Enhanced fertilization effect of a compost obtained from mixed herbs waste inoculated with novel strains of mesophilic bacteria

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Abstract

Mixed medicinal plant waste was composted with addition of novel bacterial strains belonging to the genera *Streptomyces, Paenybacillus, Bacillus* and *Hymenobacter*. The composting was followed by assessment of chemical and biological parameters including C/N ratio, loss of organic matter, phosphorous and potassium content as well as CO_2 generation and dehydrogenase activity during 164 days. The selected mesophilic bacterial starters had a potential to significantly reduce the period of mixed herb waste decomposition, from about 6 months to about 2.5 months. Based on the seed germination index of four plants (*Fagopirum esculentum, Thymus vulgaris, Cynara scolimus and Lavandula officinalis*) the germination and radial root growth of the investigated plants was improved by the inoculated compost. The germination index of all tested species on the mature inoculated composts was in average 60% higher compared to the control compost. The research indicates that the mesophilic starter addition into the herbs waste can contribute to the speed of waste decomposition and lead to the improvement of biofertilization effect of the obtained compost.

Keywords: composting, herbs waste, mesophilic bacteria, germination index, plant growth stimulation, phytotoxicity.

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Generation of lignocellulolytic waste may represent a problem due its extensive accumulation and declining possibility of its safe disposal. Disposal of large quantities of this waste causes energy, economic and environmental problems. The practice in agriculture as well as in industrial processing suggests that lignocellulolytic waste should be regarded first as a nutrient substrate rather than waste. It can be converted by different groups of microorganisms that enable natural processes of matter degradation and circulation. Many engineering systems use this microbial potential for bioconversion and bioremediation. Biological conversion of agro-waste into an added value product such as compost is an option with multiple benefits [1]. Although the composting is a spontaneous microbiological process, addition of selected microbial starters can accelerate the processes and provide standardized quality of compost. The addition of selected microorganisms could be used for preparation of multifunctional biofertilizers thus replacing the use of a significant amount of synthetic fertilizers in agriculture [2]. The additional value of the fertilizer depends on characteristics of the used microbial species and of particular importance is the inclusion of species that possess the ability to control plant pathogens. This ability can be based on production of antibiotics, hydrolytic enzymes such as chitinases, proteases, amylases or glucanases, or inductors of plant resistance [3].

During the composting process, decomposition of phytotoxic organic substances produced during the active composting stages is carried out [4]. Phytotoxicity and immaturity of compost is connected to the presence of organic acids, ammonia and ethylene oxide in earlier stages of the composting process [5]. Compost maturity can be determined by various physical-chemical and biological or microbiological parameters [6], while phytotoxicity testing usually involves determination of the seed germination index (GI) of compost extract. Following ranges had been proposed to evaluate the phytotoxicity: for $GI < 25$, the substrate is very phytotoxic, for $26 < GI < 65$ the substrate is phytotoxic, and for $66 < GI < 100$ the substrate is non-phytotoxic and can be used for agricultural purposes. However, if $GI > 101$ the substrate is characterized as phytonutrient–phytostimulant and can be used in agriculture as a fertilizer [7].

The aim of this study was to examine the effect of novel mesophilic bacterial cultures on the speed of the

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decomposition process of mixed herbs waste. According to the best knowledge of the authors, it is the first time that a complex mixture of the real industrial waste, composed of medicinal herbs debris, was used for the composting process. The decomposition process was followed by measurements of chemical and biological parameters and the quality of the obtained compost was evaluated by the germination index of four different plant species.

MATERIALS AND METHODS

Material for decomposition

A material used in this study was the mixed plant waste that was generated in 2012 during production and processing of herbs in the Institute of Medicinal Plants Research "Dr Josif Pančić", Belgrade. The mixture of waste contained over 90 species of plants (www.mocbilja.rs) and its content was classified according to the group of plant species as is shown in Table 1.

Equiseti herba (SE) waste was obtained following the processing of a single herb species, *i.e.*, the aboveground part of the horsetail (*Eguisetum*). Another type of herbal waste (SG), consisted of several representatives of the root medicinal plants ("bitter drugs"). The waste designated as "herbal drugs" (SH) includes different granulations of wasted part of plant species used in the production of tea. Waste from the extraction (E) represents mixed herbs waste obtained from the extraction process with some residual ethanol content. The selected physicochemical properties of the raw mixed composting materials were measured prior to the start of the experiment (Table 1).

All chemicals and reagents used were of analytical grade and were purchased from Sigma.

Bacterial consortium preparation

Strains used in this study are natural isolates from soil and marine sediments with different hydrolytic potentials. They are part of culture collection of the microbiological laboratory at the Department of Biochemical Engineering and Biotechnology (University of Belgrade, Faculty of Technology and Metallurgy). The strains were identified to nearest species based on morphological characteristics, and sequence of the 16S rRNA encoding gene (gene accession numbers KP715850--KP715856) which was higher than 99% for all species with exception of the strain CKS3 (with 98% similarity to closest *Hymenobacter* species). The strains were designated as: *Streptomyces spororaveus* CKS2, *Streptomyces microflavus* CKS6, *Streptomyces fulvissimus* CKS 7, *Paenybacillus chitinolyticus* CKS1, *Hymenobacter* sp. CKS 3, *Bacillus amyloliquefaciens* ssp. *plantarum* PPM3 and *Bacillus altitudinis* PPT1.

Screening for the cellulolytic potential of the strains was assessed by growth on agar plates with carboxymethyl cellulose (CMC) according to the method of Kasana *et al.* (2008) [8]. The ability to grow on the medicinal plant waste was tested on agar plates supplemented with 1, 5 or 10% of mixed medicinal plant waste prepared for composting. Fresh, overnight cultures of bacteria were strickled on the agar surface and growth was determined after 1–5 days of incubation at 30 °C. Mutual relations of the selected bacteria were tested by streak-plating technique on the appropriate agar medium (ISP1) that is convenient for fast screening of antimicrobial potential of isolates [9,10].

The cultures were prepared for composting by growing in ISP1 broth (pancreatic digest of casein 5.0 g/L; yeast extract 3.0 g/L) until reaching the titter of 10^6–10^7 CFU/ml. Liquid cultures were mixed in equal volume ratios in ISP medium and the obtained mixed population was used for inoculation of medicinal plant waste material.

Composting process

For the experimental purpose, small wooden boxes (60 cm×70 cm×90 cm) were filled with 30 kg of mixed medical plant waste. Dry plant waste (SH, SG and SE) was mixed in a ratio that is approximately close to the annual composition (Table 1). Then, dry part was mixed with the wet plant waste (E) obtained from the extraction process in the ratio of 5:1, respectively. The compost material was partially protected from the atmospheric influence by a perforated lid that allowed air exchange. Humidity of the composting material was kept at 60% throughout the composting process by

Table 1. Approximate amounts of the herbal waste generated during production and processing of mixed herbs waste, in the 2012 and mean values of physicochemical parameters of obtained mixed herbs waste

Herbal waste classes	Herbal waste quantities, %
SH Herbal drugs	74.06
E Waste from the extraction	17.05
SG Valeriane radix, Primulae radix, Gentianae radix	5.02
SE Equiseti herba	3.88

Mean values of physicochemical parameters of mixed herbs waste						
Ash, %	pH	Total P, %	Total K, %	Total N, (%	Total C, %	C/N
26.80±21.95	5.97±0.42	0.26±0.06	1.42±0.51	1.37±0.23	36.60±10.97	26.38±5.27

addition of tap water once per week (if necessary). The composts were mixed by overturning once per week. Three boxes of homogeneous plant waste were inoculated with 2% of fresh bacterial cultures (*cca*. 10^7/ml). These samples were designated as inoculated compost (IC). The controls were the boxes with the same homogenous plant waste that was composted spontaneously and was designated as the control compost (CC). Plant waste decomposition and the composting process were monitored for 164 days (approximately 6 months).

Sampling and analysis

During the composting process, sampling was performed once a week. From each box a sample was taken by mixing five sub-samples taken from different representative spots of the composting material. A part of each sample was analyzed immediately while another part was stored at –20 °C until chemical analysis. Ambient temperature and the temperature of the composting material at its center were measured and recorded once per week.

Prior to chemical analyses, samples were defrosted and dried in an air oven at 80 °C until constant weight and homogenized by grinding in a mill in 0.5–1.0 mm particles. After grinding, the samples were dried again at 80 °C until constant weight. Results of the chemical analysis are given per gram of dry-weight material.

Moisture contents of raw materials and composting mixtures were determined by automatic Moisture analyzer (MLS 65-3A, KERN, Balingen, Germany). Ash was determined in a muffle furnace by heating to 550 °C until constant weight. The organic matter (OM) of samples was calculated as the difference between ash and dry weight and expressed as a percentage [11]. The percentage of the total organic carbon (TC) was calculated by multiplying OM by 0.58. Biodegradability coefficient (K_b) was calculated using the equation:

$$K_b = \frac{100\left(OM_i - OM_f\right)}{OM_i(100 - OM_f)} \quad (1)$$

where OM_f is the OM content at the end of the process and OM_i is OM content at the beginning of the process [7].

The nitrogen content (TKN) was determined according to the semi micro Kjeldahl digestion procedure by digesting the samples with concentrated sulfuric acid (H_2SO_4) and the mixture of catalysts (100:1:1000 $CuSO_4{\cdot}5H_2O$/Se/K_2SO_4). Ammonium in the Kjeldahl digests was determined titrimetrically, following alkaline steam distillation [12]. Phosphorus level was determined using digestion with HNO_3–$HClO_4$ followed by a colorimetric method using Barton's solution [13]. Potassium was determined using digestion with HNO_3–$HClO_4$ and a flame photometer (PFP7, Jenway, UK) [13].

For pH and water-soluble carbon (WSC) determination, the extracts were prepared by liquid solid extraction of samples using distilled water (1:10). After 4 h of extraction the samples were centrifuged at 4500 rpm for 10 min and the supernatant was used for further analyses.

pH values were measured in the aqueous extract by inoLab pH 720 (Germany) [14]. The water-soluble carbon (WSC) in the aqueous extract was determined by the phenol-sulfuric acid method [15].

Dehydrogenase activity (DHase) was measured by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) according to ISO23753-1:2011 procedure. Briefly, samples of compost (5 g) were incubated for 24 h at 30 °C in 5 ml of a TTC solution (5 g l-1 in 0.1 M Tris-HCl buffer, pH 7.4). The sample was then blended with 25 ml of acetone to extract TPF, shaken in the dark for 2 h at 150 rpm and centrifuged at 4500 rpm for 5 min. The optical density of the extract was determined by a UV–Vis spectrometer (Ultrospec 3300 pro, Amersham Bioscience, Sweden) at 485 nm and DHase activity was calculated based on the standard curve constructed with TPF and was expressed as μgTPF mg^{-1} dry compost h^{-1}.

Determination of the CO_2 content was performed according to the procedure of Iannotti *et al.* (1993) [16]. Briefly, 2.5 g of dry compost sample was incubated for three days at room temperature (25 °C) in a tightly closed container with a test tube filled with 2 ml 1 M NaOH. After the incubation, the evolved CO_2 was determined by titration of remaining NaOH solution with 1M HCl.

Phytotoxicity measuring

Seed germination test is widely accepted for assessment of phytotoxicity and stability of compost [17,18]. In the present work, this test was carried out using water extracts of samples. Fresh samples were diluted in distilled water 1:10 and shaked for 1h in an orbital shaker (KS 4000i control, IKA, Werke GmbH & Co. KG, Germany) at 150 rpm. 5 ml of each extract (IC and CC) was used for wetting the sterilized Whatman No.1 filter paper in a glass Petri dish. Five ml of distilled water was used as a control. The germination index (*GI*) was calculated using 25 seeds from 4 different medicinal plants (*Fagopirum esculentum, Thymus vulgaris, Cynara scolimus* and *Lavandula officinalis*). For each medicinal plant seed, three replicates were used. Seeds were allowed to germinate in 72 h at 25 °C in the dark. After that time, the number of germinated seeds was counted and the length of the root radical was measured. The GI was calculated using the following equation:

$$GI\ (\%) = 100(G/Gc)(L/Lc) \quad (2)$$

where *G* and *L* are the germination and the root length of the samples, respectively, while *Gc* and *Lc* are the

germination and the root length of the control (distilled water), respectively.

Statistical analysis

Statistical analysis was performed using the program Origin Pro 7 (OriginLab, Northampton, MA, USA). All results are presented as the mean±standard deviation of three independent measurements. Data were analyzed using analysis of variance (one-way ANOVA) and the Tukey's test was applied to means comparisons with a level of significance of 0.01. Design Expert 8 software (Stat-ease, USA) was used for the correlation coefficient determination between selected parameters. For comprehensive multivariate statistical analysis, software Canoco for Windows 4.5 [19] was used. Redundancy analysis (RDA) was used to assess correlations between characteristics of the compost in respect to the presence or absence of bacterial inoculum during composting. The RDA figure was drawn using CanocoDraw software as implemented in the Canoco for Windows 4.5 [19] package.

RESULTS AND DISCUSSION

Screening of bacteria for the composting process

In order to increase the speed of composting we have selected cellulolytic bacteria including five novel natural isolates from forest land (*Paenibacillus chitinolyticus* CKS1, *Hymenobacter* sp. CKS 3, *Streptomyces sporoveraus* CKS2, *Streptomyces microflavus* CKS6, *Streptomyces fulvissimus* CKS 7) and two novel isolates from marine sediments (*Bacillus amyloliquefaciens* ssp. *plantarum* PPM3 and *Bacillus altitudinis* PPT1) (Figure 1a). All tested strains are mesophilic with an optimal growth temperature of approximately 28–30 °C (with exception of *B. amyloliquefaciens* ssp. *plantarum* PPM3 with the optimal growth temperature of 45 °C) and have antibacterial and antifungal properties (data not shown). Cellulolytic enzymes play an important role in natural degradation processes in which plant lignocelluloses are efficiently converted into products that can be utilized by bacteria for their growth. The addition of cellulolytic bacteria could, therefore, increase the speed of plant material degradation, and composting process.

However, not all bacteria are expected to be active utilizers of plant waste since higher plant species contain tannins that can act as antimicrobials towards various microorganisms [20,21]. Therefore, it was very important to investigate antimicrobial properties of the mixed medicinal plant waste on growth and activity of the selected cellulolytic bacteria. Our results showed that all selected bacteria were able to grow onto the agar plates with 1, 5 and 10% of mixed plant waste. The growth ability on agar plates with 10% of mixed plant waste is shown in Figure 1b. Moreover, the medicinal plant waste showed growth stimulation effect, particularly for the *P. chitinolyticus*. CKS1, *B. amyloliquefaciens* ssp. *plantarum* PPM3 and *B. altitudinis* PPT1. It has been reported that some essential oils from the medicinal plant stimulate soil respiration [22], which is most likely due to stimulation of growth and activity of beneficial soil bacteria. Since the selected cellulolytic strains that were able to grow on medicinal waste, did not show mutual antagonism, the composting starter was composed by forming mix of equal amounts of these seven bacterial strains.

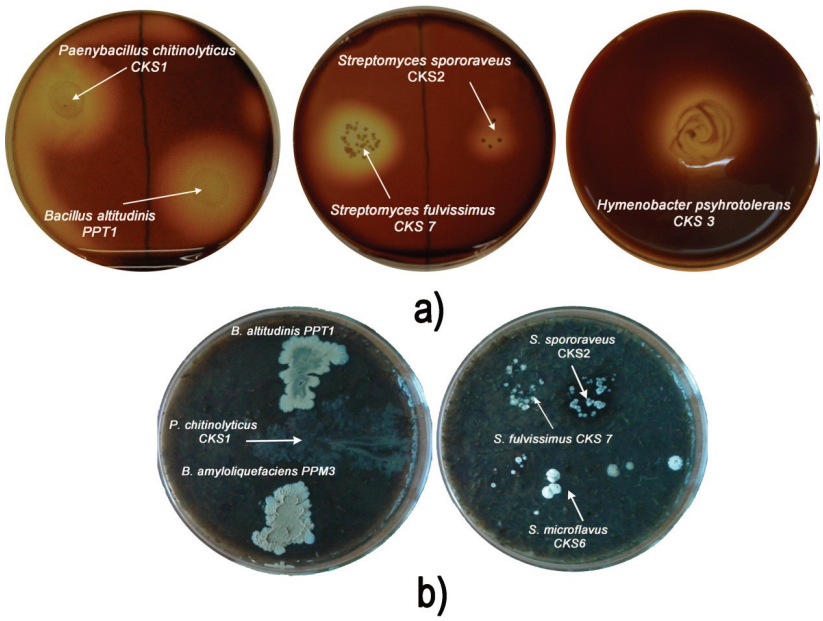

Figure 1. Selected bacterial strains. a) Cellulolytic activity of the strains on CMC agar plates; b) growth ability of the strains on agar plates containing ISP1 agar with 10% of mixed medicinal plant waste.

Monitoring the composting process

Physicochemical parameters

Temperature, moisture and pH. The results of basic physicochemical parameters are presented in Table 2. The inner temperature of the plant material was in the mesophilic range and varying from around 33 °C, over 62.3 °C and to 22 °C, at the beginning, on the 33rdday and at the end of the process, respectively. The moisture content in samples varied from 55 to 70% and at the end of the process it dropped under 50%. There were no significant differences between CC and IC samples, neither in temperature during composting nor in the moisture content (data not shown). Temperature of the compost pile depends on different factors such as: type of the material that is composted, moisture content of the material, composting procedure, season, *i.e*., ambient temperature [23]. Some studies have shown that the composting process does not necessarily reach the thermopilic phase and this could be due the effect of scale of the compost pile [24].

The initial pH value of the samples was 6.19–6.54. Over the first week of composting, pH value dropped to 5.56 for the IC but not for the CC and after that period the pH value gradually increased for both samples near to pH 8.00 (Table 2). Similar results were obtained during composting of green tea and rice bran waste [25]. The initial drop of pH is connected to the formation of organic acids in the initial stage of the composting process. On the contrary, the increase in pH in later phases of composting is a consequence of ammonia and other volatile alkaline compounds released during mineralization of proteins, amino acids and peptides.

Water soluble carbohydrates. Quantification of water soluble carbon (WSC) during the composting period showed that there is a marked increase in WSC during the first seven days of composting (Table 2) followed by a sharp decrease until the day 61. After that the concentration of WSC was relatively constant in both samples and reached 0.05 and 0.07% for IC and CC, respectively. These values of WSC are typical for the mature compost [26]. A noticeable difference in the content of WSC between the IC and the CC samples was observed only on the second sampling (day 7) that may be an indicator of the enhanced hydrolytic activity of the bacterial starter compared to the natural microflora of CC. In our previous work it was found that *P. chitinolyticus* CKS1 produce egzoglucanase [27]. In addition, the *Streptomyces* sp. CKS 6 and CKS 7 possess strong β-glucosidase activity according to the APY ZYM test (bioMerieux, data not showed). Therefore, there is a reasonable assumption that some representatives of starter consortium contribute to the enhanced hydrolysis of cellulosic materials and increase content of WSC during the first week of process. A strong negative correlation (–0.933) between WSC and pH was noticed (Table 3), which implies that the hydrolytic potential of both CC and IC microflora was more prominent at the lower pH (6.7 and 5.5, respectively).

Organic matter. In contrast to the WSC, the total organic matter (OM) gradually declined during the composting process (Table 2). The initial content of organic matter in the composting mixture was 87.62% and after 93 days, it reached values of 62 and 60% in the CC and the IC compost, with the biodegradability coefficient of 0.524 and 0.552, respectively. Although the difference in OM between the IC and CC was notable over the whole period, this difference was not statistically different. High correlation coefficient (0.800) was noticed for OM and WSC (Table 3).

Table 2. Changes in the main chemical parameters during the composting period in the compost mixture with bacterial inoculums (IC) and without bacterial inoculums (CC): pH of aqueous extract (1:10); OM - organic matter content; WSC - water soluble carbon in aqueous extract (1:10) of compost; TC - total carbon content; TKN - total Kieladal nitrogen content, C/N ratio

Parameter		Time of composting, days							
		0	7	20	33	61	93	128	164
pH	IC	6.18±0.12	5.56±0.12	7.49±0.15	7.39±0.22	8.14±0.25	7.89±0.18	7.66±0.25	7.77±0.13
	CC	6.54±0.12	6.71±0.24	7.43±0.13	7.47±0.18	8.09±0.17	7.89±0.18	7.75±0.21	7.76±0.12
OM (%)	IC	87.02±1.12	75.21±1.28	70.95±1.26	69.04±1.93	67.83±2.01	65.37±2.02	62.03±1.17	60.01±1.08
	CC	87.32±1.01	75.13±1.13	74.07±2.02	70.22±1.81	66.49±1.75	64.94±2.02	63.34±1.45	61.87±1.18
WSC(%)	IC	1.53±0.05	2.20±0.04	0.54±0.02	0.28±0.03	0.25±0.02	0.19±0.03	0.11±0.01	0.07±0.01
	CC	1.53±0.05	1.90±0.02	0.56±0.05	0.32±0.02	0.22±0.03	0.13±0.01	0.06±0.01	0.05±0.01
TC (%)	IC	50.47±0.8	43.62±0.4	41.15±0.5	40.04±0.2	39.34±0.4	37.91±0.7	35.97±0.2	34.80±0.3
	CC	50.64±0.8	43.57±0.6	42.96±0.9	40.72±1.02	38.56±0.7	37.66±0.4	36.73±0.5	35.88±0.5
TKN (%)	IC	1.36±0.16	1.83±0.15	2.18±0.12	1.95±0.13	2.04±0.14	2.32±0.11	2.37±0.13	2.42±0.11
	CC	1.36±0.12	1.46±0.14	2.07±0.11	2.29±0.12	2.24±0.11	2.39±0.13	2.09±0.12	2.23±0.11
C/N	IC	37.11±1.12	23.83±1.02	18.87±1.36	20.53±1.12	19.28±1.44	16.34±1.21	15.18±1.23	14.38±1.03
	CC	37.24±1.14	29.84±1.24	20.75±1.52	17.78±1.64	17.21±1.23	15.75±1.03	17.57±1.32	16.09±1.22

Table 3. Correlation coefficient (P<0. 05) between selected parameters

	pH	OM	WSC	TC	TKN	C/N	P	K	CO_2	DHase
OM	-0.770									
WSC	-0.933	0.800								
TC	-0.762	0.994	0.793							
TKN	0.759	-0.895	-0.832	-0.890						
C/N	-0.758	0.954	0.809	0.951	-0.970					
P	0.714	-0.928	-0.785	-0.941	0.895	-0.907				
K	0.566	-0.586	-0.560	-0.554	0.620	-0.663	0.415			
CO2	-0.325	0.255	0.357	0.275	-0.128	0.068	-0.132	0.523		
DHase	-0.269	0.075	0.363	0.077	-0.069	-0.048	-0.010	0.265	0.872	
GI Fag	0.160	-0.597	-0.198	-0.665	0.226	-0.306	0.452	-0.398	-0.716	-0.513
GI Thy	0.536	-0.858	-0.610	-0.894	0.576	-0.658	0.810	-0.132	-0.869	-0.762
GI Cyn	0.581	-0.830	-0.632	-0.890	0.570	-0.659	0.870	-0.191	-0.820	-0.688
GI Lav	0.308	-0.579	-0.291	-0.664	0.256	-0.337	0.554	-0.495	-0.757	-0.540

Total carbon and nitrogen, C/N ratio. The initial content of TC in the samples was approximately 50.64% (Table 2) and the initial content of TKN was 1.36% (Table 2). High TC content and relatively low TKN content, as well as C/N ratio of approximately 37 (Table 2) is typical for the lignocellulosic plant material [28]. High correlation coefficients between TC and OM (0.994) and between TC and WSC (0.793, Table 3) during the composting process indicated that TC comprises the majority of the total organic matter in the studied plant waste. It has been recommended that the compost mixture should be prepared so that the initial C/N ratio falls between 25 and 50. With higher C/N ratio, temperature in the compost pile may fail to rise, whereas if C/N is too low, the mixture may emit unpleasant odors [28]. During the aerobic composting process a large fraction of carbon is consumed by microorganisms and is released as carbon dioxide, while the rest is assimilated in microbial cell metabolism. Consequently, during the composting of medicinal plant waste TC content decreased rapidly, while the total N content increased and, accordingly, the C/N ratio decreased. The final C/N ratio reached values of 16.09 and 14.38 for the CC and the IC compost, respectively. The changes in the C/N ratios reflect compost stabilization [28] and this value is often used as a relative indicator of the compost maturity. Since the C/N ratio of mature compost should be 15–25 [7], it is evident that both composts produced in this study reached maturity.

The change in TKN of the composting mixture with time is strongly related to the type of substrate [26]. The medicinal plant waste is rich in cellulose, hemicellulose and lignin, but is poor in nitrogen and therefore, it is a substrate with a slower degradation rate. The relatively low initial TKN content of the compost mixture coupled with a slightly alkaline pH and a relatively low composting temperature prevent the volatilization and loss of N [28]. The observed increase of TKN can be attributed to a concentration effect, which is a consequence of the degradation of organic C compounds [26,29]. Difference between TKN in the IC and the CC at the end of the composting period was notable but not statistically significant.

Potassium and phosphorus. In both the CC and the IC compost, potassium content increased during the first 33 days of composting, after which it declined to values only slightly above the initial (Figure 2). In contrast, phosphourus content increased gradually during the composting period with a statistically significant difference between the CC and the IC (P = 0.01). This difference was the most prominent at the end of the composting process. Moldes and coworker (2007) [30] obtained similar trend in P and K changes during composting of grape marc. Phosphourus is the second limiting nutrient after N in majority of soils for crop production and many attempts had been made to produce compost with increased availability of this element [7]. During the composting process, release of organic acids to the substrate helps solubilisation of insoluble phosphorus as well as addition of N_2-fixing and phosphate solubilizing bacteria, that mineralizes organic P [31,32]. However, the relative amount of phosphorus can be limited due to its immobilization onto C-rich bulking agents resulting in slowing down phosphourus solubilisation and subsequent mineralization [33]. High negative correlation coefficient (–0.941) between P and TC that we observed (Table 3) indicates the presence of the same mechanisms in our compost. The recommended ratio of the C/P is between 120 and 240 when the C/N ratio is 30 [28]. The initial value of the C/P ratio for the medicinal plant waste was 152 and 146 for CC and IC, respectively.

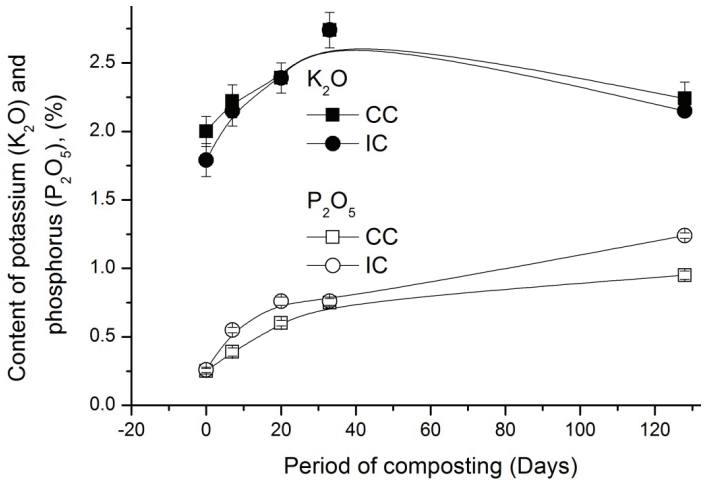

Figure 2. Changes in potassium and phosphorus contents during the composting period.

Dehydrogenase activity and CO_2 releases during composting

DH-ase activity is a measure of the total biological activity in compost and an easy method to monitor compost maturity [34]. DH-ase activity at the beginning of the composting of the IC was significantly higher than for the CC (Figure 3a). This observation indicates the effectiveness of the bacterial starter and its ability to enhance the startup of composting. A relatively high initial DH-ase activity in CC indicates high activity of the natural microbiota that is stimulated by the high WSC content (Table 2), which is also indicated by the positive correlation between these two parameters (0.363, Table 3). After the first week, DH-ase activities decrease in both, the IC and the CC until the day 33. The second pick of DH-ase increase was on the day 61. It is probably connected to the increase of microbial activity in the compost. Then, DH-ase declined up to day 93 at a level that is held constant until the end of composting without significant difference between CC and IC. The respiration rate, expressed as the release of CO_2 showed high correlation coefficient (0.872) with DH-ase activity (Table 3). Considering the low respiration rate at the end of composting, both data indicate the compost stabilization (Figure 3b). Similar results can be found in other studies [35].

Phytotoxicity of composting material

Phytotoxicity of the CC and the IC was evaluated by assessment of the seed germination index for four medicinal plant species: *Thymus vulgaris*, *Lavandula officinalis, Cynara scolimus* and *Fagopirum esculentum* (Table 4). Results have indicated that both the CC and the IC exhibit phytotoxic activity in the first stages of composting (approximately three weeks). Phytotoxicity of the IC was higher at the first and second measured points (day 7 and 20), which indicated that the IC induces a more intense degradation process. Already at the third measured point (day 33), phytotoxicity of the

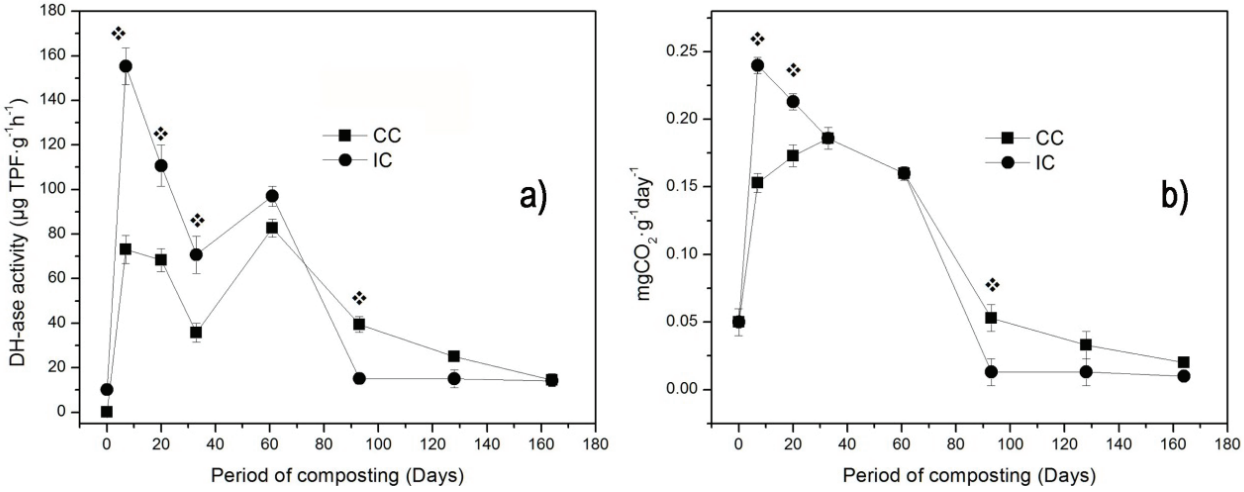

Figure 3. Changes in a) DH-ase activity and b) CO_2 release during the composting period; ❖ refers to the statistically significant difference (P=0.01).

IC was lower than that of the CC, and this trend remained until the end of the composting process.

After the day 61 the IC showed quality of acceptable and safe compost for the three tested plants (*F. esculentum, T. vulgaris* and *L. officinalis*) with *GI* above 66 (66 < *GI* < 100) [7]. Furthermore, *C. scolimus* was less sensitive to IC extract and already had GI of 80±7% at day 33. For this plant species, the IC showed the biofertilization effect already on the day 61 (*GI* 105±3%), while at the end of the composting period the *GI* reached the value of 190±3%. The mature IC acted also as a biofertilizer for the other tested plants – *GI* for *T. vulgaris* was 128±5%, for *F. esculentum* 122±6% and for *L. officinalis* 106±6%.

In contrast to the IC, the CC showed a moderate phytotoxicity after 61 days of composting, but only for *T. vulgaris* and *C. scolimus*. At the end of composting, the CC extract showed a biofertilization effect toward these two plant species (GI 119±3 and 108±3%, respectively). Sensitivity of *F. esculentum* and *L. officinalis* to the CC extract remained until the end of composting when this compost showed moderate phytotoxicity (*GI* 75±4 and 70±6%, respectively). This result shows that selection of plant species for phytotoxicity testing is very important because of differences in their sensitivities to intermediate components of the degradation process. Different sensitivities of various plant seeds on the same compost was noticed in the work of others researchers [36] (*e.g.*, Komilis and Tziouvaras (2009)).

In our work, the GI test clearly showed the difference between the CC and the IC as well as that addition of the selected bacterial starter significantly improved the quality of the obtained compost. High positive correlation coefficients between the GI and TKN and particularly P (Table 3) apparently indicate that the increase in N and P not only improved the biological value of compost but was associated with the decrease in toxicity of the medicinal plant material. It can be hypothized that activity of selected bacteria contributes to the biofertilization effect of the obtained compost [2]. This hypothesis should be confirmed in field investigations. The fate of mesophilic starters in composting of large quantities of plant waste might be questionable due to potential development of a thermophilic phase. However, investigation of López-González and coworkers (2015) [37] showed that most of mesophilic microorganisms are able to survive the thermophilic phase, and as soon as temperature declined, these bacteria started to actively grow again. Moreover, in the cooling stage, thermophilic microorganisms disappeared while the mesophilic continued degrading polymer components that were not degraded in the bio-oxidative phase.

Contribution of the bacterial starter in the decomposition process and compost quality

In order to summarize effects that the bacterial starter had on the composting process, redundancy analysis was performed. Results showed that bacterial starter addition affected the *GI* of three tested species (Figure 4). Starting from the day 33, the *GI* in the *IC* was significantly higher than in the CC, for all tested species, but *Thymus vulgaris* (Student's paired test, $p < 0.05$). More importantly, the GI of all tested species on the mature composts was in average 60% higher on the compost obtained with the bacterial starter and this difference was found to be significant ($p = 0.03$).

Moreover, the bacterial starter addition was correlated with higher DH-ase activity, higher released CO_2, higher P content, and lower pH (Figure 4). These results indicated that the speed of composting (measured by releasing CO_2 and DH-ase activity) was increased by the addition of bacterial starter, and more importantly, that bacterial starter addition had a major impact on GI of three out of four tested medicinal plants. Addition of bacterial starters transformed the compost generated from the waste medicinal plant material into a valuable biofertilizer.

CONCLUSIONS

This study presents the decomposition process of a real industrial waste, a mix of over 90 different medicinal plants. The resulting product exhibited characteristics of a stable compost, according to all physico-

Table 4. Evolution of germination index (GI) for seeds of Fagopirum esculentum, Thymus vulgaris, Cynara scolimus and Lavandula officinalis; CC-non inoculated compost; IC-inoculated compost, various superscripts indicate statistically significant differences (P=0.01)

Time of composting, days	*Fagopirum esculentum*		*Thymus vulgaris*		*Cynara scolimus*		*Lavandula officinalis*	
	IC (%)	CC (%)	IC (%)	CC (%)	IC (%)	CC (%)	IC (%)	CC (%)
7	60±4^{a}	73±6^{a}	25±1^{a}	50±2^{a}	11±8^{A}	45±5^{a}	30±4^{a}	58±5^{a}
20	20±1^{b}	32±1^{b}	21±2^{b}	48±3^{b}	35±1^{b}	38±1^{b}	16±1^{b}	24±1^{b}
33	67±3^{c}	42±3^{c}	64±4^{c}	66±6^{c}	80±7^{C}	41±2^{c}	37±4^{c}	26±3^{c}
61	68±2^{d}	59±2^{d}	88±2^{d}	72±6^{d}	105±3^{D}	78±3^{d}	75±6^{D}	29±3^{d}
93	84±4^{e}	62±5^{e}	96±4^{e}	84±8^{e}	121±4^{F}	83±4^{f}	83±7^{E}	36±2^{e}
128	102± 3^{F}	68± 5^{f}	128±5^{E}	97±4^{f}	163±6^{G}	101±6^{g}	89±3^{F}	52±3^{f}
164	122±6^{G}	75±4^{g}	175±7^{G}	119±3^{g}	190±4^{H}	108±3^{h}	106±6^{G}	70±6^{g}

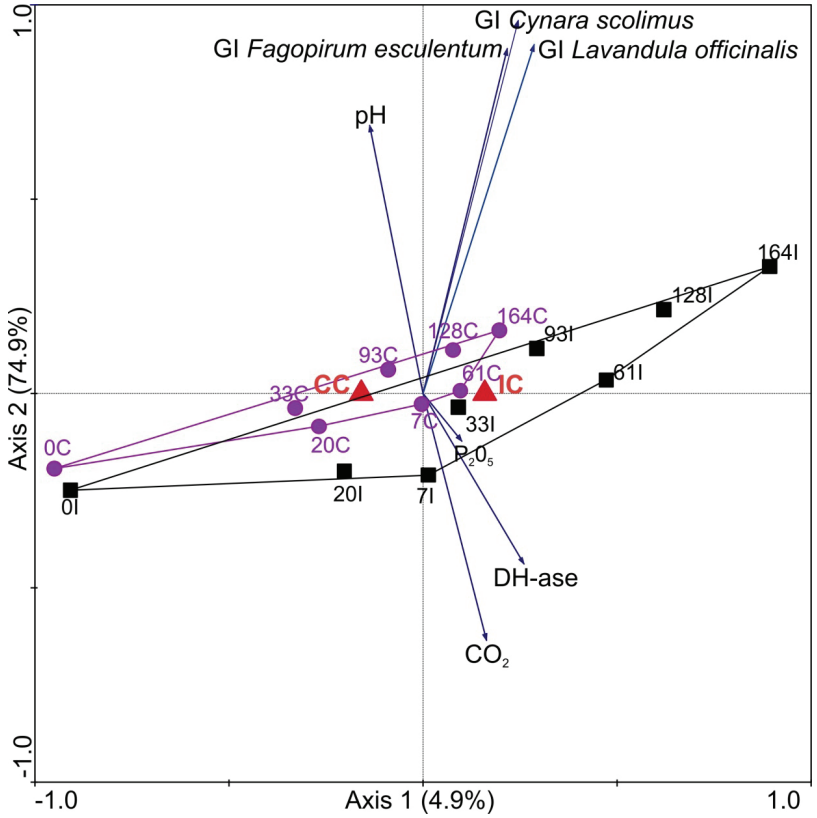

Figure 4. Redundancy analysis of the composting process with and without bacterial inoculums.

chemical and biological parameters. The selected mesophilic bacterial starters showed a potential to reduce the period of mixed herb waste decomposition, from about 6 months to about 2.5 months. In addition, a biofertilization effect to tested medicinal plants was in average 60% higher than for the non-inoculated compost. Such compost could be used in fields and it is expected that it would increase the yields of medicinal plants providing an effective way of plant waste valorization for the industry that generates this type of waste.

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IZVOD

UNAPREĐENJE EFEKTA FERTILIZACIJE KOMPOSTOM DOBIJENOG MEŠANJEM OTPADA LEKOVITOG BILJA KOJI JE INOKULISAN SA NOVIM SOJEVIMA MEZOFILNIH BAKTERIJA

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(Naučni rad)

Mešani otpad lekovitog bilja je kompostiran uz dodatak novih bakterijskih sojeva koji pripadaju rodovima *Streptomyces, Paenybacillus, Bacillus* i *Hymenobacter*. Kompostiranje je praćeno promenom hemijskih i bioloških parametara uključujući C/N odnos, gubitak organske materije, sadržaj fosfora i kalijuma, kao i oslobađanje CO_2 i dehidrogenaznu aktivnost tokom 164 dana. Odabrani sojevi mezofilnih bakterija su pokazali potencijal da značajno smanje period razlaganja mešanog biljnog otpada, sa 6 meseci na 2,5 meseca. Na osnovu indeksa klijanja semena (*GI*) četiri biljne vrste (*Fagopirum esculentum*, *Thymus vulgaris*, *Cynara scolimus* i *Lavandula officinalis*) klijavost i rast korena ispitanih biljaka je poboljšan inokulisanim kompostom. *GI* svih testiranih vrsta zrelim inokulisanim kompostom bio je u proseku 60% veći u poređenju sa kontrolnim kompostom. Istraživanje pokazuje da dodatak mezofilnih sojeva u biljnom otpadu može doprineti brzini razlaganja otpada i poboljšanju biofertilizacionog efekta dobijenog komposta.

Ključne reči: Kompostiranje • Biljni otpad • Mezofilne bakterije • Indeks klijanja • Stimulacija rasta biljaka • Fitotoksičnost