Assessment of Phytotoxicity of Potable Water Treatment Plant Sludge-Bound Compost Pellets on Seed Germination of Radish (*Raphanus sativus* L.)

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Abstract

Maintenance of compost pellet stability highly depends on the binding materials used. Potable water treatment plant sludge (WTPS) has the potential to be used as a binding material due to its high content of clay, organic matter, and nutrients. Compost and WTPS may contain heavy metals, toxic compounds, salts, and growth inhibitors and they may be phytotoxic to the plants. The present study aimed to assess the phytotoxicity of different pelleted compost using a seed germination bioassay of *Raphanus sativus* L. Pellet aqueous extracts (PAEs) were prepared from four types of compost pellets (T1: commercial compost pellet, T2: commercial integrated pellet, T3: WTPS-bound compost pellet, T4: WTPS-bound integrated pellet). The dilution sequence of PAEs at 50% and 100% concentrations were tested for seed germination in Petri dishes in a randomized design with three replicates. pH, EC, and selected heavy metal concentrations (Al^{3+}, Zn^{2+}, Cu^{2+}, and Cr^{6+}) were determined in PAEs. Relative Seed Germination % (RSG%), Relative Radicle Growth % (RRG%), and Germination Index (GI) were calculated for all the treatments after 72 hours. RSG%, RRG%, and GI showed negative correlation with EC. PAEs of T2 showed high phytotoxicity, while T4 showed less phytotoxicity at the 100% concentration level, whereas other treatments did not show any signs of phytotoxicity. All the PAEs at 50% concentration level were free from phytotoxicity. Hence, 10% WTPS on a w/w basis can be used as a binding material in pelletizing loose compost.

Keywords: Alum sludge, bioassay, compost, phytotoxicity, radish

1. Introduction

Pelleted compost is more advantageous than loose compost in terms of ease of handling and field application. This is a densified form of loose compost with or without other organic or inorganic amendments (Souri et al., 2019). Pelleted compost has several advantages, such as reduction of storage space, suitability for use with machinery, ideal for residential areas due to no dust production, greater efficiency with spreaders, convenience for long-distance transportation, suitability for long-term preservation, and the ability to add chemical components to improve pellet quality (Zafari and Kianmehr, 2014). Loose compost is compacted into cylinder-shaped, high-density pellets during pelletizing (Hettiarachchi et al., 2019). Compost pellet stability is crucial for maintaining pellet strength and durability.

For that, the incorporation of a binding material during the pelletization process is essential to maintain the stability of the finished pellet products (Nikiema et al., 2014). The compressive strength is

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increased when a proper binding material is mixed in appropriate proportions (Hettiarachchi et al., 2019). Water, clay, bee wax, bentonite clay, lime, rock phosphate, molasses, and rice flour are some of the binding materials that can be utilized in pelletizing compost (Hettiarachchi et al., 2019). As a waste product, potable water treatment plant sludge (WTPS) has the potential to be used as a binding material because it contains clay and has a hydrophilic effect. However, it has not been utilized so far as a binding material in compost pelletizing.

WTPS is a waste by-product of the potable water treatment process. The potable water is supplied by the National Water Supply and Drainage Board (NWSDB) to various regions in Sri Lanka. It currently purifies 590 million cubic meters of potable water per year. According to estimates, the volume of sludge produced during the potable water treatment process ranges from one to three percent of the raw water used throughout the treatment process (Dassanayake et al., 2015).

WTPS, commonly known as alum sludge, has caused serious environmental issues in Sri Lanka due to the lack of a proper disposal mechanism. These include environmental contamination, the risk to human health, reduction of arable land, surface and groundwater pollution, and threat to aquatic life when it discharges directly into the rivers, bare lands, or other water bodies. To manage this waste efficiently, it is important to explore the possibility of reusing WTPS. Many studies have been carried out to experiment the reuse of WTPS as a construction material such as bricks and cellular lightweight blocks (Dolage et al., 2019). Some studies have investigated the use of bulk WTPS as a soil amendment for nutrient supply. It was noted that adding bulk WTPS to soils has improved their physical, chemical, and biological properties, enabling plants to grow well (Aggelides and Londra, 2000). The amount of organic carbon contained in WTPS-amended soil is about three times higher than that of soil supplemented with inorganic fertilizers (Nyamangara et al., 2001).

The potential of using WTPS as a binding material for compost pelletizing was taken into account based on these previous findings. However, previous studies recorded that poor germination and reduction of phosphate uptake in maize resulted from the excessive application of alum sludge (Rengasamy et al., 2015). According to their recommendations, this could be avoided by treating the alum sludge before seeding. Also, growth inhibitions of alum sludge have been observed in some studies (Bugbee and Frink, 1985).

Soil fertility and microbial population could be highly affected by the influence of higher concentrations of heavy metals (Mtshali et al., 2014). Compared to sewage sludge produced by wastewater treatment plants, alum sludge has very few environmental impacts as alum sludge is relatively free from heavy metals, other hazardous organic components, and the levels of harmful pathogens in alum sludge are often substantially lower (Dassanayake et al., 2015). However, before applying alum sludge as a fertilizer and/or soil conditioner, additional treatment is necessary to decrease heavy metals to safer levels (Lalith and Perera, 2007).

Unfortunately, alum sludge has not been widely adopted in agriculture. There is limited research on determining growth-limiting variables, such as high salt ion concentrations, Aluminium toxicity, and other heavy metal toxicity in alum sludge (Dassanayake et al., 2015). Aluminium phytotoxicity is influenced by environmental factors that determine the solubility of aluminium in soil. According to Dassanayake et al., (2015), extractable Al^{3+} in alum sludge may be harmful to plants at low soil pH levels (pH <5). The other two main metals found in sludge produced by wastewater treatment plants are Fe and Zn (Lalith and Perera, 2007). According to Skene (1986), any study has not mentioned the use of alum sludge as a growth medium which could cause aluminium toxicity as a significant problem.

In the case of loose compost, there are many hazardous metals, and it is essential to know the bioavailability of these metals before using compost for plants. Standards of the compost, including maturity and stability, should be assessed before application to the soil. Plant growth correlates with the maturity of the compost, which is a measure of agronomic performance. Immature or unstable compost may have negative impacts on soil, seed germination, and plant growth due to the presence of phytotoxic substances, low oxygen dispenses, or readily available ammonium nitrogen. Emerging pollutants, such as antibiotics, organic acids (e.g. phenolic acids), metals, ammonium nitrogen, agrochemicals, and high salt concentrations can be hazardous to plants if they persist for more than their appropriate or desirable range (Mazumder et al., 2020).

Earthworm growth, collembolan reproduction and survival, and seed germination bioassay are the possible techniques used as bioassays. Seed germination bioassay is a set of physiological processes that come together during seed germination, which starts with latent dry seeds absorbing water and ends with the emergence of radicles from the seed coverings (Mazumder et al., 2020). Considering the possible negative effects which can be encountered by both materials (compost and WTPS) for plant growth, the present study aimed to conduct a germination bioassay to find out the phytotoxicity of potable water treatment plant sludge-bound compost pellets on seed germination of radish (*Raphanus sativus* L.).

2. Methodology

2.1 Producing Bulk Compost

Loose compost used in this study was produced by using cow dung, poultry layer litter, green manure, and 5% Eppawala Rock Phosphate (ERP) as raw materials in the aerobic heap method.

2.2 Binding Material

Water treatment plant sludge was collected from the Hapugala water treatment plant in Galle district, Sri Lanka (6.0797° N, 80.1964° E), to be used as the binding material for two treatment pellets. The Hapugala water treatment plant produces 129.16 m^3 of WTPS every three months as a waste product during the process of water purification from the river 'Gin Ganga'. In this process, the main coagulant is Aluminum Sulphate (Alum). The collected WTPS or alum sludge sample was air-dried, ground, and sieved through a 2 mm mesh to be used as the binding material.

2.3 Compost Pelletizing Process

Cylindrical pellets were produced by using a roller ring die-type electrical pelleting machine. The machine operated at a speed of 160 rpm and it generated heat in the range of 50-60 °C (Figure 1).

Figure 1: Roller ring die-type pelleting machine

2.4 Pelleted Compost and Aqueous Water Extracts

The experiment was based on four types of pelleted compost (T1: commercial compost pellet (100% compost), T2: commercial integrated pellet (90% compost + 10% NPK inorganic fertilizer), T3: WTPS-bound compost pellet (90% compost + 10% WTPS), T4: WTPS-bound integrated pellet (80% compost + 10% WTPS + 10% NPK inorganic fertilizer). For all treatments, the same batch of compost was used, and the ingredients were mixed on a weight-to-weight basis.

Powdered compost pellets were mixed with distilled water in a 1:10 (w/v) ratio using a mechanical shaker for one hour, and the aqueous extract was obtained by filtering the suspension (100%

PAE). A series of 50% PAE was prepared by diluting 100% PAEs. pH and EC were tested in all PAEs, and selected heavy metals (Al^{3+}, Zn^{2+}, Cu^{2+}, and Cr^{6+}) in 100% PAEs were analysed using a multi-parameter analyzer (HANNA HI 83099).

2.5 Procedure for the Bioassay

Filter papers were placed at the bottom of Petri dishes, and 3 ml of each extraction was added to moisten the filter papers. Subsequently, five radish seeds were placed in each Petri dish and covered with a lid. Distilled water was used as the control, and the same number of radish seeds were placed in separate Petri dishes.

Treatments were arranged in a complete randomized design (CRD) with three replicates. All the Petri dishes were kept in dark conditions for three days to allow germination. The germination of seeds in the control group was used to determine the appropriate stage for data collection, which occurred when more seeds germinated in the control group, and their root length reached about 3-5 cm (Figure 2).

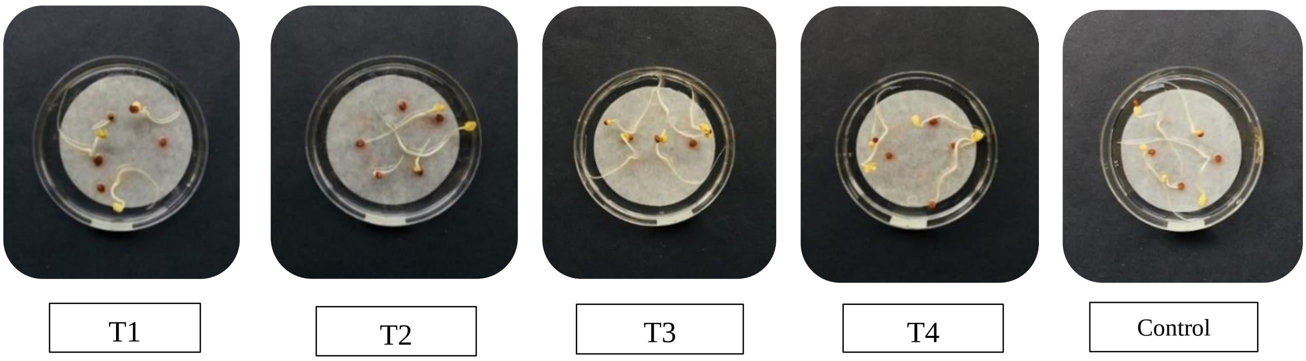

Figure 2: Germination of seeds in different treatments

Finally, the number of germinated seeds and root lengths were recorded following the method described by Emino and Warman, (2004). Relative Seed Germination % (RSG%), Relative Root Growth % (RRG%), and Germination Index (GI) were calculated based on the equations provided by Taylor et al., (2013).

$$Relative\ Seed\ Germination\ \%\ (RSG\%) = \frac{number\ of\ seeds\ germinated\ in\ the\ extract}{number\ of\ seeds\ germinated\ in\ the\ control} \times 100\% \qquad 1$$

$$Relative\ Radicle\ Growth\ \%\ (RRG\%) = \frac{mean\ radicle\ length\ in\ the\ extract}{mean\ radicle\ length\ in\ the\ control} \times 100\% \qquad 2$$

$$Germination\ Index\ (GI) = \frac{RSG\ X\ RRG}{100} \qquad 3$$

Relative Seed Germination % (RSG%), Relative Root Growth % (RRG%), and Germination Index (GI) for each PAE were calculated as per the equations given above, with distilled water serving as the control.

ANOVA was used to analyze the effect of different compost pellets on RSG%, RRG%, and GI. The Correlation analysis was done to determine the relationship between EC and the bioassay parameters of RSG%, RRG%, and GI. All the statistical analyses were performed using SAS® statistical software.

3. Results

The pH, EC, RSG%, RRG%, and GI of the PAEs in different treatments are given in Table 1.

Table 1: pH, EC, RSG%, RRG% and GI of pellet aqueous extracts

Treatment	PAE concentration %	pH	EC ($mScm^{-1}$)	RSG%	RRG%	GI
T1	50	7.0	0.23	133.33	108.75	145.00
	100	6.9	0.45	88.89	106.75	94.89
T2	50	7.2	1.74	88.89	95.01	84.45
	100	7.2	3.12	44.44	72.75	32.33
T3	50	6.8	0.18	100.00	115.17	115.17
	100	6.8	0.39	77.78	110.70	86.10
T4	50	6.8	2.34	133.33	89.92	119.89
	100	6.9	2.42	88.89	75.65	67.25

(T1: commercial compost pellet (100% compost), T2: commercial integrated pellet (90% compost + 10% NPK inorganic fertilizer), T3: WTPS-bound compost pellet (90% compost + 10% WTPS), T4: WTPS-bound integrated pellet (80% compost + 10% WTPS +10% NPK inorganic fertilizer)

According to the results, T2 recorded the lowest values for RSG% and RRG% in 100% and 50% PAEs. Additionally, T2 and T4 showed high EC values in both concentrations of PAE. The highest radicle length (RRG%) was reported in sludge-bound compost pellet treatment (T3) in both PAEs. Following that, both PAEs of T1 treatment showed high RRG%.

Furthermore, all the PAEs showed pH values ranging from 6.8 to 7.2 indicating alkaline conditions that may contribute to increasing soil pH. In both 50% and 100% PAEs, all treatments showed GI values 80 or higher, except for T2 (commercially integrated pellet) and T4 (WTPS bound integrated pellet). T2 and T4 showed GI values of 32.33 and 67.25, respectively, in the 100% PAE. All bioassay parameters showed negative correlations with EC, and a near-perfect correlation was observed between RRG% and EC. The results of the correlation analysis are given in Table 2.

Table 2: Correlation between EC and bioassay parameters

Parameter	EC (mS/cm)	*P* value
RSG%	-0.44	0.2730
RRG%	-0.98	<0.0001
GI	-0.69	0.0580

Correlation Coefficients (Spearman), N=8

In this study, water-extractable heavy metals of Al^{3+}, Zn^{2+}, Cu^{2+}, and Cr^{6+} ion concentrations were tested in 100% PAEs, and the results are given in Table 3.

Table 3: Water extractable heavy metal content (mgL^{-1}) in 100% PAEs of compost pellets

Water Soluble Heavy metals	Al^{3+} (mgL^{-1})	Zn^{2+} (mgL^{-1})	Cu^{2+} (mgL^{-1})	Cr^{6+} (μgL^{-1})
T1	0.37	0.67	<0.01	<0.01
T2	0.01	0.33	0.53	<0.01
T3	0.05	0.65	0.12	<0.01
T4	0.14	0.50	0.16	<0.01

(T1: commercial compost pellet (100% compost), T2: commercial integrated pellet (90% compost + 10% NPK inorganic fertilizer), T3: WTPS-bound compost pellet (90% compost + 10% WTPS), T4: WTPS-bound integrated pellet (80% compost + 10% WTPS + 10% NPK inorganic fertilizer)

According to the results, T1 contained a high amount of Al^{3+}, while T2 showed a low amount of Al^{3+}. The lowest Zn^{2+} concentration was recorded in T2, which consists of compost and inorganic fertilizer. However, the highest Cu^{2+} concentration was observed in T2. Additionally, T1 showed a very low amount of Cu^{2+} when compared to other treatments. All the PAEs contained minute concentrations of Cr^{6+}, which were below 0.01 µg/L.

4. Discussion

In this study, the phytotoxicity of potable water treatment plant sludge-bound compost pellets were assessed using a bioassay with radish seeds. The discussion of the results is as follows.

pH and EC are important parameters that influence seed germination (Amarasinghe and Jayaweera, 2022). Several studies have discussed the increase in EC due to the application of inorganic fertilizers, and Barral and Paradelo (2011) suggested that higher pH and EC values in compost can inhibit seed germination. In this study, the elevated EC values in the pellets may be attributed to the integration of inorganic fertilizers in the T2 and T4 pellet treatments. The salinity effects are generally considered inconsequential when EC values are less than 2 $mScm^{-1}$ (Hoekstra et al., 2002). Accordingly, PAEs of T2 and T4 may create high salinity conditions that could affect germination. The correlation results showed a negative relationship between EC and bioassay parameters which is consistent with similar findings reported by Hoekstra et al., (2002) and (Amarasinghe and Jayaweera, 2022) highlighting the significant inverse correlation of EC with RRG% and GI.

The quality of WTPS may vary depending on the water source and the flocculation process (Odimegwu et al., 2018). WTPS bound compost pellets exhibited high RRG% indicating substantial radicle length. Bugbee and Frink (1985) found that, sludge may contain phyto-stimulants that promotes radicle elongation. They also reported that alum sludge did not hinder the germination of ryegrass and suggested that the higher levels of plant nutrients, such as aluminium, chromium, magnesium, and potassium in alum sludge, were not at toxic levels. Additionally, the presence of organic matter, clay minerals, and hydroxides in the alum sludge may contribute to increased seed germination (Odimegwu et al., 2018). Similar study was done by Rigby et al, (2010) in wheat cultivation using alum sludge/WTPS found that it provided nitrogen for growth. According to the results of present study, the radicle length has increased in alum treated pellets. Thus, the addition of alum may increase the P absorption and will affect the radicle elongation. Further, Heil and Barbarick (1989) experimented the *Sorghum bicolor* L. growth in alum sludge amended soils and found that low rates (5 and 10 g per Kg of soil) had the highest yield with no P deficiency. Moreover, a study conducted by Basta et al. (2000) found that phosphorus (P) uptake in bermudagrass (*Cynodon dactylon*) grown in soils amended with WTPS in three different soil types and incorporating 200 mg P kg^{-1} did not increase the soil P but increased P in plants. However, Bugbee and Frink, (1985) reported that increasing alum sludge concentration in ryegrass reduced plant growth and plant tissue phosphorus resulting in no growth increment. Furthermore, Dayton and Basta (2001) found that applying WTPS to soil in concentrations above 10 % could lead to a reduction in crop yield and available phosphorus. Titshall et al. (2007) stated that the high application rates of WTPS employed in their research are generally not recommended. Accordingly, it is important to note that the present study did not exceed a concentration of 10 % WTPS in any of the compost pellet treatments.

All the PAEs in this study showed neutral pH values. Bugbee and Frink (1985) suggested that liquid alum sludge and dried alum sludge could increase pH by 0.5-1.0 in topsoil and act as a liming material in potting media, respectively. Some other research studies found that alum sludge improved soil pH (Moodley and Hughes, 2005; Pecku et al., 2005). Elliott and Dempsey (1991) also reported that alum sludge could be used to modify the soil pH and provide substantial amounts of micronutrients. Rengasamy et al., (2015) mentioned that alum sludge may increase crop growth and improve soil properties. Elliott and Singer (1988) found that pH has increased from 5.3 to 8.0 in *Lycospersicum esculentum* grown soil treated with WTPS.

The low values of GI observed in T2 and T4 treatments indicate a phytotoxic effect on seed germination and low radicle growth due to high EC values. Emino and Warman (2004) has classified phytotoxins as no longer present at the GI ≥80, with extreme phytotoxicity indicated by a GI of 0, while a GI ≤50 signifies high phytotoxicity. Furthermore, GI between 50 and 80 is considered as less phytotoxic. According to this classification T2 at 100% PAE showed high phytotoxicity, and T4 showed less phytotoxicity while other treatments showed no phytotoxicity.

The addition of Alum (Aluminium Sulphate) during the water treatment process can result in a higher concentration of Al^{3+} in WTPS. Even a small amount of Al^{3+} can enhance flocculation in the soil, leading to aggregate formation and improvements in various physical properties such as aeration, water movement, and available moisture holding capacity (Bugbee and Frink, 1985). Ahmed et al. (1997) reported about Al toxicity but noted that the naturally occurring soil concentrations of Al were higher than those found in the WTPS. Although WTPS was used in the present study, the concentration of Al^{+3} in PAEs did not significantly exceed allowable levels in both T3 and T4 treatments. Amarasinghe and Jayaweera (2022), reported that water extractable Cu^{2+} contents exceeding 0.04 mgL^{-1} inhibited root growth. Therefore, the reduced RRG% in the present study may be attributed to the high concentration of Cu^{2+} (0.53 mgL^{-1}). A reduction in Cd, Zn, Cu, and Ni uptake by the *Lycospersicum esculentum* shoots was observed after the application of WTPS attributing to high pH conditions in soil (Elliott and Singer, 1988). However, Lucas et al. (1994) found that the application of WTPS led to elevated concentrations of Mn and Cu in plant tissues of *Festuca arundinacea*, along with a reduction in plant yield at higher WTR rates. It also underlines that pot experiments can offer insights into how WTR affects plant growth, but these results may not directly interpret to field conditions, emphasizing the need for applying to the field conditions.

5. Conclusions and Recommendations

This present study has provided valuable insights about utilizing potable water treatment plant sludge (WTPS) as a binding material in compost pelleting and integrated pelletization, and to seek the potential effects on seed germination and radicle growth of radish (*Raphanus sativus* L.) seeds. According to this study, the highest radicle length was observed in radish (*Raphanus sativus* L.) seeds germinated in the PAEs of WTPS-bound compost pellets, despite the integration of inorganic fertilizers. Hence, integrated pellets with WTPS have the potential to provide a balanced nutrient source for seed germination and radicle elongation. Furthermore, all the compost pellets exhibited no phytotoxic effect on seed germination in 50% PAEs. This suggests that WTPS can be used as a binding material at a 10% w/w basis in compost pelleting, as well as in integrated pellets with inorganic fertilizers. This indicated the importance of optimizing the mixture ratios of WTPS in compost pelletizing. Thus, it is important to find an optimum blend of ingredients to maximize output in future studies. While this study provided valuable insights of seed germination in the presence of WTPS binding agent, it is essential to continue investigating the long-term effects of WTPS-bound pellets on crop growth, soil health, and the overall sustainability of agricultural practices. Further, it is crucial to understand the extended impacts of WTPS utilization which may contribute to environmentally friendly approaches by reducing environmental pollution through direct disposal of WTPS.

In summary, the present study concludes that WTPS can be effectively utilized as a binding material in compost pelleting with integration, contributing benefits in waste management and seed germination. Integrated pellets combining WTPS and inorganic fertilizers provides an opportunity for balanced nutrition integration for crop nutrient requirements. Thus, future research should converge on optimizing WTPS mixture ratios and assessing the long-term effects on crop growth, thereby encouraging sustainable and environmentally sensible farming practices.

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