Ecology/Écologie

Treated wastewater phytotoxicity assessment using *Lactuca sativa*: Focus on germination and root elongation test parameters

Évaluation de la phytotoxicité d'eaux de rejets via Lactuca sativa : paramètres des tests de germination et d'élongation

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A B S T R A C T

Sensitive and simple ecotoxicological bioassays like seed germination and root elongation tests are commonly used to evaluate the phytotoxicity of waste and industrial discharge waters. Although the tests are performed following national and international standards, various parameters such as the number of seeds per dish, the test duration or the type of support used remain variable. To be able to make a correct comparison of results from different studies, it is crucial to know which parameter(s) could affect ecotoxicological diagnosis. We tested four different control waters and three seed densities. No significant differences on either germination rate or root elongation endpoints were shown. Nevertheless, we found that the four lettuce cultivars (Appia, batavia *dorée de printemps*, grosse blonde paresseuse, and Kinemontepas) showed significantly different responses when watered with the same and different metal-loaded industrial discharge water. From the comparison, it is clear that a differential sensitivity scale occurs among not just species but cultivars.

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R É S U M É

Les bio-essais écotoxicologiques simples comme les tests de germination et d'élongation sont fréquemment utilisés pour évaluer la phytotoxicité de rejets industriels. Si ces tests sont réalisés selon des standards nationaux et internationaux, de nombreux paramètres (nombre de graines, durée du test, type de support…) varient. Afin de comparer correctement les résultats tirés de différentes études, il est indispensable de savoir précisément quel(s) paramètre(s) pourrai(en)t affecter le diagnostic écotoxicologique. Nous avons testé quatre eaux de contrôle et trois abondances de graines : aucune différence significative du taux de germination ou d'élongation des plantules n'a été mise en évidence. Néanmoins, nous avons montré que les quatre variétés de laitue testées (Appia, batavia *dorée de printemps*, grosse blonde paresseuse and Kinemontepas) présentaient des différences de réponse écotoxicologique, après avoir été mises en contact avec le même

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1. Introduction

Surface treatment (ST) is a very important industrial sector in Europe and in France, Franche-Comté is especially concerned. ST industry supplies a great variety of products for various industrial and domestic sectors including the motor, building, electronic, military and also clothing industries. However, their activities are energy- and water-consuming as well as highly chemically polluting. Indeed, ST is well known to be one of the largest consumers of chemicals (toxic metals known to be harmful to humans and to the environment in particular) and to generate large amounts of toxic waste water with a complex composition [1]. The main pollutants are metal ions such as Cr(III), Cr(VI), Zn, Sn, Cu, Ni, Ag and Fe, organic substances such as chloroform and polycyclic aromatic hydrocarbons, and other non-metallic pollutants such as cyanide, boron, and fluoride. Despite the efforts made to clean up their polycontaminated effluents, most commonly by physico-chemical treatment [2], industry and scientists are confronted with a great challenge: to remove the entire load of organic and inorganic pollutants to assess their ecotoxicological effects and hence move towards zero pollution discharge [3,4]. While pollutant mixtures present in discharge water after treatment are relatively easy to characterize chemically, assessing their impact on the environment is usually difficult [2]: over the past few decades, ecotoxicological methods have been developed to complete chemical analysis [5].

Bioassays are widely carried out following national and international recommendations. Some very different organisms are commonly used in ecotoxicological biomonitoring: primary producers (algae, *i.e. Pseudokirchneriella subcapitata* [6]), primary consumers (aquatic invertebrates, *i.e. Daphnia magna*, *Gammarus pulex* [7]) or secondary consumers (aquatic vertebrates, *i.e. Gambusia holbrooki* [8]). Less frequently used in comparison with faunal tests [9], toxicity studies using higher plants have however increased in recent years [11,12]. Ratsch and Johndro [13] first concluded that the inhibition of root elongation (RE) is a valid and sensitive indicator of environmental toxicity. Several articles [10,14–20] have since shown that phytotoxicity tests like seed germination rate (GR) and RE tests present many advantages as summarized in Table 1. These bioassays are simple, inexpensive and only require a relatively small amount of sample. Moreover, the seeds remain usable for a long time. The most common plant species recommended by, among others, the US Environmental Protection Agency [21], the US Food and Drug Administration [22], and the Organization for Economic Cooperation and Development [23] are cucumber *Cucumis sativus*, lettuce *Lactuca sativa* L., radish *Raphanus spp* L., red clover *Trifolium pratense* L., and

wheat *Triticum aestivum*. Previous studies [20,24] compared some of these species and recommended *L. sativa* as a bioindicator to determine the toxicity of soil and water samples.

Haugland and Brandsaeter [25] previously studied the effects of various procedures and conditions on bioassay sensitivity in allelopathic studies. They pointed out that the lack of real standardized bioassays makes comparison between studies very difficult. It is nowadays in fact the proceedings that are not standardized: even when phytotoxicological bioassays using lettuce are performed in accordance with national or international standards, multiple parameters remain variable (Table 2). Di Salvatore [10], studying synthetic solutions containing metal ions, found that lettuce GR and RE were not affected by substrate, agar agar *vs.* filter paper. However, there is no literature comparing the parameters used of industrial effluent, as that of the ST industry.

The present study is based on the assessment that proceeding parameters could affect the ecotoxicity diagnosis. Indeed, we tested three of the most variable parameters, using GR, root and total lengths as end points: control water quality, number of seeds per germination dish and lettuce cultivar.

2. Material and methods

2.1. Industrial discharge waters

During this study, discharge waters were once collected in three different surface treatment companies (denoted Co1, Co2 and Co3) in the Franche-Comté region. Effluent samples were collected at the outlet of the decontamination station of each company. The main activities of each company and the related major environmental concerns are reported in Table 3. The table also shows the

Table 1
Major advantages of phytotoxicity assays using vascular plant seeds.

Advantages of phytotoxicity tests involving seeds (seed germination rate, root elongation, etc.)[a]
Simple and very reproducible method
Applicable in situ and *in vitro*
No requirement for major equipment
Minimal maintenance costs
Seeds are self-sufficient (no adjuvants/nutrients needed in the test water)
Only small sample size required (e.g. water, effluent, soil, sediment)
No seasonality
Seeds can be easily purchased in bulk
Seeds remain viable a long time
Rapid germination

[a] Based on multiple references including [10,14,15,17].

Table 2
Non-exhaustive list of parameters that remain variable in seed germination bioassays[a].

Parameter	Examples
Cultivar	Regina; Buttercrunch; Trocadero; Divina; Iceberg; non-specified
Support	Agar agar; filter paper; germination paper; non-specified
Seed pre-treatment	Yes; no; non-specified–When yes: 10 or 30% hypochlorite solution
Temperature [in °C]	20; 24; 28; room temperature; non-specified
pH	5.5 to 8.2; non-specified
Dish	Glass; polyethylene; non-specified
Number of seeds	10; 20; 50; non-specified
Amount of sample	4 mL; 9 mL; non-specified
Duration	72 to 192 h
Control water	Distilled; deionized; milliQ; non-specified

[a]Based on multiple references including [1,5,10,13,19,20,26–30].

concentration threshold in discharge for key pollutants. Table 4 shows the characteristics of the samples studied here, taken from three different surface treatment companies (Co1S1, Co2S1 and Co3S1). The effluents are average samples, characteristic of daily activity. Each treated water sample was tested following the same concentration range: 25, 50, 75 and 100%. All dilutions were prepared in Reverse Osmosis Water (ROW).

2.2. Lettuce seeds

Four lettuce (*L. sativa L.*) cultivars were germinated: Appia (A), batavia dorée de printemps (B), grosse blonde paresseuse (GBP) and Kinemontepas (K). They were chosen among the 1500 or so commercially available cultivars. The seeds (Caillard, Avignon, France) were all kept under laboratory conditions, in the dark and shielded from large modifications of temperature and moisture [1].

Table 3
Main environmental issues encountered by the two surface treatment companies and the regulatory values (in mg·L^{-1}) for different pollutants contained in the water discharges (French law of 5th September 2006).

Company and main activity	Contaminant(s) of major concern	Threshold emission value (mg·L^{-1})
Co 1	Zn	3.5
Treatment by electrolysis	Ni	3.5
Co2	Fe	5
Plating with precious metals	Ni	2
Co3	Al	5
Surface treatment of aluminum		

Table 4
Physicochemical characteristics of three discharge waters (Co1S1, Co2S1, Co3S1) from the three industrial sites investigated in this study.

Parameter/Metal	Co1S1	Co2S1	Co3S1
pH	8.5	8.4	6.9
Conductivity	–	1730	3280
Fe	1.97	5.18	0.117
Cr	0.13	0.079	0.12
Zn	2.67	0.15	0.05
Ni	0.6	0.96	0.49

All concentrations are expressed in mg·L^{-1}, except the conductivity in μS·cm^{-1}.

2.3. Control and toxicity test

Germination rates for samples were evaluated using the French normalized method ISO 17126 [31]. Tests were conducted using 100×15 mm disposable plastic Petri dishes and two layers of filter paper. Thirty plump undamaged seeds of almost identical size were laid on the filter paper in each dish, which contained 4 mL of industrial discharge water (pH ∼ 8.4). Each condition was tested in triplicate. All dishes were kept in the dark, at 24 ± 1 °C, for seven days of exposure. As recommended by the normalized method [31], a control test with distilled water was performed in triplicate for every condition tested. Multiple parameters were tested as described in Table 5. After seven days, germinated seeds were counted (GR) using equation (1) (where GSS is the number of Germinated Seeds in the Sample and GSC the number of Germinated Seeds in the Control) and plantlet growth measured (root and total lengths; RL and TL; the total length refers to the root and hypocotyl of the plantlets).

$$GR = \frac{GSS}{GSC} \qquad (1)$$

As recommended by the normalized method [31], GR under 90% is unacceptable for control conditions. Control water pH did not skew germination test results as long as it remained between 5.5 and 9.5.

2.4. Statistical analysis

All homoscedasticities were tested using a Bartlett test as prerequisite for parametric test. The GRs were compared using the Chi^2 test and lengths (root and total) using

Table 5
Parameters assessed here.

Quality of water	Distilled water DW (pH 7.3)
	Mineral water Evian®, E (pH 7.2)
	Reverse osmosis water ROW (pH 6)
	Ultra pure water UPW (6.05)
Number of seeds per Petri dish	15
	20
	30
Cultivar of lettuce	var. Appia (A)
Lactuca sativa	var. batavia dorée de printemps (B)
	var. grosse blonde paresseuse (GBP)
	var. Kinemontepas (K)

Table 6
Comparison between germination rate (GR) and root length RL means ($\pm$ SD; $n = 3$) of *L. sativa* var Batavia dorée de printemps (B) watered with four different control waters (E, UPW, ROW, DW).

	E	UPW	ROW	DW	*P* value
GR (%)	100	99 $\pm$ 1.6	98 $\pm$ 3.1	94 $\pm$ 1.6	0.067
RL (mm)	14.28 $\pm$ 4.47	14.64 $\pm$ 5.99	17.80 $\pm$ 9.11	14.44 $\pm$ 5.79	0.147
TL (mm)	63.14 $\pm$ 4.20	61.33 $\pm$ 6.26	65.21 $\pm$ 6.18	67.73 $\pm$ 1.16	0.441

Table 7
Comparison between germination rate GR and root length RL means ($\pm$ SD; $n = 3$) of 4 lettuce cultivars watered with ROW.

	A	B	K	GBP	*P* value
GR (%)	95 $\pm$ 4.1	96 $\pm$ 2.9	93 $\pm$ 3.9	96 $\pm$ 3.4	0.357
RL (mm)	16.20 $\pm$ 5.40	25.70 $\pm$ 9.49	28.07 $\pm$ 8.16	24.49 $\pm$ 9.91	0.08
TL (mm)	57.49 $\pm$ 10.56	71.50 $\pm$ 14.55	81.05 $\pm$ 12.86	74.27 $\pm$ 15.02	0.02

Kruskal–Wallis tests, with a significance threshold of $p < 0.05$. All statistical analyses were performed with R (2.15.1) (R Development Core Team, 2013, www.r-project.org).

Dose-dependent curves and EC$_{50}$ values were calculated with Hill's model using the macro Excel Regtox free version EV 7.0.6.

Germination Index (GI) [32–35] were used to assess the response variability between lettuce cultivars. Calculations of these indexes were performed using the equations (2) where RLS is the Root Length of the Sample, RLC the Root Length of the Control.

$$GI = \frac{RLS \times GSS}{RLC \times GSC} \tag{2}$$

3. Results and discussion

3.1. Control water

Four kinds of water (distilled, mineral, reverse osmosis, and ultra pure) were used as controls. Table 6 reports the relative data of GR and RL of seeds watered with the different control waters. The assay was performed on the same cultivar of lettuce *L. sativa*. The results showed that neither GR nor root length showed significant differences. For practical reasons, the control water chosen was ROW.

We synchronically tested the potential ecotoxicological differences between the four cultivars watered with ROW. Results (Table 7) showed no statistical GR or RL differences between the different cultivars. Total lengths appeared to be different, especially Appia's total length from the three others. Differential cultivar total length was attributed intrinsic natural differences as far as root lengths were not significantly different.

3.2. Number of seeds per dish

Three seed densities (15, 20 and 30 seeds per dish; ROW) were tested, using the same lettuce cultivar (var. B). The results for the three bioassay endpoints are detailed in Table 8. It can be seen that there was no significant difference between GR (100%; 95%; 98%) and both root (17.5; 18.5; 17.8 mm) and total (62; 71; 65.4 mm) length of the plantlet grown in dishes containing 15, 20, and 30 seeds, respectively.

Weidenhamer et al. [36] reported that the number of seeds relative to the solution volume used in a seed germination bioassay was a factor in the results obtained as the amount of ferulic acid available to each seed influenced germination, rather than chemical concentration of the tested solution. It seems that there is not such a consensus about the effect of seed number on length: some report a correlation (*e.g.*, [37]) and some do not (*e.g.*, [38]). Our results show that for a 4 mL sample, the number of seeds (15 to 30 seeds) did not affect either germination or elongation. For practical reasons, the number of seeds per dish was fixed at 20.

3.3. Seed cultivar

Bioassays were conducted using a sample of raw discharge water, taken from three different surface treatment companies (Co1S1, Co2S1 and Co3S1). The characteristics of the samples are reported in Table 4. Fig. 1 shows the four different dose–response curves of the four lettuce cultivars watered with Co1S1. It can be seen that when watered with the same effluent sample, the four cultivars did not show the same ecotoxicological response. This was confirmed by the results described in Table 9, which presents the GR and GI values for every diluted

Table 8
Number of seeds per Petri dish versus germination rate GR (%), root and total lengths RL and TL (mm) of *L. sativa* var B (ROW; $n = 3$ replicates).

| Number of seeds	GR (%)	RL (mm)	TL (mm)	*P* value
15	100	17.5 $\pm$ 8.5	62 $\pm$ 12.7	
20	95 $\pm$ 4	18.5 $\pm$ 5.75	71 $\pm$ 8.75	> 0.05
30	98 $\pm$ 3	17.8 $\pm$ 9.1	65.4 $\pm$ 16.8	

Table 9
Germination rate, EC_{50} and germination index values for the four lettuce cultivars watered with the four effluent samples.

	Sample	Lettuce cultivar	Treatment (tested effluent concentration)				EC_{50}	Sensitivity Scale
			25%	50%	75%	100%		
Germination rate GR [%]	Co1S1	B	98 ± 2.4	92 ± 2.4	97 ± 2.4	45 ± 4.1	99.75	B > K > GBP > A
		K	70 ± 14.7	58 ± 11.8	42 ± 11.8	20 ± 8.2	59.15	
		A	47 ± 2.4	7 ± 2.4	2 ± 2.4	2 ± 2.4	25.11	
		GBP	83 ± 6.2	35 ± 8.2	18 ± 6.2	0	42.93	
	Co2S1	B	73 ± 2.4	93 ± 2.4	87 ± 6.2	77 ± 14.3	n.a.	B∼K∼GBP > A
		K	95	92 ± 2.5	92 ± 2.5	87 ± 2.5	n.a.	
		A	82 ± 8.5	52 ± 14.3	33 ± 6.2	28 ± 14.3	61.17	
		GBP	97 ± 4.7	97 ± 6.2	97 ± 4.7	68 ± 6.2	n.a.	
	Co3S1	B	98 ± 2.4	90 ± 4.1	88 ± 2.4	85 ± 4.1	n.a	B∼K > GBP > A
		K	97 ± 4.7	100	98 ± 2.4	92 ± 2.4	n.a	
		A	93 ± 2.4	82 ± 6.2	67 ± 6.2	35 ± 4.1	90.01	
		GBP	100	98 ± 2.4	88 ± 2.4	72 ± 4.7	n.a	
Germination index GI	Co1S1	B	1.5	1.45	1.42	0.4		B > >K > GBP > A
		K	0.96	0.67	0.33	0.13		
		A	1.12	0.13	0.03	0.05		
		GBP	0.89	0.51	0.23	0		
	Co2S1	B	1.21	1.16	1.42	1.18		B > GBP∼K >A
		K	0.73	0.98	1.01	0.98		
		A	1.23	0.74	0.59	0.32		
		GBP	1.21	1.16	1.13	0.85		
	Co3S1	B	1.01	0.77	0.7	0.53		B∼GBP > K > >A
		K	1.04	0.88	0.7	0.56		
		A	0.49	0.42	0.32	0.23		
		GBP	1.32	0.92	0.68	0.49		

sample assessed on the four lettuce cultivars, and the EC_{50} values for every sample. GR decreased with all the four raw samples. The intensity of the decrease depends on the sample (*e.g.*, var B's GR varied from 45 ± 4.1 to 93 ± 2.4%). Indeed, Charles et al. [1] showed that lettuce ecotoxicological response variability can be linked with the chemical composition of the samples, which varies on a daily basis (Table 4). Differences between GRs were significant: for example, values for undiluted Co1S1 were 2 ± 2.4%, 45 ± 4.1%, 0% and 20 ± 8.2% for var A, var B, var GBP and var K, respectively (Fig. 1). The same observation was made

considering all the tested samples and EC_{50}s (Table 4). After comparing GR and EC_{50} values, var A was found to be the most sensitive cultivar, vars K and GBP medium, and var B the most resistant to the three effluents tested. This was confirmed by comparing the GR/EC_{50} sensitive scale with the GIs sensitive scale.

Although GR (lethal endpoint) is the most commonly used endpoint, it is not the most sensitive [39]: root length (sublethal endpoint) has often proved to be a more sensitive parameter, but not as easy to measure as germination. This is the reason why GI, first defined to
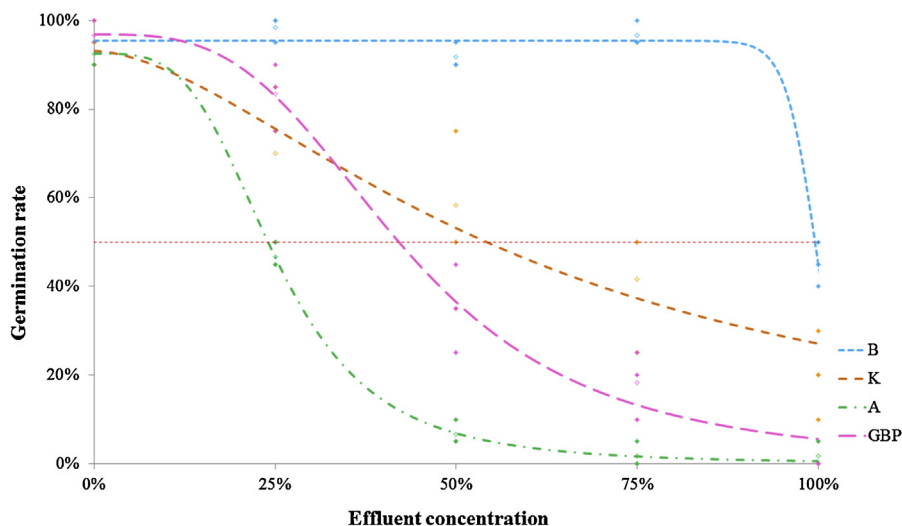
Fig. 1. Lettuce germination rate versus concentration of Co1S1 raw discharge waters for the four lettuce varieties (batavia B, Kinemontepas K, Appia A and grosse blonde parresseuse GBP).

assess compost toxicity [32], combines advantageously relative seed germination and RE measurements, generating an objective sensitivity scale, here: B > K > GBP > A, where the Appia cultivar is the most sensitive of the four.

Toxicity and ecotoxicity depend on the bioassay indicator and the endpoints considered [40]. Differences in sensitivity of plant *species* to various pollutants have been demonstrated [10,41–43]. Wang and Freekmark [17] reviewed and concluded that sensitivity varies among toxicants and taxonomic *groups* and *species*. The choice of the bioindicator variety has already been showed for species like potato or wheat. Beside the risk of toxicity underestimation when only one bioassay is used [44], Cairns and Pratt [45] concluded that the potential difference in results from one species to another may affect the extrapolation accuracy.

4. Conclusion

This study demonstrated that among multiple variable germination and elongation test proceeding parameters, control water quality and seeds density did not affect neither lettuce GR nor root or total lengths. However, when sensitivity differences among species are well known, it appears that the cultivar has a major effect in the assessment of discharge water toxicity: we suggest choosing carefully the bioindicator cultivar, and maybe carrying out rapid tests among multiple cultivar. It would be interesting in further investigations to determine which physiological phenomena (*e.g.*, metal uptake) are different, and can explain the ecotoxicological differences observed.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

References

[1] J. Charles, B. Sancey, N. Morin-Crini, P.M. Badot, F. Degiorgi, G. Trunfio, G. Crini, Evaluation of the phytoxicity of polycontaminated industrial effluents using the lettuce plant (*Lactuca sativa*) as a bioindicator, Ecotoxicol. Environ. Safe. 74 (2011) 2057–2064.

[2] B. Sancey, G. Trunfio, J. Charles, P.M. Badot, G. Crini, Sorption onto crosslinked cyclodextrin polymers for industrial pollutants removal: an interesting environmental approach, J. Incl. Phenom. Macrocycl. Chem. 70 (2011) 315–320.

[3] E.C. Directive, Directive 2000/60/EC of the European Parliament and of the Council of the 23 October 2000 establishing a framework for Community action in the field of water policy, Official Journal of the European Communities, L 327/2, 22.12.2000, Brussels, 2000.

[4] E.C. Directive, Directive 2008/105/EC of the European Parliament and of the Council of the 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and Council, Official Journal of the European Communities, L 348/84, 24.12.2008, Brussels, 2008.

[5] B. Fjallbörg, B. Li, E. Nilsson, G. Dave, Toxicity identification evaluation of five metals performed with two organisms (*Daphnia magna* and *Lactuca sativa*), Arch. Environ. Contam. Toxicol. 50 (2006) 196–204.

[6] P. Radix, M. Léonard, C. Papantoniou, G. Roman, E. Saouter, S. Gallotti-Schmitt, H. Thiébaud, P. Vasseur, Comparison of four chronic toxicity tests using algae, bacteria, and invertebrates assessed with sixteen chemicals, Ecotoxicol. Environ. Safe. 47 (2000) 186–194.

[7] J. Charles, G. Crini, F. Degiorgi, B. Sancey, N. Morin-Crini, P.M. Badot, Unexpected toxic interactions in the freshwater amphipod *Gammarus pulex* (L.) exposed to binary copper and nickel mixtures, Environ. Sci. Pollut. R. 21 (2014) 1099–1111.

[8] V. Drèze, G. Monod, J.P. Cravedi, S. Biagianti-Risbourg, F. Le Gac, Effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (*Gambusia holbrooki*), Ecotoxicology 9 (2000) 93–103.

[9] M.A. Lewis, Use of freshwater plants for phytotoxicity testing: a review, Environ. Pollut. 87 (1995) 319–336.

[10] M. Di Salvatore, A.M. Carafa, G. Carratù, Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates, Chemosphere 73 (2008) 1461–1646.

[11] G. Uzu, S. Sobanska, G. Sarret, M. Munoz, C. Dumat, Foliar lead uptake by lettuce exposed to atmospheric fallouts, Environ. Sci. Technol. 44 (2010) 1036–1042.

[12] L. Rizzo, Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment, Water Res. 45 (2011) 4311–4340.

[13] H.C. Ratsch, D. Johndro, Comparative toxicity of six test chemicals to lettuce using two root elongation test methods, Environ. Monit. Assess. 6 (1984) 267–276.

[14] A.M. Mayer, A. Poljakoff-Mayber, The germination of seeds, 4th Ed., Pergamon Press, New York, 1989.

[15] B.J. Dutka, Methods for microbiological and toxicological analysis of waters, wastewaters and sediments, National Water Research Institute (NWRI), Environment, Canada, 1989.

[16] W. Wang, Literature review on higher plants for toxicity testing, Water Air Soil Pollut. 59 (1991) 381–400.

[17] W. Wang, K. Freemark, The use of plants for environmental monitoring and assessment, Ecotoxicol. Environ. Safe. 30 (1995) 289–301.

[18] K.E. Gustavson, S.A. Sonsthagen, R.A. Crunkilton, J.M. Harkin, Ground water toxicity assessment using bioassay, chemical, and toxicity identification evaluation analysis, Environ, Toxicol. 15 (2000) 421–430.

[19] X.D. Wang, C. Sun, S. Gao, L. Wang, H. Shuokui, Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*, Chemosphere 44 (2001) 1711–1721.

[20] M.K. Banks, K.E. Schultz, Comparison of plants for germination toxicity tests in petroleum-contaminated soil, Water Air Soil Pollut. 167 (2005) 211–219.

[21] E.P.A., Ecological effects test guidelines (OPPTS 850.4200): Seed germination, root elongation toxicity test, US Environmental Protection Agency, Office of Toxic Substances, Washington, D.C, 1996.

[22] F.D.A., Seed germination and root elongation, Environmental Assessment Technical Assistance, US Food and Drug Administration Department of Health and Human Services, Washington, D.C, 1987.

[23] O.E.C.D., Terrestrial plant test; Seedling emergence and growth test, Organization for Economic Cooperation and Development, Guideline 208, Paris, 2003.

[24] W. Wang, Root elongation method for toxicity testing of organic and inorganic pollutants, Environ. Toxicol. Chem. 6 (1987) 409–414.

[25] E. Haugland, L.D. Brandsaeter, Experiments on bioassay sensitivity in the study of allelopathy, J. Chem. Ecol. 22 (1996) 1845–1859.

[26] W. Wang, P.H. Keturi, Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample, Water Air Soil Pollut. 52 (1990) 369–376.

[27] M. Kummerová, E. Kmentová, Photoinduced toxicity of fluoranthene on germination and early development of plant seedling, Chemosphere 56 (2004) 387–393.

[28] B. Fjallbörg, N. Gustafsson, Short-term bioassay responses to sludge products and leachate, Arch. Environ. Contam. Toxicol. 51 (2006) 367–376.

[29] L.F. Andrade, L.C. Davide, L.S. Gedraite, The effects of cyanide compounds, fluorides, aluminium, and inorganic oxides present in spent pot liner on germination and root tip cells of *Lactuca sativa*, Ecotoxicol, Environ. Safe. 73 (2010) 626–631.

[30] E. Schreck, C. Laplanche, M. Le Guédard, J.-J. Bessoule, A. Austruy, T. Xiong, Y. Foucault, C. Dumat, Influence of fine process particles enriched with metals and metalloids on *Lactuca sativa* L. leaf fatty acid composition following air and/or soil plant field exposure, Environ. Pollut. 179 (2013) 242–249.

[31] A.F.N.O.R., Qualité des sols–Détermination des effets des polluants sur la flore du sol–Essai de détection de l'émergence des plantules de laitue (*Lactuca sativa* L.), AFNOR ISO 17126, 2005 (in French).

[32] F. Zucconi, A. Pera, M. Forte, M. de Bertoldi, Evaluating toxicity of immature compost, Biocycle 22 (1981) 54–57.

[33] F. Zucconi, A. Monaco, M. Forte, M. de Bertoldi, Phytotoxins during the stabilization of organic matter, in: J.K.R. Gasser (Ed.), Composting of agricultural and other wastes, Elsevier, London, 1985, pp. 73–85.

[34] P. Alvarenga, P. Palma, A. Gonçalves, R. Fernandes, A. Cunha-Queda, E. Duarte, G. Vallini, Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land, Environ. Int. 33 (2007) 505–513.

[35] M.T. Varnero, C. Rojas, R. Orellana, Phytotoxicity indices of organic residues during composting, J. Soil Sci. Plant Nutr. 7 (2007) 28–37.

[36] J.D. Weidenhamer, T.C. Morton, J.T. Romeo, Solution volume and seed number: often overlooked factors in allellopathic bioassays, J. Chem. Ecol. 13 (1987) 1481–1491.

[37] G. Woodward, B. Ebenman, M. Emmerson, J.M. Montoya, J.M. Olesen, A. Valido, P.H. Warren, Body size in ecological networks, Trends Ecol. Evol. 20 (2005) 402–409.

[38] E.P. White, S.K.M. Ernest, A.J. Kerkhoff, B.J. Enquist, Relationships between body size and abundance in ecology, Trends Ecol. Evol. 22 (2007) 323–330.

[39] G.R. Leather, F.A. Einhellig, Bioassay of naturally occurring allelochemicals for phytotoxicity, J. Chem. Ecol. 14 (1988) 1821–1828.

[40] S.A. Wangberg, B. Bergström, H. Blanck, O. Svanberg, The relative sensitivity and sensitivity patterns of short-term toxicity tests applied to industrial waste water, Environ. Toxicol. Water Qual. 10 (1995) 81–90.

[41] W.E. Miller, S.A. Peterson, J.C. Greene, C.A. Callahan, Comparative toxicology of laboratory organisms for assessing hazardous waste sites, J. Environ. Qual. 14 (1985) 569–574.

[42] J.M. Thomas, J.R. Skalski, J.F. Cline, M.C. McShane, J.C. Simpson, W.E. Miller, S.A. Peterson, C.A. Callahan, J.C. Greene, Characterization of chemical waste site contamination and determination of its extend using bioassays, Environ. Toxicol. Chem. 5 (1986) 487–501.

[43] J.S. Fletcher, F.L. Johnson, J.C. McFarlane, Database assessment of phytotoxicity data published on terrestrial vascular plants, Environ. Toxicol. Chem. 7 (1988) 615–622.

[44] M.D. Hernando, A.R. Fernandez-Alba, R. Tauler, D. Barcelo, Toxicity assays applied to wastewater treatment, Talanta 65 (2005) 358–366.

[45] J.J. Cairns, J.R. Pratt, The scientific basis of bioassays, Hydrobiologia 188–9 (1989) 5–20.