Available online at www.sciencedirect.com

ScienceDirect

Bioresource Technology 99 (2008) 1450–1464

BIORESOURCE TECHNOLOGY

Decontamination of sludge by the METIX-AC process. Part II: Effects on maize growth and bioaccumulation of metals

Driss Barraoui [a], Michel Labrecque [b], Jean-François Blais [a,*]

[a] *Institut national de la recherche scientifique (INRS-ETE), Université du Québec, 490, rue de la Couronne, QC, Que., Canada G1K 9A9*
[b] *Institut de recherche en biologie végétale (IRBV), 4101, rue Sherbrooke Est, Montréal, Que., Canada H1X 2B2*

Received 19 November 2004; received in revised form 17 January 2007; accepted 17 January 2007
Available online 26 March 2007

Abstract

Given the fact that, according to our knowledge, no study has compared the agro-environmental use of decontaminated with non-decontaminated sludge, a greenhouse experiment was carried out to test the growth of maize (*Zea mays* L., G-4011 Hybrid) and bioaccumulation of metals in the presence of four different sludges (MUC, QUC, BEC and DAI), before and after their decontamination by a novel process (METIX-AC). Data showed that decontaminated sludge ameliorated plant growth and biomass production, and decreased bioaccumulation of metals, more than control soil, inorganic chemical fertilization, or conventional non-decontaminated sludge. Since chemicals used by the METIX-AC process contained S and Fe, decontaminated sludge introduced large amounts of these elements, while the overall presence of metals was reduced. Often, sludge dose also affected maize growth and bioaccumulation of metals. Overall, no toxicity to plants was noticed and bioaccumulation and transfer of many metals remained below the limits reported in the literature.

Keywords: Sludge; Decontamination; Bioaccumulation; Metal; Maize

1. Introduction

Municipal Sewage sludge (MSS) is the unavoidable by-product of municipal wastewater treatment. In the USA, Canada and Europe alone, MSS production amounts to some 20 million tons a year (dry solid basis) (Duvaud et al., 1999; EEA, 1999; USEPA, 1999). Approximately 40% of MSS is used as fertilizer on North American and Western European agricultural soils (Duvaud et al., 1999; USEPA, 1999; Spinosa and Vesilind, 2001). Spreading MSS on agricultural lands has several beneficial effects: it ameliorates soil structure (Nemati et al., 2000), phosphorus and organic matter contents (Martinez et al., 1999), increases tree growth (Labrecque et al., 1995) and helps restore the vegetal cover of contaminated sites (Brown et al., 2003).

However, MSS may contain higher levels of metals than the limits prescribed for fertilizers (Smith and Vasiloudis, 1991; USEPA, 1993; Desjardins and Brière, 1994), and these metals may be harmful to plants and organisms. In the USA, as many as 1500 wastewater treatment plants (WTP) may produce sludge exceeding US federal standards for agricultural use (USEPA, 1990). Conventional methods of sludge stabilization, such as aerobic or anaerobic digestion, are known to be inefficient in removing heavy metals (Chipasa, 2003). Consequently, the development of new technologies for sludge decontamination became inevitable, especially after the ban of ocean dumping of MSS. Numerous chemical and biological processes were experimented worldwide and have allowed the extraction of satisfactory amounts of metals from MSS (Couillard and Mercier, 1991; Blais et al., 1992; Tyagi et al., 1995; Xiang et al., 2000; Naoum et al., 2001; Cho et al., 2002; Kim et al., 2002; Lombardi and Garcia, 2002; Chan et al., 2003). In this context, a novel decontamination process (the METIX-AC) was recently developed and described

---

[*] Corresponding author. Tel.: +1 418 654 2541; fax: +1 418 654 2600.
E-mail address: blaisjf@ete.inrs.ca (J.-F. Blais).

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.01.053

Nomenclature

BEC	Bécancour sewage sludge
DAI	Daishowa pulp and paper wastewater sludge
DEC	decontaminated sludge
FER	chemical fertilization
MSS	municipal sewage sludge
MUC	montreal urban community sewage sludge
N_{AV}	available nitrogen
NDE	non-decontaminated sludge
QUC	Quebec urban community sewage sludge
SAS	statistical analysis software
SLA	specific leaf area
TKN	total Kjeldahl Nitrogen
USEPA	United States Environmental Protection Agency
WTP	wastewater treatment plant

in a publication (Blais et al., 2004), following successful tests performed at the Montreal WTP (Mercier et al., 2002). Without greatly affecting the nutrient content of MSS, the METIX-AC process significantly reduces metallic contents, odor and pathogens.

A comparative study was undertaken in order to test the agricultural use of decontaminated versus non-decontaminated sludge for the cultivation of maize (*Zea mays* L.) under greenhouse conditions. Four sludges, collected from different locations in the province of Quebec (Canada) were considered, along with non-amended and inorganically amended soils. The present data, which cover the second part of the recorded results, specifically concern the effects of dose enhancement and decontamination of sludge on maize growth parameters, grain yields and bioaccumulation of metals. A separate paper (Barraoui et al., 2007) can be consulted for data dealing with the impact of decontamination on sludge quality as well as that of sludge doses and decontamination on leaching of nutrients and metals into drainage water.

2. Methods

The materials and methods described below are agronomy-related (i.e., concern maize growth and bioaccumulation of metals), while those concerning the effects of decontamination on sludge quality and of dose enhancement and decontamination of sludge on leaching of nutrients and metals are presented in a separate paper (Barraoui et al., 2007). The reader is also referred to that paper for further information concerning the: (1) Description of the METIX-AC process for metals extraction from sludge; (2) concentration of nutrients and metals in sludge before and after decontamination; (3) preparation of pots, soil, soil–sludge mixtures and chemical (inorganic) fertilizers; (4) handmade montage used for plant irrigation and water sampling; (5) analytical methods employed for soil, sludge and drainage water characterization.

2.1. Overview of soil and sludge characteristics

Top soil was obtained from the Montréal Botanical Garden in Quebec. It was a neutral, black and loamy sand soil according to USDA classification (Donahue, 1958), and probably originated from spododols soils, since these are the most preponderant in the region studied (Brady and Weil, 2002). The estimated CEC/100 of soil was 27.1, organic matter content was 10.2% and lime indices were 71 (“K + Ca + Mg” saturation of 86.7%). The Mehlich contents of nutrients and total concentration of metals are reported, respectively, in Tables 1 and 2.

Four different sludges were obtained from the wastewater treatment plants (WTP) of Montreal (MUC: Municipal non-digested physico-chemical sludge), Quebec (QUC: Municipal non-digested mixture of primary and secondary sludge), Becancour (BEC: Municipal aerobically digested biological sludge) and Daishowa (DAI: Non-digested mixture of primary, secondary and de-inking sludge from a pulp and paper manufacturing plant); all located in the province of Quebec.

2.2. Experimental conditions

Low and high doses of each sludge type, i.e., non-decontaminated (NDE) and decontaminated (DEC) forms, were computed in order to supply soil with the respective equivalents of 80 or 160 kg/ha of available N (N_{AV}) (Table 1). The N_{AV} in sludge was approximated at “30% of TKN + nitrate + ammonium”, as recommended by the Ministry of Environment of Quebec (Environnement Québec, 2004) regarding the agricultural utilization of sludge. The weighed amount of sludge was thoroughly mixed with a quantity of soil to obtain 2 kg of sludge–soil mixture. The latter was then spread on the surface of soil previously measured into plastics pots. Three controls were considered, namely non-amended soil, (“control soil” or T0), and two chemically fertilized soils, “FER-80”or T1 (equivalent of 80 kg/ha N) and “FER-160” or T2 (equivalent of 160 kg/ha N). The levels of P and K were also fixed in the inorganic fertilizations, respectively to 55 and 75 kg/ha (FER-80 treatment) or 110 and 150 kg/ha (FER-160 treatment) (CPVQ, 1997). It should be noted that abbreviations given in Tables 3–7 correspond to codes T0 to T18 shown in Fig. 1. The experimental design contained 16 sludge amendments (4 sludge × 2 forms (NDE and DEC) × 2 doses) plus three controls (T0, FER-80 and FER-160). These 19 treatments were replicated 6 times in a partial randomized block.

Table 1
Initial nutrients content of soil versus quantities supplied by fertilizers and sludge

		Equivalent field rate		Quantities added to soil (kg/ha)						
		DM (t/ha)	N_{AV} (kg/ha)	NO_3^-–N	NH_4^+–N	P	K	Ca	Mg	S
Chemical fertilizers	FER-80		80	n/a	n/a	55	75	n/a	n/a	n/a
	FER-160		160	n/a	n/a	110	150	n/a	n/a	n/a
MUC	NDE-80	6.65	80	3.53	21.5	102	50.3	255	39.7	29.6
	NDE-160	13.3	160	7.08	43.1	203	101	511	79.4	59.2
	DEC-80	9.44	80	4.82	2.09	148	58.9	97.8	37.2	152
	DEC-160	18.9	160	9.61	4.16	295	118	195	74.5	303
QUC	NDE-80	6.32	80	3.21	23.9	60.5	39.9	144	21.8	21.8
	NDE-160	12.7	160	6.62	48.1	122	80.5	289	43.9	43.7
	DEC-80	8.94	80	4.50	3.06	92.5	52.9	59.8	27.3	121
	DEC-160	17.9	160	9.19	6.29	185	106	120	54.8	244
BEC	NDE-80	5.09	80	2.73	11.6	62.1	46.1	49.3	86.9	28.4
	NDE-160	10.2	160	5.47	23.0	125	92.2	98.8	174	57.1
	DEC-80	5.90	80	3.05	1.76	77.4	47.9	16.7	96.0	79.0
	DEC-160	11.8	160	6.10	3.37	155	96.0	33.4	193	158
DAI	NDE-80	28.8	80	15.0	9.49	26.7	35.9	669	50.0	43.9
	NDE-160	57.5	160	29.9	19.0	53.2	71.6	1335	100	87.7
	DEC-80	30.6	80	15.9	0.32	31.1	29.3	84.9	46.1	125
	DEC-160	61.1	160	31.8	0.64	62.1	58.5	169	92.1	249
Nutrients content of soil (Mehlich III kg/ha)					TKN = 0.3%	78	337	9080	761	n/a

a: the total quantities of the three forms of fertilizers (more explanation in the text).

Table 2
Total concentrations of metals in soil (mg/kg) and the equivalent field quantities supplied by sludge (kg/ha)

Conditions tested		Essential metals					Non-essential metals				
		Cu	Fe	Mn	Na	Zn	Al	Cd	Cr	Ni	Pb
MUC	NDE-80	3.10	198	1.35	25.4	3.24	279	0.04	0.66	0.21	0.48
	NDE-160	6.19	394	2.70	50.8	6.48	558	0.08	1.33	0.42	0.95
	DEC-80	1.96	430	1.25	33.4	2.20	223	0.03	0.82	0.22	0.74
	DEC-160	3.89	859	2.48	66.8	4.40	445	0.06	1.65	0.45	1.49
QUC	NDE-80	1.14	88.7	1.40	26.9	1.94	170	0.06	0.40	0.10	0.27
	NDE-160	2.29	178	2.81	54.1	3.89	342	0.11	0.79	0.19	0.56
	DEC-80	0.88	240	1.19	37.3	1.24	169	0.03	0.56	0.14	0.40
	DEC-160	1.77	481	2.37	75.0	2.48	340	0.05	1.14	0.29	0.79
BEC	NDE-80	7.77	120	1.43	23.3	2.44	114	0.01	0.74	0.72	0.55
	NDE-160	15.6	240	2.85	46.7	4.89	228	0.03	1.48	1.45	1.11
	DEC-80	2.55	197	1.19	27.9	0.96	125	0.01	1.04	0.72	0.61
	DEC-160	5.11	393	2.36	55.7	1.91	250	0.01	2.07	1.43	1.20
DAI	NDE-80	3.85	48.8	2.19	33.5	3.22	859	0.02	0.79	0.40	0.27
	NDE-160	7.68	97.3	4.38	66.8	6.42	1719	0.04	1.56	0.80	0.55
	DEC-80	4.28	88.1	0.82	35.8	1.55	670	0.00	0.84	0.53	0.37
	DEC-160	8.55	177	1.62	71.4	3.09	1339	0.01	1.66	1.06	0.76
Natural content of soil (mg/kg)		22.0	22130	394	22640	8.5	53130	0.46	48.5	16.2	16.6

2.3. Maize cultivation, growth and harvest

Maize seeds (*Zea mays* L., G-4011 Hybrid) were sown in plastic pots under greenhouse controlled conditions for about three months. This particular maize hybrid was chosen because its development requirements (i.e., 2500 thermal units) suit the weather conditions of the study zone. All pots received the same amount of de mineralized irrigation water regularly, by means of a programmed computer drip system. Plants were treated against some common insects, for example trips.

For all treatments, germination of maize seeds occurred within one week after sowing. Male inflorescence and spikes started to emerge seven to 8 weeks later. The magni-

Table 3
Selected leaf parameters at the mid-growth period

Conditions tested		SLA (g/cm^2)	N	C	Dry matter	Chlorophylls (μg/cm^2)	
			%			*a*	*B*
Control soil		133	1.16	40.0	6.80	69.1	18.0
Chemical fertilizers	FER-80	146	1.54	39.4	8.37	114	30.3
	FER-160	163	1.82	39.3	7.77	137	47.9
	NDE-80	161	1.38	39.7	8.40	103	26.7
MUC Sludge	NDE-160	180	1.73	40.1	8.85	122	30.9
	DEC-80	181	1.30	39.7	9.43	109	29.7
	DEC-160	165	1.65	39.1	8.95	135	56.9
			●			●	●
QUC sludge	NDE-80	185	1.47	39.2	7.77	108	27.4
	NDE-160	183	1.57	39.9	9.28	124	31.8
	DEC-80	183	1.46	39.8	9.37	103	27.4
	DEC-160	180	1.74	40.3	9.42	129	31.6
						●	
BEC Sludge	NDE-80	186	1.16	40.0	9.35	112	56.8
	NDE-160	168	1.73	40.3	9.78	127	36.3
	DEC-80	156	1.65	40.0	9.12	117	29.8
	DEC-160	160	1.69	40.2	9.52	148	36.8
DAI sludge	NDE-80	163	1.77	38.9	6.02	83.0	16.4
	NDE-160	149	1.75	38.6	6.17	81.8	20.2
	DEC-80	148	1.69	39.1	6.47	81.6	16.8
	DEC-160	161	1.81	38.5	6.82	86.6	22.5
					■		

Dark circles (●) and squares (■) indicate significant ($Pr > F$ less than 0.05) increasing effects of sludge dose or decontamination, respectively.

tudes of emergences of inflorescence and spike were estimated using an arbitrary scale between 0 (late or even no emergence) and 7 (pronounced emergence). Twelve weeks after sowing, a piece of the fourth leaf of each plant was sampled; then dry matter content and specific leaf area (SLA) were determined. Two weeks later, a second leaf sampling was performed to analyze nitrogen, phosphorus, potassium, and carbon as well as chlorophylls a and b. Finally, plants were harvested after a period of 14 weeks, separated into four different parts (roots, stalks, leaves and spikes) and individual weights of these parts were measured. Roots were thoroughly cleaned to remove any particles of soil. Grain yields were evaluated by counting and weighing the total number of grains per plant. Each of the four parts was dried overnight at 103–105 °C, and the content of P, K, S, Al, Ca, Cd, Cu, Cr, Fe, Mg, Mn, Na, Ni, Pb and Zn determined. Only data regarding Al, Cd, Cu, Cr, Fe, Mn, Na, Ni, Pb and Zn are presented and discussed in the following section. Data regarding nutrients are cited only for purposes of explaining the extent to which metals were absorbed by roots and accumulated in the remaining parts.

2.4. Analytical measurements

Total Kjeldahl Nitrogen was analyzed according to APHA (1999) (method 4500-N-org). Total organic carbon was analyzed on solid samples by a LECO CHNS-932 apparatus (Leco Corporation, St-Joseph, MI). Dry matter was performed at 103–105 °C (2540 B method, APHA, 1999). Specific leaf area (SLA) was calculated after determining area and dry leaf mass. Chlorophylls a and b were extracted from leaves using the sonication method (Simon and Helliwell, 1998) and quantified following Arnon's method and formulas (Arnon, 1949).

The concentrations of metals (Al, Ca, Cd, Cu, Cr, Fe, Mg, Mn, Na, Ni, Pb and Zn) as well as of P, K and S in the different maize parts were determined by atomic absorption with a simultaneous Varian ICP-AES, Vista model (Mississauga, Ontario, Canada). Solid samples were digested to 0.2 g dry samples in the presence of HNO_3, in a final solution of 5% HNO_3 (method 4.1.5.1; Van Loon, 1985). Quality controls were performed with certified liquid samples (multi-elements standard, catalog number 900-Q30-002, lot number SC0019251, SCP Science, Lasalle, Quebec, Canada) to ensure the conformity of the standards. Quality controls for maize grains, stalks and leaves were performed with solid samples of tomato leaves (standard reference material 1573a, Gaithersburg, MD 20899). Quality control for maize roots was performed with solid samples of citrus leaves (standard reference material 1572, Washington, DC 20934).

2.5. Statistical analysis

Statistical analysis was carried out using Statistical Analysis Software (SAS) (SAS, 1989). The approach consisted of performing two way analysis of variance (ANOVA) in order to test the effect of sludge dose enhancement and decontamination on the growth of maize plants and on accumulation of metals in the different maize parts. All statistical analyses were performed with a significance level of $Pr \leqslant 0.05$. When effects were found to be significant, means were compared using Tukey-Kramer's comparison tests and groupings were given. Factorial analyses were also performed following the transformation of drainage water data.

3. Results

3.1. Plant development at mid-growth period

The nutritional characteristics of maize plants at the mid-growth period are shown in Table 3. All organic amendments slightly increased the SLA, N content and dry matter of leaves, compared to control soil, and in some cases, even compared to corresponding chemical fertilizations. There were, however, no notable differences in C concentrations. Chlorophylls a and b were appreciably increased by sludge, compared to control soil, but levels were generally lower than those obtained with chemical fertilizers. In many cases, enhancement of sludge doses and/or

Table 4
Sizes (cm) and total weights (g) of the maize parts at harvest

Conditions tested		Length of		Diameter of		Weights of			
		Stalks	Spikes	Stalks	Spikes	Stalks	Spikes[a]	Leaves	Roots
Control soil		177	4.97	1.01	2.33	15.7	2.94	9.63	4.91
Chemical fertilizers	FER-80	213	8.56	1.03	3.07	17.3	5.28	11.6	5.77
	FER-160	201	9.46	1.12	3.10	24.3	5.86	12.3	8.07
MUC sludge	NDE-80	220	8.61	1.27	3.08	14.6	5.91	12.5	3.00
	NDE-160	216	10.4	1.35	3.26	18.0	8.17	13.7	4.83
	DEC-80	217	9.75	1.29	3.10	19.2	6.81	13.3	4.45
	DEC-160	210	10.2	1.39	3.14	22.0	7.56	13.3	6.27
						●			
QUC sludge	NDE-80	200	9.63	1.05	3.16	19.6	6.03	10.7	1.68
	NDE-160	216	11.2	1.33	3.43	27.3	8.54	14.2	3.94
	DEC-80	226	9.19	1.28	3.12	34.2	7.53	14.8	6.73
	DEC-160	210	7.37	1.39	2.94	24.5	6.73	14.3	8.42
				●, ■			●NDE		■
BEC sludge	NDE-80	212	10.1	1.40	3.39	21.3	7.34	14.3	4.22
	NDE-160	214	9.87	1.51	3.41	30.4	8.93	16.8	8.98
	DEC-80	213	11.1	1.42	3.34	25.7	8.41	14.1	4.98
	DEC-160	226	12.1	1.41	3.66	22.6	11.1	15.0	5.65
			■				●		
DAI sludge	NDE-80	150	5.14	0.92	2.02	17.5	3.23	8.96	6.33
	NDE-160	152	3.84	1.01	1.71	18.9	2.25	9.33	7.32
	DEC-80	157	5.06	0.99	1.89	21.4	3.13	10.0	6.07
	DEC-160	173	5.06	1.05	1.64	24.8	3.23	11.5	8.90
		■ d2		■		■			

Dark circles (●) and squares (■) indicate, respectively, significant ($Pr > F$ less than 0.05) increasing effects of sludge dose or decontamination.

[a] Weight of spike excludes grains.

Table 5
Number of spikes produced and yields of grains at harvest

Conditions tested		Number of spikes per plant[a]	Total number of grains	Total weight of grains (g)	Averaged unit weight (g)
Control soil		1	46	9.39	208
Chemical fertilizers	FER-80 F	1	113	24.9	226
	FER-160	1	113	27.0	236
MUC sludge	NDE-80	1	121	27.5	228
	NDE-160	1	167	33.4	200
	DEC-80	1	137	29.9	219
	DEC-160	1 (3)	143	32.2	236
QUC sludge	NDE-80	1	126	27.5	218
	NDE-160	1	172	37.6	221
	DEC-80	1 (3)	108	27.6	265
	DEC-160	1 (3; 4)	97	25.3	269
			●NDE	●NDE	■
BEC sludge	NDE-80	1	137	33.3	246
	NDE-160	1	162	38.7	248
	DEC-80	1 (3)	147	34.1	232
	DEC-160	1	233	57.4	249
			●	●DEC ■d2	
DAI sludge	NDE-80	1	9	2.08	254
	NDE-160	1 (0)	10	2.47	223
	DEC-80	1	4	0.82	218
	DEC-160	1 (2)	1	0.17	247

Dark circles (●) and squares (■) indicate significant ($Pr > F$ less than 0.05) increasing effects of sludge dose or decontamination, respectively.

[a] For the replicates which generated more than one spike, the number is given in parentheses. For example, in the case of QUC sludge (DEC-160 treatment), two replicates gave 3 and 4 spikes, respectively.

Table 6
Bioaccumulation of essential metals in four parts of maize (G: grains, L: leaves, S: stalks, R: roots), in mg/kg

Conditions tested		Cu				Fe				Mn				Na				Zn			
		G	L	S	R	G	L	S	R	G	L	S	R	G	L	S	R	G	L	S	R
Control soil		3.44	26.7	1.56	11.0	57.0	162	86.9	392	7.41	22.1	2.92	12.7	13.9	235	44.2	1005	31.1	58.2	21.9	13.8
Chemical fertilizers	FER-80	2.88	19.3	3.91	6.80	44.9	211	76.9	251	6.52	25.7	2.39	6.76	4.99	212	52.2	898	23.9	39.1	5.07	9.74
MUC sludge	FER-160	2.36	16.7	4.20	10.3	46.3	280	78.9	305	7.05	33.8	2.97	8.50	5.96	228	63.3	918	23.1	45.0	7.77	9.33
	NDE-80	2.84	15.4	4.36	15.0	45.5	226	75.2	293	5.86	18.8	1.60	7.03	5.45	237	56.6	1132	26.0	41.7	8.94	11.7
	NDE-160	2.56	17.6	10.5	9.92	45.9	265	88.7	229	5.48	21.9	2.08	5.42	5.18	213	167	839	27.1	47.4	12.0	9.64
	DEC-80	3.09	19.3	7.06	5.52	55.8	214	76.2	260	6.37	35.9	2.37	6.77	7.80	213	45.1	785	28.3	49.6	8.65	8.84
	DEC-160	3.11	23.0	3.19	9.66	48.1	224	76.6	259	6.47	41.7	2.67	6.63	6.89	199	14.1	706	27.9	50.7	9.79	11.8
											●, ■	■			☐						
QUC sludge	NDE-80	2.59	21.8	0.45	8.83	45.1	218	77.7	328	5.67	23.1	1.67	6.65	9.36	225	51.2	1245	26.3	43.1	6.32	10.6
	NDE-160	3.16	23.2	6.92	8.84	47.6	253	73.0	238	5.87	23.7	1.95	5.81	9.27	225	26.2	961	27.2	60.9	11.4	8.79
	DEC-80	2.76	15.4	2.54	5.52	50.9	212	83.9	257	6.37	34.1	2.65	6.21	17.8	205	148	1201	28.9	42.0	7.99	6.16
	DEC-160	3.23	18.6	3.05	6.10	51.1	222	79.7	247	6.99	36.6	3.60	6.88	9.12	176	20.7	763	29.0	55.1	11.5	9.38
				●							■	■							●	●	
BEC sludge	NDE-80	2.91	21.8	3.21	10.0	49.1	236	82.9	265	5.83	25.7	2.24	6.80	8.70	211	30.9	850	27.3	49.0	11.6	14.1
	NDE-160	3.08	19.4	9.86	10.9	50.8	252	79.2	271	6.69	45.1	3.21	7.98	6.14	187	17.6	721	27.8	51.0	11.6	11.8
	DEC-80	2.53	15.6	3.65	10.8	44.9	209	79.1	198	6.35	42.6	3.24	6.84	5.90	166	29.3	699	23.3	38.1	6.89	8.88
	DEC-160	2.42	19.8	2.79	10.9	45.5	190	77.5	244	5.86	58.6	2.56	7.90	7.36	155	26.0	740	22.1	50.2	6.46	11.7
		☐				☐					●, ■				☐			☐		☐	
DAI sludge	NDE-80	4.52	23.6	2.59	11.4	63.5	179	79.3	319	8.36	30.4	3.31	8.89	7.25	202	29.3	578	40.3	72.4	24.3	15.6
	NDE-160	3.92	20.9	7.01	8.65	50.9	190	77.9	221	7.69	43.1	3.98	7.24	7.42	202	21.6	514	33.7	81.2	31.1	21.0
	DEC-80	3.52	25.1	16.3	21.3	65.1	167	87.7	241	7.79	37.1	4.45	8.09	8.29	166	14.9	527	33.9	79.5	34.2	32.8
	DEC-160	3.33	13.5	2.41	11.5	71.4	176	80.7	252	8.41	55.8	6.64	10.6	11.1	185	30.7	480	36.1	70.2	28.7	22.0
											●, ■	●, ■			☐						

Dark circles (●) refer to significant ($Pr > F$ less than 0.05) increasing effect of sludge dose. Dark and open squares (■, ☐) indicate, respectively, significant ($Pr > F$ less than 0.05) increasing or decreasing effect of decontamination.

Table 7
Bioaccumulation of non-essential metals in four parts of maize (G: grains, L: leaves, S: stalks, R: roots), in mg/kg

Conditions tested		Al				Cd[a]	Cr				Ni				Pb			
		G	L	S	R	L	G	L	S	R	G	L	S	R	G	L	S	R
Control soil		0.59	156	21.8	520	<LD[b]	0.36	4.55	0.76	1.97	0.63	4.11	<LD	1.79	<LD	1.87	1.92	<LD
Chemical fertilizers	ER-80	<LD	188	8.98	299	0.16	0.17	4.65	0.58	0.67	0.30	3.92	<LD	0.43	0.57	2.30	1.50	1.28
	FER-160	2.62	247	9.75	377	0.16	0.17	4.35	0.64	1.17	0.31	3.48	<LD	1.42	0.30	2.23	0.80	<LD
MUC sludge	NDE-80	2.99	228	10.5	372	0.16	0.25	4.93	0.54	1.04	0.33	3.85	<LD	<LD	0.30	1.93	1.17	<LD
	NDE-160	2.13	268	23.7	252	0.16	0.17	4.57	3.38	1.03	0.51	3.39	6.81	<LD	<LD	3.02	1.05	<LD
	DEC-80	3.53	208	9.99	263	0.16	0.20	4.41	0.68	0.86	0.67	3.91	<LD	<LD	0.27	2.92	1.49	<LD
	DEC-160	<LD	227	8.25	257	0.16	0.17	4.58	0.51	1.54	0.37	4.85	<LD	2.82	<LD	2.11	0.97	<LD
QUC sludge	NDE-80	5.54	218	8.46	389	0.19	0.23	4.43	0.57	0.99	0.39	4.04	<LD	0.97	0.15	1.58	1.97	1.35
	NDE-160	3.74	254	9.08	251	0.27	0.17	4.45	0.44	1.06	1.04	4.54	1.24	<LD	<LD	2.96	1.83	<LD
	DEC-80	3.74	202	10.7	309	0.16	0.18	5.87	0.98	1.27	0.62	4.40	2.69	0.46	1.69	1.95	0.41	<LD
	DEC-160	2.98	195	9.49	260	0.19	0.31	4.69	0.62	1.21	0.84	3.56	0.78	<LD	<LD	2.30	<LD	<LD
						●, □			■									
BEC sludge	NDE-80	2.81	242	18.2	319	0.12	0.27	5.34	1.03	1.88	0.43	4.04	1.60	2.49	<LD	1.93	1.73	3.42
	NDE-160	7.69	243	11.2	310	0.16	0.20	5.39	0.78	1.35	0.45	4.09	2.27	<LD	0.32	2.14	<LD	<LD
	DEC-80	5.22	184	10.7	174	<LD	0.13	4.59	0.62	0.94	0.42	3.42	1.64	<LD	<LD	3.20	<LD	<LD
	DEC-160	3.27	154	13.6	258	0.16	0.97	4.96	1.32	1.23	0.40	3.61	<LD	<LD	0.35	2.00	3.98	<LD
			□		□	□						□						
DAI sludge	NDE-80	9.13	162	6.44	458	0.16	0.14	3.13	0.36	1.29	0.47	3.31	<LD	2.29	0.98	1.50	<LD	<LD
	NDE-160	2.24	165	7.78	274	0.16	0.11	2.79	0.41	0.89	0.40	3.47	2.07	<LD	0.55	1.57	1.49	<LD
	DEC-80	4.08	135	7.29	305	0.17	0.11	2.85	0.62	0.97	0.33	3.50	3.37	1.42	<LD	2.26	1.82	0.84
	DEC-160	3.14	128	8.37	401	<LD	0.19	2.72	0.70	0.96	0.45	3.80	<LD	<LD	0.70	1.47	<LD	<LD
								○	■									

Dark and open circles (●, ○) refer, respectively, to significant (*Pr* > *F* less than 0.05) increasing or decreasing effect of sludge dose. Dark and open squares (■, □) indicate, respectively, significant (*Pr* > *F* less than 0.05) increasing or decreasing effect.

[a] Cadmium was detected only in leaves.

[b] LD means limit of detection.

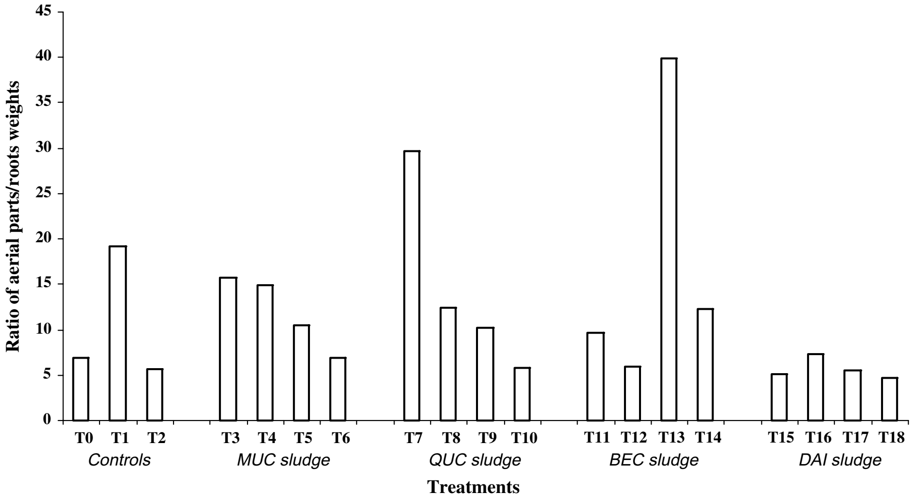

Fig. 1. Biomass/roots formation at harvest as affected by soil amendments.

decontamination slightly increased one of the mentioned parameters.

Statistically, there were no differences between low and high sludge doses and between non-decontaminated and decontaminated sludge, with respect to SLA and C content. The contents of N ($Pr > F = 0.0411$) as well as chlorophylls a ($Pr > F = 0.0067$) and b ($Pr > F = 0.0129$) were significantly increased by the enhanced MUC sludge dose, while only chlorophyll a ($Pr > F = 0.0032$) content was significantly increased by doubling the QUC sludge dose. Decontamination of DAI sludge significantly increased dry matter ($Pr > F = 0.0126$) of leaves.

3.2. Biomass production at harvest

The use of MUC, QUC and BEC sludge amendments generally tended to increase the size of stalks and spikes as well as the weight of stalks, spikes and leaves at harvest, compared to control soil and chemically fertilized soils (Table 4). The weight of roots was affected differently depending on the sludge type and whether it was decontaminated or not. With DAI sludge, an imperceptible effect or a limited decrease occurred in the cited parameters of stalks, spikes and leaves, but a more appreciable increase occurred in root weight.

Statistically, enhancing the dose of MUC sludge significantly increased the weight of spikes ($Pr > F = 0.0374$), while that of QUC sludge similarly affected stalk diameter ($Pr > F = 0.0044$) and spike weight ($Pr > F = 0.0373$) (non-decontaminated form only). Enhancing the BEC sludge dose significantly increased spike weight ($Pr > F = 0.0275$), producing the heaviest spikes in the presence of a high level of decontaminated BEC sludge (160 kg/ha N_{av}). With respect to the decontamination effect, there were significant increases in stalk diameter ($Pr > F = 0.0251$) and root weight ($Pr > F = 0.0202$) in the case of QUC sludge, while significant increases in spike length ($Pr > F = 0.0060$) occurred with BEC sludge, producing the longest spike in the presence of a high level of decontaminated BEC sludge (160 kg/ha N_{av}). Finally, DAI sludge decontamination significantly increased the length ($Pr > F = 0.0260$) (160 kg/ha N_{av}), diameter ($Pr > F = 0.0050$) and weight ($Pr > F = 0.03260$) of stalks.

3.3. Fructification and grain production

For simplification purposes, data relating to fructification (emergence of male inflorescence and spikes) is not presented, but discussed briefly below. It was observed that emergences of those components occurred late in the case of non-amended pots as well as for those amended with chemical fertilizers and DAI sludge. The earliest appearances were observed for the remaining sludge amended pots, especially decontaminated BEC sludge. Moreover, dose enhancement and decontamination of most tested sludge generally tended to accelerate emergence of the male inflorescence.

Data recorded at harvest (Table 5) indicates that, except for DAI sludge, organic amendments seemed to increase the number and total weight of grains more than control soil, and in several cases even more than corresponding chemical fertilizations. Consequently, the weakest measured parameters for grains at harvest corresponded to the DAI sludge treatments, while the highest yields were obtained with the BEC sludge amendments.

Statistically, the total number and weight of grains were significantly increased ($Pr > F = 0.0436$ and 0.0420, respectively) with dose enhancement of non-decontaminated QUC sludge. Similar increases were also noted with BEC sludge ($Pr > F = 0.0085$ and 0.0318, respectively), but the effect on the total weight of grains was restricted to the decontaminated sludge form. Decontamination of QUC sludge significantly increased the average unit weight of grains ($Pr > F = 0.0313$), while that of BEC sludge increased the total weight of grains ($Pr > F = 0.0318$), but only at a high sludge rate (160 kg/ha N_{av}). Here again, the highest total number and weight of grains were obtained with the decontaminated BEC sludge at a high rate (160 kg/ha N_{av}).

3.4. Bioaccumulation of essential metals

In the absence of any amendment, Cu, Mn and Zn tended to migrate mostly to leaves; the lowest concentrations of Cu and Mn were in stalks and of Zn in roots (Table 6). Meanwhile, Fe and Na were confined mainly to roots, and their lowest content was in grains. The use of chemical fertilizers did not affect the main target of essential metals, except for Cu, for which the lowest content was in grains rather than stalks, and Zn, which accumulated less in stalks than in roots. With few exceptions, sludge additions did not change the maize parts where essential metals tended to be stored, but provoked slight shifts. All sludge decreased Cu in leaves, but only MUC, QUC and BEC sludge also decreased its concentration in grains. As was the case with inorganic fertilizations, most of the MUC, QUC and BEC sludge amendments led to the lowest concentration of Cu in grains rather than in stalks. Moreover, all sludge increased Fe in leaves, while only MUC, QUC and BEC sludge decreased Fe content in roots, stalks and grains. Similarly, all organic amendments increased and decreased Mn, respectively in leaves and roots, but most (especially MUC, QUC and BEC sludge) also decreased this metal in stalks and grains. All sludges decreased Na in stalks, leaves and grains. Only MUC, QUC and BEC sludge decreased Zn levels in all maize parts, and the opposite was observed with DAI sludge.

Statistically, when significant ($Pr \leqslant 0.05$) increases were caused by dose enhancement or decontamination of sludge, this occurred specifically in stalks or leaves. Any significant ($Pr \leqslant 0.05$) decrease was due only to decontamination, and mainly occurred in grains. When doubling the QUC sludge dose, significant increases of Cu in stalks ($Pr > F = 0.0064$) as well as Zn in leaves ($Pr > F = 0.0285$) and stalks ($Pr > F = 0.0131$) occurred. Enhancing the doses of MUC and BEC sludge significantly increased Mn in leaves only ($Pr > F = 0.0319$ and 0.0046, respectively), while enhanced doses of DAI sludge simultaneously increased Mn in stalks ($Pr > F = 0.0068$) and leaves ($Pr > F = 0.0120$). MUC and DAI sludge decontamination significantly increased Mn in stalks ($Pr > F = 0.0129$ and 0.0209, respectively) and leaves ($Pr > F = 0.0001$ and 0.0018, respectively), but decreased Na in leaves ($Pr > F = 0.0286$ and 0.0008, respectively). Decontamination of QUC sludge also increased Mn in stalks ($Pr > F = 0.0334$) and leaves ($Pr > F = 0.0001$). Decontamination of BEC sludge significantly increased Mn, but only in leaves ($Pr > F = 0.0003$), while it decreased Cu ($Pr > F = 0.0127$) and Fe ($Pr > F = 0.0078$) in grains, as well as Na in leaves ($Pr > F = 0.0437$), and Zn in stalks ($Pr > F = 0.0064$) and grains ($Pr > F = 0.0131$).

3.5. Bioaccumulation of non-essential metals

Firstly, in the absence of any amendment, In the absence of any soil amendment, Cd was undetectable in any maize part, even leave (Table 7). Both Al and Cr accumulated less in grains, and primarily in roots and leaves, respectively. The lowest and highest Ni contents were observed, respectively, in stalks and leaves, while Pb was not detected in roots and grains, but accumulated in stalks or leaves. The use of inorganic fertilizers slightly increased Cd content of leaves, but had no apparent effect on the maize parts where the lowest and highest concentrations of the remaining non-essential metals were retrieved. Except for a few cases, sludge did not affect the main targets of non-essential metals, although they caused slight shifts in their concentrations. First, sludge tended to decrease Al in roots and stalks, and the metal became more abundant in grains, especially with some DAI sludge amendments, compared to control soil. Similarly, all sludge additions slightly increased Cd level in leaves, particularly with QUC sludge treatments, but the metal was still undetectable in the remaining maize parts. Cr and Ni contents generally decreased in roots and grains following all sludge additions, while their concentrations in leaves were slightly affected, especially with DAI sludge, which tended to decrease them. The Pb level in all maize parts showed no clear tendency following sludge additions.

Statistically, significant ($Pr \leqslant 0.05$) changes occurred mostly in leaves, then in stalks or roots, but never in grains. Dose enhancement of QUC and DAI sludge significantly increased Cd ($Pr > F = 0.0495$) and reduced Cr ($Pr > F = 0.0380$), respectively, in leaves. Decontamination of QUC sludge significantly reduced Cd in leaves ($Pr > F = 0.0331$), but increased Cr in stalks ($Pr > F = 0.0278$). BEC sludge decontamination significantly decreased Cd ($Pr > F = 0.0260$) and Ni ($Pr > F = 0.0347$) in leaves as well as Al in leaves ($Pr > F = 0.0397$) and roots ($Pr > F = 0.0215$). DAI sludge decontamination significantly increased Cr in stalks ($Pr > F = 0.0482$).

4. Discussion

The literature covers an extensive number of studies dealing with the effects of conventional sludge on plant growth as well as on the phytoavailability of naturally present and sludge-borne metals. Many studies of sludge decontamination for metals removal also exist. Yet, to our knowledge, no study has compared the agro-environmental use of decontaminated with non-decontaminated sludge. Consequently, the following discussion only deals with studies of conventional sludge.

According to data recorded in the current study, leaf parameters at the mid-growth period as well as those at harvest were never negatively affected by the use of decontaminated rather than non-decontaminated sludge. However, some of the biometric measurements corresponding to decontaminated sludge amendments exceeded those of control soil and even those of chemical fertilizations and non-decontaminated sludge amendments. Generally, the selected parameters (sizes of stalks and spikes, weight of the four maize parts) were higher for the sludge-amended rather than non-amended pots, and there were significant ($Pr \leqslant 0.05$) increases following decontamination and/or dose enhancement of a given sludge. For example, the dry matter of leaves rose with all sludge additions, and even if the lowest results were achieved with DAI sludge, decontamination of the latter significantly ($Pr \leqslant 0.05$) increased that parameter. This confirms that, by affecting the native naturally present concentrations of nutrients in soils (Kabata-Pendias and Pendias, 2001), sludge can enhance the growth of maize (Mazen, 1995) and alleviate its biomass production (Pigozzo et al., 2000). Among nutrients of primary importance, approximately 80–85% of N and K and 5% of P may be absorbed by maize from soil (Hernandez et al., 1991). The tested soil contained appreciable amounts of N, P and K (but also of Ca, Mg and S) which influence parameters of plant development, such as chlorophylls content, stalk size, biomass weight and grain yield (Hopkins, 1999). However, the use of MUC, QUC and BEC sludge seemed to ameliorate the growth conditions of maize, since, when the doses of these biosolids were enhanced, significant ($Pr \leqslant 0.05$) increases occurred in the chlorophyll content of leaves, for example. Even if the METIX-AC process significantly ($Pr \leqslant 0.05$) decreased concentrations of NH_4^+, K, Ca and Mg in decontaminated sludge (Barraoui et al., 2007), biosolids still contained satisfactory levels of these elements, and significantly ($Pr \leqslant 0.05$) higher quantities of S, because of the chemicals required by the METIX-AC process. The availability of P is particularly important for plants, because it influences stalk size (Hopkins, 1999), SLA level and secondary (adventitious) root formation (Pellerin et al., 2000). Given that P is more available when bound to mineral rather than organic phases (Vetterlein et al., 1999), and since decontaminated MUC, QUC or BEC sludge more or less increased many of the mentioned biometric parameters, the P added during decontamination probably existed in available forms.

At harvest, grain yield was significantly ($Pr \leqslant 0.05$) increased with dose enhancement and/or decontamination of QUC and/or BEC sludge. Similarly, Akrivos et al. (1999) observed an increase in maize grain yield when applying 10 t/ha of sludge. In the current study, even with lower sludge doses (namely 5.90 t/ha), decontaminated BEC sludge generated more than three times the number of grains than control soil, and approximately 1.3 times more than the equivalent rate of inorganic fertilizer. The use of DAI sludge, however, whether decontaminated or not, did not result in satisfactory grain yield, even if the applied doses exceeded 28 t/ha. This may be explained by the fact that, since it was nutritionally poor, DAI sludge supplied the lowest concentrations of nutrients to soil, and the light physical structure of that sludge caused a high loss of drainage water, and hence significant ($Pr \leqslant 0.05$) leaching of nutrients (Barraoui et al., 2007).

Given the importance of nutrient balance in soil for optimal plant growth, one may consider the ratio of aerial parts (stalks + leaves) weight to that of roots. This parameter is important, because an excess of N is known not only to delay flowering, but also to stimulate biomass production (high aerial parts/roots ratio), whereas an excess of P increases root formation (Hopkins, 1999). In general, inorganic and organic amendments increased biomass production, compared to control soil, but the lowest results were observed with DAI sludge amendments (Fig. 1). Moreover, as is well-established, an excess of nutrients reduces biomass production, and this is clearly evident in the current study: when doubling the dose of chemical fertilizers or that of MUC, QUC and BEC sludge, the ratio mentioned decreased. MUC and QUC sludge decontamination also decreased that ratio, while increases were observed with decontaminated BEC sludge, where a higher ratio was obtained with a low dose of this sludge. The most favorable conditions for optimum growth of maize were expected to involve the use of decontaminated BEC sludge; there are two reasons for this expectation. First the lowest rate that supplied the required N_{av} amount corresponded to the decontaminated form of BEC sludge. Second, this sludge rate simultaneously provided the lowest C/N ratio to soil, a ratio known to be agriculturally important for the development of plants (Brady and Weil, 2002).

With respect to the uptake and bioaccumulation of essential metals, it should be stressed that, except for a few cases, most of the MUC, QUC and BEC sludge treatments had similar effects on the levels of metals in maize part (s), and nearly all of these effects were opposite to those caused by the DAI sludge amendments. Recorded data showed that Cu, Mn and Zn tended to be stored naturally in leaves, while Fe and Na remained in roots. These trends were not greatly affected by the use of either inorganic or organic amendments. Controversy exists in the literature, however, regarding the effects of sludge additions on the accumulation of metals. For example, while increases have been reported for Cu, Fe, Mn and Zn levels in plants following sludge application (Pigozzo et al., 2000), a decrease has been observed for Mn, with no significant effects for Cu, Fe and Zn (Hernandez et al., 1991). Differences in experimental conditions probably explain such divergences.

In the current study, MUC, QUC and BEC sludge amendments decreased the Cu level in leaves and grains, compared to control soil, while enhancement of the QUC sludge dose significantly ($Pr \leqslant 0.05$) increased metal in stalks. Webber (1988) reported that for an input of 240–320 kg Cu/ha, metal may increase in all maize parts, except in grains, by up to 2 mg/kg. In particular, the low dose of decontaminated DAI sludge increased Cu in roots and stalks, respectively, by 10 and 15 mg/kg, while the remaining organic amendments led to less of an increase of Cu storage. An increase of Cu in grains (of about 1 mg/kg) was recorded only with the DAI sludge amendments. However, the use of decontaminated rather than non-decontaminated MUC, QUC and BEC sludge generally tended to decrease Cu contents in all of the maize parts. As was the case with inorganic fertilizations, high doses of all non-decontaminated sludge led to such high increases of Cu in stalks that the lowest concentration of metal was retrieved in grains rather than in stalks. More specifically, the highest input of Cu (15.6 kg/ha) was from the non-decontaminated BEC sludge at 160 kg/ha N_{av}, and this increased Cu in stalks (8.30 mg/kg), but decreased it in leaves and grains, respectively, by 7.30 and 0.36 mg/kg. When using the same rate of decontaminated BEC sludge, just 5.11 kg/ha of Cu was supplied, and metal content in stalks increased only by 1.23 mg/kg, while decreasing in leaves and grains, respectively, by 6.90 and 1.02 mg/kg. This suggests that Cu derived from decontaminated sludge was probably less phytoavailable, since its translocation toward stalks and its storage in grains were diminished significantly ($Pr \leqslant 0.05$). Furthermore, the transfer coefficient of Cu (ratio of the plant/soil concentrations), which is normally in the range of 0.01–0.05 for maize seedlings (Henning et al., 2001), was moderately high, especially for control soil, but was slightly decreased by the addition of sludge. In all cases, Cu accumulation in maize parts was much lower than its toxicity limits of 20–100 mg/kg (Hernandez et al., 1991, 1999).

The use of MUC, QUC and BEC sludge increased levels of Fe in leaves and decreased it in roots, stalks and grains, compared to control soil. The use of the decontaminated form of BEC sludge alone significantly ($Pr \leqslant 0.05$) reduced Fe in grains. Availability of Fe to plants is crucial, because it plays an important metabolic role in, for example, N fixation and enzyme synthesis (Hopkins, 1999). The significant ($Pr \leqslant 0.05$) increase of Fe in maize leaves indicates that Fe^{2+}, which was supplied to soil by decontaminated sludge (because H_2SO_4 was required by the METIX-AC process), was probably readily absorbed by roots and rapidly transferred to leaves. This is supported by the fact that roots are able to reduce Fe^{3+} to Fe^{2+} in order to facilitate metal uptake (Kabata-Pendias and Pendias, 2001). For all treatments, the transfer coefficients of Fe were extremely low. More specifically, the concentration of Fe in grains was in the range of 25–80 mg/kg, which has been reported for cereal grains (Kabata-Pendias and Pendias, 2001). Finally, as will be discussed later, the presence of high Fe levels in decontaminated sludge may depress the accumulation of other metals.

MUC, QUC and BEC sludge simultaneously increased Mn concentration in leaves, and decreased it in roots, stalks and grains, compared to control soil. Moreover, with dose enhancement and/or decontamination of one of the four sludge, Mn significantly ($Pr \leqslant 0.05$) increased in leaves and/or stalks. However, Mn increase in stalks seemed to be insufficient to inhibit Ca translocation toward the stalk apex, or to compete with assimilation of Fe and Mg by plants (Hopkins, 1999). This may be explained by the fact that Mn concentrations in soil as well as the amounts supplied by sludge were lower than those of Ca, Mg and Fe. Jarausch-Wehrheim et al. (2000) noted that Mn initially accumulates in maize roots, but is slowly transferred to aerial parts, leading to 10 and less than 5 mg Mn/kg in grains, respectively, for control and sludge amended soils. In the current study, up to 7.41 mg Mn/kg was detected in grains from control plants; MUC, QUC and BEC sludge amendments decreased its level, though was not the case with DAI sludge. The transfer coefficients of Mn were extremely low for control and all sludge amendments. Mn accumulation in all maize parts was much lower than its toxicity limits of 100–400 mg/kg (Hernandez et al., 1991, 1999).

The use of sludge amendments generally decreased Na concentration in leaves and grains, compared to control soil, while random trends were observed for Na in roots and stalks. Moreover, decontamination of MUC, BEC and DAI sludge significantly ($Pr \leqslant 0.05$) reduced Na in leaves. It is known that sodium chloride accentuates Cd uptake by plants (Kabata-Pendias and Pendias, 2001), but an extremely weak correlation was calculated between Na and Cd in leaves in the present study. The adverse effects of Na can, indeed, be counterbalanced by the presence of Ca and Mg (Brady and Weil, 2002). This probably occurred in the soil tested in this case, since it contained higher total concentrations of Ca and Mg than Na, and also because sludge supplied much greater quantities of these two nutrients than Na. Moreover, significant ($Pr \leqslant 0.05$) quantities of Na remained confined in roots, and sludge additions did not greatly affect this Na storage. This suggests that the maize plants probably used this confinement as a strategy in order to avoid an excessive accumulation of Na in their aerial parts, and thereby to prevent toxic conditions. However, whatever the maize part considered, transfer coefficients of Na were very low for both non-amended and amended soils.

The use of MUC, QUC and BEC sludge decreased Zn levels in all maize parts, compared to soil, but enhancement of the QUC sludge dose increased Zn in leaves and stalks. Except for DAI sludge amendments, our findings are supported by the work of Jarausch-Wehrheim et al. (1999), who found that Zn is transferred less from sludge treated soil to maize leaves than from other soil amendments. Addition of decontaminated BEC sludge at a low rate (80 kg/ha N_{av}) decreased Zn level in leaves by 35%, while a 37% increase occurred under similar conditions with DAI sludge. Bioaccumulations of Zn were, however, much lower than the concentrations of 500, 400 and 50 mg/kg, measured by Jarausch-Wehrheim et al. (1999), respectively, in maize roots, leaves and grains. However, the transfer coefficient of Zn to leaves exceeded normal values of 1–2 (Henning et al., 2001), specifically for the control and the chemical fertilization treatments, but MUC, QUC and BEC sludge additions appreciably decreased this transfer.

Moreover, the Zn accumulation in all maize parts was much lower than its toxicity limits of 400–1000 mg/kg (Hernandez et al., 1991, 1999). Maize is known to be moderately tolerant of Zn (Sabey, 1980; Tlustos et al., 2001), and substrates that contain high Fe levels may depress Zn uptake by maize by up to 80% (Chlopecka and Adriano, 1996). This could be the case in the present study, since the METIX-AC process significantly ($Pr \leqslant 0.05$) increased Fe in decontaminated sludge, as a result of the use of ferric chloride as an oxidant agent. Consequently, the use of decontaminated BEC sludge, for example, significantly ($Pr \leqslant 0.05$) decreased Zn in stalks and grains.

Concerning non-essential metals, data showed that Cd was detected exclusively in leaves, and that the highest level of Al was in roots, while that of Cr, Ni and Pb was in leaves. Except for a few cases, the use of inorganic and organic amendments had no apparent effect on the main targets of non-essential metals, but some minor decreases and increases were noticed.

Sludge generally transferred part of Al to leaves and grains, and reduced its content in roots and stalks. The main target of Al was roots, and this agrees with Kabata-Pendias and Pendias (2001). Although some of the recorded levels of Al in grains exceeded the normal concentration of 2.6 mg/kg (Kabata-Pendias and Pendias, 2001), Al was detected in grains in only one or two replicates out of six. Furthermore, Al transfer coefficients did not exceed 0.01 following any treatment. Al could limit Cu uptake by roots, because of the presence of toxic concentrations of Al, but this mainly occurs in acidic soils (Kabata-Pendias and Pendias, 2001). In the conditions tested, i.e., with a neutral soil pH, Al should not cause a toxic effect.

Among non-essential metals, Cd is one of the most studied, because it may limit plant productivity (Boukhars and Rada, 2000), and is readily transported through the food chain. In the present study, sludge additions (especially non-decontaminated QUC sludge) slightly increased the Cd level in leaves, and the metal was not detected in other plant parts. This corroborates the findings of Kabata-Pendias and Pendias (2001), who stated that Cd transfers rapidly from roots to aerial parts, particularly leaves. Addition of as little as 28.1 mg Cd/kg is considered to cause Mg deficiency in maize, and the latter can then delay plant growth by decreasing its photosynthetic activity (Ferretti et al., 1993). The presence of appreciable levels of Cd can also reduce the length of roots and provoke chloroplast alterations (Rascio et al., 1993). In the present study, no deficiency of Mg was observed in plants, nor was there a decrease in chlorophyll content or in the length of roots, all of which could be caused by Cd. However, it has been reported that addition of 4–7 kg Cd/ha does not affect the metal's accumulation in grains, but its content in stalks can be increased by 40–160 mg Cd/kg (Webber, 1988). In the current study, no more than 0.11 kg Cd/ha was supplied by sludge, and the metal was not detected either in stalks or in grains, and less than 0.28 mg Cd/kg was retrieved from leaves. Also, the maximum transfer coefficient of Cd was less than 0.4. It is known that Zn generally depresses the effects of Cd (Kabata-Pendias and Pendias, 2001), and this can be checked by computing the Cd/Zn ratio. This ratio was lower than 1%, and this explains why Cd values (less than 0.28 mg/kg) were less than the toxicity limit of 5 mg/kg (Kabata-Pendias and Pendias, 2001).

Cr mostly accumulated in leaves and was not significantly ($Pr \leqslant 0.05$) affected by MUC, QUC and BEC sludge additions, but DAI sludge amendments caused a decrease. All sludge amendments decreased Cr in roots and grains. If Cr exists in soil in unavailable Cr(III) form, it is not easily assimilated by plants (Sabey, 1980). Since sequential extraction of metals was not performed on tested soil and sludge, it is not possible to establish the fractional distribution of that metal precisely. However, considering that the presence of up to 736 kg Cr/ha does not affect its uptake by maize (Webber, 1988), we can suppose that the low quantities (maximum of about 2 kg/ha) of the metal supplied by sludge should not have affected its accumulation in maize, even if QUC and DAI sludge decontamination significantly ($Pr \leqslant 0.05$) increased Cr content in stalks. Moreover, recorded Cr concentrations (0.1–0.3 mg/kg for grains and 2.6–5.9 mg/kg for leaves) were mostly lower than the toxicity limits of metal in corn, which are reported to be in the range of 4–8 mg/kg (Kabata-Pendias and Pendias, 2001). The transfer coefficient of Cr to leaves reached 0.14, and did not exceed 0.04 in stalks and roots.

All sludge generally decreased Ni in roots and grains. As has been previously demonstrated (Kabata-Pendias and Pendias, 2001), leaves are the principal target of Ni, even if its level was decreased in this case by the DAI sludge amendments. As with Al in grains, Ni was detected in roots or stalks only in one or two replicates, and this suggests a high mobility of this metal. Furthermore, it was established that although Ni requirements by plants during the whole growth cycle are very low (200 ng), the metal can readily accumulate in plants, and reach 0.05–5 mg/kg (Hopkins, 1999). Without any sludge amendment, 16.2 mg/kg of Ni was measured in tested soil, i.e., it contained more than the required amounts of Ni (about 81×10^3 times the plants' needs). Slightly more than 4 mg/kg of the metal was detected in leaves of plants grown in control soil, but many sludge amendments led to some decreases, possibly because sludge supplied low amounts of metals, which may have caused a certain dilution of metal in the soil solution. Ni significantly ($Pr \leqslant 0.05$) decreased in leaves following the use of decontaminated BEC sludge. Care must be taken with Ni addition to soil, because at low doses, leaf chlorosis may occur, while at high rates and when in prolonged contact with roots, necrosis of maize leaves and loss of chlorophylls may result (Baccouch et al., 1998). In the present study, there was no chlorosis, nor any necrosis of leaves. Moreover, an addition of 536 kg Ni/ha is reported to increase its accumulation in maize stalks and grains by 1 mg/kg (Webber, 1988). The highest amount of Ni added was 1.43 kg/ha (with decontaminated BEC sludge), but this treatment decreased Ni content of grains by 0.23 mg/kg, compared to control soil, and did not increase metal in stalks. Ni toxicity level can range from 10 to 100 mg/kg, depending on the plant (Kabata-Pendias and Pendias, 2001), but much lower concentrations were observed in the present study. As mentioned above, Ni accumulated mostly in leaves, without exceeding 5 mg Ni/kg. The transfer coefficients of metal were also very low, especially for grains and roots, but attained 0.4 for leaves. Ni/Fe ratios, which have been reported to be more indicative of Ni toxicity than metal concentration (Kabata-Pendias and Pendias, 2001), were close to 0.02 with all treatments.

Pb was retrieved mostly in stalks and leaves, but generally not in roots or grains. Some sludge amendments increased Pb level in leaves, while others decreased it, but variations remained insignificant. Our results differ from previous studies that reported finding Pb stored in roots, at levels that increased parallel to its concentration in the soil solution. Poor solubility and mobility of metal (Henning et al., 1999) makes its translocation to the aerial parts very limited (Kabata-Pendias and Pendias, 2001), and only 0.45% of Pb input supplied by a given sludge may transfer to plants (Tlustos et al., 2001). The maximum rate of metal added to soil in the present study (1.49 kg Pb/ha or 42 μg Pb/pot) was supplied by the decontaminated MUC sludge at a high dose (160 kg/ha N_{av}). However, the quantity of Pb corresponding to that amendment accumulated in leaves alone reached 2300 μg, exceeding the supplied amount of 42 μg. This suggests that there was an external source of Pb contamination, possibly the insecticide sprayed a few weeks before harvesting the plants, or contamination of the medium during analyses of plants. As with Al and Ni, Pb was detected in roots, stalks or grains only for one or two replicates out of six. Moreover, the highest level of Pb input into soil (1.49 kg/ha) is still extremely low, compared to the rate of 450 kg Pb/ha reported to cause no significant impact on metal uptake by maize (Webber, 1988). Furthermore, the bioavailability of Pb should be reduced by the presence of high P levels (Sabey, 1980; Akrivos et al., 1999), since the METIX-AC process significantly ($Pr \leqslant 0.05$) increased that nutrient in decontaminated sludge. Overall, Pb content in leaves (a maximum of 3.20 mg/kg) did not exceed toxicity limits of 30–300 mg/kg (Henning et al., 1999). Moreover, the transfer coefficients of Pb were below the normal range of 0.01–0.05 (Henning et al., 2001), except for leaves, where they reached 0.18.

In addition to the previous discussion of the individual effects and dual interaction of metals and their accumulation in maize tissues, it is important to consider more complex inter-metallic reactions. Such interaction can multiply the individual effects of metals in soil (Beckett and Davis, 1982), which may lead to conditions of toxicity. The "*Zn equivalent*" principle (Sabey, 1980), which consists of summing the concentrations of "$1^*[Zn] + 2^*[Cu] + 8^*[Ni]$", is a useful interpretive tool. At a soil pH of 6.5, there should be no toxicity conditions if this sum is below 250 mg/kg. Similarly, Beckett and Davis, 1982 found no elevation of Cu, Ni and Zn toxicities if the concentration of each metal did not exceed its individual toxicity limit by 50–60%. Application of either the "*Zn equivalent*" principle or Beckett and Davis's findings to current data confirmed that addition of non-decontaminated as well as decontaminated sludge did not result in toxic conditions.

Overall, it was demonstrated that when amending soil with decontaminated rather than non-decontaminated sludge, the risk of soil enrichment by metals and the uptake of these chemicals by plants can be minimized. For example, decontamination of MUC and QUC sludge prior to their spreading may decrease Cd input into soil by 19% and 38%, respectively. Similarly, decontamination can reduce the uptake of Zn from BEC sludge by up to 50%. Data from the present study show that the use of decontaminated sludge actually decreased the uptake of several metals by roots and their ultimate translocation toward aerial parts of maize, compared to controls and to treatments using non-decontaminated sludge. This was due not only to the lower metal content in decontaminated sludge, but likely also to additional factors, such as the presence of higher P levels in the decontaminated sludge, which are known to limit the availability of metals (Sabey, 1980; Akrivos et al., 1999).

5. Conclusions

MUC, QUC, BEC and DAI sludge (non-decontaminated versus decontaminated), each of a different origin, were tested for use in maize cultivation in a greenhouse experiment, and the subsequent accumulation of metals was studied. Sludge was decontaminated by a novel process, the METIX-AC, developed in our laboratory. The experiment considered two sludge application rates as well as non-amended and inorganically amended soils having a N rate similar to that of sludge.

Decontaminated sludge stimulated maize growth better than did soil and fertilization controls, and results were similar to, or often better than those of the non-decontaminated sludge. The METIX-AC process decreased metal content far more than nutrient levels. Some beneficial elements (P, S and Fe) were increased, because of the chemicals added during decontamination. Consequently, decontaminated MUC, QUC and BEC sludge supplied soil with up to 17% of P, while metal input, e.g., Zn, decreased by up to 55%. Many of the selected biometric parameters, biomass and the produced grains were positively affected by dose enhancement or decontamination of most of the sludge. Regarding metal accumulation, decontaminated sludge significantly ($Pr \leqslant 0.05$) increased just Mn and Cr, respectively, in stalks and leaves and in stalks only. Other metal levels decreased, especially non-essential metals. Most of the significant ($Pr \leqslant 0.05$) effects of enhancing sludge dose reflected as increases in the accumulation of many essential metals. Overall, metal levels in all maize parts were inferior to toxicity limits, and transfer coefficients were low.

Decontaminated BEC sludge had the greatest impact on mid growth parameters (appearance of male and female organs, size of spikes), biomass production (ratio of aerial parts/roots weights), and yield (total number and weight of grains). This was probably due to the fact that BEC sludge supplied the lowest C/N ratio to soil. DAI sludge was the least effective treatment, both for maize growth and bioaccumulation of metals. This is probably due to the poor quality of that sludge and/or to its physical structure, which accentuated water drainage and consequently leaching of nutrients.

Field studies will be required to establish the effectiveness of the METIX-AC process for producing safer biosolids. Experiments on metals speciation, before and after sludge decontamination, as well as the use of naturally and artificially loaded sludge, could suggest useful guidelines for the agricultural recycling of these biosolids.

Acknowledgements

The present work has been conducted with the financial support of the National Sciences and Engineering Research Council of Canada, FCAR (fellowship program) and Biolix Corporation. Special thanks are addressed to Gabriel Teodorescu, horticulturist at the Montréal Botanical Garden for his supervision of the experiment, to Danielle Leblanc for her technical help and to Stéphane Daigle for his assistance with statistical analysis.

References

Akrivos, J., Kouloumbis, P., Rigas, F., 1999. Sewage sludge application on cotton and corn crops. Proceedings of the Specialized Conference on Disposal and Utilization of Sewage Sludge: Treatment Methods and Application Modalities, 13–15 October 1999. IAWQ-ECDG XIII, Athens, Athens, Greece, pp. 644–650.

APHA, AWWA, WPCF, 1999. Standard Methods for Examination of Water and Wastewaters, 20th ed. American Public Health Association, Washington, DC.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 (1), 1–15.

Baccouch, S., Chaoui, A., Elferjani, E., 1998. Nickel toxicity – effects on growth and metabolism of maize. J. Plant Nutr. 21, 577–588.

Barraoui, D., Labrecque, M., Blais, J.F., 2007. Decontamination of sludge by the METIX-AC process. Part I: effects on sludge quality and leaching of chemicals. Bioresource Technol., in press, doi:10.1016/j.biortech.2007.01.052.

Beckett, P.H.T., Davis, R.D., 1982. Heavy metals in sludge – are their toxic effects additive. Water Pollut. Control 81, 112–119.

Blais, J.F., Tyagi, R.D., Auclair, J.C., 1992. Bioleaching of metals from sewage sludge by sulfur-oxidizing bacteria. J. Environ. Eng. Div. ASCE 118 (5), 690–707.

Blais, J.F., Meunier, N., Sasseville, J.L., Tyagi, R.D., Mercier, G., Hammy, F., 2004. Hybrid chemical and biological process for decontaminating sludge from municipal sewage sludge. US patent, No. 10,060,277.

Boukhars, L., Rada, A., 2000. Plant exposure to cadmium in Moroccan calcareous salty soils treated with sewage sludge and wastewaters. Environ. Technol. 21, 641–652.

Brady, N.C., Weil, R.R., 2002. The Nature and Properties of Soils. Prentice Hall, Upper Saddle River, New Jersey, USA, 881 p.

Brown, S.L., Henry, C.L., Chaney, R., Compton, H., DeVolder, P.S., 2003. Using municipal biosolids in combination with other residuals to restore metal-contaminated mining areas. Plant Soil 249, 203–215.

Chan, L.C., Gu, X.Y., Wong, J.W.C., 2003. Comparison of bioleaching of heavy metals from sewage sludge using iron- and sulfur-oxidizing bacteria. Adv. Environ. Res. 7 (3), 603–607.

Chipasa, K.B., 2003. Accumulation and fate of selected heavy metals in a biological wastewater treatment system. Waste Manag. 23 (2), 135–143.

Chlopecka, A., Adriano, D.C., 1996. Mimicked in situ stabilization of metals in a cropped soil – bioavailability and chemical form of zinc. Environ. Sci. Technol. 30, 3294–3303.

Cho, K.S., Ryu, H.W., Lee, I.S., Choi, H.M., 2002. Effect of solids concentration on bacterial leaching of heavy metals from sewage sludge. J. Air Waste Manag. Assoc. 52 (2), 237–243.

Couillard, D., Mercier, G., 1991. Optimum residence time (in CSTR and airlift reactor) for bacterial leaching of metals from anaerobic sewage sludge. Water Res. 25, 211–218.

CPVQ Inc., 1997. Le maïs est encore une production d'avenir. In: Compte-rendu de la Conférence du Salon de l'agriculteur, Auberge des seigneurs, Saint-Hyacinthe, Canada.

Desjardins, M.A., Brière, F.G., 1994. Caractérisation de boues d'étangs aérés facultatifs. Sci. Tech. Eau 7 (4), 45–56.

Donahue, R.L., 1958. Soils; An Introduction to Soils and Plant Growth. Prentice-Hall, Englewood Cliffs, NJ.

Duvaud, E., Mugnier, E., Gazzo, A., Aubain, P., Wiart, J., 1999. Situation du recyclage agricole des boues d'épuration urbaines en Europe et dans divers pays du monde. ADEME Editions, Paris, France, 134 p.

EEA, 1999. Waste generation and management. In: Environment in the European Union at the turn of the century. European Environmental Agency, Copenhagen, Danemark, pp. 204–226.

Environnement Québec, 2004. Guide sur la valorisation des matières résiduelles fertilisantes. Critères de référence et normes réglementaires. Environnement Québec, Direction du milieu rural, Québec, Canada, 127 p.

Ferretti, M., Ghisi, R., Merlo, L., Dallavecchia, F., Passera, C., 1993. Effect of cadmium on photosynthesis and enzymes of photosynthetic sulphate and nitrate assimilation pathways in maize (*Zea mays L*). Photosynthetica 29, 49–54.

Henning, B.J., Snyman, H.G., Aveling, T.A.S., 1999. The cultivation of Maize (*Zea mays L.*) on high sewage sludge dosages at field scale. In: Proceedings of the Specialized Conference on Disposal and Utilization of Sewage Sludge: Treatment Methods and Application Odalities, 13–15 October 1999, IAWQ-ECDG XIII, Athens, Greece, pp. 453–459.

Henning, B.J., Snyman, H.G., Aveling, T.A.S., 2001. Plant–soil interactions of sludge-borne heavy metals and the effect on maize (*Zea mays L.*) seedling growth. Water South Africa 27, 71–78.

Hernandez, T., Moreno, J.I., Costa, F., 1991. Influence of sewage sludge application on crop yields and heavy metal availability. Soil Sci. Plant Nutr. 37, 201–210.

Hopkins, W.G., 1999. Introduction to Plant Physiology, 2nd ed. Wiley, New York, NY.

Jarausch-Wehrheim, B., Mocquot, B., Mench, M., 1999. Absorption and translocation of sludge-borne zinc in field-grown maize (*Zea mays L*). Eur. J. Agr. 11, 23–33.

Jarausch-Wehrheim, B., Mocquot, B., Mench, M., 2000. Distribution of sludge-borne manganese in field-grown maize. Commun. Soil Sci. Plant Anal. 31, 305–319.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants, 3rd ed. CRC Press, Boca Raton, FL.

Kim, S.O., Moon, S.H., Kim, K.W., Yun, S.T., 2002. Pilot scale study on the *ex situ* electrokinetic removal of heavy metals from municipal wastewater sludge. Water Res. 36 (19), 4765–4774.

Labrecque, M., Teodorescu, T.I., Daigle, S., 1995. Effect of wastewater sludge on growth and heavy metal bioaccumulation of two *salix* species. Plant Soil 171, 303–316.

Lombardi, A.T., Garcia, O., 2002. Biological leaching of Mn, Al, Zn, Cu and Ti in an anaerobic sewage sludge effectuated by *Thiobacillus* *ferrooxidans* and its effect on metal partitioning. Water Res. 36 (13), 3193–3202.

Martinez, G.A., Guzman, J.L., Vazquez, M.A., Rivera, L.E., Gonzalez, A., 1999. Chemical and physical properties of two tropical soils treated with sewage sludge compost. J. Agr. Univ. Puerto Rico 83, 103–121.

Mazen, A.M.A., 1995. Assessment of heavy metal accumulation and performance of some physiological parameters in *Zea mays* L. and *Vicia faba* L. grown on soil amended by sewage sludge resulting from sewage water treatment in the state of Qatar. Qatar Univ. Sci. J. 15, 353–359.

Mercier, G., Blais, J.F., Hammy, F., Lounès, M., Sasseville, J.L., 2002. A decontamination process to remove metals and stabilize Montreal sewage sludge. Sci. World J. 2, 1121–1126.

Naoum, C., Fatta, D., Haralambous, K.J., Loizidou, M., 2001. Removal of heavy metals from sewage sludge by acid treatment. J. Environ. Sci. Health Part A Toxic Hazard. Substan. Environ. Eng. 36 (5), 873–881.

Nemati, M.R., Caron, J., Gallichand, J., 2000. Stability of structural form during infiltration: laboratory measurements on the effect of de-inking sludge. Soil Sci. Soc. Am. J. 64, 543–552.

Pellerin, S., Mollier, A., Plenet, D., 2000. Phosphorus deficiency affects the rate of emergence and number of maize adventitious nodal roots. Agr. J. 92, 690–697.

Pigozzo, A.T.J., Gobbi, M.A., Lenzi, E., Luchese, E.B., 2000. Effects of the application of sewage sludge and petrochemical residue in maize culture as source of micro-nutrients on soils of Parana state. Braz. Arch. Biol. Technol. 43, 143–149.

Rascio, N., Dallavecchia, F., Ferretti, M., Merlo, L., Ghisi, R., 1993. Some effects of cadmium on maize plants. Arch. Environ. Contamin. Toxicol. 25, 244–249.

Sabey, B.R., 1980. The use of sewage sludge as a fertilizer. In: Handbook of Organic Waste Conversion. Van Nostrand Reinhold Company, New York, NY.

SAS Institute Inc., 1989. SAS/STAT user's guide. Release 6, vol. 1, 4th ed. SAS Institute Inc., Cary, NC.

Simon, D., Helliwell, S., 1998. Extraction and quantification of chlorophyll A from freshwater green algae. Water Res. 32 (7), 2220–2223.

Smith, R., Vasiloudis, H., 1991. Importance, determination and occurrence of inorganic chemical contaminants and nutrients in South African municipal sewage sludge. Water South Africa 17, 19–30.

Spinosa, L., Vesilind, P.A., 2001. Sludge into Biosolids, Processing, Disposal and Utilization. IWA Publishing, Alliance House, London, 424 p.

Tlustos, P., Balik, J., Dvorak, P., Szakova, J., Pavlikova, D., 2001. Zinc and lead uptake by three crops planted on different soils treated by sewage sludge. Rostlinna Vyroba 47, 129–134.

Tyagi, R.D., Blais, J.F., Auclair, J.C., 1995. Simultaneous sludge digestion and metal leaching in semi-continuous process. US patent, No. 5,454,948.

USEPA, 1990. ASCII Format Databases for the 1988 National Sewage Sludge Survey. United States Environmental Protection Agency, Cincinnati, Ohio, EPA PB93-500403.

USEPA, 1993. Standards for the use and disposal of sewage sludge. 40 CFR Parts 257, 403 and 503, Final Rule. United States Environmental Protection Agency, Cincinnati, Ohio.

USEPA, 1999. Biosolids generation, use and disposal in United States. Municipal and Industrial Solid Waste Division, Office of Solid Waste. United States Environmental Protection Agency, Washington, DC, EPA530-R-99-009, 81 p.

Van Loon, J.C., 1985. Selected Methods of Trace Metal Analysis: Biological and Environmental Samples. Wiley, New York, NY.

Vetterlein, D., Bergmann, C., Huttl, R.F., 1999. Phosphorus availability in different types of open-cast mine spoil and the potential impact of organic matter application. Plant Soil 213, 189–194.

Webber, M.D., 1988. Contrôle de la concentration des métaux lourds dans les sols après épandage de boues d'égout municipales: l'approche canadienne. Sci. Tech. Eau 21, 45–51.

Xiang, L., Chan, L.C., Wong, J.W., 2000. Removal of heavy metals from anaerobically digested sewage sludge by isolated indigenous iron-oxidizing bacteria. Chemosphere 41, 283–287.