Bioaccumulation of Chemical Elements in Vegetables as Influenced by Application Frequency of Municipal Solid Waste Compost

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Bioaccumulation of Chemical Elements in Vegetables as Influenced by Application Frequency of Municipal Solid Waste Compost

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ABSTRACT

Municipal solid waste (MSW) compost is used to enrich soils by virtue of its bio-physicochemical properties. But undesirable accumulation of chemical elements can reduce soil quality and cause food safety issues. A 5-year field study was carried out to investigate the impact of Compost Quality Alliance tested MSW compost application frequency (annual, biennial and no-compost) on soil quality and chemical elements accumulation in edible portions of lettuce (*Lactuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green bean (*Phaseolus vulgaris* cv. Golden Wax). Analysis of soil showed that chemical elements were highest in annual followed by biennial, but less in control (no-compost) and fallow soils. Soil background levels of chemical elements influenced the concentrations of iron and manganese in green bean, aluminum in green bean and beets, and barium in carrot, beets, and lettuce. Cadmium concentration in beets, lettuce, and green bean grown in annual plot was increased by 48%, 52% and 62%, respectively while carrot recorded 56% increase in the biennial plot compared to no-compost. Bioaccumulation factors < 1 for all the essential and non-essential trace elements in all the plant species except boron and molybdenum. However, lettuce showed a higher tendency to accumulate cadmium, rubidium, and strontium. Overall, the health risk for human consumption is low. Although long-term annual application of compost to vegetables seemed safe for human consumption, it is necessary to continuously monitor potential chemical elements accumulation, particularly non-essential trace elements in soils and plants.

Keywords: green bin compost, organic farming, trace element, essential nutrients, bioaccumulation factor

INTRODUCTION

Bioconversion of municipal solid waste (MSW) to compost for agricultural use is an effective and efficient waste management strategy, which enhances soil rejuvenation (Horrocks et al. 2016). However, long-term continuous application of MSW compost is of profound concern to environmentalists and farmers due to the likelihood of chemical elements accumulation, particularly toxic elements in soils, foods, and water bodies. Generally, chemical elements are categorized as: (1) essential macro-elements, which are required in large amounts by plants for growth and development e.g., nitrogen (N), phosphorus (P) and potassium (K); (2) essential trace elements, which are required in small amounts by plants to maintain metabolic processes e.g., manganese (Mn), sulfur (S) and iron (Fe); and (3) non-essential trace elements such as mercury (Hg), Lithium (Li) and arsenic (As) that are toxic to plants at low concentrations. Usually, accumulation of these chemical elements in plant tissues is dependent on soil management practices, inherent soil properties, chemical composition of the compost feedstock, type, and nature of the element and the genotypic characteristics of the plant (Dumontet et al. 2001). Thus, the level of elemental uptake and their accumulation in plant tissues can vary with different plant species.

The elemental composition of compost feedstock determines the chemical properties of the final compost and its impact on soil properties (Jodar et al. 2017). It was recently shown that the source of compost i.e., seafood waste, kitchen or MSW determined the chemical composition of compost (Abbey et al. 2018), while the frequency of MSW compost application significantly influence plant metabolome and lipidome (Abbey et al. 2021). Municipal solid wastes are dominated by food and non-food materials including paper and paper boards, metals, and plastics, all of which contain chemical elements (USEPA 2017). For instance, high amounts of non-essential trace elements

such as silver (Ag), antimony (Sb), chromium (Cr), Fe, zinc (Zn) and copper (Cu) in MSW were attributed to contamination from industrial and urban wastes, and consumer products with trace elements such as Ag nanoparticles (Margui et al. 2016; Gebeyehu, and Bayissa 2020). Therefore, compost regulations and standards have been established to ensure trace elements do not exceed established limits for crop production (CCME 2005). The Compost Quality Alliance (CQA), a voluntary program, was established by the Compost Council of Canada in association with compost producers to employ standardized testing procedures and uniform operating protocols to increase customer confidence in compost selection and utilization. However, the bioaccumulation impacts of CQA-tested MSW compost is largely understudied and as such, its potential for enhancing soil and crop quality is not realized.

Studies on the overall impact of annual and biennial MSW compost on chemical elements, particularly trace elements accumulation in field-soils and edible vegetables are sporadic and *ad hoc*, although MSW compost is recognized as a source of essential plant nutrients. A 5-year application of MSW compost increased soil content of Zn, Cu, Pb, Cr, nickel (Ni) and cadmium (Cd), irrespective of the rate of application but did not influence trace elements concentrations in squash leaves (*Cucurbita maxima*; Warman et al. 2009). Other studies also explained that plants such as spinach (*Spinacea oleracea*), lettuce (*Latuca sativa*), cabbage (*Brassica oleracea* var. capitata), and radish (*Raphanus sativus*) have the natural tendency to accumulate high amounts of Cd, Zn and Cu from soils in their edible portions (Intawongse and Dean 2006; Zhou et al. 2016; Waheed et al. 2019). The negative impact of continuous application of compost on soils, plants and human health were reported by Gebeyehu and Bayissa (2020) but not long-term variation in application frequency of MSW compost, which was the focus of the present study. They reported that elemental accumulation in cabbage was greater than tomato (*Solanum lycopersicum*).

Excessive accumulation chemical elements also referred to as bioaccumulation poses food safety concerns because they can be toxic to consumers as previously reported (Dumontet et al. 2001; Kabata-Pendias 2010; Guerra et al. 2012). The question therefore is: what will be the bioaccumulation factor (BAF) of essential macro-elements, essential trace elements and non-essential trace elements in edible plant tissues when the application frequency of MSW compost is varied? BAF is a measure of chemical elements accumulation in edible portions of plants relative to the amount present in the soil. Baker and Walker (1990) categorized field crops into excluders, accumulators and hyperaccumulators using BAF rating. Plants with BAF rating of chemical elements below 1 were categorized as excluders, plants with rating between 1 – 10 were categorized as accumulators and those with BAF rating >10 were categorized as hyperaccumulators.

Based on previous studies by Reddy et al. (2018), it was expected that BAF of plants will be higher with continuous application of MSW compost over the long-term. This study, therefore, hypothesized that biennial application of CQA-tested MSW compost will have lower BAF of plants and less health hazard compared to annual treatment. As such, the objective of the study was to determine the extent to which chemical elements accumulate in field-soil and edible portions of four different species of vegetable plants after five years of annual, biennial and no CQA-tested MSW compost applications. Potential health risk to consumers of the harvested vegetables was also assessed at the end of the 5th-year as described by Gebeyehu and Bayissa (2020). The targeted vegetable plants were lettuce (*Lactuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax). These were the main vegetable plants grown and marketed through

Community Supported Agriculture model by Agaard Farms, MB with the potential to accumulate trace elements in their edible portions.

MATERIALS AND METHODS

Study Location and Material Input

The 5-year field research was performed in Agaard Farms, Brandon, MB, Canada (Longitude 99° 56' 59.9892" W Latitude 49° 50' 53.9916" N; altitude: 409 m above sea level) between the fall of 2015 and 2019. The climate of Brandon falls under dominant to moderate cool, Boreal, sub-humid continental (MAFRD 2010). The soil in Agaard Farms belongs to the Newdale series and characterized by an Orthic Black Chernozem solum on moderate to strong calcareous, loamy morainal till of limestone, granitic and shale origin (MAFRD 2010). CQA tested municipal solid waste compost was donated by the City of Brandon waste management facility. Soils from fallow plots were also examined. Seeds of lettuce cv. Grand Rapids, beets cv. Detroit Supreme, carrot cv. Nantes and green beans cv. Golden Wax were purchased from a local greenhouse nursery (The Green Spot, Brandon, MB, Canada) for the study.

Climatic Conditions

The mean monthly climatic conditions during the growing season for the five years of the study can be found in Table S1. Mean monthly temperature increased from May to August as the season progressed from spring to summer throughout the study period between 2015 and 2019. Planting was usually done after the frost-free days in mid to late May during which night temperatures were low ranging from 0.4°C in 2019 to 4.9°C in 2016 compared to warmer nights in the months of June, July, and August irrespective of the year. The highest amount of precipitation was recorded in the warmest months of June and July throughout the 5 years of the study. The highest precipitation was recorded in June followed by July and the least was recorded in August.

Precipitation was negligible in August 2016, leading to extensive use of drip irrigation to reduce water-deficit stress effect on plant growth and development.

Field Preparation and Planting

The experimental field was ploughed and tilled to a depth of approximately 15 cm in the spring of every year of the research period, before planting. The dimension of the experimental field was 80 m x 50 m, and was further divided into three blocks, each of dimension 20 m x 10 m. Each block was subsequently subdivided into three 6 m x 3 m treatment plots to facilitate control, annual and biennial compost treatment in each block. In that regard, each block contained a total of 9 treatment plots. Separation between blocks was 2 m while that between treatment plots was 1 m. Annual and biennial plots were uniformly applied with 0.0137 m³ (approximately, 5 t/ha at a bulk density of 650 kg/m³) of CQA-tested municipal solid waste compost in the fall of every year for five years prior to planting in the spring of the following year. The compost was mixed in the soil during the tilling process. No extra compost nor fertilizer was added throughout the growing season.

Planting commenced after the last frost-free day between May 20th and 30th of each year when the soil temperature is above 10°C. Seeds were directly sown in single rows in each treatment plot spaced 75 cm apart. Carrot seeds were drilled, bean seeds were sown 20 cm apart, and the beets and lettuce seeds were sown 10 cm apart within the rows. The plants were irrigated every day using drip irrigation system when there was no rain or when soil moisture content was low. Weeding was done manually using a hand-hoe. No synthetic chemical fertilizer or pesticide was applied to the soil or plants. Young green lettuce leaves, carrot roots, beets tubers and green bean pods were harvested in batches at edible maturity stage between 45 to 60 days after sowing.

Soil Analysis

Soil samples from the individual plots were randomly collected ($n = 10$) at the peak harvesting time in August using a portable soil auger, from 20-cm depth where most of the root mass were found. Soil samples were also collected at the start of the growing season. Composite samples (300 g) of the soils were taken for each treatment plot per replication (i.e., 3 treatments $\times$ 3 blocks = 9 treatment plots). The soil samples were sent to the A & L Canada Laboratories Inc., London, Ontario for the analysis of organic matter, cation exchange capacity, potential concentration of hydrogen (pH) and mineral elements, and the Inorganic Analytical Chemistry laboratory at RPC, Fredericton, NB, Canada, for complete elemental analysis. The soil samples were air-dried before passing through 2-mm sieve. A portion of each soil sample was digested according to the Environmental Protection Agency (USEPA) method 3050B (Standard Operation Procedures #4.M19). The resulting solutions were analyzed for chemical elements by inductively coupled plasma- mass spectrometry/inductively coupled plasma optical emission spectroscopy (ICP-MS/ICP-ES) using EPA method 200.8/EPA 200.7 (Standard Operation Procedures #4.M01/4.M29). For the nitrate-nitrite analysis (as nitrogen), portions of the soil samples were leached in dilute potassium chlorate (KClO_3) for 1 hr. The leachate solutions were filtered and analyzed calorimetrically (Cataldo et al. 1974).

Plant Tissue Analysis

Edible portions of the lettuce, carrots, beets, and green beans were harvested and oven-dried at 65°C for 48 hrs or until a constant weight in a 52100-10 Cole-Parmer mechanical convection oven dryer (Cole-Parmer Instrumental Company, Vernon Hills, Ill., USA). The dried plant tissues were ground using a hammer mill and screened through a $53\text{-}\mu\text{m}$ sieve. The individual samples were put in a falcon tube to an approximate volume of 60 mL before shipping to RPC Laboratory for

further sample preparation and analysis. Portions of the samples were prepared by microwave-assisted digestion in nitric acid (SOP 4.M26). The resulting solutions were analyzed for chemical elements by ICP-MS (SOP 4.M01). Nitrates and nitrites (as nitrogen) concentration of the plant tissue samples were analyzed using the method described for the soil samples above.

Experimental Design and Data Analysis

The soil treatments (annual, biennial and no-compost) and plants were arranged in a single factor randomized complete block design with three replications. The four plant species were treated independently. Due to the large sample size and limited resources, composite samples of at least 15 (i.e., 5 samples x 3 replications) per treatment for soil and plant tissue were collected, and analyses were done in duplicates and the average was presented as the result. Graphs were plotted using Microsoft Excel 2016. The bioaccumulation factor (BAF) which is the ratio of the total chemical elements in the edible portions of the individual vegetable plant species to the total chemical elements in the soil, was computed as *Bioaccumulation Factor* =

$$\frac{\text{Total concentration in edible vegetable part (mg/kg)}}{\text{Total concentration in soil (mg/kg)}}.$$

Health Risk Assessment

The health risk associated with the consumption of vegetables that have bioaccumulated Cd, molybdenum (Mo), Zn, Cu, and boron (B) was evaluated using estimated daily intake (EDI) and hazard quotient (HQ).

Estimated daily intake

The estimated daily intake (EDI) of non-essential trace elements in the lettuce, beets, carrot, and green beans were determined as described by Gebeyehu and Bayissa (2020).

$$EDI = \frac{Ef \times ED \times FIR \times CM \times Cf \times 0.001}{BW \times TA}$$
 where Ef = exposure frequency (365 days/year), ED = exposure duration (life expectancy of 83.8 years for both sexes at age 65 per average lifespan in Canada (Statistics Canada 2020), FIR = average food consumption (5 serving of vegetable which is equivalent to 400 g) (WHO 2003; Statistics Canada 2017), Cf = concentration conversion factor (0.085) for vegetable fresh to dry weight and CM = chemical element concentration (mg/kg dry weight) (Rattan et al. 2005), BW = average normal body weight (72.8 kg) for adults (Health Canada 2018; Statistics Canada 2020), TA = average exposure time (83.8 years x 365 days), and conversion factor = 0.001 (Rattan et al. 2005).

Hazard quotient

Hazard quotient (HQ) as described by Rattan et al. (2005), is an estimation of carcinogenic human health risk associated with ingestion of vegetable that have bioaccumulated trace elements.

$$HQ = \frac{EDI}{RfD_o} \dots \dots \dots (3)$$

where EDI = estimated daily trace element intake of the population in mg/day or per body weight (kg) and RfD_o = oral reference dose (i.e., Cd, Mo, Cu, Zn, B and strontium (Sr) were 0.005, 0.04, 0.3, 0.001, 0.2 and 0.6 mg/kg/day, respectively) (USEPA 1992a; 1992b; 2004; 2005; 2013). A vegetable is presumed safe for consumption if HQ <1, while HQ >1 indicate high health risk.

RESULTS AND DISCUSSION

MSW Compost Chemical Elements

The ranges of chemical elements concentrations for the three batches of CQA-tested MSW compost used in the study is presented in supplementary Table S2. We found that the concentrations of the elements did not vary much i.e., within ±15% margin. In general, the

concentrations of the chemical elements in the first batch of the CQA-tested MSW compost pile increased by the 5th-year of the study. For instance, Fe and Cu, calcium (Ca), magnesium (Mg), B, total N and K rose by approximately >1000%, 940%, 740%, 240%, 96% and 40%, respectively in year 5 with reference to the 1st-year (data not reported). The chemical elements in the MSW compost noticeably enhanced the essential macro-elements and both essential and non-essential trace elements concentrations in the applied soil. A wide range in chemical composition of MSW compost have been reported (Warman et al. 2009; Jodar et al. 2017) like our compost, and this can be ascribed to the diversity in chemical composition of the municipal solid waste compostable materials. The varied chemical elements altered the chemical composition of the soils to an extent that was dependent on the frequency of application i.e., annual versus biennial. The non-essential trace elements in the MSW compost were below the maximum limit for category A compost (CCME 2005), which suggested that the CQA-tested MSW compost met the CCME compost quality guidelines, and it was suitable for agricultural use.

Soil Chemical Elements

Supplementary Table S3 showed slight change in soil pH from year 1, which remained consistent throughout the five years of study. Organic matter content and all the chemical elements drastically increased in soils that were applied with MSW compost compared to no-compost (control), particularly the annually applied soils. The most outstanding elements were P, K, Na, and Al. Table 1 showed a varied range of concentrations for individual soil chemical elements for the different treatments i.e., annual, biennial, fallow and no-compost by 5th-year as demonstrated by the standard deviations. For instance, the standard deviations and coefficient of variations for beryllium (Be), Cd, B and Cr for example, were within narrow ranges but wider ranges for Ca, Mg, K, Al, and Fe.

By the 5th-year of the study, total N in the annual-soil was 150% more than what was found in the biennial-soil, and more than 400% that of the no-compost while N in the fallow soil was below the reporting limit of 10 mg kg⁻¹ (Fig 1). The K in the annual-soil was 85% more than that of biennial-soil and the biennial-soil was approximately, 284% more than that of no-compost soil.

Fig. 1. Macronutrients and micronutrients accumulation in control and fallow soils and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. The soil belongs to the Newdale series and classifies as Orthic Black Chernozem solum. Error bars represent standard deviation. Value for potassium represents multiples of 10 and values for iron and aluminum represent multiples of 100.

Also, K increased by 46% in the fallow soil compared to the no-compost soil. There was also a slight increase in Mg and Ca of approximately 35% and 26% in the annual-soil compared to the biennial-soil, respectively; and 84% and 36% compared to the no-compost soil, respectively. However, Mg and Ca in the fallow soil was reduced by more than 26% and 61% respectively, compared to the no-compost soil. Therefore, the study revealed that the essential macro-elements N and K doubled in addition to the remarkable increases in Mg and Ca due to annual MSW compost application compared to the biennial application. Other studies reported the abundance of soil nutrients such as N, K, Ca, and Mg upon addition of MSW compost to soil (Warman et al. 2009; Jodar et al. 2017), which aligned with the present study. Increases in some of the chemical elements such as K, Fe and Mn in the fallow soil compared to no-compost soil can be attributed to regenerative capacity of the fallow soil while the low concentration of soil N, Ca and Mg in the soil pool could be due to less mineralization in the fallow soil (Neilson and Calderon 2011). Also,

soil Ca and Mg can be highly susceptible to loss by erosion or transformation under fallow conditions as reported by Baumhardt et al. (2015).

Soil essential trace elements such as Fe and Mn ranged from 7670 - 9330 mg kg⁻¹ and 400 - 605 mg kg⁻¹, respectively (Table 1). Fe was increased by 6.8%, 9.6% and 21.6% in the annual-soil compared to the biennial, fallow, and no-compost soils. Similarly, Mn increased respectively by 50% and 22% in the annual-soil and biennial-soil compared to the no-compost soil, and by 30% and 8% compared to the fallow soil (Fig 1). Positive change was also observed for Al with 24% increase in the fallow soil and 16% in the annual-soil compared to the no-compost soil. But Al was not altered in the biennial-soil. These results suggested that the contribution of the native soil trace elements was based on the inherent differences in bio-physicochemical characteristics of the no-compost and the fallow soils. Thus, the marginal to low accumulation of these trace elements in the fallow soil suggested that there was minimal contribution from background soil due to low soil organic matter content compared to contribution from the MSW compost application (Haluschak et al. 1998; MAFRD 2010).

Comparatively, B, Cu, Mo, and Zn concentrations in the annual-soil rose by 116%, 225%, 300% and 110%, but rose by 50%, 125%, 150% and 43% in the biennial-soil compared to the no-compost soil, respectively (Fig 2 A-D). The essential trace elements concentrations reported in the present study were in accordance with previous studies by Achiba et al. (2009) and Warman et al. (2009) in which MSW compost application led to appreciable increases in soil B, Mo, Zn and Cu concentrations. The average soil Mo, Zn, and Cu were below the Canadian Council of Ministers of the Environment (CCME) acceptable limits i.e., 5, 200 and 63 mg kg⁻¹, respectively except for B which exceeded 2 mg kg⁻¹ (CCME 1991a; 1991b; 1997; 2018). The global average for soil B concentration ranged from 5 to 30 mg kg⁻¹ and most leafy and root vegetable plants are moderately

responsive to imbalance in soil B. Typically, B, Mo, Zn and Cu availability to plants increase as soil organic matter decomposes and mineralization progresses (Antoniadis et al. 2019). This makes compost addition to agricultural soils very important.

Fig. 2 A -D. Trace elements accumulation in control and fallow soils and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. The soil belongs to the Newdale series and classifies as Orthic Black Chernozem solum. Error bars represent standard deviation. Values for barium, zinc, strontium and vanadium represent multiples of 10 and values for beryllium, cadmium, uranium and molybdenum represent multiples of 0.1.

Non-essential trace elements such as barium (Ba) and Cd were moderately increased in the annual-soil by 19.4% and 24.7%, and in the biennial-soil by 12.9% and 18%, respectively compared to no-compost and fallow soils (Fig 2A-D). Sr (3.7 kg/kg) and rubidium (Rb) (10.6 mg/kg) were highest in the annual-soil while Be, As and vanadium (Va) were similar for all the other soils. There were moderate (45% and 18%) to high (188% and 76%) concentrations of Li and lead (Pb) in the annual-soil and the biennial-soil, respectively, compared to the no-compost soil. The difference between the no-compost and the fallow soils was mainly due to significant differences in concentrations of Pb, Sr and Rb. Compost contains varied and complex chemical compounds and myriad of microbial populations and biochemical activities (Jodar et al. 2017). As such, it is expected that the different frequencies of MSW compost application will result in varied biophysicochemical activities in the soil. These activities were the main influencers of the composition of chemical elements and their availability in the different soils. A study linked the

amount and availability of non-essential trace elements in soils to soil acidity, organic matter content, precipitation, and leaching (Jeske 2013; Ermokhin et al. 2019). With regards to essential and non-essential trace elements in soils, the concentrations reported in this study were within the acceptable range (Haluschak et al. 1998; CCME 1997; 2005; 2007; 2013; 2015) and thus, there was no soil trace element phytotoxicity effect at the end of the 5th-year of the study.

The trend for plant growth and yield were annual > biennial > no-compost applied green beans, lettuce, beets, and carrot plants (data not presented). Biomass carbon accumulation determined by dry mass, leaf dry matter content and specific stem density were significantly increased in the control plants compared to MSW compost treatments. The reason could be the latter switched to adaptation and survival modes in the absence of adequate nutrients, especially nitrogen and imbalance in other nutrients.

Chemical Accumulation in Vegetables

Macro-elements

The means and standard deviations for essential macro-elements in the green bean, lettuce, beets, and carrot over the 5-year period are presented in supplementary Table S2. There were large variations of the chemical elements in the vegetables as influenced by variations in treatments and plant responses. The ranges for macro-elements N, Ca, Mg and K in the vegetables were 4850, 12010, 4480 and 75,300 mg kg⁻¹, respectively as further demonstrated by the respective standard deviations (Tables 2).

Annual-green bean N, K and Ca were respectively increased by 14.3%, 39.4% and 2.3%, but Mg was reduced by 10.6% compared to the biennial-green bean (Table 3). All these macro-elements were reduced in the no-compost green bean except Ca, which was increased by 6.6% in the latter compared to the mean for the annual- and the biennial-green bean. No-compost green bean had

the least N, K and Mg. The annual-carrot accumulated 200 mg kg⁻¹ tissue N while biennial- and no-compost green bean accumulated less than 100 mg kg⁻¹ (Table 3). Annual-carrot had 13.2% more K than biennial-carrot but Mg and Ca were respectively reduced in the former by 16.5% and 6% compared to the latter. The no-compost carrot had the least K, Mg and Ca. For beets, N was below the reporting limit of 100 mg kg⁻¹ regardless of the treatment (Table 3). Nonetheless, K, Mg and Ca were increased by 35%, 4.7% and 13.8% in the annual-beets, respectively compared to the biennial-beets while the no-compost beets recorded the least values. It was obvious the concentrations of the macro-elements in the lettuce were remarkably higher than all the other three vegetables (Table 3). Annual-lettuce had 308.3% N and 42.9% K more than the biennial-lettuce; while the biennial-lettuce had 8.3% Mg and 5.6% Ca more than the annual-lettuce. However, the highest Mg and Ca in lettuce was recorded by the no-compost treatment. The no-compost lettuce had 22.4% Mg and 9.8% Ca more than the annual-lettuce and 19.1% Mg and 3.8% Ca more than the biennial-lettuce.

The overall increase in lettuce and green bean N irrespective of treatment may be an indication of growth and photosynthetic demand for N in above-ground part (i.e., leaf and pods, respectively) of the plants. Comparatively, carrot and beets seemed to require less N but more Ca and Mg in their roots. The lettuce N reported in this study was 12% higher than those obtained following application of 200 kg N ha⁻¹ inorganic fertilizer to pot-grown lettuce (Liu et al. 2014). The difference in the N can be ascribed to differences in treatments and growing conditions and lettuce variety, which can influence nutrients uptake and accumulation. Based on nitrate accumulation rating for vegetables (Santamaria 2006), the N in annual-lettuce and annual green bean were very high followed by biennial-, and then their no-compost counterparts. Beets and carrots that received annual, biennial, and no compost applications were rated very low except for the annual-carrot.

Overall, the results of the present study agreed with nitrate-N accumulation capacity of carrot, which was reported as low by Santamaria (2006). The same can be said of the beets as well. Horrocks et al. (2016) reported that N-release rate of MSW compost can reach up to 0.5% by the 4th-year after application. This implied that cumulative annual and biennial MSW compost application over the five year period contributed to increased soil nutrients, particularly for the annual-soil. This cumulative effect made nutrients more available for the vegetable plants. Soil N and K are derivatives of the mineralization process, which is a function of soil organic matter (Morra 2019). As a result, soils that annually received compost rich in organic matter enriched the soils and plants with desirable macro-elements.

Essential trace elements

The essential trace elements Fe, Zn, Ba, Al, B, Cd, cobalt (Co), Cu, nickel (Ni) and Mo in the green bean were similarly influenced by the annual and biennial compost applications but Mn was not altered (Fig 3 A-D). Fe, Al and B were predominantly elevated in no-compost green bean by 15.2%, 73.4% and 21.5%, respectively compared to the respective mean values for the same essential trace elements in annual- and biennial-green bean.

Fig. 3 A - D. Trace elements accumulation in green beans (*Phaseolus vulgaris* cv. Golden Wax) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

No-compost green bean had the least Cd and Ni. Compared to the no-compost green bean, Ni increased by approximately 21.2% in annual- and biennial-green bean. Annual-carrot had

remarkably the highest essential trace elements Zn, Al and Cu i.e., 12.5%, 64.8% and 15.2% respectively, compared to the mean values for the biennial- and no-compost carrot (Fig 4 A - D).

Fig. 4 A -D. Trace elements accumulation in carrot (*Daucus carota* cv. Nantes) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

Overall, Mn, B and Mo increased slightly in annual-carrot by 8.2%, 6% and 12.6%, respectively compared to the biennial-carrot and the least in the no-compost carrot. However, biennial-carrot recorded the highest Fe and Ni by about 79% and 150%, respectively compared to the annual-carrot. The concentration of Co was similar in both annual- and biennial-carrot.

The increase in concentrations of Fe, Mn, Zn, Cu and Mo in the edible portion of the annual-beets were 10.3%, 14.5%, 37.6%, 41.7% and 40%, respectively compared to the biennial-beets (Fig 5 A-D).

Fig. 5 A- D. Trace elements accumulation in beets (*Beta vulgaris* cv. Detroit Supreme) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

On the other hand, the no-compost beets had 31.4% more Al than the biennial-beets, and the latter also had 12.9% more Al than the annual-beets. It was found that neither the annual nor the biennial treatments affected Co nor Ni concentration in beets. For lettuce, Fe and Al were significantly enhanced by annual compost application i.e., 40.5% and 35%, respectively compared to biennial-

lettuce; while the biennial-lettuce recorded the highest concentration of Ni i.e., 168.4% compared to the annual-lettuce (Fig 6 A-D). Additionally, Mn, Mo and Cu were similar in the annual- and biennial-lettuce but higher than those in the no-compost lettuce with the exception of Cu, which was higher in the biennial-lettuce. The only essential trace element that was not altered by the treatments was B.

Fig. 6 A - D. Trace elements accumulation in lettuce (*Latuca sativa* cv. Grand Rapids) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

Overall, the mean beets and carrot essential trace elements concentrations across the MSW compost treatments was higher than the means for lettuce and green bean. The wide range of concentrations for these essential trace elements can be ascribed to differences in effect of frequency of MSW compost application. Essential trace elements concentrations in vegetables can be associated with soil trace elements concentration and soil organic matter content (McLean and Bledsoe 1992; Kwiatkowski and Harasim, 2020). Additionally, it appeared that the genotypic characteristics of the plant also determined the type and amount of essential trace element accumulated through different physiological pathways that characterize each plant species.

Non-essential trace elements

Non-essential trace elements i.e., Ba, Cd, Cr, Li, Pb, rubidium (Rb) and Sb concentrations in the four vegetables ranged from 0.19 mg Pb/kg to 66.2 mg Ba/kg as presented in supplementary Table S2. Unlike the macro-elements, the non-essential trace elements had a narrow ranges and standard deviations. The potentially toxic trace elements Sb and Pb were respectively increased in biennial-

green bean by 24.2% and 300% compared to annual-green bean, and 44% and 100% compared to no-compost green bean (Fig 3 A-D). On the other hand, Cr, Ba, Rb and Li were respectively increased in annual-green bean by 500%, 16.5%, 49.7% and 300% compared to biennial-green bean. The no-compost green bean had the least concentration of non-essential trace elements with the exception of Rb, which was 19.2% higher than that of the annual-green bean and 20.9% higher than that of the biennial-green bean. For the carrot, Rb, Pb, Li and Cr were respectively increased by 21.4%, 60%, 50% and 11.1% in annual-carrot compared to biennial-carrot while Cd was increased by 17.3% in biennial-carrot compared to annual-carrot (Fig 4 A-D). It was not clear why the Li in the no-compost carrot was not different from that of the annual-carrot but 50% higher than that of the biennial carrot. The no-compost carrot had the highest Ba at a concentration of 71.9% more than the average for the annual- and biennial-carrot. Sb was not altered by frequency of MSW compost application but slightly higher (i.e., 3.1%) in the no-compost carrot.

Compared to the other three vegetables, it appeared beets accumulated less non-essential trace elements (Fig 5 A-D). Rb, Cd, Pb and Cr concentrations were generally low in the beets, irrespective of treatment differences. There were increases of about 21.8% in Rb and 19.4% in Cd for annual-beets compared to biennial-beets. Sb increased in the no-compost beets by about 28.6% and 26% respectively compared to annual-beets and biennial-beets. Rb, Li, Cd and Pb were respectively increased by 2.7%, 10.8%, 30.9% and 40% in annual-lettuce compared to biennial-lettuce (Fig 6 A-D). On the other hand, Ni and Ba in biennial-lettuce increased by 184.2% and 33.5%, respectively compared to annual-lettuce. Sb was 24.5% higher in the biennial-lettuce than the annual-lettuce, but not the no-compost lettuce. Sb was the only non-essential trace element that was elevated in no-compost lettuce by 47.4% compared to annual-lettuce and 11.3% compared to biennial-lettuce. In general, changes in the concentrations of Pb and Cd were low i.e., an average

of <0.2 mg/kg dry edible portion of lettuce with the highest value in annual-lettuce. Cr was not significantly altered by MSW compost application.

Overall, the high accumulation of non-essential trace elements in green beans and lettuce was due to annual MSW compost application, and may suggest an affinity for non-selective absorption of the analyzed non-essential trace elements by the vegetable plants as previously explained by Achiba et al. (2009) and Robinson et al. (2018). Typically, indiscriminate absorption of non-essential trace elements is a characteristic of vegetables in the Asteraceae family, which includes lettuce (Manzoor et al. 2018). The results of our studies validated the assimilation and accumulation of non-essential trace elements in edible portions of lettuce, beets, green bean and carrot. Background soil concentration of non-essential trace elements influenced some of the elevated non-essential trace elements in the vegetables grown without compost application as previously reported by Haluschak et al. (1998) and Intawongse and Dean (2006). Generally, the trace elements were within ranges previously reported in other plants following application of MSW compost (Haluschak et al. 1998). In this study, the relationship between vegetable and soil non-essential trace elements was positive and significant ($r \geq 0.74$; $P < 0.05$) (data not presented).

Bioaccumulation of Trace Elements in Vegetables

Bioaccumulation of chemical elements determines the extent to which plants have absorbed and stored these chemical elements in tissues, and further gives an indication of the safety of these plants for human consumption. Our present results indicated $BAF < 1$ for most of the trace elements, which means less uptake and accumulation of these trace elements in edible portions of the lettuce, beets, carrot, and green bean irrespective of the MSW compost application frequency (Table 4). A $BAF < 1$ suggested that these vegetables are excluders and have low capacity to bioaccumulate trace elements (Reddy et al. 2018). Similar observations were made previously by

Antonious et al. (2017). They reported BAF values <1 for edible beets and bean following compost application. The low BAF was attributed to the influence of MSW compost, concentration and interaction of other soil chemical elements, soil characteristics, and the genetic characteristics of the plant (Tasrina et al. 2015). Some of the trace elements, particularly B and Mo had BAF >1 in all the vegetables irrespective of treatment (Table 4). The interaction and balance between some of the soil trace elements influence their availability and uptake by plants. For instance, the interaction between Cd and Zn are well documented (Chaney et al. 1994; Adamczyk-Szabela et al. 2020). The high transferability of Cd from soil to lettuce was similarly reported by Chang et al. (2014) and Waheed et al. (2019). They explained that addition of MSW compost to soil promotes Cd adsorption by the formation of soluble complex with inorganic and organic ligands as it associates with cationic elements and compounds in the compost. However, the different vegetable plants responded differently, and that is why Cd had a relatively high BAF in lettuce irrespective of the soil treatment including no-compost lettuce compared to the low BAF in the other vegetables. Another evidence is that Va was below the limit of detection in all the vegetables except lettuce although it was present in appreciable amount in the soil. Mo had the highest BAF value of 16.3 in the no-compost green bean (Table 4). Apart from B and Mo, which had the highest BAF ranging from 1.5 to 4.1 and 1.1 to 16.3 respectively, the other trace elements were within acceptable levels with moderate concern for lettuce bioaccumulation of Cd, Rb and Sr (Table 4). The no-compost vegetables tended to have higher BAF for most of the trace elements. Green bean and lettuce were the highest bioaccumulators of Mo and B followed by beets and then carrot. The deficiency of essential trace elements such as Mo and B in foods globally is a grave public concern. As such, it is good news that MSW compost can biofortify vegetable plants with B and Mo so long as it falls within acceptable health limit.

According to Fedorkova et al. (2012), soil organic matter forms complexes with trace elements leading to the formation of organometallic compounds that tend to reduce the availability of the bound trace elements. This could be the mechanism that led to the reduction in BAF for all the four the vegetables despite annual MSW compost application.

Health Risk Assessment of Bioaccumulated Trace Elements in Vegetables

Human health risk from the bioaccumulated trace elements was assessed using models for estimated daily intake (EDI) (Table S4) and health hazard quotient (HQ) (Table S5). Total EDI for B, Mo, Cu, Zn, Cd and Sr as affected by MSW compost application frequency and responses of the different plant species were determined for an adult who consumes 400 g vegetable per day. Interestingly, the EDI was calculated to be less than 10^{-5} mg/day for all the trace elements analyzed (data not presented). Similarly, the HQ values for the trace elements assessed were all less than 10^{-4} for the risk of non-carcinogenic effects (data not presented). Therefore, both EDI and HQ values suggested minimal health risk upon consumption of 400 g of the vegetables i.e., green bean, beets, carrot, and lettuce per day (WHO 2003; Statistics Canada 2017). HQ data vary from one country to another and may depend on baseline information for the crop, and environmental safety. However, similar results were obtained for vegetables grown with irrigation wastewater in a long-term study in Ethiopia (Woldetsadik et al. 2017).

CONCLUSION

The present study demonstrated the importance and positive impact of long-term application of MSW compost in agriculture. Apparently, the major concern expressed by the public over long-term use of MSW compost and the likelihood of soil contamination and food safety related diseases were not observed in our study. Although soil chemical elements were remarkably increased by the 5th-year of the present study, the absorption and bioaccumulation of chemical elements,

particularly potentially toxic trace elements in edible portions of green bean, lettuce, carrot and beets were not found. Overall, annual MSW compost application enhanced soil and harvested plants macro-elements, and essential and non-essential trace elements more than the biennial application. A comprehensive crop rotation program could influence chemical element availability in the soil and plant uptake. The EDI and HQ models indicated that all the vegetables were safe for consumption. More importantly, there was no carcinogenic risk associated with the selected trace elements in the vegetables. Soil Pb, Ni, Cr, Li, and Ni were at least 10, 7, 4 and 10 times lesser than the acceptable levels in soil and CCME guidelines. Therefore, the four vegetables investigated in the present study were safe to consume but this may not be the case in a much longer term i.e., > 5 years. The concern over Cd, Sr and Rb with propensities to accumulate in lettuce must be investigated. Furthermore, continuous monitoring of trace elements in fields with long term compost application should be considered. Additionally, future studies might want to assess the regenerative potentials of soil impacted by trace elements from MSW compost.

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Table 1. Selected descriptive statistics for concentrations of chemical elements across fallow, annual, biennial and no-compost plots in the 5th-year of the study

Trace element	Maximum (mg kg ⁻¹)	Minimum (mg kg ⁻¹)	Mean (mg kg ⁻¹)	Standard deviation (mg kg ⁻¹)	CV ²
Nitrogen ³	50.0	10.0	22.5	18.9	0.78
Calcium	22000.0	6120.0	1540.0	6688.0	0.43
Magnesium	5920.0	2360.0	3970.0	1541.0	0.39
Potassium	3610.0	940.0	1968.0	1171.0	0.60
Aluminum	4540.0	3650.0	4018.0	443.0	0.11
Arsenic	3.0	2.0	2.8	0.5	0.18
Barium	111.0	89.0	99.5	10.3	0.10
Beryllium	0.2	0.2	0.2	0.0	0.01
Cadmium	0.3	0.2	0.2	0.1	0.20
Boron	13.0	6.0	8.7	3.2	0.37
Chromium	9.0	6.0	7.8	1.3	0.16
Cobalt	3.6	2.9	3.3	0.3	0.09
Copper	130.0	4.0	8.0	3.9	0.49
Iron	9330.0	7670.0	85640.0	688.0	0.08
Lead	9.1	3.4	6.6	2.6	0.40
Lithium	4.8	3.3	3.9	0.7	0.17
Manganese	605.0	400.0	497.1	85.4	0.17
Molybdenum	0.8	0.2	0.4	0.3	0.68
Nickel	10.0	8.0	9.0	0.9	0.09

Rubidium	10.6	6.7	8.6	1.6	0.19
Strontium	37.0	14.0	26.5	9.5	0.36
Uranium	0.5	0.4	0.5	0.1	0.10
Zinc	59.0	28.0	39.8	13.8	0.35
Vanadium	16.0	14.0	14.8	1.0	0.06

¹Newdale series and classifies as Orthic Black Chernozem solum: ²CV, coefficient of variation;

³Nitrate and nitrite as nitrogen.

Table 2. Selected descriptive statistics for chemical elements across all vegetable plants (lettuce (*Lactuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) in the 5th-year of the study

Trace element	Maximum (mg kg ⁻¹)	Minimum (mg kg ⁻¹)	Mean (mg kg ⁻¹)	Standard deviation (mg kg ⁻¹)
Nitrogen ¹	4900.0	50.0	804.0	1388.0
Calcium	13600.0	1590.0	5563.0	4591.0
Magnesium	5930.0	1450.0	2861.0	1462.0
Potassium	96600.0	21300.0	38479.0	21917.0
Aluminum	172.0	4.7	39.4	60.1
Boron	24.9	18.0	21.7	2.7
Barium	72.9	6.7	27.1	18.2
Cadmium	0.3	0.01	0.1	0.1
Chromium	3.7	0.2	1.4	1.4
Cobalt	0.2	0.02	0.1	0.1
Strontium	36.4	7.6	16.1	9.7
Vanadium	0.7	0.1	0.2	0.3
Zinc	37.1	15.1	23.3	7.2
Copper	8.5	4.8	6.0	1.1
Iron	364.0	29.0	110.3	114.3
Lead	0.2	0.01	0.1	0.1
Lithium	1.7	0.01	0.5	0.6

Manganese	95.8	13.8	39.0	31.3
Molybdenum	7.6	0.5	2.8	2.6
Nickel	5.1	0.2	1.7	1.2
Rubidium	19.1	4.4	8.6	5.9

¹Sum of nitrate and nitrite as total nitrogen.

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Table 3. Average macro-elements concentrations in lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) as affected by variations in frequency of municipal solid waste compost application in the 5th-year of the study

Macro-element	Green bean (mg kg ⁻¹)			Carrot (mg kg ⁻¹)			Beets (mg kg ⁻¹)			Lettuce (mg kg ⁻¹)			
	AN	BI	NC	AN	BI	NC	AN	BI	NC	AN	BI	NC	CV ²
Nitrogen ¹	1600	700	600	200	<100	<100	<100	<100	<100	4900	1200	200	0.81
Potassium	34850	25000	23700	35200	31100	28300	34700	25600	21300	96600	67600	37800	1.75
Magnesium	2395	2650	2360	1580	1840	1450	2220	2120	2210	4600	4980	5930	1.95
Calcium	4090	4000	4310	3160	3350	3080	1810	1590	2270	12400	13100	13600	1.21

¹Nitrate and nitrite as nitrogen; ²CV, coefficient of variation; AN, BI and NC are annual, biennial and no municipal solid waste compost application, respectively.

¹CV, coefficient of variation; AN, BI, and NC are annual, biennial, and no municipal solid waste compost application, respectively.

Table 4. Bioaccumulation factor of essential and non-essential trace metals in edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) as affected by variations in frequency of municipal solid waste compost application in the 5th-year of the study

Element	Green bean			Carrot			Beets			Lettuce			CV ¹
	AN	BI	NC	AN	BI	NC	AN	BI	NC	AN	BI	NC	
Aluminum	0.001	0.001	0.002	0.004	0.003	0.003	0.004	0.005	0.006	0.052	0.045	0.048	1.41
Barium	0.092	0.084	0.072	0.195	0.234	0.428	0.282	0.392	0.784	0.172	0.243	0.253	0.73
Boron	1.481	2.067	3.833	1.915	2.611	3.967	1.492	2.000	3.050	1.785	2.644	4.100	0.37
Cadmium	0.078	0.074	0.067	0.532	0.678	0.556	0.320	0.291	0.300	1.320	1.096	1.200	0.82
Chromium	0.200	0.038	0.100	0.111	0.113	0.083	0.044	0.038	0.033	0.411	0.438	0.600	1.04
Cobalt	0.035	0.042	0.038	0.009	0.010	0.007	0.009	0.010	0.014	0.050	0.042	0.052	0.68
Copper	0.369	0.633	1.250	0.492	0.589	1.450	0.654	0.667	1.225	0.454	0.700	1.825	0.54
Iron	0.007	0.007	0.010	0.004	0.006	0.004	0.005	0.004	0.005	0.039	0.030	0.034	1.02
Lead	ND	0.007	0.006	0.008	0.008	0.012	0.003	0.007	0.018	0.021	0.025	0.053	0.94
Lithium	0.017	ND	0.006	0.019	0.015	0.030	0.129	0.154	0.209	0.363	0.403	0.297	1.00
Manganese	0.032	0.037	0.050	0.028	0.031	0.035	0.056	0.058	0.077	0.150	0.187	0.205	0.81

Molybdenum	9.275	15.100	16.300	1.338	1.900	3.450	1.138	1.300	2.600	5.163	8.680	10.600	0.85
Nickel	0.183	0.167	0.163	0.133	0.167	0.075	0.133	0.144	0.025	0.211	0.567	0.325	0.73
Rubidium	0.623	0.551	0.796	0.604	0.659	0.696	0.533	0.580	0.772	1.802	2.325	2.537	0.71
Strontium	0.239	0.393	0.281	0.349	0.457	0.489	0.265	0.357	0.467	0.668	1.168	1.348	0.66
Vanadium	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.047	0.043	0.050	0.08
Zinc	0.366	0.538	0.675	0.297	0.385	0.539	0.508	0.545	0.657	0.629	0.755	1.139	0.38

AN, BI and NC are annual, biennial and no municipal solid waste compost application, respectively.

ND is not determined for tissue chemical element concentration below laboratory reporting limit.

Fig. 1. Macronutrients and micronutrients accumulation in control and fallow soils and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. The soil belongs to the Newdale series and classifies as Orthic Black Chernozem solum. Error bars represent standard deviation. Value for potassium represents multiples of 10 and values for iron and aluminum represent multiples of 100.

Fig. 2 A -D. Trace elements accumulation in control and fallow soils and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. The soil belongs to the Newdale series and classifies as

Orthic Black Chernozem solum. Error bars represent standard deviation. Values for barium, zinc, strontium and vanadium represent multiples of 10 and values for beryllium, cadmium, uranium and molybdenum represent multiples of 0.1.

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Fig. 3 A - D. Trace elements accumulation in green beans (*Phaseolus vulgaris* cv. Golden Wax) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

Fig. 4 A -D. Trace elements accumulation in carrot (*Daucus carota* cv. Nantes) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

Fig. 5 A- D. Trace elements accumulation in beets (*Beta vulgaris* cv. Detroit Supreme) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.

Fig. 6 A - D. Trace elements accumulation in lettuce (*Lactuca sativa* cv. Grand Rapids) and changes with the annual and biennial application frequency of Compost Quality Assurance tested municipal solid waste (MSW) compost. Error bars represent standard deviation.