PAPER

The effect of biosolid/soil composition on growth and uptake of zinc (Zn) by broccoli (*Brassica oleracea* var.) under greenhouse conditions†

Kefa K. Onchoke, [ID] *[a] Brett P. Horalan Jnr.[a] and Robert B. Friedfeld[b]

Wastewater sludge (also known as biosolids) is commonly applied to agricultural and forestry land, offering the advantage of recycling nutrients and organic matter from the waste material back to the land. This study investigated the influence of biosolids on the uptake of Zn by broccoli (*Brassica oleracea* var.), a commonly consumed vegetable, grown in biosolid-amended soil compositions, by using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDX). Broccoli was grown in soil amended with composted wastewater sludge at five different compositions (0, 25, 50, 75, and 100% wt/wt) treated with 100 ppm Zn at the beginning. *Brassica oleracea* var. (broccoli) plants from pot experiments were harvested after 30 days and Zn concentrations in plant parts (root, stem, and leaf) were analyzed by using flame atomic absorption (FAA) spectroscopy. Harvested biomass increased by 141–454% in comparison to the control (zero% wt/wt biosolid). The best growth was apparent at 25% (w/w)–75% (biosolid wt/wt), with Zn concentrations in plant parts found in the order $[Zn]_{root} > [Zn]_{shoot} > [Zn]_{leaf}$. SEM/EDX and FTIR spectral data show the presence of carboxyl functional groups that can bind Zn. The investigation shows that biosolids influence the yield and root-to-shoot-leaf transfer of Zn.

Environmental significance

Zinc (Zn) is an essential micronutrient in biological processes. Here, the role and influence of wastewater sludge (biosolids) on the uptake of Zn when biosolids are applied as an amendment to soil are studied. The study uses FTIR, SEM/EDX and XRD to characterize soil and sludge and AAS to determine concentrations of Zn, providing advances to current imaging methods. Through calculation of translocation factors, implications for the continued use of biosolids in the environment, specifically on vegetable plants, are discussed. The results suggest that biosolids can potentially enhance the translocation of Zn into plants. The study also considers future investigations into possible uptake properties at the molecular level and multi-interelement approaches to consider various mechanisms for Zn uptake at different growth stages.

1 Introduction

Zinc (Zn) is an essential micronutrient that plays beneficial roles in biological processes. It is a cofactor in several metalloenzymes and transcription factors and is a structural component of ribosomes and some t-RNA synthetases.[1,2] Zinc is also important for photosynthesis, maintaining the cell membrane integrity, growth and reproduction of plants, cell division, cell growth, wound healing, the breakdown of carbohydrates, chlorophyll biosynthesis, gene expression, signal transduction, and plant defence systems.[3] Zinc may be supplied and transported into the plant system through various mechanisms, including the anatomical structure of conducting tissue, soil pH, and soil content.[4] Deficiency of zinc in plants often results in poor chloroplast development and function.[4,5] The availability of Zn in plants is therefore important for the development and function of plant parts.

Various ways of introducing Zn to supplement and boost plant growth are often sought. The addition of Zn to soils through use of fertilizers and application of biosolids has been investigated.[6,7] The nitrogen-rich Composted Wastewater Sludge (CWS or MSW) is one such medium.[1] However, the application and reuse of biosolids can either tie or increase the metal content in plant parts.[8–10] Although Zn concentrations in biosolids in the USA are below regulated USEPA guidelines,[11,12] their continued application may increase Zn amounts in soils and plant parts.[12]
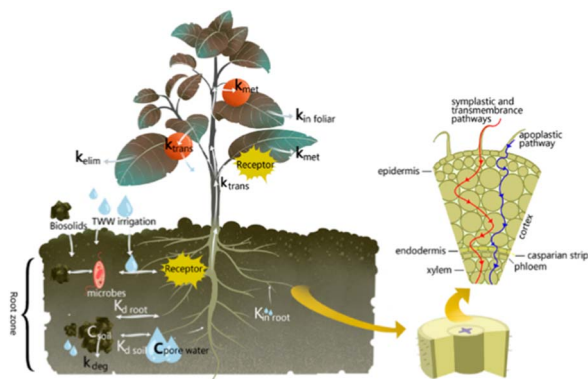
CWS is a dewatered undigested sludge that has gone through the process of composting with a bulking material added to enhance soil aeration.[13] The process of composting involves the decomposition and stabilization of organic compounds under controlled aerobic and thermophilic conditions.[2] Composting

aDepartment of Chemistry & Biochemistry, Stephen F. Austin State University, Box 13006 – SFA Station, Nacogdoches, Texas, 75962-13006, USA. E-mail: onchokekk@sfasu.edu

bDepartment of Physics, Engineering and Astronomy, Stephen F. Austin State University, 1901 Raguet St North, Box 13044, Nacogdoches, Texas 75962, USA

Scheme 1 Fate and transport processes of metal ions or pollutants in the soil–biosolid–plant system. Abbreviations: C_{soil}, concentration in soil; $C_{porewater}$, porewater concentration; $K_{d\ soil}$, soil/water partition; $K_{d\ root}$, root/water partition; K_{deg}, degradation in soil; $K_{in\ root}$, uptake into root, $K_{in\ foliar}$, foliar uptake; K_{trans}, translocation in plant, K_{met}, in-plant metabolism; K_{elim}, potential loss from plant (adopted with permission from Qiuguo Fu, et al., Environ. Sci. Technol., 2019, 53, 14083−14090).[19]

tends to increase the complexation of heavy metals in organic waste residuals, limiting their solubility and potential bioavailability in soil.[14] Irshad et al.[14] found that the composting process limited Zn solubility from 22.3 (mg kg^{-1}) to 14.8 (mg kg^{-1}) after 100 days. Vaca et al.[15] found that the tendency for Zn sorption follows the trend: sewage sludge > compost > sewage sludge-soil > compost-soil ≫ soil, and depends on soil pH. Kalavrouziotis et al.[16] found that Cu and Zn solubilities are reduced at high pH values. The restriction in the use of CWS stems from its tendency to contain heavy metals, including Zn. High Zn concentrations in plants result in wilting and death. Other issues encountered with sludges are due to the tendency of heavy metals to leach into the surrounding soil as leachates. Hoagland solution[17] is often applied to samples to assuage any potential deficiencies for plant growth.

Several studies have examined the uptake of Zn in vegetables with varying solution concentrations. A number of processes are involved in the uptake of metals, pollutants or pharmaceuticals,[18] including various distribution equilibria shown in Scheme 1. However, fewer studies have investigated the influence of biosolids on the uptake of Zn by broccoli at different biosolid–soil ratios. Therefore, the objective of this study was to examine the ability of biosolids to influence the uptake of Zn by Brassica oleracea var.italica (broccoli) at five soil–CWS ratios, namely, 0% (wt/wt), 25% (wt/wt), 50% (wt/wt), 75% (wt/wt), and 100% (wt/wt). Given the high affinity of biosolids to withhold metals, we sought to find out whether CWS will deprive the broccoli or enhance the uptake and translocation of Zn to plant parts (root, stem or leaves).

2 Materials and methods

2.1 Soil and compost characterization, preparation, and treatment of composted wastewater sludge (CWS)

Soil samples were collected from a local farm in Nacogdoches in East Texas (USA). The samples were air-dried, passed through a 2-mm sieve to remove debris and/or roots, and crushed and ground to fine powder with a mortar and pestle. Composted wastewater sludge (CWS, also known as biosolids) was obtained from the Neches Compost Facility (NCF) in Jacksonville, Texas, located near the Neches River on HWY-79 West of Jacksonville. The NCF markets and sells CWS under the trade name Soil Therapy Compost™ (STC). The physicochemical and mineralogical analyses of CWS are reported in previous ref. 2, 9, 11 and 20 and in Section 3. The analysis was carried out using inductively coupled plasma optical spectroscopy (ICP-OES), X-ray powder diffraction (XRPD), SEM/EDX and TGA. Soil physicochemical and mineralogical characteristics were analysed using powder X-ray diffraction (PXRD) and SEM/EDX.

Sieved soils were carefully homogenized before mixing with STC. A total of 300 g of soil–STC (wt/wt%) compositions per pot was weighed. Each soil-CWS composition was performed in triplicate. The amended soil–CWS mixture was left to stabilize for three days prior to planting seeds.

2.2 Culture media, pot experiments, harvesting, and biomass measurements

Broccoli (Brassica oleracea var.) was grown on standard soil and soil–CWS media in a greenhouse. Five different soil–compost compositions viz., 0%, 25%, 50%, 75%, and 100% (wt/wt) were used in the experiment. Soil–CWS ratios were treated with Hoagland solution three days prior to sowing seeds and every three days during growing stages.

Broccoli seeds (Brassica oleracea var.) were purchased from local Walmart stores (Nacogdoches, Texas, USA). The experiment was carried out in a greenhouse, with natural light at a temperature range of 15 to 35 °C. Pots were frequently watered with nanopure water, as needed, to keep them close to the water field capacity during the growing cycle. Four seeds were planted per pot for each of the soil–CWS mixtures. After 30 days, plants were harvested and separated into roots, shoots, and leaves, weighed and then placed in an oven for drying at 60 °C for 24 h. The dried plant material was then stored in air-tight plastic bags till analysis, weighed to within 500 ± 0.001 mg, and prepared for acid digestion. The samples were later prepared for instrumental and spectroscopic analysis. The pH of soil and CWS was measured using USEPA Method 9045D.[21]

2.3 Instrumentation and spectroscopic analysis

The surface morphology of the biosolids and soil was examined with a JEOL-JSM 6100 scanning electron microscope equipped with a Horiba energy dispersive X-ray spectrometer (SEM/EDX). The elemental composition of the air-dried samples was determined with EDX for 120 seconds at 40 kV using a SiriusSD detector kept at −20 °C. To minimize electron charging, biosolids and soils were coated with palladium/gold using an Automatic Platinum Sputter Coater System (Quorum Q150RS).

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS FTIR) spectra of soil and STC in the 230–4000 cm^{-1} region were acquired on a silicon carbide abrasive pad (4 cm^{-1} resolution) with a PerkinElmer Spectrum 100 spectrometer equipped with a Ge/CsI beam splitter and a DTGS detector. Four

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Environ. Sci.: Adv., 2025, 4, 912–920 | 913

to eight scans or greater were collected in DRIFTS (Kubelka–Munk) mode. Powder XRD (PXRD) analysis was carried out following a previously reported procedure.[11] PXRD analysis was performed in the 2θ range between 2–90° on a Bruker AXS D8 Advance diffractometer equipped with an X-ray tube (Cu K$_\alpha$ radiation: $\lambda = 1.54060$ Å, 40 kV, and 40 mA) using a Ni filter and a one-dimensional LynxEye detector at a scanning speed of 2° min^{-1} and a step size of 0.0125° with a count time of 1 s/step. The generator was operated at 40 kV and with a 40-mA current.

2.4 Digestion of plant biomass

The samples were digested using the USEPA Method 3050B procedure.[22] The samples were digested in 5 mL aliquots of 4 M conc. nitric acid (HNO$_3$) in digiTUBES (SCP Science, https://www.scpscience.com/) at 95 °C with a DigiPREP till 2.5 mL solution remained. Addition of 1-mL aliquots of 30% H$_2$O$_2$ was performed till effervescence subsided. The solution was then reheated at 95 °C till 2.5 mL remained and cooled to room temperature. Then 2–2.5 mL of nanopure water was pneumatically pipetted. The remaining solution was filtered through a 0.45 μm filter using a filtration apparatus (https://www.scpscience.com/).[2,9] The filtered solution was diluted to 50 mL with nanopure water. The same digestion protocol was followed for analysis of standards and sample plant weights. The samples were thereafter diluted, digested, and diluted 5 times.

2.5 Determination of Zn concentrations in plant parts

A Shimadzu 6800 spectrometer with flame atomic absorption spectrometry (FAA) was used to determine Zn concentrations *via* calibration curves at $\lambda_{Zn} = 213.9$ nm. A stock standard of 1000 mg L^{-1} was automatically diluted to appropriate concentrations. Reagent blanks and internal standards were run in triplicate for Zn analysis.

2.6 Validation and quality assurance of data

The results were validated by using the atomic absorption spectrophotometric measurements, employing a Shimadzu 6800 FAA spectrometer. Known standard solutions were run along with the samples for validation and verification. The recoveries for Zn in spiked blanks were within 94% to 105% and in the quality control samples were within ± 5% of known concentrations. For further verification, calibration curves were drawn, and correlation coefficients of $r^2 = 0.9988$ or better were calculated.

3 Results

3.1 Physicochemical analysis of soil and sludge

Table 1 shows the mean pH of the soil and CWS samples. The pH of the soil falls in the range of 6.3–6.7. This is comparable to WHO or USEPA guideline standards in the range of 6.5–8.5.[23] The CWS shows a lower pH in the range of 4.81–5.4 and is comparable to that measured at the Neches Compost facility (https://www.anra.org/services-projects/compost/neches-compost-facility/). The physicochemical soil characteristics from Nacogdoches soil and CWS are summarized in Table 1. The Deep East Texas soils are classified as fine, mixed, semiactive,

thermic Rhodic Paleudalfs.[24] Average organic carbon (%), % C/N ratio, NH$_4^+$–N, and % organic matter (OM) were 2.85 ± 0.21, 20.34 ± 1.04, 151.2 ± 18.9 and 5.71 ± 0.41 and 23.60 ± 0.28, 11.09 ± 0.10, 596.2 ± 4.1 and 47.20 ± 0.57 in soil and CWS, respectively (Table 1). The measured values are comparable and similar to those determined at the Neches Compost Facility that produces CWS in East Texas.[25] The NH$_3^+$–N values (Table 1) are higher than those reported at WWTP, with values of 74–90 mg L^{-1}.[26] Macroelements Ca, Mg, Na, and K are present in the soil A horizon, although typically deficient of Al, Si, Mn, Na, C, and S. Table 1 shows that soil and CWS contain K (0.8 and 3700 mg kg^{-1}), Ca (4.4 ± 2.6 and 3640 cmol kg^{-1}), Mg (1.5 ± 0.7 cmol kg^{-1} and 0.74), % organic matter (5.71 ± 0.41 and 47.2 ± 0.57), and total % N (0.140 ± 0.003 and 2.13 ± 0.01 wt%). Measured phosphorus concentrations in mg kg^{-1} were 6.1 ± 0.1 mg kg^{-1} (soil) < 13 666 ± 956 mg kg^{-1} (CWS). The electrical conductivity was determined to be 12.5 μs cm^{-1} in soil *vis-à-vis* 1464 μs cm^{-1} in CWS (Table 1).

3.2 Soil characterization using FTIR and SEM/EDX spectroscopy

3.2.1 FTIR. Fig. 1A and B show the FTIR spectra (DRIFTS) of soil and CWS (STC), respectively. The broad bands at ~3400–3700 cm^{-1}, with shoulder peaks at 3702 cm^{-1} and 3624 cm^{-1}, correspond to the presence of amino ν(N–H), ν(O–H) of phenolic compounds, and carboxylic acid stretching bands.[27] In addition, bands observed at 3398 cm^{-1} are attributed to the aromatic C–H functional groups.[28] In the 2700–3000 cm^{-1} region, bands observed at 2957 cm^{-1}, 2931 cm^{-1}, and 2856 cm^{-1} are ascribed to ν(C–H) asymmetric/or symmetric stretches in acyclic alkanes.[28] The bands observed at 1612 cm^{-1}, 1652 cm^{-1}, 1689 cm^{-1} may be ascribed to the C=O or C–H in-plane bending of acyclic or aromatic compounds, phenolic OH groups (1420 cm^{-1}), as well as carboxyl and carbonyl CO (1720 cm^{-1}) absorptions.[15] The peak at 1415 cm^{-1} may be due to the –COOH stretching vibrations. The peaks observed in the 1300–1375 cm^{-1} and 1500–1575 cm^{-1} regions may be indicative of the presence of nitrated aromatic compounds.[29–32] The presence of bands at 1097–920 cm^{-1} may be ascribed to silicates and SiO$_3$ (ref. 2–33) absorptions. The peaks at

Table 1 Physicochemical analysis of soil and CWS samples (ND = not determined). Where no standard deviation is given, only one sample measurement was made

Parameters	Soil	CWS
pH	6.51 ± 0.23	5.1 ± 0.26
Conductivity at 25 °C μs cm^{-1}	12.05	1464
Organic carbon (%)	2.85 ± 0.21	23.60 ± 0.28
% C/N ratio	20.34 ± 1.04	11.09 ± 0.10
% organic matter (OM)	5.71 ± 0.41	47.20 ± 0.57
Phosphorus (mg kg^{-1})	6.1 ± 0.1	13 666 ± 956
Potash (K$_2$O)%	ND	0.44
Zinc (mg kg^{-1})	ND	315–435
Ca (cmol kg^{-1})	4.4 ± 2.6	3640
K (mg kg^{-1})	0.8	3700
NH$_4^+$–N	151.2 ± 18.9	596.2 ± 4.1
% N	0.140 ± 0.003	2.13 ± 0.01
Iron oxide (% wt)	24.8 ± 3.6	ND
Magnesium (%) (cmol kg^{-1})	1.5 ± 0.7	0.74

914 | *Environ. Sci.: Adv.*, 2025, **4**, 912–920

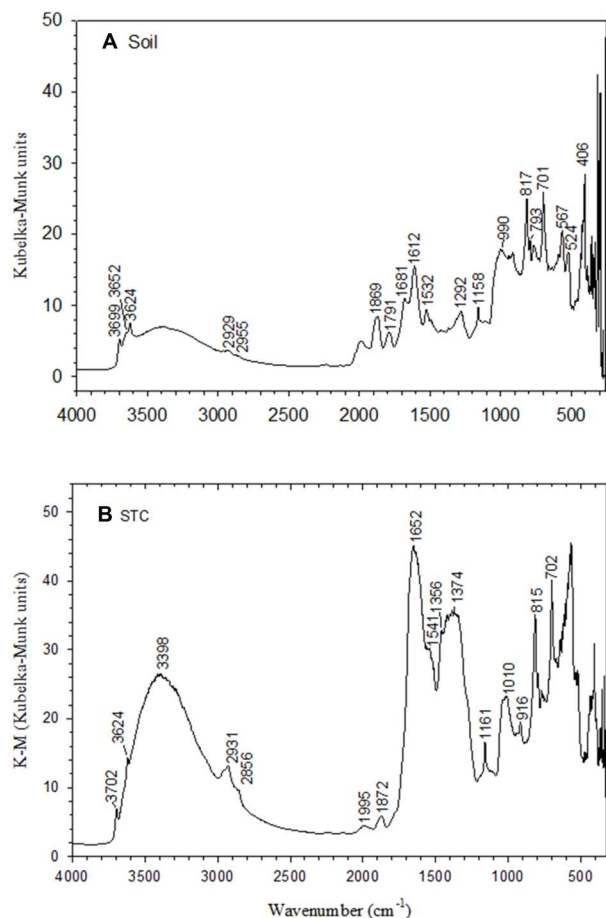© 2025 The Author(s). Published by the Royal Society of Chemistry

Fig. 1 FTIR (DRIFTS) spectra of soil (A) and (B) Soil Therapy Compost (STC) from the Neches Composting Facility, acquired at 4 cm^{-1} resolution.

1040 cm^{-1}, 815 cm^{-1}, and 450 cm^{-1} may be ascribed to Si–O–Si stretching, Si–O bending and Si–O rocking frequencies, respectively.[34] The peaks at 916 and 866 cm^{-1} are attributed to the Al–OH deformation bands of the kaolinite sheet and the probable presence of hematite.[35,36]

3.2.2 SEM/EDX analysis of soil and STC. Fig. 2A and B show SEM micrographs for STC and soil, respectively. The corresponding EDX analysis for elemental composition is depicted in Fig. 3A and B. The micrographs of soil show elemental particle sizes in the range 20–50 mm. Similarly, STC particle size diameters were observed in the range of 25–350 mm.[2]

The elemental composition of STC and soil was further examined by EDX (Fig. 3A and B). The EDX analysis (Fig. 3B) shows that soil is composed of oxygen (O), aluminum (Al), carbon (C), iron (Fe), and silicon (Si) with traces of Ca, Na, Mg, K, Cu, N, Cr, Mn, and Ti.

The elemental composition concentration in STC follows the order: O (32.2 ppm) > C (26.0 ppm) > Si (10.4 ppm) > Al (8.7 ppm) > Ca (5.8 ppm) > Fe (5.5 ppm) > P (4.6 ppm) > S (3.3 ppm) > K (1.5 ppm) > Mg (0.9 ppm) > Na (0.5 ppm) ≈ Ti (0.5 ppm) > Cl (0.1 ppm). It is averred that the presence of C and O is indicative of the presence of functional groups such as carboxylic and hydroxyl groups that enhance the binding of Zn and its uptake
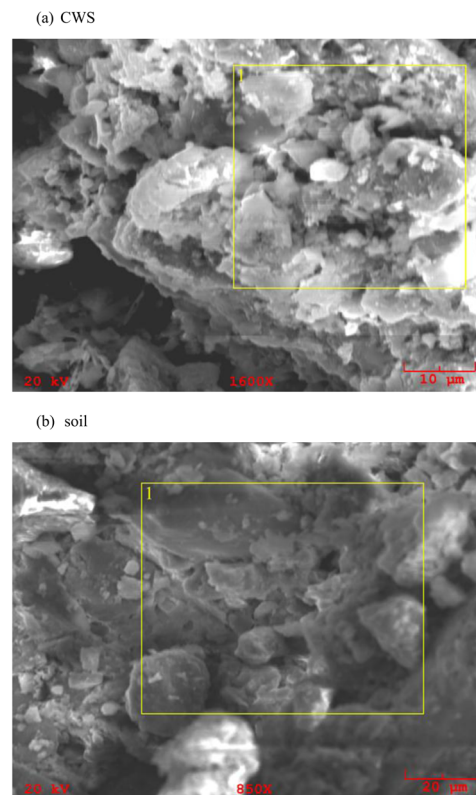

Fig. 2 SEM micrographs of (a) sludge (CWS), and (b) soil.
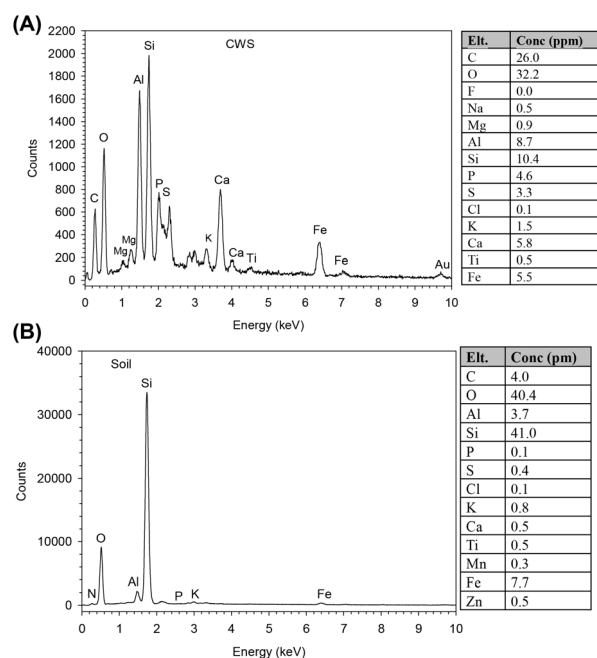

Fig. 3 EDX spectra showing elemental composition of (A) soil therapy compost (STC) and (B) soil. The EDX spectrum was acquired at a magnification of 1600× and 850×, respectively, an accelerating voltage of 20 kV, and a filament current of 200 A. The elemental concentration of CWS and STC is shown alongside each spectrum.

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Environ. Sci.: Adv., 2025, **4**, 912–920 | **915**

by plant parts (Fig. 1). Furthermore, elemental mapping analysis (ESI, Fig. SI†) showed that P, K, O, and Fe are spread throughout the soil sample matrix, thus indicating the presence and availability of the elements to the plant roots.

3.2.3 Mineralogical crystalline phases in CWS and soil.

Fig. 4A and B show the powder X-ray diffraction patterns of CWS and soil, respectively. The reported diffraction patterns, d-spacings and intensities, calculated with the program DIFFRAC.EVA,[38] show sharp peaks indicative of the crystalline nature of the biosolid material. Through the use of TOPAS software and Rietveld analysis, the crystalline and the powder diffraction data in biosolid sludge were previously indexed with corresponding h, k, l parameters.[37] The peaks of highest intensities at $2\theta = 26.64°$ and $2\theta = 6.20°$ are ascribed to quartz (SiO_2) and vermiculite, respectively. The PXRD data reveal other crystalline components present, including alunogen, aluminite, actinolite, andalusite, chamosite, clinoptilolite, vermiculite, kaolinite, gypsum ($CaSO_4$·$2H_2O$), annite mica, borax, palygorskite M, hexahydrate, and minerals of lower abundance.[37]

3.3 Effect of STC–soil amounts on plant biomass

Fig. 5 depicts dry biomass amounts after 30 days. An increase in growth and biomass is evident in the biosolid (CWS) treatment
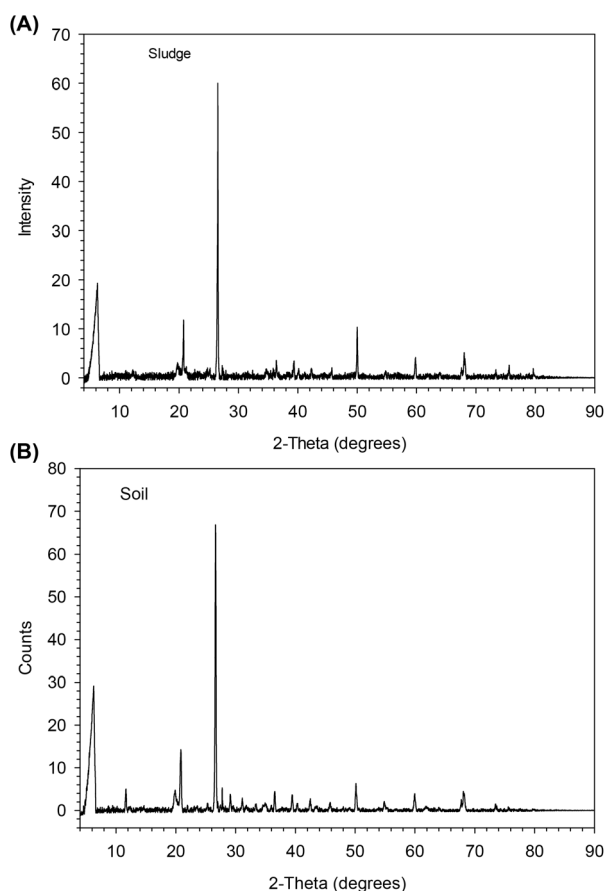

Fig. 4 XRD patterns of (A) sludge and (B) soil. The assignment of peaks in sludge (STC) is given in ref. 11 and 37 with d-spacing and h, k, l values. The 2θ peak values of soil are remarkably similar to those of STC.
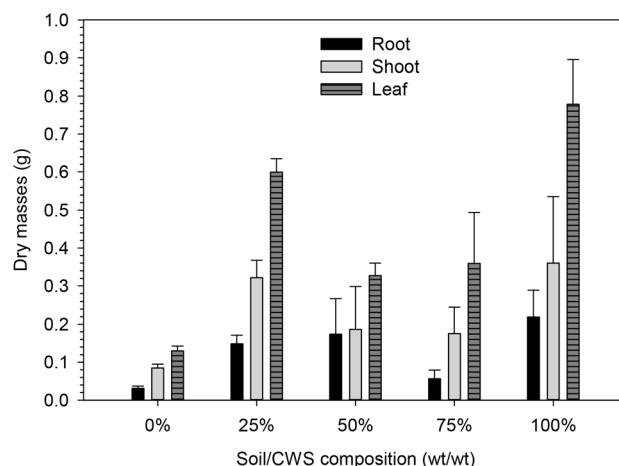

Fig. 5 Biomass yields of root, shoot and leaf of broccoli grown in five different soil–biosolid compositions (wt/wt). All soil/CWS compositions were measured in triplicate ($n = 3$).

vis-à-vis the control (0% CWS wt/wt) in all pots. It is also noted that the total biomass of plant parts follows the trend: leaf biomass 100% (wt/wt) > 25% (wt/wt) > 50% (wt) ≈ 75% (wt/wt) > control (0% biosolid wt/wt treatment) (Fig. 5). Similar increased effects of growth were observed in broccoli (*Brassica oleracea* var. *Botryti* cv. Marathon) species when sludge is added[39] to peat as a growth media. Other studies show that sludge could be used up to 66.7% by volume without negative effects on plant growth.[39]

In general, a significant increase was observed in the dry and fresh weights of broccoli root, stem and leaf with increasing CWS. The greatest growth is noted in the 25% (wt/wt) to 100% (wt/wt) compositions. The biomass yields *vis-à-vis* the control (0% wt/wt biosolid treatment) increased by 373.6%, 180.33%, 141.28%, and 454.12% in 25% (biosolid wt/wt), 50% (biosolid wt/wt), 75 % (biosolid wt/wt) and 100% (biosolid wt/wt) treatments, respectively. The higher biomasses observed in 25% (biosolid wt/wt), 75% and 100% (biosolid wt/wt) results from the biomass of leaves at the stage of harvest, the extensive root systems, and the soil particles within the soil-sludge interstitial spaces. The increase in N and P supplied by CWS further contributes to increased biomass, especially in leaves at each % CWS. The yields of the root, shoot and leaf are: 38.87-times, 38.14-times, and 46.33-times in 25% treatment; 55.97-times, 22.06-times, and 25.27-times in 50% treatment; 1.82-times, 20.78-times, 27.75-times in 75% treatment; 7.29-times, 4.269-times, 6.01-times in 100% biosolid treatment from the biosolid treated pots *vis-à-vis* the control (0% wt/wt CWS).

3.4 Concentration of Zn in plant parts

Fig. 6 shows Zn concentrations and uptake in plant parts at pH 6.51 ± 0.225 grown in increasing biosolid composition (CWS). An increase in applied biosolid amounts showed an increase in Zn amounts in the order: $[Zn]_{root} > [Zn]_{leaf} > [Zn]_{shoot}$. The concentrations of Zn in the plant samples were in the range of 3.38 ± 0.59 to 39.304 ± 7.258 mg g^{-1} in roots, 1.681 ± 0.716 to 7.882 ± 0.696 mg g^{-1} in shoots, and 2.366 ± 0.336 to 18.714 ± 2.380 mg g^{-1} in leaves.
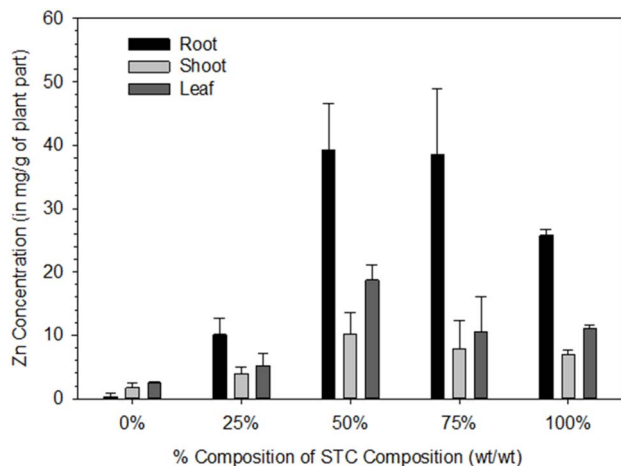
Fig. 6 Zinc concentration (mg g^{-1}) in broccoli root, leaf, and shoot upon addition of biosolids to soil (100 ppm of Zn was added to each soil/CWS 3 days prior to the start of the experiment).

Fig. 7 Change in pH with the increase in biosolid (CWS) composition.

The increase in CWS treatment shows a concomitant increase in Zn in roots compared to shoots or leaves. Compared to the control, more Zn was taken up by each plant part. The Zn concentrations were found in the order [Zn]$_{root}$ > [Zn]$_{leaf}$ > [Zn]$_{shoot}$. The increase in [Zn] in shoots and leaves is in agreement with Mishra *et al.*[40] where edible plants were grown in smelter-contaminated soils, in soybean plants[41] and shoots of chamomile (*Matricaria chamomilla* L.) plants.[42]

These findings are comparable to previous studies on other vegetables grown in sludge. Intawongse and Dean[43] observed that Zn accumulated in leaves in lettuce, spinach, radish and carrot grown on compost that had previously been contaminated at different treatment amounts. Grejtovský *et al.*[42] found that chamomile (*Matricaria chamomilla* L.) accumulates Zn in its shoot system and all its organs *vis-à-vis* the soil. In other pot experiments, it was also observed that Zn accumulation in plant parts increased 2 to 3-times in two varieties of hemp plants (*Cannabis sativa*).[1]

3.5 Effect of pH on Zn uptake

Fig. 7 shows the changes in soil–CWS pH under different biosolid treatments. The pH decreased with an increase in CWS treatments in the range 6.88–5.02 in 25% (wt/wt), 50% (wt/wt), 75% (wt/wt) and 100% (wt/wt). The pH in the sludge-treated soil was slightly lower than in the control and very similar in all the treatments during this period. This finding agrees with many studies that demonstrated a decrease in soil pH with the addition of organic waste or biosolids.[44,45] The decrease in pH may be attributed to the presence of organic acids exuded from the root system.[46]

4 Discussion

4.1 Translocation factors (TFs)

Translocation factors (TFs) are calculated from the metal concentrations measured in the different plant organs (stems, roots and leaves), as follows:
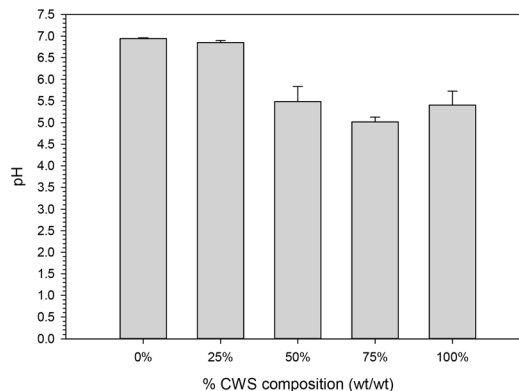
$$TF = \frac{C_{plant\ part\ in\ g\ (DW)}}{C_{root\ in\ g\ (DW)}}$$

where C corresponds to analyte concentration(s) in the stem or leaf, C_r corresponds to the Zn concentrations in the root, and DW is the dry weight. The TF is therefore calculated as the ratio between the Zn concentration in the leaf or shoot and Zn concentration in the root system. This ratio explains the ability of a plant to translocate heavy metals from the roots to the stem and leaves.[9] A ratio greater than 1 indicates the plant's ability to act as a bioaccumulator. Table 2 shows calculated translocation factors (TFs) (shoot-root or leaf-root ratios). Moreover, there is an increase in Zn concentrations in the plant parts with an increase in biosolid composition. Nevertheless, the translocation factors for Zn in all treatments at pH value 4.09–6.92 were less than 1.0. The low Zn TFs suggest selective mechanisms for the Zn transport system in broccoli plants from roots to the leaves.

4.2 Environmental implications

This study shows that biosolids enhance Zn uptake into plant parts, as demonstrated when compared to the control experiments (0% wt/wt biosolid treatment). The translocation of Zn into the shoot and the leaves (Table 1) shows that biosolids do not sequester Zn in the roots. These findings implicate mechanisms (symplastic *vs.* apoplastic transport) through which Zn is translocated in broccoli parts compared to other plants. These results suggest that using biosolids has beneficial effects that could be used to enhance uptake of Zn into plant parts, and thus potentially enhancing the translocation of Zn into plant parts. Thus, biosolid's abilities to increase Zn uptake may consequently be regarded as enhancers of Zn into plant parts. Although studies show the sequestration of Zn in the roots,[47] more work is needed to address the long-term uptake of Zn at the root interfaces and/or surfaces, characterization of chemical Zn-complexes formed, as well as the potential Zn uptake enhancement or potential transformation over longer time periods.

4.3 Limitations of the study

In this study, CWS enhanced the uptake of Zn into plant parts in broccoli. The time allowed for plant germination and growth

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Environ. Sci.: Adv., 2025, **4**, 912–920 | **917**

Table 2 Zn translocation factor (TF) of broccoli plants cultivated after initial Zn spiked treatment (100 ppm = 0.1 mg g^{-1}) at pH 6.20

Soil-CWS composition (wt/wt)	TF (shoot/root)	TF (leaf/root)
0%	4.973	7.00
25%	0.385	0.512
50%	0.258	0.512
75%	0.205	0.274
100%	0.270	0.429

may be pertinent for such studies. Furthermore, the various stages of growth and harvest may be important in future investigations, as this would affect the efficiency of storage and translocation.[12] Additionally, imaging techniques such as transmission electron spectroscopy (TEM) at each stage of growth would yield information on Zn uptake into the roots, shoots or leaves and the enzymes involved in transport and translocation mechanisms. In this respect, more work on more plant species may be examined to establish prevalent mechanisms for Zn translocations into plant parts.

Potential benefits of using CWS are derived from the presence of micro- and macronutrients and nitrogen.[48] With the increased presence of N, an increased Zn correlation was found. Higher N levels correlated with increased plant yield. Notably, CWS has higher amounts of Ca, Mg, and Na² available post composting treatment. These essential macronutrients are vital for plant growth. It is our view that more synergistic correlations between elements may be necessary in understanding underlying mechanisms between the macro- and microelements (including Zn) and the enzymes produced at each stage. This will begin to unravel how CWS exerts its effects upon the uptake of Zn by broccoli.

5 Conclusions

The effects of CWS and the accumulation of Zn in broccoli (*Brassica oleracea* var.) were studied. The results show different amounts of Zn accumulated in different broccoli parts. The following conclusions can be drawn from the study. Firstly, the amount of applied CWS affects different amounts of Zn concentrations in broccoli root, shoot or leaf. Increases in Zn concentrations in plant parts were greater in soil amended with sludge. The application of 25–50% (wt/wt) CWS amounts resulted in the greatest increased biomass. Secondly, more Zn was taken up by plants amended with CWS than in the control (100% soil wt/wt). This finding is reasonable as CWS contains N and P, both building blocks for the growth of plants as shown by SEM/EDX analysis.

The influence of CWS composition is not fully understood as it may depend on many factors, such as the different harvesting stages. We thus suggest that consideration of various operative mechanisms be examined to determine Zn uptake levels at different growth stages. The findings from this study are crucial and shed light on the influence of CWS on Zn's supply to the environment. Additionally, this study shows that soil amended with CWS increased growth of plant parts *vis-à-vis* non-

amended soils. These results merit further investigations into metal–CWS interactions.

Ethical statement

This is an original article that did not use other information, which requires ethical approval.

Consent to participate

All authors participated in the preparation of this article.

Consent for publication

All authors have given consent to the publication of this article.

Data availability

Additional data that support the findings of this study are available upon reasonable request from the corresponding author. Supporting data are available in the ESI section.†

Author contributions

Kefa K. Onchoke (KKO): project administration, conceptualization, resources, methodology, investigation, data curation, formal analysis, validation, writing – first draft, writing – original draft, writing – reviews and editing, visualization, investigation, supervision, funding acquisition. Brett Horalan: formal analysis, writing – first draft, validation, investigation. Robert Friedfeld: validation, investigation, resources (instrumentation), visualization, data curation.

Conflicts of interest

There are no conflicts to declare.

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