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Abstract

Textile industry is considered as one of the important factors of the economic growth in Tunisia. However, this prominent role has certainly some drawbacks mainly represented by the huge amounts of textile wastewaters generated that become a real menace to nature. Many previous studies showed the purifying potential of some activated sludge and bacteria (*Pseudomonas putida*) to decolourize textile effluents. However, in many cases, decolourization of wastewaters is not necessary associated with detoxification, generating a real risk for the ecosystem in general. We evaluated in this work the induced toxicity of a textile effluent before and after its treatment with activated sludge followed by *P. putida*, using a battery of biotests. This study proved the detoxifying power of the activated sludge according to most of ecotoxicity tests. The treatment with *P. putida* did not improve the quality of the effluent; on the contrary, it could increase its toxicity. *Daphnia magna* and *Raphidocelis subcapitata* appear to be the most sensitive organisms in assessing eventual toxicity caused by this kind of wastewaters.

Keywords

Textile effluent Activated sludge *P. putida* Ecotoxicological tests *D. magna R. subcapitata*

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Introduction

At the global level, textile industry represents an important factor of economic growth for many countries. This sector has always been to Tunisia, one of the most interesting of the domestic industry, because of its quality of products, the number of generated job positions and its high export potential as the fifth European Union supplier clothing in 2010 (Ministry of Industry, Energy and Mines). The textile industry, particularly dyeing and finishing phases, consumes a wide range of chemicals that are very harmful for humans (Ben Mansour et al. 2009a) and certainly for environment (Banat et al. 1996). In this context, several attempts have been made at the national and international scale by the concerned ministries or industries to set centres which treat water coming from different production lines, prior to discharge into nature (river, ground, sea…). However, the heterogeneity of the composition of products used in dyeing (dyes, salts, additives, detergents and surfactants) makes it extremely difficult to achieve satisfactory wastewater quality levels, imposed by the international environmental standards. Conventional physical and chemical techniques of treatment such as activated carbon, membrane filtration and application of ozone

often still non-specific, weakly effective (O'Neill et al. 2000; Silveira et al. 2009) or very expensive (Robinson et al. 2001; Jadhav et al. 2007; Ben Mansour et al. 2012).

Recently, many attempts have been made to obtain higher wastewater quality by replacing conventional methods currently applied (physical or chemical) with biological treatments which are more effective and less expensive (Dogruel et al. 2006; Lofrano et al. 2013; Phugare et al. 2011). That is why an increasing number of textile industries through the world adopted biological treatment of wastewaters before the discharge in the environment. Using activated sludge often allows the total decolourization of effluents, resulting from tannery processes and significantly decreases their chemical oxygen demand (COD) values ("Société Industrielle de Textile" SITEX 2012).

Moreover, since recently, a strain of *Pseudomonas putida* mt-2 has been described to be efficient in decolourization and detoxification of several effluents (Silveira et al. 2009; Ben Mansour et al. 2011, 2012, 2013), we studied the effect of the combined treatment (sludge and then *P. putida*) on the toxicity of these industrial effluents.

This work aims to evaluate the efficiency of a combined treatment, by both an industrial activated sludge and *P. putida*, of a textile effluent sample, compared to that obtained only with activated sludge. We determined the toxicity of each sample, using a battery of seven ecotoxicological tests, including organisms belonging to different trophic levels: (i) aquatic organisms, which were selected for their high sensitivity towards different pollutants of textile effluents as reported by the literature, the alga *Raphidocelis subcapitata* (Sponza 2002), the aquatic plant *Lemna minor* (Casieri et al. 2008; Tigini et al. 2011; Malachová et al. 2013), two crustacean species *Daphnia magna* (da Silva et al. 2012; Verma 2008) and *Artemia franciscana* (Libralato et al. 2010; Matthews 1995) and the bacterium *Vibrio fischeri* (Casieri et al. 2008; Tigini et al. 2011; Malachová et al. 2013) and (ii) dicotyledonous plants, *Cucumis sativus* and *Lepidium sativum* (Tigini et al. 2011), which are important bioindicators of treated wastewater safety aimed to be reused for irrigation, in countries having problems of lack of water. We based the choice of these tests on the work of Tigini et al. (2011), who selected the most sensitive organisms in the treatment of tannery effluents.

Materials and methods

Effluent samples

Three effluent samples were studied (E_1, E_2 and E_3). Two effluent samples E_1 (containing Reactive Blue HEGX, Reactive Yellow S3-R and Direct Black VB) and E_2 were kindly provided by a textile industrial company located in Ksar Hellal (eastern centre of Tunisia) in April 2012. E_1 was sampled from the homogenization tank in the wastewater treatment plant (WWTP) of a textile company. E_2 was sampled after the activated sludge was used in the same WWTP. E_3 was obtained from the treatment of E_2 with *P. putida* mt-2 that revealed to be effective in the treatment of dyes and effluents as described by Ben Mansour (2011, 2012, 2013). The pH, COD, biochemical oxygen demand (BOD) and conductivity of the tested effluents are summarized in Table 1.
Table 1

Physico-chemical characteristics of the textile effluents

	E_1	E_2	E_3
pH	12.54	8.34	7.95
COD (mg/L)	2200	160	2180
BOD (mg/L)	1200	28	223
Conductivity (µS/cm)	6662	6589	6630

P. putida culturing and biodegradation process

The *P. putida* mt-2 (DSM 3931) strain used for the decolorization assays was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). *P. putida* was grown at 30 °C in 250-mL flasks, containing 50 mL of medium, under rotary shaking incubation at 200 rpm. The growth medium contained yeast extract (10 g/L), peptone (3 g/L) and glucose (5 g/L). After cultivation of *P. putida* mt-2 on an enzymatic inductive medium (nutrient broth supplemented with 10 % of textile wastewater), the exponential phase culture (~1.5 g/L of dry cells) was centrifuged (3500 rpm for 10 min), then, cells were harvested and transferred into a second flask (100 mL in a 500-mL flask) containing only effluent. Biodegradation of textile effluents was conducted in flasks at 30 °C under oxygenated condition assured by agitation (200 rpm) and continuous air injection. After incubation of *P. putida* with effluent mt-2 during 24 h, the supernatant was obtained by centrifugation (3500 rpm, 10 min, 4 °C) for being used further in toxicity studies.

Ecotoxicity tests

Alga test

The alga test was performed according to the standard UNI EN ISO 8692:2005 (ISO 2005) using a monospecies culture of *R. subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O.M. Skulberg (ex *Selenastrum capricornutum* Prinz). The test was performed in triplicate, in 24 multiwell plates, with a working volume of 2.5 mL per well (culture media composition and final concentration were mentioned in Appendix) and with at least six sample dilutions as described by De Schepper et al. (2010). The abiotic control (effluent without algal inoculum) was performed with one repetition. The biotic control (algal without effluent) was performed with six repetitions. After 72 h of incubation at 23 °C under 8000-lx light intensity, well samples were homogenized, in order to resuspend the algal cells, and their absorbances were spectrophotometrically measured at 633 nm (TECAN infinite M200, Austria). Since the presence of residual concentration of bacteria *P. putida*, coming from the treatment of E_3 interfered with the spectrophotometer measurements. The sample was previously filtered with a 0.2-µm filter. Absorbance values were converted into algae concentrations, thanks to a linear regression line previously plotted. Results were plotted on a dose–effect chart.

L. minor test

This test evaluates the effect on the growth of an aquatic plant, *L. minor* L. The test was performed in triplicate in 250-mL glass beakers, with a working volume of 150 mL and with a 1/15 diluted sample in 1/10 modified Steinberg medium (Appendix) according to the standard ISO SO/WD 20079 (ISO 2001). Distilled water added with 1/10 modified Steinberg medium was used as a control. Ten fronds of *L. minor* (two or three fronds per colony) of similar size were used as inoculum. The test was carried out in a climatic exposure test cabinet, calibrated at 25 °C, with

continuous light (8000 lx), for 7 days. At the end of the experiment, frond number and plant dry weight were used to calculate the growth inhibition rate with respect to the control average according to standard procedures

Phytotoxicity tests

Two dicotyledonous plants, *C. sativus* L. and *L. sativum* L., were used for phytotoxicity tests, according to the standard method UNICHIM N.1651 (ISO 2003). The seeds (90 % germination warranty) were purchased from Ingegnoli S.p.A. (Milan, Italy).

For both species, ten seeds were placed in 9-cm Petri dishes, containing 5 mL of the tested sample and a filter paper (Whatman No. 1). The control was performed in four replicates, using distilled water. The seeds were incubated for 72 h in the dark at 25 °C. At the end of the test, the germinated seeds were counted, and root elongation was measured according to standard procedures.

D. magna test

The test was carried out according to the UNI EN ISO 6341:2012 using *D. magna* Straus (Cladocera, Crustacea) (ISO 2012). The test was performed in 25-mL glass beakers with a working volume of 20 mL, using at least six dilutions in freshwater standard solution (Appendix) of each effluent. Each dilution was incubated with five animals in four replicates. Control (freshwater without effluent) was performed with four repetitions. Immobile animals were counted after 24 h, and the response was given as percentage of mobile animals with respect to the control. Results were plotted on a dose–effect chart.

A. franciscana test

A. franciscana test was performed by means of miniaturized kit (ARTOXKIT M) purchased from Ecotox LDS (Cornaredo, MI, Italy) according to the ECOTOXICOLOGICAL METHOD 8060 of APAT-IRSA in 2003. Brine shrimp cysts were hatched in standard seawater at 25 °C and constant lighting (3000–4000 lx) for 30 h. The bioassay was performed in a 24 multiwell test plate. Ten instars II–III larvae were incubated in 1 mL of sample in each well, at 25 °C in darkness. Larval mortality was estimated after 24 h by counting the dead larvae (i.e., those which exhibited no movement, during 10-s observation). Serial dilutions of each effluent sample were assayed (100, 50, 25, 12.5, 6.3 and 3.1 %). Three replicates per dilution were considered. Results were plotted on a dose–effect chart.

Luminescent bacteria test

Luminescent bacteria tests were performed according to the standard UNI EN ISO 11348-3, using the Microtox system (Microtox Model 500; Microbics Corp., USA) with an automatic record of the luminescence (ISO 2007). Freeze-dried marine luminescent bacteria (*V. fischeri* strain NRRLB-11177) were bought from Ramcon A/S (Birkeroed, Denmark). All dose–response curves were plotted with eight effluent dilutions, each in duplicate and with four controls. The luminescence intensity in all cuvettes was measured before and 15 min after addition of the wastewater samples. Automatic colour correction was performed when necessary. Toxicity was expressed as the effective concentration that inhibits the bacterial growth by 50 % (EC_{50}) by means of the probit statistical model, as the toxicological units which corresponds to $100/EC_{50}$, as the lowest observed effect concentration (LOEC) and finally as the no observed effect concentration (NOEC).

Synthetic index determination

The outputs of ecotoxicity tests that allowed calculating of EC_{50} were condensed with a synthetic index developed by UNICHIM N. 1651 (2003) (Tigini et al. 2011). The method allows the comparison of the outputs of batteries, in which the same tests are performed, by calculating the toxicity score of the battery (BTS) as the mean of the relative toxicity of each test ($RT_{endpoint}$). These last values are expressed as a percentage, as follows:

$$RT_{endpoint}=100-100\cdot[\log(C\cdot EC_x)\cdot R\cdot S]_{max}-[\log(C\cdot EC_x)R\cdot S]_{endpoint}[\log(C\cdot EC_x)R\cdot S]_{max}$$

where C is a statistical corrective ($C = 2$ if the EC_x is higher than 100 %; $C = 1$ if the EC_x and its 95 % confidence limits are lower than 100 %), S is a score depending on the considered endpoint (mortality = 8, bioluminescence = 7, development = 6, reproduction = 5, growth = 4, genotoxicity = 3, mutagenicity = 2, behaviour = 1), and R is the rank of toxic concentrations, and it is assigned from the lowest concentration to the highest one.

Moreover, the risk score of the battery (BRS), expressed as a percentage, has been calculated according the following formula:

$$BRS=\text{mean of the } RT_{endpoint}\cdot[(\sum RT_{endpoint}+\text{consistency})/\sqrt{N}](\sum RT_{endpoint}/\sqrt{N}) \quad (2)$$

where N is the number of total endpoints; consistency is the half of total endpoints to which non-significant endpoints are subtracted. The consistency indicates the agreement rate among different endpoints: it is high (positive value) if all tests give results in agreement with each other; on the contrary, it is low (negative value) if the tests are discordant. The role of the consistency is to increase or decrease the risk score, according to the number of significant endpoints.

Moreover, the relevance of the battery has been calculated according to the following formula:

$$\text{Total relevance } \%=100\cdot(S_{mean})/8 \quad (3)$$

The BTS is converted in a scale based on the expert judgment as follows: BTS ≤5 % = negligible toxicity; 5 % < BTS ≤ 20 %, consistency ≤0 = moderate toxicity; 5 % < BTS ≤ 20 %, consistency >0 = high toxicity; BTS >20 % = very high toxicity; and BTS >50 % = extremely high toxicity. As well, the risk score is converted in the following scale: BRS ≤5 % = negligible risk, 5 % < BRS < 10 % = moderate risk, 5 % < BRS < 20 % = high risk, BRS >20 % = very high risk and BRS >50 % = extremely high risk.

Eventually, this method allows the calculation of the EC_x and its confidence limits for the battery as follows:

$$\text{Battery } EC_x=10^{(\text{mean}(\log EC_x))} \quad (4)$$

$$\text{Battery } L_{low}=10^{(\text{mean}(\log \text{ of the lower confidence limit}))} \quad (5)$$

$$\text{Battery } L_{up}=10^{(\text{mean (log of the upper confidence limit)})} \quad (6)$$

Only the tests showing a significant toxicity (i.e., calculable EC_x) contribute to the estimation of the battery EC_x.

Statistical analysis

EC_{50} and its confidence limits of each ecotoxicity test were calculable using a probit statistical model. Student's *t* test was used to compare the efficacy of each treatment vs. control. Difference was considered significant when $P < 0.05$.

Results

Ecotoxicity tests

The toxicity obtained when exposing *L. minor*, *C. sativus* and *L. sativum* to E_1, E_2 and E_3 was expressed as growth inhibition percentage with respect to the control (without effluent) (Table 2). E_1 was the most toxic effluent towards *L. minor* regarding frond and biomass development, with growth inhibition percentage of 33.9 and 52.8 %, respectively. Inhibition rate decreases significantly when *L. minor* was exposed to the same effluent treated by the activated sludge (E_2). Actually, the inhibition percentages obtained were 21.1 and 10.14 % for fronds and biomass development, respectively. Results obtained with E_3 did not show any considerable difference relatively to those obtained with E_2 effluent. The inhibition percentage was 21.3 % for frond number, while any inhibition percentage was shown for biomass development (Table 2).

Table 2

Inhibition percentages resulting from the exposure of *L. minor*, *L. sativum* and *C. sativus* to effluents

	Effluent 1	**Effluent 2**	**Effluent 3**
L. minor frond number	33.9 ± 4.9**	21.1 ± 5.8*	21.3 ± 9.2*
L. minor biomass development	52.7 ± 25.0*	10.1 ± 4.2	NI
L. sativum germination	100 ± 0***	20 ± 1.6*	17.5 ± 1.1***
L. sativum root elongation	100 ± 0***	91.2 ± 0.6***	91.3 ± 0.4***
C. sativus germination	45.7 ± 0.7***	22.9 ± 6.0**	25.7 ± 20.5
C. sativus root elongation	96.1 ± 0.3***	77 ± 1.2***	74.3** ± 4.1

NI no growth inhibition was showed

Significant difference obtained with $*P < 0.01$; $**P < 0.001$; $***P < 0.0001$

Germination and root elongation of *L. sativum* were totally inhibited, with respect to the control, when the plant was exposed to E_1. Their inhibition slightly decreases; when the species was exposed to E_2, inhibition rates were 20.0 and 91.2 %, respectively, and when exposed to E_3, inhibition rates were 17.5 and 91.3 %, respectively (Table 2).

C. *sativus* seems to be less sensitive to the toxicity induced by E_1 (45.7 and 96.1 % for germination and root elongation, respectively) compared to that of *L. sativum*, which was totally inhibited. On the contrary, results obtained by the exposition of *C. sativus* to E_2 and E_3 effluents confirmed those obtained by *L. sativum* (Table 2).

D. magna revealed a very high sensitivity towards E_1 ecotoxicity (Fig. 1). Daphnids exposed to over than 10 % E_1 exhibited 100 % mortality, and the estimated EC_{50} and toxic units (TU) were 137.0 % and 0.73, respectively. This high toxicity totally disappeared when animals were exposed to E_2. Indeed, this effluent did not cause any mortality even when animals were exposed to a not diluted effluent (Fig. 1). Instead, E_3 exhibited a high toxicity towards *D. magna*. In fact, daphnids exposed to over than 75 % E_3 exhibited 100 % mortality and the estimated EC_{50} and TU were 61.5 % and 1.6, respectively.

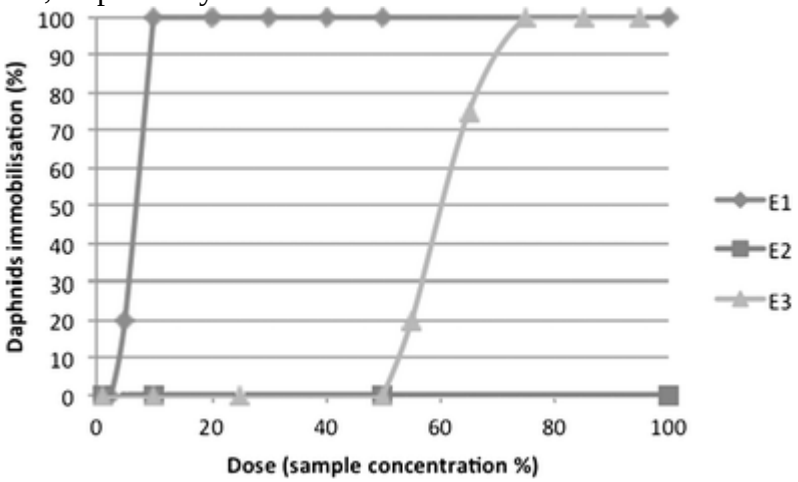

Fig. 1

Percentage of *Daphnia magna* immobilisation effect caused by effluents E1, E2 and E3. *E1* effluent 1, *E2* effluent 2, *E3* effluent 3

A. franciscana revealed the highest rate of toxicity with 40.0 % of larvae immobilisation exposed to E_1 50 % effluent dilution. This toxicity totally disappeared when animals were exposed to E_2, than slightly increased when exposed to E_3 (6.7 % immobilisation) at the same dilution rate. For none of the wastewater, it was possible to calculate the EC_{50} because toxicity was always lower than 50 % (Fig. 2).

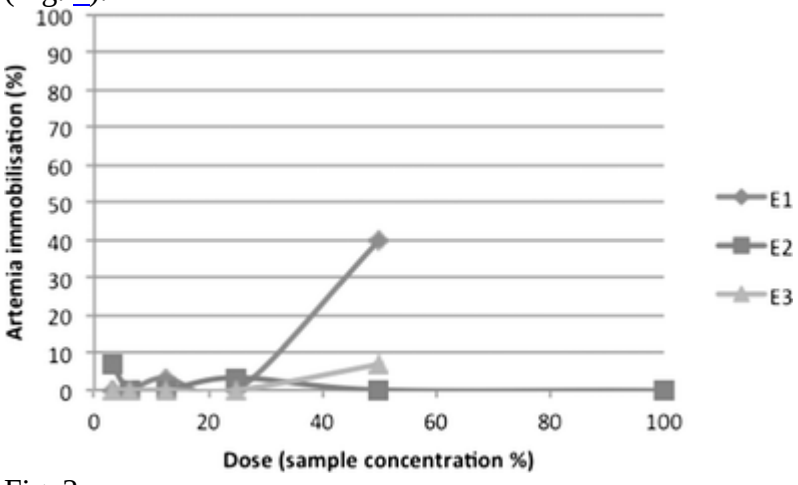

Fig. 2

Percentage of *Artemia franciscana* immobilisation effect caused by effluents E1, E2 and E3

Toxicity of the tested effluents was also evaluated against *V. fischeri*. The results of Microtox test are reported in Table 3. Toxicity of E_1 effluent was determined after automatic correction because of the intensity of its colour: it was the most toxic effluent with an EC_{50} of 3.0 % and TU_{50} value of 33.1. Effluent treated by activated sludge (E_2) did not exhibit any toxicity towards the bacterium, whereas E_3 showed an EC_{50} of 8.2 % and TU_{50} of 12.2 (Table 3).

Table 3

Toxicity of effluents against *V. fischeri* expressed as EC_{50}, TU, NOEC and LOEC

	E_1	**E_2**	**E_3**
EC_{50}	3.0	nd	8.2
TU	33.1	nd	12.2
NOEC	0.3	nd	0.8
LOEC	0.9	nd	2.6

EC_{50} the effective concentration that inhibits 50 % of the bacterial growth, *TU* the toxicological units = $100/EC_{50}$, *LOEC* the lowest observed effect concentration, *NOEC* no observed effect concentration

Toxicity results using the alga are reported in Fig. 3. E_1 was the most toxic sample towards *R. subcapitata*, with an EC_{50} of 8.7 % (TU = 11.5) and a growth inhibition percentage of 69.3 % at the highest testable dose (12.5 % *v*/*v*). The toxicity decreased when alga was exposed to E_2, with an EC_{50} of 45.8 % (TU = 2.2) and a growth inhibition percentage of 55.0 % at the highest testable dose (50 % *v*/*v*). Toxicity further decreased when alga was exposed to E_3 with an EC_{50} of 83.6 % (TU = 1.2) and a growth inhibition percentage of 46.0 % at the highest testable dose (80 % *v*/*v*).

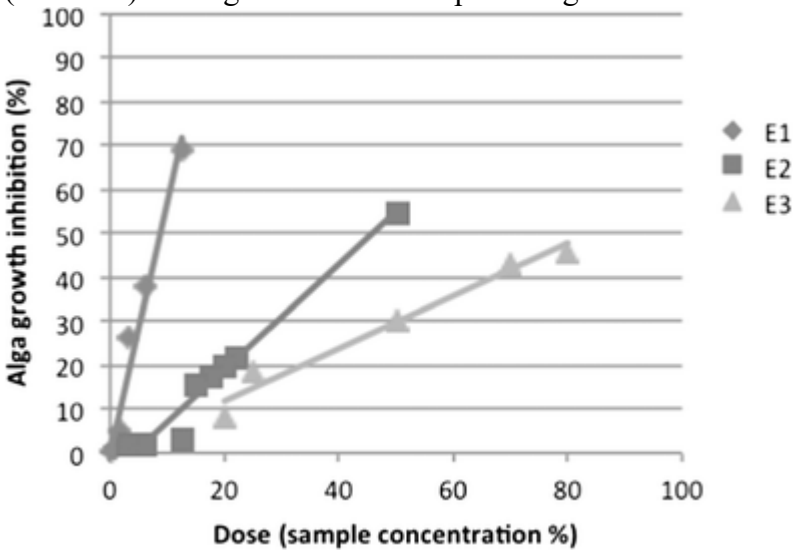

Fig. 3

Growth inhibition percentage of alga exposed to E1, E2 and E3

Synthetic index determination

The tests included in the synthetic index elaboration were *D. magna*, *A. franciscana*, *R. subcapitata* and *V. fischeri* (Fig. 4). According to the elaboration with the synthetic index, the E_1 effluent was characterized by extremely high toxicity and risk (both values were higher than 50 %). The consistency of the battery was 13 % and relevance was 90 %.

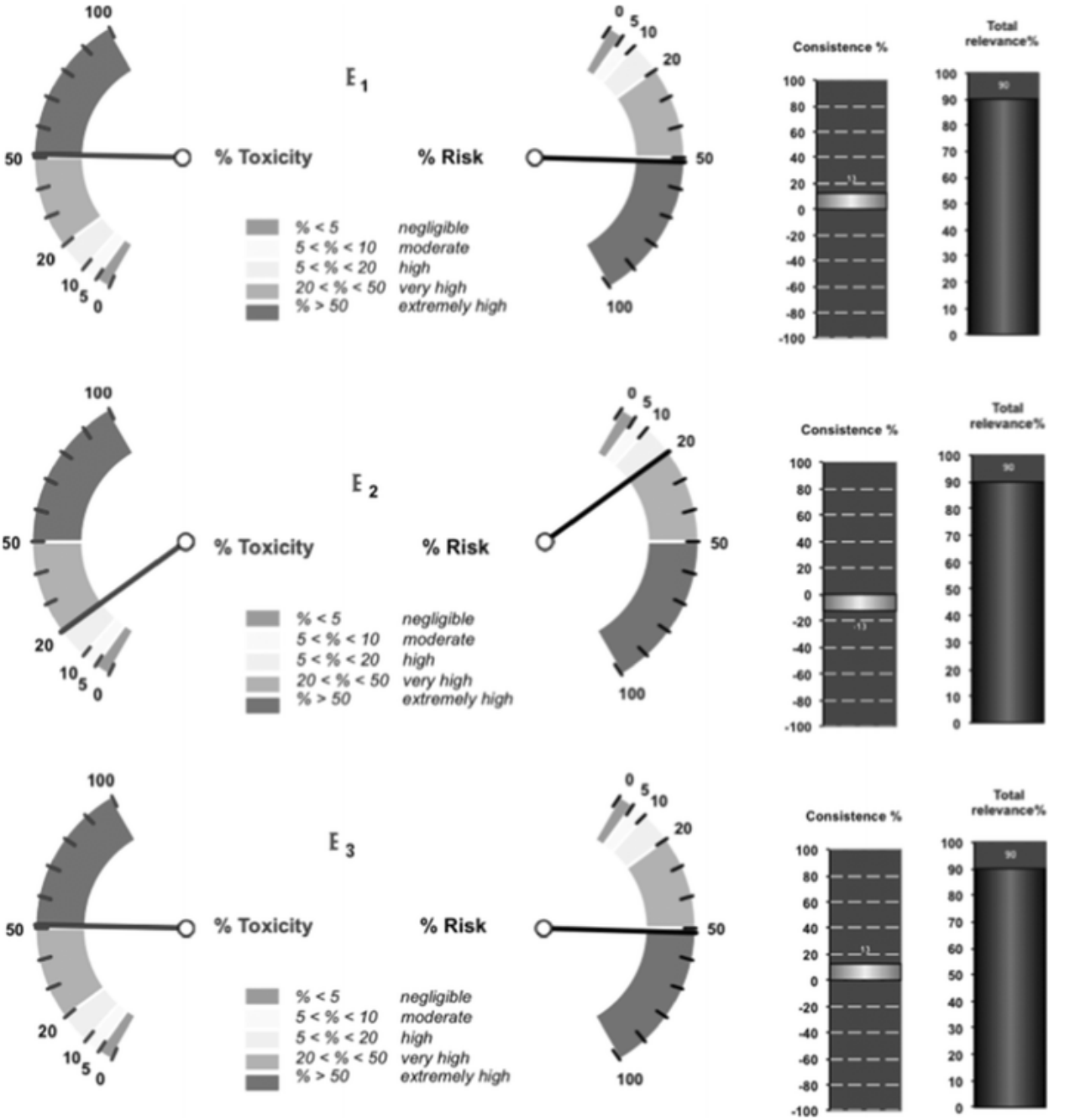

Fig. 4

Toxicity and risk scores, consistency and total relevance of the battery for E1, E2 and E3, according to the synthetic index. *BTS* toxicity score of the battery, *BRS* risk score of the battery

When applied to E_2, the test battery decreased to very high toxicity and risk (value = 20 %), whereas E_3 increased again the toxicity and risk recorded by the battery to extremely high (higher than 50 %).

Discussion

Ecotoxicity of effluents and effectiveness of treatments

It appears from our results that untreated sample E_1 which was characterized by high values of COD (2200 mg/L) and pH (12.54), as well as an intense colour, was the most toxic effluent towards all the tested species. In absence of precise chemical analysis from the WWTP, we can only hypothesize that the toxicity of the E_1 could be due, at least partially, to azo dyes used in the production line of this industry and probably present in their native or modified forms in the effluent released. In fact, azo dyes and/or their derivatives were reported by several authors to be genotoxic, mutagenic (Ben Mansour et al. 2009a, b, c, 2011), teratogenic (Chen 2006) and also carcinogenic (Moller and Wallin 2000; Moawad et al. 2003; Umbuzeiro et al. 2005).

The toxicity and risk assessment by means of the synthetic index pointed out an extreme hazard associated to this effluent. It is interesting to note that the same index applied to spent dye baths (with higher dye concentration) showed a lower risk (Tigini et al. 2011). This is mainly due to the higher relevance (90 %) and the consistency (13 %) of the present tests. Actually, a high consistency indicates the agreement rate among different endpoints.

Since the output of ecotoxicity tests were elaborated in different manners according to the performed procedures, the variation of the toxicity due to the treatments of activated sludge (E_2) and *P. putida* (E_3) with respect to the untreated effluent (E_1) was expressed in different ways, depending on the organism. The variation of toxicity detected by tests performed using samples without dilution was expressed as variation of the endpoint inhibition at 100 % sample dose. On the contrary, for tests performed with diluted samples, the toxicity variation was expressed as variation of TU (Table 4).

Table 4

Toxicity variation after the treatment with activated sludge (E_2) and *P. putida* (E_3)

	Variation of E_2 with respect to E_1 (effect of activated sludge) (%)	**Variation of E_3 with respect to E_2 (effect of *P. putida* treatment) (%)**	**Variation of E_3 with respect to E_1 (effect complessive treatment) (%)**
L. minor frond number inhibition at 100 % dose	−38	1	−37
L. minor biomass development inhibition at 100 % dose	−81	−100	−100
L. sativum root elongation inhibition at 100 % dose	−9	0	−9
L. sativum germination inhibition at 100 % dose	−80	−13	−83

	Variation of E_2 with respect to E_1 (effect of activated sludge) (%)	Variation of E_3 with respect to E_2 (effect of *P. putida* treatment) (%)	Variation of E_3 with respect to E_1 (effect complessive treatment) (%)
C. sativus root elongation inhibition at 100 % dose	−20	−4	−23
C. sativus germination inhibition at 100 % dose	−50	12	−44
D. magna (TU)	−100	2	−98
A. franciscana (TU)	−84	−100	−100
V. fischeri (TU)	−100	12	−63
P. subcapitata (TU)	−81	−45	−90

The activated sludge used in these experiments exhibited almost always a high detoxification power, from 9 % (*L. sativum*) to 100 % (*D. magna* and *V. fischeri*), compared to E_1: six out of ten endpoints indicated a toxicity reduction higher than 80 %. Three out of ten endpoints indicated a toxicity reduction between 20 and 50 %. This is confirmed by the positive value of the consistency and the relevance of the test battery. The activated sludge treatment decreased the risk associated to the textile effluent; actually, the BRS of E_2 was lower than E_1. This result is a consistent indicator of the detoxifying capacity of the tested activated sludge, which is in accordance with the literature, which indicated that activated sludge is effective in reducing textile effluent toxicity (Anastasi et al. 2012; Giorgetti et al. 2011; Araújo et al. 2005). The ecotoxicity decrease was proportional to the decrease of chemical parameter (pH, COD and BOD), confirming the effectiveness of bioremediation power of the activated sludge.

As *P. putida* was proved to be efficient in detoxifying textile effluents containing azo dyes or their derivatives (Ben Mansour et al. 2011), we applied this treatment after that with activated sludge, which resulted in effluent E_3. The toxicity variation due to *P. putida* treatment with respect to the activated sludge showed that the additional treatment with *P. putida* caused a toxicity variation between +12 % (*C. sativus*) and −100 % (*L. minor* and *A. franciscana*). Five out of ten endpoints showed unchanged or increased toxicity with respect to E_2, whereas the other five endpoints showed a decrease of the toxicity. Thus, in this case, *P. putida* does not seem to improve the water quality of the wastewater. This is confirmed also by the chemical analyses, which pointed out an increase of COD and BOD. Despite the toxicity recorded by the single tests never returned at the level of E_1 (Table 4), the synthetic index showed the same toxicity and risk score, which characterized the initial condition of E_1 effluent. The study of metabolites resulting from the biodegradation process with *P. putida* could give elements to understand the toxicity increase obtained when using this process.

Sensitivity of different organisms

Among the organisms tested towards non-diluted effluents, *L. sativum* was the most sensitive organism towards the three effluents (considering the root elongation as endpoint). Only in two cases, *C. sativus* was more sensitive, even if not significantly, than *L. sativum* (seed germination against E_2 and E_3).

Among the organisms tested towards non-diluted effluents, *D. magna* was the most sensitive towards E_1 toxicity, *R. subcapitata* was the most sensitive towards E_2, and *V. fischeri* was the most sensitive towards E_3; it is not easy to understand the reason why these organisms recorded a sensitivity change after the two treatments. This phenomenon should be ascribed to change in effluent composition due to the biodegradation by activated sludge and *P. putida*, which can produce metabolites with different toxic effect towards the test organisms. We should also hypothesize that enzymes excreted by *P. putida* may break *V. fischeri* membrane, and this induces bacteria cell death (Filloux 2004). In fact, Jayammal and Sivakumar (2013) reported better antibacterial activity of *Pseudomonas* species than some synthetic antibiotics, against different microorganisms such as *Vibrio* species.

D. magna test has been pointed out as particularly sensitive to surfactants (Tigini et al. 2011). On the contrary, the alga was reported to be sensitive to dye pollution (Chapman 2000; Whitehouse 2001; Novotný et al. 2006; Tigini et al. 2011).

L. minor and *V. fischeri* exhibited low sensitivity towards tested wastewaters. The results about *L. minor* are in accordance with previous studies (Cleuvers and Ratte 2002; MacRae 2003; Marinho-Soriano et al. 2011; Tigini et al. 2011) which reported that *L. minor* is not or weakly sensitive to this kind of effluents in spite of the high toxicity levels of these latter.

However, a characterization of the chemical composition of the three samples may add important informations to the comprehension of their behaviour, as far as we have not enough indications about the variability of samples. In fact, only COD, BOD, pH and conductivity were determined. Thus, we cannot draw a general conclusion about the relevance of treatment efficiency versus sample variability.

Conclusion

This study revealed that the analyzed activated sludge was able not only just to decolourize textile wastewater, but also to significantly detoxify it. For that, we used a battery of organisms previously reported to be highly sensitive in detecting wastewater toxicity. Further treatment with *P. putida* does not improve the quality of the effluent, but it increases its toxicity, indicating that the bacterium should be active in the transformation of some effluent components. A possible application of this treatment before inoculating the activated sludge could be investigated in order to achieve a complete bioremediation of the effluent. In fact, the efficiency of such process was established by Anastasi et al. (2012), who have reported that a fungal step treatment followed by a sludge treatment of textile wastewaters exhibits better detoxifying effect.

This study confirms that *D. magna* and *R. subcapitata* can be good indicators for textile wastewater toxicity comparing with *A. franciscana* and *L. minor*.

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Appendix

Table 5

Culture media for *R. subcapitata*, *L. minor* and *D. magna*

Ecotoxicity test	Culture media (mg/L)
R. subcapitata test	NH_4Cl (15); $MgCl_2 \cdot 6H_2O$ (12); $CaCl_2 \cdot 2H_2O$(18); $MgSO_4 \cdot 7H_2O$ (15); KH_2PO_4 (1.6) $FeCl_3$ $6H_2O$ (0.064); Na_2 EDTA $\cdot$ $2H_2O$ (0.1) H_3BO_3 (0.185); $MnCl_2 \cdot 4H_2O$ (0.415); $ZnCl_2$ (0.003); $CoCl_2 \cdot 6H_2O$ (0,0015); $CuCl_2 \cdot 2H_2O$ (0.00001); $Na_2MoO4 \cdot 2H_2O$ (0.007) $NaHCO_3$ (0.050) Modified Steinberg solution
L. minor test	KH_2PO_4 (90); KNO_3 (350); K_2HPO_4 (12.6); $MgSO_4 \cdot 7H_2O$ (100); $Ca(NO_3)_24H_2O$ (295); $MnCl_2 \cdot 4H_2O$ (0.18); H_3BO_3 (0.12); $Na_2MoO_4 \cdot 2H_2O$ (0.044); $ZnSO_4 \cdot 7H_2O$ (0.18); $FeCl_3$ $6H_2O$ (0.76); Na_2 EDTA $\cdot$ $2H_2O$ (100) Standard culture media
D. magna test	$CaCl_2 \cdot 2H_2O$ (195.87); $MgSO_4 \cdot 7H_2O$ (82.20); $NaHCO_3$ (64.80); KCl (5.80); Na_2SeO_3 (0.002)

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