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Industrial Engineering

THE ANALYSIS OF PATHOGENIC MICROORGANISM CONTAMINATIONON LITTERFALLCOMPOST USING THREE ACTIVATORS AT UNIVERSITAS AL AZHAR INDONESIA

Tastaptyani Kurnia Nufutomo[1], Irawan Sugoro[1, 2], Nita Noriko[1], Dewi Elfidasari[1]

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Abstract-**This study aims to determine the effect of three activator typesdue to the number of microorganisms on the green waste and to determine the bacterial contamination, such as *Salmonella Shigella*, *Streptococcus, Staphylococcus, Coliform* and Fungi on green waste compost at Universitas Al Azhar Indonesia (UAI). The amount analysis of microorganisms is carried by the drop test on various medium, such as PCA, SSA, BP, ES, McConkey and PDA. The identification of *Coliform* is performed with biochemical activity assay and MPN test in LB broth and BGLB medium. The result of this study shows that there is no increasing amount of microorganisms with the role of three activator types. A fine commercial fertilizer still have a colony of *Salmonella Shigella, Streptococcus, Staphylococcus, Coliform* and fungi with a few number of these bacteria.**

Keywords *-Microorganism contamination, Compost, UAI green waste.*

I. INTRODUCTION

Universitas AlAzharIndonesia(UAI) produces organicwastein the form of litterfall. Litterfall is managedtobecompostedwith microorganisms. Microorganismhas an important rolein helpingthe maturation ofcompost. Therefore, the researcher usesthreeactivators, such asmanure,commercialcompostand artificial fertilizers from UAI studentstoassist thedecomposition oflitterfallcompost.

Inthe composting processsomequality standardsmust comply with StandarNasional Indonesia inmanufacturing organic fertilizer(SNI: 19-7030-2004) [1]. In order tomaintainand producegoodcompost, something that is need to be considered is the number of *Coliform*and*Salmonella-Shigella* microorganisms, witheach value of <1000MPN/ gand<3MPN/g.

In addition, there are bacteriaand other microorganismsthat must be consideredduring the process ofcomposting, such as*Staphylococcus*, *Streptococcus*, and fungi. These pathogenic bacteriaandfungiare notincluded inISObecause theywilldieduringthe composting process.

To determine the contamination ofmicroorganisms, the researcher conductsand analysisin order tomaintain thequality of compostlitter.

II. RESEARCH METHODS

Litterfall Compost

An artificial fertilizer that is used in this study is compost which is produced at Universitas Al Azhar Indonesia. The researcher uses three activators, such as manure (cow dung), commercial fertilizers, artificial fertilizers from UAI students, and has to put them into a 40L bucket which has holes on the edges to ease aeration process. There also homogenization process of these materials once a

week. Samples are taken from the week 7, 14, 21, 28, and 35for an analysis of pathogenic bacteria and fungi at the laboratory.

Enumeration of Bacteria and Fungi

1g sampleis inserted intoyellow tube. Mix it with 0.85% of NaCl and do homogenization in vortex. After that, the mixtureof litterfallcompost and0.85%NaCl are diluted to10^{-10}. Insert these materials into an *ependorf*with thehelp ofmicropipette. The production of fungi and bacteria are processed with an autoclave ata temperature of121^0Cabout 1½hoursina petri dish. A standard medium PCAandselectivemediums, such asSSA(*Salmonella Shigella*), BP(*Staphylococcus*), ES(*Streptococcus*) andPDA(fungi) are used in this research. Aftera coldmedium, the samples are implantedwith10µl of micro pipetteinto those mediums by drop test, and incubate them for 24hours at a temperature of 37^0C. A colony is calculatedto determine the numberof its fungi and bacteria.

***Coliform*Isolation**

Coliformbacteria which aregrown inmediumMc. Conkey are isolatedinto *EosineMethyleneBlue*(EMB), and are incubatedfor 1-2daysat a temperature of37^0C.

The Identification of Coliform Bacteria in EMB Medium

The identificaation of *Coliform* may be determined by its color which is shown in Eosine Methylene Blue (EMB).

Biochemical Activity Test

The test isperformedwith RapidIdentificationKitREMEL. IsolatesofEMBmedia are inserted andhomogenized into *ependorf*. Add 2ml ofreagentREMEL and observe the color changes and matchwithbiochemical tests. Record the test resultsand save it to a computer program, ERICREMELrapidone.

MPN Test

There are two MPNtests. First, the testmedia useLBBrothandBGLBmedia. The media of LBBrothandBGLB are transferredinto three tubes due to thelevel ofdilutionfrom10^{-1}, 10^{-2}, 10^{-3}withdurhamtube. The resultsof this methodcan be seenwith the presence ofbubbleindurhamfor eachtest due to the level ofdilution. Recordthe test resultand match it with (APM) *Coliform*[2].

Data Analysis

The obtained data are the total number of bacteria and the amount of *Salmonella Shigella, Staphylococcus, Streptococcus, Coliform,* and fungi from moist compost. The number of fungi and bacteria are analyzed with one-way ANOVA ($P \leq 0.05$) using SPSS 19.

III. RESULT AND DISCUSSION

Enumeration of Bacteria

The result of enumerationforeach treatment of A (manure), B (commercialcompost), C(artificial fertilizers) andK(water) on PCA mediumPCAshow afluctuatingpattern of growthduringincubationbecause theheterogeneous and complex constituentmaterials of compost. ANOVAstatistical analysisshowsthatthe addition ofactivatordoes not affectthe amount ofbacteria duringcomposting($P \leq 0.05$)because theresult is notsignificantly difference.

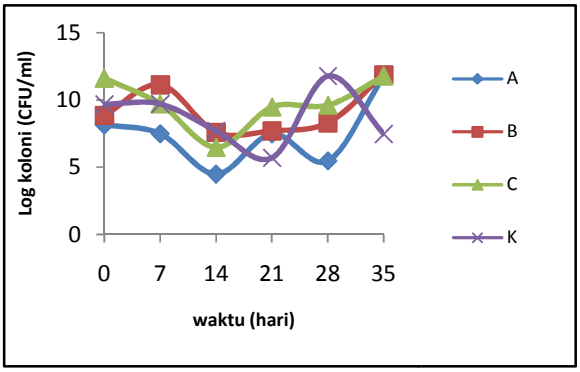

Figure 1. Bacterial growth based on the treatment of A (manure), B (commercial compost), C (UAI students-made compost) and K (litterfalland water) using PCA medium

The initial number of bacteria in figure 4.1 is occurred in the treatment of A, B and C with 8.15 CFU / ml, 8.85 CFU / ml, 11.6 CFU / ml and K 9.96 CFU / ml. The number of bacterial colonies has increased as the addition of each activator. Treatment A and C have decreased on the 7th day

because its bacteria degrade the complex carbohydrates, such as lignin, cellulose and hemicellulose. It is due to the role of bacteria in the process of compound decomposition into a simpler form. However, fungi are capable to decompose difficult materials [3].

The increasing cell is occurred after the 14th day to the end of incubation on the 35th day as a result of the nutrient degradation in which complex carbohydrates become simple. It is caused by provided nutrients, such as simple carbohydrates which are found in commercial compost. As a source of nutrients is reduced, there is a decline in the number of cells and the cell will prepare to degrade complex carbohydrates, such as lignin, cellulose and hemicellulose in litterfall.

The change of bacterial growth curve is influenced by several environmental factors because it requires adaptation. Environmental factors that affect the rate of decomposition is the balance of C / N (Carbon and Nitrogen), composting temperature, acidity (pH) and aeration, humidity (RH), the structure of raw materials, the size of raw material, and homogenization.

Enumeration of Fungi

The result of fungoid enumeration for each treatment shows a fluctuating pattern of growth during the incubation period. The statistical result shows that the addition of activators does not affect the number of fungi during the composting process ($P \leq 0.05$) as a result is notsignificantly difference.

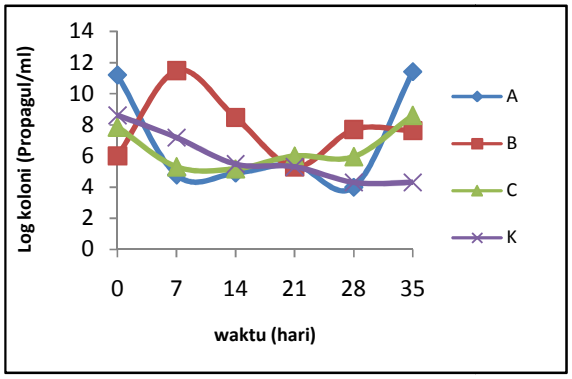

Figure 2. The growth of fungi from the treatment of A, B, C, and K using PDAmedium

The growth offungishowsthe number ofinitialfungalcolonieson treatment of A,B, andCare11.2propagules/ml, 6 propagules/ml, 7.8propagules/ml. TreatmentA is greater thanthe controlwhich is only8.6CFU/ml. The number offungi colony at the beginning ofincubation(7th – 14th day) has increasedintreatment Bin contrast to thenumber offungiincontrol. It is caused bytreatment B, in which its commercialfertilizers are already adapted toenvironmental conditions while the other treatments are not.

The number of colony has decreased in TreatmentB on day 21 because of its complexnutrients. All treatments in the process ofincubationon the 35th day have increased compared tothe amount ofcontrol with4.3propagules/ml. The amountofA,BandCare 11.4propagules/ml, 7.6propagules/ml, 8.6propagules/ml. These are due tothe interaction offungiandbacteria in decomposing complexnutrients. Thefluctuation of fungiduring the compostingis caused by the effect ofbacteria. It can be seenin Figure1.

Enumeration of Pathogenic Bacteria

Enumerationof *Staphylococcussp*

The result of *Staphylococcussp.* enumerationshowsafluctuatinggrowth due to the environmental factors, such as temperature,pHandaeration. The statistical resultshowsthe addition ofsomeactivatorsdoes not affect thenumber of bacteriaduringthe composting process($P \leq 0.05$)because it is not significantly difference.

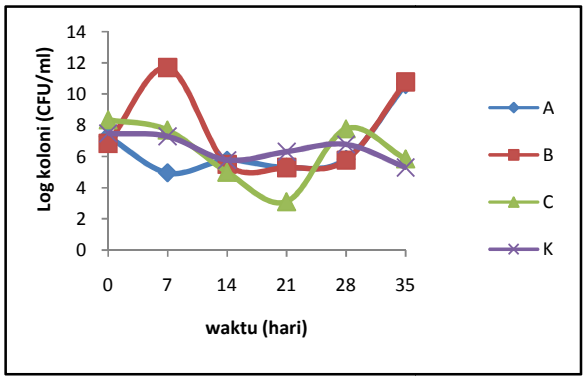

Figure 3 The Growth of *Staphylococcussp* in A, B, C, and K using B P medium

The graphic above, it shows the initial numberof Staphylococcus sp. colonies on eachtreatment of A,BandC. Each treatment hasa highamount of

colonythan the control. The amount of eachtreatment is 7.3CFU/ml, 6.8CFU/ml, 8, 3CFU/ml. The value of coloniesin controlis 7.4CFU/ml. The results ofincubation Ain the 7th day has decreased as the number of those coloniesadapt the medium environmentand thelack of nutrientsin A.The composition ofAcontainslitterfallwithcow feces while *Staphylococcussp.* in B, C, and control has increased. This is due tothe availability ofnutrients in themediumof each treatment.

*Staphylococcussp.*on the incubation14 – 21 is decreased intreatment B, Cand controlbecause the nutrientsare notavailablein themedium. However, in treatment A, it begins to increase becausethe availability ofnutrients inthe medium,and is influencedby environmental factors. At the 28thincubationday, the number of *Staphylococcussp.* colonies has risen as the role reactivation ofthese bacteria. *Staphylococcus sp.* also has a roleto degrade available nutrients.

At theend of day 35 for incubation time, the numberof bacterial coloniesat eachtreatment of A,Band C, andcontrolsare 10.6CFU/ml, 10.7 CFU/ml, 5.8CFU/ml, and 5.3CFU/ml. The population of each treatmenthas increased, except control.

Enumeration of *Streptococcus sp*

Streptococcus sp. has a fluctuatinggrowthwhich has been affectedby environmental factors, such as temperature,pHandaeration. The statistical resultshows the addition ofsomeactivatorsdoes not affect the increasing numberof pathogenic bacteria duringthe composting process($P \leq 0.05$)because it has noreal difference.

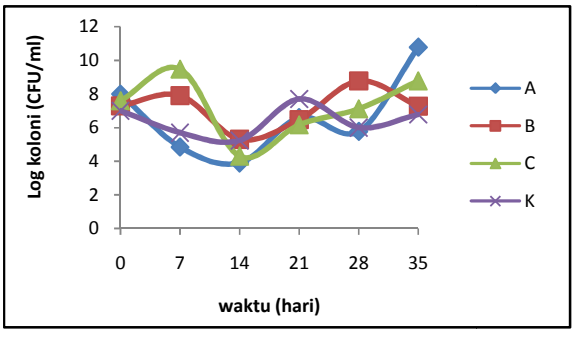

Figure 4. The growth of *Streptococcus sp.* with A, B, C, and K using ES medium

The initial amount of bacteria for each treatment and control is 8 CFU / ml, 7.3 CFU / ml, 7.4 CFU / ml and 7 CFU / ml. This fluctuating growth may be due to some environmental factors that support the growth of *Streptococcus sp.*

At the incubation day 7, the Streptococcus colonies have increased in B and C because the condition of decomposers are optimal, and the addition of activators that has been achieved as a source of acclimatization and the consortium of degrading bacteria. However, the stage of anaerobic process has not been progressed in which it may change the condition of environment and affect the growth of bacteria.

Streptococcus sp. colonies are decreased on the 14th incubation day due to unavailability of nutrients and anaerobic absence of O_2 when the composting process. On the day 21 – 35 , the growth of *Streptococcus sp.* colonies of each treatment has fluctuated. The final amount of Streptococcus A, B, C and control are 10.7 CFU / ml, 7.27 CFU / ml, 8.7 CFU / ml and 6.7 CFU / ml.

The increasing number of *Streptococcus sp.* colonies may occur because a part of its colonies have to adapt its decomposer or the changes of environmental conditions which foster the growth of colonies as well as the interaction between Streptococcus sp. with other microbial, and will establish a synergism.

Enumeration of *Salmonella-Shigella*

Salmonella-Shigella has a fluctuating growth because it is affected by environmental factors, such as temperature, pH and aeration. The statistical result shows that the addition of activators does not affect the increasing number of pathogenic bacteria during the composting process ($P \leq 0.05$) because it has no real difference.

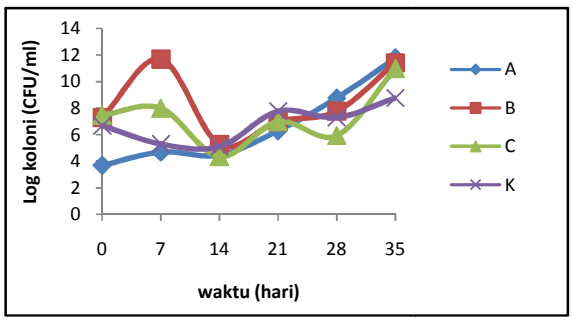

Figure5.The growth of *Salmonella-Shigella*fromA, B, C, and K with SSAmedium.

The early number of SS coloniesin A,B, C, and controlsare 3.6CFU/ml, 7.3CFU/ml, 7.4CFU/ml and 6.6CFU/ml. The numberof SS in BandCis higherthan control. This maybe caused bythe adaptationof bacteria tovariousenvironmental factors whether its colonies will be increasedor remain static.

By looking the graphic above, the growth of coloniesshows a fluctuating improvement from the 7^{th} – 35^{th} of incubationday.The final amountof SS coloniesinA,B, C, and controlare 11.7CFU/ml, 11.4 CFU/ml, 11 CFU/ml, 8.7CFU/ml.

Enumerationof*Coliform*

*Coliform*has afluctuatinggrowththat is affectedby environmental factors, such as temperature,pHandaeration. The statistical resultshowsthat the addition ofactivatorsdoes not affect the increasing numberof pathogenic bacteria duringthe composting process($P \leq 0.05$)because it has noreal difference.

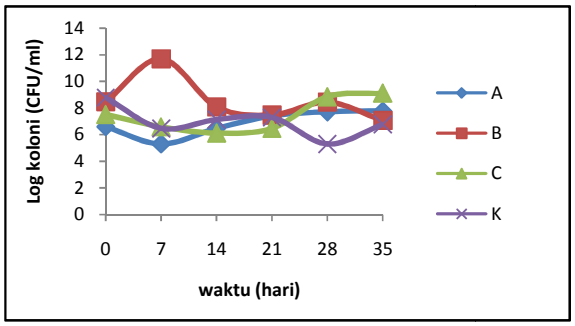

Figure6.The growth of *Coliform*inA, B, C, and KwithMc. Conkey medium.

The early amount of *Coliform*coloniesinA,B, C, and controlare 6.6CFU/ml, 8.4CFU/ml, 7.5CFU/ml, 8.7CFU/ml. The value ofcolonies in controlis higher thanthe number of bacteriaat eachtreatment because it mayhavebeen contaminatedwith*Coliform* beforeadding the activators.

The result of *Coliform*enumerationfrom 7 – 55 incubationday, it has a fluctuatinggrowth because of its increasinganddecreasing number ofcolonies. It may occur asthe adaptationofbacteria with its changingenvironmental factorsas well asthe availability ofnutrients. The total amount of *Coliform* atthe end ofA,B, C, and controlare 7.7CFU/ml, 7.07CFU/ml, 9.1CFU/ml, 6.7CFU/ml.

From the analysisof biochemical tests, the dominant*Coliform* in this waste compostis a pathogenic bacteria, *Serratiamarcescens*. These bacteria maybe pathogenicto humansand beneficentduringthe composting process. During the composting process, *Serratiamarcescens*may producechitinoliticenzymesandisoenzymes**[4].**

The Comparison of Bacteria and Fungi Enumeration on Moist and Dry Compost Comparisonin EachMedium

Enumerationin PCA Medium

The process of dry compostis conducted tocompare the resultsof compost enumeration (moistcompost) and the end of composting(dry compost).

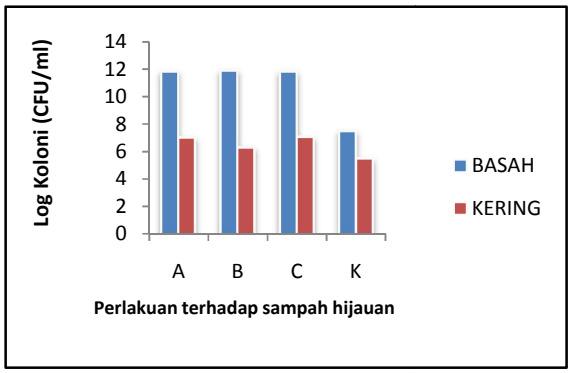

Figure7.The comparison ofbacterial on moist anddrycomposts for A, B, C, andK treatments inPCAmedium.

The growth of colony during wet compost, it will remain higher than control for each treatment of A, B, C. However, if the compost is dried, its result shows at leastthe growthof bacteriain thetreatment ofBcontaminationas6.2CFU/mlcomparing to 5.4CFU/ml, and other treatments. It showsthat the colony growth has decreased inB treatment because a long period of drying with sunlight.

Enumerationin PDA Medium

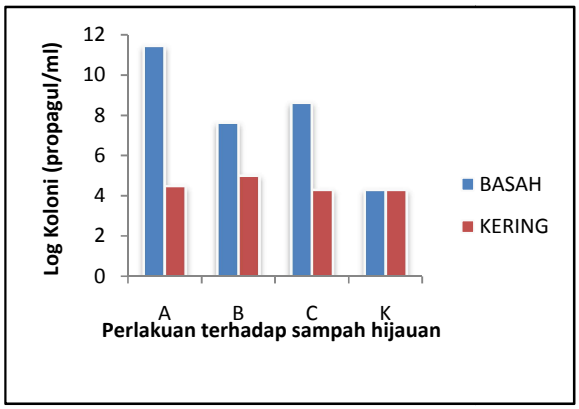

Figure 8. The comparison of fungi on wet and dry composts for A, B, C, and K treatments in PDA medium

Enumerationin BP Medium

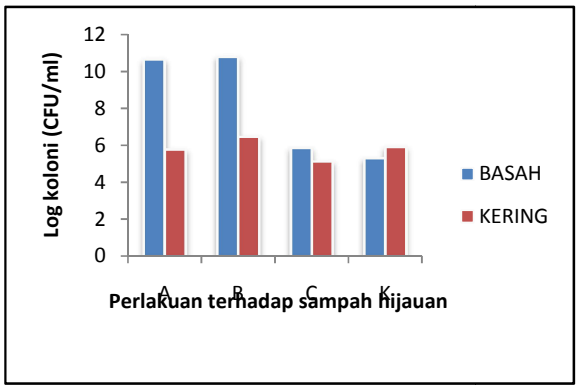

Figure 9. The ratio of *Staphylococcus* between wet and dry compost for A, B, C, and K treatments in BP medium

The high number of colonies in wet condition are A and B but C has decreased due to its dry compost and has a small number of colony than control. The amount of bacteria in dry compost of C treatment is 5.11 CFU / ml while its control is 5.3 CFU / ml. The declining number of *Staphylococcus* may be caused by the death of these bacteria in thermophylic phase during maturation process or due the process of drying with sunlight.

Enumeration in ES Medium

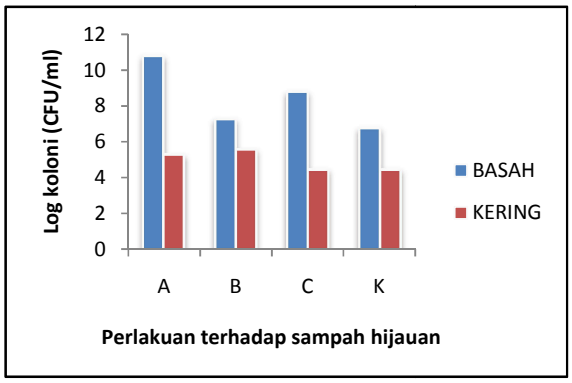

Figure 10. The comparison of *Sreptococcus* on wet and dry compost for A, B, C, and K treatments in ES medium.

The increasing number of *Sreptococcus* colony for A and C treatments in ES medium occurs in wet compost but its decreasing number is also occurred in C due to its dry compost *Sreptococcus* and is equal to control in 4.47 CFU / ml. The declining number of *Streptococcus* may be caused by the death of these bacteria in thermophylic phase during the ripening process or due to the process of drying with sunlight.

Enumerationin SSA Medium

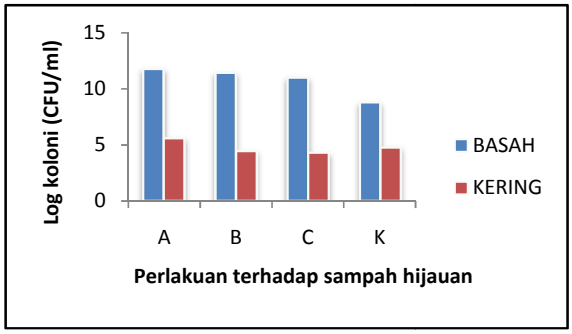

Figure 11. The ratio of *Salmonella Shigella* on wet and dry compost dry for A, B, C, and K treatments in SSA medium

A number of*Salmonella-Shigella*colony has increased due to A and B treatments in wet compost but it also has been decreased with its value of 4.3CFU/ml in C treatment comparing to controls which is only 4.77CFU/ml.

This may bedue tothedeath ofSalmonella-Shigelladuring the process ofmaturation witha high temperature. In addition, it also may becaused bythe death ofbacterial cell during the process of drying with sunlight.

Enumerationin Mc. Conkey Medium

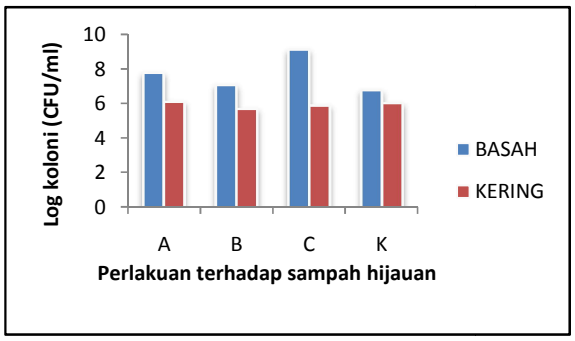

Figure 12. The comparison of *Coliform* bacteria on wet and dry compost for A, B, C, and K in Mc. Conkey medium

A number of *Coliform* has increased due to its wet compost in C treatment and control with 9.11 CFU / ml and 6.7 CFU / ml but it has decreased in B treatment comparing to other treatments and has a value below the control. The number of bacteria is 5.6 CFU / ml and 6.04 CFU / ml for control.

MPN Test

Table 1. MPN Test

Treatments	The Result of APM *Coliform* Abalysis			SNI 19–7030–2004
	Week 1	Week 3	Week 5	*Coliform* Bacteria
A	> 2400	> 2400	75	1000 MPN/gr
B	> 2400	> 2400	64	
C	> 2400	> 2400	75	
K	> 2400	> 2400	43	

Based on theanalysis of MPN, it can be seenthat thequality of litterfall composton first and thirdweek, the number ofColiformexceeds the standard of SNIincomposting. It maybe causedby the production oflitterfallcompost which is not good orcaused bysome environmental factors. On the fifth week all treatmentsarebelow the standard ofSNI1000MPN/g. This may bedue to decreasingactivity of*Coliform* during the process ofdecomposition. This litterfall compostcan be used because its SNI standard.

The Results of Macro and Micro Drying Compost

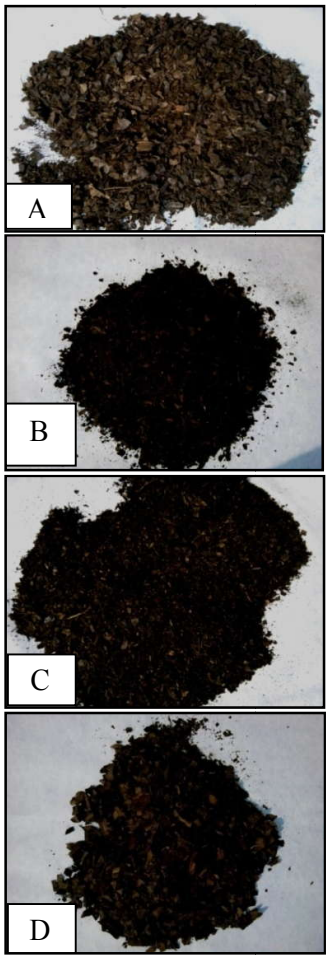

Figure13. The result of drycompostwith various macro activators (A, B, C, and D).

Litterfall compost of A treatment is a mixture ofmanure but the result shows that this compost cannot be used to fertilize crops. Meanwhile, B compost is a mixture of litterfall and commercialfertilizer which may have beenused tofertilize the plants becauseit containsmacro andmicroelements. C compostis a mixture oflitterfall and UAIstudents-made compost inBATAN. It is qualified bySNI19-7030-2004**[5].** DCompostis a mixture oflitterfall and water. The draining result of D compostcan be considered ashumusand if it is used on crops, it mayprovide fewnutrients becauseof its natural compost.

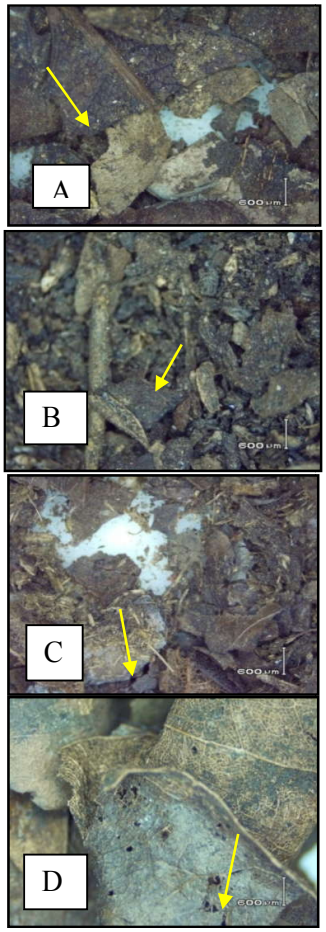

Figure14. The result of drycompostusing various micro activators (A, B, C, and D)

It can beseen some crushedleaves from variousmediums. This isdue tothe composting processwith bacteria andfungiin decomposer. It shows the differences between the leaf whether it uses treatment or not.

IV. CONCLUSION

An addition of three activators does not affect the amount of fungi and bacteria in litterfallcompost.The final result ofmicroorganism analysis on litterfall compost at Universitas Al Azhar Indonesia with three types ofactivators, such as manure,commercialfertilizer, and UAI students-made fertilizersin BATAN. By those activators, the colony growth ofSalmonella-Shigella, Streptococcus, Staphylococcus, Coliformandfungi are found.

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PROCEEDING INTERNATIONAL SEMINAR ON SCIENCE TECHNOLOGY INNOVATION 2012 UNIVERSITY OF AL AZHAR INDONESIA, JAKARTA OCTOBER 2- 4 2012 THE ANALYSIS OF PATHOGENIC MICROORGANISM CONTAMINATIONONN LITTERFALLCOMPOST USING THREE ACTIVATORS AT UNIVERSITAS AL AZHAR INDONESIA Tastaptyani Kurnia Nufutomoo1, Irawan Sugoro1, 2, Nita Noriko1, Dewi Elfidasari1 1Departement of Biology (Biotechnology) Faculty of Science and Technology University of Al Azhar Indonesia, Jl. Sisingamangaraja, Jakarta 12110 2Laboratory of Microbiioollooggyy,, Badan Tenaga Nuklir, in Patir BATAN, Pasar Jum'at, Lebak Bulus Email : tastaptyani @gmail.com Abstract-This study aims to determine the effect of three activator typesdue to the number of microorganisms on the green waste and to determine the bacterial contamination, such as Salmonella Shigella, Strepttooccooccccuuss,, Staphylococcus, Coliform and Fungi on green waste compost at Universitas Al Azhar Indonesia (UAI). The amount analysis of microorganisms is carried by the drop test on various medium, such as PCA, SSA, BP, ES, McConkey and PDA. The identification of Coliform is performed with biochemical activity assay and MPN test in LB broth and BGLB medium. The result of this study shows that there is no increasing amount of microorrggaanniissmmss with the role of three activator types. A fine commercial fertilizer still have a colony of Salmonella Shigella,

Strepttooccoocccccuuss,, Staphylococcus, Coliform and fungi with a few number of these bacteria. Keywords -Microorganism contamination, Compost, UAI green waste. I. INTRODUCTION U niversitas AlAzharIndonesia(UAI) produces organicwastein the form of litterfall. Litterfall is managedtobecompostedwith microorrggaanniissmmss.. Microorganismhas an important rolein helpingthe maturation ofcompost. Therefore, the researcher usesthreeactivators, such asmanure,commercialcompostand artificial fertilizers from UAI studentstoassist thedecomposition oflitterfallcompost. Inthe composting processsomequality standardsmust comply with StandarNasional Indonesia inmanufacturing organic fertilizer(SNI: 19-7030-2004) [1]. In order tomaintainand producegoodcompost, something that is need to be considered is the number of ColiformandSalmonella-Shigella microorganisms, witheach value of <1000MPN/ gand<3MPN/g. In addition, there are bacteriaand other microorganismsthat must be consideredduring the process ofcomposting, such asStaphylococcus, Streptococcus, and fungi. These pathogenic bacteriaandfungiare notincluded inISObecause theywilldieduringthe composting process. To determine the contamination ofmicroorganisms, the researcher conductsand analysisin order tomaintain thequality of compostlitter. II. RESEARCH METHODS Litterfall Compost An artificial fertilizer that is used in this study is compost which is produced at Universitas Al Azhar Indonesia. The researcher uses three activators, such as manure (cow dung), commercial fertilizers, artificial fertilizers from UAI students, and has to put them into a 40L bucket which has holes on the edges to ease aeration process. There also homogenization process of these materials once a THE ANALYSIS OF PATHOGENIC ..... (Tastaptyani Kurnia Nufutomo, Irawan Sugoro, Nita Noriko, Dewi Elfidasari) 36

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Tastaptyani Kurnia Nufutomo[1], Irawan Sugoro[1, 2], Nita Noriko[1], Dewi Elfidasari[1]

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Abstract-**This study aims to determine the effect of three activator typesdue to the number of microorganisms on the green waste and to determine the bacterial contamination, such as *Salmonella Shigella*, *Streptococcus, Staphylococcus, Coliform* and Fungi on green waste compost at Universitas Al Azhar Indonesia (UAI). The amount analysis of microorganisms is carried by the drop test on various medium, such as PCA, SSA, BP, ES, McConkey and PDA. The identification of *Coliform* is performed with biochemical activity assay and MPN test in LB broth and BGLB medium. The result of this study shows that there is no increasing amount of microorganisms with the role of three activator types. A fine commercial fertilizer still have a colony of *Salmonella Shigella, Streptococcus, Staphylococcus, Coliform* and fungi with a few number of these bacteria.**

Keywords -Microorganism contamination, Compost, UAI green waste.

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