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Faculty of Mathematics and Natural Science
University of Brawijaya

MESSAGE FROM RECTOR OF BRAWIJAYA UNIVERSITY

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Dean Faculty of Mathematics and Natural Sciences
And
All participants

Assalamualaikum Warahmatullahi Wabarakatuhu

First of all let us pray to Allah the Almighty for His blessings bestowed to all of us that today we can all be here to attend the The 4th annual Basic Science International Conference and the 5th International Conference of Global Resources Conservation.

It is indeed an honor and privilege for me to welcome you warmly at this Conference. My great appreciation to all of you the distinguished participants of this important event, and trust that this conference will be an valuable input to the empowerment of Applying science to conserve our nature. I would like to take this opportunity to offer my appreciation to Dean Faculty of Mathematics and Natural Sciences, and committee from Biology Department University of Brawijaya who have organized this Seminar.

Distinguished Guests, Ladies and Gentlemen,

University of Brawijaya (UB) as oneof the leading university in Indonesia with its mission of World Class University, should take actions to participate in conserving our nature where we are living inside through innovation of science. We encourage all of academician here, as the backbone of nation building for continuous learning to save our planet for best interest of human being and living thing. Therefore, We should work together, across institutions and across discipline. We should start thinking how to be leader and control the world with our resources, knowledge and technology to achieve an equitable welfare.

Distinguished Guests, Ladies and Gentlemen,

UB with combination between International standard and local culture has been educating communities who have made positive impacts in their Communities-throughout Indonesia. Hence we warmly welcomes collaborative works of mutual partnership with many other institutions; especially universities, industry, government and other institution; both at national and international level. Moreover UB has dedicated itself to be a world class university. Based on spirit we belief that only with international partnership able pursue multinational

connectivity on business, established in the higher education institution, for future generations. In this case, this occasion is really necessary to initiate cooperation beyond national border to supply comprehensive knowledge to be a winner in the global competition.

Distinguished Guests, Ladies and Gentlemen,

Science, technology and education will determine the well-being of people and nations in the future. Therefore, academician and scientist as Scholar who will bear the future of this nation have to work hard, and the ability to create brilliant innovation to supply environmental technology to solve human problem. UB rely on and encourage increased international partnership, as well as greater staff mobility. The partnership, it will be able to supports economic and social development. In Another word, whatever challenges that will arise, it should be challenges for all of us, and it should be solved with partnership.

As a conclusion, I propose to strengthen our collaboration to create synergism at any levels, especially all stakeholders, locally, regionally, as well as internationally. By working together all over the world through sharing information and resources, we can make the bright future. Finally, let us hope that the fruitful points aimed from this conference will develop the new concept and networking based on science and technology to save our nature.

During the organization and execution of this conference, and the one I am currently opening "The 4th annual Basic Science International Conference and the 5th International Conference of Global Resources Conservation".

Ladies and gentlemen, I hereby wish you a fruitful Conference.

Wabillahitaufiqwalhidayah, Wassalamualaikumwarahmatullahiwabarakatuh

Thank you for your attention.
Malang, February 15th, 2014

Rector,

PREFACE

All praises are due to Allah, God Almighty, Who made this annual event of successful of " The **"4th Annual Basic Science International Conference 2014** ***in conjunction with*** **The 5th International Conference on Global Resource Conservation 2014, both annual** scientific events organized by the Faculty of Mathematics and Natural Sciences, Brawijaya University.

In this year, the conference took a theme of **"Applying science to conserve nature"**. These conferences are concerned about our current challenge on how to explore, utilize and apply our knowledge and science to conserve water, soil, earth, air, plants, animals and microorganisms that involved multi disciplines. As a conjunctive conferences, these covered a wide range of topics on basic sciences: physics, biology, chemistry, mathematics and statistics as well as conservation biology and applied science.

The conference in 2014 was the continuation of the preceding conferences initiated in 2011 as the **International Conference on Basic Science (ICBS)** and **International Conference on Global Resource Conservation initiade in 2010. Therefore** the proceeding was also divided into two books, each with, each with a different ISSN. The proceedings were also published in electronic forms that can be accessed from BaSIC website.

I am glad that for the first time both types of publication can be realized. These international conferences are held to to increase dissemination of applying science to conserve natural resources, to present new research findings, ideas and informations and to discuss topic related to conference theme and to develop collaboration among multi discipline sciences and to find potential young researchers. This is in line with university vision as a World Class Entrepreneurial University.

I am grateful to all the members of the program committee who contributed for the success in farming the program. I also thank all the delegates who contributed to the success of this conference by accepting our invitation and submitting paper for presentation in the scientific program. I am also indebted to PT. Fajarmas, PT. Makmur Sejati, KPRI, CV. Gamma, PT. Tata Bumi Raya and CV. Enseval Medica Prima for their support in sponsoring this event.

I hope that you enjoy in this seminar. We wish for all of us a grand success in our scientific life and for the coming conferences and even better.

Thank you,

Malang, February 2014

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Relationship Model Anomaly Harvested Rice With a Weighted Rainfall Index in Buru Maluku Using Bootstrap Aggregating Mars Methods to Predict the Forecast Error Rates Harvested Area and Rice Production

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***Abstract*— Seasonal climate variations is one of the main causes of the diversity of crop production in Indonesia. Long drought and drought causing crop failures and food shortages that could affect agricultural production and food security. The indicator is a decline in acreage, harvested area and production declined sharply when climate irregularities. The magnitude of the impact caused by climatic irregularities cause we need a model that connects the harvested area with indicators of climate anomalies that can do the proper planning and anticipation measures early in order to avoid the risk of crop failure. Buru as the largest rice -producing areas in the provinces of Maluku course is expected to avoid the risk of crop failure in order not to disrupt the supply of rice. Data Collection and forecast rice production annually conducted by the Central Statistics Agency (BPS). BPS forecast model but has not entered a climatic factor, while the climate affect rice production. This research used the bootstrap aggregating MARS method to model anomaly rice harvested area with a weighted rainfall index to predict the error rate forecast harvested area and rice production. From the analysis using the best models of replication bagging MARS 150 times in the first period (January-April) and 200 times in second period (May-August) and third period (September-December) obtained an error rate forecast harvested area and rice production respectively by 5.72 % and 6.81 % .**

Keywords*— *Anomaly Area harvested, weighted rainfall index, MARS, Boostrap Aggregating, rice production

I. INTRODUCTION

Rice is the main food for the people of Indonesia, which provides seasonal income and employment for rural communities. Rice production has increased since 1970, but the harvest is particularly vulnerable to climate variability of extreme events: El-Nino and La-Nina. In the event of the El - Nino rice production has decreased quite dramatically, as in 1991, 1994, and 1997. Similarly, in the La-Nina (1995) also decreased rice production [8].When the national rice supply is insufficient and a decline in production, the import policy is often done. The problem is the need to forecast future production decline (extreme weather events), so the anticipation can be done. That requires models that accurately forecast rice production in order to support national food security. Data Collection and forecast national rice production every year conducted by the Central Statistics Agency and the Ministry of Agriculture. Forecasts made by the province were calculated based on time series data and the provinces are not based on the sum of the forecast district/city level. Production per province is obtained by multiplying the harvested area clean with a yield per hectare per unit of harvested area clean for every sub round (4 monthly: sub round 1 (January-April), sub round 2 (May-August), and sub round 3 (September-December) [5]. production and harvested area in a year (January to December) is obtained from the sum of production and harvested area for three sub round. Yield to each hectare is the yield each hectare in the form of tile results each unit of harvested area.

II. REVIEW OF LITERATURE

2.1 . MARS

MARS is an implementation technique popularized by [4] for solving regression problems with the aim of predicting the response variable values of a number of predictor variables. MARS is an approach to the development of Recursive Partitioning Regression (RPR) which still has the drawback that the resulting model is not continuous at the knots. MARS model is used to overcome weaknesses in the model generate the RPR is continuous at knots. In spline modeling, the first step is to determine the points of data or a change in the pattern of behavior is called the point knots. The selection of knots in MARS using forward and backward algorithms. The selection of the model using a forward step taken to get the maximum number of base functions with base selection criteria function is to minimize Average Sum of Square Residual. To parsimony concept (simple model) performed a backward step is selecting base functions of forward

stage by minimizing the value of the Generalized Cross - Validation or GCV [4].

The minimum GCV as a criterion for determining knots are as follows:

$$GCV(M)=\frac{\left(\frac{1}{N}\right)\sum_{i=1}^{N}\left(y_i-\hat{f}_M(x_i)\right)^2}{\left(1-\frac{C(M)}{N}\right)^2}$$

where

M : The number of base functions

$C(M)$: The number of parameters in the model $=\text{trace}\,(\mathbf{B}(\mathbf{B}^{\mathbf{T}}\mathbf{B})^{-1}\mathbf{B}^{\mathbf{T}})+1$

$\mathbf{B}$: matrix of base function

N : The number of data

y_i : Value of the response variable

$\hat{f}_M(x_i)$: Estimated value of the response variable on M base functions.

From forward and backward, MARS models obtained as follows:

$$\hat{f}(x)=a_0+\sum_{m=1}^{M}a_m\prod_{k=1}^{K_m}\left[s_{km}\left(x_{v(k,m)}-t_{km}\right)\right]_+$$

where

a_0 : main of base function

a_m : coefficients of m-base functions

M : maximum of base function

K_m : degree of interaction

s_{km} : ± 1

$x_{v(k,m)}$: independent variables

t_{km} : Point knots of independent variables $x_{v(k,m)}$

Algorithms for MARS models are as follows:

1. Starting with a simple model involving only constant base functions.
2. Finding space of base functions, for each variable and for all knots are possible, and add it to minimize the prediction error.
3. Repeat steps 2 to obtain a model that has maximum complexity.

Finally, in the last stage, the trimming procedure is applied in which the base functions are not significantly removed to obtain the minimum GCV.

2.2. Bagging Mars

Bagging method was first used by Breiman (1994). Bagging is used as a tool to form a more stable classifier. Bagging predictors is a method to generate multiple versions of a predictor and use it to aggregate predictors. Multiple versions of the bootstrap replication is formed by a set of data.

Defined a set data £ consists of $(y_n, x_n), n=1,\ldots,N$ where y a numerical response or a class label. If x the input is then y predicted by $\varphi(x,£)$, where $\varphi(x,£)$ is a predictor. To gain a better predictor performed bootstrap replication $\{£_k\}$ is then called $\{\varphi(x,£_k)\}$. Performed totally B-times of bootstrap replication so that $\{£^{(B)}\}$ from £ where $\{£^{(B)}\}$ resampling with replacement and established predictors of $\{\varphi(x,£^{(B)}\}$.

Bagging MARS algorithm is as follows.

1. Taking bootstrap *n* samples of set data £ with *n*-repetitions to each aggregate variables in each observation.
2. MARS modeling sets data $£_B$ bootstrap sample results.
3. Test the model generated in step 2.
4. Repeating steps 1-3 as much as *B*-times (bootstrap replication).
5. Obtain the best model.
6. Forming bagging MARS models of the average of each parameter at each sampling to B-times.

To obtain better results then the bootstrap replication is done as much as possible.

2.3. Weighted rainfall index and anomalies Harvested Rice

Weighted rainfall index (weighted rainfall index: WRI) developed in Australia by Stephen, et al (1994). This index is compiled based on monthly rainfall data is weighted. WRI which can be used in the modeling is that the WRI weighted system has been modified by Sutikno (2008). The modification is written as follows.

$$WRI_{t,D}=R^*_{t,D}\frac{LT_t}{L_{s\tan dard}}$$

where,

$$R^*_{t,D}=\sum_{j=1}^{m}\frac{A_j}{A}R_j,\ A=\sum_{j=1}^{m}A_j$$

Description:

$R^*_{t,D}$: Area weighted rainfall Regional weather forecast region (DPM) / DPM revision in the region/district/city

m : a large area of DPM

A_j : Total area of *j*- DPM

LT_t : Plant area at *t*-month

$L_{s\tan dard}$: standard area for rice crops in the regional

j : DPM regional (1,2,3, ..., m)

t : Months (1, 2, ..., 12)

D : Regional

Model anomalies rice harvested area to each period ($AnLP_p$) with a weighted rainfall index (WRI) is as follows.

$$AnLP_{pi} = WRI_{1i} + WRI_{2i} + WRI_{3i} + WRI_{4i}$$

From the equation above three equations obtained for each of the following.

$$AnLP_{1i} = WRI_{1i} + WRI_{2i} + WRI_{3i} + WRI_{4i}$$
$$AnLP_{2i} = WRI_{5i} + WRI_{6i} + WRI_{7i} + WRI_{8i}$$
$$AnLP_{3i} = WRI_{9i} + WRI_{10i} + WRI_{11i} + WRI_{12i}$$

where

i = 1, 2, 3,..., n (n is the number of observations)
p = 1, 2, 3 (Period)
$AnLP_1$ = harvested area Anomaly in first period (January to April)
$AnLP_2$ = harvested area Anomaly in second period (May to August)
$AnLP_3$ = harvested area Anomaly in third period (September to December)
WRI_1, ..., WRI_4 indicates a weighted rainfall index first until the fourth month in a first period (WRI_1 = in January, WRI_2 = in February, WRI_3 = in March, WRI_4 = in April).
WRI_5, ..., WRI_8 indicates a weighted rainfall index first to fourth month in a second period (WRI_5 = in May, WRI_6 = in June, WRI_7 = in July, WRI_8 = in August).
WRI_9, ..., WRI_{12} indicates a weighted rainfall index first to fourth month in a third period (WRI_9 = in September, WRI_{10} = in October, WRI_{11} = in November, WRI_{12} = in December).

III. METHOD OF ANALYSIS

Methods of data analysis performed in this study can be explained as follows.

1 . Identification of data include the identification and the relationship between WRI and $AnLP_p$ that can be shown on the scatter plot.
2 . To model based anomaly harvested area weighted rainfall index for the data in- sample using the bagging MARS method with the following stages.
- In MARS models for the first sets data
  - Determine the maximum base functions
  - Determine the maximum number of interactions
  - Determine the minimum number of observations between knots
  - Determine the number of degrees of freedom
- Getting the best MARS models for the initial set of data based on the value of the smallest MSE and GCV.
- Getting the significant variables of the best MARS models for the initial set of data.
- Perform bagging of the pair response variable and the predictor variables were significant from the best MARS models for data sets beginning with 50, 100, 200, and 250 bootstrap replication.
- Perform MARS modeling on each sample-B bootstrap replication with the maximum number of base functions, the maximum amount of interaction and the minimum number of observations between knots is equal to the maximum number of base functions, the maximum amount of interaction and the minimum number of observations between knots at best MARS models for data sets beginning.
- Getting MSE at each sampling B bootstrap replication.
- Getting MSE bagging of the average MSE at each sampling to B
- Bagging MARS model obtained is the best MARS models for the initial data sets. This is because the value of changing each knots for each replication so that the estimated parameters can not be averaged.

3. Counting rice production forecast for the year to out -sample of data as follows.
- Calculating forecast rice harvested area forecast results by adding the anomalous area harvested to each period results of MARS best modeling with an average area harvested during a certain period (2002 to 2008).
- Suspect productivity to each period using the average productivity of the last five years (2007 to 2011).
- Calculating forecast rice production to each period.

$P_p = Pro_p \times LP_p$ dengan p = 1,2,3
P_p : forecast of production in the p-period
Pro_p : forecast of productivity in the p-period
LP_p : forecast of harvested area in the p-period
- Getting forecast of rice production for the year which is the sum of the third period of the forecast.
- Comparing rice production forecast results have been obtained with the MARS method with the actual value of rice production from BPS issued last three years (2009 to 2011).

IV. DISCUSSION

4.1. Identification Data

To model the relationship between the anomalous area harvested and predictor variables weighted rainfall index first identified patterns of relationship between the two. Identification of relationship pattern is very necessary to know the exact model in modeling the relationship between the two variables.

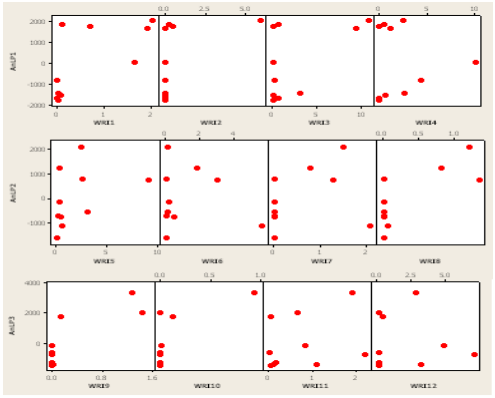

Figure 1. Scatterplot AnLP and WRI in Buru

Figure 1 shows that the pattern of relationships between AnLP and WRI appears no linierity clear pattern not even have a specific pattern. Likewise, the direction of the relationship is positive or negative. This suggests that AnLP in first period (January to April), second period (May to August), and third period (September-December) are not affected by these two variables are linearly so necessary to find the possibility of non-linear statistical models.

4.2. *Relationship between the model anomaly rice harvested to each Period (AnLPp) and weighted rainfall index (WRI) using Bagging MARS method.*

In this study bagging MARS method is applied in modeling the anomalous area harvested per period as the response variable and weighted rainfall index as a predictor variable. The data used for modeling can be quite small, namely 10 years since year 2002 to year 2011, so it needs to be done in preparing the model resampling methods. Resampling method used is that a bagging sampling with replacement for a data set consisting of the response variable and the predictor variables (significant base functions).

4.2.1. *Relationship between the model anomaly rice harvested area (AnLP) and the weighted rainfall index (WRI) in first period using bagging MARS method.*

A. MARS Model

Initial step of MARS modeling done by trial and error on the maximum base function (BF), maximum interaction (MI), minimum number of observations between knots or minimum observation (MO), and the number of degrees of freedom (DB) until an optimal model is obtained with the MSE and GCV minimum. [4] suggest a maximum number of basis functions of two to fourth times the number of predictor variables. Maximum interaction one, two, or three with a consideration if more than three will result in a very complex model. As well as the minimum distance between knots or knots as the minimum observation between 0, 10, 20, 50, and 100.

Table 1. Trial and Error Model MARS a first period in Buru

BF	MI	MO	DB	R^2	MSE	GCV
8	**1**	**0**	**1**	**0.507**	**544216.122**	**1523805.55**
8	2	0	1	0.204	1240372.80	3088471.75
8	3	0	1	0.204	1240372.80	3088471.75
12	1	0	1	0.507	544216.122	1523805.55
12	2	0	1	0.204	1240372.80	3088471.75
12	3	0	1	0.204	1240372.80	3088471.75
16	1	0	1	0.507	544216.122	1523805.55
16	2	0	1	0.204	1240372.80	3088471.75
16	3	0	1	0.204	1240372.80	3088471.75

Table 1 shows the value of R^2, MSE, and GCV in combination BF, MI, MO, and DB. based on the criteria of goodness of the model, selected models with minimum MSE and GCV. From the above results it can be concluded the model with a combination of BF = 8, MI = 1, MO = 0, and DB = 1 is the best model. The best MARS model is as shown below.

$$\hat{f}(x) = 1031{,}325 + 985{,}757BF_1 - 9439{,}66BF_4$$

where

$$BF_1 = \max(0, WRI_1 - 0{,}088) = \begin{cases} 0, & if\ \ WRI_1 \le 0{,}088 \\ (WRI_1 - 0{,}088), & if\ \ WRI_1 > 0{,}088 \end{cases}$$

$$BF_4 = \max(0, 0{,}251 - WRI_2) = \begin{cases} 0, & if\ \ WRI_2 \ge 0{,}251 \\ (0{,}251 - WRI_2), & if\ \ WRI_2 < 0{,}251 \end{cases}$$

From the best MARS models can be interpreted that each increase of one unit of the base functions 1 (BF_1) can increase rice yields broad anomalies in the period 1 at 985.757 if weighted rainfall index in January (WRI_1) more than 0.088 mm, with a base of other functions that go assumed to be constant in the model. Meanwhile, for each increase of one unit of the base function 4 (BF_4) can reduce anomalies rice harvested area of 9439.66 a first period if the weighted rainfall index in February (WRI_2) of less than 0.251 mm with base other functions are included in the model held constant. The next best model obtained from two predictor variables were entered into the model, which is a weighted index of rainfall in February (WRI_2) and weighted rainfall index in January (WRI_1) based on the relative variable importance table. Percentage of contribution weighted rainfall index in February (WRI_2) and weighted rainfall index in January (WRI_1) are shown in Table 2 below.

Table 2. Percentage of contributions of each variable in the first period

Variable	Contribution
WRI_2	100 %
WRI_1	57,711 %

B. MSE calculations on models of bagging MARS

MARS modeling of the data sets obtained MSE Value in the first period is 544,216.122. To minimize the error variance performed on the data resampling. Table 3 below shows the results for the first period bagging MARS in Buru.

Table 3. Results of bagging MARS in first period

Bootstrap replication	average value of MSE	Decrease in the value of MSE
25 times	19511,6	524704, 522
50 times	3566,25	540649,872
100 times	3993,14	540222, 982
150 times	**1538,90**	**542677, 222**
200 times	2413,91	541802,212

Table 3 gives the smallest MSE value of the information obtained during the boostrap replicate as much as 150 times. thus it can be concluded that the best results obtained in the replication boostrap bagging as many as 150 times. Bagging models can lower the MSE value of the data model that is equal to the initial set of 544216.122 be 1538.90 or in other words bagging can reduce the value of MSE of 542677.222 of the initial data sets.

4.2.2. Relationship between the model anomaly rice harvested area (AnLP) and the weighted rainfall index (WRI) in second period using bagging MARS method.

A. MARS Model

Trial and error to BF, MI, MO, and DB MARS modeling in second period are shown in Table 4.

Table 4. Trial and Error Model MARS a second period in Buru

BF	MI	MO	DB	R^2	MSE	GCV
8	**1**	**0**	**1**	**0.261**	**637497.059**	**1147494.92**
8	1	0	2	0.088	637497.059	1416660.57
8	1	0	3	0.088	637497.059	1416660.57
12	1	0	1	0.261	637497.059	1147494.92
12	1	0	2	0.088	637497.059	1416660.57
12	1	0	3	0.088	637497.059	1416660.57
16	1	0	1	0.261	637497.059	1147494.92
16	1	0	2	0.088	637497.059	1416660.57
16	1	0	3	0.088	637497.059	1416660.57

From Table 4 it is seen that the best MARS model is a combination of BF = 8, MI = 1, MO = 0, and DB = 1. It can be seen from the MSE and the smallest GCV among others, are respectively 637497.059 and 1147494.92 so the best MARS model is as shown below.

$$\hat{f}(x) = -566{,}237 + 1654{,}87BF_1$$

where

$$BF_1 = \max\left(0, WRI_8 - 0{,}22\times10^{-7}\right) = \begin{cases} 0, & if\ \ WRI_8 \le 0{,}22\times10^{-7} \\ \left(WRI_8 - 0{,}22\times10^{-7}\right), & if\ \ WRI_8 > 0{,}22\times10^{-7} \end{cases}$$

This model can be interpreted that each increase of one unit of the base functions 1 (BF_1) can increase rice yields broad anomaly in second period for 1654.87 if weighted rainfall index in August (WRI_8) more than 0.22 x 10-7 mm, on the base of other functions in the model are held constant. Furthermore, Table 5 looks only variable weighted rainfall index in August (WRI_8) are included in the model. So important variable scores for weighted rainfall index in August (WRI_8) worth 100%, which means the variable weighted rainfall index in August has a dominant influence on anomalous rice harvested area in second period (May-August).

Table 5. Percentage of contributions of each variable in the second period

Variable	Contribution
WRI_8	100 %

B. *MSE calculations on models of bagging MARS*

The best MARS models between anomalous rice harvested area weighted rainfall index in second period provide information that model has a MSE is 637,497.059, with a significant predictor variables are weighted rainfall index in August (WRI_8). Table 6 below shows the average MSE in second period.

Table 6. Results of bagging MARS in second period

Bootstrap replication	average value of MSE	Decrease in the value of MSE
25 kali	13521,9	623975,159
50 kali	15068,9	622428,159
100 kali	7378,52	630118,539
150 kali	4284,14	633212,919
200 kali	**3017,05**	**634480,009**

Table 6 provides information that bootstrap replication earned 200 times average value of the smallest MSE is 3017.05, So based on the above results it can be concluded that the results obtained by bagging the best with an average value of the smallest MSE is a bootstrap replicate as much as 200 times. With replication as much as 200 times, bagging can reduce MSE of the initial set of data models is 637497.059 be 3017.05 or in other words bagging can reduce the value of MSE of 634480.009 from the initial set of data models.

4.2.3. *Relationship between the model anomaly rice harvested area (AnLP) and the weighted rainfall index (WRI) in third period using bagging MARS method.*

A. MARS Model

Table 7 gives information based on that value the smallest MSE and GCV is combination of BF=8, MI=1, MO=0, and DB=1. So that model with the combination is the best model. This model is shown below.

$$\hat{f}(x) = 2355{,}804 - 24391{,}732BF_2$$

Where

$$BF_2 = \max(0, 0{,}141 - WRI_9) = \begin{cases} 0, & if\ \ WRI_9 \ge 0{,}141 \\ (0{,}141 - WRI_9), & if\ \ WRI_9 < 0{,}141 \end{cases}$$

Table 7. Trial and Error Model MARS a third period in Buru

BF	MI	MO	DB	R^2	MSE	GCV
8	**1**	**0**	**1**	**0.761**	**453362.558**	**816052.711**
8	2	0	1	0.749	453362.558	858437.928
8	3	0	1	0.749	453362.558	858437.928
12	1	0	1	0.761	453362.558	816052.711
12	2	0	1	0.749	453362.558	858437.928
12	3	0	1	0.749	453362.558	858437.928
16	1	0	1	0.761	453362.558	816052.711
16	2	0	1	0.749	453362.558	858437.928
16	3	0	1	0.749	453362.558	858437.928

From the best model can be interpreted that each increase of one unit of the base function 2 (BF_2) can reduce anomalies rice harvested area in the period 3 of 24391.732 if weighted rainfall index in September (WRI_9) of less than 0.141 mm, with bases other functions assumed to be constant in the model. Later in the third period, based on table 8 weighted rainfall index in September (WRI_9) contributes 100%, which means the variable weighted rainfall index in September to have a dominant influence on anomalous rice harvested area in the third period.

Table 8. Percentage of contributions of each variable in the third period

Variable	Contribution
WRI_9	100 %

B. *MSE calculations on models of bagging MARS*

The best MARS models between anomalous rice harvested area weighted rainfall index for the third period provides information that the model has a MSE is 453362.558 significant predictor variables are weighted rainfall index in September (WRI9). Table 9 below shows the average MSE results of bagging MARS in third period.

Table 9. Results of bagging MARS in third period

Bootstrap replication	**average value of MSE**	**Decrease in the value of MSE**
25 kali	11418,2	441944,358
50 kali	9452,84	443909,718
100 kali	3224,57	450137,988
150 kali	1213,38	452149,178
200 kali	**961,970**	**452400,588**

Table 9 provides information that the bootstrap replication earned 200 times the average of the smallest MSE value is 961.970, so it can be concluded that the results obtained by bagging the best with an average value of the smallest MSE is a bootstrap replicate as much as 200 times . With replication as much as 200 times, bagging can reduce MSE of the initial set of data models for 453362.558 be 961.970 or in other words bagging can reduce the value of MSE is 452,400.588 from the initial set of data models.

4.3. *Rice Production forecast*

To evaluate the model and see the level of reliability that is formed, it can be seen from the average forecast error rates for harvested area and production of rice in the year 2009 to the year 2011. Rice production Forecast to each period is the multiplication of the value of the harvested area forecast productivity. Used to estimate the productivity of the average productivity value over the last five years . While the forecast harvest

area was obtained from the sum of the forecast anomalies rice harvested area with average area of rice crop. Of the best MARS models which have been obtained from the previous analysis can be calculated models forecast values for harvested area and production of rice to each period as shown in Table 10.

Table 10. forecast Value of harvested area and rice production to each period (using models MARS)

Period	year	harvested area		production		(Abs(Δ)/Akt)x100 %	
		actually	forecast	actually	forecast	harvested area	production
First period	2009	3897	2747,045	148086	104563,5	29,50%	29,39%
	2010	1393	863,342	52934	32862,2	38,02%	37,92%
	2011	2293	2569,57	87134	97808,2	12,06%	12,25%
					average	26,53%	26,52%
Second period	2009	5628	4818,40	242004	203153,5	14,38%	16,05%
	2010	2371	3033,99	101953	127919,4	27,96%	25,46%
	2011	4280	5037,54	184040	212392,8	17,69%	15,40%
					avcrage	20,01%	18,07%
Third Period	2009	3320	3806,9	119520	136203,8	14,66%	13,95%
	2010	3569	3830,9	128484	136203,8	7,33%	6,67%
	2011	4901	3830,9	176760	136203,8	21,83%	22,45%
					average	14,61%	14,36%

Δ = Actually – forecast

Table 10 provides information based on the results of the forecast error for the harvested area and rice production to each period look distinctly average prediction error rate is at least third periods respectively 14.61% and 14.36%, followed by a period of 2 each by 20.01% and 18.97%, as well as period 1 respectively 26.53% and 26.52%. BPS and the Ministry of Agriculture every year to data collection and forecast rice production in Indonesia is divided into three periods, namely from January to April, May to August, and September to December. Harvested area of each period obtained from the amount of harvested area in the first month until the fourth month in a period. Production and harvested area in one year (January to December) is obtained from the sum of production and harvested area for three periods. So the forecast results for the year and harvested area of rice production MARS models as shown in Table 11 below.

Table 11. Forecast value of harvested area and rice Production Harvested to each year

year	harvested area (Ha)		production (ton)		(Abs (Δ) / Akt.) x 100 %	
	actually	forecast	actually	forecast	harvested area	production
2009	12845	11372,36	509610	439746,59	11,46%	13,70%
2010	7333	7728,27	283371	298837,10	5,39%	5,45%
2011	11474	11438,05	447934	442286,77	0,31%	1,26%
average					5,72%	6,81%

Δ = Actually – forecast

Based on the results of table 11 forecasts to each year for harvested area and rice production of MARS models can be calculated the average error rate forecast harvested area of rice by 5.72 % while the average error rate for rice production forecast is 6.81 %. Average error rate of rice production forecast issued BPS to each province ranged from 5% to 10 %.

V.CONCLUSIONS

Results of rice harvested area forecast MARS model in Buru has an error rate forecast is 5.72 %. As for rice production forecast results have an error rate of 6.81 %. In accordance with an error rate forecast of rice production issued BPS ranging between 5 %-10 %, it can be said rice production forecast errors in Buru is in accordance with the rate specified BPS.

REFERENCES

[1] Breiman. L, Bagging Predictor. Technical report 421. Statistics department of the University of California, 1994.
[2] Efron. B. and Tibshirani. R.J, An Introduction to the Bootstrap, Chapman & Hall, Inc.,New York, 1993.
[3] Eubank. R. L, Spline Smoothing and Nonparametric Regression, Mercel Dekker, New York, 1988.
[4] Friedman. J. H, Multivariate Adaptive Regression Splines , Rev Tech Report 102, Department of Statistics Stanford University Stanford , California, 1990.
[5] Kartini. A. Y, Bagging MARS for Developing forecast Models of Anomalies Area Rice Harvested of Weighted Rainfall Index in Ngawi [thesis], Graduate Program, Sepuluh Nopember Institute of Technology, Surabaya, 2010.
[6] Sutikno, Statistical Downscaling GCM Outcomes and Utilization for Rice Production Forecasting (Disertation), Bogor, Graduate Program, Bogor Agricultural University, 2008.
[7] StatSoft ., Multivariate Adaptive Regression Splines (MARSplines) , Copyright StatSoft , Inc, 2000.

Sequential Pattern Discovery of Deoxyribonucleic Acid (DNA) for Cancer Patients Using PrefixSpan Algorithm

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***Abstract*— The high rate of mortality is caused of cancer disease due to it is effect of uncontrolled growth of cells and mutations. It effects many research to be conducted for early detection of the desease. One of them is a study and analysis of the molecular level is done by combining molecular biology, mathematics and informatics which is known as bioinformatics. A method in the field of informatics that can be used to find the pattern of Deoxyribonucleic Acid (DNA) sequence databases as a constituent amino acids of the p53 gene is called Sequential Patterns Mining Discovery. This method is a process of extracting data to generate knowledge about the series of events that has the appearance of a certain frequency. This research is proposed PrefixSpan algorithm to discovery the patterns due to it has a high performance in computation time. The test is implemented using several threshold, such as minimum support (minimum frequent of sequence in the whole transaction) and sequence length in each transaction database. It takes from DNA sequence databases of cancer patients in each the same exon. The test result is obtained patterns or motifs of DNA sequences for the patients. The performance measure shows that the average support is high and stable in the range of 0.8. This number means that the frequent of thus pattern is high. Another performance measure is lift ratio which has average value more than 1 . This value shows that the generated patterns have high relationship and dependency.**

***Keywords*— cancer, bioinformatics, DNA, sequential pattern, PrefixSpan**

I. INTRODUCTION

CANCER is classifed as mallignant and deadly disease. Generally, it is caused of gene mutation, i.e. p53 and it effects to change of p53 protein sequence [1]. This protein consists of a combination of 20 amino acids which are synthesized by ribosomes are formed based on the genetic code of the Deoxyribonucleic Acid (DNA). If the DNA is mutated, then the protein composition will be incorrect. Continuously, it is effect to variety of diseases and disorders such as cancer. Therefore, early detection can be conducted by analizing of protein sequence in the blood test. The most frequently altered gene of P53 or TP53 mutations is found in human cancers. There are 30,000 somatic mutations of various cancer types in TP53 database which is collected over 20 years. Recently, the methodology of sequencing cancer genome impacts on the healing and data management [2]. According Soussi, p53 mutation analysis of the pattern has become essential to investigate the cause of cancer. His test result shows that infrequent of gene mutation is associated with the normal activity of the p53 protein [2].

The high rate of mortality which is caused by cancer, it makes early detection of this disease. A field that is expected to provide his role is bioinformatics. This field is a knowledge discipline that combines the study of molecular biology, mathematics and information technology (IT). It is defined as the application of computational tools and analysis to capture and interpret molecular biology data . Molecular biology itself is also an interdisciplinary field in molecular level of life sciences [3]. Bioinformatics has a very important role, including the data management for molecular biology, especially DNA sequences and has huge volume of genetic information. One of IT field that is used to find the DNA patterns of cancer patiens is *Sequential Pattern Discovery*. Sequential pattern discovery is a part of data mining task that generates knowledge about the series of events that have a frequency of occurrence that exceeds a specified threshold value [4]. The pattern is expected to use as biomaker of cancer disease. This research is proposed PrefixSpan algorithm to discovery its pattern of DNA sequence database. This algorithm is a method of Sequential Pattern Discovery which has high performance of computational time [5].

II. SEQUENCING GENOME

DNA sequencing is a process of determining three million nucleotide bases order which consist of adenine, guanine, cytosine and thymine (A, T, G ,C) in a DNA molecule. However, sequencing genome is the determination of the nucleotide sequence of DNA bases in the genome or in the body of an organism . Sequencing results are expressed in the form of a sequence of letters nucleotide bases in particular DNA, for example AGTCCGCAGGCTCGGT. Sequencing genome is always compared to coding process, whereas the sequencing process is not only defining a code, but also analogous genome sequence of letters from a mysterious language. It has an important and specific meaning.

Furthermore, the sequence alignment is the process of setting two or more sequences so that the sequences similarities can be detected. Sequence alignment line is interpolated (usually with the sign "-") such that the columns contain characters that are identical or similar between the sequences. Here is an example of the alignment of short sequences of DNA from two different DNA, "ccatcaac" and "caatgggcaac" (the "|" indicates a match or matches between the two sequences) are shown in Fig. 1.

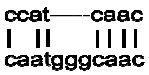

Fig. 1. Matching two sequences

The mismatch in alignment is associated to the mutation, but the gap (the sign "-") is associated to the insertion or deletion. In addition, sequence alignment is also used to find similarity in sequence databases.

III. DATA MINING

Data mining is a method of multiplying the data to obtain the hidden information. Defining a wide range of data mining [7]:

- It is an untrivial decomposition of dataset into potential information implicitly.
- It is mining and analysis by an automatic or semi-automatic utility from huge data to find patterns that have meaning.
- Data mining is also part of the knowledge discovery in databases (KDD)

Briefly, there are two methods of data mining to implement the role, include [6]:

- Prediction Method
  It is using some variables to predict unknown values or future values of other variables.
- Method Description
  It seeks human-interpretable pattern that can explain certain data.

3.1. Sequential Pattern Discovery

Sequential pattern mining is a data mining process that generates knowledge about the sequence of events that have an occurence frequency that exceeds the threshold value [4]. Sequential pattern is a derived patten from the association rules, because both indicate the relationship between events. The difference is that the sequential pattern of events focused on finding patterns of evennt that appear after another event, but association rules is a pattern of events that occurently appear with other events.

3.2. PrefixSpan Algorithm

PrefixSpan (Prefix-Projected Sequential Pattern Growth) is a development approach that uses an algorithm to search for sequential pattern sequences. PrefixSpan will seek frequent sequences of the elements and then develop the sequences by adding elements one by one. As a result, the additional sequence is still the previous sequence. This way is not necessary to generate and test for candidates. The pseudocode of PrefixSpan algorithm is shown in Fig. 2 [5].

```
Input: sequence database, minimum support
Output: A complete set of sequential pattern
Method: Call prefixspan({},(),S).
Subroutine: Prefixspan(α, l, S|α)
Parameter: α: suatu seqential pattern, l:
length α,
S|α: projected database, if α ≠(); if it is not
then sequence database is S
Method:
 1. Scan S|α once, find set frequent itemset,
    so that:
     a. b can be combined to last element from
        α to construct sequential pattern; or
     b. <b> can be added to α for constructing
        squential pattern
 2. For each item b is appear, add to α for
    constructing a sequential pattern α', and
    output α';
 3. For each α', construct α' projected
    database S|α', and call PrefixSpan (α',
    l+1, S|α').
```

Fig. 2. Pseudocode of PrefixSpan Algorithm

3.3. Evaluation

In order to know the performance of syatem, there are some measurements such as support, confidence, lift ratio and accuration rate.

- Support
  It is probability of frequent itemset in whole transaction. It is ratio of transaction which consists of an itemset as shown in Eq. 1[4].

$$support = P(A \cap B) = \frac{total\ transacton\ whcih\ contains\ the\ both\ \ item\ A\ dan\ B}{total\ transaction} \quad (1)$$

- Confidence
  It is measurement which shows relation between two items conditionally. Confidence is stated is Eq 2.[4].

$$Confidence, C(A \rightarrow B) = \frac{\sigma(A \cup B)}{\sigma(A)} \quad (2)$$

  Where:
  - $\sigma(A \cup B)$ = the number of itemset in all transactions
  - $\sigma(A)$ = the number of antacedent in transaction

- Lift ratio
  Lift ratio is a measure to know the strength of constructed rule of sequential pattern mining algorithm. The value of lift ratio is between 0 and unlimited. It can be defiined as below: [8]
  - If lift ratio value is less than 1, then it means that rule antacedent will be effect to negative of rule consequent.
  - If lift ratio value is equal to 1, then the rule is frequent but it is independent.
  - The others, if lift ratio value is more than 1, then it means that the rule antacedent will

be effect to positive of rule consequent. It is recommended value.

The formula of lift ratio is stated in Eq. 3 and Eq. 4.

$$Expected\ Confidence, EC\ (A \rightarrow B) = \frac{\sigma(B)}{m} \quad (3)$$

$$Lift = \frac{Confidence}{Expected\ Confidence} \quad (4)$$

where:

- $\sigma(B)$= the number of *consequent* in transaction
- m = the number of transaction

IV. METHODOLOGY

The research is conducted to find the pattern of DNA sequence for breast cancer patients. In the inial state, it is required to prepare data set of DNA sequences database, including some threshold, such as sequence length and the minimum support. Then, it is applied PrefixSpan algorithm to find the sequence pattern. In order to know the strength of pattern, it is evaluated by finding support, confidence and lift ratio values.

The sequence pattern procedure using Prefix Span algorithm is shown in Fig. 3. The initial step is the sequence numbering, which is based on transaction id and sorted by length of string. The next step is reading the database. Get the DNA sequence patterns and calculate how long a frequency of occurrence that satisfies the minimum support. The next step, the search space is based on its prefix and to find the projected database as well as the sequence pattern. Find a subset of the sequence pattern and to count its frequency. If the frequency of occurrence is greater than or equal to the minimum support, then it is added a postfix to the previous prefix. Repeat these steps recursively until there is no longer a subset of pattern sequences found.

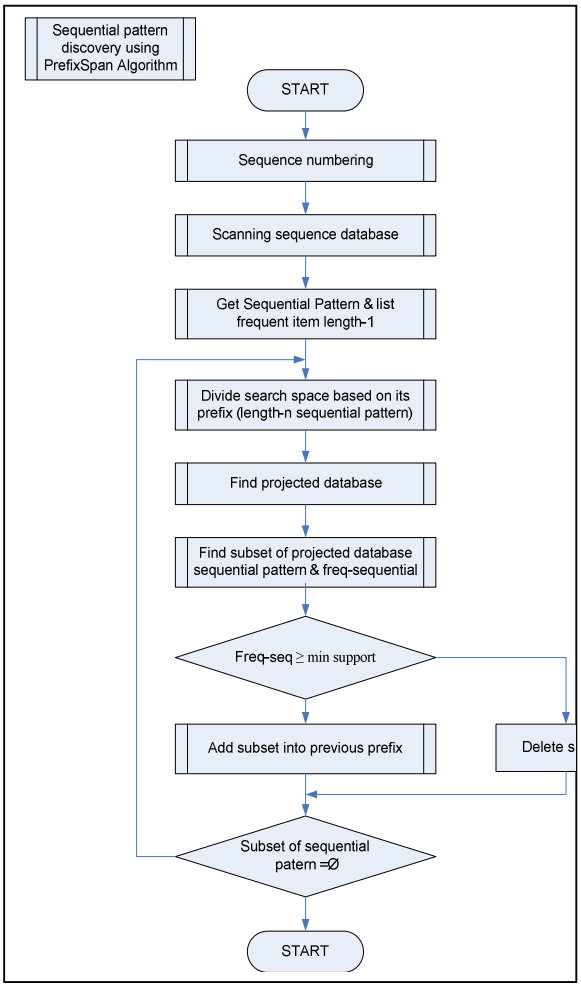

Fig.3. Flowchart of Sequential Pattern Using PrefixSpan Algorithm

V. Experimental Result and Analysis

In the experiment, it is used DNA sequence database which is taken from 7th exon of ten humans as is shown in Table 1. The Exon is a subset of DNA sequence.

Table 1. Ilustration of DNA sequence database
(Homo sapiens breast cancer anti-estrogen resistance 3 (BCAR3), transcript variant 4, mRNA

Human #	DNA sequences
1	gttggctctgactgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
2	gttggctcaggactgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
3	gttggctctgactgtaccaccatcaactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
4	gttggctcggactgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
5	gttggctctgactgccaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
6	gttggctctgactgtaccaccatcaactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
7	gttggctcggactgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
8	gttggctctgacttgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag
9	gttggctctgagctgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccag

Furthermore, the interface of application program is shown as in Fig. 4.

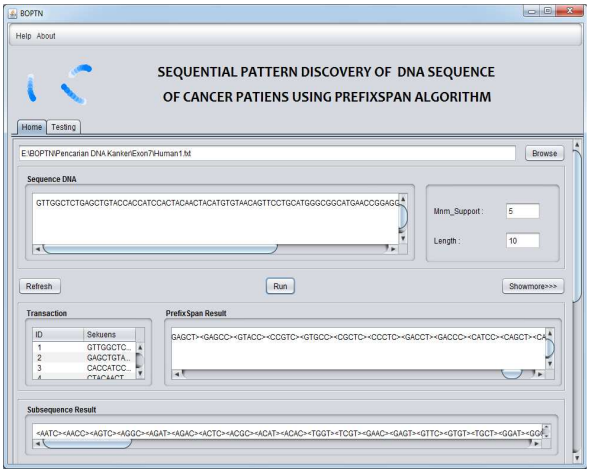

Fig. 4. Interface of Sequential Pattern Discovery Aplication Program of DNA Sequence of Cancer Patients

5.1. Experimental Result

There are several thresholds which are used such as minimum support and sequence length. As a result, it is found various patterns. As illustration, it is given minimum support count=7 and sequence length=10 which is applied to 7th exon of human#1, then the resul is as shown in Fig. 5.

<aac><gtt><ggt><ggc><gct><ctt><cgt><cgc><cct><gtac><agtc><gtgc><ctac><actc><ctgc><atcc><gatc>
<agcc><accc><catc><gacc><gtcc><cacc><ctcc>

Fig. 5. A sequencial pattern result of DNA patients

A pattern is <aac> or a→a→c which is shown in Fig.5. It means that there is pattern which is composed from sequence a is followed by a and the latest is c. This pattern is occured at least seven times in each ten sequences or transactions of the DNA sequence.

5.2. Dicussion and Analysis

In order to know performance of the system, it is evaluated based on measurement of support, confidence, and lift ratio. First, the test is applied to the training data which results several DNA sequential patterns of 7th exon for human #1, human #2, human #3, human #4, and human #5. There are two evaluation scenario of data training patterns, such as various minimum support and sequence length.

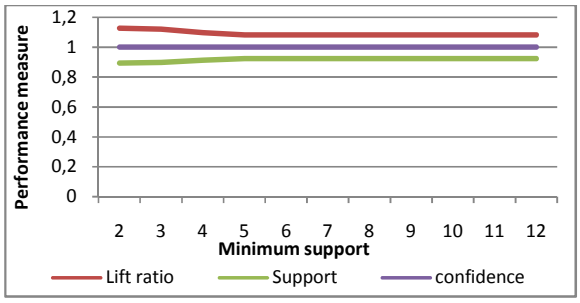

Fig.6. Performance measure of DNA sequence for human #1 (by various minimum supports)

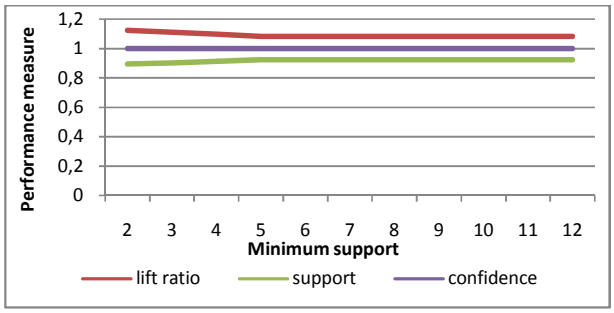

Fig 7. Performance measure of DNA sequence for human #2 (by various minimum supports)

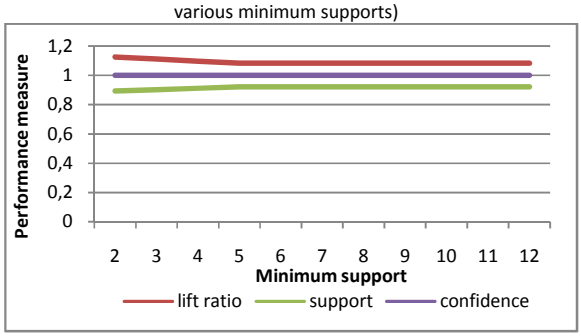

Fig. 8. Performance measure of DNA sequence for human #3(by various minimum supports)

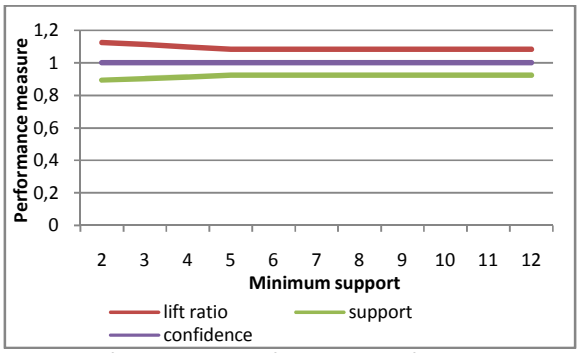

Fig. 9. Performance measure of DNA sequence for human #4 (by various minimum supports)

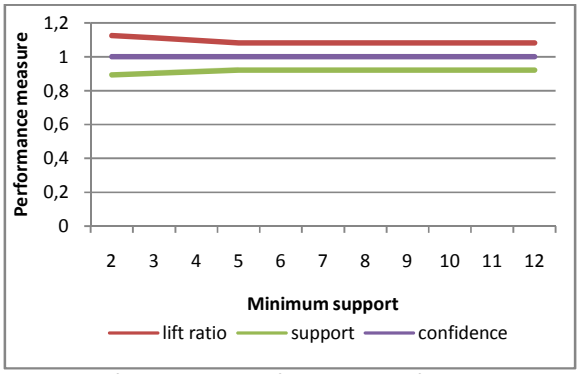

Fig. 10. Performance measure of DNA sequence for human #5 (by various minimum supports)

The five graphs in Fig. 6 up to Fig. 10 show that the performance measurement including lift ratio and support of the constructed pattern tends to be stable and the best at minimum support in the range 6 and 11 on the sequence length 10. The both measurements almost close to 1. Also, for the confidence is always at 1. It means the resulted paterns have highly strength and corrrectness

Then, the second test is using various sequence length with the same minimum support = 7. After it is applied this scenario to five humans, the various patterns are obtain with the performance results as shown in Fig. 11 up to Fig. 15. Almost all graphs on the Fig. Show that lift ratio and support is stable and the best performance at the sequence length in the range 6 up to 12. However, the confidence value is not depend on the sequence length. It is always at 1.

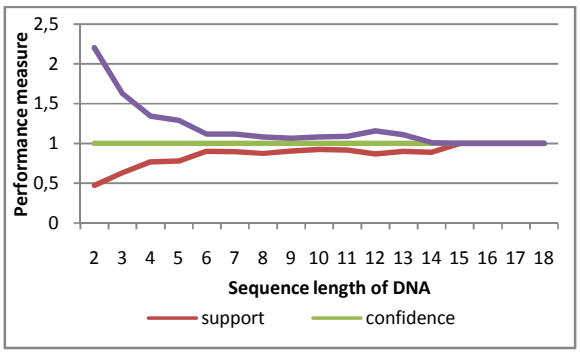

Fig 11. Performance measure of DNA sequence for human #1 (by various sequence length)

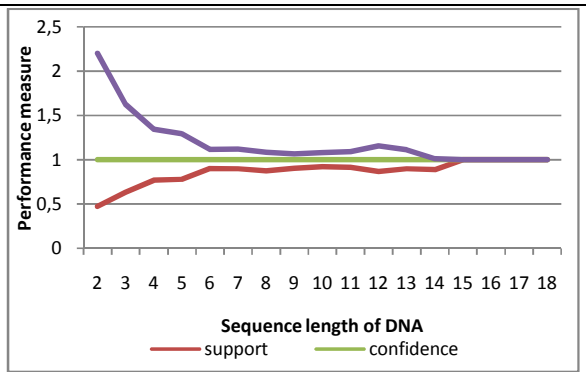

Fig.12. Performance measure of DNA sequence for human #2 (by various sequence length)

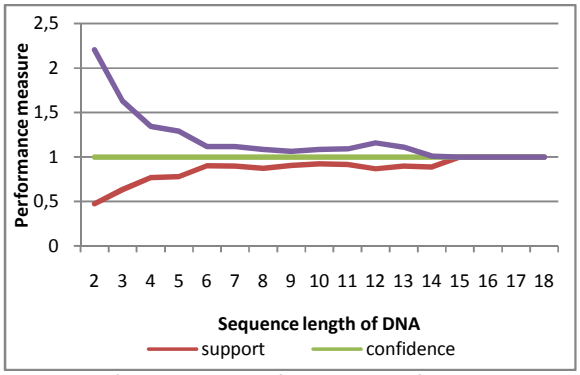

Fig. 13. Performance measure of DNA sequence for human #3 (by various sequence length)

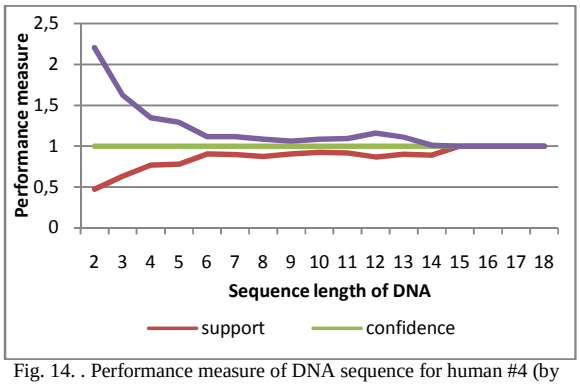

Fig. 14. . Performance measure of DNA sequence for human #4 (by various sequence length)

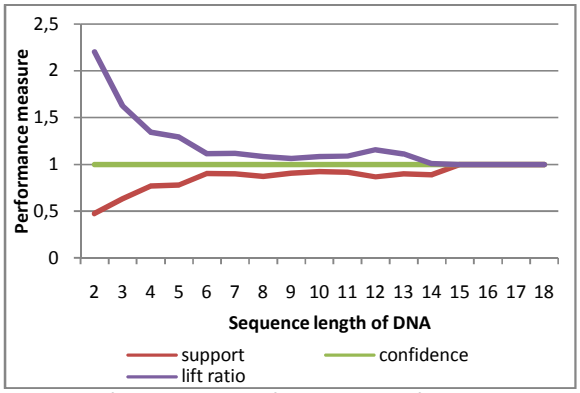

Fig 15. Performance measure of DNA sequence for human #5 (by various sequence length)

VI. CONCLUSION

Sequential pattern discovery is a method in data mining task to find the pattern on DNA sequence database of patient's cancer disease. By giving the threshold such as various minimum support and length of sequence, it is obtained the different sequence patterns. In order to know reliability of the system, it is used three performance measures, such as support, confidence, and lift ratio. The experiment results show that almost all the support value is closed to 1. It means that the probability of co-occurrence for sequence item in the pattern is high. Also, the confidence value is always 1.Furthermore, the lift ratio is almost always more than 1. This means that the patterns which consist of items are dependent each other. Therefore, the patterns can be used to define the DNA sequence characteristic of the patient's cancer desease.

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REFERENCES

[1] Pustai L., Lewis, C., & Yap, E. (1996). *Cell Proliferation in Cancer- Regulation Mechanisms of Neoplastic Cell Growth*. Oxford: Oxford University Press

[2] Thierry Soussi. 2011. *TP53 Mutations in Human Cancer: Database Reassessment and Prospects for the Next Decade*. Adv Cancer Res 110: 107-139

[3] C. Sander (2001) Bioinformatcs challenges in 2001. Bioinformatics 17, 1-2.

[4] Han, J., & Kamber, M. (2006). *Data Mining: Concepts and Techniques* (2nd ed.). San Francisco: Morgan Kaufmann Publishers

[5] Jian Pei, Jiawei Han, Behzad Mortazavi-Asl, Jianyong Wang, Helen Pinto, Qiming Chen, et al. (2004) "*Mining Sequential Patterns by Pattern Growth: The PrefixSpan Approach"*. IEEE Transaction on Knowledge and Data Engineering, vol. 16, nos. 11, pp. 1041-4347.

[6] Pei, Jiawei Han, Behzad Mortazavi-Asl, Helen Pinto, Qiming Chen, Umeshwar Dayal, et al. (2001) "*PrefixSpan: Mining Sequential Patterns Efficiently by Prefix-Projected Pattern Growth*," Proc. 2001 Int'l Conf. Data Eng. (ICDE '01), pp. 215-224

[7] Pang-Ning Tan, Michael Steinbach and Vipin Kumar. (2006). *Introduction to Data Mining*, Pearson Education

[8] Fomby, Tom. 2011. *Association Rules (Aka Affinity Analysis or Market Basket Analysis).* Departemen of Economics Southern Methodist University Dallas. Texas.

Investigation on Leaves' Response of *Putri Malu* (*Mimosa pudica* L.) Respects to Light Intensity

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***Abstract*—Investigation has been carried out to the leaves of *Puteri Malu* (*Mimosa pudica L.*) that is growing wildly in tropical countries such of Indonesia. The well-known characteristic observed from this plant is the responses of their leaves (going to close) when any external stimulation such of touch, wind pressure introduced to the plant together with hanging down the stem (petiole) from a normal position. The interesting phenomenon will be reported in this research is the movement of leaves from a close to open condition for a certain percentage when any changes of light intensity introduced meanwhile the petiole is at a normal position (hanging up). The measurement was done at room temperature (relative humidity of 63 %) using a visible camera that allows to record the leaves' movement continuously and real time. A bulp lamp that produces polychromatic spectra was used to illuminate the environment intensity of 200 lux. This spectra is aimed to mimic a spectra produced by the sun. From the observation, the leaves will open completely (100 %) from closed completely takes about 60 minutes linearly. Further potential application could be developed from this phenomenon.**

Keywords*—Mimosa pudica*, leaves' movement, sensor, petiole, polychromatic

I. INTRODUCTION

*P*uteri Malu (*Mimosa pudica* L.) is a seismonastic plant growing wildly in many tropical areas such of Indonesia. Puteri Malu is a member of species *Mimosaceace*, this plant grows up to 30 cm high and length up to 40 cm as shown in Fig. 1. Thorn growing along the branch's skin at a periodic distance [1]. Each petiole grown by many small leaves pair that could move along the axis to close and open among their pairs. The well-known phenomenon observed from the plant is the mechanical movement of leaves to close while the stem (called petiole) is hanging down in response to wind, vibration and touch as a defense mechanism for protection from animals and some insects [**2**]. After some times, the petiole will hang up back together with movement of the leaves to open up (from closed) at a normal position of petiole. The petiole movement is believed to be controlled by turgorins [**3**] by means of reducing water loss. This water molecules mobility from a certain position to another by the external stimulation is the main factor of mechanical movement of petiole.

Fig. 1. *Mimosa pudica* is growing in the nature as a wild plant in the area of Yogyakarta, Indonesia.

Study on the potential use for medicine of this plant has been done by researchers for vatious purposes. Kokane *et.al.* [**4**] have investigated the healing activity of root of *Mimosa pudica*. There are still many other works of a medical treatment that have been done using this part of plant. Beside great potential could be found from this plant. This exploration is encouraging for any treatment that could open up the technological concept of back to nature. This term is not only used by the people in producing medicine products, nutrition industries, cosmetics, and renewable energy.

Another interesting study on Mimosa pudica is the work of Volkov *et.al.* [2]. They investigated the mechanical movements of petioles induced by the electrical stimulation by a low electrical voltage and charge. From their experiment, they found that voltage and electrical charge are responsible for the electro-stimulated closing of a leaf.

Another research group has been studying the xylem vessels organization of *Puteri Malu* using X-ray CT and histological imaging [5]. In this paper we report another unique phenomenon of the leaves that will close and open along the axis respects to the change of environment intensities, meanwhile the petiole at a normal position (hang up).

II. EXPERIMENT AND METHOD

Plant of *Puteri Malu* used for the experiment was grown using a hydrophonic technique by immersing the plant together with their roots into the water in a bottle as shown in Fig. 2. The plant of health condition was used for the experiment. During the time the plant was kept in the normal environment by exposing sun light in the day when the plant (sample) was not performed for the experiment. This was done to maintain the natural habit of plant and keeping the plant for freshness in open air.

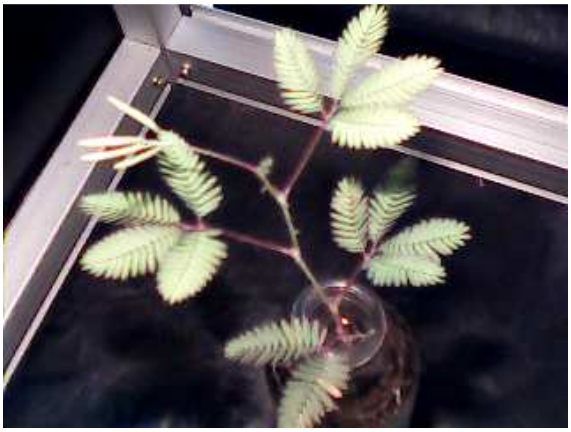

Fig. 2. Top view image of *Puteri Malu* used for the observation

For this experiment, selected observation was done onto the certain leaf from the plant to make easy and accurate the results. Some leaves were also cut it down from the plant without disturbing the natural habit of plant. The environment temperature was of 25 ºC at a relative humidity of 63 %. During the observation, the petiole of the plant was in a normal position (standing/hang up). The plant was kept in a dark box made of wood that is painted for dark / black color to minimize any scattering light may occur as shown in Fig. 3. To introduce light illumination to mimic a natural light from the sun, the bulb lamp (40 W) was used. The lamp produces a light spectrum at a visible region wavelength as this was measured using a spectrometer.

To measure the intensity of light source illuminate the environment, a lux meter was used. This meter was positioned near the leaves being measured. The distance between the light source and leaves is 18 cm. For the observation, light intensity of 200 Lux was kept constant.

Observation to leaf movement of *Puteri Malu* was done by using a visible camera mounted in the box to allow monitoring continuously and real time of any mechanical change of leaf. Change of leaf's position was recorded during the observation time. By using this method, a consistency observation could be obtained that will be calculated later for the percentage change of leaf opening.

When the leaf is open completely, the cross width of leaf printed by a black line (guided by the arrow) on the picture is used as a maximum value (reference). For any change of cross section top area of leaf (measured its cross distance) due to the observation time, the percentage was taken by dividing the value of reference.

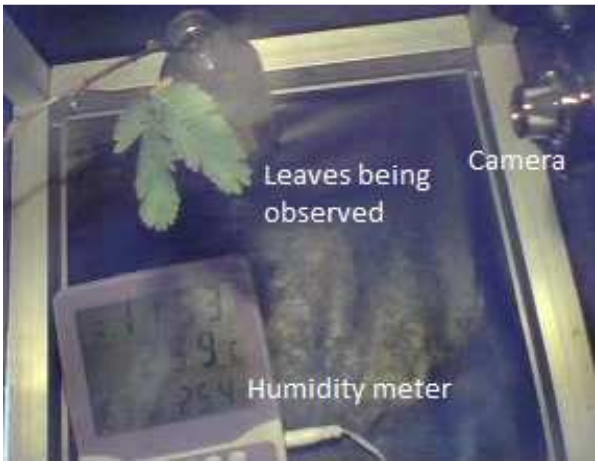

Fig. 3. Measurement set up of *Puteri Malu* in the box.

III. RESULTS AND DISCUSSION

From the measurement done to the leaves of *Puteri Malu*, the results obtained are given in Fig. 4. That figure shows some performances of leaves opening at different observation time. For calculation, pictures recorded were measured each leaf's width at a selected position consistency for all measurement. Black lines are plotted on the picture to show the axis movement between two leaves to perform closed and open. A white line printed on the leaf indicates the axis that allow pairs of individual leaf perform the movement to close and open, *vice versa*.

From the observation done to the leaf of *Puteri Malu* for the leaf closed (lamp is off) and then illumination was introduced. It was found a relationship between percentage of leaf opening and the observation time as shown in Fig. 5. The relationship is considerably linear although a few data point are miss out the curve. The initial observation as can be seen in that figure is not 0 % because when the leaves are closed, there is a width to be considered. This movement phenomenon is interesting as we can find a quantitative results and qualitative, as well to elaborate the physical phenomenon shown by the plant.

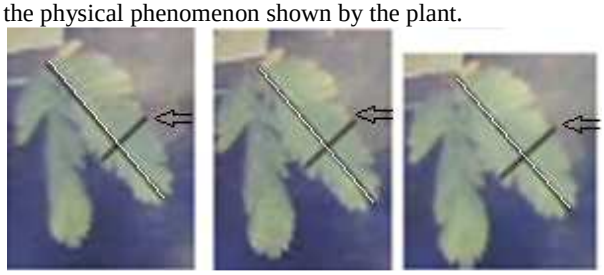

Fig. 4. Black line shows (guided by the arrows) the width of leaf (selected) when opening used to calculate the percentage opening of leaf by observation time.

The leaf starts to open after 5 minutes and then open wider until the leaf open completely. In the open completely, the percentage is observed to be 100%. The leaf performs open completely after 60 minutes light illumination from the closed condition. In this condition the leaves are not closed perfectly (notated as ~ 0 %). The interesting phenomenon found from the measurement is the linearity of the leaf change from closed to open completely. Change of leaf movement was occurred for all leaves experienced the illumination. Position of petiole in standing performance shows us that only the light intensity affect the leaf movement. Well-known phenomenon observed from this plant is closed leaf movement that will occur when any touch introduced to the plant. In this condition, the petiole is at drop position that is different position of petiole of the above phenomenon.

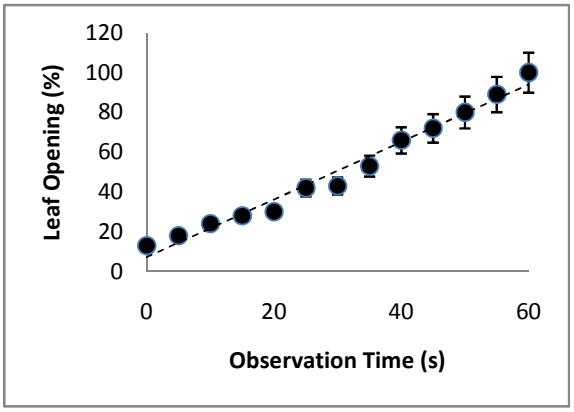

Fig. 5. Change of percentage of leaf opening respect to observation time when any light illumination introduced from dark environment.

IV. CONCLUSIONS

From the experiment done to leaf movement of *Puteri Malu*, it was obtained some interesting physical phenomenon of percentage of leaf opening from dark to light environment at 200 lux. Leaf movement to open completely was achieved after 60 minutes. This quantitative value shows any scientific explanation regarding this living plant to be used for any possible applications beside this phenomenon is encouraging for knowledge exploration particularly the potential of wild plant in tropical country of Indonesia for applications.

ACKNOWLEDGMENT

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REFERENCES

[1] Graf, A.B., 1992. *Hortica*, Roehr Co., East Rutherford, New Jersey.
[2] Volkov A.G., Foster, J.C., Ashby T.A., Walker R.K., Johnson J.A., Markin, V.S., (2010), '*Mimosa pudica*: Electrical and mechanical stimulation of plant movements, *Plant, Cell and Environment*, 33: 163–173.
[3] Ueda, M., Yamamura, S. (1999), Leaf-Closing Substance of *Mimosa pudica* L.; Chemical Studies on Another Leaf-movement of Mimosa II., *Tetrahedron Letters*, 40 : 2981 – 1984.
[4] Kokane, D.D., More, R.Y., Kale, M.B., Nehete, M.N., Mehendale, P.C., Gadgoli, C.H. (2009), Evaluation of wound healing activity of root of *Mimosa pudica*, Journal of Ethnopharmacology, 124: 311-315
[5] Lee, S.J., Song, K., Kim, H.K., Park, J. (2013). 'Microscopy Research and Technique, X-ray CT and histological imaging of xylem vessels organization in *Mimosa pudica*, 76(11) : 1204-1212.

EFFECTIVENESS OF *Eichornia crassipes* Mart (Solm.) AND *Salvinia molesta* Mitchell TO ABSORB CHROMIUM HEAVY METAL IN LIQUID WASTE OF BATIK INDUSTRY

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***Abstract*—Batik industry was one of waste producer from the coloring process. One of pollutant which brought trough the liquid waste of batik industry was heavy metal such as chromium (Cr) which known as toxic to water organism. One of waste management method which could be used is phytoremediation. There are two kinds of aquatic plant that were used in this research namely *Eichornia crassipes* Mart (Solm.) and *Salvinia molesta* Mitchell. This research were aimed to observe chromium (Cr) absorptioneffectively of E. *crassipes*, S. *molesta* and combination both of them in different concentrations of liquid waste. This research was done experimentaly by randomized block design, there were two treatments factor which were observed namely concentration of liquid waste in plants media (0%, 25%, and 50%) and the kind of plant factor, E. *crassipes*, S. *molesta* and combination both of them. This research was done with 3 repetitions so there were 27 research units. The research parameters that were observed are chromium accumulation in the root and chromium deficiency level in plants media. Hipothesist test from variance of treatments to reduce chromium level in different concentrations of liquid waste was analyzed by ANOVA and followed by Tukey test. Secondary data were temperature and pH of plants media. The result showed that the most effective treatment in chromium accumulation (Cr) is combination of *Eichornia crassipes* Mart (Solm.) and *Salvinia molesta* Mitchell. The best conditions for optimal Cr reduction was plants media with 25% concentration, with the results of the percentage reduction of 67.97%.**

***Keywords*—Eichornia crassipes, Salvinia molesta, phytoremediation, chromium**

I. INTRODUCTION

Batik industry is one of the largest liquidwaste from the dyeing process. Waste generated from the batik dyeing is non - biodegradable organic compounds, which caused environmental pollution, especially aquatic environments [1].

The content of industrial liquid waste in the form of batik organic substances, suspended solids, phenols, heavy metals such as chromium (Cr), cadmium (Cd) and lead (Pb), fat oils and dyes [2].

Cr levels in batik industrial liquid waste was 260.27 ± 2.15 ppm, this concentration has exceeded the threshold of heavy metals, which only amounted to 1 ppm. While Cd of 0.131 ppm and Pb of 0.7810 ± 0.0269 ppm, both still meet the standard of Cdis 0.1 ppm and 1 ppm of lead.

Water bodies that have been contaminated by excess Cr ions concentration can lead to death for water organism and can caused a variety of diseases, such as lung cancer, liver and kidneys damage, and other health problems such as weakened of immune system, genetic material changes, and death [3].

Waste from industrial production of batik is not processed first, but directly discharged into water bodies (water) so as to pollute the waters.Waste management is an effort that must be done right. Phytoremediation is an innovative liquid waste treatment technology. Phytoremediation can be an alternative technology in dealing with pollution, which in practice depends on a synergistic relationship between plants and naturally, microorganisms and their environment [4].

Floating plants such as water hyacinth (*Eichorniacrassipes* Mart (Solm.)) and salvinia (*Salviniamolesta* Mitchell) had a low potential economic value as phytoremediation agents [6]. Floating plants had two mechanisms in the absorption of heavy metals contained in the water bodies, there are (1) passively with the help of sunlight and transpiration, and (2) actively through the process of absorption that occurs in the plant body [7].

This research based on the ability of aquatic plants water hyacinth and salvinia to decrease levels of the Cron batik industrial liquid waste in phytoremediation process. The purpose of this research was to determine the effectiveness of the absorption of chromium heavy metals by water hyacinth, salvinia, and combinations

both of water hyacinth and salvinia on the different concentrations of batik industrial liquid waste.

I. MATERIALS AND METHODS

This research was experimental because it had a repetition of the treatment group and the controls, samples were taken at random and homogeneous. The research was conducted in Green house of C3 building, Department of Biologi, Science Faculty, State University of Surabaya in July-August 2013. Cr level analysis conducted in Chemical Laboratory of Brawijaya University, Malang and Chemistry Laboratory State University of Surabaya.

The target of this research is water hyacinth from Technological Institute of Surabaya lake and salvinia from the Dead River in Porong, and liquid waste from batik home industry of Jetis, Sidoarjo. This research used a randomized block design with two treatment factors, namely the type of plant (water hyacinth, salvinia and combinations both of them) and growing media concentration (0%, 25%, and 50%). Initial concentrations of Cr in the waste concentration of 0% = 0 ppm, 25% = 10.225ppm, and 50% = 14.531ppm. This treatment was repeated 3 times. The experiment used glass aquarium *20 cm ×30 cm ×40 cm. Heavy plant used was 80 grams. 80 grams of water hyacinth, 80 grams of salvinia and for the combination treatments were 40 grams of salvinia and 40 grams of water hyacinth.*

Research phases include the step of acclimatization, the manufacture of plants media and data retrieval. Acclimatization conducted for 10 days using distilled water in 4 -liter aquarium and giving Hoagland solution as much as 1:10. Making plants media begins by taking liquid waste in accordance with the existing concentration. Concentration of 25% for the addition of liquid waste as much as 1 liter and 3 liters of distilled water, for 50% additional 2 liters liquid waste and 2 liters of distilled water, for media 0% using 4 liters distilled water. After that, 80 grams of cultivated plants in growing media in accordance with the research design for 21 days.

Cr test on each of the roots was done after 21 days. Measured temperature and pH of the plants media everyday. Measurement of decreased levels of Cr contained in the plants media, there are the number of Cr levels before and after treatment. Measurement of Cr levels found in plants water hyacinth and salvinia before treatment, there are before the process of acclimatization. The method used to prepare the analysis of the Cr in roots was the wet destruction, then analyzed using *Atomic Absorption Spectrophotometry* (AAS).

Measurements carried out after the addition of Cr treatment, there are after exposure for 21 days, samples were taken in plants exposed to media with the addition of waste. In this research, observation of environmental factors that included was temperature and pH of the plants media.

Data obtained in the form of Cr level changes in the ANOVA test done to see the difference in each treatment, and if the results are significant, and then followed by Tukey test (honestly significant difference) to see the best treatment, while the physic- chemical factors in descriptive analyzed.

II. RESULTS

The research data presents that decreased Cr levels is the most optimal treatment with the plants media in concentration liquid waste by 25% and the combination treatment, with an average percentage 67.97 % of Cr, and Cr decrease in waste that already meet the standard quality standard (1 ppm). lowest decreased was in treatment with water hyacinth in liquid waste with concentration of 50%, a decreased percentage 35.78 and Cr contained in the liquid waste is the highest there was 4.40 ppm. Accumulation of Cr levels in various treatments was difference. In combination treatment can accumulate Cr higher than the treatment of water hyacinth and salvinia, there was increase of 6.95 ppm and 6.97 ppm. In the treatment concentration, concentration of media with 50% can absorb Cr with high accumulation respectively 5.20 ppm, 6.15, and 6.97 ppm (see Table 1).

Table 1. Average level of chromium accumulated in the root of plants decreased in plants media with varying concentrations of chromium

Kinds of plant	Consentrations of Cr in media	Mean total amount of Cr accumulation in root (ppm)	Mean total amount of Cr retained in culture media (ppm)	%-age of Cr decreased in culture media
Water hyacinth	0%	0.00±0.00	0.00±00	0
	25%	4.21±0.76	4.01±0.36	41.17
	50%	5.20±0.13	4.40±0.86	35.78
Salvinia	0%	0.00±0.00	0.00±00	0
	25%	5.46±0.48	2.12±0.13	53.40
	50%	6.15±0.06	3.38±0.01	42.32
Combination	0%	0.00±00	0.00±00	0
	25%	6.95±0.02	0.68±0.23	67.97
	50%	6.97±0.67	1.61±1.06	47.96

Note: The initial concentration of Cr in the waste concentration of 0% = 0 ppm, 25% = 10.225ppm, and 50% = 14.531ppm.

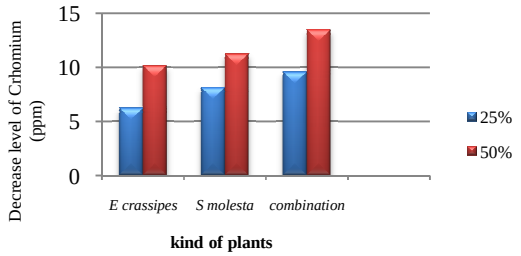

Figure 1. Average level Cr decreased in the plants media after treatment for 21 days.

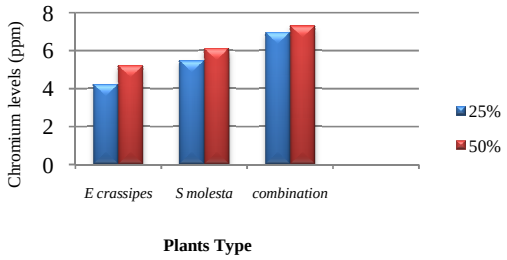

Figure 2. Average Cr accumulation in aquatic plants.

Table 3. Average of temperature in plants media

Times	Treatment		
	Control	25%	50%
Beginning	28°C	31°C	32°C
7 days	28°C	30°C	32°C
14 days	27°C	29°C	29°C
21 days	27°C	27°C	28°C

Table 3 presents the decrease of temperature in all treatments, the first 7 days of treatment the decrease of temperature is 0.333°C, in the treatment of 14 days the decrease of temperature is 27°-28°C, and the 21-day treatment the decrease of temperature is 3°C. The control treatment, the temperature changed is only on day-14, this is caused because the temperature was stabilized at control media. Temperatures on other treatments decreased and almost the same as the temperature of the control treatment.

Table 4. pH rate of the plants media at the beginning of treatment and after treatment for 21 days

	Initial pH of Plants at concentration			pH on the 21st day at concentration		
Plants type	0%	25%	50%	0%	25%	50%
Water hyacinth	7	6	5.9	7	6.56	6.43
Salvinia				7	6.63	6.53
Combination				7	6.7	6.63

Table 4 explains that pH in plants media change in 21 days. pH was increased in all treatments. Initial pH in every treatment was different, in control treatment was 7. in concentration of 25% was 6 and in concentration of 50% was 5.9. In all control treatment, pH was not changed, its stabile from beginning treatment. In concentration of 25% pH changed from 6 to 6.56 for water hyacinth, from 6 to 6.63 for salvinia and from 6 to 6.7 from combination. In concentration of 50% pH was increased. The pH changed from 5.9 to 6.6.43 for water hyacinth, from 5.9 to 6.53 for salvinia and from 5.9 to 6.63 for combination.

The longer of detention time. the greater pH plants media is. The smaller chromium levels, the greater pH is (more neutral) and greater levels of pH, the smaller chromium is (more acidic).

II. DISCUSSION

Decreased levels of Cr in liquid waste of batik is due to the absorption of chromium heavy metals by water hyacinth and salvinia. *Chromium h*eavy metals can be absorbed by plants because of differences in the concentration of chromium ions between the two types of media. there are media in plant tissue and plants media.

This concentration difference would result in a transfer or mass transfer of chromium diffusion and osmosis. wherein the high concentration of plants media will move towards the low concentration plant tissues. Water hyacinth and salvinia has an adjustment mechanism to pollutants polluted. so water hyacinth and salvinia was able to live in plants media containing chromium heavy metals [7].

Absorption and accumulation of chromium by water hyacinth and salvinia had three continuous process. namely chromium absorption by roots. translocation of Cr from the roots to the other plant parts. and localization of chromium on the part of certain cells in order not constrain the plant metabolism [8].

Cr ions with water in the xylem vessels will move in a tube toward the stem xylem vessels and eventually leading to the leaf xylem vessels [8].In xylem (xylem in leaf vascular tissue). water is drawn into the cells of the leaves and along the walls of the cell. Leaf mesophyll cells in direct contact with the atmosphere through the system extensively from intercellular airspaces. Water evaporates (evaporated) from a thin layer of air cavity. then the water vapor out of the leaf through the stomata pores [10].

Accumulation of heavy metals to plant roots through a ligand in the membrane transport roots. then it will form a complex metal transport that will penetrate the xylem and continue heading to the leaf cells (cytoplasm and tonoplasm). Transport removable and acceptor ligand metal complexes accumulate in the vacuole [11].

Increased levels of chromium in the water hyacinth and salvinia roots was caused by the accumulation in the root parts. Cr ions in form of inorganic salts absorbed by the roots that have great ability in the process of absorption of heavy metals compared with other parts of the plants body. because the roots serve as an organ of absorbing and channeling nutrients to the other parts [11]. The composition of the roots can collect particles dissolved in water [12].

Plants undergo tolerance in the way; metals accumulate in plants after forming complexes with other elements or compounds. with the help of a binder compound is one that is Phytochelatin 2-8 peptides

containing the amino acid cysteine in the center of the molecule and glutamic acid and glycine at the end of the opposite. Phytochelatin function forms a complex with heavy metals in plants and serves as the detoxification of heavy metals on plants. Phytochelatin formed in nucleus which then passes through the endoplasmic reticulum (ER), golgi apparatus, secretors vesicular to get to the cell surface. When met with Cr and other heavy metals Phytochelatin will form bonds at the end of the sulfide sulfur in cysteine and form a complex that Cr compounds and other heavy metals will be carried to the plant tissue [7].

Each plant has a different ability to absorb and survive in a variety of heavy metals. Especially in places contaminated with more than one type of metal. There are certain species called hyper-accumulator plants that absorb much higher amount of pollutants than other species. Hyper-accumulator plants capable of concentrating metals in the biomass in unusually high levels. Most plants accumulate metals such as nickel at 10mg/kg dry weight (DW) (equivalent to 0.001%). but the metal hyper-accumulator plants able to accumulate up to 11% from dry weight (DW). Heavy metal content limits contained in the biomass of a plant that can be called hyper-accumulator vary depending on the type of heavy metal [13]. Hyper-accumulator plants to absorb chromium heavy metals capable of ≥ 1000mg/kg. This caused the plant is still fresh and experienced good growth during the research (current exposure) [14].

In conjunction with the use of plants as agents of recovery polluted environment, there are presents the prerequisites namely accumulation rate should be high even at low environmental levels of contaminants, the ability to accumulate high levels of contaminants, the ability to accumulate several kinds of metals, grows rapidly, high biomass production, and pest and disease resistant [9].

Both water hyacinth dan salvinia had met the prerequisites as good remediator plants. The difference in the absorption effectiveness of both types of plant roots is caused by the more amount of salvinia root fibers, form the roots meet the water column for each node are formed so that the roots can absorb mineral nutrients and metals chromium by weight more than water hyacinth. Root fibers in water hyacinth is considerably less than the number of salvinia root fibers.

Physical environment factors affect plant life and the rate of chromium accumulation. such as pH and water temperature. pH of water affect biochemical processes in water [15]. At the beginning of treatment before planted aquatic plants, pH of the medium at a concentration of 25% at 6 and at 50% concentration at pH 5.9 both show that acidic pH values because many contain heavy metals chromium. But after 21 days of treatment the pH to increase, in each approaching neutral pH at 6.56, 6.43, 6.63, 6.53, 6.63, and 6.7. This is due to the high pH levels causing chromium ion binds with H_2O to form H_2 + CrO. Cr ions in the water then turned into CrO [16].

Increased pH also occurs because of the bond between the ligand that is alkaline with acidic ion Cr. Effect of pH raising is directly decrease the solubility of heavy metals, because pH raising alters the stability of the carbonate form into hydroxide which forms a bond with the particles in the liquid waste batik[16], pH raising causes precipitated of heavy metals [17].

Inside the roots plant changes the pH by the roots [18]. The pH increase caused by the process of photosynthesis, denitrification, organic nitrogen breakdown and sulfate reductions [19]. The present of water hyacinth dan salvinia in the plants media, pH becoming increasing as the results of research, in each decade the pH of media increased from acidic to near - neutral. These things indicated that the presence of aquatic plants can give a good effect on the environment. Remedioator plants have a positive role in the remediation process, one of which is a driving factor and facilitator of microscopic organisms increase the efficiency of biodegradation of pollutants [20].

In addition the pH changed, temperature is also changing. Temperature decreased in all plants media after treatment. The decreased in temperature caused by the direct interaction of plants that give the effect of microclimates in the rhizosphere. The growth rate of plants running at maximum speed when environmental factors favor, namely pH and temperature were the optimum conditions (suitable), so absorbtion of Cr would be more optimal [21].

III. CONCLUSION

The research concluded that the treatment is most effective in absorbing chromium heavy metals is a combination treatment both of water hyacinth (*Eichornia crassipes* Mart (Solm.)) and salvinia (*Salvinia molesta* Mitchell). The best conditions for optimal Cr reduction was plants media with 25% concentration, with the results of the percentage reduction of 67.97%. In other treatments showed accumulation of Cr in lower level, there were in treatment of water hyacinth and salvinia in the 50% concentration of plants media.

REFERENCES

[1] Sumat. 2012. *Bioremidiasi Logam Berat Timbal (Pb)*. http://repository.usu.ac.id. (2012. December 12).

[2] Decree ofHead Governor DIY No. 281/1998. 1998. *Baku Mutu Limbah Cair Untuk Industri Tekstil*. http://bapedalda.go.id.(2012. December 11).

[3] Nikarita P. 2011. *Kromium*. http://biodiversitas.mipa.uns.ac.id. (2013. February 13).

[4] Siregar UJ. Chairil AS. 2010. *Fitoremediasi: Prinsip dan Prakteknya Dalam Restorasi Lahan Paska Tambang di Indonesia*. http://data.dppm.uii.ac.id. (2012. December 11).

[5] Eddy S. 2009. Pemanfaatan Teknik Phytoremediation pada Lingkungan Tercemar Timbal (Pb). *MASA Bina Kampus Bina Umat*. 2: 6-13.

[6] Eddy. 2008. *Pengolahan Eceng Gondok sebagai Bahan Baku Tas*. http://balittanah.litbang.deptan.go.id. (2012. December 11).

[7] Puspita UR. Asrul SS. Nuning VH. 2011. Kemampuan Tumbuhan Air Sebagai Agen Fitoremidiator Logam Berat Kromium (Cr) yang Terdapat pada Limbah Cair Industri Batik. *Jurnal Berkala perikanan terubuk*. 39: 58-64.

[8] Sallysbury FB. Ross CW. 1995. *Fisiologi Tumbuhan Jilid I*. Bandung: ITB.

[9] Priyanto. Prayitno. 2012. *Fitoremediasi Sebagai Sebuah Teknologi Pemulihan Pencemaran. Khususnya Logam Berat*. http://ltl.bppt.tripod.com. (2013. February 11).
[10] Boyer JS. 1970. Leaf enlargement and metabolic rates in corn. soybean. and sunflower at various leaf water potentials. *Plant Physiology*. 46: 233–235.
[11] Fitter AH. Ray KH. 1991. *Fisiologi Lingkungan Tanaman*. Jogja: Gadjah Mada University Press.
[12] Widyningsih. 2012. *Pengaruh Variasi Biomassa Eceng Gondok (Eichornia crassipes) Terhadap Kandungan Krom (Cr) Limbah Cair Industri Sablon "Temenan" Monjali Yogyakarta*. *Skripsi*. Unpublished. Yogyakarta: Universitas Negeri Yogyakarta.
[13] Baker AJM. Reeves RD. Hajar ASM. 1999. Heavy metal accumulation and tolerance in British populations of the metallophyte Thlaspi caerulescens J.&C. Presl (Brassicaceae). *New Phyto*. 127: 61-68.
[14] Rahma A. 2013. *Penyebaran Tanaman Hyper-accumulator*. http://ayurahma9298.wordpress.com. (2013. November 13).
[15] Fitriyah. 2011. Potensi Kayu Apu (*Pistia stratiotes* L.) Sebagai Bioabsorber Timbal (Pb) Dalam Air. *Skripsi*. Unpublished. Surabaya: State University of Surabaya.
[16] Palar H. 2008. *Pencemaran dan Toksikologi Logam Berat*. Jakarta: Rineka Cipta.
[17] Norpian LS. 2011. Teknik Uji Cepat untuk Identifikasi Pencemaran Logam Berat Tanah di Lahan Apel Batu. *Thesis*. Published. Malang: Brawijaya University.
[18] Tsabitul MF. Aunurohim. Tutik N. 2013. Efektivitas Kombinasi *Salvinia molesta* dengan *Hydrilla verticillata* dalam Remediasi Logam Cu pada Limbah Elektroplating. *Jurnal Sains dan Seni POMITS*. 2: 240-245.
[19] Kholidiyah N. 2010. Respon Biologis Tumbuhan Eceng Gondok sebagai Biomonitoring Pencemaran Logam Berat Cadmium (Cd) dan Plumbum (Pb) pada Sungai Pembuangan Lumpur Lapindo. Kecamatan Porong. Kabupaten Sidoarjo. *Skripsi.* Unpublished. Malang: Islam State University of Maulana Malik Ibrahim.
[20] Syahputra R (2005). Fitoremediasi Logam Cu dan Zn dengan Tanaman Eceng Gondok (*Eichhornia crassipes* (Mart) Solms.). *Logika*. 2: 147-152.
[21] Subahar TSS. *Biologi*. Jakarta: Quadra

PRODUCTIVITY AND STABILITY OF CASSAVA PROMISING CLONE

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***Abstract*—The aim of the study was to know the productivity and stability of cassava promising clones. Analysis of stability is based on the regression. The experiments were done on mineral soil in nine environments in Pati, Probolinggo, Malang, Lampung Timur, Lampung Tengah and Lampung Selatan during 2009-2012. The experiments were done using a randomized complete block design, three replications. The plot size was a 5 m x 5 m. Plants distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P_2O_5 + 60kg K_2O/ha. The clones used were CMM 03001-10, CMM 03020-2, BIC 556, BIC 499, BIC 280, BIC 180, CMM 03013-11, and CMM 02048-6, as promising clones, as well as UJ3 as released varieties. Parameter recorded was fresh tuber yield of seven month old plants. The study showed that all clones tasted were stable clones based on regression. Mean of fresh tuber yield of CMM 03001-10 over locations and years was the highest (31.75 t/ha), 11 % higher than UJ3, equal to IDR 2,377,500,-/ha, if the price of cassava tuber is IDR 750,-.**

***Index Terms*—cassava promising clones, fresh tuber yield, productivity, stability**

I. INTRODUCTION

SELF suficiency in food is the major problem that is often discussed in excecutive institute and legislative institute. One of ways that can be done to solve the that problem is by promoting the food diversification programs. Cassava plants are potential plants for the food diversification programs. The productivity of cassava in farmer level is still low. Increasing productuvity can be done by using the best variety. The number of released varieties are limited for the farmer, so development of new varieties should be promoted. There are eleven released varieties of cassava, some of them are Adira 1 and UJ3. Adira1 and UJ3 are varieties that can be harvested earlier than the others. Adira 1 was released in 1978, this variety is not bitter. UJ3 was released in 2000. Until now, Adira 1 was still planted by farmers in Magetan, and Sampang, and many farmers plant UJ3 in Lampung.

In Indonesia, cassava is planted in dry areas with varied environmental condition. When some clones are tested in different environments, then the result will be two possibilities. The first, the ordinal of the yield of clones tested is the same between environments. The second, the ordinal of the yield of clones tested is different between environments. Most cassavas in Indonesia are planted in Eat Java, Lampung, Central Java, and West Java. Type of land of East Java, Lampung, West Java is dry land, while that of lampung is acid dry land. Howeler [1] reported that most cassavas in Indonesia are grown in Alfisol 25%, Ultisol 22%, Entisol 20%, and Inceptisol 18%. In East Java and Central Java, most cassavas are planted in C2, C3 and D3 climate type which there are 2-6 dry months. In Wesat Java and Lampung, most cassavas are planted in B and C2 climate type which there are 2-3 dry months.

There are a few methods to analyze the stability of genotypes for multi-locations trials, one of them is method that was proposed by Eberhart and Russel [2] Many scienties used that method for analysis of stability, few of them are Adie *et al.* [3], Rasyad *et al.*[4], Sumarno and Sutisna [5], and Lestari *et al.* [6].

Some promising clones have been resulted from previous cassava breeding activities. These clones are needed to be tested in various locations/environments conditions before releasing the superior ones as new varieties. The aim of this study was to know the productivity and stability of cassava promising clones.

II. MATERIALS AND METHODS

The experiments were conducted in nine environments in Pati, Probolinggo, Malang, Lampung Timur, Lampung Tengah and Lampung Selatan during 2009-2012. The experiments were done using a randomized complete block design, three replications. The plot size was a 5 m x 5 m. Plants distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P_2O_5 + 60kg K_2O/ha. The clones used were CMM 03001-10, CMM 03020-2, BIC 556, BIC 499, BIC 280, BIC 180, CMM 03013-11, and CMM 02048-6, as promising clones, as well as UJ3 as released varieties. Parameter recorded was fresh tuber yield of seven month old plants. Tuber yield was analyzed using MSTAT (Michigan Statistic), version C software (released by Michigan State University) to obtain the combined analysis of variance. Stability analysis based on the technique of Eberhart and Russel (1966) was used.

III. RESULTS AND DISCUSSION

Analysis of variance for the 9 clones, six locations, 2009-2012 for fresh tuber yield are shown in Table 1. The clones x locations interaction were significantly different for fresh tuber yield. Because the clones x locations interaction were significant, so analysis of stability of clones should be done to know the stability and adaptability. Genotype interaction with environment produces phenotype that is natural law. This phenomenon was also reported by Sholihin [7] and [8], and Sundari *et al.* [9].

Tuber yield of clones during 7 months in Lampung Tengah 2011 ranged from 23.60 – 42.47 t/ha, with mean of 31.51 t/ha (Table 2). Tuber yield of UJ3 was the highest. Tuber yield of CMM 03001-10 were similar to UJ3. Tuber yield of clones in Lampung Timur 2011 range 21.38 – 30.13 t/ha, with mean 26.09 t/ha. Tuber yield of CMM 02048-6 was the highest, however they were not different significantly. Tuber yield of clones in Lampung Selatan 2011 ranged from 22.03 – 33.33 t/ha, with mean of 29.15 t/ha. Tuber yield of UJ3 was the highest. Tuber yield of CMM 03001-10, CMM 03020-2, BIC 556, BIC 499, BIC 280, BIC 180, and CMM 03013-11 were similar to UJ3. Tuber yield of clones in Probolinggo 2011 ranged from 17.16 – 31.51 t/ha, with mean 25.28 t/ha. Tuber yield of CMM 03001-10 was the highest. Tuber yield of CMM 03020-2, BIC 556, BIC 180, CMM 02048-6, UJ3 were similar to CMM 03001-10. Tuber yield of clones in Pati 2010 ranged from 26.7 – 35.76 t/ha, with mean 31.56 t/ha. Tuber yield of CMM 03020-2 was the highest. Tuber yield of CMM 03001-10, CMM 03020-2, BIC 499, BIC 280, BIC 180, and CMM 03013-11 were similar to CMM 03020-2. Tuber yield of clones in Malang 2011/12 range 29.41 – 45.36 t/ha, with a mean of 37.93 t/ha. Tuber yield of BIC 280 was the highest. Tuber yield of CMM 03001-10, CMM 03020-2, BIC 499, and BIC 180 were similar to BIC 280. Tuber yield of clones in Lampung Timur 2010 range 19,81 – 26.67 t/ha, with mean 22.79 t/ha. Tuber yield of CMM 03001-10 was the highest, however they were not different significantly. Tuber yield of clones in Lampung Tengah 2010 range 14.14 – 29.2 t/ha, with a mean of 19.10 t/ha. Tuber yield of CMM o3001-10 was the highest. Tuber yield of BIC 180 was similar to CMM 03001-10. Tuber yield of clones in Lampung Selatan 2010 range 11.10 – 21.7 t/ha, with a mean of 19.06 t/ha. Tuber yield of UJ3 was the highest. Tuber yield of CMM 03001-10, CMM 03020-2, BIC 556, BIC 499, BIC 280, and BIC 180 were similar to UJ3.

The mean of the fresh tuber yield of CMM 03001-10 over locations and years was the highest (31.75 t/ha), and it was significantly different to UJ3. A range of the fresh tuber yield, mean of the fresh tuber yield, mean square of standard deviation (S_{di}^2), and coefficient of regression of cassava promising clones are shown in Table 3. The mean of the fresh tuber yield of CMM 03001-10 over locations and years was the highest (31.75 t/ha), 11 % higher than UJ3, equal to IDR 2,377,500,-/ha, if the price of cassava tuber is IDR 750,.

Mean square of standard deviation (S_{di}^2) of all clones tested were not significantly different from zero (0), a coefficient of regression (bi) of all clones/varieties tested were not significantly different from one (1). So, all clone/varieties tested were stable clones. There are two possibilities in which cassava can buffer to varying environment conditions. One is that a clone is a hybrid, and second that it has genetic potential to perform well irrespective of the environment where they are grown. Sholihin [10] reported that environmental factors which are important in determining stability of cassava clone/variety based on the tuber yield in nine months were soil pH of subsoil, maximum air temperature in 1 month after planting, the minimum air humidity in four months after planting, number of rainy days in 1 and 2 months after planting, total rain fall 9 months after planting, the minimum air temperature 3 months after planting.

Information on HCN content is important in determining the appropriate use of cassava. Cassava varieties with HCN content > 50 ppm is not suitable for fried or steamed cassava. Sholihin [11] reported that HCN content of CMM 03001-10 was 35.34 ppm, mean that this variety can be used for fried or steam cassava. In addition, the color of tuber of CMM 03001-10 is white, so it is suitable for the fried cassava chips. In terms of resistance to mites, CMM 03001-10 was moderate resistant to mite reported by Sholihin *et al.* [12]. Red mites are an important insect pest in Cassava especially in Java.

TABLE I
COMBINED ANOVA FOR 9 CASSAVA CLONES, 6 LOCATIONS, 2009-2012. FOR TUBER YIELD

Source	Degrees of Freedom	Mean Squares
Environment (E)	8	1053.238**
Error (a)	18	78.83
Clones (C)	8	185.000**
C x E	64	42.014*
Error (b)	144	29.835
Coefficient Variation (%)		20

TABLE II
FRESH TUBER YIELD (T/HA) OF CASSAVA CLONES/VARIETIES IN NINE ENVIRONMENTS, 2010-2012.

No	Clone/variety	Lampung Tengah 2011	Lampung Timur 2011	Lampung Selatan 2011	Probolinggo 2011
1	CMM 03001-10	41.84 a	26.71 a	32.29 a	31.51 a
2	CMM 03020-2	23.60 c	24.08 a	27.71ab	24.36 abc
3	BIC 556	28.85 bc	24.88 a	31.20 a	29.25 ab
4	BIC 499	30.42 bc	21.46 a	30.05 ab	22.14 bc
5	BIC 280	30.17 bc	28.04 a	30.21 ab	20.85 bc
6	BIC 180	33.85 ab	28.88 a	29.60 ab	29.64 ab
7	CMM 03013-11	27.44 bc	21.38 a	25.92 ab	17.16 c
8	CMM 02048-6	24.97 c	30.13 a	22.03 b	24.65 abc
9	UJ3	42.47 a	29.25 a	33.33 a	27.93 ab
	mean	31.51	26.09	29.15	25.28

V**= 1 % significantly different

TABLE 2
CONTINUED

No	Clone/variety	Pati 2010	Malang 2011/12	Lampung Timur 2010	Lampung Tengah 2010
1	CMM 03001-10	33.80 ab	42.75 ab	26.67 a	29.2 a
2	CMM 03020-2	35.76 a	41.74 abc	19.81 a	15.76 b
3	BIC 556	26.70 b	34.39 bcde	22.68 a	19.95 b
4	BIC 499	27.65 ab	42.94 ab	20.56 a	20.08 b
5	BIC 280	32.67 ab	45.36 a	21.73 a	19.58 b
6	BIC 180	34.13 ab	40.30 abcd	22.32 a	20.72 ab
7	CMM 03013-11	34.68 ab	27.41 e	24.50 a	15.91 b
8	CMM 02048-6	30.09 ab	33.63 cde	22.97 a	14.14 b
9	UJ3	28.59 ab	32.82 de	23.89 a	16.60 b
	mean	31.56	37.93	22.79	19.10

V**= 1 % significantly different

TABLE 2
CONTINUED

No	Clone/variety	Lampung Selatan 2010	Mean
1	CMM 03001-10	20.94 a	31.75 a
2	CMM 03020-2	19.58 ab	25.82 cd
3	BIC 556	19.59 ab	26.39 bcd
4	BIC 499	17.29 ab	25.84 cd
5	BIC 280	21.70 a	27.81 bc
6	BIC 180	19.64 ab	28.79 b
7	CMM 03013-11	11.10 b	22.83 e
8	CMM 02048-6	19.33 ab	24.66 de
9	UJ3	22.39 a	28.58 bc
	mean	19.06	26.9
	C.V. (%)		20

V** = 1 % significantly different

TABLE 3
MEAN OF THE FRESH TUBER YIELD, MEAN SQUARE OF STANDARD DEVIATION, AND COEFFICIENT OF REGRESSION OF CASSAVA PROMISING CLONES

No	Clone/variety	Mean of the fresh tuber yield t/ha	Coefficient of regression $(b_i)^a$	Mean square of standard deviation $(S^2d_i)^b$
1	CMM 03001-10	31.745	0.989 ns	**-13.700 ns**
2	CMM 03020-2	25.822	1.191 ns	**-13.270 ns**
3	BIC 556	26.386	0.728 ns	**-23.189 ns**
4	BIC 499	25.842	1.188 ns	**-19.690 ns**
5	BIC 280	27.812	1.220 ns	**-18.298 ns**
6	BIC 180	28.787	1.079 ns	**-27.292 ns**
7	CMM 03013-11	22.832	0.908 ns	**-3.626 ns**
8	CMM 02048-6	24.658	0.789 ns	**-14.040 ns**
9	UJ3	28.584	0.908 ns	**0.334 ns**

Note : a. ns : not significantly different from 1,
b. ns : not significantly different from 0,

REFERENCES

[1] Howeler, R.H. 1992. Agronomy research in the Asia cassava network - an overview 1987-1990, *In* Howeler (Ed.), Cassava breeding, agronomy and utilization research in Asia, Proc. 3rd regional workshop held in Malang, Indonesia, Oct 22-27 1990. CIAT, Thailand. p.260-285.

[2] Ebberhart, S.A. and Russel, W.L. Stability parameters for comparing varieties, *Crop Sci*. 6, pp. 36-40. 1966.

[3] Adie,M.M, H Soewanto, T Agus, J.S. Wahono,G.W.A. Susanto, dan N Saleh. 2010. Potensi hasil, stabilitas dan karakter agronomic galur haraan kedelai berbiji besar. Jurnal Penelitian Pertanian Tanaman Pangan. 29 (1), pp. 9-20, 2010.

[4] Rasyad, A., G.M.E. Manurung, and D.A.Van Sanford. 2011. Genotype x environment interction and stability of yield components among rice genotypes in Riau Province, Indonesia. SABRAO Journal of Breeding and Genetics 18(1), pp 102-111, 2011.

[5] Sumarno and E. Sutisna. Identification of rice (Oryza sativa L.) varieties suitable for dry season and wet season planting. Indonesian Journal Agricultural Science. 11(1), pp. 24-31, 2010.

[6] Lestari, A.P., B. Abdullah, A. Junaedi, and H. Aswidinnoor. Performance of grain quality and aroma of aromatic new plant type promising rice lines. Indonesian Journal Agricultural Science. 12(2), pp. 84-93, 2011.

[7] Sholihin. The genotypes x environment interaction for starch yield in nine months of cassava promising clones. Indonesian Journal Agricultural Science. 10(1), pp 12-18, 2009.

[8] Sholihin, AMMI model for interpreting clone - environment interaction in starch yield of cassava. HAYATI Journal of Bioscience. 18 (1), pp 21-26, 2011.

[9] Sundari T, K. Noerwijati, and IMJ Mejaya. Hubungan antara komponen hasil dan hasil umbi klon harapan ubikayu. Jurnal Penelitian Pertanian Tanaman Pangan. 29 (1), pp. 29-35, 2010.

[10] Sholihin. Analisis interaksi genotipe x lingkungan untuk hasil ubi ubikayu dengan model AMMI. Proseding seminar nasional, Pemuliaan berbasis potensi dan kearifan lokal menghadapi tantangan global, Peripi Komda Banyumas, Totok A *et al.* (Ed.), pp.510-520, 2011.

[11] Sholihin. Produktivitas klon-klon harapan dan varietas ubikayu umur genjah di Pati serta responnya terhadap busuk umbi. Proseding seminar nasional 2011, Implikasi teknologi budidaya tanaman pangan meuju kemandirian pangan nasional, Fakultas Pertanian Universitas Muhammadiyah Purwokerto, Watemin *et al.* (Ed.), pp.680-686, 2011.

[12] Sholihin, N. Saleh, B.S. Radjit, T. Sundari, T.S. Wahyuni, dan I.M.J Mejaya. Perakitan varietas dan perbaikan sistem produksi ubikayu umur genjah, sesuai untuk pangan dan industri dengan potensi hasil 40 - 60 t/ha. 2011

Shoot Multiplication and Rooting Induction in *Carica pubescens* Lenne & K. Koch (Mountain Papaja)

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***Abstract*—An efficient micropropagation protocol in *Carica pubescens* Lenne & K. Koch known as an endemic species at Dieng highland and has a great commercial importance has been developed. In the present study the influence of growth regulator of culture medium on shoot multiplication and rooting induction was investigated. For shoot multiplication, lateral shoot explant was chosen. The research was carried out using a completely randomized factorial design with two factors. Different concentrations of 6-benzylamino purine (BAP) (1.0, 2.0, 3.0 and 4.0 µM) and gibberelic acid-3 (GA3) (0.1, 0.2, 0.3, and 0.4 µM) were added to Murashige and Skoog (MS) medium. The best shoot multiplication was showed on MS medium containing 2.0 µM of BAP and 0.3 µM of GA3. After 3 months of culture shoot initiation has been observed in all explants with 5.3 number of shoot per explant, and 3.9 cm shoot height. Further shoot multiplication in successive subcultures was also possible. For rooting induction, the research was conducted using a completely randomized design with one factor. Medium MS supplemented with 2 ppm (9.8 µM) of 3-indolebutyric acid (IBA) proved to be the best one for rooting of shoots, producing root initiation at 16 days after rooting, 4.50 number of roots per shoot and 7.57 cm root length. This protocol opens up the prospect of *in vitro* conservation of this endemic species.**

***Keywords*—*Carica pubescens*, Root Induction, Shoot multiplication,**

I. INTRODUCTION

Carica pubescens Lenne & K. Koch (syn. *Vasconcellea pubescens*) belongs to the Caricaceae. Currently, only about 33 thousands of *C. pubescens* plants were cultivated in Dieng (private communication with district government of Wonosobo, Central of Java, 2011), mainly concentrated at altitude of 2400 m above sea level like Sembungan and Sikunang village. These villages are few areas where *C. pubescens* could grow well and bears fruit optimally with a high antioxidant capacity [1]. *C. pubescens* possesses great economic potential due to their benefits as food and medicine materials. Its habitus is similar to *C. papaya* but the fruits are smaller than it (Fig. 1a) and have a strong characteristic flavor. The fruit mainly used to produce preserved fruit. An additional feature of this species is its ability to produce latex with a high level of papain, an important and valuable proteolytic enzyme in industrial and pharmaceutical [2].

Because of the economic potential and associated with its extensive usability, germplasm preservation is important strategy to guarantee not only the conservation, but also their sustainable use. *In vitro* conservation needs a large quantity of
shoots which can be induced to form roots.

This study aims to develop *in vitro* shoot multiplication and root induction protocol of *C. pubescens*. The multiplication of shoot and induction of root are greatly influenced by plant growth regulators content in the medium. Increase in volume associated with shoot multiplication and adventitious root growth must be the result of either cell division or cell enlargement, or both. Cytokinin was believed as division cell regulator and auxin known to be involved in cell enlargement. The requirement of type and concentration of each of plant growth regulator varies with different species [3]. Previous study showed that addition of auxin and cytokinin into Murashige and Skoog (MS) (1962) medium was not effective in shoot multiplication of *C. pubescens* (data no shown). Therefore, this study would examine the use of gibberellins (especially gibberelic acid-3 or GA3), and cytokinin (especially 6-benzylamino purine or BAP) in inducing shoot multiplication. The roles of GA3 related to photomorphogenesis are in stem elongation and leaf differentiation [3].

Auxin has been recognized as a controlling factor in rooting induction since a long time [4]. Of the several types of synthetic auxin, indole butyric acid (IBA) was more effective for producing roots of woody plants than the others [5].

II. MATERIALS AND METHODS

Shoot multiplication

The objectives of this step study were to examine the effects of BAP and GA3 supplementing into MS medium on shoot multiplication of *C. pubescens*and and to determine the concentration of BAP and GA3 capable of proliferating shoot maximally.

Laterally shoots of 2 to 3 cm long of stem branch (diameter 1 cm) were obtained from selected plants 5 years old tree growing in Sikunang village, Dieng. These trees were selected on the basis of their healthy, number and characteristics of fruits. The shoot explants were washed with soft detergent and tap water, followed by 3-4 rinses with distilled water. To prevent browning, the explants were soaked in 150 ppm ascorbic acid solution, and then soaked in 70% alcohol for 3 minutes. The surface disinfectant of explants was accomplished by dipping explants in 6% (v/v) solution of commercial bleach for 15-20 minutes with 0.1% tween 20. Afterwards, the explants were rinsed five times with autoclaved distilled water under a laminar air flow (LAF) cabinet which had been sterilized by UV irradiation for 30 minutes and spraying 70% alcohol. Before planting in the multiplication medium, sterile explants were grown on MS medium without growth regulators to control contamination for 4 days, and then transferred to a basal medium, that was MS medium supplemented with 2µM BAP, 0.5µM IBA and0.3µMGA3.

Effects of adding concentration of BAP and GA3 in the MS medium on growth and multiplication of *C. pubescens* shoot were carried out in a completely randomized factorial design experiment with two factors, that were concentration of BAP and GA3. Each of the experimental unit consisted of two shoots, cultured in one culture vial (Fig. 1b). Four replicates were prepared for each combination of the BAP and GA3 concentration.

The explants were, aseptically, placed on agar-solidified MS medium containing 3% sucrose varied in plant growth regulators combinations. MS medium were supplemented with a combination of BAP and GA3 at concentrations of 1.0, 2.0, 3.0, and 4.0 µM and 0.1, 0.2, 0.3 and 0.4 µM, respectively. All media were adjusted to pH 5.8 at 25°C with KOH and HCl and were autoclaved for 20 minutes at 121°C (15 PSI nominal steam pressure) after the addition of growth regulators as required. As much as 25 ml medium was poured into each of the culture vial. The cultures were incubated in a 24 h light at 15 ± 2°C and were illuminated with fluorescent light at 1000 lux. The explants were cultured in each for a total of three months period, and were transferred onto fresh medium every month.

Shoot multiplication responses recorded were percentage of explants growing shoots, number of axillary shoots formed from explant, shoot length (cm), and number of leaves per shoot. Data were statistically analyzed in a completely randomized design with four replicates. The data were analyzed by Analysis of Variance and Duncan's multiple ranges test (DMRT) using SAS statistical analysis program System for Windows 9.0.

Root Induction

The aims of this second step study were to examine the effect of IBA supplementing into MS medium on root induction of *C. pubescens* and to determine IBA concentration capable of inducing root maximally.

Effects of IBA supplementing into MS medium on root induction was carried out in a completely randomized design experiment with one factor, that was concentration of IBA. Each of the experimental unit consisted of one shoot, cultured in one culture vial. Eight replicates were prepared for each of the IBA concentration. To induce roots from the selected shoots, all regenerated shoots (2-3 cm in height and with 1-2 leaves) were selected and cultured on MS medium supplemented with 2 ppm, 4 ppm, 8 ppm IBA for 1 week. After cultured on root induction on MS-IBA medium, the shoots were transferred onto MS medium added by active charcoal until they develop into plantlets.

Root induction responses recorded were time of root emergence, root length (cm), and root number. Data were statistically analyzed in a completely randomized design with four replicates. The data were analyzed by Analysis of Variance and DMRT using SAS statistical analysis program System for Windows 9.0

III. RESULTS AND DISCUSSION

Shoot multiplication

BAP concentrations in MS medium greatly influenced multiplication of *C. pubescens* shoot explants. This cytokinin type at 2-4 µM concentration resulted in good responses of explants for shoot induction (87.5-100%). The maximum shoot number per explant, shoot length and leaf number per shoot were observed at 2 µM BAP. The medium supplemented with BAP more than 2 uM inhibited shoot multiplications (Tab. 1). The inhibition of shoot multiplication was in line to the result obtained in *Phaseolus angularis* that its bud growth and shoot multiplication were stimulated by reducing the BAP concentrations from 5.0 to 2.5 µM after 3 weeks [6].

Cytokinins have been proved to overcome apical dominance, release lateral buds from dormancy and promote shoot formation [3]. Stimulation of multiple shoot formation by BAP has been reported in several plant species, including *Ocimum basilicum* [7], *Galax urceolata* [8], and *Aloe vera* [9].

TABLE 1
EFFECT OF PLANT GROWTH REGULATORS (BAP AND GA3 ALONE) ADDED INTO MS MEDIUM ON SHOOT MULTIPLICATION OF *C. pubescens*

PGR	Percentage of explants growing shoots (%)	Shoots number/ explant	Shoot length (cm)	Leaves number / shoot
B1	62.5 b ± 21.6	1.32bc ± 0.56	3.05 b ±0.55	2.4 c ±0.44
B2	100.0 a ± 0	2.32 a ± 1.67	3.42 a ±0.39	3.8 a ±1.35
B3	87.5 a ± 21.6	1.57 b ± 0.57	2.90 bc ±0.43	2.0 d ±0.64
B2	87.5 a ± 21.6	1.57 b ± 0.57	2.82 bc ±0.37	2.1 d ±0.54
G1	87.5 a ± 21.6	1.50 b ± 0.53	2.55 c ±0.45	2.0 d ±0.17
G2	87.5 a ± 21.6	1.65 b ± 0.52	3.07 b ±0.13	2.6 c ±0.21
G3	100.0 a ± 0	2.15 a ± 0.53	3.07 b ±0.52	2.9 b ±1.34
G4	62.5 b ±21.6	1.50 b ± 0.84	2.90 bc ±0.51	2.5 c ±1.51

- Data represent mean ± standard error of two explants per treatment in four replicated experiments. Means within a single column

followed by the same letter are not significantly different according to DMRT at p = 0.05

- PGR (plant growth regulators) treatment: B1, B2, B3 and B4: concentration of BAP 1 µM, 2 µM, 3 µM and 4 µM; G1, G2, G3 and G4: concentration of GA3 0.1 µM, 0.2 µM, 0.3 µM and 0.4 µM respectively

Supplemented of GA3 into MS medium had also significant effect on shoot multiplication of *C. pubescens*. The GA3 at 0.1-0.3µM concentration resulted in good response of explants for shoot multiplication (87.5-100%). The maximum shoots number per explant and leaves number per shoot were observed at 0.3 µM GA3 (Tab. 1). Gibberelins have showed a broad spectrum of physiological effect in plants. Because of their role in cell wall formation and cell expansion they involved in dormancy breakage [10], lateral branching and young leaf growth [11]. This result was in accord with finding from previous study on *Lotus corniculatus* L. [12].

The lower and higher GA3 concentrations than 0.3 µM in MS medium were ineffective to induce shoot multiplication (Tab. 1). As a phytohormone, GA3 promotes the growth optimally at a relatively low concentration, so at below and above of the optimum concentration, the growth will be inhibited [3].

Of the 16 combinations of BAP and GA3 tested, the combination of BAP 2 µM + GA3 0.3 µM and BAP 2 µM + GA3 0.4 µM-treated explants achieved highest regeneration than those other combinations. The two combinations of treatments yielded maximum regeneration (100%) and maximum number of multiple shoots (3.0-5.3 shoots per explant). Shoots developed in this medium also were longest (3.6-3.9 cm) and generated maximum number of leaves (4.7- 5.3 (Tab. 2, Fig. 1c).

TABLE 2
EFFECT OF GROWTH REGULATORS (BAP AND GA3 COMBINATION) ADDED INTO MS MEDIUM ON SHOOT MULTIPLICATION OF *C. pubescens*

PGR	Growing shoot (%)	Shoot number / explant	Shoot length (cm)	Leaves number
B1G1	50 b ± 17.7	1.0 c ± 0.71	2.2 c ± 1.18	1.7 c ± 0.43
B1G2	50 b ± 0.0	1.0 c ± 0.71	3.0ab ± 0.37	2.3bc ± 0.43
B1G3	100a ± 0.0	2.3 b ± 0.83	3.3 a ± 0.39	2.3bc ± 0.83
B1G4	50 a ± 17.7	1.0 c ± 0.00	3.7 a ± 0.36	3.0 b ± 0.71
B2G1	100a ± 0.0	1.7 b ± 0.43	3.0ab ± 0.35	2.0 c ± 0.71
B2G2	100a ± 0.0	1.3 c ± 0.43	3.0ab ± 0.46	2.7 b ± 0.43
B2G3	**100a** ± 0.0	**5.3 a** ± 0.43	**3.9 a** ± 0.15	**5.3 a** ± 0.83
B2G4	**100a** ± 0.0	**3.0 a** ± 0.71	**3.6 a** ± 0.28	**4.7 a** ± 0.83
B3G1	100a ± 0.0	1.0 c ± 0.00	2.0 c ± 0.21	2.0 c ± 0.71
B3G2	100a ± 0.0	2.3 b ± 1.09	3.0ab ± 0.41	2.7 b ± 0.69
B3G3	100a ± 0.0	2.0 b ± 0.71	2.6 b ± 0.46	2.3bc ± 0.83
B3G4	50 b ± 17.7	1.0 c ± 0.00	2.0 c ± 0.82	1.0 d ± 0.71
B4G1	100a ± 0.0	2.3 b ± 0.43	3.0ab ± 0.62	2.3bc ± 0.83
B4G2	100a ± 0.0	2.0 b ± 0.71	3.3 a ± 0.46	2.7 b ± 0.83
B4G3	100a ± 0.0	1.0 c ± 0.00	2.7 b ± 0.45	2.0 c ± 0.71
B4G4	50 b ± 17.7	1.0 c ± 0.00	2.3 c ± 0.38	1.3cd ± 0.43

- Data represent mean ± standard error of two explants per treatment in four replicated experiments. Means within a single column followed by the same letter are not significantly different according to DMRT at p= 0.05
- PGR (plant growth regulators) treatment: B1, B2, B3 and B4: concentration of BAP 1 µM, 2 µM, 3 µM and 4 µM; G1, G2, G3 and G4: concentration of GA3 0.1 µM, 0.2 µM, 0.3 µM and 0.4 µM respectively

Fig.1 The habitus of *Carica pubescens* and *in vitro* shoot multiplication. (a) A *C. pubescens* plant grown at Dieng. Bar = 15 cm (b) Two explants after cultured on multiplication medium for 1 month. Bar = 0.7 cm. (c) High rate of shoot multiplication on MS medium supplemented with BAP 2 µM + GA3 0,3 µM after cultured 3 months. Bar = 0.5 cm.

Previous finding have also indicated the role of BAP and GA3 in *Rosa* shoot multiplication. The highest multiplication rate of *R. canina* and *R. rubiginosa* (4.1 shoots per one explant) and *R. dumalis* (2.9 shoots per one explant) was obtained when shoots were multiplied on an MS medium supplemented with 1 µM BA and 1.5 µM GA3 [13].

Although it was difficult to quantify healthy of shoot but we have observed that shoots obtained from explant cultured in MS medium supplemented with the optimally concentrations of cytokinin and gibberelin (BAP 2 µM + GA3 0.3 µM or BAP 2 µM + GA3 0.4 µM) were generally healthier (better vigor) than those obtained from the other concentrations. This indicates that multiplication response depends upon the type and concentration of plant growth regulator used.

Root Induction

Elongated shoots (3–4 cm) were excised and placed on full-strength MS medium supplemented with various concentrations of IBA for induction of roots. These media gave developed roots within 16–30 days. Application of IBA at 2 ppm resulted fastest rooting than the higher concentration (Tab. 3). Supplementing of IBA at 2 ppm into MS medium might have triggered the early anticlinal cell division and root primordia formation than higher concentration [14].

TABLE 3
EFFECT OF IBA CONCENTRATIONS IN MS MEDIUM ON ROOTING INDUCTION OF *C. pubescens*

IBA concen-tration (ppm)	Root emergence (days)	Root induction (%)	Root number	Root length (cm)
2	16.75 a ± 1.71	100 ± 0	4.50 a ± 0.93	7.57 a ± 1.43
4	24.88 b ± 0.92	100 ± 0	2.25 b ± 0.43	2.18 b ± 0.57
8	29.75 c ± 0.85	100 ± 0	1.50 b ± 0.50	1.27 b ± 0.36

Data represent mean ± standard error of one explant per treatment in eight replicated experiments. Means within a single column followed by the same letter are not significantly different according to DMRT at p= 0.05

All of the shoot explants capable to generate roots at a high frequency (100%) in MS medium containing 2, 4

and 8 ppm IBA, but in medium containing 2 ppm IBA the number of roots was highest (4.50) The greater IBA concentration, the lower number of roots (Tab. 3, Fig. 2). The result showed that auxin is essential to induce rooting in the *C. pubescens* microcutting as no rooting was observed in the absence of IBA (data not shown).

IBA produced the longest roots (7.57 cm) also at concentration of 2 ppm (Tab. 3, Fig. 2). The results also revealed that root length tend to reduce with higher than optimum concentration of IBA. Like other developmental processes, root cell elongation involves sequential changes in levels and/or activity of enzymes. The enzymes involved in cell enlargement processes are triggered by the auxin [3].

Indole-3-acetic acid (IAA) is the most abundant naturally or endogenous auxin and IBA is classified as a synthetic auxin [3]. Process of root formation in cuttings involves the activity of peroxidase, IAA oxidase, and phenolics [15]. Exogenous IBA induce changes in activities of peroxidase and IAA oxidase and in contents of phenolics allowing the establishment of the favourable endogenous hormone balance. The metabolism of the exogenous IBA, especially its combination with phenolic compounds, has been considered in relation to the promotion of adventitious rooting [16].

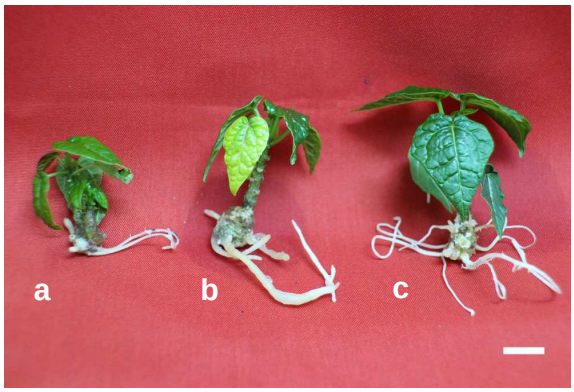

Fig 2. Formation of roots of shoots cultured in MS medium supplemented with (a) 8 ppm, (b) 4 ppm, and (c) 2 ppm IBA. Bar = 1 cm

Compared to stems, roots may require a less concentration of auxin to grow [17]. Therefore root growth is strongly inhibited by higher level of auxin because at high level, it induces the production of ethylene, a root growth inhibitor [18], and induces the higher level of degradative metabolites in tissues which blocking the regeneration process [19]. Besides these, specific exogenous IBA to endogenous IAA ratio may be important for plant development [20], and the application of exogenous IBA of 2 ppm might shift the balance to promote root development of *C. pubescens*.

The stimulatory effect of IBA of root formation has been reported in many other plant species. Exogenous IBA had a significant positive effect on the rooting responses of woody plants such as date palm [21], apple [22], and olive cultivar 'Moraiolo' [23].

IV. CONCLUSION

Supplementing BAP and GA3 into MS medium proved to be suitable plant growth regulators for multiplication of *C. pubescens*. The best shoot multiplication of *C. pubescens* was achieved in MS medium containing 2.0 µM of BAP and 0.3 µM of GA3. IBA 2 ppm were also proved to be the best rooting hormone in term of root emergence rate, root number and root length. This protocol can be used to regenerate a large number of rooting shoots as materials of *C. pubescens in vitro* conservation.

REFERENCES

[1] A.N. Laily, Sunarto, and Sugiarto, "Characterization of Carica pubescens in Dieng Plateau, Central of Java based on morphology, antioxidant capacity and protein binding pattern," *Bioscience*, vol. 4, no. 1, pp. 16-21, March 2012.

[2] B. Carrasco, P. Avilla, J. Prez-Diaz, P. Munoz, R. Garcia, B. Lavandero, A. Zurita-Silva, J.B. Retamales, and P.D.S. Caligari, "Genetic´ structure of highland papayas (*Vasconcellea pubescens* (Lenne´ et C. Koch) Badillo) cultivated along a geographic gradient in Chile as revealed by Inter Simple Sequence Repeats (ISSR)," *Genet Resour Crop Evol*, vol. 56, pp. 331–337, August 2008.

[3] L. Taiz and E. Zeiger, "*Plant Physiology*" 5th ed., Sunderland, Massachusetts, USA: Sinauer Associates Inc., Publishers, 2010, ch. 19, 20, 21.

[4] E. Tanimoto, "Regulation of root growth by plant hormones: Roles for auxin and Gibberellin," *Crit. Rev. Pl. Sci.*, vol. 24, no. 4, pp. 249-265, 2005.

[5] J. Wynne and M.S. McDonald, "Adventitious root formation in woody plant tissue: The influence of light and indole-3-butyric acid," *In vitro Cell Dev. Biol.*, vol. 38, no. 2, pp. 210-212, 2002.

[6] S.V. Mohamed, J-M. Sung, T-L Jeng, C-S Wang, "Organogenesis of *Phaseolus angularis* L.: high efficiency of adventitious shoot regeneration from etiolated seedlings in the presence of N6-benzylaminopurine and thidiazuron," *Plant Cell Tiss Organ Cult*, vol. 86, pp. 187–199. July 2006.

[7] S. Saha, T. Dey, and P. Ghosh, "Micropropagation of *Ocimum kilimandscharicum* Guerke (Labiatae)," *Acta Biologica Cracoviensia Series Botanica*, vol. 52, no. 2, pp. 50–58, 2010.

[8] G. Yang, X. Shen, R. Jackson, and Z. (C) Lu, "Factors affecting *in vitro* seed germination and shoot proliferation of galax *[Galax urceolata* (Poir.) Brummitt]". *Aust. J. Crop Sci.* vol. 7, no. 11, pp. 1766-1771, 2013.

[9] S. Zakia, N.J. Zahid, M. Yaseen, N.A. Abbasi, A.A. Hafiz and N. Mahmood, "Standardization of micropropgation techniques for Aloe vera: A pharmaceutically important plant," *Pak. J. Pharm. Sci*., vol.26, no.6, pp.1083-1087, Nov. 2013.

[10] A.K. Huttly & A.L. Phillips, "Gibberellin-regulated plant genes," *Physiol. Plant*., vol. 95, pp. 310–317, 1995.

[11] C.M Fleet and T. Sun, "A DELLAcate balance: the role of gibberellin in plant morphogenesis," *Current Opinion in Plant Bio.*, vol. l, 8, pp. 77–85, 2005.

[12] R. Nikolic, N. Mitic, S. Ninkovic , B. Vinterhalter, S. Zdravkovic´-Korac, and M. Nes˘kovic´, "Gibberellic acid promotes *in vitro* regeneration and shoot multiplication in *Lotus corniculatus* L.," *Plant Growth Regul*, vol. 62, pp. 181–188, August 2010.

[13] B. Pawłowska, "The effect of BA and GA3 on the shoot multiplication of *in vitro* cultures of Polish wild roses," *Folia Hort*., vol. 23, no.2, pp. 145-149, 2011.

[14] J.L. Muller, "Indole-3-butyric acid in plant growth and development," *Plant Growth Reg*., vol. 32, no. 2-3, pp. 219-230, 2000.

[15] E. Caboni, M.G. Tonelli, P. Lauri, P. Iacovacci, C. Kervers, C. Damiano, and T. Gaspar, "Biochemical aspects of almond microcuttings related to *in vitro* rooting ability," *Biologia Plant*, vol. 39, pp. 91-97, 1997.

[16] A. Qaddoury and M. Amssa, "Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date Palm offshoots," *Bot. Bull. Acad, Syn* . vol. 45, pp. 127-131, 2004.

[17] M. Kollmeier, H.H. Felle and W.J. Horst, "Is basipetal auxin flow involved in inhibition of root elongation," *Pl. Physiol*., vol.

122, pp. 945-956, 2000.
[18] J.F. Hausman, "Changes in peroxidase activity, auxin level and ethylene production during root formation by poplar shoots raised," *In vitro. Plant Growth Reg.*, vol. 13, no. 3, pp. 263-268, 2003.
[19] C.M. Baker and H.Y. Wetzstein, "Influence of auxin type and concentration on peanut somatic embryogenesis," *Plant Cell Tiss. Organ Cult.*, vol. 36, no. 3, pp. 361-368, 2004.
[20] E. Epstein and J. Ludwig-Mu¨ller, "Indole-3-butyric acid in plants: occurrence, synthesis, metabolism, and transport," *Physiol. Plant*, vol. 88, pp. 382–389. 1993.
[21] A. Qaddoury and M. Amssa, "Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date Palm offshoots," *Bot. Bull. Acad, Syn.*, vol. 45, pp. 127-131, 2004
[22] T. Sharma, M. Modgil, and M. Thakur, "Factors affecting induction and development of *in vitro* rooting in apple rootstock", *Indian J. of Exp. Biology*. Vol 45, pp 824-829, Sept. 2007.
[23] A. Ali A, T. Ahmad, N.A. Abbasi, I.A. Hafiz, "Effect of different concentrations of auxins on *in vitro* rooting of olive cultivar Moraiolo", *Pak. J. Bot.* vol 41, no 3, pp 1223-1231, 2009.

SODIUM CARBOXYMETHYL CELLULOSE (Na-CMC) FROM CORN COB

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Abstract*—In Indonesia, corn is an agricultural commodity which is also the second staple food after rice. Corn's grain is mainly used, while the corn cob is thrown away. Actually, the corn cob is potential due to its cellulose content which can be reach 45%. This cellulose can be converted to sodium carboxymethyl cellulose (Na-CMC). Na-CMC is one of cellulose derivatives that are widely used in food, pharmaceuticals, cosmetics, paints, detergents, ceramics and paper industries. It is used as a thickener, emulsion or suspension, stabilizer and as a binder. Na-CMC can be synthesized from plant cellulose through alkalization and carboxymethylation process, in which NaOH and sodium chloroacetate (SCA) was used as reagents. This study applied variation concentration of the reagents i.e NaOH 20, 30, and 40% (w/v), whereas SCA concentration such as 48, 60, and 72% (w/v). A 15 g of cellulose was dissolved in 300 mL of isopropyl alcohol. Alkalization process was undergoing for 2 hour at temperature 27 ^{o}C. While carboxymethylation reaction was conducted for 3 hour at temperature 55 ^{o}C. The reaction provided 27.25% of corn cobs cellulose and 73.88% yield after extraction. The process was optimum using 20% (w/v) of NaOH and 72% (w/v) of SCA. It produced Na-CMC with 58.61% purity, yield 133.44 gram Na-CMC/cellulose; pH of a solution 1% Na-CMC 7.87; degree of substitution 1.001; and viscosity of solution 2% Na-CMC 1163cP.

***Keywords*—corn cobs, cellulose, Na-CMC, alkalization, carboxymethylation**

I. INTRODUCTION

Sodium carboxymethyl cellulose or sodium carboxymethyl cellulose (Na-CMC) as shown in Figure 1 is one of the cellulose derivatives which widely used in the food industries, pharmaceuticals, cosmetics, paints, detergents, ceramics and paper (Winarto, 1995). The use of the Na-CMC is as a thickener, stabilizer and emulsion or suspension as a binder. Na-CMC can be synthesized from plant cellulose (Achmadi in M. Nurnayny, 2008) by substituting the hydroxyl groups on the anhydroglucose unit of the cellulose with a group carboxymethyl of monochloroacetic acid or sodium chloroacetate through several stages of reaction i.e alkalization and carboxymethylation (Kirk & Othmer, 1967).

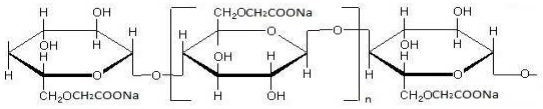


Figure 1. The molecular structure of Na-CMC

Cellulose-containing plants such as rice, beans, wheat and corn are as potential raw material for Na-CMC. The corn cobs still have a fairly high content of lignocellulosic i.e 45% cellulose, 35% hemicellulose and 15% lignin (Howard et al, 2003). Therefore, it is possible that potential corn cobs as raw material Na-CMC, so as to expand the utilization of corn processing.

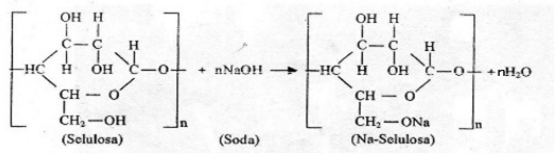
Cellulose is a polymer of carbohydrates or polysaccharides which is composed of units of the formula $C_6H_{10}O_5$ anhydro glukopyranose. Cellulose bounds by ß-1, 4 glycosidic form linear polymer chains with a uniform chain structure. Hemicellulose is a polymer that is structurally similar to cellulose (homologenus). Hemicellulose is a branched molecule that contains only 150-200 monomer (Mulcahy, 1996 in the Ambriyanto, KS, 2010). Lignin is an organic material of plant cell wall constituent of high-level, predominantly in the carrier's network (Glazer and Nikaido, 2007). Lignin as a biomass is composed of units called lignol, consisting of aryl propanol arranged on aromatic compounds and three-carbon chain. Lignol is structurally highly related to the amino acids phenylalanine and tyrosine. Lignol is a derivative of the amino acids phenylalanine and tyrosine. Lignin is more hydrophobic than cellulose and hemicellulose (Ahmed et al., 2001).

Things to consider in the manufacture of Na-CMC are alkalization and carboxymethylation reactions because these reactions will determine the characteristics of the resulting Na-CMC. Alkalization reaction is done before carboxymethylation using NaOH. It will activate -OH groups in cellulose molecules and serves as a developer. This will facilitate the deployment of cellulosic carboxymethylation reagent diffusion. The process uses reagent Sodium Chloroacetate (SCA). The amount reagent used SCA will affect the substitution anhydroglucose units in cellulose (Pribadi T., 1985). Increasing the amount of alkali used may increase dissolved SCA, thus simplifying and accelerating the diffusion of SCA to the reaction center i.e hydroxy group (Yuniarti and Setiawan, 1998). The role of both

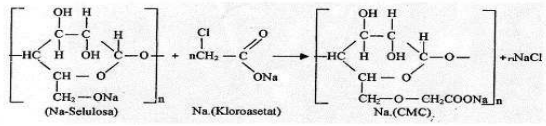
carboxymethylation and alkalization reagent in this process will determine the quality of the resulting Na-CMC (Y. Setiawan, 1990).

Therefore, this study examined the optimization of reaction conditions in the manufacture of cellulose Na-CMC corn cobs. Optimization of reaction conditions was done by varying the amount reagent NaOH and SCA on alkalization reaction and carboxylation reaction. The reaction of Na-CMC manufacture of which is as follows (Kirk & Othmer, 1967):

Alkalyzation:



Carboxymethylation



Side reaction

$nClCH_2COONa$ + nNaOH → $nHOCH_2$-COONa + nNaCl

Na.(Choloracetate) (soda) Na-Glycolate

II. METHODOLOGY

The research was carried out in several stages: raw material preparation, corn cob delignification, cellulose extraction and Na-CMC production. Na-CMC production was done through the stages of alkalization, carboxymethylation, neutralization, filtration, purification and drying. Corn cobs powder was used as raw materials for alkalization reaction. Calculation the yield of cellulose derived from corn cobs and the yield of Na–CMC were done as well. Analysis Na-CMC characteristics include purity, pH, solution 1 % Na-CMC, degree of substitution, viscosity of 2 % solution of Na-CMC and water content.

Corn cob cellulose with a weight of 15 g was mixed with 300 ml of 2-propanol, while 10 g NaOH in 50 ml of distilled water was prepared. They were together reacted for 2 hours (27^{o}C) while kept stirring. Then the mixture was mixed with 24 g of SCA that has dissolved in 50 ml of distilled water and let the reaction for 3 hours (55^{o}C) while kept stirring. Then neutralization is done by adding acetic acid to a pH of 7. The mixture was filtered to separate the residue and the filtrate. The residue was purified by adding 100 ml of methanol, then it was dried at 60^{o}C temperature for 2 hours to form Na-CMC .

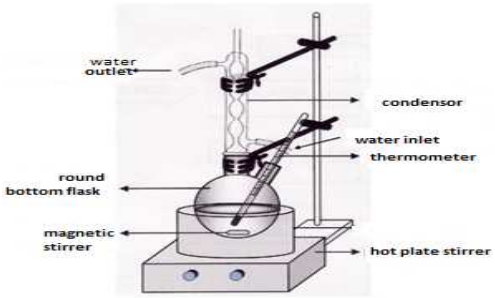


Figure 2. Schematic of experimental tools

III. RESULTS

In this research, Na-CMC manufacture of corn cob cellulose by using a variation of the use of reagents and reaction alkalization reaction carboxymethylation ie NaOH (20%, 30%, 40% w / v) and SCA (48%, 60% 72% w / v). Alkalization reaction was carried out at a temperature of 27 ° C for 2 hours while carboxymethylation reaction was carried out at 55 ° C for 3 hours. The study began with producing cellulose which was the raw material of manufacture of Na-CMC. Cellulose was obtained from corn cobs through a two-stage separation process, the stage delignification and cellulose extraction stage. Before going through the stages of corn cob was dried and grinded into to 60/80 mesh.

The yield of cellulose obtained by comparing the weight of gained cellulose by the weight of used corn cobs powder. In this stage the yield obtained was 23.73% cellulose.

Where the cellulose obtained from the separation process to obtain holocellulose from delignification stage and phase extraction to obtain cellulose. The research has isolated 27.25% corn cobs cellulose and 73.88% after extracted. Corn cob size reduction of up to 60/80 mesh was aimed to expand the raw material contact surface with reagents. Size reduction causes physical and chemical changes in the polymer cellulose. These changes will lead to changes in the molecular structure as well as a reduction in the degree of polymerization (Irrawaddy in Resita , 2006).

Cellulose is a long chain polymer molecules that have a strong structure and high molecular weight, this causes the low solubility of cellulose. The first phase, 60/80 mesh corn cobs powder was delignified using NaOCl to remove lignin which was still attached to the cob of corn . Size reduction and delignification caused the dissolution of a long polymer chains into shorter polymer chains and separate the lignin from the cellulose .

At this stage NaOCl was chosen as a solvent because of NaOCl containing hypochlorite ions which are capable to break carbon bonds and the structure of lignin. Soaking the corn cobs in 1 % NaOCl for 5 hours at room temperature was to dissolve the lignin .

Cellulose extraction was done with the addition of NaOH . NaOH here served to extract hemicellulose resulting in separation of cellulose with hemicellulose . NaOH was used by 15 % (%w/v) due to the concentration

of NaOH can properly separate the cellulose from hemicellulose.

Purity of Na-CMC is generally allowed is in the range of 65% - 99.5% (SNI, 1995). Purity of Na-CMC shows the use of Na-CMC produced for the purposes of food or non-food. In this study, the purity of the resulting Na-CMC is determined using the procedures of ASTM D 1439-72 and the results can be seen in Figure 3.

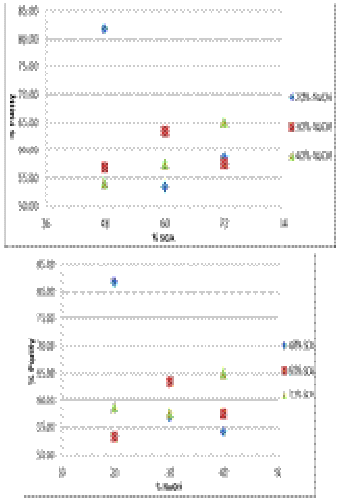


Figure 3. The effect of the addition of NaOH and SCA to the purity of Na-CMC

From Figure 3, it can be seen that the purity of Na - CMC produced by the addition of each reagent SCA and reagent NaOH, It had a great purity value when using 20 % NaOH and 48 % SCA. This suggests that the product formed is Na - CMC with the presentation of 81.84 % purity. Then after the addition of reagents SCA, purity becomes down and this may be due to the formation of side products is directly proportional to the use of excess NaOH and SCA reagents where these reagents could not react further with existing cellulose. Using 30 % NaOH, purity value has increased 60 % in the use of SCA and fell back in line with addition of SCA reagent. Using 40 % NaOH, purity has increased with addition of SCA, but purity is still less value compared to the value of purity in use 20 % NaOH, and 48% SCA.

From Figure 3, the purity NaCMC for each reagent addition of NaOH while SCA fixed, showed declining values. This impairment seen from start NaOH reagent usage growing even though there is an increase at some point, but not significant and tend to fall. In general it can be seen that the purity of Na - CMC produced ranges between 53%-81%. This suggests that the utility of Na - CMC for non-food purposes.

NaOH reagents used in the alkalization reaction serves to activate the - OH groups in cellulose molecules and serve as developer of cellulose molecules. This will facilitate the deployment of cellulosic SCA reagents to diffuse and substituting anhydroglucose units in cellulose because the bonds between cellulose molecules, especially the hydrogen bonds weakened and disconnected to later replaced with carboxymethyl groups. Increasing the amount of alkali used may increase dissolved SCA thus simplifying and accelerating the diffusion of SCA to the reaction center i.e -OH group .

The purity (53% -81%) is rather low, indicating that there were other products besides Na-CMC formed of two reactions. By products of this reaction include NaCl formed due to the reaction between Na-cellulose with SCA and adverse reaction of NaOH and the SCA which did not react with cellulose. Besides these side reactions resulting from Na-glycolic. Cellulose content of 73.88% indicates that there is other components in the raw material. The other components include hemicellulose or lignin insoluble cellulose separation process from raw materials. Lignin and hemicellulose can react with the reagents NaOH and SCA during alkalization reaction and karboksimetilasi reactions to form other by-products that can reduce the purity of Na-CMC.

From Figure 4. it can be seen that with the addition of reagents SCA on the use of a fixed NaOH reagent, yielding Na-CMC is decreasing in use (30% NaOH, 72% SCA) and (30% NaOH, 72% SCA) and the rest showed a trend of increasing mainly be seen in the use of 40% NaOH.

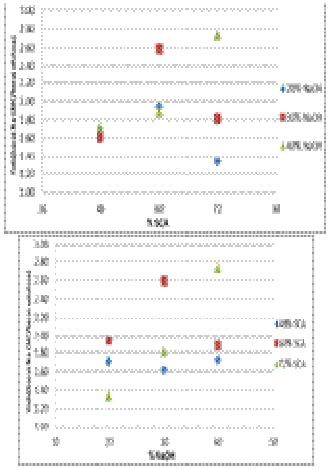


Figure 4. The effect of the addition of NaOH and SCA to yield Na-CMC

From Figure 4, the yield of Na-CMC for addition of NaOH reagent while SCA fixed, it has a tendency of decreasing in consumption 30% NaOH-48% SCA) and 40% NaOH-60% SCA, and then increased significantly in use 40% NaOH,-72% SCA. The use of cellulose fixed amount of 15 grams of the cellulose content of 73.88 % was treated with NaOH (20 %, 30 %, 40 % w/v) to form Na - cellulose alkalization reaction stage. Then Na - cellulose which had been formed reacted with SCA (48 % , 60 % , 72 % w/v) to form Na-CMC. Formation of Na - CMC alkalization of the reaction and the reaction in addition to primary products carboxymethylation, this reaction also produced by products that NaCl and Na - glycolic because the amount of excess reagent used. In addition to the views of the cellulose content only 73.88 % of the 15 grams of cellulose, it is certain that there are other components that join react that causes the purity of Na - CMC is not too high .

The pH value of Na-CMC stated in the SNI of 6 to 8.5. Below pH Na-CMC produced can be seen in Figure 5.From Figure 5, it can be seen the value of Na-CMC pH produced by the addition of reagents SCA while NaOH fixed will reduce at 60% addition of the SCA. From this figure, the pH value is generated for addition of NaOH while SCA fixed showed relatively minor changes. However, the use of 60% of SCA are pH (< 7) of a

solution of 1 % Na - CMC and pH values tend to increase along with the addition of NaOH. This can occur because during the process of neutralizing the excess of acid used. pH on Na - CMC product obtained can actually be set when karboksimetilasi neutralization process after the reaction is complete. The decline in pH may decrease Na - CMC solution viscosity due to the polymer is rolled or turned into granular. Na-CMC polymer coiled lead group substituted (carboxymethyl group) into a roll as well, consequently the length of the polymer chain Na - CMC be shortened . This will reduce the solubility and viscosity of Na - CMC in water.

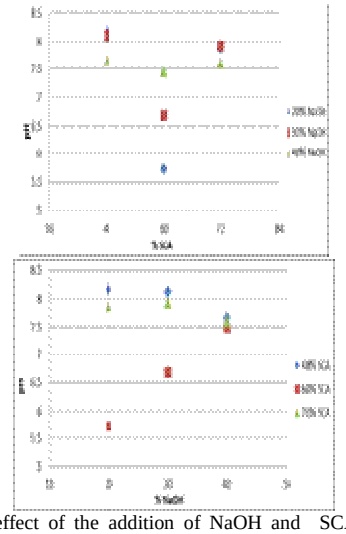


Figure 5. The effect of the addition of NaOH and SCA to pH 1% solution of Na-CMC

Analysis of the degree of substitution (DS) was conducted to determine the number of hydroxy group (-OH) on each anhydroglucose units substituted by carboxymethyl groups (-CH2COONa). Here the value of DS Na - CMC produced in Figure 6.

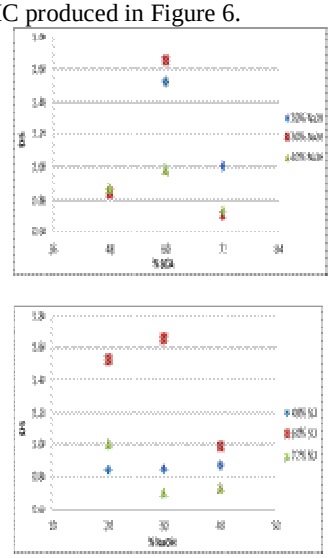


Figure 6. The effect of the addition of NaOH and SCA to DS Na-CMC

From Figure 6, it is known that the addition of SCA reagent while NaOH is fixed, DS will reach the lowest at addition 72% SCA. In the other hands, the addition NaOH will slightly increasing DS and decreasing at some conditions (40% NaOH-60% SCA) dan (30% NaOH-72% SCA). Cellulose has hydrogen bonds that leads to low solubility. In the structure of cellulose per anhydroglucose unit ($C_6H_{10}O_5$) has three-OH groups that are ready to be replaced by other compounds. As a result of the inclusion compounds of reagent replacement SCA (group-CH_2COONa) in the cellulose chain, thus changing the molecular structure of water or other solvent compounds can enter and dissolve the cellulose polymer.

The more SCA that dissolves in cellulose the higher value of DS, but at usage of great SCA will decrease the DS. It is because decomposition of the sodium salt of SCA which will reduce the substituted groups.

Viscosity is one of the quality requirements to be met by the product of Na-CMC. Viscosity solution of 2% Na-CMC should greater than 25 cP (Hercules, 1999). The viscosity of a solution of 2 % Na - CMC generated by the addition of SCA and a fixed NaOH has a value of > 25 cP . The use of 48 % and 60 % of SCA SCA has a viscosity greater value when adding NaOH reagent. For the same treatment that the more the number of SCA will increase the viscosity of Na-CMC. At 20 % NaOH-72% SCA looks the highest viscosity, this can happen due to the-OH group in cellulose substituted with groups CH_2COONa reaches the optimum. The longer chain molecules of Na - CMC the more viscous the solution. Na - CMC viscosity value is influenced by the pH and the amount reagent dissolved SCA at carboxymethylation stage, so that when many of the -OH group is substituted by a group CH_2COONa the more long - chain molecules. With using 20% w/v NaOH and 72% w/v of SCA (% w/v), it reached the optimum viscosity of solution 2% Na-CMC i.e 1163cP.

IV. CONCLUCION

Based on the results of research and discussion, it can be concluded as follows:

1. Separation of cellulose from corn cob through a phase of delignification and cellulose extraction can increase levels of cellulose
2. Characteristics of Na-CMC generated goes into a set of technical quality SNI include purity, degree of substitution and pH
3. The optimum conditions are making of Na-CMC on the use of 20% NaOH and SCA 72% (% w / v) with 58.61% purity analytical results, yield of 133.44 grams Na-CMC/selulosa, the pH of a solution of 1% Na-CMC 7.87, the degree of substitution of 1.001, a viscosity solution of 2% Na-CMC 1163 cP and 41.9% moisture content
4. Na-CMC product produced is Na-CMC for non-food items

REFERENCES

[1] Z. Ahmed, H. Banu, M.M Rahman, F. Akhter, and M.S. Haque. *Microbial activity on the degradation of lignocellulosic polysaccharides*. Journal of biological sciences. 1(10):993-997. 2001.

[2] K.S. Ambriyanto. *Isolasi dan Karakterisasi Bakteri Aerob Pendegradasi Selulosa dari Serasah Daun Rumput Gajah(Pennisetum purpureum Schaum)*. Surabaya: FMIPA, Institut Teknologi Sepuluh Nopember. 2010.

[3]A.N. Glazer, and H. Nikaido. *Microbial biotechnology: fundamentals of applied microbiology, second edition.* Cambridge:USA, 2007.

[4]R.L. Howard, E. Abotsi, E.L. J. van Rensburg, and S. Howard. *Lignocellulose Biotechnology: Issue of Bioconversion and Enzyme Production*. African J. of Biotech. Vol 2(12), 602-619. 2003.

[5]Y. P. Kencana dan Y. Setiawan. Berita Selulosa, , *Karboksimetil Selulosa (CMC) Dari NBKP dan Pulp Bagas*. Bandung : Balai Besar Selulosa. Maret 1998, hal 7 – 11

[6]*R.E. Kirk*,. and D.F. *Othmer*. *Encyclopedia of Chemical Engineering Technology*. New. York: John Wiley and Sons Inc. *1967*

[7]M. Nurnayny. *Eterifikasi Selulosa Ampas Sagu untuk Pembuatan Karboksimetil Selulosa [Tugas Akhir]*. Makassar: Teknik Kimia, Politeknik Negeri Ujung Pandang. 2008

[8]T. Pribadi. *Pembuatan dan Pemurnian Sodium Karboksimetil Selulosa (CMC). Bandung* : Balai Besar Selulosa. Berita Selulosa. Desember 1985, XXI No.4, hal 135 - 140,

[8]E.T. Resita. *Produksi Selo-Oligosakarida dari Fraksi Selulosa Tongkol Jagung oleh Selulase Trichoderma viride*. Bogor: FTP, Institut Pertanian Bogor. 2006.

[9]Y. Setiawan, dan M. Pramono. *Penelitian Pengaruh Suhu Reaksi Alkalisasi dan Suhu Reaksi Eterifikasi Terhadap Karakteristik Carboxy Methyl Cellulose (CMC).* Bandung : Balai Besar Selulosa. Berita Selulosa, Juni 1990, XXVI No.2, hal 33 – 37,

[10]SNI 06-3736-1995. *Natrium Karboksimetil Selulosa Teknis.* Dewan Standarisasi Nasional

[11]F.G. Winarto. *Kimia pangan dan gizi*. Gramedia pustaka utama. Jakarta. 1995.

Automation Systems for Regeneration Process of Demin Water to Maintain Quality Control using Programmable Logic Controller

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***Abstract*—The development of technology and the increasing demand for the quality, making all production systems in automotive manufacturing company should go well and a small error rate. Similarly, the control system that controls the plant utilities regeneration process of demin water. Demin water regeneration process is repeated water washing process to be demin water, which will be used in preventing rust on the body of the car, should be in good condition. The timing on regeneration demin water is still done manually by the operator, in which fewer mistakes are made by an operator in setting the time will lead to the difference of time of a predetermined time standard. This will lead to the greater difference in time that happens, so it will affect the final quality of the demin water. Therefore, to overcome these problems it is necessary to make a new control system in the form of automation in the process of regeneration of demin water. Control device used is a PLC (Programmable Logic Controller) that uses Omron PLC Sysmac CPM - 1A. In making this new control system has been carried out field observations , literature study , interviews with operators , group leaders , supervisors , analysis -related, design and manufacture of electrical and programs as well as some testing . The automation system can prevent operator error in setting the time manually with a time difference of standard is 0 minutes. Subsequently, the final quality demin water be guaranteed with no air bubbles marked when the water is heated to 100 º C.**

***Keywords*— regeneration process, demin water, automation, PLC (Programmable Logic Controller)**

I. INTRODUCTION

In a company engaged in automotive manufacturing, especially the four-wheeled vehicles assemble (Authorized Automotive General Assembler), there is a production process that includes: Welding, Painting and Assembly. The development of technology and the increasing demand for quality, making all production systems in these companies should go well with a small error rate. Good production process should be supported by the control system operated in accordance with the standard of work that has been determined.

Similarly, the control system that controls the plant utilities demin water regeneration process. Demin water regeneration process is repeated water washing process to be demin water, which will be used in preventing rust on the body of the car, should be in good condition. Things that affect the final quality of the process are setting the time on every stage of the process. Accuracy in the timing of this process is not always in accordance with the standards specified time. Occurs due to the time difference the timing is still operated manually by the operator accuracy in estimating time corresponding to the standard, the greater the difference in time that happen it is very influential on the quality of the end result of demin water.

To overcome these problems then, it is necessary to turn a new control system that can control the time at this stage of the regeneration process automatically demin water. The control system is made to be flexible (if it will be improving the suitability or modification), available in the market, and must consider the affordability aspect, namely by using a PLC (Programmable Logic Controller) which uses PLC OMRON Sysmac CPM - 1A. Substitution control system is expected to cope with the possibility of error by the operator in setting the time manually, so there is no time difference occurs and the accuracy of the final quality demin water is guaranteed.

This study will discuss how to design and make the control system to run the regeneration of demin water without the need to set the time manually, which is adapted to the sequential process and the safety of work processes, as well as how the regeneration of demin water is controlled by PLC Omron Sysmac CPM - 1A, lapse of time can lessen the occurrence of the desired standard. Previously, we have researched about control system by using PLC [1, 2, 3, 4, and 5].

II. BASIC THEORY

A. Understanding Demin Water

Demin water is water that is made of a water purification process and free from minerals dissolved in water. Minerals dissolved in the water is a smaller number of minerals in solution, some examples are salt and sugar.

In the laboratory water with aqua demin-called distilata, its function is to wash laboratory equipment or as a mixer / solvent chemicals. In the automotive world demin water commonly called water batteries whose utility is to add water to the battery. Due to the nature of the minerals in the demin water very small then it can not lead to a "surge" on batteries and can extend the battery life of the vehicle. In addition demin water can also be used to charge the vehicle radiator so the radiator free from rust and can be used in the pre-treatment process at the plant painting (paint area) to prevent rust on the body of the car.

B. Water Purification Process (Demineralization)

Water purification process there is some kind of one of them is by way of demineralization / binding of the chemical elements. Demineralization process is the removal of minerals dissolved in the water, positive and negative charges in the water are bound to chemicals called cations and anions. Demineralization works according to the principle of ion exchange. Demin Plant generally consists of two ion exchange tank, the tank swap cations for H + ions and anions tank to exchange OH - ions. How it works demin plant are as follows:

- Cation exchange positive ions in water such as Ca, Mg, Na with H + ions.
- Water discharged from acidic cation.
- Anion exchange of negative ions in water such as Cl, SO4, SiO2 with OH - ions.
- If Conductivity (ion concentration measurement tool) gained ≥ 5 microseconds said saturated units.
- If the unit is already saturated necessary to regenerate / re-washing process with chemicals such as NaOH and HCL that the ion exchange process is still going well.

C. The regeneration Mechanism Demin Water

In general, the regeneration process is driven by demin water solenoid valve, solenoid valve on the magnetic field generated is used to drive the valves or valve solenoid valve that serves as a water faucet in the open condition. So when the electrified coil occurs around the magnetic field between two solenoids and solenoid core is given that can move freely up and down, core or cores made of materials that can be pulled by a magnet so that when the magnetic field around the coil core is attracted to above .

Core that can move up and down is connected to the valve or valve so that if the valve core and above interested also attracted significant upward solenoid valve in an open state. Conversely, if the current in the coil is switched off then the magnetic field around the coil will also be lost and the iron core moves down as pressure mounted by spring force opposite to the direction of the magnetic field coils. This spring force pushing the valve cover and block the flow rate, so that the solenoid valve will be closed.

III. DESIGN

A. Sequential process of regeneration work demin water before repair

Regeneration demin water process in automotive manufacturing company consists of 4 steps; include regeneration of cation / anion, slow rinse cation / anion, cation fast rinse, fast rinse anion. Each step has a different time standards and standard sized mixing of different chemical compounds.

Step 1 (Regenerating Cation / Anion)

The first step in the process is the demin water regeneration process Cation / Anion. This process is operated by making sure all the inputs and outputs of a process that does not include the regeneration of demin water off, as follows:

- Close the faucet to fill the tank and valve of PAM
- Turn off the switch for the pump of PAM, CO2, and for the contents to the tank
- Turn off the switch operations waste
- Conductivity already reached 5μs

Step 2 (Slow Rinse Cation / Anion)

The second step in the regeneration of demin water is Slow Process Rinse Cation / Anion. This process is operated after the regeneration of the cation / anion has been completed, as well as ensuring the input and output of the regeneration of the cation / anion has been off. After that, give input via selector switch for step 2 and Pump 3 contained in the panel, then takes place using the compound NaOH washing with 200 litres, 430 litres HCL, and Demin Water 1500 litres. This mixing process lasted for 50 minutes and then discharged to the leaching process Waste Water Treatment (WWT) to process waste operations. The goal of Slow Rinse cation / anion is to wash the rest of HCL and NaOH are there in the cation / anion, this process uses 4 valve includes valve 51, valve 85, valve 83, and valve 49.

Step 3 (Fast Rinse Cations)

The third step in the process of regeneration is the Fast Rinse Demin water cations. This process is operated after the slow rinse cation / anion has been completed, as well as ensuring the input and output of a process of slow rinse cation / anion has been off. After that, give input via selector switch for step 3 and Pump 3 contained in the panel, then a process of leaching by demin water 10000 litres. This mixing process lasted for 10 minutes and then discharged to the leaching process Waste Water Treatment (WWT) to process waste operations. The purpose of Fast Rinse cation is to eliminate the remnants of the regeneration solution trapped in the cation resin. This process uses two valves, i.e: valve 48, and valve 52. In this process, setting valve 48 still operated manually by the operator by turning the valve. It is because the automatic valve is damaged and can not replace the maintenance and repair due to a series of automated data documentation lost of previous PLC UN 11.7. Therefore the valve is replaced with a manual valve.

Step 4 (Fast Rinse Anion)

The fourth step in the process of regeneration is the Fast Rinse Demin water anions. This process is operated after the Fast rinse cation has been completed, as well as ensuring the input and output of the cation has been fast rinse off. After that, give input via selector switch for step 4 and Pump 3 contained in the panel, then a process of leaching by Demin Water 6000 litres. This mixing process lasts for 60 minutes or until the above 5 microseconds Conductivity and pH of approximately 6-7. Then, discharged to the leaching process Waste Water Treatment (WWT) in process waste operations. The purpose of Fast Rinse Anion resin is to wash, where the process is using the 2 valve covers valve 86, and valve 82. In this process, setting valve 82 is the same as the third step, which is still operated manually by the operator by turning the valve. It is because the automatic valve is damaged. It can not replace the maintenance and repair due to a series of automated data documentation lost of previous PLC UN 11.7, therefore the valve is replaced with a manual faucet.

B. Problems in Regeneration Process Demin Water

Regeneration process Demin Water produced directly in automotive manufacturing company plant division 1 utility area. The control system in the regulation time in the regeneration of the demin water previously operated automatically using PLC UN 11.7 produced Durr, but the PLC is damaged. It can not be repaired and is not produced anymore; the data and drawing electrical circuit PLC system demin water regeneration panel has been lost. It is difficult to carry out maintenance repairs if there is a problem, so the control system using PLC was changed to use a manual system to move the selector switch solenoid valve.

Manual control system on demin water regeneration process consists of 4 steps with different time settings complicate the operator in demin water regeneration process manually. Experienced operator Idle Power Man, because it requires that the operator is always in the area until the regeneration is complete, this is due to the time that has been standardized to be precise, so the accuracy of the final quality demin water regeneration should be guaranteed. In addition, operators are also easily influenced by the decrease in concentration at work, feeling tired, and other circumstances, so it is in fact based on the data obtained in the field, the average time has been estimated by the operator is not always accordance with the standards specified time.

It does not affect the final quality demin water, due to the difference in the time difference with the standard specified time is still small, but the longer the control system is used, the greater the possibility of operator error in estimating the time and the greater the difference in the time that happens so very influential on the quality the end result of demin water. It required a replacement engine control system using this type of control system that is flexible, available at the company, and can control the regeneration of demin water automatically.

C. The Spesifications

In turn and manufacture of new control systems on the regeneration of the demin water, automotive manufacturing company has determined the specifications required to make the control system tailored to the needs of the working process of the regeneration area demin water, which is as follows:

- Substitution control system in the regeneration process demin water using a control device such as a PLC (Programmable Logic Controller).
- The control system uses PLC can be used to operate the machine automatically demin water regeneration. As well as the addition of a manual system this is controlled directly by the PLC.
- If necessary modifications or turn on the PLC system, not complicate maintenance and does not exceed 1.5 hours.
- System timing in the regeneration process by timer dkontrol demin water contained in the PLC.
- The timing is controlled by the PLC is in the process of regeneration of demin water must comply with the standard of work that is 150 minutes.
- PLC used is contained in the company and in accordance with the number of inputs and outputs that will be used is Sysmac CPM Omron PLC - 1 A with type 30 CDR - A - V1, which has 30 terminal I / O, I / O modules can be plus, current 0.6 A, has a 100-240 volt AC Operational voltage and the output voltage 24 volts DC.
- Process Automation in demin water regeneration can be done with only 2 input. Ie Button Start and Auto Switch.
- The system uses PLC should be able to drive the output of the solenoid valve with AC voltage of 24 volts.
- Can be integrated with other systems.
- It takes a warning sign for the operator if the process has been completed.
- The process of regeneration work to be sequential demin water during operation using the control system.

D. Design Concept

To meet the above specifications, we design a concept in the making of a new control system using PLC automation. Figure 7 shows an illustration concept design using PLC control circuit.

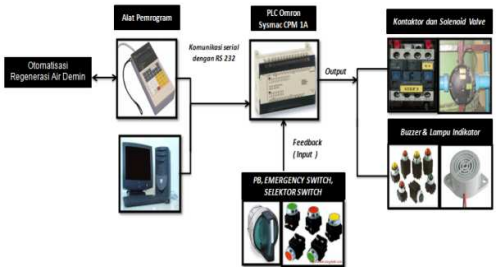

Fig. 1. Illustration of the concept of control circuit design using PLC

PLC control system made by Omron Sysmac CPM - 1A is programmed with a PC or console. The working principle of what happens when an operator operate the regeneration of demin water, the operator presses the push button voltage source and switch the auto- regeneration and pressing the push button auto to give input and command on the PLC to move / turn on the output of solenoid valves, contactors, lamps indicator, and buzzer [5, 6, 7, 8, 9, and 10].

The order is then received by PLC Omron CPM - 1A Sysmac programmed via PC with software such as CX - Programmer or console is a tool programmer's use language mneumonik. The order will be processed by the PLC. If the command line with the PLC program will execute to turn off or turn on the output.

Once the execution is done then the PLC reads the status of the output is dead or flame. Then the PLC will provide information on the output of the solenoid valve, contactor, indicator lights, and a buzzer, which indicates that the output status in accordance with the commands that have been programmed. The design of the tool is expected to solve problems that occur in the process of regeneration demin water to avoid possible errors in setting the time manually by the operator and allows an operator to operate the machine without experiencing idle man power. Based on the specification of the required field and then be made to the draft concept design demin water regeneration process controller with specification tools:

- Using Omron PLC Sysmac CPM - 1A with type 1 and 30 CDR-A-V 220volt AC operating voltage and the output voltage is 24 volts DC.
- Using 7 pieces of contactor as the output device specification voltage 220-230 volts.
- Using 1 selector switch for automatic or manual mode, and 4 selector switch, as the input of demin water regeneration process manually but not directly controlled by a PLC which has a voltage of 220 volts.
- Using the Push Button 1, for the input modes operate automatically in demin water regeneration process with a voltage of 220 volts.
- Using the Buzzer and light indicator with a voltage of 220 volts as well as the output device, a warning sign for the operator.
- Using the CX - Programmer 8.1 as the software and hardware in the console as programming.
- Using RS232 DB9 serial connector to connect to either a PC programming tool or consoles.

IV. TESTING

Programs that have been made to go through the testing stage, either the hardware or its software. The purpose of the test itself is to find a variety of potential causes of system failure or. Basically the manufacture of control systems using PLC, the largest percentage of system failures comes from the input PLC, actuators and wiring connections, rather than a failure caused by an internal error of the PLC itself. Therefore, the test is not only focused on testing the program but also the testing of input and output devices and other external factors. Tests conducted in this thesis is once a week for three months after a change of control systems. Here is some of the testing that has been done.

A. Testing of Cycle Process

In this process cycle testing conformance testing program that has been created to cycle processes and specifications desired program.

B. Difference in standard time after repairs

The main objective in conducting the turn system controls the regeneration process is the demin water to prevent or eliminate the possibility of a lapse of time of labor standards that have been determined.

V. CONCLUSIONS

Automation of the process of regeneration of demin water designed and fabricated using Omron PLC Sysmac CPM-1A is functioning properly with the timing is controlled directly by the PLC, and in accordance with the sequential and safety of the work process. The programming use Omron PLC CPM-1A Sysmac by ladder language and mneumonik with the help of a PC or a console programmer tool. Omron PLC Control System using CPM-1A Sysmac 7 input activates a push button, selector switches and 11 outputs to activate the motor contactors, solenoid valve, indicator light, and buzzer. Through timing of PLC controlled directly by the lapse of time which happens to be 0 minutes and the final quality assured demin water with no air bubbles marked when water is heated 100 º C.

REFERENCES

[1] Ardi, S., Pratama, R. A., Power Saving on Piston washing Machine Modification by Utilizing Air Pressure and Programmable Logic Controller, Prosiding Seminar Nasional SciETec 2012, ISBN 978-602-97961-1-7, pp. TE15-1 – TE15-5.

[2] Ardi, S., Prasetyo, D., Design of Inspection Tool for Checking The Existence and Position of Hole Stopper Piston 5D9 Using Sick Inspector Camera at Automation Center Bosh Cutting & Engraving Machine, pp. C-77 – C-80, Proceeding SNEEMO 2011, 2011, ISBN 978-602-19043-0-5.

[3] Ardi, S., Hidayat, M., Azhari, Y., Design of Sensory Station System for Modular Mechatronics System at Politeknik Manufaktur Astra, Prosiding SNPPTI 2011, ISSN 2086-2156, pp. 246 - 249, 2011.

[4] Ardi, S., Lin Prasetyani, Reza Guntur Budianto, "Pokayoke Control System Design using Programmable Logic Controller (PLC) on Station Final Check Propeller Shaft", Halaman: C-74 – C-80, Proceeding Annual Engineering Seminar 2013, ISBN: 978-602-98726-2-0

[5] Ardi, S., Alfan Subiantoro, Design Control System for Radio Battery Function Checking using Programmable Logic Controller (PLC), AICST 2012, Page: 185 – 194, Proceeding The 2nd ACIKITA International Conference on Science and Technology (AICST), 2012, ISBN: **978-602-18102-1-7.**

[6] Rullan, A., Programmable Logic Controllers versus Personal Computers for Process Control, Computers ind. Engineering, Nos 1-2, pp. 421-424, 1997.

[7] J. Swider, G. Wszolek, W. Carvalho, Programmable controller designed for electro-pneumatic systems, Journal of Materials Processing Technology 164-165 (2005), pp. 1459-1465.

[8] G. Valencia-Palomo, J.A. Rossiter, Programmable logic controller implementation of an auto-tuned predictive control based on minimal plant information, ISA Transactions 50 (2011), pp. 92-100.

[9] Wang, R., Song, X., Zhu, J., Gu, M., Computers in Industry 62 (2011), pp. 23-31.

[10] Bayindir, R., Cetincevic, Y., A Water pumping control system with a programmable logic controller (PLC) and industrial wireless modules for industrial plants-an experimental setup, ISA Transaction 50 (2011), pp. 321-328.

Development of somatic embryos and secondary somatic embryos of Cassava

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***Abstract*— Cassava (*Manihot esculenta* Crantz) as staple food is an important tuber crop especially in the tropic region. Several researches in cassava have been reported to develop improved and high-yielding cassava cultivars. Cassava accession 433 is one of ICABIOGRAD breeding collection. This genotype has high carbohydrate and starch content (over 80%) which is promising as raw material for bio-fuel. However, the utilization is still rare and therefore appropriate conservation methods needed to maintain this useful genotype. Cassava is a vegetatively propagated crop, thus seed storage is not a feasible option for conservation. Also, in vitro storage via slow growth is limited by periodic subculture which leading to somaclonal variation. Cryopreservation of embryonic calli could be logical choice for long term conservation. The successful of cassava somatic embryogenesis is vary depend on the genotype, explants sources and hormones composition in media. This experiment tried to develop somatic embryogenesis of cassava in several media enriched with proline, ABA or copper sulphate ($CuSO_4$) as treatments. Development of somatic embryos and secondary somatic embryos were observed on maturation medium supplemented with $CuSO_4$.**

Keywords*— Somatic embryogenesis, ABA, proline, $CuSO_4$, *Manihot esculenta.

I. INTRODUCTION

Somatic embryogenesis is an efficient and reproducible regeneration system for propagation, transformation and long term conservation via cryopreservation [1-4]. According to [5], callus formation and somatic embryogenesis in cassava is vary depend on the genotype, explants sources and hormones composition in media.

It has been proposed that PGRs (Plant Growth Regulators) and stress play a central role in mediating the signal transduction cascade leading to the reprogramming of gene expression [6], induced either unorganized callus growth or polarized growth leading to somatic embryogenesis [7]. ABA is considered as a stress hormone and growth inhibitor, but recent evidence suggested that it plays an active role in root and shoot growth as well as maturation and germination of the embryos [8].

Enhancement of embryo development and germination with respect of cassava somatic embryogenesis also widely reported with exogenous ABA application [8,9]. Abscisic acid (ABA) is well known to be effective in regulating the maturation of embryos as well as allowing embryos to store carbohydrate, reserves and also enhance desiccation tolerance of a diverse range of other species [10].

Another report on stimulation of auxin-induced somatic embryogenesis by proline has a great impact on development of somatic embryos and secondary somatic embryogenesis [11-12]. It was postulated that proline acts as an active solute [13] and as an enzyme protectant [14]. Pronounced changes in proline accumulation were noticed during different stages of somatic embryos of chickpea. The role of proline in differentiating cultures was examined in pea plants. Free proline may act as an osmoticum, a nitrogen storage pool, source of $NADP^+$, which is necessary for rapidly growing embryos [12]. The mediation of the cellular redox potential that results from proline accumulation is likely to have a large effect on the flux through redox-sensitive biochemical pathways like pentose phosphate pathway [15].

In recent years, plant regeneration via somatic embryogenesis induced with supplementary of micronutrients such as Ag^+, Co^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+} have received considerable attention [16,17], as well as in cassava [18,19,20,21,22].

In this experiment, we reported response of proline, ABA and $CuSO_4$ supplemented in maturation medium to induced maturation and germination of somatic embryogenesis of cassava.

II. MATERIAL AND METHODS

A. Plant Material

The in vitro collection of cassava accession no. 433 obtained from ICABIOGRAD field collection. Explants have been cultured in vitro and were maintained on medium containing MS [23] basal salts and vitamins supplemented with 30 g/l sucrose, incubated at a temperature of 24–25 °C under a 16/8 h (light/dark) photoperiod with light provided by white fluorescent tubes at an intensity of 800 μmol $m^{-2}s^{-1}$.

Young leaf with adaxial side up and stem from in vitro plantlets were dissected approximately size of 0.5x0.5 cm and cultured on CIM (Callus Induction Medium): MS basal medium, MW vit., picloram 25 ppm, NAA 25 ppm, casein hydrolisat 100 ppm and sucrose 30 g/l, solidified with phytagel 2.5 g/l [24]. Friable and embryonic callus, characterized by yellowish-green friable callus, were used as explants on study of somatic embryos development

(Fig 1).

Fig. 1. Cassava friable embryogenic callus (FEC) induced on callus induction medium, used for study on development of somatic embryos.

B. Somatic embryos development and maturation

Friable embryogenic calli (FECs) obtained from callus induction medium (CIM) then transfer into several treatments on somatic embryos development and maturation medium:

Treatment 1. Callus cultured on MS basal medium supplemented with MS vitamin, BA 0.5 ppm, 2.4 D 0.5 ppm, ABA (5, 10 ppm) and sucrose 30 g/l, solidified with 2.5 g/l phytagel. pH adjusted to 5.8 before autoclaved for 30 minuted 121°C.

Treatment 2. Callus cultured on MS1 medium (MS basal medium supplemented with MS vitamin, BA 0.5 ppm and casein hydrolysat 300 ppm, sucrose 30 g/l). Either prolin 100 ppm and kinetin (1 ppm, 2 ppm) or prolin 200 ppm and kinetin (1 ppm, 2 ppm) were added to the MS1 media as treatments. Phytagel 2.5 g/l added as gelling agent to solidified medium. pH adjusted to 5.8 before autoclaved for 30 minuted 121 ^{0}C.

Treatment 3. Callus cultured on IM medium (MS basal medium supplemented with MS vitamin, $CuSO_4$ 0.5 ppm and sucrose 20 g/l, solidified with 1.5 g/l phytagel). Picloram 12 ppm or 2.4 D 11 ppm added as treatments. pH adjusted to 5.7 before autoclaved for 30 minuted 121°C.

All treatments incubated at a temperature of 24–25 °C under 16/8 h (light/dark) photoperiod with light provided by white fluorescent tubes at an intensity of 800 µmol $m^{-2}s^{-1}$. A completely randomized experimental design was used. Each treatment consisted of three replications. Number of embryonic callus and matured embryos (%) scored 1 month after treatments.

C. Germination and regeneration of somatic embryos

Germination induced on *MM-1 medium* (MS basal medium supplemented with CS vit. (myo inositol 100 ppm, thiamin HCl 1 ppm, pyridoxine 1.5 ppm, nicotinic acid 1.5 ppm, 2 ppm glycine), BAP 0.1 ppm, $CuSO_4$ 0.25 ppm + sucrose 30 g/l + phytagel 2.5 ppm) and *MM-2 medium* (MS basal medium supplemented with BAP 0.1 ppm + prolin 100 ppm + kinetin 1 ppm + casein hydrolisat 300 ppm). pH adjusted to 5.7 before autoclaved for 30 minuted 121°C.

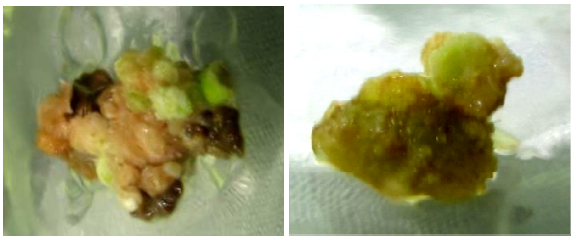
All treatments incubated at a temperature of 24–25°C under 16/8 h (light/dark) photoperiod with light provided by white fluorescent tubes at an intensity of 800 µmol $m^{-2}s^{-1}$. Number of plantlets regenerated scored 1 month after treatments.

III. RESULT

A. Somatic embryos development and maturation

A.1. The effect of ABA treatments

In this experiment, none of globular-friable calli on both ABA treatments was able to develop into advance somatic embryo structures. Observation until 4-6 month after transfer to ABA medium showed then calli turned green and compact (Fig. 2).



(a) (b)

Fig. 2. Cassava callus on maturation medium supplemented with ABA at: (a) 5 ppm; (b) 10 ppm.

A.2. The effect of proline and kinetin

In this experiment, addition of prolin and kinetin on maturation media also reduced number of friable callus which some of them developed into compact green callus. The effect of proline and kinetin to percentage of compact and green callus were given in Fig. 3.

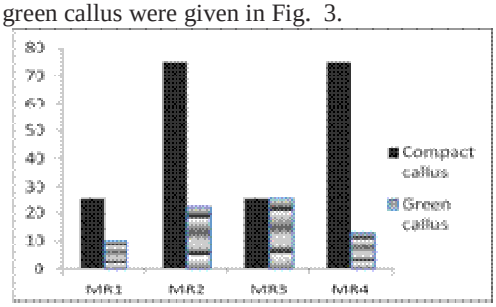
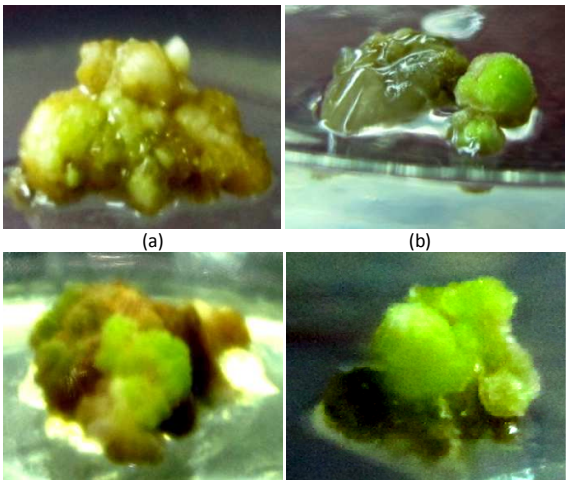


Fig. 3. Percentage of compact callus and green callus induced on MR1= Proline 100+kin 1; MR2= Proline 100+kin 2; MR3= Proline 200+ kin 1; MR4= Proline 200+kin 2) ppm

Treatment with higher concentration of kinetin (2 ppm) induced growth of compact callus whereas the highest green callus induced on media supplemented with proline 200 ppm and kinetin 1 ppm (MR3). No advance somatic embryo structures developed from globular callus were observed on this treatment, whereas green callus then emerged from all treatments, induced an organogenesis development (Fig. 4).

In our study, friable callus on both treatments (ABA and proline) were enabled to proliferate beyond the pre-globular stage, or more over, callus were break up into non-embryogenic callus and hard brown callus.

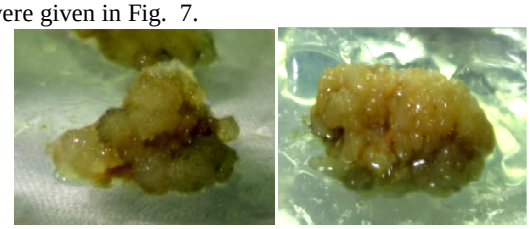


(a) (b) (c) (d)

Fig. 4. Compact and green callus induced on: (a) MR1= Proline 100+kin 1; (b) MR2= Proline 100+kin 2; (c) MR3= Proline 200+ kin 1; (d) MR4= Proline 200+kin 2) ppm.

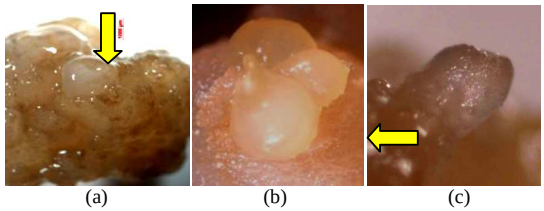
A.3. The effect of copper sulphate ($CuSo_4$)

Initiation of embryonic callus was found at 2 months after treatment on IM-1 and IM-2 medium (Fig. 5). But only IM-1 medium (MS medium supplemented with MS vit., $CuSO_4$ 0.5 ppm, picloram 12 ppm, sucrose 20 g/l and solidified with phytagel 1.5 g/l) could induced development of different somatic embryos structures (Fig. 6). Average number of somatic embryos per explants (efficiency scores) induced at 2 months on IM-1 medium were given in Fig. 7.

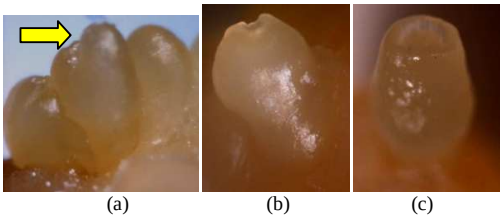


(a) (b)

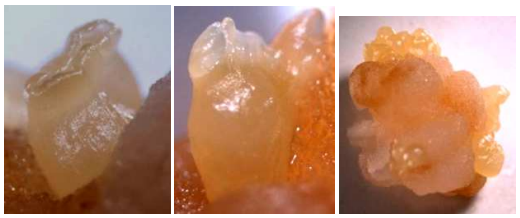
Fig. 5. Embryonic callus produced on induction medium supplemented with: (a) picloram 12 ppm (IM-1); (b) 2.4 D 11 ppm (IM-2).



(a) (b) (c)



(a) (b) (c)



(g) (h) (i)

Fig. 6. Development of somatic embryos structure of cassava: (a) Early development of globular somatic embryos; (b) late globular-early heart stages; (c) early heart stage; (d) embryos at heart stage; (e) late heart-early torpedo stage; (f) torpedo stage; (g) early cotiledonary stage; (h) cotiledonary stage with leaf primordial; (i) callus clumps with different stage of embryos development

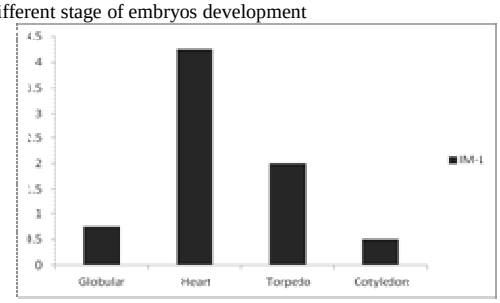
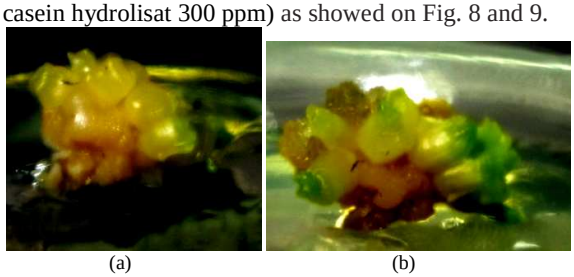


Fig. 7. Efficiency scores of somatic embryos produced on IM-1 medium ((MS medium supplemented with MS vit., $CuSO_4$ 0.5 ppm, picloram 12 ppm, sucrose 20 g/l and solidified with phytagel 1.5 g/l)

B.1. Germination and regeneration of somatic embryos

Germination embryos and regeneration were observed on both MM-1 medium (MS medium + CS vit. + BAP 0.1 ppm + CuSO4 0.25 ppm) and MM-2 medium (MS + MS vit + BAP 0.1 ppm + prolin 100 ppm + kinetin 1 ppm + casein hydrolisat 300 ppm) as showed on Fig. 8 and 9.



(a) (b)

Fig. 8. Germination and regeneration: cassava somatic embryos (a); horn-shape plantlets (b), induced on MM-1 medium (MS medium + CS vit. + BAP 0.1 ppm + CuSO4 0.25 ppm).

(a) (b)

Fig. 9. Germination and regeneration: cassava somatic embryos (a); horn-shape plantlets (b), induced on MM-2 medium (MS + MS vit + BAP 0.1 ppm + prolin 100 ppm + kinetin 1 ppm + casein hydrolisat 300 ppm).

Efficiency scores of somatic embryos in germination medium were given in Fig. 10.

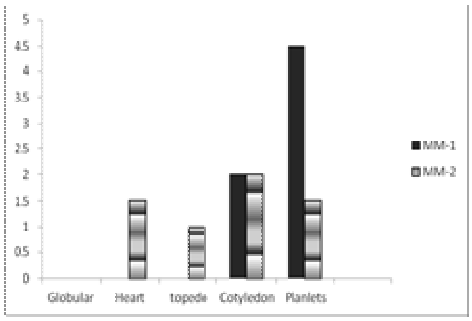
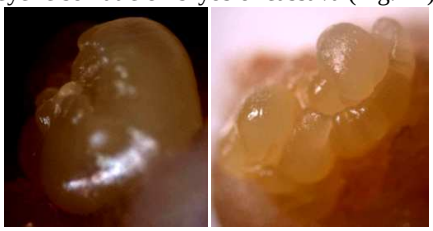


Fig. 10. Efficiency scores of somatic embryos and plantlets regenerated on MM-1 medium (MS medium ,CS vit., BAP 0.1 ppm, CuSO4 0.25 ppm) and MM-2 medium (MS, MS vit, BAP 0.1 ppm, prolin 100 ppm, kinetin 1 ppm, casein hydrolisat 300 ppm).

B.2. Cyclic somatic embryos

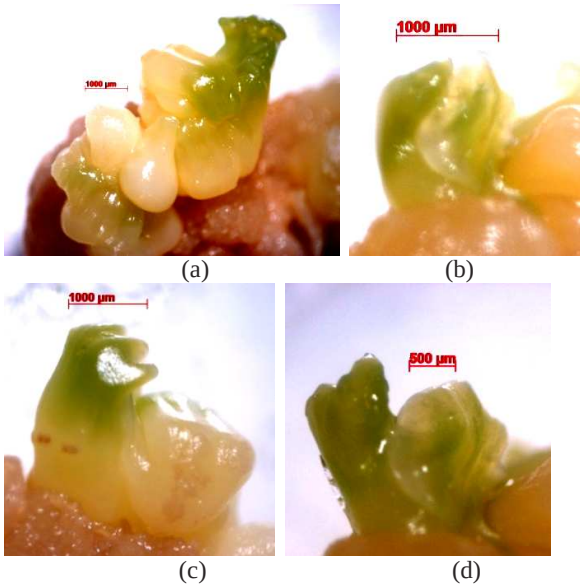
In this study, second subcultured on maturation medium (IM-1) supplemented with picloram 12 ppm, induced cyclic somatic embryos of cassava (Fig. 11).



(a) (b)

Fig. 11. Cyclic somatic embryos: embryos emerged (a,b) from maturing somatic embryos, induced at 2nd sub-culture on IM-1 medium.

Development and germination of secondary embryos produced 20 days after transferred on MM-2 medium (MS medium + BAP 0.1 ppm + prolin 100 ppm + kinetin 1 ppm + casein hydrolisat 300 ppm.) as shown on Fig. 12.



(a) (b)

(c) (d)

Fig. 12a-d. Development and germination of cassava embryos on MM-2 medium.

IV. DISCUSSION

There is hypothesis that somatic embryogenesis is an extreme stress response of cultured plant cells [7]. ABA in some experimental has been reported to induce somatic embryogenesis [25] and believed to act as 'stress hormones' in plants [10].

The stresses and appearance of ABA are essential for the acquisition of the embryogenic competence, which is essential in early stages of somatic embryogenesis formation [10,26]. Proline, accumulated in drought-stressed plants, synthesized in plant which is regulated by ABA [27, 28]. Statement of [12], combination of cytokinin and prolin often stimulate somatic embryogenesis, Agreed with [29] that a low concentration of cytokinin supplemented on the culture medium tends to stimulate the initiation of embryogenic cultures in most species.

In this study we found that addition of ABA, proline and kinetin without or with low concentration of auxin in medium could not improved development of somatic embryos. According to [30], the initial high auxin shock to somatic cells induced to synthesize gene products necessary to complete the globular stage of embryogenesis while the subsequent low concentration of auxin turns off a number of genes so that the embryogenic programme can be proceed. [11] also found that induction of somatic embryogenesis in sugarcane was influenced by 2.4 D concentration in a pronounced way.

2.4 D added in ABA treatment medium did not induced further development of friable-embryogenic calli on this experiment, as well as [31] stated that compact non-friable browning of 2.4 D induced calli likely to inhibit further development and maturation of globular embryos in embryogenic calli.

Picloram and 2.4 D added into maturation medium with copper sulphate 0.5 ppm induced embryonic callus formation. In this study we reported development of somatic embryos on medium supplemented with copper sulphate and picloram. Addition of copper sulphate in cassava maturation medium improved embryogenic competence and reduced the maturation time [19]. Further study [31] also found that picloram enhanced early maturation of primary somatic embryo while 2.4 D delayed embryo maturation.

Primary somatic embryos induced secondary somatic embryos by further sub-culturing on auxin-containing medium [9,20,32]. Secondary somatic embryos also found in this study after 2nd sub-cultured on medium supplemented with 11 ppm picloram. Cyclic embryogenesis system can be established either in liquid or solid medium, where the embryos rarely pass the torpedo stage until transferred to germination medium [32].

V. CONCLUSION

The present of auxin still required in early somatic embryo development of cassava accession 433. Somatic embryos developed and matured on IM-1 medium (MS medium supplemented with MS vit., $CuSO_4$ 0.5 ppm, picloram 12 ppm, sucrose 20 g/l and solidified with phytagel 1.5 g/l) and produced cyclic embryogenesis at 2nd subcultured on medium supplemented with 11 ppm 2.4 D. Embryos successfully regenerated into plantlets on both MM-1 medium (MS medium + CS vit. + BAP 0.1 ppm + CuSO4 0.25 ppm) and MM-2 medium (MS + MS vit + BAP 0.1 ppm + prolin 100 ppm + kinetin 1 ppm + casein hydrolisat 300 ppm).

REFERENCES

[1] K. J. J. M. Raemakers, J. Bessembinder, G. Staritsky, E. Jocobsen, R.G.F. Visser, G. O. Young, "Induction, germination, and shoot development of somatic embryos of cassava," *Plant Cell, Tissue and Organ Cult*. Vol. 33, pp. 151-156, 1993.

[2] N.J. Taylor, M.V. Masona, R. Carcamo, T. Ho, C. Schopke, C.M. Fauquet. "Production of embryogenic tissue and regeneration of transgenic plant in cassava (*M. esculenta*),". *Euphytica*, vol. 120, pp. 25-34, 2001.

[3] J. Puonti-Kaerlas. "Recent advances on cassava biotechnology", *AgBiotechnol news inf*. Vol. 9, pp. 259-330, 1997.

[4] J. Zhou, G. Fu-Xing, M.K. Razdan.. Somatic embryogenesis and germplasm conservation of plants. In: *Conservation of plant genetic resources in vitro*, vol 2, Razdan, M.K and E.C. Cocking, *Eds*., Application and Limitations Science: Plymouth, 2000, pp. 167-192.

[5] E. Sudarmonowati, R. Hartati, T. Taryana. "*Produksi tunas, regenerasi dan evaluasi hasil ubi kayu (M. esculenta) Indonesia asal kultur jaringan di lapang*," Natur Indonesia, vol. 4, no. 2, pp. 96-108, 2002.

[6] S. V. Arnold, I. Sabala, P. Bozhkov, J. Dyachok, L. Filonova, "developmental pathways of somatic embryogenesis," *Plant Cell, Tissue and Organ Culture*, vol. 69, pp.233-249, 2002.

[7] Duditis, D, Gyorgye, L. Bogre, L. Bako. "Molecular biology of somatic emryogenesis," In Thorpe T.A., *Ed*., *In vitro embryogenesis in plants*. Kluwer Academic Publishers, Dordrecht, Boston, London. 1995. pp. 267-308.

[8] Danso, K. E. and B.V. Ford-Llyod. "The effect abscisic acid and sucrose on post thaw embryogenic competence and subsequent plant recovery from embryogenic calli of cassava," *American-eurasian j.agric & environ.sci*, vol. 3, no. 4. Pp. 663-671, 2008.

[9] J. Groll, D.J. Mycock, V.M. Gray, S. Laminski. "Secondary somatic embryogenesis of cassava on picloram supplemented media," *Plant Cell, Tissue and Organ Culture*, vol. 65, pp. 201-210, 2001.

[10] M. A. Zavattieri, A. M. Frederico, M. Lima, R. Sabino, B. Arnholdt-Schmitt. (2010, January). Induction of somatic embryogenesis as an example of stress-related plant reactions, *Electronic Journal of Biotech* [Online],13(1).Available:http://www.ejbiotechnology.info/content/vol13/issue1/full/4.

[11] N. K. Gill, R. Gill, S S Gosal. "Factors enhancing somatic embryigenesis and plant regeneration in sugarcane (*Saccharum officinarum* L.)," *Indian journal of biotechnology*. vol. 3, pp. 119-123. 2004.

[12] P. Das. "Mass cloning of Rose and Mussaenda, popular garden plants, via somatic embryogenesis," *Hort. Sci. (Prague)*, vol. *37*, no.2, pp. 70–78, 2010.

[13] G. R. Stuart, J.A. Lee. "The role of proline accumulation in halophytes," *Planta*, vol. 120, pp. 279–289, 1979.

[14] B. Schobert, H. Tschesche, "Unusual solution properties of proline and its interaction with protein," *Biochemistry Biophysics Acta*, vol. 541, pp. 270–277, 1978.

[15] S.K. Ghanti, K.G. Sujata, S. Rao, M. Udayakumar, Kavi, P.B. Kishore. "Role of enzymes and identification of stage-specific proteins in developing somatic embryos of chickpea (*Cicer arietinum* L.)," *In Vitro Cellular and Developmental Biology-Plant*, vol. *45, pp.* 667–672. 2009.

[16] A.K. Sahrawat, S. Chand, "Stimulatory effect of copper on plant regeneration in indica rice (*Oryza sativa* L.)," *J.Plant Physiol*, vol. 154, pp. 517–522, 1999.

[17] U. Kowalska, K. Szafranska, D. Krzyzanowska, W. Kiszczak, R. Gorecki, K. Janas, K. Gorecka, "Efficient of increased copper ion content in the medium on the regeneration of androgenetic embryos of carrot (Daucus carota L.)," Acta Agrobotanica, vol. 65, no. 2, pp. 73-82, 2012.

[18] J. Groll, D.J. Mycock, V.M. Gray, "Effect of medium salt concentration on differentiation and maturation of somatic embryos of cassava (Manihot esculenta Crantz)," *Ann. Bot*, vol. 89, no. 5, pp. 645-648, May, 2002.

[19] Danso, K. E and B. V. Lloyd. "Induction of high-frequency somatic embryos in cassava for cryopreservation," *Plant Cell Rep*, vol. 21, pp. 226-232, 2002.

[20] L. Saelim, S. Phansiri, S. Netrphan, M. Suksangpanomrung, J. Narangajavana. "Optimization of in vitro cyclic somatic embryogenesis and regeneration of the Asian cultivars of Cassava (*M. esculenta*) for genetic manipulation system," *Global Journal of Biotech and Biochemistry*, vol. 1, pp. 7-15, 2006.

[21] T. Feitosa, J.L.P. Bastos, L.F.A. Ponte, T.L. Juca, F.A.P. Campos. "Somatic embryogenesis in cassava genotypes from the northeast of Brazil," *Brazilian archives of biological technology*, vol. 50, pp. 201-206, 2007.

[22] C. B. Rossin, M.E.C. Rey. "Effect of explants sources and auxins on somatic embryogenesis of selected cassava (*M. esculenta*) cultivars," *South African Journal of Botany*, vol. 77, pp. 59-65, 2011.

[23] T. Murashige, F. Skoog. "A revised medium from rapid growth and bioassay with tobacco tissue cultures,".*Physiologia Plantarum*, vol. 15,pp. 473-497, 1962.

[24] S. Diantina, D. Efendi, I. Mariska. "Effect of Medium and Explant source on cassava callus induction," in *Proc.International Conference, The 4th Green Technology*. Malang, East Java. Indonesia, 2013 (to be published).

[25] M. Nishiwaki, K. Fujino, Y. Koda, K. Masuda, Y. Kikuta. "Somatic embryogenesis induced by the simple application of abscisic acid to carrot (Daucus carota L.) seedling in culture," *Planta*, vol. 211, no. 5, pp. 756-759, Oct. 2000.

[26] A. Kikuchi, N. Sanuki, K. Higashi, T. Koshiba, H. Kamada. "Abscisic acid and stress treatment are essential for the acquisition of embryogenic competence by carrot somatic cells," Planta, vol. 223, no. 4, pp. 637-645. 2006.

[27] P.D. Hare,W.A. Cress, J. Van Staden. "Proline synthesis and degradation: a model system for elucidating stress-related signal transduction," *J. Exp. Bot*, vol. 50, pp. 413-434, 1999.

[28] E. Abraham, G. Rigo, G. Szekely, R. Nagy, C. Koncz, L. Szabados. "Light dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabiodpsis," *Plant Mol. Biol*., vol. 51, pp. 363-372, 2003.

[29] E.F. George, M.A. Hall, G.J. De Klerk, *Eds*., "Plant Propagation by Tissue Culture 3rd Edition, 2008. Vol. 1. Springer, Dordrecht, The Netherlands, pp. 335-354.

[30] J.L. Zimmerman. "Somatic embryogenesis: A model for early development in higher plants," *Plant Cell Rep*, 5: 1411-1423. 1993

[31] K. Danso, W. Elegba, V. Oduro, P. Kpentey. "Comparative study of 2.4 D and picloram on friable embryogenic calli and somatic embryogenesis development in cassava (*Manihot esculenta* Crantz)," *International Journal of Integrative Biology*, vol. 10, no.2, pp. 94-100, 2010.

[32] M. Makwarela, C. Rey, "Cassava biotechnology, a southern African perspective," *Biotechnology and Molecular Biology*, vol. 1, no. 1, pp. 2-11, April 2006.

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The Effect of CO_2 Concentration in Modified Atmosphere Package and Storage Length on Meat Quality of Broiler

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Abstract

The study was conducted to determine the effect of CO_2 concentration and storage length and their interaction in modified atmosphere package on meat quality of broiler. High density of polyethylene plastic, CO_2, 0_2 and N_2 were used as the experimental materials. The experimental design was 4 x 4 factorial patterns with completely randomized design. The CO_2 concentration of 25%, 50% and 75% were the first factors, and storage length of 0, 7, 14 and 21 days were the second factors. The results showed that CO_2 concentration affected (P<0.01) total bacterial number, meat tenderness, and water-holding, capacity, water content, color score, visual score and odor score; but did not affect the pH of meat. The storage length affected (P<0.0 I) water content, total bacterial number, pH, tenderness, cooking loss, water-holding capacity, color score, visual score and odor score of the meat. There were interactive effect (P<0.05) between treatments on total bacterial number, meat tenderness and on water-holding capacity, respectively. The higher CO_2 concentration and the shorter storage length would make a better quality of the broiler meat persistence.

Keywords: CO_2 concentration, storage time, modified atmosphere package

I. INTRODUCTION

The meat has a high nutritional value. It contains water, protein, fat, vitamins and minerals that cause the flesh susceptible to damage due to bacterial activity. In addition to nutrients, bacteria also require a suitable environment for growth is moisture, temperature, pH and the presence or absence of oxygen (Lawrie, 1995). Meat contaminated with bacteria will undergo physical and chemical changes that have occurred and unwanted decay (Cont and Cuningham, 1993). Many methods are used for preservation of fresh meat to extend the shelf life for example drying, cooling / freezing, currying and sweetening. Preservation by using freezing difficult to apply, especially meats that are marketed by way of the display and also require high operational costs. One way that yields satisfactory preservation is preservation with packaging. Packaging is one of the widely used preservation method for extending the shelf life of meat, non-toxic and does not damage the physical properties of fresh meat.

Packaging with atmosphere modified is a way of packaging that contains different gases to the outside thus slowing the rate of respiration, reducing the growth of microorganisms and slows the enzymatic breakdown. The gases that are commonly used for packaging Modified atmosphere is N_2, O_2 and CO_2. CO_2, a gas that is bacteria static and fungi static used to extend the shelf life of meat. White meat chicken is a lighter color with a low myoglobin content so as not to become discolored if packed in a modified atmosphere. Therefore, there is need for research on modified atmosphere packaging with CO_2 concentration and storage time on total bacterial, physical, sensory testing and gas analysis on broiler meat. This study aimed to assess the effect of CO_2 concentration, storage time, and modified atmosphere packaging interactions in the broiler meat quality.

II. MATERIALS AND METHOD

Research Procedures

Broiler chickens as much as 24 tails are cut and processed into carcass. Chicken meat samples split into 2 parts left and right, each hemisphere is used as a sample. Parts of the breast meat is weighed and then put in a plastic bag. All samples were vacuum packed, 12 samples of which are used as vacuum treatment, 12 samples for concentrations of 25 % CO_2, 12 samples for concentrations of 50 % CO_2 and 12 samples for 75 % CO_2 concentration.

Mixing gases

To mix the gas as required, first be aware of volume mixing tube by filling the tube filled with water until the water drains out through the exit that indicates the tube has been filled. Gas entered through an air inlet and water inlet channel is closed. Air density is less than water to the surface and the air gets pushed out past the water drains out and placed in a flask so as to know the volume of the incoming gas mixture composition and adjusted to the volume of gas passing views flask with the volume of water out.

Incorporating a mixture of gases in the sample. Samples were first given a piece of rubber patches on the outside and the inside, and then the sample is introduced and vacuumed with vacuum-tool. Gas mixture in the mixing tube is injected into the sample on the piece of rubber to the road push in the air out with water in a mixing tube so pressing out the air passing through the outlet into the sample.

Parameters observed

This study used a modified atmosphere packaging with treatment factors of CO_2 concentration and storage time. The concentration of CO_2 is composed of : vacuum packaging, packaging with a mixture of 25 % CO_2, O_2 N_2 5 % and 70 % Packaging with a mixture of 50 % CO_2, O_2 5 % and 45 % N_2 and 75 % CO_2, O_2 5 % and 20 % N_2. All samples were stored at 4 ° C and analyzed on days 0, to 7, to 14 and to 21, which is treatment duration of storage. Parameters were observed: total bacteria,

moisture content, pH value, and tenderness, cooking shrinkage, water holding capacity, sensory tests include color, smell and texture.

Data Analysis

Data were analyzed using analysis of variance of factorial completely randomized design with CO_2 concentration factor (vacuum, 25 % CO_2, 50 % CO_2 and 75 % CO_2) and storage time factor (day 0, day 7th, day 14th and to 21st) and all treatment was repeated 3 times. If there is a difference between treatments, subsequent Duncan's multiple range test (Astuti, 1980).

III. RESULTS AND DISCUSSION

Water levels

The mean total bacterial counts of broiler meat with a long treatment and storage of CO_2 concentration in the atmosphere modified packaging presented in Table 1. The results of variance analysis of CO_2 concentration and significant effect ($P < 0.05$), and storage time was highly significant ($P < 0.01$) the moisture content of broiler meat. Interaction between CO_2 concentration and storage time was not significant effect.

Absence of interaction between the effects of CO_2 concentration and duration of storage of broiler meat in modified atmosphere packaging because of the combination of the two treatments did not affect it. CO_2 gas is widely used as a preservative for meat and meat products because they have influence as bacteria static and fungi static. High CO_2 concentration over inhibit the growth of bacteria causing water levels to fall. High concentration of CO_2 will lower water levels due to the ability of the protein binding of water is higher when compared with low CO_2 concentrations. This is in accordance with the opinion.

The water content increased linearly with the length of storage time. The presence of nutrients, water content and the value of ' *aw* ' high lead easily overgrown bacteria to degrade proteins in meat. Webster et al. (1982) stated that there will be degradation during storage proteins into fragments of protein, soluble in water. One example of the degradation of amino acids produces water is a serine and threonine.

During the decomposition process occurs storage proteins and fats that produces water so the longer the storage further increase the water content Beside degradation proteins and fats, it also occur carbohydrate degradation give water. Soeparno (1992) states that microorganism aerobic growth on the surface of the meat, for example Pseudomonas, fungi and yeasts can degrade sugars into CO_2 and H_2O. Water that is produced may also increase the water content of meat.

TABLE 1.
MEAN MOISTURE CONTENT (%) BROILER CHICKEN MEAT WITH CO_2 CONCENTRATION TREATMENT AND LENGTH OF STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 Concentration	**0**	**7**	**14**	**21**	**Mean**
Vacuum	75,43	77,21	77,40	78,53	77,14^{y}
CO_2 25%	75,70	77,10	77,37	78,83	77,002 ^{y}
CO_2 50%	75,51	76,82	76,86	77,99	76,79 ^{y}
CO_2 75%	75,27	75,74	76,28	76,99	76,07^{x}
Mean	75,48'	73,72b	76,98^{b}	77,83^{b}	

$^{a.b}$ Different superscript at the same row showed the real difference (P<0,01)
$^{x.y}$ Different superscript at the same row showed the real difference (P<0,05)

Total Bacterial

The mean total bacterial broiler meat with a long treatment and storage of CO_2 concentration in the modified atmosphere packaging presented in Table 2. The result of variance analysis and the CO2 concentration and storage time showed highly significant differences ($P < 0.01$) and the interaction of both treatments showed significant differences to total bacteria on broiler chicken meat.

TABLE 2.
THE MEAN TOTAL BACTERIA (LOG COLONY / G) BROILER MEAT WITH CO_2 CONCENTRATION TREATMENT AND LENGTH OF STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Mean
Vacuum	4,473^{j}	6,49^{l}	8,11°	9,56°	7,1 i ^{Y}
CO_2 25%	4,231 ^{j}	6,42 ^{l}	7,12m	9,17°	6,74Y
CO_2 50%	4,25 ^{j}	5,65k	6,80'	8,57w	6,32'
CO_2 75%	4,49 ^{j}	5,48k	6,49'	8,31°	6,19'
Mean	4,36^{b}	6,01s	7,13'	8,90d	

[a.b,c,d] Different superscript at the same row showed the real difference (P<0,01)
[x.y] Different superscript at the same coloumn showed the real difference (P<0,01)
[j,k,l,m,n] Different superscript at the same coloumn and row showed the real difference (P<0,01)

There is a significant interaction between CO_2 concentration and storage time and treatment on total broiler meat bacteria. Total bacteria are influenced by the concentration of CO_2 because CO_2 serves as bacteria static and the longer storage of bacterial growth is high because of the CO_2 concentration decreases with storage time. The higher the concentration of CO_2 would inhibit the growth of bacteria, as well as the shorter the storage time in total bacteria will also be low.

Low CO_2 concentration will raise the total bacteria. At storage, the concentrations of CO_2 75% total value of the lowest bacteria because CO_2 is a bacteria static and fungi static so that growth can be inhibited battery. Richard (1987) stated that CO_2 has inhibiting properties of bacteria and fungi, when the concentration reaches 20 %.

The longer the storage will increase the total microorganisms, especially on environmental conditions that are rich in nutrients. Total bacteria increased with increasing storage time according to the bacterial growth curve.

Physical Characteristics of Meat: pH value

The mean pH values of chicken meat with a long treatment and storage of CO_2 concentration in broiler meat in the packaging atmosphere modified presented in Table 3.

TABLE 3
. THE MEAN PH VALUE BROILER CHICKEN MEAT WITH CO2 CONCENTRATION TREATMENT AND LENGTH OF STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Means
Vacuum	5,98	6,05	6,25	6,42	6,17
CO_2 25%	5,85	6,09	6,21	6,30	6,11
CO_2 50%	6,02	6,01	6,32	6,30	6,17
CO_2 75%	5,69	5,93	6,28	6,34	6,10
Means	5,92[a]	6,02[a]	6,26[ab]	6,35[b]	

[a.b,] Different superscript at the same row showed the real difference (P<0,01)

The result of CO_2 concentration and analysis of variance showed no significant differences, while storage time showed highly significant differences ($P < 0.01$) and the interaction did not significantly affect the pH value of broiler meat.

There is no interaction between CO_2 concentration and storage time on the pH value of broiler chicken meat because only storage duration are affected while the CO_2 concentration had no effect, so that there is a combination between the two treatments.

High CO_2 concentration had no significant effect on the pH value. In theory, an increase in the concentration of CO_2 will lower the pH value. This is because the increase in CO_2 concentration will increase the lipid solubility and form carbonic acid (Smith et al., 1992). Formation of carbonic acid reaction scheme postulated by Brody (1989) is as follows:

$$CO_2 + H_20\ H\ CH_2O,\ H\ 1\text{-}1 + + HCO_3^-$$

Increased CO_2 concentrations are high in modified atmosphere packaging will lead to more and more CO_2 is dissolved in water to form carbonic acid so much that causes the meat to have a low pH value. The pH value of meat in this study did not change significantly with treatment concentrations of CO_2 This is due to the formation of carbonic acid only occurs on the surface of the tissue and not to enter into the sample used for the measurement of pH value is taken not only on the surface so that the network does not affect the pH value.

Retention will raise the pH value. This is because the storage time will affect the increase in total bacteria in meat. Bacteria were grown to degrade proteins and fats because the bacteria are able to produce extracellular protease and lipase enzyme that produces, among others, ammonia, in dole and H_2S.

Physical Characteristics of Meat: tenderness

Tenderness is one of the physical properties were measured after the meat is cooked by the ease of chew time without loss of viable tissue properties. The mean value of broiler meat tenderness at the treatment concentration and duration of storage of CO_2 in modified atmosphere presented in Table 4.

TABLE 4.
MEANS TENDERNESS (KG/CM^2) BROILER CHICKEN MEAT WITH CO_2 CONCENTRATION TREATMENT AND LENGTH OF STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Means
Vacuum	1,61[n]	1,59[n]	1,04[k]	0,04[j]	1,27[x]
CO_2 25%	1,57[n]	1,55[n]	1,16[1]	1,16[l]	1,36[y]
CO_2 50%	1,56[n]	1,55[n]	1,28[m]	1,23[lm]	1,38[y]
CO_2 75%	1,56[n]	l,4[mn]	1,41[mn]	1,34[m]	1,43[y]
Means	1,57[b]	1,50[b]	1,22[a]	1,14[a]	

a,b,c,d Different superscript at the same row showed the real difference (P<0,01)
x,y Different superscript at the same coloumn showed the real difference (P<0,01)
j,k,l,m,n Different superscript at the same coloumn and row showed the real difference (P<0,01)

The results of variance analysis of CO_2 concentration and storage time and their interactions showed highly significant effect (p < 0.0 1) in the value of broiler meat tenderness.

There is a very real interaction between CO_2 concentration and storage time on tenderness. The high concentration of CO_2 will decrease tenderness because bacterial growth is inhibited and short storage will also reduce the softness caused by a number of bacterial and enzymatic activity remains low.

CO_2 concentrations very significant effect on the value of tenderness. Increasing the value of tenderness allegedly because of the treatment of high CO_2 concentrations will affect the metabolism in the flesh. Inhibited bacterial growth so that the degradation of carbohydrates, protein and fat will decrease and increase the value of tenderness. Mooha - lee et al. (1996) stated that the beef in modified atmosphere containing oxygen, nitrogen or carbon dioxide and other gases will reduce shrinkage and improve tenderness.

The longer the storage of the average value decreased tenderness. The decline in value is due to the tenderness of the storage time affects the degradation of proteins, including collagen protein which is the determining factor of the tenderness of meat.

Physical Characteristics of Meat: Cooking Losses

Cooking shrinkage was defined as fluid is lost during cooking and is an indicator of the nutritional value of meat associated with determining the value of the juice and the liquid content of meat and meat nutrition (Soeparno, 1992). The mean value of broiler meat cooking shrinkage treatment with long storage of CO_2 concentration in Modified atmosphere packaging is presented in Table 5. Results of analysis of variance treatment of CO_2 concentration, storage time and their interaction also showed significant effect (P <0.01) to the value of broiler meat cooking shrinkage.

TABLE 5.
COOKING MEANS SHRINKAGE (%) BROILER CHICKEN MEAT WITH CONCENTRATION TREATMENT AND LENGTH OF STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Rerata
Vacuum	$16,53^{j}$	$18,99^{jk}$	$24,59^{m}$	$28,64^{n}$	$22,19^{y}$
CO_2 25%	$16,82^{j}$	$19,51^{k}$	$22,09^{1}$	$29,97^{n}$	$22,09^{y}$
CO_2 50%	$16,27^{j}$	$19,45^{k}$	$19,63^{k}$	$21,00^{k}$	$19,19^{x}$
CO_2 75%	$17,51^{j}$	$17,38^{j}$	$18,55^{j}$	$21,92^{k1}$	$18,84^{x}$
Means	$16,78^{a}$	$18,83^{a}$	$21,22^{o}$	$8,90^{c}$	

a,b,c,d Different superscript at the same row showed the real difference (P<0,01)
x,y Different superscript at the same coloumn showed the real difference (P<0,01)
j,k,l,m,n Different superscript at the same coloumn and row showed the real difference (P<0,05)

There is a very real interaction between CO_2 concentration and storage time on cooking shrinkage values. At concentrations of CO_2 which is low, a higher total bacteria. The bacteria will degrade the protein and soluble protein causes shrinkage cooking so high, while in the longer storage time storage will increase the total bacteria will degrade proteins that cooking shrinkage will also be high.

Treatment concentrations of CO_2, affect the value of cooking shrinkage significantly. In vacuum packaging will increase bacterial activity in decomposing meat proteins lead to the ability to withstand water drops so that the meat becomes loss the water contains. Soeparno (1992) stated that low protein degradation causes shrinkage values lower cooking. Smith et al. (1992) stated that the high concentration of CO_2 will cause decrease the muscle in "drip loss".

Treatment generally longer storage can increase the value of cooking shrinkage. Increasing the value of cooking shrinkage is caused due to long storage will increase the enzymatic activity of proteins that affect the structure of the protein 's ability to bind water into a weak other compounds, further increasing the value of cooking shrinkage. This is in accordance with the opinion Soeparno (1992) which states that the value of cooking shrinkage increases with increasing storage time.

Physical Characteristics of Meat: Power water holding

Water holding capacity of meat is defined ability to hold his own in the presence of water pressure or outside influence. Means shrinkage values cook chicken with long treatment and storage of CO_2 concentration on broiler meat in modified atmosphere packaging presented in Table 6.

Results of analysis of variance treatment CO_2 concentrations showed significant differences while storage time showed a significant influence (P < 0.0 1), while the interaction did not significantly affect the value of the water holding capacity of broiler meat in modified atmosphere packaging.

TABLE 6.
MEANS POWER TIE WATER (%) BROILER CHICKEN MEAT WITH TREATMENT CONCENTRATIONS OF CO_2 AND OLD STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Means
Vacuum	46,23	39,79	35,25	31,67	38,23[x]
CO_2 25%	45,62	41,65	38,35	37,03	40,66[y]
CO_2 50%	45,42	42,45	40,94	37,45	41,57 [y]
CO_2 75%	45,53	42,82	41,08	38,95	42,09 [y]
Means	45,70[a]	41.68[b]	38,91[c]	36,27[d]	

[a,b,c,d] Different superscript at the same row showed the real difference (P<0,01)
[x,y] Different superscript at the same coloumn showed the real difference (P<0,05)

Interaction between CO_2 concentration and storage time was not significantly different to the water holding capacity. This suggests that in this study the water holding capacity of meat is not affected by a combination of both factors.

Rate of CO_2 concentration increased linear with water holding capacity of broiler meat in modified atmosphere packaging. At long treatment will occur storage protein degradation by microorganisms that thrive on meat that causes the release of water bounded protein so the water holding capacity decreased. Soeparno (1992) stated that the water holding capacity is influenced by several factors, including the duration of storage.

Sensory properties: Color

Consumers generally prefer and interested in the color as a main component to determine the quality of food before other factors are taken into account visually. The color factors apparently first noticed and sometimes very decisive as the identification of loss of quality. Means of broiler meat color with long treatment and storage of CO_2 concentration in the modified atmosphere packaging presented in Table 7.

TABLE 7 .
SCORE MEANS MEAT COLOR OF BROILER CHICKENS WITH CO_2 CONCENTRATION TREATMENT AND LENGTH STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

	Length of Storage (day(s))				
CO_2 concentration	0	7	14	21	Means
Vacuum	4,76	4,57	4,23	4,16	4,43[y]
CO_2 25%	4,83	4,67	4,50	4,40	4,60 [y]
CO_2 50%	4,77	4,27	4,10	4,00	4,28 [y]
CO_2 75%	4,97	4,10	4,00	3,50	4,10 [y]
Means	4,79[b]	4,40[a]	4,21[a]	4,02[a]	

[a,b,] Different superscript at the same row showed the real difference (P<0,01)
[x,y] Different superscript at the same coloumn showed the real difference (P<0,01)

Results of analysis of variance treatment of CO_2 concentration treatment, significant, length of storage highly significant (P<0,01) while interaction scores did not significantly affect the color . The interaction between treatment concentrations of CO_2, and storage time had no significant effect on the scores of color, although the concentration of CO_2, the real effect and storage time was highly significant, but the combination had no significant effect.

Scores color change from colorless neutrals decreased to darkness. At a concentration of 75 % CO_2 to form the darkest color, this is due to the formation of metmyoglobin high . Metmyoglobin formed due to low O_2 and high CO_2. In this study, the longer the storage of O_2 concentration increased with lower CO_2 concentrations. High CO_2 concentration causes inhibition of respiration as much, remaining less oxygen which causes the formation of metmyoglobin more so the color is darker.

The longer the storage in modified atmosphere becomes darker flesh color. Ledward (1970) stated that the beef is stored in modified atmosphere packaging with high CO_2 concentrations and low O_2 concentration increased metmyoglobin formation, due to the increased oxidation causes the color to red brown.

Sensory properties: Appearance

Means scores of broiler meat appearance with CO_2 treatment and storage time on broiler meat in modified atmosphere packaging presented in Table 8. Results of analysis of variance treatment of CO_2 concentration, significant, long storage treatment was highly significant (P < 0.01), while interaction scores did not significantly affect the appearance . Not achieved interaction between treatment and storage time of CO_2 concentration on the appearance of the score. CO_2 concentration and storage time affects the appearance of the score, but the interaction of the two treatments was not influence it.

TABLE 8.

SCORE MEANS APPEARANCE MEAT CHICKEN BROILER WITH CO_2 CONCENTRATION AND LENGTH OF TREATMENT STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

CO_2 concentration	Length of Storage (day(s))				
	0	7	14	21	Means
Vacuum	5,00	4,7 3	4,47	4,43	$4,66^{x}$
CO_2 25%	5,17	4,60	4,40	4,30	$4,62^{x}$
CO_2 50%	5,13	4,97	4,60	4,40	$4,77^{y}$
CO_2 75%	5,13	4,90	4,80	4,43	$4,84^{y}$
Means	$5,03^{a}$	$4,88^{a}$	$4,57^{b}$	$4,43^{b}$	

a,b, Different superscript at the same row showed the real difference (P<0,01)

x,y Different superscript at the same coloumn showed the real difference (P<0,01)

The concentration of CO_2, which would raise the high score of the appearance on the surface of the meat for bacteria to evolve so inhibited mucus produced, is also low. Purnomo el al. (1992) stated that food damage often caused by the presence of bacterial growth on the surface of foods that contain high water content. Changes that occur in the form of tire and stinging flavor accompanied the onset of mucus on the surface of the food. The duration of storage of broiler meat will lower the score the appearance that the meat will be slimier. The longer the storage will increase the total bacteria that degrade components of meat.

Sensory properties: Smell

Means scores odor by treatment with CO, and storage time on broiler meat packing in atmosphere modified presented in Table 9. The analysis result of variance significant concentration of CO_2 treatment, storage time treatment was highly significant (P <0.01), while interaction scores did not significantly affect the smell.

TABLE 9

. SCORE MEANS BROILER CHICKEN MEAT SMELL THE TREATMENT OF CO_2 CONCENTRATION AND OLD STORAGE IN MODIFIED ATMOSPHERIC PACKAGING

CO_2 concentration	Length of Storage (day(s))				
	0	7	14	21	Means
Vacuum	5,10	4,30	4,07	3,57	$4,26^{x}$
CO_2 25%	5,07	4,43	4,27	3,70	$4,37^{x}$
CO_2 50%	5,13	4,53	4,47	3,80	$4,48^{x}$
CO_2 75%	5,07	4,80	4,53	4,17	$4,64^{y}$
Means	$5,09^{a}$	$4,52^{a}$	$4,33^{b}$	$3,81^{b}$	

a,b,c Different superscript at the same row showed the real difference (P<0,01)

x,y Different superscript at the same coloumn showed the real difference (P<0,05)

In vacuum packaging occurs very foul odor when compared with the concentrations of other packaging. The stench of high CO_2 concentrations also occur in the lowest, this is caused by the presence of spoilage bacteria. The stench is caused due to volatile compounds. Varnam and Shutherland (1995) stated that the stench of the chicken meat due to the presence of H_2S gas, dimethyl sulfide, dimethyl disulfide, methyl acetate, ethyl acetate, heptadiene, methanol and ethanol.

Changes during storage odor are caused by the growth of bacteria that produce foul smell. At high protein foods such as meat will be easily overgrown bacteria produce extracellular enzymes prosthesis in (Alur et al ., 1988) . These protease enzymes will use meat proteins for nutrition. Protein will be parsed into a polypeptide compound, oligopeptides and amino acids (Schlegel, 1994) . Free amino acids are converted formation by hydrolase enzymes that cause changes in flavor due to the onset of NH_3 and H_2S gases (Miller et al ., 1990) .

IV. CONCLUSIONS

Conclusion

High concentration of CO_2 will lower the water content, total bacteria, and tenderness, cooking shrinkage, color score, score and score smells appearance, increase water holding capacity and no Trans change the pH value of broiler meat in modified atmosphere packaging. Retention will increase the water content, total bacteria, pH, cooking losses, water holding capacity, color score, score the appearance, the smell of meat and meat tenderness of broiler decline in modified atmosphere packaging. Interaction between CO_2 concentration and storage time affects only the total bacteria, tenderness, and shrinkage cook chicken broiler modified atmosphere packaging.

Suggestion

The use of CO_2 concentration 75 % and 50 % in broiler meat in modified atmosphere packaging can still be consumed until day 14.

REFERENCES

[1] Alur, M.D., D.P. Nerkars and V. Venugopal. 1988. Growth and protease secretion by spoilage bacteria : Influence of nitrogen fractions of proteinaceous food on Aeromonas hydrophyla. J. Food Sci. 53 : 243 —246.

[2] Astuti, M. 1980. Experimental Design and Statistical Analysis. Part Animal Husbandry Faculty UGM. Yogyakarta.

[3] Brody. A.L. 1989. Controlled/Modification Atmosphere/Vacuum Packaging of Food. Food and Nutrition Press Inc. Trumbul Connecticut.

[4] Cant, N.A. and F.E. Cuningham. 1983. Standard and experimental method of identification and enumeration. Dalam : Methods of Preservation of Poultry Product. In the Microbiology of Poultry Meat Product. Academic Press Inc. New York.

[5] Lawrie, R.A.. 1995. Meat Science. U.I. Press Jakarta. (Translated by A. Parakkasi dan Y. Amwila).
[6] Miller, M.T., D.B. Bawcom, C.K. wu, M.K. Meade and C.B. Ramsey. 1990. Microbiology of hot-fattrimmed beef. J. Anim Sci. 73 : 1368-1371.
[7] Mooha-lee, J. Sebranek and F.C. Parrish Jr. 1996. Accelerated post-mortem aging of beef utilizing electron-beam irradiation and modified atmosphere packaging. J. Food Sci. 61 : 133-136.
[8] Purnomo, H., M.C.H. Padaga, I.Suryo dan Purwadi. 1992. Technology Basic Livestock. Animal Husbandry Faculty, Universitas Brawidjaja, Malang. (unpublished).
[9] Richard, I. 1987. Modified atmosphere packaging. In F.A. Payne Modern Processing Packaging and Distribution System for Food. Blackie and Sons Limited Bishopbirggs. Glasgow.
[10] Schlegel, H.G. 1994. Allgemeine Microbiologie.. Gadjah Mada University Press. Yogyakarta. (Translated by R.M.T. Baskoro).
[11] Smith, J.P. B. Simpson and H. Ramaswamy. 1992. Modified atmosphere packaging, principles and application. Dalam : Hui Y.k. *Encyclopedia* Food and Technology. John Willey and Sons Inc. New York.
[12] Soeparno. 1992. Meat Science and Technology. Gadjah Mada University Press. Yogyakarta.
[13] Webster, C.E.M., F.A. Munesz-Gonzales and D.A. Ledward. 1982. Role of lipid and glycerol in determining the shelf-life of glicerol desorbed intermediate moisture meat products. J. Meat Sci. 6 : 191-198.

CONSERVATION BIOLOGY

RESOURCE POTENTIAL OF SNAKE FRUIT (*Salacca zalacca* var Amboinensis) AND CANARY (*Canarium amboinense*) IN THE LIFE OF SERAM ISLAND SOCIETY, MOLUCCAS

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***Abstract*—Strategic issues in this research are food security and poverty alleviation in the life of society in Ceram island who called themselves Alune People and Wemale People. They stated that snake plant and canary are endemic and native plants in this region. This is because both of these commodities have been around since ancient times can not be separated in their lives and cultures. Thus the potential of the plant needs to be known for the economic development of this region, but also the tradition of community life should be studied in relation to both commodities through traced study in ethnobotany. Found in the field in addition to the ivory-colored snake fruit fruits, is also red snake fruit. The issue of food security and poverty alleviation can then be solved by strengthening the development of agribusiness basis of snake fruit and canary, where the idea is an effort to strengthen the bargaining position of the society in Ceram Island in Maluku where traditional farming systems are still being practiced. This means that this two commodities is growing through the mediation of the animals in the forest and there is no action by human cultivation. Snake fruit commodity in Ceram island has been established by the Decree of the Minister of Agriculture republic of Indonesia in 2003 as National Superior variety, but the fact is that the development of the cultivation is poor and production could not be relied upon as an industrial raw material. The objectives of this research was to make both commodities as superior commodities which have economic added-value that deserved by Ceram communities.**

***Keywords*—food security, snackfruits, canary, seram island**

I. INTRODUCTION

Society as dynamic social problems continue to be influenced by both external and internal factors . Case study in ceram island tried to present how resistanc of indigineous people (Alune and Wemale People) reacts to enclose various external influences, starting with the presence of migrants from Java island in 1954 at Kairatu . On the other hand, Ceram people feel threatened because their property right to their land had been deprived due to geopolitical issues that are not necessarily well understood by neither the migrants nor by the Ceram people. Later on, the presence of forest exploitation by logging companies and plywood mills . Are there benefits to Ceram people ? They have not been able to work in this industrial sector, and eventually ceram people become isolated and inferior.

Understanding society and solving social problems is not a simple concept . This means that participation of universities as Unpatti is needed to give more significantly contribution through research and community service that is well planned and aplicable.

Ceram people who live in the mountains have abundant natural resources such as canary and snake fruit commodities. Both of these plants grow wild in the forest. Although in 2003 the Ministry of Agriculture Republic of Indonesia has been given an official certificate to “Salak Riring” as national superior variety, however what does it mean to them? if it does not provide a significant economic impact. All Ceram people have a dream that when canary and snake fruit became famous for having a distinctive flavor with good packaging. Pillar agribusiness from upstream to downstream must be built to ensure the stock of market demand, good product quality and guarantee of an open market for their products. The emergence of supermarkets in Ambon city and soon will be reaching Ceram island is an economic opportunity that must be anticipated. It will be linked to the concept and model of community empowerment that should be pursued seriously.

Recently multidisciplinary research is needed to solve many environmental problems which intersect with social problems. Issues that has not been completely addressed until now are the problems of poverty and food security. Both of these issues will greatly affect the pattern of environmental management in both rural and urban areas. The objectives of this research was to find a package of technologies that able to improve the productivity and product quality, such as the appropriate technologies to unshelled canary fruit and how to make

snake fruit commodity towards snake fruit chips industry. Then build agribusiness pillar of canary and snake fruit that start by managing the upstream production of raw materials as well as setting up downstream with the post-harvest technologies based on domestic industry.

This study was expected to provide benefits for building families economic and poverty alleviation . In addition it will also provide reinforcement to the concept of food security in the community so that snake fruit and canary can provide energy to the community to sustainthe level of food sufficiency.

II. METHODS

This research is a review of participatory acts (participatory action research). In this case, this research used a systemic approach with focused discussion, field observation and in-depth interviews. Questionnaire instruments were used in the survey. Focussed discussions was conducted by interviewing government officials , traditional leaders and key respondents in each kinship group. This was done to look for information related to customs and land tenure which generally not certified yet customarily recognized as well as social economic capitals which are still subsistence. Aspects being observed this research were Ethnobotany, Culture techniques (Land Systems , Plant Cultivation , Agroclimate) and Post Harvest Technology. Observations were also done on Economic and Social aspects and data collection of the potencial of commodities

III. DATA ANALYSES AND INTERPRETATION

These five research areas (Uweth , Buria , Riring , Rumahsoal and Lohiasapalewa villages) included in the District Taniwel, West Seram Regency, Maluku Province. Located in the mainland and only separated by a river and mountains. The research area is located in isolated mountainous areas.

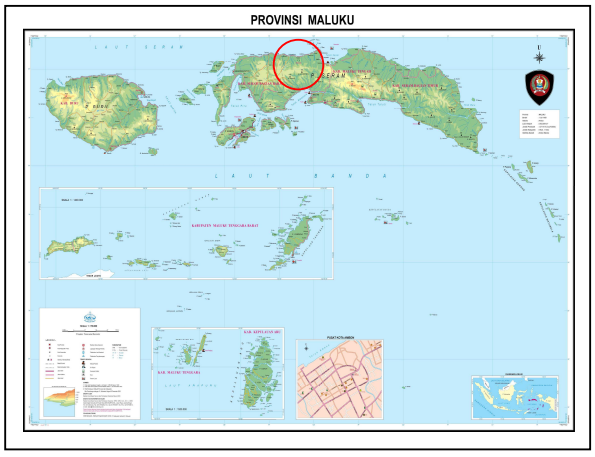

Figure 1. Map of Moluccas; Field Study Seram Island

Accesibility of a region is affected the region development significantly, where regions with high levels of accessibility has a faster rate of development compare to the region with lower accessibility. Accessibility to the research site is difficult since it should be reach by walking in long distance, except for Uweth village which located on the coast within 3 (three) Km to the capital of the Taniwel District.

Population in the study area are mostly natives of the Alune tribe. Data of registered population in 2011, with a population density of 18.98 people per km^2.

TABLE 1.
POPULATION DENSITY IN RESEARCH AREA IN 2011

Village	Household	Number of population		Total
		Male	Female	
Uweth	75	205	167	372
Buria	120	312	350	662
Riring	172	393	352	745
Rumahsoal	47	134	127	261
Lohiasapalewa	58	157	137	294

Based on the observed data in the field in the village monograph, the percentage of the working age population in the Buria village showed the highest number followed by Riring, Uweth , Rumahsoal and Lohiasapalewa villages . The population of productive age in the study area is very large , however it can not be denied that not all residents of this age are working or have a job . Education of the head of family in the region dominated by the primary school level . Agriculture is the main livelihood of the population in the study area , while the non-agricultural sector occupied only by a small portion of the population in the region [6].

Social relations in the society is still well established, it could be seen from the culture of helping each other, which is still maintained until now. Traditional institutions in the form of local knowledge in the study area which are still carried from generation to generation is environmental ”SASI”. In practice, the opening of SASI by farmers is marked by a splash of water fortified with prayers by religious leaders (pastors) with rules that have been agreed institutionaly. Thus the people in the study area generally have awareness of and benefit from the natural resources available. This does not mean that society / the locals closed to the presence of technology or changes comes from outside, but the people believe the natural environment should be maintained for the sustainability of biological resources for future generations [3].

Canary is one of native commodity in Maluku province with huge potency. The problems encountered today is the unavailability of sufficient data to describe the distribution of its potential in the Maluku . This research was conducted as an effort to develop a canary potential in a few villages in the mountainous region in the district of Taniwel, West Seram Regency.

Data collection of canary potential was done by purposive sampling based on the distribution of canary found at the reseacrh sites. Lines of observations was made and adjusted to the local topography . In those lines of observations a plot size of 20 meters x 20 meters was made and the dimensions of canary tree including diameter and height were measured and recorded.

Based on the results of an inventory conducted at the research site, it was found that the most canaries tree was found in Riring village as many as 81 trees/ha, followed by 57trees/ha in Uweth village. Distribution of the least canary trees was found in the village of Rumahsoal as much as 23 trees / ha. Distribution of tree diameter measured on research site ranged from 11.5 cm to 134.4 cm and the height of tree without branches can reach 30

meters. The calculation of the volume of canary trees showed that volume of tree without branches ranged from 36.621 m3/ha to 298.882 m3/ha. Data inventory of potential locations of canary trees in the study area was presented in Table 2.

Table II. Inventory of Potency of Canary Tree in Research Area

No	Village	Number of Tree (N/ha)	Diameter (cm)	Heihgt (m)	Volume*) (m^3/ha)
1.	Uweth	57	11,5 – 122,5	10 – 30,4	79,011
2.	Buria	28	20 – 105	9 – 25	36,621
3.	Lohiasapalewa	50	21,5 – 63,5	5,5 – 12,6	44,309
4.	Rumahsoal	23	54,2 – 149,8	7,3 – 22,5	232,265
5.	Riring	81	42,5 – 134,4	10,6 – 22,7	298,882

*) Tree volume without branches.

It had been shown in table 2 that the canary trees found in research areas have a large size, so that the utilization of timber and fruit will be very valuable [1]. However, the sustainability of results and continuity of production should be maintained , through silvicultural measures in accordance with the carrying capacity of the land.

(a) (b)

Figures 2. Canary (a) and Snake Fruit (b)

Snake fruit is one of essential commodity for mountain communities in the study areas. Data collection of snake fruit potency was collected using purposive sampling by considering the presence of plant distribution at the study site. In the areas where snake fruit tree were found Lae plots of 20 meters x 20 meters or 400 m^2 was made and observations was done on the trees.

From the results of the inventory of snake fruit in research area, the highest number of trees of 293 clumps was found in Rumahsoal village , followed by 225 clumps in Buria village. The lowest number of trees of 48 clumps was found in Lohiasapalewa village in Uweth village snake fruit trees were not found. When viewed from the average number of clumps per hectare , Riring village had the highest number which was 3450 and 4000 clumps / ha , followed by Rumahsoal village as many as 1465 clumps / ha . Lowest number of clumps found in the village of Lohiasapalewa which was 415 clumps/ha . Inventory data of potency of snake fruit tree at the study site is shown in Table 3 [5].

TABLE III..
INVENTORY OF POTENCY OF SNAKE FRUIT TREE IN RESEARCH AREA

No	Village	Number of Tree (Clumps)	Number of Clumps per Plot*)	Average Number of Clumps (R/ha)
1.	Uweth			
2.	Buria	225	20 – 100	1406
3.	Lohiasapalewa	48	1 – 79	415
4.	Rumahsoal	293	42 – 73	1465
5.	Riring	138**); 160***)	138**; 160***)	3450**); 4000***)

*) Size of sample plot (20 x 20) m, **) Red Snake Fruit, ***) Ivory Snake fruit

Potency of snake fruit tree in Lohiasapalewa village was low due to the distribution of plants which was very few , and only owned by a few farmers which were parents or ancestral heritage. On the other hand, most people have not been interested in planting and cultivating of snake fruit crop [4].

Conclusion

Ceram People in inland of Ceram island is a shifting land communities, who are forest gatherers, living isolated and completely depend on nature. Nature is a laboratory of their lives in the past , present and in the future .

Ceram people living in harmony with nature . Knowledge to manage environment was carried on from generation to generation by verbal description only. Obedience to nature form point of view that the universe is something which gave inspiration to deliver the concept of holistic and concept of totality in managing resources and their interactions with other living things. That means that the whole concept of the life space that mountain , beach and the sea are inseparable . This understanding begins with their very strong views to the concept of mountain and wate , which deliver the concept of an orderly way of life and to continue to be believed from one generation to the next.

From long fallow cultivation, it appears that they have the wisdom to manage natural resources as a source of food for life. The diet of Ceram people describe that they are relatively not lack of food . Even the type of food consumed have high carbohydrate (sago) , protein (pork , Kusu , deer , shrimp and fish) as well as other vitamins (vegetables) .

If the measure of poverty used is based on the ability to fulfill family food consumption , then the ceram people is not included in the category of the poor. Poverty of Ceram people as a autcton resident and so did other alokton population residing on the island of Seram, is a feeling of isolated due to mechanisms , systems , and regional and national government policies which in turn makes them shackled in that isolation . Yet the reality on the field proves both autocton and alocton residents are people who are not isolated , because they are always in touch with the outsider because of the trade . For example, they can communicate regularly with inter-island traders and other migrants who came freely use sea transportation.

So the traditional economic pillars of Ceram people is relatively strong and indigenous kinship system is still maintained because of their orientation to the cosmology world. Ceram island in the shape of a woman body and all of her organs is representatived by territory which is

clearly divided and controlled by each kinship . That way it will ensure the orderly of their lives for the sake of exhausting work to the life of the island called Ceram . Obedience to the tradition because there is a strong accord that is the orientation of the whole universe and not partial so all must be protected simultaneously and at the same time also constructed system of norms to regulate the whole order of life . Therefore, the traditional economic pillars of Ceram people increasingly strengthened so that they become obedient to the their customs.

REFERENCES

[1] A.K.A. Dandjouma, C. Tchiegang, J. Fanni and M. Parmentier, Changes in *Canarium schweinfurthii* Engl. oil quality during microwave heating . Resecrah Paper. *European Journal of Lipid Science and Technology*. 108 (5): 429-433, 2006.

[2] E. Kriswiyanti, I. K. Muksin, L. Watiniasih Dan M. Suartini, Pola Reproduksi Pada Salak Bali (*Salacca zalacca* Var. *Amboinensis* (Becc.) Mogea). Jurnal Biologi XI (2): 78-82. 2008

[3] M.J. Pattinama, dkk, Studi Bina Mulia ke Lautan di Pulau Seram, Provinsi Maluku : Mempelajari Kearifan Lokal Orang Seram Mengelola Lingkungan Darat dan Laut, Laporan Penelitian Hibah Bersaing, Unpatti-Dikti. 2007.

[4] T. Sudaryono, S. Purnomo, M,. Cultivar distribution and estimation of area development of Salacca (in Indonesian). Penel. Hort., 5, 1-4. 1993

[5] M.J. Pattinama, H.E.P. Leimena, A. Boreel and J.K.J. Laisina, Pengembangan Agribisnis Komoditas Kenari (*Canarium amboinense*) dan Salak (*Salacca zalacca* var Amboinensis) Sebagai Upaya Memantapkan Posisi Tawar Ekonomi Serta Memperkuat Ketahanan Pangan Bagi Masyarakat Wilayah Pegunungan di Maluku. Laporan Penelitian Penprinas MP3EI. 2012

[6] M.J. Pattinama, A. Boreel, H.E.P. Leimena, and J.K.J. Laisina, Pengembangan Agribisnis Komoditas Kenari (*Canarium amboinense*) dan Salak (*Salacca zalacca* var Amboinensis) Sebagai Upaya Memantapkan Posisi Tawar Ekonomi Serta Memperkuat Ketahanan Pangan Bagi Masyarakat Wilayah Pegunungan di Maluku. Laporan Penelitian Penprinas MP3EI. 2013.

Inventorization of Edible Macrofungi from The Tropical Rainforest Ecosystem of Meru Betiri National Park East Java

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***Abstract*—Indonesia is a tropical country with very high biodiversity rate, including the biodiversity of macrofungi. Macrofungi is one of the biological resources which play important roles in human life. Some kinds of macrofungi are edible with such characteristics as having nutritional value, fruit body, and non-poisonous. High protein value makes macrofungi an ideal source of food. Beside of protein, they may also contain vitamin B complex and some mineral salt from the elements of Ca, P, Fe, Na, and K. This research aimed to inventory edible macrofungi in Meru Betiri National Park East Java that can be used as alternative food sources. The method that used in this research was the explorative method with descriptive analysis. The sampling process took place alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java. The tropical rainforest ecosystem that was reached from Sukamade Resort had the geographic coordinates S 8º 27' 08" and E 113º 48' 42" then going south for around 100 metres and turn west, going into the tropical rainforest ecosystem until around three kilometres. The collecting of macro fungi samples was limited until three metres left and right side from the tropical rainforest trail. The result of this research showed there were 45 different genera and 17 of them are edible. Those 17 genera are *Auricularia, Bovista, Coltricia, Coprinus, Creprodetus, Mycena, Flamulina, Kuehneromyces, Laccaria, Lactarius, Leucocoprinus, Piptoporus, Polyporus, Psathyrella, Pycnoporus, Sarcoscypha,* and *Tremella*.**

***Keywords*—Edible Macrofungi, Meru Betiri National Park, Biodiversity, Tropical Rainforest**

I. INTRODUCTION

INDONESIA is a tropical country with high biodiversity of flora and fauna. The presence of tropical rainforest in Indonesia highly contributes to flora and fauna biodiversity in Indonesia [7,14]. Aside of flora and fauna, Indonesia also has high diversity of fungi. One example of common fungi is macrofungi, there are approximately 200,000 species of macrofungi in Indonesia considering the humidity and tropical temperature that are supporting the growth of macrofungi [7].

Meru Betiri National Park East Java, with the geographic coordinates position S 8º 22' 16" - S 8º 32' 05" and E 113º 37' 51" - E 113º 57' 06" is one of the national parks located in East Java, Indonesia [1,17]. Meru Betiri National Park East Java has tropical rainforest ecosystem zone [18] which has natural condition supporting biodiversity of macrofungi because of its humidity and high rain precipitation. The potential of macrofungi found in the tropical rainforest ecosystem hasn't been known much.

The potential of macrofungi in general is its function as decomposer, but some macrofungi are edible and therefore potential for food source [8]. Some macrofungi are edible because it has fruit body, not poisonous, and has nutritional value [9]. Edible macrofungi has high protein with complete amino acid including the essential amino acid which human needs [8] and also contains vitamin B complex [4,8,13] and some mineral salt from the elements of Ca, P, Fe, Na, and K [6,8,10,15].

Biodiversity and potential of macrofungi in Indonesia is still hasn't known much. The inventorization edible macrofungi alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java aims to make records of macrofungi which can be utilized as alternative food source.

II. MATERIALS AND METHODS

Inventorization is done for 2 days, started from February 7 - 8 2013 in Meru Betiri National Park East Java. The selected location for macrofungi sampling is alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java. That area reached from Sukamade Resort which has geographic coordinates S 8º 27' 08" and E 113º 48' 42" then going south for around 100 metres and turn west, going into the tropical rainforest ecosystem until around 3 kilometres. Macrofungi sampling is limited by 3 metres on the lefside and rightside of the trails.

The instruments used in this research are digital camera, knife, cutter, scoop, labels, plastic bags, stationery, calipers, determination and characteristic sheets, latex gloves, soil tester, magnifying glass, the book *The Complete Encyclopedia of Mushrooms* by Keizer (1998) and *The Great Encyclopedia of Mushrooms* by Lamaison and Polese (2005) and also some journals to facilitate observation and identification. The materials used for identification is all the macrofungi found alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java.

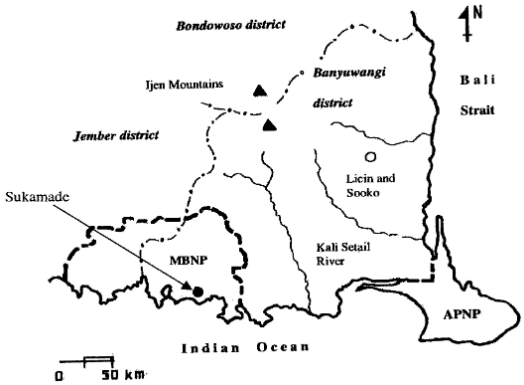

Fig. 1. Eastern Part of Java, MBNP is Meru Betiri National Park East Java [18].

Samples were photographed with standard comparator such as ruler and calipers. The environmental parameters where the sample grows were also noted, for example substrates (trees, decomposed woods, and soil), soil's pH, and substrate temperature.The samples were collected by knife or cutter for macrofungi living in a decomposed tree, whereas for macrofungi living on the decomposed remains above soil is collected using scoop. After the sample is collected, it is placed in a labeled plastic bag.

The collected macrofungi were classified by its fruit body such as cup fungi, puffball fungi, stick fungi, jelly fungi, bracket fungi, and coral fungi. Then, further identification with the character of each macrofungi and type of growth substrates was conducted to determine its genus. The macrofungi edibility potential is known by reffering to *The Complete Encyclopedia of Mushrooms* by Keizer (1998) and *The Great Encyclopedia of Mushrooms* by Lamaison and Polese (2005).

III. RESULT AND DISCUSSIONS

The inventorization of edible macrofungi alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java found 76 identified isolates and 4 unidentified isolates because of the characters were not matching to the references. Those 76 isolates were classified into their respective genera, resulting 45 different genera.

TABLE 1
List of Macrofungi Genera Alongside The Trail of The Rainforest Area of Meru Betiri National Park East Java

Number	Genera	Total
1	*Aleuria*	1
2	*Aphyllophorale*	1
3	*Ascocoryne*	1
4	*Auricularia**	1
5	*Bjerkandera*	1
6	*Bovista**	1
7	*Bulgaria*	1
8	*Clavulina*	1
9	*Clavulinopsis*	2
10	*Collybia*	1
11	*Coltricia**	5
12	*Coriolopsis*	1
13	*Cortinarius*	1
14	*Coprinus**	1
15	*Creprodetus**	1
16	*Flamulina**	1
17	*Ganoderma*	3
18	*Geastrum*	1
19	*Helvella*	1
20	*Heterobasidion*	1
21	*Hirneola*	1
22	*Kuehneromyces*	1
23	*Laccaria**	1
24	*Lactarius**	2
25	*Leucocoprinus**	1
26	*Marasmiellus*	1
27	*Marasmius*	3
28	*Megalocystidiu*	1
29	*Meruliopsis*	1
30	*Mycena**	4
31	*Oligoporus*	1
32	*Peroneutypa*	1
33	*Pholiota*	1
34	*Piptoporus**	1
35	*Polyporus**	3
36	*Psathyrella**	1
37	*Pycnoporus**	1
38	*Rosellinia*	1
39	*Sarcoscypha**	1
40	*Skeletocutis*	1
41	*Stereum*	1
42	*Trametes*	14
43	*Tremella**	1
44	*Typhula*	4
45	*Xylaria*	4
	*Edible macrofungi	

Edible macrofungi has fruit body, not poisonous, and has nutritional value [9]. From the alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java, the edible macrofungi were found are *Auricularia, Bovista, Coltricia, Coprinus, Creprodetus, Mycena, Flamulina, Kuehneromyces, Laccaria, Lactarius, Leucocoprinus, Piptoporus, Polyporus, Psathyrella, Pycnoporus, Sarcoscypha,* and *Tremella.*

Generally macrofungi contain 90% water and 10% dry matter. Protein content varies between 27 and 48%, carbohydrates are less than 60% [5], and low-fat [2,8] only consisting of 2-8% of the dry weight [5,8]. High protein contents are very ideal as a food source for it contains every essential amino acid the body needs [8]. Macrofungi proteins contain all nine amino acids essential for human and they are especially rich in lysine and leucine, which are lacking in most staple cereal foods [12]. Furthermore, it is known that the protein content of macrofungi is about twice that of vegetables and four times that of oranges [3].

The fruit body of edible macrofungi is a great source of vitamin B complex [4,8,13]. Vitamin B complex consists of riboflavin ($B_{2)}$, niacin (B_3), thiamine (B_1), biotin, folic acid, and vitamin B_{12}. 100 grams of fresh macrofungi gives more than 25% vitamins needed by an adult per day. Macrofungi is unique because it contains vitamin B_{12} which not every vegetable has. In each gram of

macrofungi contain 0.32 to 0.65 mg vitamin B_{12}. Niacin is essential for forming enzymes needed to transform sugar into energy, also maintaining body tissues to keep healthy. Riboflavin is needed to process nutritions such as vitamin $B_{6,}$ niacin, and folic acid to a simpler form which can be utilized by the body [8].

Macrofungi cultivation in Indonesia is relatively advanced compared to other countries such as China, Japan, Taiwan, France, Italy, United States, and others. Currently, more than 15 types of macrofungi have been cultivated in the world. In Indonesia, there are some types of macrofungi that have been known and cultivated, such as *Volvariella*, *Agaricus*, *Pleuarotus*, *Auricularia*, *Lentinus*, *Flamulina*, *Velutives*, and *Grifola* genera [16]. From alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java, it is found that 17 genera have potential for food source. This fact increased the information about diversity of edible macrofungi which can be cultivated as alternative food sources.

IV. CONCLUSION

Genera of edible macrofungi alongside the trail of the tropical rainforest ecosystem of Meru Betiri National Park East Java are *Auricularia*, *Bovista*, *Coltricia*, *Coprinus*, *Creprodetus*, *Flamulina*, *Kuehneromyces*, *Laccaria*, *Lactarius*, *Leucocoprinus*, *Mycena*, *Piptoporus*, *Polyporus*, *Psathyrella*, *Pycnoporus*, *Sarcoscypha*, and *Tremella.*

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REFERENCES

[1] Aliadi, A. *Community Forestry Supporting Resilience in Meru Betiri National Park, Indonesia*. Lembaga Alam Tropika Indonesia.2005.
[2] Buswell, J. A. Lignocellulolitic Enzyme Profiles of Edible Mushroom Fungi. *World J. Microbiol. Biotechnol.*12: 537-542.1996.
[3] Chan, H. K. M. Consumption of Edible Mushrooms in Hong Kong. *Mushroom Newsletter for the Tropics* 1(4): 5-10. 1981.
[4] Chang, S. T., Buswell, J. A. Mushroom Nutriceuticals. *World J. Microbiol. Biotechnol*. 12: 473-476.1996.
[5] Crisan, E. V., Sands, A. 1978. *Nutritional value.* In: S. T., Chang and W. A., Hayes (Eds). *The Biology and Cultivation of Edible Mushrooms*. London, Academic Press Inc. pp. 137-165.
[6] Fasidi, I. O. Studies on Volvariellaesculenta (Mass) Singer: Cultivation on Agricultural Wastes and Proximate Composition of Stored Mushrooms. *Food Chem*., 55: 161-163. 1996.
[7] Gandjar, I., Syamsurizal, W. *Mikologi Dasar dan Terapan.* Jakarta: Yayasan Obor Indonesia. 2006.
[8] Ingram, Stephanie. *The Real Nutritional Value of Fungi.* United Kingdom: Cancer Research UK. 2002.
[9] Keizer, G. J. *The Complete Encyclopedia of Mushrooms.* The Netherland: Rebo International. 1998.
[10] Kuforiji, O. O., Fasidi, I. O., Odunfa, S. A. Nutritive Value of Pleurotus tuber-regium Cultivated on Different Agro-industrial Wastes. *Nig. J. Microbiol*., 17: 63-67.2003.
[11] Lamaison, J. L., Polese, J. M. *The Great Encyclopedia of Mushrooms*. Germany: Konemann. 2005.
[12] Li, G. S. F., Chang, S. T. *Nutritive value of Volvariella volvacea.* 1982. In: *Tropical Mushroom. Biological Nature and CultivationMethods*. Ed. Chang, S. T., Quimio, T. H. The Chinese University Press. Hong Kong.
[13] Mattila, P. K., Konko, M., Eurola, J., Pihlava, J., Astola, L., Vahteristo, V., Hietaniemi, J., Kumpulainen, N., Valtonen, V., Piironen, V. Contents of Vitamins, Mineral Elements and Some Phenolic Compounds in The Cultivated Mushrooms. *J. Agric. Food Chem*. 49: 2343-2348.2000.
[14] Nandika, D. *Hutan Bagi Ketahanan Nasional*. Surakarta: Muhammadiyah University Press. 2005.
[15] Ogundana, S. K., Fagade, O. E. Nutritive Value of Some Nigerian Edible Mushrooms. *Food Chem.*, 8: 263-268.1982.
[16] Suharjo, Enjo. *Budidaya Industri Jamur Konsumsi di Indonesia.* Pusat Penelitian dan Perkembangan Peternakan Kementerian Pertanian. Temu Teknis Nasional Tenaga Fungsional Pertanian 2006.
[17] Taman Nasional Meru Betiri. *Statistik dan Laporan Tahunan Taman Nasional Meru Betiri.* Jember: Taman Nasional Meru Betiri. 2000.
[18] Whitten, T., Soeriaatmadja, R. E., Affif, S. A. *The Ecology of Indonesian Series Volume II: The Ecology of Java and Bali*. Singapore: Periplus Editions (HK) Ltd.1996.

COMPARATIVE STUDIES OF THE WATERBIRD DIVERSITY FROM FAMILY SCOLOPACIDAE AND CHARADRIIDAE ON WONOREJO CONSERVATION AREA IN SURABAYA

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***Abstract*— Birds are one class of Animalia that have high biodiversity in Indonesia, especially water birds (shorebird) as an active migratory birds from the northern hemisphere to the southern hemisphere, and Indonesia is one of the migration paths of traffic and always used as a stopover place while preparing for the journey to the next. One of the places in Indonesia is in a conservation area on Wonorejo, Surabaya where traversed by migratory birds, especially from Scolopacidae and Charadriidae because both families are the largest and most diverse group of birds shorebird. To obtain these data, this study used the inventory data through birding activities and Point Count method so that it was not directly participating in conservation activities and maintaining and also taking care of existing ecosystems. It was also expected as a diversity comparable data at any location that is used (there were between Bosem-Dermaga pond and Gajahan pond) so as to know the factors that affect and cause the loss of natural habitat as well as the existence of water birds. The results showed that Dermaga-Bosem pond had a higher level of diversity than Gajahan pond diversity based on the value obtained. The evaporation ponds in their inactive period presented low similarity with other ponds because when the ponds was evaporated and downs, the bird species abundance was more higher with highly domination. However when the ponds in their active period, could be presented high similarity because based by high tide or some people was fishing there.**

***Keywords*— shorebird, migration, inventory, conservation.**

I. INTRODUCTION

Surabaya East Coast region, one of which includes Wonorejo, has been designated as a conservation area by the local government in 2005, the implementation for the conservation of mangrove forests have not been fully realized, it is the development of human settlements and the first realization is expanding to a change in land use is an area that initially only conservation of mangrove forest and then converted into a tourist area accoding to the laws of no. 22, 1999. In fact, Wonorejo region is one of the Important Bird Areas or regions that play an important role in the conservation of birds in the area, especially waterbirds.

This requires data collection, especially water birds of the family Scolopacidae and Charadriidae because this family includes two groups of birds scaffolding and always dominate populations of waterbirds during migration season. This collection serves as conservation of water birds at once biomonitoring and bioindicator of environmental pollution.

The aims of this study is to study the differences in the location of the area that is the area that shows the level of diversity Wonorejo different, to determine the level of diversity in the location of plots: Dermaga-Bosem pond and Gajahan pond in the region, and to determine the factors affecting the changes in the level of diversity at these locations.

II. MATERIAL AND METHODS

Data collection was conducted using Point Count and use binoculars and monoculars with overland. The data is taken from the number of birds in the family Charadriidae and Scolopacidae encountered at each specified location which amounted into 2 post. Observations in each post that will randomly determined using a maximum of 15 minutes. Retrieval of data in three locations to be observed will be done twice, with each data collection was done every Saturday since 13 April 2013 until 18 May 2013 in the morning around 5:00 am-9:00 pm and in the afternoon around 16:00 pm-18:00 pm. So in each of the target where the research will be conducted six times the retrieval of data. Here's the map:

Fig. 1. Description of the location in Wonorejo Conservation Area used in the study: (A) Dermaga-Bosem pond and (B) Gajahan pond

III. RESULTS AND DISCUSSION

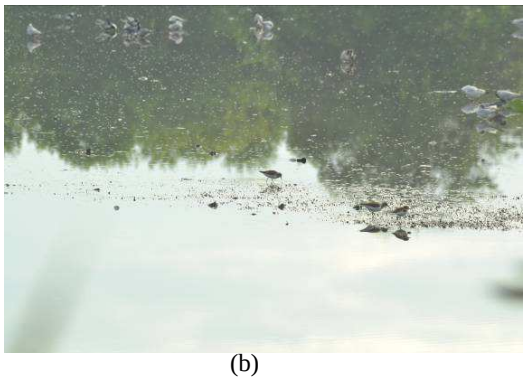
Based on the observations that have been carried out for 6 times, has obtained the following data:

TABLE 1.
THIS TABLE SHOWS THE DIFFERENCES NUMBER OF INDIVIDUALS PER SPECIES BETWEEN ON DERMAGA-BOSEM POND AND GAJAHAN POND

Species of Birds	Number of individual each spesies on	
	Dermaga-Bosem pond	Gajahan pond
Javan Plover	243	53
Kentish Plover	32	0
Pacific Golden Plover	1	0
Lesser Sand Plover	1	0
Common Sandpiper	7	5
Wood Sandpiper	2	0
Rufous-necked Sandpiper	534	19
Long-toed Stint	2	0
Whimbrel	1	272
Bar-tailed Godwit	0	7
Marsh Sandpiper	0	4
Total	823	360

Ponds during periods of inactivity in production presented low similarity with other ponds because when the ponds was evaporated and downs, the bird species abundance was more higher with highly domination. However when the ponds in their active period, could be presented high similarity because based by high tide or some people was fishing there (Figure 2). The high similarity was caused by few communities still seek food in same place day by day and other reason is the place has much abundance food that specific with some bird communities, for example, Gajahan pond was being a favourite place for Whimbrel (in Indonesia, it called Gajahan) because of their specific abundance food like mollusca and some crab. These was contrast with Dermaga-Bosem pond that it had much abundance food but not too specific so that a lot of communities birds taken this place for seek food. In this case, the birds seek food with activity levels were high such as Javan Plover, Kentish Plover, Rufous-necked Stint, and Whimbrel.

(a)



(b)

Fig. 2. Description of the location in Wonorejo Conservation Area used in the study: Dermaga-Bosem Pond (a) during inactive period showed more higher abundance of bird species, contrast with Gajahan Pond (b) during active period showed less for its abundance of bird species because of high tide.

Other reason which affect the diversity is high tide and how much human activity around the ponds.

High tide on pond made some birds which have short leg or body than the tide such as Javan Plover, Kentish Plover, Lesser Sand Plover, Common Sandpiper, Wood Sandpiper, Long-toed Stint, Rufous-necked Sandpiper, and Common Sandpiper (especially if they have tarsometatarsus) can't stay so that they also cannot seek food and rest for a while. Otherwise, Whimbrel and Bar-tailed Godwit have length in their leg and body so that they can stay, seek food, and also rest for a while easily.

Human activity around the ponds like fishing when the ponds in active period does also disturb the present of birds because some bird especially shorebirds have high sensitive around their environment, it usually because of human activity. In addition, Lafferty *et al.* (2013) mentioned that the abundance and richness of shorebirds and the richness of other waterbirds was lower where human activity was high. In fact, the construction was happening when this study was going on 27 April 2013. This activity made disturbance around the ponds so that abundance of shorebirds was lower because the birds was far away from these (Figure 3).

Fig 3. The construction was going on 27 April 2013 that meant for made a canal near river through Wonorejo Conservation Area so that it would reduce flood in Surabaya.

In both pond, it also found 4 species of migratory birds that are rarely found in the pre-breeding season to get back to the breeding areas like Pacific Golden Plover (*Pluvialis pulva*) and Lesser Sand Plover (*Charadrius leschenaultii*) on Dermaga-Bosem pond; Bar-tailed Godwit (*Limosa lapponica*) and Whimbrel (*Numenius*

phaeopus) on Gajahan pond. Their present in Dermaga-Bosem pond and Gajahan pond also made affect to this study.

They generate diverse data and different from each other. This is confirmed by the calculation of diversity indices on both the ponds. Diversity indices used by *Lee et al.* (1978), namely:

$$H = -\sum \frac{ni}{N} ln \frac{ni}{N}$$

H = Bird diversity index
ni = Number of individuals of each species
N = Total all species

At Dermaga-Bosem pond, valued diversity index 0.86, while the Gajahan pond worth 0.83. Diversity Index on Gajahan pond lower than Dermaga-Bosem pond. Both of these pond have a diversity index (H) is very low based on the value of diversity index (H) according to the criteria of *Lee et al.* (1978), namely:

1. If H > 2.0 = High
2. If 1.6 < H < 2.0 = Medium
3. If 1.0 < H < 1.5 = Low

The low index is caused by several factors, such as fewer birds found scaffolding (migratory bird season passes due to the research carried out in the pre-breeding season and breeding), tide gauges often happens that cover the land sludge which is used as a place search for food by birds, and human activity is high as some people were fishing there and a few events held there with a clear voice pollution levels high.

IV. CONCLUSIONS

In this study, Dermaga-Bosem pond had amount to 823 whereas Gajahan pond had amount to 360. The difference number in that ponds showed different levels of diversity can be seen from the results of index.

At Dermaga-Bosem pond had a higher level of diversity than Gajahan pond diversity index based on the value obtained.

Factors does affect the diversity index of birds of the family Charadriidae and Scolopacidae include tide and human activity. For the migration season, included in the category of factors caused by human error, which is included in human activities.

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REFERENCES

[1] Anonymous, 1999, *Important Bird Area (IBA) Java-Bali,* Bird Life International
[2] Holmes, D. & Nash. S., 1999, *LIPI-Seri Panduan Lapangan Burung-Burung di Jawa dan Bali*, Jakarta: Puslitbang Biologi-LIPI
[3] Lafferty, K. D., Rodriguez, D. A., and Chapman, A., Temporal and spatial variation in bird and human use of beaches in southern California, *SpringerPlus* 2013 2:38
[4] Lee, C. D., S. B. Wang & C. L. Kao, 1978, *Benthic Macroinvertebrate and Fish as Biological Indicator of Water Quality with Reference to Community Diversity Index*, In Quano E.A.R., Developing Countries, The Asian Institute of Technology, London
[5] MacKinnon, J., 1995, *Panduan Lapangan Pengenalan Burung-Burung di Jawa dan Bali*, Yogyakarta: Gadjah Mada University Press
[6] MacKinnon, J., Karen P, Bas Van Balen, 2010, *Burung-Burung di Sumatra, Jawa, Bali dan Kalimantan (Termasuk Sabah, Serawak, dan Brunei Darussalam)*, Jakarta: Puslitbang Biologi-LIPI
[7] McCrary, M. D., Pierson, M.O., 2000, Influence of human activity on shorebird beach use in Ventura County, California. In: Brown, D. R., Mitchell, K. L., Chang, H. W. (eds) Fifth California Islands Symposium, OCS Study, MMS 99-0038, Santa Barbara, CA, pp 424-427
[8] Peterson, R.T., 1986, *Burung*, Jakarta: Tira Pustaka
[9] Geering A., Agnew L., and Harding S., 2007, *Shorebirds of Australia*, Australia: Csiro Publishing

Willingness to Pay to Avoid Deforestation and Forest Degradation: Evidence from Berau District Indonesia

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***Abstract*— In this paper, Contingent Valuation Method (CVM) is applied to estimate local people' willingness to pay (WTP) to avoid deforestation and forest degradation in a watershed in Berau District, East Kalimantan, Indonesia. A total of 410 respondents in 10 villages were interviewed in face-to-face surveys with double-bounded dichotomous choice used as elicitation method. Nonparametric estimation of WTP for lower bound, intermediate bound, and upper bound is Rp50,044, Rp61,236, and Rp72,428, respectively per household for onetime payment. Aggregating the WTP to Berau District results in total benefits from the proposed program is Rp1,901 million to Rp2,752 million. While the WTP estimates seem miniscule, local people supports toward a contingent scenario to avoid deforestation and forest degradation is very high, i.e. 96.61% of respondents agree to the scenario. In addition, several socioeconomic variables, i.e. income, age, gender, occupation, household size, tribe, type of village, migration status and length of stay in the respective village are included in interval data model to assess determinants of the WTP. The results show that respondents who have higher income and have occupation outside extractive sectors, i.e. not as farmer, hunter or fisherman, have higher probability to have positive WTP.**

***Keywords*— willingness to pay, deforestation, forest degradation, socioeconomic, contingent valuation method.**

I. INTRODUCTION

INDONESIA forests are enormous, represent 52% of land area, which are the third largest tropical forests after Brazil and Democratic Republic of the Congo [1]. Unfortunately, deforestation and forest degradation are evident. Drivers for deforestation are complex, ranging from institutions to macroeconomic factors, involving changing forests to other land use, e.g. agriculture, mining, infrastructure, and urban expansion [2], [3]. On the other hand, forest degradation does not completely alter forest use; instead it reduces services provided by forests. Drivers of forest degradation include selective timber extraction, fire, and shifting cultivation [4], [5]. Seen from costs versus benefits point of view, it suggests that benefits from clearing or decreasing forests are bigger than associated costs. Thus, it calls for valuation of forests.

Specifically, this paper focuses on valuation of forests for local people living in or around the forests. As mentioned before, the people may responsible for forest degradation by means of shifting cultivation, charcoal production, fuel wood, or grazing practice [3], [4]. Thus, this study estimates value of forests for local people as well as determinants of local people' support, if any, to a program aimed at avoiding deforestation and forest degradation.

II. MATERIAL AND METHODS

A. Contingent Valuation Method

Contingent Valuation Method (CVM) is a valuation method used to elicit value of good or service which is contingent upon scenario presented to the respondents. In environmental economics literatures, it falls in stated preference method category which directly asks respondents' stated preference toward goods and services, especially for those not sold in the market. Theoretically, CVM exploits Hicksian's compensation surplus, i.e. minimum willingness to accepted (WTA) compensation to ensure utility unchanged without a certain good or service; or maximum willingness to pay (WTP) to ensure consumption of a certain good or service. Thus, there is a trade-off between utility in the condition of with/without or increasing/decreasing in quantity or quality of certain good or service and sum of money offered in the scenario. Complete exposition of theoretical backgrounds can be read in CVM textbooks such as [6], [7], and [8].

While at the beginning this method was criticized for its potential biases, by now CVM is accepted in academia as well as in policy formulation. CVM is now widely used not only to value environmental goods and services, but also cultural, sport, and health services. Recent debates about this method can be seen for example in [9], [10], and [11].

B. Questionnaire Designs

In this study, to elicit local people WTP to avoid deforestation and forest degradation, a contingent scenario is developed. The scenario is built upon information that some foreign governments, companies, NGOs and individuals are willing to fund a project aimed to avoid deforestation and forest degradation. To supplement the fund, every family in the surrounding forests is expected to contribute for the project. To increase consequentiality and thus increase validity [9], [12], respondents were told that their votes are very important for decision to continue or cancel the program.

Respondents were then asked whether he/she is willing to pay a certain amount of money for a project to avoid deforestation and forest degradation in Segah watershed. Elicitation method is double bounded dichotomous choice with five bid offers, i.e. Rp10,000; Rp 25,000; Rp50,000; Rp75,000 and Rp100,000. The bid offers previously were pretested in two villages in Samarinda City and Berau

District. In double-bounded dichotomous choice method, if a respondent answered "yes" to first offered bid, then a higher bid offer was presented. On the other hand, if a respondent answered "no" to the first offers then a lower bid offer is presented.

In addition to WTP question, respondents were also asked about his/her acceptance of the scenario as well as socio-economic information, i.e. income, age, gender, education, employment, household size, number of school age, number of senior age, tribe, length of stay and migration status. Other variables were also obtained from the respondents. However, due to limited space, this paper only reports impact of socio-economic variables.

C. Surveys Implementation

Face-to-face surveys were conducted in 10 villages in Segah Subdistrict and Teluk Bayur Subdistrict of Berau District, East Kalimantan, Indonesia in August and September 2011. Sampling method was done through several steps. Firstly, ten villages were deliberately chosen to reflect three types of villages: Dayak villages, transmigration villages, and mix villages. In the chosen village, quota sampling was applied to most visible factor, i.e. gender. Subsequently, the interviewers randomly chose respondents in the respective hamlets. In total there are 410 successful interviews. However, there are also 26 protests responses in terms of "no-no" answers to both WTP answers and missing responses. As standard approach, protest responses are deleted from further analysis. Therefore, there are 384 valid responses.

D. Method of Analysis

To estimate mean WTP, nonparametric method is applied due to conservatism. In addition, since question method is double-bounded dichotomous choice it produces interval data of WTP for each respondent. To analyze the interval data, an interval data method proposed by [13] is applied. Specifically, analysis is implemented by using *doubleb* command [14], [15] in Stata release 11. This command assumes linear function of WTP and normal error terms.

III. RESULTS AND DISCUSSION

A. Descriptive Statistics

Among 384 respondents, 96.61% (n = 371) agree to the scenario to avoid deforestation and forest degradation, while 2.60% (n = 10) disagree, 2 respondents answered "do not know", and 1 is missing value. This data shows that respondents very highly support for the proposed program. This is not surprising due to the fact that majority of respondents (76.07%, n = 257) perceived that benefits from forests are bigger than costs, while 4.15% (n = 15) answered benefits equal costs, and 8.59% (n = 33) answered "do not know" and missing answers.

Descriptive statistics for respondents are presented in Table I. It can be seen that average income is about 1 million per month, average age is about 42 years, substantial amount of respondents have extractive jobs, and substantial amount of respondents are Dayak tribes.

The respondents' characteristics are comparable to Berau District and East Kalimantan in terms of gender and education variables at 5% level of significance, but not for age and tribe variables. This is due to sampling method which is deliberately chooses several villages in remote areas of Berau District in which Dayak tribes are majority. In addition, the study chooses head of household as interviewees which results in oversampling of senior respondents. Since representativeness of the sample data allow for aggregating mean WTP across relevant areas.

TABLE I
SOCIO-ECONOMIC VARIABLES

Variable	Explanation	Mean	Std. Dev.	Min.	Max.
income	Monthly, categorical, 1: ≤0.5 m.; 2: 0.5 m.-1 m.; 3: 1 m. -2 m.; 4: 2 m. - 3 m.; 5: 3 m -4 m.; 6: 4 m.- 5 m.; 7: >5 m.	2.988	1.408	1	7
age	Continous, in years	41.742	14.879	17	100
male	Gender, Binary, 1: male, 0: female	0.526	0.499	0	1
maxedu-category2	Binary, 1: secondary school & up, 0: noschool & elementary	0.435	0.496	0	1
occupation	Binary, 1: farmer, hunter or fisherman, 0:others	0.657	0.475	0	1
hhsize	Household size, continous data	4.773	2.002	1	14
havebaby	Binary, 1: have baby, 0: do not have	0.432	0.496	0	1
schoolage	Number of childreen at elementary & secondary school, continuous data	1.128	1.056	0	6
havesenior	Binary, 1: have senior age, 0: do not have	0.247	0.432	0	1
tribedayak	Category, 1: Dayak, 0: others	0.272	0.445	0	1
tribejava	Category, 1: Java, 0: others	0.368	0.483	0	1
stay	Length of stay, continous, in years	20.015	13.232	0.083	100
migration1	Binary, 1: indigenous, 0: migrants	0.201	0.401	0	1
mixvillage	Category, 1: stay in mix village	0.354	0.479	0	1
transvillage	Category, 1: stay in transmigration village	0.401	0.491	0	1
wtp1Xagree	Interaction variable between first bid offer & yes/no	45.331	33.519	0	100

Source: Survey result
Note: income in million rupiah (Rp)

B. Nonparametric Mean WTP

Distributions of answers for both first and second bid offers are presented in Table II. As seen in the table, in

general the higher the bids the lower the percentage of yes answers, which is consistent to standard economic theory.

To estimate nonparametric mean, the probability of

TABLE II
DISTRIBUTIONS OF WTP ANSWERS

Bids	First WTP			Second WTP			Total % Yes
	No	Yes	% of Yes	No	Yes	% of Yes	
0	0	7	n.a.	0	7	n.a.	n.a.
5,000	n.a.	n.a.	n.a.	6	2	25	25.00
10,000	8	59	88.06	4	2	33.33	83.56
25,000	6	71	92.21	23	44	65.67	79.86
50,000	8	80	90.91	24	61	71.76	81.50
75,000	15	66	81.48	33	52	61.18	71.08
100,000	7	57	89.06	15	54	78.26	83.46
200,000	n.a.	n.a.	n.a.	35	22	38.60	38.60
Total	44	340	88.54	140	244	65.34	76.04

Source: Author calculation

"yes" answer for each bid level is multiplied by lower bound, intermediate, or upper bound interval [7], [16]. The results of estimation show that, as expected, lower bound mean is the lowest and upper bound mean is the highest with intermediate mean in the middle, such that Rp50,044.13 (lower bound) < Rp61,236.30 (intermediate bound) < Rp72,428.48 (upper bound).

To estimate welfare improvement caused by the proposed program, an aggregation to respective area is computed. Aggregation is computed by multiplying the means with number of households in the area (i.e. 2,835 in 10 sample villages and 42,912 in Berau District) and adjusted with percentage of respondents who are willing to pay, i.e. 88.54%. For 10 sample villages the value stretches from Rp125.62 million to Rp181.80 million for onetime payment. For Berau District, the value ranges from Rp1,901.39 million to Rp2,751.87 million. Since there is no previous valuation study in the area, we cannot compare these estimates to judge their appropriateness. Comparing intermediate WTP bound and average monthly income, it turns out that average WTP represents 2.05% of monthly income, which is larger than average WTP in developing countries, i.e. 0.2 to 0.4% of household income [17].

C. Determinants of WTP

The regression results of probability of answers to the first and second WTP offers as dependent variables and socio-economic variables as independent variables are presented in Table III. Overall, the model is significant and almost has acceptable explanatory power, i.e. 9.49%. As suggested in [7], standard for R^2 in CVM studies in 10%. Furthermore, it can be seen that variables of income, occupation, and interaction variables between first WTP and "yes" answers to the first bid are significant determinants of WTP.

TABLE III
DETERMINANTS OF WTP

Variables	Coefficients	Standard Error	Significant
income	3.548	1.546	**
occupation	-10.320	4.502	**
wtp1Xagree	1.517	0.062	***
constant	20.598	6.742	***
Number of obs.	341		
Log Likelihood	-252.087		
Prob. > chi2	0.0000		
R-squared	0.0949		

Source: Author calculation
Note: **: significant at 5%, ***: significant at 1%

Income has positive sign, as expected, which means that respondents with higher income have more probability to elicit positive WTP. This is very important sign for validity of the model.

variable has negative sign which means that respondents who have occupation in extractive sectors, i.e. as farmer, hunter, and fisherman have less probability to elicit positive WTP. This result is surprising in the sense that it is generally assumed that people who get more benefits will be willing to pay more. A possible explanation for this result is that people who relying their livelihoods on the forests are afraid that the proposed program will restrict their access to the forest resources.

Lastly, interaction variable is positive and significant which signals of anchoring effect, i.e. the second WTP answers are influenced by the first WTP offers. This result is in line with previous study, e.g. [18], which argues that the double-bounded dichotomous choice is unlikely in everyday purchase decision which may lead to unexpected result.

Compared Table I and Table III it turns out that scores of variables are insignificant determinants of WTP, i.e. age, gender, education, tribe, length of stay, migration status, household size, whether have school age children, whether have elderly, tribe, and proxy for respondents' location. Although the socio-economic variables listed in Table I are common variables used in previous CVM studies, it is not uncommon that some variables are significant in some studies while insignificant in other studies. However, among the insignificant variables, tribe variable warrants further comment. It is generally assumed that local people are naturalist and thus more willing to pay more for a conservation programs. Previous studies in Ethiopia [19], Vietnam [20] and Indonesia [21] found negative relationship between ethnic and WTP, whereas studies in Spain and French [22], [23] found positive relationship between ethnic with WTP. One possible explanation for this results is that the indigenous people have pragmatic behaviors [24], [25] which may due to tough living condition faced by them and their responses to deteriorated forest condition exploited by extern actors.

IV. CONCLUSION

Local people in the sample villages generally are willing to pay for a program to avoid deforestation and forest degradation. Although in terms of monetary the contributions may be regarded as small, nevertheless majority of respondents are supportive to the program. The implication is that a forest conservation program in the area has a high probability to succeed if taken into account character of local people as rational actors.

The minor contribution may reflect low income earned by respondents. Thus, for future research it is advisable to compare WTP in monetary and WTP in terms of nonmonetary contributions, such as time, labor, or in kind, e.g. [19], [26], [27] especially for cash strapped respondents.

This study observes anchoring effect between first and second WTP. Implication for future research is that it is better to use elicitation method by single-bounded dichotomous choice rather than by double-bounded dichotomous choice. Single-bounded dichotomous choice resembles referendum style, i.e. eliciting onetime "yes" or

"no" response to the offered bid. Although double-bounded dichotomous choice method is statistically more efficient [28], the benefit is less meaningful if anchoring bias is observed.

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REFERENCES

[1] FAO, *State of the World's Forests 2011*. Rome: Food and Agriculture Organization of the United Nations, 2011.

[2] D. H. Wibowo and R. N. Byron, "Deforestation mechanisms: A survey," *International Journal of Social Economics*, vol. 26, no. 1/2/3, pp. 455-474, 1999.

[3] N. Hosonuma, M. Herold, V. de Sy, R. de Fries, M. Brockhaus, L. Verchot, A. Angelsen and E. Romijn, "An assessment of deforestation and forest degradation drivers in developing countries, " *Environmental Resource Letters*, vol. 7, pp. 1-12, 2012.

[4] M. Skutsch, A. B. Torres, T. H. Mwampamba, A. Ghilardi, and M. Herold, "Dealing with locally-driven degradation: A quick start option under REDD+," *Carbon Balance and Management*, vol. 6, no. 16, pp. 1-7, 2011.

[5] C. Erni, "Shifting to blame? Southeast Asia's indigenous peoples and shifting cultivation in the age of climate change," *Indigenous Affairs*, vol. 1, no. 09, pp. 38-49, 2009.

[6] R. C. Mitchell and R. T. Carson, *Using Surveys to Value Public Goods: The Contingent Valuation Method*. Washington, USA: Resource for the Future, 1989.

[7] I. J. Bateman, R. T. Carson, B. Day, M. Hanemann, N. Hanley, T. Hett, … and J. Swanson, *Economic Valuation with Stated Preference Techniques: A Manual*. Cheltenham, UK/Northampton, MA, USA: Edward Elgar, 2002.

[8] T. C. Haab and K. E. McConnell, *Valuing Environmental and Natural Resources: The Econometrics of Non-Market Valuation*. Cheltenham, UK & Northampton, MA, USA: Edward Elgar, 2002.

[9] C. L. Kling, D. J. Phaneuf, and J. Zhao, "From Exxon to BP: Has some number become better than no number?" *Journal of Economic Perspectives*, vol. 26, no. 4, pp. 3-26, 2012.

[10] J. Hausman, "Contingent valuation: From dubious to hopeless," *Journal of Economic Perspectives*, vol. 26, no. 4, pp. 43-56, 2012.

[11] T. C. Haab, M. G. Interis, D. R. Petrolia, and J. C. Whitehead, (2013*). From Hopeless to Curious? Thoughts on Hausman's "Dubious to Hopeless" Critique of Contingent Valuation*. Department of Economics Working Paper No. 13-07. Boone, NC, USA: Appalachian State University, 2013.

[12] C. A. Vossler and S. B. Watson, "Understanding the consequences of consequentiality: Testing validity of stated preferences in the field," *Journal of Economic Behavior and Organization*, vol. 86, pp. 137-147, 2012.

[13] M. Hanemann, J. Loomis, and B. Kanninen, "Statistical efficiency of double-bounded dichotomous choice contingent valuation," *American Journal of Agricultural Economics*, vol. 73, no. 4, pp. 1255-1263, 1991.

[14] A. Lopez-Feldman, *DOUBLEB: Stata module to compute Contingent Valuation using Double-Bounded Dichotomous Choice*. Available: http://ideas.repec.org/c/boc/bocode/s457168.html Accessed November 13, 2013.

[15] A. Lopez-Feldman, *Introduction to Contingent Valuation Using Stata*. MPRA Paper No. 41018, 2012.

[16] B. Kriström, "A non-parametric approach to the estimation of welfare measures in discrete response valuation studies," *Land Economics*, vol. 66, no. 2, pp. 135-139, 1990.

[17] D. Whittington, "What have we learned from 20 years of stated preference research in less-developed countries?" *Annual Review of Resource Economics*, vol. 2, pp. 209-236, 2010.

[18] R. C. de Faria, R. Y. Matsuhita, J. M. Nogueira, and B. M. Tabak, "Realism versus statistical efficiency: A note on contingent valuation with follow-up queries," *Atlantic Economic Journal*, vol. 35, pp. 451-462, 2007.

[19] W. Girma and F. Beyene, "Willingness to contribute to collective forest management: Evidence from Godere in the Gambela Region of Ethiopia," *The Journal of Socio-Economics*, vol. 41, pp. 79-86, 2012.

[20] M. Ahlheim, O. Frör, A. Heinke, N. M. Duc, and P. V. Dinh,). *Labour as a utility measure in contingent valuation studies: How good is it really?* FZID Discussion Paper No. 13-2010. Stuttgart, Germany: Center for Research on Innovation and Services, University of Hohenheim, 2010.

[21] C. Okten and U. O. Osili, "Contributions in heterogeneous communities: Evidence from Indonesia," *Journal of Population Economics*, vol. 17, pp. 603-626, 2004.

[22] D. Hoyos, P. Mariel, and J. Fernandez-Macho, "The influence of cultural identity on the WTP to protect natural resources: Some empirical evidence," *Ecological Economics*, vol. 68, pp. 2372-2381, 2009.

[23] A. Longo, D. Hoyos, and A. Markandya, "Willingness to pay for ancillary benefits of climate change mitigation," *Environmental and Resource Economics*, vol. 51, pp. 119-140, 2012.

[24] L. Feintrenie and P. Levang, "Local voices call for economic development over forest conservation: Trade-offs and policy in Bungo, Sumatra," *Forests, Trees and Livelihoods*, vol. 20, no. 1, pp. 35-49, 2011.

[25] P. L. Levang, E. Dounias, and S. Sitorus, "Out of forest, out of poverty?" *Forests, Trees, and Livelihoods*, vol. 15, no. 211-235, 2005.

[26] P. Shyamsundar and R. A. Kramer, "Tropical forest protection: An empirical analysis of the costs borne by local people," *Journal of Environmental Economics and Management*, vol. 31, pp. 129-144, 1996.

[27] L. T. Hung, J. B. Loomis, and V. T. Thinh, "Comparing money and labour payment in contingent valuation: The case of forest fire prevention in Vietnamese context," *Journal of International Development*, vol. 19, pp. 173-185, 2007.

[28] J. Richer, "Willingness to pay for desert protection," *Contemporary Economic Policy*, vol. 13, no. 4, pp. 93-104, 1995.

Identification of Vegetation Diversity for Keeping the Quality of Slope Around Dengkeng Watershed in Klaten Central Java

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***Abstract*—Central Java has approximately 128 watershed that flow through 174 rivers, but until 2008, five to eight river areas are in very critical condition. Dengkeng, is one of the most critical watershed because of highest erosion level and high potential for landslide. Efforts to prevent landslide around the slope is necessary. One of them conducted by increasing vegetation diversity in this area. The purpose of this research is to identify biodiversity of vegetation to keep quality of slope around Dengkeng watershed. This research is conducted in 4 observational station located in water catchment areas of Dengkeng watershed that is in Gantiwarno, Wedi, Tlingsing Cawas, and Serenan Juwiring. Research was done by the survey method that is look at monitoring station. The emphasis is largely confined to observation on biodiversity vegetation without counting density of every type of vegetation. Result of this study showed that from Dengkeng watershed, found 60 tree species with the number of individuals reaches 6610 and also 94 lower crop community (LCC) species with the number of individuals reaches 39017. Result of the analysis index on biodiversity Shannon-Weinner tree species to the Dengkeng River watershed shows that the diversity index of tree in Dengkeng watershed of 6.27. The value is showing that on biodiversity trees in Dengkeng watershed entered the high category (H'>3). Results of the analysis index on biodiversity Shannon-Wienner to species LCC in Dengkeng watershed shows that the LCC diversity index of 7.47. The value is showing that on biodiversity trees in Dengkeng River watershed entered the high category (H>3).**

Keywords—Dengkeng, slope, vegetation, the landslide

I. INTRODUCTION

WATERSHED is a land area that is unity of rivers and tri-butary that has a function accommodate, stor, and also brought water that comes from rainfall on the lake or naturally that limits on the land and the borders a topographical area dealing with a separation in the sea to the water that is still influenced by such land activity[1]. Watershed is a complex megasystem built by physical system, biological, and people. The role each component and the relationship between the components determine the quality of watershed. Each component have their own distinctive characteristics components and its existence are not stand-alone but related to other components to form a united ecological system[2]. Watershed ecosyste, has many components consisting human, animals, vegetation, soil, climate, and water. Each component have their own distinctive characteristics and its existence does not stand alone, but asso-ciated with other components to form a united ecological sys-tem. Component of watershed ecosystem are related to the quality of watershed. One of the following components that determine watershed quality is diversity of vegetation.

Vegetation is one of the main component ecosystem in watershed ecosystem. One of the role of green land around the watershed area is as a component that buffer erosion and drought[3]. Vegetation has a significant influence to the slope stability, which is due to wind currents vegetation on the slopes will have an effect on reducing security factor slope that will eventually be smashed trees that grow on the banks in the surrounding areas. Another influence of vegetation is increasing the burden of slopes, adding to pressure to shift workday style, encouraging or style counteract vegetation will add stability corner slope. The roots of plants will be able to add cohesion that would prevent a landslide. Vegetation according to Susanto is a unity of plants that are usually made of some species live together in one place. Furthermoe, vegetation is the entire plants from an area that functions as an area covering of land[4]. Encircle vegetation can be influenced by physical conditions of land that there are some of them are potentially topography of land.

Natural vegetation that grows on a region is a reflection of climate change, the land, topography and the height that interact each other in complex. Each type of plants need a environment that are specific to be able to grow and develop with good. Changes and variation certain environmental conditions will give impact to the structure and composition of the crops mainly in terms of plenty, the pattern deployment, the association with other types and a growth rate that is different from other species[5].

There are several reasons to the need for the vegetation in this case of forests in a region, including in water catchment area. Pudjiharta said that the upstream forest cover with good 80-85% of the total mainstream is derived from the base that is supported by mainstream slowly from their *zone of aeration*, and the rest is directflow[6]. This statement explained that the existence of forests in the upstream will manage or control, the majority of total flow (80-85%) that comes from the basic (base*flow*) amounting (15-20%) comes from the direct (*direct runoff*).

This mutual relationship between forest vegetation and availability water resources in the watershed

ecosystem have a significant influence, so forest conservation and component environment around the watershed to indication sustainable environmental services is produced, one of which water resources. The presence vegetation that developed by the community, especially the evapotranspirasinya low have contributed to help water supplies, especially stock exchange a sponge that absorb and retain rain so much more slowly and evenly, reduce the tendency flood in the day of rain downpour and deliver water is constantly in the dry season so that it was able to maintain stability water debit in areas downstream and certainly affect the production of various companies in the area downstream[7].

Role of vegetation according to Suryatmojo[8] is a dynamic, that will change seasonally as well as yearly. In the state vegetation that has been steadily, change in the role vegetation may be visible only seasonally adjusted, according to the pattern spread hujannya. The role of forests to water management in the region of watershed area among others connected with: (1) interseption; (2) evapotranspiration; (3) ability to control humid forest land; and (4) control the water yield.

Central Java has a suitable natural condition, climate, and topography that provide many advantages for potential resources society esspecially abundant supply of water. Central Java, has flowed into 128 watershed and flow through 174 rivers, which is divided into 10 rivers area. One of watershed is Dengkeng watershed. Dengkeng is located in Klaten regency and it is a tributary of Bengawan Solo. This river flow through approximately 6 district that are Prambanan, Gantiwarno, Wedi, Bayat, Cawas, and Karangdowo. Dengkeng made conspicuous in a few years because it's critical condition, sedimentation so often caused flood threat. Therefore, its needed efforts to overcome sedimentation DAS Dengkeng besides efforts done manually normalization with dredging. This research will try to reveal the importance on biodiversity vegetation in maintaining the quality DAS Dengkeng.

Issues raised in this research is how to identify on biodiversity of vegetation as an effort for maintaining the quality of slope around Dengkeng watershed. Purpose of this research are: (1) to identify diversity of vegetation that could be found in the slope around Dengkeng watershed; and (2) understand the implications on vegetation biodiversity to the slope quality around Dengkeng watershed.

II. MATERIAL AND METHODS

This research qualitative descriptive research carried out in September to December 2013. Research conducted in the four monitoring station which is located in Dengkeng watershed in Gantiwarno, Wedi, Tlingsing Cawas and Serenan Juwiring. Methods used in this research are survey methods that is to look at vegetation that could be found in the slope around Dengkeng watershed in every monitoring station. Each monitoring station, made 30 quadrat measuring 10 x 10 m^2 for trees vegetation and 30 quadrat size of 1 x 1 m^2 for *lower crop community* (LCC) vegetation. The layout determination harvesting taken by *purposive sampling*. Every slot observation observed kinds of trees and LCC program as well as in the count of embracing. data analysis will be done descriptive both quantitative and qualitative research. Quantitative analysis done by counting index diversity of species good trees to know level as well as LCC program on biodiversity vegetation around the Dengkeng watershed. Qualitative analysis form species composition editor vegetation in Dengkeng watershed and the role on biodiversity vegetation in maintaining slope stability and preventing soil erosion around Dengkeng watershed. Diversity of species can be described every region with the Shannon-Wienner index (Barbour, 1987) as follows.

$$H' = -\sum \frac{ni}{N} \log \frac{ni}{N}$$

Note:

H' : Shannon-Wienner biodiversity index
Ni : Number of individuals of a species of i
N : Number of total individual all kinds of

The amount index index diversity of species Shannon-Wienner according to is defined as follows: 1) the $H' > 3$ shows high diversity; 2) the H', $1 \leq H' \leq 3$ medium diversity; and 3) the $H' < 1$ shows low diversity[9].

III. RESULT AND DISCUSSION

A. Composition of Vegetation Around Dengkeng Watershed

On the survey of trees vegetation, in 4 monitoring station around Dengkeng watershed found 60 tree species with the number of individuals reached 6610 individuals. While LCC species which was found as many as 94 species with the number of individuals reached 39017 individuals. This result is completely stated in Table 1 and Table 2.

Based on the result in Table 1, there are 3 tree species with the largest number of individuals that are *Tectona grandis* (Jati), *Switenia mahagoni* (Mahoni), and *Pterocarpus indicus* (Angsana). While the result in Table 2 shows that 3 LCC spe-cies with the largest number of individuals that are *Blumea sessiliflora*, *Galingsoga parviflora*, and *Euphorbia hirta*. Composition of vegetation both tree and LCC in each monito-ring station is very different. Composition of vegetation gives a high self-control space from every kind of plant community building blocks as a result interaction organic components and biotic[9]. Each monitoring station have different types of species and number of individuals. These difference determined mainly by unknown environment in each monitor-ing station.

TABLE 2
LCC SPECIES IN SURROUNDING DENGKENG WATERSHED

No	Species	Number of Individuals
1	*Acalypa heterophylla*	124
2	*Acalypa indica*	165
3	*Adenostema lavenia*	112
4	*Ageratina riparia*	30
5	*Ageratum conizoydes*	1434
6	*Amaranthus spinosus*	182
7	*Amorphophalus campanulatus*	482
8	*Anasthropus compressus*	1243
9	*Andrographis paniculata*	50
10	*Andropogon acciculatus*	869
11	*Andropogon contortus*	66
12	*Andropogon intermedius*	24
13	*Artenisia repens*	254
14	*Artropurpurea sp*	38
15	*Axonopus affinis*	86
16	*Axonopus compressus*	1062

17	*Bergia oryzetorum*		55
18	*Bidens tripartita*		105
19	*Blumea sessiliflora*		3125
20	*Borerria occymiodes*		889
21	*Borerria sricta*		60
22	*Caladium tricolor*		126
23	*Catharanthus roseus*		393
24	*Centrocema plumiria*		481
25	*Clitoria ternatea*		1739
26	*Colocasia esculenta*		80
27	*Commelia nudiflora*		80
28	*Cucumis sativus*		79
29	*Curcuma xanthorrhiza*		25
30	*Cynodon dactylon*		104
31	*Cyperus difussus*		707
32	*Cyperus eragrostis*		7
33	*Cyperus globasus*		22
34	*Cyperus rotundus*		2010
35	*Desmedium rotundifolium*		653
36	*Desmodium heterophyllum*		14
37	*Digitaria ascendens*		181
38	*Digitaria sanguinalis*		95
39	*Duranta erecta*		304
40	*Eclipta prostata*		207
41	*Elephantopus scaber*		2344
42	*Eleusine indica*		520
43	*Eragrostis amabilis*		432
44	*Eriochiola decumbeus*		5
45	*Euphorbia hirta*		2158
46	*Festuca arundinacea*		428
47	*Acalypa heterophylla*		124
48	*Fimbrystilis annua*		1010
49	*Fimbrystilis ovata*		651
50	*Flemingia lineata*		87
51	*Galinsoga parviflora*		2855
52	*Glioscorea esculenta*		300
53	*Hewettia sublobata*		148
54	*Hyptis brevipes*		428
55	*Hyptis suaveolens*		347
56	*Ilysanthes ciliata*		45
57	*Imperata cylindrica*		44
58	*Indigofera viscosa*		111
59	*Ipomea crasicaulis*		83
60	*Ipomea obscura*		111
61	*Ischaemum timurence*		192
62	*Justicia pectoralis*		2048
63	*Lauria obchordata*		49
64	*Lavatera olbia*		104
65	*Leersia hexandra*		278
66	*Lindernea crustacea*		373
67	*Lindernia dubiata*		72

TABLE 1
TREE SPECIES IN SURROUNDING DENGKENG WATERSHED

No	Species	Local Name	Number of Individuals
1	*Acacia auriculiformis*	Akasia	393
2	*Abrus precatorius*	Saga	1
3	*Alangium javanicum*	-	2
4	*Albizzia falcata*	Sengon	425
5	*Alstonia scholaris*	Pule pandak	7
6	*Altingia excelsa*	-	10
7	*Annona muricata*	Sirsak	49
8	*Annona squamosa*	Srikaya	12
9	*Artocarpus altilis*	Sukun	2
10	*Artocarpus heterophyllus*	Keluwih	15
11	*Artocarpus integra*	Nangka	1
12	*Averrhoa blimbii*	Belimbing wuluh	2
13	*Bauhinia purpurea*	Bunga kupu-kupu	21
14	*Caesalpinia bonducella*	-	23
15	*Caesalpinia pulcherrima*	Bunga merak	102
16	*Carica papaya*	Pepaya	101
17	*Cassia siamea*	Johar	102
18	*Ceiba pentandra*	Randu	108
19	*Centella asiatica*	-	2
20	*Citrus histrix*	Jeruk purut	2
21	*Cococ nucifera*	Kelapa	24
22	*Colocasia esculenta*	Talas	90
23	*Dalbergia latifolia*	Sana	178
24	*Delonix regia*	Flamboyan	95
25	*Dimocarpus longan*	Kelengkeng	48
26	*Elaeocarpus obtusus*	-	12
27	*Erythrina variegata*	Dadap	2
28	*Eucalyptus alba*	Kayu Putih	1
29	*Ficus benjamina*	Beringin	101
30	*Gnetum gnemon*	Melinjo	112
31	*Guazumma ulmifolia*	Jati belanda	148
32	*Hibiscus tiliaceus*	Waru	23
33	*Jatropa curcas*	Jarak pagar	45
34	*Justicia gendarussa*	Tetehan hijau	2
35	*Leucaena glauca*	Lamtoro	276
36	*Mangifera indica*	Mangga	82
37	*Mannihot utilissima*	Singkong	383
38	*Maranta arundinacea*	-	3
39	*Melia azedarach*	-	39
40	*Musa paradisiaca*	Pisang	25
41	*Parkia speciosa*	Petai	132
42	*Persea americana*	-	2
43	*Pithecelobium dulce*	Asam londo	262
44	*Polyalthia longitosa*	Glodogan	23
45	*Psidium guajava*	Jambu biji	33
46	*Pterocarpus indicus*	Angsana	476
47	*Riccinus communis*	Jarak	27
48	*Samanea saman*	Trembesi	454
49	*Schima wallichii*	-	67
50	*Schleichera oleosa*	-	51
51	*Ficus retusa*	Bibis	1
52	*Sterculia foetida*	-	10
53	*Streblus asper*	-	2
54	*Switenia mahagoni*	Mahoni	852
55	*Syzigium aquea*	Jambu air	101
56	*Syzigium cumini*	Duwet	93
57	*Tectona grandis*	Jati	939
58	*Terminalia catapa*	Ketapang	2
59	*Vigna sinensis*	Kacang panjang	14
TOTAL			6610

TABLE 2
LCC SPECIES IN SURROUNDING DENGKENG WATERSHED

No	Species	Number of Individuals
68	*Mimosa invisa*	85
69	*Mimosa pudica*	29
70	*Mirabilis jalapa*	49
71	*Momordica charantia*	708
72	*Nardus stricta*	185
73	*Oplismenus burmanii*	20
74	*Oxalis corniculata*	349
75	*Paederia foetida*	13
76	*Panicum miliaceum*	40
77	*Paspalum conjugatum*	43
78	*Paspalum scorbiculatum*	141
79	*Paspalum setaceum*	42
80	*Passiflora foetida*	262
81	*Peperomia pellucida*	16
82	*Phylantus urinaria*	4
83	*Physalis minima*	115
84	*Richardsonia brasiliensis*	199
85	*Ruelia tuberosa*	7
86	*Scindapsus aureus*	45
87	*Spermacoce exilis*	407
88	*Stacitarpeta jamaicensis*	7
89	*Stacitarpheta indica*	177
90	*Tridax procumben*	422
91	*Uraria lagopodioides*	1189
92	*Urena lobata*	171
93	*Waltheria americana*	188
94	*Widelia montana*	1119

This is supported by a report which said that environmental factors affect on biodiversity living beings comprising an area (both bone density and wealth species) include vegetation and fauna[10]. Environmental factors including soil temperature, pH, and nutrient

content on the ground. In addition, Krebs [11] (2009) and Ardhana[12] said that conditions in their natural habitat plants also have an effect on to the plants community. The distribution of plants was largely affected by a moisture gradient that was controled by elevation, slope, soil texture, sand, and also the speed of winter and summer wind[13]. Good soil environments can provide plants with sufficient nutrients and available water, and topographic characteristics are closely associated with local climate, which greatly impacts plants. Plants community will be interacted with all factors in the environment, both organic and biotic factors[12]. All factors existing in an ecosystem interact on each other and the result is things can be seen in it[14].

B. Diversity of Vegetation Around Dengkeng Watershed

Diversity indices are better measure of the species diversity of a forest and more informative than species counts alone. According to the result of the diversity indices of Shan

Results of the analysis index on biodiversity Shannon-Wienner tree species to the DAS Dengkeng shows that the index on biodiversity/biological (H') vegetation trees in DAS Dengkeng of 6.27. The value is showing that on biodiversity trees in DAS Dengkeng entered the category was (H->3). When viewed the index in each biological monitoring station so great index biological in Gantiwarno sub-district of 0.63, Wedi 1.7975, Tlingsing Cawas 1.0628, and Serenan Juwiring of 1.0639. These results show that in monitoring station Wedi, Tlingsing Cawas, and Serenan Juwiring tree species diversity in the category are abundant and ($1 \leq H' \leq 3$), while in monitoring station Gantiwarno sub-district in ketegori abundant low/little ($H' < 1$). A comparison index biological trees in every monitoring station in DAS Dengkeng can be seen in the Figure 1.

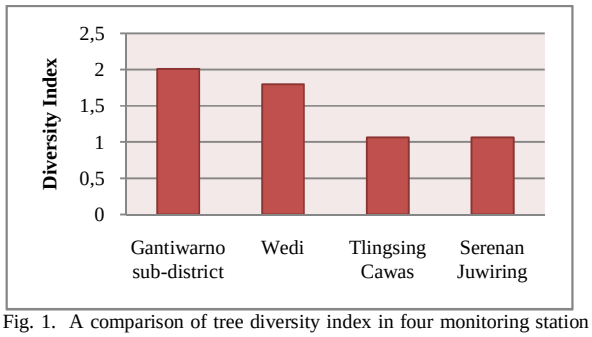

Fig. 1. A comparison of tree diversity index in four monitoring station in Dengkeng Watershed.

Result of analysis in biodiversity index to LCC species in Dengkeng watershed showed that biodiversity index of LCC is 7,47. The value show that on biodiversity of LCC was in high category. Comparison of diversity index in every monitoring station can be seen in Figure 2.

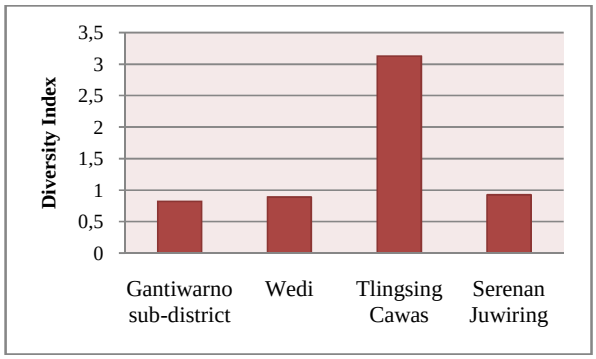

Fig. 2. A comparison of LCC diversity index in four monitoring station in Dengkeng Watershed

Diversity index describe wealth of species in Dengkeng watershed. Diversity is correlated with the number of different species[15]. There is an increasing number of species that found then the value on biodiversity index will be more than. Habitats tend to have a number of species more or more diverse are likely have a higher diversity of species[16]. This was added by Parker and Malcolm (1995) that the structure vertical regrowth forest structure of the trees will produce variations and in various form live vegetation.

C. Role of Vegetation in Supporting Quality of Slope Around Dengkeng Watershed

Results of the study showed that as a whole on biodiversity vegetation good trees and LCC program around DAS Dengkeng check-in the criteria on biodiversity. But if seen in every monitoring station, there are high on biodiversity is different. The vegetation around DAS Dengkeng known through analysis of vegetation can be used as the criteria in determining quality tebing tinggi so that it can be used to monitor the occurrence of soil erosion and landslides in around DAS Dengkeng. This is supported by research results that stated vegetation have an effect on to the ability to land hold water so that can prevent erosion and landslides in around cliff[17]. Arrijani[18] also said that vegetation and the grass is thick vegetation types that effective in holding erosion if compared to the plants overlapping take turns, cotton plantations and maize crop. Roots of various plants can increase significantly and land stability metaphor role anti-erosion[18].

The roots vegetation good trees and LCC program can improve stability and land a significant role as an anti-erosion. Vegetation can reduce soil erosion not only through reduced limpasan (*runoff*) but also reduce transmission capacity surface waters. price reduction sediment higher than the price reduction *runoff*. A reduction in runoff vegetation of 30.8 percent and the vegetation grass of 5.6 percent while average sediment reduction of 88.8 percent in vegetation forest and 77.4 percent in vegetation grass[19].

IV. CONCLUSION

1. Result of the research showed that from Dengkeng water-shed found 60 tree species with the number of individuals reached 6610 individuals. 3 species with the largest number namely *Tectona grandis*, *Switenia mahagoni*, and *Pterocar-pus indicus*.
2. From Dengkeng watershed also found 94 LCC species with total number of individuals 39017. 3 species with

the largest number namely *Blummea sessiliflora*, *Galingsoga parviflora*, and *Euphorbia hirta*.

3. Results of the analysis index on biodiversity Shannon-Wienner tree species to the DAS Dengkeng shows that the index biological trees in DAS Dengkeng of 6.27. The value is showing that on biodiversity trees in DAS Dengkeng entered the category was (H->3). If it was seen in the index biological monitoring station so the amount each index biological in gantiwarno sub-district of 0.63, wedi 1.7975, Tlingsing cawas 1.0628, and serenan juwiring of 1.0639.
4. Results of the analysis index on biodiversity Shannon-Wienner to species LCC program in DAS Dengkeng shows that the index biological LCC program in DAS Dengkeng of 7.47. The value is showing that on biodiversity trees in DAS Dengkeng entered the category was (H->3). If it was seen in the index biological monitoring station so the amount each index biological in gantiwarno sub-district of 0.8191, wedi Tlingsing cawas 0.886, 3.129, and serenan juwiring of 0.9248.
5. The vegetation around DAS Dengkeng known through analysis of vegetation can be used as the criteria in determining quality tebing tinggi so that it can be used to monitor the occurrence of soil erosion and landslides in around DAS Dengkeng. Vegetation have an effect on to the ability to land hold water so that can prevent erosion and landslides in around cliff.

REFERENCES

[1] Undang-Undang Nomor 7 Tahun 2004 tentang Sumber Daya Air. Jakarta: Kementerian Lingkungan Hidup.

[2] BPDAS. 2010. *Pengertian dan Pengelolaan DAS*. (Online), (http://bpdas.dephut.go.id) Diakses pada 30 Desember 2012.

[3] Susanto, W. 2012. *Analisis Vegetasi pada Ekosistem Hutan Hujan Tropis untuk Pengelolaan Kawasan Taman Hutan Raya Raden Soerjo (Wilayah Pengelo-laan Cangar-Kota Batu).* (Online), (wayansusantoshut.blogsot.com), Diakses pada 30 Maret 2013.

[4] Maryantika, N., Lalu, M.J., Andie, S. 2010. *Analisa Perubahan Vegetasi Ditinjau dari Tingkat Ketinggian dan Kemiringan Lahan Menggunakan Citra Satelit Landsat dan Spot4 (Studi Kasus di Kabupaten Pasuruhan).* (Online), (reposi tory.its.ac.id/bitstream/...pdf), Diakses pada 30 Maret 2013.

[5] Kusmana, C. 1989. *Phitososiologi Hutan Hujan Pegunungan Gunung Gede Pangrango Jawa Barat*. Laporan Penelitian. Bogor: FH IPB.

[6] Pudjiharta, A. 2008. Pengaruh Pengelolaan Hutan pada Hidrologi. *Info Hutan*. V(2): 141-150.

[7] Njurumana, G.N.D. 2006. *Kunjungan Silang Pengelolaan DAS Cidanau Terpadu Provinsi Banten*. Kupang: Balai Litbang Kehutanan Bali dan Nusa Tenggara.

[8] Suryatmojo, H. 2010. *Peran Hutan sebagai Penyedia Jasa Lingkungan*. Yogyakarta: UGM.

[9] Fachrul, M. F. 2007. *Metode Sampling Bioekologi*. Jakarta: Bumi Aksara

[10] Gupta, N., R.C., Sharma, A.K., Tripathi. 2008. Study of bio-physico-chemical parameters of Mothronwala swamp, Dehradun (Uttarakhand). *Journal of Environmental Biology*. 29(3): 381-386..

[11] Krebs, C.J. 2009. *Ecology the Experimental Analysis of Distribution and Abundance 6th*. Benjamin Cummings, Pearson. USA.

[12] Ardhana, I.P.G. 2012. *Ekologi Tumbuhan*. Bali: Udayana University Press

[13] Tilaki, G.A.D.T., H.N., Nasrabad, J., Abdollahi. 2011. Investigation of Relationship between Vegetation, Topography, and Some Soil PhysicoChemical Characteristics in Nodushan Rangelands of Yazd Province (Iran). *International Journal of Natural Resources and Marine Sciences*. 1(2): 147-156.

[14] Reyhan, M.K., F., Amiraslani. 2006. Studying the Relationship Between Vegetation and PhysicoChemical Properties of Soil, Case Study: Tabas Region, Iran. *Pakistan Journal of Nutrition*. 5(2): 169-171.

[15] Paramitha, I.G.A.A.P.; I Gede, P.A.; Made, P. 2010. Keanekaragaman Anggrek Epifit di Kawasan Taman Wisata Alam Danau Buyan Tamblingan. *Jurnal Metamorfosa*. I(1): 11-16.

[16] Sujalu, A.P. 2008. Analisis Vegetasi Keanekaragaman Anggrek Epifit di Hutan Bekas Tebangan, Hutan Penelitian Malinau (MRF)-CIFOR. *Media Konservasi*. 13(3): 1-9.

[17] Wang, C.; Chuan, Y.Z.; Zhong, L.X.; Yang, W.; Huanhua, P. 2013. Effect of Vegetation on Soil Water Retention and Storage in a semi arid Alpine Forest Catchment. *Journal of Arid Land*. 5(2): 207-219.

[18] Arrijani. 2006. *Korelasi Model Arsitektur Pohon dengan Laju Aliran Batang, Curahan Tajuk, Infiltrasi, Aliran Permukaan dan Erosi*. Bogor: Sekolah Pascasarjana Institut Pertanian Bogor.

[19] Migguo, Z.; Cai, Q.; Chen, H. 2007. Effect of Vegetation on Runoff-Sediment Yield Relation ship at Different Spatial Scales in Hilly Areas of the Loess Plateau, North China. *Acta Ecologica Sinica*. 27(9): 3572-3581.

DIVERSITY INDEX OF HOLOTHUROIDEA (ECHINODERMATA) AT CASED TRADITIONAL GOLD MINING AREA, LAMPON BANYUWANGI DISTRIC

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Abstract— **While the gold amalgamation activity was stopped and the tailing haven't discharged in this region, but the effect of mercury pollution still be remaining for unpredictable times. Lampon is the estuary and rocky shore that have many macrobenthic such as *Holoturoidea*. *Holothuroidea* can be used as bioindicator to detected mercury effect in this region. *Holothuroidea* as detrivore and beach cleaner to organic detritus. They are important to balanching intertidal ecosystem. Existence of *Holothuroidea* in Lampon must be tracing time after time. We were using the Shannon-Wiener Index to predict the diversity of Holothuroidea in this site, but we were also using Domination Indeks to know the kind species that most dominating to the other species. Based on the observations, can be found in 12 species Lampon *Holothuroidea*. *Holothuroidea* species encountered in Lampon namely: *Holothuria leucospilota, Holothuria cinerascens, Holothuria arenicola, Cucumaria* sp1, *Cucumaria* sp2, *Cucumaria* sp3, *Leucosynapta* sp, and *Pentacta quadrangularis*. Environment parameters at both in the normal range of quality standards**

Keywords—Mercury, diversity, Holothuroidea

I. INTRODUCTION

Lampon estuary was one of traditional gold mining area at Banyuwangi District. Activity logging have been stopped since 2011 . However, the effect of residual mercury logging still be impacting to biota around this area . Initial research has shown that bioaccumulation of mercury in the gastropods *Nerita argus* have reached 3.03 ppm , while the *Terebralia sulcata* up to 3.10 ppm. *Nerita argus* hepatopancreas in the region have experienced a very severe atrophy [1]. An ecosystem is a functional unit of any size that composed of biotic and abiotic elements [2]. Many organisms in an ecosystem are associated to each other for the purpose of feeding, spawning and also perform as a refuge from predators. These elements was interact to each other. If there is one element of the disorder, will affect to the other elements. Bentos are aquatic organisms that live on the seabed, rivers or lakes of the tidal limit to the deepest downs [3], [4]. In the transport cycle and changes in the form of pollutants, described the benthic biota has a role in the degradation process into other forms even be bioacumulator pollutants [5]. In an environmental assessment, a decrease or change in species diversity can be used as an indicator of environmental pollution. *Holothuroidea* is a species of many benthic organisms that also live in the coral reef coastal region which aims to find food. *Holothuroidea* is also one of the benthic environment is often used as bio-indicators . Based on the above understanding , there needs to be more careful observation and assessment of the diversity of benthic especially in coastal Lampon *Holothuroidea* species .

II. MATERIAL AND METHODS

The research was conducted in traditional gold mining region Lampon estuary. Lampon administratively located in District Pesanggaran Banyuwangi Regency, East Java. Lampon Geographical Location : 8° 37'05 .39 " S 144° 05'11 .46 " E. Rainfall: 1000 - 2500 mm/year. The samples that used were all species of the class *Holothuroidea* at Lampon intertidal zone. Some of the tools used is: rollmeter, paralon quadrant plot size of 1x1 meters, handcounter, tweezers, shovel, logbook, stationery, plastic bags, paper labels, flakon, bottled water samples. The tools used to measure environmental parameters: thermometer, DO - meter, pH - meter, salinometer (hand - refractometer), glass cup, stopwatch , GPS, rollmeter. Ecological data sampling based on purposive random sampling method . Primary transect line along the coast. Specified secondary transects perpendicular to the direction of the sea from the primary transect. At each beach transect lines set 3 secondary, each 250 meters. At each transect line drawn 10 secondary sampling plots with a size of 1 x 1 m. Distance of 10 meters of each plot. Data was analyzed by descriptive quantitative and qualitative. Determination of the Shannon diversity index using the formula Wiener. It also determined the value of the frequency , density , dominance index, and the importance value index. Data is analyzed and interpretated, and described quantitative [6].

III. RESULT AND DISCUSS

Environmental conditions affect the existence of an organism in an area. *Holothuroidea* has a very importnant role in the stability of ecosystems in the intertidal zone. Lampon is the remain area of gold amalgation activity, is still predicted contaminated by mercury waste are buried in logging around waste disposal. By tracking the accumulation of mercury in 2011-2012 tailings pile of leftover logging in this region is high enough [1]. *Holothuroidea* diversity in Lampon indicate that this region can still support their existence in their habitat.

Holothuroidea species that can be encountered in Lampon shown in Table 1.

TABLE1.
THE *HOLOTHUROIDEA* SPECIES DIVERSITY IN THE FORMER LOGGING LAMPON BANYUWANGI REGENCY (RAINY SEASON)

No.	Species	Sum	F	fr	d	Dr	np	pi
1	*Holothuria leucospilota*	125	**66,67**	**52,63**	2,08	73,96	**126,6**	0,74
2	*Holothuria cinarecens*	1	6,67	5,26	0,02	0,59	5,85	0,01
3	*Holothuria arenicola*	6	20	15,79	0,1	3,55	19,34	0,04
4	*Holothuria atra*	0	0	0	0	0	0	
5	*Leucosynapta* sp	2	6,67	5,26	0,03	1,18	6,45	0,01
6	*Cucumaria* sp 1	35	26,67	21,05	0,58	20,71	41,76	0,22
7	*Cucumaria* sp 2	0	0	0	0	0	0	0
8	*Cucumaria* sp 3	0	0	0	0	0	0	0
9	*Pentacta quadrangularis*	0	0	0	0	0	0	0
10	Sp x1	0	0	0	0	0	0	0
11	Sp x2	0	0	0	0	0	0	0
12	Sp x3	0	0	0	0	0	0	0
	SUM	169	126,67	100	2,82	100	200	1

Note: Date of data taken in 25th July 2013; f = frekuensi; fr = frekuensi relatif; d = density; dr = relative of density; np = importance value; pi = ni/N = sum of individual plot divided by the total amount of individu; Sp X = unidentified speciment

Enermous Species discovered in Lampon is *Holothuria leucospilota*. In the intertidal Stone Shore Meru Betiri (control zone) *Holothuria leucospilota* is also very easy to find. Comparison of mercury bioaccumulation between samples *Holothuria leucospilota* Lampon and Stone Shore Meru Betiri is estimated as the evidence that mercury contamination can not be eliminated quickly from the area of the former logging gold Lampon (Table 6).

Fig 1. *Holothuroidea* species in Lampon and Stone Shore TNMB *Leucosynapta* sp (top left); *Holothuria leucospilota* (bottom left); *Holothuria cinarescens* (top right) and *Cucumaria* sp 1 (bottom right).

The number of *Holothuroidea* species that can be found in the intertidal zone Lampon (Table 1.) As many as 12 species. Some species which exist in Lampon are not found in intertidal zone Stone shore national Park Meru Betiri. The absence of some of these species, presumed because they are not sampled when the data is taken. However, *Leucosynapta* sp is the species that appear significantly different in number. Based on the type of estuary and volcanic sediments at both locations of observation, *Leucosynapta* sp assumed can be found in large quantities. However, *Leucosynapta* sp is very rare in Lampon, whereas in Stone Shore national park Meru Betiri, *Leucosynapta* sp can be found easily because the number are abundant (Table 2.). Based on the above data *Leucosynapta* sp can be a measurement that mercury contamination can affect the existence *Leucosynapta* sp in mercury polluted areas.

TABLE 2.
THE DATA ON THE ENVIRONMENTAL PARAMETERS AND STONE SHORE LAMPON TNMB . LOCATION COORDINATES: (S 08 ° 37'18 .6 "E114 ° 05'19 .0") TIME: 14:00 TO 16:00 HRS LOW SPRING TIDE (25TH JULY-23TH AUGUST 2013)

Parameter	Average		Units
	Lampon	National Park (TNMB)	
DO air	5,36 ± 0,055	5,56 ± 0,261	ml/g
DO water	5,84 ± 0,182	6,26 ± 0,904	ml/g
Suhu udara	32 ± 0,158	35,36 ± 1,119	C°
Water temperatur	28,54 ± 0,288	26,6 ± 0,548	C°
Salinity	34,6 ± 0,894	33,4 ± 2,702	‰
pH Water	9,1 ± 0,071	9,06 ± 0,089	
Sediment temperatur (low tide)	27,4 ± 1,517	29,4 ± 0,894	C°
Tidal Range	109,8 ± 13,35	112,8 ± 10,96	cm

The results of measurements of environmental parameters (Table 3.) Environmental conditions in Lampon can be said fullfill environmental quality standards in the marine waters of Indonesia. Although there is a difference in the measurement results, the possibility of changes following the daily fluctuations are very likely to occur, both in Lampon and Stone shore Meru Betiri. According to dicission of Minister of Environment No. 51, 2004, states that the sea water quality standards some measurable range of environmental parameters: temperature 28 ° -32 ° C, pH 7 to 8.5, dissolved oxygen (DO)> 5, salinity up to 34 ‰. This value is within the range of natural, normal condition means an environment, vary every time (day, night, season) with a note that the change is allowed up to 10% concentration of the seasonal average. Temperature changes obtained up to <2° C of temperature naturally, the change is allowed up to 0.2 pH units, allowed a change up to <5% the maximum average of salinity [9].

TABLE 4.
THE *HOLOTHUROIDEA* SPECIES DIVERSITY IN THE FORMER LOGGING GOLD LAMPON BANYUWANGI REGENCY (SUMMER)

No.	Species	Sum	F	fr	D	Dr	np	pi
1	*Holothuria leucospilota*	49	**46,67**	43,75	**0,82**	32,03	75,78	0,32
2	*Holothuria cinarecens*	0	0	0	0	0	0	0
3	*Holothuria arenicola*	9	13,33	12,5	0,15	5,88	18,38	0,06
4	*Holothuria atra*	0	0	0	0	0	0	0
5	*Leucosynapta* sp	2	6,67	6,25	0,033	1,31	7,56	0,01
6	*Cucumaria* sp 1	90	33,33	31,25	1,5	58,82	**90,07**	0,59
7	*Cucumaria* sp 2	0	0	0	0	0	0	0
8	*Cucumaria* sp 3	0	0	0	0	0	0	0
9	*Pentacta quadrangularis*	0	0	0	0	0	0	0
10	Sp x1	3	6,67	6,25	0,05	1,96	8,21	0,02
11	Sp x2	0	0	0	0	0	0	0
12	Sp x3	0	0	0	0	0	0	0
	SUM	153	106,67	100	2,55	100	200	1

Note: retrieval date of data October 7, 2013; f = frequency; fr =relative frequency = d = density; dr = relative density; np = importance value; pi = ni / N = number of individual plots divided by the total number of individuals; Sp X = unidentified specimens

Changes in environmental conditions between the summer and rainy season is not striking . Fluctuation range of changes in environmental parameters are within normal limits . Although summer temperatures are higher (± 2-3 ° C) than in the rainy season , these conditions can be tolerated by bioindicator (*Holothuroidea*) at the location of observation . This is proved through the species found when the data is collected in the summer (Table 4 . And Table 5). During summer , *Holothuria leucospilota* still dominate in Lampon although the critical number has decreased . The importance value index of *Holothuria leucospilota* decline from 126.60 % to 75.8 % in the summer . The decrease is presumed because *Holothuria leucospilota* move to more hidden niche for avoiding heat , so it is not seen in the summer. There is high increasing number of *Cucumaria* sp 1 in the summer at Lampon . The importance value index of *Cucumaria* sp 1 rose to 90.07 % from 41 , 76 % . Individuals Cucumaria sp 1 were found in the summer quite a lot from the previous data retrieval in the rainy season . However , *Cucumaria* sp 1size in the summer tend to be small . Environmental factors fluctuate every day , especially during the seasons changing.

TABLE 5.
THE *HOLOTHUROIDEA* SPECIES DIVERSITY IN STONE SHORE MERUBETIRI NATIONAL PARK (SUMMER)

No.	Species	Sum	F	fr	D	Dr	np	Pi
1	*Holothuria leucospilota*	136	**60**	45	2,27	36,36	81,36	0,36
2	*Holothuria cinarecens*	2	6,67	5	0,03	0,53	5,54	0,005
3	*Holothuria arenicola*	0	0	0	0	0	0	0
4	*Holothuria atra*	0	0	0	0	0	0	0
5	*Leucosynapta* sp	225	40	30	3,75	**60,16**	90,16	0,602
6	*Cucumaria* sp 1	10	20	15	0,17	2,67	17,67	0,027
7	*Cucumaria* sp 2	0	0	0	0	0	0	0
8	*Cucumaria* sp 3	0	0	0	0	0	0	0
9	*Pentacta quadrangularis*	0	0	0	0	0	0	0
10	Sp x1	0	0	0	0	0	0	0
11	Sp x2	0	0	0	0	0	0	0
12	Sp x3	1	6,67	5	0,02	0,27	5,27	0,003
	SUM	374	133,33	100	6,23	100	200	

Note: data retrieval date October 20, 2013; f = frequency; relative frequency fr = d = density; dr = relative density; np = importance value; pi = ni / N = number of individual plots chopped i divided the total number of individuals; Sp X = unidentified specimens.

Scheiner and Willig (2008) review the basic principles of understanding about community of ecology, stated there are seven principles: heterogeneity of distribution of organisms; interaction of organisms, contingency; heterogeneity of environmental factors; limited and heterogeneous resources; death of the organism as well as the process of evolution [7]. These principles evolve and become the foundation of mind about, distribution, abundance, dominance and the presence of a population in the range of space and time. Density or population density is the number species by area . Many types that have fluctuating quantity by year, but it has not known much yet about the cause and similarity of density in the same year . Density fluctuations are more common , does not mean extinct in habitat if the density is very low . The existence of individual organisms in nature is strongly associated with the physical environment (abiotic) and with other biota [8]. *Holothuroidea* Existence calculated on the value of the frequency, population density , species dominance, importance value index of species in former mining areas Lampon. The results of this analysis gives an overview of some of the species that exist in mining areas, both in the dry and rainy season.

TABLE 6.
DIVERSITY INDEX AND DOMINANCE INDEX OF *HOLOTHUROIDEA*

Ecological Paramether	LAMPON		Merubetiri National Park (TNMB)	
	Rainy Season	Summer	Rainy Season	Summer
Diversity Index (H')	0,33	0,42	0,44	0,35
Dominance Index (ID)	0,59	0,45	0,42	0,49

Note: H ': <= 1 low diversity; H': 1-3 = medium diversity; H:> 3 high diversity; ID dominated close to 0 = dominated by 1 species; ID close to 1 = dominated by 2 or a few species

Based on the calculation of diversity index and dominance index, it can be assumed that the diversity of species in Lampon and national park is the same (rather low), while dominance index showed that in both locations are equally dominated by more than one species. Dominated species in both areas is *Holothuria leucospilota*. However *Leucosynapta* sp can be an indicator that the existence in Lampon is not as good as in national park, because *Leucosynapta* sp is very rare found in Lampon. Many factors affected the existence of the organism in a place, one of which is blackened environmental conditions including materials that affect their existence in that place. Mercury contamination can be suspected as a cause of low *Leucosynapta* sp number in Lampon.

IV. CONCLUSION

Diversity of species in Lampon is low and dominated by more than one species (*Holothuria* sp *leucospilota* and *Cucumaria* 1). *Leucosynapta* sp is an indicator that the species is rarely found in Lampon although with similar characters can be found abundantly in national park Meru Betiri. It is Needed to recite deeply of other factors that cause the absence of *Holothuroidea* in Lampon.

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REFERENCES

[1] Susintowati. 2012. *Bioakumulasi merkuri dan struktur komunitas Gastropoda di kawasan bekas penggelondongan emas muara sungai Lampon, Banyuwangi*. Naskah Tesis. Universitas Gadjah Mada. Yogyakarta.

[2] Nybakken, J.W. 1988. *Marine biology: An ecological approach*. GM 212 88.018.

[3] Abercrombie, M., Hickman, M., Johnson, M.L., Thain, M. 1993. *Dictionary of Biology*. 8th Edition. Penguin Books Ltd. England.

[4] Romimohtarto, K. dan Juwana, S. 2007. *Biologi laut: Ilmu Pengetahuan tentang biota laut*. Penerbit Djambatan. Jakarta.

[5] Connell, D.W., Miller, G.J., 2006. *Kimia dan ekotoksikologi pencemaran*. UI-Press. Jakarta.

[6] Odum, E.P. 1993. *Fundamentals of ecology*. Third Edition. Saunders College Pub. Diterjemahkan oleh : Samingan, T. & Srigandono, B. UGM Press. Yogyakarta.
[7] Scheiner, S.M. and Willig, M.R. 2008. A General Theory of Ecology. *Theor.Ecol*. 1:21-28. DOI 10.1007/s12080-007-0002-0.
[8] Brewer, R. 1993. *The Science of Ecology*. Second Edition. Saunders College Pub. Tokyo
[9] Susintowati and N. Nurchayati, "Mercury Pathology Effect to Spicules Density of Holothuroidea (Echinodermata) at Ceased Lampon Traditional Gold Mining, Banyuwangi District", (accepted for publication) in *Saintek* Journal, Kopertis Wilayah VII Jawa Timur. 2013.

POSTER

Diversity of Ophiuroids in Intertidal Zone of Porok and Kukup Beach Gunung Kidul Yogyakarta

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***Abstract*— Indonesia is an archipelago country which has a high diversity of marine life. One of the abundant marine lives in Indonesia is Ophiuroidea Class members. While the numbers are abundant, but the data on variability has not been studied. Therefore, it is necessary to study the diversity of Ophiuroidea Class members on a beach as the preliminary study. The study was conducted in Kukup Beach and Porok, Gunungkidul Yogyakarta. Those two beaches are located adjacent but Kukup Beach is more famous than Porok Beach. Kukup beach has more tourists than Porok Beach. The study was conducted by the method of handpicking, and then samples obtained were fixed and preserved in 70% alcohol. Specimens were then identified by looking at the morphology and character matched with identification key. The results obtained from this study are 12 types of two families, Ophiotrichidae and Ophiocomidae. Member of the Ophiotrichidae family is *Macrophiothrix* sp. Members of Ophiocomidae family are *Ophiomastix annulosa, Ophiocoma scolopendrina, O. schoenleini, O. erinaceus, O. brevipes, O. wendtii, O. latinanxa, O. pica, O. anaglyptica, O. dentata,* and *O. valenciae*. Of the names above, Porok Beach has 11 species found, and in Kukup beach there are 4 species found. The diversity of animal species of Opiuroidea in Porok beach is higher than Kukup beach.**

***Keywords*—Ophiuroidea, Diversity, Porok Beach, Kukup Beach, Intertidal**

I. INTRODUCTION

Indonesia is an archipelago that has great potential for waters with high biodiversity, especially in the marine aspect. Indonesia is also included to the Coral Triangle, an area with high marine biodiversity [15]. The richness of marine life in Indonesia spread from the intertidal zone to the deep sea. In the intertidal zone, some beaches have a unique ecosystem with its own character. The beaches which has a distinct character are Porok and Kukup beach. Both of these beaches have a different character due to the number of visitors who come. Porok beaches have fewer visitors compared to Kukup . Porok and Kukup Beach is located in Kemadang, Tanjungsari District, Gunung Kidul, Yogyakarta. Gunung Kidul is known to have beaches with white sand and beautiful scenery and some beaches have been opened as a tourist attraction[10] , such as Kukup beach. On the left of Kukup Beach, there lies Porok Beach. This beach has not been opened as tourist attractions, but has been utilized as a research area. One group of marine life in Indonesia is Echinodermata , Ophiuroidea particularly. Class Ophiuroidea is a group of marine life that has five flexible arms and scaly so-called brittle stars [6]. Some of these species living on the seabed (benthic) [12]. These animals are vulnerable to predators, so they always lurks by releasing two of the five arms to capture food at sea level [4]. This animal is easily found because it can live in a wide variety of marine habitats such as: sand, mud, dead coral, coral ecosystem, seagrass and algae ecosystem, etc [5] [11] [12] [17]. They also have some association with algae and seagrass community such as: feeding, nurturing, and to be shelter [4] [16]. Animals members of this class have a high abundance values, but the data about their diversity is not much known. The purpose of this study was to determine the diversity of animal species contained members Ophiuroidea Porok Beach and Kukup Beach. This research results is expected to be a source of information and a basis for further research. In addition, data that have been obtained are expected to be useful for the government and local communities in the areas of business management and development in Porok Beach and Kukup Beach.

II. METHODS

The method is divided into two parts: specimens collection and data analysis. Specimen collection process was done in the intertidal area of Porok and Kukup Beach, Yogyakarta. Specimen collection process carried out during low tide at April 24 and May 26. Kukup beach is located near Porok Beach. In figure 1, Kukup Beach is on the left Porok Beach. Both areas are separated by cliffs and fields. Figure 1 shows the sampling site.

Figure 1. The sampling location, Kukup beach (blue oval), and Porok Beach (red circle) [2]

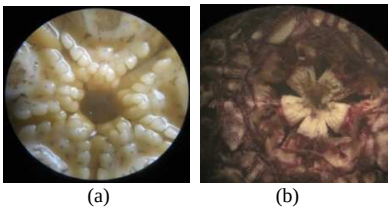
Sampling was conducted on April 26 and May 24 during the day and night in order to get optimal results. Sampling was conducted with purposive sampling

methods. Additional trap was also mounted on a variety of substrates within a specified period. The speciments catched then preserved by these steps: First, the specimen to be relaxed with MgCl2 73 mg dissolved in 1 liter of sea water so that the specimen did not move much when fixed. When relaxed, the specimen body was formed like a comet, with the arms in one direction. Then it is fixed by soaking in 70% ethanol. The specimen is preserved into 70% ethanol.

Identification was done by looking at the morphological characters of specimens. In class Ophiuroidea identification to do with the rate of guidebooks [14], and to the species level using the reference of [7] and [9]. The characteristics of the body those were used for identification include: tooth position papillae, the shape of scales on the arms, the location of the gonads, the articulation of the arm cavity, the number of tentacle scale, style body color, ratio of the sleeve length to diameter of the central disc, and so on.

III. RESULT AND DISCUSSION

Gunung Kidul south coasts are included in the beach that characterized with quite big waves. This caused the animals that live in this area had also been adapted to the coastal conditions. One such animal is the members of Class Ophiuroidea. Animals members of this class had much podia, so it could stick and hid in the cavity. It made Ophiuroidea to be survive in the intertidal zone of the beach. In Table 1. it can be seen that the specimen came from the Family of Ophiotrichidae and Ophiocomidae. Both of these parts are the characters of Order Ophiurina [14]. Ophiotrichidae and Ophiocomidae, those family members don't have branched or distinctly bent arms. Most of the spikes on the arm are perpendicular to the arm. Disk surface is usually covered with spine, granules or skin. In the oral part of the mouth are surrounded by 5 teeth [14]. The differences between Ophiotrichidae and Ophiocomidae are written in references [8] and [14]. By figure 2 one of the difference between the two Family is based on the form of their mouths.



(a) (b)

Figure 2. The appearance of the mouth of each (a) Ophiocomidae and (b) Ophiotrichidae.

Figure 3. *Macrophiothrix* sp., Member of Ophiotrichidae

Macrophiothrix sp. is a member of Ophiotrichidae Family [13] [14]. This genera have very long arms with small central disc and less proportional when compared to the length of his arm. Long arms are used to capture the food or prey that lies far away [13], and the spines on the arms are slender and pointy. These animals are very easy to break their arms when they are theaten by predators so that they can hide the rest of the body in cavity of dead coral. These animals are sensitive to light, so he likes the habitat in such dark cavity of dead coral colonies [1] [3] [7]. To get these animals, it was required to break the reef. However, at the time of sampling, it was not performed because it was feared that the coral breaking would damage habitat of *Macrophiothrix* sp.

Most specimens found came from the Family Ophiocomidae, they are Ophiomastix and Ophiocoma. In general, both the character is not much different family.

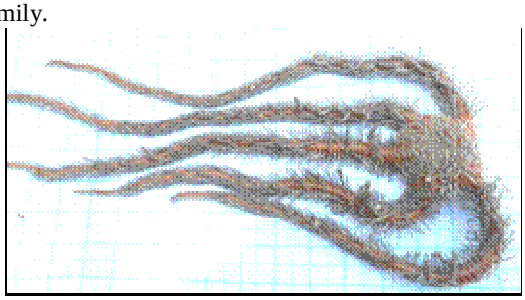


Figure 4. *Ophiomastix annulosa*, member of Ophiocomidae

TABEL 1.

OPHIUROIDS FOUND IN POROK AND KUKUP BEACH, AND THEIR CLASSIFICATION.

No	Class	Order	Family	Genera	Species
1	Ophiuroidea	Ophiurina	Ophiotrichidae	Macrophiothrix	*Macrophiothrix* sp.
2			Ophiocomidae	Ophiomastix	*Ophiomastix annulosa* (Lamarck, 1816)
3				Ophiocoma	*Ophiocoma pica* (Muller & Troschel, 1842)
4					*O. schoenleini* (Muller & Troschel, 1842)
5					*O. erinaceus* (Muller & Troschel, 1842)
6					*O. latinanxa* (Murakami, 1943)
7					*O. brevipes* (Peters, 1851)
8					*O. scolopendrina* (Lamarck, 1816)
9					*O. wendtii* (Koehler, 1907)
10					*O. anaglyptica* (Ely, 1944)
11					*O. dentata* (Muller & Troschel, 1842)

The central disc of *Ophiomastix annulosa* surface is spiny. The form of central disc usually pentagonal or five-sided and colored reddish-brown that appears darker than the arms. The arms are also spiny, and some spines are. This specimen was found at the coral ecosystem during the day. At the time of sampling these animals were found in coral ecosystems. According to the reference [12] those animals could also be found in sea grass beds in the calm waters. They are omnivorous animals that eat detritus, algae, and small animals such as crustaceans [12].

The most common genera found in both beaches was from Ophiocoma genera. The species member which present was 9 Species. Those species are found in dead coral with algae (DCA) ecosystem and sandy-surfaced area.

Figure 5. *Ophiocoma scolopendrina*, member of Ophiocoma genera

Ophiocoma scolopendrina has some variations by the color and stripe motives. The distinguishing character of this type of animal lies on their major body color, dark brown on the dorsal and pale onthe ventralpart. Cigar-shaped thorns on the arms amount to no more than four on each arm segment. Body color and pattern on the center of the disc varies. However this type of animal is still recognizable from the amount of each segment arm spines and thorns like a cigar shape [11]. Kukup Beach has environmental conditions better than Porok Beach. This type of animal found in Kukup beach and Porok.

TABLE 2.
OPHIUROIDS FOUND IN EACH KUKUP AND POROK BEACHES.

No.	Name	Kukup Beach	Porok Beach
Family Ophiotrichidae			
1	*Macrophiothrix* sp.	-	+
Family Ophiocomidae			
2	*Ophiomastix annulosa*	-	+
3	*Ophiocoma scolopendrina*	+	+
4	*O. schoenleini*	-	+
5	*O. erinaceus*	+	+
6	*O. brevipes*	-	+
7	*O. wendtii*	-	+
8	*O. latinanxa*	-	+
9	*O. pica*	+	-
10	*O. anaglyptica*	+	+
11	*O. dentata*	-	+
12	*O. valenciae*	-	+

"+": present; "-": absent

Table 2 explain the species found in both beach. From the table, Kukup beach had only four species while Porok beach had eleven. Based on the results of the sampling that had been done, diversity in Porok Beach was higher than Kukup Beach. Generally this was caused by poor environmental conditions at Kukup beach. One of the factors that cause a decreasement in the quality of the environmental conditions in Kukup Beach was the presence of many tourists. The tourists spent time at the beach with lots of activity. One of the activities that the tourists did is capture marine life. This led to the decreasment of diversity of animal species, especially Ophiuroidea members.

At the time of sampling, Ophiuroidea Class members in Porok Beach were easier taken than animals at Kukup beach. This might be due to differences in lifestyle habits on both coasts . In Kukup Beach, these animals were accustomed to the presence of stress in the form of tourists who catch these animals so Ophiuroidea in Kukup Beach had been able to adapt well to avoid predators. An example of adaptation was to move quickly when there was a threat. While at the Porok beach,

Beach, there were not too many threats because there were no tourists visiting. This causes these animals did not get used to moving quickly to avoid enemies and predators. This caused the animals Ophiuroidea members in Beach Porok were easier to sample.

This different biodiversity was also able to be caused by the quality of the beach, from the food, interactions and shelter aspect. At Kukup Beach, the visitor caught not only Ophiuroids but also any other animals, such as fishes, crustaceans and mollusks. Those animals are the prey of some ophiuroids. Beside, those animals did some interaction with Ophiuroids. When the visitor caught those animals it means the Ophiuroids lost their preys. The visitor also unintentionally broke the algae and seagrass community. It could decrease the food and shelter for the Ophiuroids since Ophiuroids often associate with algae and seagrass [4] [16].

IV. Conclusion

Diversity of members in the class Ophiuroidea Porok Beach (11 species) is higher than in Kukup Beach (4 species). The difference is caused by different environmental conditions. In future research needs to be conducted and collected data about the abundance ratio Ophiuroidea Class members on both coasts and its relation to environmental conditions.

Acknowledgment

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References

[1] Aizenberg, J., A. Tkacenko, S. Weiner, L. Addadi, and G. Hendler. "Calcitic Microlenses as a part of the photoreceptor system in brittlestars". *Nature*. Vol 412. 2001, p. 819

[2] Anonim. (2013 March 28). [online] available: http://www.wikimapia.com/pantai-kukup/

[3] Aziz, A. "Habitat dan Zonasi Fauna Ekhinodermata di Ekosistem Terumbu Karang". *Oseana*. 21(2). 1996. Pp: 33-43

[4] Azis, A. dan Soesetiono. "Asosiasi antara bintang mengular dengan algae. Oseanografi". ISBN 979-8105-42-7. 1998.

[5] Birkeland, C. *The Influence of Echinoderms on Coral Reef Communities. In: M. Jangoux, J. M. Lawrence (Eds.). Echinoderms Studies 3*. Rotterdam: Balkema. 1989. p. 79

[6] Campbell, N. A., J. B. Reece, L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky, & R. B. Jackson. *Biology Eighth Edition*. . San Francisco: Pearson Benjamin Cummings. 2008. Pp: 673

[7] Clark, A. M., Rowe, F. W. E. *Monograph of shallow-water indo-west pacific echinoderms.* London: British Museum, 1967, pp. 117-119.

[8] Fujita, T. *Echinodermata. In, national museum (ed.), collection building management for natural history*. Tokyo : Tokai University Press. 2003. Pp 32-38

[9] Guille, A. P, and J. L. Menou. *Handbook of the sea stars, sea urchins, and related echinoderms of new caledonia lagoon*. Institut francais de scherche scientifique pour le developpement en cooperation. Paris: (penerbit). 1986. Hal...

[10] Jati, A. W. N. *Biozonasi echinodermata di pantai drini, gunung kidul, Yogyakarta*. Biota vol 1 (2): 13-21. 1996. p 16

[11] Perwitasari, A. *Keragaman jenis bintang laut mengular (ophiuroidea) pada zona intertidal pantai watukarung kecamatan pringkuku kabupaten pacitan jawa timur*. Seminar. Faculty of Biology, Universitas Gadjah Mada, Yogyakarta. 2011

[12] Susetiono. *Lamun dan fauna teluk kuta, pulau Lombok.* LIPI press. Jakarta. 2007. Hal. 90

[13] Susetiono, R. A. K. *Keanekaragaman jenis ophiuroidea di kawasan pasang surut pantai sdelatan, gunung kidul.* Naskah seminar. Fakultas Biologi, Universitas Gadjah Mada, Yogyakarta. 2011.

[14] *Western pacific sampling protocol and Ophiuroids taxonomy training workshop. LIPI-Nagisa*. 2010.

[15] WWF. (2012 September 29) [online] available: http://wwf.panda.org/what_we_do/where_we_work/coraltriangle

[16] Yusron, E. *Biodiversitas fauna ekhinodermata di perairan selat lembeh, bitung-sulawesi utara*. Oseanologi dan Limnologi di Indonesia Vol. 35 (2): 225-237. 2009. p 226

[17] Yusron, E. *Keanekaragaman jenis ophiuroidea (bintang mengular) di perairan wori, minahasa utara, sulawesi utara*. Makara, Sains Vol 14 (1) 2010 pp: 75-78

Increasing of Soil Enzyme Activities by Addition of Yeast Extracted from Semi-solid Waste of Alcohol Industry

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***Abstract*—Recently, the development of alcohol industry rapidly increased causes increasing of waste product. Waste from the alcohol industry consists of liquid and solid waste. These wastes production usually dumped into landfill or river. These semi-solid wastes still contain yeast from the production, so there is likely to be used again. The yeast contain could be extracted by autolysis process. Not has been yet known the potential of yeast extract from wastes as an additive organic matter for soil. So, this research aims to study the potency of yeast extract as an organic matter by testing the activity of soil enzymes.**

In this research, waste yeast extracts was added to sterile soil with concentration of 0; 2.8; 5.6; 11.2; and 22.4 g kg soil^{-1}. The activity of soil enzyme on Phonska 0.46 g kg^{-1} sterile soil and non-sterile soil also determine for comparison. Effect of yeast extract was determined by testing the urease activity, nitrification activity and cellulase activity in each treatment every week for 28 days. The results obtained were tested using analysis of variance two-way anova.

The conclusion of this research is the yeast extract from industrial waste alcohol increase soil enzyme activity (urease activity, nitrification activity, and cellulase activity). The addition of yeast extract 22.4 g kg^{-1} soil gave best increased of soil enzyme activity.

***Keywords*— waste, alcohol-industry, soil enzyme activities.**

I. INTRODUCTION

THE development of alcohol industry that so rapid today, besides provide economic benefits, but also gave a negative impact on the environment due to the discharge of waste [1]. This waste is usually disposed to landfill or into the river so that it can degrade the quality of the environment [2].

Reed [3] had been reported that semi-solid wastes of alcohol industry still contain yeast, so there is likely to be used again by several methods. The autolysis process of waste alcohol will produce yeast extract which is rich in nitrogen, vitamins and organic compounds. When applied to the soil, these yeast extract can stimulate plant growth. Several studies also reported that application of organic compounds (organic manure) in soil could increased microbial biomass, microbial diversity, and plant yields [4], [5]. In addition, yeast extract can be used as an ingredient in the growth medium for microorganisms [6]. As an organic compounds, yeast extract could be used as soil organic compounds.

The addition of organic matter to the soil may provide additional substrate for soil microorganisms [7]. This organic material will be biodegraded by enzymatic processes of microorganisms, so usually a fertile soil which rich in organic material will contain more diverse microorganisms and high enzymatic activity [8]. Several important enzymes are soil urease enzyme, cellulase enzymes, enzymes that play a role in nitrification, β-glucosidase, dehydrogenase, protease, and fosfomonoesterase [9]. Not has been yet known the potential of yeast extract from wastes as an additive organic matter for soil. So, this research aims to study the potency of yeast extract as an organic matter by testing the activity of soil enzymes.

II. MATERIALS AND METHODS

The experiments were performed at green house of Biology Laboratory. Before use, each soil sample was air-dried and crushed to pass a 2 mm screen. Yeast extract were prepared from semi-solid waste of alcohol industry and precipitated overnight. Precipitate was twice washed with distilled water until it looks like paste. Yeast paste was put into container and resuspended with distilled water (1:1, v/v). These suspensions were adjust at pH 7 with baking soda, and then incubated 6 days in agitation (10 rpm). These extract of yeast autolisat were dried at 70°C, 24 hours. Dried yeast extracts were crushed to make powder.

Thirty-five 3kg soil samples were filled into 3kg plastic bag and mixed according the treatments. The treatments were three controls (dried soil, soil + phonska 0.46g kg^{-1} dried soil, and sterile soil) and four treatments (soil + 2.8, 5.6, 11.2, and 22.4 g yeast extract kg^{-1} dried soil). Soil samples were sterilized as described by Powlson and Jenkison [10]. All treatments were replicated five times. Samples were taken every week and analyzed for pH [11], the content of organic C and total N [12], and soil bioactivity.

Soil bioactivity was characterized by bioassay for hydrolytic enzyme activity in air-dried soil samples. Urease (EC 3.5.1.5) activity was assayed according to the modified spectrometric method at λ 420 nm [8]. Determination of urease activity was modified according to Kandeler and Gerber (1988) equation, and expressed in μg NH_4-N g^{-1} soil dry weight hour^{-1}[13] Activity of nitrification was assayed according to the modified Keeney and Nelson (1982) [11] spectrometric method at λ 410 nm, and expressed in μg N-NO_3^- g^{-1} soil dry weight. Cellulase activities were determined according to Schinner *et al.* 1996 spectrometric method at λ 540 nm, based on glucose forming. Its activity was expressed in unit g^{-1} soil dry weight [8].

Statistical analysis

The confidence limits of the data were based on two–way analysis of variance by *ANOVA* (in case of significant interactions) to determine the significant differences between treatment means. Standard

deviation (*sd*) and correlation were calculated at level of statistical significance $P < 0.05$ to determine correlation between total C, N, rasio C/N, soil pH, and soil enzymes activities.

III. RESULTS AND DISCUSSION

Our analysis revealed that initial condition of soil samples were in the range 4.6-4.8 of pH and low of total C and N. Initial total C and N were 1,26% – 1,79% and 0,13% – 0,27%, respectively. Based on chemical characteristic of soil, these soil samples categorized in low-quality. However, after the addition of yeast extract, C/N ratio of soil samples were ranged from 6:1 to be 10:1. These C/N ratio can be categorized into the C/N ratio which is good for agriculture. According Hakim et al (1986), value of C/N ratio is good for agricultural land ranges from 8:1 to 15:1, or an average of 10:1 to 12 [14]. These results indicated that yeast extracted from semi solid waste of alcohol industry can be used as a substitute of organic matter for agricultural soil.

Results of statistical analysis also showed that there are significant interaction effect of the addition of yeast and incubation time on the activity of the urease enzyme in the soil (P<0,05). In general, the addition of yeast can increase the activity of the urease up to 14 days. Although the urease enzyme activity then decreased until the end of the study (Figure 1), but the decline is thought to be caused by the reduction of organic matter in the soil. These reduction of organic content in the soil is due to the decomposition of organic matter. Mailani (2006) proposed that the cause of the decline in enzyme activity is decreasing of organic matter availability for microorganisms play an important role in the activity of the enzyme urease [15].

The results of the analysis showed that urease activity of soil which added 22.4 g yeast extract gave the highest urease activity.

High or low levels of urease activity is highly dependent on the presence of organic matter, the change of seasons, the population of soil microorganisms and the reaction products which is catalyzed by urease ie urea and ammonium [16]. High content of organic matter will increase the activity of the enzyme as a direct influence on the increase in microbial activity. The content of organic matter in the soil can be improved by the addition of organic matter [17].

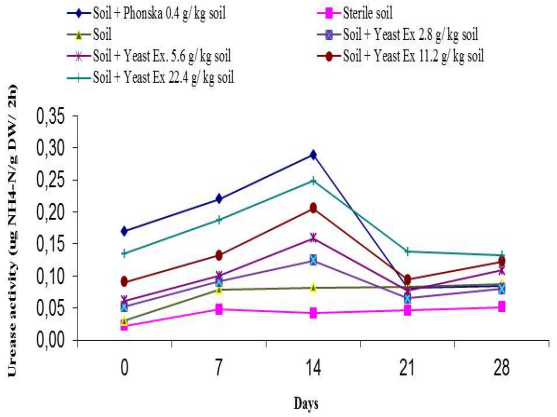

Fig 1. Urease activity (µg N-NH_4.g $^{-1}$dw.h^{-1}) in the soil with or without yeast extract addition during 28 days

As well as the effect of the addition of yeast extract on urease activity, the addition of yeast extract also increases the activity of nitrification (Figure 2) and cellulase (Figure 3). Results of statistical analysis also showed that there are significant interaction effect of the addition of yeast and incubation time on the activity of nitrification and cellulase enzyme in the soil (P<0,05).

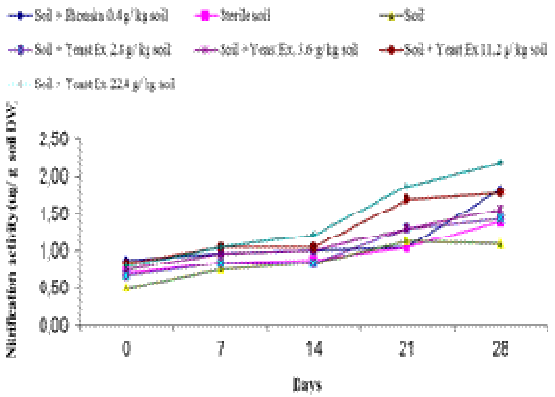

Fig 2. Nitrification activity (µg g^{-1} dw) in the soil with or without yeast extract addition during 28 days

According to Hakim *et al* (1986), an increase of nitrification activity occurs due to several factors such as pH, addition of organic matter, temperature, soil moisture is optimal, and the C/N ratio [14]. These condition will increase microbial growing on the soil and the nitrification activity will running well. Brady and Weil (2002) also explains that the concentration of NO_3 in the soil is determined by the amount of organic material supplied or the rate of nitrification in the soil [18]. Nitrifying bacteria have the same nutrient requirements with higher plants. Provision of macro and micro nutrients will encourage the nitrification activity.

Results of statistical analysis also showed that the addition of yeast significantly affect the activity of cellulase enzymes and the observation time. However, based on the calculation results obtained show that the enzyme activity is low. According to Fauzi (2008), cellulase enzyme activity in the soil is low compared to the value of the activity of the enzyme urease and nitrification activity [19]. Despite the low activity, however in the treatment of soil + yeast extract 22.4 g/kg soil has a highest cellulase enzyme activity than other treatments. The results of the analysis showed that there are significantly differences (P> 0.05) on each of both treatment and control.

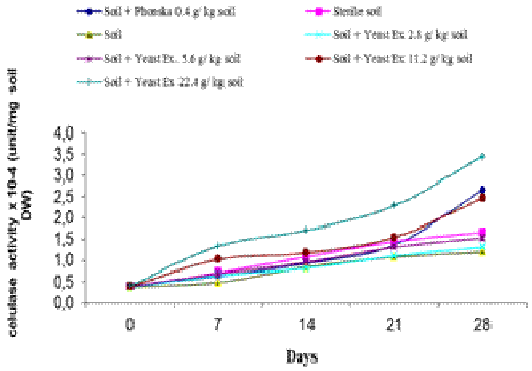

Fig 3. Celulase activity (unit µg^{-1} soil dw) in the soil with or without yeast extract addition during 28 days

Cellulase enzyme activity in the soil generally has a low value due to the slow degradation of cellulose in the soil. Cellulose in soils derived from plant material and overhaul fraction derived from fungi and bacteria in the soil. Degradation of cellulose in nature is very slow due to extracellular enzymes that hydrolyze cellulose is a special product that is only produced by certain fungi and bacteria [20].

IV. CONCLUSION

From these results it can be concluded that the yeast extract of the semi-solid waste of alcohol industry increases the microbial enzyme activity (urease, nitrification activity, and cellulase) in the soil. The highest enzyme activity (activity of urease, nitrification activity, and activity of cellulase) in soil found in soil + yeast extract dose of 22.4 g kg^{-1} soil

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REFERENCES

[1] L. R. Balia (2004). *Potensi dan prospek yeast (khamir) dalam meningkatkan diversifikasi pangan di Indonesia*. Fakultas Peternakan, Universitas Padjajaran Bandung. [Online]. Available: http://www. pdf4free.com

[2] Supraptini, "Pengaruh limbah industri terhadap lingkungan di Indonesia," *Media Litbang Kesehatan*, vol. XII no 2, 2002.

[3] G. Reed, "Use of Microbial Cultures : Yeast products," *Food Tech*. vol. 35, p. 89, 1981

[4] H.Y. Chu, T. Fujii, S. Morimoto, X.G. Lin, K. Yagi, J.L. Hu, J.B. Zhang, "Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil," *Appl. Environ. Microbiol*. vol. 73, pp. 485–491. 2007

[5] W. Zhong, T. Gu, W. Wang, B. Zhang, X. Lin, Q. Huang, and W. Shen, "The effects of mineral fertilizer and organic manure on soil microbial community and diversity," *Plant Soil*, vol. 326, pp. 511–522, 2010

[6] EURASYP (European Association for Specialty Yeast Products). Autolysed Yeast. 2010. Available: http://www.eurasyp.org/public. levure.autolyse.screen).

[7] A. Pera, G. Vallini, I. Sireno, M.L. Bianchin, and M. de Bertoldi, "Effect of organic matter on rhizosphere microorganism and root development of Sorghum plants in two different soils," *Plant & Soil*. vol. 74, pp. 3-18. 1983.

[8] F.E. Schinner, E. Kandeler, R. Ohlinger, and R. Margesin. *Method in Soil Biology*. Berlin: Springer-Verlag. 1996.

[9] W.A. Dick, L. Cheung, and P. Wang, "Soil acid and alkaline phosphatase activity as pH adjusmnt indicators," *Soil Biol. Biochem*. vol. 32, pp. 1915-1919. 2000.

[10] S.W. Bartock, "Steaming is still the most effective way of treating contaminated media," *Greenhouse Manager*. vol. 110, pp. 88-89, 1993.

[11] Alef, K, and P, Nannipieri. *Principles of soil microbiology and molecular biology*. London : Academic Press Limited. 1995.

[12] K.E. Eriksson, R.A. Blanchette, and P. Ander. *Biodegradation of cellulose. In Microbial and Enzymatic Degradation of Wood and Wood Components*. New York : Springer – Verlag. 1990.

[13] E. Kandeler and H. Gerber, "Short-term assay of soil urease activity using colorimetric determination of ammonium," *Biol. Fertil. Soils*. vol. 6, pp. 68-72, 1988

[14] N. Hakim, Nyakpa, M.Y. Nugroho, S.G. Diha, M. Amin, dan G.B. Hong. *Dasar-dasar Ilmu Tanah*. Lampung: Universitas Lampung. 1986.

[15] Mailani. *Aktivitas enzimatik dan respirasi pada tanah tercemar pestisida yang diberi serbuk jerami dan bakteri pendegradasi nitril*. [skipsi]. Bogor: MIPA, IPB. 2006.

[16] T. Askin and R. Kizilkaya, "The spatial variability of urease activity of surface agricultural soils within an urban area," *J. Central European Agriculture*. vol 6, pp. 161-165, 2005.

[17] R.N. Wandansari, *Aktivitas urease pada beberapa tanah di Indonesia*. [skripsi]. Bogor : Fak. Pertanian, IPB. 2006

[18] N. C. Brady and R. R. Weil. *The Nature and Properties of Soils*. 13th Edition. New Jersey: Pearson Education, Inc. p. 960. 2002.

[19] A. Fauzi, *Analisa kadar unsur hara karbon organik dan nitrogen di dalam tanah perkebunaan kelapa sawit Bengkalis Riau*. [skripsi] Medan: MIPA, Universitas Sumatera Utara. 2008

[20] A. Kanti. *Penapisan khamir selulolitik Cryptococcus* sp. *yang diisolasi dari tanah Kebun Biologi Wamena, Jaya Wijaya, Propinsi Papua*. Bogor : Bid. Mikrobiologi, Puslit Biologi-LIPI. 2000.

Population Dynamic of Parasitic Plant *Viscum articulatum* Burm.f. in Purwodadi Botanic Garden

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***Abstract*— *Viscum articulatum* is a parasitic plant and used as potential medicinal plant. The species also grows as hyperparasite on the other species of parasitic plant. The research aims to study population dynamics and the plant diversity hosts of *Viscum articulatum* in Purwodadi Botanic Garden on April to September 2013 by cruising methods and literature study on the previous research of the parasitic plants in the garden. The results showed that *Viscum articulatum* only found as hyperparasite on another parasitic mistletoe *Dendropthoe pentandra*. The population number increased following the increasing of host plant number and time. The species, genera and family of the host trees increased from 20 species, 14 genera and 13 families in 2005 to 23 species, 19 genera and 17 families in 2013. The dominant host tree was *Cassia fistula* L.**

***Keywords*: population, *Viscum articulatum*, host, parasite**

I. INTRODUCTION

Parasitic plants grown by attaching to and absorb water, nutrients and food from their host plant. The parasitic plants account for about 1 % of flowering plants, with more than 3,000 species distributed in 16 families [7]. Among these species, mistletoes (Family Loranthaceae and Viscaceae) are widely recognized companies as an ecologically important functional group [5]. Types of the parasites can be categorized as obligative, facultative, hemiparasitic and hyperparasite. The facultative parasites or hemiparasites have photosynthetic organs such as leaves to meet the need of growth as commonly found on Viscaceae and Loranthaceae. On the hyperparasites, the parasite attaches to another parasite, usually on family Loranthaceae [3] such as *Viscum orticulatum* which attached to the parasite *Dendrophtoe pentandra* (21). Both macro and micronutrients by the parasitic plant absorbed from the host plants reported by [10]. The accumulation of the nutrients in the parasite *Viscum articulatum* was higher than that in its host and proximal branches of the host tree. [21] reported that the parasite caused growth inhibition, damage and death of distal branches until 30 %.

Although the parasites known as harmful plant species, the plants has long been known as a source of traditional medicine [4], [1], [6] such as *Viscum articulatum* been used by the Chinese as a hypertension drug [20]; anticancer [13], diuretic [12], antioxidant [25], antiulcer [14], antiepileptic [9], immonodulatory[23].

Viscum articulatum contains triterpenoids (betuline, oleanolic acid, lupeol stearate, palmitate lupeol, lupeol acetate, a- amyrin, lupeol Betulinic acid), flavonoids, steroids (ß-sitosterol), saponins, and glycosides, tannic acid, ceryl oleonolate and mesoinositol [2].

Viscum articulatum belongs to family Viscaceae which is found in the tropics from to eastern India to Vietnam and to the south in Malesia region and Australia [3]. The parasite has been found in Purwodadi Botanic Garden since the nineteen years ago [16], and its population and the hosts plants tend to increase. The parasite population is dynamic by time and other factors such as seeds dispersal by birds and increasing number of the host plants [17]. [18] reported that the parasites population and their hosts on Rutaceae and medicinal plants in Purwodadi Botanic garden increased in 2005-2013. This research aims to study the population dynamics and the hosts of the parasitic plant *Viscum articulatum* in Purwodadi Botanic Garden.

II. MATERIALS AND METHODS

The research was conducted in Purwodadi Botanic Garden in April - September 2013 by cruising methods [15] in the area of about 85 hectares and literature study on the research of the parasitic plants in the garden. The tree hosts and primary parasites where *Viscum articulatum* attached to were recorded. The population parameters calculated included the growth rate [11] and frequency of *Viscum articulatum* as followed:

$$GR \text{ (growth rate)} = \frac{P(t_2) - P(t_1)}{(t_2 - t_1)}$$

$P(t_2)$ = population number of *Viscum articulatum* at the end observation

$P(t_1)$ = population number of *Viscum articulatum* at early observation

t_2-t_1= time priods t_2-t_1 (year)

$$\text{Frequency} = \frac{\text{Number of } \textit{Viscum articulatum} \text{ found}}{\text{The total number of primary host parasite which is the main host of parasite is } \textit{Dendrophtoe pentandra}}$$

III. RESULTS AND DISCUSSION

A. Population

Total population of *Viscum articulatum* and its main host, *Dendrophtoe pentandra* in 2013 increased respectively by 46,64 % and 48,38 % compared to 2006 (Table 1). Whereas the total population of *Dendrophtoe pentandra* as the primary host and the parasite *Viscum*

articulatum in 2006 respectively 198 and 31 specimens then in 2013 increased respectively to 290 and 46 specimens (Table 1). This is due to the spread of seeds by birds and increasing the number of the host plants during the period 2006-2013. [21] reported that the birds play an important role in the seeds dispersal of the parasites such as *Dendrophtoe pentadra* and *Viscum articulatum*. The birds belong to the family Dicacidae [24], especially 'bird peppers' (*Dicaeum* spp.) [19], which eat the fruits. The fruit is berry, white fleshy, sweet taste, single seed, covered with a sticky substance such as rubber *viscin* that is not digested in the digestive system of the birds and will be wasted with faeces or the seeds eaten were wasted moment. The seeds that falls on a tree branches or twigs will stick, germinate and become new plants, however not all the seeds can germinate and grow well. The seeds can plastered and germinated on the leaf surface, but they can not develop because the leaves will fall.

TABLE 1.
THE POPULATION AND TREE HOST OF *VISCUM ARTICULATUM* AND MAIN *DENDROPHTOE PENTANDRA* IN 2006-2013.

Year	Population		Frequency *)	Host tree number	
	Dendrophtoe pentandra	*Viscum articulatum*	*Viscum articulatum*	*Dendrophtoe pentandra*	*Viscum articulatum*
2006	198	31	0,156	52	20
2013	290	46	0,158	86	26
growth (%)	46.64	48.38		65.34	30.00
growth rate (plant.year^{-1})	13,14	2,14		4,857	0,857

The growth rate (GR) of *Viscum articulatum* is lower than its host plant *Dendrophtoe pentandra*, which reached 2,14 plants.year$^{-1,}$ whereas *Dendrophtoe pentandra* 13,14 plants. year^{-1}. This high GR of *Dendrophtoe pentandra* facilitated the mistletoe become the most dominant parasite in Purwodadi Botanic Garden [22], [21], [17], [18], so it has more ability and posibility to grow and to develop than other parasites such as *Macrosolen tetragonus, Scurulla atropurpurea* and *Viscum ovalivolium.*

Whereas the lower GR on *Viscum barticulatum* was caused by less attractive fruits. Fruit flesh thin, small in size and it has seed germination type easily removed when it rains or more sensitive to the environment. Shade leaves barriers of the host tree plants and the primary host *Dendrophtoe pentandra* can also interfere the attachment of the parasite seeds on their host plants. Dependence on a particular host can also be a factor ofthe slow GR.

TABLE 2.
THE MORTALITY OF *VISCUM ARTICULATUM* ON SOME HOST TREE SPECIES IN 2013

No.	**species**	**Number**	**Mortality(%)**
1	*Cassia fistula*	15	32,61
2	*Hydnocarpus sumatrana*	1	2,17
3	*Cassia garrethiana*	1	2,17
4	*Tectona grandis*	1	2,17
5	*Scolopia spinosa*	1	2,17
6	*Lagerstroemia thorellii*	1	2,17
Dye		**22**	47,83

Note : The population of Viscum articulatum is 46 psecimens

B. Host plants

All of the parasites require hosts for their life. Differences in time, type and characters of each parasite can cause differences in species composition of the host species. Table 3 and Figure 1 show that during the period 2005-2013 there were differences in the composition and secondary host plant species where *Viscum articulatum* were found. Over a period of 8 years between 2005-2013 the host treem increased 6 species, 7 genera and 5 family. The host plant species were predominant by *Cassia fistula* that found of 18 specimens. Some tree host species were new host that is not found before as many as 11 species of trees such as *Averrhoa carambola, and Ceiba pentandra, Dillenia hilippensis* (Table 4).

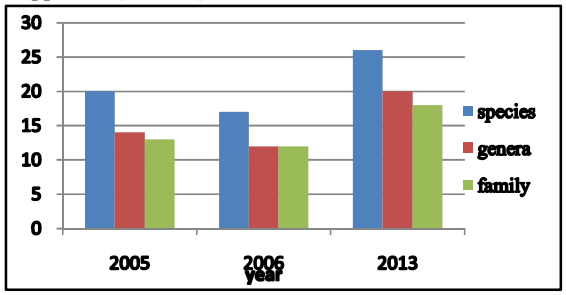

Figure 1. The host trees composition of *Viscum articulatum*

TABLE 3.
THE COMPOSITION OF SPECIES, GENERA, AND FAMILY OF THE HOST TREE PLANTS IN 2005 – 2013

year	species	genera	family
2005	20	14	13
2006	17	12	12
2013	26	21	18

In this study *Viscum articulatum* as hyperparasitic was only found on *Dendrophtoe pentandra* as the primary host (Table 5). [22] reported this parasite ever encountered on the parasite *Macrosolen tetragonus* that hosted on *Mammea* tree cf. *Odorata, Ficus callos*, *Ficus microcarpa* and *Ficus racemos*a and [21] reported the presence of *Viscum articulatum* also discovered on *Macrosolen tetragonus* growing on *Ficus microcarpa* and *Ficus fistulosa*. This is presumably due to the death of the host and the parasite. At the time of this study the presence of the parasite was not found. [18] reported that many *Macrosolen tetragonus* found growing attached to the parts of plants the family Moraceae such as *Ficus racemosa*, *Ficus religiosa* and *Streblus asper*, however there have been no *Viscum articulatum* which is hosted on this parasite.

TABLE 4.
THE TREE HOSTS PLANT OF *VISCUM ARTICULATUM* IN 2005-2013 IN PURWODADI BOTANIC GARDEN

No.	Species	Family	2005	2006	2013
1	*Aegle marmelos*	Rutaceae	v	v	
2	*Albizia procera*	Mimosaceae	v		
3	*Averrhoa bilimbi*	Averrhoaceae			v
4	*Barringtonia asiatica*	Lecythidaceae	v		
5	*Cassia fistula*	Caesalpiniaceae	v	v	v
6	*Cassia garrethiana*	Caesalpiniaceae	v	v	v
7	*Ceiba pentandra *)*	Bombacaceae			v
8	*Dilenia philippensis *)*	Dilleniaceae			v
9	*Dillenia pentagyna*	Dilleniaceae	v	v	v
10	*Diospyros blancoi*	Ebeneceae		v	v
11	*Diospyros malabarica*	Ebeneceae	v	v	v
12	*Euodia sp. *)*	Rutaceae			v
13	*Ficus fistulosa*	Moraceae		v	
14	*Ficus callosa*	Moraceae	v		v
15	*Ficus microcarpa*	Moraceae	v	v	
16	*Ficus paniiflora*	Moraceae	v	v	
17	*Ficus religiosa*	Moraceae	v		v
18	*Ficus superba*	Moraceae	v	v	v
19	*Ficus variegata*	Moraceae	v		v
20	*Garcinia dulcis (Roxb.) Kurz.*	Clusiaceae	v	v	v
21	*Glochidion sp3 *)*	*Euphorbiaceae*			v
22	*Holoptelea integrifolia Planch. *)*	*Urticaceae*			v
23	*Hydnocarpus sumatrana *)*	Flacourtiaceae			v
24	*Ixora longifolia*	Rubiaceae	v	v	
25	*Lagerstroemia thorelii*	Lytheraceae	v		v
26	*Lagerstromea floribunda*	Lytheraceae		v	
27	*Lagerstromea loudonii *)*	Lytheraceae			v
28	*Mangifera indica*	Anacardiaceae	v	v	v
29	*Osmanthus fragrans *)*				v
30	*Pithecellobium dulce*	Mimosaceae		v	v

31	*Saccopetalum Horfieldii*	Annonaceae	v	v	v
32	*Scolopia spinosa (Roxb.) Warb. *)*	Flacourtiaceae			v
33	*Stelechocarpus burahol *)*	Annonaceae			v
34	*Tectona grandis*	Verbenaceae	v	v	v
35	*Terminalia catappa *)*	Combretaceae			v

Note: (v) = *Viscum articulatum* was found ; ( ) = not found ; *) = new record

TABLE 5.
THE PRESENCE OF *VISCUM ARTICULATUM* ON OTHER PARASITES IN PURWODADI BOTANIC GARDEN

No.	Species	2005	2006	2013
1	Macrosolen tetragonus (Blume)Miq.	v	v	
2	Dendrophthoe pentandra (L.)Miq.	v	v	v
3	Scurrula atropurpurea (Blume)Danser			
5	Viscum ovalifolium DC.			

Note: (v) = *Viscum articulatum* was found ; ( ) = not found ;

IV. CONCLUSION

Viscum articulatum was found as hyperparasite on the parasite *Dendrophtoe pentandra.* The population of parasitic plant *Viscum articulatum* and its primary host *Dendrophtoe pentandra* in Purwodadi Botanic Garden increase between 2005–2013 each 46,64 % and 48,38 %. The frequency and population growth rate of *Viscum articulatum* respectively 0,158 in 2013 and 2,14 plants.year^{-1} during 7 years. The species, genera and family of the host trees of this parasite increased from 20 species, 14 genera and 13 families in 2005 to 23 species, 19 genera and 17 families in 2013. The dominant host tree is *Cassia fistula* L.

REFERENCES

[1] A.B. Chozin,Wahjoedi, and Pudjiastuti,"Informasi penelitian botani dan fitokimia tanaman benalu", Warta tumbuhan obat Indonesia, 4(4), 1998, pp. 1-2.

[2] Anonymous, 2013. Taka*Viscum articulatum* Burm. f., leafless mistletoe. Philippine Medicinal Plant. http://stuartxchange.com/Taka.html,. acessed 20 January 2014

[3] B.A. Barlow, " Viscaceae" in Flora Malesiana, Series I, Vol.13, C. Kalkman,, D. W Kirkup, H.P. Nooteboom, P.F. Stevens , and W.J.J.O de Wilde Eds. Rijkherbarium,1997, pp 403-442.

[4] C. Kirana, 1996" Bioavtive compounds isolated from scurulla oortiana parasitizing tea plant *Camelia chinensis*", Thesis of master or agriculture program, Adelaide university. 1996, Adelaide.

[5] D.M., Watson, " Mistletoe - a keystone resource in forests and woodlands worldwide" Annual Review of Ecology and Systematics , 32, 2001, pp. 219-249

[6] F.I. Windadri and J.S.Rahajoe, " Keanekaragaman jenis benalu di pulau Jawa" *Warta tumbuhan obat Indonesia*, 4(4), 1998, pp. 25-29.

[7] J. Kuijt,"The biology of parasitic flowering plants". Univ. California Press, Berkeley, 1969, 246 pp

[8] Khwaja, T.A., Dias, and C.B., Pentecost," Recent studies on the anticancer activities of mistletoe (*Viscum album*) and its alkaloids" *Onconology* 43, 1986, pp 42-50.

[9] K.M. Geetha, P. V. V. S. Bhaskara Gopal, and V. Murugan " Antiepileptic activity of aerial parts of Viscum articulatum (Viscaceae) in rats " Geetha, K. M.; Bhaskara Gopal, P. V. V. S.; Murugan, V. / Journal of Pharmacy Research;Dec2010, Vol. 3 Issue 12, p2886

[10] K. Tennakoon, W. Chak, L.L.L. Im, and J. Bolin.

[11] "Mineral nutrition of the hyperparasitic mistletoe *Viscum articulatum* Burm.f.(Viscaceae) in tropical Brunei Darussalam", *Plant Specie Biology* vol.29, 2014, pp 101–107

[12] L.Agustina, Analisis Tumbuh Tanaman. Jurusan Budidaya Pertanian. Fakultas Pertanian. Universitas Brawijaya. Malang. 1988, p 46.

[13] N. Jadhav, C.R Patil, and K.B Chaudhari, "Diuretic and natriuretic activity of two mistletoe species in rats ", *Pharmacognosy Research*, Vol.2.Issue 1, 2010, pp. 50-57

[14] R. E Mutha, R.D. Shimpi, and R.B. Jadhav , " Study of preliminary anticancer potential of some hemiparasite plants" IJPRD, 2010, PUB, ARTI, VOV-2, ISSUE-1, 2010,

[15] R.Naganjaneyulu, C.K.Ashok Kumar, G.Avinash Kumar, M.Deena Dalith and D.Jamal Basha, "Antiulcer activity of viscum articulatum burm f. (viscaceae) ," International Journal of Innovative Pharmaceutical Research." (2)2, 2011,pp.139-143.

[16] Rugayah, E.A. Widjaja, and Pratiwi," Pedoman pengumpulan data keanekaragaman flora" Bogor, Pusat penelitian Biologi-LIPI , 2004.

[17] Soejono," Inventarisasi pohon inang benalu Di Kebun Raya Purwodadi Pasuruan, Jawa Timur" *Seminar Nasional Biologi IX*, Universitas Gadjah Mada, Yogjakarta, 1995.

[18] Solikin,"Tumbuhan inang benalu pada suku Rutaceae di Kebun Raya Purwodadi", Prosiding Seminar Nasional Pendidikan dan Saintec., E. Sugiyarto, Mahajoeno,S Chalimah dan H Julendra (eds), Prodi Biologi FKIP UMS. 12 Mei 2013, pp. 186-194.

[19] Solikin, 'Parasitic palnts on medicinal plants: Study in Purwodadi Botanic Garden", International Seminar on Forest and Medicinal Plants for Better Human Welfare, Puslitbang Peningkatan Produktivitas Hutan Balitbang Kehutanan, . Bogor, 9-10 September 2013

[20] S. Pitojo,"Benalu hortikultura: Pengendalian dan pemanfaatan", Trubus agriwidya, Wildl.Monogr. Ungaran , 86 ,1996, pp. 1–66

[21] S.S. Bachhav, M.S. Bhutada , S.D. Patil, B. Baser, and K.B. Chaudhari KB," Effect of *Viscum articulatum* Burm. (Loranthaceae) in Nω-nitro-L-arginine methyl ester induced hypertension and renal dysfunction., *J.Ethnopharmacol.*142(2), 2012,Elsevier Ireland Ltd : pp 467-73.

[22] Sunaryo, E. Rahman, and T. Uji,"Kerusakan Morfologi Tumbuhan Koleksi Kebun Raya Purwodadi oleh Benalu (Loranthaceae dan Viscaceae)", *Berita Biologi*, Vol 8(2), 2006, pp. 129-139.

[23] T. Uji and Samiran, "Keanekaragaman jenis benalu dan Tumbuhan inangnya di Kebun Raya Purwodadi, Jawa Timur" Laporan Teknik. Bidang Botani, Bogor Puslit Biologi_LIPI., 2006, pp 269-277.

[24] Tzu-Li Lu, Jing-Yuan Chuang, Jai-Sing Yang, Shau-Ting Chiu, Nai-Wan Hsiao, Mei-Chen Wu, Shih-Hsiung Wu, and Ching-Hsiang Hsu , "Production of Active Nonglycosylated Recombinant B-Chain of Type-2 Ribosome-Inactivating Protein from Viscum articulatum and Its Biological Effects on Peripheral Blood Mononuclear Cells ,*Evidence-Based*

[25] W.M. Van Leeuwen, "On the biology of some javense Loranthaceae and the role birds play in their life history" *Beaufortia* (4)41, 1954, pp. 103-207.

[26] Yu-Jen Kuo, Yu-Ching Yang, Li-Jie Zhan et al , "Flavanone and Diphenylpropane Glycosides and Glycosidic Acyl Esters from Viscum articulatum ", *J. Nat. Prod*., 73 (2), 2010, pp 109–114

The Influence Of Explant Sterilization Method And 2,4-Dichlorophenoxy Acetic Acid (2,4-D) On The Callus Formation Of Various Explants Of Red Betel (*Piper crocatum*)

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***Abstract*— Due to its various secondary metabolites, Red betel (Piper crocatum) possesses a great potential as a multifunctional drug which is conventionally obtained by extraction procedure directly from the plant, and constrain large land for plant cultivation. Plant tissue culture technology is an attractive alternative to overcome such problems, because it does not require large field, much less raw material, and possible of continuous production. This study herein report the influence of chemical sterilization method and addition of growth regulation 2,4-D toward callus formation. The results showed that the most appropriate sterilization method for leaf explants was soaking of explants in 70% ethanol for 6 minutes followed by soaking in 2% sodium hypochlorite solution for 10 minutes, while the suitable sterilization method for stem and stalk explants was soaking the explants in 70% ethanol for 10 minutes, then in 25 NaClO solution for 15 minutes. The best medium formulation for callus induction was MS medium supplemented with 2.5 ppm of 2,4-D. The best part of red betel used for callus induction is leaf.**

Keywords: ***Piper betel*, sterilization, callus.**

I. INTRODUCTION

P roclaiming free of infectious diseases in Indonesia is still impossible, due to the high cases of infection suffered by many Indonesian. Common procedure preventing such kind of disease is usage of antibiotics. However, prolonged and uncontrolled uses of antibiotics cause resistance and arising of side effects, hypersensitivity, allergic, and immune system disorders [1]. According to WHO (2008), more than 80% of the world's population believe the effectiveness of herbal traditional remedies [2].

Currently the needs of drug material increase constantly, therefore the research of producing active ingredients methodology, especially in the industrial scale is a challenge. Herbal bioactive components are usually derived from the secondary metabolites and obtained through extraction process. Producing such bioactive components required a lot of raw material and consequently a high cost of plant cultivation. Plant tissue culture technique is an alternative answer to produce bioactive compounds, because it does not require a lot of land for plant cultivation, lots of materials, and the possibility to produce the bioactive compounds continuously [3.4]. However, the crucial step of the plant tissue culture is the explant preparation because each plant requires a typical sterilization process for the callus formation. There is no a general sterilization method applicable for all kind of plants.

Red betel (*Piper crocatum*) is a plant possessing potential as a multifunctional drug, such as antimicrobial, hepatitis, tuberculosis, diabetes mellitus, gout, kidney stones, cholesterol, and high blood pressure [5.6]. Alkaloid contents of the red betel play an important role in the healing process of such diseases. According to the backgrounds mentioned above, herein we report the influence of sterilization method and variation of concentration of 2,4-D as growth hormone for callus induction and subsequently their secondary metabolites contents.

II. MATERIALS AND METHODS

A. Plant Material.

The red betel plant was purchased in the flower market in Surabaya and determined by the Department of Biology, Faculty of Science and technology, Airlangga University – Indonesia.

B. Medium Culture

The medium culture used in this research was made according to Murashige & Skoog (1962). The pH of the medium was 5.5, and was adjusted by adding of HCl or KOH. Subsequently different concentration of 2,4-D was added as growth hormone in medium solidified with agar.

C. Explants Preparation and sterilization methods

The explants used in this research were from the leaves, stalks, and the stem of red betel. The explants were first cleaned, soaked in the detergent solution, and then rinse with running water until free of detergent. The explants were then sterilized in three different sterilization methods, that were soaked in 10% sodium hypochlorite solution for 10 min (method A), soaked in 70% ethanol for 6 minutes and then 2% sodium hypochlorite solution for 10 minutes (method B), and soaked successively in 70% ethanol for 10 minutes and 2% sodium hypochlorite solution for 10 minutes (method C). It was then rinsed with sterile aquadest three times for 10 minutes. This step was performed in sterile laminar air flow cabinet.

D. Callus Formation

The planting of the explants was carried out under aseptic condition in a sterile laminar air flow cabinet. About 10 ml of medium culture was poured into a sterile bottle, closed tightly and then sterilized using autoclave (1 atm, 121^0C, 15 minutes), then cooled until room temperature. Subsequently the explants were planted in the medium culture for 4 weeks, observed of their color, texture, and the weight of the formed callus. The variations of the medium formulation are as followed: medium I: free hormone MS medium (K0); medium II: MS + 0.5 ppm 2,4-D (K1); medium III: MS + 1 ppm 2,4-D (K2); medium IV: MS + 1.5 ppm 2,4-D (K3); medium V: MS + 2 ppm 2,4-D (K4); medium VI: MS + 2.5 ppm 2,4-D (K5). The test was replicated six times for each treatment.

III. RESULTS AND DISCUSSION

A. Optimization of explant Sterilization Method

Explants sterilization can be carried out whether mechanically or chemically [7]. Chemical sterilization is appropriate to be applied for soft explants, such as red betel. Therefore we used chemical sterilization method in this research. To determine the suitable sterilization method for the callus formation of the red betel, various combination of surface sterilizing agents and duration /time of sterilization were carried out. The results of the influence of sterilization method were displayed in Table 1.

TABLE 1.
THE EFFECT OF VARIOUS STERILIZATION METHOD ON VARIOUS EXPLANTS OF RED BETEL.

Sterilization method	Leaves			Stems			Petiols		
	Treatment	Sterile explant (%)	contamination	Treatment	Sterile explant (%)	contamination	Treatment	Sterile explant (%)	contamination
A	K0	50	fungi	K0	0	fungi	K0	0	Fungi
	K1	50	fungi	K1	0	fungi	K1	0	Fungi
	K2	0	fungi	K2	0	fungi	K2	0	Fungi
	K3	0	fungi	K3	0	fungi	K3	0	Fungi
	K4	0	fungi	K4	0	fungi	K4	0	Fungi
	K5	0	fungi	K5	0	fungi	K5	0	Fungi
B	K0	100	----	K0	0	fungi	K0	0	Fungi
	K1	100	----	K1	0	Bacteria, fungi	K1	0	Fungi
	K2	100	----	K2	0	fungi	K2	33.33	Fungi
	K3	100	----	K3	0	fungi	K3	0	Fungi
	K4	100	----	K4	0	fungi	K4	50	Fungi
	K5	100	----	K5	0	fungi	K5	16.67	Fungi
C	K0	100	----	K0	100	----	K0	0	----
	K1	100	----	K1	100	----	K1	0	----
	K2	100	----	K2	100	----	K2	0	----
	K3	100	----	K3	100	----	K3	0	----
	K4	100	----	K4	100	----	K4	0	----
	K5	100	----	K5	100	----	K5	0	----

Sterilization method	Treatment	Sterile explant (%)		
		Leaves	Stems	Petiols
A	K0	50	0	0
	K1	50	0	0
	K2	0	0	0
	K3	0	0	0
	K4	0	0	0
	K5	0	0	0
B	K0	100	0	0
	K1	100	0	0
	K2	100	0	33.33
	K3	100	0	0
	K4	100	0	50
	K5	100	0	16.67
C	K0	100	100	0
	K1	100	100	0
	K2	100	100	0
	K3	100	100	0
	K4	100	100	0
	K5	100	100	0

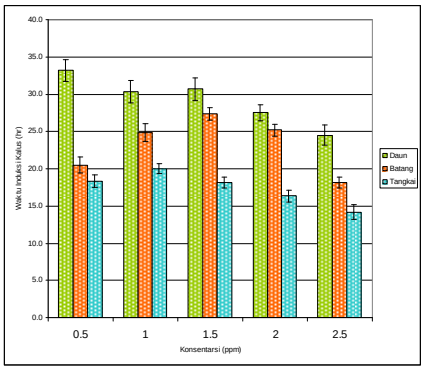
The results showed that 10% sodium hypochlorite solution alone as sterilizing agent was not effective to reduce contamination, whereas combination of ethanol and sodium hypochlorite successively as sterilizing agent in the different soaking time greatly reduced contamination. The dominant contaminants were fungi.

B. Callus induction

Callus is a mass of undifferentiated cells formed by continuous cell division and triggered by plant growth regulators [8]. The various explants of red betel growing in the MS medium added by 2,4-D in various concentration were able to induce the cell proliferation leading to callus formation.

On leaves explants, the initial response such as curving and wrinkle of the explants was observed on the fourth day after the planting (Fig 1).The fastest time for callus induction was achieved in 24.5 days for leaf, 18.2 days for stem, and 14.2 days for stalk explants growing in MS medium with the addition of 2,4-D of 2.5 ppm (Fig. 2). The resulting callus was white brownish with compact texture. However, explants grew in the MS medium without 2,4-D addition, no callus induction was observed (Fig 4). This evidence is in accordance with the statement that the presence of growth regulator in plant tissue culture plays a very important role [7.9]. Moreover, the observations indicated that callus induction time are varied for leaf, stem, and stalk respectively. Beside the presence of plant growth regulator, the callus induction depends also from the age physiology of the tissue, the season of the plant was isolated, parts of the plant used, and the type of the plant. That concentration of 2,4-D of 2.5 ppm were able to induce callus formation of patchouli leaves within 13.33 days [10]. The presence of growth regulator in plant tissue culture plays a very important role [7.9].

Figure 1. Morphology of red betel leaf explants on MS medium + 2,4-D 2.5 ppm on the 4th day after planting.



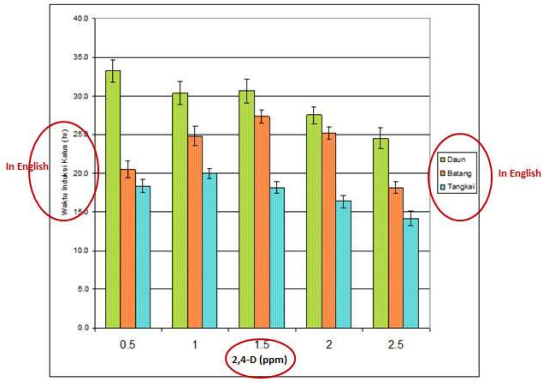


Figure 2. Callus induction on leaves, stems and petiols explants of Red betel at various 2.4-D concentrations.

Figure 3. Morphology of red betel leaf callus on MS medium + 2.4-D 2.5 ppm aged 8 week

C. Fresh weight of callus

Growth is a universal concept in biology and is a result of the integration of various biochemical reactions, biophysical events, physiological processes, and interaction of plant body with external factors [11]. The parameters which is usually used to determine growth are plant height, stem diameter, leaf width or fresh mass of plants. In this study, the parameter used to determine the growth is the fresh weight of callus [12].

Various concentration of growth regulator 2,4-D gave different responses in callus fresh weight (Figure 4). Based on observations, leaf explants produced the highest fresh weight of callus compared to the explants from stem and stalk, and it shows that each kind of explants response in different rate to the growth regulator. That the fresh weight of *Catharanthus roseus* callus growing in MS medium containing 2 ppm of 2,4-D was 1.06 g [13] Our study resulted that the highest callus fresh weight was achieved by leaf explants grown in MS medium containing 2 ppm 2,4-D.

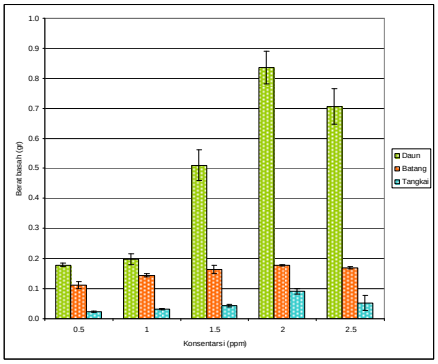


Figure 4. Fresh weight of callus of leaves, stems, and stalks explants at various concentrations of 2.4-D.

IV.CONCLUSION

From the study, it can be concluded that all kind of explants are able to form callus, which is the highest fresh weight was produced from leaf explants. Callus produced from in vitro culture can be used as an important alternative source of secondary metabolites.

ACKNOWLEDGEMENT

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REFFERENCES

[1] E.C. Schinor, M.J Salvador, I.Y Ito, and D.A Dias. 2007. Evaluation of The Antimicrobial of Crude Extracts and Isolated Constituens from Chresta scapigera. *Brazilian Journal of Microbiology. Vo. 38. p: 145-149*

[2] Misra. 2009. Antibacterial Activity of Ethanol Extract of Andrographis panniculata.*Indian J. Pharm Sci 71: 436-438*

[3] B Deus and MH Zenk. 1982. Exploitation of Plant Cell for the Production of Natural Compounds. *Biotechnol Bioeng 24:1965-1974*

[4] A Stafford, P Morris, and MW Fowler. 1986. Plant Cell Biotechnology: A Perspective. *Enzym Microbial Tech 8: 578-597*

[5] M Safithri dan F Fahma. 2008. Potency of *Piper crocatum* Decoction as an Antihiperglycemia in Rat Strain Sprague dawley. *Hayati Journal of Biosciences. P 45-48*

[6] F Zubier, K Mramono, S Widaty, H Nilasari, M Loiuisa, dan R Y osana. 2010. Efikasi Sabun Ekstrak Sirih Merah Dalam Mengurangi Gejala Keputihan Fisiologis. *Majalah Kedokteran Vol 60. Nol 1*

[7] EF George, MA Hall. and G Jan De Klerk 2008. *Plant Propagation by Tissue Culture.* 3 rd Edition. Springer Dodrecht. The Netherlannds.

[8] DE Evans, C JOD oleman, and A Kearns. 2003. *Plant cell Culture.* Bios Scientific Publishers. New York.

[9] Zulkarnain. 2009. *Kultur Jaringan*. Bumi Aksara. Jakarta
[10] S Amin, M Ariyanti, A M rief, dan D.Kurniawan 2007. Induksi Kalus dari Daun Nilam Kultivar Lhoksemauwe, Sidikalang, dan Tapak Tuan dengan 2,4D. *Zuriat Vol 18. No.2*
[11]Stern. 2003. *Introductory Plant Biology*. Mc Graw Hill Companies
[12] FB Salisbury dan CW Ross. 1995. *Fisiologi Tumbuhan*. Edisi III. ITB Bandung
[13] D Pandiangan dan N Nainggolan. 2006. Respon Pertumbuhan dan Kandungan Katarantin Kalus *Catharanthus roseus* Yang Diberi Asam 2,4-Diklorofenoksi Asetat. *Eugenia 12(8): 184-195*\

"BORNEO PRIMA", MANDARIN CULTIVAR FOR LOW AND WET AREA FROM KALIMANTAN INDONESIA

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***ABSTRACT*--In Indonesia, most of the mandarin cultivar (*Citrus reticulata*, Blanco) with orange color developed grows at high land areas of 700 - 1200 m above the sea level. Trees grown under this altitude force the rind fruit color change to green-yellowish with more pale flesh. Evaluation to five selected trees has been conducted in order to choose the best one based on the horticulture performances. The selected mother tree then were freed from Huang long Bin pathogen, Citrus Tristeza Virus, and other viruses by shoot-tip grafting followed by indexing. In 2014, 3 years after the viruses free planting material were produced, more than 200.000 trees or 400 ha have been planted by citrus growers at regency of Kutim, Kukar, Paser, Nunukan, Bulungan and Berau in province of East Kalimantan/Borneo where this mandarin cultivar was found, and it will reach 4000 ha at 2015. "Borneo Prima" or Borneo Best, the promising mandarin variety which will be become a mascot of East Kalimantan has round shaped fruit, yellow to orange color rind, easily pealing and good balance sweet and sour taste, and it's well adapted to low and wet areas. The crop requirement, agro ecological zones and its related to the other selected cultivars of Indonesian mandarin by finger printing (DNA-test) were discussed in this paper.**

Keywords : ***Citrus reticulata*, selection, description, low and wet areas, agro ecological zones, developing area**

I. INTRODUCTION

Indonesia is rich in varieties of citrus with local names, both of which endogenous or introductive varieties which have been already well adapted in the local agro-climate, such as Siam Pontianak (West Kalimantan), Siam Medan (North Sumatra), Keprok SoE (East Nusa Tenggara), Pamelo Nambangan (East Java), lime Borneo (East Kalimantan) and many other types which each has its own superiority. Almost 85% of citrus plantation in Indonesia are Siam cultivar (tangerine) while for mandarin cultivar only around 10% and the rest are pummelo (*Citrus grandis*), lime (*Citrus aurantifolia*), *Citrus hystrix* and others. In 2009, citrus fruit production in Indonesia reached 2,131,765 tons from 60,190 ha of productive orange trees [1].

The productivity is fluctuated because of climate changes and disease incidence mainly caused by Huang Long Bin desease in some centre area production. Import of citrus fruit especially of mandarin group tent to increase yearly and in 2010, it reached 160.255 ton with valued USS 143.392.444. The important citrus imported countries are China, Australia, Thailand, Pakistan, United State, South Africa, and others from South America.

For the last five years, Department of Agriculture has a program of developing mandarin with yellow-orange color rind to imported substitute. In Indonesia, most of the mandarin cultivars with best color performance found at the high area with altitude of more than 700 m above the sea level and dry climate. There are view number of mandarin cultivars with yellow-orange rind grown in low and dry land area for example mandarin of Tejakula in north of Bali, Selayar mandarin from Selayar island of South Sulawesi, and Madura from Madura island of East Java. The present of Borneo Prima, a mandarin cultivar which well adapted to low and wet area give a promising to citrus growers for developing this mandarin to a larger area in Kalimantan.

II. MATERIAL AND METHODS

A. Mother tree selection and description

Borneo Prima mandarin plants discovered by accident in the citrus orchard belong to grower namely Sarmin garden in the village of Tanjung Labu, Rantau Pulung district, East Kutai regency, and province of East Kalimantan. This location lies in the coal mining area named PT Kaltim Prima Coal (KPC). Based on the information from the owner and field officers of local agriculture, tree health, and horticultural performance at field, ten trees was chosen for further evaluation. The evaluation based on the tree horticultural performance, productivity and fruit quality components, shown that tree numbered 2 has been designated as a mother tree of Keprok Borneo Prima. Borneo Prima means Borneo Best, and Borneo is another name of Kalimantan, it is a big and famous island of "orangutans" bordered to Serawak and Sabah, Malaysia.

Tree architecture of Borneo Prima is not different to other mandarin cultivar trees. Fruit shape is oblate, yellow-orange color and thick rind, good aroma, smooth fruit texture, and delicious. Like other mandarin cultivars, this Borneo Prima is also susceptible to Citrus Virus Tristeza (CTV) and Huang Long Bin disease. Because it has thicker fruit skin, the fruit flies like them. The agro ecological zone of the site where a mother tree of Borneo Prima found is AIVx2 - low and wet area good for woody plants. The yearly pattern of rainfall, humidity, maximum and minimum temperature of climatologically station closed to the mother tree found.

The climatologically station at coal mining location is closer to the site of mother tree of Borneo Prima to

those of Tanjung Bara. Based on result, the pattern of yearly rainfall in that location is tropical type. Rainy comes to September and it's increasing till December then slowly decreasing to August. The humidity for all the year is relatively high and constant to 75-80%. Temperature at district of Rantau Pulung is high with maximum temperature reached 36-38º C and 21-22º C for minimum temperature. The interesting one is the amplitude or the different between maximum and minimum temperature is high enough and it reached more than 11ºC caused the bright color develop well. It looks that this temperature condition which made Borneo Prima mandarin trees produced an attractive fruit rind color.

As mention above, Indonesia has a lot number of good local mandarin varieties planted spread to the whole country especially in high land area; and it has only less than three lowland mandarin varieties as Tejakula, Selayar and Sioumpu. In order to understanding the genetic relationship between Borneo Prima and others famous mandarin varieties, fingerprinting or DNA-test were conducted by Polymerase Chain Reaction (PCR) based and it used *Random Amplified Polymorphic DNA* (RAPD)[2,3,4]. Detection of DNA band was conducted by bio documentation system, and the genetic relationship among the mandarin varieties was analyzed by dendrogram.

Primer OPA17 was able to identify sequences homologous to the germ plasma has been identified and reproduce homologous loci sequences on different intensity and frequency. Based on the DNA bands pattern produced, it shown that the primer OPA17 RAPD has been amplified to most of the citrus genome with varying number of bands (polymorphic) among the varieties.

III. RESULT AND DISCUSSION

A. Related to Other Mandarin Varieties

Mandarin Borneo Prima has different band pattern of DNA (loci) to those of SoE , Ponkan, Tejakula, Selayar, Pulung, Batu55 mandarin. Borneo Prima has no locus on the size of 600 bp, while other types of mandarins have it. Mandarin of Borneo Prima and Selayar screen has almost the same banding pattern of DNA except at the size of 600 bp, while other varieties showed variations in the DNA bands except Keprok Garut and Batu55 show mono morfis pattern. Borneo Prima and Selayar mandarin has a difference of about 15 %, while Batu 55, Pulung, and Garut has a closer relationship with differences of 5-10%. All mandarins analyzed have 40-90 % similar.

The technology of molecular markers (DNA) capable of identifying common plant DNA from one genera, species and inter-species, and can be used to create genetic identity, diagnosis heterogeneity, and to ensure or maintain the propagation plant material in many variations. Polymorphism detected by RAPD marker (and RFLP) are caused mainly by differences in nucleotide sequence of base substitution or deletion / insertion. Amplification results showed that all varieties of citrus orange genotype selection based on RAPD markers can indicate the true to types of the proposal of new genotypes of a variety as it has been done on pummelo[5], tangerine[6] and Apple[7] and identification of plant propagation material derivation[8].

B. Producing of citrus nursery stockand areas for developing

After selected as a mother tree of Borneo Prima mandarin, some budded tree from selected tree were brought to the Tissue Culture laboratory at Citrus and Sub tropical Fruit Research Institute in Batu, East Java. The plants then were freed from viruses by shoot-tip grafting method followed by indexing of HLB, CTV, CVEV, CEV, CPsV, and CTlV. The viruses free of mother tree of Borneo Prima then propagated and planted under insect proof screen houses for Foundation Blocks and Multiplication Blocks before the bud sticks come to the citrus nurseries. Based on this national scheme for producing certificate citrus nursery stocks, since last 3 years , the nurserymen at East Kalimantan have been produced more than 200.000 stocks covered 400 ha of citrus area and planted to the six regencies of East Kalimantan those were Kutim, Kukar, Paser, Nunukan, Bulungan and Berau[9, 10].

Actually all areas with agro ecological zone of IVax2 are suitable for developing of Borneo Prima mandarin, not only for area located in East Kalimantan but also the whole area of this country. Area with amplitude or has different maximum and minimum temperature more than 10º C were preferred in order to develop the yellow-orange color rind and better fruit quality. The provincial government has a program to develop Borneo Prima mandarin to up 4000 ha at the year of 2015 and establish new screen house and improve existing facilities to produce such number of nursery stocks needed by citrus growers in East Kalimantan.

IV. CONCLUSION

Borneo Prima or Borneo Best is a mandarin for low and wet area found in village of Tanjung Labu, district of Rantau Pulung, Regency of East Kutai, East Kalimantan, Indonesia. It produce yellow-orange color fruit rind, good texture and delicious. It will give better performance if they planted an area with amplitude more than 10º C. This good promising variety will planted by citrus growers guided from provincial government of East Kalimantan to more than 6000 ha at 2015 for import substitute goal.

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REFERENCES

[1] BPS, 2010. Produksi buah-buah Indonesia. http://www.bps (27 Oktober 2011)

[2] Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18, 6531-6535.

[3] Santos, JB Dos, J. Nienhuis, P. Skroch, J. Tivang, M.K. Slocum. 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among Brassica oleracea L. genotypes. Theor Appl Genet 87 : 909–915

[4] Rajapakse S, L.E. Beltho, G. He, A.E. Estager, R. Scorza, I. Verde, R.E. Ballard, W.V. Baird, A. Callahan, R. Monet, A.G. Abbott. 1995. Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90 : 503–510

[5] Agisimanto, D dan A. Supriyanto. 2007. Keragaman Genetika Pamelo Indonesia Berdasarkan Primer Random Amplified Polymorphic DNA. Diusulkan untuk diterbitkan pada Jurnal Hortikultura

[6] Agisimanto, D., C. Martasari, dan A. Supriyanto. 2007. Perbedaan Primer RAPD dan ISSR dalam Identifikasi Hubungan Kekerabatan Genetik Jeruk Siam (*Citrus suhuniensis* L. Tan) Indonesia. Diusulkan untuk diterbitkan pada Jurnal Hortikultura

[7] Supriyanto, A., D. Agisimanto, T. Purbiati, N.F. Devy, M.E. Dwiastuti. 2006. Genotype analysis of vegetative propagated of Citrus using RAPD Primers. Journal of Horticulture Vol. 16. No.1. (ISSN 0853-7097)

[8] Anonymous, 2010. Profil Pengembangan Kawasan Hortikultura Kalimantan Timur. Dinas Pertanian Tanaman Pangan Kalimantan Timur. 51p.

[9] Anonymous, 2008. Road Map and Rancang Bangun Pengembangan Kawasan Hortikultura,Kabupaten Berau, Kalimantan Timur. Dinas Pertanian Kalimantan Timur. 30p

PUERARIN INHIBIT VASCULAR CELLULAR ADHESION MOLECULE-1(VCAM-1) AND TUMOR NECROSIS FACTOR-A (TNF-A) EXPRESSION ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECS) AFTER INDUCED BY LEPTIN *IN VITRO*

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***Abstract*— Peurarin is isoflavonoid compound that has a role as anti-inflammatory agent. VCAM-1 and TNF-α are inflamatory cytocines. Leptin is 16 kD polypeptide, it was secreted from adipose cells and has potential role on macrophage accumulation. Determination of puerarin to inhibited VCAM-1 and TNF-α Of HUVECs after treated by Leptin. The HUVECs were divided into two group that were (1) induced by leptin 0 ng/ml for 6 hours then incubated with puerarin (0.5, 25, 200, 525 □M) for following 6 hours. The immunocytochemistry were employed by mouse monoclonal antigen antibody VCAM-1 as primer antibody and anti-mouse VCAM-1 as secondary antibody. The TNF-α concentration were applied by using human TNF-α ELISA kit, TNF-α was read by ELISA reader based on cell endothelial culture medium. The data were analyzed by one-way ANOVA test and followed by Tukey test. The result showed that leptin 25 ng/ml were giving positive impact to increased the VCAM-1 expression (2.68±0.15)% compared to leptin 0 ng/ml (0.54±0.15)% and peurarin 200 and 525 □Minhibited the TNF-α concentration.**

***Keywords*— HUVECS, Leptin, Peurarin, TNF-α, VCAM-1**

I. INTRODUCTION

Endothelial cells are the largest cells tissues that construct human body, endothelial cells are involve in many kind of physiological system, such as cardiovascular, endocrine, immune systems, etc. Injured endothelial will be giving inflammation occurred and physiological dysfunction [1]. Human Umbilical Vein Endothelial Cells(HUVECs) are one of the established cell culture that useful for supporting system for in vitro study on physiological aspect for understanding many cellular mechanism.

Pueraria lobata is one of plants that has known containing potential-isoflavon agent. Comparing with soybean and more other 68 leguminous, Pueraria lobata is the highest isoflavon producer. The isoflavon is present in root of pueraria lobata, that are: puerarin, daidzin, formonetin, as well as glucosidals C and O and others. That compounds were related to antioxidants and others pharmacological effects [2-6]. Peurarin is active compound that was call isoflavonoid fitoestrogen. It is isomer from derivates of polyphenolic antioxidant. The compound has scavenging activity character's, so it uble to inhibit inflammation and endothelial dysfunction trough inhibition of NF-κB activity pathway [7-9]. Peurarin as antioxidant has two function that are: primer antioxidant, as atomic hydrogen donor, this compound will be quickly giving hydrogen atom to lipid radicals compound (R*,ROO*) or convert to more stable compound. Secondary antioxidant is inhibition of auto-oxidation rate [10].

Leptin is chemical compound which is believed stimulating inflammation and endothelial dysfunction, it was secreted from adipose tissue. It is known that Leptin was inducting reactive oxygen species (ROS) as mitochondrial super-oxide anion which was produced by endothelial cells through increasing fatty acid oxidation[11,12].

It is present two kind of vascular cellular adhesion molecule-1(VCAM-1) that are VCAM-1 and VCAM 1b which is classified on endothelial cell adhesion molecule-1 (ELAM-1), HUVECs were expressed the VCAM-1 through NF-κB activity pathway under suppressed of ROS. However, ROS accumulation will be activating transcription factor of NF-κB, this transcription factor will be stimulating anti-inflammatory cytokines such as tumor necrosis factor-α (TNF-α). So that, HUVECs which were exposed by leptin will be giving positive impact to increasing the expression of VCAM-1 and TNF-α and it will be inhibited expressed after peurarin treatment.

II. MATERIAL AND METHODS

The experiment had been done on July 2007 to January 2008 in Physiology and biomedical laboratory, Faculty of medicine and animal cell Culture laboratory Faculty of Science Brawijaya University. The umbilicus

tissues were resulted from Caesar surgery patient from SaifulAnwarHospital. There were isolated with cord solution, PBSA and collagenase method [13]. The endothelial cell were centrifuge 1000 rpm/minutes for 8 minutes, resuspended on culture medium, then transfer to 0.2% gelatinized 24 well culture plate and incubated on CO_2% with 37^oC and maximum humidity for 30 minutes, it was HUVECs. The confluent HUVECs were washed with serum free media and medium exchange. Then HUVECs were exposed with 0ng/ml and 25 ng/ml of Leptin for 6 hours. The HUVECs were washed then treated with 0, 5µM, 25µM, 200µM, and 525µM of peurarin for 6 hours.

The HUVECs were washed on PBS pH 7.4 tree times and fixated on absolute methanol 10 % (v/v) for 20 minutes, then washed on PBS pH 7.4 tree times for 5 minutes. HUVECs were dropped by sodium azide 0.02% (w/v), and then washed 5 minutes tree times. The HUVECs were dropped with H2O2 on PBS solution for 10 minutes. Then blocking with 5% FBS containing 0.25% Triton X for 1 hour. HUVECs were washed on PBS, and then incubated primer antibody on serum 1: 2000 (mouse Monoclonal VCAM-1) for 24 hours on 4 ^oC.HUVECs were transfer to room temperature then washed tree times, and then dropped with SA-HRP for 40 minutes, and treated with cromogen DAB (3,3 Diaminobenzenedine tetrahydrocloride), counterstaining with mayer hematoxilen for 10 minutes. The HUVECs were washed by water and aquadest respectively, finally the HUVECs were observed under microscope inverted. The VCAM-1 expressed cells were counted and each unit of treatment were replicated tree times, The data were analyzed by one-way ANOVA test and followed by Tukey test.

TNF-α concentrations were measured by ELISA method with human TNF-α assay kit [14]. Then absorbance standard and sample values were read by ELISA readers on OD 492 nm. TNF-a sample concentration value were statically analysed by one-way ANOVA test and followed by Tukey test.

III. RESULT AND DISCUSSIONS

Base on the result of the experiment the expression of VCAM-1 showed that HUVECs were not exposed by leptin (0 ng/ml) and peurarin (0 µM) were expressed 0.54% VCAM-1, and treated 5, 25, 200, and 525 mM peurarin were expressed 0.92%, 1.23%, 0.77%, and 0.85% respectively. The result showed that VCAM-1 were expressed tend to increase after peurarin treatment (see table 1).

TABLE 1:
VCAM-1 EXPRESSED HUVECS FOLLOWING LEPTIN AND PEURARIN INDUCTION.

Dose of Puerarin (µM)	VCAM-1 expressed cells (%)	
	Leptin 0 ng/mL	Leptin 25 ng/mL
0	$0,54 \pm 0,15^{a}$	$2,86 \pm 0,15^{d}$
5	$0,92 \pm 0,40^{ab}$	$1,92 \pm 0,05^{bcd}$
25	$1,23 \pm 0,38^{abc}$	$2,12 \pm 0,26^{cd}$
200	$0,77 \pm 0,36^{a}$	$2,08 \pm 0,25^{cd}$
525	$0,85 \pm 0,34^{a}$	$2,22 \pm 0,69^{cd}$

Statistical analyzed were showed that leptin were giving significant impact for VCAM-1 expression ($p<0.05$), it was indicated that endothelial cells were expressed VCAM-1after treated with leptin. [15] explained that 25 ng/ml of leptin will be affecting to endothelial cell culture. This statement also supported by Boulomie [11] who said that endothelial cells treated with leptin will stimulate the increasing of ROS, then ROS were affecting cellular dysfunction through activating transcription factor NF-κB cytokine [16].

The result showed that the effect of peurarin on endothelial cells after 25 ng/ml leptin induction were decreasing of VCAM-1 expression, VCAM-1 is biomarker for endothelial dysfunction. Leptin is cytokine secreted from adipose tissue, in high concentration leptin giving significant impact to produce ROS on endothelial cells. ROS through ERK1/2 activation will inducted CRP. CRP will be induce VCAM-1 expression through protein-kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), tyrosin kinase and NFkB [17].

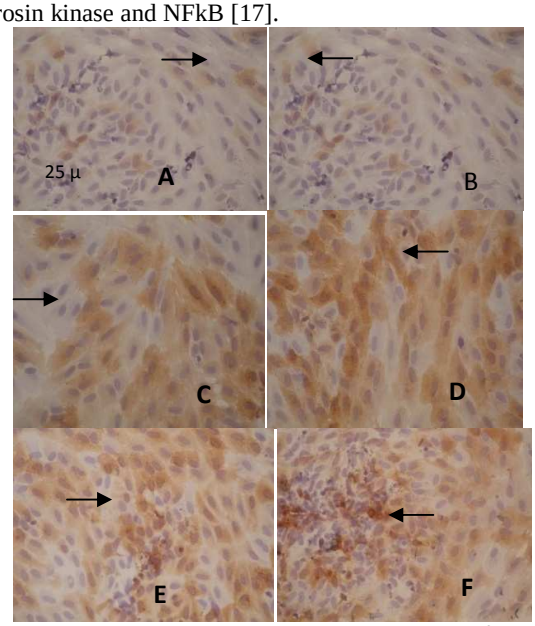

Fig 1: Immunocytochemistry VCAM-1 expression (magnification 400X) with different treatment: (A. Leptin 0ng/ml+ peurarin 0 µM; B Leptin 25 ng/ml; C.Leptin 25 ng/mL+ puerarin 5µM; D.Leptin 25 ng/ml+ puerarin 25µM; Leptin 25E.Leptin 25 ng/ml+ puerarin 200 µM; F .Leptin 25 ng/ml + puerarin 525 µM. VCAM-1 expression marked by arrow).

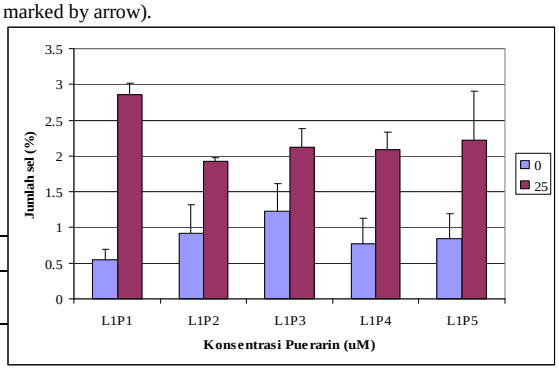

Figure 2: The effect of puerarin n VCAM-1 expression

The result showed that The HUVECs have been expressed VCAM-1 tend to decreased on puerarin 5µM treatment as compare with 25 µM, it was indicated that CRP half-life at about 20 hours [18] So that CRP have

not activated formation NF-κB yet further adation of puerarin. However, leptin treatment giving positive to VCAM-1 expression. [11] axplained that the expression of VCAM-1 will be presence at the time of 30 minutes on leptin 10 ng/ml. But the result showed that puerarin treatment were not significantly affected to decreasing the expression of VCAM-1 ($p>0.05$). The result also indicate that the puerarin will be giving positive impact on NF-κB after 24 hours exposure [19]

The result showed that all puerarin treatment to HUVECs culture were affected to decreased to TNF-α concentration. Base on data's, it was showed that Th HUVECs without treatment the concentration of TNF-α were 58.328±28.6 pg/ml, and exposed by puerarin 5;25; 200; 525 µM were35.114 ± 8.556; 16.614 ± 1.198; 20.9 ± 7.142 and36.115 ± 2.71 pg/mL respectively. These datas were nor significantly different ($p>0.05$).

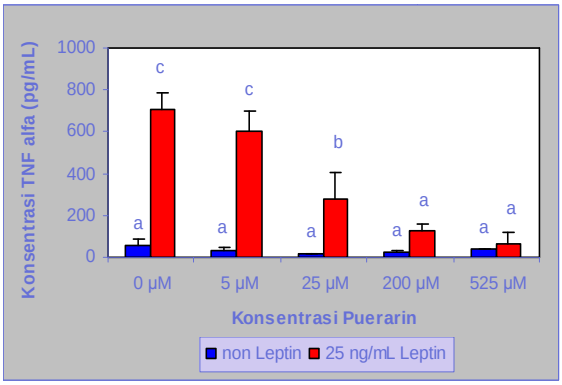

Figure 3: The effect of puerarin on TNF-a expression in HUVECs.

The research result showed that puerarin were affecting to inhibited TNF-a expression after the HUVECs were exposed by leptin. It was indicated that puerarin has potential role as agent to protected the endothelial cells destruction causing by ROS or other free radicals.

REFERENCES

[1] Silvestro, A., Olivia G dan Brevetti G. 2002. Intermitten Claudication and endothelial Dysfunction. *J. Immunology*

[2] Gao, G.Y., D.J. Li dan W. M. Keung. 2003. Synthesis of Daidzin Analogues as Potential Agents for Alcohol Abuse. *Boorg. Med. Chem*. (11): 4069-4081.

[3] Mazur, W. 1998. Phytoestrogens in food- In Bailliere's Clinical Endocrinology and metabolics: Phytoestrogens. *Bailliere Tindall* (12): 98-112.

[4] Meezan, E., Elizabeth M. Meezan., K. Jones., R. Moore., S. Barnes dan Jeevan K. Prasain. 2005. Contrasting Effects of Puerarin and Daidzin on Glucose Homeostasis in Mice. *J. Agric Food Chem.* (53): 8760-8767.

[5] Prasain, J.K., K. Jones., N. Brissi., R. Moore., J.M. Wyss dan S. Barnes. 2004. Identification of Puerarin and its Metabolites in rats by Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrofotometry. *J. Agric. Food Chem.* 2004, 52: 3708-3712.

[6] Zhu, J.H., X.X. Wang., J.Z. Chen., Y.P. Shang., X.G. Guo dan J. Sun. 2004. Effect of Puerarin on Number and Activity of Endothelial Progenitor Cells from Peripheral Blood. *Acta Pharmacol. Sin* (22): 1045-1051.

[7]. Altavilla, D., Antonino Saitta., Mariarosaria Galeano dan Giovanni Squadrito. 2001. The Phytoestrogen α-Zearalenol Reverses Endothelial Dysfunction Induced by Oophorectomy in Rats. *Lab Invest*, 81:125–132.

[8] Xu, M.E., Xiau S.Z., Sun Y.H., Zheng X.X., Qu-Yang dan Guan C. 2005. Abstract of The Study of Anti-metabolic Syndrome Effect of Puerarin in Vitro. *Life Sci* 4; 7(25): 3183-3196.

[9] Trilaksani, W. 2003. Antioksidan:Jenis, Sumber, Mekanisme Kerja dan Peran terhadap Kesehatan. *Term Paper Intoductory Science Philosophy (PPS702).* IPB. Bogor.

[10] Boulomie, A., H. C. A. Dexler., M. Lavontan dan R. Busse. 1999. Leptin the Product of *Ob* gene, promotes angiogenesis. *Circ. Research* (70): 593-599.

[11] Boulomie, A., Takeshi Marumo., Max Lavontan dan Rudi Busse. 1999. Leptin Induces Oxidatives Stress in Human Endothelial Cells. *FASEB J.* (13): 1231-1238.

[12] Packard, R. R. S dan Peter Libby. 2008. Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction. *Clinical Chemistry* 54(1):24-38.

[13] Assay Design, Inc. 2006. Human TNF-α Enzyme Immunometric Assay Kit. www.assaydesign.com. Tanggal akses 6 Mei 2007.

[14] Khotimah, H. 2003. Pengaruh Vitamin E dan Vitamin C terhadap Release Endothelial Derived Relaxing Factor (REDR) Kadar MDA dan Kepadatan Sel Endotel HUVECs yang dipapar Glukosa Tinggi. Tesis Program Pasca Sarjana. Universitas Brawijaya. Malang.

[15] Brian R., BR. Clapp., GM.Hirschfield. 2005. Inflammation and Endothelial Function Direct Vascular Effects of Human C-Reactive Protein on Nitric Oxide Bioavailability. Circulation 111:530-1536.

[16] Kawanami D., K. Maemura., N.Takeda., T. Harada., T.Nojiri., T.Saito., I.Manabe., Y.Imae., R.Nagai. 2007. C-reactive protein induces VCAM-1 gene expressionthrough NF-kappaBactivation in vascular endothelialcells. University of Tokyo. Japan.

[17] Ding, M. P., F. Feng., H. T. Hut. 2007. Effect of Puerarin on Expression of Nuclear Factor Kappa B After Cerebral Ischemia/ Reperfusi in Rats. Departement of Medicine, ZhejiangUniversity. China

Sea Holy (*Acanthus ilicifolius*) Viability Test on Heavy Metal: Lead (Pb) and Cadmium (Cd)

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***Abstract*— The development become in increased human activity in various sectors, such as: industrial, residential, agricultural, and other sectors. Human activity in the development that not consider the environmental aspects, impact on improving the quality and quantity of waste that will contaminate various environmental parts. Heavy metal pollution is a serious attention, if absorbed and accumulate in the human body can be disorder to health and cause death. Heavy metals are toxic pollutants in all environmental parts, such as Pb (lead) and Cd (cadmium). Plant species of Acanthus ilicifolius an annual shrub, woody, single leafy, spiny, flowering compound, fruit ovoid, contain four seed each fruit. Distribution in the tropics, especially Indonesia, often found along the edge of the river with the sea, and in the mangroves near the coast. Can grow to 500 m above sea level. The study aims to determine the viaability of *Acanthus ilicifolius* on heavy metals (Pb and Cd). Research conducted at the laboratory scale in greenhouse of Environmental Engineering - ITS. Methods with range finding test use three replications, observation gradually over two weeks to determine the letal concentrations of heavy metal. Result showed that *Acanthus ilicifolius* cant live in concentration for lead (Pb) and cadmium (Cd) is 8000 mg/L and 300 mg/L.**

***Keywords*— Seaholy (*Acanthus ilicifolius*), Lead (Pb), Cadmium (Cd).**

I. INTRODUCTION

HUMAN population growth and development resulted in increased human activity in various sectors development, such as industrial, residential, agricultural, and other sectors. The increasing human activities in various sectors, often increasing the amount of waste pollutants. Wastewater pollutants that enter into the environment can disrupt and harm the environment. The impact of environmental pollution, both directly or indirectly affect the human body health and cause systemic disorder. Pollutants enter the human body through contact with the skin, respiratory and digestive organs. Therefore, if the pollutant is not managed properly will cause environmental pollution, endangering the health and life, both human and other organism.

Heavy metal pollution is a serious attention, because can absorbed and accumulate in the human body can be detrimental to health [1] and in some cases cause death. Heavy metals can accumulate in the environment and moves from one medium to another medium [2]. So heavy metal can contaminants in all environmental media (multi-media pollutant). Heavy metals are not needed by organism because it is toxic such as Pb (lead), and Cd (cadmium). Pb and Cd has a widespread distribution and a major cause of environmental pollution and health problems [3].

The application of science and technology to assess and prepare solutions to environmental problems using plants known as Phytotechnology. Phytotechnology used to expand understanding of the importance of plants and their role in human life and environmental systems. The phytotechnology concept is focused on plants as environmental technology that able to solve environmental problems. In a review of the technology and process, phytotechnology clarify show nature-based approach to environmental problem solving. This is the balance technology between man-made process and natural-plants process, as representation of how the two processes to overcome various environmental problems [4].

Phytotechnology study based on transformation of substances in the ecotoxicological effects. Ecotoxicology have common with the study of environmental toxicology in products such as the negative effects of substances on living organisms and treatment outcome for substance restrictions. However, the difference in the method of exposure substances, size substances, carrier substances and objects organism. So that needs to know that the negative effects of a substance as health preservation and sustainability of life organism [5].

Ecosystem areas that are exposed and become the estuary of waste, especially wastewater pollutants from industrial and urban wastewater that enter the river, and leading to estuary into the sea. The estuary area is a coastal area with mangrove ecosystem. Surabaya city has coastal and marine areas are broad, which rivers and estuary ecosystem which is formed by a mixture of fresh water and sea water with a unique ecosystem and water salinity fluctuates. This ecosystem is called the mangrove ecosystem, which has a flow of water with nutrients important impact on the productivity of coastal areas. However, mangrove areas near to industrial and rural is an area that often polluted from industrial and domestic wastewater.

Acanthus ilicifolius is chosen because this plant live

ini mangrove ecosystem, that make environmental balance, but not many reasearch studies looking at the viability this plant to clear wastewater pollutants. The selection of this species based on locallity or location which is a native species, naturally found in nature and some certain conditions releted in phytotechnology. Therefore, testing the viaability of *Acanthus ilicifolius* to heavy metal contaminants interesting to do. This study aims to determine the concentration of waste pollutant from heavy metals: lead (Pb) and cadmium (Cd), which cause to the death of *Acanthus ilicifolius*. The information generated is expected to be the basis of phytoforensic research and develop knowledge about the potential plants diversity in the environment phytotechnology.

II. METHOD

This reasearch is a preliminary study to trace concentrations of waste pollutants on plants in phytotechnology. The study was conducted on 20 to 27 December 2013 and 7 to 14 January 2014, with laboratory scale at greenhouse and laboratory Environmental Sanitation and Phytotechnology (SILFI/Sanitasi Lingkungan dan Fitoteknologi) Environmental Engineering Department - ITS. *Acanthus ilicifolius* are native plants, natural found in estuary and aquatic plants type emergent that have roots, stems and leaves are clear part. Plants were placed in a cylindrical plastic container with a diameter of 8 cm and a height of 15 cm as shown in Figure 1.

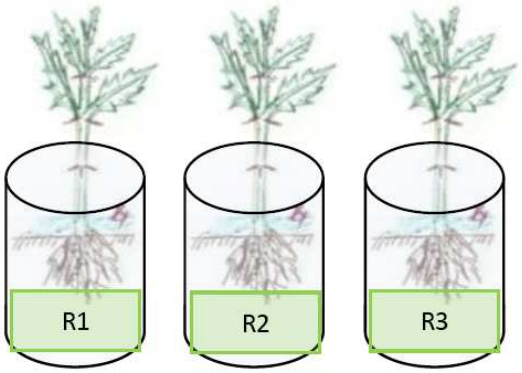

Figure 1. *Acanthus ilicifolius* experimental models in the Range Finding Test (R1= replication first, R2= replication second, R3= replication third).

Planting medium used sand and water, with a quantity of 500 grams sand and 100 ml water, with a 100 ml fertilizer two weeks before exposure. Waste pollutants made artificial such as heavy metals derived from a standard solution of Pb and Cd. Testing with Range Finding Test (RFT) to establish pollutant concentration that causes death in plants. RFT conducted with three replications of two phases, the first phase at a concentration of 500, 1000, 1500, and 2000 mg/L for Pb and Cd, while the second phase at a concentration of 4000, 6000, 8000, and 10,000 mg/L for lead (Pb) and concentration 100, 200, 300, and 400 mg/L for cadmium (Cd). Observations RFT for 7 days (a week) exposure, if not show the changes continued for 7 days (a week) forward. Data obtained and then discussions with outlines and process based on the literature. This are presented in Table and Figure.

III. RESULT AND DISCUSSION

Heavy Metals

Metals are classified as heavy metal when weighing up to 5 grams or more on 1 cm^3, so that it can be said that the heavy metal weighs 5 times greater than the weight of water [3]. According to the characteristics of heavy metals classified as having the specific gravity greater than 4 (Palar, 1994) or 5 [6].

Heavy metals can be divided into two categories. Toxic heavy metal, that heavy metal which is not at all needed in the body of living organism, because in small concentrations already toxic, such as Pb, Cd and Hg; and essential heavy metal, which is a heavy metal that is needed by the body of living organism in limited concentration and generally small, becouse that heavy metal use to growth and development of body cells as well as in biochemical processes. If the needs of a very small amount is not met then it would be fatal for the survival of living organism, but if the amount over can be toxic to the body, such as Cu, Zn, Ni, and Cr.

Lead in scientific language called Plumbum and symbolized by Pb. Pb in the group IVA metal group of chemical elements in the periodic table, has an atomic number 82 with an atomic weight of 207.2 [7]. Metallic bluish gray, with high density, soft metal properties, the active chemical properties that can be used to coat metals and low melting point (327.5°C).

Pb is multi-media pollutant for environmental. Among the toxic heavy metals, Pb have distribution wide and primary cause of environmental pollution as well as health problems [8, 9]. Pb is a heavy metal that physiological not need by plants and animals, and can not be degraded. So remediation of heavy metal contamination is done with physically, chemically and biologically [10].

Pb can enter the plant tissue through the leaf surface and root system. While in animals and humans, Pb entry through food and drink as well as through breathing and skin penetration. Pb accumulation in the human body will cause a variety of health effects, including bone loss, reproductive system damage, brain damage, central nervous system toxicity [11], gives the effect on enzyme activity [1], the synthesis hemoglobin, the nervous system, urinary system, reproductive system, and endocrine system [7].

Cadmium (Cd) is a soft metal, silvery white, has the atomic number 48 with an atomic weight of 112.41, is heat resistant and highly resistant to corrosion. So it is good to mix the manufacture of ceramics, enamel, plastic and coating the iron and steel plate [3]. The use of Cd and chemistry compounds commonly found in the dyeing industry, photographic, photoelectric, photoconductor, and others [12]. In addition it is also widely used in small industry such as processing of bread, fish, food, beverages, textiles and others [7].

Cd including heavy metals are dangerous because high risk for blood vessel. Cd effect on humans in the longterm and can accumulate in the body, especially the liver and kidneys. Similarly with Pb, Cd can cause poisoning chronic and acute, which gives effect to the bones, lungs, blood, heart, and reproductive system [7].

Toxic heavy metals such as Pb and Cd can enter the human body through the digestive tract / gastrointestinal

/ ingestion, lung/inhalasi, skin / other topical and parenteral path [13]. Plants have the ability to absorb heavy metals from the environment [14]. Cd more easier accumulated in plants than Pb. Experiments on the absorption of natural vegetation to Pb and Cd concentration was 8 mg/L [15]. On leaf toxic concentrations of Cd and Pb is 5-30 ppm and 30-300 ppm [8].

According to Regulation Law, Surabaya Act No. 2 Year 2004 on water quality management and water pollution control, East Java Governor Act No. 413 Year 1987 on management and water quality in East Java, Goverment No. 82 Year 2001 on management of water quality and water pollution control, Pb and Cd concentration limits that are accept in the environment is 0.03 mg/L and 0.01 mg/L. While Environmetal Ministry Act No. 51/ Kep-MENLH/1/1995 on standar industrial wastewater, Pb concentrations required 0.1 mg/L.

In fact, in Kenjeran channel Surabaya containing Cd average of 5.89 ppm in sediment and 0.09 ppm on water [16]. In research [17] this channel Cd: 0.02 mg/L in water and 3.80 mg/L in the sediment, while in the estuary 0.03 mg/L on seawater and 0.48 mg/L in sediment as well as 0,21 mg/L in the organism (shellfish).

Plant Acanthus ilicifolius (Seaholy)

This plant call Deruju in local name (Indonesia), with synonyms is *Acanthus volubilis* Wallich (1831). Acanthus plant are herb, erect or reclining shrubby, with mostly perennial herbs. Leaves decussate, simple, undulate to pinnatifid, rarely entire, margins often spiny, rigid, without cystoliths, dark green and shiny; petiole present; stipules absent, but often with a pair of spines from the leaves in the stipular position. Inflorescence a terminal uninterrupted spike. Flowers bisexual, asymmetrical; bracts imbricate, ovate, large; bracteoles in 2 pairs, oblong; calyx 4-partite, lobes imbricate, 2 outer ones larger, corolla tube short, horny, upper lip absent, lower lip elongate, obtusely 3-lobed; stamens 4, slightly didynamous, filaments stout, attached to the corolla throat, anthers 1-celled, linear oblong, bearded along 1 margin; ovules 2 in each loculus, style slender, stigma 2-fid. Fruit a capsule, erect, oblong to ellipsoid. Seeds 4, orbicular, muricate, glandular. Seedling with hypogeal germination. Morphological habitus can be seen in Figure 2.

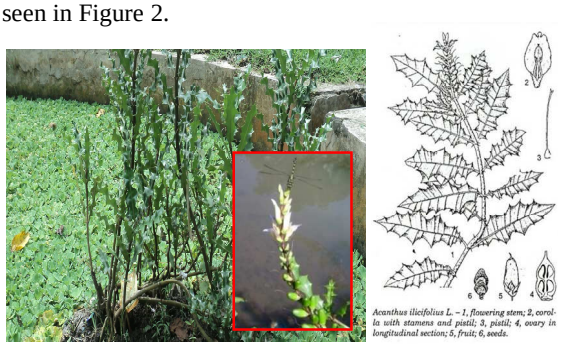

Figure 2. *Acanthus ilicifolius* habitus and illustrations.

Grown in Asia tropic and Africa to northern Australia, often found from southern India and Sri Lanka to Indo-China and Indonesia, especially in Java and Madura. Habitat along the estuary of the river and lake, waterfront, commonly found in soil and mangrove swamps close to the beach. Able to grow to a height 500 m above sea level[18, 19, 20].

Acanthus ilicifolius viability with Pb and Cd

Acanthus ilicifolius know the viability from Range Finding Test (RFT) with done in two stages. RFT first determined the same exposure concentration for Pb and Cd are: 500, 1000, 1500, and 2000 ppm. in 6 days all plant dead for Cd concentration at all, so for the second RFT use concentrations of Cd below 500 ppm. In contrast to Pb concentrations all plant are life, so continued observation for two weeks, and did not any change and death. Therefore the second RFT for Pb concentrations above 2000 ppm.

In the second RFT within 6 days plants dead for Pb concentrations at 8000 and 10,000 ppm, whereas for concentrations Cd at 300 and 400 ppm. Plants that live on Pb and Cd continued observation for two weeks if did not show signs of death. So that the concentration of Pb and Cd deadly to *Acanthus ilicifolius* at 8000 ppm and 300 ppm, more details can be seen in Table 1.

TABLE 1.
THE CONCENTRATION OF PB AND CD EXPOSURE

Concentration	Pb	Cd
0 ppm (control)	0	0
RFT 1		
500 ppm	0	100
1000 ppm	0	100
1500 ppm	0	100
2000 ppm	0	100
RFT 2		
100 ppm	-	0
200 ppm	-	0
300 ppm	-	100
400 ppm	-	100
4000 ppm	0	-
6000 ppm	0	-
8000 ppm	100	-
10000 ppm	100	-

Note: 1 ppm (part per miliar) = 1 mg/L.
- = not test, 0 = life, 100 = death.

Acanthus ilicifolius plant used or test have the same criteria. Derived from vegetative propagation (cuttings) trunk diameter 0.5-0.8 cm, age 40-50 days, 15-20 cm height, root length> 10 cm, and the number of leaves 2-6. Observation of Pb and Cd exposure waste as can be seen in Table 1 *Acanthus ilicifolius* change morphologically. Showed morphological changes to the death of the leaves are not erect, the plant looks weak stem, and leaf discoloration. The changes can be seen in Figure 3.

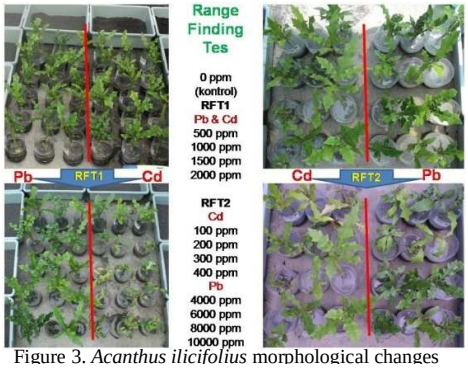

Figure 3. *Acanthus ilicifolius* morphological changes

The morphological changes also supported with observation of high plants and number of leaves that live after a week exposure. Observations on the second RFT, can see in Table 2.

TABLE 2.
THE NUMBER OF LEAVES AND HEIGHT PLANT

Concentration	Test	0 day	6 day
Pb			
4000 ppm	R1	10 cm	16 cm
		6/6 leaf	6/6 leaf
	R2	12 cm	17 cm
		4/6 leaf	6/8 leaf
	R3	15 cm	21 cm
		6/4 leaf	8/6 leaf
6000 ppm	R1	10 cm	16 cm
		6/6 leaf	6/6 leaf
	R2	13 cm	18 cm
		6/6 leaf	8/6 leaf
	R3	16 cm	21 cm
		6/0 leaf	10/0 leaf
Cd			
100 ppm	R1	14 cm	14 cm
		0/6 leaf	0/6 leaf
	R2	12 cm	15 cm
		3/4 leaf	6/6 leaf
	R3	18 cm	18 cm
		0/6 leaf	0/6 leaf
200 ppm	R1	12 cm	12 cm
		6/4 leaf	6/4 leaf
	R2	14 cm	13 cm
		4/4 leaf	4/4 leaf
	R3	21 cm	20 cm
		6/0 leaf	6/0 leaf

Note: R = replication - , a/b leaves
a = branch right, b = branch left.

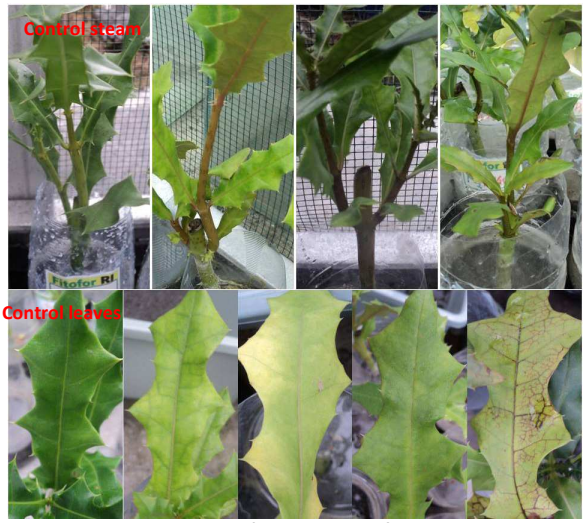

Figure 4. Stem and leaves of *Acanthus ilicifolius* are discolorated compared with control .

Based on Table 2 known that concentrations exposure that are not lethal, for Pb are increase the growth. It seen from the height of branch / stems and number of leaves. Whereas for Cd concentrations, can inhibit the growth of branch stems and leaves. Besides morphological changes in Acanthus ilicifolius plants heavy metal exposed, lead (Pb) and cadmium (Cd) can be easily seen, as in Figure 4. Pb exposure on leaf veins and stems brownish-black to blackish green leaves. While Cd exposure in the leaves more pale green to yellowish.

IV. CONCLUSION

Result show that Acanthus ilicifolius (seaholy) death in letal concentration for 8000 mg/L in lead (Pb) and 300 mg/L in cadmium (Cd). Cd concentrations although not lethal but inhibit the growth of stems and leaves, in other hand Pb concentrations did not show inhibit indication but accelerating growth. In addition to the color change of Pb exposed to brownish-black on leaf veins and blackish green on stem, but Cd exposed that green pale to yellowish before die.

Suggestion: it is necessary for further research on heavy metal concentrations of Pb and Cd contained in each part of plants such as leaf, steam and root.

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REFERENCES

[1] Widowati, W., Sastiono, A., and Rumampuk, R.J. 2008. Effects Metals Toxic: Prevention and Control Pollution. ANDI Publisher. Yokyakarta.

[2] Wang, L.K., Chen, J.P., Hung, Y.T. and Shammas, N.K. 2009. Heavy Metals in the Environment. CRC press. New York.

[3] Darmono. 1995. Metals in Biological Systems of Organism. Indonesia University. Jakarta.

[4] Mangkoedihardjo, S., and Samudro, G. 2010. The Applied Phytotechnology. Graha Ilmu. Yogyakarta.

[5] Mangkoedihardjo, S., and Samudro, G. 2009. Ecotoxicology Technosphere. Guna Widya. Surabaya.

[6] Soemirat, J. 2003. Environmental Toxicology. Gadjah Mada University Press. Yokyakarta.

[7] Palar, H. 1994. Contamination and Toxicology of Heavy Metal. Rineka Cipta. Jakarta.

[8] Allowey, B.J. and Ayres, D.C. 1997. Chemical Principles of Environmental Pollution. 2nd Edition. Blackie Academic and Professional Chapman and Hill. London.

[9] Cunningham, S.D. and Berti, W.R. 1993. Remediation of Contaminated Solid With Green Plants: an overview. In Vitro Cell. Dev. Biol. 29 . page 207-212.

[10] Hindersah, H., Kalag, M.A. and Muntalif, S.B. 2004. Accumulation of Pb and Cd Tomato Fruit that Planted on Dry Land Mud Containing of Domestic Wastewater Treatment. Environmental Engineering. ITB. Bandung.

[11] Amriani. 2011. Bioaccumulator Heavy Metal: Lead (Pb) and Zinc (Zn) On The shell blood (*Anadara Granosa* L.) and The shell mangrove (*Polymedosa bengalensis* L.) in the waters of Kendari Bay. Environmental Science Thesis. Diponegoro University. Semarang.

[12] Darmono, 2006. Environment and Pollution: relationship with toxicology of metal compounds. Indonesia University. Jakarta.

[13] Ahmad, R. 2004. Environmental Chemistry. ANDI Publisher. Yogyakarta.

[14] Irwan A., Noer, K., and Yenny E. 2008. Studies absorption Metal Cd, Ni, and Pb Concentration With Variations In Roots, Stems and Leaves of Spinach Plants. Lambung Mangkurat University, Banjarmasin.

[15] Neis, U. and Bittner, A. 1989. Wastewater Use. Obor Indonesian Foundation. Jakarta.

[16] Lembahingtyasadjeng, F.H. 2003. Review of Heavy Metal Cadmium (Cd) at Kenjeran Channel Effect of Industrial Waste and Strategy Management. Environmental Engineering Thesis. ITS. Surabaya.

[17] Hasan, I. 2005. Dynamic Modeling System for Regional Ecosystem Management of Kenjeran Coastal for Heavy Metal Pollution of Cadmium (Cd). Environmental Engineering Thesis. ITS. Surabaya.

[18] Hidayat, Yuzammi, S., Hartini, S., and Astuti, I.P. 2004. Aquatic Plants of Bogor Botanic Garden (Vol.1 No.5). Bogor Botanic Garden. Bogor.

[19] Backer, C.A. and Bakhaizen, R.C. 1963. Flora of Java. The Rijksherbarium. Netherlands.

[20] vanValkenberg, J.L.C.H., and Bunyapraphatsara. 2002. Plant Resources of South-East Asia No. 20 (2): Medical and Poisoning Plant 2. PROSEA Foundation. Bogor.