Post-digestate composting benefits and the role of enzyme activity to predict trace element immobilization and compost maturity

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Corresponding Author:	Biyensa Gurmessa Marche Polytechnic University ITALY
First Author:	Biyensa Gurmessa
Order of Authors:	Biyensa Gurmessa
	Stefania Cocco
	Amanda J. Ashworth
	Ester Foppa Pedretti
	Alessio Ilari
	Valeria Cardelli
	Flavio Fornasier
	Maria Letizia Ruello
	Giuseppe Corti
Abstract:	The current study evaluated the quality of agricultural waste digestate by composting or co-composting with biogas feedstock (maize silage, food processing waste, or poultry litter). Temperature, phytotoxicity, C/N ratio, water extractable trace elements and 14 enzyme activities were monitored for 90 days. Temperature dropped earlier in digestate and maize silage co-composting pile, reducing time to maturity by 20 days. Composting and co-composting reduced phytotoxicity and C/N ratio, but increased immobilization of Al, Ba, Fe, Zn, and Mn at least by 40% in all piles. All the enzyme activities, except arylsulfatase and α-glucosidase, were increased at the maturity phase and negatively correlated with organic matter content and most of trace elements. Post-digestate composting or co-composting with biogas feedstock is a promising strategy to improve digestate quality for fertilizer use, and selected enzyme activities can be indicators of compost maturity and immobilization of trace elements.

07/07/2021

RE: Submission of revised paper

Dear Prof. HUU HAO NGO,

Thanks for inviting us to resubmit our manuscript after revision. We have carefully considered reviewers' comments and made changes to the manuscript, and we believe the current version of the manuscript is significantly improved. We have also modified the title a little bit. Revisions are made following points raised by reviewers, and response is provided under each inquiry. We used red font to indicate the changes we made in the manuscript.

Best regards,

Biyensa

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Reviewer #1: This paper studied the quality of post-digestate compost quality and dynamics of enyme activities on predicting compost maturity and trace elements immobilization. The experiments were carefully carried out, and good results were obtained. This research is of great signification to the utilization of solid digestate. Therefore, the paper is acceptable for publication in Bioresource Technology. However, before acceptance, please consider the following comments and suggestions.

Response: We are grateful to the reviewer for the revisions and suggestions for the manuscript. We have made significant changes to the manuscript following the points of improvements requested by this Reviewer.

1. The contents of manuscript should be checked again, especially the grammar and punctuation.

Response: Thanks. This paper has been thoroughly checked throughout the manuscript and corrections were made where necessary.

2. Awkward sentence make reader confused.

Response: Since we have made major changes to the manuscript, sentences were seemingly confusing have been changed. However, if reviewer specifies which statement needs to be corrected, we will address it in the next round of review.

3. A clear and concise graphical abstract is needed to show readers the main information of the research work.

Response: Thanks. A graphical abstract is now included to this revised manuscript.

4. The format of reference should be carefully checked again.

Response: References have been thoroughly checked and corrections are made where necessary, according to the guidelines.

Reviewer #2: The manuscript number BITE-D-21-03871 entitled "Enzyme activities predict trace elements immobilization and compost maturity during agricultural waste digestate composting" by Gurmessa et al looks at impact of co-composting on post-digestate quality. The study also probes into some fourteen enzyme activities. The presented work addresses one of the important problems of repurposing anaerobic digestion waste (digestate) which is a need of an hour. However, in my opinion, the presented manuscript does NOT seem fit for publication because of the following reasons:

Response: We are thankful to the reviewer for the complements and review suggestions. We admit there were mess up in sections order and apologize for the inconvenience. We have carefully addressed the suggestions by the reviewer and the manuscript now complies with BITE author guidelines . Authors hope that the amended manuscript it deemed more accessible.

1. The manuscript does NOT seem to have followed BITE author guidelines:

Response: Thanks! We have reviewed and made changes to manuscript to comply with the BITE author guidelines.

2. The structure of the manuscript seems a little unusual with first section being "Material and Methods".

Response: Right! We have re-organized the paper accordingly.

3. The sub-section numbering seems totally messed up

Response: This is also corrected in the current version of the MS; thank you for your attention to this.

4. Abstract is poorly written:

Response: Thanks. Abstract is re-written in the current version with significant improvements.

5. Phrases like "The current study set out to.." seem a little unusual from academic writing pointing of view

Response: This is changed to "This study evaluated…" in the revised MS.

6. Abstract demands at least a cursorily glimpse at the methodology adapted - which seems lacking here.

Response: Thanks! We have included the following into the abstract to briefly show the method:

"The current study evaluated the quality of agricultural waste digestate by composting or co-composting with biogas feedstock (maize silage, food processing waste, or poultry litter). Temperature, phytotoxicity, C/N ratio, water extractable trace elements and 14 enzyme activities were monitored for 90 days."

7. There is no closing sentence(s) on future directions, likely impact etc

Response: Thanks! We have now included concluding remark at the end of the abstract:

"Post-digestate composting or co-composting with biogas feedstock is a promising strategy to improve digestate quality for fertilizer use, and selected enzyme activities can be indicators of compost maturity and immobilization of trace elements."

8. Conclusion is also poorly written with NO concrete/quantifiable information.

Response: Conclusion is now rewritten, and significant changes are made. Some quantifiable information is included. In addition, number of words is reduced to less than 100.

9. Too much information compressed in figures 1-5 making data comprehension challenging. Authors might consider splitting them in multiple sub-figures and present only relevant information upfront. For instance, in my opinion, Fig. 5-a could better suit in the supplementary rather than mainframe. This might also help readers grasp information in Fig. 5-b and c effectively.

Response: Thanks. As suggested, authors moved Fig.5 A to supplementary files. Accordingly, Fig 5 now has only two sub-figures. We understand the concern of the reviewer, however, keeping all Fig.4 together is important for reader to understand relationships or differences between activities of the different enzymes. However, if it still is of interest to separate, we can make this change in the next round of revision.

Reviewer # 6: Please carefully check if the paper meets the following points. NON-COMPLIANCE OF THESE POINTS WILL CAUSE A SIGNIFICANT DELAY OF THE PUBLICATION AND MIGHT LEAD REJECTION OF THE MANUSCRIPT.

Response: Thanks. We have checked all the points and made corrections (e.g., conclusions part) where and the paper meets the requirements. We have made changes to conclusions part which previously did not meet the requirement.

- No E-supplement figures and tables (e.g. Fig S1..Table S1,.etc.) quoted in text directly;
  - ➢ Checked. There are no E-supplement figures quoted as Fig S1, Table S1, etc.
- Maximum five (including tables/figures/sub-figures) in Supplementary Material;
  - ➢ Checked. Only one supplementary material is provided.
- Only ONE PERSON as corresponding author;
  - ➢ Only one person is provided as corresponding author for the paper.
- No abbreviations in the title;
  - ➢ There is no abbreviation in the title.
- Maximum 150 words in Abstract;
  - ➢ Abstract is limited to 143 words.
- Maximum 100 words in Conclusion;
  - ➢ This is limited to 100 words.

- Combined results and discussion;
  - This is in accordance.
- Maximum 85 characters, including space for each item of highlights;
  - Checked. Every highlight does not exceed 85 characters including space
- No list of abbreviations or Nomenclatures, except huge number of abbreviations, i.e. in kinetic and modelling works;
  - Checked. No list of abbreviations provided.
- No conventional spectra (X-ray, FTIR, UV, NMR, etc.), SEM photographs, one column simple data table, biochemical (having chemical structures) pathways, simple one-line drawing;
  - Checked. No item of these types is provided in the manuscript.
- No usage of first person (we, our, us);
  - Checked. No usage of such pronouns is found in the manuscript.
- No full justification (e.g. no usage of a constant right-hand margin);
  - Checked. Full justification avoided.
- Maximum 6 Tables and 6 Figures in Research paper,
  - The paper conforms this limitation: has only 5 figures and 3 tables.
- Numbered references in the list of References (to check if followed the limit set by BITE, i.e., Short Communication – Max. 25; Research Article – Max. 50; Review Article- Max. 150);
  - Checked. Number of references is 43.
- No usage of non-English references;
  - Checked. Non-English reference is not used.
- No usage of the Track Changes feature in Microsoft Word in the revised version
  - Checked. Track changes are avoided.

Highlights

- Pilot level aerobic co-composting of digestate and biogas feedstocks was conducted.
- Composting reduced C/N ratio, release of trace elements, and phytotoxicity.
- Enzyme activities were influenced by OM content and release of trace elements.
- Enzyme activities could be compost maturity indicators.

Post-digestate composting benefits and the role of enzyme activity to predict trace element immobilization and compost maturity

Biyensa Gurmessa[1*], Stefania Cocco[1], Amanda J. Ashworth[2], Ester Foppa Pedretti[1], Alessio Ilari[1], Valeria Cardelli[1], Flavio Fornasier[3], Maria Letizia Ruello[4], Giuseppe Corti[1]

[1]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, 60131 Ancona, Italy

[2]USDA-ARS, Poultry Production and Product Safety Research Unit, 1260 W. Maple St. Fayetteville, AR 72701, USA

[3]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (C.R.E.A.-R.P.S.), 34170 Gorizia, Italy

[4]Department of Materials, Environmental Sciences and Urban Planning, Università Politecnica delle Marche, 60131 Ancona, Italy

*Corresponding author: biyensa@gmail.com

Abstract

The current study evaluated the quality of agricultural waste digestate by composting or co-composting with biogas feedstock (maize silage, food processing waste, or poultry litter). Temperature, phytotoxicity, C/N ratio, water extractable trace elements and 14 enzyme activities were monitored for 90 days. Temperature dropped earlier in digestate and maize silage co-composting pile, reducing time to maturity by 20 days. Composting and co-composting reduced phytotoxicity and C/N ratio, but increased immobilization of Al, Ba, Fe, Zn, and Mn at least by 40% in all piles. All the enzyme activities, except arylsulfatase and α-glucosidase, were increased at the maturity phase and negatively correlated with organic matter content and most of trace elements. Post-digestate composting or co-composting with biogas feedstock is a promising strategy to improve digestate quality for fertilizer use, and selected enzyme activities can be indicators of compost maturity and immobilization of trace elements.

Keywords: C/N ratio; compost maturity, food processing waste, maize silage, poultry litter

1. Introduction

In Europe, solid digestate, a byproduct of biogas production, is often directly used as fertilizer although legal status differs among member States. Some have policies that encourage direct use as fertilizer (Beggio et al., 2019; Tambone et al., 2015), while others do not consider it a fertilizer because of possible associated environmental risks such as ammonia emissions, odor, and high content of volatile fatty acids (Nkoa, 2014; PiotrZeng et al., 2016). Low nutrient supply and load of trace elements are other disadvantages associated with direct usage of digestate as fertilizer (Kupper et al., 2014; Torres-Climent et al., 2015). Digestates, especially those originating from manure or a mixture of manure and food processing wastes, can also be source of weeds, pathogens, pharmaceutical residues, and antibiotic resistant genes (Gurmessa et al., 2020).

Trace element loading with digestate at the farm-level could be high owing to continuous land applications as fertilizer (Kupper et al., 2014), thereby posing environmental risks. To avoid these and improve the quality of digestate for its use as an amendment, post-digestate treatment has been proposed (Bustamante et al., 2012; Rehl and Müller, 2011). It can be one possible treatment that has advantages in terms of increasing nutrient content, attenuating trace elements release, reducing the volume of biomass, and mitigating overall environmental risks. For instance, Karwal and Kaushik (2020) found significant reduction of Cd, Cu, Pb, and Zn contents with composting.

Thus, effective post-digestate composting may be sought for industrial level composting, which could involve the use of an appropriate co-composting material that is inexpensive and locally available. Moreover, it is essential to understand the benefits of composting digestate in terms of nutrient release, immobilization of trace elements, and phytotoxicity reduction. In addition to the commonly known compost monitoring indicators such as temperature, C/N ratio, pH, total solids content, total heavy metals content, and germination index (Tang et al., 2020), there is lack of knowledge on the dynamics of enzyme activities during post-digestate composting, which could be indicators of compost functioning, fate of trace elements, and maturity (a ready to use stable compost). Enzyme activities during composting were reported to be one of the best indicators of compost quality and maturity when the activities tend to increase (Wan et al., 2020) or become stable (Mondini et al., 2004) during the maturity phase. However, findings were not consistent, and variations can be due to the differences in the sources of composting materials, the composting period, and enzyme activities.

Thus, the current study is aimed at understanding the role of post-digestate composting and co-composting with biogas feedstock on the final compost quality in terms of nutrient release, immobilization of trace elements, and phytotoxicity. The study is also aimed at investigating: *i*) the role of co-composting materials on post-digestate compost quality and maturity, *ii*) the trends of enzyme activities during composting and their use as proxy to define maturity of post-digestate compost, and *iii*) the relationship between enzyme activities and soluble nutrients and trace elements content during composting.

2. Materials and Methods

2.1. Composting, monitoring, and sampling

The digestate and co-composting materials were obtained from an industrial biogas plant in the Marche region, Italy. Digestate used is a byproduct of anaerobic digestion from a biogas plant that uses 10% poultry litter (85:15 chicken manure: wheat straw ratio; moisture ≈58%) and 90% of other biomass sources such as maize silage and food processing wastes (byproducts of cereal mill and fruit).

Five composting piles, 300 kg each (on wet basis) were prepared as follows: 1) solid digestate only (D00); 2) solid digestate + food processing waste (DCB); 3) solid digestate + maize silage (DMS); 4. solid digestate + poultry litter (DPL); 5) solid digestate + maize silage + poultry litter (DMP). Solid digestate was the target composting material and constituted 80% of the total biomass (w/w) of the piles; the other 20% was made by the co-composting material. The mix-ratio is described in Table 1.

The composting materials were mixed thoroughly and stacked inside high-density polyethylene boxes, each having a volume of 1 m^3. To allow air movement, four holes of 3 cm diameter were drilled at 5 cm and 50 cm from the base on the corners of the box: two from one side and two on the opposite side. Two hard plastic tubes of 2 m length, each with holes every 20 cm, were inserted diagonally from the bottom hole of one corner to the upper hole of the other corner of the box. Piles were turned weekly during the thermophilic phase, every two weeks during the mesophilic period, and every three weeks during the maturity period, following standard industry procedures. Composting lasted for 90 days, and temperature was monitored daily from 4 to 5 o'clock p.m. using a temperature probe (A.M. Leonard Backyard Compost Thermometer) 30 cm depths in each pile; temperature monitoring stopped after 50 days of composting, when similar readings were observed across piles.

Three 1 kg samples were collected from every pile at ≈20 cm depths at 0, 7, 14, 21, 35, 49, 70, and 90 days of composting. The samples were dried at 40 °C and ground until it passed all through a 2 mm sieve. For each fragmented sample, an aliquot of about 100 g was stored at 4 °C for < 10 days to be analyzed for enzyme activities, while the rest of the sample was stored at room temperature for the physicochemical analyses.

2.2. Main physicochemical characteristics

The pH was determined potentiometrically in H_2O (1:8 w/v) after one night of contact time. Total solid (TS) content was estimated as the fraction of dry mass remaining after samples were dried at 105 °C for 24 h. The organic matter (OM) content was determined as the loss on ignition at 550 °C until a constant weight was reached (Heiri et al., 2001). Total C and N were determined by the dry combustion method using a CHNS analyzer (EA-1110, Carlo Erba Instruments, Milan, Italy).

2.3. Enzyme activities

The activities of 14 enzymes were determined according to the method described in Cardelli et al. (2019). Briefly, 150 mg of specimen was placed in a 2-ml Eppendorf tube with glass beads containing 1.2 ml of 50 mM tris–HCl solution at pH 7 containing 2% lysozyme as a desorbing protein. The tube was then subjected to bead-beating (3 min, 30 strokes s^{-1}) using a Retsch MM400 mill (Haan, Germany) and centrifuged for 5 min at 20,000 *g*. Enzyme activities were analyzed fluorometrically in microplates using 4‐methyl‐umbelliferyl and 7‐amino‐4‐methyl coumarine conjugated surrogate substrates (Sigma, St. Louis, MO, USA) at three pH ranges in three different solutions. Activities of acid phosphomonoesterase, α-glucosidase, arylsulfatase, β-glucosidase, β-galactosidase, cellulase, chitinase, glucuronidase, and xylosidase were determined in 200 mM MES (morpholineptansulfonic acid) solution at pH 5.8; whereas, the activities of leucine aminopeptidase, lipase nonanoate-esterase, pyrophosphatase-phosphodiesterase, and phosphodiesterase were determined in 100 mM tris–HCl solution at pH 7.5. The alkaline phosphomonoesterase activity was determined in 100 mM tris–HCl solution at pH 9.0.

2.4. Water-extractable elements

Ten g of each sample was added to 100 ml of distilled water (1:10 w/v) and shaken for about 1 h. Then, the suspension was centrifuged for five minutes at 300 *g* and the solution was filtered using a Whatman 42 filter. The amounts of macronutrients (Ca, K, Mg, P, and S) and trace elements (Al, Ba, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) in the extracted solution were determined using Inductively Coupled Plasma Spectroscopy.

2.5. Phytotoxicity test

Germination index (GI) was used to evaluate the phytotoxicity of the original solid digestate (experimental control) and of the composting digestate at 7, 21, 51, and 90 days of composting. The experiment was conducted in a completely randomized design with three replications. One g of digestate or composting material was added to 5 ml deionized water in a 50 ml tube and shaken for 2 hours; the suspension was then centrifuged for 5 minutes at 300 *g*, and the solution filtered through a Whatman 42 filter. The germination test was conducted on petri dishes of 8.5 cm diameter using cress (*Lepidium sativum* L.) seeds, which are highly recommended because of their high sensitive to even low toxicity (Luo et al., 2018). Two Whatman™ 1442-125 grade 42 filter papers were moistened by applying 1 ml of the extract solution, whereas deionized water was used as a control. One filter paper was placed on the petri dish and 10 seeds were placed per dish. Then, the second filter paper was used to cover seeds to protect them from moisture loss. All Petri dishes were closed, sealed to avoid moisture loss, and incubated in the dark at temperature of 25 °C. Germinated seeds were counted after 48 hours of incubation, and root length was measured using a digital caliper. GI was estimated as the percentage of seeds germinated multiplied by the average root length in the treated Petri dishes in relation to the number of seeds germinated multiplied by the average root length in the control Petri dishes.

2.6. Statistical analysis

Statistical analysis and visualizations were conducted using R packages. Pearson's correlation analysis was conducted and plotted between enzyme activities and other chemical compositions using the CorLevel package in R. Redundancy analysis (RDA) was conducted using Vegan package (Oksanen et al., 2019) in R for Windows, v. 4.0.1. and plotted using ggpot2 to understand the relationship between enzyme activities (response variables) and pH, OM, total C, and water extractable macronutrients and trace elements (environmental variables). The environmental variables were log transformed, whereas response variables were Hellinger transformed. PERMANOVA test was run to evaluate the statistical significance of the interaction effects of the environmental variables on response variables. The significance of composting on release and immobilization of elements were computed as the additional amount released or the amount reduced in the final composts as percentage of the initial concentration.

3. Results and discussion

3.1. Physicochemical characteristics of the digestate and co-composting materials

In Table 2, characteristics of digestate and co-composting materials are reported. The solid digestate had sub-alkaline pH (8.1), which was similar to previously studied digestate originating from feedstock mixtures containing pig-slurry, but higher than that of cattle slurry (Alburquerque et al., 2012a). In contrast, the pH of poultry litter was neutral, while it was slightly acidic for maize silage and food processing waste. Digestate and maize silage had the lowest TS content

(38-41%), while food processing waste was the greatest (94%). OM content was the highest in food processing waste and maize silage, but the organic C content was similar in the four materials. Digestate mean C/N ratio was ≈26 (similar to that of maize silage), and exceeded the limit of <25 in use in several countries including Italy (Tambone et al., 2015) for direct use as fertilizer. This result was greater than that of by Alburquerque et al. (2012b), indicating possible variations among the different sources of digestate. Digestates with C/N values greater than 20, meaning with excess of degradable organic C, could lead to immobilization of N (Teglia et al., 2011a), and may undermine the agronomic benefits of direct use of digestate. The lowest C/N ratio of the poultry litter was due to greater total N content. Water extractable macro-nutrients and trace elements mainly abounded in the poultry litter. Instead, digestate showed the highest content of Fe and the lowest of Mg, P (together with maize silage), and Ba.

3.2. Changes of Temperature, pH, OM, total N, C/N, and GI during composting

In Fig. 1, temperature, pH, OM, total C, total N, C/N ratio, and GI changes are presented. These variables govern compost dynamics over the composting period and are also indicators of maturity. Except for D00, temperatures of all other piles increased to above 60 °C in the first two weeks of composting, but dropped quickly for DMS, thus reducing time to maturity by about 20 days compared to the control. The thermophilic phase lasted for about 49 days for D00, DCB, DPL, and DMP, and maturity was delayed. The pH decreased from alkaline to neutral in the control pile, while it increased from slightly acidic to neutral in DCB. The pH changes in other piles were minimal. Both total C and OM showed a decreasing trend in all the piles, but the rate was lowest in D00 compared to the rest of the piles, suggesting the significance of the co-composting materials. Total N increased throughout the composting period with higher rates in all the piles with co-composting materials compared to the D00 pile. The C/N ratio decreased over the experimental period in all piles, reaching the value of 17 or lower in the final composts, indicating compost maturity.

GI increased in all piles over the composting period, but reached more than 60% only in DCB, DMS, and DMP, suggesting the significance of co-composting for reducing phytotoxicity of digestate. However, even after 90 days of composting, compared to the other piles, GI of D00 did not reach a satisfactory level, indicating the possible occurrence of phytotoxicity, especially when sensitive crops or vegetables are grown using digestate as fertilizer. A previous study also reported possible ecotoxicity of digestate (Tigini et al., 2016), and our results suggest post-composting digestate, and co-composting materials may help mitigate phytotoxicity. A mature compost should have GI of 50% or more (Bernal et al., 2009), but it is commonly recommended to be 60% or more, which is generally believed to be indicator for a low toxicity level (Tambone et al., 2015), although there are differences among the seed types used for the test. The relatively low GI found in this study was similar to previous findings can be linked to the sensitivity to toxicity of the cress seeds used for the test, which is high even at low toxicity levels (Luo et al., 2018).

3.3. Composting effect on macronutrients release

The trends of water extractable values showed disparities among macronutrients during the composting period (Fig. 2). K and P showed an increasing trend in all the piles, while Mg showed an increasing trend only in D00. Instead, Ca displayed an inverse relationship in DCB and DPL, with few changes observed in D00. S concentration was reduced in DPL, while little changes were observed in the other piles.

Total N had increasing trends in all the piles and increased by about 30% in DPL and more than 50% in the other piles, suggesting a benefit from co-composting materials in reducing loss or increasing mineralization of N during composting compared to the control. Previous research reported the recovery of N and other macro nutrients from wastes following composting (Rai and Suthar, 2020). Water extractable (available) was P enriched by 25, 30, 51, and 62% in the final composts for DCB, DMP, D00, and DMS, respectively (Table 3). On the other hand, DPL had little effect on P release, despite it having the highest amount of available P. Previous studies reported increases in available P release with composting, although the rate varies depending on the composted materials and the composting time (Sharma et al., 2018).

In the current study, S content in the DPL pile was the greatest, although it gradually decreased over the composting period, suggesting the main source of S was poultry litter. The decreased in S content in all the piles over the composting period may be linked to the loss in the form of H_2S (Blazy et al., 2014). S breakdown during composting (and subsequent odor release) is sought to be mitigated to reduce S loss and pollution risk.

3.4. Composting effect on trace elements release

Water extractable contents of Ba, Cu, Fe, Mn, Ni, and Zn showed a decreasing trend in all the piles (Fig. 3), indicating immobilization with increased OM stability during composting. More than 60% of Al, Ba, Fe, Mn, and Zn was immobilized with composting across the piles (except Al for DPL), while it was >40% for Ni; Cu showed the maximum immobilization (57%) in D00. In contrast, Pb showed little change, and Cd was enriched for all treatments except DPL (Table 3). Concentrations of water extractable Al, Ba, Cu, Fe, Mn, Ni, and Zn were reduced by 16-93, 74-95, 25-55, 75-89, 60-74, 42-70, and 66-85%, respectively. DPL was the least effective in reducing Al, Cu, and Fe release compared to the other treatments. This may be ascribed to the lower C/N ratio compared to the other piles (Wu et al., 2017). Cd level increased after the first week of composting in all the piles, except DPL, and remained constant until the maturity period. Pb mineralization or immobilization was little in all piles, suggesting composting digestate did not influence the release or immobilization of Pb. This could also be because of the relatively little concentration found in the co-composting and digestate materials.

Immobilization of trace elements is one of the benefits of composting. However, like the case of Cd in the current study, mineralization of trace metals could also be possible with post-digestate composting (Miaomiao et al., 2009). A previous study by Awasthi et al. (2020) reported that composting was effective in immobilizing Cu and Zn with biochar as a bulking agent, indicating the importance of co-composting materials. Our study also gives an insight that stabilizing digestate with post-composting potentially stabilizes the material and reduces the release of trace elements, but also suggested careful selection of co-composting materials for optimization of nutrient digestate nutrient levels.

3.5. Effect of composting on enzyme activities

Among the 14 enzyme activities evaluated, arylsulfatase and xylosidase activities were either absent or very low in all piles over the composting period (Fig. 4). In contrast, acid phosphomonoesterase, alkaline phosphomonoesterase, chitinase, and pyrophosphate/phosphodiesterase showed clear increasing trends in all piles during the 90 days of composting, with the greatest values achieved during the maturity phase, thus implying their role in depolymerizing the most complex polysaccharides of the composting materials. The greatest activity was found for leucine aminopeptidase (11886 nmol kg^{-1} h^{-1}) in DMS during the second week of composting, followed by lipase nonanoate-esterase (9379 nmol kg^{-1} h^{-1}) and alkaline phosphomonoesterase (7250 nmol kg^{-1} h^{-1}) in DCB during the maturity phase. Acid phosphomonoesterase, α-glucosidase, β-galactosidase, β-glucosidase, glucuronidase, cellulase, and chitinase reached their peak values during the thermophilic phase, whereas pirophosphate and alkaline phosphomonoesterase, reached their peak during the maturity phase. Increased levels of activities of these latter two enzymes, indicates depletion of major unstable organic matter components (Herrmann and Shann, 1993), and may be indicators for stability of composts.

Some of our results agreed with the findings of Karwal and Kaushik (2020), Tiquia (2002), and Herrmann and Shann (1993), who conducted composting experiments with mixtures of buffalo dung and fly ash, manure, and municipal solid waste for 90, 154, and 90 days, respectively. In contrast, Castaldi et al. (2008) found a decreasing trend for dehydrogenase, urease, protease, and cellulase at maturity phase during municipal solid waste composting that lasted for 40 days. Ge et al. (2020) reported reduced activities of both alkaline and acid phosphatase during 60 days of cattle manure composting, but the trend of cellulase activity was consistent with our findings. This shows that enzymatic activity may serve as an indicator of compost maturity and stability, but with extended length of composting period (≥ 90 days) and when the most degradable organic materials are exhausted.

There were variations among the different co-composting materials. Interestingly, enzymatic activities in DPL changed little over the composting period, indicating the possible presence of inhibitory substances in the poultry litter. Increases in the activities of different enzymes like acid phosphomonoesterase, alkaline phosphomonoesterase, β-glucosidase, β-galactosidase, chitinase, and pyrophosphate/phosphodiesterase activities indicated maturity of composts, but their functional roles are different and, thus, they can be taken indicators of different chemical processes taking place during the composting process. For instance, cellulase are important indicators for cellulose degradation, which is expected to rise during the maturity stage of composting as easily degradable organic substances have already been degraded by microbes at the early stage of composting (Li et al., 2020). Cellulase had the highest activity in D00 and maintained levels until the end of composting, thus implying low availability for microbes.

3.6. Correlations and RDA analysis

Alkaline phosphomonoesterase, Chitinase, Glucuronidase, Leucine aminopeptidase, and Pyrophosphate/phosphodiesterase, and activities had strong positive correlation (p<0.05) with available P. Most of the enzyme activities had a negative correlation with C/N ratio, S, and most of the trace elements except Cd. E-supplementary data of this work can be found in online version of the paper. The correlation was moderately strong with Al, Ba, Cu, Fe, Ni, Mn, and Zn, indicating that an increase of trace elements release during the early period of composting might inhibit enzyme activities (Aponte et al., 2020), which further suggests increased enzyme activities at later composting stages could be indicators of stability and immobilization of trace elements. A similar strong negative correlation of trace elements with enzyme activities was reported to occur during a cow manure vermicomposting by Malley et al. (2006) . However, in this study, positive correlations were found between Cd and all enzyme activities, implying that it could be rather stimulatory. Unlike all the other trace elements, Pb showed weak and positive correlations, possibly because Pb release was not affected during composting. RDA analysis of the enzyme activities as response variables and pH, OM, C/N ratio, and macro-nutrients as environmental factors showed a variance of 70.8 explained by RDA1 and RDA2 (Fig. 5B). The PERMANOVA test conducted showed the contribution of variance explained by RDA1 was significant (p<0.01), but RDA2 was not. In Fig. 5C, trace elements explained 69.5% of total enzyme activity variation with RDA1 and RDA2. The overall results showed the significance of organic matter dynamics and the release or immobilization of elements in predicting the enzyme activities.

4. Conclusions

Post-digestate composting and co-composting reduced C/N ratio to 11-17. Phytotoxicity was reduced and greatest GI (76%) was obtained by co-composting with food processing waste. Up to 90% trace elements immobilization was found, and better results were obtained by co-composting with maize silage and food processing waste. Many of the 14 enzyme activities were low or absent in digestate but increased in the final composts. Alkaline phosphomonoesterase, and Pyrophosphate/phosphodiesterase had strong negative correlations (p<0.05) with Ba, Cu, Fe, Mn, Ni, and Zn. The overall findings suggest the significance of post-digestate co-composting in improving quality and enzyme activities as compost maturity index.

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Table 1. Composition of the compost piles.

Pile	Composition	Mix ratio (w/w)
D00	Solid digestate only	-
DCB	Solid digestate + food processing waste	4:1
DMS	Solid digestate + Maize silage	4:1
DPL	Solid digestate + Poultry litter[¥]	4:1
DMP	Solid digestate + Maize silage + Poultry litter[¥]	8:1:1

[¥] The poultry litter was 85:15 chicken manure: wheat straw ratio; moisture ≈58%.

Table 2. Physicochemical properties of digestate and the co-composting materials (Mean ± SD) (n = 3). For each parameter, mean values with different letters significantly differ ($p < 0.05$).

Variable	Digestate	Food processing waste	Maize silage	Poultry litter	*P*-value
pH	8.1 ± 0.0a	5.8 ± 0.1d	6.2 ± 0.0c	7.2 ± 0.1b	<0.001
TS (g kg^{-1})	384 ± 42c	941 ± 45a	413 ± 17c	565 ± 44b	<0.001
OM (g kg^{-1})	837 ± 2b	956 ± 1a	958 ± 0a	828 ± 0c	<0.001
Total C (g kg^{-1})	390 ± 35	416 ± 3	415 ± 6	387 ± 2	0.15
Total N (g kg^{-1})	15.1 ± 1.0b	18.2 ± 1.1b	16.1 ± 1.6b	35.3 ± 3.4a	<0.001
C/N ratio	25.8 ± 2.7a	22.9 ± 1.3a	25.8 ± 2.2a	11.0 ± 1.1b	<0.001
Ca (g kg^{-1})	3.0 ± 0.3c	0.8 ± 0.0d	6.0 ± 0.3b	12.6 ± 0.2a	<0.001
K (g kg^{-1})	141.0 ± 6.4b	36.2 ± 0.9d	83.5 ± 4.3c	259.6 ± 5.3a	<0.001
Mg (g kg^{-1})	2.9 ± 0.1d	4.2 ± 0.1c	7.7 ± 0.2b	15.3 ± 0.1a	<0.001
P (g kg^{-1})	10.2 ± 0.2c	17.9 ± 0.5a	10.2 ± 0.1c	14.4 ± 0.2b	<0.001
S (g kg^{-1})	11.7 ± 0.3b	2.0 ± 0.1d	4.4 ± 0.1c	57.5 ± 0.3a	<0.001
Al (mg kg^{-1})	108.2 ± 4.0b	145.9 ± 10.5a	113.1 ± 6.0b	143.1 ± 4.3a	<0.001
Ba (mg kg^{-1})	0.1 ± 0.0c	1.7 ± 0.5b	1.3 ± 0.3b	11.5 ± 0.3a	<0.001
Cd (mg kg^{-1})	0.8 ± 0.1b	1.2 ± 0.1ab	0.7 ± 0.1b	1.6 ± 0.3a	0.001
Cu (mg kg^{-1})	39.2 ± 1.5b	10.2 ± 0.3c	9.1 ± 0.2c	130.1 ± 0.4a	<0.001
Fe (mg kg^{-1})	1525.0 ± 65.9a	93.0 ± 4.9c	60.6 ± 3.1c	552.2 ± 1.5b	<0.001
Mn (mg kg^{-1})	28.8 ± 1.0b	20.7 ± 0.4b	25.9 ± 2.5b	141.9 ± 5.8a	<0.001
Ni (mg kg^{-1})	11.6 ± 0.1b	1.5 ± 0.2c	0.3 ± 0.3d	44.1 ± 0.4a	<0.001
Pb (mg kg^{-1})	36.7 ± 3.4	34.4 ± 2.4	35.4 ± 1.8	36.6 ± 2.6	0.672
Zn (mg kg^{-1})	95.3 ± 14.6b	41.1 ± 0.3c	4.3 ± 0.1d	258.0 ± 20.6a	<0.001

TS = total solid; OM = organic matter on a dry mass basis.

Table 3. Immobilization and release of trace elements and nutrients expressed as a percentage of the amount immobilized or released over the composting period from the concentration of the mix (mean ± SD) (n = 3). Negative values indicate immobilization, positive values indicate release.

Pile	D00	DCB	DMS	DPL	DMP
Ca	-9.1 ± 4.5	-53.5 ± 3.0	-22.3 ± 0.6	-50.9 ± 2.2	-33.0 ± 6.2
K	28.3 ± 3.9	51.3 ± 4.8	28.4 ± 2.8	-1.0 ± 1.5	-3.3 ± 1.2
Mg	124.2 ± 5.7	-38.5 ± 0.2	7.2 ± 1.5	-19.0 ± 2.4	-22.9 ± 3.6
P	50.7 ± 2.7	25.4 ± 3.0	61.8 ± 3.1	-1.5 ± 0.4	29.8 ± 6.7
S	-52.9 ± 1.6	2.6 ± 2.3	-47.7 ± 1.1	-18.1 ± 0.5	-32.4 ± 2.3
Total N	26.1 ± 16.2	89.8 ± 16.4	92.9 ± 14.2	36.7 ± 6.8	64.0 ± 15.6
Al	-93.4 ± 0.3	-94.6 ± 0.3	-93.6 ± 0.4	-16.2 ± 6.6	-93.2 ± 1.5
Ba	-74.8 ± 1.0	-95.3 ± 0.8	-91.3 ± 1.4	-97.7 ± 0.0	-93.8 ± 0.1
Cd	586.0 ± 76.1	475.9 ± 23.7	593.2 ± 83.9	-34.3 ± 8.6	528.9 ± 77.9
Cu	-55.7 ± 2.2	-47.9 ± 4.1	-43.2 ± 2.4	-25.7 ± 1.7	-49.4 ± 0.2
Fe	-89.3 ± 0.5	-82.3 ± 0.6	-86.5 ± 0.4	-75.3 ± 0.5	-85.9 ± 0.5
Mn	-74.6 ± 0.9	-60.6 ± 1.9	-68.6 ± 0.5	-72.6 ± 1.2	-72.3 ± 0.4
Ni	-42.5 ± 4.9	-42.6 ± 2.3	-40.1 ± 5.2	-70.4 ± 0.9	-71.0 ± 5.7
Pb	3.8 ± 16.8	-0.6 ± 4.7	-1.7 ± 8.4	-3.2 ± 10.2	-10.8 ± 1.8
Zn	-85.5 ± 2.2	-70.9 ± 3.9	-66.3 ± 4.1	-93.9 ± 1.0	-73.8 ± 2.5

D00 = solid digestate only; DCB = solid digestate + food processing waste; DMS = solid digestate + maize silage; DPL = solid digestate + poultry litter; DMP = solid digestate + maize silage + poultry litter.

Figure captions

Fig.1. Trend of temperature, pH, total C, C/N ratio, organic matter (OM), and germination index (GI) during the composting period. D00 = solid digestate only, DCB = solid digestate + food processing waste, DMS = solid digestate + maize silage, DPL = solid digestate + poultry litter, DMP = solid digestate + maize silage + poultry litter.

Fig. 2. Dynamics of macro-nutrients during the composting period. D00 = solid digestate only, DCB = solid digestate + food processing waste, DMS = solid digestate + maize silage, DPL = solid digestate + poultry litter, DMP = solid digestate + maize silage + poultry litter.

Fig. 3. Changes in the content of trace elements during the composting period. D00 = solid digestate only, DCB = solid digestate + food processing waste, DMS = solid digestate + maize silage, DPL = solid digestate + poultry litter, DMP = solid digestate + maize silage + poultry litter.

Fig. 4. Enzyme activity dynamics over the composting period. acP: acid phosphomonoesterase; alkP: alkaline phosphomonoesterase; alfaG: α-glucosidase; aryS: arylsulfatase; betaG: β-glucosidase; betaGAL: β-galactosidase; bisP: phosphodiesterase; cell: cellulase, chit: chitinase; leu: leucine aminopeptidase; nona: lipase nonanoate-esterase; piroP: pyrophosphate/phosphodiesterase; uroni: glucuronidase; xilo: xylosidase. D00 = solid digestate only, DCB = solid digestate + food processing waste, DMS = solid digestate + maize silage, DPL = solid digestate + poultry litter, DMP = solid digestate + maize silage + poultry litter.

Fig. 5. Redundancy analysis of enzyme activities in relation to environmental factors (A: pH, C/N ratio, OM, and macro-nutrients; B: trace elements). acP: acid phosphomonoesterase; alkP: alkaline phosphomonoesterase; alfaG: α-glucosidase; aryS: arylsulfatase; betaG: β-glucosidase; betaGAL: β-galactosidase; bisP: phosphodiesterase; cell: cellulase, chit: chitinase; leu: leucine aminopeptidase; nona: lipase nonanoateesterase; piroP: pyrophosphate/phosphodiesterase; uroni: glucuronidase; xilo: xylosidase. D00 = solid digestate only, DCB = solid digestate + food processing waste, DMS = solid digestate + maize silage, DPL = solid digestate + poultry litter, DMP = solid digestate + maize silage + poultry litter.

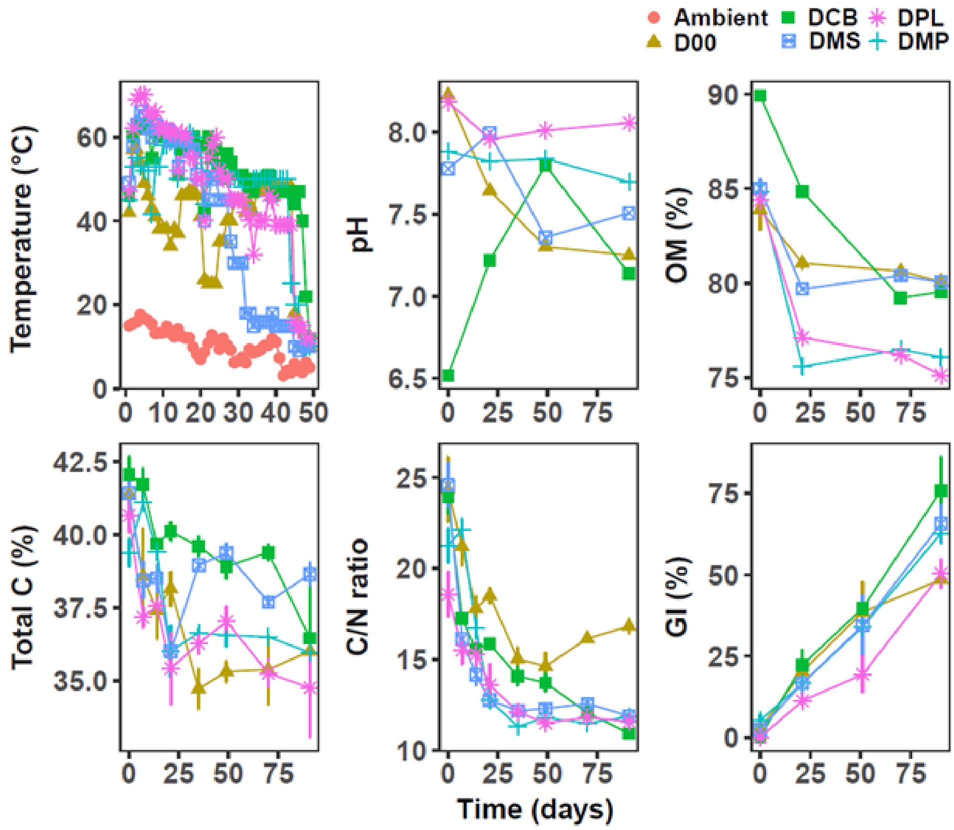

Ambient
DCB
DPL
D00
DMS
DMP
Temperature (°C)
pH
OM (%)
Total C (%)
C/N ratio
GI (%)
Time (days)

Fig. 1.

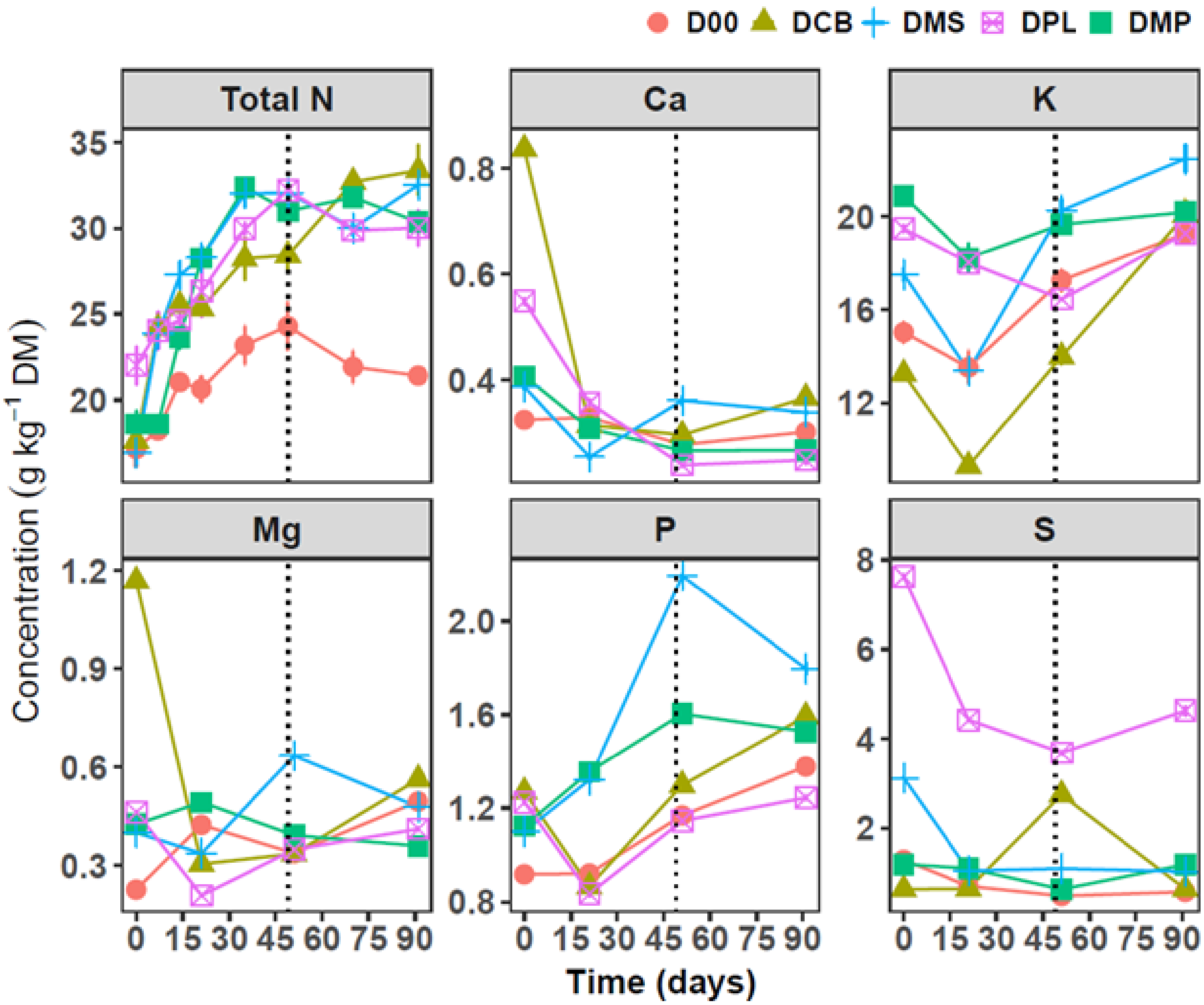
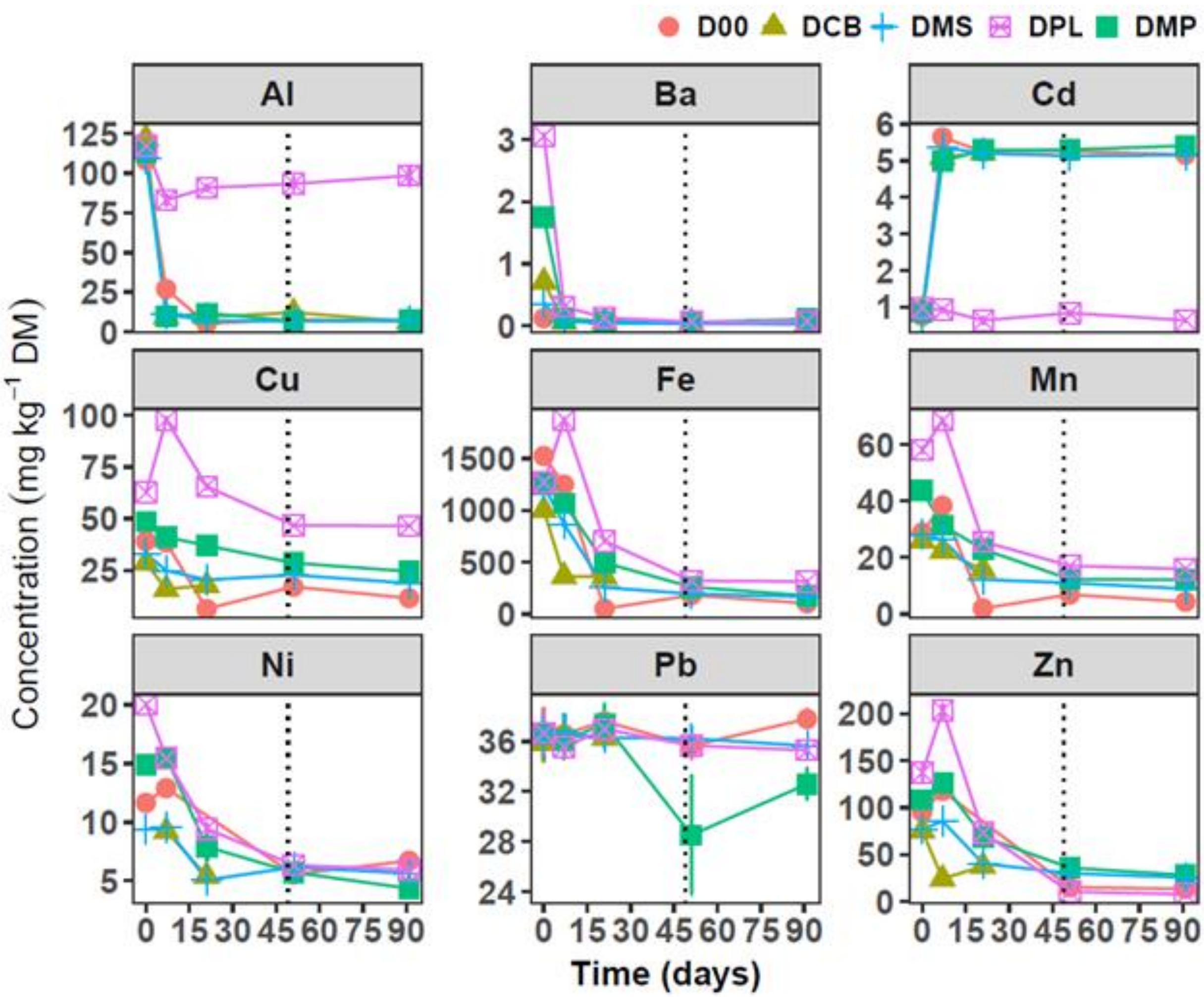

D00
DCB
DMS
DPL
DMP
Total N
Ca
K
Mg
P
S
Concentration (g kg^{-1} DM)
Time (days)
0 15 30 45 60 75 90
35
30
25
20
0.8
0.6
0.4
20
16
12
1.2
0.9
0.6
0.3
2.0
1.6
1.2
0.8
8
6
4
2

Fig. 2.



D00
DCB
DMS
DPL
DMP
Al
Ba
Cd
Cu
Fe
Mn
Ni
Pb
Zn
Concentration (mg kg^{-1} DM)
Time (days)

Fig. 3.

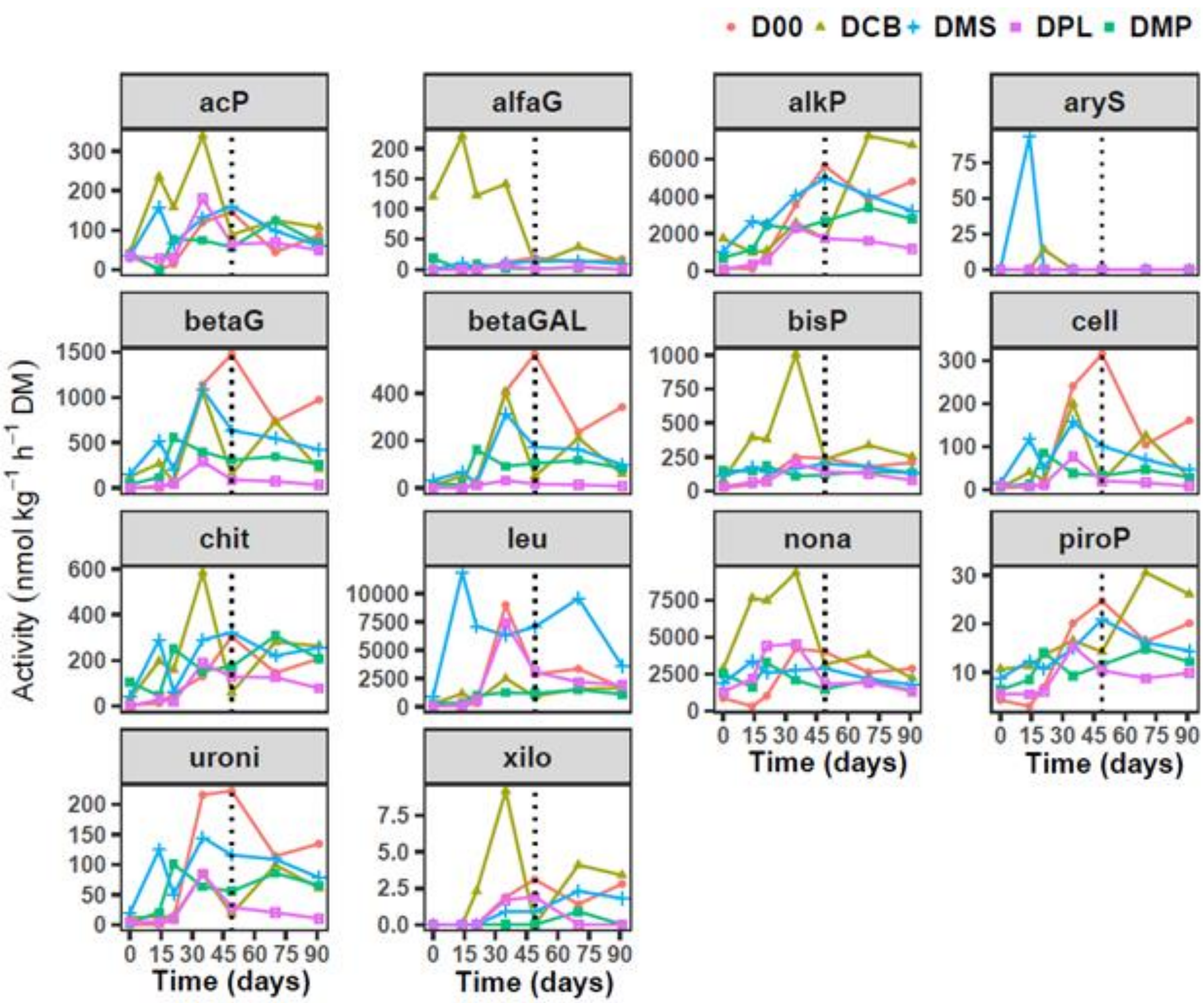

D00
DCB
DMS
DPL
DMP
acP
alfaG
alkP
aryS
betaG
betaGAL
bisP
cell
chit
leu
nona
piroP
uroni
xilo
Activity (nmol kg^{-1} h^{-1} DM)
Time (days)

Fig. 4.

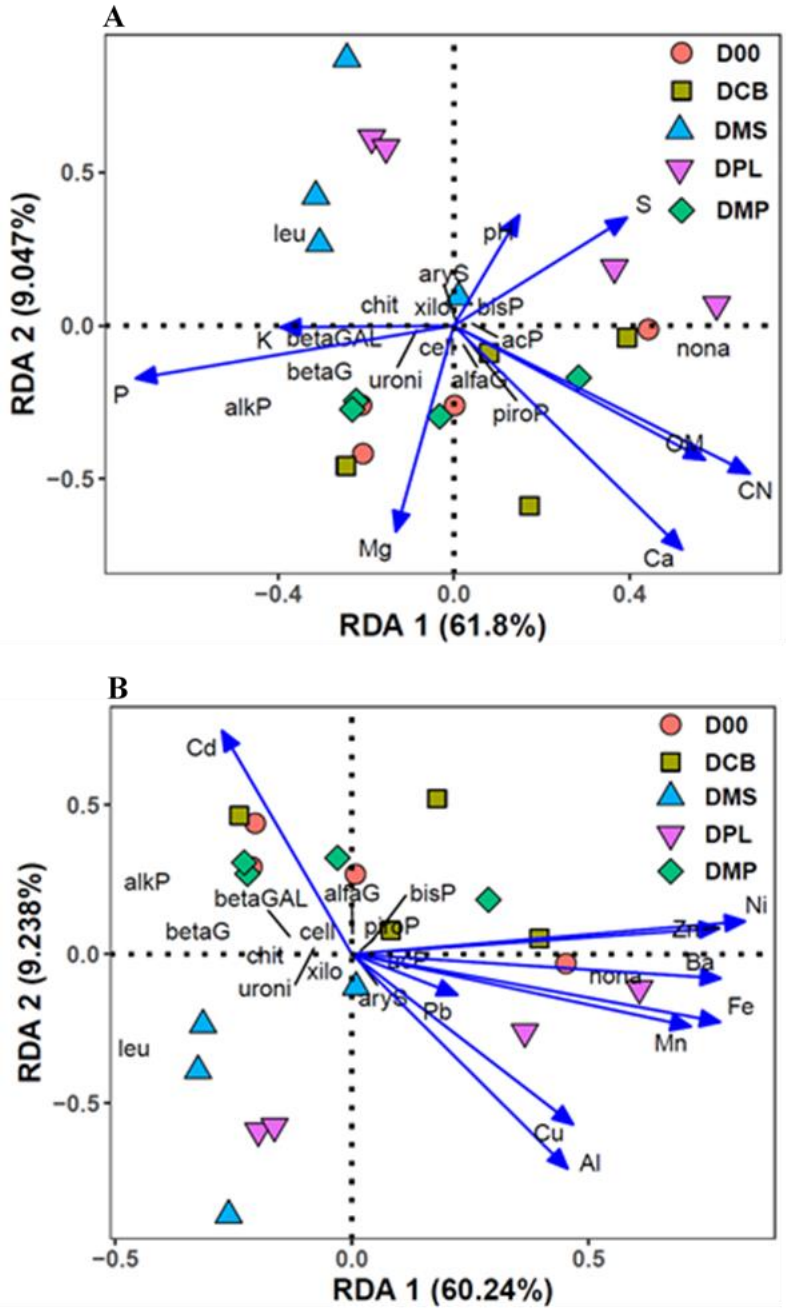
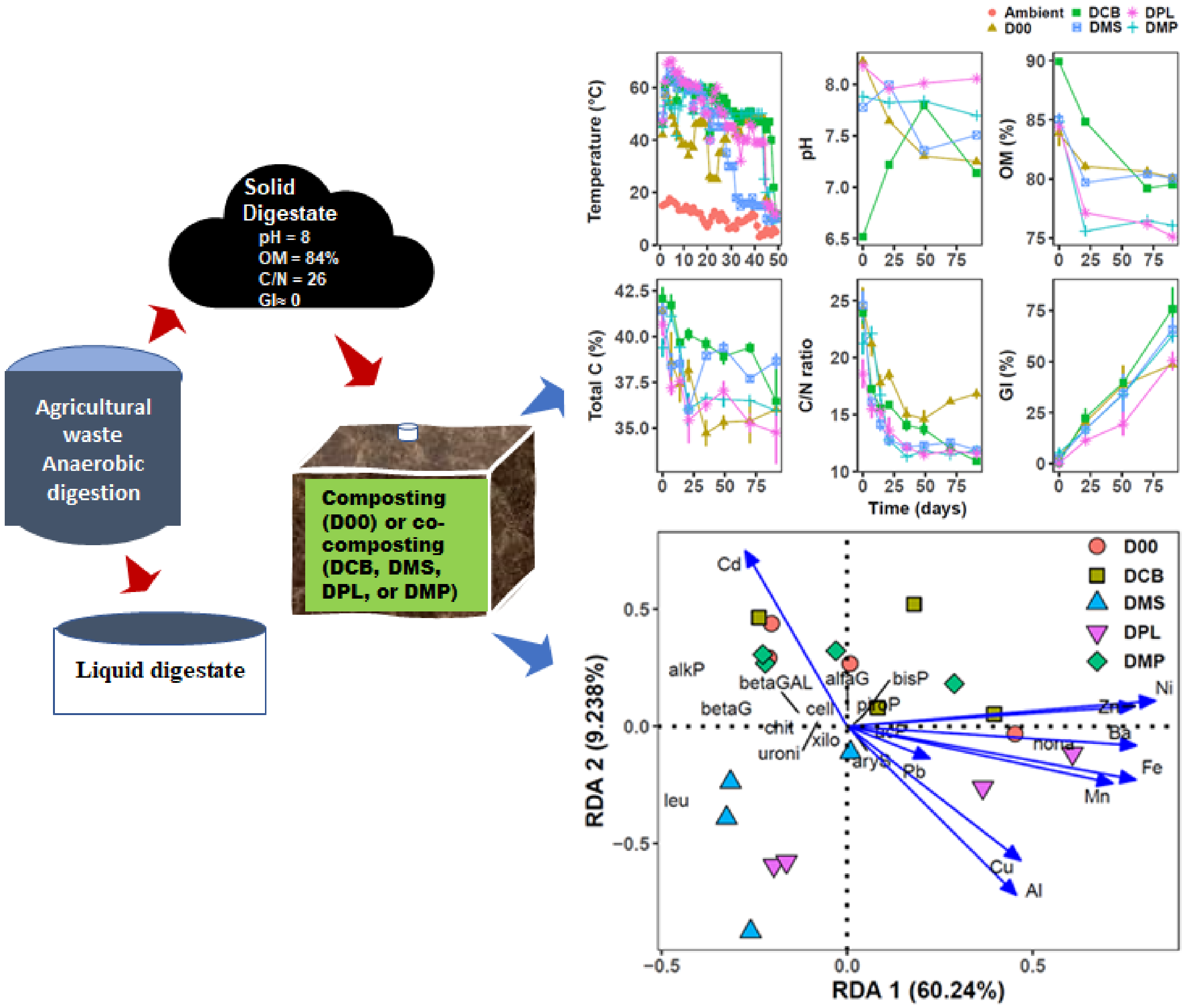

A
D00
DCB
DMS
DPL
DMP
RDA 2 (9.047%)
RDA 1 (61.8%)
0.5
0.0
−0.5
−0.4
0.0
0.4
leu
ph
S
aryS
chit
xilo
bisP
K
betaGAL
acP
cell
betaG
uroni
alfaG
P
alkP
piroP
nona
OM
CN
Mg
Ca
B
D00
DCB
DMS
DPL
DMP
RDA 2 (9.238%)
RDA 1 (60.24%)
0.5
0.0
−0.5
0.0
0.5
Cd
alkP
betaGAL
alfaG
bisP
betaG
cell
chit
xilo
uroni
aryS
Pb
Ni
Zn
Ba
Fe
Mn
nona
leu
Cu
Al

Fig. 5.

Graphical Abstract



Post-digestate composting benefits and the role of enzyme activity to predict trace element immobilization and compost maturity

Biyensa Gurmessa[1*], Stefania Cocco[1], Amanda J. Ashworth[2], Ester Foppa Pedretti[1], Alessio Ilari[1], Valeria Cardelli[1], Flavio Fornasier[3], Maria Letizia Ruello[4], Giuseppe Corti[1]

[1]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, 60131 Ancona, Italy

[2]USDA-ARS, Poultry Production and Product Safety Research Unit, 1260 W. Maple St. Fayetteville, AR 72701, USA

[3]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (C.R.E.A.-R.P.S.), 34170 Gorizia, Italy

[4]Department of Materials, Environmental Sciences and Urban Planning, Università Politecnica delle Marche, 60131 Ancona, Italy

*Corresponding author: biyensa@gmail.com

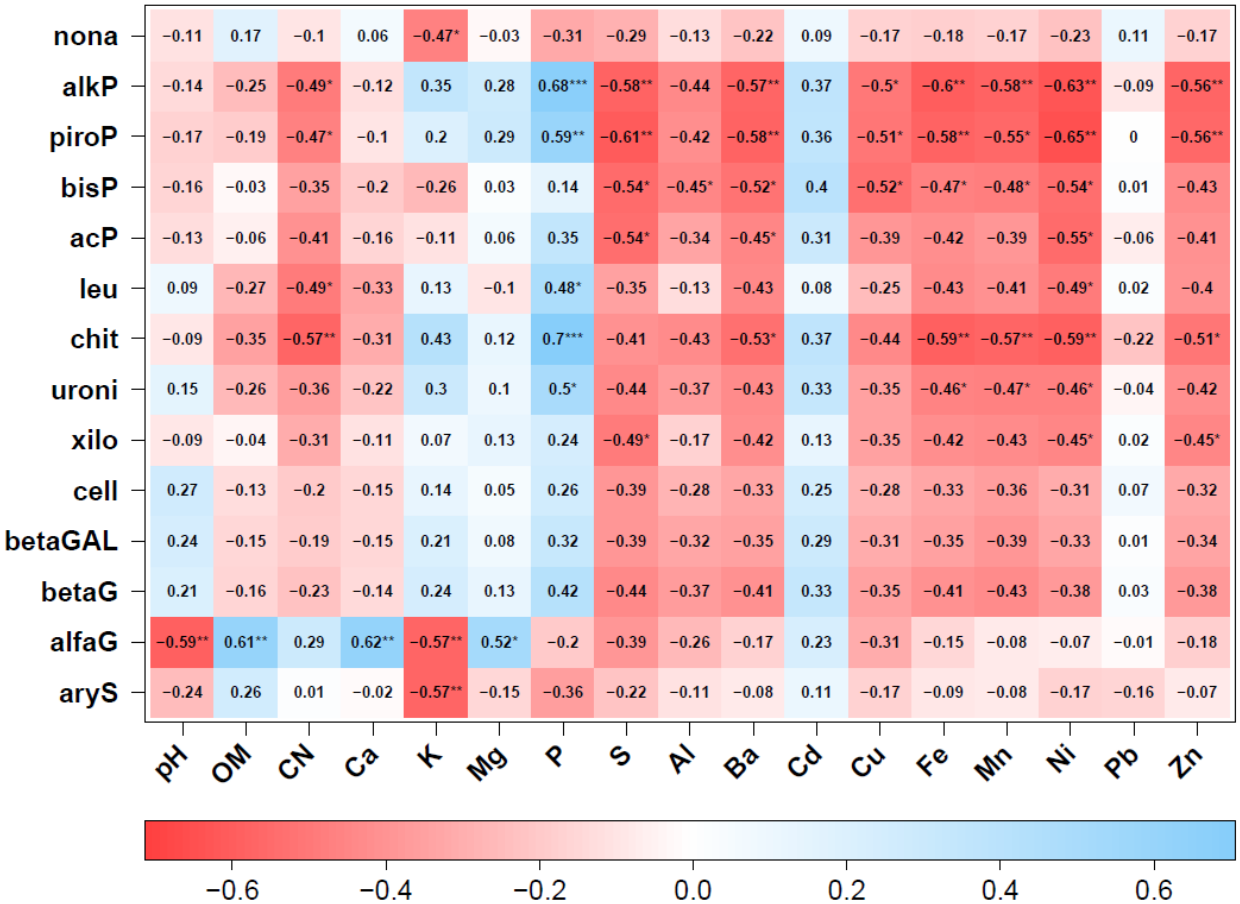


Fig. Pearson's correlation between enzyme activities and water extractable elements. Correlation coefficients indicated with *, **, and *** were statistically significant (p<0.05, p<0.001, and p<0.001, respectively).

Biyensa Gurmessa: Conceptualization, Methodology, Software, Data curation, Visualization, Writing original draft preparation. **Stefania Cocco**: Writing-Reviewing, **Amanda J. Ashworth**: Writing-Reviewing, **Ester Foppa Pedretti**: Supervision, Design, Conceptualization. **Alessio Ilari**: Design, conceptualization. **Valeria Cardelli**: Analysis, Investigation, **Flavio Fornasier**: Analysis, Investigation. **Maria Letizia Ruello**: Analysis, Writing-reviewing. **Giuseppe Corti**: Writing-Reviewing, supervision

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: