ELSEVIER

Journal of Hazardous Materials A138 (2006) 438–447

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Toxicity studies in a chemical dye production industry in Turkey

Delia Teresa Sponza *

Dokuz Eylul University, Engineering Faculty, Environmental Engineering Department, Buca-Kaynaklar Campus, Izmir, Turkey

Received 29 April 2005; received in revised form 25 May 2006; accepted 26 May 2006
Available online 22 July 2006

Abstract

This study investigated the acute toxicity of chemical dye production industry wastewaters by traditional and enrichment toxicity tests and emphasized the importance of toxicity tests in wastewater discharge regulations. The enrichment toxicity tests are novel applications indicating whether there is potential toxicity or stimulation conditions. Different organisms were used including bacteria (floc-*Zoogloea ramigera* and coliform-*Escherichia coli* bacteria), algae (*Chlorella vulgaris*), fish (lepistes-*Poecilia reticulate*) and protozoan (*Vorticella campanula*) to represent four tropic levels. The toxicity test results were compared with chemical analyses to identify the pollutants responsible for the toxicity in the effluent wastewater samples. Toxicity of the effluents could not be explained by using physicochemical analyses in four cases. The results clearly showed that the use of bioassay tests produce additional information about the toxicity potential of industrial discharges and effluents.

Keywords: Chemical dye production industry; Traditional acute toxicity; Enrichment toxicity test

1. Introduction

Some organic and inorganics at toxic levels have been detected in industrial discharges resulting in plant upsets and discharge permit violations [1]. The conventional approach to controlling harmful chemicals in the aquatic environment is to use a set of global physical–chemical and biochemical parameters. Chemical procedure alone cannot provide sufficient information on the potential harmful effects of chemicals on the aquatic environment. The toxic effects of unknown and often undetermined substances in complex mixture or with possible synergistic effects among compounds to wastewaters can be detected only by toxicity testing. Biological toxicity testing is now a rapidly expanding field involving numerous bioanalytical techniques developed and applied to organisms which are at different tropic levels [2–5]. Various countries are now using toxicity tests as part of their water quality monitoring program (in the Netherlands in the United States in the United Kingdom since 1996 [6–12]. In Turkey chemical-specific monitoring is used, but toxicity testing was not included in the regulations. Only the fish toxicity test, based on the toxicity dilution factor (TDF), which indicates the toxicity, was included in the Turkish Water Pollution and Control Regulation [21]. Other conventional toxicity tests (for instance, bacteria, algae, *Daphnia*, protozoan, etc.) were not taken into consideration for industrial discharges in these practices.

Conventional toxicity tests using *Daphnia magna*, Microtox, Biosensor, Eclox and Toxalert tests are routinely used to assess the toxicity of industrial samples. Many of these toxicity tests, however, time consuming and use higher organisms as test species, which is ethically undesirable [11,13]. The enrichment toxicity tests containing bacteria could be valuable screening tools for identifying and categorizing toxic effluents together with acute toxicity tests [2,14]. The assessment of whether complex substances present a risk to organisms in the environment can most accurately be accomplished by acute and enrichment toxicity tests [15]. Even though in some cases the effluent quality is not violating the discharge limits, the wastewater could shows toxicity. However, toxicological monitoring does not provide direct indications of the specific cause(s) of toxicity. Yet knowing the cause of toxicity is a key need for efficiently controlling effluent toxicity.

The chemical industry contained colors including red, blue, green, brown and black through the production of different color containing dyes. The use of organic dyes has increased in the last years mainly by their low cost variety of colors. The wastewater containing residual amounts odd dyes have intense color [16].

* Tel.: +90 232 412 71 19; fax: +90 232 453 11 53.
E-mail address: delya.sponza@deu.edu.tr.

0304-3894/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2006.05.120

The aim of this study was the evaluation of the toxicity of wastewater from the chemical dye production industry, emphasizing the incorporation of acute toxicity parameters into Turkish Environmental Regulations in order to protect receiving ecosystems. Causality was established by linking the concentration of stressor (possible toxicants) with observed effect such as mortality. As part of the monitoring program, enrichment toxicity tests were performed using bacteria to categorize toxic discharges. Additionally, conventional toxicity tests were carried out to assess the toxicity of chemical dye production industry wastewater for four-test organisms. Through this study the chemical analysis results were compared to enrichment and conventional toxicity test results to confirm and define Cr^{6+}, color, Cd^{2+}, Zn^{2+}, Fe^{3+}, Pb^{2+} and total hydrocarbon as the cause of acute toxicity to test organisms. Furthermore, the sensitivity of toxicity tests was compared.

2. Methods

The samples taken from the effluents of the treatment plants of the chemical dye production industry were analysed for chemical, biochemical and toxicity parameters during 9 months from May 1997 to February 1998. The existing treatment plant of chemical industry consisted from a mechanical, chemical and biological treatment. A conventional activated sludge unit was used following the chemical treatment unit. The volume of activated sludge unit was 8500 m^3 and the flow rate of this wastewater was 8000 m^3 day^{-1} which has been brought in an equalization tank before pre-chemical treatment. The existing activated sludge plant consisted of an aeration and setting tank connected in series with the recycled sludge introduced to the aeration basin. $FeCl_3$ and polyelectrolite were used as coagulant compounds in chemical treatment reactors. The effluent of the post-settling tank was discharged to a mouth of a river nearby to a bay.

Protozoa (*Vorticella campanula*), algae (*Chlorella vulgaris*), fish (lepistes-*Poecilia reticulate*) and bacteria (coliform-*Esherichia coli* and floc-*Zoogloea ramigera*) were used to indicate four different trophic levels for investigation of the toxicity of effluent wastewater. Short-term definitive traditional acute and enrichment toxicity tests were performed and compared. The results of toxicity tests were compared with the chemical analysis results to determine the causality of toxicity to microorganisms in wastewater.

2.1. Traditional acute toxicity tests

These bioassays were performed to detect the relative toxicity of wastewater to selected microorganisms taken from the effluent of the treatment plants. In the determination of viable numbers of bacteria, protozoan, algae and fishes, the effluent wastewater samples were diluted and inoculated with the aforementioned microorganisms in a special medium containing all the necessary substances for growth. They were incubated at an appropriate temperature and counted according to membrane filter technique [17]. Initial bacteria colony concentration was measured, then the number of bacterial colonies was monitored for 24 and 48 h of exposure period and the concentrations which affected 50% of the organisms tested in different volumes of effluent (EC_{50} values as w/v) were calculated. All tests were performed in triplicate.

2.1.1. Bacteria toxicity

Since the number of floc (*Z. ramigera*) and coliform (*E. coli*) bacteria are significantly high and predominantly responsible for organic degradation in biological waste treatment, two bacteria groups were chosen for use in the toxicity tests. Total coliforms were isolated by membrane filtration on *m-Endo* broth [17]. A cellulose-acetate membrane filter is used in this method. The nominal pore sizes of the membrane filters were 0.45 μm. Appropriate dilutions of each wastewater samples were filtered. The membranes were placed on absorbent pads soaked with media *m-Endo* broth and incubated at, 37.5 °C for 48 h. Floc forming bacteria were isolated and counted by membrane filtration. FF Protease-peptone yeast broth soaked pads were used as media and incubated at 27 °C for 3 days [18]. The total coliform was isolated from contaminated drinking water with municipal wastewater containing no organic and inorganic toxic pollutants. The floc bacteria was isolated from the municipal wastewater treatment in İzmir containing no toxicants.

2.1.2. Fish toxicity

Effluent wastewater taken from the treatment plants was diluted in certain volume percentages (between 1 and 100%) and the mortality of lepistes (*P. reticulate*) was monitored after 48 h of incubation period at ambient temperature [17]. Lepistes was purchased from an aquarium in İzmir.

2.1.3. Algae toxicity

C. vulgaris were identified and enumerated under a microscope on filtered and immersion coated membranes following the end of a 3-day incubation period at 21 °C in mineral-algae medium soaked membranes containing Al^{3+}, Fe^{3+} salts and inorganic nitrogen and phosphorous [19]. Algae was obtained from the Aegean University Department of Biology cultured in natural water not polluted environments.

2.1.4. Protozoan toxicity

V. campanula was taken from the Zoology Department of the Science Faculty of Aegean University cultured in natural water. This microorganism was inoculated into the diluted effluent wastewater varying between 1 and 100% and incubated for 24 h at 21 °C. Motility or viable cells of *V. campanula* were assessed for lack of toxicity [19].

2.1.5. Assessment of acute toxicity

All traditional acute toxicity test results are expressed in EC_{50}, which means that this is the concentration which affected 50% of the organisms tested in different volumes of effluent. All concentrations are given in volume percentage of the effluents [20,21]. The higher this result, the less acutely toxic the sample. For example, 100% effluent is undiluted effluent. In order to indicate that the raw effluent was not diluted due to the absence of toxicity the percentage concentration of effluent wastewater

Table 1
Assessment of conventional acute toxicity tests

Volume percent of effluent wastewater (EC_{50}, w/v)	Acute toxicity evaluation
<1	Acutely toxic
1–10	Moderately acutely toxic
10–100	Minor acutely toxic
>100	Not acutely toxic

was shown as 100%. When the effluent is diluted ten times, the expression is 10%. If this is the result of the test, the effluent is acutely toxic for 50% of the test organisms at a dilution of 10 (see Table 1).

2.2. Enrichment toxicity tests

The enrichment test is based on the growth of *Enterobacter aerogenes* in a chemically defined minimal growth medium. This bacteria was isolated from drinking water. The presence of a toxic agent or a growth promoting substance will alter the 48 h population by an increase or decrease of 20% or more when compared to control. Depending on their concentration some unknown toxic chemicals cause mortalities to occur in microbial population [17]. Twenty-one-milliliter aliquots of wastewater samples taken from effluent were added to flasks B, C, D and E containing different type of substrate medium. These media were; control, effluent wastewater sample taken from treatment plant and nitrogen and phosphorus containing flasks. Flask A was the control and contained sufficient amounts of nitrogen, carbon, phosphorus sources and distilled water for organism growth without wastewater samples. Flask B contained nitrogen, carbon, phosphorus sources and wastewater sample taken from the effluent. If the wastewater added to this flask did not contain toxic substances, substrate limiting or unlimiting conditions would be suitable for maintaining bacterial growth. For this reason flask B indicated the "unknown wastewater". Flask C did not contain carbon or nitrogen sources. In this case, if the wastewater added to this flask contained sufficient carbon and nitrogen bacterial growth would occur. This indicated that the wastewater contained "available food" for microbial reproduction. Flask D contained carbon and phosphorus sources with wastewater sample but did not contain a nitrogen source. If the wastewater added had a sufficient amount of nitrogen source bacterial growth would occur. For this reason this flask indicated the "nitrogen source". Flask E contained nitrogen and phosphorus sources with wastewater sample but did not contain a carbon source. In this flask, the limiting factor was the carbon source. In this case, if the wastewater contained enough carbon the bacteria number would increase and it indicated the "carbon source". All flasks were also inoculated with 1 ml of *E. aerogenes* bacteria isolated from the biological treatment plant of industries. The total liquid volumes of the flasks were 30 ml. Appropriate dilutions were carried out in the *E. aerogenes* suspension to arrive at a specific density range between 30 and 100 viable colony forming units/ml in every flask. Cell densities below this range result in ratios that are not consistent, while densities above 100 cells/ml result in decreased sensitivity to nutrients. All flasks were incubated at room temperature for 1 week. At the end of the incubation period the number of *E. aerogenes* bacteria were counted by filtering them through membrane filters with a pore size of 0.45 μm and then they were incubated on mFC broth soaked pads at 44.5 °C. All tests were done in triplicate.

The composition of sodium citrate, salt mixture and phosphate buffer solutions that were used as a growth medium in enrichment tests was as follows (concentrations of constituents are given in brackets as $mg\,l^{-1}$ for 1000 ml distilled water): $Na_3C_6H_5O_7{\cdot}2H_2O$ (580) for the sodium citrate solution; $(NH_4)_2SO_4$ (1200) for the ammonium sulfate solution; $MgSO_4{\cdot}7H_2O$ (520), $CaCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$ (460) and NaCl (5000) for the salt mixture; KH_2PO_4 (2700), KH_2PO_4 (2700) and $MgSO_4$ (2800) for the phosphate buffer solution. pH was adjusted to 7.0 with 1N NaOH in all solutions.

The enrichment tests were assessed based on the ratio of *E. aerogenes* numbers in the flask B to control flask A as summarized below [17].

2.3. Assessment of enrichment toxicity test results

2.3.1. For growth inhibiting substances

A B/A ratio below 0.8 indicates the possible presence of toxic substances in wastewater. If this occurred, the impact of possible toxic substances was evaluated.

2.3.2. For growth stimulating substances

When the B/A ratio exceeds 1.2, it may be assumed that growth-stimulating substances are present in wastewater. This procedure is extremely sensitive up to ratios 3.0. When the B/A ratio is between 1.2 and 3.0; C/A, D/A and E/A ratios are not necessary. Nutrient excess or deficiency in terms of nitrogen and carbon is not significantly important due to bacterial growth in flask B.

2.3.3. For growth limiting substances

If the number of microorganisms in flask B is higher than control (flask A), B/A ratio could go above 0.8 and it vary between 0.8 and 1.2. In this case it could not be said to be due to the effect of toxic substances but may be because of some nutrients present which limit the bacterial growth in wastewater. If this occurred nutrient excess or deficiency impact were assessed.

2.4. Toxicity dilution factor (TDF)

Toxicity dilution factor indicates the volume of wastewater which is diluted with dilution water. The toxic effect is determined using fish (lepistes-*P. reticulate*) as test organism and applying various degrees of wastewater dilutions. For example, TDF = 3 means that in wastewater which is diluted by the factor 1:2 and all fish survive a 48 h period. In other words, toxic effect can be determined proportionally with the dilution volume in which the wastewater is diluted with a dilution liquid. Accordingly, the minimum dilution volume in which all fish stay alive, is TDF. In this experiment an aquarium with sufficient aeration, diluted wastewater and fish (lepistes-*P. reticulate*) was used. At

the end of 24 and 48 h, the dilution in which all fish were alive was observed and accepted as the appropriate dilution ratio [22].

2.5. Chemical and biochemical analysis

Cr^{6+}, Cd, Pb^{2+}, Fe^{3+} and Zn^{2+} were measured by following Standard Methods [17]. Color was measured in filtered samples as spectral absorption intensity by a Unicam spectrophotometer at 597 and 254 nm. pH was measured by a digital pH meter. The total hydrocarbons were measured spectrophotometrically [17]. The values reported in the figures were the averages of triplicate analyses with standard deviations indicating the variance of the samples.

2.6. Statistical analysis

Differences of sensitivity score (an index indicating the most and the least sensitivity level of organisms) between microorganisms determined in conventional acute toxicity test were examined by performing a non-parametric Kruskal–Wallis test followed by a Mann–Whitney *U*-test [23,24]. The Kruskal–Wallis test was used to compare the sensitivities in toxicity response between bacteria, algae, fish and protozoa. The Mann–Whitney *U*-test was used to evaluate the relationship between paired microorganism groups. All results are reported at a significance level of $p \leq 0.10$ [24]. The statistical package used for analysis was SPSSWIN in Microsoft Windows™ [25]. Multiple regression analysis between *y* and *x* variables was performed using the SPSSWIN in Microsoft Windows™. The multiple regression analysis was used to find out the correlations between *x* and *y* variables. The linear correlation was assessed with r^2. r^2 is the correlation coefficient and reflects statistical significance between dependent and independent variables.

3. Results and discussion

Table 2 shows the Turkish Receiving Water Discharge Standards for chemical dye production industry effluents. This regulation does not contain limitations for some parameters such as Cr^{6+}, color, Cd^{2+}, Zn^{2+}, Fe^{3+}, Pb^{2+} and total hydrocarbon for this industry discharge. Effluent water analysis showed that the chemical parameters measured were violating the discharging limits and exhibited higher toxicity on some days in the effluents.

Table 2
Receiving water discharge standards in chemical dye production industry

Parameters	Chemical dye production industry[a]
COD ($mg\,l^{-1}$)	200
BOD_5 ($mg\,l^{-1}$)	50
TSS ($mg\,l^{-1}$)	60
pH	6–9
TDF	3
Cr^{6+} ($mg\,l^{-1}$)	0.03
Cd ($mg\,l^{-1}$)	0.02
Zn ($mg\,l^{-1}$)	0.09
Pb ($mg\,l^{-1}$)	0.01
Colour	
Fe ($mg\,l^{-1}$)	0.12
Total hydrocarbon ($mg\,l^{-1}$)	0.20

[a] Composite sample taken in 2 h.

3.1. Toxicity tests

The conventional and enrichment toxicity analyses were conducted in parallel from May 1997 to February 1998 in the chemical dye production industry effluents. During this period, algae, bacteria, protozoan and fish were used as test organisms representing four different trophic levels. Table 3 shows all acute toxicity and enrichment test results for the 9 months of sampling. Throughout these analyses, no significant difference in activity was observed among the two organisms except the protozoa and algae. The EC_{50} (%) values obtained from traditional toxicity tests were compared to B/A (the B/A ratio define the number of bacteria grown on wastewater to control) values obtained from enrichment toxicity tests. Enrichment toxicity test results (B/A ratios) showed similar results to those obtained from acute toxicity tests in the effluent samples. In other words, all the results obtained from the acute definitive toxicity tests were confirmed by the results obtained from the enrichment tests in which *E. aerogenes* was used instead of coliform, floc bacteria, algae, fish and protozoan.

Definitive acute tests in the chemical dye production industry showed that EC_{50} (%) value was generally between >80 and >100% for protozoa and algae in the effluents. Similar results were obtained by Slabbert and Venter [2], for the pulp-paper industry effluents. The protozoa and algae tests, in terms of effective concentration range (as EC_{50} (%)) of the chemical dye production effluents, were determined at between 80 and >100% (except on days 10 and 80 for protozoan and on days 130 and 170 for algae) for all the samplings made. Contrarily, the study carried out by Tonkes et al. [21], with 17 effluents containing wastewater from the oil refinery and chemical industry showed that algae is the most sensitive toxicity test. Algae showed acute toxicity in 13 out of 17 effluents. Bacteria and fish showed toxicity 8 and 7 times in 17 different samplings [21].

The fish toxicity test results varied between 0.2 and 100 as percent values of effective concentration for the effluents. Bacterial toxicity test results ranged between 0.02 and >100% and 0.07 and >100 as percent of effective concentrations for the coliforms and floc bacteria, respectively, in the effluents. Similar results were determined by Slabbert and Venter [2], in kraft-mill effluents.

Toxicity test results in the chemical dye production industry effluents showed potential toxicity depending on the chemical composition of the wastewater on seven 190 days. This toxicity could be attributed to a high concentration of Pb (6–9 $mg\,l^{-1}$), dyes as color (spectral absorption intensity, SAI = 3 m^{-1}), Cr^{6+} (5–6 $mg\,l^{-1}$), Cd (6–7 $mg\,l^{-1}$), TDF (8–9), Fe^{2+} (8–9 $mg\,l^{-1}$), Zn (10–12 $mg\,l^{-1}$) and total hydrocarbon (10–12 $mg\,l^{-1}$) in the effluent wastewaters (see Table 3 and Figs. 1 and 2). The multiple regression analysis between Pb, color, Cr^{6+}, color, Cd, Fe^{3+}, Zn, total hydrocarbon and TDF in the effluent samples showed a very strong linear correlation ($r^2 = 0.87$, $p = 0.005$).

Table 3
Toxicity test results in chemical dye industry effluents (mean; $n = 3$)

Days/industry	EC_{50} values (%, v/v)					Enrichment toxicity test (B/A ratio)	Remarks of B/A ratio	General results
	Coliforms	Floc bacteria	Fish	Algae	Protozoan			
1	89	95	90	>100	>100	2.19	Growth stimulation	Not acutely toxic
10	84	93	88	100	70	2.39	Growth stimulation	Not acutely toxic
20	85	80	90	98	99	2.34	Growth stimulation	Not acutely toxic
30	0.02	0.53	0.64	85	>100	0.03	Potential toxicity	Acutely Toxic
40	0.05	0.09	98	90	87	0.10	Potential toxicity	Acutely toxic
50	0.08	1	3	>100	>100	0.7	Potential toxicity	Acutely Toxic
60	99	88	77	>100	89	2.58	Growth stimulation	Not acutely toxic
70	>100	>100	>100	>100	>100	2.01	Growth stimulation	Not acutely toxic
80	23	34	19	95	70	1.34	Growth limiting nutrient	Minor acutely toxic
90	99	85	99	100	>100	2.13	Growth stimulation	Not acutely toxic
100	>100	100	100	100	>100	2.45	Growth stimulation	Not acutely toxic
110	6	4	3	89	100	0.9	Growth limiting nutrient	Moderately toxic
120	0.06	0.07	80	93	90	0.24	Potential toxicity	Acutely toxic
130	0.8	0.3	0.2	72	>100	2.59	Potential toxicity	Acutely Toxic
140	99	89	79	100	>100	2.41	Growth stimulation	Not acutely toxic
150	87	99	90	99	100	2.64	Growth stimulation	Not acutely toxic
160	0.39	0.09	0.65	86	95	0.29	Potential toxicity	Acutely toxic
170	0.40	0.12	0.8	64	90	0.40	Potential toxicity	Acutely toxic
180	9	7	8	90	100	0.97	Growth limiting nutrient	Moderately toxic
190	67	27	18	89	99	0.88	Growth limiting nutrient	Minor acutely toxic

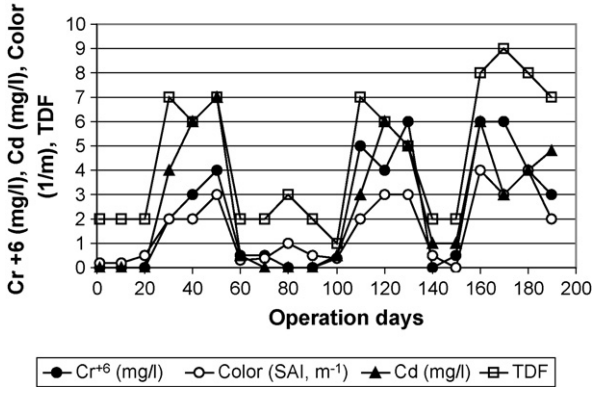

Fig. 1. Variation of Cr^{6+} (mg/l), color (SAI, m^{-1}), Cd (mg/l) concentrations and TDF levels in chemical industry dye effluents.

Although the bioassays have a useful role in identifying the toxicity, it is important to determine the cause of the toxicity. It was found that several chemicals at different concentrations caused toxicity on aquatic organisms. Cd and Pb are ubiquitous

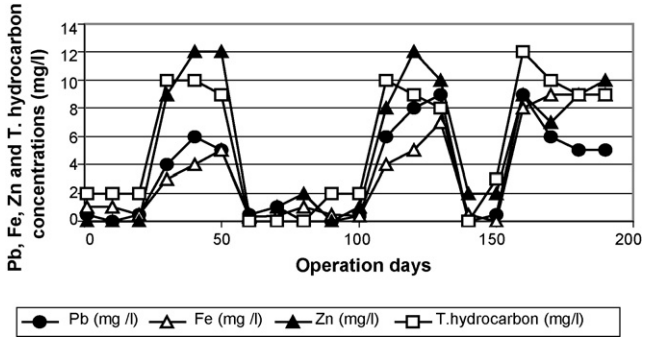

Fig. 2. Variation of Pb (mg/l), Fe (mg/l), Zn (mg/l) and total hydrocarbon (mg/l) concentrations in chemical dye production industry effluents.

in the environment and is hazardous at high levels. The addition of 1000 $\mu g\,l^{-1}$ Pb and Cd caused a decrease of bacterial abundance after 48 h. Similarly protozoan abundance diminished in the beginning, however after 48 h an important reduction respect to the control at 96 h was obtained [26]. Acute toxicity test revealed Cd LC_{50} values at 48 and 96 h were 0.064 and 0.030 $mg\,l^{-1}$, respectively, using *Tubifex tubifex* in polluted sediments [27]. Process industries such as battery manufacturing, metal plating and chemical industries are prime source of lead pollution. Current EPA drinking water discharge standard for Pb are 0.05 $mg\,l^{-1}$ [28]. According to Indian Standard Institution the tolerance limit for discharge of Pb to land surface is 0.1 $mg\,l^{-1}$ [29]. In a study performed by Chinni and Yallapragada [30] it was found that 79 and 68 decreases in biochemical constituents (total protein and total lipids) was observed when the past larvae *Penaceus indicus* was exposed to subletal concentration of lead (1.44 ppm) for 30 days.

Chromium occurs in higher concentration in the wastes from electroplating, paints, dyes, paper and chemical industries. Over exposure of chrome workers to chromium dusts and musts has been revealed to corrosion of the skin and the respiratory tract. Ingestion may cause epigastria pair, nausea and severe diarrhea. Maximum contaminant level of Cr^{6+} for the drinking water is 0.05 $mg\,l^{-1}$ [31,32]. The assessment of toxic interactions of heavy metals and bioavailability must be based on levels of specific chemical forms, rather than on total element levels [33]. The widespread use of chromium in various industries had resulted in the discharge of large quantities in the environment. Cr^{6+} exerts toxic effects in biological systems arising from the possibility of free diffusion across cell membranes and strong oxidative potential. Cr^{6+} and Cr^{3+} in chemical plating industry effluents were found to be in the range 25–100 and 5–50 $\mu g\,l^{-1}$, respectively. Cr^{6+} dominates in effluents from metallurgical and metal-finishing industries and Cr^{3+} exists mainly in tannery,

textile and decorative plating industry wastes. Both DNA and membrane damages were obtained in the toxicity of 2–5 mg l^{-1} of Cr^{6+} in vivo and in vitro studies [34]. Chromium induces the oxidative stress that results in oxidative deterioration of biological macromolecules. Clastogenic damage was observed in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) at the end of 30 days of exposure period to effluent concentrations of 2, 4 and 16% in a leather industry treatment plant [35] with respective IC_{50}-24 h values of 0.15 and 3.36 expressed as dilution ratios.

In a study performed by Jobbagy et al. [36] it was found that weak, small flocs with a characteristic lack of filament in an activated sludge treatment plant of the chemical industry depends to the toxic and changing characteristics of the wastewater by high Cr^{6+} and Pb concentrations. Acute toxicity tests performed by Cd, Cu^{2+}, Zn and Ni in *D. magna* showed that the IC_{50} values are 0.065, 0.105, 12.5 and 8 mg/l, respectively, at 20 °C temperature [37]. As the Cr^{6+} concentration increased from 5 to 200 mg l^{-1} the specific substrate utilization rate decreased to 7.10×10^2 and 1.61×10^2 mg mg^{-1} h^{-1} in activated sludge samples [37]. After 8 days of testing a reduction in growth of freshwater plant *Salvinia natans* with 10–2 mg l^{-1} Cd^{2+} and 4.48 mg l^{-1} Cu^{2+} were obtained. Furthermore, necrosis produced by cadmium on *Sphaerotilus natans* was observed [37].

Synthetic dyes which are used extensively in textile, paper, printing and chemical dye industries can be classified mainly as azo, anthraquinone, vat, phtalocyanine, indigo, polymethilene, carbonium and nitro dyes [38,39]. Most of these dyes are manufactured through different stages involving nitraration, reduction, halogenation, amination, sulfonation, diazotization and oxidation. In a study performed by Pintar et al. [40] showed that the chemical bleaching effluent containing yellow brown color is toxic for 30 min. *D. magna* and *Vibrio fisheri* after treated by catalytic wet air oxidation. Sponza [41] showed that the effluent of pulp-paper industry effluents is toxic to coliform, floc bacteria and fish (EC_{50} values (%, w/v) are 0.6, 0.17 and 1 when the color and AOX concentrations are higher). Minke and Rott [42] showed that bleaching effluents containing dyes includes high levels of AOX and heavy metal concentrations. The Fe^{3+} concentrations in the affected and unaffected leaves of helophyte *Glyceria fluitans* were 32.8 and 6.2 μmol g^{-1} DW, respectively, resulting in brown necrotic spots on 67% of the leaves where groundwater table was highest in iron contaminated soils [43].

Acute toxicity tests on chemical dye production effluent showed that the bacteria and fish were the most sensitive organisms (the lowest EC_{50} values were 0.02 and 0.2%, respectively). High mortalities in the bacteria and fish toxicity tests indicated the presence of Pb (6–9 mg l^{-1}), dyes as color (SAI = 3 m^{-1}), Cr^{6+} (5–6 mg l^{-1}), Cd (6–7 mg l^{-1}), TDF (8–9 mg l^{-1}), Fe^{2+} (8–9 mg l^{-1}), Zn (10–12 mg l^{-1}) and total hydrocarbon (10–12 mg l^{-1}) at high concentrations in effluents (see Table 3 and Figs. 1 and 2).

In general, the chemical dye production wastewater effluent contained relatively high levels of potentially toxic chemicals, indicating that effects were probably due to a combination of pollutants. The actual cause of toxicity originates from the high concentrations between Pb, color, Cr^{6+}, Cd, Fe^{3+}, Zn and total hydrocarbon (Figs. 1 and 2).

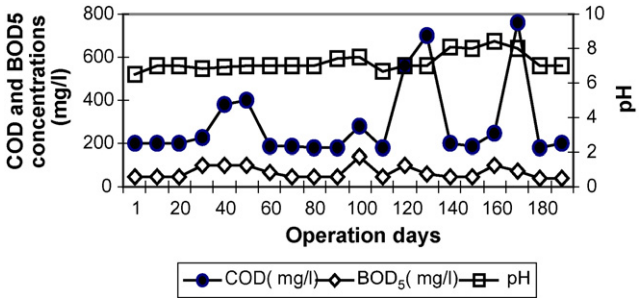

Fig. 3. Variation of COD (mg/l), BOD_5 (mg/l) concentrations and pH levels in chemical dye production industry effluents.

The effluent quality depicted by Cr^{6+}, Pb, color, Cd, Fe^{3+} and total hydrocarbon concentrations exhibited an increase of at least 30% on days 30, 40, 50, 110, 120, 130, 160, 170, 180 and 190 compared with days 1, 10, 20, 70, 80, 140 and 150 in the chemical dye production industry (see Figs. 1 and 2). Although in some cases the effluent wastewater quality achieves the prescribed quality; the toxicity test results indicate a potential toxicity, demonstrating that toxicity test should be included in discharge standards regulations. For instance, although the COD, BOD_5, pH and TDF values are lower than permissible discharge limits in this industry on days 80, 110, 180 and 190; the toxicity test results showed the presence of moderately and minor acute toxicity (see Fig. 3 and Tables 2 and 3).

Thus, it can be concluded that the reasons for this are a lack of knowledge of the composition, and a lack of acute toxicity data for the substances which are known to be present.

The general classification of all effluents based on both traditional and enrichment tests can be summarized as follows: seven effluents are acutely toxic, two effluents are moderately acutely toxic, two effluents are slightly acutely toxic and seven effluents are not acutely toxic in the chemical dye production industry. Moderate and slight acute toxicity was observed on certain days depending on of Pb dyes as color, Cr^{6+}, Cd, Fe^{2+}, Zn and total hydrocarbon levels in the aforementioned industry.

This classification was carried out according to assessment of the conventional toxicity test. Since the B/A ratios and EC_{50} (%) values increased as the concentrations were reduced in all chemical analysis the mortalities also decreased. In general, the multiple regression analysis between toxicity test results (B/A ratios and EC_{50} (%) values; *y* variables) and chemical parameters (*x* variables) showed that there was a very strong linear correlation to each other in all industries ($r^2 = 0.83$, Durbin–Watson statistic = 1.26). It was observed that both B/A ratios and EC_{50} (%) values decreased with increasing toxicities (depending on mortalities) and concentrations in analytical parameters in the studied industry.

3.2. Sensitivity of toxicity tests

In order to compare the toxicity tests, a sensitivity index was constructed by taking into consideration only the results of traditional acute toxicity tests for each organism tested. A score

Table 4
Toxicity test results chemical dye production industry effluents (mean; $n = 3$)

Days/industry	Assessment of sensitivity				
	Coliforms	Floc bacteria	Fish	Algae	Protozoan
1	1	3	2	4	5
10	1	3	2	5	4
20	2	1	3	4	5
30	1	2	3	4	5
40	1	2	5	4	3
50	1	2	3	4	4
60	4	2	1	5	3
70	1	1	1	1	1
80	2	3	1	4	4
90	2	1	2	3	3
100	1	1	1	1	1
110	3	2	1	4	5
120	1	2	3	5	4
130	4	2	1	3	5
140	3	2	1	4	4
150	1	3	2	4	5
160	2	1	3	4	5
170	2	1	3	4	5
180	3	2	1	4	5
190	3	2	1	4	5
Sum	38	37	41	76	86

of "1" was assigned to the most sensitive test for each sample down to 5 for the least sensitive. Table 4 shows the sensitivity assessment for every organism used in acute toxicity tests.

The EC_{50} values measured for protozoa and algae differed significantly from bacteria (coliform and floc bacteria), and fish in the decline of sensitivity scores and exhibited lower mortalities in the effluents (Kruskal–Wallis test statistic 15.65, $p \leq 0.05$ for algae, and Kruskal–Wallis test statistic 17.09, $p \leq 0.05$ for protozoan). The Kruskal–Wallis test statistics showed that protozoa and algae have higher EC_{50} values and lower sensitivity scores than other groups. In other words, both protozoa and algae tests were differed significantly from the remaining microbial groups is being less sensitive. The coliforms and floc bacteria had a similar response to toxicity and mortalities (Mann–Whitney U-test statistic = 0.678, $p = 0.10$). The response to toxicity of coliform bacteria and fish is different in all the industries. However, the difference is not significant (Mann–Whitney U-test statistic = 0.870, $p = 0.05$). The coliform bacteria had lower EC_{50} values than protozoa and these differences were significant (Mann–Whitney U-test statistic = 16.540, $p < 0.10$). The response to toxicity of coliform bacteria and algae was found to be different and this difference was significant (Mann–Whitney U-test statistic = 8.23, $p = 0.05$). The floc bacteria had higher EC_{50} values than the algae and the difference was significant (Mann–Whitney U-test statistic = 8.92, $p \leq 0$). The sensitivity score of floc bacteria was higher than fish but the difference was not significant (Mann–Whitney U-test statistic = 0.94, $p \leq 0.05$). The sensitivity score of floc bacteria was higher than protozoan and this difference was significant (Mann–Whitney U-test statistic = 13.09, $p \leq 0.05$). The sensitivity score of fish was different from algae and the differences were significant (Mann–Whitney U-test statistic = 10.81, $p \leq 0.10$). The EC_{50} values measured for protozoa was higher then algae and this difference was not significant (Mann–Whitney U-test statistic = 0.75, $p \leq 0.10$). The sensitivity score of fish was higher than protozoa and this difference was significant (Mann–Whitney U-test statistic = 9.75, $p \leq 0.05$).

According to the statistical analysis results, in a sample comparison of the sensitivity among all trophic levels, the protozoa and algae test seems to be less sensitive than the bacteria and fishes tests. In other words, the protozoa (*Vorticella*) and algae (*Chlorella*) were found to be very resistant. It was shown that the coliform bacteria test was not as sensitive on days 70, 100 and 190 floc bacteria and fish also showed similar results on days 70. It should be pointed out, however, that the bacteria, algae and fish tests are reference standards used world-wide for toxicity testing and represent one of the trophic level tests required in the toxicity evaluation.

From the toxicity tests it can be seen that different organisms influenced different by the chemical dye effluent wastewater. This could be attributed to the presence of sensitive and resistant organisms and the interactions between heavy metals reducing the number of organisms. The initial coliform–floc bacteria, algae and protozoan in 10 l water were 2×10^7 and 8×10^7 colony, 30 algae, 30 protozoan and 10 fish, respectively. The number of coliform, floc bacteria, algae, protozoan and fish in 10 l water varied between 10^6 and 10^7 colony, 28–30 algae, 27–30 protozoa and 6 fish if the wastewater is not acutely toxic. When moderate toxicity was observed the number of coliform–floc bacteria, algae, protozoan and fish in 10 l water varied between 10^2 and 10^3 colony, 25 algae, 22 protozoa and 2 fish, respectively. When minor acute toxicity was showed in 10 l water the number of coliform–floc bacteria, algae, protozoan and fish were 10^1–10^2 colony, 20 algae, 19 protozoan and 2–3 fish, respectively. The number of coliform, floc bacteria, algae, protozoan and fish were 0 colony, 16 algae, 17 protozoan and 0 fish, respectively, in 10 l water, when acute toxicity was observed. From the number of organisms it was observed that the bacteria and fish are more sensitive organisms while the protozoan and algae were the resistance organisms. On days 20–40 and 100–140 the high Cd, Zn and low Cr, Fe, Pb concentrations does not significantly affect the growth of *V. campanula* and *C. vulgaris*. Zn has been shown to reduce the Cd uptake to lepistes [44] In algae *C. vulgaris* uptake of Cd was completely inhibited by 0.01 mg Fe l^{-1} [45]. In this study the combined effects of Zn and Cd to protozoan *V. campanula* gave lower mortality than would expected if there were no interaction between metals [45]. The resistance of protozoan and algae could be attributed to these results. The toxic impact of Cd to aquatic organisms depends on the presence of Zn, notably in *Z. ramigera* [45,46]. The sensitivity of floc bacteria could be attributed to these results. Zn can behave synergistically with Pb or Fe alone in combination in *C. vulgaris* resulting in low mortalities in algae [45]. This result shows the resistance of algae.

From Table 3, it would appear that the protozoan and algae tests would be potential surrogates since their overall sensitivity is significantly lower than the bacteria and fish tests. However, both the traditional and enrichment tests are affected by

the presence Pb dyes as color, Cr^{6+}, Cd, Fe^{2+}, Zn and total hydrocarbon in the chemical dye production effluent samples. Greater sensitivity would be obtained by using coliforms bacteria, floc bacteria and fish for chemical dye production industry effluents where protozoan and algae tests displayed greater resistance.

The effects of mixed heavy metals were investigated by some researchers and different results was obtained: Zn has been shown to reduce the Cd uptake to lepistes [44]. In algae *C. vulgaris* uptake of Cd was completely blocked by 0.2 mg Mn l^{-1} and inhibited by 0.02–5 mg Fe l^{-1} [45]. Mo, Pb, Zn and Co had no affect the nutrient uptake by the fishes ([47], Megharaj et al., 2003). Cd was the most toxic of heavy metal tested particularly on coliforms [48]. The toxic impact of Cd to aquatic organisms depends on the presence of Zn, notably in floc bacteria [46,47]. In a study of the combined effects of Zn and Cd to protozoan gave lower mortality than would expected if there were no interaction between metals [46]. Zn is adsorbed strongly by ferric hydroxide on Mn and the mixed of these metal cause mortality in fishes [49]. Zn can behave antagonistically in combination with Cu and synergistically with Pb or Fe alone in combination in algae *C. vulgaris* [45]. Zn toxicity was lowered by Mo and increased by Co or Se [50]. The acute toxicity of Pb to the algal growth may be due to its faster uptake by the test algae, while the relatively mild toxicity of copper and zinc may be due to their slow uptake [51]. In this study when the mixed Cd, Zn concentrations are high and mixed Cr, Fe and Pb concentrations are low (between days 20–40 and 100–140) generally acute toxicity was observed in more sensitive (coliforms, floc bacteria and fish) organisms depending to interactions between heavy metals. The resistant organisms protozoan and algae were low influenced from the effect of mixed heavy metal concentrations. Furthermore, the genus and the species of the used organisms affect the toxicity [52].

3.3. Sensitivity ranking

To explain the sensitivity of test results based on the toxicity, a table was constructed ranking the samples in order of toxicity. Sensitivity ranking indicates the sum of toxicity response of every microorganism used in conventional and enrichment toxicity tests. Organisms representing five trophic levels were classified according to traditional and enrichment test results. The comparison of toxicity response and sensitivity ranking was assessed in Table 5. The acute toxicity classification of an effluent should always be based on the results of testing all trophic levels at least once.

Table 5
Sensitivity ranking for chemical dye production industry

Organism	Sensitivity ranking			
	Very toxic	Moderately toxic	Slightly toxic	Not toxic
Coliform bacteria	7	2	9	2
Floc bacteria	6	2	9	1
Algae	0	0	16	4
Fish	4	3	11	1
Protozoan	0	0	11	8

Acute toxicity could be explained on the basis of chemical data with only 16 effluents in the pulp-paper industry. Under these conditions, the multiple regression analysis between toxicity test results and chemical parameters showed a very strong linear correlation to each other ($r^2 = 0.88$, Durbin–Watson statistic = 1.02). In four cases this was not possible. Under these conditions, the multiple regression analysis between toxicity test results and chemical parameters showed a weak linear correlation to each other ($r^2 = 0.59$, Durbin–Watson statistic = 4.52) (on days 80, 110, 180 and 190).

In acute toxicity the microorganisms they do not have enough time for acclimation to toxicants in wastewater since the incubation period with wastewater is 24–48 h. The high mortality level in fish, floc and coliform bacteria and low mortality in protozoan and algae could be attributed to the interaction between high level of Zn and Cd and to the interactions between low level of Cr, Pb and Fe resulting in acute, moderate, minor toxicities and no toxicity, respectively, between days 20–40 and 140–140. Furthermore, the genus and species used in the toxicity studies affect the toxicity effects of heavy metals.

The results of this research indicate that the discharges from the treatment plants show a different toxicity response from day to day. It can be concluded that the studied acute toxicity and enrichment tests produce valuable and additional information about the toxic characteristics of both treated and untreated discharges when compared to chemical analysis alone. In particular, the data obtained from the enrichment toxicity tests suggest nutrient deficiencies and growth stimulation conditions besides potential toxicity. It has become clear that the chemical specific approach produces enough information for only a limited number of complex effluent samples.

4. Conclusions

Studies on wastewater effluents indicated that all the toxicity tests have a viable role to play in water quality monitoring and control in the rural areas. The studies demonstrated that there is no single method that can constitute a comprehensive approach to aquatic life protection. For this reason, toxicity tests containing the sensitive microorganisms should be applied in battery form so those tests can complement each other and chemical analysis. According to the results of this study, bacteria and fish toxicity tests could be developed as promising techniques for control of chemical dye production industry wastewater to receiving aquatic ecosystems. Furthermore, the results of this study showed that enrichment toxicity tests are practical, cost effective and accurate tests in the determination of potential effects.

The results of this study showed that it is not possible to speculate on the mode of the toxic action due to the difficulties involved in the detailed characterization of the chemical content of wastewater. All effluents, which are tested for acute toxicity, were characterized as thoroughly as possible by physical and chemical analysis. This is needed to gain more insight into the possible causes of toxicity. For this reason, in this

study, the causality was established by correlating the chemical parameters with the observed effect in all tested industrial effluents. The concentrations of possible toxicant were attributed to the observed toxicity in the effluents. When the Cd, Zn are high and Cr, Fe and Pb concentrations are low (between days 20–40 and 100–140) generally acute toxicity was observed in more sensitive organisms and no toxicity or moderate, minor toxicities were shown in resistant organisms depending to interactions of heavy metals and kind of choosen organism type.

Studies on wastewater effluents indicated that all the toxicity tests have a viable role to play in water quality monitoring and control of the effects of pollutants on aquatic ecosystem. The studies demonstrated that there is no single method that can constitute a comprehensive approach to aquatic life protection. For this reason, toxicity tests containing the sensitive microorganisms from several trophic levels should be applied in battery form so those tests can complement each other and chemical analysis.

A methodology for wastewater characterization employing a single substance or a specific chemical substance could not give convenient results for determination the wastewater toxicity. The toxicity of effluents discharged into the environment should be effectively monitored and a toxicity test should be applied to water quality legislation to give recommendations concerning a strategy for regulatory toxicity control of different industrial branches.

Pb, dyes as color, Cr^{6+}, Cd, Fe^{2+}, Zn and total hydrocarbon originating from the chemical dye production processing discharges to receiving media should be reduced by the application of technology in the treatment plant, thus preventing toxicity in the receiving media. Where environmentally significant discharges of uncharacterized complex organic chemicals exist, a clearly defined toxicity limit should be specified, along with the appropriate form of toxicity test to be used, and the toxicity of effluents from wastewater treatment plant installations should be strongly controlled.

Toxicity test should be incorporated into receiving water discharge standards and the existing receiving water discharge standards should be reviewed to include the toxicity tests. In other words, toxicity tests should be imposed on effluent discharge standard regulations. Evaluation of toxicity or persistency of wastewater is essential for their hazardous impact and risk assessment.

Acknowledgment

The authors are grateful to the Research Laboratories from the industries for contribution to the chemical analysis.

References

[1] C.-Y. Chen, J.-N. Chen, S.-D. Chen, Toxicity assessment of industrial wastewater by microbial testing method, Water Sci. Technol. 39 (1999) 139–143.
[2] J.L. Slabbert, E.A. Venter, Biological assay for aquatic toxicity testing, Water Sci. Technol. 39 (1999) 367–373.
[3] C. Blaise, G. Sergey, P. Wells, N. Bermingham, N. Van Coillie, Biological testing-development, application and trends in Canadian Environmental Protection laboratories, J. Tox. Assess. 3 (1988) 385–406.
[4] J.L. Slabbert, Guidelines for Toxicity Bioassaying of Water and Effluents in South Africa, Contact Report for the Water Research Commission, Project no. K5/358/0/1, Division of Water Technology, Pretoria, South Africa, 1996.
[5] J.L. Slabbert, Improved bacterial growth test for rapid water toxicity screening, Bull. Environ. Contam. Toxicol. 37 (1986) 565–569.
[6] C.M.H. Beckers-Maessen, Toxicity Tests in WWO Law Regulatory Framework, RIZA Document 94, 1994, 0171X.
[7] EPA, Technical Support Document for Water Quality Based Toxic Control, Office of Water, Washington, DC, 1991, EPA/505/2-90-001.
[8] NRA, The Toxicity-Based Consent, a Step Towards More Effective Control of Complex Effluents Within the UK, NRA Information Leaflet, 1993.
[9] NRA, Direct Toxicity Assessment, a Step Towards Better Environmental Protection Within the UK, NRA Information Leaflet, 1994.
[10] NRA, Aquatic Toxicity, Control and Assessment, an Update on Current Research and Development, NRA Information Leaflet, 1995.
[11] P. Whitehouse, P. Dijk, The precision of aquatic toxicity tests: the implications for the control of effluents by direct toxicity assessment, in: J.F. Tapp, J.R. Wharfe, S.M. Hunt (Eds.), Toxic Impacts of Wastes on the Aquatic Environ, Cambridge, UK, 1996.
[12] I. Jonhson, J. Wharfe, D. Tinsley, R. Boumphery, Toxicity Based Consents Pilot Study, Technical Report, UK, 1996.
[13] M. Farre, D. Barcelo, Characterization of wastewater toxicity by means of a whole cell bacterial biosensor using *Pseudomonas putida* in conjunction with chemical analysis, Water Res. 371 (2001) 467–473.
[14] J.R. Wharfe, D. Tinsley, The toxicity based and the wider application of direct toxicity assessment to protect aquatic life, J. Chart. Inst. Water Environ. Man. 9 (1995) 526–530.
[15] D. Schowanek, K. Fox, M. Holt, F. Schroeder, GREAT-ER: a new tool for management and risk assessment of chemicals in river basin, Water Sci. Technol. 43 (2001) 179–185.
[16] N. Pizzolato, S. Armando, B. Voly, The dyes produced from the textile industries, J. Chem. Tech. Biotechnol. 19 (2003) 8276–8345.
[17] APHA, AWWA, WPC, Standard Methods for the Examination of Water and Wastewater, 16th ed., Washington, DC, USA, 1992.
[18] P.R. Dugan, D.C. Lundgren, Isolation and identification of floc bacteria in activated sludge systems, Appl. Microbiol. 8 (1968) 357–361.
[19] M.T. Pelezar, E.C.S. Chan, Laboratory Experiences in Microbiology, third ed., McGraw-Hill Book Company, USA, 1972.
[20] J.W. Canton, Catch-Up Operation on Old Pesticides: An Integration, RIVM-Report no. 678 80 1002, 1991.
[21] M. Tonkes, J.J. de Graaf, J. Graaansma, Assessment of complex industrial effluents in the Netherlands using a whole effluent toxicity approach, Water Sci. Technol. 39 (1999) 55–61.
[22] Turkish Water Pollution Control Regulation, In Official Gazette Numbered 19919 and Dated 4 September, 1992.
[23] W.J. Conover, Practical Non-Parametric Statistics, Wiley, New York, 1971.
[24] S. Siegel, Non-Parametric Statistics for the Behavioral Scientist, McGraw Hill, New York, 1956, p. 185.
[25] J.H. Zar, Biostatistical Analysis, second ed., Prentice Hall, Englemoood Cliffs, 1984.
[26] G.F. Leborans, Y.O. Herrrro, Toxicity and bioaccumulation of lead and cadmium in marine protozoan communities, Ecotoxicol. Environ. Safety 47 (2000) 266–276.
[27] M.L. Bouche, F. Habets, N. Biagianti-Risbourg, G. Vernet, Toxic effects and bioaccumulation of cadmium in the aquatic oligochaete *Tubifex tubifex*, Ecotoxicol. Environ. Safety 46 (2000) 246–251.
[28] A. Croffman, S. Peterson, D. Brookins, Removing lead from wastewater using zeolites, Water Environ. Technol. 5 (1992) 54–59.
[29] ISI, Drinking Water Specifications IS: 10500, 1991.
[30] S. Chinni, N. Yallapragada, Lead toxicity on growth and biochemical constituents in post larvae of *Penaceus indicus*, Mar. Environ. Res. 50 (2000) 103–106.
[31] V.K. Gupta, M. Gupta, S. Sharma, Process development for the removal of lead and chromium from aqueous solutions using red

mud—an aluminium industry waste, Water Res. 35 (5) (2001) 1125–1134.
[32] S.D. Faust, O.M. Aly, Adsorption Processes of Water Treatment, Butterworth, Stoneham, MA, 1987.
[33] W.F. Pickering, General strategies for speciation, in: A.M. Ure, C.M. Davidson (Eds.), Chemical Speciation in the Environment, Blackie Academic and Professional Press, London, 1995, pp. 9–31.
[34] S.J. Stohs, D. Bagchi, E. Hassoun, M. Bagchi, Oxidative mechanisms in the toxicity of chromium and cadmium ions, J. Environ. Pathol. Oncol. 20 (2001) 77–88.
[35] M.D.L. Easton, G.M. Kruzynski, I. Solar, H.M. Dye, Genetic toxicity of pulp mill effluent on juvenile chinook salmon (*Onchorhynchus tshawytscha*) using flow cytometry, Water Sci. Technol. 35 (1997) 347–355.
[36] A. Jobbagy, N. Nemeth, R.H. Altermath, W. Samhaber, Encouraging filament growth in an activated sludge treatment plant of the chemical industry, Water Res. 34 (2) (2000) 699–703.
[37] S. Semsari, H.A. Amar, Z. Badeni, B. Benayad, study of the inhibition of certain trophic levels by the presence of metal cations in water, Desalination 150 (2002) 177–188.
[38] E.N. Abrahart, Dyes and Their Intermediates, Edward Arnold Ltd., London, 1977.
[39] R. Anliker, Ecotoxicology and dyestuffs—a joint effort by industry, Ecotoxicol. Environ. Safety 3 (1979) 59–74.
[40] A. Pintar, M. Bessan, P. Gallezot, J. Gibert, D. Martin, Toxicity to *Daphnia magna* and *Vibrio fisheri* at kraft bleach plant effluents treated by catalytic wet air oxidation, Water Res. 38 (2004) 289–300.
[41] D. Sponza, Application of toxicity tests into discharges of the pulp-paper industry in Turkey, Ecotoxicol. Environ. Safety 54 (2003) 74–86.
[42] R. Minke, U. Rott, Anaerobic treatment of split flow wastewater and concantrates from the processing industry, Water Sci. Technol. 40 (1998) 169–176.
[43] E.C.H. Lucassen, A.J.P. Smolders, J.G.M. Roelofs, Increased groundwater levels cause iron toxicity in *Glyceria fluitans* (L.), Aquat. Bot. 66 (2000) 321–327.
[44] E. Fulladosa, J.C. Murat, I. Villaescusa, Study on the toxicity of binary equitoxic mixtures of metals using the luminescent bacteria *Vibrio fischeri* as a biological target, Chemosphere 58 (5) (2005) 551–557.
[45] S. Megharaj, P. Albina, Y. Samuel Y., Inhibitions of heavy metals to microorganisms Proc, Biochem. 29 (2003) 45–62.
[46] A.J. Miao, W.X. Wang, Cadmium toxicity to two marine phytoplankton under different nutrient conditions, Aquat. Toxicol. 8 (2) (2005) 114–126.
[47] V. Utgikar, B. Chen, Y. Chaudhary, H.H. Tabak, J. Haines, Acute toxicity of heavy metals to acetate-utilizing mixed cultures of sulfate-reducing bacteria: EC_{100} and EC_{50}, Environ. Toxicol. Chem. 20 (2001) 2662–2669.
[48] S.S. Sharma, S. Kaul, A. Metwally, K.C. Goyal, I. Finkemeier, Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status, Plant Sci. 166 (5) (2004) 1287–1295.
[49] B.T. Muyssen, A. Karel, A.C. De Schamphelaere, Mechanisms of chronic waterborne Zn toxicity in *Daphnia magna*, Aquat. Toxicol. 77 (4) (2006) 393–401.
[50] K. Vig, M. Megharaj, N. Sethunathan, R. Naidu, Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review, Adv. Environ. Res. 8 (1) (2003) 121–135.
[51] F. Tasneem, C. Meenakshi, K.J. Umesh, Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium Spirulina platensis-S5, Ecotoxicol. Environ. Safety 12 (2006) 45–62.
[52] R. Guan, W.X. Wang, Comparison between two clones of *Daphnia magna*: effects of multigenerational cadmium exposure on toxicity, individual fitness, and biokinetics, Aquat. Toxicol. 76 (3–4) (2006) 217–229.