



## Effect of metal content from sewage sludge on the growth of *Orthosiphon stamineus*

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### Abstract

Municipal sewage sludge can be used as fertilizer as it contains a lot of nutrients. By focusing on copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) contents in municipal sewage sludge from primary oxidation ponds and in plant tissue sections, this study attempts to explain the relationship between plant yield and metal concentrations. Raw sludge samples were collected, air-dried, and ground to powder form at the beginning of fieldwork. The metal concentrations of sewage sludge were copper 6.9 mg/kg; iron 330.2 mg/kg; manganese 6.7 mg/kg and zinc 9.1 mg/kg. *Orthosiphon stamineus* (cat's whiskers) was selected and the plant received a different quantity of sludge application ranging from 1 g to 4 g per plant weekly for six consecutive weeks while observations were done for eleven weeks. Physical parameters such as shrub width and height were monitored to determine plant growth. Through this study, the determined optimum sewage sludge dosage for cats' whiskers was between 2 to 3 g per plant.

### Keywords :

Sewage sludge, metal content, *Orthosiphon stamineus*, plant growth

## 1 Introduction

The United States oversaw 54% of sludge and biosolids in the land application and distribution while in Europe, 36.4% of the bio-solids were used in agriculture (Sohaili, Zaidi, and Loon 2012; Stoffella and Kahn 2001). There are many beneficial effects of the application using composted sewage sludge to several kinds of soils including agricultural soils (Fernandes, Bettiol, and Cerri 2005). Previous literatures have evidenced the use of various economically relevant species as experimental models such as barley (Sohaili, Zaidi, and Loon 2012; Epstein 2002), corn (Warman and Termeer 2005; Chen et al. 2008), sweet paper (Casado-Vela et al. 2007), and wheat (Bose and Bhattacharyya 2008; Chandra et al. 2009).

Many agricultural soils may have higher levels of metals than normally found in natural soils as the result of atmospheric deposition and application of fertilizers, pesticides, and sewage sludge itself (Thapa et al. 2021). According to Asati, Pichhode, and Nikhil (2016), adequate levels for most of the metals will not cause toxicity and hazards to plant. Molybdenum (Mo), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) are among the plant's essential micronutrients that are present in sewage sludge. Ojuederie and Babalola (2017) reported that these metal nutrient elements have high biological activity and participate well in metabolic reactions of the plant.

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Application of sewage sludge can provide a quality improvement on the soil properties and promotes rapid growth of plant (Thapa et al. 2021; Singh and Agrawal 2010). Nevertheless, Smith (2009) reported that increment in the metal concentrations and changes in the distribution of metals in soil amended with sewage sludge in the long term is reported to increase the concentrations of heavy metals in the tissue of the plants growing. Application of sewage sludge can provide a quality improvement on the soil properties and promotes rapid growth of plant (Singh and Agrawal 2010, Thapa et al. 2021). Nevertheless, Smith (2009) reported that increment in the metal concentrations and changes in the distribution of metals in soil amended with sewage sludge in the long term is reported to increase the concentrations of heavy metals in the tissue of the plants growing.

Cu functions as a co-factor in various enzymes and Cu-based protein pigments in plants. According to Pandey (2018), chlorophyll production and respiration are important plant functions that will acquire Cu uptake. The deficiency of it can cause growth reduction and abnormally dark coloration in rootlets. As for Fe, it is required by all plants since it is an essential component for the formation of chlorophyll, fatty acid metabolism, growth hormones, and photosynthetic electron transport. Besides that, Fe which is one of the prosthetic groups in enzymes is often the activator for most of the metabolic pathway. Lack of Fe will inhibit the growth and yield of a plant (Rout and Sahoo 2015). On the other hand, Mn serves as a catalyst component in enzymatic and physiological reactions in plants (Pandey 2018). It is responsible for the formation of key metalloproteinase to facilitate photosynthetic activities and secondary metabolism. It is also noted that the relationship between Mn and Fe is generally inversely proportionate as Mn in high concentration may reduce the availability of Fe to plants (Sharma

2006). As for Zn, it accumulates in different parts of the plant, from roots that have the most concentration to the fruits which had the least concentration. According to Akter et al. (2017), Zn is responsible for influencing the spike size, flower production, flower quality, and the growth of the plant. Prasad, Sajwan, and Naidu (2005) had reported that all these different metals will compete with one another during the plant uptake process.

Therefore, the primary concern of the study was to determine the content of micro nutrients namely Cu, Fe, Mn, and Zn which were used by the plants as catalytic agents in the metabolic processes concerned with growth and synthesis. A medicinal herb with different edible parts, *Orthosiphon stamineus* (cat's whiskers) were grown as part of the experiment in measuring the efficiency of sewage sludge. The objectives of this study were to determine the metal nutrient availability in sewage sludge and to see its impact in terms of growth as it is being applied on *Orthosiphon stamineus*.

## 2 Materials and method

The sludge samples were collected twice at random points with random depth along the perimeter of the primary oxidation pond at each sampling session were then being air-dried from 24 to 72 hours until a maximum allowable moisture content of 6.2% was reached. The sludge samples were then ground to fine powder to pass the sieve analysis size of 0.3 mm and stored in a big plastic container at a ventilated room.

### 2.1 Experiment loading

There was altogether a total of 10 rows with 8 samples of cats' whiskers in each row. All these row samples received 5 groups of different treatments as shown in Figure 1. Treatment applications of all groups were done around the stem base of the plants. The application of treatment started at week 1 and ended at week 6. There would be no further treatment application from week 7 to week 11. This was to allow the evaluation of the post-treatment effects of the plants.

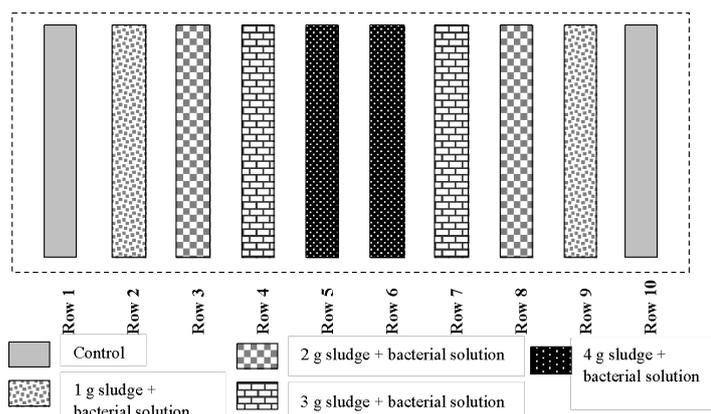


Figure 1 Arrangement of experiment loadings

### 2.2 Chemical measurement

All samples were required to be digested before being analyzed. Modified acid digestion from Environment Protection Agency (1996) Method 3050B was used throughout the experiment as this method is designed to dissolve almost all elements in sediments, soils, and sludge.

The digestion process requires 1 g of the sample that will be further analysed to be oven-dried for 24 hours. The sample was then transferred to a 250 mL Griffin beaker. 10 mL of nitric acid (HNO<sub>3</sub>) 50-70% were added into the beaker and the solution was

heated to 95 °C ± 5 °C for 10 to 15 minutes and then allowed to cool. Then, 5 mL of nitric acid 50-70% were added and the solution was heated again. Successive addition of nitric acid and heating was performed until no brown fumes were further generated. The solution was then allowed to evaporate to approximately 5 mL without boiling to prevent complete drying. After that, the solution was allowed to cool. After the sample has been cooled, 2 mL of distilled water followed by 3 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) 30% were added into the solution. The mixture was again heated at the same temperature as previous. Successive additions of 1 mL hydrogen peroxide until to the maximum limit of 10 mL and heating were performed until the violent bubbling effect was minimal or the general solution appearance remained unchanged. The solution was then allowed to evaporate and to cool as previously. Lastly, the solution was diluted to 100 mL with distilled water after cooling and filtered using Whatman No. 41 filter paper to remove any particulates in the solution. The filtered solution was then ready for further analysis. Sludge and plant samples that had been digested can be analysed for metal contents.

### 2.3 Physical growth measurement

Shrub height and width of *Orthosiphon stamineus* or cat's whiskers were measured from the start until the end of the experiment. The shrub height was measured from the soil surface to the nearest leaf node at the flower stalk of 3 random visually tallest branches and the average value was taken. The shrub width was measured randomly 3 times from the nearest leaf node at the flower stalk of one of the branches spanning across the other branch. A bull's eye bubble was used to ensure that the measuring tape was perpendicular against the line of gravity.

## 3 Results and discussion

Cat's whiskers (*Orthosiphon stamineus*) plant growths were measured physically and chemically. For the physical part, weekly and cumulative growths for both plant height and width were measured. As for chemical analyses, it was done on 3 plant components namely root, stem, and leaf to determine the metal contents. The estimated total metal loading in applied sewage sludge on each treatment group is summarised in Table 1.

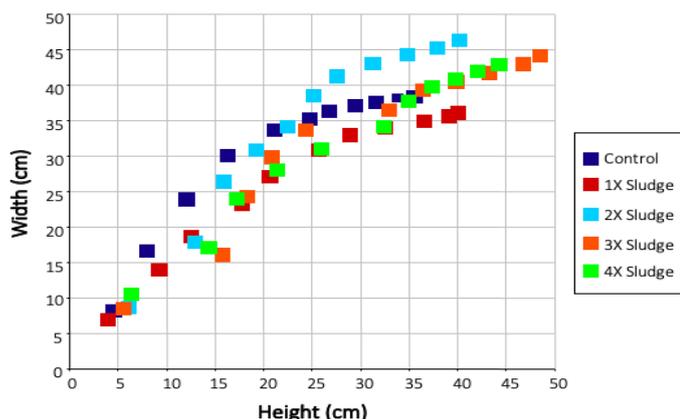
Table 1 The value of X for each of the concentration

Category	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Control	/	/	/	/
1X (1 g sludge + bacterial solution)	6.9	330.2	6.7	9.1
2X (2 g sludge + bacterial solution)	13.8	660.4	13.4	18.2
3X (3 g sludge + bacterial solution)	20.7	990.6	20.1	27.3
4X (4 g sludge + bacterial solution)	27.6	1320.8	26.8	36.4

### 3.1 Cat's whiskers height and width growth combined evaluation

All 10 rows of cat's whiskers shrub were categorized into 5 treatment groups namely as control and a combination of 1 g, 2 g, 3 g, and 4 g of sludge with 5 mL of bacterial solution each respectively. The 11-week shrub heights and widths of different treatment groups were then recorded, and the weekly changes were determined. To obtain a more accurate representation of the entire

shrub, a combination analysis of shrub height and width was required. Figure 2 showed the distribution of shrub height and width pairs categorized by treatment types.



**Figure 2** Distribution of shrub height and width pairs categorized by treatment type

Generally, the experiments have shown that sludge application increased plant growth in comparison with the control and the difference was often significant. It is noted that in Figure 2, plants receiving 2 g sewage sludge with a bacterial solution and 3 g sewage sludge with bacterial solution achieved maximum growth in terms of shrub height and width compared to other treatment groups. Contrary, further greater treatment of applied sludge at which 4 g sewage sludge with bacterial solution did not display the highest growth. This strongly indicated that the shrubs have reached the growth limit at the application level of 3 g sewage sludge as shrubs receiving that amount, displayed significant growth compared to others. This was also because the spaces between each plant have been completely used up, therefore, heading saturation level. As

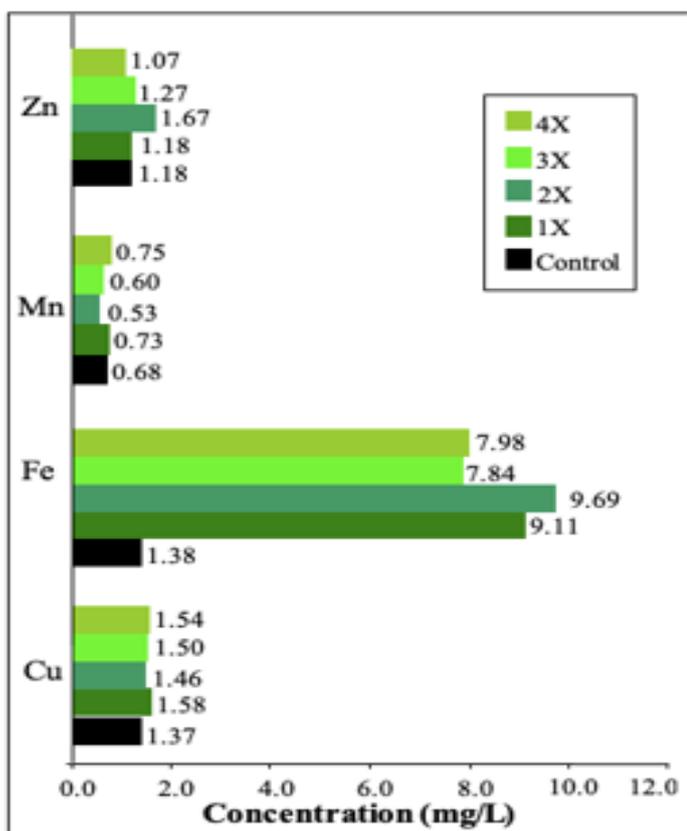
for the treatment of 1 g sewage sludge with bacterial solution, both shrub height and width indicated lower growth compared to other treatments. This was due to the lack of micro nutrients either by Fe, Zn, Cu, or Mn.

Physically, there were no distinctive growth patterns to be identified throughout the entire experiment. All shrubs were either having a significant height or significant width. Generally, all treatment groups except for shrubs receiving 2 g and 3 g sewage sludge with bacterial solution respectively showed lower growth, possibly showing signs of metal deficiency as well as reaching growth limitation.

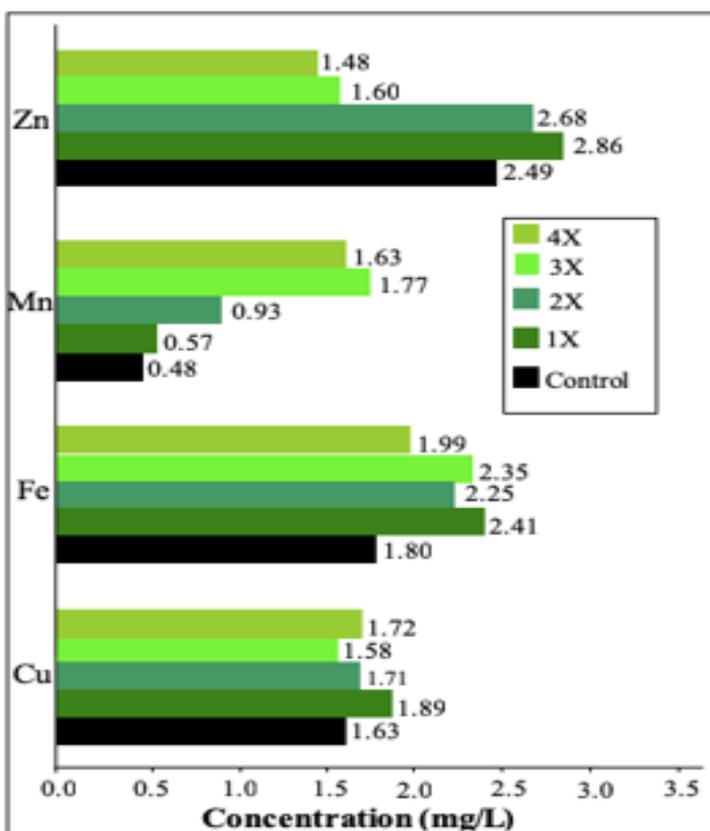
### 3.2 Metal composition in cat's whiskers

Metal analyses were done on 3 parts of the shrub namely root, stem, and leaf. Each shrub component was tested on the concentration of Cu, Fe, Mn, and Zn. The results are displayed according to each shrub segment in Figure 3a, 3b, 3c, while Figure 3d shows the summation of metal composition in cat's whiskers.

At the root level, the most significant changes in concentration within treatment groups were led by Fe followed by Cu, Zn, and Mn. The root is the main component of the plant that actively uptakes the micronutrient, particularly Fe (Pavlíková et al. 2002). This was through the mechanism at which the protons ( $H^+$ ) and reductants were being released by the root itself, hence increasing the Fe solubility (Pavlíková et al. 2002). Treatment type of 2 g sewage sludge with bacterial solution had uptake the most Fe which was 9.69 mg/L followed by 9.11 mg/L of 1 g sewage sludge with a bacterial solution. However, greater treatment of 3 g and 4 g sewage sludge with bacterial solution had shown opposite results where the concentrations of Fe were lower. This might be due to the reduction in root development that can deteriorate the uptake process (Pavlíková et al. 2002). On the other hand, the concentration changes of Cu and Zn among the treatment groups however do not indicate any distinctive pattern.



(a)



(b)

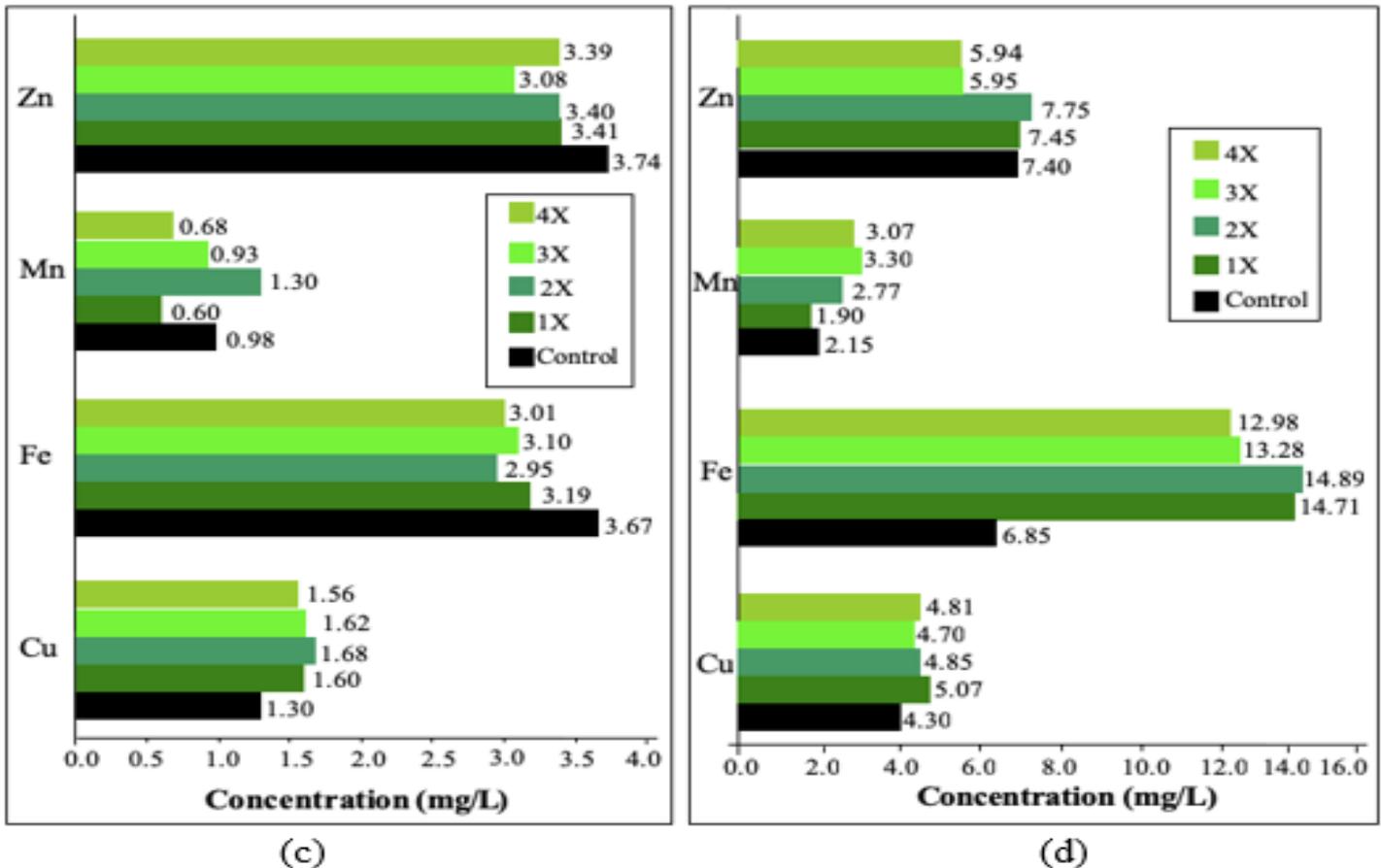


Figure 3 Metal concentration at (a) root level, (b) stem level, (c) leaf level, and (d) root-stem-leaf level

At stem and leaf levels, the most significant change of concentration within treatment groups was Mn. According to Yang, Deng, and Li (2008), Mn will normally accumulate more on the leaf, followed by stem and finally root. They then cited that capacity of the Mn to transfer from roots to the aerial parts is much higher. The percentage of Mn stored in leaf and stem had been evidenced to be higher than root (Yang, Deng, and Li 2008). Mn is involved in photosynthesis, respiration, and as an activator of some enzymes in the tricarboxylic acid cycle. The presence of Mn as a micronutrient is proved to be capable of altering the chlorophyll content of the plant (Yang, Deng, and Li 2008; Eisler 2000; Sharma 2006). At stem level, shrubs receiving 3 g sewage sludge with bacterial solution display a significantly higher Mn concentration of 1.77 mg/L compared to other treatment groups. This then indicated that the concentration had mainly contributed to the development of shrub height as being shown in Figure 2 previously.

Meanwhile, at the leaf level, shrubs receiving 2 g sewage sludge with bacterial solution show a higher Mn concentration of 1.30 mg/L. Thus, it was indicated that the amount of concentration had contributed to the development of shrub width as being shown in Figure 2. Instead of Mn, Zn concentration has also shown significant changes within treatment groups at the stem level. This observation is aligned with Fahad et al. (2014) who reported that both Zn and Mn are the main micro nutrients that are well contributed to plant growth. The highest Zn concentration of 2.86 mg/L was during the application of 1 g sewage sludge with a bacterial solution. As the amount of sludge applied to the cat's

whiskers increased, the concentration of Zn that was uptake decreased. On the other hand, there were no significant changes of all metal concentrations between various treatment groups at leaf level except Mn. Ideally, metal concentrations for various treatment groups should either be constant, gradually increasing, or decreasing within a logical range unless the shrubs enter the state of potential toxicity.

### 3.3 Metal interaction in cat's whiskers

The ratio of metal pairs that can potentially cause stress within the shrubs is summarized in Table 2. Based on the table, the increased ratio of Fe:Mn and Cu:Mn, as well as decreased ratio of Mn:Zn which resulted at the level of application of 1 g sewage sludge with bacterial solution are likely indicates that the shrubs were experiencing metal stress environment due to the potential Mn deficiency. This observation can be attributed by the high environmentally available Fe and Cu ions. Deficiency of the Mn will abort the development of cat's whiskers growth in terms of shrub height and width. This is consistent with the findings displayed in Figure 2 and aligned with reported findings by Shenker, Plessner, and Tel-Or (2004), Prasad, Sajwan, and Naidu (2005), and Nayek, Gupta, and Saha (2010). Metal ratios for plants receiving both 2 g and 3 g sewage sludge with bacterial solution respectively show inconsistent patterns, suggesting that metals were inter-replaceable in plant enzymatic pathways (Sharma 2006).

**Table 2** Total metal distribution and ratio in cat's whiskers

Component	Parameter	Control	1X	2X	3X	4X
Total Metal	Cu	4.30	5.07	4.85	4.70	4.81
	Fe	6.85	14.71	14.89	13.28	12.98
	Mn	2.15	1.90	2.77	3.30	3.07
	Zn	7.40	7.45	7.75	5.95	5.94
Total Metal Ratio	Cu / Fe	0.63	0.34	0.33	0.35	0.37
	Cu / Mn	2.00	2.67	1.75	1.42	1.57
	Cu / Zn	0.58	0.68	0.63	0.79	0.81
	Fe / Mn	3.18	7.74	5.38	4.03	4.2
	Fe / Zn	0.92	1.97	1.92	2.23	2.19
	Mn / Zn	0.29	0.25	0.36	0.55	0.52

## 4 Conclusion

Cat's whiskers were grown as part of the experiment to evaluate the performance of sewage sludge application at different loadings. Physically, shrubs receiving 3 g sewage sludge with bacterial solution displayed optimum growth but chemically shrubs receiving 2 g sewage sludge with bacterial solution displayed optimum metal contents as sewage sludge application of 2 g and above might cause potential metal stress to the shrubs. Therefore, the optimum loading of sewage sludge based on the observed and recorded findings throughout this experiment was set between 2 g and 3 g sewage sludge with the bacterial solution. Through this experiment, it can also be concluded that Mn deficiency caused by high Fe concentration can also occurred in plants thus, showing slower and stunted growth.

## Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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