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Identification of Phytotoxic Levels of Copper and Nickel in Commercial Organic Soil Amendments Recycled from Poultry Farms and Municipal Wastes

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Abstract

Commercial-scale recycling of agricultural and municipal wastes into organic soil amendments facilitates safe disposal of waste and reduces environmental contamination. However, phytotoxicity of commercial organic amendments to crops is a major concern to farmers. Consistent with this, commercial chicken manure and Milorganite (recycled from municipal waste) were found to be phytotoxic. Chicken manure aqueous extract contains 10.8 ppm Cu and 0.7 ppm Ni. The level of Cu and Ni in Milorganite is lower. The current study identified an aqueous solution containing 5 ppm Cu, lower than in chicken manure aqueous extract, was highly phytotoxic to mustard seeds germination. Therefore, phytotoxicity of chicken manure is in part due to Cu. An aqueous solution containing 1 ppm Ni was not phytotoxic; whereas 0.125 ppm Ni was phytotoxic when 62.5 ppm Na, which is nontoxic, was added to the solution. Therefore, synergistic effects of chemicals in the organic amendments may induce phytotoxicity.

Keywords Organic amendment · Phytotoxicity · Copper · Nickel · Seed germination · Mustard

Safe disposal of waste originating from municipal and animal production farms is a major challenge worldwide (Bolan et al. 2010; Hoornweg et al. 2013; Nollet et al. 2007). Commercial-scale recycling of agricultural and municipal wastes into organic soil amendments and fertilizers is the best approach to reduce environmental contamination and pollution. In addition, compared to inorganic fertilizers, organic soil amendments recycled from agricultural and municipal wastes increase the organic matter content of agricultural soils and improve soil health (Ferreras et al. 2006; Hargreaves et al. 2008).

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Commercial organic soil amendments recycled from agricultural and municipal wastes are commonly used in the USA. Hazardous chemicals such as lead and mercury were not detected in aqueous extracts of commercial organic soil amendments, such as chicken manure and dairy manure (recycled from agricultural wastes), and Milorganite (recycled from municipal wastes) (Kebrom et al. 2019). Guidelines that restrict the use and disposal of chemicals hazardous to humans may have contributed to the insignificant level of mercury and lead in the organic soil amendments (Tchounwou et al. 2012). However, the phytotoxicity of organic soil amendments recycled from agricultural or municipal wastes to crops is a major concern to farmers. In particular, the higher rate of application of organic amendments to meet the nitrogen requirements of crops may simultaneously increase the level of the other plant nutrients and heavy metals in the organic amendments beyond the maximum safe level for seed germination and plant growth (Cogger et al. 2011, 2016; Oladeji et al. 2019; Pokorska-Niewiada et al. 2018; Sethy and Ghosh 2013). Therefore, evaluating the phytotoxicity of commercial organic soil amendments and identifying potentially phytotoxic levels of plant micronutrients and heavy metals will help to develop strategies to alleviate phytotoxicity.

We recently evaluated phytotoxicity of chicken manure, Milorganite, and dairy manure using the seed germination bioassay (Kebrom et al. 2019). Aqueous extracts of chicken manure and Milorganite, but not dairy manure, were found to be phytotoxic to the germination of collard greens (*Brassica oleracea*) seeds. The level of Na in the aqueous extracts of chicken manure, Milorganite, and dairy manure was 442 ppm, 63 ppm, and 36.3 ppm, respectively. However, the germination of collard greens seeds was not affected by 1000 ppm Na containing aqueous NaCl solution (Kebrom et al. 2019). The level of heavy metals such as Zn, Cu, and Ni was higher in chicken manure and Milorganite than in the non-phytotoxic dairy manure aqueous extracts (Kebrom et al. 2019). The level of Zn, Cu, and Ni in chicken manure aqueous extract is 8.1 ppm, 10.8 ppm, and 0.7 ppm, respectively; and the level of these elements in Milorganite aqueous extract is 0.4 ppm, 0.77 ppm and 0.53 ppm, respectively. Zn, Cu, and Ni are essential micronutrients required in a small quantity for the growth and development of plants (Borkert et al. 1998; Hall 2002; Pokorska-Niewiada et al. 2018; Seregin and Kozhevnikova 2006). However, a higher level of these elements inhibits seed germination and seedling growth (Paradelo et al. 2010; Parlak 2016; Pokorska-Niewiada et al. 2018; Tiquia 2010; Tiquia et al. 1996). Therefore, phytotoxicity of chicken manure and Milorganite aqueous extracts could be due to the relatively higher levels of Zn, Cu, or Ni.

There is variation among plant species in their response to the level of Zn, Cu, or Ni in a growth media (Pokorska-Niewiada et al. 2018). Thus, it is possible that the germination of collard greens seeds could be more sensitive than other plant species to chicken manure and Milorganite aqueous extracts. Mustard (*Brassica juncea*) is a close relative of collard greens in the genus *Brassicaceae*. Also, the size of collard greens and mustard seeds is comparable. To validate the observed phytotoxicity of chicken manure and Milorganite to collard greens, in the current study, we investigated the germination of mustard seeds in chicken manure and Milorganite aqueous extracts. Also, we evaluated if the level of Zn, Cu, or Ni, and synergistic effects with Na, in chicken manure and Milorganite is phytotoxic to the germination of mustard seeds.

Materials and Methods

Mustard seeds (variety 3157U Florida Broad Leaf) were obtained from Twilley seed company (Hodges, SC). Phytotoxicity of chicken manure (Medina) and Milorganite aqueous extracts to mustard seeds was evaluated using the seed germination bioassay as described in Kebrom et al. (2019). Briefly, to prepare the aqueous extracts, 1 g chicken manure or Milorganite was mixed with 10 mL deionized water (1:10 w/v) and incubated on a rotary shaker for 1 h. The mixture was centrifuged at 5000×*g* for 15 min, and filtered through 0.4 µm membrane filter. The seed germination test was conducted by incubating ten mustard seeds in 100-mm diameter and 25-mm height Petri dishes on Whatman filter paper wetted with 4 mL of various concentrations of chicken manure or Miloragnite aqueous extracts.

To study the effect of Zn, Cu, and Ni on the germination of mustard seeds, Zinc chloride ($ZnCl_2$) (Sigma-Aldrich), Copper(II) chloride dihydrate ($CuCl_2$) (Honeywell Fluka), and Nickel chloride ($NiCl_2$) (Sigma-Aldrich) aqueous solutions of various strengths were prepared. The concentration of Zn, Cu, and Ni in the aqueous solutions was verified using radial view of Inductive Coupled Plasma Optical Emission Spectrometer (ICP-OES, Agilent ICP-5100) equipped with Agilent SP4 autosampler, standard DV torch (1.8 mm ID injector), concentric sea spray nebulizer type, standard double-pass glass cyclonic spray chamber, and supplied with ultrapure argon gas. Calibration standard solutions of 5 mg L^{-1}, with calibration range 0–500 ppm, for the ICP-OES analysis were prepared from single element (Zn, Cu, and Ni) (Sigma) and multi-element standard solutions (Agilent). The following default instrumental parameters were used: 1.2 kW radio frequency power, 12 L min^{-1} plasma gas flow rate, 1.0 L min^{-1} auxiliary gas flow rate, 1.0 L min^{-1} sample uptake rate, 0.7 L min^{-1} nebulizer gas flow rate, 5 s read time, and wavelength of 213.857 nm for Zn, 231.604 nm for Ni, and 327.395 nm for Cu. All solutions were analyzed three times. The correlation coefficients for the calibration curves was >0.9990, with a readback error of $<0.5\%$. The calculated level of Zn, Cu, and Ni in the stock solutions was 480.8 ppm, 373.1 ppm, and 226.4 ppm, respectively, and the results of ICP-OES was 480 ppm, 376 ppm and 226.5 ppm. The working solutions were prepared using the ICP-OES results.

The mustard seed germination experiments were conducted in 0 ppm (deionized water, control), 1 ppm, 5 ppm, 10 ppm, and 50 ppm aqueous solutions of Zn, Ni, or Cu. Aqueous solutions containing either $NiCl_2$ and NaCl (Ni–Na) or $NiCl_2$, $ZnCl_2$, $CuCl_2$ and NaCl (Ni–Zn–Cu–Na) were prepared to investigate synergistic effects of Ni, Zn, Cu, and Na on the germination of mustard seeds. The Ni–Na aqueous solution was prepared using 1 ppm Ni and 500 ppm Na; and the Ni–Zn–Cu–Na aqueous solution was prepared using 1 ppm Ni, 5 ppm Zn, 10 ppm Cu, and 500 ppm Na. These concentrations were chosen based on the level of these elements in the aqueous extract of chicken manure (Kebrom et al. 2019). The level of all these elements in Milorganite aqueous extract was lower than in chicken manure aqueous extract. The seed germination bioassay was conducted by incubating ten mustard seeds in each Petri dish on Whatman paper wetted with 4 mL of various concentrations of Zn, Ni, Cu, Ni–Na, or Ni–Zn–Cu–Na aqueous solutions.

The seed germination test was conducted at 25°C for 72 h. At the end of the experimental period, the germinated seeds were counted, and their radicle lengths were measured. A seed with a radicle length of 2 mm or higher was considered as germinated. Results of the seed germination experiments were analyzed following the established methods described in Luo et al. (2018). First, the number of germinated seeds and the radicle length of each germinated seed in each of the four biological replicates were determined. Next, the Relative Seed Germination (RSG), Relative Radicle Growth (RRG), and Germination Index (GI) of each biological replicate were calculated using the following equations:

$$RSG = \frac{\text{Number of germinated seeds in tretament}}{\text{Number of germinated seeds in deionized water (control)}} \times 100\%$$

$$RRG = \frac{\text{Radicle length of germinated seeds in treatment}}{\text{Radicle length of germinated seeds in deionized water (control)}} \times 100\%$$

$$GI = RSG \times RRG \times 100\%$$

The results shown in the figures are mean of four biological replicates (N = 4) ± standard error (SE) of the mean, calculated using the equation $SE = SD/\sqrt{N}$ (SD = standard deviation). The GI values were used to determine phytotoxicity. GI values below 80% indicate phytotoxicity, and GI values above 100% indicate the growth-stimulating effects of treatments (Barral and Paradelo 2011). RSG and RRG were used to see if a change in GI, and thus the effect on germination stimulation or inhibition, was due to seed germination or radicle growth or both.

Results and Discussion

The phytotoxicity of chicken manure and Milorganite to collard greens was identified using the seed germination bioassay (Kebrom et al. 2019). To verify the observed phytotoxicity, we investigated the germination of mustard seeds in chicken manure and Milorganite aqueous extracts. As shown in Fig. 1, the RSG, RRG, and GI of mustard seeds incubated with 100% chicken manure or Milorganite aqueous extracts was 0%. The GI of mustard seeds incubated in very low concentration (12.5%) of chicken manure or Milorganite aqueous extracts was 25.7% and 45.3%, respectively, due to inhibition of radicle growth. These results confirm the phytotoxicity of chicken manure and Milorganite even at lower concentrations.

Phytotoxicity of chicken manure and Milorganite aqueous extracts was associated with higher levels of heavy metals such as Zn, Ni, and Cu (Kebrom et al. 2019). The concentration of these heavy metals was low in the non-phytotoxic aqueous extracts of dairy manure. Zn, Ni, and Cu are essential nutrients required in a small quantity for normal plant growth and development; however, excessive amount of these nutrients could induce phytotoxicity and interfere with plant growth (Borkert et al. 1998; Hall 2002; Hassan et al. 2019; Parlak 2016; Pokorska-Niewiada et al. 2018; Seregin and Kozhevnikova 2006). Therefore, we investigated the germination of mustard seeds incubated with aqueous solutions of Zn, Cu, or Ni. Since the level of Zn, Ni, and Cu in chicken manure was higher than in Milorganite, a range of dilutions spanning the concentration of Zn, Ni, or Cu in chicken manure aqueous extracts were used for the seed germination experiments.

The average level of Zn in chicken manure and Milorganite aqueous extracts is 8.1 ppm and 0.4 ppm, respectively (Kebrom et al. 2019). The mean GI of mustard seeds incubated with 1 ppm or 5 ppm Zn containing aqueous $ZnCl_2$ solution was higher than the GI in deionized water (0 ppm Zn) (Fig. 2). The results indicate Zn at a concentration lower than 10 ppm stimulates radicle growth. The 10 ppm Zn solution did not inhibit seed germination. Phytotoxicity of Zn to the germination of mustard seeds was observed at 50 ppm

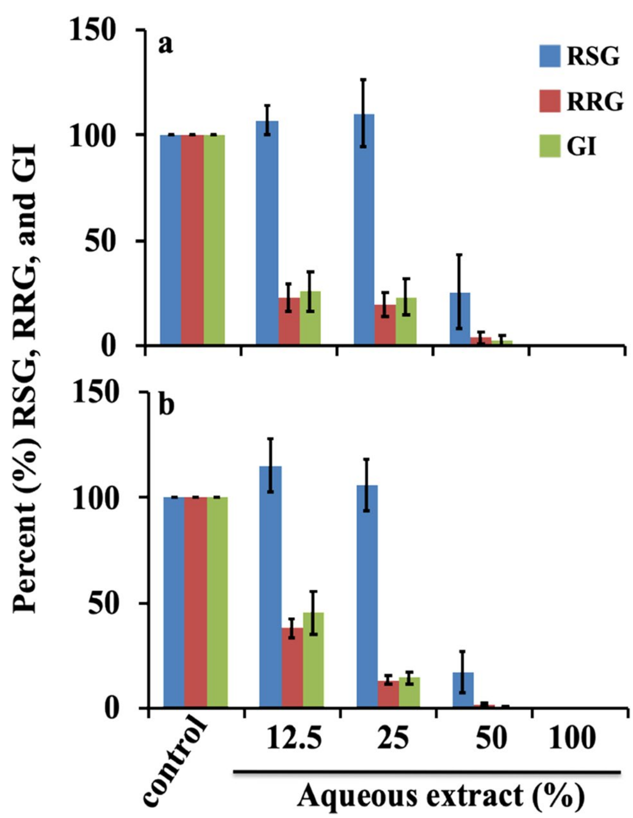

Fig. 1 The germination of mustard seeds in chicken manure (**a**) and Milorganite (**b**) aqueous extracts. *Control* deionized water, *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

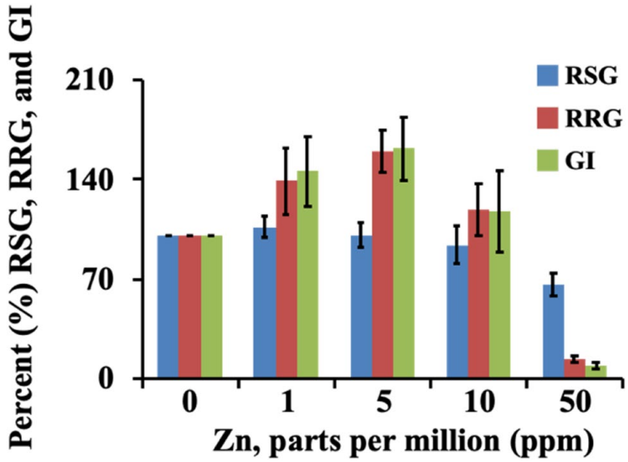

Fig. 2 The germination of mustard seeds in $ZnCl_2$ aqueous solutions. *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

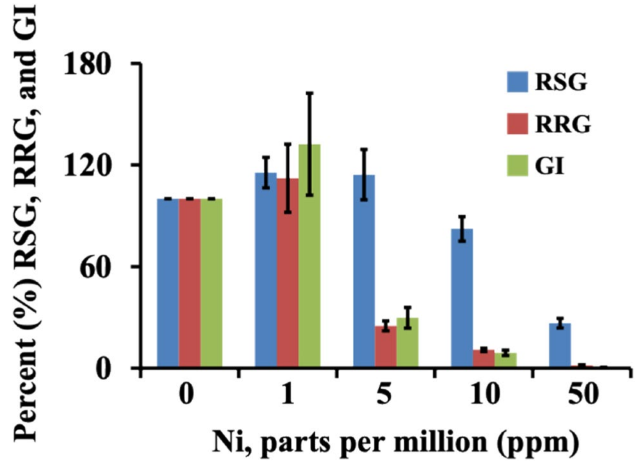

Fig. 3 The germination of mustard seeds in $NiCl_2$ aqueous solutions. *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

Zn, due to inhibition of both seed germination and radicle growth. Also, 50 mg L^{-1} (50 ppm) Zn was found to be phytotoxic to the germination of meadow cress (*Cardamine pratensis*, L.) and garden cress (*Lepidum sativum*, L.) seeds (Paradelo et al. 2010; Pokorska-Niewiada et al. 2018). Therefore, the level of Zn in chicken manure (8.1 ppm) or Milorganite (0.4 ppm) may not be phytotoxic to mustard seeds.

The average level of Ni in chicken manure and Milorganite aqueous extracts is 0.7 ppm and 0.53 ppm, respectively (Kebrom et al. 2019). Incubation of mustard seeds with 1 ppm Ni containing aqueous $NiCl_2$ solution did not inhibit the germination and radicle growth of mustard seeds (Fig. 3); whereas 5 ppm Ni, much higher than the level in chicken manure and Milorganite, was highly toxic. The combined effects of Ni and NaCl is more toxic to the growth of mustard plants than either Ni or NaCl alone (Yusuf et al. 2012). The level of Na in chicken manure and Milorganite aqueous extract is 442.2 ppm and 63 ppm, respectively (Kebrom et al. 2019). Interestingly, 1000 ppm Na containing aqueous NaCl solution did not inhibit the germination of collard greens seeds (Kebrom et al. 2019). Therefore, we investigated the synergistic effects of Ni and Na to the germination of mustard seeds. First, we prepared an aqueous solution containing 1 ppm Ni in $NiCl_2$ and 500 ppm Na in NaCl (Ni–Na). The germination test was then conducted in a range of aqueous dilutions (12.5%, 25%, 50%, and 100%) of the Ni–Na aqueous solution.

The average GI of mustard seeds in 100% Ni–Na solutions (1 ppm Ni and 500 ppm Na) was 45.6%, which indicates high phytotoxicity mainly due to a reduction in RRG (Fig. 4). The average GI in 12.5% Ni–Na (0.1 Ni and 62.5 Na) was also lower than 80%, indicating phytotoxic effects of a lower level of Ni–Na mixture to the germination of mustard seeds. Therefore, it appears that synergistic effects of Ni and Na may contribute to the phytotoxicity of chicken manure and Milorganite.

The average concentration of Cu in chicken manure and Milorganite aqueous extracts is 10.8 ppm and 0.77 ppm, respectively (Kebrom et al. 2019). The level of Cu phytotoxic to the germination of meadow cress and garden cress seeds was between 5 ppm and 30 ppm (Paradelo et al. 2010; Pokorska-Niewiada et al. 2018). In the current study, the mean GI of mustard seeds incubated with 1 ppm Cu was 72%, due to inhibition of radicle growth (RRG) (Fig. 5). Therefore, Cu could be phytotoxic at lower than 5 ppm. The mean GI of mustard seeds in 10 ppm Cu was 2.6%, due to a reduction in both RSG and RRG (Fig. 5). However, the GI of mustard seeds in 100% chicken manure aqueous extract, containing 10.8 ppm Cu, was 0% (Fig. 1). Therefore, the strong phytotoxicity of chicken manure aqueous extracts

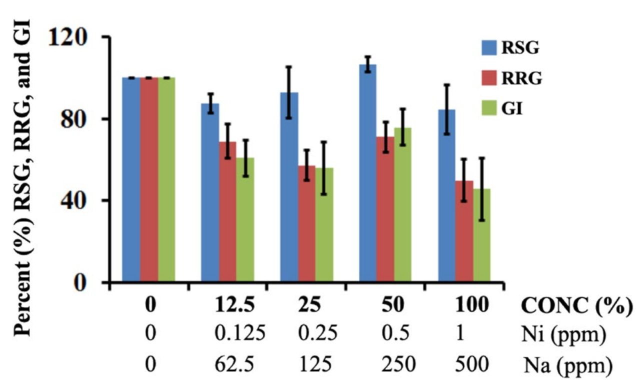

Fig. 4 The germination of mustard seeds in aqueous solutions containing $NiCl_2$ and NaCl. *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

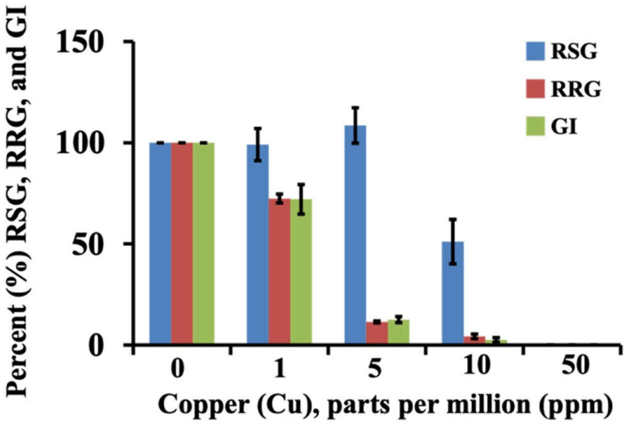

Fig. 5 The germination of mustard seeds in $CuCl_2$ aqueous solutions. *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

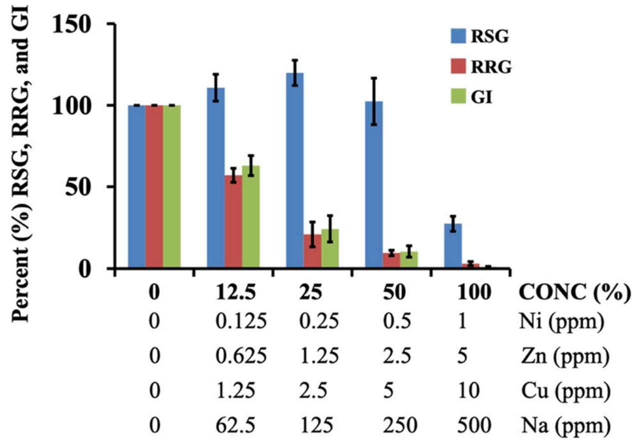

Fig. 6 The germination of mustard seeds in aqueous solutions containing $NiCl_2$, $ZnCl_2$, $CuCl_2$, and NaCl. *RSG* Relative Seed Germination, *RRG* Relative Radicle Growth, *GI* Germination Index. Data are mean ± SE. N = 4

may not be exclusively due to Cu. Also, the high phytotoxicity of Milorganite may not be due to Cu. Therefore, we investigated synergistic effects of Ni, Zn, Cu, and Na to the germination of mustard seeds.

The synergistic effect was investigated using an aqueous solution containing 1 ppm Ni, 5 ppm Zn, 10 ppm Cu, and 500 ppm Na (Ni–Zn–Cu–Na). The level of these elements in the aqueous solution is similar to the level in chicken manure aqueous extracts: 0.7 ppm Ni, 8.1 ppm Zn, 10.8 ppm Cu, and 442.2 ppm Na. The 5 ppm Zn, lower than the level in chicken manure aqueous extracts, was used because of its germination-stimulating effects (Fig. 2), thus to see if Zn antagonizes the radicle growth inhibitory effect of 10 ppm Cu in the aqueous solution.

The mean GI of mustard seeds incubated with 10 ppm Cu containing Ni–Zn–Cu–Na aqueous solution was 1% (Fig. 6). This is lower than the mean GI (2.6%) in 10 ppm Cu containing aqueous $CuCl_2$ solution (Fig. 5). In contrast to our predictions, 5 ppm Zn in the Ni–Zn–Cu–Na aqueous solution did not antagonize phytotoxicity. Interestingly, the mean RSG (27.4%) and the mean RRG (3.1%) in 10 ppm Cu containing Ni–Zn–Cu–Na aqueous solution were also lower than the mean RSG (51.1%) and the mean RRG (4.3%) in 10 ppm Cu containing aqueous $CuCl_2$ solution (Figs. 5 and 6). These results indicate, compared to Cu alone, the toxicity increased in Ni–Zn–Cu–Na containing aqueous solution, possibly due to Ni and Na. However, the 27.4% RSG in 100% Ni–Zn–Cu–Na versus 0% RSG in 100% chicken manure aqueous extract (Fig. 1) indicates as yet additional unidentified chemicals contributing to the strong phytotoxicity of chicken manure. Similarly, although a low level of Ni and Na might result in mild phytotoxicity (Fig. 4), the high phytotoxicity of 100% Milorganite aqueous extracts (Fig. 1) indicates other unidentified chemicals contributing to the strong phytotoxicity of Milorganite.

In summary, the current study identified that phytotoxicity of chicken manure could be in part due to the high level of Cu. Also, the synergistic effects of Ni and Na may contribute to the phytotoxicity of chicken manure and Milorganite. The high level of Cu in chicken manure could be due to its use as a supplement in poultry diet to improve feed efficiency and growth of broilers, and to protect chickens from communicable diseases (Gadde et al. 2017; Lu et al. 2017; Samanta et al. 2011; Yazdankhah et al. 2014). Limiting the use of Cu in poultry feed may significantly reduce the phytotoxicity of chicken manure. In addition, Cu could be reduced through appropriate composting procedures (Tiquia et al. 1996). However, the current study indicates there are as yet unidentified additional chemicals contributing to the high phytotoxicity of chicken manure and Milorganite. Therefore, more research is needed to gain an in-depth knowledge of phytotoxicity of organic soil amendments and develop strategies for their safe use on agricultural soils (Komilis 2015). Also, the current study demonstrates the need for further research on the synergistic effects of potentially phytotoxic chemicals on seed germination and plant growth.

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