Ecotoxicity tests using the green algae *Chlorella vulgaris*—A useful tool in hazardous effluents management

Aurora Silva, Sónia A. Figueiredo, M. Goreti Sales, Cristina Delerue-Matos

ABSTRACT

The treatment efficiency of laboratory wastewaters was evaluated and ecotoxicity tests with *Chlorella vulgaris* were performed on them to assess the safety of their environmental discharge.

For chemical oxygen demand wastewaters, chromium (VI), mercury (II) and silver were efficiently removed by chemical treatments. A reduction of ecotoxicity was achieved; nevertheless, an EC50 (effective concentration that causes a 50% inhibition in the algae growth) of 1.5% (v/v) indicated still high level of ecotoxicity.

For chloride determination wastewaters, an efficient reduction of chromium and silver was achieved after treatment. Regarding the reduction of ecotoxicity observed, EC50 increased from 0.059% to 0.5%, only a 0.02% concentration in the aquatic environment would guarantee no effects.

Wastewaters containing phenanthroline/iron (II) complex were treated by chemical oxidation. Treat- ment was satisfactory concerning chemical parameters, although an increase in ecotoxicity was observed (EC50 reduced from 0.31% to 0.21%).

The wastes from the kinetic study of persulphate and iodide reaction were treated with sodium bisul- phite until colour was removed. Although they did not reveal significant ecotoxicity, only over 1% of the untreated waste produced observable effects over algae.

Therefore, ecotoxicity tests could be considered a useful tool not only in laboratory effluents treatment, as shown, but also in hazardous wastewaters management.

Keywords: *Chlorella vulgaris*, Ecotoxicity, Environmental management, Laboratory wastewaters

1. Introduction

The implementation of new treatments for hazardous effluents is currently under research. Most of them are focused in the reduc- tion of the harmful species, both organic and inorganic, in order to accomplish legal regulations. Legal parameters do not always achieve the need for reducing the concentration of some uncom- mon pollutants. Moreover, there is a lack of information about the expected reduction of environmental impact achieved after treat- ment.

According to the European Community directive 2000/60/EC [1] and subsequent updating, 2006/11/EC [2] and 2008/32/EC [3] all water bodies must be protected and preserved. In order to improve the water quality and guarantee the survival of all the species of aquatic organisms the biodiversity of ecosystems should be protected, and therefore quality concerning ecotoxicological char- acteristics is also demanded.

Thus, an impact of disposed effluents in nature should take account of further evaluations, including tests with different species of organisms, since each one may have a different sensi- bility. The environmental toxicity test system ideally consists of a primary producer (e.g., an alga), a primary consumer (e.g., an aquatic arthropod), a secondary consumer (e.g., a fish) and perhaps a tertiary consumer (e.g., a bird), in order to represent the typical aquatic system [4].

Ecotoxicity tests may evaluate the effluent toxicity level upon its environmental discharge. These tests can provide relevant infor- mation for improvement of techniques that may ensure reduced potential hazard of contaminants to aquatic ecosystems [5]. The ecotoxicity tests include the evaluation of the synergistic, antag- onistic, and additive effects of all the chemical, physical and biological components, which may affect adversely the physiolog- ical and biological functions of the test organism. These tests are versatile because they could also be used to identify wastewaters that are biostimulatory and may cause nuisance growth of algae, aquatic weeds, and other organisms of higher trophic levels [6].

In this work, some laboratory wastewaters were studied. This kind of wastewaters was selected because it is generated worldwide and most of it contains hazardous species in high concentra- tions. Although most of the laboratory wastewaters are considered hazardous wastes, there is no specific guideline to their proper dis- posal/treatment.

Their rejection without any treatment would have strong neg- ative impact in the aquatic environment, considering both the laboratory wastes hazardous characteristics and the significant quantities produced, which depends on the institution dimension. The total amount of collected wastes in our institution is higher than 800 L per year [7]. This production lies in the category of small quan- tity generator, according to the Environmental Protection Agency (EPA) [8].

Colleges and universities in particular have problems with their laboratory wastes, due to the wide variety of wastes generated and because they contain nearly every hazardous chemical listed by EPA. Furthermore, their composition changes with every new research project and experiment. These facts make the proper haz- ardous laboratory waste management a complex and expensive task [9].

The most common disposal approaches are the reduction of quantity and/or toxicity before discharging in the public sewage system. This requires previous permission and concentration lim- its are imposed by the local authorities. It is also possible to treat laboratory wastewaters, after a proper segregation in order to avoid chemical incompatibilities or undesirable reactions. This treatment can be performed by the producer or by an authorized treatment company.

One of the laboratory wastes tested was the chemical oxygen demand (COD) determination effluent, in which potassium dichro- mate in sulphuric acid medium is used as oxidizing agent, together with silver and mercury sulphate, making the subsequent effluent one the most hazardous produced in laboratory. Since this param- eter is determined regularly, the effluents are produced in large amounts.

Taking account of the huge variety of chemicals that could be present, they must be collected and treated separately. This strat- egy minimizes chemical interferences [7,10]. The specific treatment applied to each laboratory effluents is described below. The chemi- cal characterization and the ecotoxicity evaluation, before and after treatment, were performed for each studied effluent, providing information about the efficiency of the treatment and the potential impact upon environment of the treated wastewater.

A multispecies test was not considered, because it is more expensive and time consuming. So the first approach was a short- term test using microalgae, Test Guideline 201 [11]. This test is easy to perform and offers a fast response to the wastewaters, within 72 h.

Algae were chosen as test organisms after considering several factors: they belong to the first level of the trophic chain, so any disturbance in their dynamics might affect the ecosystem higher levels; they are also very sensitive to changes in their environment and present the advantage of having a short life cycle, allowing the evaluation of toxic effects over several generations; the tests with unicellular organisms, show a greater reprodubility, reliability and robustness than multicellular tests of organisms [4,12]. The unicel- lular green alga, *Chorella vulgaris*, was used as test organism because it has got a good sensitivity to toxicants [13] and these algae are easily cultured in laboratory, so these tests can also be considered economical.

2. **Materials and methods**

2.1. Laboratory wastewaters

Several laboratory wastewaters were used in this study:

(A) wastewaters from chemical oxygen demand (COD) determi- nation: COD measures the amount of matter oxidised by potassium dichromate in acid medium, and was determined according to American Public Health Association, method 5220 [14];
(B) wastewaters from chloride determination after Mohr's titra- tion: classical titration with silver nitrate where the end-point is reached after silver chromate precipitation [15];
(C) wastewaters produced after spectrophotometric determina- tion of iron with 1,10-phenanthroline, following American Public Health Association, method 3500 [14];
(D) wastewaters from kinetic studies of persulphate and iodide reaction [16].

A 500 mL representative sample of each effluent was taken from the 5 to 50 L vessels where they were collected—these are the type (i) samples. After chemical treatment of the wastewaters col- lected, representative samples type (ii) were taken. Each laboratory effluent had a different treatment that will be described below, in Section 3, and then all effluents were neutralized.

The treated effluents were characterized with regard to the European Community Directives 2000/60/EC [1] and subsequent updating, 2006/11/EC [2] and 2008/32/EC [3].

The characterization was performed following the analytical methods indicated in Standard Methods [14]. For analysis by atomic absorption, samples were previously acidified using nitric acid, until a pH lower than 2 was obtained, and no modifiers were used. Silver, total chromium, and iron were evaluated (method 3111B) using a PerkinElmer AAnalyst 200 (Singapore) flame atomic absorption equipment. For silver an oxidant air/acethylene flame was used; the detection was accomplished at 328.1 nm for the working range of 0.1–3 mg/L. Total chromium was atomised in a highly reducing air/acethylene flame; a 357.9 nm wavelength was used for the working range of 0.05–2 mg/L. Iron determi- nation was performed in an oxidant air/acethylene flame; the detector wavelength used was 248.3 nm for the working range of 0.05–2 mg/L.

Mercury was measured by cold vapour generation coupled to atomic absorption spectrophotometry (method 3112B) in a Zeenit 650 Analytikjena (Germany) with hydrates generator. This method involved the reduction to elementary mercury vapour by tin (II) chloride in aqueous (ultrapure water) solution of suprapure chloridric acid solution. Atomization was performed at room tem- perature. Detection was made at 253.7 nm and the working range was 0.10–10 μg/L.

Chromium (VI) was determinated by the diphenylcarbazide col- orimetric method (method 3500-Cr B), using a single beam Jenway 6100 (United Kingdon) spectrophotometer. The absorbance was measured at 540 nm in 1 cm light path plastic cells. The optimised analytical range was 0.1–1 mg/L.

2.2. Bioassay

2.2.1. Test organism

The ecotoxicity tests were carried out with the freshwater uni- cellular green algae *Chlorella vulgaris*.

The test organism was cultured in laboratory under asep- tic conditions. A new culture was started weekly by aseptically transferring 1–2 mL of stock culture to a 50–100 mL of new culture medium (the nutrient medium is described below), in order to adapt the algae to the test conditions and ensure that the algae are in exponential growth phase when they are used to inoculate the test solutions. The stock cultures were kept at 21 ± 2 °C, under cool white fluorescent lighting, during 4 days. Agitation was performed by filtrated air bubbling. Each stock culture was examined with an optical microscope, Nikon Alphaphot-2 YS2, to ensure that there are no contaminating micro- organisms.

2.2.2. Nutrient medium

The medium for the algal growth inhibition test was pre- pared in accordance with OECD Test Guideline 201 [11] using deionised water with conductivity lower than 5 μS cm^{-1} and suit- able nutrients (from four sterilised stock solutions). The final nutrient medium solution has a pH value around 8.

2.2.3. Test procedure

The test was carried out based on the OECD 201 Guideline, updated in 2006 [11].

The inoculum of the green algae, *C. vulgaris*, provided a concentration around 10^6 cells/mL in each test flask. Initial biomass did not exceed 0.5 mg/L as dry weight, allowing exponential growth through the incubation period, without risk of nutrient depletion. Aseptic techniques were used in the algal cultures, handling and extreme care was exercised to avoid contamination. A laminar air flow chamber FASTER, model two-30, and a sterilization chamber AJC, model Uniclave 88, were used.

A set of five different effluent concentrations (usually, 0.02, 0.1, 0.2, 1 and 5%, v/v) and a control were used for each sample tested. The dilution water was the culture medium, in order to avoid nutri- ent limitation.

The test conditions are summarised in Table 1 [11]. The test flaks position was randomised and changed daily [6].

The growth of the population was measured in terms of changes in cell density, evaluated by optical density at 440 nm [17], using a Shimadzu UV-2101 PC spectrophotometer [6,11]. A linear rela- tionship was verified between cell counts and biomass versus optical density. The optical microscope was used for cell counts. The biomass was determinated by filtration over a 0.45-μm mem- brane (GN-6 Metricel Grid, Pall Corporation) followed by drying until constant weight.

The pH was also evaluated in the beginning and after 72 h, its variation should not exceed 1.5. This parameter was evaluated by means of a Crison® CWL/s7 combined glass electrode connected to a decimilivoltammeter Crison®, pH meter, GLP 22.

For validation of ecotoxicty tests performed, a reference toxicant, the potassium dichromate, was also tested in the same conditions [6].

2.2.4. Data analysis

The optical density values obtained were transformed in cell density (cells/mL) using the linear experimental relation previously determined.

The acceptability criterion considered was variability less than 20% among replicates.

The statistic analysis of results was done as suggested by EPA [6]. Normality (Shapiro–Wilk's test) and homogeneity of variance (Bartlett's test) were formally tested, since they are the underlying assumptions of the Dunnett's procedure. Since these assumptions were met, the endpoints were determined by the parametric tests.

Table 1
Summary of test conditions [6,11].

Test type	Static non-renewal
Temperature	21–24 ± 2 °C (maximum variation = 3 °C)
Light quality	"Cool white" fluorescent lighting 6000–10,000 lx
Light intensity	
Photoperiod	Continuous illumination
Test chamber size	150 mL
Test solution volume	100 mL
Test concentrations	5 and a control
Test duration	72 ± 2h
Endpoint	Growth (optical density)
Sampling	Test beginning and final

The LOEC (lowest observable effect concentration) and NOEC (no observed effect concentration) values for growth were obtained using this hypothesis test approach.

The EC50 (effective concentration that causes a 50% inhibition in the algae growth) was calculated using a point estimation tech- nique, the linear interpolation method.

Due to the use of a linear interpolation technique to calculate an estimate of the EC50, standard statistical methods for calculating confidence intervals are not applicable. This limitation is avoided by the bootstrap method as proposed by Efron [18] for deriving point estimates and confidence intervals [19].

The width of the confidence intervals calculated by the bootstrap method is related to the variability of the data. The 95% confidence interval was calculated using a specific software, ICPIN program [20].

3. Results and discussion

Several laboratory wastewaters were tested: (A) from COD determination, (B) from chloride determination after Mohr's titration, (C) from determination of iron with 1,10-phenanthroline, and (D) from kinetic studies of persulphate and iodide reaction. Each laboratory effluent had a different treatment that will be described below, in this section.

In order to compare untreated and treated wastewaters, the same chemical parameters were determined in both samples (i) and (ii) of the same effluent. The relevant chemical parameters and the maximum allowed values for discharge are presented in Table 2.

In the ecotoxicity tests performed, the initial and final optical density values obtained at 440 nm were transformed in cell den- sity (cells/mL) using the experimental linear relation obtained: (cell density) = $1.15 \times 10^7 \times$ (absorbance at 440 nm), with a square cor- relation factor of 0.996. The algal growth results, obtained by the difference between final and initial cells densities, followed the sta- tistical analysis procedure, already described in Section 2.2.4, to estimate CE50, LOEC and NOEC endpoints.

The treatment applied and the characterization of each effluent are presented and discussed below, in separate sections.

3.1. Wastewaters from COD determinations

The use of potassium dichromate in sulphuric acid medium as oxidizing agent, in COD determinations, together with silver and mercury (II) sulphate makes this effluent one of the most hazardous produced in laboratory. Moreover, being one of the parameters frequently used to quantify organic matter in wastewaters, their effluents are produced in large amounts. These determinations pro-

Table 2
Concentrations of the pollutants present in the laboratorial wastewaters from COD (A), chloride (B) and iron (C) determinations, and (D) kinetic studies of persulphate iodine reaction, before (i) and after treatment (ii), and maximum allowed discharge values [1–3].

Sample	Parameter	Concentration (mg/L)		Discharge limits (mg/L)
		Type (i)	Type (ii)	
A	Ag	2520	1.3	–
	Cr (total)	860	0.11	2
	Cr (VI)	389	<0.1	0.1
	Hg	400	39	0.05
B	Cr (total)	1280	0.59	2
C	Fe	1.5	<0.05	2
D	Colour	Detectabl	Non-	Non- (1/20

duce wastewaters – sample A(i) – with high levels of chromium (VI), mercury and silver (Table 2). Other chemical species such as sulphate, chloride and calcium are also present, however their lev- els were not considered harmful, when compared with the heavy metals present, therefore their concentrations were not evaluated. The chemical treatment applied to sample A(i) meant a signif- icant removal from aqueous media of the previous metals. Firstly, silver was removed by precipitation, as silver chloride, with com- mercial sodium chloride. In this reaction, some mercury might also be removed in the form of mercury (II) chloride. The solid phase was separated by filtration. Then sodium bisulphide was added to the liquid phase to reduce chromium (VI) to (III) (solution colour changes to green). Chromium was removed by precipitation as chromium (III) hydroxide, and separated by filtration. In order to reduce mercury concentration potassium iodide was added to pre- cipitate mercury in the form of mercury (II) iodide, separated by filtration. At the end, neutralization was performed using sodium hydroxide or chloridric acid solutions, and a representative sample type A(ii) was taken.

The chemical characterization of both samples, A(i) and A(ii), is presented in Table 2 and shows that the chemical treatment produced removal efficiencies higher than 99.9% relatively to sil- ver, chromium and chromium (VI), achieving the public discharge requirements. About 90% of the mercury in sample A(ii) was removed after chemical treatments.

Ecotoxicity tests were performed for both samples A(i) and (ii) and the results are shown in Fig. 1A.

Ecotoxicological evaluations point out an enhanced growth inhi- bition of *C. vulgaris* facing increasing concentrations of sample, for both samples A(i) and A(ii). The lower concentration tested (0.02%) led to 56% inhibition meaning that EC50 is lower than this value, and could not be determined.

The chemical treatment of sample A(i) promoted a significant reduction in the inhibition rates, with a reduction of 58% for a con- centration level of 1%, corresponding to effective concentrations of chromium, mercury and silver of 1.1, 390 and 13 ppb, respectively. Since photosynthesis has been shown to be very sensitive to heavy metals, more than other metabolic process in green algae [21], the observed effects for sample A(i) may be related to the combined effect of the presence of mercury, chromium and silver. The data analysis of the ecotoxicity tests to samples A(i) and A(ii) are given in Table 3.

Globally, ecotoxicological evaluations revealed the harmful effect of sample A(i) towards ecosystem, as it was expected consid- ering the high concentration of mercury, chromium and silver. The chemical treatment applied to sample A(i) reduced significantly its adverse effect upon environment but it was insufficient to ensure a safe disposal, once a 0.02% concentration still produces observ- able effects. This observation is in agreement with the high level of mercury measured in sample A(ii), 39 mg/L (Table 2), which amongst heavy metals, exhibits a high toxicity to photosynthesis [22].

So a new treatment step is currently under development, in order to improve the mercury removal in the effluent, namely an end line filtration by a granular activated carbon fixed bed column. Alternatively a chitosan (the second most abundant biopolymer, after cellulose) bed could be an efficient and economical treatment, as suggested by Leong et al. [23].

3.2. Wastewaters of chloride determination by Mohr's method

Mohr's method is widely used in laboratories all over the world, being commonly accepted as the reference method to analyse chlo- ride in waters for human supply.

Wastewaters generated by chloride determination, sample B(i), contains high levels of chromium (VI) and silver. Other species, such as chlorides, nitrates, carbonates, and potassium were also present; since they were not considered toxic, their concentrations were not evaluated.

Chemical treatment of these wastewaters provided a decrease in both silver and chromium concentrations, as shown in Table 2, and are lower than those allowed for public discharge.

In the chemical treatment of sample B(i) [24], chloridric acid solution was added to dissolve the silver chromate precipitate and then silver was precipitated as silver chloride. The solid phase was separated by filtration. Sodium bisulphide was added to the liquid phase to reduce chromium (VI) to (III), which was then removed by precipitation in the form of chromium hydroxide, followed by neutralization, as in COD wastewaters treatment. Finally, a repre- sentative sample B(ii) was taken.

The chemical characterization presented for both samples, B(i) and B(ii), relative to chromium and silver levels is presented in Table 2. The chemical treatment performed produced removal effi- ciencies of chromium higher than 99.9%. The final concentration of chromium was 0.59 mg/L, which meets the discharge limits, being most of it in the form of chromium (III) – less toxic than chromium (VI) – due to the chemical reduction performed. For silver, the 95% efficiency achieved, corresponding to a 0.15 mg/L final concentra- tion, might guarantee a safe discharge.

Ecotoxicity tests were performed for both samples B(i) and (ii) and the results are shown in Fig. 1B.

Ecotoxicological evaluations of sample B(i) showed a similar behaviour to sample A(i), which is most probably correlated to high levels of metals in solution, most particularly chromium (VI), with a 1280 mg/L concentration.

The statistic analysis of results from the ecotoxicity test to sample B(i), except the 5% concentration that originated a 100% inhibition, showed a normal distribution and an homogenous vari- ance. Due to the high inhibitions obtained in the tests only the EC50 value could be estimated (Table 3).

The chemical treatment established for sample B(i) was able to reduce the original toxicity of Mohr's titration wastewaters, once a 53% reduction in the ecotoxicity to *C. vulgaris* was observed for a 0.2% concentration level, when comparing samples B(i) and (ii).

The statistic analysis of results from the ecotoxicity test to sam- ple B(ii) are presented in Table 3.

Wastewaters produced after chloride determination, sample B(i), were found toxic with regard to *C. vulgaris*, a similar behaviour to that observed with sample A(i). The chemical treatment per- formed was effective and legal concentrations for discharged were reached; however, according to the results of the ecotoxicity test performed, only a 0.02% concentration of the treated effluent in the aquatic environment, would be considered safe for discharge, in spite of its low concentrations of silver and chromium. This behaviour was also common to other test organisms, showing high ecotoxicity to metals, especially to silver [25]. This treatment might be improved by ionic exchange.

Despite being considered not toxic, nitrate excess might increase the algal growth in the tests, leading to lower inhibition rates than the ones observed without nitrate.

3.3. Wastewaters from spectrophotometric determination of iron

Phenanthroline's method is often used in laboratories to analyse iron in natural waters and treated waters, as an alternative to atomic absorption spectrophotometric determinations.

This effluent contains an orange complex obtained after reaction of iron (II) with 1,10-phenanthroline, in the presence of hydroxy- lamine that reduces all iron in solution to its divalent state. It also contains sodium acetate to provide a suitable pH. Concentrations of iron, phenanthroline and hydroxylamine were expected to be 1.5×10^{-4}, 1.0×10^{-2}, and 1.0×10^{-3} (w/w), respectively. Organic

compounds are present in very low concentrations, so their contri- bution to the organic load of the effluent is not significant. Therefore colour and iron removal were the main objectives of the following treatment: an oxidative cleavage of phenanthroline with potassium permanganate was used to destroy the coloured complex. This oxi- dation reaction might produce by-products, carbonyl derivates, of unknown chemical structure. They present higher solubility than their parent structure, which in many cases induces a decreased toxicity to living beings. The treatment ended with the addition of sodium hydroxide waste solution for manganese (introduced by the oxidising agent) and iron removal in the form of hydroxides followed by neutralization with chloridric acid solution.

Considering legal parameters imposed, the most relevant parameter to control was iron concentration, which is presented in Table 2, for samples C(i) and (ii), meaning a 97% reduction after treatment and corresponding to a 50 µg/L of iron concentration in sample C(ii), accomplishing legal limits.

The results of ecotoxicity tests, for samples C(i) and (ii) (Fig. 1C), reveal an extraordinary high ecotoxicity towards *C. vulgaris*, namely, for the lowest concentration tested, 0.32%, inhibition rates of 58% and 75% were obtained, respectively.

Due to the high inhibition rates obtained both before and after treatment, LOEC and NOEC could not be determined. The high level of phenanthroline in solution may explain the toxicity of sample C(i). Though no quantitative data on the ecologic effect of this com- pound are available [26], it is considered harmful for humans.

The microalgae growth was more inhibited in sample C(ii) than in sample C(i). This suggests that the chemical treatment applied to sample C(i) led to an increased effluent toxicity. This feature may be related to the several carbonyl compounds in solution, possi- bly presenting higher toxicity than phenanthroline itself, and to manganese ions generated by the oxidant, permanganate.

Moreover, algae are known to adsorb some organic micropollu- tants, others are metabolised, originating in most cases inoffensive products, but sometimes the resulting product presents higher tox- icity than the parent compound [27].

Thus, the high ecotoxicity observed is most probably a com- bined effect of the previous factors. It is also suggested by some authors that phenanthroline in the presence of an oxidising agent has harmful effects at cellular level [28].

Although oxidative treatments are often applied to wastewa- ters containing complex molecules, such as phenanthroline [26], considering the high ecotoxicity levels verified after treatment, this treatment was abandoned and a new one is presently under research.

3.4. Wastewaters from kinetic studies of persulphate and iodide reaction

Another laboratorial waste studied in this work is originated by the kinetic study of persulphate and iodide reaction [16] which is one of the several experiments often performed in the Chemical Engineering graduation courses. The reaction products are sulphate and iodine, which reacts with thiosulphate in a secondary reaction forming iodide. These wastewaters do not include harmful compo- nents, oppositely to the other studied effluents.

As no hazardous compounds are present in this wastewater, the treatment performed to sample D(i) was very simple: a filtration to separate the sulphur-based solid and a neutralization of the liquid phase. If necessary, some sodium bisulphite was added to reduce iodine and remove the blue colour given by starch indicator. At this stage, a representative sample D(ii) was taken.

Ecotoxicity tests were performed for samples D(i) and (ii) and the results are shown in Fig. 1D.

The data analysis of the ecotoxicity tests to samples D(i) and D(ii) are shown in Table 3.

EC50 could not be estimated for sample D(i) and once no sig- nificant inhibition was observed for the maximum concentration tested, 10%. After treatment, inhibitions were also low, hence EC50 could not be estimated for sample D(ii).

Comparing the ecotoxicity evaluations before and after treat- ment, a slight increase of toxicity was observed, possibly indicating that the addition of sodium bisulphite increased the effluent tox- icity. Although the concentrations range tested, from 0.1% to 10%, were not harmful to *C. vulgaris* growth, higher concentrations of this effluent might have adverse effects. It is considered safe to dis-

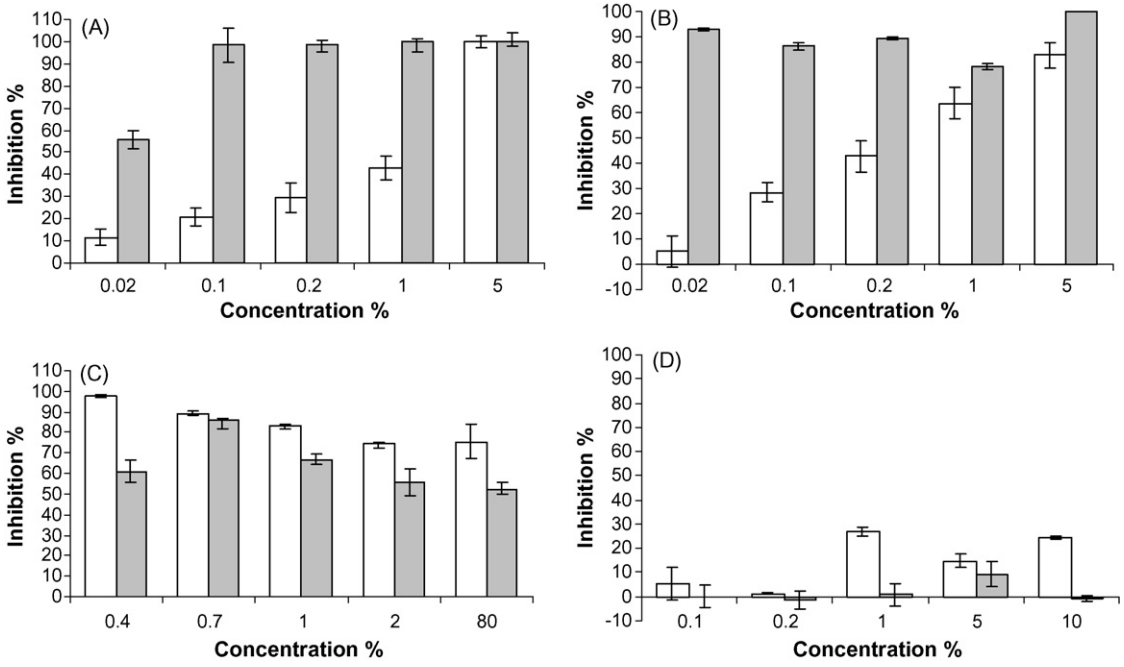

Fig. 1. Variation in optical density before (•) and after treatment (D), for samples of wastewaters: (A) CQO, (B) chloride and (C) iron determinations and (D) kinetic studies of persulphate iodine reaction.

Table 3
Data analysis of the laboratorial wastewaters ecotoxicity tests from COD (A), chlo- ride (B) and iron (C) determinations, and (D) kinetic studies of persulphate iodine reaction, before (i) and after treatment (ii).

	Sample	EC50% (v/v) 95% Confidence interval % (v/v)	LOEC % (v/v) (v/v)	NOEC %
A(i)	<0.02	ND[a]	ND[a]	ND[a]
A(ii)	1.5	1.3–1.6	ND[a]	ND[a]
B(i)	0.059	0.058–0.060	ND[a]	ND[a]
B(ii)	0.5	0.2–0.7	0.1	0.02
C(i)	0.31	0.29–0.54	ND[a]	ND[a]
C(ii)	0.21	0.20–0.26	ND[a]	ND[a]
D(i)	ND[b]	ND[b]	5	1
D(ii)	ND[b]	ND[b]	1	0.2

ND: not determined due to: [a]high inhibition rates observed; [b]low inhibition rates observed.

charge the treated effluent when the water receiving body ensures a concentration below 0.2%.

4. Conclusions

The search for new treatments of hazardous effluents is mainly focused in the concentration reduction of the harmful species, organic or inorganic, in order to accomplish legal regulation. These are not enough demanding towards some specific pollutants that, alone or in combination, may be toxic to aquatic life.

In this work, some laboratory wastewaters produced in our chemical engineering teaching institution were studied. The concentration of the most hazardous compounds was determined in the effluents, before and after treatment. Ecotoxicity tests were also performed, in order to evaluate if the treatments implemented were effective, considering both their efficiency and ecotoxicolog- ical impact.

For COD wastewaters, as the compounds responsible for the most part of the wastes toxicity (chromium (VI), mercury and sil- ver) were removed by chemical treatments, a decrease in the algal growth inhibition was observed for treated wastes of COD, but a LOEC of 0.02% and a CE50 of 1.5% (with a 95% confidence interval, 1.3–1.6%), indicated high ecotoxicity levels, due to an unsatisfactory mercury removal. Amongst heavy metals, mercury presents a high toxicity to photosynthesis [22]. The 90% efficiency obtained for mer- cury removal is now under improvement by means of adsorption technology.

For chloride determination wastewaters, containing high levels of chromium and silver, the same reduction of toxicity was observed after the chemical treatment. The EC50 increased from 0.059% (with a 95% confidence interval, 0.058–0.060%) to 0.5% (with a 95% confidence interval, 0.2–0.7%) indicating high ecotoxicity levels, even after the efficient removal of chromium and silver, achieving respectively the final concentrations of 0.59 and 0.15 mg/L, which are below the legal discharge limits. A similar behaviour was also observed with *Lemna minor* L., which exhibits high ecotoxicity to metals, especially to silver [25].

However, only a 0.02% concentration of treated effluent, in the aquatic environment, would guarantee no effects towards *C. vulgaris*.

Although the treatment of wastes containing phenanthro- line/iron (II) complex was satisfactory concerning the iron removal, reaching a 16 ppb concentration, a slight increase in ecotoxi- city was observed; the EC50 was reduced from 0.31% (with a 95% confidence interval, 0.29–0.54%) to 0.21% (with a 95% confidence interval, 0.20–0.26%). This high inhibition rate, after treatment, may be explained by the presence of by-products formed during the oxidation treatment process or metabolization com- pounds of phenanthroline by *C. vulgaris* that present higher toxicity than phenanthroline itself, so a different treatment is now under research. Moreover, it is suggested by some authors that phenan- throline in the presence of an oxidising agent has harmful effects at cellular level [28].

The treated wastes resulting from the kinetic study of persul- phate and iodide reaction did not reveal significant ecotoxicity. However, only a 1% concentration of the untreated waste would not produce observable effects over the algae. The decrease in the LOEC value after treatment, from 5% to 1% concentration, indicates an increase in toxicity, suggesting that care should be taken relatively to the use of sodium bisulphite during the treatment.

This study leads to the conclusion that a treated effluent may present very low concentrations of pollutants, accomplishing the discharge legislation parameters, and may still be toxic to the aquatic ecosystems, even considering the dilution rate inherent to discharge. This may occur when the treated wastewaters contain toxic species as organic micropollutants, most of them resistant to conventional treatments.

The use of *C. vulgaris*, as test organism, was considered an economical and easy strategy to implement and guarantee a safe disposal of the treated wastes in the aquatic environment. Considering the wide variety of laboratory wastes and their specific treatments, these ecotoxicity tests provide further information that may help in the selection and improvement of non-conventional wastewaters treatments, especially for hazardous effluents that need the development of particular treatments. Therefore, ecotox- icity tests could be considered a useful tool not only in laboratory effluents treatment, as it was shown, but also in hazardous waste- waters management.

Acknowledgements

The authors acknowledge TRELAB (Tratamento de REsíduos de LABoratório), the waste management group responsible for collection and treatment of effluents from teaching laboratory experiments.

References

[1] European Community, Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy, Official Journal of the European Communities, 23rd October 2000.

[2] European Community, Directive 2006/11/EC of the European Parliament and of the Council on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community, Official Journal of the European Communities, 15th February 2006.

[3] European Community, Directive 2008/32/EC of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Com- munity action in the field of water policy, as regards the implementing powers conferred on the Commission Official Journal of the European Communities, 11st March 2008.

[4] I.C. Shaw, J. Chadwick, Ecotoxicity testing, TEN 2 (3) (1995) 80–85.

[5] A. Fernandez, C. Tejedor, F. Cabrera, A. Chordy, Assessment of toxicity of river water and effluents by the bioluminescence assay using *Photobacterium phos- phoreum*, Water Res. 29 (1995) 1281–1286.

[6] U.S. EPA (United States Environmental Protection Agency), Short-term methods for estimating the chronic toxicity of effluents and receiving waters to fresh water organisms (EPA-821-R-02-013), 4th ed., Washington DC, USA, 2002.

[7] I. Serra, A. Silva, S. Morais, C. Delerue-Matos, M.G. Sales, I.B. Martins, Sustainable use of resources and waste management in chemical laboratories, Electron. J. Environ. Agric. Food Chem. 2 (2003) 337–342.

[8] U.S. EPA (United States Environmental Protection Agency), Managing haz- ardous waste generated in laboratories, OH, USA, 2005 (available on-line at www.epa.state.oh.us/dhwm/ in

October 2008).
[9] J.A. Herrera-Meliáne, E.T. Rendón, J.M.D. Rodríguez, A.V. Suárez, C.V. Campo,
J.P. Peñ a, J.A. Mesa, Incidence of pretreatment by potassium permanganate on hazardous laboratory wastes photodegradability, Water Res. 34 (16) (2000) 3967–3976.
[10] M.G.F. Sales, C. Delerue-Matos, I.B. Martins, I. Serra, M. Silva, S. Morais, A waste management school approach towards sustainability, Resour. Conserv. Recycl. 48 (2006) 197–207.

[11] OECD (Organisation for economic co-operation and development), Freshwater alga and cyanobacteria, growth inhibition test, Test Guideline 201, 2006.
[12] I.C. Shaw, J. Chadwick, Principles of Environmental Toxicology, Taylor & Francis, London, UK, 1998.
[13] J. Ma, F. Lin, R. Zhang, W. Yu, N. Lu, Differential sensitivity of two green algea, *Scenedesmus quadricauda* and *Chlorella vulgaris* to 14 pesticides adjuvants, Eco- tox. Environ. Safe. 58 (2004) 61–67.
[14] APHA (American Public Health Association), in: A.E. Greenberg, L.S. Clesceri, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastew- ater, APHA, Washington DC, USA, 2005.
[15] J. Mendham, R.C. Denney, J.D. Barnes, M.J.K. Thomas, Vogel's Textbook of Quan- titative Chemical Analysis, 6th ed., Longman, UK, 2002.
[16] S.J. Formosinho, Fundamentals of Kinetics Chemical Reaction, Fundaç ão Caloust Gulbenkian, Lisboa, Portugal, 1982, in Portuguese.
[17] F. Carvalho, L. Guilhermino, R. Ribeiro, F. Gonç alves, A.M.V.M. Soares, METIER (modular ecotoxicity tests incorporating ecological relevance). II. Ecotoxicity of poorly water soluble compounds: concentration versus dose, Arch. Environ. Contam. Toxicol. 29 (1995) 431–434.
[18] B. Efron, The Jackknife, the Bootstrap, and other resampling plans, CBMS 38, Soc. Industr. Appl. Math., Philadelphia, USA, 1982.
[19] A.H. Marcus, A.P. Holtzman, A robust statistical method for estimating effects concentrations in short-term fathead minnow toxicity tests, Battelle Washing- ton Environmental Program Office, U.S. EPA (Environmental Protection Agency), Contract No. 69-03-3534, Washington DC, USA, 1998.
[20] T.J. Norberg-King, A linear interpolation method for sublethal toxicity: the inhi- bition concentration (ICp) approach (Software version 2.0), U.S. EPA (United States Environmental Protection Agency), 1993.
[21] G. Samson, J.C. Morisette, R. Popovic, Copper quenching of the variable fluorescence in *Dunaliella tertiolecta*. New evidence for a copper inhibi- tion effects in PSII photoinhibitory, Photochem. Photobiol. 48 (1988) 329– 332.
[22] C.M. Lu, C.W. Chau, J.H. Zhang, Acute toxicity of excess mercury on the photosyn- thetic performance of cyanobacterium, *S. platensis*—assessment by chlorophyll fluorescence analysis, Chemosphere 41 (2000) 191–196.
[23] S.T. Leong, S. Muttamara, L. Preecha, L. Hung-Ta, Assessment of treatment alter- natives for laboratory COD wastewater: a practical comparison with emphasis on cost and performance, Environ. Monit. Assess. 74 (2002) 11–25.
[24] I. Serra, M. Silva, S. Morais, I.B. Martins, C. Delerue-Matos, M.G. Sales, Treat- ment of Mohr's waste while teaching in chemical engineering, Proceedings of the 9th International Chemical Engineering Conference, CEE015 (2005) 508– 509.
[25] B. Naumann, M. Eberius, K.-J. Appenroth, Growth rate based dose–response relationships and EC-values of ten heavy metals using the duckweed growth inhibition test (ISO 20079) with *Lemna minor* L. clone St, J. Plant Physiol. 164 (12) (2007) 1656–1664.
[26] M.R. Silva, A.G. Trovó, R. Nogueira, Treatment of 1,10-phenanthroline laboratory wastewater using the solar photo-Fenton process, J. Hazard. Mater. 146 (2007) 508–513.
[27] P. Mouchet, Algae reactions to mineral and organic micropollutants, ecological consequences and possibilities for industrial scale application: a review, Water Res. 20 (4) (1986) 399–412.
[28] F.A.C. Furtado, N.R. Asad, A.C. Leitão, Effects of 1,10-phenanthroline and hydro- gen peroxide in *Escherichia coli*: lethal interaction, Mutat. Res.: DNA Repair 385 (3) (1997) 251–258.