Environ Sci Pollut Res
DOI 10.1007/s11356-017-0103-2

RESEARCH ARTICLE

Effects of municipal solid waste- and sewage sludge-compost-based growing media on the yield and heavy metal content of four lettuce cultivars

Concetta Eliana Gattullo[1] **· Carlo Mininni**[2] **· Angelo Parente**[2] **· Francesco Fabiano Montesano**[2] **· Ignazio Allegretta**[1] **· Roberto Terzano**[1]

Received: 9 March 2017 / Accepted: 4 September 2017

Abstract Compost has been recently suggested as an alternative to peat for the preparation of growing substrates in soilless cultivation systems. However, some physico-chemical properties of compost may reduce plant performance and endanger the quality of productions, in particular for possible heavy metal accumulation in edible parts. This study aims at evaluating the suitability of a municipal solid waste compost (MSWC) and a sewage sludge compost (SSC) as components of growing media for the soilless cultivation of lettuce (*Lactuca sativa* L.). Heavy metal content of SSC complied with legislation limits but, in MSWC, it exceeded (Cu, Pb) or was very close (Cd, Zn) to safe limits. A greenhouse experiment was carried out by cultivating four lettuce cultivars ("Maximus," "Murai," "Patagonia," and "Aleppo") in pots containing a mixture of MSWC and perlite (MSWC + P), SSC and perlite (SSC + P), or peat and perlite (peat + P), the latter used as control. Plant biometric parameters measured after 72 days of growth revealed that the yield of plants cultivated on SSC + P was similar to control plants, independently of the cultivar. Conversely, MSWC + P suppressed in general the biomass production, especially for Murai and Patagonia cultivars. Compared to peat + P, both compost-based substrates reduced the leaf accumulation of heavy metals, with a major effect in Maximus plants. The levels of Cd and Pb in the edible part were always below the safe limits imposed by European regulation. Therefore, risks of heavy metal intake in food chain associated with the replacement of peat with compost in the growing media are negligible, even when a compost with a significant amount of heavy metals is used. Besides compost quality monitoring, also an appropriate varietal choice is crucial to obtain good yields and safe products.

Responsible editor: Philippe Garrigues

✉ Angelo Parente
angelo.parente@ispa.cnr.it

[1] Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy

[2] C.N.R.-National Research Council, Institute of Sciences of Food Production, Via Amendola 122/O, 70126 Bari, Italy

Keywords Compost · Trace elements · Food safety · Leaf accumulation · Leafy vegetables · Root accumulation · Soilless system

Introduction

Municipal solid waste composts and sewage sludge composts are widely used in agriculture as organic soil amendments (Smith et al. 2001; Castro et al. 2009; Diacono and Montemurro 2010; Morra et al. 2010) and as growing media in substitution of peat for soilless horticultural productions (Ingelmo et al. 1998; Hicklenton et al. 2001; Perez-Murcia et al. 2006; Cai et al. 2010). Peat is an expensive and non-renewable resource; thus, its partial or total replacement with compost or other organic matrices has been taken into consideration in the last two decades (Raviv 1998; Benito et al. 2005; Abdelrahman et al. 2017). Compost is inexpensive and easily available, and its production is estimated to increase in the next years due to the increasing collection of separated biowastes. As an example, recycled biowastes in Italy are expected to increase approximately by 50% until 2020 (CIC 2014). Moreover, the replacement of peat with a nutrient-rich material like compost may be advantageous to reduce fertilization rates (Wilson et al. 2001).

Despite the large number of beneficial properties of compost, which are supported by a robust scientific literature (Hargreaves et al. 2008), high salt content, unsuitable physical properties (low porosity, high density), and variable quality and

Published online: 20 September 2017

composition may limit its use for the preparation of growing substrates (Mininni et al. 2015). In addition, during the composting process, dangerous elements like heavy metals may concentrate due to the microbial degradation of part of the organic matter and the loss of volatile solids (Smith 2009). If this concentration process is not controlled (e.g., through the choice of heavy metal-poor feedstocks and the use of composting conditions promoting the leaching of metals), the concentration of heavy metals in compost may exceed the safe limits set by the worldwide legislation in order to preserve human health.

Once entered in the environment, heavy metals are persistent (being non-biodegradable) and, even in trace concentrations, may seriously endanger living organisms. Indeed, the use of heavy metal-rich composts in agriculture may cause the accumulation of trace metals in plants and the consequent intake by livestock and humans through the food chain (Petruzzelli 1996). Some heavy metals (e.g., Cu, Fe, Mn, Zn, Mo) are essential, in small amounts, for the normal growth of plants and animals, and may cause physiological dysfunctions either when present in excess or in deficiency (Alloway 2013). Other elements (e.g., Pb, Hg, Cd) are non-essential and toxic even at very low concentrations. Since plant uptake is one of the main pathways through which heavy metals enter the food chain (Antonious and Kochhar 2009), a careful monitoring of metal content in vegetables is required. One possible way to reduce the uptake of heavy metals by plants is the use of species or cultivars with a reduced tendency to absorb or translocate specific toxic metals (Michalska and Asp 2001). Other approaches are based on the reduction of metal bioavailability in the growing medium through different agronomical strategies, such as pH control and addition of organic matter (Baldantoni et al. 2016; Yao et al. 2016).

This study aimed at evaluating the potential use of two different composts, one characterized by a moderately high heavy metal content and the other with a low heavy metal content (compared to limits fixed by Italian legislation), as substrate components for the soilless cultivation of lettuce (*Lactuca sativa* L.). The response of lettuce plants to the two compost-based growing media was evaluated in terms of both yield parameters and heavy metal accumulation in leaves and roots. Lettuce was selected for this study because it is one of the most consumed leafy vegetables in raw form and the first salad crop cultivated and commercialized internationally, with a worldwide production of 25 millions of tons (FAO 2014). As reported in several studies, lettuce is a heavy metal-sensitive species (Zubillaga and Lavado 2002; Benzarti et al. 2008; Di Salvatore et al. 2008); thus, it can be used as indicator for evaluating the physiological effects caused by heavy metals present in the growing substrate. Since plant response to heavy metals depends also on variety, four lettuce cultivars were tested with the further aim of identifying variety/ies with high yield and low tendency to accumulate heavy metals, suitable to grow also on "bad quality" substrates.

Materials and methods

Growing substrates and plant material

A commercial compost obtained from the organic fraction of municipal solid wastes (MSWCs) was provided by an Italian private composting plant. The main chemical characteristics of the MSWC were pH 7.8 and electrical conductivity (EC) 2.8 dS m^{-1}, both determined on 1:10 (*w*/*w*) compost:water (Costello and Sullivan 2014), C_{org} 261 g kg^{-1} (Springer-Klee method), total N 16.6 g kg^{-1} (Kjeldahl method), and C/N ratio 15.7. A sewage sludge compost (SSC) was obtained by mixing 55% sludge (a mix of urban and agro-industrial sludges, the latter coming from wine, olive oil, and processing tomato industry), 25% urban green wastes (pruning and yard trimming residues), and 20% beached posidonia (*Posidonia oceanica* L. Delile) residues, on fresh weight basis. The composting process took place in piles (length 11.3 m, width 5 m, height 1.5 m) over 3 months. During the bio-oxidative phase (30 days), the windrow was injected with forced ventilation. During the subsequent curing phase (70 days), piles were periodically mechanically turned and water was added to maintain the moisture content near 60%. The main characteristics of the SSC were pH 7.6, EC 2.6 dS m^{-1}, C_{org} 310 g kg^{-1}, total N 22.5 g kg^{-1}, and C/N 13.8. Both composts showed the absence of phytotoxicity according to Italian regulations (Italian Directive no. 75/2010).

Both composts, once sieved at 1 cm, were mixed with perlite (Agrilit® 3, Perlite Italiana, Corsico, Italy) in order to obtain two growing substrates (% *v*/*v*): 50% MSWC + 50% perlite (MSWC + P) and 50% SSC + 50% perlite (SSC + P). In addition, a control substrate was prepared by mixing 50% peat and 50% perlite (peat + P). The peat used was a commercial product made up of 50% blond peat and 50% dark peat (Brill Type 3 Special, Agrochimica S.p.A., Bolzano, Italy).

Seeds of lettuce were obtained from Rijk Zwaan Italia s.r.l. (Calderara di Reno, Italy). Four cultivars belonging to different lettuce types were used for the experiments: romaine type "Maximus" (*L. sativa* L. var. *longifolia* L.), iceberg type "Patagonia" (*L. sativa* L. var. *capitata* L.), red oak leaf type "Murai," and green lollo type "Aleppo" (*L. sativa* L. var. *crispa* L.).

Characterization of growing substrates

Preliminarily to plant growth experiments, heavy metal content in the two composts, peat and perlite, was determined. Each substrate component was dried at 65 °C until reaching a constant weight and milled in an agate mortar. A plastic X-ray fluorescence sample cup (Fluxana, Germany) was filled with the pulverized sample, closed with a thin polypropylene membrane (Premier Lab Supply, USA), and analyzed for heavy metal content using a portable X-ray fluorescence spectrometer (XRF Analyzer NITON XL3t GOLDD, Thermo Scientific). Data quality was evaluated by analyzing NIST standard reference material 1573a (tomato leaves), BCR-CRM 038 (coal fly ash), and CCRMP TILL-4 (soil). In order to quantify those elements below the detection limit of XRF, each material was further analyzed for heavy metal content by inductively coupled plasma–atomic emission spectrometry (ICP-AES; Thermo iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, MA, USA), after acidic digestion of samples. For this purpose, an aliquot of 300 mg of dried material was weighed in a PTFE-TFM liner and pre-digested overnight with 7 mL HNO_3 (69.0%) and 1 mL H_2O_2 (30%) (TraceSELECT®, trace analysis reagents, Sigma-Aldrich). Afterwards, samples were digested in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria), according to the following program: 8 min to reach a power of 800 W, held for 8 min; 8 min from 800 to 1000 W, followed by 7 min at 1000 W; 6 min from 1000 to 1200 W, followed by 7 min at 1200 W; and final cooling for 25 min. Digested samples were diluted to 25 mL with deionized water, filtered with Whatman® 42 filter paper, and stored at 4 °C until analysis. Total concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined by ICP-AES, selecting the following emission wavelengths for the quantification: Cd 228.8 nm, Co 230.78 nm, Cr 267.7 nm, Cu 324.7 nm, Fe 259.9 nm, Mn 257.6 nm, Ni 231.6 nm, Pb 220.35 nm, and Zn 206.2 nm. The calibration curve was built using a blank solution (HNO_3 + H_2O_2) and a multi-element calibration standard at the concentration of 2 mg L^{-1} (Certipur® ICP Multi-element standard solution IV, Merck KGaA, Darmstadt, Germany). Instrument detection limit was calculated for each analyte as three times the standard deviation of ten replicates of the blank. The certified multi-element standard was analyzed every ten samples to check the correctness of quantification.

Each growing substrate (peat + P, MSWC + P, SSC + P) was analyzed, before use, also for the dry bulk density (BD), air capacity (AC), water-holding capacity (WC), and total porous space (TPS) according to the European Standard 13041 (1999). Water extracts of each growing substrate were obtained using a substrate:water ratio of 1:1.5 (*v*/*v*), according to the method reported by Sonneveld et al. (1974). EC, pH, and ionic composition of water extracts were also determined. In particular, the concentrations of N-NO_3^-, N-NH_4^+, K^+, S-SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, and Cl^- were measured by ion chromatography (Dionex DX 120, Dionex Corporation, Sunnyvale, CA), using an electrochemical detector (Dionex ED50). Cations were separated on IonPack CS12A column by elution with methane sulfonic acid (MSA) 20 mN at the flow rate of 1 mL min^{-1}. Anions were separated on IonPack AS14 column using, as eluents, aqueous solutions of Na_2CO_3 3.5 mM and $NaHCO_3$ 1 mM, at the flow rate of 1 mL min^{-1}. The data were processed with the software Dionex PeakNet® 5.22.

Experimental design

The experiment was conducted in a polymethacrylate greenhouse at "La Noria" experimental farm of the CNR–ISPA located in Mola di Bari, Italy (latitude 41° 03′ N, longitude 17° 04′ E; 24 m a.s.l.).

Lettuce was sown on September 30, 2013, using polystyrene plug trays (160 cells per tray, cell diameter of 2.5 cm, and volume of 21 mL) pre-filled with the peat substrate added with 1 kg m^{-3} PIG-MIX fertilizer (14N–16P–18K). After placing one seed per cell, the cell was covered with vermiculite. Germination and growth of seedlings were performed, setting the minimum temperature in the greenhouse at 10 °C. The seedlings were irrigated daily by means of a mobile sprinkler system. Starting from the second week of growth, seedlings were fertigated weakly with a nutrient solution containing (mg L^{-1}) 150 N (140 N-NO_3^- and 10 N-NH_4^+), 50 P, 200 K, 124 Ca, 45 Mg, 94 S, 1.12 Fe, 0.24 Cu, 0.03 Mn, 0.13 Zn, 0.27 B, and 0.05 Mo. An aliquot of the nutrient solution was filtered with Whatman® 42 filter papers and analyzed by ICP-AES to exclude the presence of heavy metals other than those initially added. Seedlings at the third true-leaf stage (35 days old) were transplanted into 4.5-L plastic pots filled with the growing substrate (peat + P, MSWC + P, SSC + P). Pots were placed on troughs of 6 m length × 26 cm width × 6 cm side height, which were covered with a polyethylene film and placed on a slope of 2%. Plants were grown one per pot, obtaining a final density of 7.2 plants m^{-2}. Plants were fertigated with nutrient solution using one drip emitter per pot (2 L h^{-1}) in order to ensure, for each growing substrate, a moisture level close to the water-holding capacity and a runoff of about 20% of the supplied nutrient solution. The experiment was carried out following a randomized block design with three replications and five pots per experimental unit.

Plant analyses

After 72 days of growth, when plants reached the commercial size for the fresh market, one randomly selected plant per experimental unit (three plants per treatment) was harvested in order to determine leaf number, leaf area (Li-Cor LI 3100 area meter, Biosciences, Lincoln, NE-USA), leaf fresh and dry weight (by placing samples in a ventilated oven at 65 °C), and head fresh weight. SPAD index was also measured by a chlorophyll meter (Konica Minolta Spad 502, Tokyo, Japan), and results were expressed as the average of ten readings on five leaves of different plants.

The entire root apparatus and the first six edible basal leaves were sampled from other four plants, abundantly flushed under tap water in order to remove any impurity, then rinsed with deionized water, and dried at 65 °C until reaching a constant mass. Successively, the plant material was ground (≤1 mm) in a cutting-grinding mill (IKA MF 10B, Labortechnik, Staufen, Germany) and processed for the determination of heavy metal content by ICP-AES as described above for the growing substrates.

Statistical analysis

A factorial two-way analysis of variance (ANOVA) was performed using the Statistica software package version 10.0 (StatSoft, Tulsa, OK, USA). Means were separated by the Fisher's protected least significant difference considering a significance level of $P = 0.05$.

Results and discussion

Characteristics of the growing substrates

Heavy metal content of composts, peat and perlite, is reported in Table 1. As expected, both composts were characterized by higher concentrations of heavy metals compared to peat. While heavy metal content of SSC complied with the maximum limits allowed in Italy (Italian Directive no. 75/2010), Cu and Pb levels in MSWC were higher than the admissible values. Moreover, Cd and Zn concentrations in MSWC were very close to legislation constraints. The agricultural use of organic matrices in the European Union (EU) is still now regulated by national legislations. However, an attempt has been made to set standard quality parameters for composts and digestates all over EU, as reported in the Third Working Document for End-of-waste Criteria (Saveyn and Eder 2014). When heavy metal concentrations of MSWC and SSC were compared with EU limits, MSWC exceeded the values allowed for Cu, Pb, and Zn, whereas SSC exceeded slightly the value allowed for Cu. The high concentrations of heavy metals in MSWC depended mainly on the chemical composition of the initial feedstocks. It is well ascertained that municipal solid waste composts are rich in heavy metals and, among them, Zn, Pb, and Cu are generally present in the largest amounts (Smith 2009).

The physical properties of the growing media, reported in Table 2, revealed that compost-based substrates were characterized by higher values of bulk density and moderately lower values of total porosity than the control substrate. Nevertheless, the three substrates showed a good porosity and bulk density, as compared with an ideal growing medium (Abad et al. 2001). The porosity determines the rate at which air (oxygen) can move through the substrate (Jayasinghe et al. 2010). Air capacity of the three substrates was higher than the ideal range; conversely, water-holding capacity was lower (Abad et al. 2001). In general, a limited water-holding capacity could be managed through an appropriate irrigation; on the contrary, a low air capacity could be not (Fonteno 1996). High air capacity imposes short fertigation turns and the use of small volumes of nutrient solution to avoid leaching problems (Benito et al. 2006). Moreover, high porosity and air capacity could represent an advantage for plant growth by providing more suitable gaseous exchange for the root system, thus reducing risks of plant hypoxia (Cocozza et al. 2011).

The higher pH values of MSWC + P and SSC + P compared to peat + P (Table 3) are in accordance with previous experiments (Perez-Murcia et al. 2006; Ribeiro et al. 2007; Bustamante et al. 2008). Possibly, high pH is related to the higher content of alkaline elements in compost with respect to peat which, on the contrary, is usually acidic (Mininni et al.

Table 1 Heavy metal content of peat, perlite, municipal solid waste compost (MSWC), and sewage sludge compost (SSC) used in the experiments

Materials	Cd mg kg^{-1} dry weight	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Peat	<LOD	0.7 ± 0.1[a]	2.0 ± 0.1[a]	24 ± 3	2,620 ± 19	100 ± 7	<LOD	5.0 ± 0.3[a]	22 ± 2
Perlite	<LOD	40 ± 11	25 ± 7	<LOD	5,240 ± 33	410 ± 13	<LOD	<LOD	24 ± 2
MSWC	1.4 ± 0.1[a]	2.4 ± 0.1[a]	23 ± 9	276 ± 5	10,170 ± 43	440 ± 14	18 ± 1[a]	154 ± 3	491 ± 6
SSC	0.5 ± 0.0[a]	5.4 ± 0.0[a]	64 ± 9	128 ± 4	15,470 ± 54	520 ± 15	37 ± 8	29 ± 0[a]	302 ± 5
Maximum admissible limits for organic amendments									
Italy[b]	1.5	–	0.5 Cr(VI)	230	–	–	100	140	500
EU[c]	1.5	–	100	100	–	–	50	120	400

Data reported (mean ± standard deviation; $n = 3$) were determined by X-ray fluorescence (XRF) or ICP-AES analyses. <LOD below ICP-AES detection limit, corresponding to (mg kg^{-1}) 0.02 Cd, 0.55 Cu, 1.81 Ni, 0.59 Pb

[a] Result of ICP-AES analysis

[b] Italian directive on fertilizers (D.L. 75/2010)

[c] Third Working Document for End-of-waste criteria on Biodegradable waste subject to biological treatment (Saveyn and Eder 2014)

Table 2 Main physical and hydrological characteristics of the three growing substrates used in the experiment

Substrate	AC % *v*/*v*	WC	TPS	BD kg m^{-3}
Peat + P	47 ± 1	44 ± 1	91 ± 1	147 ± 7
MSWC + P	54 ± 2	30 ± 3	84 ± 1	273 ± 17
SSC + P	37 ± 2	49 ± 3	86 ± 1	261 ± 19
Reference values[a]	20–30	55–70	>85	<400

Mean ± standard deviation; $n = 3$

Peat + P 50% peat + 50% perlite (*v*/*v*), used as control; *MSWC + P* 50% municipal solid waste compost + 50% perlite (*v*/*v*); *SSC + P* 50% sewage sludge compost + 50% perlite (*v*/*v*); *AC* air capacity; *WC* water-holding capacity; *TPS* total porous space; *BD* dry bulk density

[a] Abad et al. (2001)

2015). Moreover, the two compost-containing substrates were characterized by considerably higher EC values than the control substrate (Table 3). This was principally caused by the higher ionic content of compost-based media, especially Na^+, K^+, and Cl^-. The higher EC of MSWC + P, with respect to SSC + P, was possibly due to its higher content in Cl^- and K^+. The macronutrient content of MSWC + P and SSC + P was much higher than that of peat + P (Table 3). As observed in numerous experiments, compost-based substrates have higher concentrations of nutrients than peat-based substrates (Sánchez-Monedero et al. 2004; Grigatti et al. 2007; Mininni et al. 2015). In MSWC + P, higher concentrations of N (both N-NO_3^- and N-NH_4^+) and K^+ and lower concentrations of S-SO_4^{2-}, Ca^{2+}, and Mg^{2+} were found as compared to SSC + P. The different nutrient composition of the substrates might be taken into account in the preparation of the nutrient solution in order to limit the use of mineral fertilizers.

Plant analysis

Cultivar, substrate, and their interaction affected plant growth (Table 4). With regard to the cultivar, Murai and Aleppo plants (red oak leaf and green lollo type, respectively, both *var. crispa* L.) were on average characterized by significantly lower values of leaf area, leaf fresh weight, head weight, and SPAD index compared to Maximus and Patagonia (romaine and iceberg types, respectively, belonging to var. *longifolia* L. and var. *capitata* L.). Differences in growth rates may be related to the specific morphology and behavior of the four cultivars tested. For instance, the absence of a real head in Murai and Aleppo cultivars possibly caused the lower biomass production. Differences in SPAD values may be also ascribed to varietal diversity (Wilson et al. 2002). Among the four cultivars, Maximus produced on average the best yield, as proved by all the biometric measurements.

With regard to the growing substrate, the two compost-based media affected differently the lettuce growth (Table 4). Compared to control plants, lettuce cultivated on MSWC + P showed, on average, a significant reduction of all the biometric parameters except for leaf dry weight, which appeared higher. Conversely, plants cultivated on SSC + P grew similarly to control plants. The high salinity and heavy metal content of MSWC possibly hindered the plant growth, being lettuce very sensitive to salinity, as well as to Pb and Zn toxicity (Stevens et al. 2003). Conversely, the lower salinity and heavy metal content of SSC, along with a possible biostimulating effect already reported for posidonia-based composts (Cocozza et al. 2011; Mininni et al. 2012), might have promoted the biomass production. The values of SPAD in general decreased in plants grown on both compost-based substrates with respect to plants grown on peat + P; however, this reduction was moderate (Table 4). The SPAD response was most likely related to plant stress induced by higher pH and EC values of MSWC- and SSC-based substrates compared to control growing medium (Ronga et al. 2016).

Leaf area, head weight, and SPAD values varied significantly depending on the interaction between cultivar and substrate (Table 4). Indeed, despite the general suppression of lettuce growth caused by MSWC + P, Maximus and Aleppo plants cultivated on this substrate showed values of leaf area (Fig. 1a), head weight (Fig. 1b), and SPAD index (Fig. 1c) similar to the respective control plants. On the opposite, Murai and Patagonia plants grown on MSWC + P were characterized by lower values of leaf area and head fresh weight compared

Table 3 Chemical properties of aqueous extracts (1:1.5 *v*/*v*) of the substrates used in the experiment

Substrate	pH	EC[a] (dS m^{-1})	N-NO_3^- mg L^{-1}	N-NH_4^+	K^+	S-SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	Cl^-
Peat + P	6.7	0.47	11 ± 1	3 ± 1	26 ± 1	37 ± 3	40 ± 2	9 ± 1	37 ± 2	50 ± 13
MSWC + P	8.2	8.25	448 ± 15	46 ± 11	1624 ± 69	53 ± 1	132 ± 4	45 ± 1	799 ± 30	1688 ± 33
SSC + P	7.4	6.74	304 ± 10	23 ± 3	693 ± 11	221 ± 11	328 ± 23	103 ± 5	771 ± 31	1490 ± 30

Mean ± standard deviation; $n = 3$

Peat + P 50% peat + 50% perlite (*v*/*v*), used as control; *MSWC + P* 50% municipal solid waste compost + 50% perlite (*v*/*v*); *SSC + P* 50% sewage sludge compost + 50% perlite (*v*/*v*)

[a] Electrical conductivity

Table 4 Effect of cultivar (cv), growing substrate (s), and interaction between cultivar and substrate (cv × s) on growth parameters and SPAD index of lettuce plants grown for 72 days on soilless growing media based on peat or composts in mixture with perlite

Cultivar	Leaves				Head weight	SPAD
	Number	Area (cm^2 $plant^{-1}$)	FW (g $plant^{-1}$)	DW (%)	(g)	
Maximus	34 a	5087 a	234 a	4.0 a	430 a	40 a
Murai	33 a	4226 b	211 bc	3.8 a	284 b	23 c
Patagonia	22 c	5222 a	231 ab	3.4 b	418 a	32 b
Aleppo	30 b	3978 b	209 c	3.9 a	290 b	17 d
Substrate						
Peat + P	30 a	4749 a	229 a	3.6 b	393 a	30 a
MSWC + P	28 b	3906 b	206 b	4.0 a	274 b	27 b
SSC + P	31 a	5231 a	229 a	3.8 ab	399 a	27 b
Significance						
cv	***	***	*	**	***	***
s	**	***	*	**	***	**
cv × s	ns	**	ns	ns	*	**

Peat + P 50% peat + 50% perlite (*v/v*); *MSWC + P* 50% municipal solid waste compost + 50% perlite (*v/v*); *SSC + P* 50% sewage sludge compost + 50% perlite (*v/v*); *FW* fresh weight; *DW* dry weight

*, **, ***Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; *ns* not significant. Figures in columns followed by the same letter are not statistically different ($P \leq 0.05$)

to plants of the same cultivars grown on peat + P. These results prove that an appropriate varietal choice is crucial to overcome problems connected to the use of a non-ideal compost as component of growing media. In addition, for all the cultivars tested, plants grown on SSC + P showed values of leaf area (Fig. 1a) and head fresh weight (Fig. 1b) statistically comparable to the respective control plants. This outcome is the evidence that a good quality compost like SSC can be used, even at a high concentration, instead of peat for the preparation of growing substrates without any risk for the quantitative yield of lettuce. The measurement of SPAD index revealed that Maximus and Patagonia plants grown on SSC + P and MSWC + P, respectively, were characterized by lower values of SPAD compared to the other tested substrates, while no differences among treatments were observed in Murai and Aleppo (Fig. 1c).

Heavy metal concentrations measured in leaves and roots of lettuce plants after 72 days of growth are reported in Table 5. Values of Co, Pb, and Ni concentrations in leaves and roots were not reported, being below the detection limit (0.14, 0.59, and 1.81 mg kg^{-1} for Co, Pb, and Ni, respectively).

The accumulation of heavy metals in the edible part, except for Cd, was significantly affected by the cultivar. Among the four cultivars tested, Patagonia showed in general a slightly lower tendency to accumulate heavy metals, especially Cr and Cu. Similarly, Crews and Davies (1985) demonstrated that the accumulation of heavy metals by lettuce plants is under the varietal control.

Heavy metal accumulation in leaves strongly depended on the substrate type. The control substrate peat + P, despite presenting the lowest content of heavy metal, caused the highest metal accumulation in the edible part, except for Cu which appeared more concentrated in plants cultivated on MSWC + P. These results are in accordance with Mininni et al. (2015), who found higher levels of Cd, Cr, Fe, and Mn in basil plants grown on peat compared to plants grown on compost-based media, despite the considerably lower concentrations of these elements in the peat used. The addition of compost to the growing substrate tends to reduce the metal bioavailability through pH increase, as reported in numerous previous researches (Eklind et al. 2001; Perez-Murcia et al. 2006; Mininni et al. 2015). Moreover, the high salinity of the two compost-based media (Table 3) may have caused a restriction to metal uptake by plants, including heavy metals (Marschner 1995).

The leaf accumulation of Cr, Fe, Mn, and Zn was significantly affected by the interaction between cultivar and substrate (Table 5). The accumulation of Cr was clearly higher in Maximus and Murai plants cultivated on peat + P than in the same cultivars grown on compost-based substrates; conversely, Cr accumulation in Patagonia plants was statistically similar for treatments peat + P and SSC + P, and in Aleppo cultivar, it was independent of the substrate type (Fig. 2a). Leaf tissue concentration of Fe was always higher in plants grown in peat + P if compared with the two compost-based substrates, and this tendency was much more pronounced in Maximus than in any other cultivar (Fig. 2b). A similar effect was observed in the case of Mn concentration in leaves (Fig. 2c). Leaf concentration of Zn was higher in Maximus and Murai plants grown on peat + P in comparison with the same cultivars grown on compost-based substrates, but in Aleppo cultivar, this effect disappeared (Fig. 2d). Also, Patagonia plants cultivated on SSC + P showed levels of Zn statistically similar to the corresponding control plants (Fig. 2d).

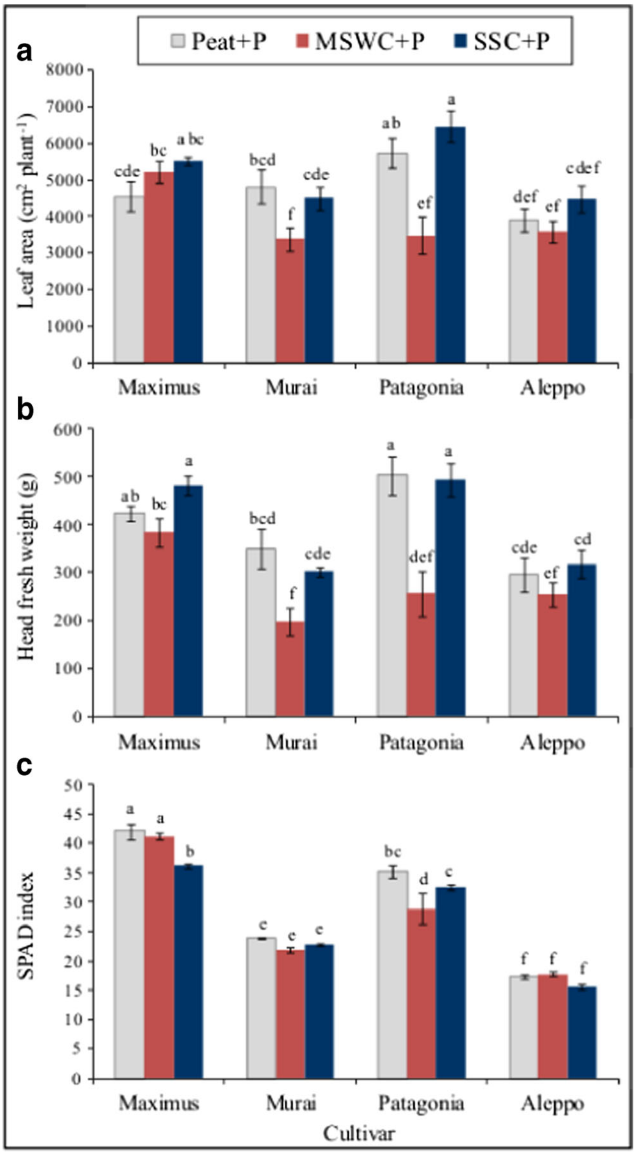

Fig. 1 Leaf area (**a**), head fresh weight (**b**), and SPAD index (**c**) of four lettuce cultivars grown for 72 days on peat or composts in mixture with perlite. The bar on each column indicates the standard error ($n = 3$)

To evaluate the potential risk of heavy metal intake by humans through the food chain, the concentrations of metals measured in leaf tissues were compared with safe limits imposed by EU regulation (Commission Regulation no. 1881/2006). For leaf vegetables, these limits are established only for Pb and Cd and correspond to 0.1 and 0.2 mg kg^{-1} fresh weight, respectively. In our study, the levels of Pb were always lower than 0.02 mg kg^{-1} fresh weight, which corresponded to the ICP-AES detection limit calculated on plant fresh weight basis. The concentrations of Cd varied in all the samples between 0.002 and 0.01 mg kg^{-1} fresh weight, again below the safe limits. Therefore, the substitution of peat with the two composts for the preparation of the growing medium seems not to pose risks for human health with regard to heavy metal content, despite the heavy metal content of both composts was considerably higher compared to peat. In one case (MSWC), heavy metal concentration was even above the limits imposed by legislation for Cu and Pb (and very close for Cd and Zn). These results are very promising, also considering the high rate of compost added to the growing substrate.

Along with the reduced assimilation of toxic elements, lettuce plants grown on the two compost-based media showed a reduced

Table 5 Effect of cultivar (cv), growing substrate (s), and interaction between cultivar and substrate (cv × s) on the heavy metal concentrations in leaves and roots of lettuce plants grown for 72 days on soilless growing media based on peat or composts in mixture with perlite

	Leaves					
	Cd	Cr	Cu	Fe	Mn	Zn
	mg kg^{-1} dry weight					
Cultivar						
Maximus	0.20	0.29 b	6.4 ab	103 a	81 a	95 b
Murai	0.16	0.38 a	7.3 a	73 b	57 b	91 b
Patagonia	0.13	0.21 c	5.6 b	84 b	68 b	89 b
Aleppo	0.14	0.26 bc	7.0 a	79 b	68 b	122 a
Substrate						
Peat + P	0.18 a	0.39 a	6.1 b	126 a	101 a	125 a
MSWC + P	0.22 a	0.21 b	7.5 a	60 b	62 b	87 b
SSC + P	0.07 b	0.26 b	6.3 b	68 b	43 c	86 b
Significance						
cv	ns	*	*	*	*	*
s	*	*	*	*	*	*
cv × s	ns	*	ns	*	*	*
	Roots					
	Cd	Cr	Cu	Fe	Mn	Zn
	mg kg^{-1} dry weight					
Cultivar						
Maximus	<LOD	2.0	14	187	28	127 b
Murai	<LOD	1.5	17	160	25	138 b
Patagonia	<LOD	1.5	12	145	30	125 b
Aleppo	<LOD	2.4	19	212	30	223 a
Substrate						
Peat + P	<LOD	2.1	14	163	43 a	174 a
MSWC + P	<LOD	1.3	16	174	29 b	152 b
SSC + P	<LOD	2.2	17	190	13 c	133 c
Significance						
cv	–	ns	ns	ns	ns	*
s	–	ns	ns	ns	*	*
cv × s	–	ns	ns	ns	ns	ns

<LOD below detection limit (0.02 mg kg^{-1} Cd)

Peat + P 50% peat + 50% perlite (*v*/*v*); *MSWC + P* 50% municipal solid waste compost + 50% perlite (*v*/*v*); *SSC + P* 50% sewage sludge compost + 50% perlite (*v*/*v*)

*Significant at $P \leq 0.001$, respectively; *ns* not significant. Figures in columns followed by the same letter are not statistically different ($P \leq 0.05$)

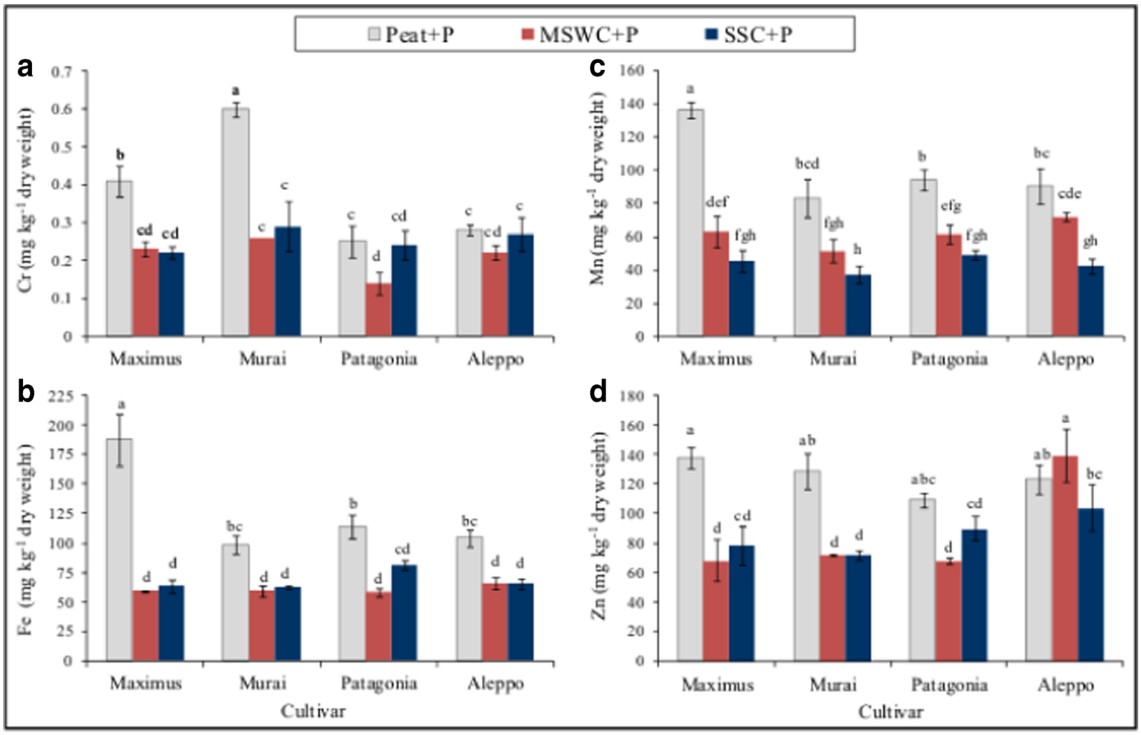

Fig. 2 Concentrations of Cr (**a**), Fe (**b**), Mn (**c**), and Zn (**d**) measured in leaves of four lettuce cultivars grown for 72 days on peat or composts in mixture with perlite. Values with the same letter are not significantly different ($P = 0.05$). The bar on each column indicates the standard error ($n = 3$)

accumulation of Fe, Mn, and Zn (Fig. 2b–d), which are considered essential elements both for plants and humans. The lower accumulation of Fe and Mn could explain the lower SPAD values measured in plants grown on compost-based media (Table 4), since SPAD meter readings assess the severity of leaf chlorosis associated with Fe deficiency, leaf color (Papasavvas et al. 2008), and leaf chlorophyll content (Massa et al. 2016) which, in turn, depends on Mn availability (Marschner 1995). However, this aspect may be easily managed through an appropriate fertigation control; thus, it does not preclude the use of compost as growing substrate component.

When the results of biometric parameters and heavy metal accumulation in leaves were considered simultaneously, Maximus appeared the most suitable cultivar for the cultivation on compost-based media. Indeed, it was characterized by very high yields, levels of unessential heavy metals (e.g., Cd, Pb) below the limits imposed by the legislation, and higher contents of essential elements (e.g., Fe and Mn) compared to the other cultivars. Also, Patagonia cultivar may be suitable for cultivation on substrates containing "good quality" composts, like SSC; however, its yield performance may be strongly reduced if a low-quality compost (like MSWC) is added to the growing medium.

The accumulation of heavy metals in lettuce roots was very moderate, especially with regard to non-essential elements (Table 5). Indeed, Cd, Co, Ni, and Pb were below the detection limits, and Cr levels did not exceed 3.3 mg kg^{-1} dry weight (value representing Cr concentration in roots of Aleppo plants grown on SSC + P). The root accumulation of heavy metals did not vary significantly between the cultivars, with the only exception of Zn, which appeared more concentrated in roots of Aleppo plants. Similarly to what observed in leaves, the growing medium affected the accumulation of Mn and Zn in roots; in particular, peat + P determined the highest accumulation, and SSC + P the lowest.

The concentrations of Cr, Cu, Fe, and Zn were higher in roots than in leaves, revealing the scarce translocation of these elements from roots to shoots. The same behavior was reported for Cr by Zayed et al. (1998), as well as for Cu by Ginocchio et al. (2002). Conversely, Cd and Mn were accumulated more in leaves than in roots. The high potential of lettuce for Cd uptake and allocation in leaves is well ascertained (Peijnenburg et al. 2000; Greger et al. 2007; Baldantoni et al. 2016) and may pose risks for human health. However, this study demonstrates that even the compost containing a Cd concentration very close to the maximum admissible limit (i.e., MSWC) may be used at a high dose (50% *v*/*v*) as component of growing substrate for the cultivation of lettuce without producing significant Cd accumulation in edible parts.

Conclusions

The replacement of peat with a sewage sludge compost in the preparation of growing substrates for the soilless cultivation of lettuce limited the accumulation of heavy metals in the edible part, without reducing the yield performance of plants. Even the use of a municipal solid waste compost characterized by high levels of heavy metals (Cd, Cu, Pb, and Zn) posed no risks for human health, since it reduced the heavy metal accumulation in leaves compared to the peat-based substrate. The high pH values of composts most likely lowered the bioavailability of heavy metals and, consequently, their uptake by lettuce roots. The addition of municipal solid waste compost to the growing medium reduced the growth of Murai and Patagonia plants, possibly because of its high salinity and heavy metal content. Among the four cultivars tested, Maximus appeared the most suitable for soilless cultivation on compost-based substrates. Indeed, it was characterized by high yields (even when cultivated on the substrate containing the municipal solid waste compost), low accumulation of toxic heavy metals (Cd and Pb), and higher accumulation of essential elements (Fe, Mn, and Zn) compared to the other cultivars. Therefore, this study proves that composts may be advantageously and safely used for the preparation of growing substrates for the soilless cultivation of lettuce, and represent a good alternative to peat, also when they contain a significant amount of heavy metals. In this last case, cultivar selection is extremely important to preserve plant yield and lettuce quality.

Acknowledgements This work was supported by the Italian research project PON02_00186_3417512 "*Strumenti Innovativi per il Miglioramento della Sicurezza Alimentare: Prevenzione, Controllo, Correzione*" (S.I.Mi.S.A.). X-ray analyses were performed at the "Micro X-ray Lab" of the University of Bari.

References

Abad M, Noguera P, Bures S (2001) National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. Bioresour Technol 77:197–200

Abdelrahman HM, Ceglie FG, Awad FA, Tittarelli F (2017) Growth responses of organic tomato seedlings to N liquid fertilizers and compost-amended growing media. Compost Sci Util 25:62–69

Alloway BJ (2013) Heavy metals and metalloids as micronutrients for plants and animals. In: Alloway BJ (ed) Heavy metals in soils: trace metals and metalloids in soils and their bioavailability, 3rd edn. Springer, Netherlands, pp 195–209

Antonious G, Kochhar T (2009) Mobility of heavy metals from soil into hot pepper fruits: a field study. Bull Environ Contam Toxicol 82:59–63

Baldantoni D, Morra L, Zaccardelli M, Alfani A (2016) Cadmium accumulation in leaves of leafy vegetables. Ecotox Environ Safe 123:89–94

Benito M, Masaguer A, De Antonio R, Moliner A (2005) Use of pruning waste compost as a component in soilless growing media. Bioresour Technol 96:597–603

Benito M, Masaguer A, Moliner A, De Antonio R (2006) Chemical and physical properties of pruning waste compost and their seasonal variability. Bioresour Technol 97:2071–2076

Benzarti S, Mohri S, Ono Y (2008) Plant response to heavy metal toxicity: comparative study between the hyperaccumulator *Thlaspi caerulescens* (ecotype Ganges) and nonaccumulator plants: lettuce, radish, and alfalfa. Environ Toxicol 23:607–616

Bustamante MA, Paredes C, Moral R, Agullo E, Perez-Murcia MD, Abad M (2008) Composts from distillery wastes as peat substitutes for transplant production. Resour Conserv Recy 52:792–799

Cai H, Chen T, Liu H, Gao D, Zheng G, Zhang J (2010) The effect of salinity and porosity of sewage sludge compost on the growth of vegetable seedlings. Sci Hortic 124:381–386

Castro E, Manas P, De las Heras J (2009) A comparison of the application of different waste products to a lettuce crop: effects on plant and soil properties. Sci Hortic 123:148–155

CIC (Italian Composting and Biogas Association) (2014) Country report on biowaste collection and recycling in Italy. http://wwwcompostit/attachments/article/861/ECN%20Country%20report%20on%20biowaste%20collection%20and%20recycling_webpdf) Accessed 3 March 2017

Cocozza C, Parente A, Zaccone C, Mininni C, Santamaria P, Miano TM (2011) Comparative management of offshore posidonia residues: composting vs. energy recovery. Waste Manag 31:78–84

Commission Regulation (EC) n. 1881 (2006) of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. pp 35

Costello RC, Sullivan DM (2014) Determining the pH buffering capacity of compost via titration with dilute sulfuric acid. Waste Biomass Valor 5:505

Crews HM, Davies BE (1985) Heavy metal uptake from contaminated soils by six varieties of lettuce (*Lactuca sativa* L.) J Agric Sci 105: 591–595

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agron Sustain Dev 30:401–422

Di Salvatore M, Carafa AM, Carratù G (2008) Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: a comparison of two growth substrates. Chemosphere 73: 1461–1464

Eklind Y, Ramert B, Wivstad M (2001) Evaluation of growing media containing farmyard manure compost, household waste compost or chicken manure for the propagation of lettuce (*Lactuca sativa* L.) transplants. Biol Agric Hortic 19:157–181

European Standard 13041 (1999) Soil improvers and growing media—determination of physical properties—dry bulk density, air volume, water volume, shrinkage value and total pore space. European Committee for Standardization, Brussels

FAOSTAT (2014) FAO statistic division data archives http://www.fao.org/faostat/en/#data/QC . Accessed 8 March 2017

Fonteno WC (1996) Growing media: types and physical/chemical properties. In: Reed DW (ed) Water, media and nutrition for greenhouse crops. Ball Publishing, Batavia, IL, pp 93–122

Ginocchio R, Rodriguez PH, Badilla-Ohlbaum R, Allen HE, Lagos GE (2002) Effect of soil copper content and pH on copper uptake of selected vegetables grown under controlled conditions. Environ Toxicol Chem 21:1736–1744

Greger M, Malm T, Kautsky L (2007) Heavy metal transfer from composted macroalgae to crops. Eur J Agron 26:257–265

Grigatti M, Giorgioni ME, Ciavatta C (2007) Compost-based growing media: influence on growth and nutrient use of bedding plants. Bioresour Technol 98:3526–3534

Hargreaves JC, Adl MS, Warman PR (2008) A review of the use of composted municipal solid waste in agriculture. Agric Ecosyst Environ 123:1–14

Hicklenton PR, Rodd V, Warman PR (2001) The effectiveness and consistency of source-separated municipal solid waste and bark composts as components of container growing media. Sci Hortic 91: 365–378

Ingelmo F, Canet R, Ibanez MA, Pomares F, García J (1998) Use of MSW compost, dried sewage sludge and other wastes as partial substitutes for peat and soil. Bioresour Technol 63:123–129

Italian Directive 75 (2010). Decreto Legislativo 29 aprile 2010, n.75. Riordino e revisione della disciplina in materia di fertilizzanti, a norma dell'articolo 13 della legge 7 luglio 2009, n. 88. Gazzetta

Ufficiale n. 121 del 26 maggio 2010. (Legislative Decree 29 April 2010, No 75. Published on Italian official gazette No. 121, 26 May 2010)

Jayasinghe GY, Liyana Arachchi ID, Tokashiki Y (2010) Evaluation of containerized substrates developed from cattle manure compost and synthetic aggregates for ornamental plant production as a peat alternative. Resour Conserv Recycl 54:1412–1418

Marschner H (1995) Mineral nutrition of higher plants. Academic, New York

Massa D, Prisa D, Montoneri E, Battaglini D, Ginepro M, Negre M, Burchi G (2016) Application of municipal biowaste derived products in hibiscus cultivation: effect on leaf gaseous exchange activity, and plant biomass accumulation and quality. Sci Hortic 205:59–69

Michalska M, Asp H (2001) Influence of lead and cadmium on growth, heavy metal uptake, and nutrient concentration of three lettuce cultivars grown in hydroponic culture. Commun Soil Sci Plant Anal 32: 571–583

Mininni C, Grassi F, Traversa A, Cocozza C, Parente A, Miano T, Santamaria P (2015) *Posidonia Oceanica* (L.) based compost as substrate for potted basil production. J Sci Food Agric 95:2041–2046

Mininni C, Santamaria P, Montesano FF, Parente A, Abdelrahman HM, Cocozza C (2012) Posidonia-based compost as a peat substitute for lettuce transplant production. Hortscience 47:1438–1444

Morra L, Pagano L, Iovieno P, Baldantoni D, Alfani A (2010) Soil and vegetable crop response to addition of different levels of municipal waste compost under Mediterranean greenhouse conditions. Agron Sustain Dev 30:701–709

Papasavvas A, Triantafyllidis V, Zervoudakis G, Kapotis G, Samaras Y, Salahas G (2008) Correlation of SPAD-502 meter readings with physiological parameters and leaf nitrate content in *Beta vulgaris*. J Environ Prot Ecol 9:351–356

Peijnenburg W, Baerselman R, de Groot A, Jager T, Leenders D, Posthuma L, Van Veen R (2000) Quantification of metal bioavailability for lettuce (*Lactuca sativa* L.) in field soils. Arch Environ Contam Toxicol 39:420–430

Perez-Murcia MD, Moral R, Moreno-Caselles J, Perez-Espinosa A, Paredes C (2006) Use of composted sewage sludge in growth media for broccoli. Bioresour Technol 97:123–130

Petruzzelli G (1996) Heavy metals in compost and their effect on soil quality. In: De Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The composting science part I. Blackie Academic and Professional Editor, Glasgow, pp 212–223

Raviv M (1998) Horticultural uses of composted material. Acta Hortic 469:225–234

Ribeiro HM, Romero AM, Pereira H, Borges P, Cabral F, Vasconcelos E (2007) Evaluation of a compost obtained from forestry wastes and solid phase of pig slurry as a substrate for seedlings production. Bioresour Technol 78:3294–3297

Ronga D, Pane C, Zaccardelli M, Pecchioni N (2016) Use of spent coffee ground compost in peat-based growing media for the production of basil and tomato potting plants. Commun Soil Sci Plant Anal 47: 356–368

Sánchez-Monedero MA, Roig A, Cegarra J, Bernal MP, Noguera P, Abad M, Antón A (2004) Composts as media constituents for vegetable transplant production. Compost Sci Util 12:161–168

Saveyn H, Eder P (2014) End-of-waste criteria for biodegradable waste subjected to biological treatment (compost & digestate): technical proposals. Publications Office of the European Union, Luxembourg, p 308. https://doi.org/10.2791/6295

Smith DC, Beharee V, Hughes JC (2001) The effects of composts produced by a simple composting procedure on the yields of swiss chard (*Beta vulgaris* L. var. *flavescens*) and common bean (*Phaseolus vulgaris* L. var *nanus*). Sci Hortic 91:393–406

Smith SR (2009) A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ Int 35:142–156

Sonneveld C, Van den Ende J, Van Dijk PA (1974) Analysis of growing media by means of a 1: 1½ volume extract. Commun Soil Sci Plant Anal 5:183–202

Stevens DP, Mclaughlin MJ, Heinrich T (2003) Determining toxicity of lead and zinc runoff in soils: salinity effects on metal partitioning and on phytotoxicity. Environ Toxicol Chem 22:3017–3024

Yao A, Wang Y, Ling X, Chen Z, Tang Y, Qiu H, Ying R, Qiu R (2016) Effects of an iron-silicon material, a synthetic zeolite and an alkaline clay on vegetable uptake of As and Cd from a polluted agricultural soil and proposed remediation mechanisms. Environ Geochem Health. https://doi.org/10.1007/s10653-016-9863-8

Wilson SB, Stoffella PJ, Graetz DA (2001) Compost-amended media for growth and development of Mexican heather. Compost Sci Util 9: 60–64

Wilson SB, Stoffella PJ, Graetz DA (2002) Development of compost-based media for containerized perennials. Sci Hortic 93:311–320

Zayed A, Lytle CM, Qian JH, Terry N (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206:293–299

Zubillaga MS, Lavado RS (2002) Heavy metal content in lettuce plants grown on biosolids compost. Compost Sci Util 10:363–367