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Ecotoxicity of cyanide complexes in industrially contaminated soils

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ABSTRACT

This study deals with acute and chronic ecotoxicity of leachates from industrially contaminated soils. Analyses focused on cyanides (complex and free forms) to study their possible involvement in leachates toxicity. No acute toxicity on the Microtox and 48 h-*Daphnia magna* tests was found in leachates collected over 18 months, but a high chronic toxicity was recorded on the reproduction of *Ceriodaphnia dubia* (EC50-7 d = 0.31 ± 0.07%) and on the algal growth of *Pseudokirchneriella subcapitata* (EC50-72 h = 0.27 ± 0.09%). Ceriodaphnids were as sensitive to free cyanide as to complex forms (EC50-7 d as CN^- = 98 μg/L, 194 μg/L and 216 μg/L for KCN, $Fe(CN)_6K_3$ and $Fe(CN)_6K_4$, respectively). The EC50-72 h of KCN to *P. subcapitata* (116 μg/L) as CN^- was also of the same level as the EC50-72 h of potassium ferricyanide (127 μg/L) and ferrocyanide (267 μg/L). Complex cyanides explained a major part of the toxicity of leachates of the soil. On the other hand, cyanide complexes had no effect on survival of the earthworm *Eisenia fetida* up to 131 mg CN^-/kg, while potassium cyanide was highly toxic [EC50-14 d as CN^- = 74 μg/kg soil].

Thermodesorption treatment eliminated a majority of cyanides from the soil and generated much less toxic leachates. Complex cyanides must be integrated into environmental studies to assess the impact of multi-contaminated soils.

1. Introduction

Past industrial activities, such as mining, coal and iron exploitation for energy or steel production, have generated wastelands and historically contaminated soils in most developed countries. In Europe, vast programs have been launched to inventory contaminated sites and define action plans for management and treatment. National inventories indicate that metals, hydrocarbons and mineral oils are the harmful contaminants most frequently found in soil and groundwater at investigated sites. The north-east of France with its past activities of coal and iron extraction, is especially concerned as it has multiple-contaminated soils with high levels of metals and organic pollutants such as polycyclic aromatic hydrocarbons (PAHs).

In a previous work, a multidisciplinary and long term study was carried out to assess the fate and environmental impact of contaminants from a coke plant soil. A field pilot study was designed to evaluate the efficiency of phytoremediation and thermodesorption. Thermodesorption was carried out to eliminate the major part of PAHs, which are considered as pollutants of high concern due to their carcinogenic properties. Investigations targeted metals and polycyclic aromatic hydrocarbons specifically to determine their mobility and their toxicity in untreated and treated soils. An integrated approach associating physicochemical analyses and ecotoxicity studies was applied for risk assessment.

Results indicated that the bioavailability of PAHs and metals was low in these aged soils [1]. No toxicity was recorded on invertebrate terrestrial species despite high levels of metals and hydrocarbons in the untreated soil [2]. On the other hand, high chronic toxicity of soil leachates was recorded on freshwater microalgae and microinvertebrates. In contrast, leachates of the thermodesorbed soil were much less toxic. As the thermal treatment eliminated most PAHs from the soil (Table 1), these pollutants were suspected of being responsible for aquatic toxicity recorded in leachates of the untreated soil. Yet, none the 16 PAH pollutants measured in leachates, nor metals could explain toxicity to aquatic species of the untreated and phytoremediated soils [3]. Therefore, further investigations appeared necessary to identify soil pollutants harmful to aquatic life.

The present study was undertaken over 18 months after the previous investigations reported above. It aimed to characterize the unknown pollutants responsible for leachate toxicity and thermodegradable. A review of the processes applied in coke plants led us to consider cyanide complexes resulting from waste and gas purification by iron treatment as possible contaminants responsible for the toxicity to microalgae and microinvertebrates.

Ferro- and ferricyanide complexes constitute the major forms of cyanides in soils as a result of wastes produced after iron

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Table 1
The main physico-chemical characteristics of both contaminated soils before and after thermal desorption (mean ± sd, standard deviation).

	Untreated soil		Thermodesorbed soil	
	Mean	sd	Mean	sd
Soil texture				
Clay %	12.8	0.2	10.4	0.05
Silt %	23.9	1.5	19.4	0.1
Sand %	64.8	1.7	70.1	0.21
pH	7.10	0.03	7.38	0.01
C/N	26		62.0	1.4
Organic carbon %	7.25	0.25	5.8	0.2
Organic matter %	12.5	0.4	10.1	0.4
Heavy metals (mg/kg soil d.w.)				
Arsenic (As)	60.75	6.13	74.50	5.45
Cadmium (Cd)	2.72	0.06	2.14	0.08
Chromium (Cr)	346	24	393.75	23.08
Cobalt (Co)	27.3	0.7	27.18	0.17
Copper (Cu)	104.5	8	109.25	1.71
Mercury (Hg)	4.0	0.7	1.54	0.24
Nickel (Ni)	155	91	102.25	2.06
Lead (Pb)	669	26	672.75	23.04
Zinc (Zn)	2647	143	2745.00	26.46
PAHs (mg/kg soil d.w.)				
Naphthalene	31	5	2.9	0.5
Acenaphthylene	1.7	0.2	2.9	0.1
Acenaphtene	84	58	0.8	0.04
Fluorene	59	25	2.6	0.1
Phenanthrene	173	55	9.3	0.3
Anthracene	62	10	6.4	0.1
Fluoranthene	260	55	15.7	0.4
Pyrene	203	35	10.6	0.4
Benzo[a]anthracene	125	18	8.2	0.3
Chrysene	108	13	5.4	0.5
Benzo[b]fluoranthene	107	14	9.1	0.5
Benzo[k]fluoranthene	63	8	3.7	0.8
Benzo[a]pyrene	107	14	8.2	0.5
Dibenzo[a,h]anthracene	12	2	4.9	0.1
Benzo[g,h,i]perylene	66	7	4.9	0.2
Indeno[1,2,3-cd]pyrene	77	11	10.7	0.4
Σ 16 PAH US-EPA	1539	314	106.3	3.6

treatment in coke and gas manufacture plants [4]. These complexes are persistent, while subjected to decomposition in UV light [5]. They are non-mobile in acidic soils, but their solubility increases with neutralisation and alkalinisation of soils [6]. They are far less toxic to mammals compared with free cyanide, even at high levels of exposure [7,8]. Yet, little is known of their toxicity to invertebrates.

In the present study, cyanides were analyzed in leachates from untreated and thermodesorbed soils generated by rainwaters in situ collected over eighteen months. Both acute and chronic bioassays were used to assess ecotoxicity of leachates. The Microtox and 48 h-*Daphnia magna* tests were applied for acute effects. Chronic toxicity to freshwater species was evaluated by population growth of the microalgae (*Pseudokirchneriella subcapitata*) and by reproduction of the microinvertebrates (*Ceriodaphnia dubia*). In parallel, chronic toxicity of cyanide chemicals (free and complex cyanides) to these aquatic species was assessed in order to study the plausibility of cyanide involvement in leachate aquatic toxicity. Potassium ferrocyanide and potassium ferricyanide were studied as models of soluble complex cyanides released from alkaline soils, and potassium cyanide as a model of free cyanide. Toxicity of these compounds to earthworms (*Eisenia fetida*) was also evaluated to provide information about the sensitivity of terrestrial species to cyanides that is lacking in data bases.

The present ecotoxicity results support the conclusions that cyanide complexes are the main pollutants responsible for aquatic toxicity recorded in soil leachates. This work underlines the high risk of soils contaminated with these complexes for environmental and human health, once conditions favoring their mobility and decomposition are met. This study points to the need to include cyanide complexes in the list of priority pollutants in routine analyses of industrially contaminated soils and their leachates, especially in the case of soils selected for requalification.

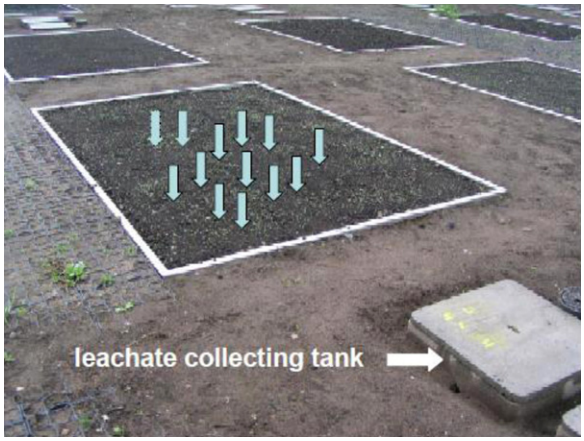

Fig. 1. Photo of a soil plot (2 m wide × 3 m long × 0.7 m deep) connected to a tank allowing the collection of leachates generated by rain water percolating through the tested soil (GISFI experimental station).

2. Materials and methods

2.1. Sampling and analyses

A multicontaminated soil was excavated from a former coking plant in the Lorraine Region (north eastern France) in 2004. Part of the soil (30 tons) was treated by thermo desorption at a temperature of 650 °C in a mobile unit. The untreated soil and the thermodesorbed soil were transferred to the experimental station of GISFI (group for integrated studies on industrial wastelands), located at Homécourt in the same country (49°2136N, 5°99608E) (http://www.gisfi.fr). Several tons of the soils (thermodesorbed or untreated) were homogenized, sieved at 80 mm mesh size, and used to fill in situ lysimeter plots in 2005.

Each lysimeter plot (2 m width × 3 m length × 0.7 m depth) was equipped with a system allowing the collection of leaching water in a stainless steel tank (Fig. 1). The leaching water collection tanks were emptied every week or after abundant rain so as to ensure that fresh leachates would be sampled for analyses in the following days. Leachates collected were stored in the dark at 4 °C until physicochemical and ecotoxicological analyses were undertaken within 48 h. After decantation and filtration through 0.7 mm porosity glass filters, leachates were analyzed for organic and inorganic contaminants including total and free cyanides and for ecotoxicity bioassays carried out on the same samples.

Representative soil samplings were carried out for analyses; soils were mixed and sieved at 4 mm for contaminant analyses.

2.2. Chemical characterisation

The PAH congeners (US-EPA) were analyzed in soil samples and leachates according to the AFNOR XP X33-012 [9] and the NF EN ISO 17993 [10] standard methods, respectively, using fluorimetric detection. Acenaphthylene was quantified using diode array. Total PAH concentrations reported in the present study corresponded to the sum of the concentrations of the 16 PAHs analyzed. Heavy metals in soils and leachates were analyzed as described in Bonnard et al. [11]. Cyanide concentrations were measured in soil samples according to ISO 11262 [12] and in leachates according to ISO 14403

[13]. Physico-chemical properties of soil such as total organic carbon, pH, grain size, texture and moisture content were measured according to ISO standards by INRA, the French National Institute for Agricultural Research (http://www.lille.inra.fr/las, Arras, France).

2.3. Chemicals tested

Potassium cyanide KCN (CAS 151-50-8) purity 98%, and potassium ferrocyanide also called potassium hexacyanoferrate II, $K_4[FeCN_6]$, $3H_2O$ (CAS 13943-58-3) 98% purity were purchased from VWR (Fontenay-sous-Bois, France). Potassium ferricyanide (potassium hexacyanoferrate III) $K_3[FeCN_6]$ CAS 13746-66-2) 99% purity were provided from Merck (Darmstadt, Germany).

All inorganic chemicals tested were of high quality grade (>99% purity). Calcium chloride ($CaCl_2$, $2H_2O$), magnesium chloride ($MgCl_2$, $6H_2O$), potassium chloride (KCl), sodium chloride (NaCl), magnesium sulphate ($MgSO_4$, $7H_2O$) and sodium sulphate (Na_2SO_4) were purchased from Merck (Darmstadt, Germany). Calcium sulphate ($CaSO_4$, $2H_2O$) and calcium nitrate were provided by VWR (Fontenay-sous-Bois, France). Potassium nitrate was purchased from Sigma Aldrich (Steinheim, Germany).

2.4. Aquatic toxicity tests

The acute toxicity tests were performed by measuring mobility inhibition of the crustacean *D. magna* according to ISO 6341 [14], and luminescence inhibition of the bacterium *V. fischeri* (Microtox® 500 system, Microbics©) according to ISO NF EN 11348-3 [15]. Chronic toxicity tests measured parameters indicating population development: reproduction of *Ceriodaphnia dubia* over 7 days according to the AFNOR NF T90-376 method [16] and growth of the freshwater alga *P. subcapitata* in a 3-day batch culture according to ISO 8692 [17]. In the *C. dubia* reproduction test, the water used for culture and testing was a mineral water, a mixture of *Evian–Volvic* mineral drinking waters (1/4: volume/volume). The effects of light and of open flasks used in the test procedure was evaluated by conducting the *C. dubia* test in the dark and/or in closed flasks in parallel to the standard test (a light <300 lux, 16 h/8 h photoperiod, open containers).

2.5. Terrestrial organism toxicity tests

Acute toxicity tests with *E. fetida* were carried out according to the ISO 11268-1 standard method [18]. The pH was adjusted to 7.0 ± 0.5 with $CaCO_3$ to be representative of the studied soils. Earthworm tests were carried out at a soil moisture content of 40–60% water-holding capacity and during exposure, all test vessels were kept at $20 \pm 2\,^{\circ}C$ in a light (400–500 lux) dark cycle of 16 h/8 h.

2.6. Statistical analysis and data expression

Toxicity results were expressed as effective concentration values reducing by 10% and 50% (EC10 and EC50) the endpoints measured compared to controls. Toxicity endpoints, used for the invertebrates, Microtox and algal tests, were estimated using the bootstrap method in the REGTOX Excel® macro (E. Vindimian, Cemagref, Montpellier, France), available from http://www.normalesup.org/~vindimian/fr_index.html. A Student *t*-test was performed to compare the distribution of the EC*x* values obtained through the bootstrap method.

All statistical analyses were performed with Statistica for Windows ($P < 0.05$; STATISTICA version 6.0 for Windows, Statsoft, Tulsa, OK).

Table 2
The main physico-chemical characteristics of leachates from the coke plant soil before and after thermodesorption treatment (mean ± standard deviation).

	Untreated	Thermodesorbed
pH	7.3 ± 0.7	7.4 ± 0.8
Conductivity (mS/cm)	2.7 ± 0.2	2.6 ± 0.2
Corg (mg/L)	65 ± 16	9.5 ± 4
SO_4 (mg/L)	1467 ± 36	1351 ± 35
NO_3 (mg/L)	99 ± 34	7 ± 3
Cl (mg/L)	10.4 ± 7	7.2 ± 2
Heavy metals (μg/L)		
As	<5	<5
Cd	<1	<1
Co	36 ± 7	<5
Cr	8 ± 2	8.7 ± 2.8
Cu	16 ± 4	5 ± 2
Ni	6.6 ± 1.5	6.7 ± 2.8
Pb	<1	<1
Zn	13.5 ± 2	20.0 ± 14.5
PAHs (μg/L)		
Naphthalene	0.079	
Anthracene	0.014	
14 other PAH	<LOQ	
16 PAHs		<LOQ
Cyanides (mg/L)		
Total cyanide	46.5 ± 14.5	0.20 ± 0.086
Free cyanide	0.28 ± 0.2	<0.02

LOQ: limits of quantification (see text).

3. Results

3.1. Physicochemical analyses of leachates from the untreated and thermodesorbed soils

Leachates of the untreated and thermodesorbed soils presented common characteristics, namely a high conductivity and high concentrations of sulphates and chlorides of the same order of magnitude (Table 2). The inorganic elements, such as metals, were found at approximately the same concentrations in both leachates, with concentrations below the limits of quantification for As, Cd and Pb, and lower in leachates of the thermodesorbed soil compared to the untreated soil for Co and Cu. The leachate pH values indicated low alkalinity, around 7.3 for the untreated soil and 7.4 for the thermodesorbed soil. Organic carbon and nitrogen constituents were present at lower concentrations in leachates of the treated soil, as a result of the thermal treatment.

Most PAHs concentrations were below limits of quantification (LOQ) in both samples (0.005 μg/L for acenaphthene, phenanthrene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene; 0.10 μg/L for acenaphthylene; 0.02 μg/L for fluorene, pyrene, and benzo(g,h,i)perylene). Only low concentrations of naphthalene (0.079 μg/L) and anthracene (0.014 μg/L) were measured in leachate samples of the untreated soil.

3.2. Cyanide concentrations in soils and leachates

Samples of the untreated soil contained high levels of total cyanides 131 ± 41 mg/kg d.w., but low concentrations of free cyanides (0.22 ± 0.01 mg/kg d.w.) near the quantification threshold, indicating that almost all cyanides can be attributed to metal-complexed cyanides. No free cyanide could be found in the thermodesorbed soil and complex cyanides were below the limits of quantification (LOQ < 0.2 mg/kg d.w.).

In leachates of the untreated soil, concentrations of total cyanides were 46.5 ± 14.5 mg/L over the study period. Free cyanides represented less than 1% of total cyanides in most cases (Table 2).

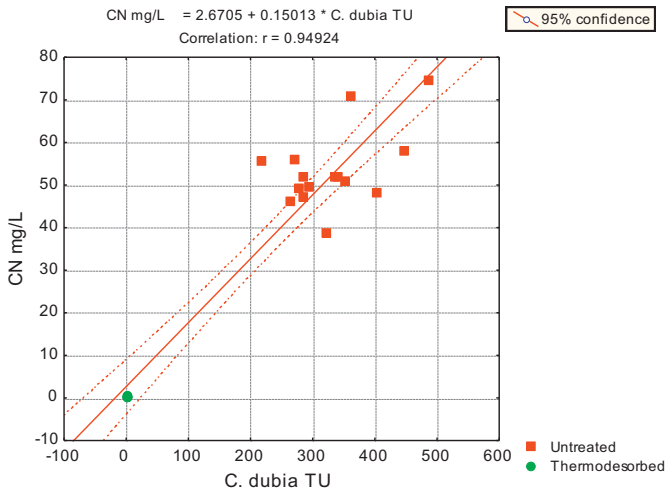

Fig. 2. Correlation between the concentration of total cyanide and toxicity expressed as toxic units (TU) on reproduction of *Ceriodaphnia dubia* ($r = 0.949$).

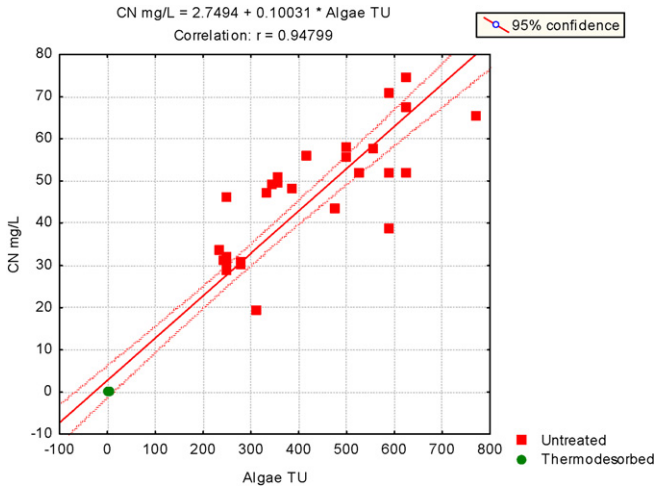

Fig. 3. Correlation between the concentration of total cyanide and toxicity expressed as toxic units (TU) on population growth of *Pseudokirchneriella subcapitata* ($r = 0.947$).

On the other hand, leachates from the thermodesorbed soil contained less than 0.5 mg/L of total cyanides, and no free cyanide was measured (below LOQ of 0.02 mg/L).

3.3. Aquatic toxicity of leachates

In leachates of the untreated soil, no acute toxicity was recorded using the Microtox or 48 h-*D. magna* immobilisation tests. On the other hand, high chronic toxicity was registered on microalgae and microinvertebrates throughout the study period, as expressed by the leachate concentrations inhibiting algal growth or invertebrate reproduction by 50% (EC50), which were inferior to 1% in all samples.

The EC50-72 h values inhibiting growth of the microalgae *P. subcapitata* suspensions by 50% were $0.27 \pm 0.09\%$ as evaluated from 28 samples collected over 18 months (Table 3). The mean EC50-72 h value expressed as toxic units (TU = 100/EC50%) corresponded to 420 ± 157 TU.

Regarding toxicity to reproduction of *C. dubia*, the EC50-7 d values of leachates of the untreated soil were $0.31 \pm 0.07\%$, which corresponded to a mean of 339 ± 81 TU ($n = 16$) (Table 3).

Only a few number of leachates from the thermodesorbed soil could be collected ($n = 8$), due to a high water retention capacity of the treated soil compared to the untreated one. In leachates of the thermodesorbed soil, algal toxicity was much lower. In half of the leachate samples, no toxicity to microalgae was recorded (EC50 > 90%), and in the other half, the algal toxicity was low with EC50-72 h values of $54 \pm 18\%$. Toxic units were <2 TU.

As for Ceriodaphnia reproduction, toxicity of leachates of the thermodesorbed soil was also low. No toxicity was registered in half of the leachate samples (EC50 > 90%), while in the other half, the mean EC50-7 d was $65 \pm 19\%$. The toxic units were <1.5 TU.

Toxicity of leachates to microalgae and microinvertebrates was analyzed in relation to total cyanide concentrations measured in the same samples. All data from the untreated and thermodesorbed leachates were taken into account. The TU for the non toxic samples were entered as equal to 1. The total cyanide concentration was entered as equal to 0.02 mg/L when below LOQ. The graphs correlating toxic units and total cyanides for microinvertebrates (Fig. 2) and for microalgae (Fig. 3) indicated a high degree of correlation in both cases, with a correlation coefficient r of 0.949 for invertebrates and 0.947 for algae.

3.4. Toxicity of cyanide chemicals to microalgae and microinvertebrates

The chronic toxicity of potassium cyanide (KCN), potassium ferricyanide ($Fe(CN)_6K_3$) and ferrocyanide ($Fe(CN)_6K_4$) to *P. subcapitata* and *C. dubia* was determined by means of the standard methods used to assess leachate toxicity.

The EC50-72 h values expressed as CN^- (with a 95% confidence interval) recorded on the microalgae *P. subcapitata* were 116 (112–125) µg/L for KCN, 158 (154–163) µg/L for $Fe(CN)_6K_3$ and 283 (270–297) µg/L for $Fe(CN)_6K_4$ (Table 3).

The EC50-7 d values expressed as CN^- (with 95% confident interval) inhibiting the reproduction of *C. dubia* by 50% were 98 (83–111) µg/L for KCN, 194 (181–208) µg/L for $Fe(CN)_6K_3$ and 216 (189–244) µg/L for $Fe(CN)_6K_4$ (Table 3).

In addition to testing in standard conditions, the *C. dubia* reproduction tests of cyanide complexes were carried out in dark rooms, the other experimental conditions remaining unchanged, to determine if light used in standard method influenced the toxicity of the ferro- and ferricyanides through a photodecomposition. The results indicated no difference in the EC50 values in darkness or under light of the standard method (Table 3).

The same experiment, i.e. comparison of toxicity in the dark or in standard test conditions (<300 lux) was carried out on a leachate randomly selected after sampling. The results indicated that the EC50 values to Ceriodaphnia reproduction of the leachate containing 25 mg/L total cyanides were identical in both conditions (0.47% in the dark and 0.48% in standard conditions).

3.5. Toxicity of cyanide chemicals to earthworms

The toxicity study of $Fe(CN)_6K_3$ and $Fe(CN)_6K_4$ to the *Eisenia fetida* earthworm showed that no lethality was recorded after 14 days at concentrations in soils of up to 131 mg/kg d.w. expressed as CN. This concentration corresponded to the mean value of total cyanides in the untreated soil studied (Table 3).

On the other hand, KCN was much more toxic, as was shown by the EC50-14 d value expressed as CN, which was 74 µg/kg soil (d.w.) with a 95% confidence interval 61–80 µg/kg soil (d.w.). The EC10-14 d value inducing 10% mortality was 56 (32–71) µg/kg soil (d.w.). These values indicate KCN toxicity at concentrations about 2000 times lower than the NOEC of the two complexes.

Table 3
The chronic ecotoxicity of leachates from the untreated and treated soils and of cyanide chemicals to aquatic and terrestrial species. EC values are given as means ± sd (standard deviation) for leachates and mean with a 95% confidence interval (CI) for chemicals.

Sample	Species	Leachate origin or chemical tested	Exposure duration	Effect criteria[a]	Concentration in test medium	
					% of leachate mean ± sd	CN μg/L (aquatic) or μg/kg (soil) mean (CI)
Leachates	*Pseudokirchneriella subcapitata*	Untreated soil	72 h	EC50	0.27 ± 0.09%	
		Treated soil	72 h	EC50	54 ± 18%	
	Ceriodaphnia dubia	Untreated soil	7 d	EC50	0.31 ± 0.07%	
		Treated soil	7 d	EC50	65 ± 19%	
Cyanide chemicals	*Pseudokirchneriella subcapitata*	KCN	72 h	EC50		116 (112–125)
				EC10		55 (28–71)
		$K_3Fe(CN)_6$		EC50		158 (154–163)
				EC10		133 (91–141)
		$K_4Fe(CN)_6$		EC50		283 (270–297)
				EC10		142 (125–169)
	Ceriodaphnia dubia	KCN	7 d	EC50		98 (83–111)
				EC10		42 (18–52.4)
		$K_3Fe(CN)_6$	7 d	EC50		194 (181–208)
				EC10		137 (111–161)
			7 d dark	EC50		173 (149–212)
				EC10		71 (25–113)*
		$K_4Fe(CN)_6$	7 d	EC50		216 (189–244)
				EC10		61 (40–114)
			7 d dark	EC50		231 (208–256)
				EC10		88 (45–185)
	Eisenia fetida	KCN	14 d	EC50		74 (61–80)
				EC10		56 (32–71)
		$K_3Fe(CN)_6$	14 d	NOEC		131,000
		$K_4Fe(CN)_6$	14 d	NOEC		131,000

[a] Toxicity is expressed as the concentration of the tested sample in the test medium inhibiting the algal growth of *Pseudokirchneriella subcapitata* and the reproduction of the cladoceran *Ceriodaphnia dubia* in aquatic bioassays, and inducing lethality of the earthworm *Eisenia fetida* in soil bioassays by 10% (EC10) or 50% (EC50). NOEC: no observed effect concentration (no lethality).
* Significantly different to the EC10 value obtained in standard experimental conditions ($P < 0.05$).

4. Discussion

The present work was conducted in situ in realistic conditions of soil exposure to natural climatic events. The leachates collected were generated by rainwaters percolating through the soils studied. We measured free and complex cyanides in leachates from both a multi-contaminated soil originating from a former coke plant and from the same soil treated by thermodesorption. The leachate toxicity of the two soils was evaluated over 18 months. No acute toxicity was noted with the Microtox and *D. magna* tests, but a high chronic toxicity to microalgae and microinvertebrates was registered in leachates of the contaminated soil. Toxicity inhibited algal division and consequently population growth of *P. subcapitata* at very low leachate concentrations (EC50-72 h = 0.27 ± 0.09%). Toxicity for the microinvertebrates *C. dubia* resulted in inhibition of reproduction and disturbance of populations at the same levels of concentrations as for algal inhibition (EC50-7 d = 0.31 ± 0.07%). This chronic toxicity was of the same order of magnitude as the toxicity registered during the three years prior to the present study [3]. On the other hand and in accordance with the previous results, few toxic effects were recorded in the leachates of the thermodesorbed soil.

4.1. Pollutants responsible for the toxicity of leachates

In the contaminated soil and the corresponding leachates, total and complex cyanides were present at relatively high levels, while concentration of free cyanides was low (<1%). Chronic toxicity of leachates appeared to correlate with cyanides, as attested by high coefficients of correlations (Figs. 2 and 3). Obviously, correlations are not proof of cause-effect relationships but indicators of links requiring to be elucidated.

The fact that concentrations of cyanides reducing by 50% the reproduction of *C. dubia* or the algal division were of the same level in diluted leachates and in solutions of ferrocyanide or of ferricyanide, indicated that cyanides were responsible for the toxicity registered with leachates. Total cyanides inhibited by 50% the reproduction of *C. dubia* at mean concentrations of 0.144 mg/L in leachates. This concentration was close to the EC50-7 d values of potassium ferricyanide (0.194 mg/L) and of potassium ferrocyanide (0.216 mg/L) measured on this aquatic species and it represented about 70% of the EC50 values of potassium Fe–CN complexes.

As for algal toxicity, total cyanide inhibited by 50% the algal growth of *P. subcapitata* at mean concentrations of 0.127 mg/L in leachates. This concentration represented 65% of the EC50-72 h value of potassium ferricyanide (0.158 mg/L) and 60% of the EC50 value of potassium ferrocyanide (0.283 mg/L).

Cyanide complexes alone explained most, but not all the toxicity to invertebrates and algae. Other pollutants in the leachates may have also contributed to alter ceriodaphnid reproduction and algal growth, but to a much lesser extent than cyanides. The concentrations of salts, metals and PAHs were low in leachates of the untreated soil and therefore unlikely to produce toxicity by themselves (Table 1). Indeed the concentration of each pollutant in leachates at the EC50s (mean values) on *C. dubia* (Table 5) and on *P. subcapitata* (Table 6) was much lower than their EC10, NOEC or LOEC values or their effective concentrations. As none of these pollutants alone could induce toxicity at their concentration in leachates, it may be put forward that other pollutants not yet identified and/or their combined effects may have also contributed to the toxicity not explained by cyanides.

The present work is the first to provide chronic toxicity data of free and complex cyanides to the freshwater cladoceran *C. dubia* and the microalgae *P. subcapitata* and to study their toxicity to earthworms. Metal-complexed cyanides are often considered to be non-toxic chemical species, although they have the potential to release free cyanides under certain environmental conditions. As it is admitted that cyanide toxicity is driven by the free cyanide forms (sum of HCN and CN^-) known to inhibit cell respiration

Table 4
The ecotoxicity of cyanide chemicals to freshwater and marine aquatic species (fish, invertebrates and microalgae) expressed as CN. The 95% confidence interval (CI) is given when possible.

	Tested compound	Exposure duration	Effect	Endpoint	Concentration μg CN/L (CI)	Reference
Fish						
Acanthopagrus butcheri[a]	NaCN	96 h	Survival	LC50	70 (67–73)	[27]
	$K_3Fe(CN)_6$	96 h	Survival	LC50	1730	
	$K_4Fe(CN)_6$	96 h	Survival	LC50	20,500	
Carassius auratus	NaCN	96 h	Survival	LC50	318	[28]
Lepomis macrochirus	NaCN	48 h	Survival	LC50	134	[28]
Lepomis macrochirus	HCN	289 d	Egg production	NOEC	<5	[29]
		42 d	Fry survival	NOEC	15	
Macquaria novemaculeata	NaCN	96 h	Survival	LC50	109 (107–112)	[27]
	$K_3Fe(CN)_6$	96 h	Survival	LC50	2830	
	$K_4Fe(CN)_6$	96 h	Survival	LC50	285,000 (218,000–372,000)	
Pimephales promelas	NaCN	8 d	Survival	LC50	114	[28]
Pimephales promelas	HCN	256 d	Reproduction	NOEC–LOEC	12.9–19.6	[30]
Salvelinus fontinalis	HCN	144 d	Reproduction	NOEC–LOEC	5.7–11.2	[31]
Invertebrates						
Asellus communis	HCN	112 d	Growth	NOEC–LOEC	29–40	[26]
			Reproduction	NOEC–LOEC	29–40	
Ceriodaphnia dubia	KCN	7 d	Reproduction	EC50	98 (83–111)	This study
				EC10	42 (18–52.4)	
	$K_3Fe(CN)_6$	7 d	Reproduction	EC50	194 (181–208)	
				EC10	137 (111–161)	
	$K_4Fe(CN)_6$	7 d	Reproduction	EC50	216 (189–244)	
				EC10	61 (40–114)	
Chlamys asperrimus[a]	NaCN	48 h	Survival	LC50	28.6 (27.7–29.5)	[24]
	$K_3Fe(CN)_6$	48 h	Survival	LC50	128 (123–134)	[24]
	$K_4Fe(CN)_6$	48 h	Survival	LC50	686 (649–726)	[24]
Cyclops viridis	NaCN	96 h	Survival	LC50	158	[32]
Daphnia magna	NaCN	96 h	Survival	LC50	90	[33]
Gammarus fasciatus	NaCN	96 h	Survival	LC50	900	[33]
Gammarus pseudolimnaeus	HCN	83 d	Growth	NOEC–LOEC	21–32	[26]
			Reproduction	NOEC–LOEC	16–21	
Algae						
Nitzschia closterium[a]	NaCN	72 h	Population growth	EC50	57 (51–61)	[25]
				LOEC	10	
	$K_3Fe(CN)_6$	72 h	Population growth	EC50	127 (113–142)	
				NOEC–LOEC	31–62	
	$K_4Fe(CN)_6$	72 h	Population growth	EC50	267 (238–301)	
				NOEC–LOEC	31–62	
Pseudokirchneriella subcapitata	KCN	72 h	Population growth	EC50	116 (112–125)	This study
				EC10	55 (28–71)	
	$K_3Fe(CN)_6$		Population growth	EC50	158 (154–163)	
				EC10	133 (91–141)	
	$K_4Fe(CN)_6$		Population growth	EC50	283 (270–297)	
				EC10	142 (125–169)	
Scenedesmus quadricauda	KCN	8 d	Population growth	LOEC	30	[34]

[a] Marine species.

through inactivation of cytochrome oxidase, research focused on these chemical species and ecotoxicity data concerned mainly free cyanides [23]. Few studies on marine invertebrate and algal species have been published [24,25]. Despite studies on fish [19], plants [20,21] and wildlife vertebrates [22], little knowledge is available about chronic cyanide toxicity to invertebrate species that would be worth protecting and considering in environmental risk assessment.

4.2. Toxicity of cyanide chemicals to aquatic species

The freshwater invertebrate *C. dubia* appeared to be quite sensitive not only to KCN, but also to the cyanide complexes. Sensitivity was comparable to the one of other aquatic invertebrates. The EC10-7 d value of 42 (18–52) μg/L for KCN to *C. dubia* reproduction was in the range of the NOEC and LOEC values of HCN on the freshwater isopod *Asellus communis* (29 and 40 μg/L, respectively), and on the lotic amphipod *Gammarus pseudolimnaeus* (16 and 21 μg/L, respectively) [26] (Table 4). The EC10 value of KCN on *C. dubia* did not call into question the final chronic value of 22 μg/L established from fish data and used to derive freshwater chronic criteria of 5 μg/L [35].

Ceriodaphnia appeared to be much more sensitive to the toxicity of cyanide complexes than the marine fish species *A. butcheri* and *M. novemaculeata* [27], while sensitivity was of about the same order of magnitude as larvae of the marine doughboy scallop *Chlamys asperrimus* [24] (Table 4).

The toxicity of cyanide complexes to *C. dubia* was due to their intrinsic toxicity and not to dissociation into free cyanide in the test medium. Indeed, the *Ceriodaphnia* tests carried out in the dark gave the same response as in standard conditions using a faint illumination (below 300 lux).

Results on the freshwater microalgae *P. subcapitata* also indicated the high toxicity of the three cyanide salts – KCN and potassium ferri and ferrocyanide – to algal growth. The EC50 values on *P. subcapitata* were quite close to the EC50-72 h values on the marine diatom *Nitzschia closterium* of 57 (51–61) μg/L for NaCN, 127 (113–142) μg/L for potassium ferricyanide, and 267 (238–301) μg/L for potassium ferrocyanide, as found by Pablo et al. [25] (Table 4). For both algal species, toxicity followed the order: NaCN or KCN > $K_3Fe(CN)_6$ > $K_4Fe(CN)_6$. As the light intensity in the

Table 5
Comparison of each pollutant concentration at the mean EC50-7 d of leachates (that inhibited reproduction of *Ceriodaphnia dubia* by 50% in the present study) with literature data on the same compounds tested individually.

	Concentration corresponding to EC50-7 d *C. dubia*	Literature data Reproduction		Reference
Chloride (Cl^- mg/L)	0.03	NOEC	786	[37]
			1020 (NaCl)	[38]
Nitrate (NO_3^- mg/L)	0.3	NOEC	7.1–56.5	[39]
Sulphate (SO_4^{2-} mg/L)	4.54	LOEC	899	[40]
Cyanide (CN^- mg/L)	0.144	EC50 (as CN^-)		
		KCN	0.098 (0.083–0.111)	This study
		$K_3Fe(CN)_6$	0.194 (0.181–0.208)	
		$K_4Fe(CN)_6$	0.216 (0.189–0.243)	
Heavy metals (μg/L)				
As	<0.01	NOEC	570	[41]
Cd	<0.002	EC50	7.2	[42]
Cr	0.008	NOEC	18.2	[43]
Co	0.127	NOEC–LOEC	6.25–12.5	[44]
Cu	0.05	EC50	1.8 (1.6–2.1)	[45]
Ni	0.157	EC20	4	[46]
Pb	<0.002	EC50	5.1 (3.5–7.5)	[45]
Zn	0.04	EC50	21.8 (11.5–30.3)	[45]
PAHs (μg/L)				
Naphthalene (μg/L)	0.24×10^{-3}	NOEC-7 d	510	[47]
Anthracene (μg/L)	0.04×10^{-3}	EC10-7 d	>3.4	[48]

standard algal test is at least 6000 lux, decomposition of complexes into alkaline cyanides at a pH of the algal medium of 8.3 can be suspected. Yet, if photodecomposition would occur, this would have little incidence on toxicity results, because toxicity of free and complex cyanides is of the same order of magnitude on *P. subcapitata*. There was no possibility of carrying out an algal test in the dark for comparison, because no growth would be possible.

Overall, results attested that ferrocyanide and ferricyanide solutions are hazardous to microalgae and microinvertebrates. In the long term, running waters solubilising iron-cyanide complexes of contaminated soils may endanger aquatic species in rivers.

4.3. Toxicity of cyanide chemicals to terrestrial invertebrates

The iron-complexed cyanides tested were not toxic to the *E. fetida* earthworm as shown by a NOEC ≥ 131,000 μg/kg d.w. of soil, expressed as CN^-. This contrasts with the high sensitivity of the same species to free cyanide (EC50-14 d as CN^- = 74 μg/kg d.w. of soil). Free cyanide dramatically affected survival at low concentrations, about 2000 times lower than NOEC of ferro- and ferricyanide. The low toxicity of complex cyanides on earthworms explains why no lethality had been recorded when the contaminated soil was tested on this earthworm species. No toxicity was found on the collembolae *Folsomia candida* either [3]. The latter *E. fetida* results are in line with soil physico-chemical analyses which revealed that complex cyanide species are the main cyanide forms in the contaminated soil.

In databases and scientific literature, there is no information on earthworm sensitivity to any cyanide chemical that would allow comparison with our results. Our study is the first to provide data on free and complex cyanide chemicals to one earthworm species.

It is noteworthy that terrestrial invertebrates are quite differing from aquatic invertebrates regarding their sensitivity to potassium

Table 6
Comparison of each pollutant concentration at the mean EC50-72 h of leachates (that reduced growth of *Pseudokirchneriella subcapitata* by 50% in the present study) with literature data on the same compounds tested individually.

	Concentration corresponding to EC50-72 h *P. subcapitata*	Literature data Population growth		References
Chloride (Cl^- mg/L)	0.03	EC50	>15	This study
Nitrate (NO_3^- mg/L)	0.27			
Sulphate (SO_4^{2-} mg/L)	3.9	EC50	>1250	This study
Cyanide (CN^- mg/L)	0.127	EC50 (as CN^-)		This study
		KCN	0.116 (0.112–0.125)	
		$K_3Fe(CN)_6$	0.158 (0.154–0.163)	
		$K_4Fe(CN)_6$	0.283 (0.270–0.297)	
Heavy metals (μg/L)				
As	<0.01	EC50	>100	[49]
Cd	<0.002	EC50	46	[50]
Cr	0.007	EC50	535 (280–789)	[43]
Co	0.11	NOEC	10–15	[51]
Cu	0.04	EC50	10	[50]
		EC50	47	[52]
Ni	0.136	NOEC	45	[53]
Pb	<0.002	EC50	500	[54]
Zn	0.036	EC50	90	[50]
PAHs (μg/L)				
Naphthalene (μg/L)	0.21×10^{-3}	EC15-7 d	5000	[55]
		NOEC-72 h	>4300	[47]
Anthracene (μg/L)	0.04×10^{-3}	EC10-22 h	1.5	[56]

ferro- and ferricyanide. The explanation for the lack of effects of these soluble iron cyanide complexes in soil invertebrates would require further investigations.

In coke plant soils, various iron-iron cyanide solids have been reported [23]. The most important of these solid Fe–CN species is $Fe_4[FeII(CN)_6]_3$, a ferric ferrocyanide complex commonly known as Prussian Blue, originating from cleaning the gas produced in coke ovens and industrial gas works with iron hydroxides. The ferric ferrocyanide complex $Fe_4[FeII(CN)_6]_3$ is hardly soluble under acidic conditions, but dissolution is favored under alkaline conditions [6,36]. The pH slightly above 7 of the contaminated soil may have favored dissolution of ferrocyanides and production of ferricyanides in oxic conditions [23]. The present work underlines that the soluble iron cyanide complexes are threatening aquatic species, but not soil invertebrates.

4.4. Environmental quality standards for soil and water pollution

The present study stressed that not only free cyanides in soil, but also complexed cyanide forms should be taken into consideration in regulations regarding toxicity of leachates to invertebrates and microalgae. As far as free cyanide concentrations are considered, the concentrations recorded in the soil studied were far inferior to environmental quality standards (EQS) of free cyanides in soils established by regulations of many countries and institutions. The EQS are generally established according to the soil use, sensitive or less sensitive, or of an agricultural, commercial or industrial use. Free cyanides measured in the contaminated soil studied here were below the thresholds fixed for sensitive use by different regulations, 0.9 mg/kg soil in Canada [57], 0.4 mg/kg soil in Sweden [58], 1 mg/kg and 20 mg/kg as target and intervention values, respectively, in The Netherlands [59] and 50 mg/kg soil in France [60].

The Swedish and Dutch regulations alone determined EQS for total and complex cyanides. According to Swedish regulations, the EQS for total cyanides are 30 mg/kg soil for a sensitive use and 120 mg/kg soil for less sensitive land use. The Dutch regulation takes pH into consideration since ferric ferrocyanide complexes are slightly soluble at pH <5 in soils, and the intervention values for complex cyanides were 50 mg/kg at pH >5 and 650 mg/kg soil at pH <5. In the present work, contaminated soil with a pH around 7 was not in line with these requirements and consequently it seemed necessary to eliminate the complex forms. Investigations of the thermodesorbed soil and its leachates ensured that the thermal treatment had been efficient in eliminating cyanides and other thermodegradable pollutants. The thermodesorbed soil fulfilled EQS requirements of safety for cyanides.

5. Conclusion

The present study was conducted in environmentally relevant conditions of soil percolation to evaluate the ecotoxicity of leachates from a coke plant soil. The coke plant soil appeared to be hazardous to freshwater microinvertebrates and microalgae as a result of leaching by rain water. The removal of cyanide complexes explained a major part of the aquatic toxicity of leachates generated from the untreated soil.

Ecotoxicity of free and complex cyanide chemicals assessed on the microinvertebrate *C. dubia*, and the microalgae *P. subcapitata* used in testing indicated a high sensitivity to cyanides, even in a complex form. The high chronic toxicity of complex cyanides recorded on the freshwater species tested contrasted with the slight effects recorded in earthworms.

Unlike free cyanide, cyanide complexes were not toxic to earthworms, explaining the lack of lethal effects of the coke plant soil to soil invertebrates. Therefore, the lack of toxicity to terrestrial invertebrates of an industrial soil contaminated with cyanide complexes does not guarantee innocuity to aquatic species.

Not only free cyanides but also complex cyanides of industrially contaminated soils should be considered as environmentally hazardous pollutants requiring systematic control in wasteland management.

The management and remediation of industrially contaminated soils should take into consideration cyanide complexes that endanger aquatic species when conditions favoring their solubility are met. Although expensive, thermal treatment of the contaminated soil was revealed to be efficient in eliminating thermodegradable pollutants and most of the aquatic toxicity of leachates through degradation of cyanide complexes.

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References

[1] P. Vasseur, M. Bonnard, F. Palais, I.C. Eom, J.L. Morel, Bioavailability of chemical pollutants in contaminated soils and pitfalls of chemical analyses in hazard assessment, Environ. Toxicol. 23 (2008) 652–656.
[2] M. Bonnard, S. Devin, C. Leyval, J.L. Morel, P. Vasseur, The influence of thermal desorption on genotoxicity of multipolluted soil, Ecotoxicol. Environ. Saf. 73 (2010) 955–960.
[3] S. Ouvrard, C. Barnier, P. Bauda, T. Beguiristain, C. Biache, M. Bonnard, C. Caupert, A. Cébron, J. Cortet, S. Cotelle, M. Dazy, P. Faure, J.F. Masfaraud, J. Nahmani, F. Palais, P. Poupin, N. Raoult, P. Vasseur, J.L. Morel, C. Leyval, In situ assessment of phytotechnologies for multicontaminated soil management, Int. J. Phytoremediation 1 (2011) 1549–7879.
[4] T.L. Theis, T.C. Young, M. Huang, K.C. Knutsen, Leachate characteristics and composition of cyanide-bearing wastes from manufactured gas plants, Environ. Sci. Technol. 28 (1994) 99–106.
[5] S. Asperger, Kinetics of the decomposition of potassium ferrocyanide in ultraviolet light, Trans. Farad. Soc. 48 (1952) 617–624.
[6] J.C.L. Meeussen, M.G. Keizer, W.H. Van Riemsdijk, F.A.M. De Haan, Dissolution behavior of iron cyanide (Prussian blue) in contaminated soils, Environ. Sci. Technol. 26 (1992) 1832–1838.
[7] World Health Organization (WHO), Hydrogen cyanide and cyanides: human health aspects. Concise International Chemical Assessment Documents 61 (2004) Geneva. http://www.inchem.org/documents/cicads/cicads/cicad61.htm.
[8] N.S. Shifrin, B.D. Beck, T.D. Gauthier, S.D. Chapnick, G. Goodman, Chemistry, toxicology, and human health risk of cyanide compounds in soils at former manufactured gas plant sites, Regul. Toxicol. Pharmacol. 23 (1996) 106–116.
[9] Association Française de Normalisation (AFNOR XP X33-012), Characterisation of sludges-determination of polynuclear aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) (2000).
[10] International Standard Organization (ISO 17993), Water Quality – Determination of 15 polycyclic aromatic hydrocarbons in water by HPLC with fluorescence detection after liquid liquid extraction (2002).
[11] M. Bonnard, I.C. Eom, J.L. Morel, P. Vasseur, Genotoxic and reproductive effects of an industrially contaminated soil on the earthworm *Eisenia fetida*, Environ. Mol. Mutagen. 50 (2009) 60–67.
[12] International Standard Organization (ISO 11262), Soil quality – Determination of cyanide (2003).
[13] International Standard Organization (ISO 14403), Water quality – Determination of total cyanide and free cyanide by continuous flow analysis (2002).
[14] International Standard Organization (ISO 6341), Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test (1996).
[15] International Standard Organization (ISO NF EN 11348-3), Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test, part 3) (1999).
[16] Association Française de Normalisation (AFNOR NF T90-376), Qualité de l'eau Détermination de la toxicité chronique vis-à-vis de *Ceriodaphnia dubia* en 7 jours. Essai d'inhibition de la croissance de la population (2000).
[17] International Standard Organization (ISO 8692), Water quality – Freshwater algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum* (1996).

[18] International Standard Organization (ISO 11268-1), Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*) – Part 1. Determination of acute toxicity using artificial soil substrates (1993).
[19] R.W. Gensemer, D.K. DeForest, R.D. Cardwell, D. Dzombak, R. Santore, M. Stewart, Reassessment of Cyanide Ambient Water Quality Criteria: An Integrated Approach to Protection of the Aquatic Environment, Water Environment Foundation, WEFTEC.06, 2006, pp. 5709–5718.
[20] M. Larsen, A.S. Ucisik, S. Trapp, Uptake, metabolism, accumulation and toxicity of cyanide in willow trees, Environ. Sci. Technol. 39 (2005) 2135–2142.
[21] N. Bjarnholt, M. Laegdsmand, H.C.B. Hansen, O.H. Jacobsen, B.L. Møller, Leaching of cyanoglucosides and cyanide from white clover green manure, Chemosphere 72 (2008) 897–904.
[22] S.R. Griffiths, G.B. Smith, D.B. Donato, C.G. Gillespie, Factors influencing the risk of wildlife cyanide poisoning on a tailings storage facility in the Eastern Goldfields of Western Australia, Ecotoxicol. Environ. Saf. 72 (2009) 1579–1586.
[23] D.A. Dzombak, R.S. Ghosh, G.M. Wong-Chong, Cyanide in Water and Soil: Chemistry, Risk and Management, Taylor & Francis/CRC Press, Boca Raton, FL, 2006.
[24] F. Pablo, R.T. Buckney, R.P. Lim, Toxicity of cyanide, iron-cyanide complexes, and a blast furnace effluent to larvae of the doughboy scallop, *Chlamys asperrimus*, Bull. Environ. Contam. Toxicol. 58 (1997) 93–100.
[25] F. Pablo, J.L. Stauber, R.T. Buckney, Toxicity of cyanide and cyanide complexes to the marine diatom, *Nitzschia closterium*, Water Res. 31 (1997) 2435–2442.
[26] D.M. Oseid, L.L. Smith Jr., The effects of hydrogen cyanide on *Asellus communis* and *Gammarus pseudolimnaeus* and changes in their competitive response when exposed simultaneously, Bull. Environ. Contam. Toxicol. 21 (1979) 439–447.
[27] F. Pablo, R.T. Buckney, R.P. Lim., Toxicity of cyanide and iron-cyanide complexes to Australian bass *Macquaria novemaculeata* and black bream *Acanthopagrus butcheri*, Aust. J. Ecotoxicol. 2 (1996) 75–84.
[28] R.D. Cardwell, D.G. Foreman, T.R. Payne, D.J. Wilbur, Acute toxicity of selected toxicants to six species of fish. US EPA. Duluth, MN. EPA-600/3-76-008. In INERIS, Cyanures et dérivés. INERIS–DRC-03-47020-00DR054.doc (2006).
[29] G.L. Kimball, L.L. Smith Jr., S.J. Broderius, Chronic toxicity of hydrogen cyanide to the bluegill, Trans. Am. Fish Soc. 107 (1978) 341–345.
[30] D.T. Lind, L.L. Smith Jr., S.J. Broderius, Chronic effects of hydrogen cyanide on the *Fathead minnow*, J. Water Pollut. Control. Fed. 49 (1977) 262–268.
[31] W.M. Koenst, L.L.J. Smith, L.L.S.J. Broderius, Effect of chronic exposure of brook trout to sublethal concentrations of hydrogen cyanide, Environ. Sci. Technol. 11 (1977) 883–887.
[32] S.K. Sarkar, Toxicity evaluation of sodium cyanide to fish and aquatic organisms: effects of temperature, Sci. Cult. 56 (1990) 165–168.
[33] W.S. Ewell, R.O. Kringle, J.W. Gorsuch, K.A. Robillard, R.C. Spiegel, Simultaneous evaluation of the acute effects of chemicals on seven aquatic species, Environ. Toxicol. Chem. 5 (1986) 831–840.
[34] G. Bringmann, R. Kühn, Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test, Water Res. 14 (1980) 231–241.
[35] U.S. Environmental Protection Agency, Ambient Water Quality Criteria for Cyanide. EPA 440/5-84-028, U.S. Environmental Protection Agency, Office of Water, Criteria and Standards Division, Washington, DC, 1985.
[36] T. Mansfeldt, H. Leyer, K. Barmettler, R. Kretzschmar, Cyanide leaching from soil developed from coking plant purifier waste as influenced by citrate, Vadose Zone J. 3 (2004) 471–479.
[37] U.M. Cowgill, D.P. Milazzo, The sensitivity of two cladocerans to water quality variables: salinity and hardness, Arch. Hydrobiol. 120 (1990) 185–196.
[38] M.A. Aragão, E.V. Pereira, Sensitivity of *Ceriodaphnia dubia* of different ages to sodium chloride, Bull. Environ. Contam. Toxicol. 70 (2003) 1247–1250.
[39] J.A. Camargo, A. Alonso, A. Salamanca, Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates, Chemosphere 58 (2005) 1255–1267.
[40] D.J. Soucek, Comparison of hardness- and chloride-regulated acute effects of sodium sulphate on two freshwater crustaceans, Environ. Toxicol. Chem. 26 (2007) 773–779.
[41] BKH – Update toxiciteitsgegevens voor vier stoffen in het kader van MILBOWA. Versie maart (1995). In Arsenic et ses dérivés inorganiques. 2010. In INERIS – DRC-09-103112-11453A. Version 4.
[42] A. Sofyan, D.J. Price, W.J. Birge, Effects of aqueous, dietary and combined exposures of cadmium to *Ceriodaphnia dubia*, Sci. Tot. Environ. 385 (2007) 108–116.
[43] S. Rodgher, E.L.G. Espíndola, The influence of algal densities on the toxicity of chromium for *Ceriodaphnia dubia* Richard (Cladocera, Crustacea), Braz. J. Biol. 68 (2008) 341–348.
[44] N.K. Nagpal, Technical Report, Water quality guidelines for cobalt. Water Protection Section Water, Air and Climate Change Branch Ministry of Water, Land and Air Protection. Victoria, BC (2004).
[45] N.L. Cooper, J.R. Bidwell, A. Kumar, Toxicity of copper, lead, and zinc mixtures to *Ceriodaphnia dubia* and *Daphnia carinata*, Ecotoxicol. Environ. Saf. 72 (2009) 1523–1528.
[46] J. Keithly, J.A. Brooker, D.K. Deforest, B.K. Wu, K.V. Brix, Acute and chronic toxicity of nickel to a cladoceran (*Ceriodaphnia dubia*) and an amphipod (*Hyalella azteca*), Environ. Toxicol. Chem. 23 (2004) 691–696.
[47] INERIS, Naphtalène – Fiche de données toxicologiques et environnementales des substances chimiques Version N° 4.0-DRC-10-109974-00932A (2010).
[48] INERIS, Anthracène. Fiche de données toxicologiques et environnementales des substances chimiques Version N° 2-1-DRC-01-25590-01DR035.doc (2005).
[49] L. Duester, H.G. van der Geest, S. Moelleken, A.V. Hirner, K. Kueppers, Comparative phytotoxicity of methylated and inorganic arsenic- and antimony species to *Lemna minor*, *Wolffia arrhiza* and *Selenastrum capricornutum*, Microchem. J. 97 (2010) 30–37.
[50] P. Vasseur, P. Pandard, D. Burnel, Influence of some experimental factors on metal toxicity to *Selenastrum capricornutum*, Toxic. Assess. 3 (1988) 331–343.
[51] TNO 1992. Cobalt. Nederlandse Organisatie voor Toegepaste geowetenschappen (Netherlands organization for Applied Scientific Research). IMW-R-92/277. In, Cobalt et ses dérivés. INERIS (1992) DRC-02-25590-02DF55.doc.
[52] 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. Saf. 47 (2000) 186–194.
[53] Janssen Pharmaceutica, The effect of nickel chlorure on the growth of the unicellular green alga (*Selesnastrum capricornutum*). (1993), In Nickel et ses dérivés. INERIS–DRC-02-25590-02DF44.doc.
[54] T.J. Monahan, Lead inhibition of chlorophycean microalgae, J. Phycol. 12 (1976) 358–362.
[55] Q. Kong, L. Zhu, X. Shen, The toxicity of naphthalene to marine *Chlorella vulgaris* under different nutrient conditions, J. Hazard. Mater. 178 (2010) 282–286.
[56] Canadian Council of Ministers of the Environment (CCME), Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health, 2007, http://www.ccme.ca/assets/pdf/rev_soil_summary_tbl_7.0_e.pdf.
[57] W.R. Gala, J.P. Giesy, Photo-induced toxicity of anthracene to the green alga, *Selenastrum capricornutum*, Arch. Environ. Contam. Toxicol. 23 (1992) 316–323.
[58] Swedish Environmental Protection Agency (SEPA), Generic Guideline Value for Contaminated Soils, 2008, http://www.swedishepa.com/en/In-English/Menu/Operations-with-impact-on-the-environment/Remediation-of-contaminated-areas/Classification-and-risk-assessment/Generic-guideline-values-for-contaminated-soils/.
[59] Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieu (VROM), Dutch Intervention Values and Target Values – Soil Quality Standards, Ministry of Housing, Spatial Planning and the Environment, The Netherlands, 2001, http://www.ContaminatedLand.co.uk/std-guid/dutch-l.htm.
[60] Bureau des Ressources Géologiques et Minières (BRGM), Gestion des sites (potentiellement) pollués. Annexe 5C révision du 9 décembre 2002. Valeurs guides en matière de pollution des eaux et des sols (2002).