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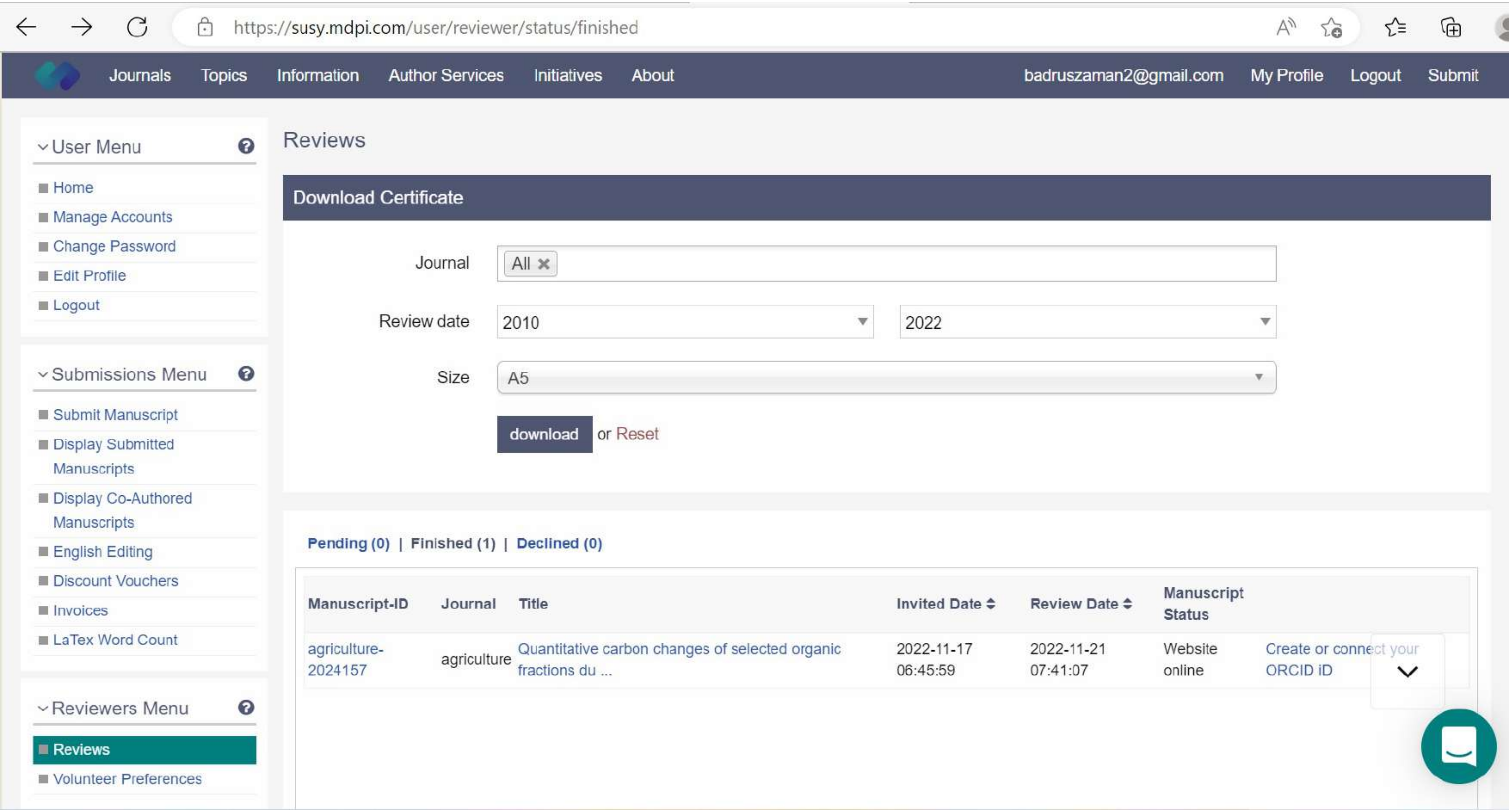

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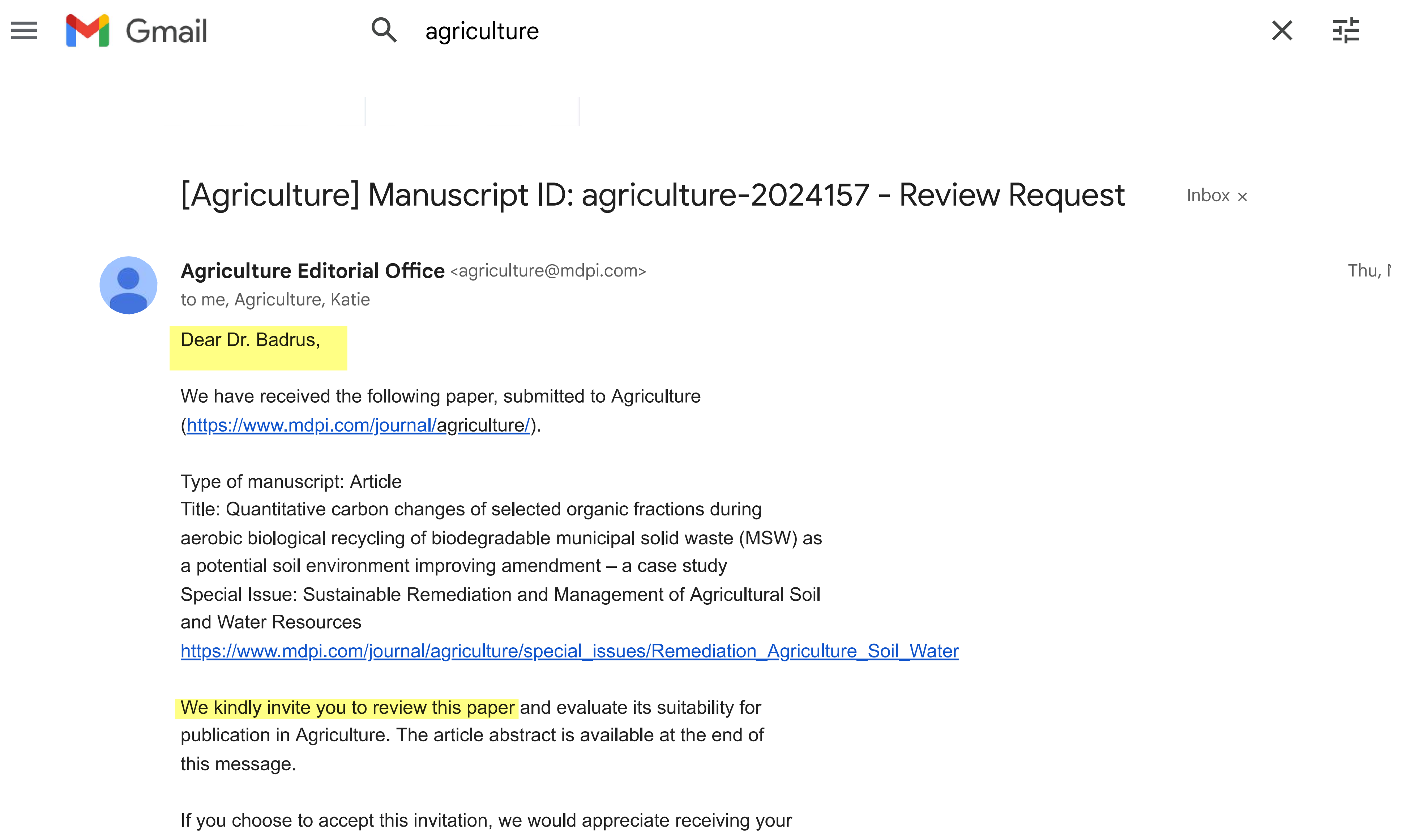

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We have received the following paper, submitted to Agriculture (https://www.mdpi.com/journal/agriculture/).

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Article

Quantitative carbon changes of selected organic fractions during aerobic biological recycling of biodegradable municipal solid waste (MSW) as a potential soil environment improving amendment – a case study

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Abstract: The aim of the investigations was to determine the quantitative changes of selected organic compounds during composting of municipal solid wastes (MSW). The object of the study was a differently matured compost produced according to open pile/windrow semi-dynamic technology from selectively collected biodegradable municipal solid waste. During the experiment, temperature and moisture of the composted wastes were monitored. In the collected samples - taken from differently matured compost - total organic carbon (TOC) and total nitrogen (TN) were determined. The organic matter fractionation method described by Stevenson and Adani et al. was adopted, which allows to determine the carbon content of the following groups of organic compounds: hydrophobic (HSC), hydrophilic (WEOC), acidophilic (CAC), cellulose (CCEL), core-HA (ligno-humic, CALK) and residual carbon (non-hydrolysing, CR). The TOC and TN content, as well as the origin and quality of the starting materials, allow the product tested to be classified for fertiliser purposes. The most intense changes were observed during the thermophilic composting phase. In spite of the optimal technological conditions of the process, the predominance of the CR and CCEL fraction was observed, and the share of humic compounds did not exceed 30% TOC. The investigated compost met legal, ecological and economic criteria for products of biological recycling, thus can be used as a good organic amendment to improve soil organic matter balance, stimulate soil biodiversity and carbon sequestration.

Keywords: humic substances; compost quality; municipal waste; transformation of organic compounds

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1. Introduction

Soil organic matter plays a crucial role in carbon capture and storage. Overall, soil management and utilization should result in maintain soil quality, fertility and productivity especially as stocks of soil organic carbon have declined in many agricultural systems all over the world. In the last decades there has been a growing interest in the use of alternative fertilizers such as brown coal, biochar, composts produced from segregated biodegradable waste or biomass, as well as other organic amendments, in agricultural and horticultural production. Soil organic amendments are a good source of stable organic carbon and, due to the presence of good-quality nutrients, can be efficiently used to improve the balance of organic matter, enhance carbon sequestration and stimulate activity of soil biodiversity [1,2]. On the other hand, the increasing amount of waste requires a reasonable strategy in its management. It focuses mainly on: quantity and toxicity reduction, recycling and reuse, and energy recovery [1,2]. Urban waste management and processing is currently a priority in the environmental policy of many countries. As a consequence of increasing pro-ecological awareness of citizens and governing elites, development of those fields of science and technology, which allow reasonable waste management, has been observed [3]. Based on the recent studies [4,5,6], the most important factors determining the waste management method were defined and characterised: qualitative composition (morphology), chemical, physico-chemical and biological properties, collection method (selective or non-selective collection), legal and economic aspects. Municipal solid waste is characterised by variability of morphological composition and physico-chem-biological properties depending on the level of civilisation development of the population, economic diversity, urbanisation and infrastructure, type of buildings and the size of settlements [7,8]. Furthermore, many studies indicate that the average chemical composition of municipal waste, and especially the content of individual groups of organic compounds, is difficult to determine. Their quantity ranges within a very wide limits and depends on many factors [9,10]. It is assumed that on average, in the biodegradable, organic part of municipal waste, carbohydrates and lignin constitute 40% - 80%, proteins 1% - 15%, and fats and hydrophobic substances 5% - 30% of dry mass [4,5].

Carbohydrates are considered to be the most common compounds in organic matter (OM) on earth and therefore predominate in the biodegradable compounds of composted waste [11,12, 13]. Their properties, in particular susceptibility to biotransformation processes, result mainly from the presence of hydroxyl groups and are strictly dependent on the structure and degree of polymerisation [8,10,14]. Mono- and disaccharides are the primary source of carbon and energy and are involved in most of the metabolic processes conducted by micro-organisms during composting. In addition, they have the ability to bind nitrogen and phosphorus compounds and thus become one of the main organic donors of these elements [8,14]. In contrast, polysaccharides, highly polymerised compounds whose structure is stabilised by hydrogen bonds, are classified as very stable substances with low chemical activity. The most important is cellulose, the proportion of which can achieve above 50% of the organic matter of the composted mass [13,14,15]. Since the intensity of cellulose transformation depends on the microbial activity of the composting microorganisms, it is most often considered as an organic carbon and energy store [13,14].

According to the current understanding, lignin is decomposed into phenolic monomers under the action of hydroxyl radicals, which are then converted by oxidation to quinone monomers. The resulting monomers combine with other compounds to form supramolecular polymers which can be transformed into humic substances (HS) by biochemical changes [15,16,17,18]. Moreover, due to its strong polymerization, lignin is very resistant to biotransformation processes. During composting, its transformations depend on the intensity of extracellular enzymatic processes initiated by fungi and actinomycetes. Due to their chemical and structural similarities, both lignin and humic acids belong to the group of natural biopolymers able to condense under variable environmental conditions. In its active form, lignin is a component of the lignin-humic complex which, due to its properties, can bind or unbind important compounds for humification processes, such as biphenyls, phenylcoumarins, diarylpropane, ether and glycerol [12,13,15].

Proteins are the most essential component of all living cells and their properties depend primarily on the chemical structure (sequence of amino acids) and the position of peptide chains in relation to each other and in space [15,16]. Taking into consideration the transformation processes of proteins during composting, it is more reasonable to classify proteins according to the chemical properties that determine their solubility in water and their ability to combine with other substances [19,20]. Spheroproteins are considered to be among the most reactive. As water-soluble proteins, they are a very rich source of nitrogen and carbon. Many authors indicate [7,8,19] the biotransformation products of spheroproteins are incorporated into humic acid structures and contain most of the nitrogen attached to them during humification processes. The biotransformation of complex proteins composed of a protein part and a non-protein component (metal cations, lipids, phosphates, etc.) is more difficult and complicated, while the resulting products may provide, in addition to nitrogen and carbon, additional substances derived from the non-protein part [14,16,18].

Another group of organic compounds occurring in composted municipal waste are hydrophobic substances of the nature of lipids, waxes, tars, resins [7,8,9,21,22]. The transformation processes of this group of compounds during composting are not precisely recognised. Some authors indicate that their biotransformation products may be transitional substances in the synthesis of humic compounds [13,14]. Among all hydrophobic substances present in composted waste, the biotransformation of fats was best identified and described [21,22,23]. As a result of hydrolysis in an alkaline environment, some components may be activated, mainly glycerol and alkaline salts of fatty acids. In this form they are biochemically active and may undergo further transformations. Since it is difficult to trace the transformation of fatty acids to humic substances, some authors [13,14,22] consider that their main role is to supply microorganisms with the energy they need to humify other types of organic compounds.

The heterogeneous and polymorphic nature of the substrates contained in composted municipal waste makes it difficult to develop a universal model of composting processes and a model of humic acids molecules [15,16,24]. A widely used classical procedure was to analyse the fractions obtained by alkaline extraction of the solid samples tested [18,24,25]. This method has recently been questioned by some authors [26]. Critics have claimed that alkali-extractable fractions should be considered as the laboratory artifacts, hence unsuitable for studying qualitative and quantitative changes and function of humic substances, especially in the field conditions. Despite these controversial opinions, the classical approach to HS research is strongly supported in contemporary literature [27,28,29,30] and recommended by the International Humic Substances Society (IHSS) [31].

An important aspect of municipal waste composting is the quality control system for the substrates used, the composting technology and the quality of the final products obtained [1,3,32,33]. One of the most advanced documents regulating these issues internationally is the recently accepted European Union (EU) Circular Economy Package [34]. The formal and legal solutions proposed therein, in conjunction with other studies [5,6,33], aim to regulate the production of fertilisers from municipal waste (UWC), standardise their quality standards (certification) and provide direction for the development of future legislation on UWC management.

Regardless of the applicable law and scientific theories on humification and humic compounds, when assessing the usefulness of products resulting from the Mechanical-Biological Treatment (MBT) of waste, both origin and quantitative changes in specific physical, chemical and biological properties should be taken into consideration. Therefore, the aim of this study was to investigate the quantitative changes in selected organic compounds during the composting of municipal solid waste (MSW).

2. Materials and Methods

The subject of the study was differently matured compost produced from selectively collected biodegradable substances present in municipal waste from the Zabrze agglomeration (Upper Silesia, southern Poland, population: 173.5×10^3 inhabitants). Composting was carried out according to the open pile/windrow semi-dynamic technology [35,36].

2.1. Design of the working experiment

The total duration of the experiment was 15 weeks. The collected biodegradable municipal waste contained approximately 50% (by mass) urban green waste (UGW) and 50% domestic kitchen waste (DKW). The waste was mechanically mixed to increase homogenisation and the prepared material was shaped into piles (dimensions L x W x H = 18.0 m x 2.0m x 1.5m). Samples for analysis were taken from fresh mixed material and from the pile after 14, 28, 45, 56, 70, 90 and 107 days of composting. Samples were taken from 3 different locations regardless of the compost maturity stage, and the total number of samples was 24. Collected materials were air-dried, ground and sieved through a 2.0 mm diameter. The temperature of the composted waste was monitored daily, while the moisture content was monitored every five days. The pile was mechanically turned every 5 days for the first 8 weeks and every 10 days between the 9th and 15th week of the experiment. To maintain the moisture content at the optimum level [7,8], after 1, 4, 6 and 8 weeks the pile was watered down to approximately 48-50% H_2O.

2.2. Basic chemical analyses.

In collected materials the following determinations were performed: content of total organic carbon (TOC) and total nitrogen (TN) using Vario Macro Cube CN analyser (Elementar Analysensysteme GmbH, 63505 Langenselbold Germany). The device has been calibrated for the determination of organic carbon and total nitrogen according to 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene organic analytical (BBOT-OAS) No. B2044 (Elemental Microanalysis Ltd, UK), certificate No. 314878 [37]. Inorganic carbonate carbon (IC) was eliminated by acidifying the solid samples with hydrochloric acid (Cp = 3%) according to the procedure recommended by the instrument manufacturer [S1]. The determined organic carbon and total nitrogen contents were compared with standards for chemical parameters for product qualification and compost quality assessment in the EU [35].

2.3. Detailed chemical analyses

The organic matter fractionation method described by Stevenson [16] and Adani et al. [24,38] was adopted, which allows different groups of organic matter to be extracted from solid samples by appropriately selected reagents (Figure 1).

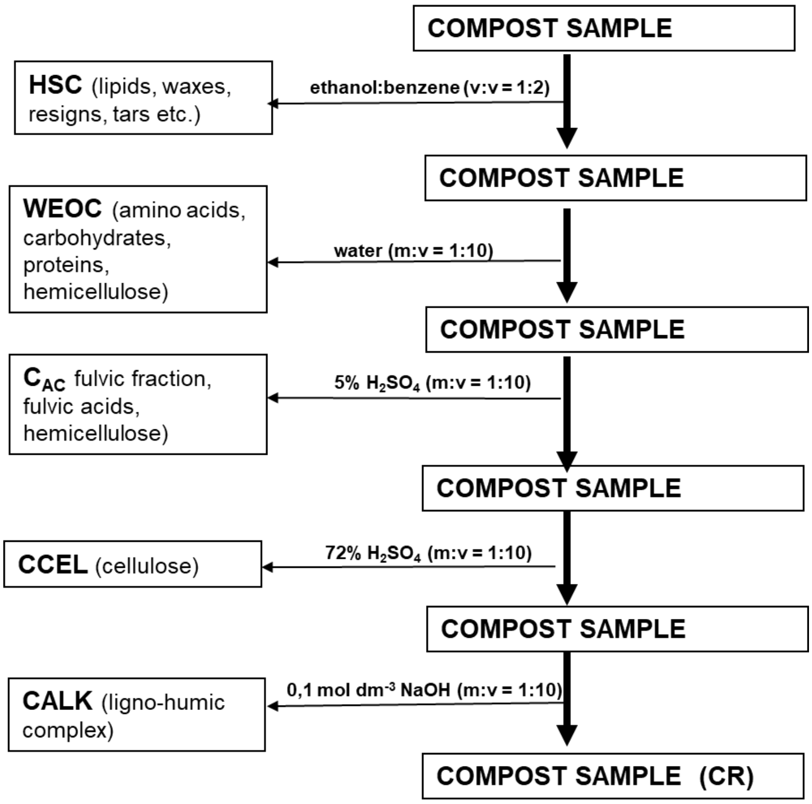

Figure 1. Simplified diagram of extraction method by Stevenson [16] and Adani et al. [24,38].

Based on the applied procedure, the quantitative changes of organic carbon of the following groups of compounds were determined:

- Carbon of hydrophobic substances (HSC): extracted by means of an ethanol and benzene mixture (1:2 v/v) the Soxhlet extractor (extraction time: 4 hours). After extraction, samples were dried in controlled condition at temperature 40 °C for 24 h to evaporate extractant. HSC was calculated as the difference in organic carbon before and after extraction [21];

- Water-extractable organic carbon (WEOC): determined in centrifuged aqueous solution 5 g ÷ 50 ml (1÷10 m÷v) after dynamic [39] extraction (three repetitions, intensity: 40 rpm, extraction time: 4 h per repetition, centrifugation: 4000 rpm 10 min^{-1});

- Carbon extracted with 5% H_2SO_4 (C_{AC}): determined in centrifuged acid solution 5 g ÷ 50 ml (1÷10 m÷v) after dynamic extraction (three repetitions, intensity: 40 rpm, extraction time: 4 h per repetition, centrifugation: 4000 rpm 10 min^{-1});

- Cellulose carbon extracted with 72% H_2SO_4 (C_{CEL}): two-stage extraction: the acid stage 5 g ÷ 50 ml (1÷10 m÷v) - three repetitions in acid, intensity: 40 rpm, extraction time: 4 h per repetition, centrifugation: 4000 rpm 10 min^{-1} and the neutralisation stage with water 5 g ÷ 50 ml (1÷10 m÷v) - three repetitions, intensity: 40 rpm, extraction time: 1 h per repetition, centrifugation: 4000 rpm 10 min^{-1}. C_{CEL} content determined the summed solutions of the both stages.

- Carbon of the ligno-humic complex extracted with 0,1 M NaoH dm^{-3} (CALK): determined in centrifuged alkaline solution 5 g ÷ 50 ml (1÷10 m÷v) after dynamic extraction (three repetitions, intensity: 40 rpm, extraction time: 4 h per repetition, centrifugation: 4000 rpm 10 min^{-1});

- Residual carbon (CR): non-hydrolysing organic carbon and humin fractions [40] remaining in the sample, calculated acc. to the formula: CR = TOC – (HSC + WEOC + CAC + CCEL+CALK).

The organic carbon content of mentioned extracts was determined with a Vario Macro Cube CN analyser (Elementar Analysensysteme GmbH, 63505 Langenselbold Germany) with the hardware attachment for solutions.

2.4. Statistical Analyses

Most of the results presented in this paper are averaged values (arithmetic mean) based on results from all replicates obtained during chemical analyses. The results obtained from chemical analyses (three repetitions for each parameter at each time) were statistically processed using ANOVA package of Statistica 13 software. The correlation matrices for selected parameters were performed (Table 1), the LSD (significance level <0.05) was calculated for the determined chemical properties for a 107-days period, mean and standard deviation (3 repetitions for each parameter) for replicates at defined sampling times.

3. Results and discussion

3.1. Changes in temperature and humidity of the composted waste.

The intensity of OM transformation and decomposition processes during composting depends on temperature and humidity which affect microbiological activity in the composted material [7,8,40]. The average temperature of initial mixture was about 30.3°C and after 34 days it reached the value of 55°Cv – the lower limit of the thermophilic phase.

This phase with an average temperature >55 °C lasted 4 weeks (between 34th and 62nd days) and the highest average daily temperature (61.8°C) was observed on the 47th day of composting (Figure 2). After this period, a regular drop in temperature to average 27.8°C degrees was observed (107th day).

Many authors indicated [7,8,41,42] that a water content of 40 to 60 % H_2O in the composted mass is the most optimal for successful composting processes. Analysis of the changes in this parameter (Figure 2) indicated that the moisture of the investigated compost was in this range, regardless of the thermal phase of the process. However, it should be noted that moisture deficiencies have been corrected on several occasions. This indicates the need for regular monitoring of parameters during the biological, aerobic processing of municipal biodegradable waste and to intervene when negative phenomena occur.

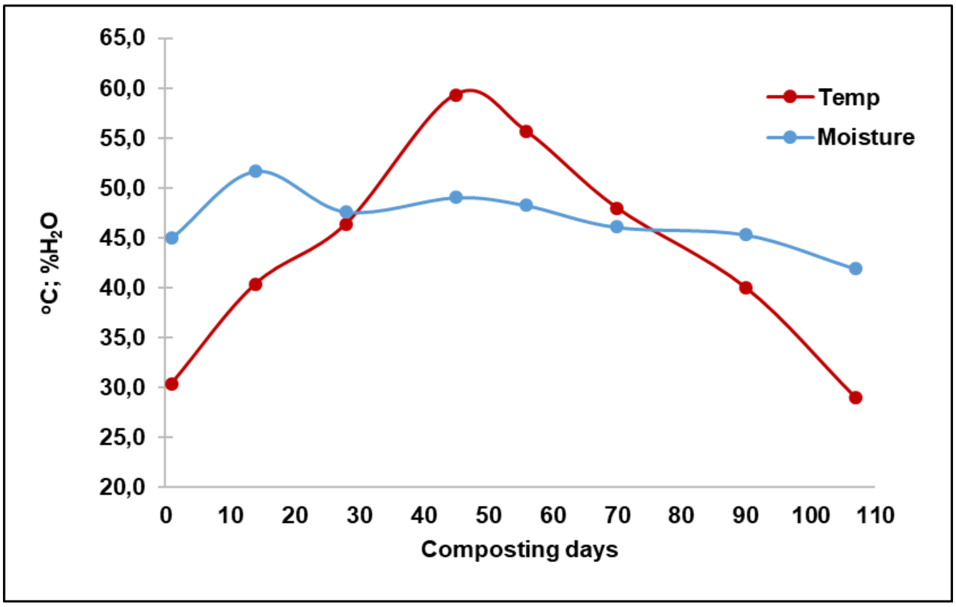

Figure 2. Changes in: a) temperature and b) humidity during composting of municipal waste.

Both temperature and moisture levels showed a significant influence on the quantitative changes of most of the analysed groups of organic compounds (Table 1). The obtained results confirm previous studies [8, 9, 14, 17], which showed that the mentioned parameters supports the maintenance and / or stimulates physico-chemical and chemical processes as well as the activity of microorganisms responsible for the dynamics of organic compounds transformation.

Table 1. Correlation coefficients between investigated chemical properties and basic composting parameters (time, temperature moisture) in the studied samples.

Parameter	Time	Temperature	Moisture	TOC	TN	HSC	WEOC	CAC	CCEL	CALK	CR
Time	-	ns	-0.587	-0.974	0.915	-0.861	-0.930	-0.927	-0.980	0.895	-0.951
Temperature	ns	-	0.593	-0.575	0.519	-0.583	-0.494	-0.682	ns	ns	ns
Moisture	-0.587	0.593	-	0.496	0.487	ns	ns	0.510	0.529	0.603	0.517
TOC	-0.974	-0.575	0.496	-	-0.932	0.897	0.949	0.930	0.960	-0.934	0.992
TN	0.915	0.519	0.487	-0.932	-	-0.884	-0.950	-0.929	-0.866	0.924	-0.914
HSC	-0.861	-0.583	ns	0.897	-0.884	-	0.956	0.829	0.879	-0.924	0.853

WEOC	-0.930	-0.494	ns	0.949	-0.950	0.956	-	0.936	0.914	-0.979	0.915
CAC	-0.927	-0.682	0.510	0.930	-0.929	0.829	0.936	-	0.881	-0.912	0.906
CCEL	-0.980	ns	0.529	0.960	-0.866	0.879	0.914	0.881	-	-0.880	0.928
CALK	0.895	0.512	0.603	-0.934	0.924	-0.924	-0.979	-0.912	-0.880	-	-0.915
CR	-0.951	-0.542	0.517	0.992	-0.914	0.853	0.915	0.906	0.928	-0.915	-

significant at $p<0.05$; ns – not significant;

3.2. Changes in TOC, TN and TOC/TN ratio during composting of MSW.

The most suitable and very widely used parameters describing the biotransformation conditions during composting [41,43,44,45] are the quantitative changes of TOC and TN in different phases of this process and the accompanying changes of TOC/TN ratio (Figure 3a-c).

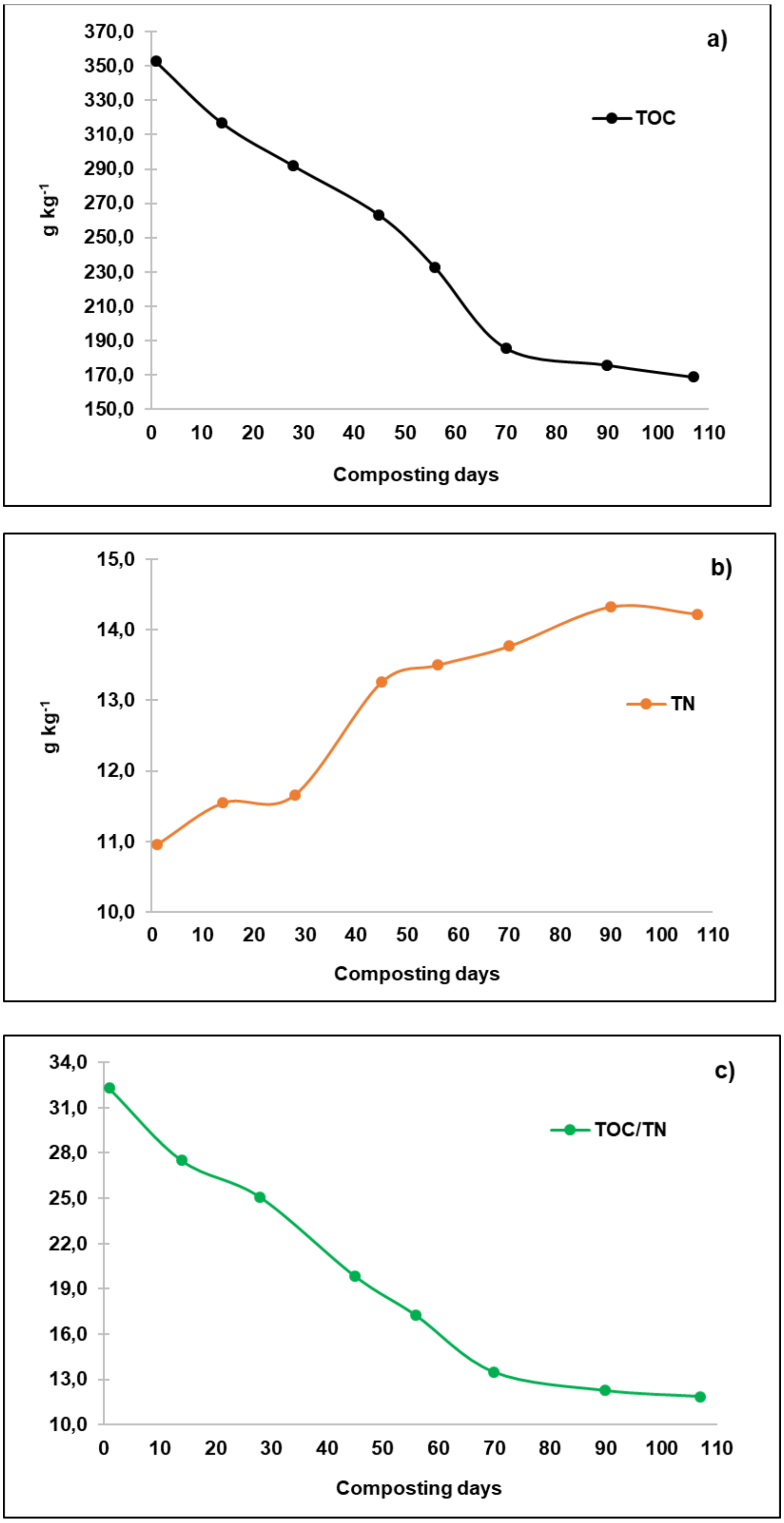

Figure 3. Changes in; a) TOC and b) TN and c) TOC/TN during composting of MSW (markers: measured values; lines: mean values).

Based on the results obtained, during the 15 weeks of the experiment, the TOC content decreased from 352.66 to 168.73 g kg^{-1} , while the amount of TN increased from 10.75 to 14.23 g kg^{-1} (Figures 3a and 3b). The most intense decrease in TOC content and increase in TN content were observed during the first 8 weeks of composting. These changes were statistically correlated with temperature and moisture content (Figure 2, 3a and b, Table 1) and were associated with the thermal phases of composting, particularly the thermophilic phase. The observed changes in TOC and TN contents are compatible with the results of previous studies [8,10,32,41,43]. It should also be noted that for both TOC and TN, a stabilisation phase was observed after 70 days of the experiment (Figure 3a and b, Table 2)

The study also indicated a decrease in the TOC/TN ratio (Figure 3c). Although the changes in the values of this parameter were determined by changes in TOC and TN content, but the processes involved in carbon mineralisation during composting had the greatest influence [41,46,47].

Table 2. Changes in means and standard deviations for replicates at defined sampling times and LSD (p,0,05) for 90-day period of investigated chemical properties.

Composting time	**Parameter**	**TOC**	**TN**	**HSC**	**WEOC**	**CAC**	**CCEL**	**CALK**	**CR**
[days]						**[g kg-1]**			
1	**Mean**	352.66	10.95	19.41	29.85	18.73	85.43	28.02	171.22
	St. dev	12.70	0.41	0.71	0.54	0.34	1.55	0.51	11.81
14	**Mean**	316.93	11.55	11.41	23.37	18.26	77.49	30.44	155.96
	St. dev	1.46	0.54	0.66	0.49	0.38	2.08	0.64	2.43
28	**Mean**	292.08	11.66	9.99	19.14	19.31	71.89	35.50	136.25
	St. dev	2.77	0.49	0.07	0.57	0.57	3.02	1.05	2.91
45	**Mean**	263.31	13.26	6.26	7.53	12.08	69.59	41.76	126.10
	St. dev	2.49	0.11	0.11	0.28	0.12	1.22	1.30	2.53
56	**Mean**	232.85	13.50	5.70	7.01	10.46	65.82	44.84	99.02
	St. dev	1.45	0.28	0.21	0.27	0.24	1.80	0.38	2.18
70	**Mean**	185.68	13.77	5.13	5.22	8.92	61.73	45.38	59.30
	St. dev	1.65	0.26	0.27	0.28	0.62	1.57	0.67	0.10
90	**Mean**	175.70	14.33	4.46	2.81	9.39	54.84	45.92	58.27
	St. dev	0.72	0.21	0.18	0.20	0.52	1.06	0.59	0.80
107	**Mean**	168.73	14.23	4.05	2.13	6.55	49.55	45.05	61.39
	St. dev	1.14	0.25	0.09	0.21	0.57	0.76	0.48	0.35
LSD		16.62	1.21	1.31	1.34	1.59	4.76	2.64	15.77

Additionally, it should be noted that the analysis of the intensity of changes in the value of TOC/TN index (Figure 3c) indicated that the studied product reached maturity (TOC/TN≤12) after 90 days (TOC/TN=11.9), which suggests optimal technological conditions of the process [7,8,41,43]

Regardless of the determined TOC and TN content and the technology used, the EU international fertiliser regulations define the principles for the use of biodegradable municipal waste as a substrate for the production of organic or organic-mineral fertiliser substances or soil substitutes [2,3,32,34]. Therefore, the possibility of using the investigated product as a component of alternative soil conditioners and/or substrates for agricultural, remediation and environmental purposes should be considered. [1,3,5,34,48,49]

3.3. Changes in HSC, WEOC, CAC, CCEL, CALK and CR contents of the composted waste.

Although the TOC content decreased by over 50% during the 107 days of the experiment (Figure 3a), the intensity and direction of changes in the carbon content of the extracted organic compounds varied (Figure 4a and b). Analysis of the results showed that, except for the CALK fraction, the organic carbon of the other fractions analysed decreased with the progression of the composting time (Table 1).

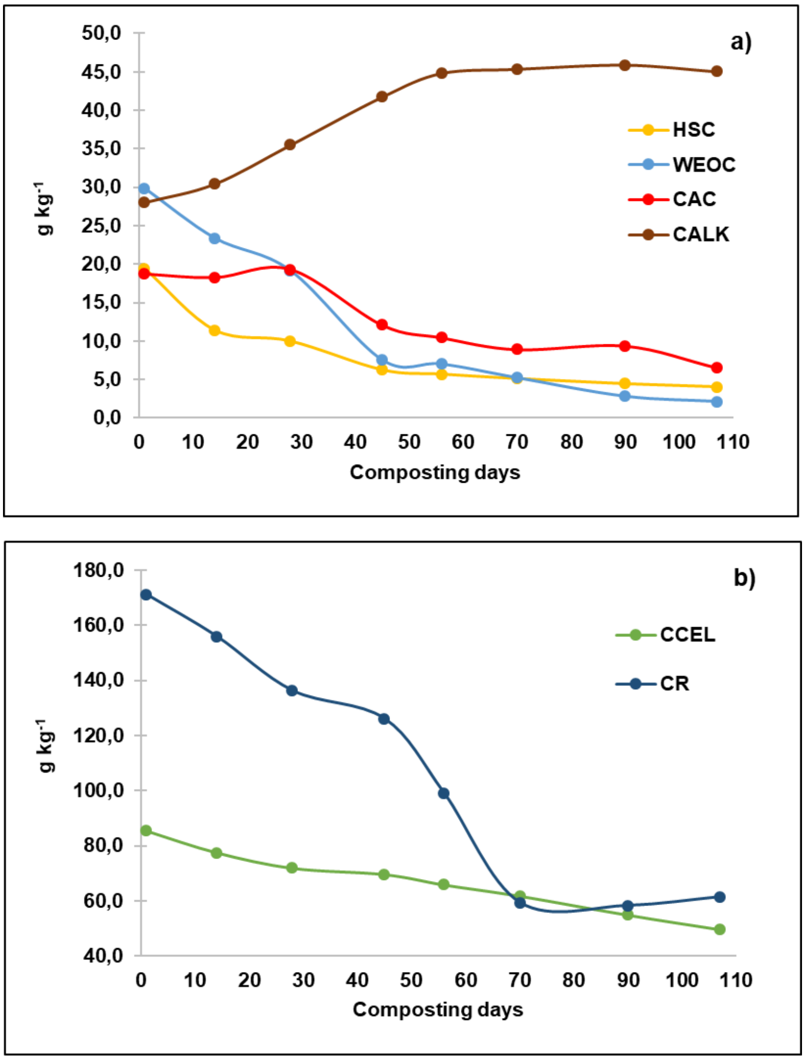

Figure 4. Changes in: a) HSC, WEOC, CAC and CALK, and b) CCEL and CR during composting of MSW (markers: measured values; lines – mean values).

Changes in the C contents of HSC, WEOC and CAC fractions showed a significantly negative correlation (Table 1) with composting, temperature while CAC, CCEL, CALK and CR indicated a significantly positive correlation with the moisture content. Furthermore, the quantitative changes in the HSC, WEOC fractions were highest up to the 47th day and CR up to the 70th day of the experiment, which may be related to the intensive biological-chemical-physical processes in the thermophilic and in the early 2nd mesophilic phase (Figure 4a and b). These results are in accordance with the studies of Ryckeboer et al. [14], Bekier et al. [21] and Amir et al. [50], especially in the intensity of transformation of hydrophobic compounds (HSC). The authors indicated that the intensity of decomposition of hydrophobic substances, was significantly correlated with composting parameters, mainly temperature and time (Table 1). Similar correlations for water-extractable organic compounds were described in the experiments of Kałuża-Haładyn et al. [51,52] and Jamroz et al. [39]. Also, other authors [17,19,44] indicated the organic components of WEOC are the main, available and important source of carbon and energy for microorganisms involved in composting processes. For this reason, quantitative changes of this fraction were observed during the intense thermophilic phase. However, the intensity of the decrease in WEOC content (Figure 4a) may also be caused by the leaching of these fractions from the composted MSW with the effluents. Analysis of the decrease in CR content (Figure 4c), represented mainly by humin, with the progress of composting, did not show a significant influence of temperature on the intensity of these changes (Table 1 and 2). Regardless of the extraction and/or isolation methods used and the biological activity of the environment, CR fractions represent the highest part in TOC content, as confirmed by the studies of Kononova [53], Stevenson [16] and Weber et al. [40]. However, it should be noted that the interpretation of the biotransformation of fractions very resistant to microbial activity, is a major scientific challenge. The lack of solubility, difficulty to determine structure and complex interactions with the mineral components make research on this fraction much more complicated.

The study showed systematic decrease in in the organic matter content represented by C_{AC} and C_{CEL} (Figure 4a and 4b) during MSW composting. While the stability of the C_{CEL} fraction, regardless of the thermal composting phases (Table 1) has been confirmed by the experimental results of Ryckeboer et al, [14], Amir et al. [50], Bekier et al. [21,48] and Kałuż-Haładyn et al. [51], the decrease in C_{AC} content was significantly correlated with all composting parameters. However, it should be noted that the intensity of C_{AC} changes is in accordance with the work of other authors [54,55]. Senesi et al [56] and Jerzykiewicz [57] indicated that transformations of small-molecule, mostly aliphatic compounds representing this fraction may have crucical influence on the direction of humification processes and the quality of organic matter obtained during composting. Furthermore, studies by Chefetz et al [9], Chen et al [54], Koivula and Hänninien [55] indicated that the contents of acidophilic organic carbon increase in the thermophilic phase and achieves a stabilisation and then remains on a decreasing trend. Therefore, the significant correlations of CAC fraction transformations with time and moisture content are appropriate, indicating the suitable quality of the substrate used for composting and the optimal process parameters.

The study indicated an increase in the CALK content during composting (Figure 4a). This fraction is commonly classified as lignino-humic compounds, obtained by biotransformation of lignino-protein complexes [15,16,18]. Therefore, the organic compounds contained in the CALK extract should be considered as a mixture of the proper humic substances also defined as a core-HS. The results obtained are in accordance with the performed studies confirming the increase of HS content in composts [8,9,17,40]. The increase of CALK content with composting time, moisture and temperature was observed (Table 1). Furthermore, the dynamics of quantitative changes in carbon of this fraction indicated a stabilisation after the thermophilic phase (from day 56) with a slight increasing trend until the end of the experiment (Figure 4a, Table 2).

It should be noted that the significantly negative correlations of time and temperature with the content of hydrophobic fractions, WEOC, C_{CEL} and CR (Table 1) are in agreement with the studies of other authors [7,21,23,39,54] indicating that hydrolysis products formed in thermophilic phase should be considered as a substrate in mesophilic biotransformation processes and formation of HS.

3.4. Changes in the share of organic carbon of the extracted fractions in relation to the TOC.

Considering the different durability of the organic compounds contained in the waste to the biotransformation processes during composting, many authors indicates the necessity to express the percentage of organic carbon of the extracted substances in relation to the TOC content [16,21,25,39,55]. This method of interpreting allows one to trace which compounds and to what extent determine the organic carbon pool in the different composting phases. The study showed the CR fraction was dominant both in quantity and in share of TOC (Figure 4c and 5). Furthermore, this relation was observed in all samples tested, regardless of the compost maturity stage. It should be noted that, despite the significant decrease of CR content (Figure 4c), the organic carbon of this fraction represented the largest resource of total organic carbon in the composts studied. The observed phenomena are confirmed by studies performed by other authors [8,9,39,54].

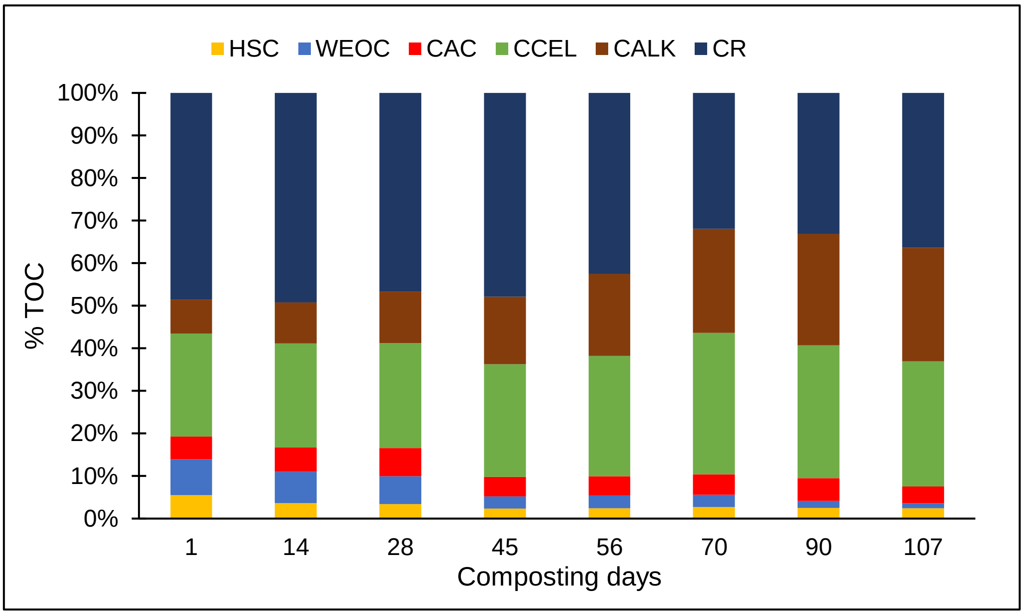

Figure 5. Changes in the share of organic carbon of the extracted fractions in relation to the TOC during the experiment.

A different interaction was observed for the carbon of the CAC and CCEL fractions. Despite a systematic quantitative decrease in the cellulose and acidophilic fractions, an increase in the share of CCEI and very irregular changes in CAC were observed (Figures 4a, 4b and 5). While for cellulose these changes are acceptable [14,48,51], for the CAC fraction the results are unusual and difficult to explain, indicating the need for research into the transformations of small-molecule acidophilic compounds during composting.

The results obtained confirm previous studies [17,21,39,51] indicating that both the carbon content of the hydrophobic and WEOC fractions and their share of TOC (Figures 4a and 5) decrease with the duration of composting. Furthermore, in samples composted for 107 days, the summed share of the HSC and WEOC fractions did not exceed 3.5% of TOC, indicating that the maturation and stabilisation of the composted waste was correct [17,38,39].

The results confirmed the formation and accumulation of CALK (core-HS) compounds. A quantitative increase in CALK carbon content resulted in an increase in the share of this fraction in TOC. These phenomena occurred with different dynamics, and the most intense changes were observed between 28 and 70 days of composting (Figure 4a and 5). In the last 30 days of the experiment, these processes stabilised (Table 2), which indicates the optimal course of the composting process and indicates the maturity of the final product [15,16,18,41].

4. Summary and conclusions

Both the type of technology and the selected physico-chemical properties of the substrate indicate optimal composting conditions, enabling a positive evaluation of the experiment. The applied technical solutions stimulated the formation and accumulation of core-HS compounds. The relatively low content (Figure 4a) and share of HSC and WEOC fractions in TOC (Figure 5), the dominance of CR and CCEL compounds and the optimal TOC/TN ratio may result from the properties of the substrate [9,17,41,45]. Furthermore, the results and observations obtained indicate that the studied compost produced from selectively collected biodegradable waste can have a wide spectrum of applications [1,2,4,34,58,59,60]. In addition, the lack of substances considered to be contaminants is an advantage of the tested product [3,4,5]. Based on the results obtained, it can be stated that the final product *studied*, resulting from the applied mechanical-biological treatment (MBT), confirmed the benefits of biological, aerobic methods for recycling organic compounds contained in the waste [1,3,32,33].

Considering the results obtained and the interpretation of the observed transformations, the following conclusions can be drawn: (1) The quantitative changes in total carbon and total nitrogen, observed during composting, showed significant correlations with time, temperature and moisture content of the composted mass; (2) The most intense changes of organic carbon contents were observed in the hydrophilic, core-HS and residual fractions; (3) The composting conditions, the technology applied and the properties of the substrate stimulated both the quantitative increase in CALK and the share of this fraction in TOC; (4) The study showed that the highest share of organic carbon in the final product was incorporated in the residual, cellulose and core -HS fractions, respectively; (5) The contents of TOC, TN, the origin and quality of the substrate used allow to qualify the studied product as organic or organic-mineral fertilizer; (6) The investigated composts from selectively collected biodegradable municipal waste meet legal and ecological and criteria for products of biological recycling, thus can be used as a good organic amendment to improve soil organic matter balance, stimulate soil biodiversity and carbon sequestration. Further investigations determining soil and plant response to the compost application are needed to confirm its benefits as an attractive option for soil amendment.

Author Contributions: Conceptualization J.B., E.J; methodology J.B., E.J.; validation E.J., M.D., I.C-P,.; formal analysis J.B. E.J.; investigation J.B.; resources J.B., E.J, M.D.; data curation J.B., E.J, I.C-P.; writing—original draft preparation J.B.; writing—review and editing E.J., A.K, M.D, I.C-P.; visualization J.B.; supervision E.J., I.C-P. ; project administration J.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Supplementary Materials

S1. File VMC manual.pdf., Chapter 6, 91-101.

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Thu,

Dear Dr. Badrus,

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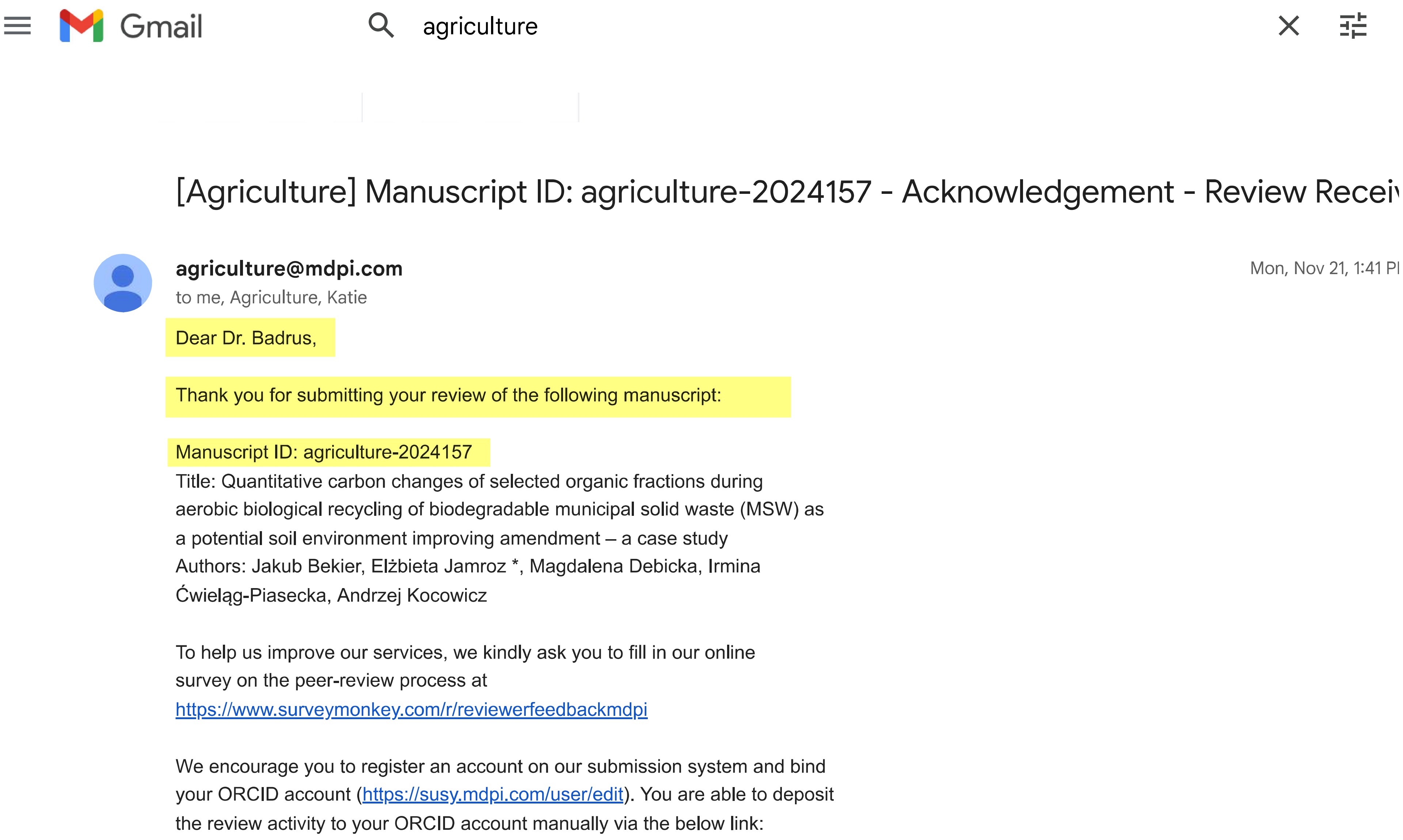

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Thank you for submitting your review of the following manuscript:

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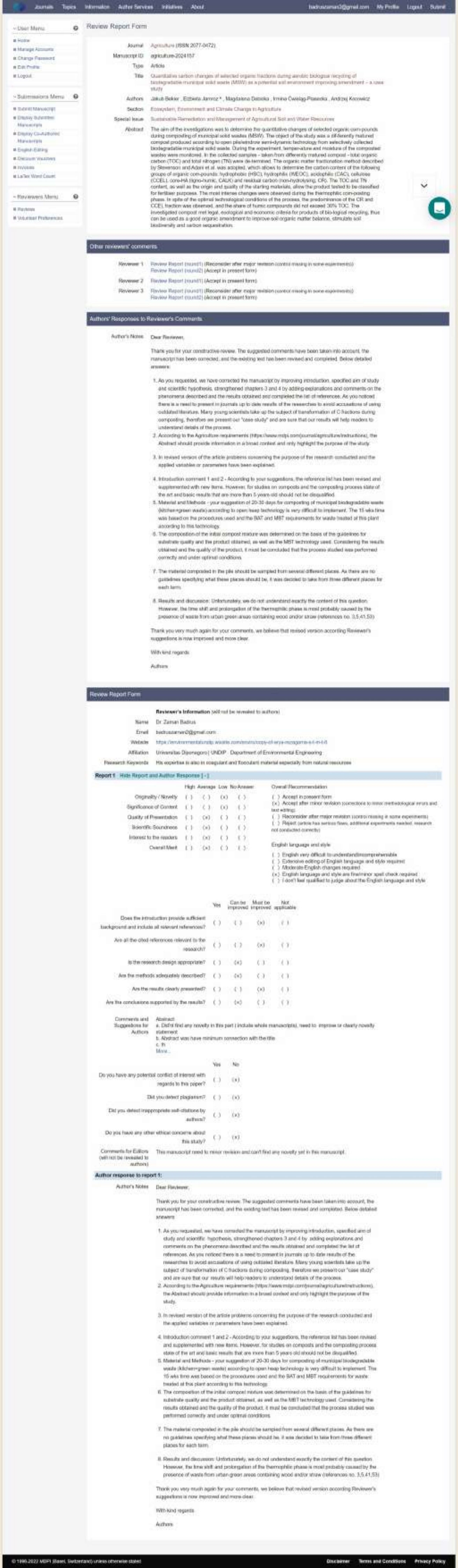

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Journal	Agriculture (ISSN 2077-0472)
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Type	Article
Title	Quantitative carbon changes of selected organic fractions during aerobic biological recycling of biodegradable municipal solid waste (MSW) as a potential soil environment improving amendment – a case study
Authors	Jakub Bekier , Elżbieta Jamroz * , Magdalena Dębicka , Irmina Ćwieląg-Piasecka , Andrzej Kocowicz
Section	Ecosystem, Environment and Climate Change in Agriculture
Special Issue	Sustainable Remediation and Management of Agricultural Soil and Water Resources
Abstract	The aim of the investigations was to determine the quantitative changes of selected organic com-pounds during composting of municipal solid wastes (MSW). The object of the study was a dif-ferently matured compost produced according to open pile/windrow semi-dynamic technology from selectively collected biodegradable municipal solid waste. During the experiment, temper-ature and moisture of the composted wastes were monitored. In the collected samples - taken from differently matured compost - total organic carbon (TOC) and total nitrogen (TN) were de-termined. The organic matter fractionation method described by Stevenson and Adani et al. was adopted, which allows to determine the carbon content of the following groups of organic com-pounds: hydrophobic (HSC), hydrophilic (WEOC), acidophilic (CAC), cellulose (CCEL), core-HA (ligno-humic, CALK) and residual carbon (non-hydrolysing, CR). The TOC and TN content, as well as the origin and quality of the starting materials, allow the product tested to be classified for fertiliser purposes. The most intense changes were observed during the thermophilic com-posting phase. In spite of the optimal technological conditions of the process, the predominance of the CR and CCEL fraction was observed, and the share of humic compounds did not exceed 30% TOC. The investigated compost met legal, ecological and economic criteria for products of bio-logical recycling, thus can be used as a good organic amendment to improve soil organic matter balance, stimulate soil biodiversity and carbon sequestration.

Other reviewers' comments

Reviewer 1	Review Report (round1) (Reconsider after major revision (control missing in some experiments)) Review Report (round2) (Accept in present form)
Reviewer 2	Review Report (round1) (Accept in present form)
Reviewer 3	Review Report (round1) (Reconsider after major revision (control missing in some experiments)) Review Report (round2) (Accept in present form)

Authors' Responses to Reviewer's Comments

Author's Notes

Dear Reviewer,

Thank you for your constructive review. The suggested comments have been taken into account, the manuscript has been corrected, and the existing text has been revised and completed. Below detailed answers:

1. As you requested, we have corrected the manuscript by improving introduction, specified aim of study and scientific hypothesis, strengthened chapters 3 and 4 by adding explanations and comments on the phenomena described and the results obtained and completed the list of references. As you noticed there is a need to present in journals up to date results of the researches to avoid accusations of using outdated literature. Many young scientists take up the subject of transformation of C fractions during composting, therefore we present our "case study" and are sure that our results will help readers to understand details of the process.
2. According to the Agriculture requirements (https://www.mdpi.com/journal/agriculture/instructions), the Abstract should provide information in a broad context and only highlight the purpose of the study.
3. In revised version of the article problems concerning the purpose of the research conducted and the applied variables or parameters have been explained.
4. Introduction comment 1 and 2 - According to your suggestions, the reference list has been revised and supplemented with new items. However, for studies on composts and the composting process state of the art and basic results that are more than 5 years old should not be disqualified.
5. Material and Methods - your suggestion of 20-30 days for composting of municipal biodegradable waste (kitchen+green waste) according to open heap technology is very difficult to implement. The 15 wks time was based on the procedures used and the BAT and MBT requirements for waste treated at this plant according to this technology.
6. The composition of the initial compost mixture was determined on the basis of the guidelines for substrate quality and the product obtained, as well as the MBT technology used. Considering the results obtained and the quality of the product, it must be concluded that the process studied was performed correctly and under optimal conditions.
7. The material composted in the pile should be sampled from several different places. As there are no guidelines specifying what these places should be, it was decided to take from three different places for each term.
8. Results and discussion: Unfortunately, we do not understand exactly the content of this question. However, the time shift and prolongation of the thermophilic phase is most probably caused by the presence of waste from urban green areas containing wood and/or straw (references no. 3,5,41,53)

Thank you very much again for your comments, we believe that revised version according Reviewer's suggestions is now improved and more clear.

With kind regards

Authors

Review Report Form

Reviewer's Information (will not be revealed to authors)

Name	Dr. Zaman Badrus
Email	badruszaman2@gmail.com
Website	https://environmentalundip.wixsite.com/enstro/copy-of-erya-muzgorna-s-t-m-t-t
Affiliation	Universitas Diponegoro (UNDIP . Department of Environmental Engineering
Research Keywords	His expertise is also in coagulant and flocculant material especially from natural resources

Report 1 Hide Report and Author Response [-]

	High	Average	Low	No Answer
Originality / Novelty	( )	( )	(x)	( )
Significance of Content	( )	( )	(x)	( )
Quality of Presentation	( )	(x)	( )	( )
Scientific Soundness	( )	(x)	( )	( )
Interest to the readers	( )	(x)	( )	( )
Overall Merit	( )	(x)	( )	( )

Overall Recommendation
( ) Accept in present form
(x) Accept after minor revision (corrections to minor methodological errors and text editing)
( ) Reconsider after major revision (control missing in some experiments)
( ) Reject (article has serious flaws, additional experiments needed, research not conducted correctly)

English language and style
( ) English very difficult to understand/incomprehensible
( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	( )	( )	(x)	( )
Are all the cited references relevant to the research?	( )	( )	(x)	( )
Is the research design appropriate?	( )	(x)	( )	( )
Are the methods adequately described?	( )	(x)	( )	( )
Are the results clearly presented?	( )	( )	(x)	( )
Are the conclusions supported by the results?	( )	(x)	( )	( )

Comments and Suggestions for Authors

Abstract
a. Did'nt find any novelty in this part (include whole manuscripts), need to improve or clearly novelty statement
b. Abstract was have minimum connection with the title
c. Th
More...

	Yes	No
Do you have any potential conflict of interest with regards to this paper?	( )	(x)
Did you detect plagiarism?	( )	(x)
Did you detect inappropriate self-citations by authors?	( )	(x)
Do you have any other ethical concerns about this study?	( )	(x)

Comments for Editors (will not be revealed to authors): This manuscript need to minor revision and can't find any novelty yet in this manuscript.

Author response to report 1:

Author's Notes

Dear Reviewer,

Thank you for your constructive review. The suggested comments have been taken into account, the manuscript has been corrected, and the existing text has been revised and completed. Below detailed answers:

1. As you requested, we have corrected the manuscript by improving introduction, specified aim of study and scientific hypothesis, strengthened chapters 3 and 4 by adding explanations and comments on the phenomena described and the results obtained and completed the list of references. As you noticed there is a need to present in journals up to date results of the researches to avoid accusations of using outdated literature. Many young scientists take up the subject of transformation of C fractions during composting, therefore we present our "case study" and are sure that our results will help readers to understand details of the process.
2. According to the Agriculture requirements (https://www.mdpi.com/journal/agriculture/instructions), the Abstract should provide information in a broad context and only highlight the purpose of the study.
3. In revised version of the article problems concerning the purpose of the research conducted and the applied variables or parameters have been explained.
4. Introduction comment 1 and 2 - According to your suggestions, the reference list has been revised and supplemented with new items. However, for studies on composts and the composting process state of the art and basic results that are more than 5 years old should not be disqualified.
5. Material and Methods - your suggestion of 20-30 days for composting of municipal biodegradable waste (kitchen+green waste) according to open heap technology is very difficult to implement. The 15 wks time was based on the procedures used and the BAT and MBT requirements for waste treated at this plant according to this technology.
6. The composition of the initial compost mixture was determined on the basis of the guidelines for substrate quality and the product obtained, as well as the MBT technology used. Considering the results obtained and the quality of the product, it must be concluded that the process studied was performed correctly and under optimal conditions.
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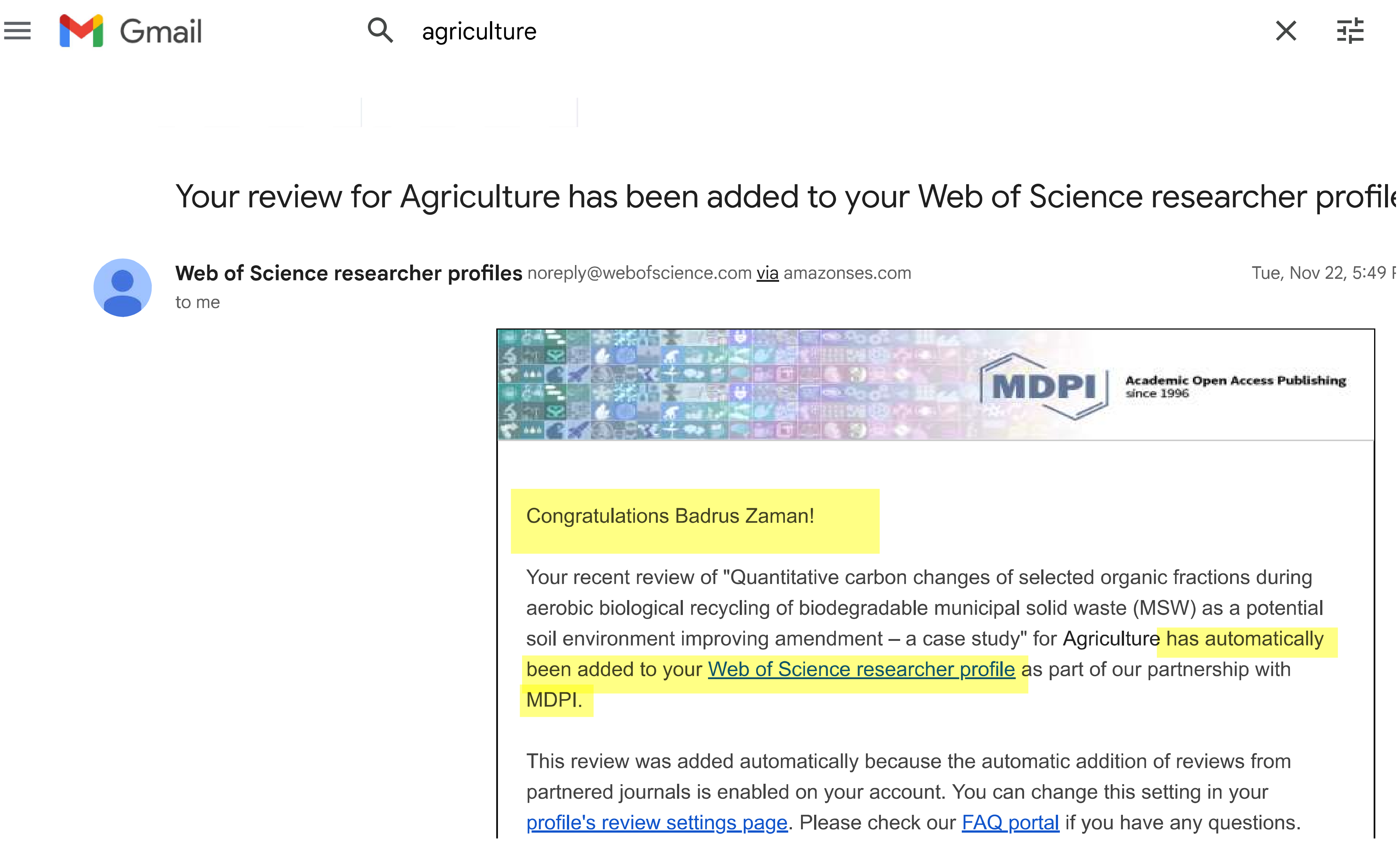
Authors



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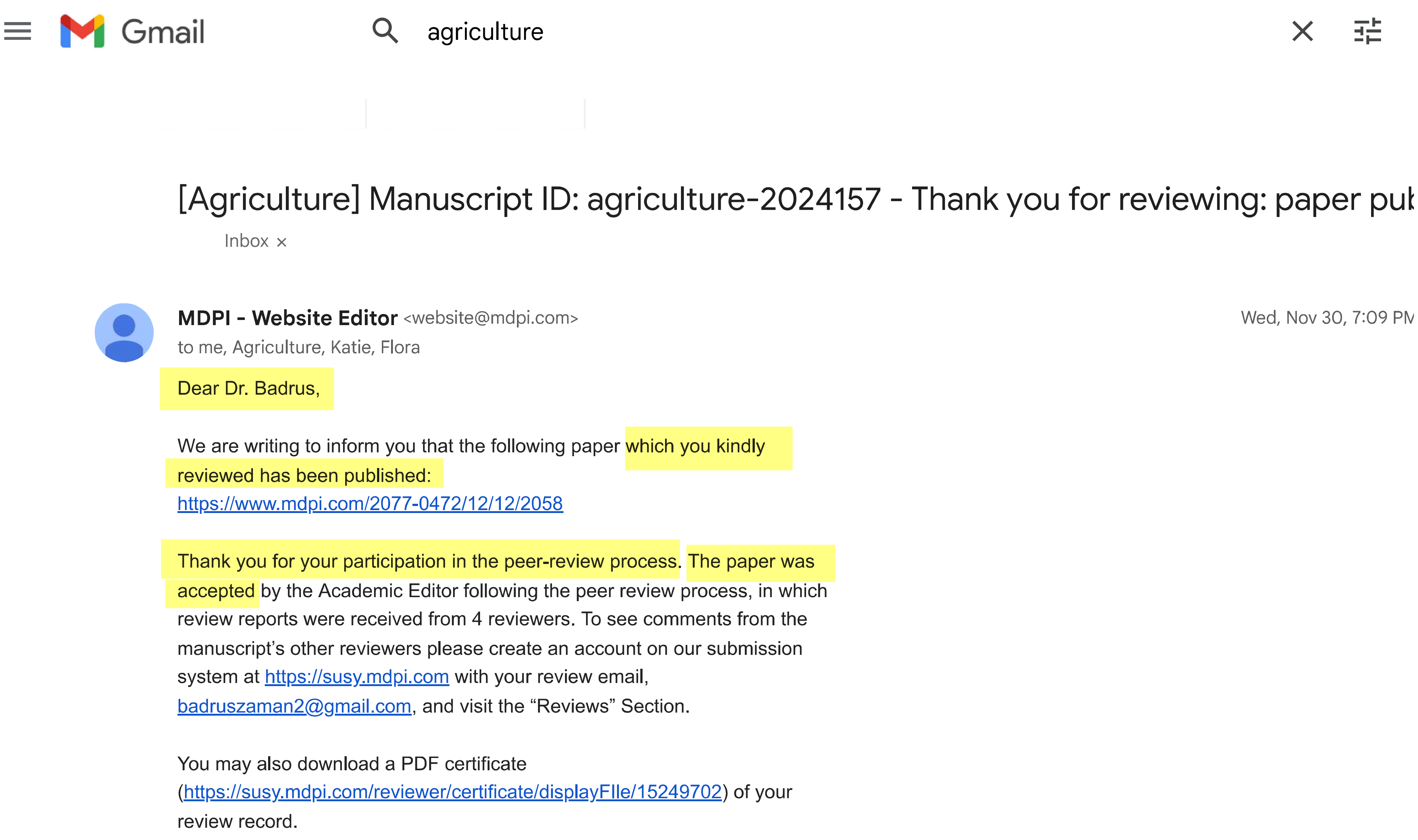
to me

Tue, Nov 22, 5:49 P

Congratulations Badrus Zaman!

Your recent review of "Quantitative carbon changes of selected organic fractions during aerobic biological recycling of biodegradable municipal solid waste (MSW) as a potential soil environment improving amendment – a case study" for Agriculture has automatically been added to your Web of Science researcher profile as part of our partnership with MDPI.

This review was added automatically because the automatic addition of reviews from partnered journals is enabled on your account. You can change this setting in your profile's review settings page. Please check our FAQ portal if you have any questions.



[Agriculture] Manuscript ID: agriculture-2024157 - Thank you for reviewing: paper pu

MDPI - Website Editor <website@mdpi.com>
to me, Agriculture, Katie, Flora

Wed, Nov 30, 7:09 P

Dear Dr. Badrus,

We are writing to inform you that the following paper which you kindly reviewed has been published:
https://www.mdpi.com/2077-0472/12/12/2058

Thank you for your participation in the peer-review process. The paper was accepted by the Academic Editor following the peer review process, in which review reports were received from 4 reviewers. To see comments from the manuscript's other reviewers please create an account on our submission system at https://susy.mdpi.com with your review email, badruszaman2@gmail.com, and visit the "Reviews" Section.

You may also download a PDF certificate (https://susy.mdpi.com/reviewer/certificate/displayFIle/15249702) of your review record.