

Assessment of toxic response of *Lactuca sativa* to compost extracted from agri-food waste

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Abstract:

Purpose: In this study, we aim to investigate the suitability of five samples of compost extracted at various concentrations by testing for their phytotoxic response on *Lactuca sativa* L. We analyze relevant parameters with regards to the quality of the compost. By doing this, we hope to contribute to the attainability of clean, high-value organic fertilizer from local food waste production.

Method: We set up a mini composting pilot where raw materials were classified, chopped into bits, and finally mixed. They include residues of *Solanum tuberosum* (potatoes), *Zea mays* (corn), *Musa Paradisiaca* (bananas), *Allium cepa* (onions), *Lactuca sativa* (lettuce), *Daucus carota* (carrots), *Solanum lycopersicum* (tomatoes), as well as fruits such as *Passiflora edulis* (passion fruit), *Carica papaya* (papaya). Composting was run over a period of 90 days. We then measured several bromatological analyses of compost. For phytotoxicity tests of compost relative seed germination (RSG), relative radicle growth (RRG) and Germination Index GI on *L. sativa* were measured.

Results: The prepared compost showed the following parameters: pH (8.6), OM (21.4%), C/N (11.59), EC (7.24 dS/m), Total N (1.72%), P (325.6 ppm), Al (0.1 cmol.kg⁻¹) K (41 cmol.kg⁻¹), Ca (8.12 cmol.kg⁻¹), Mg (5.62 cmol.kg⁻¹), Na (0.76 cmol.kg⁻¹). Minor elements appeared in acceptable ranges, and do not pose any risk. Besides, the compost showed a germination rate of 112.6% on *L. sativa*, which allowed us to validate its high maturity.

Conclusion: The compost produced stands out for its quality and efficiency, due to its low phytotoxicity, specific mixture of the materials and the processing method. The mature compost produced represents a valuable material for the acidic and nutrient-poor soils of the Colombian Piedmont.

Keywords: Composting; C/N ratio; Germination index; Valorization; Wastes bio-fertilizer; Sustainability

1. Introduction

The generation of agri-food waste is a growing problem worldwide, especially in Latin America. One-third of the food produced globally is wasted, equivalent to 1.3 billion tons per year (Sahoo et al. 2023). In Latin America, 15% of food production is lost or wasted, equivalent to 127 million tons per year (Roy et al. 2023). Restaurants are one of the main sources of agri-food waste in the world

(Sehnm et al. 2022). This problem not only has environmental consequences, such as greenhouse gas emissions and pollution of soil and water, but also economic and social implications. For example, unequal access to food has been highlighted in (Horton 2017; Hatjiathanassiadou et al. 2019; Kassim et al. 2022); while persisting high levels of hunger and malnutrition in Latin America are documented by (Espinosa-Cristia et al. 2019), which illustrates concerning food insecurity in the region. In 2020, the impact of the

COVID-19 pandemic has intensified this problem, as food waste continues to increase while access to staple food is challenging for the most vulnerable populations (Aldaco et al. 2020; Ellison and Kalaitzandonakes 2020).

To create a more sustainable food system, we must develop strategies and policies to decrease agri-food waste and improve practices across the production chain, from harvest to consumption (Smetana et al. 2018; Tanveer et al. 2021; Kour et al. 2023). Environmental solutions for agri-food waste, such as energy valorization through biofuels or biogas generation via anaerobic digestion, can significantly mitigate its negative impact (Lorenzo et al. 2022; Ayala-Parra et al. 2017; Mahmudul et al. 2022). Waste treatment technologies, like source separation and recycling, recover valuable materials from agri-food waste (Ciccullo et al. 2021). Circular economy approaches suggest reusing and recycling waste, creating bioplastics, and using organic waste to make new food (Tanveer et al. 2021). To curb food waste in the supply chain, strategies include enhanced packaging design, quality management systems, and sustainable farming practices (Vargas-Pineda et al. 2019; Aramyan et al. 2021).

We aim to highlight agronomic valorization, this practice consists of the use of waste as fertilizer or compost, improving soil fertility and reducing the need for chemical fertilizers (Puglia et al. 2021; Bulgari et al. 2023). Composting is a biological method that is used to transform organic remains of various materials into a relatively stable product. The practice of this method has been increasing in recent years and it is an effective choice for improving productivity and soil quality and recovering biodegradable waste (Guermoud et al. 2009).

The compost produced from organic waste is increasingly used in horticultural production, as an organic amendment

or substrate for seedlings (Pellejero et al. 2015). Organic wastes can be processed to produce a high value resource for agricultural production and for the restoration of degraded ecosystems. Therefore, compost can be used as a valuable amendment for agriculture and landscaping (Corato 2020). Compost incorporation is relevant to soil because it enriches soil's organic matter content, and this constituent plays an essential role not only to minimize nutrient losses but also to preserve or restore soil quality (Vargas-Pineda et al. 2019). While it is a widely used practice in organic waste management; if the compost is not mature enough will promote negative effects on the environment (Schaub and Leonard 1996; Azim et al. 2017; Waqas et al. 2023; Tiquia 2000; Luo et al. 2018; Mahapatra et al. 2022; Yasmin et al. 2022). Immature compost can affect soil quality, reduce microbial activity, and decrease nutrient uptake by plants (D'Hose et al. 2014; Chaoui et al. 2003). In addition, it can emit greenhouse gases such as methane and nitrous oxide during its decomposition (Chau et al. 2003).

Hence, ensuring mature compost as a safe soil amendment is crucial. To assess maturity, the Germination Index (GI), introduced by Zucconi et al., gauges compost's impact on seed germination (Zucconi et al. 1981). This test, an affordable and efficient bioassay, determines compost toxicity before use (Luo et al. 2018). *Lactuca sativum*, noted by Oleszczuk as sensitive to compost and sewage sludge substances, is proposed for the GI measurement (Oleszczuk 2008).

In equatorial areas such as the region of Villavicencio (Colombia), oxisols are the dominant types of soils. They are characterized by an acidic pH (4 – 5.5), high concentration of heavy metals, Al and Fe toxicities and low cation exchange capacity (CEC), which limits the availability of nutri-



Figure 1. Agri-food waste consumed daily in restaurant of Universidad de los Llanos, Colombia.

ents for plants, resulting in lower yields (Trujillo-González et al. 2022). In these acidic conditions, organic fertilizers contribute to increasing fertility by providing essential nutrients such as phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sulfur (S) and nitrogen (N) (Vargas-Pineda et al. 2019). In this framework, this work presents an alternative compost material as an effective solution to the problem of organic waste management in the acid soils of the Colombian Piedmont.

2. Material and methods

The experiment was carried out in the field and laboratory in western Colombia in South America ($4^{\circ} 04' 24.8''$ N, $-73^{\circ} 34' 56.2''$ W) at an altitude of 418 m above sea level, with an average temperature of 25°C , a rainfall of 4050 mm, and a relative humidity of 75%.

2.1 Composting process

Compost was prepared from the 30 kg of agri-food waste. The raw material was collected from Universidad de los Llanos in Colombia. The mixture consists of residues like peelings, husks and other unwanted parts of *Solanum tuberosum* (potatoes), *Zea mays* (corn), *Musa Paradisiaca* (bananas), *Allium cepa* (onions), *Lactuca sativa* (lettuce), *Daucus carota* (carrots), *Solanum lycopersicum* (tomatoes), as well as fruits such as *Passiflora edulis* (passion fruit), *Carica papaya* (papaya) (Fig. 1).

The composting process was performed in a 50 L capacity rotary drum composter. The rotary drum composter was made by modifying a recycled plastic drum and providing equidistant holes of 10 mm to facilitate air circulation inside the drums, similar to that proposed by Manu et al. (Fig. 2) (Manu et al. 2016). The composting process was carried

out for 90 days under a cover in an open space to allow for natural aeration.

2.2 Sampling and physicochemical analysis

Homogenous samples were taken from different points of the rotary drum and preserved in the refrigerator prior to being analyzed. Duplicates were used for quality assurance. We conducted the composting trial for 90 days (from Aug 11th, 2022, to Nov 19th, 2022). Temperature monitoring was carried out every eight days using an Extech 39240 thermometer with a range of -40 to 200°C and an accuracy of ± 2 . Previously, in chemical analysis of the compost, it was subjected to a digestion in nitric acid, hydrochloric acid, and hydrogen peroxide. The pH was measured with a potentiometer in a 1:1 soil: water mixture. The electrical conductivity (EC) was determined by stirring 20 g of the compost in 100 ml distilled water (1:5 w/v) for 15 min; OM was determined by the Walkley-Black method and calculated by multiplying the total carbon by 1.724; available phosphorus was calculated by Bray-Curtis method (IGAC 2006). Exchangeable bases (Ca, Mg, Na, K) were extracted with ammonium (pH 7.0) (IGAC 2006); Ca, Mg Na and K were also quantified by atomic absorption spectrophotometry (AAS) (IGAC 2006). Minor elements (B, Cu, Mn, Fe, Zn) of compost were determined by the Diethylene Triamine Penta Aceticacid (DTPA) method (Lindsay and Norvell 1978). Heavy metals (Cd, Cu, Fe, Pb, Mn, Zn) were determined by AAS by flame (Air-Acetylene, Environmental Protection Agency - EPA 3050B, 3111B SM). For quality control, blank samples were analyzed periodically every ten samples.



Figure 2. Rotary drum composter model.

2.3 Phytotoxicity tests of compost

The phytotoxicity was evaluated by the measurement of GI in *Lactuca sativa* L. seeds (Zucconi et al. 1981). GI was determined by comparing the number of germinated seedlings in a substrate treated with compost, to the number of germinated seedlings in a control substrate. This methodology made it possible to determine the effect of compost on soil and plant quality.

The procedure consists of germinating, in a petri dish, 10 seeds of *Lactuca sativa* L. with the different samples studied. The compost was sterilized, and five extracts were prepared in w/v ratios of 1:5, 1:10, 1:15, 1:20, 1:25, which correspond to the treatment 1 (T1) treatment 2 (T2), treatment 3 (T3), treatment 4 (T4) and treatment 5 (T5) respectively. The extracts were shaken at 200 rpm for 60 minutes and filtered through 0.45 μm pore size membrane. 10 mL of the filtrate was added to Petri dishes (9 cm diameter) containing 20 *Lactuca sativa* L seeds on top of a sheet of filter paper as support. Four repeated Petri dishes were used for each sample, providing a total of 80 test seeds (Fig. 3). Controls with 10 mL of distilled water were used as reference. Seeds were placed in a binder incubator at $20 \pm 0.31^\circ\text{C}$ for 96 h in dark conditions. A completely randomized block experimental design was carried out with four repetitions for each treatment.

Relative Seed Germination (RSG), relative radicle growth (RRG) and GI were measured (Zucconi et al. 1981; Tiquia 2000; Varnero et al. 2007), the indices were calculated by means the Equations 1, 2 and 3 respectively.

$$\text{RSG} = \left(\frac{\text{Number of germinated seeds (extract)}}{\text{Number of germinated seeds (control)}} \right) \times 100 \quad (1)$$

$$\text{RRG} = \left(\frac{\text{Total radicle length of germinated seeds (extract)}}{\text{Total radicle length of germinated seeds (control)}} \right) \times 100 \quad (2)$$

$$\text{GI} = \left(\frac{\text{RSG} \times \text{RRG}}{100} \right) \quad (3)$$

For the result's interpretation it was applied the following criteria established by Zucconi et al.: $\text{GI} \leq 50\%$ high presence of phytotoxic substances; GI between 50% and 80% moderate phytotoxic substances; $\text{GI} \geq 80\%$ There are no phytotoxic substances, or they are in very low concentration (Chau et al. 2003).

2.4 Data analysis

The results of phytotoxicity tests were processed using SPSS software version 23.0 (IBM SPSS Inc., Chicago). Shapiro Wilk test, analysis of means and Tukey test were carried out. The mean differences were compared at the 95% confidence interval level (p -value < 0.05).

3. Results and discussion

3.1 Characterization of the finished compost

Composting was run over a period of 90 days, which, according to Jusoh et al. and Bohórquez et al., is the appropriate time to achieve a high transformation and low moisture content of the organic material (Jusoh et al. 2013; Bohórquez et al. 2014). During the development of the composting process, we observed the four stages of the process known as: (i) mesophilic fermentation, (ii) thermophilic fermentation (iii) cooling step and (iv) maturity stage. Fig. 4, shows the temperature variations registered during the process. In the first stage, mesophilic fermentation occurs, it is characterized by an increase in the compost temperature to approximately 40°C . Mesophilic microorganisms, which thrive between 15°C and 35°C , initially consume the carbohydrates present in organic matter (Sánchez et al. 2017; Sayara et al. 2020). This phase was completed within the first 10 days of the process. Notably, a reduction in the waste volume was observed in the absence of any unpleasant odors or pests.

The second stage, i.e. thermophilic fermentation, begins when microbial metabolic activity, which generates exothermic reactions resulting in a temperature increase ranging

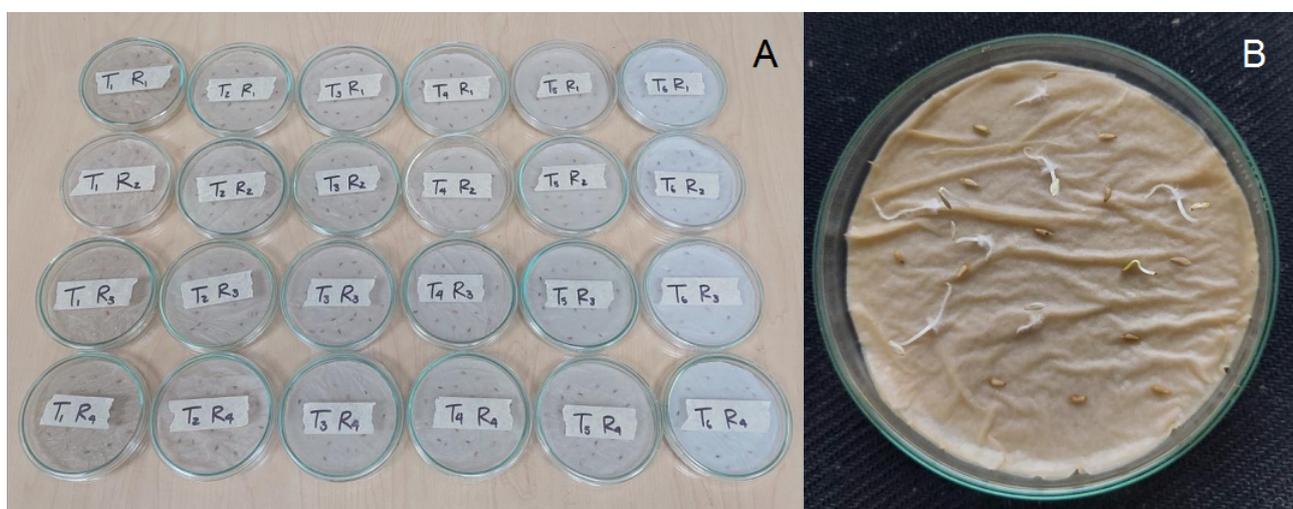


Figure 3. A) Experimental design of the toxicity test in Petri dishes. B) Germinated seeds on *L. sativa* of one of the treatments.

Table 1. Chemical analysis of compost with minor elements (DTPA method) and heavy metals (EPA 3050B). *Minor elements (DTPA) - Heavy metals (total content).

Parameter	Value	Parameter	Value	*Minor elements	Value (mg kg ⁻¹)	*Heavy metals	Value (mg kg ⁻¹)
pH 1:1 ater:soil	8.6	P (ppm)	325.6	B	9.89	Cd	< 0.6
EC (dS m ⁻¹)	7.24	Al cmol(+)/kg ⁻¹	0.10	Cu	4.80	Cu	< 18
Total N %	1.07	K cmol(+)/kg ⁻¹	41	Mn	71.25	Fe	16556
OM %	21.4	Ca cmol(+)/kg ⁻¹	8.12	Fe	235	Mn	236
C %	12.41	Mg cmol(+)/kg ⁻¹	5.62	Zn	98.75	Pb	< 18
C/N ratio	11.59	Na cmol(+)/kg ⁻¹	0.76	S	166.63	Zn	433.4

from 40 to 60°C (Insam and Bertoldi 2007; O'Connor et al. 2021). The thermophilic fermentation was successfully reached within the initial 25 days, during which mesophilic microorganism activity is reduced. Additionally, thermophilic microorganism activity is enhanced allowing the degradation of complex compounds, e.g. proteins. This stage is also crucial to eliminate pathogens (Neklyudov et al. 2008).

The cooling or third stage begins with a decrease in temperature until its equilibrium with the environment (ambient temperature, 25°C). This temperature reduction is facilitated by the decrease in energy within the compost and the reactivation of mesophilic microorganisms, which continues to break down residual sugars (Zeng et al. 2010; Awasthi et al. 2020). Finally, during the maturation stage, compost temperatures reach 26°C and the metabolic activity decreases. The importance of these stages lies in the fact that compounds that are difficult to degrade are broken down to form precursors of humic substances. In this stage, there is approximately a 40% volume reduction (Vélez-Sánchez-Verín et al. 2008) which is in accordance with our observations. The physicochemical nature conditions of the mature compost are shown in Table 1. It has been reported that the

relatively high pH values found (8.6) could affect seed germination (Siles-Castellano et al. 2020). However, it helps to counteract the acid soils of the Piedemonte Llanero de Colombia region that reach 4.8 in cultivation areas (Trujillo-González et al. 2022). OM content of the final product was 21.4%, this value is below that reported by Vargas-Pineda et al. (Vargas-Pineda et al. 2019) in a similar study for the same region and for the ranges described in the literature (30 – 60%) (Fialho et al. 2010). Nevertheless, this final product could be suitable for acid soils with OM levels between 1.5 and 2.2%, which are common values found in the region's soil. N concentrations in any of its forms allow determining the quality of the compost (Siles-Castellano et al. 2020). The total N values were 1.07%, like those obtained by Khater (Khater 2015), and they are considered as acceptable for agri-food waste compost.

The EC value was 7.24 dS m⁻¹, this EC is within the range of agricultural plant residues (4.6 – 18.7 dS m⁻¹) (Chang et al. 2007). In general, composting leads to a reduction in the electrical EC associated with raw materials. This decrease is primarily caused by the release of volatile organic sulphur compounds, the precipitation of mineral salts e.g., phosphates and ammonia magnesium and sulphur ions. Ad-

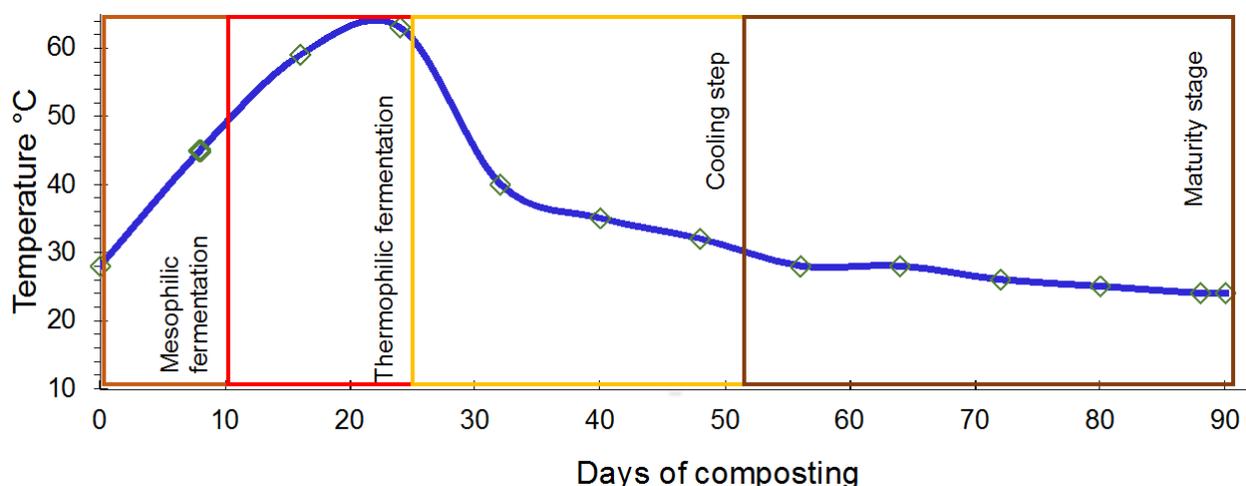


Figure 4. Changes in temperature during composting process.

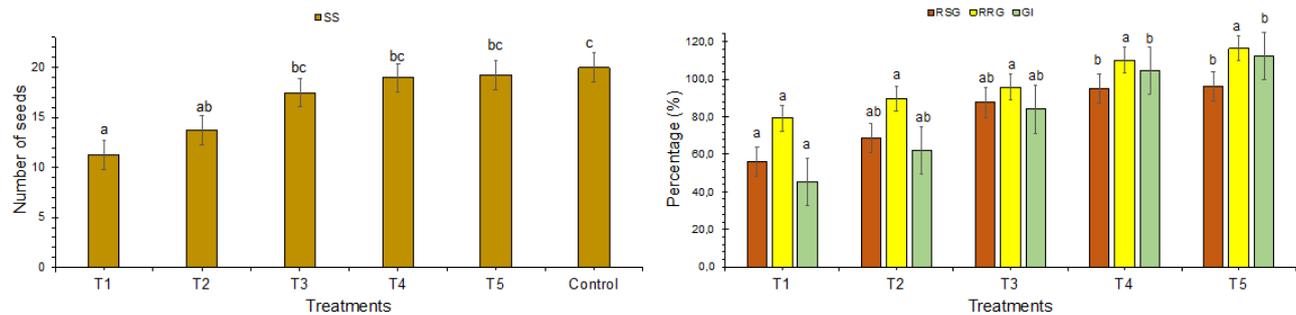


Figure 5. (left) Average results and analysis of means for SS: Sprouted Seeds; (right) Average results and analysis of means for RRG: Relative Seed, Germination, RRG: Relative radicle growth and GI: Germination Index. Equal letters (a and b) indicate the absence of statistically significant difference between the types of treatments.

ditionally the microbial consumption of salts and leaching of compost piles during the decomposition process (Chang et al. 2007; Wu et al. 2010) reduce the EC. Regarding the C/N ratio, it reached 11.59, this value is lower than that obtained by Khater and Rosen et al., who found that C/N ratios in the range of 15:1 to 20:1 are ideal for ready-to-use compost (Khater 2015; Rosen et al. 1993). The available P was considered high in a compost, while Al, K, Ca, Mg and Na showed similar concentration levels to those reported by (Weber et al. 2007; Ait-El-Mokhtar et al. 2022).

The decomposition of organic matter during anaerobic digestion and composting processes can result in an increase in the concentration of heavy metals due to mass loss and volume decrease (Zheng et al. 2022). However, we found values of Pb, Cu below the detection limits (Cd 1 mg kg^{-1} , Cu and Pb 5 mg kg^{-1}), while the concentrations for Zn and Fe were higher.

3.2 Phytotoxicity tests

Fig. 5 shows the results of the phytotoxicity tests, their respective descriptive statistics and analysis of means. According to the Shapiro Wilk test, the results of each of the applied tests presented normal behavior in the data. In the Sprouted Seeds test, the results showed an inverse trend, i.e. as the concentration of the extract decreases, the number of germinated seeds increases. This was verified by the control with all the germinated seeds. With regard to the T1 treatment, the average number of germinated seeds was 11.3. In addition, the treatments T3, T4 and T5 did not show statistically significant differences with respect to the control, which indicates that the germination inhibitor compounds probably did not act on the seeds.

The germination ratios found in our trials were higher than 60%. Also, these values are higher than those reported by (Orlina et al. 2023; Rozas et al. 2023). The RSG relates the germination percentage of the germinated seeds of the different treatments with respect to the germinated seeds in the control. This index showed that the treatments T2, T3, T4 and T5, did not show significant statistical differences in contrast to T1, which represented the extract with the highest concentration. While in the RRG index there was no statistically significant differences in any of the treatments. However, the highest radicle growths occurred at the lowest concentrations, as shown in treatments T3, T4 and T5 which

could demonstrate that the growth-promoting compounds acted at this concentration. The GI results showed that the treatments T3 (83.68%), T4 (104.6%) and T5 (112.16%) exceeded 80%. According to Zucconi et al. criteria, we can assume an extremely low concentration or the absence of phytotoxic substances (Zucconi et al. 1981). Regarding the treatment T1 (44.63%), it presented a high percentage of phytotoxic substances, while T2 treatment showed moderate phytotoxic substances.

4. Conclusion

This study demonstrates the suitability of the compost obtained from peeling-type waste from restaurants of Universidad de los Llanos, Colombia. Through the phytotoxicity tests carried out with *Lactuca sativa*, we discovered a source of high-quality compost. This material represents a valuable alternative to contribute to the management of agri-food waste. We postulate that this material can be highly convenient for its application in the acidic and nutrient-poor soils from Colombian and from other regions of the world. The importance of composting as a circular economy strategy that contributes to sustainable food security is also highlighted. Next steps will consist of studying its performance in Colombian soils.

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Compliance with ethical standards

Conflict of interest: The authors declare that there are no conflicts of interest associated with this study.

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