Changes of chemical and biological parameters during vermicomposting of kitchen biowaste with an emphasis on pathogens

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Summary

Food and kitchen biowaste can be an environmental and economic problem if not managed properly. The aim of this study was to uncover the effect of an outdoor vertical-flow vermicomposting system with continuous feeding by kitchen biowaste on pathogens in relation to the biological and chemical properties. After one year of outdoor vermicomposting the chemical and biological stability of vermicompost was found. Pathogens were reduced by 66%, while Salmonella spp. was not present at all. With the increasing age of the vermicompost, the total microbial biomass and its components (fungi, actinobacteria, G+ and G- bacteria) as well as enzymatic activity declined. The average activity of enzymes such as β-D-glucosidase, phosphatase, sulfatase, lipase, chitinase, cellobiohydrolase, in the bottom layer was less by 53% compared to the top layer. The moisture and EC decreased to 48% and 722 $\mu S \cdot cm^{-1}$, respectively. On the other hand, the pH increased up to 8.0. The decreasing trends of C_{tot} and N_{tot} correlated positively with their available forms such as DOC, $N\text{-}NO_3^-$, and $N\text{-}NH_4^+$, with their proportions of the total content at 1.1%, 0.9%, and 0.3%, respectively. Among other nutrients, Ca_{tot}, K_{tot}, and Fe_{tot} exhibited the greatest contents (identically 1.3%) followed by Mg_{tot} (0.35%) and P_{tot} (0.3%). The proportion of the available contents of K, Fe, Mg, and P constituted on average in all of the three layers 39%, 2%, 12%, and 16%, respectively, of the total content.

Keywords: *vermicomposting, earthworms, kitchen biowaste, layers, pathogens, chemical and biological properties*

Introduction

Only 2/3 of the food products produced each year are utilized, which equates to 1.3 billion tons of food which is unnecessarily wasted[1]. In the countries of the European Union, food losses amount to 88 million tons per year, which has a negative impact on the use of primary resources, individual segments of the environment, and ultimately, the economy[2]. Food waste contains a wide range of chemical substances. The chemical composition is affected by the proportion of individual foods. On average, it contains 60% starch, 30% lipids, and 10% proteins[3,4].

Besides the avoidance of food loss or waste, a proper re-utilization seems to be the only responsible way of dealing with wasted food today and in the future[5]. Food and kitchen biowaste can be an environmental and economic problem if not managed properly[6]. The food waste produced from restaurants and canteens form a major component of the putrefying organic waste that ends up in landfill sites. High moisture, the requirement for a bulking substrate, and low pH are the main problems encountered with food or kitchen biowaste composting or vermicomposting[7,8]. Therefore, especially, constant care with moisture management and a suitable ratio of carbon and nitrogen is required for successful processing[9].

The earthworms are involved in the vermicomposting process of biowaste by creating small corridors through which the air can flow. Using microorganisms and their enzymes, they digest organic substances, creating vermicompost with a high agronomic value[10]. From the operational point of view, vermicomposting unlike classical composting is a mesophilic process. With the use of temperatures above 35 °C the earthworms will leave the processed biowaste or die. The elevated temperature maintained for some time is a key factor in classical composting for reduction of human pathogens such as *E. coli, Salmonella* spp., helminths, and various human viruses. However, elevated temperatures of 70 °C have adverse effects on the rate of decomposition because the activity of microorganisms decreases[11]. The sensitivity of the earthworms to high temperature can be solved by the initial thermophilic pre-composting without the presence of earthworms and subsequent vermicomposting[12]. However, this combination may not be possible for technical, spatial, or economic reasons. Previously, there was a view that vermicomposting itself was capable of reducing or completely eliminating the number of pathogenic microorganisms[13,14]. The mechanisms by which this occurred are not fully known. These are direct influences (mechanical disruption using ingestion and grinding, microbial assimilation, enzymatic digestion, effect of microbial antagonists and antimicrobial substances produced by the earthworms) and indirect influences (phagocytic activity with the participation or absence of earthworms, production of antimicrobial substances such as humic acids, the stimulation of endemic or other microbial species leading to competition). The most likely mechanism is probably a combination of these effects[15]. It was reported that the inactivation of the pathogenic load largely depends on the earthworm species involved in vermicomposting, because individual species have different abilities to eliminate pathogens[16].

The aim and at the same time novelty of this study was to uncover the effect of an outdoor vertical-flow vermicomposting system with continuous feeding by kitchen biowaste on pathogens in relation to the biological and chemical properties.

Experimental part

Experimental setup

The experiment was conducted in three concrete boxes each with a ground plan 2 m x 2 m placed in outdoor conditions at the Research Institute of Agricultural Engineering, Prague, Czech Republic (50°5'11.327"N, 14°18'7.522"E).

The bedding of grape marc with earthworms (*Eisenia andrei*) was placed first. Next 10 cm layers of kitchen biowaste were added every 3 weeks. Adding a thin layer prevents the warming which the earthworms are sensitive to. To reduce evaporation, the surface was covered with special fabrics which allowed for air exchange while retaining water. If necessary, the vermicomposted material was irrigated to maintain 80 – 85% moisture content which is optimal for earthworms[17]. After one and half years from the beginning of the experiment, samples were taken up from the cross profiles of different depths, and the average ages above the bedding from each of the 3 boxes in the three replications was as follows:

A: 0 – 20 cm, 1 – 6 months
B: 20 – 40 cm, 6 – 12 months
C: 40 – 60 cm, 12 – 18 months

The weight of each primary sample was about 5 kg. The resulting vermicompost sample without earthworms was divided into 3 parts, each of 1 kg, and treated as required for laboratory analyses. One part of the vermicompost sample was stored for a brief period at 4 °C, the second part was dried at 35 °C to a constant weight, the third part of the vermicompost sample was frozen at -20 °C and then lyophilized.

Biological and chemical analyses

Pathogenic microorganisms were determined in the fresh sample by cultivation on selective diagnostic media and confirmed by biochemical tests[18]. The contents of the containers with the tested

substrates were first weighted, diluted, and homogenized. Ten-fold diluted suspensions of samples were used for cultivation of microorganisms. Dehydrated complete preparations (HiMedia) were used for the initial suspensions, dilutions, and culture media. Thermotolerant total coliform bacteria were determined by capturing on the surface of the solid soil with lactose. M-FC agar (Membrane Fecal Coliform Agar) was used as the culture medium. This agar is selective for thermotolerant coliform microorganisms and contains lactose, bile salts and aniline blue. After culturing for 24 hours at 43 ± 1.0 °C, characteristic blue colonies were counted. At least 5 characteristic colonies were inoculated onto nutrient agar for subculture for 24 hours at 36 ± 1.0 °C for oxidase testing. Subsequent confirmation was performed by a negative oxidase assay. To determine the number of *Escherichia coli* (*E. coli*), selected colonies from M-FC agar were inoculated onto 4-methylumbelliferyl β-D-glucuronide-saturated medium (MUG) and cultured for 4 hours at 37 ± 1.0 °C. *E. coli* showed a light blue fluorescence under a UV light emitting radiation of 360 nm wavelength. Enterococci were cultured on solid medium with sodium azide and 2,3,5-triphenyltetrazolium chloride, which reduced the bacteria to red formazan, causing the characteristic colony staining. Incubation was carried out for 4 hours at 36 ± 1.0°C and then 24 hours at 43 ± 1.0 °C. Colonies were confirmed on bile-esculin-azide agar by culturing for 4 hours at 43 ± 1.0 °C. For positive or negative finding of *Salmonella* spp. non-selective cultivation in peptone water was performed for 20 hours at 36 ± 2.0 °C, selective growth in Rappaport-Vassiliadis medium for 24 hours at 41 ± 1.0°C, and growth in soil with selenite and cystine for 24 hours at 36 ± 1.0 °C, followed by cultivation on solid selective soil - phenol red and brilliant green agar and xylosol-lysine-deoxycholate agar. After incubation of the cultures for 24 hours at 36 ± 1.0 °C, growing colonies were subjected to confirmation by biochemical assays.

Samples for the phospholipid fatty acid (PLFA) analysis were extracted in triplicate using a mixture of chloroform-methanol-phosphate buffer (1:2:0.8; v/v/v) from the lyophilized samples. Analyses were done by the Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic. The extracts were analyzed by tandem gas chromatography-mass spectrometry (GC-MS; 450-GC, 240-MS Varian, Walnut Creek, CA, USA). Methylated fatty acids were identified according to their mass spectra using a mixture of chemical standards obtained from Sigma-Aldrich, Prague, Czech Republic and Matreya LLC, USA. The gram positive (G+) bacteria biomass were quantified as the sum of i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0. The gram negative (G-) bacteria were determined by sum of 16:1ω7, 18:1ω7, cy17:0, cy19:0, 16:1ω5. The 10Me-17:0, 10Me-18:0, and 10Me-16:0 were used for actinobacteria biomass. For the total biomass of the bacteria, the acids for G+, G-, actinobacteria, and also 16:1 ω 9, 17:0, 10Me-18:0, cy19:0 were used. The 18:2ω6,9 was used to determine the fungal biomass. The total biomass was quantified as the sum of all of the markers together with 16:0 and 18:1ω9[19].

The enzymatic activity of β-D-glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, and leucine aminopeptidase were measured in 96-well microplates. 0.2 g of lyophilized vermicompost was extracted with 20 mL of acetate buffer (pH 5.0, c = 50 mmol·L^{-1}) in an Erlenmeyer flask. The mixture was homogenized using the Ultra-Turrax (IKA Labortechnik, Germany) for 30 s at 8,000 rev/min. Individual enzyme activities were measured in four replications. 200 µL of the homogenized sample was pipetted into appropriate wells in the microplate and then 40 µL of substrate was added. The microplate was placed in an incubator heated to 40 °C for 5 min, and after the fluorescence was measured using the Tecan Infinite® M200 (Austria). The microplate was again placed in the incubator for 2 h and the fluorescence was measured again[20]. For the calibration curve 1.0 mmol·L^{-1}, 0.1 mmol·L^{-1}, 0.01 mmol·L^{-1} methylumbellyferol, and 0.1 mmol·L^{-1}, 0.01 mmol·L^{-1} 7-aminomethyl-4-coumarin were used.

The pH, oxidation-reduction potential (ORP), and electrical conductivity (EC) were measured in the fresh samples. Measurements were conducted on samples mixed with deionized water (1:5 w/v wet basis) using a WTW pH 340 i (pH and ORP) and WTW cond 730, respectively according to BSI EN 13037[21]. The CHNS Vario MACRO cube analyzer (Elementar Analysensysteme GmbH, Germany) was used for the determination of total carbon (C_{tot}) and nitrogen (N_{tot}) in the dried samples. The total contents of P, K, and Mg were determined by decomposition utilizing digests obtained by pressurized wet-ashing (HNO_3 + H_2O_2) of dried sample in a closed system with microwave heating (Ethos 1, MLS GmbH, Leutkirch im Allgäu, Germany). The contents of ammonium nitrogen ($N\text{-}NH_4^+$), nitrate nitrogen

($N\text{-}NO_3^-$), dissolved organic carbon (DOC), and the available portions of P, K, and Mg were determined in CAT solution (0.01 mol·L^{-1} $CaCl_2$ and 0.002 mol·L^{-1} diethylene triamine pentaacetic acid (DTPA)) at the rate of 1:10 (w/v), according to the International BSI EN 13651[22]. The $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and DOC contents in the extracts were measured using the SKALAR SANPLUS SYSTEM® (the Netherlands). The total and available element concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia).

Statistical analysis

The results are presented as the mean values of three replicates. Nonparametric Kruskal-Wallis ANOVA ($P \leq 0.05$), including testing for normality and homogeneity of data, were performed using STATISTICA 12 software (StatSoft, Tulsa, USA).

Results and discussion

Total coliforms, enterococci, and *E. coli* were the most represented pathogens. *E. coli* is generally found in animal waste[23] and food waste[24]. *Salmonella* spp. was absent during vermicomposting (Figure 1).

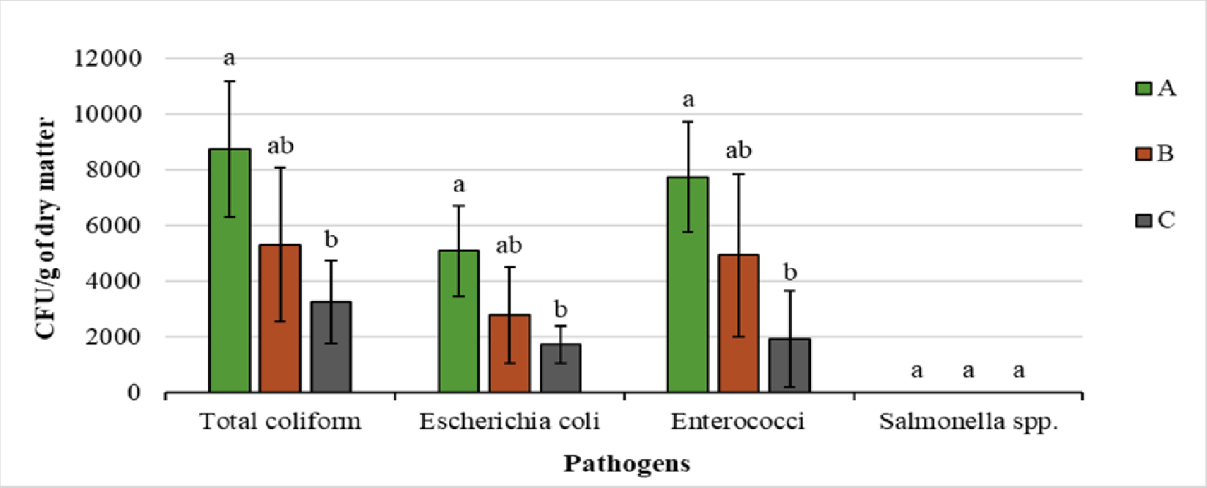

Figure 1: Distribution of bacterial pathogens in layers A, B, and C during vertical-flow vermicomposting. The values are the means ± SD (n=3). Different letters indicate significant differences within a group (Kruskal-Wallis test, P≤0.05)

A: 0 – 20 cm, 1 – 6 months; B: 20 – 40 cm, 6 – 12 months; C: 40 – 60 cm, 12 – 18 months

All pathogens showed the greatest values in layer A (total coliform bacteria: 8,733 CFU/g of dry matter; *E. coli*: 5,100 CFU/g of dry matter; enterococci 7,733 CFU/g of dry matter) and the least values were in layer C (total coliform bacteria: 3,267 CFU/g of dry matter; *E. coli*: 1,733 CFU/g of dry matter; enterococci 1,913CFU/g of dry matter). The oldest layer C contained the least amount of total coliforms, *E. coli*, and enterococci by 63%, 66%, and 75%, respectively, compared the young layer A. Statistically significant differences were found between these two layers (A and C) for all pathogens (except Salmonella spp., which was not present in all layers). In another experiment, apple pomace substrate was artificially inoculated with *E. coli*, *Salmonella* spp., thermotolerant coliform bacteria, and enterococci to assess the contribution of earthworms *Eisenia andrei* during the selective reduction of the pathogens. The inner side of the intestinal tract of earthworms was changed by the artificial bacterial load. On the contrary, intestinal activity, reproduction, and earthworm weight were not affected by the addition of pathogens. The earthworms reduced the amount of *E. coli* significantly more (63-fold, 77-fold, and 840-fold) than enterococci (6-fold, 36-fold, and 7-fold) after 2, 4, and 6 weeks, respectively[25]. The authors explain the rapid reduction of *Salmonella* spp. by the upregulated expression of two pattern recognition

receptors, which bind lipopolysaccharide-binding protein, lipopolysaccharide, and coelomic cytolytic factor. Similarly, other authors found a reduction of pathogens during the vermicomposting of the organic fraction of municipal solid waste using *Eudrilus eugeniae*, even greater than during in-vessel composting[26]. In the case of total coliforms and *E. coli* a 3.12 log and 6.14 log reduction, respectively, was found for food waste after 10 weeks of vermicomposting. Both composting and vermicomposting generated Salmonella-free products. They speculated that the absence of *Salmonella* spp. in vermicompost might be caused by the stimulation of an endemic microflora that reduces the pathogens. In another study, *Salmonella spp*. decreased by 97.8% to 99.9% compared to the control without earthworms[27]. Khwairakpam and Bhargava[28] exhibited a reduction of 99% in total coliforms using *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus* after 6 weeks of vermicomposting of sewage sludge. Yadav et al.[29] reported 100% removal of both total and fecal coliforms during 60 days of vermicomposting of source-separated human feces using *Eisenia fetida*. Complete sanitization of *E.coli*, *Salmonella* spp., and *Shigella* spp. during 60 days of vermistabilization of various agricultural wastes, such as rice straw, sugar cane, and vegetable wastes amended with cow dung was reported by Kumar and Shweta[30]. Swati and Hait[31] summed up the results of 15 experiments with different feedstock and concluded that reduction varied from 85 to 100% for total coliforms, 93 to 100% for fecal coliforms, 99.7% for fecal enterococci, 97.66 to 100% for *E. coli*, and 85.7 to 100% for *Salmonella* spp. In contrast to previous studies, Cao et al.[32] found that the *E. coli* levels decreased slightly during the first 75 days, while increased levels were observed after 90 days of both lab and field experiments with mixtures of vegetables, fruits, and paper. At the end of the experiments, *E. coli* levels were in the same orders of magnitude as in the initial samples. The results of their study showed that the pathogen reduction in vermicomposting is uncertain, probably due to low temperature and the insufficient air circulation. The mechanism for pathogen inactivation might vary with different feedstock and experimental conditions, which requires improved understanding to consolidate the pathogen inactivation mechanisms in the vermicomposting process.

During vermicomposting, the pathogens enter the food chain of earthworms and the predator-prey relationship is established, resulting in the rapid sanitization of wastes. The major sources of nutrients in the diet of earthworms include fungi and protozoa, whereas bacteria are of the least importance[23]. It is known that actinobacteria and fungi metabolize products that have antibacterial effects on the composting process[33]. In the current study, the proportion of the sum of fungi and actinobacteria in the total microbial biomass among the layers (10.7% ±1.3) was similar (Figure 2).

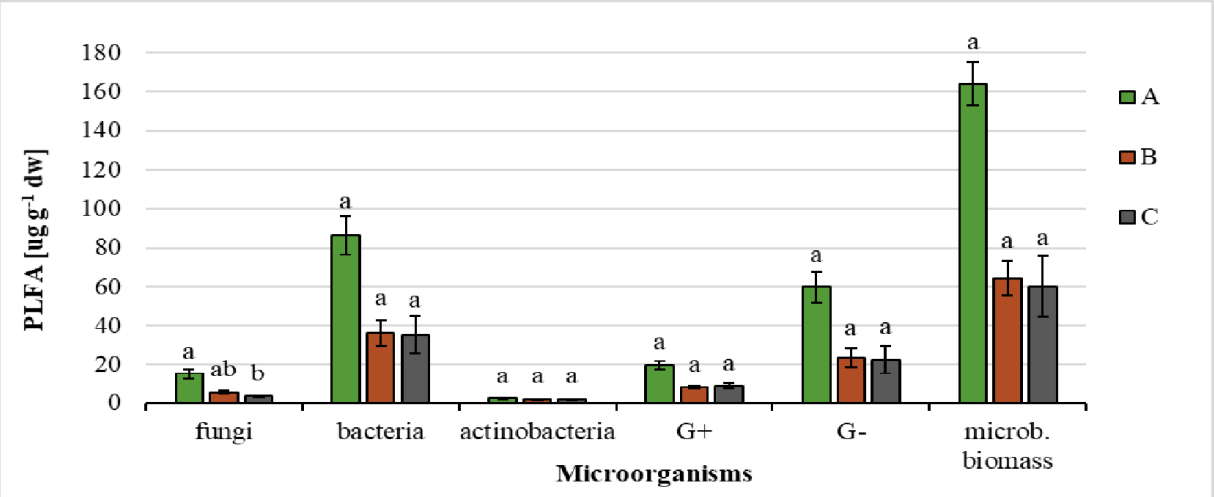

Figure 2: Changes in fungal PLFAs, bacterial PLFAs, and total microbial PLFAs biomass in the layers of the vertical flow vermicomposting system. The values are the means ± SD. Different letters within a group indicate significant differences (Kruskal-Wallis test, P≤0.05)
A: 0 – 20 cm, 1 – 6 months; B: 20 – 40 cm, 6 – 12 months; C: 40 – 60 cm, 12 – 18 months

However, with the increasing age of the vermicompost, the total microbial biomass and its determined components (fungi, actinobacteria, G+ and G- bacteria) declined. The bacteria, especially G- bacteria were the most represented in vermicompost. The greatest content of G- bacteria (59.8 $\mu g \cdot g^{-1}$ dw) was found in the youngest layer A as well as the total microbial biomass. The least total microbial biomass and its components were found in the oldest layer C, with the exception of the G+ bacteria which had the lowest value (8.3 $\mu g \cdot g^{-1}$ dw) in layer B. No statistically significant differences between the layers were found in microbial biomass, except the fungi PLFAs, where a statistically significant difference between layer A and layer C was found. The bacterial/fungal ratio increased with the age of the layers (layer A: 5.7; layer B: 6.4; layer C: 9.9). The greatest microbial biomass content was in layer A (164.2 $\mu g \cdot g^{-1}$ dw) and it was more than two times greater than the least content of microbial biomass (60.1 $\mu g \cdot g^{-1}$ dw, which was found in layer C), but no statistical difference was found. Swati and Hait[31] compared five vermicomposted wastes from the point of view of total bacterial, fungal, and actinomycetes population[31]. The greatest content of all the populations was found in the case of herbal pharmaceutical waste (initial total bacterial population: 9,500 x 10^7 CFU/g, final total bacterial population: 15,000 x 10^7 CFU/g, initial total fungal population: 120 x 10^4 CFU/g, final total fungal population: 400 x 10^4 CFU/g, initial total actinomycetes population: 10,000 x 10^4 CFU/g, final total actinomycetes population: 20,000 x 10^7 CFU/g). The total bacterial population was found during the vermicomposting of paper mill sludge, and the fungal and actinomycetes populations were found in the case of toxic weeds. Since the above results were determined by another procedure, they cannot be compared with the results of this study. Aira et al.[23] found 70-fold, 9-fold, and 1.5-fold total, bacterial, and fungal PLFA during the vermicomposting of cow manure compared food waste in this study.

The greatest values of all enzymatic activities were measured in the youngest layer A (Table 1) and the least values were for ß-D-glucosidase (448.3 µmol MUFG$\cdot h^{-1} \cdot g^{-1}$), acid phosphatase (587.4 µmol MUFP$\cdot h^{-1} \cdot g^{-1}$), arylsulphatase (15.0 µmol MUFS$\cdot h^{-1} \cdot g^{-1}$), lipase (2,233.7 µmol MUFY$\cdot h^{-1} \cdot g^{-1}$) in the oldest layer C, and for chitinase (16.4 µmol MUFN$\cdot h^{-1} \cdot g^{-1}$) and cellobiohydrolase (8.6 µmol MUFG$\cdot h^{-1} \cdot g^{-1}$) in layer B.

Table 1: Enzymatic activity of different layers (A-C) in the vertical-flow windrow vermicomposting system

Layer	**A**	**B**	**C**
β-D-glucosidase [µmol MUFG$\cdot h^{-1} \cdot g^{-1}$]	775.9 ± 252.3 ^{a}	683.4 ± 224.4 ^{a}	448.3 ± 256.3 ^{a}
Acid phosphatase [µmol MUFP$\cdot h^{-1} \cdot g^{-1}$]	1707.5 ± 309.6 ^{a}	884.1 ± 154.1 ^{ab}	587.4 ± 57.7 ^{b}
Arylsulphatase [µmol MUFS$\cdot h^{-1} \cdot g^{-1}$]	36.9 ± 16.7 ^{a}	28.8 ± 10.5 ^{a}	15.0 ± 4.3 ^{a}
Lipase [µmol MUFY$\cdot h^{-1} \cdot g^{-1}$]	2977.3 ± 804.4 ^{a}	2452.4 ± 325.1 ^{a}	2233.7 ± 166.2 ^{a}
Chitinase [µmol MUFN$\cdot h^{-1} \cdot g^{-1}$]	66.1 ± 65.1 ^{a}	16.4 ± 8.6 ^{a}	26.8 ± 14.6 ^{a}
Cellobiohydrolase [µmol MUFC$\cdot h^{-1} \cdot g^{-1}$]	43.1 ± 24.2 ^{a}	8.6 ± 7.1 ^{a}	14.6 ± 8.6 ^{a}
Alanine aminopeptidase [µmol AMCA$\cdot h^{-1} \cdot g^{-1}$]	n. d.	n. d.	n. d.
Leucine aminopeptidase [µmol MUFG$\cdot h^{-1} \cdot g^{-1}$]	n. d.	n. d.	n. d.

Values are the means ± SD (n=3). Different letters in a superscript indicate significant differences (Kruskal-Wallis test, P≤0.05). n. d. = no detection
A: 0 – 20 cm, 1 – 6 months; B: 20 – 40 cm, 6 – 12 months; C: 40 – 60 cm, 12 – 18 months

The most active enzymes were lipase and acid phosphatase, which exhibited multiple times greater activity than the least active enzymes (arylsulphatase, cellobiohydrolase, and chitinase). The activity of ß-glucosidase in the experiment done by Fernández-Gómez et al.[34], who vermicomposted sheep manure for 210 days using *E. fetida*, increased (from 1000 µg PNP·g^{-1}·h^{-1} to 6000 µg PNP·g^{-1}·h^{-1}) initially, but after 90 days of vermicomposting decreased (to 500 µg PNP·g^{-1}·h^{-1}). They also measured the activity of acid phosphatase, which decreased from 4000 µg PNP·g^{-1}·h^{-1} to 1000 µg PNP·g^{-1}·h^{-1} as in our study. The same trend was also observed in a vermicomposting study of winery waste (vinasse biosolids mixed with vine shoots) in laboratory conditions[35]. The activity of ß-glucosidase increased from 10,000 µg PNP·g^{-1}·h^{-1} to 20,000 µg PNP·g^{-1}·h^{-1} after 8 weeks of vermicomposting, and then decreased to 5000 µg PNP·g^{-1}·h^{-1}. The activity of phosphatase only decreased with the time of vermicomposting (from 80,000 µg PNP·g^{-1}·h^{-1} to 10,000 µg PNP·g^{-1}·h^{-1}). The activities of alanine and leucine aminopeptidase were below the detection limit. Only one enzymatic activity, in which a statistically significant difference (between layer A and C) was found, was acid phosphatase activity.

The greatest moisture content (Table 2) was found in the youngest layer (layer A), and it was similar in the vermicompost from grass clipping, shredded paper, and kitchen waste done by Nair et al[9] using *Lumbricus rubellus* and *Eisenia fetida* earthworms[9].

Table 2: Select parameters of different layers (A-C) in the vertical-flow windrow vermicomposting system

Layer	**A**	**B**	**C**
Moisture [%]	52.4 ± 3.9 ^{a}	51.3 ± 1.3 ^{a}	50.5 ± 3.2 ^{a}
pH/H_2O	7.6 ± 0.2 ^{a}	7.8 ± 0.2 ^{a}	7.9 ± 0.1 ^{a}
EC [µS·cm^{-1}]	999 ± 231 ^{a}	813 ± 125 ^{a}	783 ± 54 ^{a}
ORP [mV]	254 ± 5.8 ^{a}	260 ± 2.4 ^{a}	257 ± 4.9 ^{a}
C_{tot} [%]	21.3 ± 2.6 ^{a}	21.1 ± 2.7 ^{a}	17.9 ± 1.3 ^{a}
C/N	13.7 ± 1.2 ^{a}	14.8 ± 1.8 ^{a}	13.0 ± 1.2 ^{a}
N-NH_4^+ [mg N·kg^{-1}]	94.3 ± 38.2 ^{a}	52.5 ± 32.6 ^{a}	38.3 ± 11.0 ^{a}
N-NH_4^+/N-NO_3^-	0.7 ± 0.4 ^{a}	0.3 ± 0.2 ^{a}	0.4 ± 0.1 ^{a}
DOC [mg C·kg^{-1}]	2530 ± 417 ^{a}	2184 ± 105 ^{a}	2118 ± 102 ^{a}

Values are the means ± SD (n=3). Different letters in a superscript indicate significant differences within a parameter (Kruskal-Wallis test, P≤0.05).
A: 0 – 20 cm, 1 – 6 months; B: 20 – 40 cm, 6 – 12 months; C: 40 – 60 cm, 12 – 18 months

In this experiment, the moisture decreased with the age of the layers, but was not statistically significant. The pH value ranged from 7.6 (layer A) to 7.9 (layer C), which is slightly less than in experiments done by Hanc and Pliva[7], and Nair et al.[9], where the pH values were about 8.5. The EC decreased with the age of the layers, which is also confirmed by Majlessi et al.[36], who vermicomposted pre-composted food waste for 7 weeks in plastic bags using *Eisenia fetida*. The ORP fluctuated between 248 mV (layer A) to 262 mV (layer B). The C_{tot} was the greatest in layer A (on average 21.3%), but the greatest C/N ratio (on average 14.8) was found in layer B. The C_{tot} value decreased with the age of the vermicompost, as well as in the vermicompost from food waste in a study by Arancon et al.[37], where the C*tot* value of the mature vermicompost was 19.5%. The reduction of the C/N ratio can be influenced by the enzymatic activity[9], which was the greatest in layer A (see above). The decreasing trends of C_{tot} and N_{tot} correlated positively with their available forms such as DOC, N-NO_3^-, and N-NH_4^+. The greatest

$N\text{-}NH_4^+$ and $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio were found in layer A, and these values decreased with the age of the layers (Table 1). The $N\text{-}NH_4^+$ value in the experiment done by Hanc and Pliva[7], who vermicomposted kitchen waste with wood chips for 5 months using *Eisenia*, first dropped from 160 mg $N{\cdot}kg^{-1}$ to 40 mg $N{\cdot}kg^{-1}$. However, after 3 months of vermicomposting this value increased slightly (to about 50 mg $N{\cdot}kg^{-1}$). The least DOC (2118 mg $C{\cdot}kg^{-1}$) value was observed for layer C, and the greatest DOC value (2530 mg $C{\cdot}kg^{-1}$) was seen in layer A. With respect to the total contents of elements, K and Fe were the most represented (Figure 3).

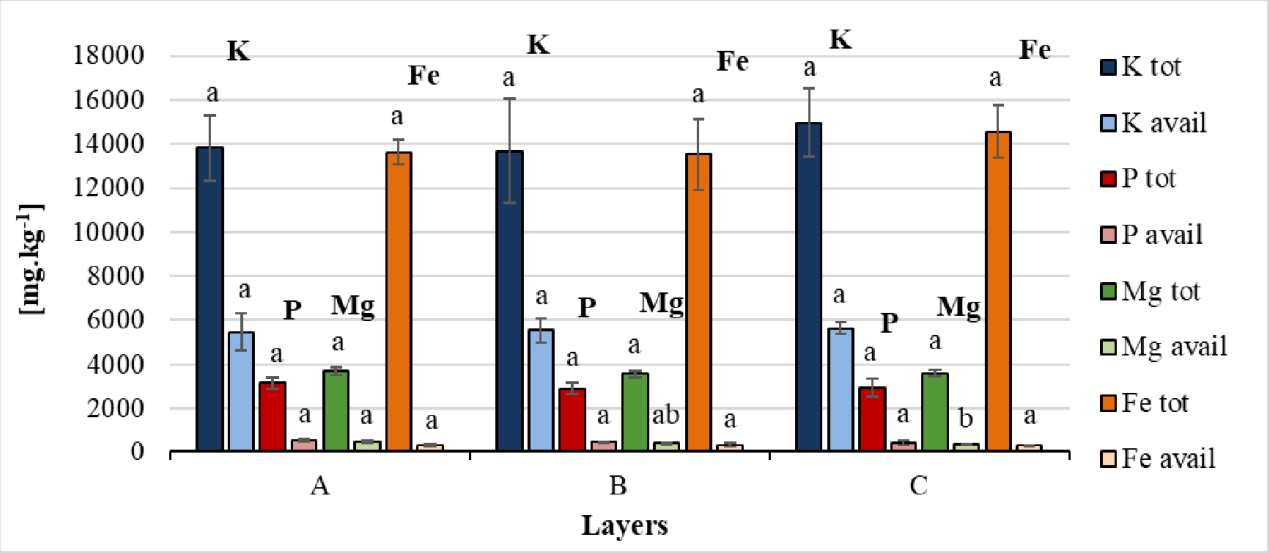

Figure 3: Changes in total and available K, P, Mg, and Fe contents [mg·kg^{-1}] in layers A, B, and C of the vertical-flow vermicomposting system. The values are the means ± SD (n=3). Different letters indicate significant differences within a nutrient (Kruskal-Wallis test, P≤0.05). A: 0 – 20 cm, 1 – 6 months; B: 20 – 40 cm, 6 – 12 months; C: 40 – 60 cm, 12 – 18 months

The greatest total contents of these two elements (K: 14,971 mg·kg^{-1}; Fe: 14,563 mg·kg^{-1}) were measured in the oldest layer (layer C). On the other hand, the total contents of P (3,150 mg·kg^{-1}) and Mg (3,692 mg·kg^{-1}) were the greatest in the youngest layer (layer A) (Figure 3). The total contents of these nutrients were greater in the vermicompost from food waste in the experiment by Arancon et al.[38] (P: 27,000 mg·kg^{-1}; K: 92,000 mg·kg^{-1}; Mg: 4,364 mg·kg^{-1}; Fe: 23,264 mg·kg^{-1}). The proportion of the available contents of K, P, Mg, and Fe constituted on average in all three layers 39%, 16%, 12%, and 2%, respectively, of the total content. The greatest percentage of the available P and Mg were in layer A (P: 17.1% of the total content; Mg: 12.8% of the total content), and of the available K and Fe were in layer B (K: 40.6% of the total content; Fe: 2.4% of the total content). The least percentage of available nutrients were in the oldest layer (layer C), but there were not statistically significant differences between the layers, except layers A and C in the available content of magnesium. Compared to the study done by Hanc and Pliva[7], our study contained a lesser percentage of available phosphorus by 54% and magnesium by 17%. The percentage of available potassium was greater in our experiment by 22%, which could be due to the addition of wood chips in the case of the experiment by Hanc and Pliva[7].

Conclusions

Although select chemical parameters did not differ significantly in individual layers, the stability of vermicompost from kitchen biowaste was found after one year of outdoor vermicomposting. This was evident from the assessment of the biological parameters. The average activity of enzymes such as β-D-glucosidase, phosphatase, sulfatase, lipase, chitinase, cellobiohydrolase, in the bottom layer was less by 53% compared to the top layer, and the pathogens were reduced by 66%, while *Salmonella* spp. was not present at all.

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Změny chemických a biologických parametrů během vermikompostování kuchyňského bioodpadu, s důrazem na patogeny

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Souhrn

Gastroodpad a kuchyňský bioodpad se mohou stát environmentálním a ekonomickým problémem, pokud s nimi nebude řádně nakládáno. Cílem této studie bylo zjistit vliv venkovního vermikompostování v systému průběžného krmení žížal kuchyňským bioodpadem na patogeny ve vztahu k biologickým a chemickým vlastnostem. Po jednom roce venkovního vermikompostování byla zjištěna chemická a biologická stabilita vermikompostu. Obsah patogenů byl snížen o 66 %, zatímco Salmonella spp. nebyla vůbec přítomna. S přibývajícím stářím vermikompostu klesala celková mikrobiální biomasa a její složky (houby, aktinobakterie, bakterie G+ a G-) a také enzymatická aktivita. Průměrná aktivita enzymů, jako je β-D-glukosidáza, fosfatáza, sulfatáza, lipáza, chitináza, cellobiohydrolasa, byla ve spodní vrstvě o 53 % menší než ve vrchní vrstvě. Vlhkost poklesla na 48 % a měrná vodivost na 722 $\mu S \cdot cm^{-1}$. Hodnota pH se zvýšila na 8,0. Klesající trendy C_{tot} a N_{tot} korelovaly pozitivně s jejich dostupnými formami, jako jsou DOC, $N\text{-}NO_3^-$ a $N\text{-}NH_4^+$, přičemž jejich podíl na celkovém obsahu činil 1,1 %, 0,9 % a 0,3 %. Nejvyšší obsahy byly zjištěny u Ca_{tot}, K_{tot} a Fe_{tot} (shodně 1,3 %) následované Mg_{tot} (0,35 %) a P_{tot} (0,3 %). Podíl dostupného obsahu K, Fe, Mg a P představoval v průměru ve všech třech vrstvách 39 %, 2 %, 12 % a 16 % z jejich celkového obsahu.

***Keywords**: vermikompostování, žížaly, kuchyňský bioodpad, vrstvy, patogeny, chemické a biologické vlastnosti*