IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Influence of *Trichoderma viride* on Growth, Cocoon Production and Biodegradation Efficiency of *Eisenia fetida* During Vermicomposting

Diksha Pathania[1]& Neha Salaria*[1]

[1] Arni School of Basic Sciences and biotechnology, department of life Sciences

Arni University, Kangra (H.P.), India.

Abstract

This research aims to determine the effect of *Trichoderma viride* inoculation on the development, cocoon formation, and biodegradation capacity of the epigeic earthworm *Eisenia fetida* when undergoing vermicomposting of organic waste materials. A total of treatment combinations was The whole procedure was conducted in 150 days with samples being collected at periodic intervals (0, 45, 80, 120 and 150 days). Parameters like physicochemical properties such as pH, OC, TN, available phosphorus (P), K and C:N ratio were considered.

Analysis of results revealed that inoculation with T. *viride* increased the rate of decomposition, decreased organic carbon content up to 75% and enhanced nutrient contents. Biological parameters like biomass production and cocoon formation in E. *fetida* were also improved in inoculated treatments.

It is evident that the use of microbial inoculants with T. *viride* improves the efficiency of vermicomposting and hence, can be considered as a sustainable solution for organic waste management.formulated using various types of organic materials with or without T. *viride* inoculation.

Keywords: *Trichoderma viride*, *Eisenia fetida*, vermicomposting, cocoon production, biodegradation

1. Introduction

Vermicomposting can be considered a highly environment-friendly technology that uses the combined efforts of earthworms and microorganisms for transforming organic materials into useful compost (Singh *et al.*, 2020). Not only does vermicomposting contribute significantly towards efficient waste management, but it also helps in improving soil fertility and enhancing crop yield through the production of good-quality organic fertilizer (Lirikum *et al.*, 2022). Many epigeic earthworms have been used in vermicomposting; however, *Eisenia fetida* has received much attention owing to its exceptional ability to survive under different environmental conditions, excellent reproductive capability, fast growth, and higher efficiency in breaking down various organic materials, especially kitchen and agricultural wastes.

In this regard, the inoculation of microbial culture is regarded as one of the most important factors in the rapid formation of compost. For instance, the fungus *Trichoderma viride*, known for its ability to decompose cellulose and lignin, is one of the microorganisms that can be utilized in increasing the efficiency of composting (Wang *et al.,* 2024). The fungus is a source of diverse extracellular enzymes, such as cellulase, hemicellulase, and lignin degradation enzymes, among others, that contribute to the decomposition of refractory compounds like cellulose, hemicellulose, and lignin into simple units.

It is important to note that the synergy between the earthworms and the microorganisms plays a significant role in making the process of vermicomposting effective (Domínguez *et al*.,2009). The earthworms break down and introduce air into the organic waste, thus increasing its surface area for the actions of the microorganisms to occur. Microorganisms continue to decompose the material by breaking down its biochemical elements. The result is the increased mineralization of nutrients such as nitrogen, phosphorous, and potassium.

Even though the functions of each earthworm and microbe have been established separately through scientific experiments and investigations, there is a lack of literature in which the effect of *Trichoderma viride* has been considered in isolation from other microbes. The reason is because the majority of the studies focus on combinations of different types of microbes rather than single species. Hence, in order to fill this knowledge gap and establish the effect of using *Trichoderma viride* in combination with *Eisenia fetida* during vermicomposting of organic waste, the purpose of this study was to assess the effect of inoculating *Trichoderma viride* in relation to growth, reproduction, and biodegradation ability of *Eisenia fetida*.

2. Materials and Methods

2.1 Experimental Site

The experiment was conducted at Arni University, Himachal Pradesh, in a controlled environment to ensure the best performance of the vermicomposting system (Kumari & Salaria, 2023). The temperature range inside the experimental setup was kept between 24 and 30 degrees centigrade, which is an optimum temperature for the growth, reproduction, and metabolism of epigeic worms like *Eisenia fetida*. Similarly, the relative humidity level was maintained between 65% and 75%, thus ensuring an optimum moist condition suitable for effective microbial activities and earthworm survival, which have a high tendency of desiccation.

Even though this system is not entirely controlled, similar to that of an incubator in the laboratory, some measures were taken to ensure that no environmental disturbances occur during the experiments. For instance, the experimental units were kept in a shaded area with good ventilation. Also, there was periodic checking of temperature and moisture levels and spraying of water when necessary.

2.2 Experimental Design

This experiment was performed following the Completely Randomized Design (CRD) approach to examine the effectiveness of vermicomposting in different treatment conditions (Mahmud et al., 2018). CRD design was chosen for this purpose since it is ideal for laboratory-based investigations with minimal fluctuations in the environment.

Four sets of treatments were developed, each having two replications. Thus, eight plots were used during the experimentation period lasting 150 days.

In this investigation, four treatment methods were developed to examine the impact of microbial inoculation involving Trichoderma viride, combined with organic substrate and earthworm species *Eisenia fetida*.

Different combinations of treatments were developed based on organic waste substrates with and without *Trichoderma viride*. The information provided in the table below:

Treatment Code	**Substrate Composition**	**Earthworm Species**	**T. *viride* Application**
T1	Organic waste mixture (e.g., kitchen waste + cow dung)	*Eisenia fetida*	Absent
T2	Organic waste mixture (e.g., kitchen waste + crop residues)	*Eisenia fetida*	Absent
T3	Organic waste mixture (same as T1)	*Eisenia fetida*	Present
T4	Organic waste mixture (same as T2)	*Eisenia fetida*	Present

- **T1 and T2 (Control Treatments):** These treatments consisted of organic waste substrates processed using *Eisenia fetida* without the addition of *Trichoderma viride*. They served as control setups to evaluate the natural vermicomposting efficiency (Singh *et al.*, 2020).
- **T3 and T4 (Inoculated Treatments):** These treatments were similar to T1 and T2 but were supplemented with *Trichoderma viride*. The fungal inoculant was added to enhance microbial activity, accelerate decomposition, and improve nutrient mineralization (Singh *et al.*, 2020).

2.3 Substrate Preparation

Organic substrates used for vermicomposting, which include kitchen waste, cow dung, and agricultural waste, were sourced locally and prepared before experimentation (Bajal *et al.*,2019). Kitchen waste comprised peels from vegetables, residues of fruits and other biodegradable foods, while agricultural waste included dried plant leaves and other agricultural wastes. Freshly sourced cow dung acted as a source of nitrogen that will be added to help balance the carbon content in other substrates.

These wastes were manually separated to exclude non-biodegradable material such as plastic materials, glass, and metals (Pattnaik & Reddy, 2010). The substrates were cut to small sizes (around 2-5 cm). Pre-decomposition of the substrate mix was conducted for 15 days using the aerobic method. Optimal moisture levels (60-70%) were achieved by sprinkling water on the substrates every three to four days to enhance aeration, which will allow microbial degradation of complex organic compounds.

This process also helped minimize heat generated by microorganisms, thereby reducing the risks associated with high temperatures during decomposition.

2.4 Earthworm Inoculation

Ten mature clitellates of the *Eisenia fetida* species were deliberately chosen for introduction into each treatment setup at the onset of the experiment. The developed clitellum showed that the worms had reached sexual maturity and thus would be biologically consistent throughout the experiments. In preparation for the experiment, the earthworms were adapted to laboratory conditions before being introduced into the substratum medium (Pattnaik & Reddy, 2010).

2.5 Physicochemical Analysis

The physicochemical properties of vermicompost were analyzed to evaluate compost maturity, nutrient enrichment, and overall quality. Standard analytical methods were followed for all parameters.

1. pH and Electrical Conductivity (EC)

The pH value of the samples of vermicompost was determined through a digital pH meter in a 1:10 (w/v) compost-to-distilled water extract (Majlessi et al., 2012). The pH value is an important parameter when it comes to microorganisms' activity and compost stability. On the other hand, electrical conductivity (EC) of the compost extract was measured using a conductivity meter. It can be used to evaluate composting process by determining the content of dissolved salts in the compost.

2. Organic Carbon (OC)

The content of organic carbon in the compost was estimated using the Walkley and Black dichromate oxidation technique (Bremner & Jenkinson, 1960). It serves as an important parameter when it comes to estimating organic matter content and composting maturity level. Organic carbon content in the compost is being reduced throughout vermicomposting because of microorganism respiration and mineralization.

3. Total Nitrogen (TN)

Total nitrogen content was estimated using Kjeldahl digestion method (Matejovic, 1995). Nitrogen is one of the major macronutrients that is required for plants' nutrition and development. Increased nitrogen content in the vermicomposting process is related to microbial activity, nitrogen fixation, and concentration effect.

4. Available Phosphorus (P)

Available phosphorus levels were measured based on the Olsen technique (neutral and alkaline soils) or Bray technique (acidic soils) (Mustapha et al., 2022). The levels of available phosphorus increase in vermicompost due to the mineralization of organic phosphorus and activity of microorganisms like phosphate solubilizers.

5. Available Potassium (K)

Potassium levels were measured using flame photometry following the decomposition of the compost samples with an acid (Tabbasum et al., 2021). Potassium plays an important role in the metabolic processes in plants. The available potassium levels increase in vermicompost because of the decay of organic material and the formation of mineral salts.

6. Carbon to Nitrogen Ratio (C:N Ratio)

The C:N ratio was computed through the division of organic carbon levels by the nitrogen level in the compost samples. It is one of the most significant indices of compost quality and maturity. A low C:N ratio (usually under 20:1) means good decomposition and maturity of the compost sample.

2.6 Biological Parameters

There are several biological parameters that can help assess the performance of earthworms and effectiveness of the vermicomposting process. They include the biomass gain, growth rate, and cocoon production.

1. Biomass Gain

The biomass gain refers to the change in body weight of the earthworms during the experimental period (Hartenstein,1984). This parameter is an indicator of suitability of the substrate used in the experiment as well as good growth conditions for earthworms. It was calculated based on the formula:

Biomass Gain (g) = Final biomass – Initial biomass

2. Growth Rate

The growth rate is used to measure the speed of growth of the body of the earthworms in biomass per day (Hartenstein,1984). The growth rate of each group of earthworms was calculated according to the formula:

Growth Rate (mg/worm/day) = (Final weight – Initial weight) / (Number of days × Number of worms)

3. Cocoon Production

The number of cocoons produced by earthworms during the period of the experiment was assessed as another indicator of their reproductive potential (Bhattacharjee & Chaudhuri, 2002)

2.7 Statistical Analysis

All data collected under the different treatments were subjected to statistical analysis and presented as mean ± SD for describing the data trends and variation. Statistical software was used in data analysis for accurate and reliable results.

One-way analysis of variance (ANOVA) was done to check the significance of differences between the means of the treatments. ANOVA was chosen since this technique allows comparisons of more than one treatment means at once through an experiment involving just one factor (Komarek *et al.*, 2020).

3. Results and Discussion

3.1 Physicochemical Properties

Physico-chemical properties of vermicomposts are the key parameters that characterize compost maturation, fertilizing properties, and quality. In this case, considerable differences were found between substrates treated both with and without *Trichoderma viride*, suggesting high significance of this microorganism for composting process and nutrients transformation.

pH Variations

Initially, the pH of untreated substrate was rather high (7.8-8.2), demonstrating slightly alkaline nature of fresh waste organic materials (Song *et al*., 2018). Following the composting process, the pH was decreased to 7.2-7.5 in the absence of *Trichoderma viride* and even lower to 6.9-7.1 in the presence of this microorganism.

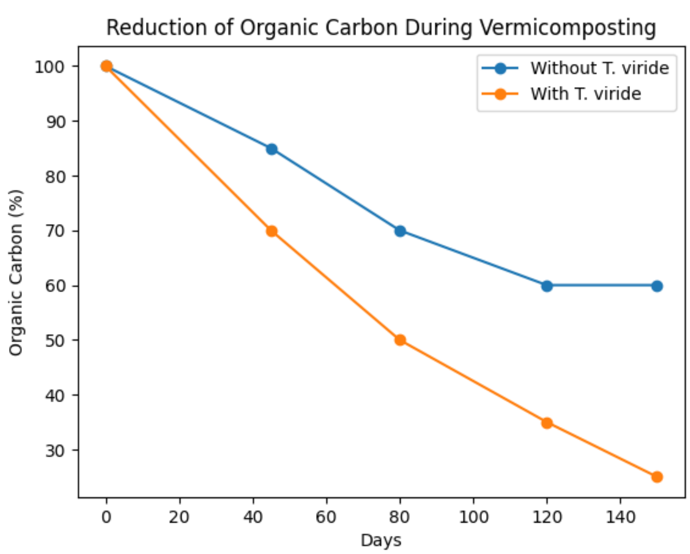
This gradual trend toward neutral pH is a well-known sign of compost stabilization. Such a decrease in pH values is explained by an elevated rate of substrate mineralization owing to organic acids production and intensive respiration in *Trichoderma viride* treatments. Neutral pH favors plants' growth and development, confirming the quality of produced compost.

Organic Carbon (OC) Reduction

The initial content of organic carbon was relatively high because of the presence of undecomposed organic material (Lim et al., 2015). There was a marked decrease in the OC content during the process of vermicomposting:

- 30-40% for the control
- 60-75% for the treatments containing *Trichoderma viride*

The sharp decrease in the OC content can be attributed to vigorous biological degradation, wherein carbon is released in the form of CO_2 through respiration. The higher degree of reduction in the T. *viride* treatments indicates its efficiency in decomposing complex organic materials, including cellulose and lignin, using enzymatic action.



Total Nitrogen (TN) Enhancement

The total nitrogen content steadily rose throughout the course of composting as follows:

• Initially low in fresh waste material

• Increased moderately in absence of T. *viride*

• Became highly concentrated with the use of T. *viride*

This increased nitrogen concentration occurs because of:

• Mineralization of organic nitrogen

• Addition of nitrogen via earthworm secretions and excretion products and microorganisms

• Lower carbon content resulting in higher nitrogen concentration

This rise in nitrogen content in the presence of T. *viride* indicates enhanced compost quality.

Phosphorus and Potassium (P & K)

Both phosphorus and potassium were significantly improved:

- Below optimal in initial raw substrate
- Moderate when not using T. *viride*
- Significantly increased when T. *viride* was used

The increase in phosphorus could be attributed to the solubility of phosphate, whereas the increase in potassium is caused by the decomposition of organic materials. Both nutrients are important for plants, thereby showing T. *viride*'s importance in the process.

C:N Ratio Reduction

One of the most effective parameters in evaluating compost maturity is the C:N ratio. In the present investigation:

- High initial C:N ratio (implying immature organic waste)
- Decreased by about 50% in absence of T. *viride*
- Decreased by about 75% in presence of T. *viride*

The drastic decrease in the C:N ratio with respect to T. *viride* is indicative of rapid humification process. Lower value of C:N ratio (less than 20) denotes the maturity of compost.

Decomposition Rate

The overall rate of decomposition proved considerably higher for treatments containing *Trichoderma viride*. The reasons include:

- Elevated efficiency in breaking down complex compounds through enzymes
- The synergic effect of earthworms (*Eisenia fetida*) and microbe inoculation
- Higher microbial activity resulting in quick decomposition of organic materials

Parameter	Initial	Without *T. viride*	With *T. viride*
pH	7.8–8.2	7.2–7.5	6.9–7.1
OC (%)	High	↓ 30–40%	↓ 60–75%
TN (%)	Low	Moderate	High
P & K	Low	Moderate	High
C:N Ratio	High	↓ 50%	↓ 75%

3.2 Growth and Cocoon Production of *Eisenia fetida*

Performance of growth and breeding of *Eisenia fetida* was considerably affected by the use of the microbial inoculum *Trichoderma viride*. When compared with *Eisenia fetida* without *Trichoderma viride*, it was observed that there were considerable variations in biomass formation and growth rates.

Parameter	Without *T. viride*	With *T. viride*
Biomass Increase	Moderate	High
Cocoon Production	60–70	80–90
Growth Rate	Moderate	Enhanced

Biomass Increase

Without the inoculation with *Trichoderma viride*, there was only a slight increase in biomass among earthworms. However, the application of the inoculant considerably improved biomass increase. Such a significant change may be explained by the fungus' ability to break down organic substrates through the secretion of cellulases and ligninases.

Cocoon Production

The reproductive function of *Eisenia fetida* was much better developed when *Trichoderma viride* was used. While in the controls there were 60-70 cocoons produced, with T. *viride* there were 80-90 cocoon formed. Such a difference suggests that the microbe inoculation created a more favorable environment conducive to reproduction.

Growth Rate

Without the inoculation, the growth rate of *Eisenia fetida* appeared to be average, while in the case of *Trichoderma viride*, a considerable enhancement was noticed. Due to the symbiosis of worms with beneficial microbes, it is possible to achieve faster nutrition and rapid growth.

3.3 Biodegradation Efficiency

Enhanced organic matter degradation as a result of inoculation:

Inoculating *Trichoderma viride* enhanced the organic matter degradation process in a way that the rate at which complex organic materials such as cellulose, hemicellulose, and lignin were broken down was enhanced (Wang et al., 2021). The degradation rates were enhanced to ensure efficiency in reducing organic waste material.

Enhanced enzyme activity:

There was enhanced enzyme activity in respect of some key extracellular enzymes such as cellulase, protease, and ligninase. The enzymes were very effective in the breakdown of complex organic materials.

Nutrient mineralization increase:

Through the symbiotic effects of earthworms and *Trichoderma viride*, there was nutrient mineralization. There was enhanced mineralization of nutrients such as nitrogen, phosphorous, and potassium. This resulted in high quality vermicompost.

3.4 Compost Quality

Color: Dark brown

Dark brown or near-black coloration of the compost comes from complete decomposition and humification, which means that the complex organic molecules have turned into stable humus material (Singh & Longkume, 2018).

Texture: Granular

Compost must be granular and crumbly in nature just like the soil. The formation of this texture is achieved through the activity of microorganisms and earthworms, which decompose organic materials.

Odor: Earthy smell

Another sign of maturity is the presence of an earthy smell characteristic of soils. This smell results from a preponderance of positive microorganisms such as actinomycetes.

C:N ratio: Below 20

The C:N ratio under 20:1 points to the more advanced level of decomposition because the simple forms of carbon get decomposed at this stage.

4. Conclusion

Consequently, this research proves the possibility of increasing the efficiency of the vermicomposting process using *Eisenia fetida* with the help of *Trichoderma viride* inoculation. From the analysis of the findings, it is evident that the process of degradation accelerates in case of inoculation that can be explained by the fact that T. *viride* increases the rate of decomposition of complex organic compounds via enzymatic activity (Ahmed et al., 2019). In addition, the process is associated with the enhancement of nutritional enrichment with such macronutrients as nitrogen, phosphorus, and potassium, which means the maturity and stabilization of the product.

Besides, earthworms reveal higher fecundity and biomass growth when subjected to inoculation. The finding indicates that there are positive effects of using T. *viride* for improving the vermicompost process. Consequently, the interaction between earthworms and microbial inoculants creates favorable conditions for improving biological activity and vermicompost quality.

Thus, the implementation of *Trichoderma viride* along with earthworms for vermicomposting provides a sustainable approach to the processing of organic waste and fertilizer production.

References

1. Singh, A., Karmegam, N., Singh, G. S., Bhadauria, T., Chang, S. W., Awasthi, M. K., ... & Ravindran, B. (2020). Earthworms and vermicompost: an eco-friendly approach for repaying nature's debt. *Environmental Geochemistry and Health*, *42*(6), 1617-1642.
2. Lirikum, Kakati, L. N., Thyug, L., & Mozhui, L. (2022). Vermicomposting: an eco-friendly approach for waste management and nutrient enhancement. *Tropical Ecology*, *63*(3), 325-337.
3. Wang, S., Long, H., Hu, X., Wang, H., Wang, Y., Guo, J., ... & Yang, Q. (2024). The co-inoculation of Trichoderma viridis and Bacillus subtilis improved the aerobic composting efficiency and degradation of lignocellulose. *Bioresource Technology*, *394*, 130285.
4. Domínguez, J., Aira, M., & Gómez-Brandón, M. (2009). Vermicomposting: earthworms enhance the work of microbes. In *Microbes at work: from wastes to resources* (pp. 93-114). Berlin, Heidelberg: Springer Berlin Heidelberg.
5. Kumari, I., & Salaria, N. (2023). Effect of organic compost on the vegetative growth of ladies' finger (Abelmoschus esculentus) in sub-tropical Himalayan region of Himachal Pradesh, India. *Crop Research (0970-4884)*, *58*.
6. Mahmud, M., Abdullah, R., & Yaacob, J. S. (2018). Effect of vermicompost amendment on nutritional status of sandy loam soil, growth performance, and yield of pineapple (Ananas comosus var. MD2) under field conditions. *Agronomy*, *8*(9), 183.
7. Singh, N. K., Shahi, K., Kumar, K., & Suthar, S. (2021). Enhanced vermicomposting of leaf litter by white-rot fungi pretreatment and subsequent feeding by Eisenia fetida under a two-stage process. *Bioresource Technology Reports*, *13*, 100609.
8. Bajal, S., Subedi, S., & Baral, S. (2019). Utilization of agricultural wastes as substrates for vermicomposting. *Journal of Agricultural and Veterinary Science*, *12*, 79-84.
9. Pattnaik, S., & Reddy, M. V. (2010). Nutrient status of vermicompost of urban green waste processed by three earthworm species—Eisenia fetida, Eudrilus eugeniae, and Perionyx excavatus. *Applied and Environmental soil science*, *2010*(1), 967526.
10. Majlessi, M., Eslami, A., Najafi Saleh, H., Mirshafieean, S., & Babaii, S. (2012). Vermicomposting of food waste: assessing the stability and maturity. *Iranian journal of environmental health science & engineering*, *9*(1), 25.
11. Bremner, J. M., & Jenkinson, D. S. (1960). Determination of organic carbon in soil: I. Oxidation by dichromate of organic matter in soil and plant materials. *Journal of soil science*, *11*(2), 394-402.
12. Matejovic, I. (1995). Total nitrogen in plant material determinated by means of dry combustion: A possible alternative to determination by Kjeldahl digestion. *Communications in Soil Science and Plant Analysis*, *26*(13-14), 2217-2229.

13. Mustapha, A. A., Abdulrahman, B. L., Dawaki, M. U., & Usman, A. (2022). Comparative determination of available phosphorus at different pH levels in Nigerian Savannah soils using Mehlich, Olsen and Bray Methods. *Nigerian Journal of Soil Environmental Research*, *21*, 69-77.
14. Tabbasum, S., Akhtar, M., Sarwar, N., Tipu, M. I., Ikram, W., Ashraf, A., ... & Khan, M. R. (2021). Relative effectiveness of phosphorus and potassium along with compost and organic acids on maize crop grown in calcareous soil: A multivariate analysis. *Journal of Soil Science and Plant Nutrition*, *21*(1), 437-449.
15. Hartenstein, R. (1984). Rate of production and loss of earthworm biomass in relation to species and size. *Soil Biology and Biochemistry*, *16*(6), 643-649.
16. Bhattacharjee, G., & Chaudhuri, P. S. (2002). Cocoon production, morphology, hatching pattern and fecundity in seven tropical earthworm species—a laboratory-based investigation. *Journal of biosciences*, *27*(3), 283-294.
17. Komarek, L., Jakab, P., & Festő, D. (2020). The relationship between the crop, nutrition parameters of winter wheat and the various doses of fertilaser treatments analised by one-way anova. *Annals of the Faculty of Engineering Hunedoara*, *18*(1), 181-189.
18. Song, C., Zhang, Y., Xia, X., Qi, H., Li, M., Pan, H., & Xi, B. (2018). Effect of inoculation with a microbial consortium that degrades organic acids on the composting efficiency of food waste. *Microbial Biotechnology*, *11*(6), 1124-1136.
19. Lim, P. N., Wu, T. Y., Clarke, C., & Nik Daud, N. N. (2015). A potential bioconversion of empty fruit bunches into organic fertilizer using Eudrilus eugeniae. *International journal of environmental science and technology*, *12*(8), 2533-2544.
20. Wang, X., Cheng, H., Ye, G., Yao, F., Wang, Y., Jiao, Y., ... & Ye, D. (2021). Preparation of porous carbon based on partially degraded raw biomass by Trichoderma viride to optimize its toluene adsorption performance. *Environmental Science and Pollution Research*, *28*(34), 46186-46195.
21. Singh, R. K., & Longkumer, T. E. (2018). Compost: the black cold. *KrishiVigyan Kendra–Phek, ICAR–NRC on Mithun, Porba, Phek, Nagaland*, *112*.
22. Ahmed, M., Ahmad, S., Qadir, G., Hayat, R., Shaheen, F. A., & Raza, M. A. (2019). Innovative processes and technologies for nutrient recovery from wastes: a comprehensive review. *Sustainability*, *11*(18), 4938.