes must be sent off-site for disposal. Handling and disposal of this sludge is typically the largest single cost component in the operation of a wastewater treatment plant. Activated sludge systems require very high capital as well as operation and maintenance costs. The cost factor coupled with the recent but increasingly stringent environmental regulations and standards (Bosnic et al. 2000; Kabdasli et al. 2002), has led the industry to search for new effective treatment technologies.

With these concerns, there has been increased interest in using biological methods for remediation of different industrial wastewaters. Most studies have concentrated on the use of fungi and bacteria to

treat wastewater (McMullan et al. 2001; Tastan et al. 2010). However, additional carbon sources are required for such systems. Hence, the use of microalgae in bioremediation of industrial wastewaters is of practical interest due to their central role in autotrophic carbon dioxide fixation. The process of wastewater treatment using algae has low energy requirement, reduced sludge formation as well as GHG emission along with production of variously useful algal biomass. It has been shown to be a more cost effective method for removal of BOD, certain microbial pathogens, phosphorus and nitrogen than activated sludge process and other secondary treatment processes (Sheehan et al. 1998).

Various techniques are in place for exploiting faster growth rates and nutrient removal efficiencies of certain microalgae for the treatment/detoxification of a variety of wastewaters. During the last four decades, phycoremediation has been evaluated by numerous investigators (Oswald 1988; Mara et al. 1996; Tadesse et al. 2003; Shi et al. 2007; Chu et al 2008; Craggs et al. 2012; Mustafa et al. 2012; Dixit and Singh 2014; Posadas et al. 2014). Dunn (1998) and Dunn et al. (2013) examined the detoxification of tannery wastewater and nutrient removal using high-rate algal pond systems. Different microalgae such as species of *Oscillatoria*, *Phormidium*, *Ulothrix*, *Chlamydomona*, *Scenedesmus* have been evaluated for their ability to grow in tannery effluent and accumulate chromium (Rai et al. 2005; Balaji et al. 2015; Ajayan et al. 2015). Notable reports on the use of *Chlorella* for bioremediation of tannery wastewater are those by Chellam and Sampathkumar (2012) and by Jaysudha and Sampathkumar (2014). Both free as well as immobilized cells of *C. salina* or *C. marina* have been examined mainly for removal of nutrients in tannery wastewater. *Chlorella vulgaris* was used by Rao et al. (2011) for removal of nutrients from tannery wastewater and by Hernandez-Zamora (2015) for removal of Congo Red, an azo-dye used for dyeing cotton, jute, leather, paper, silk and wool

In view of such advantages offered by microalgae, this study aimed at evaluating the microalga, *Chlorella vulgaris* for its potential in reducing BOD, COD, sulphates, inorganic nutrients, dissolved solids and chromium in the effluent. Our results demonstrate a very highly efficient, minimal energy requiring improvement of tannery wastewater by this salt tolerant microalgal strain.

MATERIALS AND METHODS

Isolation of microalga

Salt tolerant microalga *Chlorella vulgaris* NIOCCV was used in this study. It was grown in algal culture medium (ACM, HiMedia, Mumbai) at a constant temperature of $28 \pm 0.5^\circ\text{C}$ under fluorescent illumination of $150\text{-}300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (with 10:14 h light:dark photoperiod).

Characteristics of tannery wastewater

The tannery wastewater (TW) was collected from outside the discharge point of a tannery industry located in Kanpur, in Northern India on the banks of River Ganges. Various physical and chemical parameters of the wastewater collected were analysed using standard methods in APHA (American Public Health Association, 2005).

Experimental design

Initially, the growth of NIOCCV was tested in different strengths of TW viz., 100% (no dilution), 70% (7:3), 50% (1:1), 30% (3:7) and 10% (1:9). The original TW was diluted to these strengths using tap water. Even after several trials of altering inoculum size, light periods and aeration, very poor growth was observed in the 100% and 70% TW unlike the vigorous growth (*vis-a-vis* that in standard algal culture medium) in 50%, 30% or 10% TW. Hence, it was resorted to test the bioremediation potential of this strain in 1:1 diluted TW.

Tannery wastewater was diluted to 50% (1:1) with tap water and 100 ml aliquots of this dilution were dispensed into several 250 ml Erlenmeyer flasks. Ten millilitres of exponential algal culture, standardized to an optical density (OD 620 nm) of 0.2, were inoculated into eight triplicate sets of 250 ml flasks containing 100 ml of 1:1 TW. The culture was grown for 21 days at a constant temperature of $28 \pm 0.5^\circ\text{C}$ under fluorescent illumination of $150\text{-}300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (10:14 h light:dark photoperiod). Cells from a set of three flasks each were harvested on day 0, 3, 6, 9, 12, 15, 18 and 21. Two controls (one of TW without any algae while another of standard algae culture broth (HiMedia, Mumbai) with algal cells) were included to confirm and check the effect of TW on the growth of algae during the experiment. On all sampling days, analysis of different parameters was carried out both from un-inoculated and algae-inoculated flasks with 1:1 TW.

For chlorophyll *a* measurement, cells were harvested from a 10 ml volume of filtered sample (Whatman GF/C), extracted with acetone overnight and measured spectrophotometrically (Strickland and Parsons 1968). Cells were also counted microscopically at 400X on all sampling days.

Analytical procedures

The physico-chemical parameters BOD, COD, nitrates ($\text{NO}_3\text{-N}$), phosphates ($\text{PO}_4\text{-P}$), sulphates ($\text{SO}_4\text{-S}$), total dissolved solids (TDS), total chromium (Cr) and hexavalent chromium (Cr^{6+}) were measured from the samples harvested during the experiment. Measurements of BOD and COD were made following standard methods described in APHA (2005). The BOD was determined using the 5-day BOD test and the COD, using open reflux oxidation method. TDS was determined by gravimetric method as per APHA (2005). TDS was calculated by measuring the residual weight after

drying known sample volumes filtered through 0.7µm glass microfiber filters at 180°C. The algal cells were separated from wastewater through filtration using 0.45µm glass microfiber filters, and the filtrate was used for measurement of dissolved nitrates, phosphates and sulphates. Nitrate concentrations were colorimetrically measured following the Cd column reduction method (APHA 2005). Phosphates were measured using the Ascorbic acid method (APHA 2005). Sulphates were measured following the barium chloride precipitation method (APHA 2005). For total chromium concentrations, the samples were digested with concentrated nitric acid followed by filtration through 0.45µm filter paper, while for hexavalent chromium, 0.45µm filtered samples without any pre-digestion were analyzed. The chromium contents were measured using the colorimetric Diphenylcarbazide (DPC) method (APHA 2005).

Toxicity bioassay

Brine shrimp (*Artemia salina*) bioassay (Kiviranta et al. 1991) was used to assess the reduction in toxicity in the remediated tannery effluent. Briefly, one day old *Artemia* hatchlings were placed in multi-well plates (10 nos. well⁻¹). Survival was monitored from 1 ml treated and untreated wastewater samples and seawater (positive control). The assay was conducted in five replicates. The number of dead nauplii after 24, 48 and 72 h were counted and percent survival calculated.

Statistical analysis

Statistical analyses were carried out using XLSTAT 7. One-way analysis of variance (ANOVA) was used to check the effect of *C. vulgaris* on its ability to bring down various toxic parameters from the wastewater. Subsequent pair-wise comparisons were performed using post hoc Tukey's HSD (Honestly Significant Difference) tests.

RESULTS

Characteristics of tannery wastewater

The physico-chemical characteristics of the raw tannery wastewater are presented in Table 1. The wastewater, collected from the outlets of a tannery, had dark brown colour, salinity of 15 PSU, high BOD, COD, nitrates, phosphates, sulphates, TDS and chromium concentrations. Both BOD and COD were quite high with average concentrations of 1350 (±42.5) mg.L⁻¹ and 4000 (±51.2) mg.L⁻¹, respectively. Untreated TW had very high concentrations of nitrate (0.93±0.01 mg.L⁻¹), phosphate (6.01±0.05 mg.L⁻¹) and sulphate (178.69±0.98 mg.L⁻¹). Total dissolved solids (TDS) were higher compared to total suspended solids (TSS), indicating high contents of dissolved salts in the wastewater. Total Chromium (3.22 mg.L⁻¹) concentrations in the wastewater were found to be higher than the permissible limits (<2.0 mg.L⁻¹) for discharge of effluents. Concentrations of chlorides, Ca

and Mg in the wastewater were also higher than those permitted by Bureau of Indian Standards (BIS, 1994).

Growth of *Chlorella vulgaris* in tannery wastewater

Cell counts of *C. vulgaris*, enumerated once every three days during the culture period of 21 days, increased by over 7 times the initial counts of 5×10^3 cells.mL⁻¹. Also the chlorophyll *a*, 0.25 mg.mL⁻¹ was higher on day 21 (Fig. 1). In comparison to standard algal culture medium, the growth of cells was lower in the 1:1 diluted tannery effluent.

Reduction of different physico-chemical parameters

The concentrations of physico-chemical parameters analysed from the 1:1 TW before treatment (day 0) and after treatment with *C. vulgaris* (day 21) are presented in Table 1. The concentrations of different parameters in tannery wastewater at three day intervals after treatment with *C. vulgaris* are shown in Table 2. After 21 days of treatment, the BOD level was reduced from 672 to 27 mg.L⁻¹, and the COD from 1680 to 88 mg.L⁻¹ (Table 2). In case of BOD, the reduction was 95.92%, whereas for reduction of COD, the efficiency rate was 94.74% by day 21 (Fig. 2). Similarly, significant reduction of nitrates, phosphates and sulphates occurred within 21 days (Table 2). Indeed, 100% reduction of nitrates was seen by day 6 itself. Removal of phosphates by *C. vulgaris* was as high as 91.74% by day 6 and over 99% by day 21. The sulphate concentrations in TW were reduced by 67.4% by day 21. The very high concentration of TDS of >3000 mg.mL⁻¹ in untreated wastewater was reduced to 2066 mg.mL⁻¹ by *C. vulgaris* after 12 days (Fig. 2) and to 1766 mg.mL⁻¹ by day 21 (Table 2) with an overall TDS removal of 41% (Fig. 2). Concentrations of total chromium in the tannery effluent, following treatment with *C. vulgaris* are presented against control TW (Fig. 3). Hexavalent chromium was completely transformed from the TW by day 12.

One-way ANOVA with post-hoc Tukey's HSD test showed that the concentrations of all the measured parameters decreased significantly after the treatment process of 21 days. For TDS, the reduction was significant ($p < 0.001$), and highly significant ($p < 0.0001$) for BOD, COD, NO₃-N, PO₄-P, SO₄-S, total and hexavalent chromium (Table 3).

Toxicity assay

Percent survival of brine shrimp (*Artemia salina*) nauplii/hatchlings subjected to treated and untreated effluent for 24, 48 and 72 h differed rather significantly (Table 4). For instance, in the *C. vulgaris* treated effluent, the survival of *Artemia* nauplii was 83.9% on Day 1 and increased to 100% by day 9. A positive trend was apparent in the survival of *Artemia* hatchlings in the treated effluent

when exposed for 24, 48 and 72 h. Survival was significantly less in the untreated effluent. It was atleast 28-32% lower compared to that in treated effluent.

DISCUSSION

Industrial effluent generation is on the rise in fast growing economies as India. While stringent regulatory norms are available statutorily, their implementation at fundamental levels is an obvious missing reality, in particular for tannery effluent discharges.

Concentrations of different physico-chemical parameters of the tannery wastewater were comparable to those reviewed by Durai and Rajasimman (2011). The observations of Munawar (1970) and Kannan (2006) suggest that algae grow luxuriantly with great variety and abundance in waters rich in calcium. The present data of the effluent characteristics also showed that calcium is possibly one of the favorable factors for growth of *Chlorella vulgaris* in Kanpur.tannery effluent. Besides calcium, high amounts of nitrates and phosphates and other oxidizable organic matter in the effluent also could be contributing to the growth of this microalgal strain as suggested by Murugesan and Sivasubramanian, (2005) and Burch et al. (2001).

The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are important parameters to determine the water quality. Both of these parameters are greatly affected by the pollution load resulting from tannery industries (Islam et al. 2010). BOD in TW was reduced significantly ($p < 0.001$) by 95.93% and that of COD by 94.74% after treatment. Our strain, *C. vulgaris*, was efficient in bringing down the levels to within the standards set by BIS (1994). These reductions were much higher within 21 days than those reported by Nanda et al. (2010) for *Nostoc* sp. treated TW. Their strain of *Nostoc* sp. could bring down BOD by only 57.5% and COD by only 37.8% in 28 days using 1:5 TW diluted with BG11 medium. Therefore, our approach of diluting the TW by 50% merely with tap water seems a more pragmatic and inexpensive approach. In order to achieve adequate improvement of the TW for safe discharge, the wastewater could be diluted and growing *C. vulgaris* in it for a fortnight or so would prove ecologically advantageous.

Results from this study demonstrate higher reduction of nutrients (nitrates, phosphates and sulphates) by *C. vulgaris* from TW when compared with those reported by Ajayan et al. (2015), for *Scenedesmus* sp. and Adam et al. (2015), for *Tetraselmis* sp. Remarkably, the nutrient levels were reduced to well below the maximum permissible limits of BIS.

Total dissolved solids (TDS) is an important chemical parameter of water, which mainly indicates the presence of various minerals including nitrate, nitrite, phosphate, sulphates, metallic ions, alkalis and acids in both colloidal and dissolved forms (Rahman et al. 2012; Kabir et al. 2002). The

Chlorella vulgaris strain we tested has only a moderate potential for reducing the TDS contents in the tannery wastewater when compared to the BIS (1994) permissible limit.

Most pertinently, the strain of *Chlorella vulgaris* we studied could efficiently biotransform Cr^{6+} from Kanpur tannery wastewater. This culture could achieve 100% biotransformation of Cr^{6+} from the TW in about 12 days itself. As Cervantes et al. (2001) noted in the case of *Oscillatoria*, *Phormidium*, *Scenedesmus* and *Pandorina* spp., the present isolate seems to be useful for bio-sorption studies for the removal and/or biotransformation of Cr from contaminated sources. These kinds of algae biotransform/detoxify heavy metal ions usually through the process of biosorption, adsorption and bioaccumulation (Rehman and Shakoory, 2001; Gin et al. 2002; Rehman et al. 2007). Rehman (2011) reported 68% reduction in Cr concentrations from TW in 20 days using *Euglena proxima*. In comparison to the previous results, the transformation potential of *C. vulgaris* is far superior.

Toxicity tests are indispensable tools to evaluate the quality and pollutant charge of the effluent. The brine shrimp, *Artemia salina* is a microcrustacean used frequently in toxicity studies, due to the ease of culture, short generation time, low commercial cost as dormant eggs (cysts), and cosmopolitan distribution (Vanhaecke et al. 1981). Hasegawa et al. (2014) determined the toxicity of tannery wastewater, before and after zinc oxide-assisted photocatalytic treatment, using dry shrimp eggs. Islam et al. (2014) studied the toxic effects of varying dilutions of tannery wastewater on brine shrimp nauplii.

Toxicity testing of tannery wastewater in this study, ascertained that the untreated wastewater is deleterious to the hatchlings of *Artemia salina*. The treatment of tannery wastewater with *Chlorella vulgaris* unambiguously promoted the survival of the nauplii which can be directly ascribed to the reduction of toxic effects of treated effluent on *A. salina*. The results are useful to ascertain the decrease in toxicity in *C. vulgaris* treated effluent to *Artemia* larvae. This can be attributed to the substantial reduction in concentrations of the toxicants that were in far higher concentrations than BIS (1994) permitted limits. Further, the biotransformation of toxic metals, in general, hexavalent chromium from the wastewater by *C. vulgaris* ought to be facilitating the prolonged survival. Therefore, the toxicity results of this work showed that survival of *A. salina* was an important tool to evaluate the efficacy of TW bioremediation by *Chlorella vulgaris*.

Our salt tolerant *Chlorella* strain grew the least in 100% and quite poorly in 70% TW, as explained in Methods section. This was the case even after increasing inoculum size, lengthening of light periods and elevated aeration. While the growth of this strain was far superior in the 3:7 or 1:9 TW, to ensure that the salt tolerant strain would not experience undue hypo-osmotic stress with more

diluted TW, we resorted to test its bioremediation efficiency in 1:1 TW. Since tannery effluent is a mixture of innumerable alkalies/chorides/salts, our approach of using 1:1 dilution is very likely to be effective in the reduction of COD, BOD, nutrients and chromium. Further, as can be deciphered from the growth curve, the profuse growth of *Chlorella* NIOCCV in 1:1 TW generates substantial biomass that can be useful variously, as has been proposed by Huang et al (2010), for biofuel production in particular. Notably, the accumulation of troublesome sludge in conventional treatment methods is certainly avoided by phycoremediation. Additionally, unlike the use of costly chemical medium, as done for instance by Nanda et al (2010), our approach of using fresh water for dilution ought to be beneficial for the industry in some production processes. In that, by separating the algal biomass - that could be of use as a feeder material in a variety of other uses- the treated water can be recycled in the industry itself or for controlled irrigation of forests, lawns, and other greeneries. The latter intent of the use of microalga-treated TW for irrigating the non-food crops is an eco-friendly approach of carbon sequestration. Further, as Orandi and Lewis (2013) also proposed, the rigorous recovery of accumulated metals from the algal biomass can be achieved through washes with dilute acid or via adsorption/desorption cycles of alga itself.

CONCLUSION

This study is useful to ascertain that treatment by *C. vulgaris* has significance in reducing pollution load from tannery wastewater with high efficiency. Therefore, this algal species can be used as an alternate, low cost method for treating tannery effluent before releasing into natural systems. Such methods seem to offer economic treatment and may be effective in minimizing the environmental impact. These very promising results call for elaborated studies on the use of algal species for promulgating functional phycoremediation strategies in the future.

ACKNOWLEDGEMENTS

We thank the Director, CSIR - National Institute of Oceanography for facilities and encouragement. The work was supported under the CSIR project BSC0111 'Integrated NextGen Approaches in Health, Disease and Environmental Toxicity (INDEPTH)'.

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Table 1 Physico-chemical characteristics of raw, diluted (1:1) untreated and treated tannery wastewater (TW)

Parameter	Raw TW	Untreated 1:1 TW (Day 0)	Treated 1:1 TW (Day 21)	Maximum permissible limits (BIS, 1994)
pH	7.45±0.00	7.00±0.00	7.20±0.00	5.5-9.0
Salinity (PSU)	15.00±0.30	12±0.38	10±0.25	–
Total Solids (mg.L ⁻¹) ^a	5000.00±0.50	–	–	2200
Total Suspended Solids (mg.L ⁻¹) ^a	500.00±30.40	–	–	100
Total Dissolved Solids (mg.L ⁻¹)	4333.33±288.70	3333.33±0.00	1766.70±288.68	2100
BOD (mg.L ⁻¹)	1350.00±42.50	658.40±26.42	27.33±4.04	30
COD (mg.L ⁻¹ L)	4000.00±51.20	1747.24±62.09	88.33±3.82	250
H ₂ S (S ²⁻) (mg.L ⁻¹) ^b	11.75±11.28	–	–	2
Sulphates (SO ₄ ²⁻) (mg.L ⁻¹)	178.69±0.98	75.34±4.84	25.16±2.37	1000
Nitrates (mg.L ⁻¹)	0.93±0.01	0.39±0.02	0.00	–*
NH ₃ (mg.L ⁻¹) ^b	2734.16±1.12	–	–	50
Phosphates (mg.L ⁻¹)	6.01±0.05	3.85±0.25	0.002±0.00	–
Phenols (mg.L ⁻¹)	3.80±0.58	–	–	5-50
Cr (mg.L ⁻¹)	3.22	0.88±0.03	0.00	2
Mn (mg.L ⁻¹) ^c	0.10	–	–	–
Ni (mg.L ⁻¹) ^c	0.05	–	–	3
Cu (mg.L ⁻¹) ^c	0.02	–	–	3
Zn (mg.L ⁻¹) ^c	0.08	–	–	15
Ca (mg.L ⁻¹) ^c	265.10	–	–	–
Mg (mg.L ⁻¹) ^c	33.40	–	–	–

(Standard deviation, n=3)

*no set limit

^a This parameter was not analysed in the treated TW because the algal biomass adds to the total/suspended solids

^b Untraceable in both untreated and treated TW

^c Since the concentrations were within safe limits in raw TW, they were not measured

Table 2 Effect of *C. vulgaris* in reducing toxic pollutant concentrations from TW. Means±SD in a column that have no superscript in common are significantly different from each other (ANOVA with post-hoc Tukey's HSD, p<0.05)

Days		BOD	COD	NO ₃ -N	PO ₄ -P	SO ₄ -S	Cr	Cr ⁶⁺	TDS
0	Treatment	672.02±36.79	1680.05±19.93	0.371±0.03	3.712±0.34	77.41±4.56 ^a	0.821±0.06	0.561±0.01	3000.00±577.35 ^a
	Control	658.40±26.42	1747.24±62.09	0.385±0.02	3.852±0.25 ^a	75.34±4.84 ^a	0.877±0.03 ^a	0.577±0.03	3333.33±0.00 ^a
3	Treatment	480.67±31.00	1401.67±60.37	0.146±0.03	1.455±0.09	72.06±1.91 ^{ab}	0.524±0.09 ^a	0.224±0.06 ^a	2666.70±115.47 ^{ab}
	Control	516.67±35.12	1414.17±82.53	0.271±0.01 ^a	3.707±0.09 ^{ab}	72.52±1.78 ^{ab}	0.825±0.04 ^{ab}	0.325±0.04 ^a	3066.67±254.70 ^a
6	Treatment	271.67±28.43	779.17±30.03	0.000	0.307±0.04 ^a	63.99±2.34 ^{bc}	0.453±0.10 ^a	0.053±0.01 ^a	2333.30±0.00 ^{abc}
	Control	423.33±25.17 ^a	894.50±59.14 ^{ab}	0.229±0.00 ^a	3.293±0.05 ^{bc}	64.41±0.89 ^{bc}	0.820±0.05 ^{ab}	0.242±0.06 ^{ab}	3000.00±577.35 ^a
9	Treatment	185.33±25.50	253.33±55.14 ^a	0.000	0.273±0.04 ^a	56.91±4.40 ^c	0.029±0.01 ^b	0.004±0.00 ^b	2233.30±100.00 ^{bc}
	Control	373.33±20.82 ^{ab}	777.40±48.92 ^{ab}	0.122±0.01 ^b	3.217±0.06 ^{cd}	62.92±1.12 ^c	0.819±0.04 ^{ab}	0.199±0.04 ^b	2900.00±251.66 ^a
12	Treatment	86.67±7.64 ^a	206.67±15.51 ^{ab}	0.000	0.215±0.03 ^a	44.84±4.80 ^d	0.004±0.00 ^b	0.000	2066.70±115.47 ^{bc}
	Control	366.67±11.55 ^{ab}	720.67±27.14 ^b	0.175±0.02	2.754±0.23 ^c	59.88±4.51 ^c	0.785±0.05 ^{ab}	0.088±0.01 ^c	2866.67±115.47 ^a
15	Treatment	46.67±7.64 ^a	125.00±18.03 ^{bc}	0.000	0.156±0.02 ^a	36.62±4.97 ^d	0.000	0.000	1933.30±152.75 ^{bc}
	Control	346.67±36.86 ^{bc}	626.67±86.61 ^c	0.106±0.00 ^{bc}	3.057±0.03 ^{cde}	59.80±0.68 ^c	0.737±0.07 ^b	0.023±0.01 ^c	2833.33±57.74 ^a
18	Treatment	28.33±7.64 ^a	98.80±3.54 ^c	0.000	0.070±0.01 ^a	35.48±3.54 ^{de}	0.000	0.000	1900.00±251.66 ^c
	Control	333.33±11.55 ^{bc}	548.33±27.14 ^c	0.083±0.02 ^{bc}	2.830±0.17 ^{de}	58.35±3.40 ^c	0.712±0.02 ^b	0.021±0.01 ^c	2766.67±0.00 ^a
21	Treatment	27.33±4.04 ^a	88.33±3.82 ^c	0.000	0.002±0.00 ^a	25.16±2.37 ^c	0.000	0.000	1766.70±288.68 ^c
	Control	278.33±17.56 ^c	518.90±41.26 ^c	0.065±0.01 ^c	2.250±0.15	49.01±2.86	0.685±0.07 ^b	0.019±0.01 ^c	2666.67±152.75 ^a

(Standard deviation, n=3)

Table 3 Significant differences in the removal efficiencies after treatment for each parameters (One-way ANOVA, $p < 0.05$)

	Source of variance	DF	Sum of squares	Mean of squares	F value	P value
BOD n=24	Between days	7	1192896.328	170413.761	343.881	3.16E-16
	Within days	16	7928.965	495.560	—	—
	Total	23	1200825.293	—	—	—
COD n=24	Between days	7	8553938.972	1221991.282	1139.862	2.27453E-20
	Within days	16	17152.825	1072.052	—	—
	Total	23	8571091.798	—	—	—
NO ₃ -N n=24	Between days	7	0.377	0.054	253.230	3.56942E-15
	Within days	16	0.003	0.0002	—	—
	Total	23	0.380	—	—	—
PO ₄ -P n=24	Between days	7	34.053	4.865	306.068	7.96224E-16
	Within days	16	0.254	0.016	—	—
	Total	23	34.307	—	—	—
SO ₄ -S n=24	Between days	7	7487.319	1069.617	74.234	5.26261E-11
	Within days	16	230.539	14.409	—	—
	Total	23	7717.859	—	—	—
Total Cr n=24	Between days	7	2.205	0.315	117.286	1.51693E-12
	Within days	16	0.043	0.0027	—	—
	Total	23	2.248	—	—	—
Cr ⁶⁺ - Cr n=24	Between days	7	0.837	0.120	254.923	3.38616E-15
	Within days	16	0.008	0.00047	—	—
	Total	23	0.845	—	—	—
TDS n=24	Between days	7	3696250	528035.714	7.823	0.000347
	Within days	16	1080000	67500	—	—
	Total	23	4776250	—	—	—

Table 4 Toxicity testing in *Artemia salina* exposed to untreated water and in water treated with *Chlorella vulgaris*. Control – tannery wastewater without inoculated algae; Treatment - tannery wastewater inoculated with *C. vulgaris*.

Means±SD in a row that have no superscript in common are significantly different from each other (ANOVA with post-hoc Tukey's HSD, $p < 0.05$)

Days		<i>Artemia</i> survival (%)		
		24 h	48 h	72 h
0	Treatment	83.9±1.7 ^a	81.4±5.4 ^{ab}	38.1±23.7 ^b
	Control	0.0±0.0	0.0±0.0	0.0±0.0
3	Treatment	95.8±6.4 ^a	95.8±6.4 ^a	72.5±8.8
	Control	91.4±1.5	75.9±1.4	17.6±3.5
6	Treatment	91.5±20.0 ^a	71.3±20.6 ^{ab}	21.7±9.8 ^b
	Control	78.5±2.3	69.2±6.1	4.9±3.1
9	Treatment	100.0±0.0 ^a	78.6±18.9 ^a	5.6±3.2
	Control	76.9±4.6	59.7±5.1	6.7±10.2
12	Treatment	100.0±0.0 ^a	87.2±8.9 ^a	14.1±12.8
	Control	77.4±1.5 ^a	77.9±11.2 ^a	11.6±5.6
15	Treatment	100.0±0.0 ^a	88.0±3.0 ^a	27.1±9.8
	Control	78.0±0.0 ^a	78.3±8.6 ^a	8.1±4.6
18	Treatment	100.0±0.0 ^a	90.0±3.5 ^a	18.9±9.7
	Control	76.9±4.6	64.3±12.7	3.1±4.6
21	Treatment	100.0±0.0 ^a	93.0±7.1 ^a	20.6±14.7
	Control	72.0±0.0	61.9±3.7	2.6±3.9
	Seawater	100.0±0.0 ^a	100.0±0.0 ^a	74.8±17.8

(Standard deviation, n=5)

Figure 1 Growth of *Chlorella vulgaris* in tannery wastewater

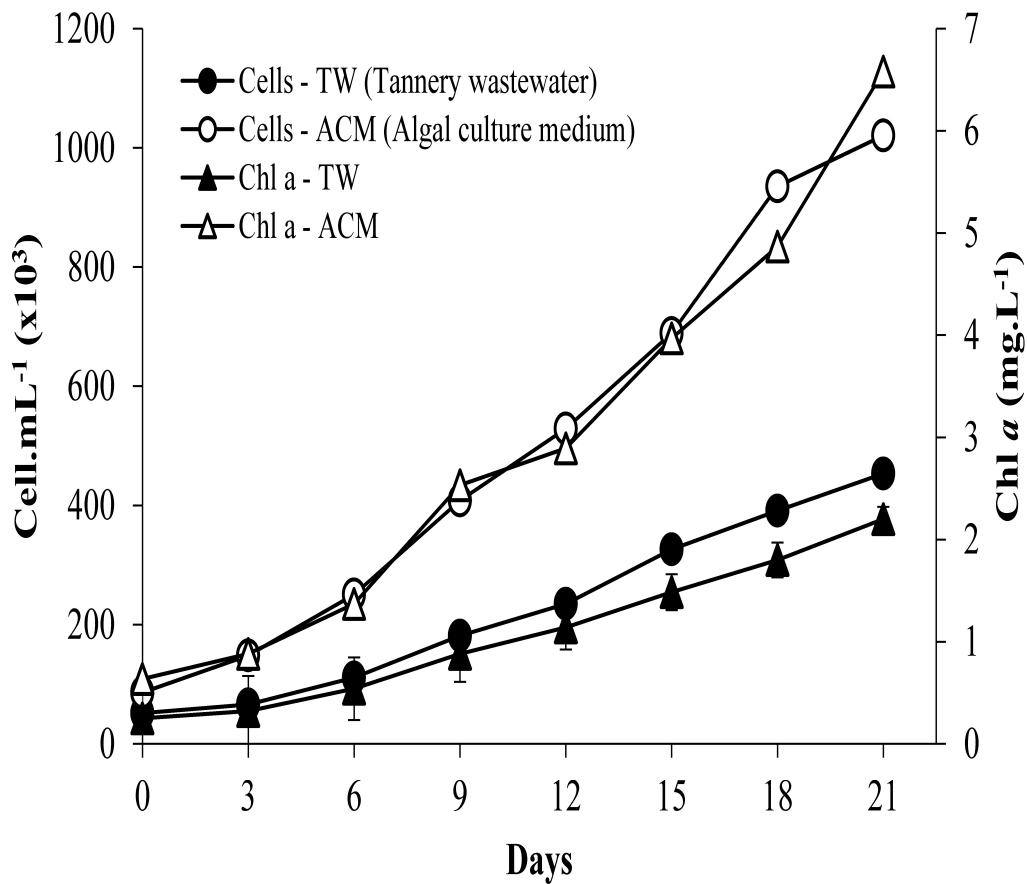


Figure 2 Reduction efficiencies for pollutants of tannery effluent after treatment with *C. vulgaris*

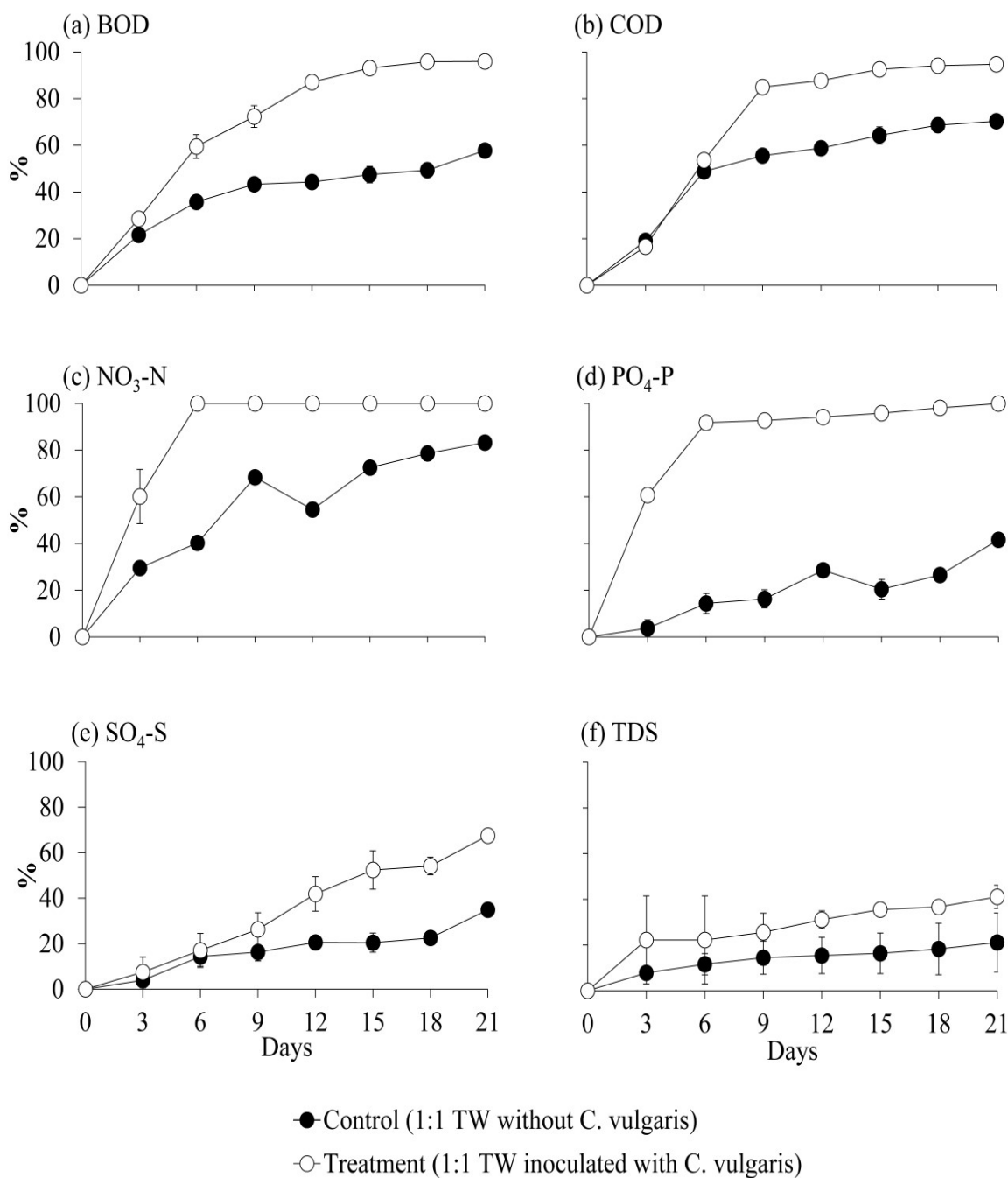


Figure 3 Reduction efficiencies for total and hexavalent chromium concentrations of TW after treatment with *C. vulgaris*

