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Response of *L. Scoparium* and *K. Robusta* to biosolids and dairy shed effluent application in a low fertility soil

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Abstract

Biosolids and Dairy Shed Effluent (DSE) can contain high concentrations of plant nutrients, making them potential resources for enhancing forest tree species growth and soil fertility. This study aimed to investigate the effects of biosolids and DSE application on the growth and nutrient uptake of *Leptospermum scoparium* and *Kunzea robusta*, while also considering the potential accumulation of contaminants. The results demonstrated that amending low-fertility soil with 2600 kg N ha^{-1} of biosolids and 200 kg N ha^{-1} of DSE positively influenced the growth of both *L. scoparium* and *K. robusta*. This improvement was evident through increased biomass production and enhanced uptake of essential elements such as calcium (Ca), potassium (K), and sulfur (S). Notably, *L. scoparium* exhibited superior growth when combined with DSE, while both species showed similar positive responses when combined with biosolids. However, it should be noted that the application of biosolids resulted in elevated concentrations of certain trace elements in the plants, whereas DSE did not. These trace elements included cadmium (Cd), copper (Cu), manganese (Mn), and zinc (Zn). Despite the increase, the levels of these elements did not exceed unacceptable thresholds. Considering the potential influence of biosolids on plant rhizodeposition, it is recommended that future studies investigate the interactions between plant roots and microbes, particularly in relation to plant element uptake. This line of research would further enhance our understanding of the underlying mechanisms involved. In conclusion, the findings suggest that the application of biosolids and DSE can effectively improve forest tree growth and nutrient uptake. However, careful management is necessary to mitigate the potential accumulation of trace elements. These results provide valuable insights for optimizing the use of biosolids and DSE in forestry practices, with potential economic and environmental benefits.

Keywords: Native plants, biosolids, dairy shed effluent, macronutrients, essential trace element, nutrients uptake.

Introduction

Biosolids and Dairy Shed Effluent (DSE) can contain elevated concentrations of plant nutrients (Di et al., 1998; Zaman et al., 1999; Antolín et al., 2005; Hawke and Summers, 2006; Haynes et al., 2009; Bai et al., 2013a,b; Cogger et al., 2013 Hedley et al., 2013; Moir et al., 2013; Paramashivam et al., 2016). The low C: N ratio of biosolids and DSE makes them a net N source, where the N and other nutrients are released slowly from these biowastes as they decompose in the soil (Gilmour et al., 2003; Murphy et al., 2007; Powlson et al., 2012). Therefore, the land application of these biodegradable materials can provide short and long-term benefits to soils (Ginting et al., 2003) and crops, which can lead to a lower requirement for mineral fertilizers. Various studies have shown positive effects of DSE and biosolids application on forest tree species, which can subsequently provide economic returns through increased biomass and soil nutrients, while avoiding

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accumulation of biosolids derived contaminants above threshold values (Zaman et al., 2002; Kimberley et al., 2004; Singh and Agrawal, 2008; Wang and Jia, 2010). The application of biosolids provides nutrients, increases organic matter, improves soil structure, enhances nutrient absorption by plants (Freeman and Cawthon, 1999; Morera et al., 2002; Antolín et al., 2005; Weber et al., 2007; Singh and Agrawal, 2008), as well as increase the number and activities of soil microbes (Rogers and Smith, 2007; Singh and Agrawal, 2008; Cytryn et al., 2011). Biosolids have been used as fertilizers or composts in land applications to improve and maintain soil productivity, stimulate plant growth and establish sustainable vegetation at mine sites (Fresquez et al., 1990). They enhance the activities of soil enzymes as well as the number and biomass of soil organisms due to its high organic matter content and nutrient availability (Lteif et al., 2007; Singh and Agrawal, 2008). Frequent applications of biosolids has positive ecosystem effects with relatively low extractable metal levels in soil and support greater plant biomass and tissue quality (Sullivan et al., 2006). Moderate application rates of biosolids to low organic matter and clay content soils enhances soil organic carbon and increases nutrient retention (Antoniadis, 2008), enhances the adsorption capacity of soil to immobilize heavy metals such as Cu, and effectively reduced Pb availability in a high Pb urban soil (Brown et al., 2003). The application of DSE, resulted in a greater and more diverse microbial biomass in soil (Hawke and Summers, 2006). In addition, the enzyme activities of root exudates of Lolium L. and Trifolium repens L., grown on Templeton sandy loam, significantly increased N mineralization due to the application of DSE (Zaman et al., 1999). Another study found that the application of DSE improved long-term soil fertility by increasing the concentration of total N, total P and plant available nutrients (Hawke and Summers, 2006). However, the application of biosolids and DSE to forest soil can result in decreased forest productivity because there is a strong dependence on the composition of biowastes, soil type and plant species (Cline et al., 2012).

I hypothesised that biosolids, but not DSE, will lead to elevated concentrations of Cd, Cu and Zn in the plants, as these elements occur at elevated concentrations in biosolids (Simmler et al., 2013). Further, I also hypothesize that fresh biosolids and DSE will enhance the growth of *L. scoparium* and *K. robusta* in low fertility soil because DSE and biosolids which high concentrations of these macronutrients (Zaman et al., 1999; Kimberley et al., 2004; Antolín et al., 2005; Hawke and Summers, 2006; Singh and Agrawal, 2008; Wang et al., 2009). The aim of the research was to assess the growth and elemental composition of the foliar part of *L. scoparium* and *K. robusta* after the application of fresh biosolids and fresh DSE, with the goal of obtaining accurate measurements.

Material and Methods

Experimental setup

The experiment was conducted at Lincoln University greenhouse facility (43°38’42.3”S 172°27’41.0”E). Low fertility soil with yellow-grey earths, mostly classified as Lismore stony silt-loam derived from Greywacke gravels and thin loess deposits from a former pine plantation of Eyrewell (Figure 1A - 43° 25’19” S, 172° 15’52”E), New Zealand, was used as planting medium. Fresh Dairy Shed Effluent (DSE) was collected from Lincoln University Dairy Farm, New Zealand (Figure 1B - 43°38’40"S, 172°26’ 32” E; 17 m asl) in January 2015. Biosolids were obtained from the Kaikoura Wastewater Treatment Plant, New Zealand (Figure 1C - 42°21'37.40"S, 173°41'27.35"E) in July 2014. The initial treatment consisted of sedimentation and anaerobic digestion in settlement ponds for 6-8 months. The key properties of soil, DSE, and biosolids used in this experiment are presented in Table 1.

Table 1. Concentration of nutrients, trace elements and contaminants in soils, DSE, and biosolids used in the present study. Values in brackets represent standard error (n=15[1]; n=6[2]; n=5[3])

Properties	Soil[1]		DSE[2]		Biosolids[3]	
pH	4.5	(0.3)	7.5	(0.01)	4.5	(0.0)
C, %	4.3	(0.4)	0.11	(0.0)	27	0.7)
N, %	0.17	(0.02)	0.02	(0.0)	2.5	(0.6)
P, %	0.05	(0.00)	0.001	(0.0)	0.50	(0.0)
K, %	0.2	(0.01)	0.002	(0.0)	0.14	(0.01)
S, %	0.03	(0.00)	0.001	(0.0)	0.87	(0.01)
Ca, %	0.2	(0.01)	0.003	(0.0)	0.63	(0.01)
Mg, %	0.3	(0.00)	0.001	(0.0)	0.30	(0.00)
B, mg kg^{-1}	5.0	(0.3)	0.04	(0.0)	27	(0.1)
Cu, mg kg^{-1}	4.1	(0.2)	0.0	(0.0)	891.0	(18.9)
Zn, mg kg^{-1}	72	(1.5)	0.08	(0.0)	1073	(27)
Mn, mg kg^{-1}	265	(15)	0.04	(0.0)	185	(4.5)
Fe, mg kg^{-1}	21121	(291)	0.05	(0.0)	14534	(92)
Cd, mg kg^{-1}	0.2	(0.01)	0.04	(0.0)	4.0	(0.1)

Thirty-six 10 L pots (25 cm in diameter with a height of 29 cm) were used. The treatments contained total of 6 L Dairy Shed Effluent (DSE) which is 220 kg N ha^{-1} equiv. and 1 kg fresh biosolids per pot, which was 2600 kg N ha^{-1} equiv. The DSE and biosolids were first homogenised thoroughly using a 100 L plastic tank and black tarpaulin respectively**.** DSE then further stored in the fridge for further application in the greenhouse. The biosolids were mixing with soils at the beginning of the experiment. For each individual pot, 1 kg fresh biosolids was mixed completely with 9 kg fresh soil using a 20 L bucket. The soil was then filled into the pot in layers to give a soil bulk density of approximately 1.3 g cm^{-3}. *L. scoparium* and *K. robusta* seedlings were obtained from Waiora Nursery Ltd., Christchurch, New Zealand. All plants were transplanted directly after all pots were filled with medium (soil and plus biosolids). The pots were arranged in the glasshouse using a randomized block design.

To avoid preferential flow, DSE was applied gently on to the soil surface of the pots which contained 9 kg of fresh soil with soil bulk density of approximately 1.3 g cm^{-3}. DSE was applied weekly (500 mL $week^{-1}$). In the first two weeks (January 12th, 2015 and January 19th, 2015), the DSE was applied daily (from Monday to Friday) of 100 mL of each application, 3 hours after irrigating the pots. During the next three weeks (Jan 26th, 2015; Feb 2nd and 9th, 2015) the DSE was applied on Monday, Wednesday, and Friday at rates of 150 mL, and 200 mL respectively. From February 2nd, 2015 to March 3rd, 2015, it was applied twice per week (Monday and Friday) of 250 mL of each application. In the last two weeks before harvesting the experiment, 500 mL of fresh DSE was applied weekly only (Mondays). Each treatment had 4 replicates. The controls received neither biosolids nor Dairy Shed Effluent. During the experiment, the pots were irrigated with measured amount of water using an automated irrigation system. Each pot received 200 mL of water twice a day over the experimental period to ensure optimal plant growth at conditions near field capacity. The temperature in the greenhouse ranged from 9 to 20°C during the night (10 pm until 6 am) and from 14°C to 28°C during the day. After 12 weeks, the above ground biomass was carefully harvested and weighed. Plant samples was dried at 70^0C until constant weight was obtained and ground using a Retch ZM200 grinder.

Soil pH was determined using pH meter (MTSE). A 10 g portion of soil of soil was mixed with 25 mL deionised water and then shaken for two hours using an end-over-end shaker (at 20 rpm). The plant-available elements were determined using a 0.05 M $Ca(NO_3)_2$ extraction (Esperschuetz et al., 2017). Concentrations of Ca, K, S, Cd, Cu, Mn, and Zn were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES - USA). Reference soil and plant material from Wageningen University, the Netherlands (International Soil analytical Exchange 921 and International Plant analytical Exchange 100) was analysed with the samples. Recoverable concentrations were 81–112% of the certified values.

Data and statistical analysis

Significant differences (α=0.05) between treatments were determined by analysis of variance, followed by Duncan post-hoc tests at *P=0.05*. The analyses were done in IBM SPSS v.22.

Results

Aerial biomass production

Figure 1 shows the cumulative biomass (g per pot) of *L. scoparium*, and *K. robusta* in combination with DSE, biosolids, and control. With the exception combination of DSE and *L. scoparium*, compared to the control, the addition of 2600 kg N ha^{-1} equiv. of biosolids and 200 kg N ha^{-1} equiv. significantly (*p≤ 0.05*) increased the cumulative biomass production of *L. scoparium* and *K. robusta.* Twelve weeks after applying treatments, significant differences were detected in the growth response of *L. scoparium* and *K. robusta* as a result of different treatments, ranking in order of biosolids > DSE > control (Figures 1 and 2).

In combination with *K. robusta*, biosolids application resulted in the highest increment (100%) of biomass, from 105 g per pot, equivalent to 21 t ha^{-1} to 210 g per pot, equivalent to 43 t ha^{-1}. In combination with *L. scoparium* by comparison, biosolids application significantly increased its biomass by 44% higher than the control, from 144 g per pot to 207 g per pot, equivalent to 41 t ha^{-1}.

DSE increased the above ground biomass of *K. robusta* by 24%, up to 135g per pot, equivalent to 28 t ha^{-1}. Whereas in combination with *L scoparium*, amending soil with DSE resulted in a significant increase of the above ground dried biomass by 29%, up to 179 g per pot, equivalent to 36 t ha^{-1}. There was a significant difference in above ground biomass between *L. scoparium* and *K. robusta* in combination with DSE (Figure 3). In combination with DSE, *L. scoparium* produced 25% higher above ground dried biomass than that of in *K. robusta.*

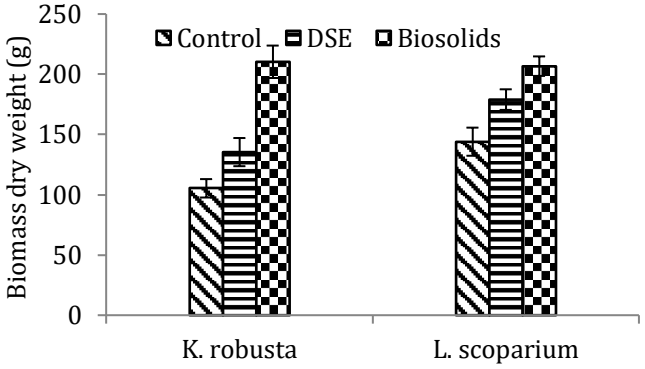

Figure 1. The cumulative biomass (g per pot) of L. scoparium, and K. robusta in combination with DSE, biosolids, and control

Figure 2. Plant growth responses under different treatments of 12 weeks experiment period under Eyrewell soil medium

Element uptake

Macronutrients

The foliar macronutrient concentrations and ratios of *L. scoparium* and *K. robusta* measured at the end of the experiment are presented in Figures 3 and 4. Compared to the control, in combination with *L. scoparium*, the application of both DSE and biosolids significantly (*p≤0.05*) increased the uptake of the concentration of foliar Ca by 21% and 29% higher than the control, respectively (Figure 5). Whereas in combination with *K. robusta,* DSE and biosolids addition resulted in 22% and 51% higher concentration of foliar Ca than control. There was no significant different of Ca uptake between DSE and biosolids treatment in combination with *L. scoparium* (Figure 3). Although in combination with *L. scoparium* and *K. robusta* there was no significant difference in N uptake between treatments, these New Zealand native species responded differently in accumulating foliar N (Figure 4). In combination with *L. scoparium*, biowastes application increased significantly increased the foliar N uptake compared to that of when combined with *K. robusta*. Amending DSE and biosolids increased the foliar N uptake of *L. scoparium* by 23% and 29%, respectively compared to *K. robusta*.

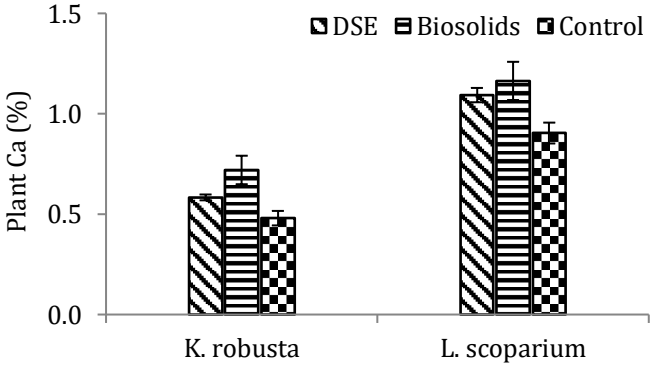

Figure 3. Total concentrations of foliar Ca (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly (p<0.05).

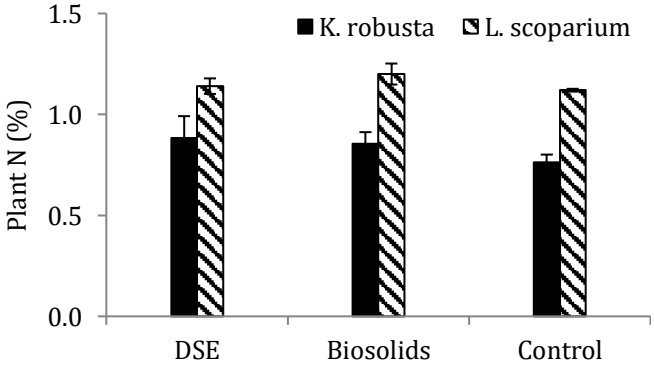

Figure 4. Total concentrations of foliar N (%) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Asterisks (*) signify significant differences between the effluents (striped bars) and controls (solid bars) at *p≤0.05*.

Micronutrients

Figure 6 shows total concentrations of foliar micronutrients (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. The application of biosolids and DSE to *K. robusta* increased the concentration of foliar Cu by 78% and 15%, whereas these treatments increased Cu in *L. scoparium* by Cu by 42 and 46%, respectively (Figure 6). Biosolids significantly increased the uptake of Zn by both *L. scoparium* and *K. robusta* by 569% and 298% respectively (Figure 6). In comparison, the DSE did not significantly change the Zn concentration in the leaves of *K. robusta* and only produced a 37% increase in *L. scoparium* (Figure 6).

K. robusta accumulated significantly (*p≤0.05*) higher Cd in the biosolids treatment**,** whereas the DSE treatment*, K. robusta* was not different to the control (Figure 7). *K. robusta* responded to the application of biowastes in related to Mn uptake. Biosolids application significantly increased (*p≤0.05*) the uptake of Mn (Figure 6B). The application of biosolids increased the concentration of foliar Mn in *K. robusta* by 71% compared to the control. In contrast, Figure 8B shows that in combination with *K. robusta*, there was no significant difference in total concentration of foliar Mn between DSE and the control. In addition, there were no significant differences of foliar Cd and Mn in both *L. scoparium* and *K. robusta* compared to the control (Figures 7 and 8).

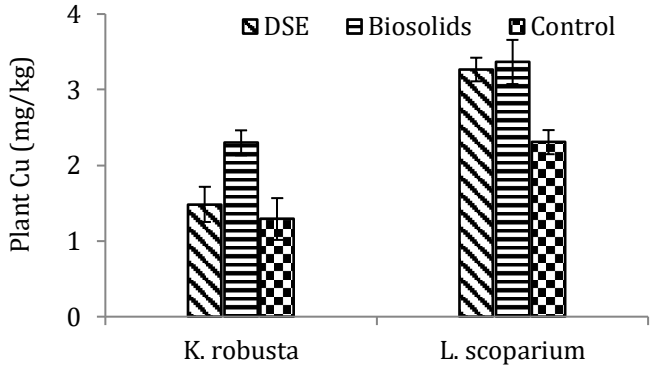

Figure 5. Total concentrations of foliar Cu (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly ($p<0.05$)

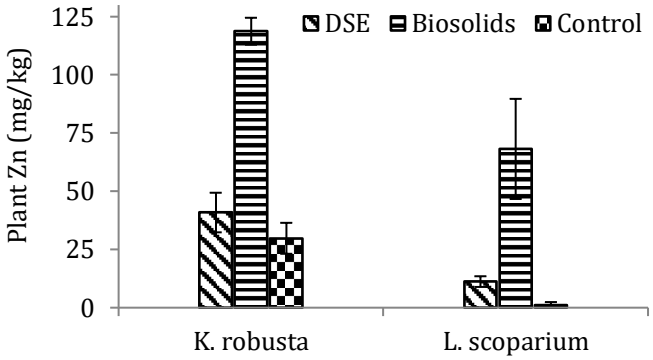

Figure 6. Total concentrations of foliar Zn (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly ($p<0.05$)

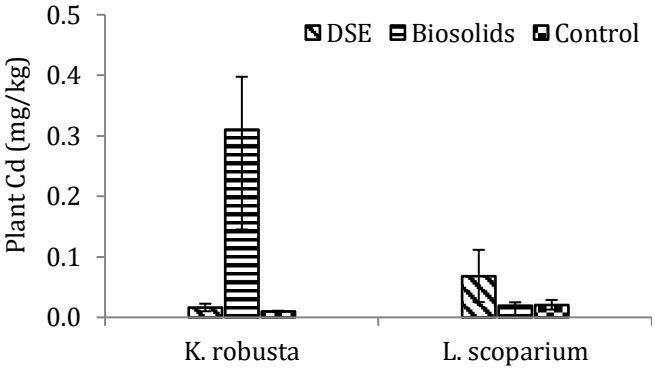

Figure 7. Total concentrations of foliar Cd (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly ($p<0.05$)

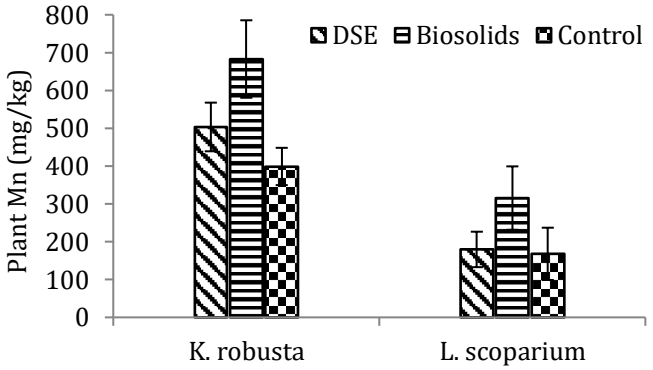

Figure 8. Total concentrations of foliar Mn (mg/kg) of *L. scoparium* and *K. robusta* measured at the end of experiment. Error bars represent the standard error of the mean. Treatment that share letters have means that do not differ significantly ($p<0.05$)

Discussion

Plant growth

The positive growth effects of biosolids and DSE may be due to their contribution of available nutrients, especially, N, P, K and S. As organic materials, amending these biowastes increased the concentration of organic C and, therefore, increased the Cation Exchange Capacity - CEC (Antolín et al., 2005; Weber et al., 2007; Brady and Weil, 2008), contributed in retaining nutrients and making them available to plants (Wong et al., 2001; Garcıa-Gil et al., 2004; Kaur et al., 2008; Delibacak et al., 2009). As a source of valuable nutrients, the application of DSE improved long-term soil fertility by increasing the plant available nutrients (Hawke and Summers, 2006). Esperschuetz et al. (2016) reported that adding 1250 kg N ha^{-1} equiv. of biosolids improved the growth of *Brassica napus* and *Sorghum bicolor* compared to the control. The effect of applying biosolids and DSE on plant growth could be related to role in stimulating root-microbe interactions processes (Khan, 2006), in which adding biowastes such as DSE to soil could provide a source of food for the microbes (Hawke and Summers, 2006). Mok et al. (2013) pointed out that other myrtaceae family members, *Eucalyptus polybractea* and *Eucalyptus cladocalyx* grown on biosolids produced high biomass. Moyersoen and Fitter (1999) and Weijtmans et al. (2007) reported that Ectomycorrhizal has been identified with *L. scoparium* and *K. robusta*.

Nutrients and trace elements in plant biomass

The application of biosolids and DSE to soil influenced nutrient cycling by increasing bioavailability and the uptake Ca, K, S, Cu, Zn, and Mn to plants. The biowastes may have increased nutrient cycling, making more nutrients available (Morera et al., 2002; Antolín et al., 2005; Murphy et al., 2007; Singh and Agrawal, 2008). Nutrients incorporated into organic matter can be consumed by bacteria, fungi, and other decomposers and transformed into plant-available forms. The present study found that the uptake of nutrients and contaminants associated with biowastes (NCAB) is species dependent. In combination with biosolids and DSE, both *L. scoparium* and *K. robusta* accumulated Ca, Cu, and Zn, whereas plant K, S, Mn, and Cd were only detected in biomass of *K. robusta.* These findings are in agreement with (Baldani and Döbereiner, 1980) and Mazzola

et al. (2002) who found that the role of plants in the availability and mobility of nutrients and contaminants associated with biowastes through root-microbes interaction is dependent on the species. Biosolids and DSE application could have stimulated root exudation (Koo et al., 2013), including organic acids, which have played an important role for solubilisation and mobilization of NCAB (Bertin et al., 2003), particularly elevating the availability of Zn (Keller and Römer, 2001; Hinsinger, 2001). Since exudate composition strongly varies with plant species (Walker et al., 2003), this can lead to different plant responses in terms of NCAB uptake.

Copper and Zn uptake by *L. scoparium* and *K. robusta* were higher than that of reported by Beshah et al. (2015) for other species. They found that the application of 65 t ha^{-1} dried biosolids significantly increased the accumulation of foliar Zn of oats (*Avena sativa*) by 280% (from 16 to 61 mg kg^{-1}) which are lower than our results of *L. scoparium* by 569% (increased from 1.2 to 68.2 mg kg^{-1}) and *K. robusta* by 298.3% (increased from 29.8 to 118.7 mg kg^{-1}). Mok et al. (2013) reported that two myrtaceae members, *Eucalyptus cladocalyx,* and *E. polybractea*, which were grown in a pot trial in heavy metal-contaminated biosolids reported that these species accumulated Cu (5.3 – 16.3 mg kg^{-1}) and Zn (215.4 – 2074 mg kg^{-1}), which were higher than this study. Another similar study showed that adding 65 t ha^{-1} dried biosolids significantly increased foliar Cu (Beshah et al., 2015). As reported by Beshah et al. (2015), both *Brassica napus* and *Avena sativa* increased herbage Cu by 100% (from 10 to 20 mg kg^{-1} and from 3.5 to 7.0 mg kg^{-1}), which was higher than the increases in this study. Prosser (2011) reported that the application of biosolids contained 0, 300, and 600 mg kg^{-1} Zn and 0, 100, and 200 mg kg^{-1} Cu within 6-month experimental period resulted in the accumulation of total foliar Cu and Zn in *L. scoparium* by 30-58 mg kg^{-1} and 79 – 140 mg kg^{-1} respectively, which were higher than our finding. In the present study, the DSE and biosolids contained somewhat lower concentrations of Cu and Zn the experimental period was shorted. Increasing the application rate and extending the experimental period could promote higher foliar Cu and Zn of this species. Although these elements were increased, the levels in all treatments were in the reported range of toxic thresholds (Broadley et al., 2007; Alloway, 2013). The lower concentration of foliar K found in *K. robusta* was probably influenced by either structural roles in cell walls and membranes or inter- and intracellular functions (Marschner, 2012). It is suspected that adding biosolids may have changed either chemical properties or growth environment of root. This condition is in agreement with (White and Broadley, 2003) who reported that the uptake of K mainly occurs via root tips.

Contaminants accumulation in the leaves

Concentrations of Cd in *K. robusta* were between 0.02 and 0.3 mg kg^{-1}, which has been reported as a normal range in plants (Alloway, 2013). The significant increase of Cd found in *K. robusta* biomass due to biosolids application compared to control, was not in the range that would pose a risk to human or animal health (Alloway, 2013; Esperschuetz et al., 2016). While the concentration of Cd in honey or essential oils were not measured, the low foliar concentrations indicates that transfer of excessive Cd into saleable plant products is unlikely. This indicates that biosolids can enhance uptake of essential trace elements in plant parts while not increasing toxic elements like Cd to levels dangerous for animal and human health. *L. scoparium* which did not accumulate increased contaminants from the biosolids treatment, may be safely amended with higher rates of biosolids.

Conclusion

The study demonstrated that amending low-fertility soil with 2600 kg N ha^{-1} of biosolids and 200 kg N ha^{-1} of DSE resulted in improved growth of both *L. scoparium* and *K. robusta*. This improvement was evident through increased biomass production and enhanced uptake of essential elements such as Ca, K, and S. Interestingly, *L. scoparium* exhibited better growth when combined with DSE, while both species showed similar positive responses in combination with biosolids. The application of biowastes also led to increased uptake of certain essential trace-elements and contaminants, but these levels did not exceed acceptable thresholds. Notably, the discrepancy in biomass increase between *L. scoparium* and *K. robusta* when combined with DSE compared to biosolids treatment suggests the stimulation of different types of mycorrhiza, associated with each respective species. This finding presents an intriguing area for future research. Additionally, since biosolids may have influenced plant rhizodeposition, it is recommended that future studies investigate plant root-microbe interactions concerning plant element uptake. Overall, the results indicate the potential of biosolid and DSE applications in enhancing plant growth and nutrient uptake. However, further exploration of the underlying mechanisms and long-term effects is warranted to fully understand the implications and optimize the use of these biowastes in agricultural practices.

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