Journal of King Saud University – Science 34 (2022) 101770

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Biochar amended compost maturity evaluation using commercial vegetable crops seedlings through phytotoxicity germination bioassay

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ARTICLE INFO

Article history:
Received 25 October 2021
Revised 8 December 2021
Accepted 12 December 2021
Available online 17 December 2021

Keywords:
Compost manure
Nutrient quality
Crop seeds
Phytotoxic effect
Germination index

ABSTRACT

Objectives: Phytotoxicity is a significant indicator of final compost product maturity and is assessed through germination bioassays using a variety of crop seeds. In this study, the final composted product extracts from a mixture of different raw materials, such as swine manure (SM), biochar (BC), and sawdust (SD) were examined for their phytotoxic effects on five different commercial vegetable crop seeds.
Methods: The final composted products were procured by applying four different treatments (i.e., SM + SD + 3%BC (T1), SM:SD + 5%BC (T2), SM:SD + 10%BC (T3), and SM:SD (C) used as a control (without nano-biochar). The effect of phytotoxicity on vegetable crop seed growth was measured using GI% (germination index), RSG% (relative seed germination), and RRE% (relative root elongation) in five commercial crops, including radish (*Raphanus sativus*), carrot (*Daucus carota*), cabbage (*Brassica oleracea*), tomato (*Lycopersicon esculentum*), and napa cabbage (*Brassica rapa*).
Results: In accordance with the phytotoxicity results, none of the treatment compost product extracts had any phytotoxic effect on the five commercial crop seeds, which agreed with our previous study on final product nutrient availability and manure maturity. Moreover, T3 (10% BC) showed the highest GI value compared to the other treatments, including the control.
Conclusions: Overall, the results confirmed that compost extract, especially 10% biochar amendment, was beneficial for commercial vegetable crops.

1. Introduction

Generally, most solid wastes are generated from human activities, which can be categorized based on hazard and composition in accordance with their source (Sharma et al., 2021; Al-Dhabi and Arasu, 2021). Usually, solid wastes are generated from commercial, domestic, industrial, and agricultural activities. Estimates suggest that more than two billion tons of solid wastes are generated on account of several anthropological functions in 2016; this excessive production has unfavorable effects on the environment (Ferronato and Torretta, 2019; Sorathiya et al., 2014). Since the 1980s, the number of livestock in Korea has steadily increased with the rise in meat consumption. According to recent statistics, meat consumption has risen from 11.3 kg per person in 1980 to 53.9 kg per person in 2018, with pork being the most preferred meat, followed by beef and chicken (Statistics Korea, 2020). High meat consumption has led to a rapid increase in livestock manure, such as swine and chicken manure. As inorganic fertilizers are more expensive and have a limited capacity in enhancing soil quality, farmers are turning to organic manure, such as chicken manure or swine manure. However, livestock manure contains notable heavy metals and phytotoxic compounds (e.g., ammonia, ethylene oxide, organic acids, phenols, salts, heavy metals, etc.) (Luo et al., 2018; Barral and Paradelo, 2011), which can enter agricultural soils through improper management of animal feed and storage systems. Cho et al. (2017) and Irshad et al. (2013) suggested that the presence of heavy metals in manure depends on the animal type, feed, and farm practices. In contrast, Yadav and Garg (2008) reported that an acceptable and limited range of heavy metal content may be essential for plant growth and crop productivity (Table 1).

Biochar is a solid, carbon-rich material produced from different waste biomass during pyrolysis ($\leq$700 °C) in an oxygen-limited

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101770
1018-3647/

Table 1
Characteristics of final compost (Ravindran et al., 2019).

Parameters (Units)	Final Compost			
	T1	T2	T3	Control
pH	8.27 ± 0.46	8.35 ± 0.48	8.4 ± 0.49	7.4 ± 0.44
EC (mS/cm)	0.86 ± 0.03	0.87 ± 0.03	0.85 ± 0.03	0.84 ± 0.03
C/N ratio	17.1 ± 0.87	16.5 ± 0.82	15.8 ± 0.79	18.9 ± 1.00
Heavy metals (mg/kg)				
Pb	32 ± 0.96	21.2 ± 0.73	15.7 ± 0.56	55.2 ± 1.79
Cd	1 ± 0.02	0.8 ± 0.01	0.69 ± 0.01	1.1 ± 0.03
Zn	423 ± 12.98	395 ± 12.91	361 ± 13.93	496 ± 17.16
Cu	179.9 ± 8.59	165 ± 6.07	153 ± 5.92	206.3 ± 8.49
Ni	11.61 ± 0.69	10.7 ± 0.67	9.47 ± 0.54	7.23 ± 0.42

Note: The data represent the mean ± standard deviation of three replicates.

environment. Recently, it has received significant attention as it known to increase plant productivity, reduce nutrient leaching, and decrease soil acidity. Several researchers have also suggested that biochar can increase soil microbial biomass, rhizobia nodulation, plant K tissue concentration, soil NPK content, and total soil carbon (C). Thus, biochar can influence plant productivity and seed germination (Novak et al., 2009). Our previous study focused on swine manure composting using different ratios of biochar, that is, 3%, 5%, and 10% for phytotoxic reduction (Ravindran et al., 2019). However, phytotoxicity germination assay is one of the most useful factors for evaluating agricultural compost quality and estimating the optimum application dosage (Roca-Pérez et al., 2013). Generally, the process of determining phytotoxicity is a sensitive and reliable plant assay, which includes planting seeds in compost and measuring phytotoxicity via germination (Boluda et al., 2011). In this study, the phytotoxicity of four different treatments of composted manure extracts was evaluated using five different commercial crop seeds, namely radish, tomato, carrot, cabbage, and napa cabbage as test crops.

2. Materials and methods

2.1. Collection of final composted material

The final composted sample was procured from four different composting treatments, which were performed in a temperature-controlled laboratory (20–22 °C) at Kyonggi University (37.3005 °N; 127.0358 °E) in Suwon, Gyeonggi Province, South Korea. Composting was performed for 50 days using food waste mixed with swine manure, chicken manure, and sawdust using different ratios of biochar, as described in our previous study (Ravindran et al., 2019).

2.2. Chemical analyses

A compost and deionized water ratio of 1:10 (w/v) was used for electrical conductivity (EC) and pH analysis (Ravindran, et al., 2017). Total carbon (TC) and total nitrogen (TN) were measured with a Trupec C/N auto analyzer using the dry combustion technique (LECO, 2003). Aqua regia (3:1v/v hydrochloric acid: nitric acid) was used in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina) to determine the total Ca, Mg, Na, and heavy metals, including Cu, Cr, Ni, Zn, and Pb.

2.3. Phytotoxicity study

A seed germination bioassay was used to determine the phytotoxicity of various composting treatments using five commercial vegetable crops. Seed germination bioassay was conducted using the Tiquia and Tam (1998) technique and final composting sample extracts were produced with distilled water at a ratio of 10:1 (Ravindran et al., 2016). Distilled water was chosen as the control. These samples were agitated for 1 h on a mechanical shaker before filtering them through Whatman® No. 1 filter paper for extract preparation. Then, a piece of Whatman® filter paper was placed in a disinfected Petri dish and soaked with 5 mL of composted sample extract. Five seeds of radish (*Raphanus sativus*), carrot (*Daucus carota*), cabbage (*Brassica oleracea*), tomato (*Lycopersicon esculentum*), and napa cabbage (*Brassica rapa*) were placed on top of the filter paper and incubated at 18 °C for 5–7 days with no light. All experiments were performed in triplicate. Germination index (GI %) was determined by counting the average root length as well as the average number of germinated seeds in every sample and comparing with the control treatment. Then, relative seed germination (RSG%) as well as relative root elongation (RRE%) were calculated, as follows (Ravindran et al., 2017):

$$RSG\,(\%) = (No.\ for\ seed\ germination\ in\ the\ compost\ extract) / (No.\ of\ seeds\ germinated\ in\ the\ control) \times 100$$

$$RRE(\%) = (Mean\ root\ elongation\ in\ the\ compost\ extract) / (Mean\ root\ elongation\ in\ the\ control) \times 100$$

$$GI(\%) = (\%RSG) \times (\%RRE)/100$$

The results were derived from a statistical analysis of three replicates (n = 3) and the computation of standard errors (SE).

3. Result and discussion

3.1. Phytotoxicity evaluation of final composted sample extracts on commercial crop seedlings

3.1.1. Effect of relative seed germination

Phytotoxicity is a well-known primary issue when applying untreated manure or immature compost to agricultural soil (Ravindran et al., 2017). In this study, toxin bioassays were performed on four different composting treatment samples to determine the level of phytotoxicity. In general, plant seeds adsorb extracts and use their nutrients for various metabolic processes, such as cell division, elongation, and differentiation (Cho et al., 2017). The results of RSG% were as follows: 90–100% for tomato; 88.8–111.1% for radish; 100–180% for carrot; 180–200% for napa cabbage, and 116.6–166.6% for cabbage (Table 2). In the case of tomato, two treatments had an RSG% value of 100; for radish, only T3 showed a value greater than 100%; three treatment values were higher than 100% in the case of carrot; for napa cabbage and cabbage, all the treatment values were above 100%. In addition, the RSG% values for T2 and T3 extracts were found to be 100–200% for all five crops. However, the RSG levels may have decreased because the extracts released hazardous chemicals and short-

Table 2
Relative seed germination (%) of different vegetable crops extract of four final compost treatments.

Extract collection treatment	Bioassay crop				
	Tomato	Radish	Carrot	Napa cabbage	Cabbage
T1	90 ± 2.7	100 ± 3	140 ± 5.6	200 ± 10	133.3 ± 5.3
T2	100 ± 5	100 ± 3	160 ± 4.8	180 ± 5.4	150 ± 6
T3	100 ± 4	111.1 ± 4.4	180 ± 9	200 ± 8	166.6 ± 5
Control	90 ± 2.7	88.8 ± 2.6	100 ± 5	180 ± 9	116.6 ± 5.8

Note: The data represent the mean ± standard deviation of three replicates.

Table 3
Relative root elongation (%) of different vegetable crop extracts of four final compost treatments.

Extract collection treatment	Bioassay crop				
	Tomato	Radish	Carrot	Napa cabbage	Cabbage
T1	136.8 ± 5.47	100 ± 3	74.8 ± 2.24	839.6 ± 25.1	296.7 ± 8.9
T2	151.0 ± 4.53	102.4 ± 5.12	95.1 ± 4.75	864.3 ± 34.5	350.4 ± 10.5
T3	158.4 ± 4.75	103.6 ± 4.14	97.84 ± 3.91	883.5 ± 44.1	354.5 ± 17.7
Control	132.2 ± 6.61	72.4 ± 2.89	57.9 ± 1.73	766.5 ± 38.3	187.8 ± 7.5

Note: The data represent the mean ± standard deviation of three replicates.

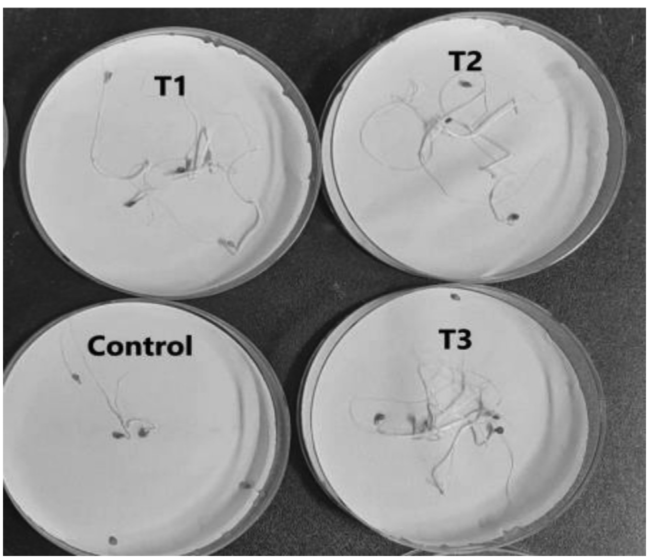

Fig. 1. Tomato seedlings growth germination bioassay using extract for compost maturity analysis.

chain fatty acids with low molecular weight, mainly acetic acid (Manjula and Meenambal, 2012).

3.1.2. Effect of relative root elongation

The range of RRE (%) results for five crop seeds from the four treatments were as follows: tomato: 132.2–158.4%; radish: 72.4–103.6%; carrot: 57.9–97.4%; napa cabbage: 766.5–883.5%; and cabbage: 187.8–354.5% (Table 3). Moreover, the RRE% values were found to be less than 100% in carrot seedlings, including T1, T2, T3, and control. The RRE (%) values for all crop seeds were found to be substantially different ($p \leq 0.05$). In all five crops, the highest RRE (%) value was reported in T3 extracts and the lowest was found in the control extract. A strong correlation was found between T3 extract's highest RRE and RSG percent values; perhaps the T3 extract contained more nutrients than the other extracts. However, RRE% could have been higher as a result of manure nitrogen content, indicating the importance of organic molecules for plant growth. Livestock manure is excellent source of organic carbon, which is crucial for enhancing soil, whereas nitrogen (N) is crucial

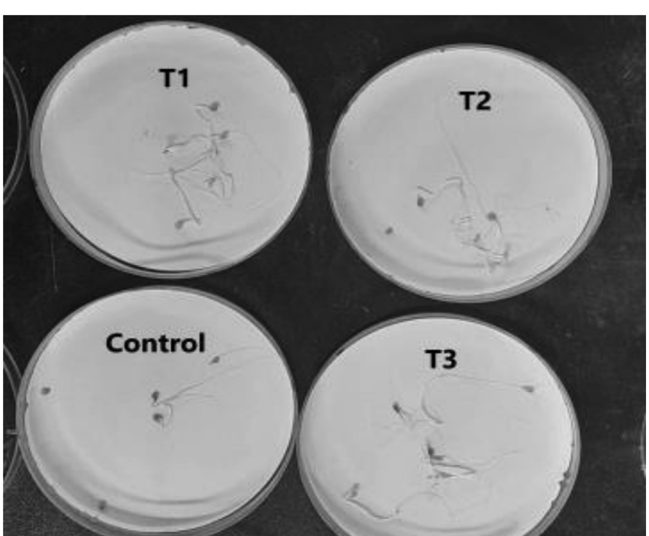

Fig. 2. Radish seedlings growth germination bioassay using extract for compost maturity analysis.

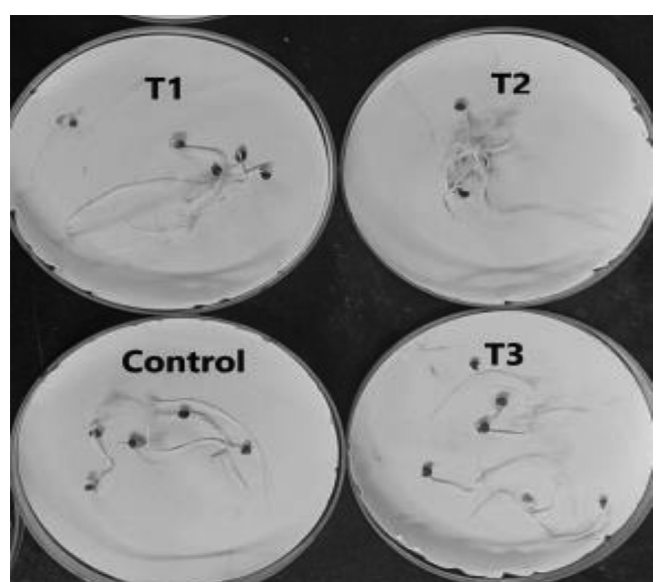

Fig. 3. Carrot seedlings growth germination bioassay using extract for compost maturity analysis.

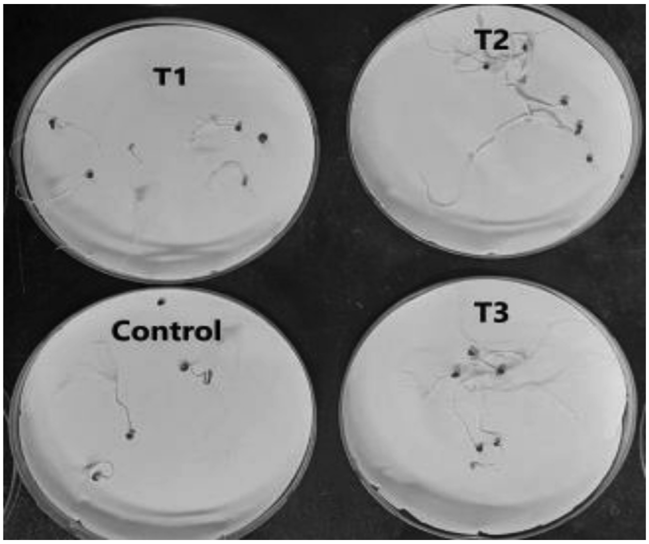

Fig. 4. Napa cabbage seedlings growth germination bioassay using extract for compost maturity analysis.

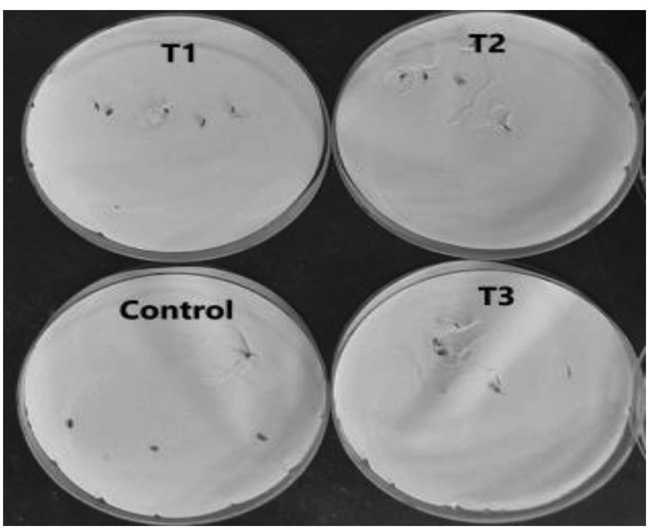

Fig. 5. Cabbage seedlings growth germination bioassay using extract for compost maturity analysis.

for plant growth. Generally, the standard range of C/N ratio for a good final composted product is 25–30 (Azim et al., 2017). On the other hand, a C/N ratio below 25 can be harmful to plants as it can lead to excessive ammonia toxicity, thereby inhibiting plant growth. Some researchers have also disclosed that in the case of manure only, a C/N ratio of less than 20 exhibits the manure existence and maturity of the manure, which is suitable for growing the plant (Ravindran et al., 2019). Cardoso et al. (2012) stated that N and P have eminent and beneficial effects on aerial and root growth. However, heavy metal contamination can change in their physiological and biochemical activities, thereby retarding their growth (Asati et al., 2016). Cho et al. (2017) also mentioned regarding the heavy metals limits (mg/kg) for manure in Korea, USA and European countries (Table 5).

3.1.3. Effect of germination index

The evaluation of germination index (GI) using seeds is a feasible indicator for denoting composted manure maturity by the process of phytotoxic assessment of seed and seedling growth (Ravindran et al., 2019). The pH and EC values also play an important role in the assessment. pH variation can affect the ability of plants to absorb nutrients and also inhibit root growth. Moreover, EC is a key determinant of soil salinity (the content of soluble salts) as well as the presence of potentially toxic salts, which may alter soil physical characteristics, and cause ion toxicity and osmotic stress. These could impact physiological processes as well as crop germination in plants (Dikinya and Mufwanzala, 2010). Generally, germination index (GI) is based on the phytotoxicity quantification using crop seedlings, RRE, and RSG in the assay phase. A GI level of less than 50% indicates mild phytotoxicity, 50%-80% indicates modest phytotoxicity, and >80% demonstrates no phytotoxicity (Ravindran et al., 2017). In the present analysis, all five crops (Figs. 1–5) showed GI of <50%, indicating no phytotoxicity. Moderate phytotoxicity (>50%, but <80%) was observed in one treatment (control) with radish and one treatment (control) with carrot only, while there was no moderate phytotoxicity seen in tomato, cabbage, and napa cabbage (Table 4). GI values greater than 80% were recorded in all four treatments for tomato, napa cabbage, and cabbage, indicating that there was limited or no phytotoxicity in the final composted extracts. Moreover, medium phytotoxicity was recorded in the control treatment for radish (64.4%) and carrot (57.9%). Thus, the highest GI value was recorded in T3 (10% BC), while the other treatments indicated sufficient maturity (Fig. 1).

Table 5
Heavy metals limits (mg/kg) for manure in Korea, USA and European countries (Cho et al., 2017).

Heavy metal	Korea limit range	European Union limit range	USA biosolids limit range
Copper (Cu)	360	70–600	1500
Cadmium (Cd)	5	0.7–10	39
Chromium (Cr)	200	70–200	1200
Nickel (Ni)	45	20–200	420
Mercury (Hg)	2	0.7–10	17
Lead (Pb)	130	70–1000	300
Zinc (Zn)	900	210–4000	2800
Arsenic (As)	45	25	41

Table 4
Germination Index (%) of different vegetable crop extracts of four final compost treatments.

Extract collection treatment	Bioassay crop				
	Tomato	Radish	Carrot	Napa cabbage	Cabbage
T1	132.2 ± 5.3	100 ± 5	119.7 ± 5.9	1533.0 ± 45.9	472.7 ± 14.1
T2	142.6 ± 4.3	103.6 ± 4.1	136.9 ± 4.1	1555.9 ± 46.6	494.5 ± 24.7
T3	151.1 ± 7.5	113.8 ± 3.4	171.3 ± 6.8	1767.0 ± 88.3	525.6 ± 26.2
Control	99.5 ± 2.9	64.4 ± 1.9	57.9 ± 2.3	579.2 ± 23.1	110 ± 3.3

Note: The data represent the mean ± standard deviation of three replicates.

4. Conclusions

The results revealed that the final composted samples using four different treatments were significant sources of nutrients. Biochar combined with swine manure had a positive impact through the in-vessel composting working process. Most of the treatments showed no phytotoxicity effects, except the control. Moreover, T3 showed the maximum GI values for all crops. Therefore, the application of biochar (10%) with swine manure and sawdust mixture treatment (T3) is important to increase the content of nutrients and reduce the level of phytotoxicity in plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by Kyonggi University's Graduate Research Assistantship 2020**.**

References

Al-Dhabi, N.A., Arasu, M.V., 2021. Effective degradation of Chlortetracycline using dual bio catalyst. Environmental Research. https://doi.org/10.1016/j.envres.2021.112339.

Asati, A., Pichhode, M., Nikhil, K., 2016. Effect of heavy metals on plants: an overview. Int. J. Appl. Innov. Eng. Manage. (IJAIEM) 5 (3).

Azim, K., Komenane, S., Soudi, B., 2017. Agro-environmental assessment of composting plants in Southwestern of Morocco (Souss-Massa Region). Int. J. Recycl. Org. Waste Agricult. 2017 (6), 107–115. https://doi.org/10.1007/s40093-017-0157-7.

Barral, M., Paradelo, R., 2011. A review on the use of phytotoxicity as a compost quality indicator. Dyn. Soil Dyn. Plant 5, 36–44.

Boluda, R., Roca-Pérez, L., Marimón, L., 2011. Soil plate bioassay: An effective method to determine ecotoxicological risks. Chemosphere 84 (1), 1–8. https://doi.org/10.1016/j.chemosphere.2011.02.013.

Cardoso, F.C.G., Marques, R., Botosso, P.C., Marques, M.C.M., 2012. Stem growth and phenology of two tropical trees in contrasting soil conditions. Plant and Soil, 269–281.

Cho, W.M., Ravindran, B., Kim, J.K., Jeong, K.-H., Lee, D.J., Choi, D.-Y., 2017. Nutrient status and phytotoxicity analysis of goat manure discharged from farms in South Korea. Environ. Technol., 1191–1199.

Dikinya, O., Mufwanzala, N., 2010. Chicken manure-enhanced soil fertility and productivity: Effects of application rates. J. Soil Sci. Environ. Manage. 1 (3), 46–54. https://doi.org/10.5897/JSSEM.9000019.

Ferronato, N., Torretta, V., 2019. Waste mismanagement in developing countries: a review of global issues. Int. J. Environ. Res. Public Health 16 (6), 1060. https://doi.org/10.3390/ijerph16061060.

Irshad, M., Malik, A.H., Shaukat, S., Mushtaq, S., Ashraf, M., 2013. Characteri[1]zation of heavy metals in livestock manures. Pol. J. Environ. Stud. 22 (4), 1257–1262.

LECO, 2003. Truspec CN Carbon/Nitrogen Determinator Instructions Manual. Joseph LECO Corporation, StUSA.

Luo, Y., Liang, J., Zeng, G., Chen, M., Mo, D., Li, G., Zhang, D., 2018. Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. Waste Manage. 71, 109–114.

Manjula, G., Meenambal, T., 2012. Evaluation of Phytotoxicity for Compost from Organic Fraction of Municipal Solid Waste and Paper & Pulp Mill Sludge. Environ. Res. Eng. Manage. 1 (59), 47–51. https://doi.org/10.5755/j01.erem.59.1.922.

Novak, J.M., Lima, I., Xing, B., Gaskin, J.W., 2009. Characterization and Their Effects on A Loamy Sand. Annal. Environ. Sci. 3, 195–206.

Ravindran, B., Mupambwa, H.A., Silwana, S., Mnkeni, P.N.S., 2017. Assessment of nutrient quality, heavy metals and phytotoxic properties of chicken manure on selected commercial vegetable crops. Heliyon 3 (12), e00493. https://doi.org/10.1016/j.heliyon.2017.e00493.

Ravindran, B., Nguyen, D.D., Chaudhary, D.K., Chang, S.W., Kim, J., Lee, S.R., Shin, JoungDu, Jeon, B.H., Chung, S.J., Lee, J.J., 2019. Influence of nanobiochar on physico-chemical and microbial community during swine manure composting process. J. Env. Manage. 232, 592–599. https://doi.org/10.1016/j.jenvman.2018.11.119.

Ravindran, B., Kumari, S.K.S., Stenstrom, T.A., Bux, F., 2016. Evaluation of phytotoxicity effect on selected crops using treated and untreated wastewater from different configurative domestic wastewater plants. Environ. Technol. 37 (14), 1782–1789.

Roca-Pérez, L., Marimón, L., Campillo, C., Ponce, L., Boluda, R., 2013. Assessing Compost Phytotoxicity Using Compost Eluates and a Compost Plate Bioassay. Acta Hortic. (1013), 95–100. https://doi.org/10.17660/ActaHortic.2013.1013.9.

Sharma, S., Sudhakara, P., Misra, S.K., Singh, J., 2021. Critical review on the solid-wastes issue: Generation, composition, disposal and their recycling potential for various applications. Journal of Physics: Conference Series, IOP Publishing 1804 (2021), 012147. https://doi.org/10.1088/1742-6596/1804/1/012147.

Sorathiya, L.M., Fulsoundar, A.B., Tyagi, K.K., Patel, M.D., Singh, R.R., 2014. Eco-friendly and modern methods of livestock waste recycling for enhancing farm profitability. Int. J. Recycl. Org. Waste Agricult. 3, 50. https://doi.org/10.1007/s40093-014-0050-6.

Statistics Korea, 2020. Meat consumption in south korea soars. http://www.koreaherald.com/view.php?ud=20201206000075..

Tiquia, S.M., Tam, N.F.Y., 1998. Elimination of phytotoxicity during Co-composting of spent Pig-manure sawdust litter and pig sludge. Bioresour. Technol. 65 (1998), 43–49.

Yadav, A., Garg, V.K., 2009. Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. J. Hazard. Mat. 168 (1), 262–268.