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Substrate preparation for Agaricus bisporus cultivation

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ABSTRACT: Mushroom cultivation needs a selected organic substrate obtained during a composting process which is, in some aspects, quite different from the classical one. The aim is to analyse chemical and physical aspects of a composting process for mushroom cultivation in order to point out the peculiar characteristics, which enable a much faster preparation of the substrate. Raw materials were straw, chicken manure, gypsum and ammonium sulphate. In a very short time (11-13 days) the process led to a well-stabilised biomass, as it was shown by humification indexes, C/N ratio trend, organic carbon oxidation and ammonia nitrogen concentration decrease.

In comparison to the classical composting procedure, a lower level of ammonia nitrogen and an organic nitrogen enrichment were present in the compost for mushroom cultivation. In addition, the high level of the substrate moisture, more than 75%, well above the limit normally recommended, has probably favoured the microbial growth rendering the process more efficient and faster.

KEYWORDS: *Agaricus bisporus;* biooxidation; C/N ratio; humification; mushroom; water loss.

1 BACKGROUND

Compost is the stabilised and sanitised product of a controlled bio-oxidative process and it is beneficial to plant growth. The composting process needs an appropriate mixing of raw materials (vegetable pruning, municipal and industrial sludge, agricultural and organic household wastes, farm-yard manure etc.). It develops through an initial, rapid phase of decomposition and then a process of humification [1].

The composting process for mushroom cultivation (in particular, cultivation of mushroom *Agaricus bisporus*) is slightly different from a normal composting process (see Table 1).

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Table 1. Comparison between traditional composting process and composting process for mushroom cultivation

Traditional method	**For mushroom cultivation**
Outdoor method	Indoor method because of faster spawn run and higher yields [10]
Phase 0: mixing of raw materials to reach favourable conditions for micro-organisms such as air and water contents, that depend on porosity.	
Initial optimum moisture content is between 40 and 60% [17]; it then reduces because of temperature increase and aeration. Good starting porosity is between 30 and 35 % and decreases during the process [22].	Since the composting process is shorter, special attention is paid to homogeneity [23]. The moisture content should be of about 75% [24].
Phase I: Thermophilic or biooxidative phase. The intense metabolic activity of microbial populations degrades most of the available organic matter (carbohydrates, organic acids, amino acids). Temperature between 60 and 70°C selects thermophilic micro-organisms and kills pathogens. Intermediate phytotoxic compounds (i.e.: acetic acid, propionic acid, ammonia…) can be released.	
Phase I is a period of uncontrolled self-heating, where temperature rises above 60°C. Italian recommended procedures for compost production suggest a temperature above 55°C for at least 3 days (D.M. 5/2/1998, [25]). This phase lasts **3-4 weeks** in natural conditions.	The mass undergoes aerobic fermentation in tunnels and easily reaches temperatures up to 82°C. The main aim of phase I is to increase bulk density. [23] Duration from **7 to 12 days.** Sometimes phase I is avoided [26]
Phase II: Maturation or humification phase	
Decomposition slowly proceeds, thermophilic microbial populations die, the respiratory activity and the oxygen demand decrease, temperature drops to 40-45°C. This phase can last **2-3 months**	The temperature inside indoor “pasteurisation” tunnels is described in Figure 1 (modified from [27]). The real phase of “pasteurisation” and hygienization, when the mass is brought to a temperature between 58 and 62°C, normally lasts **5-10 hours**., after which, temperature is lowered by ventilation; the conditioning of the mass ends in **5-10 days**. Phase II is complete when ammonia (extremely toxic for mushroom growth) is no longer detectable.

The objective is to obtain a homogeneous substrate from a physical and chemical point of view, which is selective for the growth of mushroom mycelia. This selectivity has microbiological and chemical aspects [2]. In the literature, a number of different substrates and methods of preparation have been widely discussed [3], [4] and several attempts have been made to develop a model to produce the desired mass of compost of the required composition [5].

Raw materials are normally a mixture of chicken manure and straw from wheat, rice, soybean, barley, oat, and cotton seed meal, etc. [6], [7], [8], [9], [10]. Straw is the most important source of carbon, it gives an elastic and porous structure and it guarantees adequate mass aeration; its water-holding capacity allows the compost to reach a good moisture content, without the phenomena of asphyxia. Gypsum is normally added in order to neutralise oxalic acid (produced by the mycelium) by transforming it to calcium oxalate, to reduce ammonia loss by means of ammonium sulphate formation, to reduce the greasiness that tends to develop in compost and to improve the porosity of the compost pile to enhance oxygen penetration [6].

The composting process depends on technically controlled factors related to the optimum biological activity such as raw materials’ composition, C/N ratio, temperature, oxygenation, moisture content and pH.

Other factors can characterise the biomass transformation and its quality [11]: nutrients (nitrogen, phosphorus, potassium), humification parameters, phytotoxicity, salinity, presence of pollutants etc. Most importantly, chemical-physical parameters of compost could explain nearly 90% of the variation in mushroom yield [12]. Thus, production efficiency could be improved by targeting these important parameters.

This study examined compost production for mushroom cultivation with the aim of identifying technological procedures which can be transferred to improve the process of urban waste composting. Another purpose was to test the possibility of substituting milled vegetable prunings for part of the raw materials in order to obtain a suitable substrate for mushroom production at lower costs and easier availability.

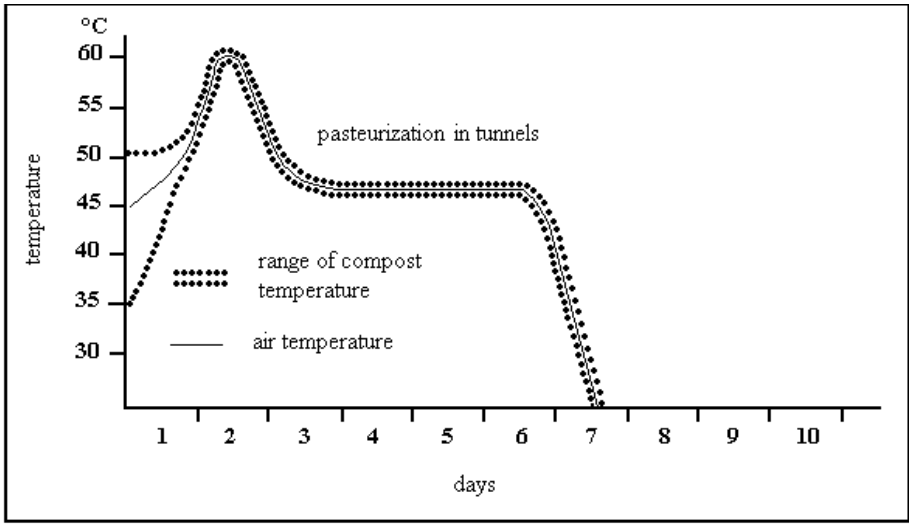

Fig. 1. Temperature trend during phase II

2 MATERIAL AND METHODS

2.1 RAW MATERIALS USED AND PHASES OF COMPOSING PROCESS

The experiment was carried out in northern Italy, on a farm doing both substrate preparation and mushroom cultivation. Three production cycles were analysed: two cycles used normal raw materials (referred to as **Cycle I** and **Cycle II**) and one cycle used urban vegetable pruning instead of straw (referred to as **Cycle III exp.**). Raw materials used in these cycles are listed in Table 2.

- *Phase 0*

Straw was mixed in the open air with half of the chicken manure, spread on a concrete surface and then trampled by an agricultural tractor in order to defiberize the straw and reduce the length of fibers. This treatment improves the straw water capacity. The remaining chicken manure was added together with the gypsum, ammonium sulfate and water. The mass was mixed again and transferred to the biooxidative cell for Phase I. This procedure lasted less than a working day.

- *Phase I*

The biooxidative room is a prism made of concrete. The floor has some holes for the ventilation. Internal walls are insulated with polystyrene panels. This room has a section of 3.4 × 3 m and it is 14 m long; loading of raw materials never exceeds the height of 2.3 m. The mass was not turned during phase I. Phase I lasted only **3-4 days**.

- *Phase II*

The mass recovered from the biooxidative cell was mixed again before starting phase II. Materials were then loaded into a 'pasteurisation' tunnel by a conveyor belt. Tunnel has a section of 4 × 4 m and it is 9 m long; materials loaded never exceed the height of 2 m. The floor is provided with holes for the ventilation, which is automatically controlled in order to maintain the correct temperature. Phase II lasted **8 days**; the real 'pasteurisation' lasts 8-9 hours and it occurs at the beginning of this phase.

- *Mushroom production process*

The compost was transferred from the 'pasteurisation' tunnel to the production rooms. The farm hosting the experiment has 10 rooms, each one having 2 rows of 6 beds placed one above the other. Beds are 1.34 × 16 m and are provided with 20 cm curbs all around. Rooms have no windows and the temperature is conditioned with forced ventilation. For each cycle, one production room was loaded. A transporting belt spread the compost along the bed length and the mycelia were sown. Compost was, then, compressed to a thickness of around 20 cm. After loading, the substrate was covered with a polyethylene film in order to reduce water loss through evaporation and maintained at 27°C during the mushroom vegetative period and at 18-19°C during the reproductive one.

After about **11-13 days**, the plastic film was taken off and around 4 cm of peat were distributed on the substrate to encourage carpophore fructification.

Only three harvests were made (others were considered of minor production). The first harvest started 20 days after covering. The following ones were separated by 8-9 days. The yield was usually about 25 kg of fresh weight per bed. Mushrooms were collected using knives to cut them at the base of the stem. Figure 2 represents the flow chart for mushroom production.

Table 2. Raw materials used in the composting process for mushroom cultivation

Raw materials	Cycle I	Cycle II	Cycle III exp.
Wheat straw (kg)	7960	7320	6360
Pruning (kg)	-	-	2660
Chicken manure (kg)	5320	5760	5560
Gypsum (kg)	480	480	480
Ammonium sulphate (kg)	70	70	70

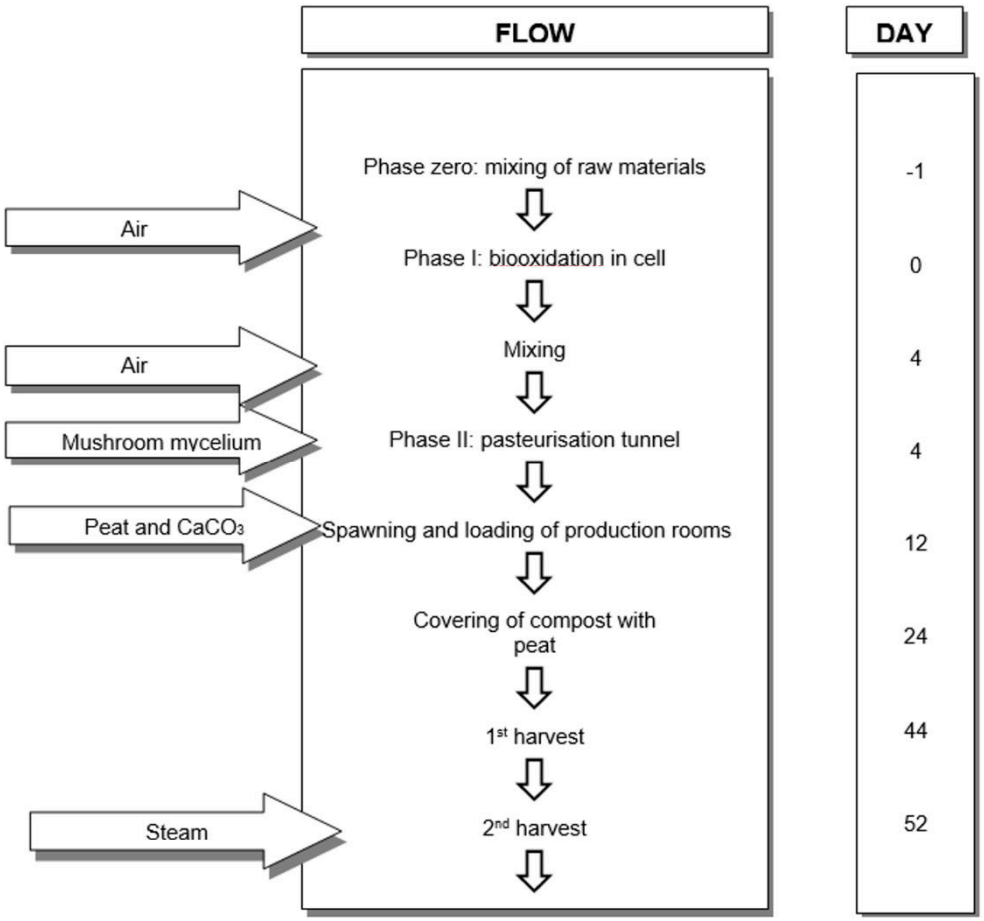

Fig. 2. Flow chart for mushroom production

2.2 EXPERIMENTATION OF SUBSTITUTIVE RAW MATERIALS

Traditional substrate used for mushroom cultivation was substituted with vegetable branches from the pruning of urban trees (see Table 2). In particular, prunings from linden trees (*Tilia cordata*) partially degraded were used in this experiment. Substrate was mixed and underwent all previously described phases.

2.3 CHEMICAL ANALYSES

A sample of substrate was taken for each cycle at the following times:

- at the beginning of Phase I after mixing the raw materials: this sample is referred to as 'Raw materials';
- at the end of Phase I, before loading the 'pasteurisation' tunnel: 'End of phase I';
- at the end of Phase II, before mycelium spawning and loading of production rooms: 'End of phase II'.

Samples were taken by using a random sampling scheme of about 7 aliquots that composed a single average sample. An average sample of the substrate ready for spawning was taken from the transportation belt at 7 different times during loading of the pasteurisation tunnel. Samples were stored at 4 °C or frozen at –18 °C for conservation.

Each sample was then divided into 2 fractions of about 400 g each (wet and dry sample), ground and used for different determinations.

Analyses were done at the Regional Agency for Environmental Protection of Veneto Region (ARPAV). The following variables were determined using standard methods (for more details see: [13]: total moisture, volatile matter and ashes, conductivity and salinity, pH, Total Organic Carbon, Extracted Carbon (TEC) and Humic Carbon (HA+FA), nitrate, nitrite, ammonia and total nitrogen, total phosphorus, total metals, Germination test.

2.4 PROCESS MONITORING

The temperature was checked during the composting process.

Phase I was normally conducted on the basis of the farmer's experience and results from the standardisation of previous working procedures.

During 'pasteurisation' and conditioning phases, three temperature probes were used for the mass control and two more for checking in- and out-air flow. The first three probes for mass control had a staff 50 cm long penetrating the compost surface, whereas the other 2 probes were positioned in the pipe of in-air and out-air of the 'pasteurisation' tunnel. The temperature was read in continuum by the probes and recorded by a microprocessor every 30 minutes as an average of the preceding measures. The computer displayed the mean, minimum and maximum temperature of the compost for the preceding 30 minutes, and the average air temperature.

2.5 DATA PROCESSING

Collected data were processed by using statistical software (SPSS and Statistica).

3 RESULTS AND DISCUSSION

The data obtained in this study were compared with a set of chemical – physical data (average values) collected by the 'Centro Agroambientale' of Castelfranco Veneto (TV) from different composting plants, sited in the Veneto region. These plants are mainly 'urban' and use urban wastes and sludge, vegetable branches, and farm-yard manure as raw materials [14].

Comparison was based on the results of Cycle I, Cycle II, Cycle III exp., and on the average values from ordinary composting plants.

Table 3, 4, and 6 report data from Cycle I, Cycle II and Cycle III exp. respectively. Table 5 reports average values of composting plants in Veneto.

3.1 COMPARISON AMONG CYCLE I, CYCLE II AND NORMAL COMPOSTING PLANTS

Firstly, Cycles I and II had a much shorter period of transformation (12-13 days), when compared with a normal urban composting process which lasts some months. In addition, a different time trend of the main parameters was also evident.

- pH: the levels for starting materials was higher than the optimum range (7.9 – 8.2). pH had the same trend during Cycle I and II, leading to a pH around 7.4 - 7.5 at the end. Urban composts (Table 5) displayed higher final values (7.8) because of the higher ammonia nitrogen concentration.
- Moisture content: both cycles were characterised by a loss of moisture during the process due to the temperature increase and aeration. However, moisture was always above 70% and therefore much higher than in a normal composting process (35.6%).

  Even in the presence of a high water content, the process for mushroom compost production did not undergo anaerobiosis. The oxygen content has to be between 5 and 15% during the biooxidative phase [15]; its demand decreases during the maturation phase. Straw has a very aerated structure and a high water retention capacity due to its hollow structure; gypsum also helps in keeping the substrate aerated and wet. The presence of water enables more intense and faster bacterial activity for organic degradation.

- Ash, dry and volatile matter: the process showed a good stabilisation and transformation of the organic fraction. It was possible to evaluate this transformation by looking at the mass balance (Figure 3): Ash content was considered constant, because it was not involved in respiration or leaching processes. Volatile matter (undergoing bacterial respiration) and water decreased during phase I and phase II. These reductions clearly showed that bacterial organic matter respiration was significant even if the time was short. Cycle II had the same trend.
- Nitrogen: as results from Tables 3 and 4 (Cycle I and II) indicate, ammonia concentration in the substrate rapidly decreased to below the detection limit (50 mg/kg d.m.). The ammonia content in compost for mushroom production was 1-2 orders of magnitude below the value found in urban composts (2645 mg/kg d.m., Table 5).

  Cycle I and II had the same nitrogen trend. Total nitrogen was always high; in Cycle I organic nitrogen (total N - ammonia N) decreased from 'Raw materials' to 'End of phase I', whereas it increased from 'End of phase I' to 'End of phase II'. The increase of organic nitrogen was 6.3% and 12% compared to the starting content and the end of phase I respectively: There was a clear enrichment in organic nitrogen during the composting process (see figure 4). Concerning Cycle II, there was a final loss of 5.7% of organic nitrogen during the process but an increase of 12.9% if compared to phase I. The origin of this organic nitrogen was the transformation of ammonia nitrogen and the incorporation of nitrogen atoms into the complex organic substrate structures from phase I to phase II (while the decrease of nitrate in Cycle II from the beginning to the end of the process seems to have no influence on organic N trend). The presence of gypsum is also critical for the organic nitrogen increase during phase II. Gypsum actually reduces ammonia loss by storing ammonia as ammonium sulphate. Biochemical processes can then incorporate this nitrogen into humic structures. This aspect deserves further investigation.

Table 3. Cycle I: results. d.m. = dry matter, n.d. = not defined, f.w. = fresh weight

Parameters	Raw materials	End of phase I	End of phase II
pH	8.04	8.05	7.4
Moisture content (%)	78.1	79.3	75.2
Ashes (% d.m.)	13.5	14.8	19.9
Salinity (meq/100 g d.m.)	104	120	111
Organic carbon (% d.m.)	41.5	40.1	36.6
Total nitrogen (% d.m.)	1.95	1.92	2.47
$N\text{-}NH_4^+$ (mg/kg d.m.)	3760	2820	<50
$N\text{-}NO_3^-$ (mg/kg d.m.)	-	-	-
C/N ratio	26.4	24.5	14.8
Total phosphorous (% d.m.)	n.d.	n.d.	0.49
Potassium (% d.m.)	n.d.	n.d.	3.19
Arsenic (mg/kg d.m.)	n.d.	n.d.	1.12
Cd (mg/kg d.m.)	n.d.	n.d.	0.17
Cr III (mg/kg d.m.)	n.d.	n.d.	4.88
Cu (mg/kg d.m.)	n.d.	n.d.	130
Hg (mg/kg d.m.)	n.d.	n.d.	0.13
Ni (mg/kg d.m.)	n.d.	n.d.	8
Pb (mg/kg d.m.)	n.d.	n.d.	<5
Zn (mg/kg d.m.)	n.d.	n.d.	141
Total Extracted Carbon (TEC) (% f.w.)	34.9	33.8	46.5
Humic Acids (HA) (% f.w.)	22.3	26.6	35.4
HA (% d.m.)	9.2	10.7	12.9
Fulvic Acids (FA) (% f.w.)	1.9	2.4	4
FA (% d.m.)	0.77	0.96	1.5
Germination Index (GI) (30%)	n.d.	n.d.	41.1
Degree of Humification (DH) (%)	69.3	85.8	84.7
Humification Rate (HR) (%)	24	29.1	39.3
Humification Index (HI)	0.44	0.17	0.18
HA/FA	11.7	11.1	8.9

Table 4. Cycle II: results. d.m. = dry matter, n.d. = not defined, f.w. = fresh weight

Parameters	Raw materials	End of phase I	End of phase II
pH	8.2	8.3	7.5
Moisture content (%)	79.9	76.7	73.7
Ashes (% d.m.)	16.1	18.2	22.7
Salinity (meq/100 g d.m.)	109	99	118
Organic carbon (% d.m.)	40.2	41.5	40
Total nitrogen (% d.m.)	2.07	2.02	2.5
$N\text{-}NH_4^+$ (mg/kg d.m.)	2240	2620	360
$N\text{-}NO_3^-$ (mg/kg d.m.)	380	250	130
C/N ratio	21.8	23.6	16.2
Total phosphorous (% d.m.)	n.d.	n.d.	0.72
Potassium (% d.m.)	-	-	-
Arsenic (mg/kg d.m.)	-	-	-
Cd (mg/kg d.m.)	n.d.	n.d.	0.34
Cr III (mg/kg d.m.)	n.d.	n.d.	3.65
Cu (mg/kg d.m.)	n.d.	n.d.	60.7
Hg (mg/kg d.m.)	-	-	-
Ni (mg/kg d.m.)	n.d.	n.d.	5.41
Pb (mg/kg d.m.)	n.d.	n.d.	3.08
Zn (mg/kg d.m.)	n.d.	n.d.	208
Total Extracted Carbon (TEC) (% f.w.)	36.1	42.9	46.7
Humic Acids (HA) (% f.w.)	24.4	28.5	34.3
HA (% d.m.)	9.8	11.8	13.7
Fulvic Acids (FA) (% f.w.)	2.52	3	1.44
FA (% d.m.)	1.01	1.26	0.6
Germination Index (GI) (30%)	n.d.	n.d.	43
Degree of Humification (DH) (%)	74.6	73.4	76.5
Humification Rate (HR) (%)	26.9	31.5	35.8
Humification Index (HI)	0.34	0.36	0.31
HA/FA	9.7	9.5	23.8

To summarise, it was experimentally demonstrated that the organic nitrogen percentage was higher and ammonia was lower in mushroom composts in comparison with urban composts (Table 5).

- C/N ratio: starting values of C/N ratios are similar to those in normal composting processes (between 25 and 35, [16] and both in Cycle I and Cycle II. There were no changes from 'Raw materials' to 'End of phase I'. During phase II the C/N ratio decreased to values around 14-16, normally assumed as stability markers [17]. They are also similar to those found in different composting plants.
- Humic substances: during the maturation phase humic substances change qualitatively and quantitatively. Humic acids (HA) rise above fulvic acids (FA) from the beginning to the end of the process; their ratio is an important parameter of evaluation [15]. Other humification parameters was also taken in consideration:

$$\mathbf{HI} = Humification\ Index = \frac{\mathrm{NH}}{\mathrm{HA} + \mathrm{FA}}$$

Where NH = Fraction of extracted organic carbon, not humified.

$$\mathbf{DH} = Degree\ of\ Humification = \frac{\mathrm{HA} + \mathrm{FA}}{\mathrm{TEC}} \times 100$$

Where TEC = Total Extracted organic Carbon.

$$\mathbf{HR} = Humification\ Rate = \frac{\mathrm{HA} + \mathrm{FA}}{\mathrm{TOC}} \times 100$$

Where TOC = Total Organic Carbon.

As a consequence of the changes in Humic acids and fulvic acids, HI (humification index) decreased during both cycles (even if values were higher in Cycle II than in Cycle I). Lower values found during Cycle I could indicate a stronger humification process. HR (humification rate) and DH (degree of humification) increased for both cycles. Values were comparable with those others of urban compost plants (Table 5).

- Salinity and phytotoxicity: Salinity is defined as the amount of salts in the organic substrate, which decreases during composting. Compost can have different uses depending on its salt content [18]. According to legislation of Veneto Region, compost salinity should be below 50 meq/100 g. Cycle I and II had similar values of salinity (110-120 meq/100 g d.m.) but were higher than values observed in ordinary composting plants (around 44 meq/100 g d.m.): this was probably due to the chicken manure's content and also to the calcium and ammonium sulphate added in compost for mushroom production.

  Phytotoxicity was measured by the Germination Index (GI) that evaluates germination and growth of roots of *Lepidium sativum*, used as a biomarker:

$$\mathbf{GI} = \frac{(\text{average number of germinated seeds} \times \text{average root length}) \text{ of sample}}{(\text{average number of germinated seeds} \times \text{average root length}) \text{ of control}}$$

  'Control' is the plant grown in pure water solution.

  As a consequence of the relatively high salinity, a degree of phytotoxicity was evident: a germination of only 40% was observed in comparison to GI > 60% for non-toxic compost.

- Phosphorous and potassium: Phosphorus and potassium contents are similar to values in urban compost (Table 5).

- Heavy metals: As mushrooms show high capacity to accumulate high levels of some heavy metals from substrates a special attention should be paid to this factor [19], [20]. In our case no worrisome values of metals in terms of toxicity was detected. There were no differences between the 2 cycles and the normal composting processes as well.

- Temperature: No temperature control was performed during phase I. Temperatures measured during phase II reflected the trend shown in Figure 1 (temperature of the mass from 40-50°C to 58-62°C and then to 50-51°C) and agreed with the values reported in the literature [21].

  In particular, Cycle II values were equal to literature data, where air and compost had a difference of only 2-4°C, while Cycle I had a higher temperature of 'pasteurisation' and a greater difference between air and compost temperature.

  Temperature control during phase II showed that thermal conditions during mushroom compost production, after a first short phase of instability, were homogeneous.

- Production: Compost, after spawning and loading the cultivation rooms, gave a final product with good commercial features. The net productivity, expressed in kg of fresh net weight per square metre of bed, was 22.13 kg/mq (10.75 kg/mq at the first harvest) in cycle I and 20.10 kg/mq in cycle II (10.10 kg/mq at the first harvest). The net weight does not consider the basal part of the mushroom, while the gross weight is calculated by adding 20% to net weight (it is 26.55 kg/mq in cycle I and 24.12 kg/mq in cycle II).

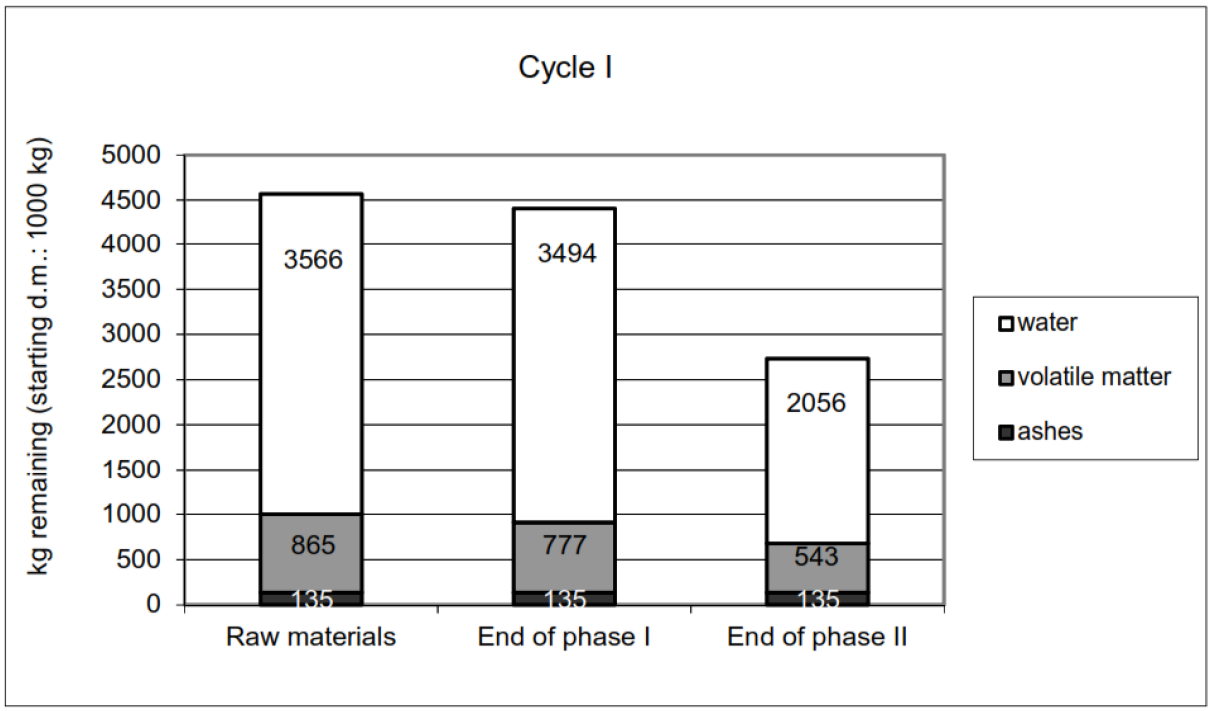

Fig. 3. Cycle I: biomass balance

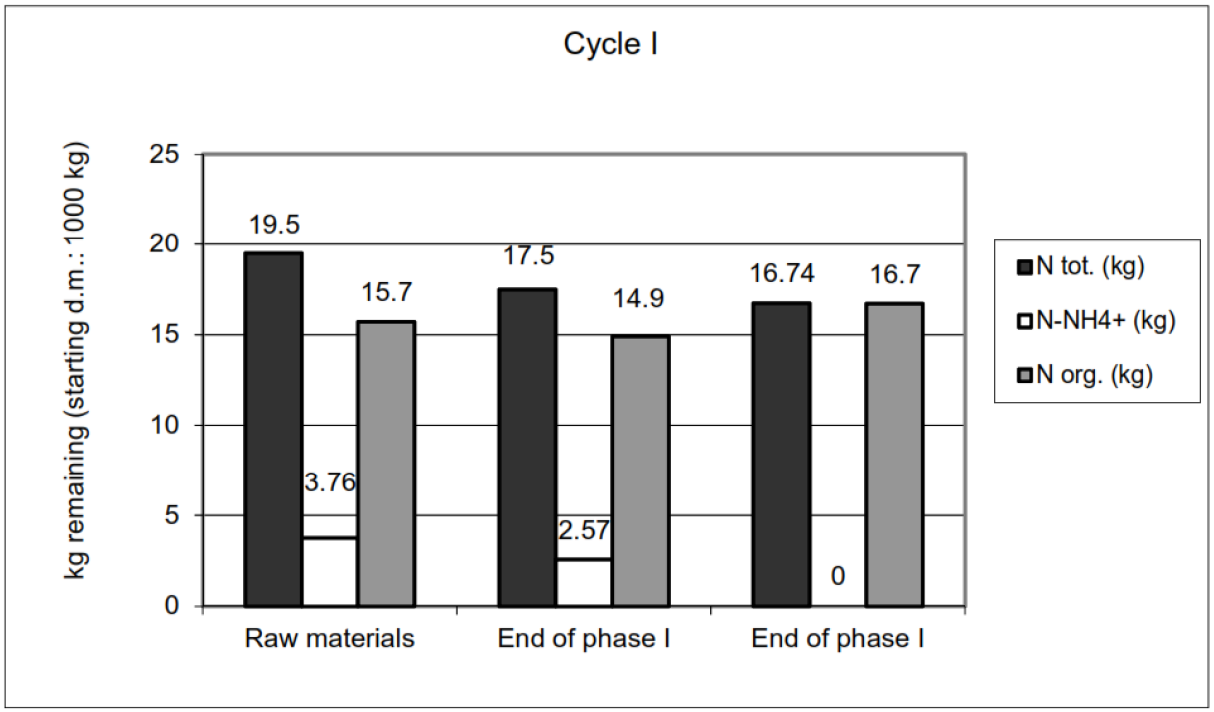

Fig. 4. Cycle I: nitrogen balance

Table 5. Average values of composting processes in urban composting plants. d.m. = dry matter, n.d. = not defined, f.w. = fresh weight

Parameters	N.	Mean	Std. Dev.	max	min
pH	130	7.88	0.74	9	5.1
Moisture (%)	130	35.6	9.4	59.7	13
Ashes (% d.m.)	130	49.4	14.7	88.5	16.8
Salinity (meq/100g)	130	43.7	35.5	265	2.5
Total Organic Carbon TOC (% d.m.)	130	24.8	7.5	44.2	9.6
N tot (% d.m.)	130	2.11	0.99	5.11	0.23
$N\text{-}NH_4^+$ (mg/kg d.m.)	103	2645	2101	9210	<50
N org (%-N tot)	104	88.4	8.6	99.8	51
C/N (TOC/N org)	117	13.8	3.4	28	8.3
P tot (% d.m.)	101	0.88	0.61	2.4	0.10
K tot (% d.m.)	36	1.16	0.58	2.87	0.16
Total Extracted Carbon (% f.w.)	118	50	7.7	73.6	32.4
Humic (HA)+Fulvic Acids (FA) (% f.w.)	118	40.5	7.7	62.2	23.5
HA +FA (% d.m.)	118	9.8	2.6	18.4	2.9
Humification Index (HI)	118	0.26	0.24	1.41	0.01
Humification Rate (HR) (%)	118	40.5	7.7	62.2	23.5
Degree of Humification (DH) (%)	118	81.5	13	98.9	41.6
Germination Index (GI) (30%)	124	68.7	35.2	130	0
As (mg/kg d.m.)	130	4.33	2.71	13.40	0.46
Cd (mg/kg d.m.)	130	0.92	0.53	4.31	0
Cr III (mg/kg d.m.)	130	64.3	70.7	540	6.8
Cr VI (mg/kg d.m.)	21	0.1	0.2	0.5	0
Cu (mg/kg d.m.)	130	130.2	73.5	536	13
Hg (mg/kg d.m.)	130	1.19	0.79	3.36	0
Ni (mg/kg d.m.)	130	26.1	15.2	98.7	4.1
Pb (mg/kg d.m.)	130	78.1	54.8	352	0
Se (mg/kg d.m.)	130	0.60	0.73	3.09	0
Zn (mg/kg d.m.)	130	420.8	230.5	1358	94.3
B (mg/kg d.m.)	130	45.5	16.5	120	17.8

3.2 COMPARISON WITH CYCLE III (EXPERIMENTATION WITH URBAN VEGETABLE PRUNING)

Data of Cycle III experiment are reported in Table 6.

Duration time was around 12 days (as seen for Cycle I and Cycle II).

The mixture of raw materials underwent a greater water loss than in the previous 2 cycles, even if the starting values of moisture were the same (final value of moisture: 68%). This is probably due to reduced water-holding capacity of the raw materials.

The increase in observed ash was also probably due to the substituted material (26% d.m. while for Cycle I and II it is around 20%).

The starting value of ammonia nitrogen was higher in those mixtures of raw materials than in normal mushroom compost materials. The final nitrogen concentration was similar to that found in the 2 cycles made with normal raw materials, and nitrogen compounds had the same trend previously observed.

There were no differences among HA, FA, TOC values and those previously observed. HR increased during the process. Both HR and DH were slightly lower than those of normal mushroom production, as it is shown in Figure 5.

Salinity was still high (more than 100 meq/100 g d.m.) and the germination index was below an acceptable threshold (GI was 28).

Temperature control of phase II gave a comparable trend with previous cycles.

In summary, no significant differences were found in the composting process and in the compost with the use of these substituted materials. Furthermore, the new substrate allowed regular mushroom mycelium development. Data obtained refer

only to the first harvest (7 kg/mq of mushrooms). Mushrooms had the same colour and shape of standard ones, but they were of bigger shape, more symmetric and proportioned. These features could naturally influence the economic value of the product.

Table 6. Cycle III exp.: results. d.m. = dry matter, n.d. = not defined, f.w. = fresh weight

Parameters	Raw materials	End of phase I	End of phase II
pH	7.9	8.4	7.4
Moisture content (%)	77.6	74.8	68.4
Ashes (% d.m.)	20.4	25.9	26.4
Salinity (meq/100 g d.m.)	122	104	104
Organic carbon (% d.m.)	39.9	37.4	39.2
Total nitrogen (% d.m.)	2.53	2.63	2.67
$N\text{-}NH_4^+$ (mg/kg d.m.)	6070	2840	230.5
$N\text{-}NO_3^-$ (mg/kg d.m.)	140	270	50
C/N ratio	20.7	15.9	14.8
Total phosphorous (% d.m.)	n.d.	n.d.	1.19
Potassium (% d.m.)			
Arsenic (mg/kg d.m.)			
Cd (mg/kg d.m.)	n.d.	n.d.	0.39
Cr III (mg/kg d.m.)	n.d.	n.d.	6.77
Cu (mg/kg d.m.)	n.d.	n.d.	61.3
Hg (mg/kg d.m.)			
Ni (mg/kg d.m.)	n.d.	n.d.	7.11
Pb (mg/kg d.m.)	n.d.	n.d.	3.95
Zn (mg/kg d.m.)	n.d.	n.d.	337
Total Extracted Carbon (TEC) (% f.w.)	33.2	40.7	41.1
Humic Acids (HA) (% f.w.)	21.4	24.3	27.2
HA (% d.m.)	8.41	9.41	10.8
Fulvic Acids (FA) (% f.w.)	2.51	2.17	2.13
FA (% d.m.)	1.97	1.67	1.69
Germination Index (GI) (30%)	n.d.	n.d.	28
Degree of Humification (DH) (%)	72	65	71.4
Humification Rate (HR) (%)	26	29.6	31.9
Humification Index (HI)	0.39	0.54	0.4
HA/FA	8.5	11.2	12.8

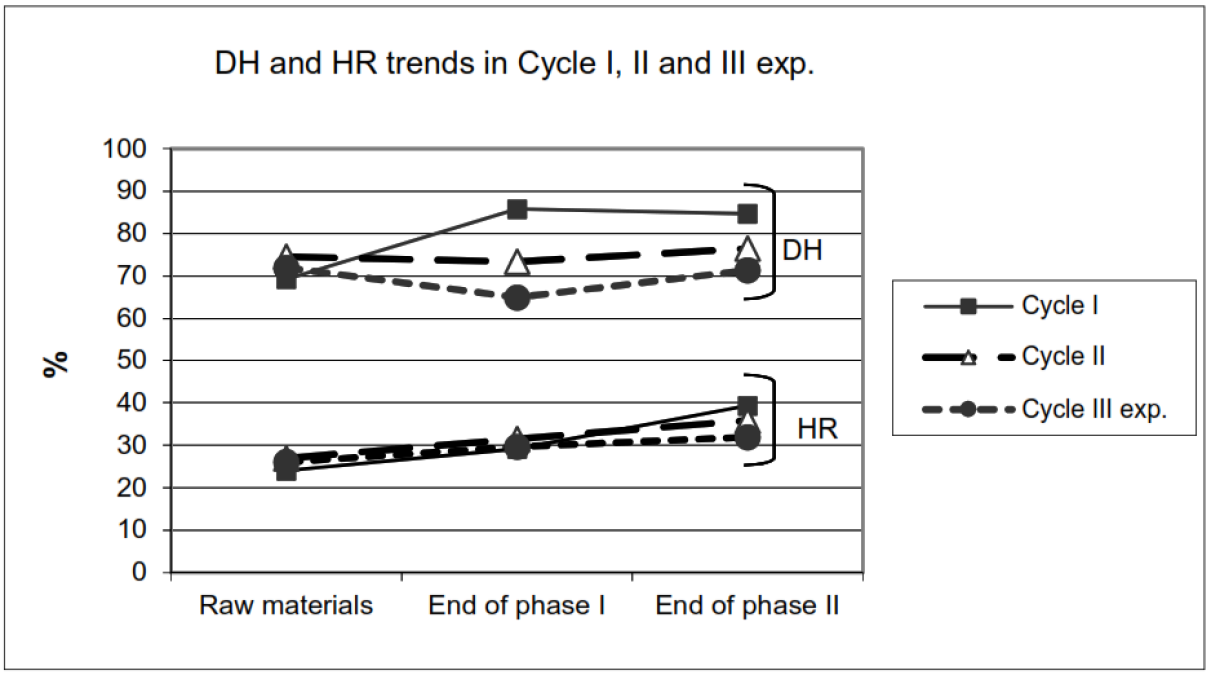

Fig. 5. DH and HR trends in Cycle I, Cycle II and III exp.

4 CONCLUSION

Some interesting analogies and differences with the composting process for urban sludge and wastes were pointed out by examining the substrate preparation process for mushroom cultivation. Results showed enhanced process efficiency, related to the use of a homogeneous and simplified type of raw materials, and the conduction of the process in closed cells.

Mushroom compost preparation is 5-6 times shorter than the urban waste composting process. Compost reaches in only 13 days chemical – physical parameters that are normally found in urban compost after 60-90 days.

Parameters of humification are comparable with those of urban composts.

Moisture content in mushroom compost is kept higher than the highest values normally found in urban composts, without showing problems due to anaerobiosis. The higher quantity of water is probably useful for the faster development of biooxidation.

Chemical characterization of the substrate ready for mycelium spawning confirmed a clear analogy with urban composts, except for a substantial difference in ammonia and organic nitrogen content. Organic nitrogen was higher than in urban composting processes while ammonia nitrogen was lower.

The addition of gypsum to the raw materials reduced ammonia nitrogen losses, while improving substrate texture, water retention and the aerobic structure of the biomass. All these features of mushroom composting could be considered to improve the urban compost production. On the other hand, the mushroom compost resulted in rather high salinity and phytotoxicity. This results from the addition of gypsum, ammonium sulphate, and in particular, chicken manure. The use of a reduced amount of gypsum in the urban composting process could guarantee the benefits seen above with acceptable increases of salinity and phytotoxicity. Experiments to test the optimum amount of added gypsum are in progress.

The thermal and moisture conditions of the process during phase II could also be applied to an urban composting process to experience a significant shortening of its duration.

The substitution of part of the straw with urban pruning showed no significant losses in productivity and a good extent of transformation. Further investigation could establish to what percentage substituting materials can be employed without compromising the quality of the product. This could be of greater importance in the case of reduced availability and therefore increased prices of this component.

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