COPPER (Cu) AND ZINC (Zn) UPTAKE BY CELERY PLANTS GROWN ON ACIDIC SOIL AMENDED WITH BIOSOLIDS

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ABSTRACT

For trace elements, such as copper (Cu) and zinc (Zn), the bioavailability of these elements, Cu and Zn, in biosolids is important because both are essential elements and both are potential contaminants when biosolids are land applied. A greenhouse study was conducted in factorial experiment based on a completely randomized design (CRD) with 4 replications on a soil treated with 4 rates of Cu (0, 50, 100, and 150 mg/kg) and 4 rates of Zn (0, 150, 300, and 450 mg/kg) on celery plants to investigate the distribution and mobility of these elements as well as growth and antioxidant changes of celery. The results of antioxidant changes were inconclusive due to irregular changes with Zn and Cu applications. However, generally the results show that Cu did not affect superoxide dismutase (SOD) or peroxidase (POD) activities in most of the treatments. On the other hand, Zn stimulated SOD and POD activities in most of the treatments. The photosynthesis

rate diminished with the applications of Cu and Zn at the rates above 100 and 300 mg/kg and increased in low Cu concentration (50 mg/kg) compared to S (soil without biosolid).

Key words: Superoxide dismutase (SOD), Peroxidase (POD), Photosynthesis rate

INTRODUCTION

The bioavailability of elements in biosolids is one of the key questions regarding risk assessment of contaminants. Copper (Cu) and zinc (Zn) bioavailability is important from two points of view: from a nutrient release perspective to improve crop growth, and also from a contamination perspective where repeated applications of biosolids may increase soil concentrations of these elements to levels that could be potentially toxic Heemsbergen *et al.*, 2010).

Scientists and the general public are concerned that heavy metal contamination will have a negative impact on the environment and human health. For these reasons, there is a global urgency to ensure that the heavy metal content of foodstuffs produced complies with the regulatory standards and compare well with those from other countries (Park *et al.*, 2011).

Due to ever-increasing production of livestock and poultry products for human consumption, a tremendous amount of biosolids are generated that are used as organic amendments as a source of nutrients and soil conditioner to improve the physical properties and fertility of the soils. As waste management applied to lands, soil is increasingly being seen as a major source of heavy metals in the human food chain, through plant.

For diffuse distribution of heavy metals remediation, amelioration of soils to minimize their 'bioavailability' is considered. Bioavailability can be minimized through chemical and

1 biological immobilization of heavy metals by inorganic and organic compounds, such as biosolids
2 (Park *et al.*, 2011). On the other hand, application of organic-rich waste material such as biosolids
3 to agricultural soils can have a beneficial effect on soil biological, chemical, and physical
4 properties, depending on the specific nature of the biosolids.

5 There are some reports which confirm the effect of biosolids as a nutrient amended to soil,
6 reducing bioavailability of some heavy metals (Basta & Chaney, 2005; Chen *et al.*, 1989; Haghghi
7 *et al.*, 2010; Park *et al.*, 2011), or increased it (Schwab *et al.*, 2007) or non effective on heavy
8 metal availability on plants (Zheljazkov and Warman, 2004). Wang *et al.* (2010) explained these
9 contradictory results in the context of the molecular weight of organic matter in soil solution, based
10 on the results of experiments with soil in which lettuce was exposed to Cu. The decomposition of
11 organic matter leads to the production of humic acid, ranging in molecular weight from hundreds
12 dalton (Da) to millions Da. Organic matter with molecular weight in excess of 1 kDa causes a
13 lower concentration of free heavy metal ions in solutions such as Cu²⁺ resulting in lower
14 availability and toxicity. However, according to Wang *et al.* (2010), organic matter with a
15 molecular weight less than 1 kDa had the opposite effect. Therefore, the molecular weight of
16 organic matter in soil solution plays a vital role in the bioavailability of heavy metals. Wang *et al.*
17 (2010) reported these results in lettuce culture exposed to Cu. McBride (2003) suggested that it is
18 plant physiology and the associated rhizosphere that affect heavy metal absorption and its toxic
19 effects on plants.

20 Heavy metals have been linked to decreased plant growth (McBride, 2003), impaired
21 photosynthesis rate, protein cleavage, DNA strand breakage by producing oxygen free radicals
22 (Upadhyay & Panda, 2009) . Fortunately, cells have defensive system, including antioxidant
23 enzymes such as superoxide dismutase (SOD) catalase (CAT), peroxidases to eliminate or reduce

the damaging effects of the deficiency or toxicity levels of these nutrient elements (Aravind & Prasad, 2003).

The present study was aimed on the biosolids application spiked with deficient and toxic levels of Zn and Cu on celery compared to control (optimum level) and to follow the SOD and POD activities and photosynthesis rate of celery as a fast growing leafy vegetable against the deficiency and toxicity levels of these elements in biosolids. Furthermore, the study was aimed on following the reactions of these metals and biosolids in soils and their influence on plant Zn and Cu uptakes.

MATERIALS AND METHODS

Anaerobically-digested biosolid samples were collected from the Palmerston North City Council Waste Water Treatment Plant sludge lagoon in New Zealand. Samples were treated, 15 days after collection, with 3 levels of Cu and Zn separately as metal sulphate salts ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, respectively) and anaerobically incubated for 117 days as described by Jeyakumar *et al.* (2008), briefly described here. The sewage sludge which was kept in air tight container for 17 days was amended with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and then was shaken for 5 minutes and rotated 10 times to reach the following concentrations, 586, 1355, and 2206 for Cu and 1179, 3837, and 5832 for Zn. The sealed container was kept air-tight and incubated for 117 days with shaking manually by hand twice a week. The concentrations of spiked Cu and Zn sulphate salts were calculated based on the soil nitrogen load rate (200 kg N/ha) such that the metal concentrations in the experimental soils were equivalent to 50, 150, and 250% of the soil metal concentration limit which is recommended by the New Zealand Water and Waste

Association (NZWWA, 2003). After 117 days of incubation, the Cu- and Zn-spiked biosolids were mixed with soil.

Anaerobically digested sewage sludge samples were collected in seven 20-L plastic cans to full capacity and kept air-tight in a shaded place. Fifteen days after collection, the sewage sludge samples (17 mg Cu L^{-1} and 73 mg Zn L^{-1}) were treated with three levels of Cu and Zn separately as metal sulfate salts ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). Each of the sealed cans was vigorously shaken five times by hand, each shaking lasted 1 min, followed by rolling of the cans on the ground to cause 10 rotations per can. The target metal concentrations (mg L^{-1}) in the metal-spiked sludges were 586, 1355 and 2206 for Cu and 1179, 3837 and 5832 for Zn. These metal concentrations were selected so that when the contents in the cans were applied to soils at the rate of 200 kg N ha^{-1} (3% N in biosolids solid phase; 6.7 t ha^{-1} of dry solids), the metal concentrations resulting in the experimental soils would be equivalent to 50, 150, and 250% of the soil metal concentration limit (100 and 300 mg kg^{-1} for Cu and Zn, respectively) recommended by the New Zealand Water and Wastes Association (NZWWA, 2003). One can without Cu or Zn addition was kept as a control. The sealed cans were kept air-tight and incubated for 117 days with frequent (twice a week) shaking by hand. Two subsamples (50 mL each) were collected from each container 0, 25, 55, and 117 days after metal addition for chemical and biological analysis. The first subsample at each time period was collected in a sterilised container, tightly sealed and stored at 4°C for dehydrogenase activity and biological oxygen demand (BOD) measurements. Gas pressure was released from each can at each of these sampling intervals.

A glasshouse trial was conducted at the Plant Growth Unit, Massey University, Palmerston North, New Zealand using celery (*Apium graveolens*) in pots containing soil (0.7 kg air-dried soil)

1 that was amended with biosolids previously spiked with three levels of Cu and Zn. The control
2 soil treatment was amended with biosolids not spiked with either Cu or Zn.

3 Soil ($\text{pH}=5\pm0.2$) was collected at a depth of 10–30 cm from each pot and was spread evenly
4 in a large tray and a calculated volume (there was a single concentration of Zn and Cu added to
5 biosolids, and the amount of biosolids added to the soil was varied to attain the desired Zn and Cu
6 concentrations) with a pre-determined concentration of spiked biosolids was poured into the soil
7 and mixed by hand to ensure uniform and thorough mixing of Cu- and Zn-spiked biosolids with
8 the acidic soil. There were two metal treatments with 4 levels of each metal: Cu (control Cu= 0,
9 Cu 1=50, Cu 2=100, Cu 3=150 mg/kg soil) and Zn (control Zn= 0, Zn 1=150, Zn 2=300, Zn 3=450
10 mg/kg soil). The unspiked biosolids (no metal added) were also mixed into the soil as a control
11 treatment, i.e. two controls were used, soil without biosolid = S and control = C = soil mixed with
12 biosolid. There were four replicated pots for each treatment with each pot containing 0.7 kg soil
13 in factorial experiment with two factors based on a completely randomized design (CRD). After
14 one week, each pot was planted with a single celery seedling obtained from a local plant nursery.
15 The pots were watered daily to approximately 80% of ‘pot field capacity’ and placed in a
16 glasshouse whose temperature was maintained at 15°C minimum (night) and 25°C maximum
17 (day).

18 The plants were harvested after 8 weeks which was considered their full maturity and
19 immediately after harvesting, soil pH was measured using a pH meter. The fresh weight of
20 harvested plant material was recorded before the plant samples were placed in a paper bag, and
21 dried at 70°C until a constant weight was recorded.

22 23 **Measurement of the Total Metal Concentrations**

A ground sub-sample of dried shoots and roots (0.1 g of each), 56 days after sowing, was digested in a digestion tube in concentrated nitric acid (10 mL) at 120°C for 3 h. The digest solution was subsequently made up to 100 mL using deionized water. The Cu concentration in solution was measured using graphite furnace atomic absorption spectrometry (GFAAS) and the Zn concentration was measured by flame atomic absorption spectrometry (FAAS). Total uptakes of Cu and Zn were calculated by multiplying the metal concentration by plant biomass (Haghighi *et al.*, 2010). The same procedure was used for determinations of the soil Cu and Zn concentrations.

The relative plant availability of biosolids-derived Cu and Zn was compared by calculating the transfer factor (TF), the ratio of the metal concentration in plant tissue to the total concentration of the metal in the soil (Zheljazkov and Warman, 2004).

The Cu and Zn concentrations of the soil were measured at the harvest time of celery (173 days after spiking biosolid with metals or D173).

Measurement of the Plant Physiological Parameters

The second fully expanded leaf was selected for enzymatic analysis according to Haghighi *et al.* (2010) procedure, briefly described here. Leaves were selected, then washed thoroughly with deionized water and homogenized with a mortar and pestle under chilled condition over ice in 50 mM phosphate buffer (pH=7). The homogenate was then centrifuged at 12,000 rpm for 10 min at 4°C and the supernatants were used for enzyme assays, namely SOD1 and SOD2 (i.e., 4 and 8 weeks after planting, respectively, or 145 and 173 days after spiking, respectively), and POD2 (8 weeks after planting).

SOD activity was assayed according to Beauchamp and Fridovich (1971) method with some modifications, briefly described here. Sub-samples of the supernatant (0.5 g) were

1 homogenized in 5 mL of extraction buffer consisting of 50 mM phosphate (pH 7.8), 0.1% (w/v)
2 bovine serum albumin (BSA), 0.1% (w/v) ascorbate, and 0.05% (w/v) β -mercaptoethanol. The
3 assay mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 μ M
4 nitrotetrazolium blue chloride (NBT), 0.025% (w/v) Triton X-100, and 0.0044 (w/v) riboflavin.
5 The photo-reduction of NBT (formation of purple formazan) was measured on a
6 spectrophotometer (DU-640; Beckman, Krefeld, Germany) at 560 nm. One unit of SOD activity
7 was defined as the amount of SOD present in the extracts causing 50% inhibition of the photo-
8 reduction of NBT.

9 POD activity was measured using Chandlee and Scandalios (1984) method with some
10 modifications, briefly described here. The reaction mixture consisted of 50 mM potassium
11 phosphate buffer (pH 6.1), 1% guaiacol (w/v), 0.4% H_2O_2 (v/v) and enzyme extract. The increase
12 in absorbance at 470 nm due to oxidation of guaiacol was measured. Enzyme activity was
13 calculated as $\mu\text{moles of guaiacol oxidized min}^{-1} (\text{g fresh weight})^{-1}$ at $25 \pm 2^\circ\text{C}$.

14 Photosynthetic rate was measured using a portable gas exchange reader (LiCor-6200,
15 Lincoln, NE) equipped with a 1/4 L fan-stirred leaf cuvette. Two fully expanded leaves of identical
16 developmental stage were used per measurement, vertically enclosed within the cuvette. Net CO_2
17 assimilation rate was automatically calculated on a leaf area basis with the LiCor-6200.

18 All data were subjected to one-way analysis of variance (ANOVA) and treatment means
19 were compared by the least significant difference (LSD) test, with $P < 0.05$ being considered
20 significantly different. Data and regression analyses were performed with Statistix version 8 and
21 regression lines were plotted in Microsoft Windows Excel. Data with a coefficient of variation $>$
22 35 was log transformed.

RESULTS

The Effect of Biosolids on Cu and Zn Absorption

Cu concentration of soil was measured at the harvest time. The results showed that in line with increasing Cu concentration spiked to biosolids, average available Cu increased in soil, but this increase was not significant among Cu1, Cu2, and Cu3. The Cu concentration of root and shoot did not change significantly in Cu1 and Cu2 compared with C (control, soil treated only with biosolids, not Cu or Zn), although their mean values increased. The highest Cu concentration in root and shoot and the highest Cu uptake of shoot were seen in Cu3. The lowest Cu concentration and uptake of shoot was in S (soil without biosolids) and the lowest Cu concentration for root was in the C treatment (Table 1).

With the Cu application treatments, the Zn absorption slightly decreased. The results revealed that in line with increasing Cu concentration, Zn absorption slightly decreased in both root and shoot compared to the C treatment (Table 1).

With spiking biosolids with Zn, Zn concentration in soil, root and shoot significantly increased. There were no significant difference in Zn concentration of shoot and root between Zn1, Zn2, and Zn3 treatments. The highest Zn concentration in root and shoot and Zn uptake of shoot was observed in Zn3. The concentration of Zinc uptaken by plants in Zn3 treatment increased by 29 µg/g dry weight per pot which is 59.7 and 9.8 times higher than the S and the C treatments, respectively (Table 2).

Cu concentration of root was not affected by different concentrations of Zn spiked to biosolids, but Cu concentration of shoot increased, although there were not significant changes seen among Zn1, Zn2, and Zn3 (Table 2).

The Effect of the Amended Biosolids with Cu on Growth, Photosynthesis Rate and Antioxidant Activity of Celery

The fresh and dry weights of shoots were not affected significantly with Cu application (Data not shown).

Photosynthesis activity was measured at two times, in the 4th and the 8th weeks of experiment. Photosynthesis rate decreased with increased Cu concentration of the media, and the lowest photosynthesis rate was observed in Cu3 treatment, although this decrease was not significant between the Cu1 and Cu2 compared to the C treatment. Photosynthesis rate in the 8th week was lower than that of the 4th week, and was not affected significantly with Cu application (Fig 1).

The authors have kept the order that first comparing treatments with C (the control, the main objective of the work), then, if there were any significant changes comparing with S. So Comparing S and C treatments, it was seen that biosolids improved photosynthesis in the 4th week and did not affect it in the 8th week (Fig 1).

SOD activity did not change with Cu application to the media compared with the C treatment in either of the two measuring times. Biosolids decreased SOD1 compared with the C treatment, however, SOD2 did not change significantly with biosolids application (Fig 2).

POD 1 activity decreased in the S treatment. POD2 decreased significantly in the Cu1 and the Cu2 treatments (Fig 3).

The Effect of the Amended Biosolids with Zn on Growth, Photosynthesis Rate, and Antioxidant Activity of Celery

1 Zn1 stimulated photosynthesis rate in the 4th and the 8th weeks of experiment by 92% and
2 40%, respectively, and then decreased in line with increased Zn concentration at both times.
3 Biosolids improved photosynthesis after 8 weeks by 75%, but did not significantly affect it after 4
4 weeks (Fig 4).

5 SOD1 and SOD2 activities increased with Zn application and improved with biosolids
6 application compared to the soil without biosolids (S). The lowest SOD1 and SOD2 activities were
7 in the S and the Zn3 treatments, respectively. The highest activities were found in the Zn2 and the
8 C treatments for SOD1 and SOD2, respectively (Fig 5).

9 In line with increasing Zn in the media, POD1 activity decreased and POD2 did not change
10 significantly. Biosolids improved POD1 activity and did not significantly affect POD2 activity
11 (Fig 6).

12 The fresh and dry weights of shoot were not affected significantly with Zn application
13 (Data not shown).

14 Soil pH was always between 4.95 and 5.26 over the entire range of the treatments, and
15 there were no significant changes found between the treatments (Data not shown).

16 17 **DISCUSSION**

18 **The Effect of Biosolids on Cu and Zn Absorption**

19 The highest Cu concentration in root and shoot and Cu uptake of shoot were seen in the
20 Cu3 treatment; this increase was almost 8 and 2.9 times in the Cu3 treatment compared to the soil
21 without biosolids (S) in plant root and shoot, respectively. This indicates that Cu was absorbed in

the celery root in higher rate than that in the shoot. The absorption of Cu in the root and the shoot compared in the S (soil without biosolids) and the C (soil with biosolids) treatments revealed that biosolids did not affect Cu absorption significantly. These results along with other research revealed that metals with organic matter have the ability to create organic functional groups to form multi-dentate complexes, thereby increasing the stability of the bond (Bradl, 2004). On the other hand, heavy metals form complexes with natural ligands such as humic and fulvic acids known as chelation, greatly improve the metal solubility and enhance their mobility. The general order of affinity of heavy metals on organic matter is as follows: $\text{Cu}^{2+} > \text{Hg}^{2+} > \text{Cd}^{2+} > \text{Fe}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Zn}^{2+} > \text{As}$ (Park *et al.* 2011). Relatively, Cu uptake increased in the C (soil with biosolids) compared with the S (soil without biosolids) treatment, but it did not change for Zn uptake in celery shoot.

The soil which was used in this research was an acidic soil; adsorption of heavy metals strongly depends on soil pH both inorganic and organic complexes. The effect of soil acidity on adsorption is manifested through two interacting factors: increasing negative surface potential on the plane of the adsorption and increasing the amount of the negatively charged metalloid species like NO_3^{-1} , SO_4^{2-} present in the soil solution. While the first factor results in lower metalloid adsorption, the second one is likely to increase adsorption. Generally, in the soil, metal uptake by plants increases with decreasing pH, while it is known to decrease with decreasing pH in nutrient culture (Park *et al.*, 2011). Therefore, in this study, the high concentrations of Cu and Zn in plants in all treatments refer to low soil pH compared to alkaline or calcareous soils.

Quantities of wet biosolids in New Zealand are approximately $1.5\text{--}2 \times 10^6 \text{ Mg year}^{-1}$ (varied moisture contents) (Park *et al.*, 2011). It is reported that biosolids used in New Zealand soils are effective in reducing phytoavailability of Cu and Cr by formation of organic metal

complexes and reduction to immobile form, respectively. However, to the best of our knowledge, there is not any report for Zn in this regard. We observed that shoot Zn concentration increased by 29 ($\mu\text{g/g}$ dry weight per pot) in Zn3 treatment which is 59.7 and 9.8 times higher than that of the S and the C treatments, respectively. This indicates that Zn uptake did not increase significantly in line with increasing Zn in the soil. Therefore, it is concluded that biosolids prevented Zn absorption in celery (Table 2) in toxic (high) level. There were not any significant changes found between the 3 treatments (Zn1, Zn2, and Zn3) on Zn uptake. Soil pH in all treatments was measured at harvest time. The soil pH values were between 4.95 and 5.26, and did not show any significant changes among treatments (Data not shown). It has been showed that each unit of increase in pH results in approximately a 2-fold decrease in heavy metal concentrations (Zn, Ni, and Cd) in the soil solution (Madejon *et al.*, 2006).

The apparent increase in the shoot and root concentrations of Cu in celery as a function of increasing the Cu concentration in the soil, and a greater concentration of Cu in roots than shoots for all treatments

Biosolids application to soil resulted in an increased in Zn and Cu concentrations in plants because of the high availability of these metals in biosolids (Illera *et al.*, 2000). With the application of biosolids spiked with Cu and Zn, the concentrations of both elements in the soil (Tables 1 and 2) increased by D173 (at the harvest time of celery: 173 days after spiking biosolids with metals), and consequently the levels of Cu and Zn in the celery shoots had also increased (Tables 1 and 2).

The present investigation indicated a slight competition between Zn and Cu absorptions by celery plants. Since Cu and Zn, both are taken up as divalent cations, their absorption can readily be inhibited. Although, Zn has already shown antagonistic effect to transition of other metals like

Cu, Fe, and Cd in the same way, (Aravind and Prasad, 2003; Luo and Rimmer, 1995) to the best of our knowledge, there are not any reports shown this effect between Cu and Zn, except biosolids application in the soil.

The Effect of Amended Biosolids with Zn and Cu on Growth, Photosynthesis Rate and Antioxidant Activity in Celery Plants

Zn and Cu are among the essential plant micronutrients. Zinc is an important component of many vital enzymes, a structural stabilizer for proteins and membranes, including DNA-binding proteins (Aravind & Prasad, 2003). Copper is involved in a wide range of biochemical and physiological processes. Moreover, Cu and Zn play an important role in electron-transfer reactions of photosynthesis in the form of plastocyanin (Upadhyay and Panda, 2009). However, excess concentrations of Zn and Cu can be harmful to plants at different physiological stages. In the present study, the photosynthesis rate diminished with the applications of Cu and Zn at the rates above 100 and 300 mg/kg soil, respectively. However, some of these changes were not statistically significant (Figs.1 and 4). On the other hand, following the biosolids effects on photosynthesis activities, we observed that the average photosynthesis activities increased from 12.9 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) to 14.3 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in Cu1 compared to S (soil without biosolids) treatment. It was clear that Cu in low concentration (50 mg/kg soil) improved photosynthesis activity and with increasing Cu concentration in the media photosynthesis activities decreased to 9.6 and 7.3 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) after 4 and 8 weeks, respectively, in Cu3 (Fig 1). Similar result has been reported for lettuce (*Pistia stratiotes* L.) exposed to Cu 0 to 100 $\mu\text{mol CuSO}_4$ (Upadhyay and Panda, 2009). Amendment of soil with biosolids at the low concentrations (Cu1 and Zn1) did not change the rate of photosynthesis of the leaves compared to the control (C). The result revealed that Cu1 increased

1 photosynthesis compared with S (soil without biosolids) treatment, but Zn1 did not affect it. It
2 appears that the original soil was deficient in Cu, but not in Zn.

3 The sufficiency range for Cu in plant leaf tissue is between 2 and 10 mg/kg (ppm) on the
4 dry matter basis, and for some plants the range between deficiency and toxicity is fairly narrow,
5 with toxicity occurring when leaf tissue Cu concentration is in excess of 15 mg/kg (ppm) (Jones,
6 2005). The sufficiency range of Zn in plant leaf tissue for most plants is 20 to 50 mg/kg (ppm) on
7 the dry matter basis. All the Cu and the Zn levels were below the phytotoxic range for plants (20-
8 40 mg kg⁻¹) according to Kabata-Pendias (2001) and Jones (2005).

9 On the other hand, it should be noted that estimates of metal bioavailability using metal
10 salts has been concluded overestimated bioavailability, especially when the experiment is carried
11 out under glasshouse conditions, conditions known to artificially increase the apparent
12 bioavailability of metals (Heemsbergen *et al.*, 2010).

13 The results of the present study showed that when biosolids are amended with Cu and Zn,
14 they are readily available that may not be in the safe range for celery in a long time. Generally,
15 organic matter increases the uptake of the micronutrients, including Fe, Zn, and Cu in plants such
16 as cucumber, melon, and bentgrass when these trace elements are at a deficient concentration due
17 to the ability of organic matter to form complex metal compounds and promote plant growth
18 (García-Mina *et al.*, 2004). Regarding the results of the present study, if Cu and Zn were applied
19 in 50% below NZ guideline for the metals in the soil amended with biosolids, these elements could
20 be in deficient range according to Jones (2005) for celery after plant analysis.

21 The tolerable upper intake levels of Zn and Cu for adults are 45 mg/day and 10,000 µg/day,
22 respectively. The maximum Zn and Cu concentrations of the shoots were 40.322±23.7 and 7.7±0.8
23 (µg/g dry weight), respectively (Sizer and Whitney, 2008).

1 According to Heemsbergen *et al.* (2010), although the use of organic amendments for the
2 immobilization of metals decreased their bioavailability, their total concentrations in the soils
3 remained unchanged. The immobilized metals may become plant available forms with time
4 through natural weathering process or breakdown of organic matter. Organic amendments that are
5 low in metals can be effectively used for celery production (Heemsbergen *et al.*, 2010).

6 Singh and Agrawal (2008) reviewed the beneficial effects of biosolids on photosynthesis
7 rate of some plants (sunflower, maize, and barely). The results of our study are in agreement with
8 those of the earlier reports where the comparison of soil without biosolids (soil-only control; S)
9 and with biosolids without amendment of Zn and Cu (control; C) showed that biosolids application
10 increased celery photosynthesis rate.

11 Other research reveals that Cu and Zn cause oxidative stress by mediating the activities of
12 antioxidative enzymes (Upadhyay and Panda, 2009; Erdei *et al.*, 2002). Copper has been shown
13 to induce the generation of reactive oxygen species (ROS) in lettuce (Upadhyay and Panda, 2009).
14 The results of the present study show some ambiguity. It seems that SOD and POD were more
15 reflected to Zn changes in celery than Cu. In line with increasing Zn concentration, POD and SOD
16 decreased compared to control plants. Zinc has a more deleterious effect than Cu on SOD and
17 POD activity. Erdei *et al.* (2002) reported the same result that Zn can stimulate SOD activity in
18 high concentration compared to other heavy metals.

20 CONCLUSIONS

21 With increasing Cu concentration in the soil, the shoot and the root concentrations of Cu
22 in celery plants increased and a greater concentration of Cu was observed in the roots than the
23 shoots.

1 With spiking biosolids with Zn, Zn concentration in soil, root and shoot significantly
2 increased and biosolids prevented Zn absorption in celery in toxic (high) level. The present
3 investigation indicated a slight competition between Zn and Cu absorptions by celery plants.

4 Regarding the results of the present study, if Cu and Zn were applied in 50% below NZ
5 guideline for the metals in the soil amended with biosolids, these elements could be in deficient
6 range for celery. Accordingly, 150 and 450 mg/kg soil of Cu and Zn spiked to biosolids and applied
7 to soil for celery cultivation cannot be hazardous for human. Even if we consider the per capita
8 consumption per day increased to almost 1000 g of celery.

9 Regarding photosynthetic and antioxidant activity affected with Cu and Zn we observed
10 that, photosynthesis rate diminished with the applications of Cu and Zn at the rates above 100 and
11 300 mg/kg soil, respectively. It seems that SOD and POD were more reflected to Zn changes in
12 celery than Cu. In line with increasing Zn concentration, POD and SOD decreased compared to
13 control plants. Zinc has a more deleterious effect than Cu on SOD and POD activity.

14 Biosolids application without spiking Cu and Zn, increased average photosynthesis
15 activities, Cu uptake increased in the C (soil with biosolids) compared with the S (soil without
16 biosolids) treatment, but it did not change for Zn uptake in celery shoot.

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