Effect of cleanup of spiked sludge on corn growth biosorption and metal leaching

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ABSTRACT

A chemical leaching process was used for the cleanup of two municipal biosolids (MOS and BES) spiked with Cd, Cu, Zn or their mixture prior to agricultural use. Non-cleaned, cleaned and washed biosolids were compared as soil amendments for corn cultivation in greenhouse. Corn growth, biosorption and metal leaching were measured. Results showed that biosolid amendments tend to produce more aerial biomass. Cleanup and washing of BES biosolid significantly increased total biomass of roots and stalks, respectively. Regarding biosorption of metals, Cd accumulated in roots (0.06-1.13 mg kg^{-1}) and leaves (0.06-0.63 mg kg^{-1}), but not in seeds nor in stalks. Larger amounts of Cu were detected in roots (10.7-18.2 mg kg^{-1}), stalks (1.29-3.78 mg kg^{-1}) and leaves (6.77-20.2 mg kg^{-1}). However, Zn was more accumulated in roots (17.9-74.9 mg kg^{-1}), stalks (6.15-17.1 mg kg^{-1}) and leaves (47.9-90.1 mg kg^{-1}). Whereas Cd and Cu decreased in the order roots > leaves > stalks, Zn decreased from leaves > roots > stalks. Cleanup and washing of MOS and BES biosolids significantly lowered biosorption of Cd (up to 84%), Cu (up to 38%), Zn (up to 63%), and other metals. Concentrations in leachate draining into outlet water varied over time, but on average were moderately low. Significant amounts of metal leached from MOS biosolid. The effects of cleanup and washing of both biosolids on biosorption and leaching depended on the initial metallic charge and the biosolid type.

Keywords

Sludge, Biosolid, Cleanup, Metal, Leaching, Corn

1 INTRODUCTION

Sewage sludge application improves physico-chemical characteristics of soil, such as organic matter content and water holding capacity, and ensures a similar, or even higher yield of cultivated plants, compared to inorganic fertilization [1-2]. A beneficial effect of sewage sludge can be observed even two years after field application, and this technique can also contribute to stabilization of the grain yield of corn [3]. Indeed, sewage sludge contains important amounts of nutrients that are indispensable to plant growth [4] and it generates very little to no environmental impact, if utilized properly [5].

One of the main problems related to the agricultural spreading of biosolid is its high heavy metal content, which may be harmful to plants, animals and humans [6-8]. Once metals are introduced into soil, they may persist there, percolate into leachate or accumulate in plants. Heavy metals can directly or indirectly affect several metabolic processes of plants, such as respiration, photosynthesis, CO_2-fixation and gas exchange [9-10]. High application of sewage biosolids could result in heavy metal uptake by plants and, ultimately, in many health problems in humans [5].

To date, numerous studies have examined the impact of different metals on plants as well as plant parts and functioning. One of the most problematic metals is Cd, which is non-essential and has no recognized metabolic role [12-14]. Figlioli et al. [11] reported a higher sensitivity of *Zea mays* to Cd than Pb. Corn is relatively less tolerant to Cd than ryegrass and cabbage, for example [15]. Yet, An [16] stated that Cd is highly immobilized by roots, and that germination of corn seeds is insensitive to Cd. In contrast, Yang et al. [17] showed that Cd can easily migrate towards corn shoots, which may explain the ultrastructural damage induced by Cd, observed especially in chloroplasts [11]. Cu is another metal whose environmental impact has been widely studied. It is an important constituent of many plant proteins and enzymes, but a high concentration of Cu may cause chlorosis, inhibition of root growth and damage to plasma membranes [18]. Iron deficiency and chlorosis of leaves are known to be direct consequences of Cu toxicity [19]. Corn is relatively sensitive to Cu excess [18,20], but is more tolerant than tomatoes [9]. A third important and widely investigated metal is Zn. It has a central role in the activity of many enzymes [21], and is absorbed by plants as the Zn^{2+} ion [22]. Plants use an in-situ complexation of Zn as a strategy to avoid harmful biochemical processes [23]. Interactions such as synergism and antagonism may exist between metals during their uptake in plants [6,21].

A common way to lower the heavy metal phytoavailability generated by the application of biosolids is to raise soil pH by adding alkaline amendments, for example, by liming biosolids prior to agricultural spreading. However, McBride and Martinez [19] reported that this practice could conjointly cause an increase of total dissolved organic matter, which in turn could enhance leachability of metals. Different types of processes (chemical, biological and electrochemical) have been proposed to eliminate toxic metals from municipal sewage sludge [24-27]. Biosolid cleanup is thus a promising tool to minimize contamination of soils and waters.

The present work used a leaching process, called METIX-AC [28], that can lower the metallic load of municipal biosolids. Two types of biosolids, one non-digested physico-chemical and the other aerobically digested, were sampled from two wastewater treatment plants in the province of Quebec, Canada. The biosolid samples were spiked with Cd, Cu, Zn or a mixture of these metals prior to their application as soil enrichment in corn cultivation. The present paper presents results related to the effect of these biosolid treatments on corn growth, biosorption of metals in plant parts and leaching of these metals into the outlet water.

2 Material and methods

2.1 Soil and sludge characteristics

The soil used in this study, a loamy-sandy soil (USDA classification) [29] with a high organic matter content (10%), originated from the Montreal Botanical Garden. Soil was sieved in order to obtain a uniform grain size (≤ 1 cm diameter). The two municipal sewage sludges tested were a physico-chemical sewage sludge from the Montreal Urban Community (MOS**)** wastewater treatment plant **(**WTP**)** and a biological sewage sludge from the Haute-Bécancour (BES**)** WTP. Their nutritional content is presented in Table 1, and amounts of metals supplied by organic amendments are provided in Table 2. More information about the characteristics of these two sludge (for example, their organic matter content) can be in Barraoui et al. [30].

Table 1 **Total solids (T.S.) and concentrations of nutrients (g kg^{-1}) in soil and sludge**[†]

Conditions tested	**T.S. (%)**	**TKN**	**NH_4^+**	**NO_3^-**	**P**	**K**	**Ca**	**Mg**	**S**
SOIL	81.6	3.00	n/a	n/a	1.04	18.0	17.8	5.72	0.32
MOS-CON	15.6	16.3	1.40	0.04	14.6	2.63	52.7	6.41	4.77
MOS-CLN	17.8	16.3	0.08 ○	0.03	16.3	4.11	44.9 ○	3.60 ○	48.2 ●
MOS-WASH	18.8	14.7	0.03	0.03	15.1	4.61	51.7	4.27	53.8
MOS-Cd-CON	14.9	14.6	1.04	0.06	12.7	6.68	61.6	7.97	6.19
MOS-Cd-CLN	26.2	11.7 ○	0.13 ○	0.01 ○	12.8	4.40	53.9 ○	3.97 ○	55.5 ●
MOS-Cu-CON	15.3	15.8	0.79	0.05	15.4	5.40	55.5	7.15	5.84
MOS-Cu-CLN	21.8	12.0 ○	0.10 ○	0.03	13.7 ○	3.98	55.7	4.22 ○	57.1 ●
MOS-Zn-CON	15.3	9.80	0.74	0.04	11.5	3.84	53.8	6.64	5.16
MOS-Zn-CLN	21.0	11.0	0.10 ○	0.02 ○	10.5	4.54	53.6	4.07 ○	58.9 ●
MOS-Mix-CON	16.6	8.91	0.37	0.03	9.96	3.66	58.0	7.04	6.13
MOS-Mix-CLN	20.3	13.2 ●	0.11 ○	0.03	13.8 ●	2.81	48.7 ○	3.55 ○	52.7 ●
MOS-Mix-WASH	20.4	12.0	0.03	0.02	10.5	3.46	49.7	3.78	51.8
C.V. (%)[‡]	n/d	6	4	9	5	9	7	7	6
BES-CON	9.78	47.2	2.96	0.10	25.0	3.24	7.69	9.54	6.46
BES-CLN	18.5	45.8 ○	1.11 ○	0.11	25.0	0.72 ○	1.05 ○	7.04 ○	14.5 ●
BES-WASH	18.4	51.7	0.10 □	0.06	26.7	0.44	0.79 □	8.00	13.9 □
BES-Cd-CON	8.68	30.5 ○	2.70	0.10	18.4	3.11	7.76	9.48	6.11
BES-Cd-CLN	23.7	28.6	0.23 ○	0.03 ○	16.9	0.70 ○	1.17 ○	8.74 ○	14.1 ●
BES-Cu-CON	8.66	32.2	3.12	0.05	16.3	3.00	8.56	11.0	6.33
BES-Cu-CLN	20.6	26.6 ○	0.26 ○	0.03 ○	14.4	0.69 ○	1.19 ○	7.91 ○	14.3 ●
BES-Zn-CON	9.98	32.8	4.55	0.04	17.6	2.70	7.72	10.4	5.70
BES-Zn-CLN	22.9	26.7 ○	0.26 ○	0.04	14.0	0.54 ○	1.10 ○	8.18 ○	15.1 ●
BES-Mix-CON	8.53	31.6	2.04	0.04	13.8	2.65	7.71	10.6	5.80
BES-Mix-CLN	20.4	30.3 ○	0.35 ○	0.03	17.9	0.72 ○	1.13 ○	8.19 ○	14.7 ●
BES-Mix-WASH	22.9	35.0	0.13 □	0.03	22.7	0.82	0.64 □	7.72	12.6 □
C.V. (%)[‡]	n/d	6	4	27	11	9	7	5	5

[†] Light and dark circles (○,●) indicate respectively significant decreases and increases, due to sludge decontamination. Light squares (□) indicate significant decreases, due to sludge washing. ‡ C.V. (coefficient of variation): to avoid overabundance of data, average concentrations of each element in the sludge group are presented. Significant effects observed for $p>F$, with F ranging from <0.0001 to 0,0409.

Table 2 Total solids of the maize parts at harvest (%)[†]

Conditions tested	Roots	Stalks	Leaves
SOIL	50.7	28.8	81.9
CHEM	59.4	29.3	67.8
MOS-CON	62.3	24.5	81.4
MOS-CLN	56.8	24.8	68.3
MOS-WASH	62.1	19.9	85.0
MOS-Cd-CON	62.2	21.1	79.2
MOS-Cd-CLN	53.4	23.8	80.9
MOS-Cu-CON	61.5	30.3	74.1
MOS-Cu-CLN	58.2	20.5	82.9
MOS-Zn-CON	59.0	22.0	74.0
MOS-Zn-CLN	62.8	27.0	77.8
MOS-Mix-CON	65.4	24.5	78.3
MOS-Mix-CLN	57.4	26.9	74.6
MOS-Mix-WASH	63.6	25.0	71.5
BES-CON	54.7	25.2	70.9
BES-CLN	57.8 ●	18.0	82.8
BES-WASH	60.0	29.4 ■	76.7
BES-Cd-CON	57.3	23.4	75.2
BES-Cd-CLN	70.1 ●	25.0	84.2
BES-Cu-CON	60.3	24.7	82.4
BES-Cu-CLN	64.6 ●	22.1	78.4
BES-Zn-CON	64.0	18.7	80.3
BES-Zn-CLN	65.3 ●	25.2	82.0
BES-Mix-CON	48.4	15.4	85.3
BES-Mix-CLN	62.0 ●	20.9	76.4
BES-Mix-WASH	68.2	30.7 ■	78.5

[†] Dark circles (●) and squares (■) indicate significant increase in the effects of decontamination and decontamination-washing of sludge, respectively. Significant effects observed for p>F, with F ranging from 0.002 to 0.0213.

2.2 Sludge spiking procedure

The objective of spiking was to determine whether our leaching process, described below, was able to clean highly loaded biosolids. For this purpose, MOS and BES biosolids were spiked individually with Cd (as cadmium nitrate), Cu (as copper chloride), Zn (as zinc chloride) or a mixture of these three metals. Additions were performed in such a way as to reach nominal concentrations of about 100, 3 000 and 5 000 mg kg^{-1}, respectively of Cd, Cu and Zn in biosolid. Following these additions, and prior to cleanup and washing, biosolids were stored and intermittently stirred for 48 h at ambient temperature, in order to redistribute exogenous metals.

2.3 Sludge cleanup and washing

Samples were collected from raw biosolids and were either spiked with metals or left in their original condition. They were subsequently cleaned using the METIC-AC process. Some of these samples were also washed. The non-cleaned biosolid treatments were compared to the cleaned and washed biosolid treatments during the present experiment.

Metals were leached from biosolids using H_2SO_4 and strong oxidants ($FeCl_3$ and H_2O_2). The efficiency of the leaching process was tested in a pilot experiment at the MOS WTP [31]. Extensive details concerning this leaching process are provided in the patent [28].

Biosolid washing, a supplementary step following the biosolid cleanup, was performed to determine its effectiveness for eliminating metals that were clustered in the interstitial water of the cleaned biosolid. Due to experimental and laboratory limitations, biosolid washing was not performed in the case of individual-metal spiked sludge. It was applied only to the non-spiked, cleaned biosolid and to the fully spiked, cleaned biosolid. Once all biosolids were ready, they were stored in a refrigerated chamber at 4°C for two weeks until they were mixed with soil.

Since the pH of cleaned and cleaned-washed biosolid was too low (pH < 3), lime was added to the biosolids before mixing with soil. This resulted in a neutral pH, which is a condition common to soil and non-cleaned biosolids.

2.4 Codification of sludge amendments

In the text and Tables, codes are used to represent the various biosolid amendments: **CON** (non-cleaned), **CLN** (cleaned), **WASH** (washed). For each of the tested biosolids (**MOS** and **BES**), the twelve amendments are referred to as:

- **CON, CLN, WASH**: non-cleaned, cleaned and cleaned-washed biosolids, respectively not previously spiked;
- **Cd-CON, Cd-CLN**: non-cleaned and cleaned biosolids spiked with Cd;
- **Cu-CON, Cu-CLN**: non-cleaned and cleaned biosolids spiked with Cu;
- **Zn-CON, Zn-CLN**: non-cleaned and cleaned biosolids spiked with Zn;
- **Mix-CON, Mix-CLN, Mix-WASH**: non-cleaned, cleaned and cleaned-washed biosolids previously spiked with the three metals mixture.

A total of 24 different types of biosolid amendments of soil were tested.

2.5 Experimental design and soil amendments

The experimental design adopted was a randomized block, with a total of 130 pots consisting of 26 treatments replicated five times each. In addition to the 24 biosolid amendments described above, two controls were prepared: a non-amended soil (**SOIL**), and a chemically fertilized soil (**CHEM**). The following chemicals were mixed for the chemical fertilization: NH_4NO_3 (34-0-0), $Ca(H_2PO_4)$ (0-46-0) and K_2SO_4 (0-0-50) to ensure N, P and K requirements were met, respectively.

Culturing medium was prepared using plastic pots (29.5 cm diameter, 30 cm height, and a loading capacity of 7-8 kg) filled with a stratum of gravel enabling leachate to percolate easily. Pots were then packed up to 5 cm below the rim only with soil (in the case of the **"SOIL"** and **"CHEM"** treatments), or only to two-thirds of pot height in the case of biosolid amendments. In the latter case, 2 kg of soil-biosolid mixture was spread on the remaining slightly less than one-third of pot height . An arbitrarily fixed amount of 45 g (dry mass basis) of biosolid was used.

Depending on the nutritional content of each sludge type (Table 1), and in order to avoid differences in corn growth potentially caused by variable nutrient availability, calculated amounts of inorganic fertilizers were added to each biosolid amended pot. The same chemicals that were used in the **"CHEM"** treatment were used here. As was recommended by a previous study [32] dealing with the nutrient requirements for optimum corn growth, exact quantities of the fertilizers were added to the biosolid-soil mixtures, in order to reach the equivalent levels of 160, 110 and 150 kg ha^{-1}, respectively of N, P and K.

2.6 Corn cultivation and harvesting

The corn was cultivated inside a climate-controlled greenhouse over a period of fourteen weeks. The corn hybrid G-4011 was selected, because it is suited to the weather conditions of the study zone (thermal needs: 2 500 degrees' day). Corn seeds were sown in plastic pots either in the presence or absence of biosolid. During the first week following sowing, seed germination was observed, and twelve weeks later, corn was harvested and divided into its constituent parts: cobs, stalks, leaves and roots. After weighing each plant part, the materials were oven dried overnight at 105°C to determine dry biomass. Subsequently, concentrations of P, K, Al, Ca, Cd, Cu, Cr, Fe, Mn, Na, Ni, Pb, S and Zn in each plant part were quantified. The harvested roots were very carefully cleaned of soil, and washed in an ultra-sound bath using demineralized water. This operation was repeated until roots appeared dirt-free. Finally, excess water was eliminated with "Kimwipes" paper, and the clean roots were air dried before being weighed and oven dried.

2.7 Irrigation scheduling and water sampling

To ensure plant irrigation requirements, demineralized water was delivered by a programmed computer-drip system. Pots containing corn plants were placed on three metallic supporting tables. All of the 130 pots were equally and regularly irrigated to maintain soil at the field capacity of water. To ensure maintenance of humidity levels, pots were verified daily using a manual hydrometer.

A "control drip" was continuously let into a separate container for two purposes: first, to check the accuracy of data collected from the previously mentioned computer-drip system in terms of the amount of entering water, and second to collect a representative sample of irrigation water.

Each week, a sample of water was taken from the "control containers" and stored at 4°C until analysis.

To collect percolating water, a plastic container was hung under each pot. Pots were set in polyethylene saucers, which were side-pierced to allow excess irrigation water to leach through a pipe inserted into the resulting holes. This system allowed water leaching from each pot to drop into the saucers, then into the hanging plastic containers. Twice a week, leaching water volumes were measured with a graduated cylinder. Following this quantification, samples of water were stored in small bottles, and conserved immediately at 4^{o}C. To constitute a composite sample, aliquots of drained water taken during three successive weeks were mixed to the corresponding leaching water volume mentioned above. Given that the total amount of irrigation and leachate was known, it was possible to estimate the portion of water lost from each pot during a given period of time.

At the end of each period, the collected composite samples of leaching water and sampled irrigation water were filtered and filtrates were subsequently analyzed for the elements described below.

2.8 Analytical

Ammonia and nitrate in the biosolid were measured by flow injection analysis colorimetry with a QuickChem FIA+, 8000 Series+ apparatus (Lachat Instruments Inc., Milwaukee, WI) (Colorimetric methods QuickChem 10-107-06-2-B (NH_4^+) and QuickChem 10-107-04-2-A (NO_3^--NO_2^-)) [33]. After acid digestion, Total Kjeldahl Nitrogen (TKN) was quantified (method: 4500-Nitrogen (organic) B) [34].

The metal contents (Al, Cd, Cu, Cr, Fe, Mn, Na, Ni, Pb and Zn) as well as Ca, Mg, P, K and S in biosolid and soil were also acid-digested, before measurement (method No. 30301) [34] using ICP-AES (Varian apparatus, Vista model). Certified samples RTS-3 (CANMET, Canadian Certified Reference Materials Project (CCRMP)) were used.

Water pH was analyzed with a Fisher ACUMET model 915 pH-meter (double-junction Cole-Palmer electrode with a Ag/AgCl reference cell). Ammonium and nitrate were quantified using a LACHAT auto-analyzer (Colorimetric methods QuickChem 10-107-06-2-B (NH_4^+) and QuickChem 10-107-04-2-A (NO_3^--NO_2^-)).

The concentrations of the metals Al, Cd, Cu, Cr, Fe, Mn, Na, Ni, Pb and Zn and the nutrients Ca, Mg, P, K and S in water and in the different corn parts were determined by atomic absorption with a simultaneous Varian ICP-AES, Vista model. Quality controls were ensured by using certified liquid samples (multi-elements standard, catalog number 900-Q30-002, lot number SC0019251, SCP Science, Lasalle, Quebec) to certify conformity between measurement apparatus.

Detection limits for Cd, Cu and Zn in soil and biosolid were respectively equal to 0.03, 0.12 and 0.18 mg kg^{-1}. In the case of leaching water, limits were equal to 0.02, 0.3 and 0.2 µg L^{-1}, respectively for Cd, Cu and Zn. The detection limits for Cd, Cu and Zn in the corn tissues were respectively 0.04, 0.27 and 0.09 mg kg^{-1}.

2.9 Statistical analysis

Analysis of variance (ANOVA) was conducted on all variables. The model measured the importance of the following effects: biosolid type (MOS or BES), spiking with metal(s) (none,

+Cd, +Cu, +Zn or +Mix) and treatments (biosolid cleanup and washing). The effect of cleanup was tested for all origins and levels of spiking, but the effect of washing concerned only non-cleaned biosolid samples which were not spiked and those which were fully-spiked (+Mix). Biosolids spiked with an individual metal (Cd, Cu or Zn) were excluded from statistical analysis of the biosolid washing effect, because they were only cleaned, and not washed. Overall, in cases where significant effects were obtained, data means were matched using Tukey's HSD. All statistical calculations were performed using SAS (Statistical Analysis Software) version 8 [35].

3 Results

Except for the nutritional status of biosolid, the remaining data that are presented and discussed are related specifically to the three heavy metals currently tested (Cd, Cu and Zn) and to some other metals for which concentrations were significantly affected by one or both of the cleanup and washing processes.

3.1 Nutritional contents of sludge

The nutrient contents of soil and sludge are presented in Table 1. The results show that soil contained high amounts of TKN, K and Ca, but low levels of P, Mg and S. Total solids were higher in MOS than in BES biosolid. However, nutritional elements were more concentrated in MES biosolid. Nitrogen compounds, particularly TKN, were 2-3 times higher in BES biosolid.

Sulfur increased in both cleaned biosolids due to the chemicals introduced in the medium during the application of the leaching process. Ca, NH_4^+ and Mg were significantly reduced following cleanup of most of the MOS and BES biosolids. Cleanup significantly lowered the levels of TKN, NO_3^- and K in most types of BES biosolid. TKN decreased only after cleanup of MOS biosolid spiked with Cd or Cu, and increased with metal mixture-spiking. Finally, the decrease of NO_3^- level in MOS biosolid was related to Cd- and Zn-spiking. In terms of percent of leaching, there were greater losses of NO_3^- and NH_4^+ 25-70% and 63-94%, respectively. Similarly, 39-59% of Mg was removed from cleaned MOS biosolid, and 73-80% of K and 85-86% of Ca from cleaned BES biosolid. In the remaining cases, no more than 26-28% of nutrients leached following the cleanup of the tested biosolid. Regarding the impact of biosolid washing, there was a significant decrease of NH_4^+, Ca and S in the BES biosolid.

3.2 Production of corn biomass

Data not shown concerning biomass yield at harvest indicated that there was no negative effect of biosolid cleanup or biosolid cleanup-washing on the dimensions, or on weights of corn leaves, cobs, stalks and roots. Moreover, there were generally no significant differences between any biosolid amendment and the control soil (SOIL). Although biosolid amendments and chemical fertilization (CHEM) generally produced plants with higher aerial parts/roots ratios (fresh weight basis) than SOIL; only MOS biosolid washing significantly increased this parameter (data not shown). On the other hand, Table 3 shows that cleanup of MOS biosolid had no significant effect on the total solids of roots, stalks and leaves, whereas significant increases in the total solids of roots and stalks were respectively due to the cleanup and washing of BES biosolid.

Table 3 **Concentration of tested metals in harvested maize parts (mg kg^{-1}) †**

Conditions tested	**Cd ‡**		**Cu**			**Zn**		
	Roots	**Leaves**	**Roots**	**Stalks**	**Leaves**	**Roots**	**Stalks**	**Leaves**
SOIL	0.08	0.06	18.2	2.16	7.06	21.3	14.0	61.0
CHEM	0.08	0.08	12.8	2.25	10.8	22.8	13.3	90.1
MOS-CON	0.10	0.09	13.9	2.08	8.74	24.5	11.7	68.0
MOS-CLN	0.11	0.08	12.1	2.37	7.99	22.2	13.2	58.0
MOS-WASH	0.10	0.08	13.2	1.48 □	9.46	27.0	12.0	78.9
MOS-Cd-CON	0.54	0.26	12.4	1.48	7.03	23.2	9.41	55.3
MOS-Cd-CLN	0.26 ○	0.14 ○	12.7	1.94	10.6	32.3	9.48	62.6
MOS-Cu-CON	0.07	0.06	15.8	2.65	7.80	17.9	17.1	58.5
MOS-Cu-CLN	0.16 ●	0.08	13.0	1.88	8.22	25.4	11.6	53.2
MOS-Zn-CON	0.10	0.08	10.7	1.70	7.96	36.6	10.8	54.9
MOS-Zn-CLN	0.10	0.07	14.6	2.26	8.16	24.6 ○	13.2	69.5
MOS-Mix-CON	0.47	0.31	14.0	1.88	7.76	30.9	12.5	73.3
MOS-Mix-CLN	0.15 ○	0.09 ○	14.0	2.30	7.89	23.9	10.6	58.2
MOS-Mix-WASH	0.15	0.08	14.1	1.67 □	10.7	24.5	10.7	57.7
BES-CON	0.13	0.09	15.0	2.06	10.5	33.4	10.6	70.4
BES-CLN	0.24	0.08	13.0 ○	1.55	7.56	41.5	7.96	54.2
BES-WASH	0.09	0.06	12.4	1.75	6.77	31.1	9.32	61.0
BES-Cd-CON	1.13	0.63	15.3	1.69	10.5	23.3	8.84	63.6
BES-Cd-CLN	0.19 ○	0.10 ○	13.8 ○	2.39	10.1	29.6	16.7	75.6
BES-Cu-CON	0.11	0.08	15.2	2.05	9.51	24.9	10.6	59.3
BES-Cu-CLN	0.13	0.08	13.4 ○	1.83	9.92	23.8	8.40	56.4
BES-Zn-CON	0.16	0.08	14.2	1.39	10.5	74.9	8.20	48.8
BES-Zn-CLN	0.09 ○	0.07	10.7 ○	1.89	8.32	31.2 ○	15.0 ●	71.8
BES-Mix-CON	0.95	0.47	15.8	1.31	11.2	65.7	13.7	64.5
BES-Mix-CLN	0.16 ○	0.10 ○	12.1 ○	1.29	9.30	24.1 ○	6.15	47.9
BES-Mix-WASH	0.14	0.09	9.76	3.78	20.2	24.1	13.0	73.5

† Light and dark circles (○,●) indicate respectively significant decreases and increases, due to sludge decontamination. Light squares (□) indicate significant decreases, due to sludge washing. ‡ Cadmium was not detected in stalks and seeds. Significant effects observed for p>F, with F ranging from <0.0001 to 0,0324.

3.3 Biosorption of tested metals in the corn parts

Data provided in Table 4 show that Cd was generally not detected in stalks, and that in the majority of cases Cd and Cu migrated principally to the roots, leading to the decreasing ranking: roots > leaves > stalks. A different behavior was noted with Zn, for which the highest concentration levels were noted in leaves, yielding the following decreasing trend: leaves > roots > stalks.

Cleanup of MOS and BES biosolids spiked with Cd (alone and in metal mixture) significantly lowered levels of this metal in roots and leaves. Similarly, cleanup significantly lowered Cd in roots in BES biosolid spiked with Zn, while an increase occurred in MOS biosolid spiked with Cu. The Cu and Zn contents in leaves were not affected by the cleanup of either biosolid. With BES biosolid, cleanup significantly lowered Cu in roots. The Zn decrease in roots was recorded following cleanup of biosolid spiked with Zn alone in both MOS and BES biosolids and with the metals mixture in the BES biosolid only. In contrast, Zn significantly increased in stalks following cleanup of BES biosolid spiked with Zn alone. There was only one case where biosolid washing had a significant effect, namely on Cu in leaves corresponding to MOS biosolid amendments.

3.4 Biosorption of other metals in the corn parts

All significant effects of biosolid cleanup, noted mostly in BES biosolid, manifested as increases in the concentration of Al, Cr, Fe, Mn and Pb in roots. Washing MOS biosolid resulted in an increase in Pb level in the leaves (Table 4). MOS biosolid cleanup increased Mn and Cr levels in roots, but the Cr increase was restricted to biosolid previously spiked with the metal mixture. In the case of BES biosolid, cleanup significantly increased metals in roots, especially Al in all BES biosolid types, Cr and Pb in biosolid spiked with Cd alone and Fe in biosolid spiked individually with Cd and Zn. Finally, non-spiked MOS biosolid washing significantly increased Pb in leaves.

Table 4 Concentrations of other metals in harvested roots and leaves (mg kg^{-1})†

Conditions tested	Roots					Leaves
	Al	**Cr**	**Fe**	**Mn**	**Pb**	**Pb**
SOIL	1990	22.1	1190	60.4	0.67	2.39
CHEM	1730	30.3	1410	67.2	0.84	2.95
MOS-CON	2070	27.9	1530	61.8	1.15	2.18
MOS-CLN	1460	16.0	1240	74.4 ●	0.77	1.65
MOS-WASH	728	11.8	791	70.4	0.70	2.67 ■
MOS-Cd-CON	1270	11.8	1080	57.6	0.88	3.10
MOS-Cd-CLN	986	18.0	1190	101 ●	1.00	2.41
MOS-Cu-CON	942	18.5	984	44.8	0.77	2.70
MOS-Cu-CLN	875	27.0	1070	97.0 ●	0.73	2.96
MOS-Zn-CON	1700	19.5	1220	60.0	0.84	2.23
MOS-Zn-CLN	1460	23.4	1390	76.0 ●	0.82	2.47
MOS-Mix-CON	1140	5.91	945	50.0	0.69	1.93
MOS-Mix-CLN	2010	24.4 ●	1490	80.0 ●	1.05	2.45
MOS-Mix-WASH	2320	26.8	1710	79.4	1.26	1.88
BES-CON	1160	17.1	1060	99.8	0.78	1.85
BES-CLN	847	16.0	956	93.8	0.89	3.20
BES-WASH	1680	9.15	1260	94.4	1.02	2.54
BES-Cd-CON	719	8.62	658	78.8	0.57	2.28
BES-Cd-CLN	2320 ●	33.7 ●	1950 ●	101	1.42 ●	3.29
BES-Cu-CON	1210	29.9	1310	91.8	0.93	2.30
BES-Cu-CLN	1240 ●	17.1	1290	105	1.05	2.37
BES-Zn-CON	774	24.0	925	105	0.72	2.76
BES-Zn-CLN	2270 ●	36.4	1800 ●	98.8	1.08	1.93
BES-Mix-CON	717	23.8	939	80.4	0.49	2.26
BES-Mix-CLN	1150 ●	12.3	969	74.2	0.69	1.59
BES-Mix-WASH	809	16.3	715	75.2	0.79	2.18

† Dark circles (●) indicate significant increases, due to sludge decontamination. Dark squares (■) indicate significant increases, due to sludge washing. Significant effects observed for p>F, with F ranging from <0.0001 to 0,0455.

3.5 Lixiviation of metals through leachate

The average concentrations of metals in leachate (Table 5) were calculated from data corresponding to the four consecutive periods (P1 to P4). All volumes of the leachate samples were taken into consideration when calculating the average concentrations of metals in solution. Statistical analyses of data were performed on raw data for each separate period. When a significant effect of biosolid cleanup was noted during a given period, it was reported in Table 5 by specifying the period of occurrence in brackets.

The data shown in Table 5 shows that the average concentrations of metals that leached from biosolid amended pots were lower (Al, Cu and Fe) or higher (Mn and Zn) than those of non-amended pots, while similar results were noted for Cd. The pH of leachate samples averaged between 7.64 and 7.98.

Table 5 data also indicates that no significant effects of washing were observed for either biosolid, whereas cleanup affected the metals leaching only from MOS biosolid amended pots. In the case of BES biosolid, only leachate pH significantly increased, from 7.66 to 7.99, during the second period, P2 (data not shown). In the case of MOS biosolid, changes were mostly noted during the first period, P1, where MOS biosolid cleanup led to a decrease of Cu in leachate for all biosolid types, of Zn for biosolid spiked with Cu, of Zn and metal mixture as well as Al and Fe for sludge spiked with the metal mixture. In contrast, MOS biosolid cleanup significantly increased the concentration of Al in leachate during the last period, P4, and of Mn during the first period, P1.

Leaching of metals over time was also studied (data not presented). Findings showed that, for all treatments, leaching of Cu and Zn were similar considering that they were high at the beginning of the experiment, and then gradually diminished with time. An inconsistent decrease in concentration was noted for Cd at the beginning, and for Cu and Zn at the end of the experiment.

Table 5 **Average concentrations of tested and other metals that leached into drainage water** ($\mu g\ L^{-1}$)[†]

Conditions tested	**pH**	**Tested metals**			**Other metals**		
		Cd	**Cu**	**Zn**	**Al**	**Fe**	**Mn**
SOIL	7.88	0.08	50.9	45.9	348	279	19.7
CHEM	7.70	0.10	36.2	40.0	248	202	43.5
MOS-CON	7.72	0.15	43.5	53.8	307	259	43.8
MOS-CLN	7.92	0.10	30.7	34.3	194	152	38.7
MOS-WASH	7.90	0.05	31.6	34.5	147	104	53.6
MOS-Cd-CON	7.82	0.13	32.3	39.2	201	147	43.9
MOS-Cd-CLN	7.73	0.13	34.5	48.4	191	161	76.6
MOS-Cu-CON	7.74	0.10	35.2	43.4	219	174	36.1
MOS-Cu-CLN	7.86	0.05	27.6	30.0 ○ (P1)	168	132	53.6
MOS-Zn-CON	7.64	0.08	39.6	40.1	290	218	20.2
MOS-Zn-CLN	7.75	0.05	32.3	40.4	194	162	35.5
MOS-Mix-CON	7.76	0.08	28.0	36.1	340	270	28.2
MOS-Mix-CLN	7.83	0.03	30.2	28.8 ○ (P1)	167 ○ (P1)	119 ○ (P1)	89.1
MOS-Mix-WASH	7.89	0.08	34.5	56.3	201	176	77.3
Decontamination effect			○ (P1)		● (P4)		● (P1)
BES-CON	7.79	0.13	34.4	45.4	223	191	64.8
BES-CLN	7.84	0.08	35.6	46.4	250	235	97.7
BES-WASH	7.79	0.08	32.9	40.1	226	173	32.5
BES-Cd-CON	7.70	0.03	36.5	32.1	179	135	43.1
BES-Cd-CLN	7.84	0.15	32.7	38.3	204	169	44.7
BES-Cu-CON	7.72	0.08	33.9	35.8	179	144	38.0
BES-Cu-CLN	7.83	0.13	34.2	46.5	232	191	110
BES-Zn-CON	7.84	0.08	32.8	38.0	226	178	54.6
BES-Zn-CLN	7.85	0.13	32.6	39.5	182	152	90.0
BES-Mix-CON	7.92	0.13	29.1	43.7	211	177	77.9
BES-Mix-CLN	7.98	0.08	30.4	45.8	220	170	38.2
BES-Mix-WASH	7.86	0.05	34.0	38.7	194	157	49.7
Decontamination effect	● (P2)						

[†] Light and dark circles (○,●) indicate respectively significant decreases and increases, due to sludge decontamination. The period (P1 to P4) during which the significant effect occurred is in brackets. Average concentrations given in the Table may be similar, but measurements corresponding to a given period can exhibit significant differences. Significant effects observed for p>F, with F ranging from 0.0010 to 0,0468.

4 Discussion

The leaching process lowered the concentrations of the heavy metals in the biosolids, even in the presence of high metal loads. The present data show that although cleanup lowered the nutritional contents of MOS and BES biosolids, satisfactory levels of many nutrients remained, thus allowing the profitable reuse of these biosolids as soil amendments. Cleanup of both raw biosolids (non-spiked) generated biosolids containing higher levels of nutrients than those desired for agricultural spreading of biosolid. These levels (g kg^{-1}, dry mass basis) were satisfactorily reached following cleanup of BES biosolid (TKN > 27, NO_3^- > 0.01, P > 18 and Mg > 5.6) and MOS biosolid (NH_4^+ > 2, K > 2.5 and Ca > 19). Washing of both biosolids had few or no significant effects on the concentration of nutrients, compared to the impact of cleanup.

Among the subsequent effects of the application of sludge containing high levels of metals are the risk of provoking limited plant growth [6], excessive biosorption of metals, which may be transmitted throughout the food chain [14], or even toxicity [36].

Regarding plant development, although not shown, our results indicated that neither the dimensions, nor weights of corn leaves, cobs, stalks and roots were negatively affected by sludge treatment. These data are consistent with those of Szymańska et al. [3], who found that biosolid fertilization, versus mineral fertilization, of corn grown for grain, did not reveal any difference regarding plant growth or development.

In our study, even after spiking MOS and BES biosolids with Cd, Cu and/or Zn, seed germination and plant growth were generally not affected. The effects of cleanup and washing on biosolid were observed mainly as an increase in the aerial parts/roots ratio of weights in the case of cleanup of MOS biosolid or in the total solids of roots and stalks, respectively following cleanup and washing of BES biosolid. The absence of important differences between any biosolid amendment and SOIL in regard to the growth performance of corn plants might be explained by the small amount of sludge that was tested (45 g pot^{-1}: the equivalent of about 7 t ha^{-1}). Although they tested lower sludge rates than ours, Szymańska et al. [3] obtained similar results, when they evaluated an application of 10 t dry mater per ha^{-1} of biosolid once every five years (mean of 2 t ha^{-1}), for field conditions of corn. To obtain significant differences compared to unfertilized soil, one has to test high sludge rates. Ilie et al. [4] observed during an experimental pot study, that sewage sludge fertilization increases corn yield significantly compared to the soil control, where the lowest yield was obtained, when starting with a rate of 200 kg N ha^{-1} (equivalent to 10 t ha^{-1}). Furthermore, Tejada et al. [37] obtained a 17% increase in corn yield with a foliar application of sewage sludge, compared to untreated samples.

The absence of significant differences we observed was probably influenced by the soil richness. Indeed, it appears that biosolid is nutritionally rich and remains able to ensure adequate corn plant development despite treatment. Detailed corn growth data shows that non-cleaned and cleaned biosolids produced strongly similar results. Greater differences between controls and biosolid amendments as well as between cleaned and non-cleaned biosolids could probably be expected if one or both of the following conditions were encountered: the soil tested did not contain such high concentrations of nutrients, and the biosolid samples were larger than 45 g pot^{-1}.

With respect to the uptake and biosorption of metals, it was shown that, in all corn parts, Zn was more concentrated than Cu, which, in turn, was more abundant than Cd. The latter was undetected in seeds and stalks, but was more highly concentrated in roots than in leaves, with a shoot/root ratio equal to 0.33. Cu showed a trend similar to that of Cd, but with a clearer distinction between the metal levels in roots versus leaves. The lowest Cu amount was found in stalks. Data showed that the metal mostly stored in plants was Zn. Leaves had the highest concentration of this metal, followed by roots and stalks. These results indicate that Zn seems to be the most mobile metal, since it appeared to be more easily transferred to aerial parts. Cd and Cu showed the opposite trend, as they were mainly confined to roots. The remarkably higher uptake of Cu by roots, along with limited translocation to shoots, concurs with previous works [9,18,38-39].

The mobility of Zn toward aerial parts of corn was confirmed by Ilie et al. [40], who showed that sewage sludge rates higher than 300 kg N ha^{-1} (equivalent to 15 t ha^{-1} of sludge) resulted in statistically significant increases of Zn content in corn kernels.

When biosolid was spiked with a given metal, its concentration increased in one or more corn plant parts, but this increase was very small in the case of Cu. The use of Cd-spiked MOS biosolid enhanced Cd absorption in roots and leaves, respectively 5 and 3 times. Similar results were obtained when BES biosolid was spiked with Cd and with the metal mixture. In the case of Cd spiking, the uptake of Cd by roots and leaves increased 9 and 7 times, respectively, while the metal mixture caused an increase in Cd uptake by 7 and 5 times, respectively. Such results, showing that Cd uptake is increased by its level in the medium, are supported by the work of Ilie et al. [40], who reported that Cd level in corn leaves increased directly proportional to the rate of sewage sludge application. Also, following the use of Zn-spiked BES biosolid, metal uptake by roots doubled. Thus, it is well established that as the total concentration of a metal in the soil increases, so does the probability of its uptake by roots. However, metal speciation is of great importance for evaluating the uptake of metals by roots as well as their possible biosorption in plant organs [38].

The effects of biosolid cleanup on the uptake and biosorption of the tested metals were far more significant for BES biosolid than MOS biosolid. Cleanup can counter the spiking-induced increase in absorption of metals. More specifically, after cleaning, the uptake of Cd and Zn by roots can be reduced by up to 84% and migration towards leaves can be reduced to 63% for BES biosolid. Although spiking biosolid with Cu (alone or in the metal mixture) did not cause an increase of Cu concentration in a given corn part, cleanup of BES biosolid caused a decrease in the metal's uptake by roots by about 10-25%. Overall, our leaching process significantly lowered the levels of Cd, Cu and Zn in both biosolids to the point that their uptake by roots, and consequently their transfer to a given corn tissue, was greatly diminished. Moreover, cleanup of BES biosolid spiked with Zn led to a decrease in the uptake of Cd and Zn by the roots. Controversy exists, however, in the published results regarding the competition that might exist between metals for uptake by plants, suggesting that Zn may compete with Cd, since they use the same transport site [6] or the difficulty of assessing alleviation of Cd toxicity by application of Zn, due to the role played by nutrients such as P [14]. As is the case for the present work, a high concentration of microorganisms exists in the sludge and in the soil used. It is very likely that these microorganisms can compete with corn, adsorbing a certain amount of metals on their surface or absorbing a small amount in their cells. These processes are among the reactions that can occur between metals and this type of ecosystem. However, it is not possible to rule on the relative importance of these processes compared to other possible mechanisms (example, adsorption of metals on organic matter or clays, or their fixation in a less reactive form such as on silicates), because this was beyond the scope of this work.

More recently, the work of Przygocka-Cyna and Grzebisz [41] showed that any increase in Fe concentration in corn grain resulted in a simultaneous decrease in Cd concentration, attesting to an antagonistic behaviour between these two elements. In fact, Przygocka-Cyna and Grzebisz [41] stated that an increased exogenous supply of Fe results in decreased uptake by plant roots. This was indeed the case in our experiments, where the concentration of Fe increased significantly in both cleaned biosolids, by 13% to 20%, as this metal is added (as ferric chloride) to biosolid during the leaching process [28].

Therefore, the increase in the availability of Cd and Zn observed due to spiking reflects an increase in their uptake by roots and translocation to leaves (Table 3). However, clean-up and washing of BES biosolid lowered the proportion of available fractions, which reduced their content in corn tissue. The increase noted for the available fractions of metals in the cleaned-washed MOS biosolid did not reflect additional biosorption in corn plants. This is probably because other metals competed for the uptake, either Mn, which increased following the clean-up and washing of all MOS biosolid types, or Fe, which increased following the clean-up and washing of several BES biosolid (Table 4), reflecting the antagonism that can occur between Cd and Fe.

The toxicity of cadmium, copper and zinc was analyzed and included comparison of the experimental data obtained in this project with published results [41]. Indeed, one previous experiment, dealing with the effect of our leaching process on the toxicity and bioavailability of metals, showed that the biosolid treatment significantly lowered the biosorption of cadmium, copper, and zinc in several exposed plant species [42]. These findings are similar to those of Al-Busaidi and Mushtaque [5], who showed that no toxicity or excessive biosorption of heavy metals occurred when sewage sludge was treated with green waste (Kala compost) to serve as soil amendment for plant cultivation.

Empirical data showed no significant decrease in root length and biomass production suggesting Cu toxicity [16,18,39,43]. Dry biomass yield of corn was not affected by Zn [44]. Similarly, Cd did not seem to cause a decrease in root and shoot biomass [15,44], and no nutritional deficiency was noted [13]. However, Lagriffoul et al. [45] stated that variations in growth and mineral content of Cd-contaminated corn seedlings are not direct consequences of Cd uptake by plants. Conversely, Figlioli et al. [11] stated that Cd induces ultrastructural damage, which is observed especially in chloroplasts. Contradictory statements reported in the literature concerning the effect of heavy metals on plant development are probably due to differences in experimental conditions. In the present study, the absence of toxic effects can also be linked to the small quantity of biosolid tested, as well as to the fact that bioavailable forms of metals, such as exchangeable fractions, were extremely low, both before and after biosolid cleanup and washing. In this context, it is well known that availability of biosolid-borne metals in soils is relatively low, because these elements are immobilized mainly as oxides [21].

Whether or not toxicity for a metal was detected in the present experiment can also be explained by comparing recorded data to published toxicity thresholds. First, the Cd level we found in any corn part was far below the toxicity limits of 4-30 mg kg^{-1} [46-47]. The phytotoxicity of Cu is very well documented but there are disagreements concerning the toxic threshold of this metal in plant tissues. The threshold can vary widely (20 to 100 mg kg^{-1}), depending on the plant [46-47], but in the specific case of corn, Fageria [48] stated that if the Cu in soil reaches 48 mg kg^{-1}, metal concentration in plant tissues attains a level of 11 mg kg^{-1}, which corresponds to the toxicity onset. Yet, Mocquot et al. [9] and Borkert et al. [20] reported that Cu toxicity to corn occurs only when metal concentration in leaf or root tissues reaches 20-21 mg kg^{-1}. McBride [38] criticized the USEPA risk assessment, which assumes that in biosolid amended soils, Cu toxicity to corn does not occur even when metal concentration in shoots reaches 40 mg kg^{-1}. In our case, Cu toxicity seemed not to occur in the presence of sludge. The highest Cu concentration was observed in roots (18.2 mg kg^{-1}) for SOIL, which initially contained 23.4 mg Cu kg^{-1}. However, all biosolid amendments lowered the Cu concentration in roots, as compared to SOIL, by up to 41%. In the case of Zn, regardless of treatment, the metal concentration in all corn parts was far below the toxicity limits of 400-1000 mg kg^{-1} [46-47].

Regarding the remaining heavy metals measured in corn parts data (not shown) indicated that Mn migrated mostly towards leaves, but was below 400 mg kg^{-1}, far below the toxic level of 2 480 mg kg^{-1} [48]. For example, Ilie et al. [40] showed that Mn increased in leaves, along with the increase of biosolid quantities in pots, while it decreased in the corn kernels. Similar to results reported by Ali et al. [18], Pb was stored more in leaves than in roots, but metal concentration was below the toxicity limits of 30-300 mg kg^{-1}, whereas Cr sometimes exceeded 4-8 mg kg^{-1} [21,47]. To explain the behavior of Pb, we could mention the work of Figlioli et al. [11], who reported the complex pattern of Pb uptake by corn parts when it enters plant roots from Pb-enriched soils, showing little translocation to the aerial parts. Otherwise, increased concentrations of Pb in aboveground tissues can be due to metal-bound dust and fine soil particles entering directly to leaves through stomata [11].

In leachate, no clear effect of BES biosolid cleanup was observed, except for a significant increase in pH. The concentrations of Al, Cd, Cu, Fe, Mn and Zn seemed unaffected when MOS and BES biosolids were washed. The significant increase in pH may have been caused by a large increase of NH_4^+ ions in cleaned BES biosolid (data not shown).

The highest concentrations of Cd, Cu and Zn in leachates from biosolid amended pots were respectively 0.15, 43.5 and 56.3 μg L^{-1}. In comparison, Gray et al. [49] measured the concentration of Cd in leachate from New Zealand pasture soils that were treated repetitively with phosphate fertilizer, and found higher concentrations: 0.35-1.57 μg Cd L^{-1}. Li et al. [50] tested mixtures of soil with unstabilized or stabilized sludge (using phosphorus based products), and measured metals (Cu, Cr and Zn) leaching into the outlet water. Total inputs of metals were lower (for Cu and Cr) or relatively close (for Zn) to our tested loads. However, Li et al. [50] reported that the levels of all three metals, Cu, Cr and Zn, in leachates attained concentrations as high as (in μg L^{-1}) 1 340, 70 and 1 060, respectively. But, we must take into account the differences in the experimental conditions between experiments. Indded, Li et al. [50] did not consider any plant uptake, but only leaching of metals from cylinders filled with biosolid-soil mixtures that were irrigated with distilled water. In our case, cleanup of biosolids as well as corn cultivation were considered, and this probably explains the gap noticed between our results and those of the mentioned research. Nevertheless, it is important to highlight the added value of current leaching process, which removed the most labile fractions of metals from biosolids, prior to their agricultural use. Our results are also supported by the recent finding of Yu et al. [51] who found that combining chemical washing with repeated phytoextraction showed considerable potential for the remediation of agricultural soils polluted with multiple metals.

Although this is beyond the scope of the present work, the metal content of the sampled leachates can be considered in relation to a strict criterion such as the guidelines for drinking water quality, in order to evaluate the risk of groundwater contamination by these leachates. Data showed that average concentrations of most metals in leachate were far below the drinking water quality guidelines of the Canadian government, the World Health Organization (WHO) and the

European Economic Community (EEC). This was particularly the case for tested metals (Cd, Cu and Zn), for which average concentrations in leachates did not exceed 5, 1 000 and 5 000 μg L^{-1}, respectively. The Fe concentration was also far below its safety guideline of 300 μg L^{-1} [52]. The mean concentrations of Fe and Zn were likewise below the respective toxic thresholds of 300 and 180 μg L^{-1}, concentrations at which these metals are detrimental to freshwater fish [53]. This was not the case for Al and Mn, which exceeded their respective limits of 200 and 50 μg L^{-1} [52]. Al content in drinking water is of great concern, because there seems to be a close correlation between the amount of this metal consumed by humans and the incidence of Alzheimer's disease [54]. In this project, the highest Al concentration (348 μg L^{-1}) was obtained for leachates of non-amended pots, but all biosolid amendments decreased the level of this metal by up to 58%. On the other hand, data not shown indicated that Pb was not detected in leachate, and Cr did not exceed the critical value of 50 μg L^{-1} [52]. Finally, data not shown indicated that leaching of the three tested metals (Cd, Cu and Zn) tended to decrease with time, leading to lower concentrations at the end of experiment. Time variation of leaching fluctuated more in the case of Cd than Cu or Zn. It can thus be stated that the quality of water that leached from pots amended with cleaned biosolid is certainly of acceptable quality for the purposes of agricultural irrigation.

The differences observed between MOS and BES biosolids in terms of biosorption and leaching of metals are probably caused by metal speciation in tested biosolids, which were differently affected by our leaching process. However, the speciation and consequent bioavailability of metals depend on many soil parameters, among which pH is of great importance. For example, application of organic matter to an acidic topsoil increases the portion of Zn that is held conjointly by Mn and Fe oxides and diminishes the remaining metal phases. At a higher soil pH, organically complexed Zn predominates and metal associates more with Fe than Mn oxides. In this study, since pH values of all biosolids were adjusted to near neutrality before use, any difference may be attributed to the biosolid matrix, and not the pH of the medium: MOS is physico-chemical biosolid, while BES is biological biosolid. Furthermore, although no measurements of metal activity were performed in this study, since this was beyond the scope of the present work, we agree with the statement of McBride [38], who specified that one has to consider the metal activity in soil solution, rather than the total metal concentration, to evaluate the phytotoxicity of metals. McBride's [38] perspective may serve as a further argument to explain the differences reported above, when comparing our results to those of Li et al. [50], notably with regard to the concentration of metals in water leaching from biosolid amended soils.

5 Conclusion

This study dealt with the effect of spiked versus non spiked biosolids on corn growth as well as on metal biosorption and leaching. Cd, Cu and Zn were added individually or in a mixture to MOS and BES biosolids. Metal concentrations in the four separate parts of corn plants were quantified at harvest: roots, stalks, leaves and seeds.

The biosolids investigated in this project contained levels of nutrients suitable for agricultural use despite having undergone cleanup and washing procedures. Micro-nutrients, such as Fe and S, may be supplied to soils from cleaned biosolid, because the leaching process requires chemicals that contain Fe and S. Results did not show a negative effect of biosolid spiking, cleanup and/or washing on corn seed germination or growth parameters. In comparison to SOIL and non-cleaned biosolid, cleaned biosolid generally enhanced aerial parts/roots ratios. Furthermore, Cd was not detected in the harvested seeds and stalks, while the Cu level was very low. Migration of Cd and Cu followed a decreasing trend: roots > leaves > stalks, while that of Zn decreased in the order: leaves > roots > stalks. The uptake and biosorption of tested metals were affected by their available fractions, which in turn, were influenced by spiking and cleanup-washing. The remaining elements drifted differently in corn parts, and effects of biosolid cleanup and/or washing were mostly related to biosolid spiked with Cd or Cu. Independently of biosolid amendments, leaching of Cu and Zn generally diminished with time, while that of Cd was more randomized. Statistical analysis of the concentrations of metals in leachate showed that most of the changes occurred because of cleanup of the MOS biosolid, while few variations were noted with BES biosolid. Concentrations of measured metals in corn tissues and in leachate were generally below their respective critical limits.

Spiking biosolid with a given metal, either alone or in the metal mixture, led to storage of this element in at least one of the corn parts and/or in leachate, but biosolid cleanup caused a significant decrease in this trend. Interestingly, the restriction imposed by the guidelines of the Quebec government regarding the agricultural spreading of BES biosolid, due to its high Cu content, does not apply if this biosolid is cleaned. In addition, knowing that some soils in the province of Quebec may contain high levels of heavy metals, the use of cleaned rather than non-cleaned biosolid should minimize, or at least delay, the risk of soil enrichment by these metals.

Finally, there are dissimilarities in the ability of MOS and BES biosolids to be cleaned and/or washed by the leaching process. These dissimilarities indicate that the proposed cleanup process depends not only on the initial total metallic charge of biosolid, but also on the biosolid type. Only MOS biosolid washing had few supplemental significant effects on the biosorption and leaching of metals. The present study should be complemented by future agri-environmental investigations in greenhouse and in the field, to compare the short-term and long-term pathways of biosolid-borne metals after spreading biosolid cleaned by our leaching process. Such studies should focus on measuring the ion activity of a given metal instead of its total concentration.

CRediT authorship contribution statement

Barraoui D. contributed to the conception and design of the study and the implementation of the experimental design in the greenhouse. He also collected data and wrote the first draft of the manuscript**.**

Blais JF. is the corresponding author and co-PI. He contributed to the conception and design of the study, and collaborated on the interpretation of the results. He revised the manuscript and prepared its electronic submission.

Labrecque M. was one of the PI involved in the conception and design of the study. He collaborated on the interpretation of the results and revision of the manuscript.

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References

[1] B. Sharma, A. Sarkar, P. Singh, R.P. Singh, Agricultural utilization of biosolids: A review on potential effects on soil and plant grown, Waste Management 64 (2017) 117-132. https://doi.org/10.1016/j.wasman.2017.03.002.

[2] M.C. Collivignarelli, M. Canato, A. Abbà, M. Carnevale Miino, Biosolids: What are the different types of reuse, Journal of Cleaner Production 238 (2019) 117844. https://doi.org/10.1016/j.jclepro.2019.117844.

[3] G. Szymanska, H. Sulewska, K. Smiatacz, Response of maize (Zea mays L.) grown for grain after the application of sewage sludge, Journal of Central European Agriculture 17(1) (2016) 139-153. https://doi.org/10.5513/jcea01/17.1.1682.

[4] L. Ilie, M. Mihalache, R.M. Madjar, C. Calin, G.V. Scaeteanu, Effect of sewage sludge amended soil on maize crop I. Influence on yield and mineral nutrition, Revista de Chimie (Rev. Chim.) 69(3) (2018) 561-566. https://doi.org/10.37358/rc.18.3.6149.

[5] A. Al-Busaidi, M. Ahmed, Treated municipal wastes: Are they contaminating or enriching the soil?, in Book: Soil contamination - Current consequences and further solutions, 2016. https://doi.org/10.5772/64962.

[6] F. Wu, G. Zhang,J. Yu, Interaction of cadmium and four microelements for uptake and translocation in different barley genotypes, Communications in Soil Science and Plant Analysis 34(13-14) (2003) 2003-2020. https://doi.org/10.1081/CSS-120023233.

[7] B.M. Cieślik, J. Namieśnik, P. Konieczka, Review of sewage sludge management:

Standards, regulations and analytical methods, Journal of Cleaner Production 90 (2015) 1-15. https://doi.org/10.1016/j.jclepro.2014.11.031.

[8] A. Raheem, V.S. Sikarwar, J. He, W. Dastyar, D.D. Dionysiou, W. Wang, M. Zhao, Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: A review, Chemical Engineering Journal 337 (2018) 616-641. https://doi.org/10.1016/j.cej.2017.12.149.

[9] B. Mocquot, J. Vangronsveld, H. Clijsters, and M. Mench, Copper toxicity in young maize (*Zea mays* L.) plants : Effects on growth, mineral and chlorophyll contents, and enzyme activities, Plant and Soil 182 (1996) 287-300. https://doi.org/10.1007/BF00029060.

[10] P. Bergkvist, N. Jarvis, D. Berggren, K. Carlgren, Long-term effects of sewage sludge applications on soil properties, cadmium availability and distribution in arable soil, Agriculture, Ecosystems & Environment 97(1-3) (2003) 167-179. https://doi.org/10.1016/S0167-8809(03)00121-X.

[11] F. Figlioli, M.C. Sorrentino, V. Memoli, C. Arena, G. Maisto, S. Giordano, F. Capozzi, V. Spagnuolo, Overall plant responses to Cd and Pb metal stress in maize: Growth pattern, ultrastructure, and photosynthetic activity, Environmental Science and Pollution Research 26 (2019) 1781-1790. https://doi.org/10.1007/s11356-018-3743-y.

[12] M.N.V. Prasad, Cadmium toxicity and tolerance in vascular plants, Environmental and Experimental Botany 35(4) (1995) 525-545. https://doi.org/10.1016/0098-8472(95)00024-0.

[13] L.E. Hernández, E. Lozano-Rodríguez, A. Gárate, Ramón Carpena-Ruiz, Influence of cadmium on the uptake, tissue accumulation and subcellular distribution of manganese in pea seedlings, Plant Science 132(2) (1998) 139-151. https://doi.org/10.1016/S0168-9452(98)00011-9.

[14] Y.G. Zhu, Z.Q. Zhao, H.Y. Li, S.E. Smith, F.A. Smith, Effect of zinc-cadmium interactions on the uptake of zinc and cadmium by winter wheat (*Triticum aestivum*) grown in pot culture, Bulletin of Environmental Contamination and Toxicology 71 (2003) 1289-1296. https://doi.org/10.1007/s00128-003-0230-y.

[15] X. Yang, V.C. Baligar, D.C. Martens, R.B. Clark, Cadmium effects on influx and transport of mineral nutrients in plant species, Journal of Plant Nutrition 19(3-4) (1996) 643-656. https://doi.org/10.1080/01904169609365148.

[16] Y.J. An, Soil ecotoxicity assessment using cadmium sensitive plants, Environmental Pollution 127(1) (2004) 21-26. https://doi.org/10.1016/S0269-7491(03)00263-X.

[17] X. Yang, V.C. Baligar, R.B. Clark, D.C. Martens, Influx, transport, and accumulation of cadmium in plant species grown at different Cd^{2+} activities, Journal of Environmental Science and Health Part B 30(4) (1995) 569-583. https://doi.org/10.1080/03601239509372954.

[18] N. Ait Ali, M.P. Bernal, M. Ater, Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*, Plant Soil 239 (2002) 103-111. https://doi.org/10.1023/A:1014995321560.

[19] M.B. Mcbride, C. E. Martínez, Copper phytotoxicity in a contaminated soil: Remediation

tests with adsorptive materials, Environmental Science & Technology 34(20) (2000) 4386-4391. https://doi.org/10.1021/es0009931.

[20] C.M. Borkert, F.R. Cox, M.R. Tucker, Zinc and copper toxicity in peanut, soybean, rice, and corn in soil mixtures, Communications Soil Science and Plant Analysis 29(19-20) (1998) 2991-3005. https://doi.org/10.1080/00103629809370171.

[21] H. Kabata-Pendias, A. Pendias, Trace elements in soils and plants, Boca Raton, FL, 2001.

[22] P.B. Gahan, Cell and Molecular Biology—Concepts and Experiments. 2nd edn G. Karp, John Wiley & Sons Ltd, ISBN 0□471□19279□1, 816 pp., £27.50 (1999), Cell Biochemistry and Function 17(4) (1999) 290-290. https://doi.org/10.1002/(sici)1099-0844(199912)17:4<290::aid-cbf849>3.0.co;2-p.

[23] F. Katterman, Environmental injury to plants, American Chemical Society, San Diego, CA, 1990.

[24] S. Babel, D. del Mundo Dacera, Heavy metal removal from contaminated sludge for land application: A review, Waste Management 26(9) (2006) 988-1004. https://doi.org/10.1016/j.wasman.2005.09.017.

[25] A. Pathak, M.G. Dastidar, T.R. Sreekrishnan, Bioleaching of heavy metals from sewage sludge: A review, Journal of Environmental Management 90(8) (2009) 2343-2353. https://doi.org/10.1016/j.jenvman.2008.11.005.

[26] Y. Xu, C. Zhang, M. Zhao, H. Rong, K. Zhang, Q. Chen, Comparison of bioleaching and electrokinetic remediation processes for removal of heavy metals from wastewater

treatment sludge, Chemosphere 168 (2017) 1152-1157. https://doi.org/10.1016/j.chemosphere.2016.10.086.

[27] D. Ma, M. Su, J. Qian, Q. Wang, F. Meng, X. Ge, Y. Fe, C. Song, Heavy metal removal from sewage sludge under citric acid and electroosmotic leaching processes, Separation and Purification Technology 242 (2020) 116822. https://doi.org/10.1016/j.seppur.2020.116822.

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

[29] R.L. Donahue, R.W. Miller, J.C. Shickluna, Soils: An introduction to soils and plant growth. Fifth Edition, Prentice Hall, Inc., Englewood, NJ, ISBN: 0138222886, 1983, 667 p.

[30] D. Barraoui, J.F. Blais, M. Labrecque. Cleanup of sewage sludge spiked with Cd, Cu, and Zn: Sludge quality and distribution of metals in the “soil-plant-water” system, Chemosphere 267 (2021) 129233. https://doi.org/10.1016/j.chemosphere.2020.129223.

[31] G. Mercier, J.F. Blais, F. Hammy, M. Lounès, J.L. Sasseville, A decontamination process to remove metals and stabilise Montreal sewage sludge, The Scientific World Journal 2 (2002) 349585. https://doi.org/10.1100/tsw.2002.201.

[32] CPVQ, Le maïs est encore une production d’avenir, in : Conférence sur le maïs, Québec, Canada, 1997, 48 p.

[33] A.L. Page, R.H. Miller, D.R. Keeny, Methods of soil analysis; 2. Chemical and microbiological properties, American Society of Agronomy, Soil Science Society of America (Editors), Madison, WI, ISBN: 0891180729, 1982, 1159 p.

[34] E.W. Rice, R.B. Baird, A.D. Eaton, Standard methods for the examination of water and wastewater. 23rd Edition, APHA, AWWA, WEF (Editors), ISBN: 9780875532875, 2017.

[35] SAS, SAS/STAT user's guide, SAS Institute Inc., Cary, NC, 2001.

[36] V. Ramachandran, T.J. D'Souza, Plant uptake of cadmium, zinc, and manganese from four contrasting soils amended with Cd-enriched sewage sludge, Journal of Environmental Science and Health, Part A. Toxic/Hazardous Substances and Environmental Engineering 37(7) (2002) 1337-1346. https://doi.org/10.1081/ESE-120005990.

[37] M. Tejada, B. Rodríguez-Morgado, I. Gómez, L. Franco-Andreu, C. Benítez, J. Parrado, Use of biofertilizers obtained from sewage sludges on maize yield, European Journal of Agronomy 78 (2016) 13-19. https://doi.org/10.1016/j.eja.2016.04.014.

[38] M.B. McBride, Cupric ion activity in peat soil as a toxicity indicator for maize, Journal of Environmental Quality 30(1) (2001) 78-84. https://doi.org/10.2134/jeq2001.30178x.

[39] G. Ouzounidou, M. Čiamporová, M. Moustakas, S. Karataglis, Responses of maize (*Zea mays* L.) plants to copper stress-I. Growth, mineral content and ultrastructure of roots, Environmental and Experimental Botany 35(2) (1995) 167-176. https://doi.org/10.1016/0098-8472(94)00049-B.

[40] L. Ilie, M. Mihalache, G.V. Scaeteanu, R.M. Madjar, D.R. Popovici, Effect of sewage

sludge amended soil on maize crop II. Influence on metal accumulation, Revista de Chimie (Rev. Chim.) 69(5) (2018) 1165-1172. https://doi.org/10.37358/rc.18.5.6282.

[41] K. Przygocka-Cyna, W. Grzebisz, The multifactorial effect of digestate on the availability of soil elements and grain yield and its mineral profile—the case of maize, Agronomy 10(2) (2020) 275. https://doi.org/10.3390/agronomy10020275.

[42] A.Y. Renoux, S. Rocheleau, M. Sarrazin, G.I. Sunahara, J.F. Blais, Assessment of a sewage sludge treatment on cadmium, copper and zinc bioavailability in barley, ryegrass and earthworms, Environmental Pollution 145(1) (2007) 41-50. https://doi.org/10.1016/j.envpol.2006.04.001.

[43] W. Friesl, O. Horak, W.W. Wenzel, Immobilization of heavy metals in soils by the application of bauxite residues: Pot experiments under field conditions, Journal of Plant Nutrition and Soil Science 167(1) (2004) 54-59. https://doi.org/10.1002/jpln.200320941.

[44] R.P. Narwal, M. Singh, J.P. Singh, D.J. Dahiya, Cadmium-zinc interaction in maize grown on sewer water irrigated soil, Arid Soil Research and Rehabilitation 7(2) (1993) 125-131. https://doi.org/10.1080/15324989309381342.

[45] A. Lagriffoul, B. Mocquot, M. Mench, J. Vangronsveld, Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.), Plant and Soil 200 (1998) 241-250. https://doi.org/10.1023/A:1004346905592.

[46] T. Hernández, J.I. Moreno, F. Costa, Influence of sewage sludge application on crop yields and heavy metal availability, Soil Science and Plant Nutrition 37(2) (1991) 201-

210. https://doi.org/10.1080/00380768.1991.10415030.

[47] T.A.S. Henning, B. Snyman, H.G. Aveling, The cultivation of maize (*Zea mays* L.) on high sewage sludge dosages at field scale, in: Selected Proceedings of the International Specialised Conference on Disposal and Utilisation of Sewage Sludge: Treatment Methods and Application Modalities, held in Athens, Greece, 13-15 October, 1999, A. Andreadakis, L. Spinosa (Issue Editors), IWA Publishing, pp. 453–459.

[48] N.K. Fageria, Adequate and toxic levels of copper and manganese in upland rice, common bean, corn, soybean, and wheat grown on an oxisol, Communications in Soil Science and Plant Analysis 32(9-10) (2001) 1659-1676. https://doi.org/10.1081/CSS-100104220.

[49] C.W. Gray, R.G. McLaren, A.H.C. Roberts, Cadmium leaching from some New Zealand pasture soils, European Journal of Soil Science 54(1) (2003) 159-166. https://doi.org/10.1046/j.1365-2389.2003.00495.x.

[50] S. Li, B. Fang, D. Wang, X. Wang, X. Man, X. Zhang, Leaching characteristics of heavy metals and plant nutrients in the sewage sludge immobilized by composite phosphorus-bearing materials, International Journal of Environmental Research and Public Health 16(24) (2019) 5159. https://doi.org/10.3390/ijerph16245159.

[51] X. Yu, T. Zhou, J. Zhao, C. Dong, L. Wu, Y. Luo, P. Christie, Remediation of a metal-contaminated soil by chemical washing and repeated phytoextraction: a field experiment, International Journal of Phytoremediation (2020) . https://doi.org/10.1080/15226514.2020.1840509.

[52] F. Van Der Leeden, F.L. Troise, D.K. Todd, The Water Encyclopedia, Second Edition,

Lewis Publishers, Boca Raton, FL, ISBN-10: 0873711203, 1990, 824 p.

[53] D.R. Edwards, P.A. Moore, S.R. Workman, E.L. Busheé, Runoff of metals from alum-treated horse manure and municipal sludge, Journal of American Water Resources Association 35(1) (1999) 155-165. https://doi.org/10.1111/j.1752-1688.1999.tb05460.x.

[54] R. Nilsson, Residual aluminium concentration in drinking water after treatment with aluminium or iron salts, In: Chemical water and wastewater treatment, H.H. Hahn, R. Klute (Editors), Springer, Berlin, Germany, 1992, pp. 399-410.