Carbonaceous soil amendments to biofortify crop plants with zinc

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HIGHLIGHTS

► Biosolids mixed with biochar increased the soluble Zn in soil.
► The biosolids/biochar mixture results in significantly increased Zn uptake by some crops species/varieties.
► Chenopods showed the greatest Zn uptake, followed by leafy vegetables.
► Cadmium, Cu and Pb were below guideline levels in all samples, except the leaves of spinach and beetroot.

ARTICLE INFO

ABSTRACT

Carbonaceous soil amendments, comprising mixtures of biosolids and biochar, have been demonstrated to improve fertility while reducing nitrate leaching. We aimed to determine the efficacy of a biosolids/biochar soil amendment in biofortification of vegetables with Zn, an element that is deficient in one third of humanity. We grew beetroot (*Beta vulgaris*), spinach (*Spinacia oleracea*), radish (*Raphanus sativus*), broccoli (*Brassica oleracea*), carrot (*Daucus carota*), leek (*Allium ampeloprsum*), onion (*Allium cepa*), lettuce (*Lactuca sativa*), corn (*Zea mays*), tomato (*Solanum lycopersicum*), and courgette (also called zucchini — *Cucurbita pepo*) in an unamended soil (silt loam, pH 5.6), and soil amended (by volume) with 10% biosolids, 20% biochar, and 10% biosolids + 20% biochar. The biosolids and biosolids + biochar treatments significantly increased the biomass and Zn concentration of most species, with a large interspecific variation. Beetroot showed the greatest increase, with dry weight Zn concentrations of up to 178 and 1200 mg kg^{-1} in the bulbs and leaves respectively. Cadmium, Cu and Pb were below guideline levels in all samples, except the leaves of spinach and beetroot, which slightly exceeded the World Health Organisation's maximum permitted concentration of 0.1 mg Cd kg^{-1} fresh weight. A mixture of biosolids and biochar is an effective means to biofortify crops with edible leaves as well as beetroot with Zn. Future research should investigate the efficacy of the system in other soil types and the role of biochar in the immobilisation/inactivation of organic contaminants and pathogens contained within the biosolids.

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1. Introduction

Zinc (Zn) is a trace element micronutrient for both plants and animals. One third of humanity is deficient in Zn, with various deficiency rates in various countries ranging from 4 to 73% (Hotz and Brown, 2004). In the United States, 10% of the population consumes less than half the recommended dietary allowance for Zn and is at increased risk for Zn deficiency (Ho, 2004). Zinc biofortification aims to increase the concentration of this essential micronutrient in crop plants, pre-harvest, by agronomic means or genetic modification (Cakmak, 2008).

Typically, Zn is the trace element that is present in the highest concentrations in biosolids (Smith, 2009). Many studies have shown that the application of sewage sludge (biosolids) to soil increases the Zn concentration in crop plants (Balik et al., 1998; Dudas and Pawluk, 1975; Kemp and Hemkes, 1976; Weggler-Beaton et al., 2003; Wells and Whitton, 1979). Such biofortification using biosolids has an additional benefit of reusing a waste material that has a global production in excess of 10 Mt yr^{-1} (Bradley, 2008). However, in addition to macro and micro-nutrients, biosolids also contain heavy metals, endocrine disrupting compounds, pesticides, herbicides, surfactants, and pathogenic helminths, bacteria, viruses and fungi (Krogmann et al., 1999; Singh and Agrawal, 2008). Stricter regulations and improved treatment technologies have resulted in reduced pathogen burdens and decreased concentrations of heavy metals and organic contaminants (Chaney, 1990), leaving N loading as the rate limiting factor of the addition of high-quality biosolids in some countries (Gibbs, 2003). The rates of biosolids addition used for biofortification in the aforementioned studies ranged from 600 to >3000 kg N ha^{-1}. These rates are well above the maximum permissible N-loading rates for agricultural soils, which are typically 200–400 kg N ha^{-1} (Gibbs, 2003).

Knowles et al. (2011) demonstrated that biochar, a form of charcoal that is added to soil, effectively mitigates nitrate leaching from biosolids. Biochars have been shown to reduce the mobility and other plant uptake of some contaminants found in biosolids, such as Cu (Karami et al., 2011), Cr (Dong et al., 2011), Pb (Cao et al., 2011), Cd (Beesley and Marmiroli, 2011), as well as pesticides such as atrazine (Cao et al., 2011), and other organic contaminants such as polycyclic aromatic hydrocarbons (Gomez-Eyles et al., 2011). A potential drawback of using a biochar/biosolids mixture for Zn biofortification is that biochar has also been shown to reduce Zn bioavailability to plants (Beesley and Marmiroli, 2011).

The sorptive properties of biochars are variable and are strongly influenced by the source material and pyrolysis temperature (Uchimiya et al., 2011a, 2011b). A review of the mechanisms of contaminant immobilisation by biochar is provided by Beesley et al. (in press). Ideally, the biochar should be produced from a widespread and low-cost biological residue. Such a source material is waste from the Monterey pine (Pinus radiata D. Don) timber industry. This tree has been introduced in vast areas of New Zealand (where it is the most common tree), Australia, Chile, South Western Europe and South Africa (Earle, 2010).

We aimed to elucidate whether a biochar/biosolids mixture would be as effective for Zn biofortification as biosolids alone. Specifically, we sought to determine the growth and elemental composition of *Beta vulgaris* (L.), *Spinacia oleracea* (L.), *Raphanus sativus* (L.), *Brassica oleracea* (L.), *Daucus carota* (L.), *Allium ampeloprsum* (L. [J. Gay]), *Allium cepa* (L.), *Lactuca sativa* (L.), *Zea mays* (L.), *Solanum lycopersicum* (L.), and *Cucurbita pepo* (L.) in soils amended with biosolids, biochar, a biosolids/biochar mixture, and a control soil.

2. Materials and methods

Biosolids were obtained from the Kaikoura regional treatment works, New Zealand. Some 160 kg of biosolids were homogenised using a concrete mixer and passed through a 20 mm sieve. The moisture content of the fresh biosolids was 53% (Knowles et al., 2011). The biochar was manufactured from *P. radiata* D. Don chips that were pyrolised in the absence of oxygen at a temperature of 350 °C. The biochar was crushed to give particles with a maximum diameter of 10 mm (Clough et al., 2010; Taghizadeh-Toosi et al., 2011). The chemical properties of the biosolids and biochar used in this experiment are given in Table 1. The topsoil, obtained from Parkhouse Garden Supplies Limited, Christchurch, New Zealand, was a silt loam. Table 2 gives the soil properties.

Table 1
Soil chemical properties of the biosolids and biochar. Values in brackets represent the standard error of the mean (n = 3 unless otherwise indicated). Adapted from (Knowles et al., 2011).

	Biosolids	Biochar
pH	4.1	7.8
CEC (cmol$_{(+)}$ kg^{-1})	n.d.	8.0[ab]
C (%)	28.0 (0.2)	70.6[a]
N (%)	2.7 (0.03)	0.2[a]
P (mg kg^{-1})	4683 (2)	412 (2)
S (mg kg^{-1})	6972 (43)	288 (12)
Ca (mg kg^{-1})	9818 (176)	7758 (160)
Mg (mg kg^{-1})	2204 (17)	605 (11)
K (mg kg^{-1})	4330 (67)	1713 (17)
Na (mg kg^{-1})	428 (3)	1000 (29)
Cd (mg kg^{-1})	2.8 (0.0)	0.1 (0.01)
Cr (mg kg^{-1})	32 (1.4)	2.8 (0.6)
Cu (mg kg^{-1})	561(33)	14 (5)
Pb (mg kg^{-1})	96 (3)	1.0 (0.2)
Zn (mg kg)$^{-1}$	878 (13)	16 (1.3)

n.d. = "not determined".
[a] Single analysis on homogenised material.
[b] Taghizadeh-Toosi et al. (2011).

A control and three soil treatments were prepared using a concrete mixer. Biosolids were mixed into the soil at a rate of 10% by volume (T1). Biochar was mixed into the soil at a rate of 20% by volume (T3) and a treatment was prepared (T2) incorporating 10% biosolids and 20% biochar by volume. On a weight basis, the rates of biosolids and biochar addition were 2% and 2.5% respectively.

For each soil type, 55 two-and-a-half litre plastic pots (diameter 16 cm, height 15.5 cm) were filled to a depth of 13 cm. The pots were placed in a greenhouse at Lincoln University, New Zealand and left for 2 weeks before planting. In each soil treatment, there were five replicates of 11 plant treatments, namely beetroot (*B. vulgaris* subsp. *vulgaris* L. var 'Baby Beet'), spinach (*S. oleracea* L. var 'New Zealand'), radish (*R. sativus* L. var 'Champion'), broccoli (*B. oleracea* L. var 'Shogun'), carrot (*D. carota* L. var 'Egmont Supreme'), leek (*A. ampeloprsum* var. *Porrum* (L.) J. Gay), onion (*A. cepa* var. *Cepa* L. var 'Pukekohe Long Keeper'), lettuce (*L. sativa* L. var 'Green Oak'), corn (*Z. mays* L. var. rugosa), tomato (*S. lycopersicum* L. var 'Russian Red'), and courgette (also called zucchini – *C. pepo* L. var 'Blackjack'). All plant material was sourced from Oderings Nursery, 20 West Coast Road, Yaldhurst, Christchurch, New Zealand. Beetroot, carrot, lettuce, radish, spinach, corn and courgette were grown from seed, whereas broccoli, onion, leek and tomato were grown from seedlings. Three seeds were planted per pot. After emergence, seedlings were thinned out to one per pot. Pots were arranged in a randomised block design. Planting occurred in late January 2010. In late March 2010, the corn was transplanted into 7.5 l pots (diameter 26 cm, height 20 cm). Pots were watered daily to field capacity. Any excess water drained via holes in the bottom of the pots. All plants were periodically fertilised with Ruakura solution (Table S1, Supplementary data). Because of the appearance of whitefly infection of the broccoli, these plants were sprayed with Key Pyrethrum (14 g l^{-1} pyrethrum and 56.5 g l^{-1} piperonyl butoxide in the form of an emulsifiable concentrate diluted to 5 ml l^{-1}) on March 10th and 29th and on May 3rd.

Plants were harvested upon maturation of the edible portions, which was between the 19th of March (radish) and 1st July (onion). The edible portions of the plants were excised. The root vegetables (beetroot, radish, and carrot) were peeled. The outer layer of the onions was removed. The husks were removed from the corn. Tomatoes were not peeled. The fresh weight of the edible portions was determined. Both the edible portions and the residual material was washed thoroughly with deionised water and placed in a drying cabinet at 105 °C until a constant weight was obtained. Samples were ground and stored in an airtight container until chemical analyses.

Following incubation in the greenhouse, six soil samples from each treatment as well as the control were dried, and sieved to <2 mm using a Nylon sieve. Soil C and N concentrations were measured using an Elementar Vario MAX CN analyser. Soil pH was determined using 10 g of soil and 25 ml of deionised water. The mixture was shaken, left overnight, and shaken again before determination with a pH meter (Mettler Toledo Seven Easy).

An estimation of the element solubility was made using a 0.05 M Ca(NO$_3$)$_2$ extraction after Black et al. (2012), who demonstrated this was the most effective procedure for determining metal bioavailability in biosolids-amended soil. Five grams of soil were weighed into 50 ml centrifuge tubes. Thirty millilitres of extractant were added and a suspension formed using a vortex mixer. The centrifuge tubes were shaken on an end-over-end shaker for 2 h and centrifuged at 3200 rpm for 15 min. After filtering (Whatman 52 filter paper), the samples were stored sealed containers until chemical analyses.

Pseudo-total elemental analysis was carried out using microwave digestion in 8 ml of Aristar™ nitric acid (±69%), filtered using Whatman 52 filter paper (pore site 7 μm), and diluted with milliQ water to a volume of 25 ml and stored for chemical analyses. Concentrations of Al, As, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES — USA).

Table 2

Average total N and P, and pseudo total (aqua regia) concentrations of the other elements in the growth media. The "control" was a silt loam soil. Values in brackets represent standard errors in the treatments (n = 6). Average pHs and standard error ranges were calculated using [H+]. In a row, values with the same superscripted letter are not significantly different.

	Control	T1 (biosolids)	T2 (biosolids + biochar)	T3 (biochar)
pH	5.6 (5.6–5.7)[a]	5.5 (5.5–5.6)[a]	5.6 (5.5–5.6)[a]	5.7 (5.6–5.8)[a]
C (%)	3.3 (0.4)[a]	3.7 (0.1)[a]	5.7 (0.5)[b]	5.0 (0.3)[b]
N (%)	0.22 (0.01)[a]	0.27 (0.02)[a]	0.34 (0.03)[b]	0.25 (0.00)[a]
P (mg kg^{-1})	596 (7.0)[a]	692 (30)[b]	728 (29)[b]	580 (27)[a]
K (mg kg^{-1})	2958 (411)[a]	4010 (290)[a,b]	3852 (759)[a,b]	4696 (280)[b]
S (mg kg^{-1})	245 (5.9)[a]	360 (30)[a]	583 (80)[b]	380 (33)[a]
Ca (mg kg^{-1})	5889 (389)[a]	6082 (334)[a]	6692 (216)[a]	6681 (163)[a]
Mg (mg kg^{-1})	2579 (61)[a]	2603 (47)[a]	2503 (59)[a]	2536 (96)[a]
Fe (%)	1.4 (0.0)[a]	1.4 (0.0)[a,b]	1.40 (0.0)[a,b]	1.4 (0.0)[b]
B (mg kg^{-1})	12 (0.6)[a,b]	13 (0.3)[a]	11 (0.6)[b]	13 (0.6)[a]
Mn (mg kg^{-1})	237 (4.9)[a]	240 (3.4)[a]	235 (5.2)[a]	239 (3.4)[a]
Cu (mg kg^{-1})	7.3 (0.1)[a]	20 (4.6)[b]	30 (4.0)[c]	9.0 (0.4)[a]
Zn (mg kg^{-1})	52 (1.4)[a]	69 (3.4)[b]	89 (3.7)[c]	54 (1.3)[a]
Al (%)	1.6 (0.1)[a]	1.8 (0.1)[a]	1.6 (0.0)[a]	1.8 (0.0)[a]
Na (mg kg^{-1})	392 (31)[a]	417 (27)[a,b]	480 (13)[b,c]	493 (14)[c]
Cd (mg kg^{-1})	0.28 (0.01)[a]	0.33 (0.02)[a]	0.40 (0.02)[b]	0.30 (0.02)[a]
Cr (mg kg^{-1})	15 (0.4)[a]	17 (0.2)[a]	17 (0.8)[a]	16 (0.7)[a]
Ni (mg kg^{-1})	8.7 (0.3)[a]	9.8 (0.9)[a]	9.2 (0.2)[a]	9.3 (0.4)[a]
As (mg kg^{-1})	5.2 (0.2)[a]	5.5 (0.2)[a]	5.9 (0.3)[a]	5.6 (0.3)[a]
Pb (mg kg^{-1})	20 (0.8)[a]	23 (1.2)[b]	24 (0.9)[b]	20 (0.7)[a]

Reference soil and plant material (International Soil analytical Exchange — ISE 921 and International Plant analytical Exchange IPE 100) from Wageningen University, The Netherlands, was analysed for quality assurance. Recoverable concentrations were 91%–108% of the certified values.

Data were analysed using Minitab® 16 (Minitab Inc., State College, Pennsylvania, USA). Data sets were analysed using ANOVA with Fisher's least-significant-difference post-hoc test to compare means. The level of significance was 0.05.

3. Results

3.1. Pseudo-total element concentrations in the soils

Table 2 shows the pseudo-total elemental concentrations in the control soil and the treatments. The biosolids treatments (T1 and T2) caused a significant increase in total Zn, with levels increasing by a factor of 1.3 and 1.7, respectively. The T1 and T2 treatments also had higher N concentrations. Extrapolating to field conditions, the rate of N addition for the T1 and T2 treatments was equivalent to 650 and 1560 kg N ha^{-1} (assuming the biosolids are incorporated to a depth of 10 cm and the soil has a bulk density of 1.3 g ml^{-1}). The rates of nitrogen addition in this study were close to the range 600–1200 kg N ha^{-1} where Knowles et al. (2011) demonstrated that biochar would mitigate leaching.

The concentrations of Cd, Cu and Pb were also increased in the biosolids treatments, but remained below their respective Dutch target values (often used as surrogate values in NZ) of 0.8, 36, and 85 mg kg^{-1}, respectively (Provoost et al., 2006).

The addition of biochar (T3) caused a significant increase in soil C, K, and Na. Extrapolating to field conditions (using the aforementioned assumptions), the rate of biochar addition is equivalent to 50 t ha^{-1}. This rate of biochar addition was within the range rates reported by other authors (Chan et al., 2007).

3.2. Soluble element concentrations in the soils

Table 3 shows the soluble (0.05 M Ca(NO$_3$)$_2$ extractable) concentrations of the measurable elements. Compared to the change in total Zn concentration in the T1 and T2 treatments (Table 3), soluble Zn increased disproportionately, by a factor of 4.4 and 7.5, respectively. There was also a disproportionate increase in soluble Cd and Cu.

Given that there was no change in pH (Table 2), this increase may be due to differences in the Zn speciation in the biosolids compared to the soil, although we have insufficient data to speculate on the nature of this speciation.

The addition of biochar caused an increase in soluble Na and B. The addition of biochar did not cause a significant reduction in the solubility of any of the elements measured. This is in contrast to the findings of some other authors (reviewed by Beesley et al., 2011) who report that many biochars cause a reduction in metal solubility. Interestingly, Gan et al. (2012), reported that biochar reduced the solubility of Cu, Ni and Cr, but not Zn. Given the diverse properties of biochars, one would expect a range of influences on the solubility of metals in soil.

3.3. Plant growth

All plants grew to maturity and none exhibited significant deficiency or toxicity symptoms. Table 4 shows the dry biomasses of the plants at harvesting. The biomasses of broccoli, corn, leeks, tomatoes, spinach and lettuce were not significantly affected by any of the treatments. The biomasses of the belowground vegetables, radish, beetroot, and carrot were significantly enhanced by the biowaste treatments (T1 and/or T2) and in the case of beetroot by all three treatments. This indicates that the growth enhancing effect may have been due to the physical structure of the soil, rather than the

Table 3

Average extractable (0.05 M Ca(NO$_3$)$_2$) concentrations and standard errors in the four different growth media (n = 6). The "control" was a silt loam soil. All units are in mg kg^{-1}. In a row, values with the same superscripted letter are not significantly different.

	Control	T1 (biosolids)	T2 (biosolids + biochar)	T3 (biochar)
P	0.54 (0.09)[a]	0.74 (0.10)[a,b]	1.00 (0.09)[b]	0.77 (0.17)[a,b]
K	15 (1.9)[a]	16 (3.8)[a]	22 (4.9)[a]	25 (4.1)[a]
S	8.6 (1.7)[a]	10 (1.2)[a]	35 (7.8)[b]	26 (9.5)[a,b]
Mg	14 (1.4)[a]	15 (1.3)[a,b]	18 (0.9)[a,b]	18 (1.3)[b]
Fe	0.44 (0.11)[a]	0.45 (0.08)[a]	0.47 (0.10)[a]	0.82 (0.38)[a]
B	0.11 (0.01)[a]	0.12 (0.01)[a]	0.15 (0.01)[a,b]	0.17 (0.02)[b]
Mn	3.8 (0.4)[a]	4.0 (0.4)[a]	3.6 (0.4)[a]	3.6 (0.8)[a]
Cu	0.021 (0.004)[a]	0.032 (0.004)[a]	0.053 (0.004)[b]	0.021 (0.002)[a]
Zn	0.14 (0.02)[a]	0.61 (0.14)[b]	1.05 (0.08)[c]	0.13 (0.03)[a]
Na	42 (5.1)[a]	49 (3.6)[a,b]	59 (1.2)[b]	57 (3.0)[b]
Cd	0.0015 (0.0002)[a]	0.0031 (0.0004)[b]	0.0042 (0.0002)[c]	0.0017 (0.0001)[a]

Table 4
Average aboveground (unless otherwise indicated) dry biomass (g) and standard errors in the treatments (n = 5). The "control" plants were grown in an un-amended was a silt loam soil. In a row, values with the same superscripted letter are not significantly different.

	Control	T1 (biosolids)	T2 (biosolids + biochar)	T3 (biochar)
Radish[a]	2.7 (0.2)[a]	3.9 (0.6)[a,b]	4.3 (0.5)[b]	3.9 (0.5)[a,b]
Lettuce	3.0 (1.9)[a]	2.8 (0.4)[a]	4.3 (0.5)[a]	3.8 (1.9)[a]
Spinach	4.8 (1.7)[a]	3.4 (0.9)[a]	–	2.9 (0.9)[a]
Courgette	15 (1.4)[a]	11 (1.3)[b]	16 (0.6)[a,b]	13 (0.6)[a,b]
Beetroot[a]	4.9 (1.3)[a]	10.4 (0.6)[b]	9.4 (0.4)[b]	9.4 (1.1)[b]
Tomato	17 (0.8)[a]	16 (2.6)[a]	17 (2.0)[a]	16 (2.6)[a]
Carrot[a]	3.1 (0.6)[a]	6.6 (1.7)[a,b]	7.6 (1.6)[b]	5.4 (0.7)[a,b]
Leek	7.4 (1.5)[a]	6.2 (1.7)[a]	8.2 (0.5)[a]	6.2 (0.8)[a]
Onion[a]	1.2 (0.3)[a]	1.9 (0.4)[a]	4.4 (0.8)[b]	1.0 (0.4)[a]
Corn	80 (9.5)[a]	80 (4.9)[a]	77 (8.7)[a]	90 (4.2)[a]
Broccoli	24 (5.9)[a]	28 (2.1)[a]	22 (5.6)[a]	28 (4.7)[a]

[a] Includes belowground edible portion.

addition of plant nutrients, which we ensured were sufficient in all treatments.

3.4. Effect of the treatments on Zn biofortification

Table 5 shows the effect of the treatments on the Zn concentrations in the edible portions of the plants. There was no significant increase in the Zn concentrations in onions, broccoli, and corn. In contrast, all other species showed a significant increase in the Zn concentration of their edible portions in one or both biosolids (T1 and T2) treatments. The biochar treatment (T3) resulted in a significant increase in the Zn concentrations of the radish bulb.

3.5. Fresh weight Zn concentrations in biofortified vegetables

Fig. 1 shows the Zn concentrations of the control and the biosolids + biochar treatment (T2) on a fresh weight (i.e. as eaten) basis. Fig. 1 also indicates the biofortification coefficient, defined here as the treatment/control concentration coefficient. The leafy portions of the plants, namely beetroot leaves, spinach lettuce and radish leaves had the highest biofortification coefficients whereas the reproductive structures of the plants, namely tomato (full berry), courgette (swollen ovary), corn (whole culm) and broccoli (buds before flowering) had the lowest biofortification coefficients. This is consistent with the observation that Zn is primarily transported in the xylem and therefore tends to accumulate in the leaves which are the major water sink (Broadley et al., 2007).
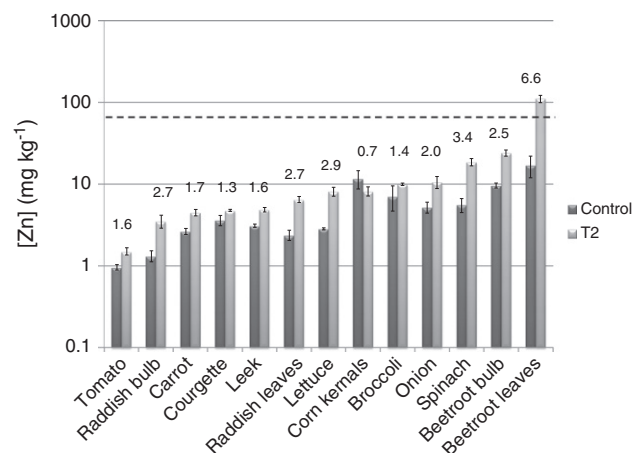

Fig. 1. Average Zn concentrations (mg kg^{-1} fresh weight) and standard errors of the control (a silt loam soil) and the T2 (biosolids + biochar) treatment (n = 5) of the edible portions of the vegetables. The numbers above the bars represent the biofortification coefficient (treatment/control concentration quotient). The dashed line represents the concentration required for the recommended daily intake of an adult male (11 mg) given a serving size of 160 g.

The plants with the highest Zn concentrations, which coincidentally had the highest biofortification coefficients, were the chenopods beetroot and spinach. A single 160 g serving of biofortified beetroot bulbs or spinach leaves could provide around half of the recommended daily intake of Zn of 11 mg.

3.6. Fresh weight concentrations of heavy metals in the plants

The maximum fresh weight concentrations of Cu and Pb in any of the plants were 1.27 and 0.046 mg kg^{-1}, respectively (Tables S2 and S3, Supplementary data). Both of these values occurred in the T1 beetroot treatments. These are well below the maximum permitted concentrations (fresh weight) of 73 and 0.3 mg kg^{-1} for Cu and Pb, respectively that are prescribed by the World Health Organisation (Bigdeli and Seilsepour, 2008). The Pb concentrations in most plants were below detection limits (<0.01 mg kg^{-1}).

Fresh weight Cd concentrations were significantly increased in the T1 treatments of beetroot, radish, onion and carrots, whereas only beetroot was significantly increased in the T2 treatment (Table 6). This indicates that the biochar may have slightly decreased the Cd uptake by some species. On a fresh weight basis, the Cd concentrations in the biofortified leaves of spinach and beetroot (Fig. 2) exceeded

Table 5
Average Zn concentrations and standard errors in the edible portions of the plants (n = 5). The "control" plants were grown in an un-amended was a silt loam soil. All units are in mg kg^{-1} on a dry matter basis. In a row, values with the same superscripted letter are not significantly different.

	Control	T1 (biosolids)	T2 (biosolids + biochar)	T3 (biochar)
Leek	15 (1)[a]	20 (5)[a]	30 (3)[b]	20 (2)[a]
Tomato	17 (1)[a]	23 (1)[b]	26 (2)[b]	16 (1)[a]
Carrot	30 (3)[a,b]	68 (13)[c]	50 (5)[b,c]	25 (1)[a]
Radish bulb	33 (1)[a]	111 (6)[b]	84 (5)[c]	54 (11)[d]
Onion	34 (6)[a]	61 (24)[a]	67 (12)[a]	44 (7)[a]
Lettuce	45 (3)[a]	62 (31)[a]	156 (17)[b]	52 (17)[a]
Radish leaves	54 (7)[a]	191 (20)[b]	140 (17)[c]	56 (11)[a]
Broccoli	55 (7)[a,b]	73 (17)[a,b]	88 (2)[b]	51 (7)[b]
Courgette	55 (3)[a]	83 (2)[b]	84 (2)[b]	54 (2)[a]
Corn kernels	57 (15)[a]	48 (4)[a]	40 (5)[a]	54 (10)[a]
Beetroot bulbs	69 (6)[a]	215 (21)[b]	178 (11)[b]	35 (4)[a]
Spinach	77 (13)[a]	177 (61)[b]	–	48 (7)[a]
Beetroot leaves	180 (25)[a]	1178 (90)[b]	1200 (74)[b]	201 (8)[a]

Table 6
Average Cd concentrations and standard errors in the edible portions of the plants (n = 5). The "control" plants were grown in an un-amended was a silt loam soil. All units are in mg kg^{-1} on a dry matter basis. In a row, values with the same superscripted letter are not significantly different.

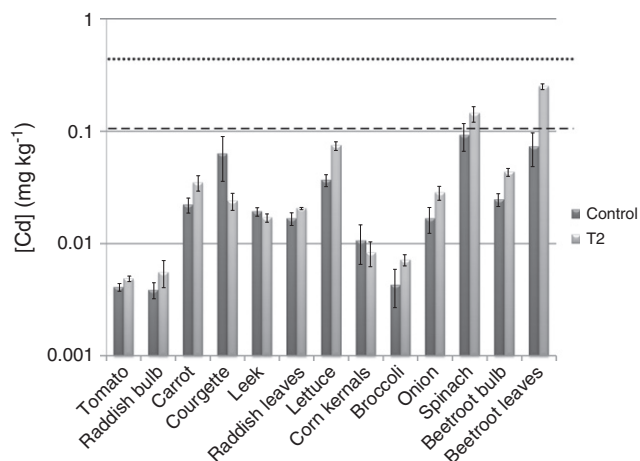|	Control	T1 (biosolids)	T2 (biosolids + biochar)	T3 (biochar)
Leek	0.10 (0.00)[a]	0.09 (0.02)[a]	0.10 (0.01)[a]	0.07 (0.02)[a]
Tomato	0.07 (0.00)[a]	0.09 (0.01)[a]	0.09 (0.01)[a]	0.07 (0.01)[a]
Carrot	0.26 (0.05)[a]	0.64 (0.13)[b]	0.39 (0.03)[a]	0.20 (0.03)[a]
Radish bulb	0.10 (0.01)[a]	0.13 (0.01)[a]	0.14 (0.02)[a]	0.11 (0.01)[a]
Onion	0.11 (0.03)[a]	0.36 (0.14)[b]	0.18 (0.03)[a,b]	0.18 (0.01)[a,b]
Lettuce	0.60 (0.09)[a]	0.75 (0.03)[a]	1.46 (0.13)[b]	0.78 (0.10)[a,b]
Radish leaves	0.38 (0.04)[a]	0.50 (0.03)[a]	0.45 (0.02)[a,b]	0.41 (0.03)[a,b]
Broccoli	0.03 (0.01)[a,b]	0.05 (0.02)[a,b]	0.07 (0.01)[a]	0.02 (0.00)[b]
Courgette	0.96 (0.40)[a]	1.68 (1.16)[a]	0.43 (0.08)[a]	0.87 (0.57)[a]
Corn kernels	0.05 (0.02)[a]	0.05 (0.01)[a]	0.04 (0.01)[a]	0.05 (0.01)[a]
Beetroot bulbs	0.18 (0.02)[a]	0.36 (0.05)[b]	0.33 (0.03)[b]	0.12 (0.01)[a]
Spinach	1.30 (0.32)[a]	1.42 (0.45)[a]	–	0.78 (0.14)[a]
Beetroot leaves	0.78 (0.12)[a]	3.41 (0.40)[b]	2.77 (0.06)[b]	1.04 (0.11)[a]

Fig. 2. Average Cd concentrations (mg kg^{-1} fresh weight) and standard errors of the control (a silt loam soil) and the T2 (biosolids + biochar) treatment (n = 5) of the edible portions of the vegetables. The dashed line represents the maximum permitted concentration in vegetables. The dotted line represents the concentration required to exceed the tolerable daily intake of 0.06 mg given a serving size of 160 g.

the World Health Organisation's limit for Cd in vegetables of 0.1 mg kg^{-1} (Bigdeli and Seilsepour, 2008). However, the amount of Cd in a 160 g portion of plant tissue was still well below the tolerable daily intake of 0.06 mg of Cd (WHO, 2007).

4. Discussion

The Zn concentration in the edible portions of tomatoes, carrots, radish (bulbs and leaves), courgette and beetroot (bulbs and leaves) were either not significantly different or significantly less in the T2 treatment compared to the T1 treatment. This indicates that the biochar may have the effect of slightly reducing the Zn uptake in some species. Nevertheless, the field application of the high rates of biosolids required for biofortification may be unacceptable unless their potential for nitrate leaching has been mitigated, for example by adding biochar.

The high Cd uptake was surprising given that the total concentration of Cd in the soil was well below even conservative threshold values (Provoost et al., 2006). When consumed, the high Zn concentrations in the leaves of the chenopods may reduce the effective toxicity of Cd by stimulating the production of metallothioneins in the liver (Klaassen et al., 1999).

We used a relatively acidic soil (pH = 5.6) in these experiments. It is likely that the biofortification coefficient would be reduced at higher pH values, where the solubility of Zn^{2+} is reduced (Robinson et al., 2009). Simply increasing the Zn concentration in vegetables may not necessarily improve human Zn nutrition. Other compounds present in the plants, such as oxalates, phytates, tannins and fibres reduce Zn uptake by the gut (White and Broadley, 2005). Cooking or other pre-processing of the vegetables before consumption may result in changes in the Zn concentration and changes in the human bioavailability of the Zn.

Our experiments tested a single cultivar of each species. It is well known that genetic variation between vegetable cultivars can result in significant differences in Zn and Cd uptake (Crews and Davies, 1985; McLaughlin et al., 1994). Alexander et al. (2006) reported no significant differences in Zn uptake between five cultivars of lettuce, onion, spinach and carrot. Carrot and spinach had significant differences in Cd uptake. Clearly, our results are therefore not applicable to all cultivars of the crop species tested.

We did not monitor the concentrations of other organic contaminants, such as triclosan or estrogens, which may have been present in the biosolids. It is possible that the risk of such pollutions is reduced

when the biosolids is combined with biochar because of sorption by the latter (Beesley et al., 2011). Similarly, the risk of human infection with pathogens from the biosolids needs to be eliminated. Here again the role of the biochar in the mixture is unknown. Heat or chemical pre-treatment of the biosolids may be necessary although this could greatly increase the cost of the operation.

5. Conclusions

Adding a combination of biosolids and biochar to an acidic soil increased the total and soluble Zn concentrations, improved plant growth and resulted in increased Zn uptake by most plants. The increase in Zn uptake is highly species dependent. Biofortified beetroot has bulb and leaf Zn concentrations that are manifold higher than those present in staple crops. However, it is unclear whether this Zn is in a form that is bioavailable to humans. Adding biosolids and biochar to soil may be an effective means of disposing of waste materials, while improving soil fertility, increasing soil carbon and potentially alleviating Zn deficiencies in humans or animals. Future work should focus on testing the performance of these amendments in other soil types, particularly those with high pHs. The role of biochar in reducing some other problems associated with biosolids such as pathogens and organic contaminants should also be investigated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.10.027.

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