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Risk of Mycotoxin Contamination in Thermophilic Composting of Kitchen and Garden Waste at Large-Scale

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Abstract: Although toxicogenic moulds have been identified in municipal waste and composting facilities, only a few reports exist on the occurrence of mycotoxins in compost. Those reports mostly concern sewage sludge as a substrate, tested only a limited range of mycotoxins, and did not monitor the production of mycotoxins during the composting process. Therefore, this study aimed to investigate whether mycotoxins are produced during composting of selectively collected kitchen and garden waste. The study was carried out at pilot scale (550 L reactor with passive aeration). Kitchen waste (59.0% *w*/*w*), garden leaves (28.2%), and wood chips (12.8%) were used as a substrate, which was sampled every five days to determine its basic physicochemical characteristics (temperature, moisture, size-fraction content, loss on ignition) and respirometric activity (AT4). The substrate and leachate samples were also tested for the content of eight mycotoxins by HPLC-MS/MS. To screen the local compost market, commercial organic-compost samples were analysed for mycotoxin contamination. The substrate was successfully stabilized after 45 days (thermophilic peak of 62.6 °C, 40.4% mass reduction, 26.9% loss of organic matter, increase in the share of particles in the smallest size fraction, AT4 of 9.82 g O_2/kg). Although the substrate was colonised by moulds at an early stage, only trace amounts of mycotoxins were detected in a few samples. Similarly, little or no mycotoxins were found in the commercial compost. Our results suggest a low risk of mycotoxin contamination in biowaste compost produced under appropriate technological conditions. Future research should focus on screening compost produced at smaller scales (e.g., in agricultural/residential compost piles) and on identifying factors associated with the risk of mycotoxin contamination in compost.

Keywords: composting; circular economy; organic recycling; risk assessment

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1. Introduction

Composting is considered an attractive and economically feasible method for recycling selectively collected municipal organic waste (biowaste), and thus contributing to the achievement of the ambitious policies (e.g., regarding the recycling targets) set out in the many frameworks for implementing a circular economy, such as the European Union Waste Framework Directive [1], the United States National Recycling Strategy [2], or the United Nations Sustainable Development Goals 2030. The composition of biowaste (and particularly that of kitchen waste) and its high moisture content favour the dynamic growth of fungi, which play an essential role in its biodegradation [3,4]. However, decaying biowaste can also be considered a potential source of toxic fungal metabolites known as mycotoxins [5].

Mycotoxins belong to a large and diverse group of secondary metabolites produced by fungi, including many mould species that frequently infect plant material and lead to contamination of crops and cereals worldwide [6–8]. Although hundreds of mycotoxins have been identified to date, only a few fungal metabolites have been the subject of toxicological research and public concern. Among these natural contaminants of food and feed, aflatoxins produced by the common moulds *Aspergillus flavus* or *A. parasiticus* are considered the most harmful, due to their hepatotoxic and carcinogenic properties. These mycotoxins are among the most abundant worldwide, along with fumonisins, trichotecenes (e.g., deoxynivalenol, T-2 toxin), zearalenone, which can be produced by various *Fusarium* strains, and ochratoxins, produced by *Penicullum*, [9]. Although many toxicogenic genera of these moulds have been identified in composting substrates and composting facilities [10,11], few studies have provided information on the presence of mycotoxins in compost [12–14]. Moreover, these publications mostly concern sewage sludge as a substrate, tested a limited range of mycotoxins, and none of them monitored the formation of mycotoxins during the entire composting process.

The limited number and scope of such studies might be due to the assumption that mycotoxins are naturally degraded to less harmful products. However, most studies on mycotoxin degradation only tested the ability of isolated strains of bacteria or fungi under in vitro conditions, not in realistic scenarios [15]. To the best of our knowledge, the ability of the composting process to degrade mycotoxins has only been tested for aflatoxin B1 [16,17]. Moreover, there are other mycotoxins that are considered chemically stable and heat resistant [18,19]. When mycotoxins are actually produced during composting, they can pose a health risk to not only wildlife or domestic animals [13] but also farmers and workers in composting facilities [10,20,21]. Thus, understanding the specific technological conditions that favour or limit the occurrence of mycotoxins in compost would help to improve worker occupational health and consumer safety.

Therefore, to address the knowledge gaps mentioned above, we aimed to assess whether selectively collected kitchen and garden waste (biowaste) has the potential to form mycotoxins during an effective composting process (i.e., in a passively aerated bioreactor at pilot scale). We focused on eight mycotoxins that we consider to be of particular concern, namely aflatoxin B1 (AFB1), deoxynivalenol (DON), fumonisin B1 and B2 (FUMs), HT-2 toxin, ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN). Towards this aim, we analysed substrate and leachate samples throughout the entire process of composting a mixture of kitchen and green waste, as well as samples from local composting facilities with large-scale installations designed to compost biowaste. The results of this study contribute to knowledge about the effect of specific composting conditions on the occurrence of moulds and the production of mycotoxins in compost, which could be of use for technologists optimizing the composting process as well as policy makers working on food-chain safety regulations.

2. Materials and Methods

2.1. Experimental Reactor

The research station was prepared in compliance with ISO 16929:2019 (Pilot Scale Composting Test) [22]. Compliance with the aforementioned standard was maintained in terms of not only reactor size (>35 dm^3) but also substrate preparation (i.e., substrate moisture > 50%, C:N ratio within the range of 20–30) and process monitoring (Figure 1). The reactor had a volume of 550 dm^3 and a height of 105 cm. The surface area of the reactor's upper floor was 85 cm by 75 cm, while that of the lower floor was 75 cm by 60 cm. The ratio of the heat exchange surface area to the volume of the reactor (SA:V) was approximately 7.3 m^2/m^3, which, according to the methodology presented by Mason and Milke [23], is characteristic of research reactors at the pilot scale.

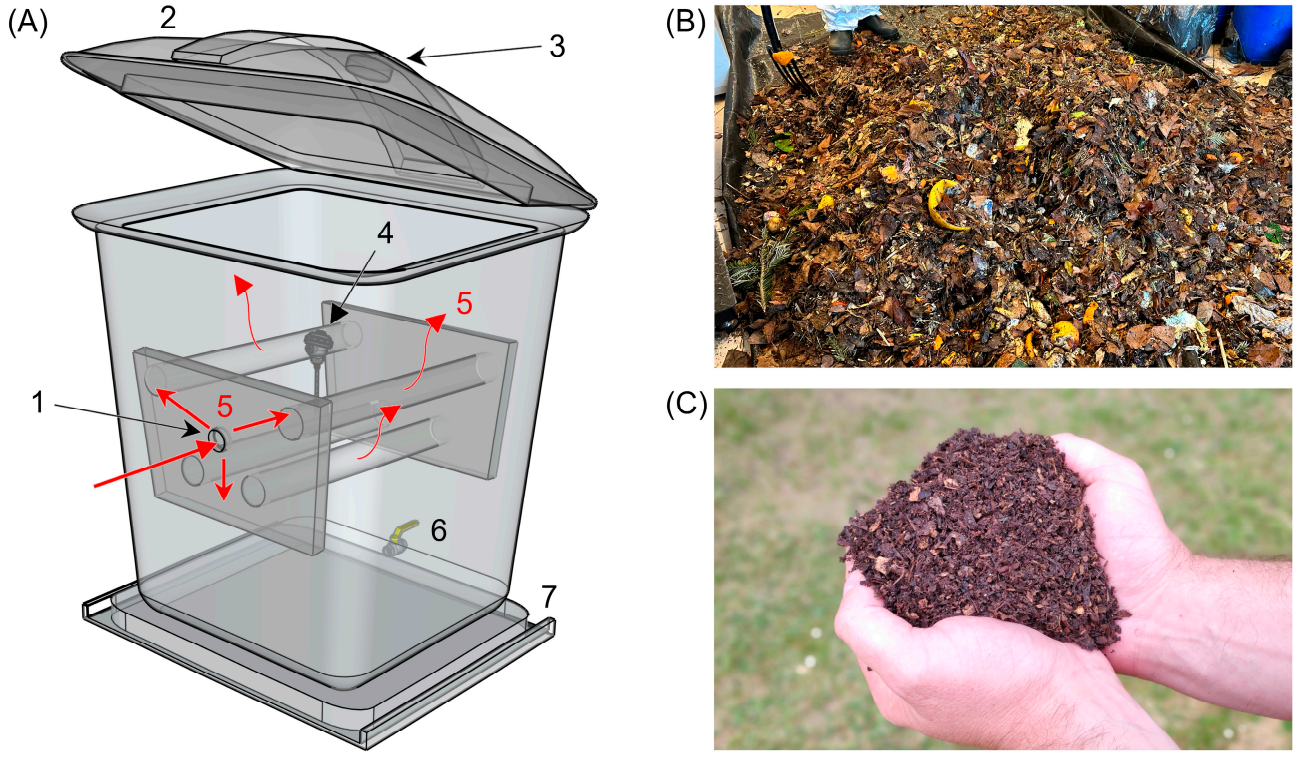


Figure 1. (**A**) Diagram of the 550 L bioreactor, (**B**) the substrate (kitchen waste mixed with leaves and gardening residuals) used for composting, and (**C**) the product (fresh compost) obtained at the end of the process. Diagram labels: 1—air inlet; 2—cover; 3—air outlet; 4—temperature sensor; 5—passive aeration system; 6—leachate outflow; 7—platform scale for measuring weight. Black arrows indicate technical elements of the bioreactor, whereas the red arrows indicate the air flow.

The research station met the requirements for self-heating reactors (<5% heat losses) due to the use of an insulating layer of polyurethane with a thickness of 7 cm. This exceeds the minimum thickness of the insulation layer for self-heating reactors as specified by Mason and Milke [23] (calculated with the formula $0.0188 \cdot e^{(0.1493[SA:V])}$, which for this reactor, equates to a minimum thickness of 5.5 cm). To limit moisture and heat losses, the reactor was covered.

The functioning of the passive ventilation system was based on the chimney effect. Aeration was facilitated through a hole with a diameter of 4 cm, which was connected to a system of perforated stainless-steel pipes. These pipes had a diameter of 3 cm and a total length of 335 cm and were positioned in the central part of the waste mass. The ratio of the volume of the aeration system to the active volume of the reactor was approximately 1:200. The air outlet was located in the reactor cover. The use of this passive aeration system eliminated the necessity for mechanical turning of the material. This system ensured an even temperature distribution throughout the entire mass of the substrate, thereby preventing temperature stratification and maintaining uniform waste processing conditions within the reactor. This effectiveness of this method has been demonstrated by our previous studies [24].

A valve was installed in the bottom part of the reactor wall to collect leachate. The reactor was equipped with a temperature sensor in the central part of the reactor. Changes in waste mass were monitored by placing the reactor on a four-sensor platform scale. The precision of the temperature and mass measurements were 0.1 °C and 0.1 kg, respectively.

2.2. Preparation and Characterization of the Substrate

As the substrate, selectively collected kitchen waste (fruit and vegetable residuals) and green waste (leaves, coniferous wood waste, and deciduous wood waste) were used. The

rationale for using this particular substrate was that rotting plant material has the highest mycotoxigenic potential. Selectively collected kitchen waste, leaves, and coniferous wood waste came from the area of the city of Olsztyn (Poland) and were delivered to the laboratory by a commercial waste collection company. The deciduous wood waste came from our university campus area and was delivered to the laboratory by the University Greenery Maintenance Department. In accordance with the municipal waste collection schedule, the kitchen waste was collected from residents once a week. Hence, this component of the substrate was no more than eight calendar days old at the time of the experiment. Before the start of the experiment, the coniferous and deciduous wood wastes were mechanically ground to a grain size of <15 mm. The use of green waste in the experiment was necessary due to the low porosity and high moisture of the kitchen waste (the bulk density and percent moisture of the kitchen waste were 750 kg·m^{-3} and 78%). Based on the moisture and shares of the individual components, the calculated bulk density and moisture of the prepared substrate were 381.7 kg·m^{-3} and 66.3%. The actual initial moisture content of the final substrate, measured analytically after its preparation, was 65.1%, and the initial C/N ratio of the substrate was 26.6. The characteristics of the individual components and the final substrate are presented in Table 1.

Table 1. Characteristics of individual components of the test substrate.

Component	Bulk Density [kg·m^{-3}]	Mass [kg]	Volume [L]	Moisture [%]	Share [% *w*/*w*]
Kitchen waste	750	115	153.3	78.0	59.0
Leaves	200	55	275.0	56.6	28.2
Deciduous wood chips *	195	15	76.9	40.2	7.7
Coniferous wood chips *	215	10	46.5	25.3	5.1
Final substrate **	381.7	195	551.8	66.3	100.0

* Wood waste mechanically ground to a grain size of <15 mm, ** estimated based on the characteristics of the individual components.

2.3. Organization of the Experiment, Description of the Analytical Methods

The composting process was rigorously monitored, focusing on the changes in the temperature and mass of the composting substrate, as well as the chemical properties of both the substrate and the generated leachate. The chemical properties of the waste were evaluated at five-day intervals to ensure a thorough analysis. For this purpose, samples averaging 775 g were collected from various locations within the reactor to ensure that they were representative of the entire reactor. These samples were mixed prior to the analyses to ensure their homogeneity and representativeness of the entire substrate mass. The analyses were then performed in triplicate to ensure the reliability and accuracy of the results. The characteristics that were analysed included the following:

(i) Percent moisture, measured by drying the sample at 105 °C for 24 h and determining the evaporated water loss by weighing the difference to an accuracy of 0.1 g (ISO 11465:1993) [25];
(ii) Share of volatile substances, measured by loss on ignition, by milling a dried sample to a size of <1 mm and heating at 550 °C for 6 h LOI (ISO 10694:1995) [26]; loss of volatiles was determined with an accuracy of 0.0001 g;
(iii) Share of total organic carbon, TOC, measured by burning a dried and milled sample at 900 °C in a Shimadzu TOC-V_{CSN} carbon analyser with an SSM-5000A module and determining the TOC by subtracting the inorganic carbon content from the total carbon content (ISO 10694:1995) [26];
(iv) Four-day respirometric activity, AT4, determined by the manometric method using a WWT Oxi-Top Control kit (Xylem Analytics Germany Sales GmbH & Co. KG; Weilheim, Germany); the analysis consisted of incubating samples for 4 days at 22 °C in the presence of a 1M NaOH solution (ISO 16072:2002) [27].

The leachate was collected each day, and the following characteristics were analysed:

(i) pH, determined by the potentiometric method (ISO 10523:2008) [28];
(ii) Chemical oxygen demand (COD), determined using the dichromate method (ISO 6060:1989) [29];
(iii) Ammonium nitrogen content, determined by steam distillation followed by titration with 0.01 N sodium hydroxide solution in the presence of Tashiro reagent as an indicator, (ISO 5664:1984) [30];
(iv) Volatile fatty acids (VFA) content, determined by steam distillation followed by titration with 0.01 N sodium hydroxide solution in the presence of phenolphthalein as an indicator (APHA 5560) [31];
(v) Phosphates, determined by a spectrophotometric method (ISO 6878:2004) [32].

Additionally, to monitor changes in the granularity of the substrate, fractional analysis was performed three times: at the start of the experiment, on the 30th day, and on the 45th day of the process. This analysis was performed manually with sieves, using the following size fractions: (i) <10 mm, (ii) 10–40 mm, (iii) 40–60 mm, (iv) 60–80 mm, (v) 80–100 mm, and (vi) >100 mm.

2.4. Calculation of the Mass Balance

The mass balance in the reactor was calculated based on a simplifying assumption [33]. Thus, it was assumed that the mass of the composted waste in the reactor at any point e (M_e) was the sum of the masses of water (W_e), organic matter (O_e), and mineral matter (I_e), according to the following equation:

$$M_e = W_e + O_e + I_e \quad (1)$$

The mass of water in the reactor was calculated as follows:

$$W_e = M_e \cdot H_e \quad (2)$$

where

W_e is the mass of water in the reactor [kg],
M_e is the total mass of the waste in the reactor [kg], and
H_e is the percent moisture of the waste in the reactor [%].
The organics mass in the reactor was calculated using the following equation:

$$O_e = (M_e - W_e) \cdot LOI \quad (3)$$

where

O_e is the mass of organic matter in the reactor [kg DM] and
LOI is the loss on ignition [% DM].

2.5. Microscopic Analysis

To assess changes in the biocenosis of the composted material, wet mount slides were freshly prepared from collected samples of the substrate and qualitatively assessed with a Nikon Eclipse 50i light microscope (Amstelveen, The Netherlands), according to a guide to mould identification [34].

2.6. Collection of Commercially Available Compost Samples

In addition to testing for the presence of mycotoxins in the composted kitchen and garden waste produced in our experiment, we were also interested in checking whether these mycotoxins were present in compost available on the local market. For this purpose, three samples, produced by two independent vendors, were purchased from local garden centres: two of biocompost (granulated organic compost) and one of biohumus (liquid vermicompost). Furthermore, two compost samples in total were collected from two local composting plants with large-scale installations designed to compost biowaste (kitchen + garden waste) under active aeration. According to the manufacturers' declarations, all

of these compost samples were produced in accordance with Polish regulations [35,36]. Additionally, each of the compost products had a certificate from the Polish Ministry of Agriculture or an equivalent European institution dealing with food chain safety.

2.7. Analysis of Mycotoxin Content

Analytical standards for aflatoxin B1 (AFB1), deoxynivalenol (DON), fumonisin B1 and B2 (FUMs), HT-2 toxin, ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN) used for chromatographic analysis were obtained from Sigma-Aldrich (Poznań, Poland). Acetonitrile, methanol, LC-MS–purity water, and reagents used in chromatographic analysis, extraction, and eluent preparation, i.e., ammonium formate (AFOR), formic acid (FA), and ammonium fluoride (AFLU), were purchased from Merck (Warszawa, Poland).

Prior to extraction, 50 g of the compost substrate sample was dried at 60 °C. To extract mycotoxins from the sample, 1 g of the solid-dried and ground material was weighed into a screw-capped falcon-type tube (15 mL), and 5 mL of the extraction solution (90% acetonitrile with 1% FA) was added. In the case of leachate samples, 1 mL of leachate was measured into a falcon tube (15 mL) and 5 mL of the extraction solution (90% acetonitrile with 10% FA) was added. The sample was then subjected to ultrasound for 30 s using a Sonics Vibra Cell apparatus, after which the samples were shaken for 30 min at 10 Hz using an Eberbach model EL.680.Q.25 shaker. After shaking, the samples were centrifuged for 10 min at 5000 rpm (4 °C) using an Eppendorf Centerfuge 5804 R centrifuge (Leipzig, Germany). The supernatant was filtered into the chromatography vials through a Teflon syringe filter with a pore diameter of 0.22 µm prior to subjection to LC-MS/MS analysis.

Importantly, due to the low recovery of ZEN from the compost samples, ZearalaTestWB immunoaffinity columns (Vicam, Nixa, MO, USA) were used to additionally purify the solid-dried compost extracts (dissolved in a mixture of 90% acetonitrile with 1% FA). Then, 5 mL of the extracts were purified using the columns according to the manufacturer's protocol and subjected to the LC-MS/MS analysis.

Determination of mycotoxin content was performed using an Agilent 1260 Infinity II chromatograph (LC) coupled to an Agilent 6470 Triple Quadrupole (MS) mass detector (Agilent Technologies, Inc., Santa Clara, CA, USA). Samples were chromatographically separated at a mobile phase flow rate of 0.5 $mL \cdot min^{-1}$ using a 2.1 × 100 mm Agilent Poroshell 120, SB-C18, 2.7 µm column. Mobile phase A was a solution consisting of H_2O, 0.2% FA, 5 mM AFOR, and 0.5 mM AFLU. Mobile phase B consisted of methanol with 0.5% FA and 0.5 mM AFLU. The analytes were tested using gradient separation. Initially, the proportion of phase B was 10% for 1 min, then it was increased linearly to 50% after 2 min. It was then increased to 95% at the 10th minute and was maintained at this level until the 14th minute. After that, it was reduced to the initial proportion of 10% and the column was conditioned in this manner for 4 min before the next sample injection was performed. Sandwich injection was used, with 40 µL of H_2O and 2.5 µL of the actual sample. The column temperature during chromatographic separation was 40 °C. The injection needle was washed with a 50% methanol solution for 3 s. The parameters of the ion source were as follows: Electrospray Ionization + Jest Stream Technology Ion Source (ESI + AJS); gas temperature, 250 °C; gas flow, 8 $L \cdot min^{-1}$; nebulizer, 45 psi; sheath gas, 350 °C; heath gas flow, 11 $L \cdot min^{-1}$; and capillary voltage, 3300 V. The Multiple Reaction Monitoring (MRM) parameters for each compound are shown in Supplementary Figure S1. Chromatographic data were collected and processed with Agilent MassHunter Workstation LC/MS Data Acquisition software for the 6400 Series Triple Quadrupole, Version 10.1, Build 10.1.67 (Agilent Technologies; Santa Clara, CA, USA).

3. Results

3.1. Behaviour of the Substrate during Composting

For technological reasons, the process was conducted until the waste was biologically stable, i.e., the AT4 value did not exceed 10 g $O_2 \cdot kg^{-1}$ DM. Thus, the composting process lasted for 45 days.

Generally, the most important indicator of composting process efficiency is the temperature, which increases as a direct result of the exothermic decomposition of organic substances in the reactor. During the experiment, the temperature peaked twice: at 63 °C on the 4th day, followed by a lesser peak at 36 °C on the 32nd day (Figure 2A).

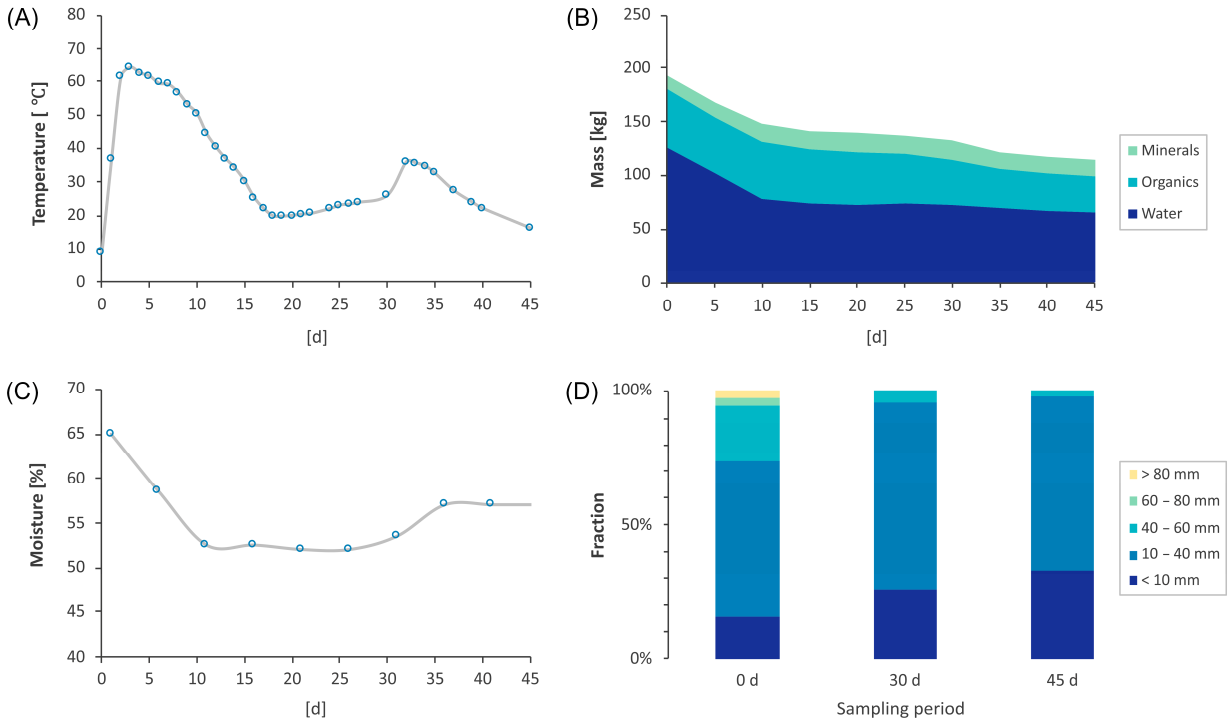


Figure 2. (**A**) Temperature, (**B**) mass, (**C**) moisture, and (**D**) size fractions of the substrate material at sampling points during the 45 days of composting in the passively aerated bioreactor.

During the 45 days of composting, the mass of the substrate decreased by 78 kg, which is equivalent to 40.37% of the total initial mass (Figure 2B). The main reason for this mass reduction was water evaporation: the changes in moisture and LOI during this period indicate that the water loss from the reactor was approximately 60 kg, and the loss of organic matter was approximately 21 kg (note that the mass of the mineral compounds increased by approximately 3 kg). The final mass balance is presented in Table 2; however, it should be noted that an accurate mass balance was not possible to calculate because some of the water in the reactor may have been produced in the process of organic matter decomposition.

Table 2. Mass balance of the process.

Timepoint	Mass [kg]	Moisture [%]	LOI [% DM]	Water [kg]	Organics [kg DM]	Minerals [kg DM]
0 d	193.20	65.00	81.00	125.58	54.77	12.85
45 d	115.00	57.00	68.00	65.55	33.63	15.82
Difference	–78.20	–8.00	–13.00	–60.03	–21.15	+2.98

While the water mass in the reactor gradually decreased, the moisture content of the waste in the reactor fluctuated. Initially, the moisture decreased from 65% to 52.5%, then, starting on the 20th day of the experiment, it slowly increased to 57% and then remained stable at that level (Figure 2C).

During the progressive decomposition of organic matter in the reactor, its particle size distribution changed. Initially, there were five size fractions: <10 mm, 10–40 mm, 40–60 mm, 60–80 mm, and 80–100 mm; however, by the 30th day of the study, there were only three:

<10 mm, 10–40 mm, and 40–60 mm (Figure 2D). Over the course of the 45-day process, the proportion of the <10 mm fraction increased from 15.5% to 32.7%, that of the 10–40 mm fraction increased from 58.3% to 70.1%, and that of the 40–60 mm fraction decreased from 20.6% to 1.8%.

3.2. Changes in Indicators of Biological Stability

During the composting process, the biological stability of the waste was assessed at five-day intervals using the change in four-day respirometric activity (AT4), the change in loss on ignition (LOI) values, and the change in total organic carbon (TOC) content. The change in AT4 served as the primary indicator of the biological activity of the waste, with the commonly accepted threshold value of <10 g $O_2 \cdot kg^{-1}$ DM set as the target for the end of the composting process. The initial AT4 value of the research substrate was 79.27 g $O_2 \cdot kg^{-1}$ DM, nearly eight times higher than the permissible level. Over the following days, this value declined relatively rapidly, reaching 15.01 g $O_2 \cdot kg^{-1}$ DM on the 10th day of the experiment and remaining relatively stable for the next 15 days. Subsequently, the AT4 value temporarily increased, reaching 20.84 g $O_2 \cdot kg^{-1}$ DM. This AT4 increase seemed to correlate with rises in the moisture (r = 0.78) and temperature (r = 0.73) of the waste. Finally, the target AT4 threshold was passed on the 45th day of the process when a value of 9.82 g $O_2 \cdot kg^{-1}$ DM was achieved (Figure 3A).

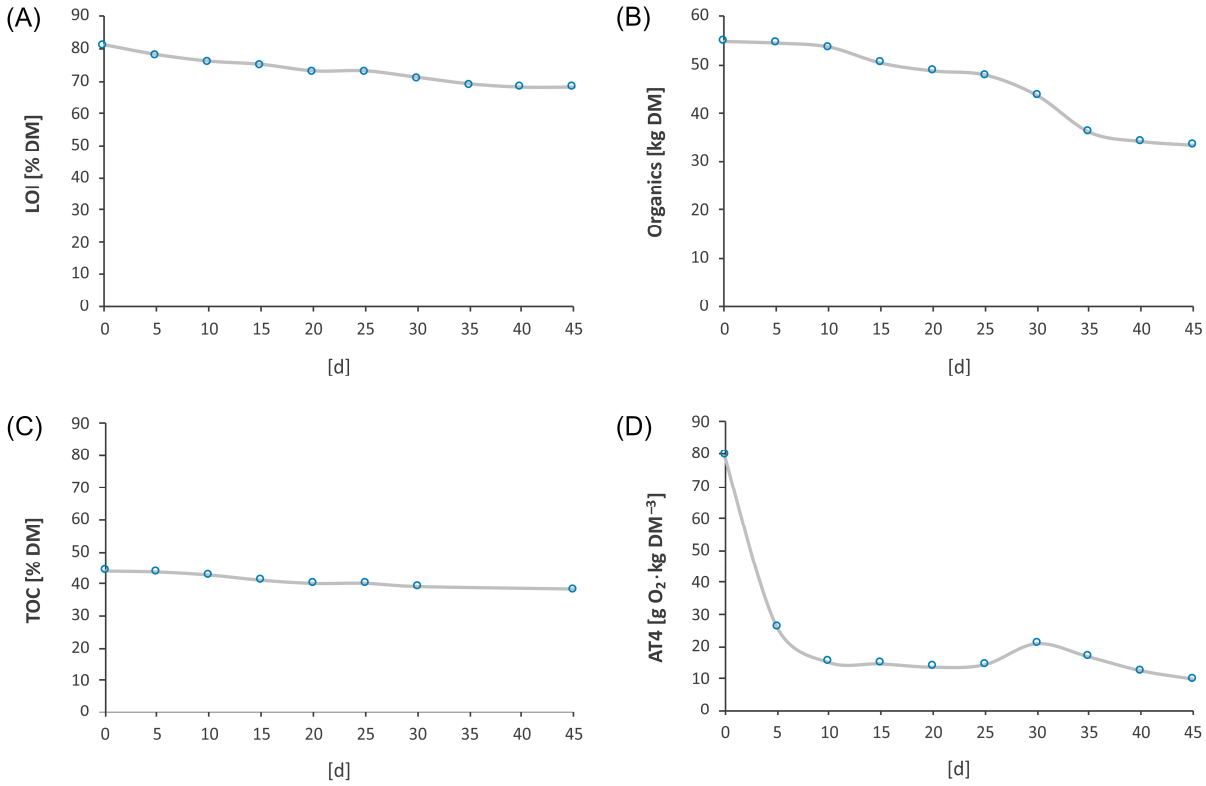


Figure 3. (**A**) Loss on ignition (LOI), (**B**) mass of organic substances, (**C**) total organic carbon (TOC), and (**D**) respirometric activity (AT4) of the substrate material at sampling points during the 45 days of composting in the passively aerated bioreactor.

LOI and TOC were additionally used for insight into the kinetics of organic matter mineralization. The proportion of organic matter, as measured by the LOI value, decreased linearly from 81% DM to 68% DM, at an average rate of approximately 0.28% DM per day, corresponding to an organic matter decrease of 21.1 kg at a rate of approximately 0.5 kg DMO per day (Figure 3B). The percentage of TOC content decreased linearly from 44.1% DM to 38.2% DM, at an average rate of approximately 0.16% DM per day, corresponding to a loss of 10.9 kg.

3.3. Chemical Measurements of Leachate

During the 45-day experiment, 1.395 dm^3 of leachate was generated, and its pH value shifted towards alkaline (Figure 4A,B). The concentration of organic compounds increased to 5680 $mg \cdot dm^{-3}$ on the 8th day of the experiment, then gradually decreased to 1160 $mg \cdot dm^{-3}$ on the 13th day of the experiment, which was the last day of leachate emission. Similarly, ammonium nitrogen, orthophosphates, and volatile fatty acid concentrations briefly increased, then decreased until leachate emission ceased. The maximum concentrations of these indicators were 126 mg $NH_4 \cdot dm^{-3}$ on the 9th day of the experiment, 55 mg $PO_4 \cdot dm^{-3}$ on the 8th day of the experiment, and 1714 mg VFA$\cdot dm^{-3}$ on the 8th day of the experiment (Figure 4C–F).

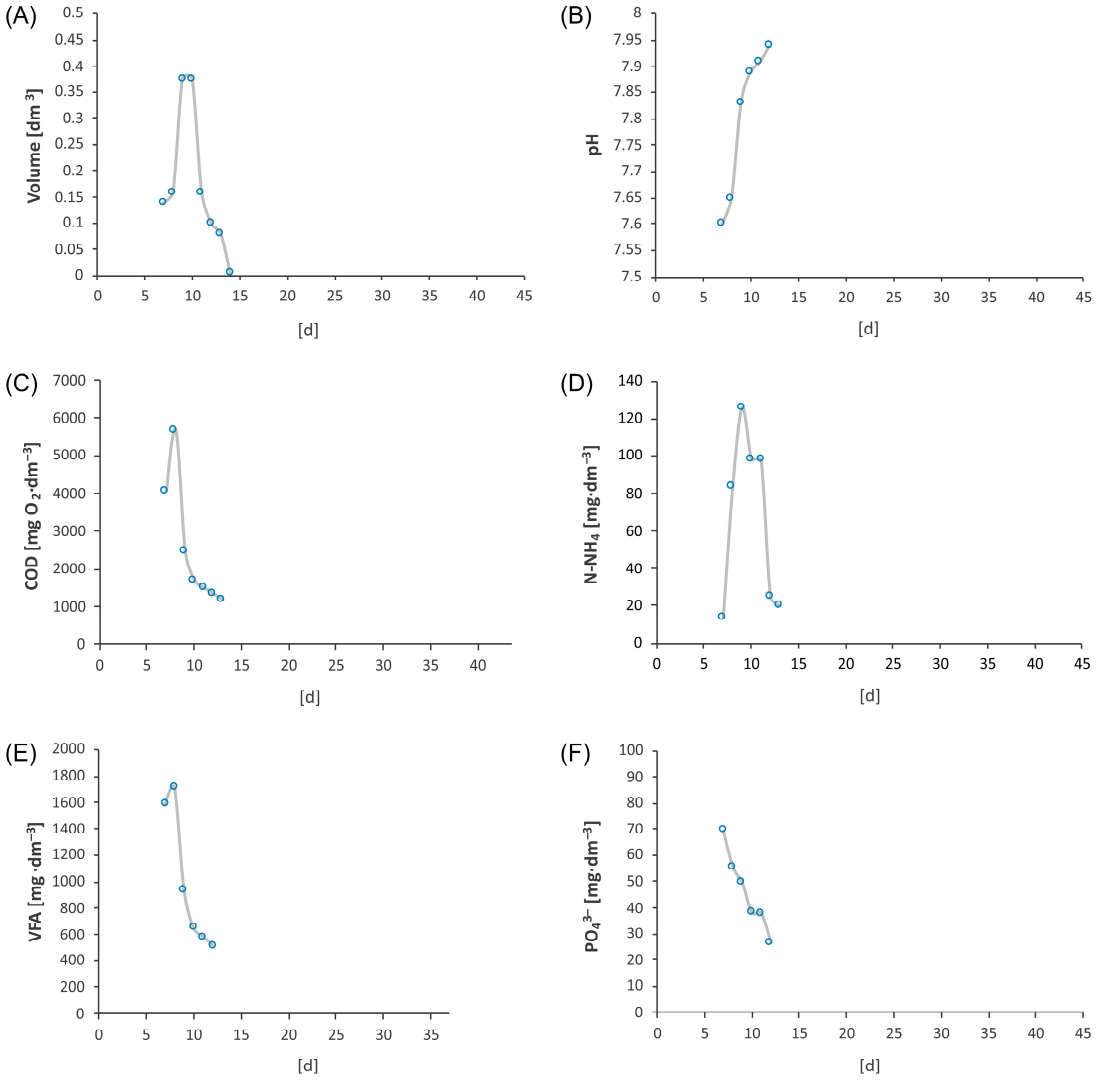


Figure 4. (**A**) Volume, (**B**) pH, (**C**) chemical oxygen demand (COD), (**D**) ammonium nitrogen (N-NH_4), (**E**) volatile fatty acids (VFA), and (**F**) phosphates (PO_4^{3-}) in leachate samples collected during the 45 days of composting in the passively aerated bioreactor.

3.4. Changes in the Biocenosis of the Composting Material

As early as 5 days after the start of composting, intensive mould growth on the substrate was evident, mainly in the form of small white crusty spots on the leaves, but occasionally also in the form of a thick white coating resembling cotton wool. The kitchen waste, in turn, was predominantly covered with a dense white or greenish layer of mould. As the experiment progressed, the mould was no longer evident on the surface of the composting material.

Microscopic analysis confirmed the presence of mould and showed dynamic changes in the composition/population of the organisms in the material during the process (Figure 5). At the first three sampling times (5, 10, 15 days), mould hyphae, sporangia, and spores predominated in the microscopic images. Among these structures, well-developed sporangia of the genus *Rhizopus* were distinguished, and vesicles of the genus *Aspergillus* were also found. In the next samples, the structure of the hyphae was less developed; in addition, protozoa (mainly free-swimming ciliates) began to appear. In the last phase of composting (35, 40 days), numerous protozoa and nematodes were observed; arthropods were also occasionally found.

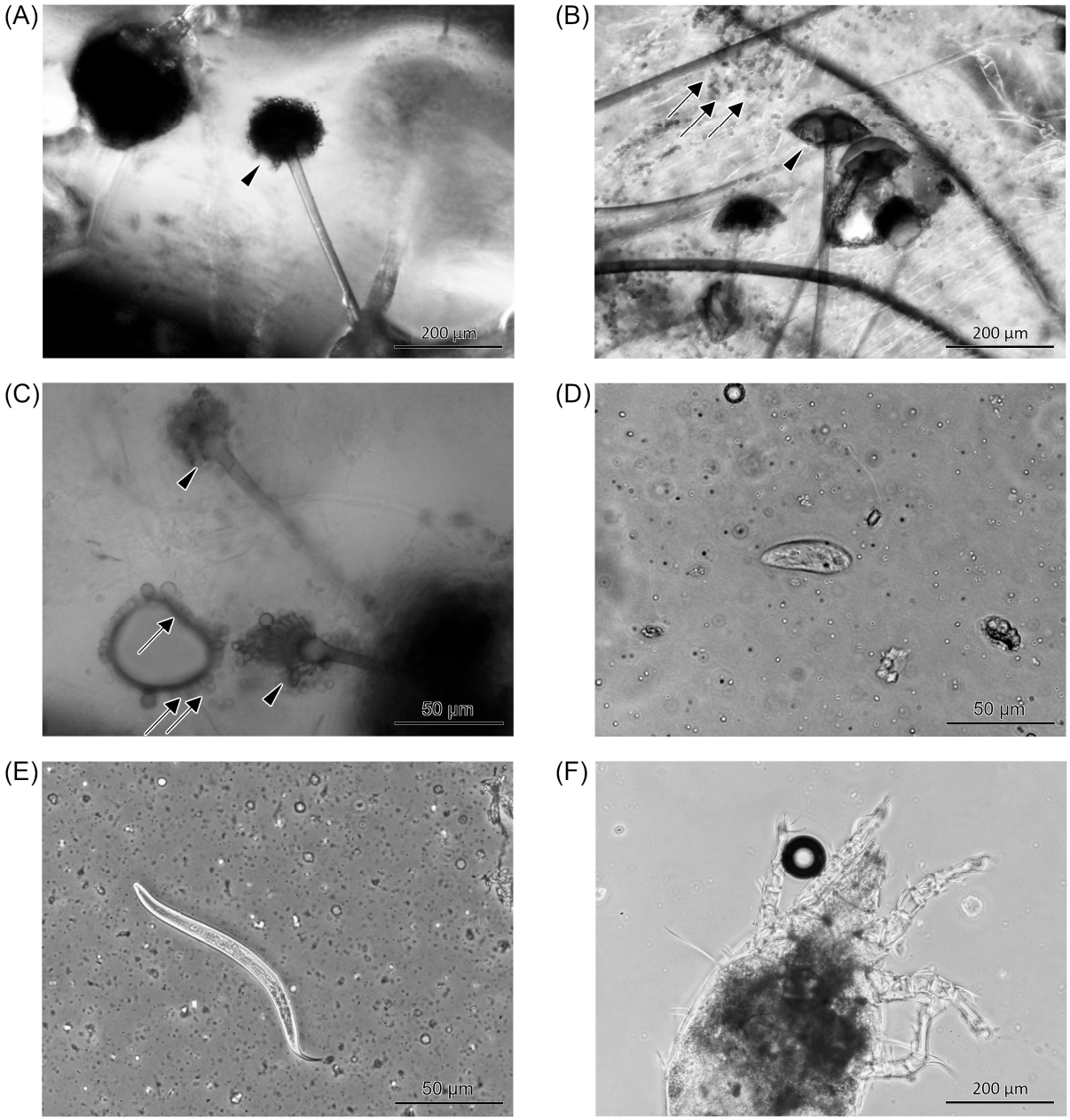


Figure 5. Microscopic images of organisms found in the material during 45 days of composting. (**A**,**B**) Sporangia (arrow heads), spores (arrows) of *Rhizopus* sp. and (**C**) conidiophores (arrow heads) and conidia (arrows) of *Aspergillus* sp. fungi observed after 10, 10, and 15 days of the process, respectively. (**D**) Free-swimming ciliates, (**E**) nematodes, and (**F**) arthropods observed after 40 days.

The presence of numerous saprotrophic organisms (including moulds) confirms the intensive biodegradation of the composted material, and the dynamic changes in the technical biocenosis indicate that the substrate matured rapidly into compost.

3.5. Mycotoxin Content

The content of selected mycotoxins (AFB1, DON, FUM B1 and B2, HT-2 toxin, OTA, T-2 toxin, and ZEN) was measured in samples of the substrate (a mixture of kitchen and garden waste) and the leachate produced during the 45 days of the composting process (Supplementary Table S1). Most of these samples did not contain any of the analysed mycotoxins at levels above our method's limit of detection (LOD, 2 $\mu g \cdot kg^{-1}$). Only a few samples contained AFB1, HT-2, FUMs, and/or ZEN, and only trace amounts of these compounds were detected. ZEN was most frequently detected, and unlike the other mycotoxins, its content in the substrate was found to be above our method's level of quantification (>5 $\mu g \cdot kg^{-1}$; LOQ), but this occurred only once. The presence of ZEN was shown by the presence of progeny ions, i.e., 319.3 → 301.2; 319.3 → 283.8, and a distinct peak at the retention time of 6.81 min (Supplementary Figure S2).

To obtain additional information about the mycotoxin contamination of commercially available compost, mycotoxin content was also determined in several samples of mature compost collected from local composting facilities or purchased from garden centres. Only trace amounts of ZEN and AFB1 (<5 $\mu g \cdot kg^{-1}$) were detected in the mature compost obtained from the composting plants and no mycotoxins were found in the commercial compost products purchased from the garden centres.

4. Discussion

4.1. Preparation of the Substrate

The technological objective of this project was to carry out the composting process under optimal conditions, i.e., those in which the interactions between the physical and biochemical properties of the substrate and the activity of the microbes would be most effective. This is usually achieved by appropriately setting the initial conditions (focusing mainly on the moisture and C/N ratio) and then maintaining them in the optimal range during the composting process [37–39]. In practice, the initial substrate composition is usually optimized by using co-substrates in appropriate weight proportions [38,40–42]. Thus, the present study used a mixture of kitchen waste (59% *w*/*w*), leaves (28.2% *w*/*w*), and wood chips (12.8% *w*/*w*) for composting; this substrate had an initial moisture content of 65% and an initial C/N ratio of 26.6. In the co-composting of kitchen waste with structural material, the recommended initial moisture content is between 50% and 65% [38,43–46] and the recommended C/N ratio is between 25 and 30 or 35 [47,48]. Therefore, the initial properties of the tested substrate were in accordance with generally accepted technological recommendations.

4.2. Behaviour of the Composting Process

Due to the exothermic nature of the decomposition of organic compounds, temperature change is the main indicator of microbial activity during composting [49]. Microbiological activity, in turn, is influenced by environmental conditions, particularly the bioavailability of organic compounds and the moisture of the composting matrix. Moreover, there is a feedback mechanism: an increase in the temperature to thermophilic conditions leads to hygienisation of the waste [50–52] and increased evaporation from the reactor/prism [53,54].

In the present study, the initial changes in the reactor were typical of composting processes: after the temperature increased relatively rapidly to thermophilic levels (64.3 °C), it decreased progressively, accompanied by decreases in the respiration activity (AT4) and moisture content. Interestingly, however, on the 30th day of the composting process, three indicators displayed marked increases: temperature from 23.9 °C to 36 °C; AT4 respirometric activity from 14.28 g $O_2 \cdot kg^{-1}$ DM to 20.84 g $O_2 \cdot kg^{-1}$ DM; and moisture

from 52% to 57%. It is assumed that the increase in moisture on the 30th day of testing caused a temporary increase in microbiological activity, which, in turn, caused an increase in the process temperature. An increase in moisture is known to induce microbial activity in compost under certain environmental conditions [55–57]. Moreover, a report by Shen et al. [46] indicates that composting of kitchen waste with an initial moisture content of 65% may also be accompanied by an increase in moisture content over the time.

In the study presented here, water loss was the main cause of the weight loss in the reactor: out of a total weight loss of 78 kg, 60 kg was due to water loss through evaporation. A temporary increase in moisture on the 30th day of the process suggests that the mass of metabolic water that was produced (0.5–0.6 g H_2O/g VS; [58]) could have exceeded the mass of water that evaporated. Mass balance calculations, however, using Equation (2), showed that the amount of water in the reactor was continuously decreasing (Figure 2B). Therefore, it should be assumed that in the tested passive aeration system, the evaporation rate was greater than the rate of metabolic water production.

Measurements of four-day respirometric activity (AT4) are commonly used to assess the biological stability of waste [59–61]. This measurement is an indicator of both microbial activity and the bioavailability of organic compounds. In the present study, the threshold for respirometric activity was set at <10 g $O_2 \cdot kg^{-1}$ DM, in accordance with generally accepted standards. In order to reach this threshold, the process lasted 45 days, during which 39% DM of organic matter and 37% DM of total organic carbon were mineralized. These results are very similar to those of other authors. For example, Kumar et al. [38] reported that, during the composting of kitchen waste, leaves, and structural material with an initial moisture content of 60%, an initial C/N ratio of 19.6, and an initial carbon content of 43.38%, organic matter was reduced by 32.8%. Similarly, Pandey et al. (2016) [62] composted food waste, horse manure, and green waste with an initial moisture content of 54% and an initial C/N ratio of 49.5 and found that the carbon content was reduced by 39%. In a recent study, green waste and food waste were co-composted in proportions similar to those in our study (60% and 40%, respectively) and with a C/N ratio between 14.28 and 20.1: thermophilic conditions were rapidly achieved, and the final product was a high-quality fertilizer [63].

The leachate that is produced during composting comes from three main sources: (1) the water content of the organic waste itself, (2) the water generated by biochemical reactions, and (3) the water added by atmospheric precipitation or adjustment of the moisture content [64]. Hence, the quantitative and qualitative characteristics of leachate generated during the composting process depend on the biochemical composition of the substrate and on the technical solutions used in the composting plant (i.e., enclosed/open composting technology). For example, leachate produced during composting of mixed municipal waste has a substantially higher concentration of COD (48 g $O_2 \cdot dm^{-3}$ in average) than that produced during composting of yard and green waste (29 g $O_2 \cdot dm^{-3}$ in average) [64]. Composting in an open system involves greater exposure to rainfall, leading to production of larger amounts of leachate with lower concentrations of total nitrogen [65]. It should be mentioned here that organic matter and nitrogen are generally considered to be the two main contaminants found in composting leachate [66,67].

Unlike the authors' previous experimental work on the composting process in passive conditions [24], in this experiment, there was no recirculation of leachate into the reactor; therefore, the amount and characteristics of the generated leachate depended on substrate saturation and the intensity of biochemical transformations in the reactor. As a result, a relatively small amount of leachate was generated (1.395 dm^3 in total), and it had relatively low COD and nitrogen concentrations (respective maximums of 5.68 g $O_2 \cdot dm^{-3}$ and 126 mg $N\text{-}NH_4 \cdot dm^{-3}$). Such peaks in leachate contaminant concentrations are rather typical and similar to those of other studies [68–71].

Taken together, these results indicate that the composting process was performed effectively from a technological point of view.

4.3. Trace Amounts of Mycotoxins found during Composting of Biowastes

Our finding of only trace amounts of AFB1, HT-2, FUMs, and ZEN in just a few samples (Supplementary Table S1) is similar to the findings of other studies, which reported little or no mycotoxin content in compost from large-scale composting facilities [12–14]. There are two likely explanations for the fact that only traces of mycotoxins were found in the substrate collected from different stages of the process and their content did not change markedly during the time of the experiment. First, any mycotoxins that were produced may have been biodegraded immediately subsequent to their production. Numerous studies have shown that pure or heterogenous microbial cultures isolated from different environments are capable of irreversibly transforming DON and other mycotoxins into less toxic compounds [15]. Composting alone or in combination with anaerobic digestion has also been shown to be an effective method of removing mycotoxins from contaminated substrates [16,17,72]. For example, 90 days of co-composting the organic fraction of municipal solid waste with corn artificially contaminated with AFB1 almost completely removed the mycotoxin [17]. On the other hand, noticeable amounts of mycotoxins may not have been produced under the conditions used in this study.

Importantly, ZEN was the mycotoxin that was most frequently detected in the samples taken in our study, and once, at the end of the biowaste composting process, its content in the substrate was high enough to be precisely quantified (>5 $\mu g \cdot kg^{-1}$; LOQ), unlike the contents of the other mycotoxins (Supplementary Table S1). These findings suggest that the production of some mycotoxins during composting could be a selective process, depending on specific bioreactor conditions that favour the development of particular taxonomic groups of moulds and/or the production of specific mycotoxins. In general, the high moisture of the kitchen waste and the conditions in which it was stored would have favoured dynamic mould growth, which was confirmed by our macro- and microscopic observations. However, the population of fungal species and strains can vary depending on the composition of the substrate and the specific conditions of the technological process, e.g., the nutrients, temperature, humidity, and pH [5,10,11,73]. Moreover, the presence of mould does not necessarily correspond to the presence of toxicogenic strains that are capable of producing mycotoxins [74]. This is supported by the fact that, even though moulds of the genera *Aspergillus* and *Rhizopus* were present in the composting substrate in our study (Figure 5), and some strains of these moulds can produce AFB1 and OTA [9], no more than trace amounts of these two fungal metabolites were found throughout the entire experiment. This is consistent with the understanding that there is no simple relationship between the growth of mould and whether it produces mycotoxins [73,75]. On the other hand, ZEN is primarily produced by moulds belonging to *Fusarium* genera, and high humidity and fluctuating moderate and low temperatures are considered the main factors that stimulate the production of this mycotoxin in grains [76]. Although the conditions that are most favourable to the production of ZEN during composting of biowastes have yet to be established, the bioreactor conditions in our study are consistent with those that are known to favour the formation of ZEN in grains.

Finally, it is also possible that there could have also been toxicogenic strains of moulds that produce mycotoxins other that the ones that were analysed in our study. For example, *Penicillum* species have been reported to produce hundreds of toxic metabolites [11,20], but analytical standards are available for only some of them. In this study, only eight of the most commonly occurring mycotoxins were investigated; thus, a broader search might have detected more mycotoxins.

Leaving aside consideration of the numerous factors that may be responsible for the production and degradation of mycotoxins, it can be stated that the technological conditions applied during the composting of kitchen and garden waste in this study did not lead to the occurrence of noteworthy amounts of the studied mycotoxins in the composted substrate. This suggests that applying appropriate technological conditions for an effective composting process may help to mitigate the potential risks of mycotoxin production.

4.4. Potential Risks Related to the Occurrence of Mycotoxins in Compost

The motivation behind our study was that, if mycotoxins are actually produced during composting, these harmful compounds could pose a health risk to not only wildlife or domestic animals [13] but also farmers and workers in composting facilities [10,20,21] or compost consumers, e.g., gardeners or florists. Therefore, in addition to examining the potential of kitchen and garden waste to form mycotoxins during composting in passively aerated conditions, we were also interested in screening for mycotoxin contamination in compost products that are provided to the local market by biowaste composting plants and garden centres. This additional survey found only trace amounts of ZEN and AFB1 (<5 $\mu g \cdot kg^{-1}$) in the mature compost obtained from the composting plants and no mycotoxins in the commercial compost products purchased from the garden centres (Supplementary Table S1). These findings indicate that there is little or no mycotoxin contamination of compost products from these sites.

There are few reports on the content of mycotoxins in compost. The most recent study screened 28 different compost piles in Canada for the presence of OTA, T-2 toxin, and ZEN [13]. Although those authors found mycotoxins in 86% of the analysed samples, and the contents often exceeded the regulatory limits for mycotoxin contamination of animal feed (i.e., OTA 5 $\mu g \cdot kg^{-1}$, T-2 toxin 100 $\mu g \cdot kg^{-1}$), they found the highest contents of mycotoxins only in compost from agricultural piles of decomposing animal feed and bedding and residential compost piles located in backyards. In contrast, they did not find T-2 toxin and OTA in compost collected from large-scale industrial piles of decomposing food waste, and they reported ZEN contents of 0.54–6.32 $\mu g \cdot kg^{-1}$ with a mean of 3.43 $\mu g \cdot kg^{-1}$ in these samples [13]. Those findings are in full agreement with our results (Supplementary Table S1). Thus, based on our findings on composting biowaste and on our survey of the local compost market, as well as the findings Murray et al. [13], we believe that the environmental and health risks related to the occurrence of mycotoxins in biowaste compost produced in large-scale installations are likely to be minimal. However, further research should investigate the factors leading to the production of mycotoxins during small-scale composting, and more screenings of agricultural and residential compost piles are necessary to understand the potential threat of mycotoxin contamination resulting from the organic recycling of biowaste.

5. Conclusions

This study found that only trace amounts of mycotoxins were present during 45-day composting of kitchen and garden waste at pilot scale. These results build on and extend the limited information that was available from studies of large-scale composting. Together, those results and ours strongly suggest that there is little risk of mycotoxin contamination in the compost produced by these facilities. The results also emphasize the importance of applying appropriate technological conditions for effective composting. These findings, however, leave open the question of mycotoxin contamination in compost produced at smaller scales (e.g., agricultural and residential compost piles). Therefore, future research should focus on screening compost produced at smaller scales and on identifying the factors most strongly associated with the risk of mycotoxin contamination in compost.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14125288/s1, Figure S1: MRM parameters of the analysed mycotoxins. Figure S2: Chromatogram confirming the presence of zearalenone in compost samples, based on the presence of progeny ions, viz: 319.3 → 301.2, and a distinct peak at the retention time of 6.81 min. Table S1. Mycotoxin content in collected samples (composting substrate and leachates collected during 45 days of composting kitchen and garden waste, and commercially available compost samples obtained from the local market).

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