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**BIOLOGICAL STABILITY ASSESSMENT OF MSW ORGANIC FRACTIONS BY**  
**MEANS OF RESPIROMETRIC AND GERMINATION TESTS**  
 --Manuscript Draft--

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<b>Abstract:</b>	Composting is the mostly applied method for recovering the organic waste producing an organic soil conditioner. Furthermore, the organic fraction of municipal solid waste (OFMSW) of unsorted waste should be treated in mechanical biological treatment (MBT) plants in view of reducing its environmental impacts. In both cases, it's essential the assessment of biological stability as well as the phytotoxicity of the final product. Aim of this work was to evaluate the maturity evolution during the composting process of OFMSW at full scale. Samples were collected from two Sicilian plants and were subjected to the following analytical measures: volatile solids (VS), dynamic respirometric index (DRI), carbon-to-nitrogen (C/N) ratio and germination index (GI). Results showed that some parameters such as pH and water content values can affect the respirometric test response and the proper activity of microorganisms responsible for biodegradation at full scale. For the unsorted waste, the DRI values suggested that depending on the initial values the stabilization duration requires to be increases prior to landfilling. DRI revealed to be effective for the assessment of the matrix stability, even if the simultaneous measurements of the different indices can provide a reliable information of biological stability and maturity of the organic matrices.
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## ORIGINAL ARTICLE

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6 **Title: BIOLOGICAL STABILITY ASSESSMENT OF MSW ORGANIC FRACTIONS BY MEANS OF**  
7 **RESPIROMETRIC AND GERMINATION TESTS**

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### Abstract

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24 Composting is the mostly applied method for recovering the organic waste producing an organic soil conditioner.  
25 Furthermore, the organic fraction of municipal solid waste (OFMSW) of unsorted waste should be treated in mechanical  
26 biological treatment (MBT) plants in view of reducing its environmental impacts. In both cases, it's essential the  
27 assessment of biological stability as well as the phytotoxicity of the final product. Aim of this work was to evaluate the  
28 maturity evolution during the composting process of OFMSW at full scale. Samples were collected from two Sicilian  
29 plants and were subjected to the following analytical measures: volatile solids (VS), dynamic respirometric index  
30 (DRI), carbon-to-nitrogen (C/N) ratio and germination index (GI). Results showed that some parameters such as pH and  
31 water content values can affect the respirometric test response and the proper activity of microorganisms responsible for  
32 biodegradation at full scale. For the unsorted waste, the DRI values suggested that depending on the initial values the  
33 stabilization duration requires to be increases prior to landfilling. DRI revealed to be effective for the assessment of the  
34 matrix stability, even if the simultaneous measurements of the different indices can provide a reliable information of  
35 biological stability and maturity of the organic matrices.

### Keywords

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45 Biowaste; Composting; Biological Stability; Phytotoxicity; Respirometric analysis

## 1. Introduction

1 Landfill is the most widely used strategy worldwide to manage municipal solid waste (MSW) [1].

2  
3 Nevertheless, landfill disposal causes several environmental hazards due to the presence of organic fraction (30-40% of  
4 municipal solid waste - MSW), such as biogas production, emission of volatile organic compounds (VOC), leachate  
5 production, vector presence (e.g. insects, rodents and birds), public health hazard, risk of explosions and plant toxicity  
6 [2, 3].

7  
8 The European Commission in view of reducing the aforementioned environmental impacts, imposes to reduce the  
9 amount of organic fraction of municipal solid waste (OFMSW) to be disposed in landfill (Directive CE/99/31). The  
10 main strategies adopted to reduce the OFMSW amount are source separation and mechanical-biological treatment  
11 (MBT). These strategies allow a valorization process aiming to the recovery of material and/or energy.

12  
13 In particular, composting is the method most commonly applied for the recovery of separately sorted organic waste [4],  
14 due to its simple implementation and operation [5, 6]. The composting process provides the biological stabilization of  
15 the organic substrate under aerobic conditions and produces a sanitized product, usually referred to as compost, with  
16 soil conditioning properties [7, 8] which can be used for improving soil quality [9, 10]. The composting process occurs  
17 in two phases: high rate composting (or active composting time - ACT) phase and curing phase. The first phase aims at  
18 degradation of organic matter, destruction of pathogens and weed seed, consequently breaking down of the phytotoxic  
19 compounds takes place. In the second phase, the compost maturation takes place [11]. The OFMSW sorted not  
20 separately is treated in mechanical biological treatment (MBT) plants in order to reduce the biodegradable organic  
21 content by subjecting the organic fraction to an aerobic stabilization process, before landfilling, thus decreasing the  
22 leachate and biogas production, or to be used for non-agronomic applications [12]. This waste “pre-treatment” is  
23 mandatory in Italy in compliance with the European Union sanitary landfill regulation imposing that final disposal must  
24 be environmental sustainable and should prevent threats to human health.

25  
26 Italian Regulations (DM 27/09/2010, updated with DM 24/06/2015) require biological stabilization of waste before  
27 landfilling. Biological stability determines the extent to which readily biodegradable organic matter has decomposed  
28 [13, 14]. It identifies the actual point reached in the decomposition process and represents a gradation on a recognized  
29 scale of values, which thus enable comparison of the process of decomposition [14]. Knowing the degree of biological  
30 stability possessed by the organic matter, not only during the aerobic biological processing but also to be found in the  
31 final products, is important for the process to be controlled effectively, for the products to be used beneficially, and in  
32 optimizing the design of the processing plant [15]. In fact, stability affects the potential for odor generation, biomass  
33 reheating, residual biogas production, regrowth of pathogens, phytotoxicity, plant disease suppression ability and  
34 process parameters such as airflow rate and retention time [16–18]. Stable compost can be considered as that which  
35 shows resistance to further decomposition [15, 19]. Unstable composts are of general concern for a number of reasons  
36 including their ability to: self-heat, which may lead to fires, generate nuisance odors, attract disease vectors and  
37 generate toxic by-products, especially under anaerobic conditions [20, 21].

38  
39 In both scenarios, the biological stability can be assessed in different ways. In the past, many analytical methods have  
40 been proposed for biological stability determination [22–25]. Among them, the respirometric techniques have been  
41 recognized as the best one [11, 26–28], to be highly effective [29] and suitable to reflect the process of organic matter  
42 biodegradation [30]. The respirometric approach consists of the measurement of O<sub>2</sub> uptake or CO<sub>2</sub> production under  
43 standardized conditions by microorganisms degrading the readily degradable organic fraction under standardized  
44 aerobic [4, 31] in a short period of time (1-4 days) [17, 31, 32]. Methods based on carbon dioxide evolution are  
45 inexpensive, but do not differentiate between anaerobic and aerobic CO<sub>2</sub> [14]; moreover, the degree of oxidation of the  
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1 organic matter affects the O<sub>2</sub> uptake per mole of the CO<sub>2</sub> produced. Therefore oxygen uptake is preferred for  
2 respirometric purposes [33–36].

3 The dynamic respiration index (DRI) represents a respirometric approach for the evaluation of waste reactivity. One of  
4 the advantages of DRI with respect to other methods proposed in the literature is in the use of a large mass (up to 13 kg)  
5 to be tested under simulated full-scale conditions [37]. As a consequence, DRI represented one of the more well-studied  
6 respirometric methods, and many applications have been proposed. DRI was used to predict potential biogas production  
7 [38] and potential odor production [39], and to calculate an index to measure the potential waste reactivity in landfills  
8 [40]. Recently, Colón et al. [27] have demonstrated the high potential of the DRI in comparing the effectiveness of  
9 different plants treating different matrices [11]. In addition, DRI, measured according to UNI/TS 11184 standard  
10 method, was adopted as the main compliance parameter to test the biological stability [41, 42]; only waste having a DRI  
11 lower than 1000 mgO<sub>2</sub>/(kg<sub>vsh</sub>h) can be landfilled.

12 In this light, the aim of the present work was to evaluate and compare the maturity evolution and the biological stability  
13 during the composting process of organic municipal solid waste at full scale coming from two different Sicilian plants:  
14 the first one was a MTB/composting plant (Plant 1), while the second one was a composting plant (Plant 2). Samples  
15 were subject to the following analytical measures: volatile solids (VS), moisture content, pH, dynamic respirometric  
16 index (DRI), carbon-to-nitrogen (C/N) ratio, seed germination and root elongation tests.

17 The latter were evaluated to assess the phytotoxicity [4], which is tightly related to biological stability, as the microbial  
18 activity of some unstable organic matter can produce phytotoxic compounds [43].

## 28 **2. Materials and methods**

### 29 *2.1 Plant description*

30 *Plant 1.* The MBT/composting plant is aimed at the treatment of the residual fractions and the unsorted waste (design  
31 MTB flow rate: 750 Mg d<sup>-1</sup>) and of the organic fraction subject to separate collection (design composting flow rate: 90  
32 Mg d<sup>-1</sup>). The mechanical-biological treatment is performed before waste landfilling, according to Italian Regulation.  
33 The process was characterized by two parallel lines, including two units of bag disruption and two waste selection units  
34 by sieving (size 130 mm). The upper sieving fraction was subject to metal separation (magnetic and non-magnetic) and  
35 could be potentially used as refuse derived fuel (RDF). However, this application was not yet introduced due to lack of  
36 facilities and consequently all of the MSW upper sieving is sent to the landfill (excepting metals that are recovered).  
37 The under sieve fraction of the two lines was gathered into one line subject to a further sieving facility (size: 70 mm);  
38 the fate of the upper sieving was the same of that previously discussed, while the under sieve fraction was subject to  
39 biological treatment. The biological treatment is applied by static piles, realized with a maximum height of almost 3 m,  
40 inside a bioreactor unit characterized by the following geometrical dimension: length: 30 m, height: 4.4 m, width: 8.7  
41 m. The residence time for the OFMSW is almost 30 days and, after assessed the design stability level, it can be disposed  
42 in the municipal landfill, close to the MBT plant.

43 In the composting line, the organic fraction is fed to a bag disruption unit, then shred and mixed with pruning scraps in  
44 order to improve the pile texture and increasing its porosity. The pile features as well as the bioreactors are identical to  
45 that above discussed. The residence time of the organic fraction inside the bioreactor is set at 30 days. The material  
46 extracted from the bioreactor is then subject to aerated static and windrow maturation, respectively. The residence time  
47 for the maturation is set equal to 60 days, for a whole composting time of 90 days. The outlet material is refined by  
48 sieving (size: 10 mm), where the under sieve fraction is the mature compost. In Fig.1 the block diagrams for Plant 1 are  
49 shown.

[FIG.1]

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2 *Plant 2.* The plant is sized for the treatment of 19.9 Mg d<sup>-1</sup> of OFMSW. Also in this case, before treatment, organic  
3 fraction is fed to a bag disruption unit, shred and then mixed with ligno-cellulosic materials. The process treatment  
4 involves an intensive biooxidation in biocells lasting 14 days, a forced aeration in a confined space for 28 days (post-  
5 composting phase) and finally a barnyard maturing on turning piles of 54 days. Biocells volume is equal to 420 m<sup>3</sup>, with  
6 a useful capacity of 300 m<sup>3</sup>; instead, piles from post-composting and maturing phases have the following geometrical  
7 dimensions: larger base 4 m, smaller base 2 m, height 3 m and 2.5m, respectively. At the end of the composting process,  
8 the raw compost is refined by a two-stage sieving (20 mm and 8 mm, respectively). Fig.2 shows block diagram for Plant  
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[FIG.2]

## 18 2.2 Analytical methods

19 The composting process was monitored for 184 days (Plant 1) and 120 days (Plant 2); these durations have been  
20 established according to the regulatory requirements (process time not less than 90 days) and the measurement  
21 frequency of biological stability. During the experimental campaign, for both plants, several samples were collected at  
22 different times in order to analyze biological stability parameters. In particular, concerning Plant 1, the experimental  
23 campaign was carried out in a winter-spring period while, for Plant 2, two sampling campaigns have been carried out  
24 (campaigns 1 and 2). Campaign 1 has been performed in a winter period; while Campaign 2 has been conducted in a  
25 summer period. For each sample, analysis for the measurement of volatile solids (VS), dynamic respirometric index  
26 (DRI), carbon-to-nitrogen (C/N) ratio and germination index have been carried out.

27 The respirometric tests on both compost and OFMSW took place in a laboratory reactor; a respirometric equipment  
28 produced by Costech International, 3022 model, with a volume equal to 25 L, was used. The analyses were carried out  
29 on 10 kg samples collected into the bioreactor and/or static piles and windrows. Before testing, density and moisture of  
30 the samples were standardized, if necessary, at values respectively lower than 0.75 kg L<sup>-1</sup> and 75% maximum water  
31 capacity. Samples were analyzed according to the UNI/TS 11184:2016 methodology (UNI/TS, 2016). Each analysis  
32 had a 96 hours duration or longer if necessary (120 hours). Temperature (T) and oxygen (O<sub>2</sub>) concentration in the air  
33 inflow and outflow were automatically measured; in particular, T was also measured within the biomass sample and the  
34 highest value reached during the test was annotated to be further correlated with the DRI value.

35 During the respirometric test, the hourly DRI (DRI<sub>h</sub>) was determined by measuring the difference in the O<sub>2</sub>  
36 concentration (mL L<sup>-1</sup>) between the respirometer inlet and the outlet airflow, and was calculated as reported by Scaglia  
37 et al. [38]:

$$38 \quad DRI_h = \frac{Q \cdot \Delta O_2}{V_g \cdot VS(DM)} \cdot 31.98 \quad [mgO_2/kg_{VS} h]$$

39 where DRI<sub>h</sub> is the hourly DRI, Q (L h<sup>-1</sup>) is the airflow rate, ΔO<sub>2</sub> (mL L<sup>-1</sup>) is the difference in the O<sub>2</sub> concentration in the  
40 inlet and the outlet air flows of the reactor, V<sub>g</sub> (L mol<sup>-1</sup>) is the volume of 1 mol of gas at the inlet air temperature, 31.98  
41 (g mol<sup>-1</sup>) is the molecular weight of O<sub>2</sub>, and VS and DM (kg) represent, respectively, the initial volatile solids and dry  
42 matter content. According to Adani et al. [31] a typical DRI<sub>h</sub> (respirogram chart) consists of a lag phase, increasing  
43 phase and peak, decreasing peak. However, depending on the nature of the matrix and involved processes, the  
44 respirogram profiles may be strongly different.

1 Phytotoxicity tests employing seed germination and root elongation were used following the APAT method (APAT,  
2 2003). *Lepidium sativum*, seeds were used for germination and growth assays on compost aqueous solutions and placed  
3 in Petri dishes (90 mm diameter) with one sheet of filter paper as support, in five replicate experiments. After the  
4 addition of 10 seeds and 1 mL of test solutions, the Petri dishes were sealed with parafilm to ensure closed-system  
5 models. The seeds were placed in a growth chamber at 27°C for 24 h. After this period, the number of seeds germinated  
6 was counted and the radical length was measured. The Index of growth (IG) was calculated by multiplying the  
7 germinated seed number (G) and length of roots (L). The Germination Index results were used to calculate the effect,  
8 expressed as percentage (GI%) with respect to the control using the following equation:  
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$$GI = \frac{G_S \cdot L_S}{G_C \cdot L_C} \cdot 100 \quad [\%]$$

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16 where S and C stands for the samples and the control, respectively. For the evaluation of the C/N ratio, the content of  
17 both total organic carbon (TOC) and nitrogen (N) were measured through a TOC-V and TNM-1 Shimadzu Analyser.  
18 VS were determined according as specified in Manual ANPA 03/2001.  
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### 21 **3. Results and discussion**

#### 22 *3.1 Results achieved for Plant 1.*

23  
24 Fig.3 shows the pattern of the DRI and  $T_{max}$  values (a) and VS and GI values (b) measured during the composting time.  
25 For sake of completeness in Tab.1 are summarized all the other variables measured. Data reported in Fig.3a reveal an  
26 initial increasing of the DRI value from 4729 to 7032 mgO<sub>2</sub>/(kg<sub>VS</sub> h) between sample at process start-up and after 33  
27 days of composting time. This result seems to be contrasting with the expected increase of the sample stability during  
28 the composting process.  
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#### 31 **[FIG.3]**

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34 Nevertheless, the low pH value (5.3) of the initial sample (composting time equal to zero) (Tab.1) could have likely  
35 inhibited the proper development of the biological process during the respirometric test thus reducing the  
36 microorganisms oxygen consumption. This low pH value could be related to the seasonal composition of the matrix  
37 subject to composting, which was rich in citrus waste. Moreover, the long storage times (almost one week) before  
38 starting the composting process could have favored the beginning of hydrolysis, thus affecting the proper development  
39 of the aerobic biological process. However, starting from composting time of 33 days, a progressive reduction of the  
40 DRI was obtained from 7032 mgO<sub>2</sub>/(kg<sub>VS</sub> h) (at day 33) to 303 mgO<sub>2</sub>/(kg<sub>VS</sub> h) (at day 184) (Fig.3a).  
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#### 46 **[FIG.4]**

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48 This behavior could be confirmed by the pattern of the observed respirogram charts reported in Fig.4. Indeed, the shape  
49 of respirogram chart at t=0 (raw matrix) which showed a very fast increase of instantaneous respiration index, followed  
50 by a rapid decrease and, again, a subsequent slow increase before the final decrease, highlighting that the aerobic  
51 process did not developed properly. In contrast, the shape of the subsequent respirogram charts was different, with a  
52 clear and regular increase of DRI during the test, followed by a net decreasing portion, suggesting in this case the  
53 proper development of the aerobic biological process. Moreover, in sample 2 it was observed a prolonged lag phase in  
54 the first part of respirometric test, likely related to the still low pH value (5.5). In sample 3, the lag phase was  
55 significantly reduced compared to sample 2, whilst it completely disappeared in sample 4. Therefore, excepting sample  
56 1, the last three samples (2, 3 and 4) were characterized by decreasing DRI values. The reduction of the maximum value  
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of hourly DRI confirmed the reduction of the biodegradable organic matter contained inside the sample and the increasing stability reached by the composted matrix, in good agreement with previous results [11].

The maximum temperature values ( $T_{max}$ ) measured during the respirometric tests reduced from 68°C (at day 33) to 19°C (at day 184) showing a similar trend of DRI associated to the decrease of biological degradation reactions, thus confirming the progressive matrix stability (Fig.3a). Moreover, reaching high temperatures ensured the matrix sanitation. In terms of VS, a reduction from 85% to 48% was observed during the composting time (Figure 3b). Conversely, a progressive increase of the GI value was obtained from 3.15% to 92% thus revealing a very quality of the mature compost, with a final value significantly higher compared to the threshold limit value of 60% imposed by the Italian Regulation (Legislative Decree n. 75/2010). The very low GI values at the beginning of the process are likely related to the production of germination inhibiting compounds such as alcohols, phenolic compound and organic acids, as highlighted by previous studies [44]. This results were well in line with what achieved by Cesaro et al. [45] (Fig.3b). Moreover, the carbon-to-nitrogen ratio decreased over time with a faster reduction in the first phase, due to the carbon mineralization. This reduction slowed down during the curing stage, according to previous results [45]. Moreover, the mineralization of organic nitrogen to ammonia contributed to the pH increase observed during experiments.

#### [TAB.1]

Fig.5 shows the correlation between the main monitored parameters during the experimental campaign:  $T_{max}$  and DRI (Fig.5a), GI and VS (Fig.5b), respectively. Higher temperature reflect higher degradation reactions and lower matrix stability, corresponding consequently to higher DRI values (Fig.5a); this result is in accordance with the study reported by Adani et al. [46]: more stable substrates reach lower temperatures than fresh substrates, as they are characterized by lower microbial activity. GI and VS showed a good correlation too (Fig.5b); indeed, as phytotoxicity decreases (increase of GI), since matrix is stable, the organic matter content of the biomass (expressed in terms of VS) decreases.

#### [FIG.5]

Tab.2 summarizes the results of the two samples analyzed for the MBT. In particular, data refer to the samples analyzed at the beginning of stabilization time (t zero (t = 0) and 34 days (t = 34), at the end of the established stabilization time. Data reported in Tab.2 show that the initial sample (t = 0) has a very high DRI value, equal to 12277 mgO<sub>2</sub>/(kgvsh), and an acid pH value (5.7). The biological reactors of the MBT plant have in charge the treatment of the under sieve of both residual fraction of separate collection and unsorted waste stream. Due to the very poor percentage of separate collection (close to 20%) in the considered urban case study, the flow stream subject to biological process was characterized by considerable amount of organic matter, thus justifying the very high value of DRI. Moreover, the low pH value has likely influenced the biological process within the MBT. Indeed, at the end of the stabilization time, which duration was set by the plant operator, the achieved DRI value of 5966 mgO<sub>2</sub>/(kgvsh) was still significantly higher than the value imposed by the Italian Regulation equal to 1000 mgO<sub>2</sub>/(kgvsh) for the waste acceptability in a landfill (Legislative Decree 3 settembre 2020 no. 121). The poor sample stability at t = 34 days was also corroborated by the very low GI value (5.13 %) and the high VS (50%) value compared to the initial sample, thus confirming that the considered sample was not acceptable for landfilling. Consequently, a longer stabilization duration should be required. The very low GI values suggested that the matrix maturity was quite low, also due to the high heterogeneity of the raw matrix (unsorted waste and residual fraction) which could promote germination inhibition. However, the observed results highlighted that a stabilization process was occurring, also confirmed by the halved value of C/N ratio, following the degradation of the organic matter content, according to what reported by Cesaro et al. [43]. From this experimental

1 study, it could be proposed that the stabilization time of 21 days imposed by local authorities in Sicily for unsorted  
2 waste and/or residual fraction from separate collection prior to landfill disposal is quite unrealistic and a prolonged  
3 duration should be proposed.  
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5 **[TAB.2]**  
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8 *3.2 Results achieved for Plant 2.*

9 Fig.6 shows respectively the trend profile of DRI and  $T_{max}$  values (Fig.6a) and VS and GI values (Fig.6b) measured  
10 during the composting time for experimental Campaign 1.  
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12 **[FIG.6]**  
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14 Referring to experimental results achieved in Campaign 1, the maximum temperature ( $T_{max}$ ) measured during the  
15 respirometric tests decreased from 52°C (at day 0) to 22°C (at day 120) following the same pattern of DRI, thus  
16 confirming the progressive matrix stability (Fig.6a) [46]. Data reported in Fig.6a reveal a decrease of the DRI value  
17 from 4940 to 200 mgO<sub>2</sub>/(kg<sub>VS</sub> h) between sample at the beginning and after 120 days of composting time, respectively.  
18 Moreover, the experimental DRI values can be well expressed by a depletion model, the latter supported by a high  
19 correlation index ( $R^2 = 0.95$ ). In general, the DRI values observed for Plant 2 during the experimental campaign 1 were  
20 slightly lower compared to what observed for Plant 1, thus suggesting an unlike behavior, probably due to the different  
21 composition of the matrix subject to the biological process. This result is confirmed by the lower  $T_{max}$  values shown in  
22 Plant 2, compared to what observed in Plant 1; indeed, the temperature increase, as above discusses, is usually  
23 associated with to the development of biological degradation reactions; therefore, the higher the amount of organic  
24 available to biodegradation, the higher will be the temperature during process. Nevertheless, the temperature values  
25 during composting are also affected by aeration mode and airflow rates and aeration excess can promote a significant  
26 water losses, thus hampering the proper development of biological process [47]. Concerning the VS and GI values  
27 (Fig.6b), also in this case it was observed a different behavior compared to what achieved in Plant 1. Indeed, for Plant 2  
28 the VS showed only a limited variation during experiments, while the GI index remained very low throughout  
29 experiments, highlighting that the organic matrix was far behind compared to the required Regulation limit, suggesting  
30 the presence of germination inhibiting compounds that hinder the maturation of the organic matrix.  
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32 Referring to Campaign 2, Fig.7 shows respectively the trend profile of DRI and  $T_{max}$  values (Fig.7a) and the trend  
33 profile of VS and GI values (Fig.7b). As notable from Fig.7a, both  $T_{max}$  and DRI maintained high values during  
34 experiments, indicating that at the end of the process, the organic matrix wasn't yet stable. In particular, after an initial  
35 increasing of the DRI value from 3851 to 9866 mgO<sub>2</sub>/(kg<sub>VS</sub> h) between sample at zero and 14 days of composting time,  
36 the latter began to decrease reaching at the end of the composting process a value equal to 5070 mgO<sub>2</sub>/(kg<sub>VS</sub> h), which  
37 is higher than the biological stability limit. The significant difference between the values obtained from the two  
38 campaigns can be attributed to their different seasonality (Campaign 1 in winter, Campaign 2 in summer). On one hand,  
39 this aspect could affect the composition of the matrix to be subject to biological process while, on the other hand, the  
40 water content of the samples analyzed was significantly different between the two campaigns (Tab.3). Indeed, the  
41 moisture values of samples collected in winter were significantly higher compared to what observed in summer;  
42 therefore, these lower moisture contents in the full scale plant during Campaign 2 could have slowed down the  
43 biological process, thus producing a dried matrix rather than a stable and mature compost. In contrast, the moisture  
44 values in Campaign 1 (winter) were more suitable for the development of microorganisms responsible for  
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1 biodegradation. Indeed, moisture is essential for the microbial metabolic processes can take place. Particularly,  
2 biological activity finds optimal conditions in an environment with a water content between 55% and 65%; while, as the  
3 humidity of the organic matrix approaches 40%, the composting process begins to be inhibited [43].  
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5 **[FIG.7]**

6 **[TAB.3]**

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10 Considering the whole composting process, it was observed a general slight decrease in the solids content (Fig.7b). The  
11 GI values also in Campaign 2 remained very low over time, in contrast with the results achieved in previous  
12 experiences [48]. A possible explanation could be found in the scarce separation of the OFMSW at the source; indeed,  
13 the latter showed poor quality due to the high presence of plastics and other non-compostable materials; therefore, these  
14 impurities might have negatively affected the phytotoxic features of the composting matrix.  
15

16 Fig.8 shows the correlation between  $T_{max}$  and DRI for Campaign 1 (a) and Campaign 2 (b), respectively.  
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19 **[FIG.8]**

20 Referring to Campaign 1 (Fig.8a), the values of biomass maximum temperature ( $T_{max}$ ), reached during the respirometric  
21 tests, and the DRI showed a good correlation (correlation index  $R^2 = 0.99$ ), this result was expected and it was in good  
22 agreement with Adani et al. [46]. Conversely, data reported in Fig.8b reveal that, although the high correlation index,  
23 DRI proved to be ineffective in describing the biological stability of organic matter.  
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#### 26 **4. Conclusions**

27 The key findings of this study are summarized as follows. For Plant 1 the results obtained for the composting plant  
28 revealed that the initial pH value may exert a relevant role for the proper evaluation of the DRI by means of  
29 respirometric tests; low initial pH value could negatively affect the real respirometric test response. Therefore, in order  
30 to avoid decrease of pH value during the matrix storage the reduction of the storage time before starting the composting  
31 process as much as possible is suggested. Moreover, the DRI revealed to be a useful index to monitor the matrix  
32 stability, in order to properly design the composting time. The results obtained for the MBT samples suggested to adopt  
33 some pH correction of the inlet sample and to establish a total stabilization time according to the initial DRI value in  
34 order to achieve the regulation limits. Finally, for MBT samples, the imposed standard for unsorted matrices is hardly  
35 achievable, especially with the suggested processing time of 21 days.  
36

37 Concerning Plant 2, DRI for Campaign 1 revealed to be an excellent indicator for the evaluation of the biological  
38 stability degree, reaching a final value at the end of the composting process equal to  $200 \text{ mgO}_2/(\text{kgvs h})$ ; while, DRI for  
39 Campaign 2 showed weaknesses in the description of the process revealing an increasing trend during the first days of  
40 composting treatment. The poor sample stability was also supported by the low GI (5.4%) and the high VS (53%)  
41 values. The different DRI values obtained during the two campaigns may be due to the different seasonality in which  
42 they were performed, as this influenced the water content of the analyzed samples, and therefore on the development of  
43 microorganisms responsible for biodegradation. Lastly, in both campaigns, GI and VS alone proved to be ineffective in  
44 describing the degradation of organic matter.  
45

46 To conclude, the achieved results showed that both initial pH and water content values can affect the real respirometric  
47 test response and the development of microorganisms responsible for biodegradation; in addition, DRI proved to be the  
48 a reliable parameter in the evaluation of biological stability. As final remark, the simultaneous measurements of the  
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different indices can provide a complete and reliable information about the biological stability and maturity of the organic matrices.

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Figure captions

1 Figure 1. Schematic layout for Plant 1: MBT (a) and composting (b) line.

2  
3 Figure 2. Schematic layout for Plant 2.

4 Figure 3. DRI and  $T_{max}$  during the composting time (a); VS and IG pattern during the composting time (b).

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6 Figure 4. Respirogram charts achieved for the different samples, in terms of instantaneous and cumulated DRI values.

7 Figure 5. Correlation between  $T_{max}$  and DRI (a) and GI and VS (b).

8  
9 Figure 6. DRI and  $T_{max}$ (a) and VS and IG (b) trend profile during the composting time for Experimental Campaign 1.

10 Figure 7. DRI and  $T_{max}$  (a) and VS and IG (b) trend profile during the composting time for Experimental Campaign 2.

11  
12 Figure 8. Correlation between  $T_{max}$  and DRI for campaign 1 (a) and campaign 2 (b), respectively.

13  
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15 Table captions

16 Table 1. pH, moisture, TS and C/N measured for the sample collected in the composting plant.

17  
18 Table 2. Measured data for the samples analyzed at the stabilization time (t) zero ( $t = 0$ ) and 34 days ( $t = 34$  days) in the  
19 MBT.

20  
21 Table 3. Water content for the samples analyzed during Campaign 1 and 2.  
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Figure 1

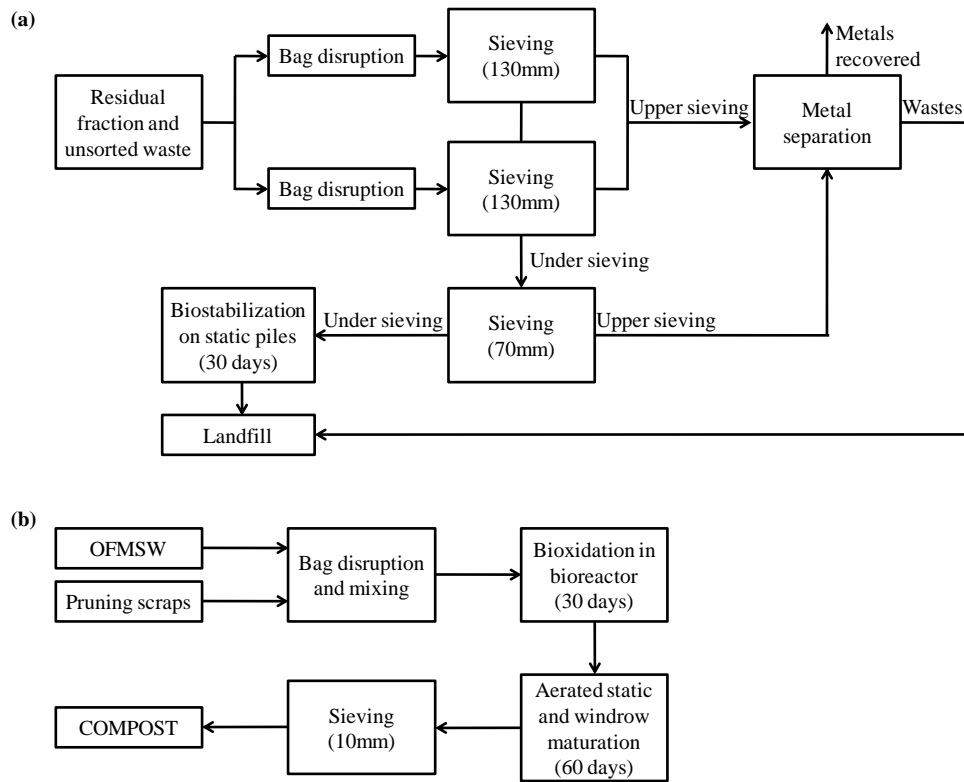
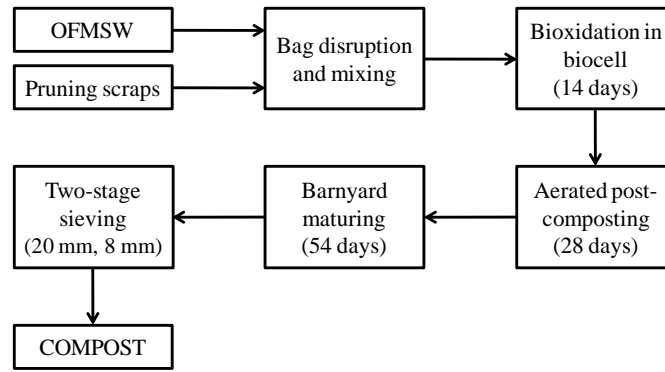


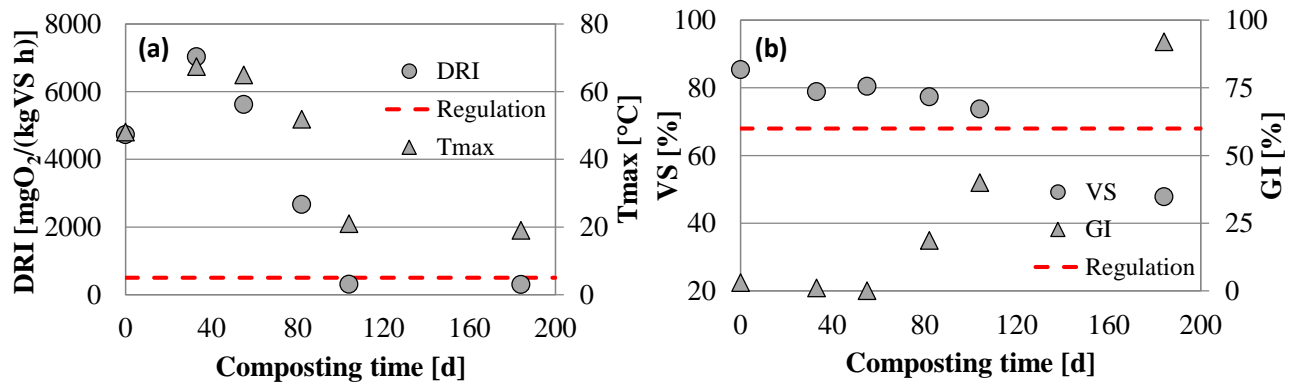
Figure 1. Schematic layout for Plant 1: MBT (a) and composting (b) line.

Figure 2



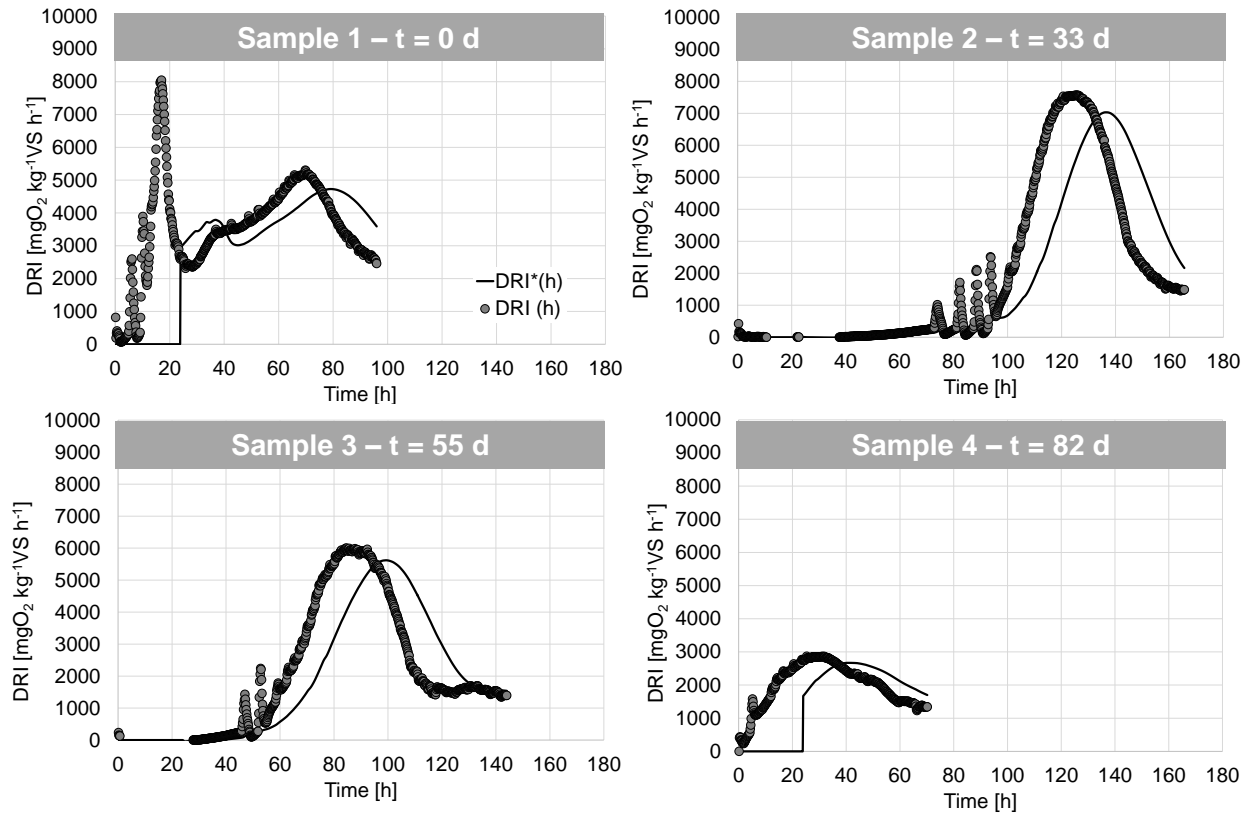
**Figure 2.** Schematic layout for Plant 2.



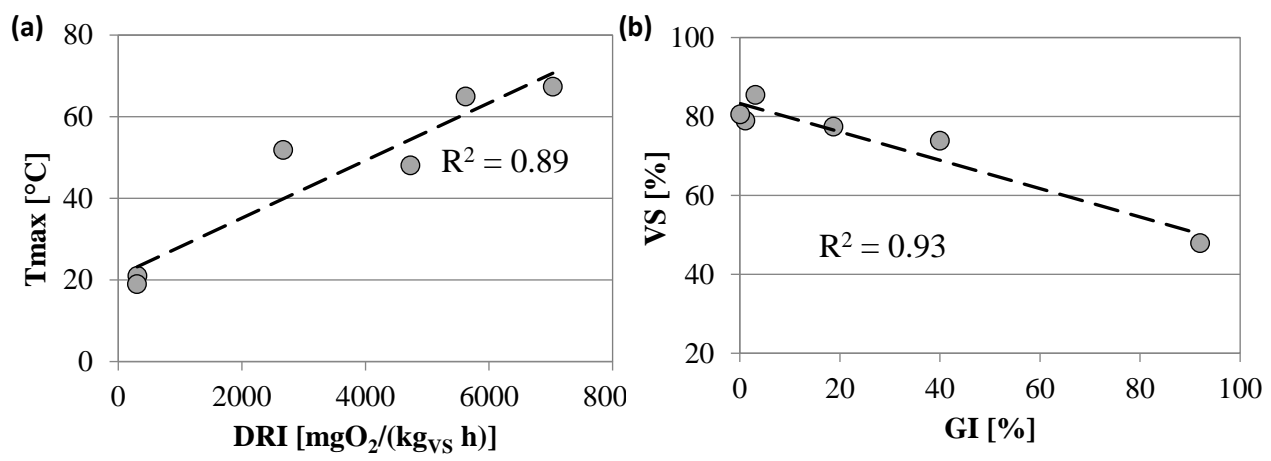


**Figure 3.** DRI and  $T_{max}$  during the composting time (a); VS and IG pattern during the composting time (b).

Figure 4



**Figure 4.** Respirogram charts achieved for the different samples, in terms of instantaneous and cumulated DRI values.



**Figure 5.** Correlation between T<sub>max</sub> and DRI (a) and GI and VS (b).

Figure 6

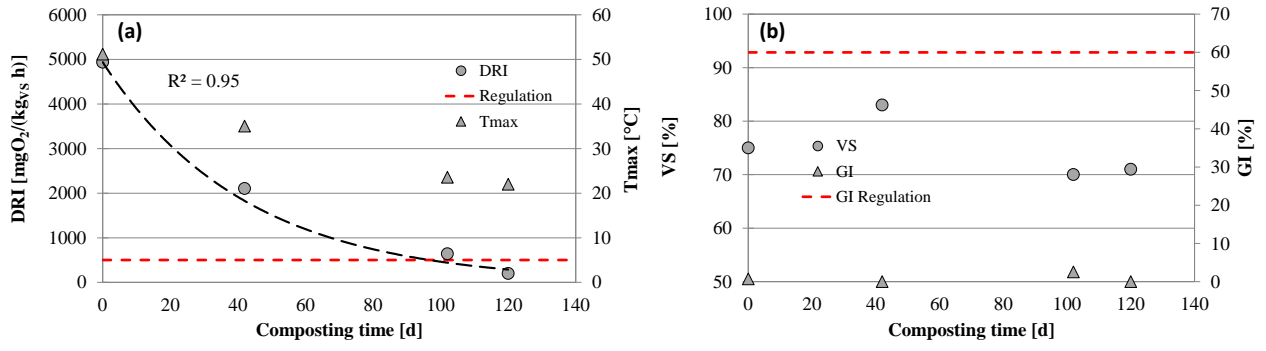
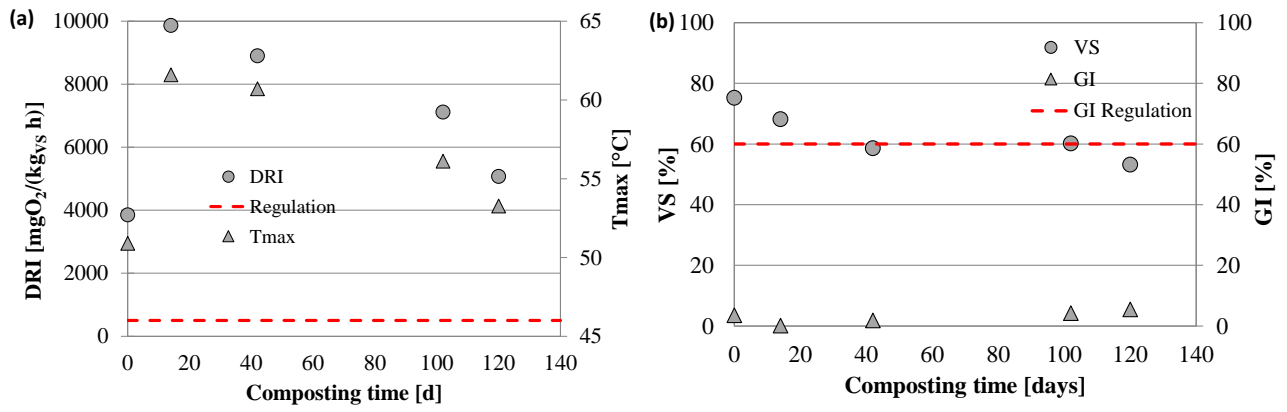
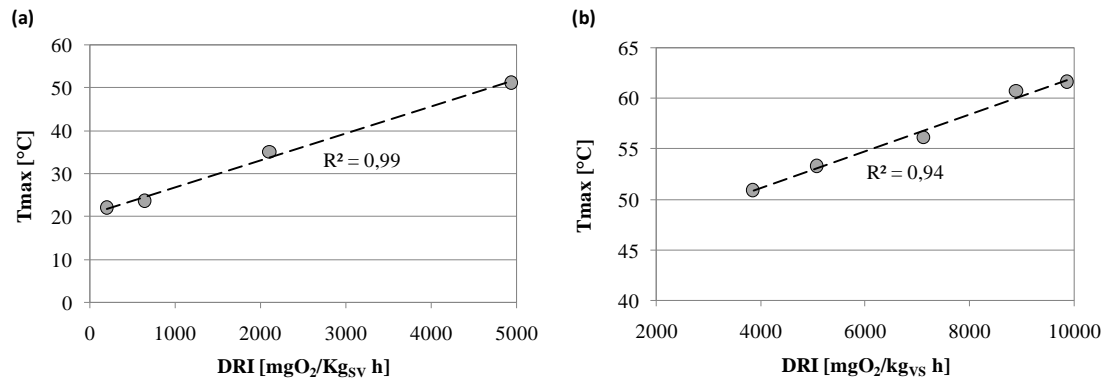


Figure 6. DRI and  $T_{max}$ (a) and VS and IG (b) trend profile during the composting time for Experimental Campaign 1.



**Figure 7.** DRI and  $T_{max}$  (a) and VS and IG (b) trend profile during the composting time for Experimental Campaign 2.

Figure 8



**Figure 8.** Correlation between  $T_{max}$  and DRI for campaign 1 (a) and campaign 2 (b), respectively.

**Table 1.** pH, moisture, TS and C/N measured for the sample collected in the composting plant.

Parameter	Symbol	Unit	Value						
			d	t = 0	t = 33	t = 55	t = 82	t = 104	t = 184
pH	-	-		5.3	5.5	6.4	7.7	7.7	8.2
Umidity	U	%		66.2	57.5	51.8	33,8	32	25
Total solids	TS	%		33.8	42.5	48.2	66.2	41.06	75.25
Carbon-to-nitrogen ratio	C/N	mgTOC/ mgTN		-	8.75	6.62	6.53	6	5

**Table 2.** Measured data for the samples analyzed at the stabilization time (t) zero (t = 0) and 34 days (t = 34 days) in the MBT.

<b>Variable</b>	<b>Symbol</b>	<b>Unit</b>	<b>Value</b>	
Duration	<b>d</b>	<b>days</b>	<b>t = 0</b>	<b>t = 34</b>
pH	-	-	5.7	7.4
Umidity	U	%	49.5	35.4
Total solids	TS	%	50.5	64.6
Total volatile solids	TVS	%	65	50
Index of growth	IG	%	0.04	5.13
Dynamic respirometric index	DRI	mgO <sub>2</sub> /kg TVS*h	12277	5966
Maximum temperature	Tmax	°C	47	48
Carbon-to-nitrogen ratio	C/N	mgTOC/mgTN	13.05	6.35



**Table 3.** Water content for the samples analyzed during Campaign 1 and 2.

<b>Moisture content [%]</b>					
	<b>t = 0days</b>	<b>t = 14 days</b>	<b>t = 42 days</b>	<b>t = 102 days</b>	<b>t = 120 days</b>
Campaign 1	60	-	49	65	55
Campaign 2	52	49.6	22	25	31