Waste Management 138 (2022) 75–82

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Toxicity and physicochemical parameters of composts including distinct residues from agribusiness and slaughterhouse sludge

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ARTICLE INFO

Keywords:
Sludge
Swine slaughterhouse
Phytotoxicity
Cytotoxicity
Genotoxicity
Co-composting

ABSTRACT

Composting is useful for treatment of residues from agribusiness, but the potential toxicity of the final compost should be evaluated before its agricultural destination. The objective of this study was to evaluate the physicochemical characteristics and the toxicity of agribusiness residues using onion seeds as bioindicators. All tested treatments were composed by sludge from a swine slaughterhouse and sawdust. Besides the control, which included no additional materials, the other treatments included aviary bedding, rice husk and residue from tobacco industries as structuring materials. After 120 days of composting, for all treatments, the temperature inside the composting piles approached the environmental temperature, the physicochemical parameters indicated that the composts were stabilized and, except for the treatment including tobacco residues, that could be used for agriculture without impairing plant germination. Although the treatments including tobacco residues and rice husk showed evidence of cytotoxicity and genotoxicity at the beginning of the composting period, that was not observed for the treatment including aviary bedding. Such potential toxicity was not observed at the end of composting for any of the tested treatments.

1. Introduction

Although raising, slaughtering, and marketing livestock is critical to provide animal protein for human populations, such processes consume many resources and generate large volumes of residues with potentially high environmental impact. According to the United Nations (2020), there is a growing need to investigate sustainable alternatives to dispose residues of agribusiness (UN. United Nations., 2020). Currently, poultry and pork are the most consumed meats worldwide (FAO, 2021). During the treatment of effluents derived from the slaughter processes of both such species, meat packing plants generate sludge, a biomass with high organic and microbial load (Mpofu et al., 2019). Due to its nutrient content, the sludge is commonly used in agriculture as a fertilizer. Nonetheless, as the sludge is not yet stabilized, it may contain several substances that are harmful for plant growth (Castillo et al., 2016; Fatunla et al., 2017; Yang et al., 2017).

Anaerobic digestion and composting are some of the major processes used for sludge treatment (Palatsi et al., 2011). Agribusiness residues can be stabilized through composting, an aerobic process that transforms immature materials in nutrient-rich composts suitable for use in agricultural crops (Sanchez-Monedero et al., 2018). Composting may be optimized with the inclusion of other residues as structuring materials in piles (Li et al, 2020). In contact with a high microbial load inside the composting piles, such residues are exposed to a thermophilic phase in which temperatures may exceed 50 °C, adjusting humidity levels, concentrating nutrients, improving the C:N ratio (Manga et al., 2021) and allowing gas exchange with the environment, reducing unpleasant odors, and contributing to reduce the toxicity (Qian et al., 2016; Mihai and Ingrao, 2018).

Sludge form slaughterhouses and sawdust are widely used in many industrial composing plants in Brazil as bulking materials. Besides, sawdust is a largely available low-cost wood processing byproduct (Sharma et al., 2018). Nonetheless, the acquisition of large volumes of sawdust may incur in relevant cost, which justifies the use of alternative structuring materials. Depending on the region, other residues may be used, such as rice husk (Meng et al., 2018), aviary beddings (Neher et al.,

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https://doi.org/10.1016/j.wasman.2021.11.032
Received 28 September 2021; Received in revised form 17 November 2021; Accepted 22 November 2021
Available online 3 December 2021

2019) and residues from tobacco industries (Wang et al., 2020), helping to reduce the environmental impact of their disposal. Compared to sawdust, such materials may present greater nutrient content, which may increase the agronomic value of the resulting compost.

However, rice husk may have relevant amounts of metals, such as Na, Al, Cu, Mg, Mn and Ti, which may cause toxicity when released in the environment at high concentrations (Lopes et al., 2017). Aviary beddings may contain residues of antibiotics commonly added to diets, which may have potentially detrimental effects (Boamah et al., 2017). Also, although the high nicotine content in tobacco residues may be toxic for plants and animals, that toxicity can be reduced adding such residues as structuring materials in composting treatments, as reported elsewhere (Okur et al., 2008). Thus, for composts including such materials to be used in agriculture, their potential toxicity should be assessed.

Several methods are available to determine the toxicity of different materials (Libralato et al., 2016). As the product resulting from composting is usually destined to agricultural use, phytotoxicity assays are recommended (Meena et al., 2021), commonly using vegetable seeds as bioindicators. Potential toxic effects on the cellular structures and on the genetic material of the bioindicators should also be evaluated (el Fels et al., 2016; Felisbino et al., 2018; Yadav et al., 2019). The evaluation of phytotoxicity, cytotoxicity and genotoxicity altogether would allow a broader understanding of the deleterious effects of those residues and of the composts resulting from their stabilization on the growth of vegetables (Ribeiro et al., 2016). On such assays, root meristems of *Allium cepa* (onion) are frequently used as bioindicators. Nevertheless, there are few studies comparing the stabilization of distinct proportions of several residues in an industrial scale, considering simultaneously their physicochemical characteristics, phytotoxicity, cytotoxicity and genotoxicity

This study was aimed to evaluate the efficiency of composting using distinct residues from agribusiness as structuring materials, by assessing the physicochemical characteristics, the phytotoxicity, the cytotoxicity and the genotoxicity of both the raw materials and the resulting composts.

2. Material and methods

2.1. Composting and raw material

The study was conducted in a covered industrial composting unit, located at the Rio Grande do Sul state, Brazil (Latitude: 29° S; Longitude: 51° W).

Sludge from a swine slaughterhouse was the primary wastewater treatment system, including eucalyptus sawdust obtained from local logging companies as a bulking material. Distinct commonly available residues from agribusiness located in the southern region of Brazil were tested as potential stabilizing components: bedding from aviaries; residues from tobacco industries; and rice husk. Such materials were mixed four distinct composting treatments, all including 7.0 m^3 of sludge:

T1: sludge and sawdust (7:12; v:v), considered as the control
T2: sludge, sawdust, and aviary bedding (7:6:6).
T3: sludge, sawdust, and tobacco residue (7:6:6).
T4: sludge, sawdust, and rice husk (7:6:6).

Since it was considered efficient in previous studies based on the resulting physicochemical stabilization (Brazy et al., 2015) and on the absence of phytotoxicity (Oliveira et al., 2018), T1 was defined as the control treatment. In T2, T3 and T4, 50% of the structuring material included other residues.

The treatments were randomly assembled in elliptical piles (Fig. 1), placed over an impermeable concrete floor with a pluvial drainage contention system, in duplicates. Each pile was approximately 20 m^3 in volume (6 m long, 4 m large and 1.8 m high). The piles were monitored for 120 days, which is the usual period of compost stabilization in industrial plants (Cayuela et al., 2009; Bohrer et al., 2020). During the first 60 days, the piles homogenized once a week. Thereafter, they were homogenized at every 25-day interval.

2.2. Sampling

The samples were collected at 20-day intervals (at days 1, 20, 40, 60, 80, 100 and 120), totaling 56 samples (4 treatments × 2 duplicates × 7 collection periods). Each sample comprised three subsamples collected from central equidistant points of the pile, which were subsequently mixed. For transport to the laboratory, the samples were cooled at 5 °C. Thereafter, the samples were frozen −20 °C.

The temperature inside the piles was registered daily using a digital thermometer attached to a rod inserted towards the center of the piles, at 60 cm depth. At each day, the recorded temperature represented the mean of three consecutive measurements. The environmental temperature was also determined daily.

2.3. Physicochemical parameters

The physicochemical parameters were determined from samples of raw material, at the beginning and at the end of the composting period (Day 1 and Day 120, respectively), considering the parameters established by the Brazilian legislation (Brasil, 2020). All measurements were conducted with equipment calibrated with standardized curves, in triplicate.

Immediately after their arrival at the laboratory, cooled samples were diluted in distilled water (1:10, m:v), for determination of both electrical conductivity and pH through digital bench equipment (MCA-150 and MPA-210, respectively, both from MS TecnoponR, Brazil).

Subsequent analyses were conducted in stored frozen samples.

Fig. 1. General view of the piles of composting treatments used in the experiment.

Humidity was determined at 105 °C in a drying oven (AOAC, 1990, method 950.01). The UV–visible spectrophotometry (660 nm) was used to determine the total P content (Huang et al., 2004). After determining the total N content through the Kjedahl (TKN) digestion method (AOAC, 1990, method 955.04) and the total organic C content through the Walkley–Black method with heating, the C:N ratio was calculated.

2.4. Phytotoxicity assays

In this assay, onion seeds (*Allium cepa*) were used as bioindicators. After 1 h in agitation, the aqueous extract of all samples (1:12,5; w/v) was filtered and placed in petri dishes containing ten seeds and incubated at 25 °C for 144 h, in the dark, along with samples including only distilled water. All phytotoxicity assays were conducted in triplicate. The germination index (GI) was determined considering the root length and the relative seed germination (Mendes et al., 2016; Gerber et al., 2017).

Cytotoxicity and genotoxicity were evaluated for samples of the raw material, for pile samples collected at Day 1 and Day 120 and for control samples with distilled water. The meristematic tissue of the apical region of the radicles was sectioned for the preparation of slides to be examined under optical microscopy at 400 X. The images were registered with a digital camera with 6-megapixel resolution. Scanning was conducted in 500 cells per slide, totaling 24,000 evaluated cells (4 treatments × 2 repeats × 2 collection periods × 3 slides × 500 cells). Cytotoxicity was expressed by the mitotic index and genotoxicity was expressed by the frequency of chromosomal aberrations (Hemachandra and Pathiratne, 2015).

The values for the phytotoxicity tests were expressed considering as a standard (control) the results using samples including only distilled water (Guidoni et al, 2021). If the mitotic index is lower than control, it indicates that the sample inhibited plant tissue development. If the result is greater than the standard, we can interpret that the sample was growth-promoting or that it caused a deleterious effect, forcing disorderly cell division and proliferation (Laughinghouse et al., 2012).

2.5. Statistical analyses

Normality was checked for all responses of interest through the Shapiro-Wilk test. Physicochemical and toxicological traits were compared across raw materials using the T-test, for responses showing normality, and using the Wilcoxon test, for those that did not follow normal distributions. The same responses were compared among treatments and periods through either analysis of variance or Kruskal-Wallis analysis of variance for non-parametric data, depending on their normality. The limits for compost quality and maturity used as references were recommended by the Brazilian legislation (Brasil, 2020). Variation in the GI during the composting period was evaluated through linear regression models, using polynomial adjustments for non-linear variation.

3. Results and discussion

3.1. Raw material characterization

The characterization of the swine slaughterhouse sludge and of all tested raw materials are shown in Table 1. The humidity was greater for the sludge than for sawdust, aviary bedding, tobacco residue and rice husk ($P < 0.05$). As such a high humidity (above 65.0%) may be excessive for composting, mixing the sludge with structuring materials with humidity equal or lower than 25.0%, such as aviary bedding, tobacco residue and rice husk, would help to reduce the humidity up to the recommended levels (Wang et al. 2020). Under those conditions, microbial growth would occur with adequate gas exchange (Ma et al., 2020). The sludge also presented the greatest content of TKN compared to the other raw materials, whereas the lowest content was observed for sawdust and rice husk (both $P < 0.05$).

Table 1
Physicochemical and phytotoxicological characterization of the raw materials from agribusiness residues.

Parameters	Sludge	Sawdust	Aviary bedding	Tobacco residue	Rice husk
		Physicochemical parameters			
Humidity (%)	67.6 ± 0.10 ^{A}	53.9 ± 0.10^{B}	25.6 ± 0.20^{C}	18.0 ± 0.90^{D}	8.0 ± 0.05 ^{E}
pH	5.4 ± 0.20^{E}	6.3 ± 0.04^{D}	9.7 ± 0.03^{A}	6.8 ± 0.02^{C}	7.7 ± 0.05^{B}
Electrical conductivity (uS/cm)	1013.8 ± 13.70^{AB}	120.4 ± 0.90^{B}	3783.8 ± 52.80^{AB}	8106.7 ± 39.30^{A}	429.7 ± 3.30^{AB}
Total Kjeldahl N (%)	5.8 ± 0.01^{A}	0.3 ± 0.02^{B}	1.8 ± 0.02^{AB}	2.3 ± 0.10^{AB}	0.4 ± 0.02^{B}
C:N ratio	4.6 ± 0.10^{D}	76.20 ± 2.70^{AB}	31.5 ± 0.40^{BC}	19.5 ± 0.70^{C}	111.7 ± 5.80^{A}
Total P (%)	2.8 ± 0.10^{AB}	0.4 ± 0.10^{AB}	3.9 ± 0.10^{A}	0.8 ± 0.04^{AB}	0.3 ± 0.04^{B}
		Toxicological parameters			
Germination index for *Allium cepa*	51.4 ± 4.3^{B}	90.1 ± 1.9^{AB}	45.3 ± 0.2^{B}	0.0	165.4 ± 2.1^{A}
Mitotic index	TN**	91.3 ± 0.1 ^{B}	TN**	NG*	182.1 ± 0.1 ^{A}
Chromosomal aberrations (%)	TN**	0.0	TN**	NG*	300.0 ± 0.1 ^{A}

*NC: Necrotic tissue, **NG: No germination.
^{A,B,C,D,E,} Means ± SEM with distinct superscripts differ in the lines by at least $P < 0.05$.

Tobacco residues presented greater electrical conductivity than sawdust ($P < 0.05$), with no further differences observed for the other tested raw materials ($P > 0.05$). The high electrical conductivity of tobacco residues may indicate an excessive content of electrolytes, that can contribute to alter the correct execution of osmosis mechanisms and enzymatic reactions by the microorganisms present in the compost (Zittel et al., 2018).

All raw materials presented pH different from each other ($P < 0.05$), with the most alkaline pH observed for aviary bedding. The sludge presented the lowest C:N ratio ($P < 0.05$) whereas the greatest ratio was observed for rice husk ($P < 0,05$). The lowest P content was observed for both rice husk and sawdust ($P < 0.05$), whereas the greatest P content occurred in the aviary bedding ($P < 0.05$), which may be since poultry excreta is commonly deposited on that substrate (Vicentin et al., 2021).

Even though the GI observed for sawdust and rice husk did not differ ($P > 0.05$), the GI observed for rice husk was greater than those of all other tested raw materials ($P < 0.05$). That agrees with studies that used rice husk as a substrate for plant germination (Meng et al., 2018; Wang et al., 2021). Rice husk also presented the greatest values for the mitotic index and the frequency of chromosomal aberrations, which may be due to the presence of toxic substances, such as arsenic (Linam et al., 2021). On the other hand, no germination whatsoever was observed in contact with tobacco residue, which could be expected since the allelochemicals present in such material interfere in structural and biochemical functions of plant tissues (Cheng et al., 2021).

3.2. Physicochemical parameters

The temperatures inside the composting piles were higher than the environmental temperature throughout the entire period (Fig. 2). All four treatments presented a similar thermophilic phase, characterized by temperatures above 60 °C until nearly the 50th day of composting (Fig. 2), indicating that sanitization occurred during that period

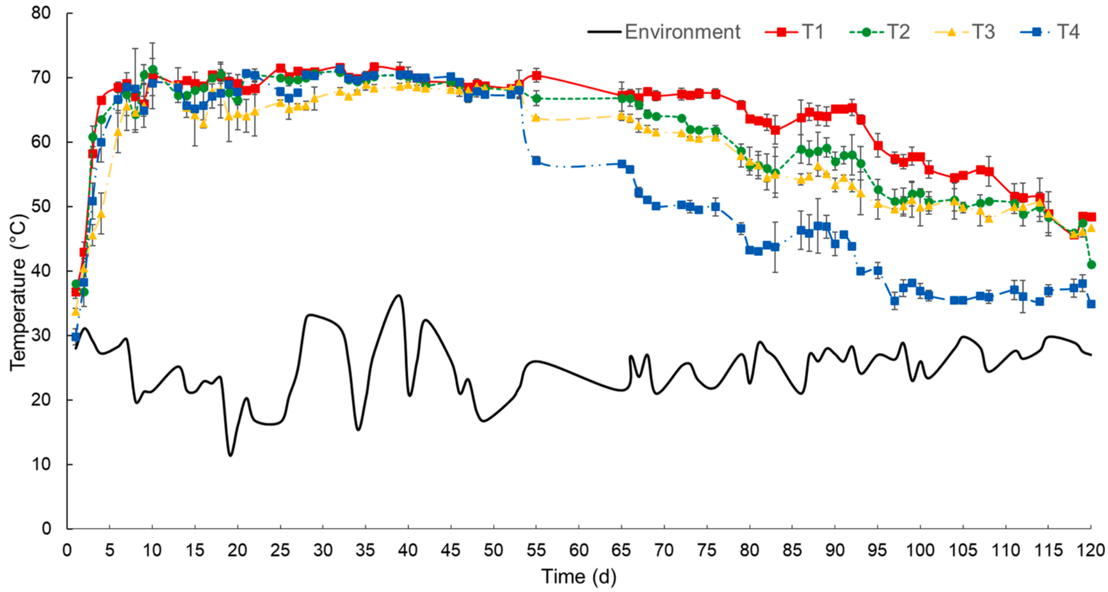

Fig. 2. Temporal temperature variation (Mean ± SD) during 120 days of composting in treatments including sludge from a swine slaughterhouse and distinct raw materials*. *T1 = Sludge:Sawdust (7:12); T2 = Sludge:Sawdust:Aviary bedding (7:6:6); T3 = Sludge:Sawdust:Tobacco residue (7:6:6); T4 = Sludge:Sawdust:Rice husk (7:6:6).

(Abdellah et al., 2021). Similar findings were reported by Kebibeche et al. (2019), with temperature of 62 °C during the thermophilic phase of composting using sewage sludge, wheat straw and sawdust as bulking materials, and by Jiang et al. (2018), in a study about composting of swine waste in which such temperature was 65.5 °C. Subsequently, all treatments presented a mesophilic phase, with reduced temperatures until the end of the composting period, although temperatures were higher for T1 and similar for T2 and T3. However, all such three treatments finished the composting period with apparently similar temperatures, near to 50 °C. The reduction in temperature was more characteristic in T4, which finished the period with the lowest temperature (lower than 40 °C), which reflects the lower humidity of this treatment at the end of composting (Table 2), likely resulting in reduced microbial activity (Onwosi et al., 2017).

At the beginning of composting (Day 1), the mean humidity for all treatments was within recommended values (Wang et al., 2015) and did not differ ($P > 0.05$) among treatments (Table 2). At Day 120, although the humidity was reduced compared to Day 1 for all treatments ($P < 0.05$), it remained within the recommendations of the current legislation (Brasil, 2020), which agrees with a study that reported humidity near to 30% at the end of composting (Evangelou et al., 2016). Across treatments, T1 presented the greatest humidity at the end of composting and T4 presented the lowest ($P < 0.05$), whereas T2 and T3 had similar humidity ($P > 0.05$). The humidity observed for T1 may reflect the great humidity originally present in the sawdust added to the sludge (Table 1), whereas, in T4, that was likely mitigated by the low humidity of rice husk.

The pH for T2 at Day 1 was alkaline (Table 2), differing from the acid pattern shown by T3 and T4 ($P < 0.05$). However, the pH for T1 was only different from that of T4 ($P < 0.05$). The pH for all treatments at Day 120 was greater than those observed at Day 1 ($P < 0.05$), although there were no differences among them ($P > 0.05$). All treatments reached greater pH at Day 120 compared to those observed at Day 1 ($P < 0.05$), which were consistent with the values recommended by the legislation. Generally, the pH is expected to be either acid or neutral at the beginning of composting and to increase at the end of the period (Lin et al., 2016). The alkalization observed as composting advanced indicates that the compost was matured (Chen et al., 2019).

In the present study, T1 and T4 presented greater electrical conductivity ($P > 0.05$) than T2 and T3 at Day 1 (Table 2). Generally, the electrical conductivity was increased at Day 120 compared to Day 1 ($P < 0.05$), except for T3, which presented similar values ($P > 0.05$). At the end of composting, greater electrical conductivity was observed for T2 and T3 than for T1 and T4 ($P < 0.05$), with T1 presenting the lowest value. Nonetheless, all treatments presented electrical conductivity at Day 120 below the maximum limit recommended for plant growth (Zittel et al., 2018). In general, the effect of salinity is negligible in the aqueous extract, with electrical conductivity inferior to 2500 uS/cm (Pampuro et al. 2017). As the electrical conductivity is the numerical expression of the conduction of an electric current through an aqueous solution (Onwosi et al, 2017), such increase during composting may be due to the formation of mineral salts, such as ammonia and phosphate ions, through transformation of organic matter (Jiang et al., 2015).

The TKN contents at Day 1 were greater for T2 and T3 ($P < 0.05$) than for T1 and T4 (Table 2), which probably reflects the amount of poultry excreta contained in the aviary bedding included in T2 (Chen et al., 2018) and the nicotine content in the tobacco residues included in T3 (Zittel et al., 2020). That same pattern of TKN content was also observed at Day 120. Nevertheless, all tested treatments resulted in a compost with TKN content above the reference levels, which is desirable considering that N is a nutrient essential for plant growth (Wang et al., 2011).

On the other hand, T1 and T4 started the composting period with greater C:N ratio ($P < 0.05$) than T2 and T3 (Table 2), although such ratio was inferior to 30:1 for all four treatments, which is desirable for the beginning of composting. A C:N ratio capable to allow appropriate development of microorganisms would be within 20:1–40:1 (Cerda et al., 2018). However, all treatments finished composting with C:N ratio inferior to 20:1, as recommended (Tran et al. 2021), with T1 presenting greater ratio than T2 ($P < 0.05$), with no other differences observed among treatments ($P > 0.05$).

The initial P content was greatest for T2 and lowest for T1 ($P < 0.05$), whereas T3 and T4 presented intermediate contents that did not differ from each other ($P > 0.05$). Hence, at the end of the composting period, all treatments presented similar P content ($P < 0.05$), indicating that such content increased in T1 while remaining constant in the other three treatments (Table 2). The final P contents observed for all treatments were similar to those reported by Wei et al. (2015), indicating that their composts may have potential agricultural use. As P is a non-renewable nutrient which is essential for cropping, losses of P during composting

Table 2
Physicochemical and toxicological parameters at the beginning (Day 1) and the end (Day 120) of the composting period in treatments including distinct residues from agribusiness.

Parameter	T1 Sludge: Sawdust (7:12)	T2 Sludge: Sawdust: Aviary bedding (7:6:6)	T3 Sludge: Sawdust: Tobacco residue (7:6:6)	T4 Sludge: Sawdust: Rice husk (7:6:6)	Reference values[1]
	Day 1				
Humidity (%)	54.2 ± 3.6^{Aa}	55.1 ± 0.4^{Aa}	48.1 ± 6.8^{Aa}	47.3 ± 0.9^{Aa}	–
pH	7.5 ± 0.2^{ABb}	8.2 ± 0.3^{Ab}	6.5 ± 0.1^{BCb}	6.2 ± 0.1^{Cb}	–
Electrical conductivity (uS/cm)	858.2 ± 211.4^{Bb}	2630.0 ± 228.9^{Ab}	3176.7 ± 413.0^{Aa}	1320.2 ± 258.2^{Bb}	–
Total Kjeldahl N (%)	2.0 ± 0.2^{Bb}	4.2 ± 0.2^{Aa}	3.9 ± 0.1^{Aa}	2.0 ± 0.1^{Bb}	–
C:N ratio	27.4 ± 1.3^{Aa}	11.3 ± 0.1^{Bb}	10.0 ± 0.5^{Ba}	29.6 ± 5.5^{Aa}	–
Total P (%)	1.1 ± 0.1^{Cb}	3.1 ± 0.3^{Aa}	2.0 ± 0.1^{Ba}	2.2 ± 0.1^{Bb}	–
Mitotic index	39.7 ± 18.4^{Aa}	64.6 ± 5.3^{Aa}	NG**	NT*	–
Chromosomal aberrations (%)	53.8 ± 33.0^{Aa}	305.5 ± 131.7^{Aa}	NG**	NT*	–
	Day 120				
Humidity (%)	36.2 ± 4.3^{Ab}	21.7 ± 0.9^{Bb}	18.5 ± 1.3^{Bb}	14.0 ± 0.1^{Cb}	≤ 50.0
pH	7.8 ± 0.1^{Ca}	8.9 ± 0.1^{Aa}	8.4 ± 0.2^{Aba}	8.1 ± 0.02^{BCa}	As stated
Electrical conductivity (uS/cm)	1570.8 ± 2.1^{Ca}	4505.0 ± 228.4^{Aa}	3742.5 ± 152.5^{Aa}	2518.0 ± 574.0^{Ba}	As stated
Total Kjeldahl N (%)	2.6 ± 0.1^{Ca}	4.0 ± 0.1^{Aa}	3.5 ± 0.1^{Ba}	3.2 ± 0.1^{Ba}	≥ 0.5
C:N ratio	12.1 ± 0.4^{Ab}	8.6 ± 1.0^{Bb}	9.6 ± 1.4^{ABa}	10.3 ± 0.1^{ABb}	≤ 20.0
Total P (%)	2.2 ± 0.1^{Aa}	2.9 ± 0.4^{Aa}	2.2 ± 0.2^{Aa}	2.9 ± 0.2^{Aa}	As stated
Mitotic index	82.5 ± 6.1^{Aa}	91.3 ± 17.2^{Aa}	94.0 ± 6.0^{Aa}	118.0 ± 34^{Aa}	–
Chromosomal aberrations (%)	200.5 ± 83.9^{Aa}	212.5 ± 52.7^{Aa}	50.0 ± 12.5^{Aa}	75.0 ± 12.5^{Aa}	–

A.B.C Means ± SEM with distinct superscripts differ among treatments by at least $P < 0.05$.
a.b.c Means ± SEM with distinct superscripts differ between Day 1 and Day 120 by at least $P < 0.05$.
*NC: Necrotic tissue, **NG: No germination.
[1] Brasil (2020).

should be avoided (Wei et al, 2016). That occurred in the present study likely because all treatments included residues originated from vegetables, which contribute positively for bacterial activity allowing retention of P in the composting pile (Wei et al., 2016).

3.3. Phytotoxicity

Although phytotoxicity assays are commonly used to evaluate the stabilization of composted materials, the natural variation in the biological response of the seeds used as bioindicators (Jagadabhi et al, 2019), likely influenced the R^2 values observed for the linear regression models used to estimate the effect of the treatments on the germination of onion seeds during the composting period, which were moderate for T1 and weaker for the other treatments (Fig. 3). Considering that a nontoxic compost with reduced risk for the environment and cropping should allow a GI equal or greater than 60% (Ortega et al., 1996; Cabañas-Vargas et al., 2005; Gómez-Brandón et al., 2008), in the present study, T4 was the only treatment that was not phytotoxic at the beginning of composting (Fig. 3). The linear regression models indicated that the GI for T1, T2 and T4 followed a cubic pattern over time ($P < 0.05$). The decreased GI observed at Day 20 for all treatments may be due to the release of phytotoxic substances during the degradation of organic matter. The GI indicated that phytotoxicity was reduced when composting reached nearly 60 days, which was maintained up until 100 days. Reduced phytotoxicity at the end of composting was also reported by Miaomiao et al. (2009). That emphasizes that recycling organic matter through composting can reduce the toxicity originally present in residues to be used as raw material (Caritá et al., 2019). However, after 100 days of composting, the GI for T1, T2 and T4 decreased up to the end of the period and the GI for T4 already indicated phytotoxicity at Day 120. At Day 120, the reduction in GI may be explained by the absorption of the beneficial fraction by the seeds, which possibly concentrated several substances that are detrimental for the germina0tion process. Those findings suggest that it may be unnecessary to extend composting for additional 20 days after Day 100, for T1, T2 and T4.

In contrast, despite showing a weak linear increase over time ($P < 0.05$), the GI for T3 indicated phytotoxicity throughout the entire composting period. As onion seeds are bioindicators of the sensitivity of vegetable cells to distinct substances (Datta et al., 2018), the toxicity observed for the tobacco residues added to T3 (Table 1), would negatively affect the division of vegetable cells (Cheng et al., 2021; Medeiros et al., 2021).

All the tested treatments presented physicochemical characteristics compatible with the requirements of the current legislation, but only T1, T2 and T4 could be recommended for use in agriculture, since the observed phytotoxicity would disqualify T3. Future studies should investigate whether that phytotoxicity could be reduced with lower inclusion of tobacco residues or through longer composting periods.

3.4. Genotoxicity

At Day 1, greater genotoxicity was observed for T3 and T4 ($P < 0.05$) compared to T1 and T2 (Table 2). As no germination occurred for seeds in contact with tobacco residues (Table 1), it was not possible to determine the mitotic index and the frequency of chromosomal aberrations at Day 1 for T3 (Table 2). Seeds in contact with sludge and aviary bedding germinated but, at the time the genotoxicity was evaluated, the tissue was necrotic (Table 1) and the mitotic index and the frequency of chromosomal aberrations for T4 at Day 1 were also not determined (Table 2). As necrosis in a type of non-physiological cell death, that may have been a biological response to exposure to certain toxic substances (Behboodi and Samadi, 2004).

Nevertheless, at Day 120, the indicators of genotoxicity were reduced for all treatments and did not differ ($P > 0.05$). Composting was beneficial for T3 and T4, reducing their genotoxicity, resulting in a compost capable to promote the development of meristematic tissue in the seeds used as bioindicators (da Silva Souza et al., 2021). The aberrant development of *Allium cepa* cells in distinct stages of division (Fig. 4) indicates that the substances present in the compost can promote disorganization in the genetic material during cell division. That is true even for agribusiness residues considered safe (Bhat et al., 2015), since genotoxic substances may resist to degradation, remaining in the compost for longer periods (Scherer et al., 2019). The frequency of chromosomal aberrations observed at Day 120 for onion seeds in T4, which included composted rice husk (Table 2), was reduced compared to the frequency observed for seeds in contact with raw rice husk (Table 1). That may have resulted from the fact that toxic metals present in the rice husk may have been complexed with the humus, becoming unavailable to be absorbed by the seed radicles during germination (Lopes et al., 2017). As far as the toxicity observed for tobacco residues (Table 1), the nicotine, which is water soluble, may be degraded by microorganisms during composting, which may reduce its detrimental effects for seed germination (Chen et al., 2016; Huang et al., 2020;

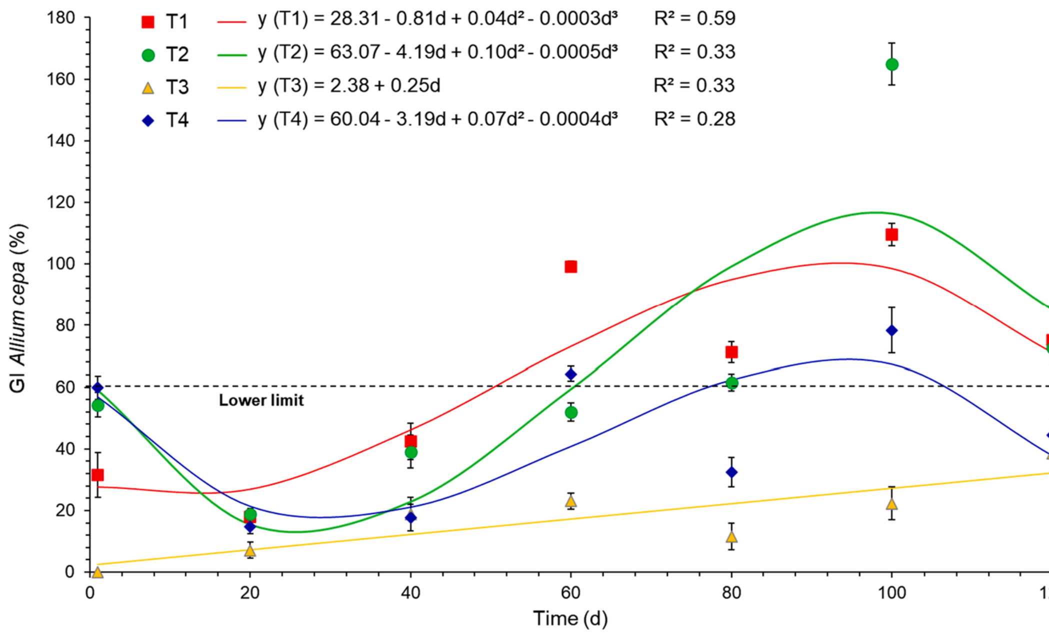

Fig. 3. Linear regression models to estimate the variation in the germination index (GI) for *Allium cepa* seeds during 120 days of composting in treatments including sludge from a swine slaughterhouse and distinct raw materials*. *T1 = Sludge:Sawdust (7:12); T2 = Sludge:Sawdust:Aviary bedding (7:6:6); T3 = Sludge:Sawdust: Tobacco residue (7:6:6); T4 = Sludge:Sawdust:Rice husk (7:6:6).

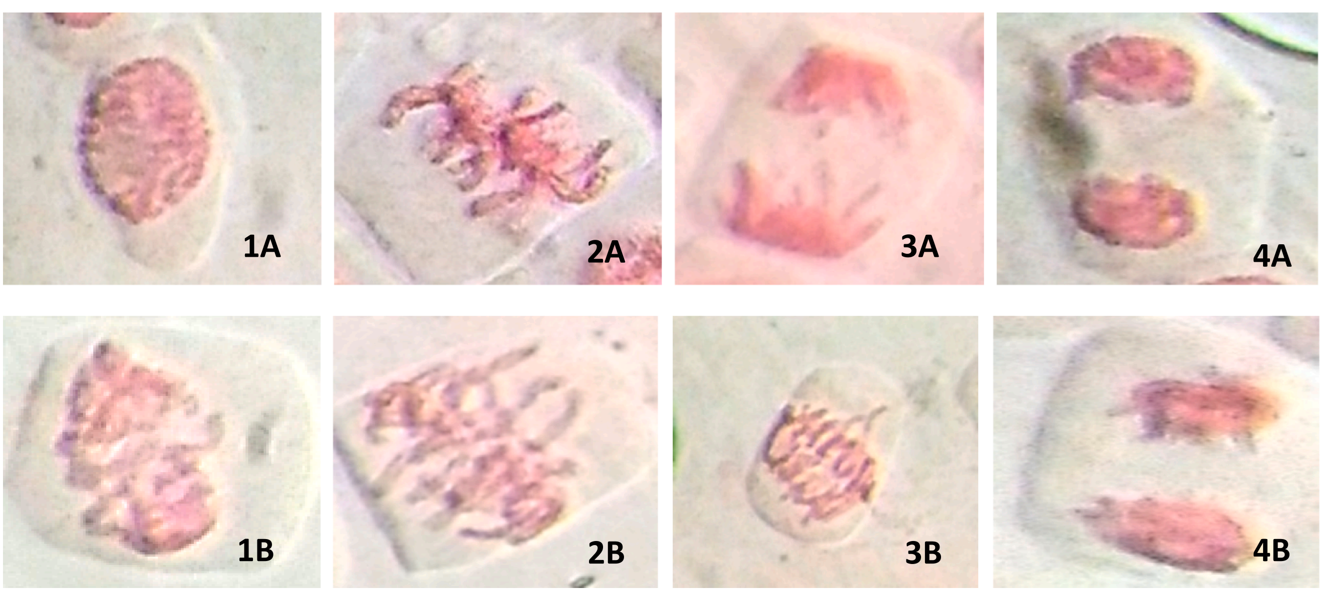

Fig. 4. Representative images of cells of *Allium cepa* submitted to phytotoxicity assays. 1A: normal prophase; 2A: normal metaphase; 3A: normal anaphase; 4A: normal telophase; 1B: prophase with isolated chromosome; 2B: disorganized metaphase; 3B: anaphase with isolated chromosome; 4B: telophase with isolated chromosome. Images observed in treatments including distinct raw materials.

Shang et al., 2021).

Generally, composting improved the characteristics of all tested residues, mitigating their genotoxicity at the end of the process (Table 2). Those findings are relevant, since onion seeds are representative bioindicators, suggesting that similar effects may occur for other vegetables (Laughinghouse et al., 2012).

4. Conclusions

Composting is a promising alternative to stabilize materials such as sludge from swine abattoirs, rice husk, tobacco residues and aviary beddings, when mixed to sawdust. All tested treatments yielded stable composts with physicochemical parameters in accordance with legal requirements. However, only the control treatment composed of sludge and sawdust and the treatments including aviary bedding and rice husk as structuring materials could be recommended for agricultural use due to their reduced phytotoxicity. For such treatments, composting could be reduced to 100 days. Complementary treatments may be necessary to reduce the phytotoxicity of the compost from the treatment including tobacco residues, either by prolonging composting beyond 120 days or by adding lower proportions of tobacco residues as bulking materials. Composting reduced the genotoxicity of all tested treatments

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.

Acknowledgements

The authors thank CAPES and CNPq for their financial support.

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