POLISH JOURNAL OF SOIL SCIENCE
VOL. LII/1 2019 PL ISSN 0079-2985

DOI: 10.17951/pjss/2019.52.1.143

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EFFECT OF FUNGAL INOCULUM APPLICATION ON CHANGES IN ORGANIC MATTER OF LEAF LITTER COMPOSTING

Received: 25.06.2018
Accepted: 19.03.2019

Abstract. The decomposition of organic matter on leaf litter substrat runs very slowly in nature resulting in the accumulation of litter in the ecosystem and has even become an organic waste that creates many problems. The research dealt with the use of lignocellulolytic fungi inoculum consisting of 3 isolates: *Aspergillus fumigatus* (cellulolytic), *A. tubingensis* (xylanolytic) and *Geotrichium* sp (ligninolytic) as a starter of leaf litter composting. The purpose of the study is to understand the pattern of humic-fulvat acid and C/N ratio on the process of composting of leaf litter with the addition of inoculum. Observations were made to the chemical changes of compost for 3, 6 and 9 weeks of composting and the data were analyzed in RM-ANOVA (Repeated Measures of ANOVA). The result shows that the best pattern of humic acid and fulvic change from the initial to final composting occurs at the *Geotrichum* spinoculum of 0.60 or 105.2% and for fulvic are of 0.55 or 56.1% of baseline. The highest ratio value of CHA/CFA at the end of observation was by consortium of *A. fumigatus* and *A. tubingensis* inoculums that was 2.94 and the lowest value was at commercial inoculum that was 0.80; and the sharpest change value also occurred in the consortium *A. fumigatus* and *A. tubingensis* inoculums of 2.20 or 297.3%. Therefore, the

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consortium isolates were capable of causing the maturity of the compost most rapidly compared to other isolates.

Keywords: leaf litter, lignocellulolytic, inoculum, consortium, composting

INTRODUCTION

The availability of mixed leaf litter as an organic substrate in urban area tends to overflow and has even become organic waste and the mixed litter is part of municipal solid waste. Municipal solid waste has caused problems around the world due to poor management (Vaverkova *et al*. 2013). Mixed leaf litter actually rich of organic materials comprises of various vegetation leaves collected from a real part of city. The materials are usually removed and hoarded away to a garbage landfills or burned. On the other hand, the condition of agriculture land has been getting worse due to nutrient loss through organic matter depletion caused by climate change (Pareek 2017). Besides, continuous cropping without application of organic or inorganic fertilizers has resulted in nutrient depletion from soils. The decline in soil fertility is attributed mainly to insufficient nutrient input compared to export through a number of pathways (FAO 2005). Those two conditions then raise problems and solutions or trends and challenges as well when they could be managed properly.

Litter mixtures derived from various vegetation plants are often overlooked and wasted. The main component of litter is the lignocellulose compound (> 60%) which is very difficult to degrade. This compound is an organic material complex which, if quickly decomposed, will provide substantial economic and ecological benefits to cultivated land.

One of the biological treatments to manage organic waste that has a bright prospect and is developing today is the method of composting. Biological processes allow for the degradation of organic compounds into compost rich in humic compounds which are indispensable for agricultural enhancement or for land reclamation (Domeizel *et al*. 2004). The major substrate for leaf litter composting is lignocelluloses difficult to decompose naturally and it has led to find a proper inoculum to initiate the decomposition. It has been recognized that the decomposition process is complex and involves many microorganisms, but attention is focused on fungal decomposer.

In this study we develop fungal inoculum to initiate decomposition in the composting process. There were applied 3 fungal inoculums, namely *A. fumigatus* (cellulolytic), *A. tubingensis* (xylanolytic) and *Geotrichum* sp (ligninolytic). The inoculum treatment was administered in single and concortium form containing two isolates. The study was designed in the form of a repeated measure of ANOVA (RM-ANOVA) with observations made in the third, sixth and ninth weeks. Observations were made to understand the pattern of humic-fulvic change and C/N ratio to asses compost maturity.

MATERIALS AND METHODS

Inoculum development

The inoculums were taken from the previous work which was derived from fungi *A. fumigatus*, *A. tubingensis* and *Geotrichum* sp which are cellulolytic, xylanolytic and ligninolytic respectively (Irawan 2014). The materials used are sorghum and paddy rice bran with a ratio of 2:1 (v/v), 4% $CaSO_4$ solution, 2% $CaCO_3$ solution (in 1 l of distilled water). Every 200 g of sorghum was added with a mixture of 50 ml $CaSO_4$ and $CaCO_3$ solution, sterilized, inoculated with each isolates and allowed to grow for 14 days. With the ready-to-use inoculum, the number of spores and the value of its CFU, with the plate count method as an illustration of its viability level, were calculated. The media plate count used was Martin's Rose Bengal Agar (glucose 10 g, pepton 5.0 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, streptomycin 30 mg, Rose Bengal 30 mg, agar 15 mg and water 1 l) (Malloch 1981). The inoculum produced consisted of 3 inoculums: inoculum A (cellulolytic), B (xylanolytic) and C (ligninolytic). The characteristics of inoculum are shown in Table 1 (Irawan *et al*. 2014).

Table 1. Character of 14 days old inocula

No.	Isolate	Fungi	Spores/ml	Viability (CFU/ml)
1	Fumigatus	cellulolytic (A)	1.5×10^9	9.6×10^9
2	Tubingensis	xylanolytic (B)	9.3×10^7	5.6×10^8
3	*Geotrichum* sp	ligninolitic (C)	4.2×10^9	8.2×10^6

Composting

Fungal consortium inoculum was made from a mixture of various combinations of single culture inoculum. Compost substrate of 3 kg (2 kg of litter and 1 kg of animal waste (Ustuner *et al*. 2009)) requires a composite inoculum of 1% (w/w) of compost weight (Gaind *et al*. 2009). The composition of the fungi consortium was prepared by combining each isolate with all the isolates present in pairs. The composition of the consortium inoculum is derived from 3 isolates A, B and C (cellulolytic, xylanolytic and ligninolytic) prepared with 2 combinations of isolates paired with each other, namely: AB, AC, and BC. Besides there were two comparison treatments consisting of treatment without inoculum (K) and commercial inoculum (X). The composting result was chemically tested for 3, 6 and 9 weeks of composting and included observations on humic acid-fulvic acid, CHA/CFA, and C/N ratio.

Humic and fulvic acid extraction

The compost material (10 g) was dissolved in 1M HCl at pH 1. This acidification process was used to separate the soluble fulvic fraction in the acid medium with the insoluble humic compound in the acid medium. The suspension was then stirred for 1 hour and the formed supernatant S1 was separated from the residue (R1) by centrifuge at a low velocity. The residue (R1) was dissolved in 1 M NaOH at pH 7, then raised pH to 13 with 0.1 M NaOH. The suspension was stirred for 4 hours in nitrogen. This alkaline medium allows the separation of the humic acids with unextracted fraction of fulvic acids. The supernatant resulting from low-speed centrifugation lowered the pH for 14 hours to pH 1 with addition of 6 M HCl and residue R2 was discarded. This process allows the humic acid to precipitate while fulvic acid remains in solution; then obtained a supernatant (S2) of centrifugation at low speed. Humic acid (HA) dissolved in residue (R3) was dissolved again with 0.1 M NaOH in nitrogen. S1 extract was filtered with 0.45 μm filter paper and fed into a column containing 1.5 ml of XAD-8 resin. The solution found at the bottom of the column was a non-humid fraction (NHF). Then the solution was eluted with 0.1 M NaOH in the column to obtain a fulvic acid solution (Domeizel *et al*. 2004).

Statistical analyses

The experiments were designed on the Completely Randomized Design with 3 replications. The data obtained were analyzed by RM-ANOVA (Repeated Measures Analysis of Variance) because it is continuous observation data of an object that interacts with treatment and time (Littell *et al*. 1998, Keselman *et al*. 2001).

RESULTS AND DISCUSSION

Humic acid

The pattern of humic acid change illustrates the open, quadratic, parabolic relationship of all treatments (Fig. 1). Humic acid levels were initially high in the third week, then decreased and increased again sharply at the end of observation in the ninth week. All the inoculum treatments caused the lowest humic levels at 5.2 weeks; the treatment of commercial inoculum X at 6.4 weeks and treatment without inoculum at 5.6 weeks; then the humic level increases until the end of the observation. In all inoculum, except X inoculum, it was shown that in the ninth week, the humic acid level was higher than in the third week. This is in accordance with some previous studies which mention the increase of humic content during composting. The best pattern of humic acid change from the initial to final composting occurs at the C treatment (*Geotrichum* sp) which is 0.60 or 105.2%.

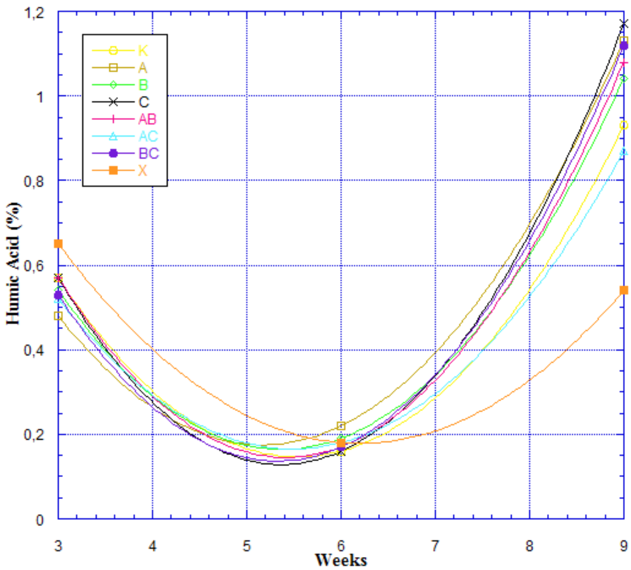

Fig. 1. Quadratic pattern of humic acid level by all inoculums

Note: K – control, A – cellulolytic inoculum, B – xylanolytic inoculum, C – ligninolytic inoculum, X – commercial inoculum

In general, humic acid levels decline at week 6, this is presumably because the process of biodegradation of organic matter is ongoing and the results begin to appear as the accumulation of humic acid at week 9. This is in accordance with Namkoong *et al*.'s (1999) study which showed a drastic decline in the early humification index composting (20 days) of foodstuffs (vegetables, cereals, meat, fish and fruits) that occur due to the humidity process of organic matter. Increased level of humic acid at week 9 is possibly due to a well-executed organic matter decomposition process, since humic is the initial phase of continued change in lignin biodegradation (Killham 1994). The occurrence of the increased level of humic acid in this study indicates that the composting process has proceeded correctly. Several other studies have also shown the same result. Ouatmane *et al*. (2000), who examined the maturity of compost from four materials: *Cedrus* sp sawdust, coffee powder, manure and household waste, found that humic acid levels of the 4 ingredients increased in the first 197 days of the 365 days of the composting process. Domeizel *et al*.'s (2004) studies on composting of green compost materials also showed increased humic acid and decreased fulvic acid during 120 days of composting.

Humic acid is derived from phenolic compounds, which are lignin degradation products, and a number of decomposition residues modified by microbes through oxidative condensation reactions to form humic acid, fulvic acid and humins, which, in turn, form a humus complex in the soil (Killham 1994).

Fulvic acid

The result shows that there are two groups of time-varying patterns of change in fulvic acid (Fig. 2). The first group describes the negative linear relationship between the fulvic acid level and the time by inoculum of A, B, C and AB consortium. The second group has an open, quadratic, parabolic relationship pattern between the fulvic acid levels and the time occurring in K (control without inoculum), X (commercial inoculum), BC and AC consortium. Inoculum groups with negative linear changes (A, B, C and AB), initially cause compost to have high levels of fulvic acid, but continue to decrease over time. The level of fulvic acid in the highest compost was originally achieved by inoculum C but had the highest rate of decreasing at the level of 0.0917% per week, so that at the end of the observation, the fulvic acid content was not relatively different from the levels when using the inoculum A, B and AB. The sharper fulvic concentration change also occurs in the treatment with C isolate inoculum of 0.55 or 56.1% of baseline.

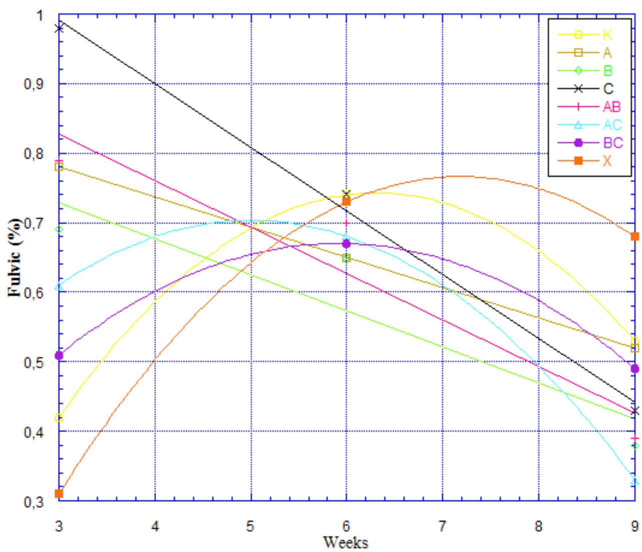

Fig. 2. Negative linear pattern of fulvic level by inoculum A, B, C and AB; and the quadratic pattern of fulvic levels by inoculum K, AC, BC and X

Note: See Fig. 1.

The pattern is probably due to that C isolate (*Geotrichum* sp) is ligninolytic fungi. Therefore, it is thought that this isolate is capable of degrading lignin compounds having large molecular size to be broken into fulvates, CO_2 and H_2O (Schnitzer and Khan 1978). Another opinion states that the fulvic is derived from the degradation of phenol which is a continuation of lignin degradation by microbes in the form of humic acid, fulvic acid and humin. Fulvic acid is a low molecular weight humic acid with oxygen content higher than in case of other humic acid (Killham 1994).

The general tendency of fulvic levels decreasing indicates a well-executed decomposition process because the existing fulvic decreases after being converted to humic acid. Benny *et al*. (1996) and Outmane *et al*. (2000), explain that the decreasing in fractional fulvic levels during the composting process is due to the rapid transformation of fulvic acid to humic acid and the biodegradation of non-humic fractions which are able to decompose.

CHA/CFA ratio

The result shows that the changing pattern of the CHA/CFA ratio is an open quadratic parabolic relationship of all treatments (Fig. 3). Levels of the CHA/CFA ratio were initially high in the third week, then decreased and increased sharply at the end of the observation in the ninth week. In all treatments, except treatment X, it was shown that in the ninth week, humic acid level was higher than in the third week. A slightly different pattern occurred in the X inoculum which showed a smaller CHA/CFA ratio (ninth week) when compared with the third week (initial), while other treatments occur otherwise.

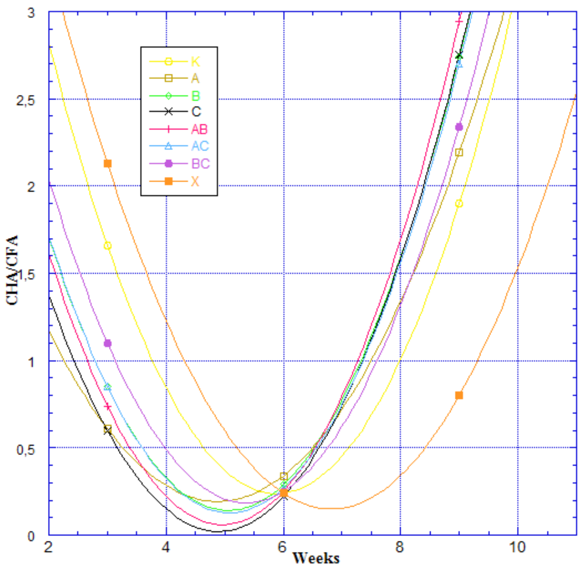

Fig. 3. Qudratic pattern of humic/fulvic (CHA/CFA) ratio levels by all inoculums

Note: See Fig. 1.

The standard CHA/CFA ratio of the mature compost is > 1.6 (Inbar *et al.* 1990). The highest ratio of CHA/CFA levels was generally achieved at the end of observation (except inoculum X). In treatment without inoculum it was 1.9; with inoculum AB it was 2.94 and with commercial inoculum X it was 0.8. The highest ratio value of CHA/CFA at the end of observation was in case of AB treatment and it was 2.94 and the lowest value was in case of treatment X that is 0.80. The sharpest change value also occurred in the AB treatment with 2.20 or 297.3%; thus, the AB isolate is capable of causing the maturity of the compost most rapidly compared to other isolates. Application of all fungal inoculums is capable of causing the maturity of compost (ratio CHA/CFA > 1.6) faster than that of control and treatment X.

C/N ratio

The result shows two patterns groups of C/N ratio changes based on time (Fig. 4). The first group shows an open quadratic parabolic relationship that occurs in the C, AC, BC and non-inoculum (K) treatments. The second group shows a positive linear relationship pattern that occurs in the treatment of commercial inoculum X and linear negatives that occur in the treatment of inoculums A, B and AB.

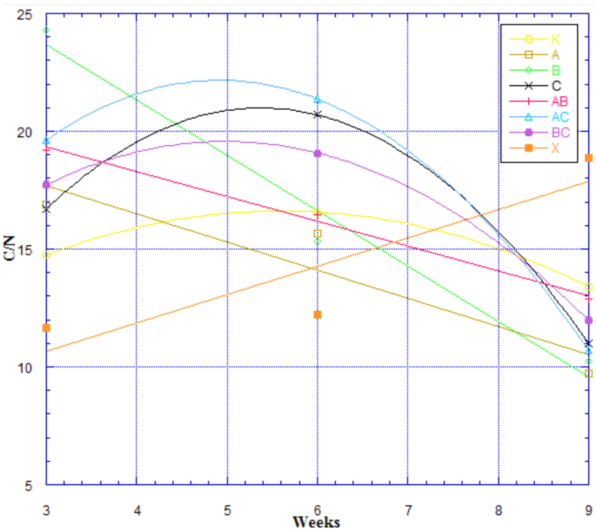

Fig. 4. Quadratic pattern of C/N ratio changes by K, C, AC and BC inoculums; and the positive/negative linear pattern of the C/N ratio by the inoculums A, B, AB and X

Note: See Fig. 1.

The first group with a parabolic quadratic-shaped open-ended relationship shows a low initial C/N ratio, then increases and decreases again at the end of ninth week of observations with a lower yield than baseline. Thus, there is an increase pattern in the mid-composting, but at the end the ratio shows a smaller value than the initial ratio.

In general, the pattern of change in C/N ratio from weeks 3–9 is quite diverse, with the C/N ratio decreasing with time of composting except treatment X. From the linear and paraboli patterns (Fig. 4), the change of C/N ratio indicated that the sharpest change was in the treatment with isolate B which was 14.07 or 57.9% from baseline. The decline in C/N ratio also occurs in all composting studies because C/N ratios are the most common indicator of compost maturity (Kumar *et al*. 2008, Beary *et al*. 2002, Gaind *et al*. 2009, Zhang *et al*. 2011, Illmer *et al*. 2007).

The decline in the C/N ratio is a pattern that also occurs in the Hassen *et al*.'s (1998) study on conducting municipal solid wastes which indicated that the stability of C/N ratio occurred at week 12. The C/N ratio in all treatment of fungal inoculum (A, B, C, AB, AC and BC) shows good results of under 12 weeks; this corresponds to the tolerance limit set as mature compost by Bernal *et al*. (1998). Too low C/N ratio also has an adverse impact on composting as it causes large emissions of ammonia gas (Itavaara *et al*. 2010).

ACKNOWLEDGEMENT

This work was supported by the Directorate General of Higher Education of Indonesia through the BPPS/BPPDN scholarship.

REFERENCES

[1] Beary, T.P., Boopathy, R., Templet, P., 2002. *Accelerated decomposition of sugarcane crop residue using a fungal–bacterial consortium*. International Biodeterioration & Biodegradation, 50: 41–46. DOI: 10.1016/S0964-8305(02)00056-2.

[2] Benny, C., Hatcher, P.G., Hadar, Y., Chen, Y., 1996. *Chemical and biological characterization of organic matter during composting of municipal solid waste*. Journal of Environmental Quality, 25: 776–785. DOI: 10.2134/jeq1996.00472425002500040018x.

[3] Bernal, M.P., Paredes, C., Sanchez-Monedero, M.A., Cegarra, J., 1998. *Maturity and stability parameters of composts prepared with a wide range of organic wastes*. Bioresource Technology, 63: 91–99. DOI: 10.1016/S0960-8524(97)00084-9.

[4] Domeizel, M., Khalil, A., Prudent, P., 2004. *UV spectroscopy: A tool for monitoring humification and for proposing an index of the maturity of compost*. Bioresource Technology, 94: 177–184. DOI: 10.1016/j.biortech.2003.11.026.

[5] FAO, 2005. *Current world fertilizer trends and outlook to 2009/10*. FAO, Rome, 43 pp.

[6] Gaind, S., Nain, L., Patel, V.B., 2009. *Quality evaluation of co-composted wheat straw, poultry droppings and oil seed cakes*. Biodegradation, 20: 307–317. DOI: 10.1007/s10532-008-9223-1.

[7] Hassen, A., Jedidi, N., Cherif, M., M'hiri, A., Boudabous, A., Van Cleemput, O., 1998. *Mineralization of nitrogen in a clayey loamy soil amended with organic waste residues*

enriched with Zn, Cu and Cd. Bioresource Technology, 64: 39–45. DOI: 10.1016/S0960-8524(97)00153-3.

[8] Illmer, P., Wagner, O.A., Mair, J., Malin, C., Farbmacher, S., 2007. *Chemical and biochemical parameters during composting of lawn clippings with special regard to the efficiency of a compost starter kit*. Compost Science & Utilization, 15: 40–46. DOI: 10.1080/1065657X.2007.10702309.

[9] Inbar, Y., Chen,Y., Hadar, Y., 1990. *Humic substances formed during the composting of organic matter*. Soil Science Society of America Journal, 5: 1316–1323. DOI: 10.2136/sssaj1990.03615995005400050019x.

[10] Irawan, B., 2014. *Organic decomposition by saprotroph fungi and fungal concortium for inoculum preparations for leaf litter composting*. Ph.D. dissertation, Gadjah Mada University, Indonesia (in Indonesian).

[11] Irawan, B., Kasiamdari, R.S., Sunarminto, B.H., Sutariningsih, E., 2014. *Preparation of fungal inoculum for leaf litter composting from selected fungi*. ARPN Journal of Agricultural and Biological Science, 9(3): 89–95.

[12] Itavaara, M., Vikman, M., Liisa, M., Vuorinen, A., 2010. *Maturity tests for composts – verification of a test scheme for assessing maturity*. Compost Science & Utilization, 18(3): 174–183. DOI: 10.1080/1065657X.2010.10736953.

[13] Keselman, H.J., Algina, J., Kowalchuk, R.A., 2001. *The analysis of repeated measures designs: A review*. British Journal of Mathematical and Statistical Psychology, 54: 1–20. DOI: 10.1348/000711001159357.

[14] Killham, K., 1994. *Soil Ecology*. Cambridge, UK: Cambridge University Press.

[15] Kumar, A., Gaind, S., Nain, L., 2008.*Evaluation of thermophilic fungal consortium for paddy straw composting*. Biodegradation, 19: 395–402. DOI: 10.1007/s10532-007-9145-3.

[16] Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. *Statistical analysis of repeated measures data using SAS procedures*. Journal of Animal Science, 76: 1216–1231. PMID: 9581947.

[17] Malloch, D., 1981. *Moulds: Their Isolation, Cultivation, and Identification*. Toronto: University of Toronto Press.

[18] Namkoong, W., Hwang, E.Y., Cheong, J.G., Choi, J.Y., 1999. *A comparative evaluation of maturity parameters for food waste composting*. Compost Science & Utilization, 7(2): 55–62. DOI: 10.1080/1065657X.1999.10701964.

[19] Outmane, A., Provenzano, M.R., Hafidi, M., Senesi, N., 2000. *Compost maturity assessment using calorimetry, spectroscopy and chemical analysis*. Compost Science & Utilization, 8(2): 124–134. DOI: 10.1080/1065657X.2000.10701758.

[20] Pareek, N., 2017. *Climate change impact on soils: Adaptation and mitigation*. MOJ Ecology and Environmental Sciences, 2(3): 00026.

[21] Schnitzer, M., Khan, S.U. (eds.), 1978. *Soil Organic Matter* (1st ed.). New York: Elsevier Science.

[22] Ustuner, O., Wininger, S., Gadkar, V., Badani, H., Raviv, M., Dudai, N., Medina, S., Kapulnik, Y., 2009. *Evaluation of different compost amendments with AM fungal inoculum for optimal growth of chives*. Compost Science & Utilization, 17(4): 257–265. DOI: 10.1080/1065657X.2009.10702432.

[23] Vaverkova, M., Toman, F., Adamcova, D., Kotovicova, J., 2013. *Verifying research of waste landfill environmental impact using bioindicators*. Polish Journal of Environmental Studies, 22(2): 313–317.

[24] Zhang, J., Zeng, G., Chen, Y., Yu, M.,Yu, Z., Li, H., Yu, Y., Huang, H., 2011. *Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting*. Bioresource Technology,102: 2950–2956. DOI: 10.1016/j.biortech.2010.11.089.