Applied Water Science (2024) 14:7
https://doi.org/10.1007/s13201-023-02059-1

ORIGINAL ARTICLE

# Influence of perlite/biosolid composition on growth and uptake of Cd and Mn by radish (*Raphanus sativus* L.) under greenhouse conditions

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Received: 16 February 2023 / Accepted: 16 November 2023 / Published online: 21 December 2023


**Abstract**
The effects of different perlite/biosolid compositions upon the uptake of Cd and Mn, and the growth of radish plants (*Raphanus sativus L*) was investigated by using inductively coupled plasma optical emission spectroscopy, and inductively coupled plasma mass spectrometry (ICP-OES and ICP-MS). Mn and Cd were added in soluble forms to perlite/biosolid compositions. Notably, Mn concentrations in different plant parts were found to increase with increase in biosolid compositions, in the order $[Mn]_{leaves} > [Mn]_{shoot} > [Mn]_{roots}$. This is plausible for Mn, in conformity with the essential role Mn plays during photosynthesis, in metabolic processes, and oxidation–reduction processes in cells. Results indicate that Mn concentrations in plant parts increased up to ~ 50% (wt/wt) perlite/biosolid application rates. In contrast the Cd uptake concentrations in plant parts decreased in the order $[Cd]_{roots} > [Cd]_{shoots} > [Cd]_{leaf}$. Thus, toxic Cd tends to be sequestered in the roots vis-à-vis Mn that is translocated to the leaves. These results suggest that radish plants sequester Cd in the roots. Biosolids therefore play an important role in sequestering and binding of Cd. The observed concomitant increase in biomass yields implicates the rich contribution of N and P from biosolids. The results from the greenhouse experiments lead to the conclusion on the role played by the biosolids in cleanup and remediations for Cd and Mn, which increased in plant parts with composted wastewater sludge—compositions.

**Keywords** Composted wastewater sludge · SEM · XRD · Plant biomass · Transfer factors

## Introduction

Enormous amounts of wastewater sludge (also known as biosolids) are produced globally from wastewater treatment plants (Du et al. 2014; Onchoke et al. 2022). Wastewater treatment facilities in the USA produce approximately $6.2 \times 10^6$ tons (dry basis) of sludge annually (Federation 2018). Wastewater sludge contains toxic heavy metals (such as Pb, Cd, Hg, and Se), essential trace metals, organic and inorganic pollutants (Onchoke et al. 2018a). The use of biosolids free of toxic chemicals is an important issue of concern for agriculture, industry, and human health (Dichiara et al. 2015). The disposal of wastewater sludge to landfills, as land restoration projects, for fertilization of lands contributes to the increased metal concentrations in the environment. In addition, natural and anthropogenic sources, including weathering, agriculture, and industrialization, increase the metal concentrations in the atmosphere (Biddau and Cidu 2017).

The search and development for new methods to sequester toxic substances in wastewater is important and essential to controlling their concentrations in the environment. Among these include use of phytoremediation plants such as ferns (Singh and Ma 2006), use of nanomaterials such as carbon nanotubes (Huang and Keller 2020; Liné et al. 2021; Patel et al. 2021) and biosolids (or sludges). The choice of any one method depends upon the strong affinity of the material and sequestration of the target metals (Gong et al. 2021; Urasa and Macha 1996). Traditionally, total metal concentrations in plants or any material have been used for assessing health risks to plants or soil or organisms. However, not all plants or materials bioaccumulate metals to the same extent (Kandziora-Ciupa et al. 2017a). Importantly, such studies ignore the fact that metals exist in different forms and the extent of their bioavailability upon application to plants varies. Thus, uptake of metals by plants and translocation to

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various plant parts may be determined by dissolution kinetics of specific pollutants.

Heavy metal uptake by plants may be controlled by various mechanisms including the role of microorganisms, soil particle size (Ajjabi and Chouba 2009; Chen et al. 2008), and pH of biosolids (Szada-Borzyszkowska et al. 2022). Other mechanisms include metal transfer by the apoplasmic pathway or symplastic transport across the root cortex to plant storage tissues (Shahid et al. 2016).

In this study, the concentrations and transfer factors of Cd and Mn in radish plants (*Raphanus sativus* L.) were investigated at pH 6.70 and 7.30. The role of pH in metal uptake, at acidic and near neutral conditions, was investigated. In addition, the effects of different perlite/biosolid composition ratios upon Cd and Mn uptake by radish plants (*Raphanus sativus L*) were evaluated. Perlite is a hydroponics growth media that contains low amounts of metals and is useful as a bulking agent. *R. sativus L.* forms a good model in experiments over short periods study times. Finally, the influence of biosolids ratios upon uptake of Cd and Mn by *R. sativus L* (radish) plants was assessed.

## Materials and methods

### Chemicals and reagents

The high-purity analytical reagents used were purchased from Fisher (Fair Lawn, NJ). Ultrapure water obtained from a Milli-Q water filtration station (18.2 MΩ.cm at 20 °C) was used in the preparation of all standards. Nitric acid (70%, ACS reagent, from Flinn Scientific Inc., Batavia, IL, USA) and hydrogen peroxide (35% wt, Sigma Aldrich, St. Louis, MO) were used in digestion of sludge samples following USEPA Method 3050B (USEPA 1996). Hoagland solution was prepared following established protocols (Hewitt 1966; Onchoke et al. 2018b).

### Characterization of soil therapy compost (STC) and perlite

Composted wastewater sludge (CWS, sold under the trade name Soil Therapy Compost, STC) were collected from Neches Compost treatment Facility (NCF) in East Texas. The CWS samples were air-dried, sifted through a mesh diameter of ≤ 2 mm, and analyzed with scanning electron microscopy/energy-dispersive X-ray analysis (SEM/EDX), inductively coupled plasma optical emission spectrometry (ICP-OES) and Fourier transform infrared spectroscopy (FTIR).

Elemental concentrations (P, K, Ca, Mg, Fe, Mn, Cu, and Zn) in STC and perlite were previously examined using ICP-OES or ICP-MS after digestion with 4 M nitric acid (Onchoke and Fateru 2021; Onchoke et al. 2022). Via ICP-OES, ICP-MS, and SEM/EDX analysis (Onchoke and Fateru 2021; Onchoke et al. 2022), perlite was shown to contain macroelements Al, Ca, Mg, Fe, K, Na, P, and S and microelements Ag, As, Ba, B, Cd, Cr, Co, Cu, Mn, Mo, Ni, Zn, and V. In addition, both STC and perlite contained 33.7 wt% C (weight percentages), % N (1.59 wt%), P, organic matter (67.40 mg/kg). Perlite contains low amounts of organic matter, P or N (Onchoke and Fateru 2021; Onchoke et al. 2022). The pH in perlite and STC was found in the range 5.3–7.2.

### Experimental design

#### Plant materials, perlite, and composted wastewater sludge (CWS)

*Raphanus sativus L.* seeds were purchased from local stores (Burpee seed Company, https://www.burpee.com/). Commercial perlite was obtained from local stores and characterized for its morphology and metal content (Onchoke et al. 2022), and confirmed in agreement with previously published data. Composted wastewater sludge (CWS) and perlite were air-dried and mixed in different proportion ratios of 0% (wt/wt), 25% (wt/wt), 25% (wt/wt), 50% (wt/wt), 75% (wt/wt), and 100% (wt/wt). Although field amounts of 25—33% (wt/wt) are recommended and practiced in field samples for soil amendments (Onchoke et al. 2018b; Siedt et al. 2021), a full range of biosolid content up to 100% (wt/wt) CWS composition) was examined. Notably, perlite/CWS ratios in this study encompass appropriate field treatment ratios (Siedt et al. 2021).

#### Plant cultivation and harvest in pot experiments

Radish seeds (*Raphanus sativus L.*) were germinated and grown in pots under greenhouse conditions at temperatures 30–40 °C in Summer 2018. Plants were supplied with Hoagland nutrient solution as described in Ref. # (Onchoke et al. 2018b). The plants were harvested at maturity after three weeks. Harvested whole plants were washed with nanopure water (18.2 MΩ resistivity). Plants were weighed immediately after harvest to obtain their fresh biomass. Thereafter, plants were oven-dried at ~ 60 °C for 48 h. Subsequently, roots, shoots, and leaves were separated, and their biomasses weighed, then ground into a fine powder, and stored in plastic containers until digestion with nitric acid and metal concentration analysis.

#### Metal analyses in radish plants

Inductively coupled plasma spectroscopy/mass spectrometry (Perkin Elmer, Elan DRC-ICP-MS equipped with a dynamic reaction cell (DRCe) was used for the analysis of Mn in plant parts. Four to 5 mL of 4 M $HNO_3$ and 2 mL of $H_2O_2$ were added to the plant samples and digested in a DigiPREP digestion block (SCP science, https://www.scpscience.com) at different temperature ranges as described in USEPA method 3050B (USEPA 1996). The Cd concentration in plant parts was analyzed by flame atomic absorption spectroscopy (Shimadzu AAS 6800) at a wavelength of 228.8 nm.

### Quality assurance, quality control, and method validation

Standard Reference Material (SRM) from SCP science (SS-2) was used for quality assurance. ICP-MS was optimized daily using a tuning solution containing Li, Y, Tl, Ce, and Co. The instrument passed the mass calibration, cross-calibration, and daily performance reports for sensitivity, stability, oxide production ratio, and doubly charged production ratio prior to sample measurement. To ensure quality assurance and quality control measures, analysis of continuous calibration verification after every 10 runs was performed. Blanks were run for every batch of sample to check for any laboratory contamination. For quality control, a Certified Reference Material (CRM, EnvironMAT, Contaminated Soil SS-2 from SCP Science Granham, NY) was run for each analytical batch. All analyses were carried out in triplicate and results reported are the mean values of replicate analyses. Table S1 shows agreement between measured values and the CRMs as analyzed by ICP-OES in the range 80% to 115%.

A validation of the ICP measurements was performed for each analyte. The limit of detection (LOD) for Hg was 0.030 ppb and for other metals in ppm (mg/kg) was determined as follows: Ag (0.001868), Al (0.002812), As (0.008147), B (0.0257), Ba (0.000469), Ca (0.5028), Cd (0.000407), Co (0.00047), Cr (0.00126), Cu (0.0043), Fe (0.00260), Hg (0.00255), K (0.2356), Mg (0.008697), Mn (0.000200), Mo (0.000408), Na (0.8436), Ni (0.001508), P (0.006847), Pb (0.00667), S (0.00829), V(0.001626), and Zn (0.0003216).

### Statistical analysis

Data presented herein is the average of at least three triplicates and is shown as mean ± standard deviations. Analyses were conducted in Excel version 22 (Microsoft Corporation, 2018. Microsoft Excel, Available at: https://office.microsoft.com/excel). Data at $p \leq 0.05$ was deemed significant during analyses. All figures were plotted using Sigmaplot 12.5 (Systat Software Inc.)

## Results

### Analysis of certified reference materials

The accuracy and precision of the procedure was first evaluated by using certified reference materials, namely, contaminated soil SS-2 (CRM, SS-2, EnvironMAT, from SCP Science Granham, NY) as previously reported (Onchoke and Fateru 2021; Onchoke et al. 2022). Good agreement between measured and certified values to ± 20% is evident (Table S1). The measured concentrations for most metals are in agreement to within 100 ± 15% for K, Ca, Mg, S, P, Al, Fe, Mn, Zn, Cu, and Mo, V, Ni, and falls within accepted CRM values. Therefore, the analytical concentrations results were considered acceptable (Table S1).

### Physicochemical and spectroscopic properties of Perlite and STC

The physicochemical properties of STC and Fourier transform infrared (FTIR) data are compared in Table 1 and Fig. 1, respectively. Thus, the pH of the biosolids is in the range 5.74 to 6.77. This is comparable to previous reports (Onchoke et al. 2018a, 2018b). Whipkern et al. (1996) and Truong et al (2018) noted that a pH range of 5.5–6.5 is ideal for many plants to adequately absorb various mineral nutrients to levels that are not too high to prevent toxicity. Notably, the measured pH is favorable for the absorption of metals into the root system. Figure 1 shows STC absorption FTIR spectral peaks at 3398 $cm^{-1}$, 2931 $cm^{-1}$, 2856 $cm^{-1}$, 1652 $cm^{-1}$, 1541 $cm^{-1}$, 1356 $cm^{-1}$, 1374 $cm^{-1}$, 1161 $cm^{-1}$, 1010 $cm^{-1}$, 916 $cm^{-1}$, 815 $cm^{-1}$, 702 $cm^{-1}$. The broad bands

**Table 1** Analysis of physicochemical properties of air-dried sewage sludge (n = 3). Where no standard deviation is given, only one sample measurement was made once

| Analyte | Results (mg $kg^{-1}$) | |
|---|---|---|
| | STC | Perlite |
| pH | 6.74 ± 0.03[b] | 5.77 ± 0.03[b] |
| C (%) | 33.70[c] | ND |
| N (%) | 1.59 | ND |
| P | 6600[c] | 4649 ± 790 |
| S | – | 2097 ± 227 |
| Total Ca | 4200[c] | 3632 ± 1035 |
| Total Mg | 700[c] | 654 ± 236 |
| Total K | 700[c] | 9337 ± 1281 |
| Organic matter | 67.40 | ND |
| $NH_4^+$-N | ND | ND |

a = reported in Ref # (Onchoke 2018a), b = This study, ND = Not determined

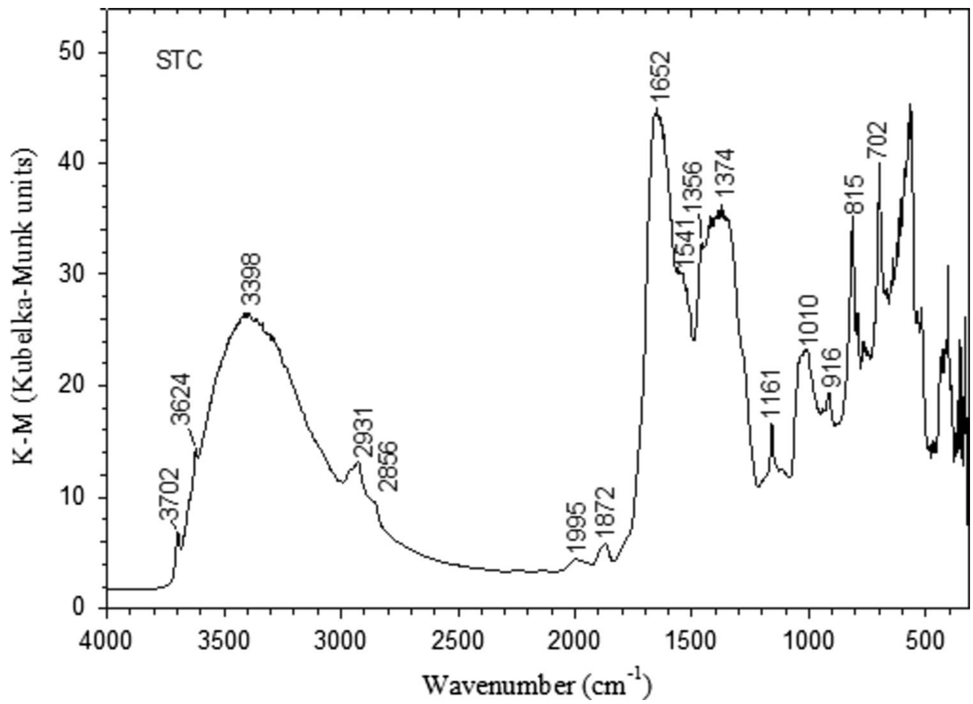


**Fig. 1** FTIR (DRIFTS) spectrum of Soil Therapy Compost (STC) from the Neches Composting Facility

at ~ 3400—3700 $cm^{-1}$ correspond to the presence of amino $\nu$(N–H), $\nu$(O–H) of phenolic compounds, and carboxylic acid bands. The absorption bands in the range 1300–1375 $cm^{-1}$ and 1500–1575 $cm^{-1}$ may be attributed to absorption of $\nu(NO_2)$. The $\nu$(C–C) and $\nu$(C-H) bands occur in the ranges 1600 and 1500–1430 $cm^{-1}$, and ~ 2800–2700 (medium) $cm^{-1}$, respectively, and 2000–1650 $cm^{-1}$ (C–H bending in aromatic compounds) (Onchoke and Fateru 2021).

## Concentrations of macro- and microelements in Perlite and STC Sludge

Previous studies (Onchoke et al. 2022) determined concentrations of macroelements and microelements (Ag, Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Ni, P, Pb, S, Se, Zn, V, Na, S, and P) in perlite (PER) and STC by using ICP-OES and ICP-MS. Perlite was found to contain low amounts of macro- and microelements. The total metal concentrations of macroelements in STC (Neches Composted Wastewater Sludge (CWS)) samples were higher than in perlite (PER) (Onchoke et al. 2018b). Figure 2 depicts an EDX spectrum and identifies elements in STC, namely, S, K, O, Fe, Mg, Al, Si, P, Ca, and Fe. Figure 3 depicts SEM micrographs of STC with particle sizes in the range 30.0 µm to 91.6 µm. In addition to its known advantages, sewage sludge also contains hazardous or potentially toxic metals that may constrain its use in agriculture such as phyto-toxicity, soil pollution, and bioaccumulation of toxic elements in food (Dar et al. 2023; Zulfiqar et al. 2022). In agreement with earlier studies, macro- and microelement concentrations were found below regulated USEPA guideline levels (Onchoke et al. 2018a).

## Effect of STC (Composted Wastewater Sludge) amounts on Plant Biomass

Figures 4a and b, and 5a and b show the total dried biomass plant biomass after harvest from 0%, 25%, 50%, 75%, and 100% (wt/wt) perlite/CWS compositions, with Mn or Cd metal amendments at pH values 6.70 and 7.30, respectively. Figure 4a and b shows increase in plant biomass vis-à-vis the control; in accord with a previous study (Onchoke et al. 2018b). Plant biomass increased up to 75% (wt/wt) compost composition. The plant biomass increased by 32.23% and 12.94% in plants grown in 25% (wt/wt), 50% (wt/wt) compost treatment vis-à-vis the control, respectively. In particular, the root, shoot, and leaf increased by 23.81%, 45.95%, and 56.32% vis-à-vis control plants at pH 6.7 (Fig. 4a). The biomass of 100% (wt/wt) CWS composition was noted to have increased as well. These results show that increasing amounts of compost treatments influenced increases in plant biomass. This is attributable to increase in nitrogen (N) and phosphorus (P) content with concomitant increased amounts of applied biosolids. This is in agreement with reported research findings (Reddy and Crohn 2018) where biomasses of okra, tomato, and chili peppers increased in plant biomass with increased use of sludge.

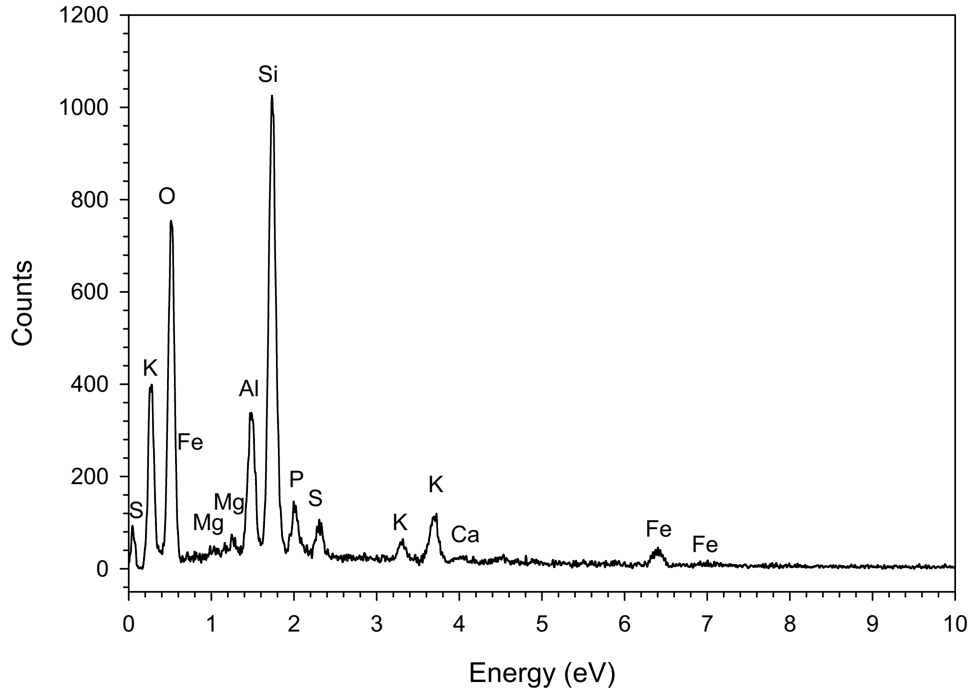


**Fig. 2** EDX elemental composition of Soil Therapy Compost (STC). EDX spectrum was acquired at a magnification of X200, an accelerating voltage of 20 kV, and filament current of 200 A

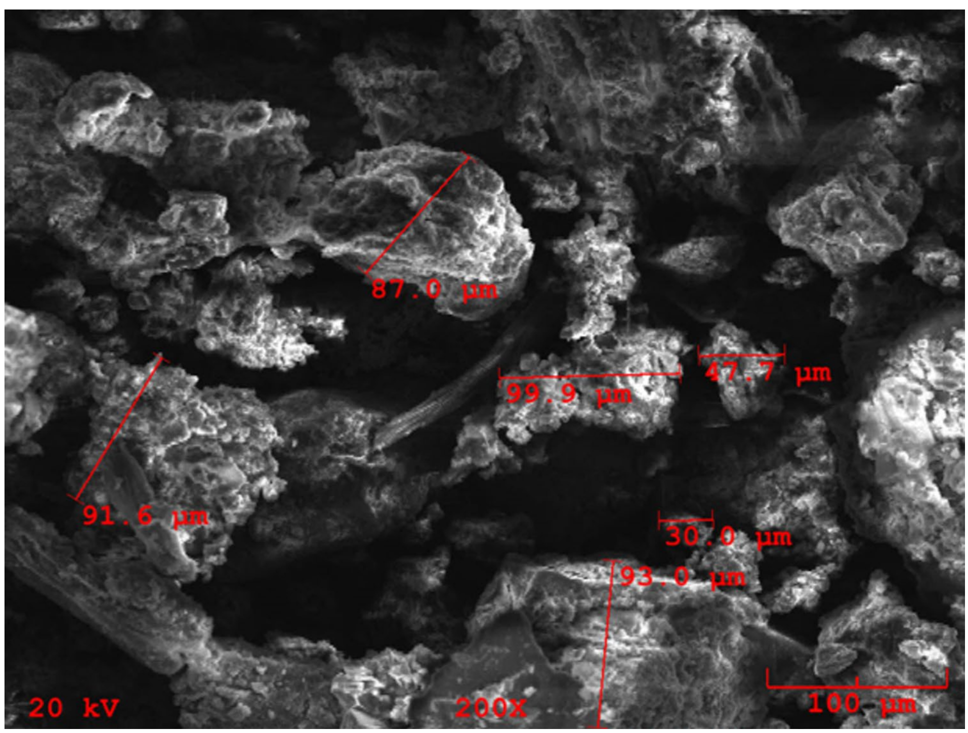


**Fig. 3** SEM micrograph for STC at a magnification of X200, an accelerating voltage of 20 kV, and filament current of 200 A. Adopted from: Onchoke, KK, Fateru OO, Friedfeld RB, Weatherford PW (2022) Evaluation and analysis of perlite and municipal wastewater sludge (biosolids) from three wastewater treatment plants in East Texas, USA. *Environmental Monitoring and Assessment.* 194: 121. 10.1007/s10661-022–09794-z. Permission granted

## Distribution of Cd and Mn in *Raphanus sativus L.* and transfer Factors

### Uptake of Cd in plant parts grown in different perlite/biosolid ratios at pH 6.70 and 7.30

Figure 6a and b displays Cd amounts in plant parts cultivated at pH 6.70 and 7.30. With increase in percent biosolid amounts (Fig. 6), Cd concentrations decreased in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$ in plants. The Cd uptake decreased by 18.75%, 12.77%, 9.0%, 2.74–18.75 (25%wt/wt, 50% (wt/wt), 75% (wt/wt), and 100% wt/

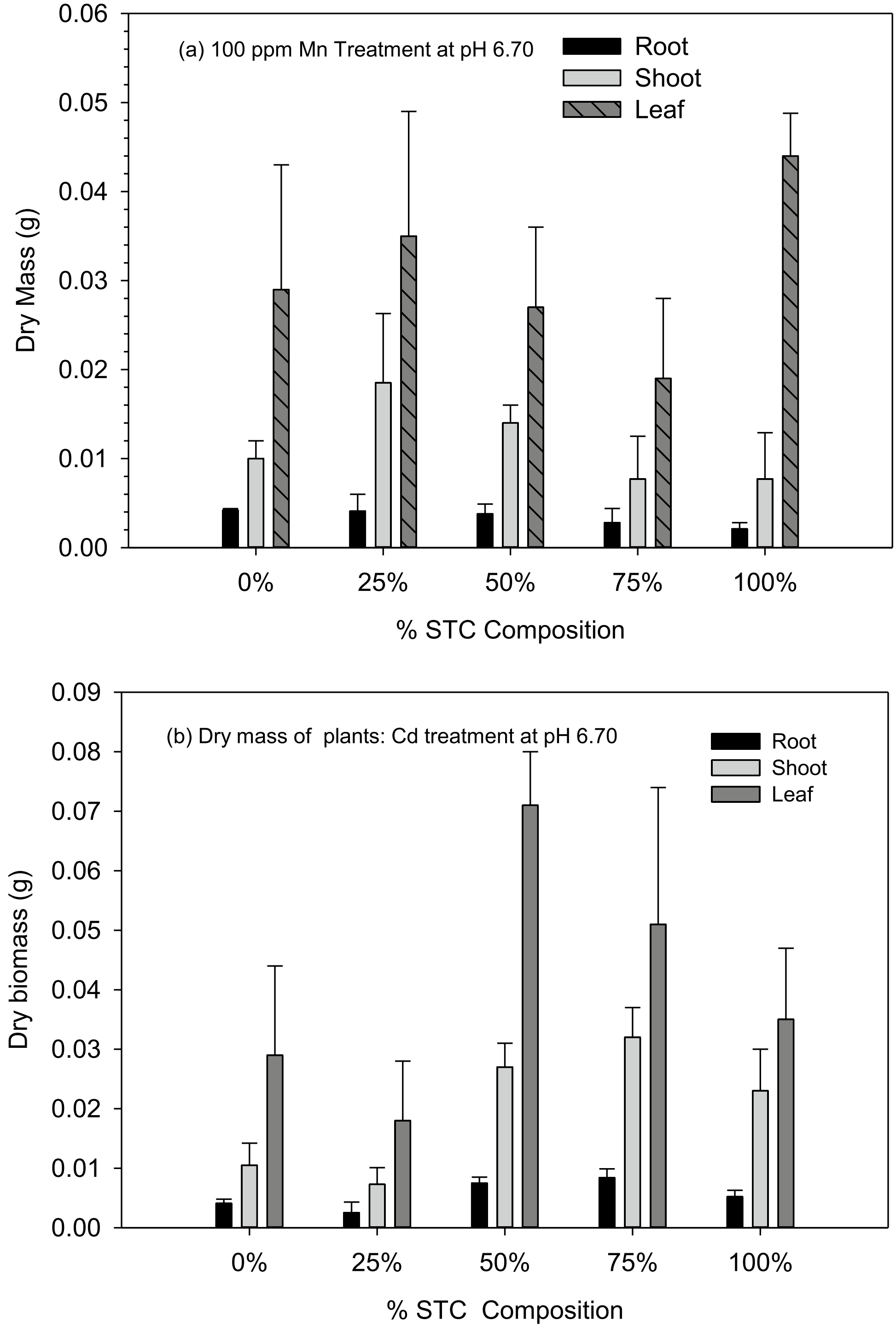


**Fig. 4** Average dry masses of rapid radish plants (*Raphanus sativus*, L.) after harvest for plants grown in various perlite/biosolids compositions at pH 6.70. Plants were harvested from biosolid/perlite treatments (wt/wt) with **a** 100 ppm Mn, **b** 100 ppm Cd treatment. Plants were harvested after 3 weeks

wt) CWS composition. This contrasts with 2.23%–4.00% CWS vis-a-vis 0% wt/wt CWS compositions in shoots, and by 0.66%–72.51% % in leaves compared to the control (0% (wt/wt)). In general, more Cd is stored in roots compared to shoot or leaves; concomitant with increase in biosolid ratios. Kandziora-Ciupa et al. (Kandziora-Ciupa et al. 2017a) found *Vaccinium myrtillus L.* and *Vaccinium vitis-idaea* L plants stored more Cd in roots in comparison with metal amounts translocated to shoots or leaves.

Cadmium (Cd) is a toxic metal with no known role in the physiology of the plant. It is thus plausible that Cd uptake and translocation to above ground parts is selective. The ability for biosolids to preferentially withhold Cd may be related to its high affinity for metals (Urasa and Macha

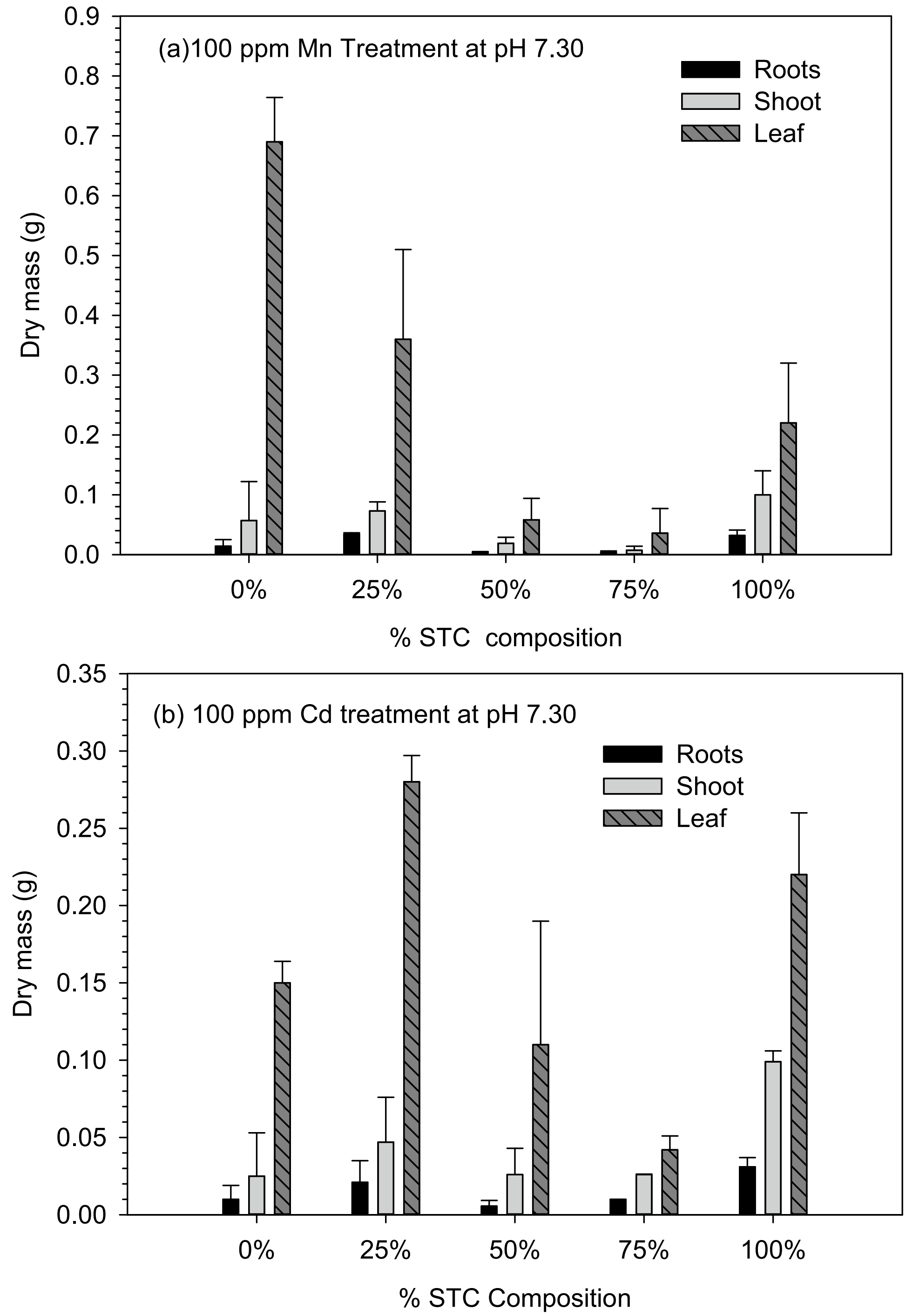


**Fig. 5** Average dry masses of rapid radish plants (*Raphanus sativus*, L.) after harvest for radish plants grown in various perlite/biosolids compositions (wt/wt) at pH 7.30. Plants harvested from biosolid/perlite treatment (wt/wt) with **a** 100 ppm Mn, **b** 100 ppm Cd treatment. Plants were harvested after 3 weeks

1999)(Onchoke et al. 2018b). A possible synergistic interaction of Cd with other metal ions may be envisaged and/or prevalent in CWS. In the present study, there is possible risk to the environment as a result of the roots's ability to bioaccumulate Cd vis-à-vis other plant parts. On the other hand, sequestration of Cd in radish roots can be viewed as environmentally beneficial. Therefore, radish plants may be used to clean up toxic Cd from soils.

Apart from hyperaccumulator plants such as lettuce (Tang et al. 2016), Cd is known to be less readily uptaken by plants (Brown et al. 1995, 1996; He et al. 2017; Zare et al. 2018). Further, evidence from scanning and/or transmission electron microscopy (SEM, TEM) studies (Qi et al. 2020; Yang et al. 2020) shows that Cd accumulates in the nodules of the root system due to the presence of carboxylic acids including butyric acids (Adeleke et al.

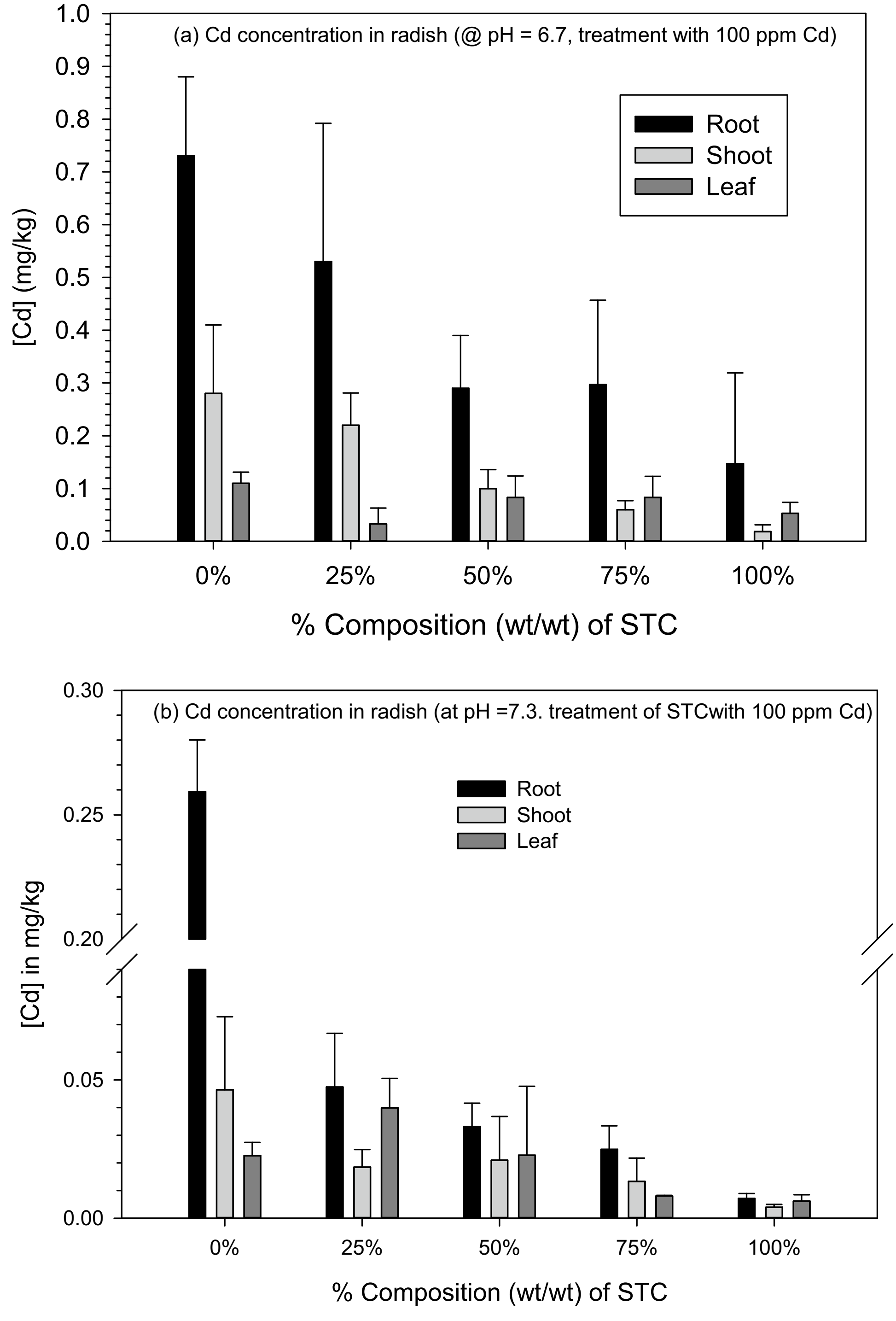


**Fig. 6** Cadmium concentration (mg/kg) in radish (*Raphanus sativus L.*) cultivated with 100 ppm Cd treatment at **a** pH 6.70, and **b** pH 7.30

2017; Choudhary et al. 2021), which enhance sorption of metals into the root system. Such findings imply that wastewater sludges contribute to suppress the uptake of Cd from being translocated to the stem or leaves.

## Uptake of Mn and Transfer Factors

Concomitant with increase in biosolid amendments, Mn concentrations in plant parts increased in the order

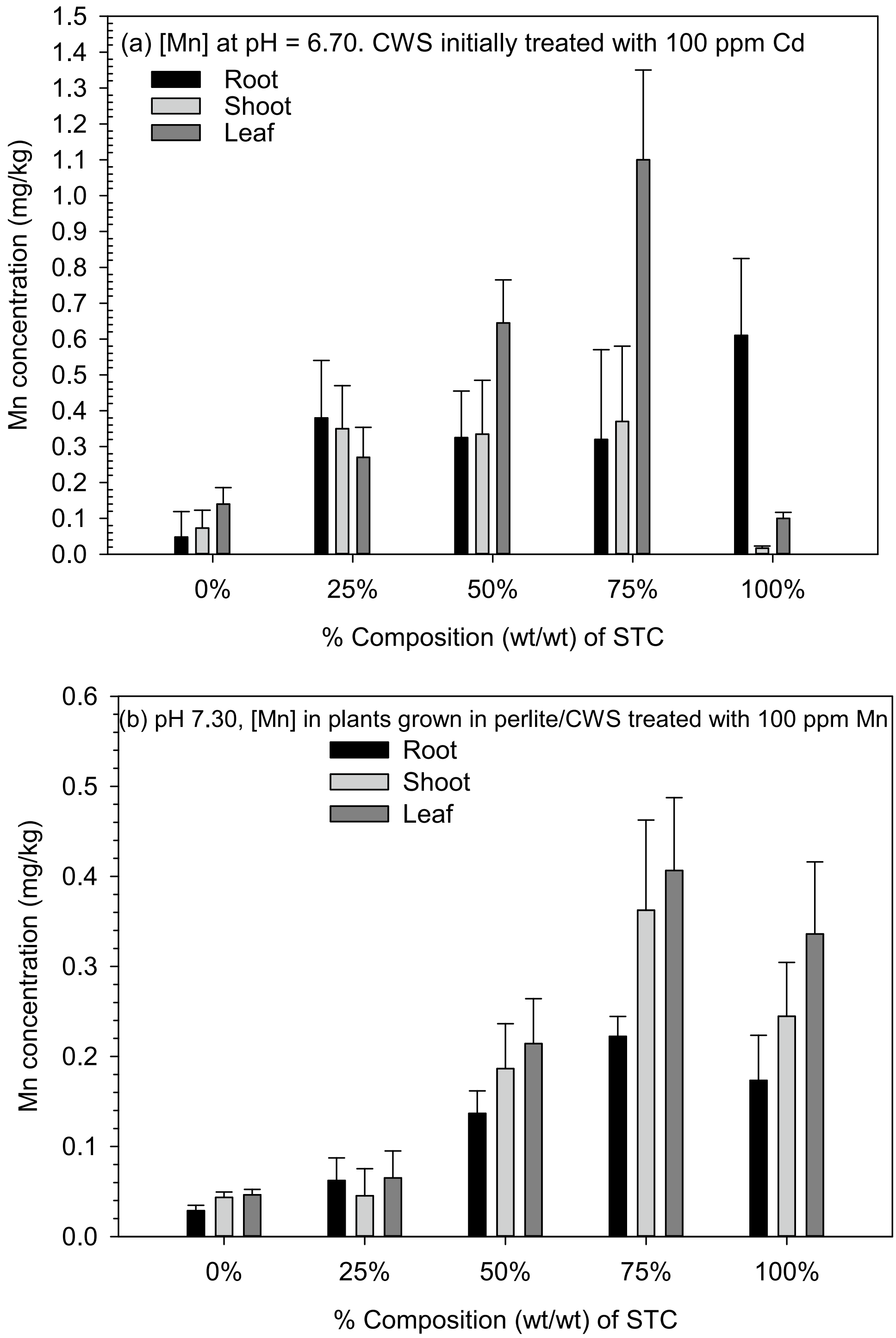


**Fig. 7** Manganese concentration (mg/kg) in radish (Raphanus sativus L.) cultivated with 100 ppm Mn treatment at pH 6.70 **a** and pH 7.30 Note: The Mn concentrations were determined per the biomass of the radishes, since there was an observed difference in the plant biomass for the triplicate samples

$[Mn]_{leaf} > [Mn]_{shoot} > [Mn]_{root}$ at pH 6.70 and 7.30 (Fig. 7a and b) This is plausible given Mn's essential role for photosynthesis in photosystem II (Carmona et al. 2010; Keren et al. 2002; Maiga et al. 2005). The Mn concentrations in leaves and shoot were about 1.61-fold to three-fold vis-à-vis roots, respectively (Table 2). The transfer factors (TF) of Mn from the soil to the plant parts were calculated as 1.6–4.6 and 1.8–3.4 in plants grown at pH 6.70 and 7.30, respectively. The transfer factors obtained are within the low to moderate contamination factor of $1 < C_F < 3$ (Sagagi et al. 2022). Notably, higher transfer factors are evident for plants grown at lower pH 6.70 vis-à-vis pH 7.30 (Table 3). A linear relationship was observed (Fig. 7) in plants grown in perlite/compost ratios at pH 6.70 composition with highest Mn amounts observed in 25–50% (wt/wt) CWS treatments.

**Table 2** Translocation factor of radish plants cultivated in initial Mn treatment (100 ppm) and Cd treatment (100 ppm) at pH 6.70

| Perlite/CWS Composition (wt/wt) | Mn TREATMENT | | Cd TREATMENT | |
|---|---|---|---|---|
| | $TF_{Mn}$ | $TF_{Cd}$ | $TF_{Mn}$ | $TF_{Cd}$ |
| 0% | 4.44 | 0.95 | 0.21 | 0.53 |
| 25% | 1.63 | 0.14 | 1.39 | 0.48 |
| 50% | 3.02 | 3.47 | 3.69 | 0.63 |
| 75% | 4.59 | 0.49 | 1.79 | 0.48 |
| 100% | 0.19 | 0.62 | 1.27 | 0.49 |

**Table 3** Translocation factor of radish plants cultivated in Mn and Cd treatments (100 ppm each at beginning) at pH 7.30

| Perlite/CWS Composition (wt/wt) | Mn TREATMENT | | Cd TREATMENT | |
|---|---|---|---|---|
| | $TF_{Mn}$ | $TF_{Cd}$ | $TF_{Mn}$ | $TF_{Cd}$ |
| 0% | 3.13 | 0.34 | 0.39 | 0.27 |
| 25% | 1.77 | 0.13 | 2.82 | 1.23 |
| 50% | 2.93 | 0.05 | 1.62 | 1.32 |
| 75% | 3.44 | 0.24 | 2.08 | 0.85 |
| 100% | 3.35 | 0.04 | 3.52 | 1.43 |

## Influence of pH upon Mn and Cd uptake by plants

### Cd Concentrations in plants cultivated in biosolids treated with 100 ppm Cd at pH 6.70 and 7.30

The influence of pH upon uptake of Cd and Mn metals was investigated at pH 6.70 and 7.30. Initially, seeds were sown in various perlite/CWS (wt/wt) ratios. Prior to planting seeds, CWS/perlite materials were watered with Hoagland solution—a plant nutrient solution which supplies essential minerals, needed for growth three days prior to sowing seeds.

The chosen pH (6.70) is comparable to pH 6.50 recommended for fertilizers for growing plants (Liu et al. 2021). Increase in amounts of applied CWS influences Cd or Mn uptake in plant parts at pH 6.70 and 7.30 (Fig. 6). Figure 6a further shows Cd concentrations in the root, shoot, and leaves of radish cultivated in 100 ppm Cd treatment at pH 6.70. Notably, there is decrease in the concentration of Cd in the roots upon addition of biosolids. The highest Cd concentrations were found in roots at 0% (wt/wt) CWS treatment. Concomitant with increase in CWS amounts was decreased Cd concentrations in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$. Radish plants grown at pH 7.30 show Cd concentration in roots < 0.3 mg Cd/kg. In general, [Cd] in plant parts decreased in the order $[Cd]_{root} > [Cd]_{shoot} > [Cd]_{leaf}$ with the highest [Cd] in the root and shoot at 0% (w/w) CWS. The Cd concentration in the leaves was below 0.04 mg/kg plant$^{-1}$. Clearly, increase in pH from 6.70 to 7.30 results in decreased Cd concentration in plant parts.

### Mn Concentrations in plants cultivated in biosolids treated with 100 ppm Mn at pH 6.70 and 7.30

Figure 7a depicts Mn concentrations in root, shoot, and leaves of radish plants cultivated with 100 ppm Mn treatment at pH 6.70. It is noted that Mn concentration in the root and shoot of radish increased from 0 to 25% (wt/wt) CWS treatment. The highest Mn concentration in the root and shoot was found at 75% (wt/wt) and 100% (wt/wt) CWS treatment. The highest Mn concentrations were found in the leaves at CWS 75% (wt/wt).

Figure 7b shows the concentration of Mn in the root, shoot, and leaves of radish plants cultivated in 100 ppm manganese treatment at pH 7.30. The [Mn] in the root, shoot, and leaves of radish plants increased from 0 to 75% (wt/wt) CWS treatment. An increase in the Mn concentration in the root is observed upon addition of biosolids. In similar trend with pH 6.70, concentrations of the Mn in radish parts were found in the order $[Mn]_{leaf} > [Mn]_{shoot} > [Mn]_{root.}$ These experiments are supported by recent reports which show that low molecular acids are produced by roots at lower pH values than at neutral pH values (Tazawa et al. 2021).

## Discussions

### Influence of sludge amendments on plant biomass

Findings from this investigation relate to the biosolid's (wastewater sludge) influence on Mn and Cd uptake by *Raphanus sativus L.* plants. CWS and perlite (a hydroponic material) were used in varying weight ratios, thus enabling assessment of the influence of wastewater sludge upon Mn and Cd uptake.

In general, it is observed that increasing application of CWS amounts resulted in increased biomass to maximum growth at 50%–75% (wt/wt) CWS composition (Figs. 4 and 5). These findings are consistent with previous studies which evaluated effects of sludge and compost on the growth of tomatoes, corn, pepper, and okra (Naz et al. 2019). Compared to controls, use of CWS resulted in increase in plant biomass at applications of 25–50% (wt/wt) by up to 40%. This growth can be attributed to higher nutrients in the compost and/or sludge, and improved aeration of the perlite (Cui et al. 2021). The effect of increased sludge amounts implicates the nutrient availability and concentrations. Studies show increased application of sludge to soils increases crop yields in corn, and barley (Agegnehu et al. 2016).

Zubillaga and Lavado (2002) performed experiments to determine heavy metal content in lettuce plants cultivated in composted biosolid. Under greenhouse conditions, lettuce plants were cultivated in varying amounts of compost biosolids (0–100% wt/wt). Notably, the use of composted biosolid resulted in a 20%–40% increment in the biomass accumulation. Experimental examination further showed Cd concentrations below detection in lettuce leaves in all treatments (Zubillaga and Lavado 2002). Garrido et. al., (2005) investigated the influence of sewage sludge in soils upon uptake of heavy metals by broad bean seeds (*Vicia faba L.*). Results showed that Cd was not detectable in the broad bean seeds. It was thus concluded that cultivation of broad beans in biosolids signified lower health or environmental risks (Garrido et al. 2005).

### Bioconcentration and translocation factors of Mn and Cd

Translocation factor (TF) is the ratio of metal concentration in the shoot to the root. This ratio explains the ability of a plant to translocate heavy metals from the roots to the stem and leaves. Tables 2 and 3 show calculated translocation factors for Mn and Cd in experiments in which radish plants were cultivated with/and without (control) 100 ppm Mn and 100 ppm Cd treatments at pH 6.70 and 7.30, respectively. The translocation factors for Mn in all treatments at both pH values were greater than 1.0, while Cd TFs were less than 1.0. The high Mn TFs implicates a selective Mn transport system in radish plants to the leaves. On the other hand, comparatively low TFs for Cd indicate differential selective mechanisms for Cd translocations in the radish parts.

### Implications of uptake mechanisms of metals

The pH changes in the area of the soil around the plant root (rhizosphere) are the most documented chemical reactions taking place at the soil–root interface (Darrah 1993; Hinsinger 2001). Research investigations on pH changes in the soil by cultivating root of beans on the surface of a marble polished plate showed that acid secretion in the roots in beans was strong enough to dissolve calcium carbonate, leaving behind visible imprints on the rock (Paul 2007). The acidic secretion of beans root was attributed to carbonic and organic acids generated by the rhizosphere microflora and roots through root respiration and exudation. Changes in pH of rhizosphere have been attributed to the release of $H^+$ or $OH^-$ ions. Hinsinger et al. (2003) found that release of charges caused by hydrogen ions ($H^+$) and hydroxyl ions ($OH^-$) counterbalances for the unbalanced cation–anion uptake at the soil–root interface as the major factor that causes root-induced pH changes in the rhizosphere. In addition, ions passing through the plasma membrane of the root cells such as organic anions released by plants also play a role in root-induced pH changes (Hinsinger et al. 2003).

The different uptake of cations and anions by plant roots is the main source of the flow of $H^+$ in the rhizosphere (Haynes 1990; Hinsinger 1998; Tang and Rengel 2003). The need to compensate for the electrical charges and regulation of cellular pH in the root cell is a major cause of uptake of cations and anions in the root cell. The pH of the aqueous part of the cytoplasm is usually maintained with a range of values around 7.30 with an efficient pH–stat system. The pH–stat system consists of both biochemical and biophysical $H^+$ exchange (Hinsinger et al. 2003). The biochemical components involve the generation and utilization of $H^+$ as a result of carboxylation and decarboxylation of organic acids in the root cell (Hinsinger et al. 2003; Tang and Rengel 2003). The pH of both the apoplasm and the cystol cannot be controlled by ATPs. The ATPs are considered to mainly act through energizing the transport of ions across the membrane which results in significant changes in pH (Gerendás and Schurr 1999). The uptake of cations is better understood with the mechanisms of ATPs (Haynes 1990). When more cations are up-taken than anions, hydrogen ion is released into the apoplasm to balance for the excess positive charges entering the cell. This results in an increase in the pH of the cytoplasm (cytosol) (54, 55). For instance, a larger uptake of $K^+$ exists than $SO_4^{2-}$ when a plant is supplied with a $K_2SO_4$ solution (Hinsinger 1998). But if more anions are up-taken than cations, hydroxyl ion, $OH^-$, will be released or hydrogen ion, $H^+$, will be taken up from the apoplasm to balance for the excess negative charge entering the cell, leading to a decrease in the pH of the cytosol. For instance, there is less uptake of $Cd^{2+}$ than $Cl^-$ when a plant is supplied with a $CaCl_2$ solution (Hinsinger 1998; Hinsinger et al. 2003). This results in a strong relationship that occurs between $H^+$ release and cation–anion balance.

## Conclusions

This study showed that radish plants (*Raphanus sativus L.*) exposed to Mn and Cd cultivated in CWS/perlite compositions tend to differentially accumulate these metals in the roots or leaves. The following conclusions are presented from this research. Firstly, increasing application biosolids up to ~ 25–50% (wt/wt) CWS composition results in increases of plant biomass. Secondly, the SEM/EDX studies show that biosolids contain elemental trace and major metals necessary for plant growth. Although the metal concentrations in biosolids are lower than the USEPA maximum ceiling concentrations, continued usage of biosolids may lead to the bioaccumulation of Mn or Cd in the environment. Thirdly, FTIR spectra shows biosolids contain –COOH in carboxylic acids, which are important in binding of metal ions—and thus contribute to the high affinity for metals. Fourthly, to avoid any contamination to the environment continuous studies are needed to ascertain the extent of heavy metal uptake from various metals.

The novelty of the conducted experiments is related to the effects of biosolid amendments on radishes (*Raphanus sativus L.*) and the uptake of Mn ad Cd into the roots, shoot, or plant parts. Despite the findings, further analysis and investigation would be needed to explain the biochemical basis of the translocation of Mn into the leaves vis-à-vis Cd in the roots.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s13201-023-02059-1.

**Acknowledgements** The authors acknowledge financial support from Stephen F. Austin State University Department of Chemistry and Biochemistry and Robert A. Welch Foundation Grant Number AN-0008. O. Fateru was supported through the Welch and SFASU Chemistry Department. Special thanks go to Nacogdoches Wastewater Treatment Plant facility personnel (David Wolfgang, and Gary Barton). We thank Professors Jared Barnes (Agriculture) and Robert Friedfeld for the use of SEM/EDX and X-ray diffractometer. Any opinions expressed in this paper are those of the authors, and therefore, no official endorsement should be inferred. We thank the Editors and anonymous reviewers whose helpful comments improved the manuscript.

**Author contributions** KKO was involved in project administration, conceptualization, resources, methodology, investigation, data curation, formal analysis, validation, writing—first draft, writing—original draft, writing—reviews and editing, visualization, supervision, funding acquisition. OOF helped in formal analysis, writing—first draft.

**Funding** The author(s) received no specific funding for this work.

**Data availability** All data generated or analyzed during this study are included in this published article [and its supplementary information files].

## Declarations

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** Not applicable.



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