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## Budapest International Research in Exact Sciences (BirEx Journal): Medical Science, Agriculture Science, Biological Science, Engineering Science and other related areas P-ISSN 2655-7835, Volume 1 No. 2, April 2019

#### **Message from the Editor**

#### Greetings Dear readers of BirEx Journal

We are happy to announce you that our Volume 1, No 2 has been published. There are 10 articles from 21 authors published in this current issue.

Dear readers, you can receive further information and send your recommendations and remarks or submit articles for consideration, please contact BirEx Journal Secretariat at the below address or email us to <a href="mailto:birex.journal@gmail.com">birex.journal.ga@gmail.com</a> or <a href="mailto:birex.journal.ga@gmail.com">birex.journal.ga@gmail.com</a>

Hope to stay in touch and meeting in our next issue.

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#### Diversity of Earthworms (Oligochaeta: Annelida) in Reclamation Land at PT. Bukit Asam (Persero) Tbk. Tanjung Enim, South Sumatera

#### Rita Oktavia

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**Abstract**: The earthworms are in the restoration program first needs to know diversity and species that have the potential to restore ecosystem function. In this study, the aim is the researcher examined the diversity of earthworms in post-mining land at various ages of revegetated land, non-revegetated land, and natural land. The research was conducted at PT. Bukit Asam (Persero) Tbk, Tanjung Enim, South Sumatra. The methodology is used the quantitative research to find the result of this research. The result is The total number of individuals earthworms, and the highest density was found on 23-year-old revegetated land and natural land.

**Keywords**: earthworm; diversity; reclamation land

#### I. Introduction

Coal mining is one of the activities of utilizing natural resources that have the potential to damage the environment, both water, soil and air. Exploitation of coal usually begins with peeling soil and cover plants, then excavating. Furthermore, coal is transported to stock piles (Sukandarrumidi 2006). After mining, the top soil is not returned to its original location or stacked for several years. The soil layer is then returned to its original location or spread to another place after mining is finished. This process causes the mixing of top soil with dumping and other mining materials (tailings), so that soil conditions become heterogeneous (Topp et al. 2001).

Restoration is the return of ecosystem functions in post-mining land. Restoration is usually focused on vegetation revegetation and more manageable soil surface fauna (Majer et al. 2007). Reconstruction of appropriate plant communities is very important in restoring damaged soil ecosystems. Efforts to restore ecosystem structure and function, cannot succeed without the ecological function of the plant community. Community improvement efforts are needed to accelerate ecological succession, recolonization of indigenous fauna and restoration of ecosystem functions (Frouz et al. 2007). Mining activities cause land to be disrupted. Soil fauna is the main focus in efforts to restore ecosystem function (Top et al. 2001).

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Earthworms are candidates for good soil fauna in the initial restoration of mining land. Sculion & Malik (2000) reported that earthworms influence the characteristics of the soil surface, especially in increasing aggregate and carbon stability. Emmerling & Paulsch (2001) also reported the ability of earthworms in land improvement efforts, namely the activity of

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digging and mixing soil (bioturbation), so that macro pores formed, regulating the movement of water and air along with other microbiota in the soil. Earthworms can increase the stability of humidity and temperature at the surface soil horizon which plays an important role in efforts to improve land.

The role of earthworms in improving soil structure, such as soil aggregate formation and soil pores, has been widely reported. Earthworms used for rehabilitation of soil texture, must meet several conditions, namely tolerant to extreme environments, have ecological plasticity, the ability to penetrate compact soil, and the ability to spread. It is recommended that the restoration efforts be used for multi-species earthworms (Garcia & Fragoso 2002).

Saptaningrum (2001) reported on post-tin mining land on Bangka island, high sand content (%) (in the first year) and gradually declined until the 15th year after being mined. Clay and dust levels are very low (%) at the beginning after mining and gradually increase until the 15th year. pH, organic C, total N, available P increase with the length of time after mining. The value of cation exchange rates increased from the 5th year after mining.

In the United Kingdom and Eastern Europe it is reported that earthworms that have survived the accumulation process at the mine opening, can re-colonize within 10-30 years (Huttl & Weber 2001). Frouz et al. (2007) reported that in Central Europe, the density of soil macrofauna in post-mining reclamation land was higher than for natural post-mining land. Earthworms were significantly different at the three ages (15, 23 and 40 years) observed. The dominant epigeic earthworms were Dendrobaena octaedra and Lumbricus rubellus. In the reclaimed land endogeic earthworms were also found. In Austria Dunger & Wanner (2001) reported the development of soil fauna in post-mining land for 46 years revegetation. The groups of epigeic earthworms found were Dendrobaena octaedra, D. rubidus rubidus and Lumbricus rubellus rubellus. Anesic groups found were Lumbricus terrestris, while the endogeic groups found were Aporrectodea caliginosa caliginosa, A. rosea rosea, and Octolastion tyrtaeum. Two species of earthworms, namely Allobophora chkorotica and Octolasion cyaneum are found in small amounts and are only found at certain times.

So far, the use of earthworms in the restoration program has not been carried out on a large scale. The use of local earthworms are in the restoration program first needs to know diversity and species that have the potential to restore ecosystem function. In this study, we examined the diversity of earthworms in post-mining land at various ages of revegetated land, non-revegetated land, and natural land. The research was conducted at PT. Bukit Asam (Persero) Tbk, Tanjung Enim, South Sumatra.

PT. Bukit Asam (Persero) Tbk. is a State-Owned Enterprise (BUMN) which was established on March 2, 1981 based on Government Regulation No. 42 of 1980. The company is based in Tanjung Enim, Muara Enim Regency, South Sumatra Province. The company is located about 3 km northwest of the city of Tanjung Enim, in Lawang Kidul District, Muara Enim Regency, South Sumatra. Geographically, the location of PT. Bukit Asam (Persero) Tbk. located at position 3042'30 "LS to 4047'30" LS and 103043'00 "BT to 103050'10". PT. Bukit Asam (Persero) Tbk. consists of two mining units, namely the Tanjung Enim mining unit (UPTE) located in Tanjung Enim and the Ombilin mining unit (UPO) located in Ombilin. PT. Bukit Asam (Persero) Tbk. has 8 Mining Authorities, one of which is the mining authority of Air Laya.

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#### II. Methodology

This research was conducted from September 2011 to January 2012 at PT. Bukit Asam (Persero) Tbk, Tanjung Enim, South Sumatra. Earthworms were collected from six types of land, namely 6-year-old revegetated land (Tembe), 12 years (MTS), 20 years (Suban), 23 years (Petai water), non-revegetation (Mahayung 1), and natural land (Mahayung 1) (Figure 1; Appendix 1).

The identification of earthworms was carried out in the Biosistematic and Animal Ecology Section of the Department of Biology, FMIPA IPB. Soil analysis was carried out in the Department of Soil Science and Land Resources of the Faculty of Agriculture, IPB. Earthworms were collected in four revegetated land types, namely revegetated land aged 6, 12, 20 and 23 years. Earthworms are also collected from non-revegetated land and natural land. At each sampling location the coordinates are measured with GPS.

The earthworms found are taken by hand sorting (Bartlett 2010). All collected earthworms are put into sample bottles which have been given 70% alcohol and labeled. Identification of specimens is based on Sim & Easton (1972) and Blakemore (2002) with the help of a stereo microscope. The environmental factors measured are pH, humidity (RH), temperature, litter dry weight, and vegetation.

The morphological and anatomical characters of the earthworm species found were described. Analysis of earthworm data includes diversity, density, relative density, and frequency of presence of a species. Worm diversity was calculated by the Margalef species richness index (DMg), Shannon-Wiener diversity index (H '), Fisher diversity index alpha ( $\alpha$ ), and Pielou (J') evenness index. The similarity of earthworm species between locations was calculated by similarity of qualitative Jaccard species (CJ) (Magurran 1987).

The formulas used are as follows:

a. Density Species (D)

D species A = 
$$\frac{\sum \text{ individual species A}}{\text{Large (m}^2)}$$

b. Relative density of species

RD species A (%) = 
$$\frac{\text{Density species A}}{\text{Sum D species}} \times 100$$

c. Relative Frequency species (Rf)

Rf species A (%) = 
$$\frac{\sum \text{plot found species A}}{\text{Sum } \sum \text{plot}} \times 100$$

d. Margaleft's Species wealth index (D<sub>Mg</sub>)

$$DMg = \frac{S-1}{\log(N)}$$

Description: S = number of species, N = number of individuals

e. Shanon-wiener's Diversity index (H')

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$$H' = \sum_{i=1}^{n} pi \ln pi$$

Description: pi = ni / N, ni = number of individuals per species, <math>N = number of individuals.

f. Fisher alpha's Diversity index

$$S = \alpha \log^e = (\frac{1+N}{\alpha})$$

g. Pielou's Average Index

$$J' = \frac{H'}{\ln S}$$

Description: N = number of individuals

h. Jaccard's Qualitativ similarity

$$CJ = \frac{j}{a + b - j}$$

Description: j = total number of species found in two habitats, a = total number of species at location a, b = total number of species at location b.

The relationship between the number of individuals with environmental analysis with Pearson (P), and principal component analysis (PCA), uses PRIMER 5 and R.2.12 programs.

The relationship between the number of individuals with environmental analysis with Pearson (P), and principal component analysis (PCA), uses PRIMER 5 and R.2.12 programs.

#### **III. Discussion**

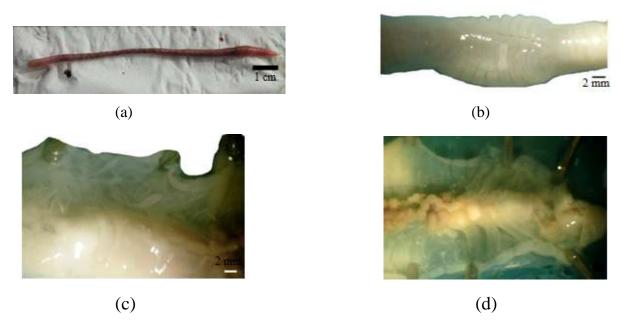
#### 3.1 Description of Earthworms

The earthworms identified are adult earthworms which are characterized by the presence of clitules in the anterior part. In this study four species of earthworms were found, namely Pontoscolex corethrurus, Polypheretima elongata, Pheretima sp, and Amynthas pauxillula. The earthworm family found was Glossoscolecidae and Megascolecidae.

#### Pontoscolex corethrurus

Pontoscolex corethrurus was observed with a body length of 6-11.5 cm (Figure 1a), prostomium prolobus type, saddle shaped clitula (Figure 1b) in segments 13-20, 14-21 or 15-22. Lumbricine seta type, which has 8 sets per segment, which is spread in the dorsal section. Dorsal holes in this species were not identified. The position of male and female holes is not clearly observed. This worm has 3 pairs of sperm holes, which are in the 6/7-7 / 8-8 / 9 segment. Hole positions are in segments 7-9. Spermases are segmented 6-8 or 7-9, lacking diverticula and accessories (Figure 1c). Spermateka P. corethrurus does not have nephridia. Stomach is in segment 6 (1d image). This species has no prostate.

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**Figure 1.** Morphology and anatomy of *P. corethrurus:* body 6-11.5 cm (a), saddle-shaped clitula (b), 3 pairs of specimens without diverticula (c), and bounch in segment 6 (d).

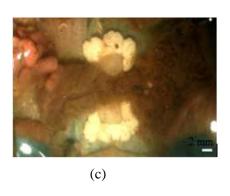
#### Polypheretima elongata

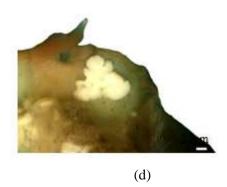
Polypheretima elongata has a body length of 7.5-11 cm, the prostomium type prolobus or proepilobus, have a ring clitulem, which is in segments 14-16 (Figure 2a). Seta perichaetine types which has more than 8 sets per segment, and spread using segments. The Single dorsal of holes are located in the middle of the segment, usually not at the top and back. Dorsal's hole in the locking process to connect to the outside environment to protect the body in the process of respiration. The first dorsal of hole is in the 10/11 segment or 12/13 in this species. It has a hole in the 18th segment and one hole in the 14th segment. This species does not have any holes. P. elongata does not have any ingredients. The stomach is located in the segment after 7/8 or 8/9 (Figure 2b). The prostate is racemose, which is equipped with a copulation pouch. P. elongata has no caeca (Figure 2c).



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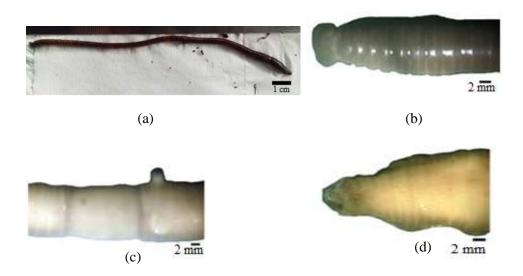




**Figure 2**. Morphology and anatomy of Pheretima sp.: prostomium epilobus (a), 4 pairs of spherical holes (b), spermas equipped with diverticula and nephridia (c), and prostate equipped with copulation bag (d).

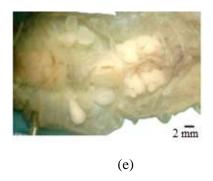
#### Amynthas pauxillula

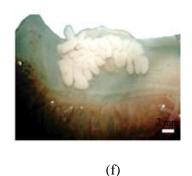
Amynthas pauxillula has a body length of 6.7-9 cm (Figure 3a), prostomium epilobus type (Figure 3b), ring-shaped clitula, in the 14-16 segment. Seta perichaetine types, usually spread in each segment. There is one dorsal hole in the center of the segment, the first dorsal hole in the 11/12 segment. This genus has a pair of male holes in segment 18, and one female hole in the 14th segment (Figure 3c). The spheres are intersegmental, holes are sensitive to 3 pairs in the ventral segment 4 / 5-5 / 6-6 / 7 (Figure 3d). The first equipment hole in the 4/5 segment. Teka pairs in three segments. Spermateka 3 pairs are located in segments to 4-6 or 5-7, equipped with diverticula, accessories, but no nephridia (Figure 3e). Stomach is between segments to 7/8 or 9/10 or 10/11. This species has a prostate, tubular racemose, without pockets of copulation (Figure 3f).



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**Figure 3**. The morphology and anatomy of Amynthas pauxillula: body length 6.7-9 cm (a), prostomium epilobus (b), 18 segment male holes and female holes in segment 14 (c), spermateka holes (d), spermens without nephridia (e), and prostate without copulation pouch (f).

#### 3.2 Diversity of Earthworms

The total number of earthworm individuals collected from the two times taken was 698 individuals. Retrieval in September found 33 individual adult worms and 186 juvenile individuals. Taking in January found 260 individual adult worms and 219 juvenile individuals. Earthworms were not found on location 5 (non-revegetated land) (Table 1). In the September collection, only Pontoscolex coretharurus (Glossoscolecidae) was found. In January, the earthworm found is Amynthas pauxillula, Pheretima sp. and Polypheretima elongata (Megascolecidae) (Table 2). The number of individual earthworms in September was 219 individuals, while in January 479 individuals.

**Table 1.** Species, number of individuals, genus of earthworms in observations in September 2011 and January 2012

Total individual / genus / Total ind		lividual
species	September	January
N (Total individual)	219	479
Total genus (individual)	1	4
Total species (individual)	1	4

**Table 2.** Species and number of individual earthworm on revegetation land; non-revegetation; and natural land

Location	Species	Earth	worm
(the field of age)	Species	∑ individual	Persentage (%)
Non-revegetation	-	-	-
R-6 years age	Pontoscolex corethrurus	4	0,015
	Amynthas pauxillula	8	0,030
R-12 years age	Pontoscolex corethrurus	45	0,173
R-20 years age	Pontoscolex corethrurus	69	0,265
R- 23 years age	Pontoscolex corethrurus	76	0,292
	Polypheretima elongata	1	0,003
	Pheretima sp.	1	0,003
Natural soil	Polypheretima elongata	41	0,157
Total	260	1	0,015

R = land revegetation

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The dominant earthworm found at the study site is Pontoscolex corethrurus which is almost found in every location. Amynthas is only found on 6-year-old land, while Polypheretima and Pheretima are found on 23-year-old land and natural land.

On revegetated land, ages 6, 12, 20, 23 years and natural land were found to be 44, 112, 196, 294 and 229 individuals respectively. The highest number of species (3 species) was found on 23-year-old revegetated land and natural land, followed on 6-year-old revegetated land (2 species), and 12 and 20-year-old revegetated land (1 species) (Table 3).

**Table 3.** Number of individuals (N), genus (G), species (S), species richness, diversity index, and earthworm evenness index (J ') on various types of land

una curun	and cartification eveniness index (3 ) on various types of faint						
			Total of I	Individual a	ınd Diversit	$\mathbf{t}\mathbf{y}$	
Parameter	Non-R	R-6	R-12	R-20	R-23	L.Natural	
		years	years	years	years		
N	0	44	112	196	294	229	875
S	0	2	1	1	3	3	4
G	0	2	1	1	3	3	4
Density	0	2,75	7	12,25	18,37	14,31	9,11
$(Ind/m^2)$							
Frequency	0	0,19	0,44	0,5	1	1	0,52
found							
$\mathbf{D}_{\mathbf{Mg}}$	0	0,52	1,27	1,32	2,63	2,76	7,23
α	0	0,72	1,65	1,67	3,63	3,91	11,51
Н'	0	0,83	1,9	1,8	2,61	2,65	3,73
J'	0	0,75	0,98	0,87	0,94	0,95	0,95

R = revegetated land, Non-R = non-revegetated land, Natural = natural land, N = number of individuals, S = species type, G = genus, DMg = species richness Margalef,  $\alpha$  = fisher diversity index, H '= Shannon-Wiener species diversity index and J '= Piolue evenness index.

The highest density of earthworms (18.37 ind / m2) was found on 23-year-old revegetated land, followed by natural land (14.31 ind / m2), and the lowest was found on 6-year-old revegetated land (2.75 ind / m2). The frequency of high earthworms (16 plots) was found on 23-year-old revegetated land and natural land. The lowest frequency of earthworms found (3 plots) was found on 6-year-old revegetated land. The results of the diversity index analysis showed that the diversity of high earthworms was found in natural land locations (DMg = 2.76,  $\alpha$  = 3.91, and H '= 2.65), followed by 23 years of revegetation (DMg = 2.63,  $\alpha$  = 3.63, and H '= 2.61). The lowest diversity of species was found on 6-year revegetated land (DMg = 0.52,  $\alpha$  = 0.72, and H '= 0.83) (Table 4). The highest evenness was found in the 12-year revegetation location (0.98), followed by natural land (0.95).

Based on the results of the analysis of species density (K), the highest density (5.93 ind / m2) in P. corethrurus on 23 years revegetated land, followed by revegetated land aged 20 years (4.31). The lowest density (0.06 ind / m2) in Polypheretima elongata and Pheretima sp., Was found on 6-year-old revegetated land. The highest relative density (97.93%) in P. corethrurus on 23-year revegetated land, followed on natural land (73.21) in Polypheretima elongata, and 6-year revegetated land (66.66%) on A. pauxillula. The lowest relative density (1.03) in Polypheretima elongata and Pheretima sp. on 23 years old revegetated land. The highest frequency was highest in P. corethrurus (81.25%) on 23-year revegetated land, followed by natural land (75%). The lowest relative frequency (6.25%) was found in A.

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pauxillula and P. elongata on 6-year-old revegetated land, and in P. elongata and Pheretima sp. on 23-year-old land (Table 4).

Species similarity between locations is known from the qualitative Jaccard value (CJ). From the results of the similarity index analysis it is known that at 12 years the revegetated land has a high species similarity (CJ = 1) with 20 years old revegetated land. The same results also occur on 23-year-old revegetated land with natural land locations (CJ = 1). The similarity of the lowest species (CJ = 0.25) was found on 6-year-old revegetated land with 23-year-old revegetated land and natural land locations (Table 5).

Table 4. Density of species (K), relative density (KR), and relative frequency (Fr) of worm

species in each study location.

1	Service	Relative	e Density and Fro	equency
Location	Species	K	KR	Fr
Non-R	P. corethrurus	-	-	-
	A. pauxillula	-	-	-
	P. elongata	-	-	-
	Pheretima Sp.	-	-	-
R-6 years	P. corethrurus	2,25	33,33	6,25
-	A. pauxillula	0,5	66,66	6,25
	P. elongata	-	-	-
	Pheretima Sp.	-	-	-
R-12 years	P. corethrurus	2,81	50	43,75
•	A. pauxillula	-	-	-
	P. elongata	-	-	-
	Pheretima Sp.	-	-	-
R-20 years	P. corethrurus	4.31	50	50
-	A. pauxillula	-	-	-
	P. elongata	-	-	-
	Pheretima Sp.	-	-	-
R-23 years	P. corethrurus	5,93	97,93	81,25
•	A. pauxillula	-	-	-
	P. elongata	0,06	1,03	6,25
	Pheretima Sp.	0,06	1,03	6,25
L Natural	P. corethrurus	0,68	19,64	75
	A. pauxillula	-	-	-
	P. elongata	2,56	73,21	43,75
	Pheretima Sp.	0,25	7,14	18,75

R = revegetated land, Non-R = non-revegetation, L. Natural = natural land, K = species density, KR = relative species density (%), Fr = Relative frequency of species (%).

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**Table 5.** Qualitative Jaccard diversity index (CJ) of earthworms between locations

Location	Similaritas Jaccard						
Location	R-6 years	R-12 years	R-20 years	R-23 years	Non-R	Natural Soil	
R-6 years	1	0,5	0,5	0,25	0	0,25	
R-12 years		1	1	0,33	0	0,33	
R-20 years			1	0,33	0	0,33	
R-23 years				1	0	1	
Non-R					1	0	
L. natural						1	

R = revegetated land, Non-R = non-revegetation, L. Natural = natural land.

Environmental factors at the study site were on 20-year-old revegetated land with a high soil pH (6.55), 12-year-old revegetated land having the lowest soil pH (6.18). High soil moisture (43.51%) in the location of natural land, the lowest humidity (25%) was found on non-regenerated land. The highest temperature (34.12oC) was found on the lowest non-revegetated land (26.4oC) at the site of natural land. Litter dry weight was highest (223.75 g) on 23-year-old revegetated land and lowest (13.75 g) on non-revegetated land (Table 6).

**Table 6.** Average environmental factors measured at the study site

Field	Similaritas Jaccard					
Factor	R-6 years	R-12 years	R-20 years	R-23 years	Non-R	Natural Soil
pН	6,29	6,18	6,55	6,35	6,37	6,30
rH(%)	34,75	31,62	29,06	42,68	25	43,51
Temp (°C)	29,81	28,95	29,37	27,43	34,12	26,4
B. s	79,25	23,68	39,78	223,75	13,75	170,40

Faculty. Ling = environmental factors, R = revegetation land, pH = soil pH, rH = soil moisture (%), temperature = soil temperature, and B.s = litter dry weight (g).

#### 3.3 Diversity of Earthworms with Environmental Factors

The results of the Pearson correlation analysis showed that the number of individual earthworms significantly negatively correlated with soil pH (p = 0.0383) and temperature (p = 0.0000030). The individual number of earthworms significantly positively correlated with soil moisture (p = 0.0000060), litter dry weight (p = 0.0035), total N (0.01) and porosity (0.05) (Table 7).

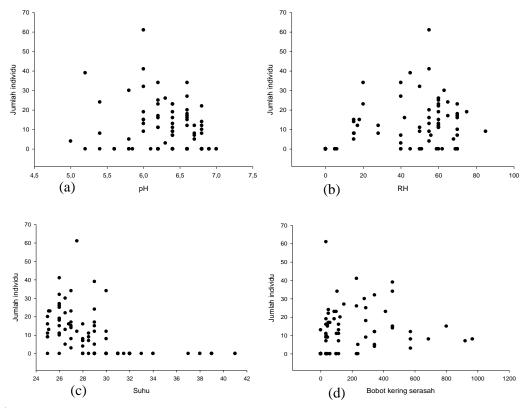
**Table 7.** Pearson Correlation (P) between the number of individual earthworms with environmental factors and their probability values (p)

Danamatan	Total individual			
Parameter	corelation	Value ( p)		
pН	-0,211	0,0383		
earth	0,444	0,0000060		
temprature	-0,494	0,00000030		
Nett	0,295	0,0035		
C-organik (%)	0,75	0,08		
N-total (%)	0,88	0,01		
sand (%)	0,67	0,14		
dast (%)	0,57	0,23		

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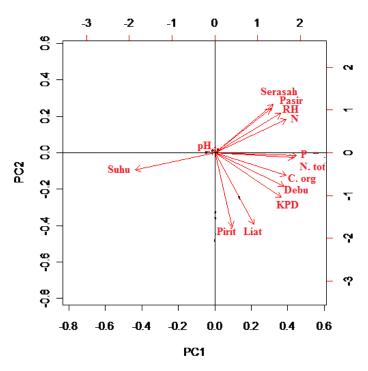
clay (%)	0,06	0,89
density (g/cm <sup>3</sup> )	0,39	0,43
Porosit (%)	0,80	0,05
Pirit (%)	-0,31	0,54

From the results of scatter plots it is known that the number of individual earthworms is found high at pH 6.0-6.5, humidity 40-55% and temperature = 26oC-27.5oC (Figure 4). The results of PCA analysis between the number of individuals and environmental factors are described in biplot (Figure 8). From the PCA's biplot is known that the number of individuals is negatively correlated with temperature and pH, positively correlated with litter, RH, porosity, N-total, organic C, dust, sand, clay, density and pyrite. The main components are 1 (PC1) and main components 2 (PC2) cover 80%.

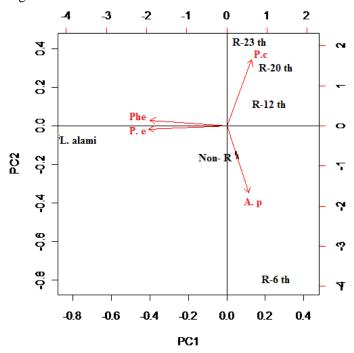


**Figure 4.** Relationship between the number of individuals to environmental factors: (a) pH, humidity (b), temperature (c), and dry weight of litter (d).

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**Figure 5.** Biplot analysis of the main components of the study variables and locations. N = number of individual worms, litter = litter dry weight, KPD = soil density, N.tot = total nitrogen, and C.org = soil organic carbon.



**Figure 6.** Biplot analysis of the main components of the study variables and locations. P.c = Pontoscolex corethrurus, A.p = Amynthas pauxillula, P.e = Polypheretima elongata, Phe = Pheretima sp ,.

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#### IV. Conclusion

PCA analysis between species and sampling locations was depicted in a biplot (Figure 9). From this biplot it is known that Amynthas pauxillula earthworm species is dominantly found on 6-year revegetated land. On revegetated land 12, 20 and 23 years the dominant species found is Pontoscolex corethrurus, and on natural land is Phretima sp. and Polypheretima elongata. They are with main component 1 (PC1) and main component 2 (PC2) covering 89%. The proportion of the main component variants can be seen in (Appendix 1).

Four species of earthworms were found, namely P. corethrurus, A. pauxillula, P. elongata, and Pheretima sp. post mining area PT. Bukit Asam (Persero) Tbk. Tanjung Enim, South Sumatra. The worms included in the epigeic group were A. pauxillula, P. elongata, and Pheretima sp., While P. corethrurus was included in the endogeic group. The total number of individuals, and the highest density was found on 23-year-old revegetated land and natural land. The highest number of species was found on 23-year-old revegetated land and natural land. Environmental parameters that significantly influence earthworms are pH, temperature, soil moisture, litter, total N and porosity.

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#### Bioaktivity Test Crude Fruit of Citrus Lime (Citrus aurantifolia) on Bacteria Escherichia coli in Vitro

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**Abstract**: The antimicrobial activity of citrus fruit crude (Citrus aurantifolia) was proven in this experiment with different concentration treatments. The purpose of this study was to determine the antibacterial power of citrus fruit crude against Escherichia coli bacteria tested in vitro. This research uses experimental method with laboratory test for citrus fruit crude using Kirby-baurier of suspectibility test method, with data obtained by using statistical test of Analysis of Varian (Anova) with complete randomized data retrieval. The results showed that of citrus fruit crude has antibacterial power to the growth of Escherichia coli bacteria. At concentrations of 25%, 50%, 75%, 100% formed inhibit zone with an average diameter of 12.6 mm, 15.4 mm, 16.6 mm, 17.3 mm. So, the higher the concentration of citrus fruit crude the better of inhibitory power.

**Keyword**: Crude; Citrus fruit (Citrus aurantifolia); Escherichia coli; Inhibition zone

#### I. Introduction

The disease caused by the development of pathogenic microbes is called infection. Infection is one of the serious health problems affecting humans. Various kinds of infections that occur in humans today can be caused by some bacteria derived from the human body itself. Such infections can cause various diseases such as diarrhea and lower respiratory tract disease or pneumonia. This disease is a disease that is often caused by pathogenic bacteria such as Staphylococcus aureus and Escherichia coli.

Escherichia coli bacteria is one of the normal flora in the human colon. In addition to the intestines of these bacteria can also be found in small amounts as part of normal flora in the respiratory system. This bacteria can infect the body if it reaches beyond the normal intestinal. Bacteria Escherichia coli is the most common bacteria causing diarrhea worldwide. The effects of diarrheal diseases can cause dehydration, especially in children and the elderly and in more severe conditions can cause acute kidney failure and changes in mental status such as confusion and headaches. Data from the Ministry of Health in 2014 shows that the prevalence of diarrhea in Indonesia 97.45% with the number of cases as many as 8,490,976 cases. The high incidence of infection and resistance to antibiotics are a major problem in the treatment of infections and a new strategy is needed in establishing alternative therapies for the treatment of infections.

Traditional medicine is a collection of beliefs, knowledge and experiences of indigenous peoples used to safeguard health, prevent, diagnose and treat physical and mental diseases. One of the traditional medicine known in Indonesia is Antibiotics. The use of natural ingredients as traditional medicine in Indonesia has also been done by our ancestors since many centuries ago. The trend of traditional drug use in the world is due to the increasing side effects of using chemical drugs. The presence of bioactive components in plants is known to have antibacterial effects. So now many tests of antibacterial effects using natural ingredients. Natural ingredients have lower side effects compared to chemical drugs, other than that cheap and easy to obtain.

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#### II. Material and Method

#### 2.1 Materials and Media

The material used for crude extract samples (Citrus aurantifolia), pure culture of bacteria Escherichia coli from Endo Agar media, Ciprofloxacin 500 mg antibiotics as positive control, aquadest, Nutrient Broth (NB) media, Nutrient Agar medium NA), and Alcohol 70%. The tools used in this research are petridish plate, reaction tube, ose needle, measuring cup, micropipet, suction pipette, oven, autoclave, incubator, Erlenmeyer flask, bunsen, stick watten, tweezers, filter paper whattman no .41, slide .

#### 2.2 Research Method

The method used in this research is experimental method. Laboratory test for Crude lemon fruit using Kirby-baurier of Suspectibility test method with disc diffusion.

#### 2.3 Bioactivity Test

The sterile cotton lidi is immersed in the germ of NB, then the cotton swab is applied on the surface of the NA medium, the procedure is repeated twice by rotating the 60o plate. The 6 mm diameter disc paper was immersed in a tube containing lime juice with concentrations of 25%, 50%, 75%, 100%, positive controls using 500 mg ciprofloxacin antibiotics and negative control using sterile aquades. Then a 6 mm disc paper is placed on the surface of the NA medium with the help of sterile tweezers with little emphasis to keep the disc paper firmly attached, then incubated at 37°C for 24 hours. Calculated the resistor zone on each of the disc paper by using the sliding term. The bioactivity test is done with 3 repetitions.

#### 2.4 Measurement of Inhibition Zones

Measurement of lime-limiting inhibition zone against Escherichia coli bacteria is by looking at the inhibit zone found on each disc at each concentration. The diameter of the inhibit zone formed can be measured by the sliding range by measuring the vertical and horizontal diameters according to the Kirby-baurier of Suspension test. The zones calculated at each concentration are compared with the classification of the inhibit zone based on Grenwood.

#### III. Result

Based on the results of research on bioactivity test of crude citrus fruit (Citrus aurantifolia) to Escherichia coli bacteria in vitro showed results that vary according to the concentration used. The drag zone results can be seen in table below.

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**Table 1.** Measurement Result of Crude Lime Zone Lime Citrus (Citrus aurantifolia)

Against Escherichia coli Bacteria

	Dia	meter Inhib	itor Zone (	mm)
Escherichia Coli Replicated	Li	me Citrus (	Concentrati	ion
	25%	50%	75%	100%
I	12,5	15,4	16,8	17,0
II	12,8	15,2	16,4	17,2
III	12,6	15,6	16,6	17,8
Average Diameter	12,6	15,4	16,6	17,3

Based on the research results obtained that crude citrus fruit (Citrus aurantifolia) has bioactivity against bacteria Escherichia coli which tested in vitro. The antibacterial effectiveness of lime fruit crude is indicated by the formation of inhibit zone around the disc paper. This inhibit zone is then measured in diameter by using the sliding term to determine the magnitude of its antibacterial power. The concentration of lemon juice used is 100%, 75%, 50%, and 25%. The concentration shown on the concentration of lime (Citrus aurantifolia) at concentration 75% yielding the average inhibitory diameter can inhibit bacteria is 16.6 mm, then compared with the classification of greenwood table is classified as a moderate growth inhibitory response. Also shown in the 100% concentration of lime (Citrus aurantifolia) that the average inhibitory zone can inhibit bacteria is 17.3 mm, the concentration has significance after being compared with the greenwood table classification that the growth inhibitory response is moderate. At concentrations of 75% and 100% are moderate because of the greenwood table classification between 16-20 mm. Of the four concentrations mentioned above that 100% concentration is the largest concentration of the average inhibit zone produced.

Based on research data shown in table 4 shows that, the concentration of citrus fruit crude (Citrus aurantifolia) has an influence on the activity of Escherichia coli bacteria. The concentration shown from 25% to 100% has increased inhibit zone.

Based on the above table, it is found that the average concentration of lime (Citrus aurantifolia) at 25% concentration has 12.6 mm inhibit zone, the area after comparison with greenwood table classification shows that the growth inhibitory response is weak. At a concentration of 50% showed that the limiting zone of lime concentration (Citrus aurantifolia) had a 15.4 mm inhibitory zone, the area compared to the drag zone classification table based on greenwood table means that the growth inhibitory response is weak. At concentrations of 25% and 50% have a weak growth inhibitory response because it is still classified between 11-15 mm.

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Table 2. Clasification Inhibitor Zona Greenwood

Average	Respon
Diameter Inhibitor Zona	Inhibitor Growed
> 20 mm	Strong Hight
16-20 mm	Medium
11-15 mm	Low
$\leq 10 \; mm$	Nothing

#### **IV.** Conclusion

Based on the results of research that has been done, it can be concluded that crude citrus fruit (Citrus aurantifolia) has bioactivity against bacteria Escherichia coli which tested in vitro. Of the concentrations tested against Escherichia coli bacteria have varied results depending on their concentration. The concentrations tested in this study were 25%, 50%, 75%, and 100%, with an average yield diameter of 12.6 mm - 17.3 mm. From the diameter obtained after comparison with greenwood table that is classified as being.

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#### Efication of Some Entomopatogen Fungus on Green Ladybug Imago (Nezara Viridula Linnaeus) (Hemiptera: Pentatomidae)

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**Abstract**: Green ladybug (Nezara viridula L.) is an important pest in some plants, including soybeans. N. viridula attacks cause a decrease in yield of up to 80%. Environmental friendly pest control studies are needed to overcome the problem of resurgence caused by N. viridula, among others by utilizing entomopathogenic fungus as their natural enemies. This study discusses about the efficacy of entomopathogenic fungi against N. viridula imago. The research is conducted at the Agricultural Agrotechnology Laboratory, Muhammadiyah University of South Tapanuli. The study uses a completely randomized design, nine treatments (control and eight isolates of entomopathogenic fungus) with three replications. The conidia density used is 108 conidia / ml. The entomopathogenic fungus application was performed on N. viridula imago. The results shows that MetKP fungus isolates are able to kill N. viridula imago with the highest mortality rate of 78.33%. The isolates of MetKM fungi and M. anisopliae caused the lowest imago mortality of 61.67%. Isolate of B. bassiana fungus had the lowest LT50 with a time of 5.66 HSA and the isolate of the MetTmM fungus had the highest LT50 with a time of 7.27 HSA. In the immature body part of N. viridula infected with fungus is enveloped by entomopathogenic fungal mycelium.

**Keywords** : Metarhizium anisopliae; Beauveria bassiana; mortality; pests; isolates

#### I. Introduction

Green ladybug (*Nezara viridula L.*) is an important pest in some plants, including soybeans. The development of green ladybugs was first reported in Ethiopia, then spread to tropical and subtropical countries in continental Europe, Asia, Africa and America (Squitier 2017). *N. viridula* comes to the soybean crop before the flowering phase to lay the eggs. Signs of *N. viridula* attack can be seen from mouth puncture marks on the skin of the pods and seeds. *N. viridula* will damage the pods and seeds until near harvest. The attack directly decreases the quality and yield of seeds (Asadi 2009). *N. viridula* attacks can cause a decrease in yield of up to 80% (Correra and Azevedo 2002).

N. viridula resurgence problems due to chemical insecticides that are used continuously can be overcome by using biological control, among others, by utilizing natural enemies, such as entomopathogenic fungus, predatory insects and parasitoid (Norris et al. 2003). Entomopathogenic fungi are one of the natural enemies that can be used as pest control agents. Entomopathogenic fungi reported to be able to infect pest insects are Lecanicillium lecanii, Metarhizium anisopliae, and Beauveria bassiana. Entomopathogenic fungi are very suitable to be chosen as bioinsecticides to control N. viridula because entomopathogenic fungus can infect N. viridula through the cuticle, in contrast to viruses and bacteria.

Biological control and utilization of entomopathogenic fungi (Prayogo et al. 2012) have various advantages, which have high reproduction, short life cycle, can form conidia that can last long in nature even in conditions that are unfavorable, relatively safe, selective, compatible with various insecticides, it is relatively easy to produce, and the possibility of

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causing resistance is very small. Therefore, it is necessary to test the efficacy of several entomopathogenic fungi against *N. viridula* as an environmentally friendly pest control.

#### II. Research Method

This research was conducted at the Agrotechnology Laboratory, Faculty of Agriculture, Muhammadiyah University of South Tapanuli. *N. viridula* for propagation was taken from paddy fields, long beans and green beans in the city of Padangsidimpuan. This study used a completely randomized design (CRD) method, with 9 treatments (control and 8 isolates of entomopathogenic fungi) and 3 replications.

#### 2.1 Propagation of Green Ladybugs (*N. viridula*)

The obtained imago group was put in a gauze cage. The group of nymphs obtained was put into a plastic box and the group of eggs obtained was inserted into the petri dish. In gauze cages and plastic boxes filled with long beans that have been washed with water to be free of synthetic insecticide residues. Furthermore, the eggs, nymphs and imago groups were maintained in the laboratory. Every day the feed is replaced with fresh beans that are still fresh.

Each nymph stage with the same age is put in a plastic box to avoid competition between the ages of the insect stage. The imago group is also put in the same screen to get the eggs produced by the imago. Imago used in the study was imago aged 1 day after changing the skin.

#### 2.2 Propagation of fungus on PDA media

The fungus isolates used in this study can be seen in table 1. Fungal isolates were grown on PDA media. The composition of PDA media used is potato 400 g, dextrose 15 g, so that 15 gr and aquades 1 L (Goettel and Inglis, 1997). PDA media are compacted in a petri dish 9 cm in diameter. The fungus was incubated for 21 days at room temperature.

**Table 1.** Isomate of Entomopathogenic Fungus

Fungus isolate	Source of Isolate
MetTrP	Metarhizium sp. from rizosfer Terong Padangsidimpuan
MetKPP	Metarhizium sp. from rizosfer Kacang Panjang Padangsidimpuan
MetKP	Metarhizium sp. from rizosfer Kedelai Padangsidimpuan
MetTmM	Metarhizium sp. from rizosfer Tomat Madina
MetJM	Metarhizium sp. from rizosfer Jagung Madina
MetKM	Metarhizium sp. from rizosfer Kedelai Madina
M. anisopliae	Center for Plant Crops and Protection (BBPPTP) Surabaya
B. bassiana	Center for Plant Crops and Protection (BBPPTP) Surabaya

#### 2.3 Preparation of Fungus Suspension for Testing

Each fungus culture is made suspension. The culture of the fungus is taken as a container by means of each cup plus 10 ml of sterile water + Tween 20 as much as 0.1 ml then scraped with a soft brush. The conidia is then put into a test tube and shaken using a vortex for approximately 60 seconds. The conidia density of each suspension was calculated by the haemocytometer Neubauer-improved until the conidia density used was 108 conidia /

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ml. The necessary conidia density is obtained by making a multilevel dilution with a sterile + tween distilled mixture (Goettel and Inglis 1997).

#### 2.4 Entomopathogenic fungus application in N. viridula Imago

The fungus suspension was applied by spraying directly on the N. viridula imago as much as 10 times the spray / experimental unit (20 imago), using a 2 ml size sprayer bottle. Each treatment was repeated 3 times. Before the application of fungi, each N. viridula imago group was put in a plastic box with a size of  $P \times L \times T = 10$ cm  $\times 10$ cm  $\times 4$ cm which had been coated with tissue. After the application of plastic fungus filled with sufficient amounts of washed beans as an imago feed supply. Then the plastic box is dripped with distilled water every day to maintain moisture.

#### 2.5 Observation parameter

The variables observed were the number of *N. viridula* who died from infection with entomopathogenic fungi, which were calculated from the time of application up to 7 days after application (HSA), and the time of death of *N. viridula* imago after sprayed with conidial fungus suspension. The percentage of imago mortality was calculated using the formula:

$$M = \frac{A}{N} \times 100\%$$

Information:

M = percentage of mortality (%)

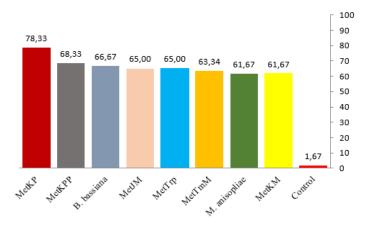
A = Number of insects that die from fungal infections

N = Number of insects tested

The data obtained was analyzed by analysis of variance and DNMRT follow-up at a real level of 5%.

#### III. Results and Discussion

The observation of the percentage of *N. viridula* immunity mortality after application of several entomopathogenic fungi isolates can be seen in Figure 1 below:



**Figure 1**. Percentage of mortality of *N. viridula* imago after the aplication of entomopathogenic fungus isolates

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**Table 2.** Mortality rate of N. viridula imago after application of fungal isolates

Fungus isolate	Instar Nymph mortality II (%)
MetKP	78.33 A
MetKPP	68.33 A B
B. bassiana	66.67 A B
MetJM	65.00 B
MetTrP	65.00 B
MetTmM	63.33 B
M. anisopliae	61.67 B
MetKM	61.67 B
Control	1.67 C

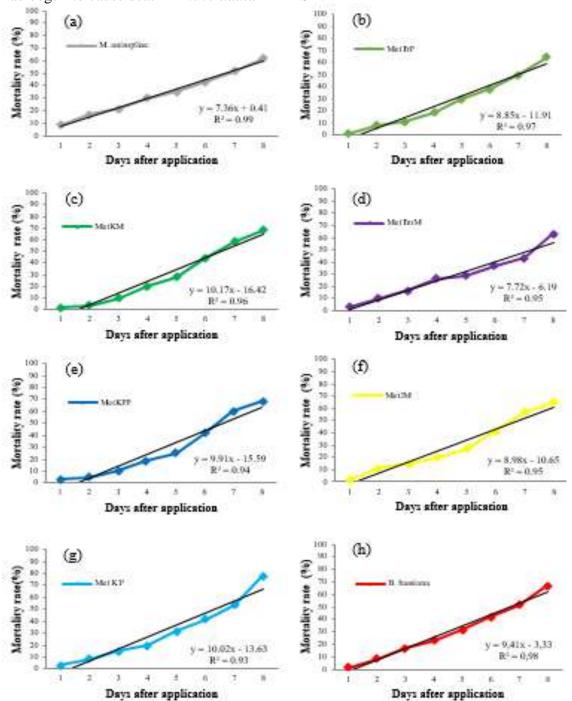
Description: Numbers followed by the same letters in the same column are not significantly different according to Duncan's test (DNMRT) at the 5% level

In table 2, the MetKP fungus isolates caused the highest mortality of *N. viridula* as large as 78.33% but not significantly different from the isolates of MetKPP (68.33%) and *B. bassiana* (66.67%). Meanwhile the fungus isolates of MetKM and *M. anisopliae* caused the lowest imago mortality of 61.67% respectively, but not significantly different from the mortality mortality due to fungi isolates MetTmM, MetTrP, MetJM, *B. bassiana* and MetKPP. The difference in mortality caused by fungus is closely related to the origin of the fungus isolates. Prayogo et al. (2005) said that growing media, virulence level, viability and pathogenicity of entomopathogenic fungi greatly determine the success of fungi in the process of infecting the host. Several factors that influence the effectiveness of entomopathogenic fungi in infecting insects are fungi, origin of fungus, conidia density, quality of growing media, type of pest controlled, application time, frequency of application, and environmental factors such as temperature, rainfall, ultraviolet light and humidity (Tengkano 2004).

In another study, Sunardi et al. (2013) reported that M. anisopliae fungus could kill *Aphis craccivora* (Hemiptera: Aphididae) by 75% at 108 / ml konidia density. Suryadi and Kadir (2007) obtained the results of the study of M. anisopliae capable of causing mortality of 46% in Nilaparvata lugens (Hemitera: Delphacidae). Prayogo (2004) also reported *M. anisopliae* infect *Riptortus linearis* (Hemiptera: Alydidae) by 40%. Furthermore, Permadi (2017) research obtained the results of *B. bassiana's* research causing mortality of *Diaphorina citri* (Hemiptera: Liviidae) of 53.33%. Ladja et al. (2011) obtained the results of B. bassiana killing 65% *Nephotettix virescens* (Hemiptera: Cicadellidae) at 108 / ml conida densities. Herlinda et al. (2012) also found that *B. bassiana* killed Paracoccus marginatus (Hemiptera: Pseudococcidae) by 75%.

The Agricultural Research and Development Agency (2012) stated that the mechanism of infection of entomopathogenic fungi begins with the attachment of fungal conidia to insect cuticles. Then konida germinate and penetrate into the insect's body. The next stage, the fungus grew and developed in hemolymph. Fungus would accelerate reproduction by separating the body of the hifa to fight the body's defenses. At the same time, antibiotic toxins produced by fungus weaken and kill insects quickly. Furthermore hyphae would grow and fill the entire body of the insect. When fungus begun to develop, insects show symptoms of pain, such as movements that are not coordinated and consequently will cause death in insects.

In Figure 2, we can see the accumulation of the daily mortality of *N. viridula* imago after application of several fungus isolates. Based on observations, the fungus isolates tested had begun to cause death in *N. virudula* in 1 HSA.



**Figure 2**. Graph of accumulated daily mortality of *N.viridula* imago due to fungal infection (a) M. anisopliae, (b) MetTrP, (c) MetKM, (d) MetTmM, (e) MetKPP, (f) MetJM, (g) MetKP, (h) *B. bassiana* 

The fastest fungus isolate causing mortality in *N. viridula* imago was *M. anisopliae* fungus isolates (5 individuals), MetTmM (2 individuals), MetKP (2 individuals), MetTrP (1 tail), MetJM (1 tail), and B. bassiana (1 tail) occurred in 1 HSA (Figure 2). Furthermore, isolates of the MetKM fungus and the new MetKPP can cause death in 2 HSAs, each with 1 tail and 2 tails. The advantages of MetKPM in the speed of shutting down host insects were influenced by the ability of these fungus isolates to produce enzymes that play a role in penetration and invasion in the insect's body. The body defenses possessed by the *N. viridula* imago also affect the time of *N. viridula* imago death. Weak body defenses from *N. viridula* imago will cause death at the beginning. Then the ability of the fungus to adjust to the body of the *N. viridula* imago to develop and obtain nutrients so as to be able to kill the *N. viridula* imago on the first day.

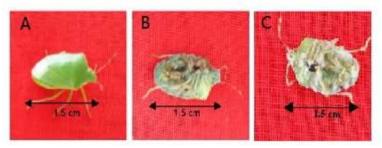
Based on the results of probit *B. bassiana* analysis, the fastest kill of 50% of *N. viridula* imago with 5.66 HSA while the fungus isolate MetTmM at the most killed 50% of *N. viridula* imago with a time of 7.27 HSA. There are several factors that cause differences in the speed of lethal *N. viridula* death by several fungi isolates used including the type of isolate, the origin of isolates, the physiology of each isolate, the amount of conidia produced by isolates, conidia sprout power, and virulence (Widayat and Rayati 1993).

Regression equation for N. viridula imago mortality due to M. anisopliae infection is y = 7.36x + 0.41 with R2 = 0.99. The formed slope value is 7.36. If the R2 value is getting bigger, then the population response to treatment is increasingly homogeneous. Each individual has a relatively similar sensitivity if the population is homogeneous (Himawati 2003). Immuno N. viridula mortality data due to infection of MetKP fungus isolates (y = 10.02x-13.63, R2 = 0.93) were fungal isolates which had the lowest  $R^2$  value. This regression equation is useful for estimating the percentage of mortality (y) obtained on a given day (x).

**Table 3.** LT50 several isolates of Metarhizium sp. in N. viridula imago

Fungus	LT50	Regression equation	$\mathbb{R}^2$
B. bassiana	5.66	y = 9.41x - 3.33	0.98
MetKP	6.23	y = 10.02x - 13.63	0.93
MetKM	6.53	y = 10.17x - 16.42	0.96
MetKPP	6.61	y = 9.91x - 15.59	0.94
M. anisopliae	6.73	y = 7.36x + 0.41	0.99
MetJM	6.75	y = 8.98x - 10.65	0.95
MetTrP	6.99	y = 8.85x - 11.91	0.97
MetTmM	7.27	y = 7.72x - 6.19	0.95

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**Figure 3**. N. virudula (A) Imago healthy, (B) Imago infected wirh fungus Metarhizium sp., (C) Imago infected wih B. bassiana fungus

In the picture above the healthy *N. viridula* imago has a bright color. Whereas *N. viridula* imago infected with fungus *Metarhizum* sp. looks covered in green mycelium. As stated by Prayogo (2006) symptoms that arise in insects infected with fungus *Metarhizium* sp. is the presence of mycelia in insects characterized by the growth of entomopathogenic fungal mycelium in parts of the body which are initially white and will then turn dark green. *N. viridula* Imago which has been infected with *B. bassiana* fungus appears to be enveloped in white mycelium.

At the beginning of the death of *N. viridula* imago there are no signs of mycelium growing in the *N. viridula* imago organ. The new fungal mycelium appears after several days of *N. viridula* imago death. Mycelia begins to grow and is found in the articulated organs, especially in the legs, then the mouthpiece, then develops on the thorax and abdominal segments. This is because the articulation organs are areas that are very flexible so that they are more easily penetrated by the conidia of the fungus.

#### **IV. Conclusion**

MetKP fungus isolates were able to kill *N. viridula* imago with the highest mortality rate of 78.33%. The isolates of MetKM fungi and *M. anisopliae* caused the lowest imago mortality of 61.67%. Isolate of *B. bassiana* fungus had the lowest LT50 with a time of 5.66 HSA and the isolate of the MetTmM fungus had the highest LT50 with a time of 7.27 HSA. In the immature body part of *N. viridula* infected with fungus is enveloped by entomopathogenic fungal mycelium.

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# The Effectiveness of *Kenikir* and Betel Leaves Extract as Bio Fungicide to the Causes of Anthracnose Disease (*Colletotrichum Capsici*) on Chili Plants (*Capsicum annum* L.) with In vitro

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**Abstract**: Effectiveness Kenikir and Betle Leaft Extraction as Biofungicide to Cause Disease Antraknosa (Colletotricum capsici) On Chili (Capsicum annuum) in In vitro. The research was done in the Laboratory Protection Plant Agriculture Faculty University of Medan Area, was held since Mei to July 2018. The research use Design Random Complete (RAL) Non Factorial with treatment F0 = negative control (PDA Media 100%) F1 = Positive control (Synthetic fungicide 0.2%), F2 = 20% kenikir+ 10%betel, F3 = 30% kenikir+ 10%betel, F4 = 40% kenikir+ 10%betel, F5 = 20% kenikir+ 20%betel, F6 = 30% kenikir+ 20%betel, F7 = 40% kenikir+ 20%betel, F8 = 20% kenikir+ 30%betel, F9 = 30% kenikir+ 30%betel, F10 = 40% kenikir+ 30%betel. The results of the study: the tested extract of kenikir and betel leaf leaves showed the same results for inhibiting the growth of colony diameter and percentage of fungi growth.

**Keywords**: Effectiveness; Kenikir and betel leaves extract; anthracnose disease

#### I. Introduction

Red chili is one of the horticultural commodities that has an important meaning because it has high economic in Indonesia, both as a commodity consumed domestically and as an export commodity. Red chili has a fairly high nutritional value generally used as a spice in cooking, medicines, cosmetics, coloring agents and industrial materials (Harpenas and Dermawan, 2011).

North Sumatra Province is one of the centers of red chili production in Indonesia. Based on data from the National Statistics Center of North Sumatra Province, it produced red chili in 2012 amounted to 197,411 tons, 2013: 161,933 tons, 2014: 147,812 tons (National Statistics Agency, 2016). When viewed from these data, red chili production experienced fluctuations in production, in 2014 the production of red chili in North Sumatra Province decreased by 14,123 tons or 8.72% compared to 2013. One of the causes of the decline in red chili production was due to anthrax. caused by *Colletotrichum* fungus and can cause crop losses of up to 65% (Hersanti et al., 2001). *Colletotrichum* mushrooms can infect the organs of red chili plants, especially the fruit. This fungal infection in red chili is characterized by initial symptoms of small spots that are blackish and slightly curved. Further attacks cause the fruit to shrink, dry and rot (Syamsudin, 2007).

Until now, most farmers still use fungicides to control the fungal pathogens. Continuous and excessive use of fungicides will result in disruption of the environmental balance and directly also very harmful to the health of consumers. One alternative in controlling anthrax disease is by using natural ingredients as bio fungicides, namely *kenikir* leaves and betel leaves.

Preliminary studies on phytochemicals of *kenikir* leaves showed the presence of active compounds of *flavonoid*, *saponin*, *terpenoid*, *alkaloid*, *tanin* and essential oils that have the potential as antimicrobials (Rasdi et al., 2010). Flavonoid compounds are polyphenol group

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compounds that have the potential as antioxidants and have other benefits, namely as an antifungal agent (Harborne & Williams, 2000).

Nurhayati's research (2006) states that treatment with betel leaf extract gives the best results in terms of suppressing the growth of colony diameter and the number of conidia of C. *Capsici*, because the administration of betel leaf extract can kill the pathogenic fungi. Triono (2017) stated that betel leaf extract is able suppress the intensity of anthracnose. Betel without fractionation and betel fractionation with water solvents is comparable to the ability of *probineb* fungicides to suppress anthracnose disease intensity.

This is in line with Lestari's research (2014) which states that betel leaf extract can inhibit the growth of *Colletotrichum capsicidan* fungi compared to synthetic fungicides. The results of the LSD test showed that the treatment of 20% concentration of betel leaf extract was proportional to the effective concentration of 1% synthetic fungicide.

The Astutiningrum (2016) study states that *kenikir* leaf extract has anti-bacterial power. *Kenikir* leaf mash extract has the smallest bacterial inhibition zone at 30% concentration of 6.76 mm and the largest inhibition zone is 7.58 mm at a concentration of 60%. Ethanol extract of the leaves of *kenikir* 30% concentration has a inhibition zone of 7.25 mm and at a concentration of 60% at 8.59 mm. In accordance with the preliminary research conducted by the author, the use of *kenikir* extract as a bio fungicide on the *Colletotrichum capsici* fungus showed that the extract of effective *kenikir* leaves began at a concentration of 40%.

Based on the description above, the authors conducted a study on the effectiveness of *kenikir* and betel leaf extract as a bio fungicide against the causes of anthracnose (*Colletottrichum capsici*) in red chili (*Capsicum annuum L*).

#### II. Research Methods

The research was conducted at the Plant Protection Laboratory of the Faculty of Agriculture, Medan Area University and University of North Sumatera (USU) Laboratory from May to July 2018.

The research was carried out by the experimental method of conducting direct experiments and conducted in vitro. This study used a completely non-factorial randomized design (Non Factorial CRD) as for the levels of concentration factors of *kenikir* and betel extract as follows:

- F0 = negative control (100% PDA media) + Colletottrichum capsici
- F1 = Positive control (Benlox 50 WP 0.2% synthetic fungicide) + Colletottrichum capsici
- F2 = PDA media with 20% kenikir leaf extract + 10% betel leaf extract + Colletottrichum capsici
- F3 = PDA Media with treatment of 30% *kenikir* leaf extract + 10% betel leaf extract + *Colletotrichum capsici*
- F4 = PDA media with 40% kenikir leaf extract + 10% betel leaf extract + Colletotrichum capsici
- F5 = PDA media with 20% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*
- F6 = PDA Media with treatment of 30% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*
- F7 = PDA media with 40% *kenikir* leaf extract + 20% betel leaf extract + *Colletotrichum capsici*

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F8 = PDA media with 20% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici* 

F9 = PDA Media with 30% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici* 

F10 = PDA media with 40% *kenikir* leaf extract + 30% betel leaf extract + *Colletotrichum capsici* 

The work procedure in this study begins with the provision of 560 grams of kenikir leaf extract and 360 grams of betel leaf extract which has been dried and mashed, then immersed with methanol 12 L solvent for kenikir and 10 L for betel for 3 x 24 hours. The solution was filtered using filter paper, then evaporated using a vacuum rotary evaporator (Buchii / R205). The filtered liquid is put together and inserted into a weighed evaporating flask, then methanol is evaporated using a rotary evaporator at temperatures (45-50) <sup>0</sup>C, rotation speed (50 - 60) rpm, and low pressure (150-200) mm Hg. After evaporation is complete, the pumpkin containing the extract is weighed and the difference between the results of the two weighing is the weight of the extract to get the concentrated solution of the extract and add aquades with a ratio of 1: 1 (b / v) after being stored in a refrigerator ( $\pm$  40C) for biological testing. The extract was then made extract dilution on the concentration treatment namely Kenikir 20% + 10% betel nut, 30% kenikir + 10% betel nut, Kenikir 40% + 10% betel nut, 20% kenikir + 20% betel nut, 20% betel nut + betel 20%,%, 40% tasting + betel 20%,%, 20% betel + 30% betel,% 30% + 30% betel nut, 40% + 30% betel nut in making agar media as much as 100 ml from each treatment. Next was isolation of Colletotrichum capsici, which was obtained from parts of the plant which showed symptoms of anthracnose attack. Then cut to size  $\pm 0.5 \times 0.5 \text{ cm}^2$  and soaked in 70% alkaline for 2.5 minutes to reduce contaminants of other organisms and then rinsed with distilled water and dried using tissue to grow in PDA media in petri dishes and incubated for  $\pm$  7 days in a 26-28oC temperature room.

After incubation then microscopic identification of conidial forms of fungi after culture was then obtained and then performed in vitro testing to determine the inhibitory test of leaf extracts of *kenikir* and betel leaves on *Colletotrichum capsici* carried out in petri dishes using the culture method of fungi, then prepared media culture according to the subsequent treatment incubation for 2 x24 hours a day then observing the parameters and the observational parameters consisted of phytochemical screening tests to analyze the bioactive content that is useful for testing anti-fungal pathogens. The phytochemical screening test of leaf powder *kenikir* and betel leaf, namely examination of *flavonoids*, *tannins*, *saponins*, *alkaloids*, *steroids* / *triterpenoids* and *glycosides*, while for culture, observation of colony diameter, and percentage of inhibition.

#### **III. Result and Discussion**

Phytochemical testing (screening) is one of the important steps in an effort to uncover the potential of plant resources. The results of the chemical content of phytochemical screening on the leaves of *kenikir* and betel leaf are in Table 1.

**Table 1**. Results of phytochemical screening on methanol extract of *kenikir* leaves (*Cosmos caudatus*) and betel leaf (*Piper betle*)

No	Phytochemical	Result	Conclusion			
NO	Test	Result	Kenikir	betel		
1	Flavonoid	Red, yellow, orange occurs in the amyl alcohol layer	+	+		
2	Tanin	Blue or blackish green occurs	+	+		
3	Saponin	Froth does not disappear as high as 1-10 cm	+	+		
4	Alkaloida	2 of 3 reagents produce the same deposits	+	+		
5	Steroid/triterpenoid	Purple or red occurs and then turns blue green	+	+		
6	Glikosida	A purple ring is formed	+	+		

Description: + (positive) = exists; - (negative) = none

Based on the results of the phytochemical screening test in Table 1 it can be seen that the leaves of *kenikir* and betel leaf contain chemical compounds of *flavonoids*, *tannins*, *saponins*, alkaloids, steroids / *triterpenoids* and *glycosides*. The results of this phytochemical screening test showed the same results with the phytochemical screening test conducted by Rasdi, et al. (2015) and Safita et al. (2017) that the phytochemical test results on *kenikir* leaf extract showed the presence of chemical compounds of *flavonoids*, *alkaloids*, *steroids* / *terpenoids*, *tannins* / *polyphenols*, *and saponins*.

Chemical compounds in these plants are known to have physiological activity as an antifungal. Anti-microbial activity of *terpenoids* by disrupting the growth and development of fungal spores due to the toxic properties of *triterpenoid* compounds (Ismaini 2011).

The extract of *kenikir* and betel leaf leaves has the potential to be anti-fungal, because it contains the active compounds of secondary *flavonoids*, *saponins*, *triterpeoid alkaloids* and *steroids* from the results of phytochemical screening tests that will be carried out for anti-pathogenic fungi causing anthracnose disease (*Colletotrichum capsici*) in red vitro (Capsicum annuum L.) in vitro.

#### **Colony Diameter**

**Table 2**. Diameter of Mushroom Colony Colletotrichum capsici in 2 to 8 days after inoculation (HSI) Treatment of Giving *Kenikir* Leaf and Betel Leaves

Treatment	Mushroom Colony Diameter Colletotrichum capsici(cm)													
Heatment	2 Hsi		3 Hsi		4 Hsi		5 Hsi		6 Hsi		7 Hsi		8 Hsi	
F0	1.23	В	1.97	В	2.72	В	3.18	В	4.00	В	4.67	В	5.2	В
F1	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F2	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F3	1	Α	1	A	1	A	1	A	1	A	1	A	1	A
F4	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F5	1	A	1	A	1	A	1	A	1	A	1	A	1	A
F6	1	A	1	A	1	A	1	A	1	A	1	A	1	A

F7	1	Α	1	Α	1	A	1	A	1	A	1	A	1	A
F8	1	Α	1	Α	1	A	1	Α	1	A	1	A	1	A
F9	1	Α	1	Α	1	A	1	A	1	A	1	A	1	A
F10	1	A	1	A	1	A	1	Α	1	Α	1	A	1	A

Description: Numbers followed by the same letters in the same column are not significantly different at the level of  $\alpha = 0.01$ 

From Table 2 the data on the diameter of the fungus colonies treated with the extract of *kenikir* leaves and betel leaves gave a very significant influence on the diameter of the fungi colony *Colletotrichum capsici*. It can be seen from the results of observations of 2-8 hsi, namely the treatment of F2 extract of *kenikir* leaves and betel leaves with concentrations (20% + betel 10%) differed very significantly from treatment F0 which was without vegetable pesticide treatment. The F2 treatment was not significantly different from the F1 treatment of *benlox* pesticide 50 WP 0.2% and treatment F3 to treatment F10. These results indicate that all test treatments have the same ability as *Benlox* 50 WP 0.2% (F1) synthetic pesticides in suppressing the growth of the *Colletotrichum capsici* mushroom colony diameter.

Based on the observations in Table 2, it can be concluded that the administration of the leaves of *kenikir* and betel leaves can inhibit the growth of the diameter of the fungus colonies. This is presumably because the leaves of *kenikir* and betel leaves contain substances that can inhibit the growth of *Colletotrichum capsici* fungi such as *flavonoids*, *tannins*, *saponins*, *alkaloids*, *steroids / triterpenoids*, and *glycosides* as evidenced by the results of phytochemical analysis test leaves of *kenikir* and betel leaves. This also affects the percentage growth inhibition of the *Colletotrichum capsici* fungus colonies.

#### **Inhibition percentage**

**Table 3**. Value of the percentage inhibition of fungi Colletotrichum capsici at 8 days after inoculation (HSI) Treatment of Kenikir Leaf Extract and Betel Leaves (%) (Transformation Results  $\sqrt{x} + 0.5$ )

Treatment	Avorago	Nota	ation
Treatment	Average	0.5	0.1
F0	0.707	b	В
F1	9.013	a	A
F2	9.013	a	A
F3	9.013	a	A
F4	9.013	a	A
F5	9.013	a	A
F6	9.013	a	A
F7	9.013	a	A
F8	9.013	a	A
F9	9.013	a	A
F10	9.013	a	A

Based on Table 3 shows the percentage value of the treatment of *kenikir* leaves and betel leaf extract gave a very significant effect on the percentage of inhibition of the *Colletotrichum capsici* fungus. It can be seen from the 8 values of inhibitory values, namely the treatment of

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F2 extract of *kenikir* leaves and betel leaves with concentrations (20% + betel 10%) different from the F0 treatment without vegetable pesticide treatment. The F2 treatment was not significantly different from the F1 treatment of *benlox* pesticide 50 WP 0.2% and treatment F3 to treatment F10. Fungal growth that does not occur due to physiological failure or tissue death

Antifungal compounds have various inhibitory mechanisms for fungal cells. Djunaedy (2008) states that antifungal compounds have a mechanism of action by neutralizing enzymes associated with invasion and colonization of fungi, damaging fungal cell membranes, inhibiting the fungal enzyme system so that it interferes with the formation of hyphae and influencing the synthesis of nucleic acids and proteins.

In line with the study of Astutiningrum (2016) states that *kenikir* leaf extract has antibacterial power at a concentration of 30% at 6.76 mm and the largest inhibition zone of 7.58 mm at a concentration of 60%. Whereas in previous studies conducted by single *kenikir* extract authors had a percentage of inhibitory power on the *Colletotrichum capsici* fungus at a concentration of 40%. This shows that *kenikir* extract has the potential as a bio fungicide. The results of the combination study of *kenikir* and betel extract as bio fungicides in F2 treatment (concentration of 20% 10% betel + betel leaf) shown in Tables 2 and 3 showed an increase in the effectiveness of inhibition of *kenikir* extract to a concentration of 20% and betel to 10%. The combination of *kenikir* extract and betel extract increases the active compound of secondary metabolites as bio fungicides including *flavonoids*, *saponins*, *tannins*, *alkaloids*, *triterpenoids* and *steroids*.

Flavonoid compounds are the largest group of polyphenol compounds. Flavonoids work by denaturing proteins to increase cell membrane permeability. Protein denaturation causes a disruption in cell formation, thus changing the composition of protein components (Wahyuningtyas, 2008). Cowan (1999) in Firdaus (2015), added that phenol compounds found in flavonoids can denaturate cell proteins and shrink cell walls causing lysis of fungal cell walls. In addition, phenolic compounds through hydroxy groups that will bind to the sulfihidril group of fungal proteins so as to be able to change the conformation of target cell membrane proteins resulting in impaired fungal cell growth can even experience death.

Saponins are water-soluble compounds and are like soap. Saponins are widespread in higher plants and have been detected in 70 plant families (Daniel 2006). Saponins are found as antimicrobials in nature. Saponin also has a function of biological activity as an antifungal (Kalaisezhiyen and Sasikumar 2012; Senthilkumar and Vijayakumari 2013). Wulansari (2009) also stated that saponin compounds have an antibacterial and anti-fungal effect. As an antifungal saponin, it results in microbial lysis cells which disrupt the stability of the cell membrane. The saponin mechanism as an anti fungus is the complex formation of saponins with sterols in the plasma membrane of fungi, then destroys semipermeable cells and causes death in fungal cells (Hoffmann 2003).

Tanin is an acidic polyphenol compound with a feeling of tightness. Tanin can be found in many plants and is spread in various plant organs such as stems, leaves and fruit. Tanin is anti-bacterial and anti-fungal. Tanin as an antifungal contributes a lot to plants to attack fungi and other microorganisms (Daniel 2006). The mechanism of tannin as an antifungal is inhibiting chitin synthesis which is used for the formation of cell walls in fungi and damaging cell membranes so that formation of fungi is inhibited (Watson and Preedy 2007).

While alkaloids are active substances from plants that function as drugs (Olivia, 2004). In general, plants that contain alkaloid compounds, physically can be identified with clear characteristics, such as gummy and bitter taste if tasted (Mustanir, 2013). According to

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Aniszewki (2007) in Gholib (2009), alkaloids are compounds that have anti-microbial activity, which inhibits esterase and also DNA and RNA polymerase, also inhibits cell respiration and plays a role in DNA intercalation.

Triterpenoid and steroid group compounds are known to have certain physiological activities as antifungal. Where the antifungal activity of terpenoids works by disrupting the growth and development of fungal spores due to the toxic properties possessed by triterpenoid compounds (Ismaini 2011).

#### IV. Conclusion

Effectiveness of extracts of kenikir leaves and betel leaves as biofungicides on the causes of anthracnose (Colletotrichum capsici) in vitro can reduce the growth of colony diameter and inhibit fungal growth. Inhibition of growth in colony diameter and percentage of fungal growth, all treatments tested for extracts of kenikir leaves and betel leaf showed the same results.

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# Effect of Heavy Metal Spread on River Flows from Gold Mining Toward Water Biota in Batang Gadis Mandailing Natal River

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**Abstract**: The purpose of this study is to find out the river water quality in terms of the spread of heavy metals found from the gold mining in Batang Gadis Mandailing Natal River and to determine the effect of heavy metal distribution on river flow from the gold mining of aquatic biota on the Batang Gadis Mandailing Natal River. This research uses descriptive exploratory method by conducting a survey first. Determination of sampling sites using purposive sampling method at the two stations that are determined. Sampling of water and sediments from each location. Water samples were taken as much as 500 mL, samples of sediment were taken at a depth of 10-15 cm from the base surface as much as 100-200 g (Mann, 1978). Examples of water biota are randomly taken at each station as much as 50-100 g, then put together into a composite sample for further analysis in the laboratory. Water quality is measured insitu include temperature, brightness, turbidity, depth, current speed, pH, DO, CO2. While the COD sample, BOD was taken to the laboratory to be analyzed by preservation using ice at a temperature of around 4 oC before observing in the UMTS biology laboratory. Data obtained, then analyzed descriptively. The results of this study are the levels of heavy metals found in the two Batang Gadis River observation stations that are equally good in water, sediment and those found in fish, namely Hg <0,0008, Cd <0,003 and Pb <0,005. Metal levels found are still below the threshold value, but need to be aware of the accumulation of these metals. The histological observations of crisp fish gills at the Bustak mine station are found to experience edema and necrosis which are strongly suspected to be caused by pollution of heavy metals found in the Batang Gadis River. The histological observations of crisp fish liver at the Bustak mine station are found to experience necrosis which is allegedly caused by heavy metal pollution found on the Batang Gadis River

Keywords: pollution; heavy metal; biota water; Batang Gadis river

#### I. Introduction

Water as a component of the environment will affect and be influenced by other components. Bad quality water will cause the environment to be bad so that it will affect the health and safety of humans and other living things. One of the bodies of water which is a wealth of water resources is a river. The river is also an easy and practical place for the disposal of waste, both solid and liquid, as a result of household activities, home industries, garments, animal husbandry, workshops, and other business ventures. With the disposal of various types of waste and waste containing various types of pollutants to water bodies, both biodegradable and non-biodegradable substances will cause more weight to be borne by the river.

Batang Gadis River is the estuary of several tributaries, the Lahantan River and the Batang Pungkut River, which originates from Gunung Kulabu, Mandailing Natal District. This river is the longest and largest river along the Kotanopan area, as for the characteristics of the Batang Gadis River which is an attraction is the clear water and large rocks neatly arranged. The results of the Atifah and Lubis (2017) study show that two observation stations

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observed showed low water quality at the Bustak Mine Station and Dalan Lidang. The low pH found in the station is due to the large number of activities of the population and gold mining activities that dispose of mining waste directly into the river in large quantities every day. The more widespread gold mining activities are worrying about the life of biota on the Batang Gadis River, especially fish. The most worrying pollution at the moment is mining activities that are increasingly prevalent in the Batang Gadis river that use engines and diesel fuel which are directly disposed of into the River (Abdullah, 2013).

Activities on the Batang Gadis River that are mostly carried out are gold mining around the river. In gold mining activities, the amalgamation process requires a process of mixing gold and mercury. According to Widodo (2011) the amalgamation process is the process of binding gold from the ore by using mercury in a tube called a bubble. The waste produced in gold mining usually contains toxic chemicals. In the mining process, mercury is used to bind gold. Ginting (1995) states that in addition to dangerous heavy metal elements, the main element that must be considered and very dangerous that is found in gold mining waste is mercury.

The entry of heavy metals mercury (Hg) and other heavy metals that have not been identified in the Batang Gadis River which can accumulate in the body of aquatic organisms living in the Batang Gadis River, especially in fish tissue. Fish absorb heavy metals through their food and directly from water by passing gills. Heavy metals can also bind to proteins throughout fish tissue, if consumed by humans for a long time they can be accumulative poisons if they cannot be decomposed by organs so that they will endanger health (Diliyana, 2008). Therefore, it is necessary to do further research on "The Effect of Heavy Metal Distribution on River Flow from Gold Mining Products to Water Biota on the Batang River Mandailing Natal Girl".

# II. Materials and Methods

This research used descriptive exploratory method by conducting a survey first. Determination of sampling sites using purposive sampling method at the two stations that were determined. Sampling of water and sediments from each location. Water samples were taken as much as 500 mL, samples of sediment were taken at a depth of 10-15 cm from the base surface as much as 100-200 g (Mann, 1978). Examples of water biota were randomly taken at each station as much as 50-100 g, then put together into a composite sample for further analysis in the laboratory. Water quality was measured with *insitu* included temperature, brightness, turbidity, depth, current speed, pH, DO, CO2. While the COD sample and BOD were taken to the laboratory to be analyzed by preservation using ice at a temperature of around 4°C before observing in the UMTS biology laboratory. Data obtained, then analyzed descriptively.

#### III. Result and Discussion

# 3.1 Heavy Metal Spread on the Batang Gadis Mandailing Natal River

The results of analysis of heavy metals found in the waters of Batang Gadis Mandailing Natal river can be seen in the table below.

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Table 1. Water Test Results of Batang Gadis Mandailing Natal River Against Metals

No	Station	Н	Note		
1,0		Hg	Cd	Pb	1,000
1	Sitamiang	< 0,0008	< 0,003	< 0,005	
2	Bustak Mine	< 0,0008	< 0,003	< 0,005	
Met	al Maximum Limit	0,05	0,01	0,03	

The results of metal content on water in Batang Gadis river were found to contain the same levels of Hg, Cd and Pb which were  $<0.0008~mg\ /\ l$ ,  $<0.003~mg\ /\ l$ ,  $<0.005~mg\ /\ l$  in two different observation stations, namely in Sitamiang and at the Bustak Mine. The levels of metals Hg, Cd and Pb found are still below the threshold value. The maximum threshold value for Hg metals was 0.05 mg / l, Cd 0.01 mg / l and Pb 0.03 Mg / l. Metals that have the highest levels of the three metals tested were Pb metal or lead which is 0.005 mg / l but still below the threshold value of 0.03 mg / l.

**Table 2.** Soil Test Results of Batang Gadis Mandailing Natal River Against Metals

No Station		Heavy Metal (mg / l)			Note
110	Sution	Hg	Cd	Pb	11010
1	Sitamiang	< 0,0008	< 0,003	< 0,005	
2	Bustak Mine	< 0,0008	< 0,003	< 0,005	

Based on results of soil analysis on Batang Gadis Mandailing Natal River at Sitamiang Station and the Bustak Mine it was obtained in the form of sandy mud with the same heavy metal content value at the two stations. The content of mercury (Hg) <0,0008, Cadmium <0,003 and Lead <0,005. Metals with the highest metal content are found in Lead metal, which is <0.005. The metal content found is still at the maximum limit of metals in the waters. Levels of Hg metal still <0,0008 still meet the Quality Standards for Quality Materials in accordance with Government Regulation No. 82 of 2001 class I. 0.001 mg / L class II. 0.002 mg / L class IV 0.005 mg / L.

According to Dahuri. Et al. (2001) that waters that are high in sediment can endanger life in the aquatic environment, including sediments which cause an increase in turbidity of water by blocking the penetration of light entering the water so that it can disrupt the life of organisms in it. Sediment is the place for the accumulation of heavy metals around the ocean waters.

**Table 3.** FishTest Results of Batang Gadis Mandailing Natal River Against Metals

No	Station	Heavy Metal (mg / l)		Note	
1,0		Hg	Cd	Pb	1,000
1	Sitamiang	< 0,0008	< 0,003	< 0,005	

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2   Bustak Mine   < 0,0008   < 0,003   < 0,005
--

The results of fish analysis of metal content in Batang Gadis river were found to contain the same levels of Hg, Cd and Pb which were <0,0008 mg / 1, <0,003 mg / 1, <0,005 mg / l in two different observation stations, namely in Sitamiang and at the Bustak Mine. The levels of metals Hg, Cd and Pb found are still below the threshold value. The maximum threshold value for Hg metals is 0.05 mg/l, Cd 0.01 mg/l and Pb 0.03 Mg/l. Metals that have the highest levels of the three metals tested are Pb metal or lead which is 0.005 mg / 1 and still below the threshold value of 0.03 mg / 1.

# 3.2 Microscopic Observation of Garing Fish Gills

Fish gills are the main respiration organ that works by the mechanism of surface diffusion of respiration gases (oxygen and carbon dioxide) between blood and water. Oxygen dissolved in water will be absorbed into the gill capillaries and fixed by hemoglobin to then be distributed throughout the body. While carbon dioxide is released from cells and tissues to be released into the water around the gills (Brown, 1962; Rastogi, 2007). Therefore, any changes that occur in the aquatic environment will directly and indirectly affect the structure and function of the gills.

The basic structure of the gills consists of the primary lamella as the main body in each gill filament and secondary lamella as a small part of the gill filaments found around the primary lamella body. In the middle part of the primary lamella there is a large duct along the primary lamella called the venous sinus filled with erythrocytes (Pinontoan, 2015).

The primary lamella is composed of chloride cells surrounded by flattened pavement cells and can be observed at the intersection between the primary lamella and the secondary lamella. Chloride cells are often located at the base of the secondary lamella. Mucus cells are a prominent feature of the gill epithelium. (Genten et al., 2009).

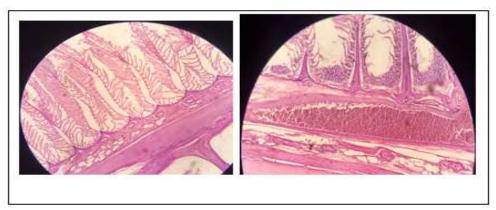


Figure 1. Histology of Garing Fish Gills at Sitamiang Station

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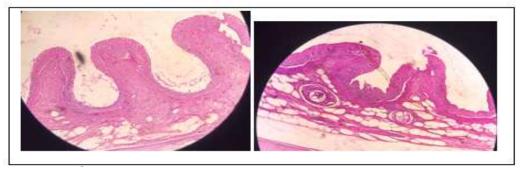


Figure 2. Histology of Garing Fish Gills at Sitamiang Station Bustak Mine

# 3.3 Microscopic Observation of Garing Fish Gills

Hydrophic degeneration is an advanced stage swelling of the liver cells where visible spaces in the cytoplasm can be seen from cells with vacuoles appearing to enlarge to push the nucleus to the edge of the cell (Triadayani et al, 2010). In addition to this picture there are also more hepatocyte cells that experience necrosis.

Hepatopankreas in fish is one of the vital organs that is very important in detoxifying substances that enter the body. Fat celling is a process of fat degeneration which is a disturbance in fat cells or excessive fat accumulation in the cytoplasm (Ramadhani et al, 2013). Characterized by the presence of vacuoles (the state between the liver cells with each other becomes stretched). Based on the study of El-Naggar (2009) who reported that the liver of Tilapia (Oreochromis niloticus) which experienced pathological changes in the form of cell fatty tissue and necrosis is the result of the liver accumulating by heavy metals (Fe, Cu, Zn, Mn, Pb, and Cd). Fat degeneration occurs because of the accumulation of fat (neutral fat) with damage to the cell nucleus and shrinking liver cell tissue (Panigoro et al., 2007). According to research by Alifia and Djawad (2000), mentioning that milkfish (Chanos chanos Forskall) exposed to lead metal results in liver degeneration.

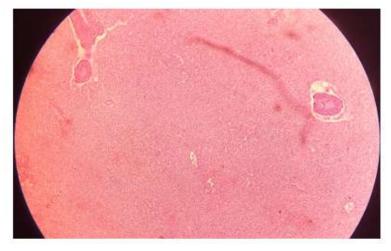


Figure 3. Histology of Liver Garing Fish at Tamiang Station

In three pictures above, it appears that there are empty cavities outside the liver cells that look like white dots. The empty cavity is caused by an enlarged vacuole. Hydrophic degeneration is the swelling of advanced liver cells where there are visible spaces in the

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cytoplasm of cells with vacuoles appearing to enlarge so that it pushes the nucleus to the edge of the cell.

In addition, in this picture there are also necrotic hepatocytes (Triadayani et al, 2010). Supported by the research conducted by Triadayani et al regarding the effect of lead metal (pb) on liver tissue of duck grouper (Cromileptes altivelis) it was found that lead metal caused hydrophic degeneration characterized by an enlarged vacuole that pushed the nucleus towards the edge.



Figure 4. Histology of Liver Garing Fish at Bustak Mine

#### **IV. Conclusion**

The conclusion of this study is that the heavy metal levels found in the two Batang Gadis river observation stations are of equal value both in water, sediment and those found in fish, namely Hg of < 0,0008, Cd of < 0,003 and Pb of <0,005. Metal levels found are still below the threshold value, but need to be aware of the accumulation of these metals. The histological observations of crisp fish gills at the Bustak mine station were found to experience edema and necrosis which were strongly suspected to be caused by pollution of heavy metals found in the Batang Gadis river. The histological observations of crisp fish liver at the bustak mine station were found to experience necrosis which was strongly suspected to be caused by pollution of heavy metals found in the Batang Gadis river.

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# The Sustainability Status if Fish Landing Port Facility Management if Ujong Baroh in West Aceh District

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Abstract: Ujung Baroh Fish Landing Port (FLP) is a type D fishing port which is an important infrastructure in small-scale capture fisheries activities. But there are some obstacles in the management of the FLP. Improper management can have an impact on the optimal utilization of facilities and activities that are not active. This is a challenge for the Regional Government of West Aceh. Therefore, Meulaboh FLP needs to be supported by a suitable management by involving the relevant agencies. Thus this research is very important to be carried out regarding the sustainability status of the optimal management of Fish Landing Port facilities. The results of the study showed that there are four dimensions that have sustainability covering the ecological dimension 57.43, economic dimensions 62.93, socio-cultural dimensions 76.08, technological dimensions 57.54, with 24 attributes while there is one institutional dimension 32.76 by having 6 attributes that do not have less sustainability in managing Ujung Baroh Fish Landing Port facilities.

Keywords: sustainability; managing; FLP; Meulaboh

#### I. Introduction

West Aceh Regency is a region of Aceh Province that has a fairly good developing fisheries sector, Ujung Baroh FLP has a good prospect because it has several advantages, including the types of economically valuable catches, such as mackerel, pomfret, skipjack, and cob. In addition, the Ujung Kalak FLP is located near to the main highway of the city of Meulaboh, which is about 200 m away and is an access to potentially marketing catches to major cities such as Blang Pidie, Nagan Raya, Banda Aceh, and Medan.

This is one of the attractions for migrant fishermen from other districts to land their catch at FLP Ujung Baroh. Unfortunately, it can be seen from the direct field observation that Ujung Baroh FLP has not been managed properly, the office buildings are feasible but not yet functioning, and the FLP technical service unit is not yet running.

Ujung Baroh Fish Landing Port (FLP) is a type D fishing port which is an important infrastructure in small-scale capture fisheries activities. But there are some obstacles in the management of FLP itself. Improper management can have an impact on the optimal utilization of facilities and activities that are not active. This has been a challenge for the Regional Government of West Aceh.

Therefore, Meulaboh FLP needs to be supported by a suitable management by involving the relevant agencies. Thus this research is very important to be carried out regarding the sustainability status of the optimal management of Fish Landing Port facilities.

# II. Research Methodology

This research was carried out at Ujong Baroh FLP, The data is gathered in two months from May to June 2018.

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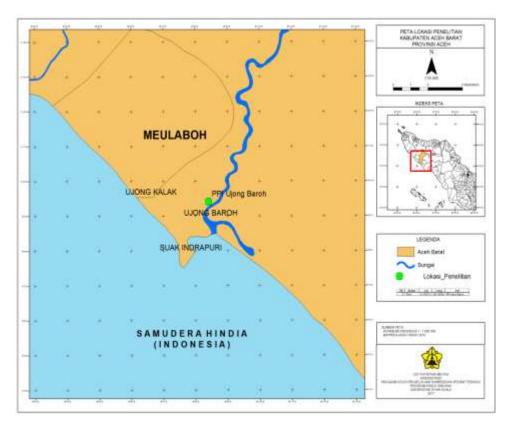


Figure. 1 Research Location Map

The method used in this study was a case study method of managing the sustainability status of Ujung Baroh Fish Landing Port Facility in West Aceh Regency. Samples taken by using the purposive sampling method were the data taken specifically from DKP, BPS, FLP Managers and fishermen.

Primary data was obtained through the interviews with relevant parties and direct observation guided by the existing questionnaire. Secondary data was supporting data obtained through information and written reports from relevant agencies.

The data analysis used in this study was the analysis of sustainability conditions by using multi dimensional scaling using RAPFISH. RAPFISH Sustainability Analysis

The sustainability analysis of the fish landing port management is carried out through several stages, namely the stages of determining the attributes of sustainable fish port fund management that cover five dimensions (ecological dimensions, economic dimensions, socio-cultural dimensions, institutional dimensions, and technological dimensions). The assessment stage of each attribute on an ordinal scale is based on continuous criteria for each dimension, oration analysis based on the multidimensional scaling (MDS) method, index compilation and the sustainability status of the fish landing port management are reviewed both in general and in each dimension (Fauzi and Anna, 2002).

The five dimensions will simultaneously influence the sustainability of the facilities and management of fish landing ports. Each of these dimensions has its own attributes and criteria which reflect the influence on the sustainability of the relevant dimensions. The various attributes and criteria used are determined based on the preferences of experts and stakeholders.

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The number of ranks for each attribute is determined by the availability of literature that can be used to determine the number of ranks. (Susilo, 2003) the attributes used to assess the sustainability of fish landing port facilities in each dimension are as follows:

The attributes that will be studied in the ecological dimension include: the condition of the aquatic environment, the degree of adaptation to fishing, fishing activities, the type of fishing gear, the volume of fishing gear, and the condition of the fish landing port. The attributes that will be studied in the economic dimension include: the regional income contribution, economic value, income level, export sales value, and number of catches. The attributes that will be assessed in the socio-cultural dimension include: stakeholder perceptions, catch equity, local wisdom, community empowerment, social togetherness, and fish landing port management policies. The attributes that will be assessed on the institutional dimensions include: aspects of the legality of the ship / fishing gear, the effectiveness of arrests, fishing facilities and infrastructure, institutional development, institutional assistance and cultural values. The attributes that will be studied in the technology dimension include: the condition of the landing site, the level of mastery of fisherman technology, the form of processing before sale, the level of utilization of information, the availability of technology, the nature and type of fishing gear.

The assessment of the scores of each attribute was analyzed in a multidimensional manner to determine one or several points that reflected the position of the marine fisheries-based business that was studied against two reference points, namely the good point and bad points. To facilitate the ordinance Rab-FA FLP uses Multi Scaling Dimensional rapfish software (Kavanagh, 2001). This rapfish software is an MDS development that is in SPSS software, the rotation process, flipping, and some sensitivity analysis has been integrated into software. Through MDS, the position of the sustainability point can be visualized in two dimensions (horizontal and vertical axis). To project these points on a horizontal line, the rotation process is carried out, with extreme extremes being given a score of 0% and an extreme point both given a score of 100%. The sustainability position of the system under study will be between these two extreme points, this value is the value of the sustainability index of marine fisheries-based business development carried out by stakeholders in West Aceh.

**Table 1.** Category of index value and sustainability status of FA FLP. Based on the analysis index value of MDS

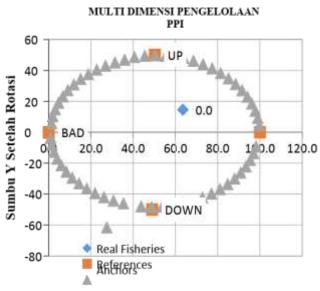
Index value of MDS		
Index Value	Category	
0-25	Bad/ Not sustainable	
26-50	Less sustainable	
51-75	Fairly sustainable	
76-100	Good/ Very sustainable	

Source: Kavanagh (2001) dalam Edwarsyah (2008)

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#### **III. Discussion**

Multidimensional Rap-facility analysis using ordination techniques through MDS method produces the continuity index value shown in Figure 10 Index value is 63.5. The results of multidimensional analysis values are included in the continuous category, because their values are at the intervals of 76-100. This index value is obtained based on 60 attributes covered by five dimensions, namely ecological dimensions (6 attributes), economics (6 attributes), socio-cultural (6 attributes), technology and institutions (6 attributes). (See figure 2)



	Sust	ainability
FLP of West Aceh	INDEX	STATUS
	63,5	Good/ Very Sustainable
	Stres: 0,1256	
	$R^2 : 0.956$	

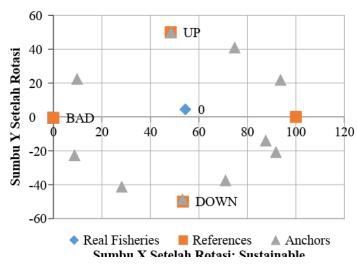
Figure 2. Multidimensional Index Analysis and Sustainability Status of FLP Management.

Based on Figure 2, it is known that the institutional dimension has a good or sustainable sustainability index. It can be seen from the conditions of management and fishing gear used which still support the continuity of its sustainability.

To see the attributes that are sensitive and contribute to the value of the sustainability index, MDS analysis is carried out with the analysis. It can be seen that the value of attribute changes to the ordination axis X. The results of the MDS analysis are presented based on each dimension used.

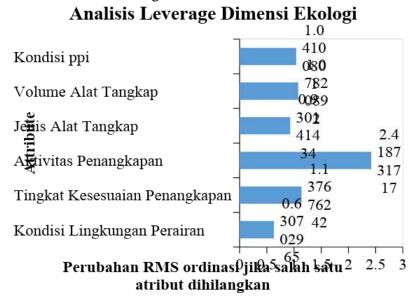
## 3.1 Ecological Dimension

The attributes used to see the effect of the ecological dimension on the sustainability of fish landing port facilities are as many as six (6) elements. Based on the results of the analysis, the ecological dimension of sustainability is 54.43, which is included in the sustainable category.



**Figure 3.** The results of the Rapfish analysis show an ecological dimension sustainability index

Then in the economical dimension there are five (5) elements that are sensitive which are influenced after leverage analysis, namely: (1) Level of fishing adjustment, (2) Catching activity, (3) type of fishing gear, (4) fishing gear volume, (5) condition of FLP. The results of all these dimensions can be seen in Figure 4 below:



**Figure 4** The role of each ecological attribute is expressed in the form of Root Mean Square (RMS).

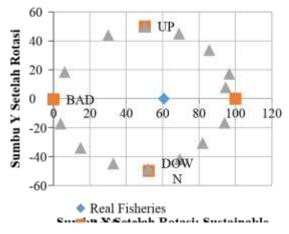
Some sensitive attributes that have an impact on sustainability such as the suitability of the current location of fish landing ports as fishing ports which are located in narrow locations and also the public whose majority of non fishermen but construction workers, teachers, civil servants and the private sector. This is very disturbing to the surrounding community from noise and odor. This is because the development of a dense location is increasingly rapidly requiring solutions to regional spatial problems.

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Other support is that the wide area of Ujung baroh fish landing port is not suitable, according to the maritime and fisheries department (2004) regarding the technical criteria for port types, fish landing bases including type D at least have at least 2 hectares of land. Because the fish landing port is in a residential area. It is necessary to improve the system of building physical facilities so that they can be maintained now and in the future. Lack of counseling about the condition of FLP which results in FLP the minimization in its usage.

#### 3.2 Economic Dimension

The economic dimension uses six (6) attributes / elements to predict the effect of sustainability on fish landing port facilities. The results of the analysis showed that the value of the sustainability of the economic dimension was 54.43 which was categorized as sustainable (see the appendix). Leverage analysis was used to see the sensitive attributes that have a sensitive influence on the sustainability index value. The results of the analysis can be seen in the following figure.

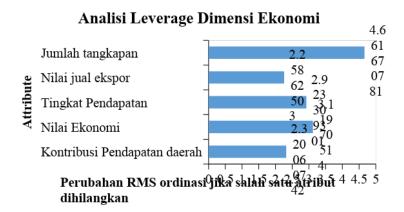


**Figure 5**. The results of the Rapfish analysis showed an economic dimension sustainability index.

Then based on the analysis shows there are six (6) variables, namely: (1) regional income contribution, (2) capture volume, (3) economic value, (4) income level, (5) export selling value (6) catch amount. In communities based market,, fish landing has met the technical criteria for port types, fish landing bases including type D which are of local marketing nature.

The number of landed fishery commodities was quite a lot and it still needed to be added to increase the economic value of the base. The running of the base activity was the amount of labor that was in accordance with the standards of the fish landing base. The existence of a fish landing base could increase the economic growth of the local community by making cheap purchases and it could also benefit from sales outside the fish landing bases (retailers). The other important thing was the consumption level of the Ujong Baroh people who was high in fish consumption, which means have a high level of consumer dependence with the existence of a fish landing base.



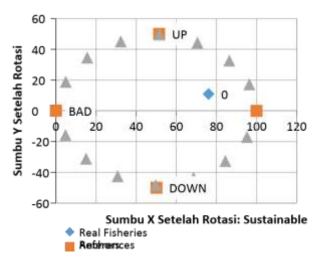


**Figure 6**. The role of each economic attribute explained in the form of Root Mean Square (RMS)

#### 3.3 Socio-cultural Dimension

The socio-cultural dimension used six (6) attributes / elements to predict the effect of sustainability on fish landing base facilities. Based on the results of the analysis, the value of the socio-cultural dimension of 76.08 including the fairly sustainable category could be seen in the appendix.

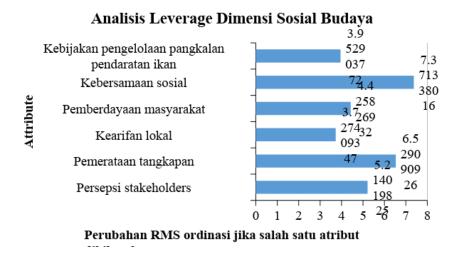
To see sensitive attributes that had a sensitive influence on the sustainability index value using leverage analysis. Based on the analysis, there were six (6), namely: (1) stakeholder perceptions, (2) equal distribution of catches, (3) local wisdom, (4) community empowerment, (5) social togetherness, (6) PPI management policies. The results of the analysis can be seen in the following figure.



**Figure 7**. The results of the Rapfish analysis which show the sustainability index of the socio-cultural dimension

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**Figure 8**. The role of each socio-cultural attribute explained in the form of Root Mean Square (RMS)

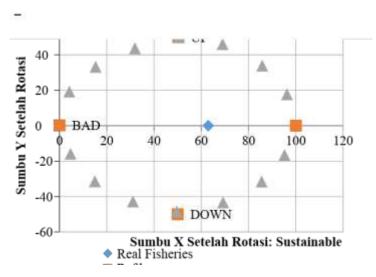
The level of social togetherness in fisheries activities was currently high and had been maximized. Very rare conflict between base and surrounding residents happened because community empowerment had been increasing. While educators in fisheries activities at the base of fish landings were still low, only up to high schools, it still needed to increase the human resources in the future. The community empowerment activities in fisheries activities at the fish landing base was important. Therefore it was necessary to educate the community to grow and develop the fish landing bases that will be able to increase community participation.

#### 3.4 Institutional Dimension

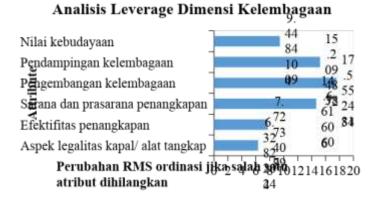
The institutional dimension used six (6) attributes / elements to predict the effect of sustainability on fish landing base facilities. Based on the analysis, the institutional sustainability values 32.76 included in the sustainability category can be seen in the appendix.

To see the attributes which were sensitive in giving sensitive effects to the value of the sustainability index using leverage analysis. Based on the analysis, there were six (6), namely: (1) legality aspects of the ship / fishing gear, (2) effectiveness of arrest, (3) fishing facilities and infrastructure, (4) institutional development, (5) institutional assistance, (6) value culture. The results of the analysis can be seen in the following figure.

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**Figure 9**. Results of Rapfish analysis that shows the sustainability index of institutional dimensions



**Figure 10**. Role of each institutional attribute explained in the form of Root Mean Square (RMS)

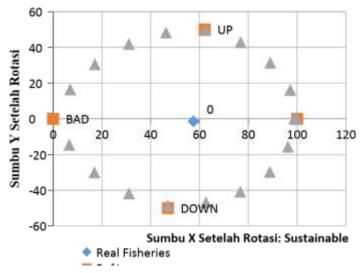
The mechanism of cooperation in the management of fish landing bases needed to be improved in government support for the construction at the fish landing base. Based on leverage analysis, data showed that the attributes of developmental development which had the highest value of 17.56, in the second position there were attributes of community companion (15.21) and the third position were attributes facilities and infrastructure with values (14.66) which had to pay more attention to sustainability Ujung Baroh PPI facility institutional dimension.

#### 3.5 Technology Dimension

The technological dimension uses six (6) scales / elements to predict the effect of sustainability on fish landing base facilities. Based on the results of the analysis, the value of

technological sustainability of 57.54 was included in the sustainable category. It could be seen in the appendix.

To see sensitive attributes which had a sensitive influence on the sustainability index value using leverage analysis, based on the analysis, there were six (6), namely: (1) the condition of landing sites, (2) the level of mastery of fisherman technology, (3) the form of processing before sale, (4) the level of utilization of information, (5) the nature and type of equipment, (6) availability of technology. The results of the analysis could be seen in the following figure.



**Figure 11**. Results of Rapfish analysis that shows the technology dimension sustainability index



**Figure 12**. The role of each technology attribute explained in the form of Root Mean Square (RMS)

It was necessary to build access to the development of technology for fish landing bases to become places of education and knowledge in the field of fisheries. It was also necessary for the builders of fisheries waste treatment technology to be processed into fish food and in order to increase the economic value of fish landing bases in the future. As well as the need to make an information technology network the level of mastery of fishermen in the future.

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Provision of physical facilities was a major supporting factor that must be available and continued to be developed for the management of fish landing bases. In terms of environmental monitoring, it was now available and needed to be increased in frequency, so that changes in the environment at the fish landing base were always monitored and recorded.

#### IV. Conclusion

Based on the results of the study, it showed that there were four dimensions that had sustainability which included the ecological dimension 57.43, economic dimensions 62.93, socio-cultural dimensions 76.08, technological dimensions 57.54, with 24 attributes while there was one institutional dimension 32.76 by having 6 attributes that had less sustainability in managing Ujung Baroh Fish Landing Base facilities.

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# The Diversity of Liverworts in the Dolok Sordang Sub-District of Sipirok, South Tapanuli, Indonesia

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**Abstract**: Research conducted in the village of Dolok Sordang bordering the Natural Reserve of Dolok Sipirok. Found 21 types of liverworts which contains the thalloid liverworts and leafy liverworts. The family has one of the highest types of variation is Lejeuneaceae (6) followed by the Lepidoziaceae (4), Radulaceae (3), Lophocoliaceae (2), Marchantiaceae (2), Plaghiocilaceae (2), Metzegeriaceae (1) and the Aneuraceae (1). These types found on the substrate and elevation. Research data will be used as a reference to see the diversity of types of liverworts in the Natural Reserve of Dolok Sipirok.

**Keywords**: diversity; liverworts; South Tapanuli; Indonesia

#### I. Introduction

Bryophyte is greenery, autotroph and a transition between cormus and thalloid plants. Moss can grow on the ground, do not have a network carrier, already has a cell that consists of cellulose (beautiful, 2009). Bryophyta is divided into 4 classes i.e. Bryopsida (Musci), Anthocerotopsida (Anthocerotae), Hepaticopsidae (Hepaticae) and Takakiopsida (Hasan dan Ariyanti, 2004).

In Bryophyte ecological role for the balance of forest ecosystems, i.e., such as peat is very dependent on the lining or cover Moss. So the existence of Lichen as the soil surface cover also affect productivity, community growth and decomposition in the forest. MOSS plants that grow on the forest floor helps reduce the danger of flooding, and able to absorb water in the dry season (Elena, 2011).

The role of the liverworts as indicators of environmental change has been reported in a variety of types of landscape (Holz and Gradstein 2005; Drehwald 2005; Larsen et al. 2007). Several Bryophytes are tolerant of metal and are able to withstand the levels of heavy metals that are toxic to other species. Heavy metals absorbed the atmosphere or substrate. Marchantia polymorpha, Solenostoma crenulata, is an example of metal tolerant populations. Scapania undulata is used to monitor water pollution (Govindapyari et al., 2010). Some other Bryophyte species has also been used as an indicator of air pollution caused by the activities of the community in the area of the settlement, which was polluted by smoke or industry (Dymytrova, 2009).

Research on Moss in Indonesia is still very little. Java has the most reports about MOSS if compared to other regions. Research on the Bryophyta is already done 150 years ago. Spearheaded by the SEAMEO Biotrop by holding training to start from 2001 to 2011. Other regions in Indonesia that already have data about the Bryophyta is Sulawesi. The latest research report that found 340 mosses, liverworts and 2 134 hornworts (Windandri, 2009).

In Sumatra, very little research regarding Moss. Found 490 species of mosses, but there were no reports of liverworts. Publications about liverworts first reported by Sandelacoste (1884) in Siregar (2015). Research on liverworts in Sumatra is performed by Siregar (2015), the location of the research includes mount Sibayak in North Sumatra Province. Found 163 different species of liverworts in 53 families and 22 ordo. Other studies conducted by Yana (2012) who reported 36 species of mosses which included 19 genus and 15 ordo.

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These data indicate that research on especially liverworts, moss is still very limited, especially in the southeastern part of North Sumatra Province.

#### II. Materials and Methode

This research was carried out in the village of Dolok Sordang area directly adjacent to the nature reserve of Dolok Sipirok. Forest area in the village of Dolok Sordang is representative of the type of vegetation is a tropical rain forest.

Beginning collection mosses using methods exploration of Ruqayah et al. (2014) that is by taking the example of moss in full (there are generation gametophytes and sporofit) as well as other required data noted as habitat, substrate, and color. Sampling collection is done by wrenching the colony following Moss substrate, then insert the paper in the envelope. Other data that needs to be noted among other habitat and substrate for its growth. Drying of specimens is performed by opening envolope. The wind dried specimens are put back in the envelope, ready to pack. Do the recording feature of the morphology, the type of substrate and elevation of each type were found.

#### III. Result

Found 21 types of liverworts contain the thalloid liverworts and leafy liverworts. The family has one of the highest types of variation is Lejeuneaceae (6) followed by the Lepidoziaceae (4), Radulaceae (3), Lophocoliaceae (2), Marchantiaceae (2), Plaghiocilaceae (2), Metzegeriaceae (1) and the Aneuraceae (1).

Liverworts were found in the village of Dolok Sordang grown on substrates that vary. 4 types of growing on the leaf (Caudalejeunea reniloba, Cololejeunea heterolobula, Cololejeunea occidentalis, Cololejeunea obtusifolia), 10 types (Riccardia limbata, Bazzania nitida, Leptoscyphus infuscatus, Mastigolejeunea virens, Metzegeria leptoneura, Plagiochila dendroides, Plagiochila gracilis, Radula Formosa, Radula javanica, Radula sumatrana) in the bark and twigs, 2 type (Marchantia emarginata, Marchantia polymorpha) in rock and soil, as well as there are 6 types of growing on bark, rocks and land (Lepidozia redacta, Lepidozia sucida, Lophocolea martiana, Schiffneriolejeunea tumida, Thysananthus convolutes).

Based on the height, the liverworts were found are at an altitude not too varied. 11 types (Caudalejeunea reniloba, Cololejeunea heterolobula, Cololejeunea occidentalis, Cololejeunea obtusifolia, Lepidozia redacta, Lepidozia sucida, Lophocolea martiana, Schiffneriolejeunea tumida, Thysananthus convolutes, Marchantia emarginata, Marchantia polymorpha) is found at an altitude of 400-800 meters above sea level, while 10 types (Riccardia limbata, Bazzania nitida, Leptoscyphus infuscatus, Mastigolejeunea virens, Metzegeria leptoneura, Plagiochila dendroides, Plagiochila gracilis, Radula Formosa, Radula javanica, Radula sumatrana) is found at an altitude of 800-1400 meters above sea level.

#### **3.2 Species Description**

# a. Bazzania nitida (Web.) Grolle

Plants green, glossy, shoots up to 1.8 mm wide. Leaves 0.9-1.3 x 0.5-0.7 mm, apex truncate, with 3 short decurved apiculi, with a vitta of 2-4 rows of wide rectangular cells

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extending nearly to leaf-apex. Underleaves not much wider than stem, 2-4-lobed to half of their length. Cell walls colorless. **Ecology:** Epiphytic or on dead wood, 1900-2100 m.

# b. Caudalejeunea reniloba

Plants 10–15 mm long, 1.8–2.2 mm wide. Cross-section of the stem with epidermis cells larger than medula cells; ventral merophyte 4 cells wide. Leaves imbricate, widely spreading. Leaf-lobe oblong, 1.2–1.5 mm long, 0.5–0.7 mm wide, margin irregularly toothed to entire near the apex; marginal cells of leaf lobe 16–18 x 12–15 μm, mid-leaf cells 45–50 x 25–26 μm, basal cells 35–45 x 35–30 μm; trigones large, cordate. Lobules 1/3 of lobe length, oblong, free margin incurved, apex with 2 teeth, first tooth elongate, with 3–4 cells long, another tooth small, 1-2 cells long. Underleaves contiguous, sometimes becoming enlarged in upper portions of shoots, orbicular to reniform, 0.35-0.5 mm long, 0.4-0.55 mm wide, bases rounded, margin entire and incurved, apex retuse and denticulate to entire. Disciform gemmae produced at the apex of shoots, 7–9 cells wide. Generative structures not seen. Ecology: found on the branch of shrub, at open place in lowland forest. Distribution: Sumatra, Borneo, Moluccas, Seram, West Irian, Papua New Guinea, Peninsular Malaysia, Philippines, Thailand, India, China, Bismark Is., Fiji, Solomon Is., Australia (Mizutani 1988; Gradstein *et al.* 2002).

# c. Cololejeunea heterolobula Tixier

Plants small, epiphyllous, densely appressed to a substrate, up to 0.8 mm wide. Stems up to 1 cm long, branched. Lobes ovate, apex rounded, 0.5 mm long and 0.3 mm wide, pseudovitta short, at base of lobe. Lobules saccate, inflated, keel straight, 0.2 mm long and 0.15 mm wide, with 2 teeth, apical tooth unicellular, hardly visible, median tooth 2-cellular, reduced lobules sometimes present. Asexual reproduction with up to 20-celled. Monoicous. Perianths ovoid, apex rounded, with ventral keels and short rostrum, 0.35 mm long and 0.2 mm wide. **Ecology:** Montane forest, epiphyllous, 2000 m.

#### d. Cololejeunea occidentalis

Plants medium-sized to large, shoots (1.5-)1.8-2 mm wide. Lobe ovate-reniform, c. 0.91.2 x 0.65-0.85 mm, apex broadly rounded, hyaline margin fimbriate, forming a border 1 row wide around most of the lobe, abruptly ending on ventral margin. Lobule ovate, 0.40.55 x 0.25-0.3, distal margin with 2 teeth, the apical tooth with a globose or club shaped hyaline papilla at the side or base of the apical cell. Chlorophyllose cells with smooth cuticle. **Ecology:** Montane forest, epiphyllous, 1700 m.

# e. Cololejeunea obtusifolia (E.W.Jones) Tixier

Synonym: *Cololejeunea pusilla* var. *obtusifolia* E.W.Jones. Plants small, shoots 0.5-0.8(-1) mm wide. Leaves approximate, spreading at 50-80° to the stem. Lobe up to 0.4-0.6 mm long, ovate, with broad rounded apex. Lobule reduced to a few (4-8) cells, 36-55 x 22-40 µm. Gemmae on non-marginal lobe cells. Autoicous. Perianth pyriform and terete, 0.4-0.6 x 0.3-0.4 mm, with 5 keels. **Ecology:** Epiphyllous in submontane and the montane forest, 1600-2000 m.

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# f. Lepidozia redacta

Plants very small and fragile, filamentous, with hair-like leaves, whitish green. Stems "zigzag"-like. Rhizoids at the base of underleaves, sparse. Leaves transverse, to succubous, leaf lobes biseriate at base, uniseriate filaments distinctly constricted at septa. Underleaves very small, 2-3-lobed. Autoicous. Perianths subcylindrical, eplicate, mouth with few bristle-like lacinia, not constricted. **Ecology:** On rocky slopes in heath forest and on decaying wood or soil in montane forest.

# g. Lepidozia sucida

Plants deep green, often forming cushions, stems pinnate or bipinnate. Main stems 2-3 cm long, sometimes stoloniform, branches regularly spaced, complanate or ascending and second. Stem leaves 3-lobed to 0.25 of their length, distant to approximate, 0.5-0.6 mm long, not much longer than wide, branch leaves 2-lobed to 0.25 of their length, oblong, much longer than stem leaves, 0.51 mm long, 1.5-2 x as long as wide, approximate, nearly longitudinally inserted, dorsal margin forming an angle of 45°-80° with the stem. Underleaves mostly 4-lobed, lobed to 0.5 of their length. Dioicous. **Ecology:** Forest floor in dense montane forest, 1800-2000

# h. Leptoscyphus infuscatus

Plants brownish, shoots 2.5-3.5 mm wide. Leaves opposite to alternate, patent, convex, rounded or oblong, 1.3-1.8 mm long, ventral margin arched, a dorsal margin less arched, shortly decurrent, apex with 1-3(-4) distant spiniform teeth, each tooth of 2-6 elongate cells. Underleaves free from the leaves or narrowly connate on one or both sides, deeply 2-lobed, the lobes longly acuminate, sinus V-shaped, with 1-4(-6) spinose teeth, short cilia or laciniae on each side. Cells hexagonal, 30-45 μm wide, walls thin, oil bodies 2-3 per cell. Dioicous. Perianths 3 x 1.5-2 mm, strongly inflated at base, mouth bilaterally compressed, truncate, shortly laciniate. **Habitat:** Epiphytic, on litter or on the ground, 1000-1500 m.

# i. Lophocolea martiana

Nees Plants medium-sized to large, shoots 2.5-3.5 mm wide. Leaves subsimetric, trapezoid, 1-1.5 mm long, alternate, dorsal base shortly decurrent, truncate at apex, 2-lobed, the lobes shortly acuminate. Cells thin-walled, 30-45 µm wide, trigones absent. Underleaves bilobed, narrowly connate with leaf base, with a tooth on either side. Autoicous. Perianths longly emergent, trigonous above, keels winged, mouth wide, trilobed, lobes laciniate. **Ecology:** On vertical rock, decaying wood and on ground in a montane rainforest, 1800-2100 m.

# j. Marchantia emarginata

Thallus narrow, ribbon like; light, yellowish, dull or dark green, with indistinct to distinct median band on dorsal surface; 2.5-5 mm wide. Margin entire, hyaline, reddish or purplish, 2-4 cells wide; thick walled; marginal cell smaller than inner cells. The epidermal cell without papillae. Epidermal pores not cruciate, 40-80 µm in diameter, bordered by 4-7 (8) rings of cells; inner opening bordered by cells with straight or convex inner walls; mucilage cavities absent or sometimes present in compact ventral tissue of thallus. Ventral surface purplish or brown, at least in the median portion; scales in 4 rows extending about

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25 to 50 % of thallus width. Median scales reddish or purplish; oil cells scattered. Appendages purplish, sometimes light red or pale brown; ovate; 5-16 (20) cells wide; apex acute or apiculate, with 1-3 cells apically; margin with sharp toothed, 1-2 (3) cells long, often curved towards base of appendage; terminal cells often lighter in colour, area (1-2.5) times smaller than that of inner cells (indistinctly smaller than inner cells). Laminal scales purplish, light red or sometimes hyaline; ovate to orbicular; apex acute or obtuse; oil cells sometimes frequent. Cupules ciliate; cilia 1-4 cells long, 1-2 cell basally; without papillae on the outer surface. Archegoniophore at apex of the main thallus. Stalk 5-14 mm long; scales purplish or light red. Scales surrounding base of stalk with appendage similar to those of median scale or with the acuminate appendage. Receptacle with distinct to indistinct rounded median projection on dorsal surface; 3.5-5 mm in diameter, deeply divided (0.7-0.9) of diameter into 5-11 lobes; nearly symmetric or asymmetric; lobe convex basally, usually broadened, twice emarginate, truncate or sometimes rounded apically; the lobe opposite the basal sinus often wider than the others. Involucres hyaline, entire. Scales of receptacle purplish or sometimes light red or hyaline, lobed or toothed, seldom entire, 3-16 cells wide; apex with a row of 3-7 cells. Spores light brown or brown. Antheridiophore at the apex of thallus. Stalk 5-14 mm long; scales purplish, sometimes hyaline. Scales surrounding base of stalk not differentiated, or with long acuminate appendage. Receptacle palmate, 4-7 mm in diameter, deeply dissected (0.5-0.7 of diameter) into 5-7 rays; asymmetric. Rays with entire or slightly crenulate margins; marginal cells smaller or hardly smaller tahn inner cells. Median cales of ventral surface of rays purplish, sometimes hyaline, with acute appendage, 2-6 cells wide with row of 1-3 cells apically. Ecology: found on soils, rocks (moist, damp or wet, shaded, semi exposed places, riversides, creeks) from 870 to 1450 m altitude. Distribution: Japan, Korea, China, India, Sri Lanka, Andaman and Nicobar Island, Thailand, Malaysia, Sumatra, Java, Borneo, Lesser Sunda Island, Bali, Moluccas, West Irian, Philippines, Marianas, Guam, New Guinea, New Britain, Solomon Island (Bischler-Causse 1989; Bischler-Causse and Piippo 1991; Song 2006; Lai et al. 2008; Chuah-Petiot 2011; Singh and Singh 2012).

#### k. Marchantia polymorpha

Thallus light or yellowish green, with distinct median band on dorsal surface. Margin crenulate. Ventral surface green or pale brown, at least in median portion, with 6 rows of scales extending over entire surface; scales reaching the thallus margin  $\pm$  visible at the margin in dorsal view. Median scales hyaline. Appendages light red to purplish; orbicular to reniform; margin with sharp, unicellular teeth. Laminal scales hyaline; apex rounded. Marginal scales hyaline or brownish; ovate to oblong; apex obtuse to rounded; margin irregularly crenulate, cell walls in upperpart with thickened angles. Cupules with ciliate lobes; outer surface with numerous papillae. Archegoniophore at apex of main thallus. Stalk up to 40 mm long; scales hyaline. Scales surrounding base of stalk hyaline with light red borders, rounded and crenulate apically, without appendage. Receptacle 8-10 mm in diameter, with 10-11 terete rays; nearly symmetric; dorsal surface without median projection. Involucres with ciliate lobe; reaching 1/3-3/4 of length of rays, hyaline, lobes 6-12 cells long with row of 3-4 cells apically, cilia 1-5 cells, 1-2 cells wide basally. Spores yellow. Antheridiophore at apex of thallus or of short lateral branched. Stalk 8-10 mm long; scales hyaline, without appendage. Receptacle peltate, 5-6 mm in diameter (young antheridium), shallowly dissected (0.1-0.2) of diameter into 6-8 lobes; almost symmetric; Budapest International Research in Exact Sciences (BirEx) Journal
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dorsal surface without papillae. Ecology: found on rocks of creek wall in exposed places, at 1500 m altitude. Distribution: Turkey, Syria, Lebanon, Israel, Iraq, Iran, Russia, USSR, Uzbekistan, Tadzhikistan, Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bhutan, China, Taiwan, Korea, Japan, Thailand, Vietnam, Malaysia, Indonesia (Java, Sumatra–new record based on the present study, Irian Jaya), Philippines, New Guinea, New Zealand, Tasmania (Bischler-Causse 1989; Söderström *et al.* 2010; Singh and Singh 2012).

# l. Mastigolejeunea virens

Plants up to 12 mm long, 1 mm wide, branched. Ventral merophyte 4 cells wide, stem 0.7 mm wide. Leaves imbricate. Leaf-lobe ovate, 0.6–0.8 mm x 0.5–0.6 mm, margin entire, apex obtuse to rounded; marginal cells of leaf-lobe 6–10 x 6 μm, mid-leaf cells 10–15 x 5–6 μm, basal cells 17–25 x 7–9 μm; trigones date; lobules 1/2 of lobe length, ovate, inflated, apex obliquely truncate with one long tooth consisting of 5–6 cells. Underleaves imbricate, cordate, margin entire, apex truncate. Generative organ not seen. Ecology: found on tree trunk at open place at lowland forest. Distribution: Sumatra (new record), Java, Borneo, Moluccas, Peninsular Malaysia, Papua New Guinea, Philippines, Thailand, Sri Lanka, Pacific Is., Australia (Gradstein *et al.* 2002).

# m. Metzegeria leptoneura

Plants green to yellowish-green. Thallus distinctly convex, margins strongly recurved to revolute, c. 10-20 x 0.7-1.2(-2.5) mm when flattened. Thallus margins with geminate, distinctly curved or falcate hairs, ventral surface of thallus lamina without hairs, ventral surface of midrib with falcate hairs. Midrib equally arched in cross-section, with 2 rows of dorsal cortical cells and 2-3 rows of ventral cortical cells, medullary cells 10-24, thick-walled. Gemmae occasional on thallus margin. Dioicous. **Ecology:** Epiphyte in montane forest, *Hagenia-Hypericum* forest and *Dendrosenecio-Lobelia wollastoni* paramo up to the alpine belt with *Alchemilla* mats, 2000-4200 m.

# n. Plagiochila dendroides

Plant yellowish to pale brown in dry specimen, 15–45 mm long, 1.7–2.2 mm wide, stem often with scale-like small leaves; branches very frequent of terminal and *Frullania*-type, forming a dendroid habit, apex of Frullania-type branches frequently elongated and becoming minute-leaved, flagelliform. Leaves remote, lobes ovate-oblong, 0.7–0.9 mm long, 0.4–0.5 mm wide; dorsal margin moderately revolute and shortly decurrent along dorsal stem midline; ventral margin shortly decurrent; apex of lobes with shallowly and asymetrically bilobed, or with 2–(3) teeth; dorsal and ventral margin entire. Leaf cells at middle portion 20–32 μm x 10–13 μm, at leaf-base 25–40 μm x 10–15 μm, at the marginal 17.5–25 μm x 10–12.5 μm; trigones indistinct, cuticle smooth. Underleaves vestigial, filiform. Androecia terminal or intercalary of branches. Gynoecia terminal on branched or intercalary with 1 or 2 innovation; bracts ovate-oblong, 1.2–1.5 mm long, 0.5–0.7 mm wide; perianth 1.5 mm long, 0.5–0.8 mm wide, margin of mouth rounded, with coarsely spinose. Ecology: found on tree trunks from lowland to lower montane forest. Distribution: Japan, Taiwan, Philippines, Malaysia, Sumatra (Mt. Singgalang, Mt. Talang), Java, Borneo, West Irian, Papua New Guinea, New Caledonia, Fiji (Inoue 1958; Inoue 1984).

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# o. Plagiochila gracilis

Plant small 10–20 mm long, 2.5–3.5 mm wide, yellowish brown in dry specimen; branches very rare, if present exclusively lateral intercalary. Leaves distant to contiguous, lobes oblong—ovate or obovate, widest at or around the middle part, 1.25–1.5 mm long, 0.6–0.8 mm wide; dorsal margin slightly revolute, long decurrent along dorsal margin; ventral margin not decurrent; apex rounded,

with 2–4 teeth wich two are often prominent (when plants are young, the two prominent teeth show a tendency toward bilobing); margin entire on dorsal side, ventral margin with 4–6 teeth on distal half; teeth on leaf margin 6–10 in total number, triangular, 2–4 cells wide at the base and 2–6 cells long. Leaf cells at middle portion 25–32.5 μm x 12.5–22.5 μm, basal cells 25–40 μm x 12.5–20 μm, marginal cells 22.5–30 μm x 12.5–17.5 μm; trigones medium sized, acute, cuticle smooth. Underleaves very vestigial, filiform, 1–2 cells wide at the base, 2–3 cells long. Generative organ not seen. Ecology: found on tree trunks from lowland to lower montane forest. Distribution: India, Buthan, Nepal, Sri Lanka, China, Japan, Taiwan, Thailand, Philippines, Sumatra (new record based on this study), Java (So 2001; Srivastava *et al.* 2006; Lai *et al.* 2008; Alam 2012).

## p. Radula formosa

Plant yellow to brownish in dry specimen, 10–15 mm long, 1.25–1.80 mm wide. Plants with numerous amentulose branches (= tiny leafy branches arising from leaf axils and hardly longer than the leaf), 0.5–0.8 mm long, with 4–6 pairs of small fusiform leaves. Leaf-lobes imbricate, widely spreading, concave, ovatel falcate, 0.7–0.9 mm long, 0.6–0.7 mm wide; margin entire, ventral margin flat, base of dorsal margin fully covering the stem; apex rounded, strongly incurved; trigones very large, nodulose, confluent, cuticle cells smooth; leaf-lobules remote, subquadrate, ca.1/2–2/3 of lobe length, widest in the lower half, apex rounded, incurved, abaxial margin straight to slightly arched, adaxial margin arched, strongly incurved toward the apex, base covering up to 1/2 of stem width; keel straight or sometimes slightly arched, not decurrent, extending at angles of about 50<sup>0</sup> with the stem, 0.3–0.35 mm long, sinus obtuse; rhizoid initial area ± convex, rhizoid not seen. Generative structures not seen. Ecology: found on tree trunks at lowland forest. Distribution: Tahiti, Ceylon, Japan, Philippines, Thailand, Malay Peninsula, Sumatra (without detailed locality), Java, Borneo, Sulawesi, Ceram, Papua New Guinea, Micronesia, Fiji, New Caledonia (Yamada 1979).

# q. Radula javanica

Plant green-yellowish in dry specimen, 10–25 mm long, 1.8–2.5 mm wide. Stem 0.1 mm in diameter, with irregularly pinnate branches. Leaf-lobes loosely to moderately imbricate, slightly convex, oblong-ovate, 1.2–1.3 mm long, 0.8–0.9 mm wide, margin entire, base of dorsal margin arched, covering 3/4 of stem width, sometimes fully covering the stem, apex rounded, not incurved; trigones small to medium sized, triangular, cuticle cells smooth; leaf-lobules remote to contiguous, ca.1/3 of lobe length, subquadrate, 0.3–0.4 mm long, 0.2–0.3 mm wide, apex obtuse, abaxial margin straight, adaxial slightly arched, base covering the stem 1/3–3/4 of the stem; keel slightly sinuate, slightly decurrent, 0.3–0.4 mm long, sinus wide, obtuse; rhizoid initial area convex, rhizoid few, brown. Dioicous. Androecia terminal on brances, with 5 pairs of bracts. Gynoecia terminal on branches with one subfloral innovation; bracts obovate, apex rounded; bract-lobule falcate-subrectangular,

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keel sinuate; perianth flat cylindric, 2.8–3.3 mm long, 1.1–1.2 mm wide, mouth two-lipped, repand. Ecology: found on tree trunks, tree branches, tree roots, and somewhat on rotten logs from lowland forest to lower montane. Distribution: Ceylon, India, Japan, Taiwan, Philippines, Thailand, Vietnam, Malay Peninsula, Sumatra (Mt. Kerinchi, Tarameast of Pajakumbuh, Mt. Sago), Java, Borneo, West Irian, New Guinea, Central and South America (Yamada, 1979; Yamada and Piippo 1989; Yamada 2000).

#### r. Radula sumatrana

Plant yellowish-green in dry specimen, 25–55 mm long, 2.5–3.2 mm wide. stem 0.15–0.20 mm in diameter, with irregularly pinnate branches. Leaf-lobes imbricate, flat, ovate, 1.5– 1.8 mm long, 1.2–1.3 mm wide, margin entire or sometimes with gemmae, ventral margin flat, base of dorsal margin covering the stem, apex rounded; trigones small, cuticle cells densely verrucose; leaf-lobules large, contiguous to covering each other at upper portion of stem and slightly remote at lower portion, ca.1/3-2/5 of lobe length, subquadrate, 0.4-0.5 mm long, 0.6–0.7 mm wide; apex narrowly to widely obtuse, abaxial margin slightly arched, adaxial margin undulate, covering the stem 1/2-3/4 of the stem width at lower portion and fully covering the stem at upper portion of stem; keel decurrent, 0.5–0.6 mm long, spreading at angles of ca.  $40^{0}$ – $50^{0}$  with the stem, sinus obtuse; rhizoid initial area convex, rhizoid few. Dioicous. Androecia not found. Gynoecia intercalary or terminal on branch with one subfloral innovation; perianth flat cylindric, ca. 3.0–3.75 mm long, 1–1.3 mm wide, mouth truncate, repand. Ecology: found on tree trunks, tree branches and rotten logs at primary and secondary forest, from lowland forest to lower montane forest. Distribution: Thailand, Sumatra (North Sumatra: Sibolangit), Java, Borneo (Yamada 1979).

#### s. Riccardia limbata

Thallus pinnate or bipinnate, usually 1-2 cm long. Main axes 5-9 cells thick, 0.5-0.9 mm wide, distinctly winged, ultimate branches, 3-4 cells thick with unistratose wings, 2-4 cells wide. Cortical cells in middle of branch  $25\text{-}35 \times 45\text{-}90~\mu\text{m}$ , branches  $12\text{-}30~\mu\text{m}$  thick in cross section. Dioicous or sometimes monoicous. Calyptra clavate, c. 2 mm long. **Ecology:** Montane forest, near rivers and streams in valleys, on wet rocks, rotting wood, tree boles, less often on soil, 1900-2400~m.

# t. Schiffneriolejeunea tumida

Plants 20–30 mm long, lebar 1.8–2.5 mm wide. Ventral merophyte 6–7 cells wide. Leaves closely imbricate. Leaf-lobe ovate to rounded, 1.4 mm x 1.3 mm, margin entire, rolled inwards along ventral and apical margin; marginal cells of leaf-lobe 9–12 x 9–10 μm, mid-leaf cells 25–30 x 12–15 μm, basal cells 35–37 x 12–15 μm; trigones cordate. Lobules 1/4–1/3 of lobe length, ovate-oblong, margin inrolled, apex truncate, with 2 teeth, first tooth larger than second tooth. Underleaves contiguous to imbricate, obovate to obcordate, 0.6–0.8 mm x 0.4–0.6 mm, apex truncate, often recurved. Androecia not seen. Gynoecia terminal on lateral branches, without innovation. Perianths obovate, with 5 rounded keels. Ecology: found on tree trunks at open place. Some populations were found on cultivated plants. Distribution: Sumatra (new record), West Irian, Papua New Guinea, Thailand, Solomon Is. (Zhu and Gradstein 2005; Kornochalert *et al.* 2012).

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# u. Thysananthus convolutes

Autoicous. Plants up to 20 mm long, 2.5–3.5, mm wide. Ventral merophyte 4-6 cells wide, diameter of stem 0.6 mm. Leaves closely imbricate. Leaf-lobe 1.5-1.6 x 0.8–1 mm, ovate, distal part recurved, asymmetric, margin entire toothed, apex rounded, recurved; marginal cells of leaf-lobe 9–10 x 6–7 μm, mid-leaf cells 25–30 x 7–10 μm, basal cells 42–47 x 10–13 μm; trigones large,cordate. Lobules 1/3–1/2 of lobe length, ovate, apex truncate, with 2 small teeth.Underleaves imbricate, spathulate, 0.6–1 mm long, 0.4–0.7 mm wide, margin toothed toward apex, lateral margin recurved, apex truncate. Androecia intercalary or terminal on short or long branches; bracts in 6–14 pairs. Gynoecia terminal onmain stem, with 1 innovation. Perianths cylindrical, 1.3–1.5 mm long, with 5 rounded keels. Ecology: found on tree trunks, rotten logs from lowland to lower montan forest. Distribution: Java, Sumatra, Borneo, Sulawesi, Moluccas, Peninsular Malaysia, Papua New Guinea, Philippines, Thailand, Solomon Is. (Haerida *et al.*2010; Kornochalert *et al.* 2012).

#### **IV. Conclusion**

Found 21 types of liverworts contains the bertalus liverworts and leafy liverworts. The family has one of the highest types of variation is Lejeuneaceae (6) followed by the Lepidoziaceae (4), Radulaceae (3), Lophocoliaceae (2), Marchantiaceae (2), Plaghiocilaceae (2), Metzegeriaceae (1) and the Aneuraceae (1). Liverworts were found in the village of Dolok Sordang grown on substrates that vary. 4 species of growing on the leaf, 10 species in the bark and twigs, 2 species in rock and soil, as well as there are 6 types of growing on bark, rocks and land. Based on the altitude, the liverworts were found are at an altitude not too varied. 11 species ares found at an altitude of 400-800 meters above sea level, while 10 species is found at an altitude of 800-1400 meters above sea level.

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# The Effect of the Mixture of Plastic Waste as a Lightweight Concrete Material

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**Abstract**: Concrete has many functions, and is also an important role in maintaining building stability and strength. By using plastic fiber as a lightweight concrete mixture material, it is expected to increase the compressive strength of the lightweight concrete, and also to reduce the negative impact of waste plastic bottles. In this study also added differences in FAS for each specimen 0.5 and 0.6. With the difference in variation in each lightweight concrete specimen, it is expected to be able to know the performance of concrete from the different variations of PET and FAS. The use of PET and FAS mixtures in lightweight concrete produces the lightest Concrete at 9% PET mixture of fine aggregate weight. In full there are two results,  $FAS\ 0.5 = 11045\ gr$ , and  $FAS\ 0.6 = 10981\ gr$ . But for the maximum weight of test specimens it cannot be said to be structural lightweight concrete because it does not meet the minimum weight requirement of 1850 Kg/m3. The Slump test on the specimen shows that the highest Slump test is in FAS 0.6 with 9% PET mixture, with slump height reaching 135 mm or 13 cm, and 125 mm or 12.5 cm in FAS 0.5 9% PET mixture. Cylinder absorbance showed that the average maximum absorption of FAS specimens was 0.5 = 1,268% and FAS 0.6 = 1,212%. The value of the lightest concrete compressive strength is the most optimum in the variation of 3% PET specimens in FAS 0.5 and variation of 6% PET specimens in FAS 0.6. that is 25 Mpa and 19 Mpa. And for the SPSS test states that there is a significant difference in the differences in the variation of PET and FAS test objects.

**Keywords**: Lightweight Concrete; FAS; PET; Variations; Test Objects.

#### I. Introduction

Nowadays there are many uses of lightweight concrete for the construction of high-rise buildings. Its own use is usually focused on making nonstructural walls or concrete parts. Lightweight concrete currently has many variations, such as concrete with ingredients added to fly-ash, coconut shells, coconut fiber, plastic fiber and much more even for general needs.

The use of lightweight concrete with additional plastic waste materials has been studied by many previous researchers. Using plastic waste as a lightweight concrete mixture material is expected to increase the compressive strength of the lightweight concrete, and also to reduce the negative impact of plastic bottle waste.

The use of plastic waste as a lightweight concrete mixture is one of the solutions to reduce the impact of increasing use of plastic waste. The nature of non-biodegradable plastics (difficult to decipher) is a problem that is still a solution to this. The use of plastic waste itself has been widely varied with other building materials, now one solution for the control of plastic waste is limited to being burned or recycled into the same material. Plastic waste combustion will cause various harmful substances that can damage health and natural conditions.

Nurmantian, Purnawan, Wibowo, (2014) the use of lightweight concrete is still intended for non-structural concrete only because generally lightweight concrete has a maximum compressive strength of 15 MPa. The solution to increase the lightweight concrete strength of foam is to add fiber to the mixture in the form of polypropylene fibers.

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There are many types of polyethylene plastic. One of them is PET (polyethylene terephthalate), Polyethylene is produced from the polymerization process of ethylene gas molecules which together form a series of molecular lengths to form a plastic (polymer). This research uses plastic waste with the type of polyethylene or commonly abbreviated as PET.

In this study also added differences in FAS for each specimen 0.5 and 0.6. It is expected that the results of this study can be an alternative to progress in the field of construction as well as one of the remedies for PET type plastic waste.

#### II. Review of Literatures

#### Concrete

Concrete is a function of its constituent material consisting of hydraulic cement, coarse aggregates, fine aggregates, water, and added ingredients. To know and study the behavior of combined elements (concrete constituent materials) requires knowledge of the characteristics of each component. Concrete has a large compressive strength while the tensile strength is small. Therefore for building structures, concrete is always combined with steel reinforcement to obtain high performance. The main characteristic of concrete, which is very strong against compressive loads, is also brittle / easily broken or damaged against tensile loads. In calculating the structure, the tensile strength of this concrete is usually ignored.

Concrete is the result of a mixture obtained by mixing Portland cement, water and aggregate (additives that vary greatly from additional chemicals, fibers to chemical building materials with certain comparisons).

If you want to make good quality concrete, in the sense that it meets more stringent requirements because of higher demands, it must be carefully considered the ways to get a good mix of concrete (fresh concrete) and hardened concrete produced is also good.

# **Lightweight Concrete**

Lightweight concrete is basically divided into several types, namely those with different weight / weight, namely lightweight concrete, heavy concrete and normal concrete, usually concrete has density according to the needs of the concrete itself. Lightweight aggregates can form light-weight concrete. The terminology of ASTM C.125 defines that light aggregate is an aggregate that produces lightweight concrete, including pumice, scoria, clay burning, perlite, slag and so on.

## **PET** waste

PET plastic waste (polyethylene terephthalate) often known by another name polyester is a thermoplastic recin polymer from the polyester group. PET is widely used in manufacturing such as beverage bottles, recin plastic, and a mixed material for making glass. PET is one of the most important ingredients in the textile industry, around 60% of PET fiber is used in the form of synthetic fibers, and bottle production reaches 30% of world demand. Usually PET is called polyester. PET consists of polymerization of ethylene terephthalate monomer units with repetition of C10H8O4 units. PET is generally recycled and given the number "1" which indicates the symbol can be recycled.

Bottles of packaging made with PET, if used too often and affected by high temperatures / heat, will cause the polymer layer to melt and release substances that are carcinogenic (toxic).

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Polyethylene terephthalate, called PET, is a high polymer derived from dehydrated ethylene terephthalate condensation. Ethylene terephthalate originates from the esterification of terephthalic acid with ethylene glycol. PET is milky white or light yellow, the polymer is very crystalline, and the surface is smooth and shiny.

In a wide temperature range with excellent physical and mechanical properties, long-term use temperature of up to 120 °C, excellent electrical insulation, even at high temperatures and high frequencies, electrical performance is still good, but corona resistance is less, Creep resistance, fatigue. Resistance, abrasion resistance, good dimensional stability.

# **Factor of Cement Water**

The cement water factor (FAS) is the weight of water divided by the weight of the low cement causing the aggregate grains to be small and the grain spacing to be short. If the cement water factor is too high then the slump will drop, this causes a decrease in compressive strength.

The cement water factor (FAS) is a ratio between the amounts of water to the amount of cement in a concrete mixture, the function of the cement water factor, namely: To allow chemical reactions that cause binding and hardening to take place, and facilitate workability. The higher the value of cement water factor, the lower the quality of the concrete strength. However the value of the cement water factor which is getting lower does not always mean that the strength of the concrete is getting higher. The value of cement water factor given is at least 0.4 and a maximum of 0.6. (Muhammad, Srikirana, 2017).

#### **Testing**

# **Concrete Tensile Strength**

Concrete compressive strength is the most important characteristic and one of the main performance of hard concrete, and is generally considered in planning concrete mixes. The concrete compressive strength is the concrete strength that holds the load in units of area.

Compressive strength is the ability of concrete to withstand how much force the force will be given in broad unity, but in the concrete still small drawings testing of concrete compressive strength can use compressive test equipment and cylindrical specimens measuring 150 mm x 300mm, by following SNI procedures or even ASTM C-39.

Several factors such as the size and shape of the aggregate, the amount of cement used, the amount of water usage, the proportion of the concrete mixture, the treatment of concrete (curing), the concrete age of the sample size and shape, can affect the compressive strength of the concrete.

The compressive strength of the concrete specimen is calculated by the formula:

$$fc' = \frac{P}{A}$$

fc : Compressive strength (kg/cm2)

P : Press load (kg)

A : Surface area of the test object (cm2)

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#### **III. Research Methods**

The research method was carried out by experimental methods carried out in the laboratory.

# **Research Materials**

- 1. 40 kg Portland Type-I cement, Semen Padang. With the condition that the cement is still tightly closed.
- 2. Smooth Aggregate in the form of River Sand from Binjai.
- 3. Aggregate Coarse Stone broken, from Tangkahan Patumbak with an average diameter of 20 mm.
- 4. Additional aggregates in the form of PET plastic fibers that have been processed into solid shapes and crushed to the size of sand.
- 5. Clean water free of organic content or water in the field PDAM.

#### **Preparatory stages**

- 1. Examination of fine aggregates (Sand), including: Test and analysis according to SNI SK namely filter analysis, water content, Saturated Surface Dry (SSD) water content, sludge content, specific gravity.
- 2. Coarse aggregate inspection, including: Test and analysis according to SK SNI, namely filter analysis, water content, sludge content, weight, density.
- 3. The PET aggregate examination includes: Test and analysis according to SNI SK namely filter analysis, water content, sludge content, fill weight, specific gravity.
- 4. Mix design with the SNI method after all data needed for the examination of the mixture material is obtained.

## IV. Results and Discussion

#### **Test Result**

The results of fine aggregate inspection can be seen in Table 1.

**Table 1.** Results of Fine Aggregate Examination

fications / nditions
< 5%
2,77 < 3,20
25 kg/m3
< 5%

Source: Research Results of 2018

The results of the rough aggregate examination can be seen in Table 2.

**Table 2.** Results of Rough Aggregate Examination

Examination	Result	Specifications / conditions
Sludge levels	0,60 %	< 5%
Sieve analysis	7,27	5,50 ~ 7,50

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Content weight	1.679 kg/cm <sup>3</sup>	$>1.125 \text{ kg/m}^3$
Absorption %	1,72 %	< 5%

Source: Research Results of 2018

The results of PET aggregate inspection can be seen in Table 3.

Table 3. Results of PET Aggregate Examination

Result	Specifications / conditions
2,58	2,20 < 2,58 < 3,20
$873,67 \text{ kg/cm}^3$	$> 1.125 \text{ kg/m}^3$
1,29 %	< 5%
	2,58 873,67 kg/cm <sup>3</sup>

Source: Research Results of 2018

The results of the mix mix of concrete mix designs can be seen as follows:

**Table 4.** Material requirements 5 specimens measuring 15x30 cm for fas 0.5

Variation	Cement	Sand	Gravel	PET	Water
variation	(kg)	(kg)	(kg)	(kg)	(Ltr)
0%	10,86	24,31	32,22	0	6,5
3%	10,86	23,58	32,22	0,729	6,5
6%	10,86	22,85	32,22	1,458	6,5
9%	10,86	22,12	32,22	2,18	6,5

Source: Research Results of 2018

**Table 5.** Material needs 5 specimens measuring 15x30 cm for fas 0.6:

Variation	Cement	Sand	Gravel	PET	Water
v arration	(kg)	(kg)	(kg)	(kg)	(Ltr)
0%	9,05	26,2	32,11	0	6,5
3%	9,05	25,48	32,11	0,788	6,5
6%	9,05	24,69	32,11	1,576	6,5
9%	9,05	23,90	32,11	2,364	6,5

Source: Research Results of 2018

Test results

**Table 6.** Results of weight of the test specimen 0.5

No	Variation	Weight of the Test Object
1	0%	11448
2	0%	11397
3	0%	11455
4	0%	11389
5	0%	11323
1	3%	11360
2	3%	11308
3	3%	11315

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4	3%	11327
5	3%	11303
1	6%	11166
2	6%	11254
3	6%	11247
4	6%	11245
5	6%	11251
1	9%	11102
2	9%	10986
3	9%	11017
4	9%	11039
5	9%	11082

Source: Research Results of 2018

**Table 7.** Results of weight of fas test object 0.6

No	Variation	Weight of the
NO	variation	<b>Test Object</b>
1	0%	11313
2	0%	11165
3	0%	11379
4	0%	11439
5	0%	11511
1	3%	11371
2	3%	11283
3	3%	11307
4	3%	11129
5	3%	11245
1	6%	11275
2	6%	11218
3	6%	11045
4	6%	11011
5	6%	11178
1	9%	10960
2	9%	10934
3	9%	11072
4	9%	10980
5	9%	10959

Source: Research Results of 2018

Based on the test results in the table above it can be seen that the addition of PET fibers tends to reduce the weight of lightweight concrete. The maximum reduction in the weight of lightweight concrete is found at 9% PET mixture of fine aggregate weight. In full there are two results, FAS 0.5 = 11045, gr, and FAS 0.6 = 10981 gr

The results of the slump test specimens can be seen in the following table:

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**Table 8.** results of 0.5 slump test results

Table 8. results of 0.5 slump test results			
No	Variation	Slump	
1	0%		
2	0%		
3	0%	110	
4	0%		
5	0%		
1	3%		
2	3%		
2 3	3%	110	
4	3%		
5	3%		
1	6%		
2	6%		
3	6%	120	
4	6%		
5	6%		
1	9%		
2	9%		
3	9%	125	
4	9%		
5	9%		

Source: Research Results of 2018

Tabel 9. Slump Results test fas 0,6

No	Variation	Slump
1	0%	
2	0%	
3	0%	120
4	0%	
5	0%	
1	3%	
2	3%	
3	3%	120
4	3%	
5	3%	
1	6%	
2	6%	
3	6%	130
4	6%	
5	6%	
1	9%	
2	9%	
3	9%	135
4	9%	
5	9%	

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Slump testing shows workability in lightweight concrete when mixing. With the results of these tests, it can be seen that the highest Slump test is in FAS 0.6 with a 9% PET mixture, ie with slump heights reaching 135 mm or 13 cm, and 125 mm or 12.5 cm on FAS 0.5 with variations of PET 9 mixture % on lightweight concrete.

The results of the absorption of specimens can be seen in the following table:

**Table 10.** Results of absorption of fas test specimens 0.5

No	Variation	Dry test weight	Heavy wet object	Absorption %
1	0%	11448	11554	0,93
2	0%	11397	11524	1,11
3	0%	11455	11543	0,77
4	0%	11389	11557	1,48
5	0%	11323	11535	1,87
1	3%	11360	11446	0,76
2	3%	11308	11435	1,12
3	3%	11315	11453	1,22
4	3%	11327	11420	0,82
5	3%	11303	11448	1,28
1	6%	11166	11321	1,39
2	6%	11254	11310	0,50
3	6%	11247	11329	0,73
4	6%	11245	11301	0,50
5	6%	11251	11334	0,74
1	9%	11102	11106	0,04
2	9%	10986	11108	1,11
3	9%	11017	11097	0,73
4	9%	11039	11085	0,42
5	9%	11082	11102	0,18

Source: Research Results of 2018

**Table 11.** Results of absorption of fas test material 0.6

No	Variation	Dry test weight	Heavy wet object	Absorption %
1	0%	11313	11529	1,91
2	0%	11165	11510	3,09
3	0%	11379	11521	1,25
4	0%	11439	11506	0,59
5	0%	11511	11515	0,03
1	3%	11371	11410	0,34
2	3%	11283	11398	1,02
3	3%	11307	11387	0,71
4	3%	11129	11387	2,32
5	3%	11245	11401	1,39
1	6%	11275	11282	0,06
2	6%	11218	11264	0,41

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3	6%	11045	11298	2,29
4	6%	11011	11256	2,23
5	6%	11178	11280	0,91
1	9%	10960	11101	1,29
2	9%	10934	11086	1,39
3	9%	11072	11081	0,08
4	9%	10980	11070	0,82
5	9%	10959	11095	1,24

Source: Research Results of 2018

Concrete absorption according to 03-0349-1989, maximum water absorption is 10%. The highest average absorption of FAS specimens was 0.5 = 1.268% and FAS was 0.6 = 1.212%, so the cylinder test material absorption met the requirements.

The results of the compressive strength test specimens can be seen in the following table:

**Table 12**. Results of absorption of fas test specimens 0.5

N.o.	Variation	Maximum load	Maximum load
No	Variation	(KN)	(Mpa)
1	0%	380	21,5
2	0%	380	21,5
3	0%	360	20,37
4	0%	370	20,93
5	0%	380	21,5
1	3%	450	25,46
2	3%	470	26,59
3	3%	450	25,46
4	3%	460	26,03
5	3%	450	25,46
1	6%	380	21,5
2	6%	440	24,89
3	6%	390	22,06
4	6%	400	22,63
5	6%	410	23,20
1	9%	300	16,97
2	9%	280	15,84
3	9%	310	17,52
4	9%	290	16,41
5	9%	310	17,52

Source: Research Results of 2018

**Table 13.** Results of absorption of fas test material 0.6

No	Variation	Maximum load (KN)	Maximum load (Mpa)
1	0%	350	19,80
2	0%	360	20,37

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3	0%	350	19,80
4	0%	350	19,80
5	0%	350	19,80
1	3%	340	20,37
2	3%	330	18,10
3	3%	340	19,24
4	3%	340	19,80
5	3%	330	20,37
1	6%	360	19,24
2	6%	320	18,67
3	6%	340	19,24
4	6%	350	19,24
5	6%	360	18,67
1	9%	280	15,84
2	9%	260	14,71
3	9%	260	14,71
4	9%	250	14,14
5	9%	260	14,71

Source: Research Results of 2018

The results show the most optimum compressive strength of cylindrical specimens in the variation of 3% PET specimens in FAS 0.5 and variations in the 0% PET test specimen in FAS 0.6 which is 25 Mpa and 19.9 Mpa.

#### V. Conclusion

From the results of research and discussion that has been done on cylindrical lightweight concrete specimens. Then the results are as follows:

- 1. The lightest weight of the concrete cylinder is found at 9% PET mixture of fine aggregate weight. In full there are two results, FAS 0.5 = 11041.40 gr, and FAS 0.6 = 10824.4 gr. It can be said that the fine PET aggregate reduces the weight of the concrete itself.
- 2. The test results of the Slump test on specimens show that the highest Slump test is in FAS 0.6 with a 9% PET mixture, i.e. with slump heights reaching 135 mm or 13 cm, and 125 mm or 12.5 cm in FAS 0.5 with a variation of 9% PET mixture on concrete. Then it can be concluded that the addition of the cement water factor in the concrete can increase the amount of water or the mortar has high (dilute) friction.
- 3. The results of the absorption of cylindrical specimens show that the average absorption of the highest FAS specimens is 0.5 = 1.268% and FAS 0.6 = 1.212%, whereas according to the maximum absorption requirements is 10%, the cylinder test specimens meet the requirements.
- 4. The value of compressive strength of lightweight concrete with PET mixture as a substitute for fine aggregate shows the most optimum compressive strength of cylindrical specimens in variation of PET test material 3% in FAS 0.5 and variation of 0% PET test material in FAS 0.6 which is 25 Mpa and 19.9 Mpa. It can be concluded that the effect of adding PET tends to increase the compressive strength of the concrete and the effect of the addition of FAS tends to reduce the value of the compressive strength of the concrete.

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# **Suggestion**

From the results of the research that has been done, the suggestions that can be conveyed for this research and subsequent research are. In making mix designs the results of mixing are done more carefully so that the mixture on the mortar can be more evenly distributed. For the use of gravel or broken stones should be reduced or replaced with lighter materials, because it affects the specific gravity of the concrete.

The PET or plastic fiber material used should use plastic seeds from the manufacturer or workmanship with a special plastic crusher machine so that the aggregate size and weight of each aggregate can be equated to facilitate workmanship.

For the development of further research can use a number of variations that are more or the same but without using broken stones as coarse aggregates. The waste plastic used can be varied to replace the cement water factor so that the best plastic material can be seen.

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# Cyber-attacks Based on Legal Requirements and International Relations of Governments

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Abstract: Nowadays, the change in the scope of power from hard power to soft power has also shifted the process of military attacks to cyberattack. Cyber-attacks, like armed attacks, must fit into the framework of humanitarian law. Therefore, government responsibilities are also determined based on these changes, and as governments have responsibilities in military strikes, their responsibilities for cyberattacks should also be identified. Therefore, the present study makes use of analytical-descriptive method based on existing documents to examine this issue. Ultimately, the present study concludes that a cyberattack could be described as armed use of force in accordance with Article 2 (4) of the United Nations Charter. On the other hand, a widespread cyberattack on the basic infrastructures that causes material damages or casualties comparable to an armed attack with conventional weapons, gives the affected government the right to seek legitimate defense. Also, governments can resort to legitimate defense in response to a cyberattack that does not amount to an armed attack but provides the settings for an impending armed attack with conventional weapons.

**Keywords**: cyberwarfare; governments; international relations; legal requirements

#### I. Introduction

The growing phenomenon of cyberattacks in the virtual world has occupied the minds of many politicians and lawyers. Therefore, many papers and lectures have been presented to offer a solution to this international threat. Perhaps in the past, land, sea and air conflicts were the most prominent examples of hostile relations among governments. But today, with the advent of various malware such as Stuxnet and Flame, various virtual networks, media, and so on threaten the security of nations. Cyberattacks, soft warfare and post-modern war are just some of the names given to these threats. Therefore, given the high potential of cyberattack, such as the ability to make changes to tax records in the stock market, sending error messages to shutdown nuclear systems, opening a dam, disrupting the air traffic system to facilitate aircraft accidents, etc., has led governments to establish a narrow and international definition of cyberattack, since achieving this definition can be an effective step in identifying these attacks and the legitimate potential responses to them (Duncan, 2007:1023).

Since these attacks may expose defense, law enforcement, banking, commerce, transportation, and scientific activities, and a large percentage of public and private sector transactions through the network to sabotage by certain individuals who gain unauthorized access to the network, they can disrupt the daily routines of a country. So, the debate on cyberattacks in cyberspace and cybercrime is on the rise every day (Lehman & Potter, 1395:207)

But the legal challenge to address such attacks is a matter of explanation of its nature and interpretation as a type of war. As in the military wars between two or more countries, the concepts of beginning and end of the war, invading and defending sides, the allies, the enemy, and even the neutral and the front are completely clear. However, in the field of cyberwarfare, these concepts are unclear. For example, the beginning and end of cyberwarfare cannot be timed; incidentally, cyberwarfare could be effective when its time is undetermined. The target country in these types of wars becomes aware of an attack when the enemy's goal is almost

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achieved and in fact, it becomes aware of the destruction and smoke caused by the attack (Libicki, 2009:170).

There is another ambiguity in this context relating to the invading country or forces. The fact is that because of the virtual nature of the space and the ambiguity in the nature and type of attack, no one can easily identify the invading country or force. The nature of cyberattack does not allow tracing the attacker. While the location of the cyberattack is not known and the dispatched virus or malware does not show any sign of the attacker. Also, due to the multiplicity of actors in the cyberspace, including states, government agencies and NGOs, terrorists, hackers, and even individuals, identification of the attacker is more difficult and complicated. It should be noted that in the cyberattacks that have taken place so far, the country or attacker have not been identified based on the cyberattack and clear evidence, but on the basis of the political atmosphere and the intentions and goals of the countries (Sanger, 2015:1).

#### II. Theoretical Framework

Perhaps, the first cyberattack was the Morris Worm case in 1988. It was one of the first known worms to disrupt cyberinfrastructure across the United States. Therefore, Robert Tappan Morris, currently a professor at the Massachusetts Institute of Technology, was the first creator of computer worms. According to him, he just wanted to measure the size of the Internet. In December 2006, NASA shut down its emails with their attached files before launching the shuttle for fear of hacking. At that time, the Business Week magazine reported that unknown foreign intruders had access to a recent US space launch program (Reverson, 2012:15).

#### 2.1 Tallinn Directive

This directive was set up in Tallinn, Estonia, in 2009 to the review the laws ruling over cyberwarfare. The project was designed by international law experts and researchers and aimed at outlining legal and law norms in these modern wars. The emphasis of this directive on cybercrime actions against cyber-equipment, for example, the use of cyber operations against a state's critical infrastructure or a cyberattack targeting the enemy's command and control systems. So, the purpose of this directive does not pivot around cyber operations against material equipment, such as air raid and bombing of the cyber-control centers. It also does not include traditional military electronic attacks, such as radio jamming. Such actions have already been defined under the law of armed conflicts. Thus, the Tallinn directive includes both international and non-international armed conflicts, and covers the laws ruling over cyberattacks and related items such as the responsibility of governments and the law of the high seas (Schmitt, 2013:16-19).

# 2.2 The position of cyberattacks from the Perspective of Banning the Use of Force and the Concept of Invasion

The subject of discussion in this section is to include cyber operations in the scope of banning the use of force in international relations. Although it should be noted that the intention to exert pressure to identify and recognize cyber operations as using military force does not suffice. However, military force is nothing but an extreme form of intervention, such as diplomatic and economic pressure, to force a victim country to submit to something. Accordingly, if cyber operations are used for this purpose, it can be easily categorized as a

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type of using force (Roscini, 2014:45). Therefore, cyber operations can include cyber-abuse to collect and monitor information, up to cyberattack, as well as from removal, tampering and modification of the software to damaging the infrastructure belonging to properties and individuals. Such a variety of actions in cyber operations has led to various opinions, including the inclusion of it as a form of using force (Roscini, 2014:52).

In this regard, the International Court of Justice, in its commentary on paragraph 4, Article 2 of the Charter which prohibits the use of force against states, has argued in the case of Nicaragua that such a ban is a customary rule of international law. Also, in a verdict regarding military operations, this court sees this clause as the cornerstone of the Charter. Therefore, cyber operations can be subject to this clause, provided that under the law of international responsibility of governments, such actions have been taken by governments or at least attributable to governments. As resorting to force does not necessarily involve using direct military force by the states or groups or individuals under their control, in cyber operations also, for example, malicious software of a rebel group and teaching how to use it can be included under the use of coercion rubric (Weller, 2015: 1112-1114).

# 2.3 Cyberattack from the Point of View of laws and Elements of War

The prerequisite for the implementation of armed conflict law is the existence of armed conflicts, which despite the growing importance of this issue, is not included in the present discussion, because no international entity has reported any cyberwarfare incident so far. The only example of the use of armed conflict law in cyber operations can be seen in during the international armed conflict between Georgia and Russia in 2008 that was used to continue the war. For example, if a hacking attack occurs after the war between the two countries, then the hackers involved in this cyberattack would effectively have the same legal status as the soldiers in the war (Gladyshev *et al.*, 2015:139).

Thus, international law on cyber operations requires military combatants to comply with regulations such as the principle of military necessity, distinction between military and civilian populations, proportionality, respect for protected individuals and objects, impartiality and the prohibition of particular warfare methods, such as the breach of treaty. Although there are doubts regarding the enforcement of cyber operations laws during armed conflicts, but disagreements over the ease or difficulty of evaluating such operations are expressed under these regulations. Failure in the definition of war and statement of various types of warfare, makes it difficult to include cyber operations in the law of war.

Thus, although there is no credible and legal definition of cyberwarfare or cyberattack in general international law, specific treaties, customary law, and doctrine, there are practical descriptions based on technology and how it operates. The United States Department of Defense has provided definitions of cyber operations concepts and actions, such as attacking computer networks, defending computer networks, and exploiting computer networks. Thus, attacking computer networks as using a computer to disrupt, degrade or destroy information available on computers and computer networks is defined as: protection, monitoring, analysis, detection and response through authorized computer network and The Ministry of Defense intelligence systems activities, and ultimately the exploitation of computer networks to collect data from a target or network or automated information systems. Overall, in the event of military conflicts, these actions can be called cyberwarfare.

Also, the International Committee of the Red Cross (ICRC), in 2005, in explaining the cyber scope in warfare and attacks in the traditional studies of international humanitarian law, stated its position on the legal applicability of international humanitarian law in cyber

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operations during armed conflict in two official texts. Similarly, the Red Cross Committee of the United Nations deems cyberwarfare and as a result, the application of international humanitarian law cited by the International Committee of the Red Cross, solely related to the co-existence and interference of armed attacks and cyber operations as follows:

"... the International Committee of the Red Cross (ICRC) drew the attention of countries to the potential implications of international humanitarian law in cyberwarfare, i.e. the attack on computer networks during armed conflict situations, which could include disastrous situations such as intervention in air traffic control systems and consequently, collision and crash of planes, interruption of urban water and electricity supply, or destruction of nuclear and chemical facilities. So, the above committee demands that all parties to the conflict comply with the international humanitarian law regarding the cyberwarfare means and methods in accordance with the principles of separation, proportionality and precaution in the attack." (Saxon, 2013: 210-220)

But the most important issue in cyber operations is the confusion about the scope and definition of the time of occurrence of this phenomenon and the term *attack* in the international humanitarian law and the *military attack* in the law of war. According to the interpretative statement of the International Committee of the Red Cross (ICRC) that takes the term *attack* equivalent to military action, the attack on cyber-networks must definitely take place in this context, so the title *attack* can be applied to it. But the Red Cross, in its recent statement mentioned the uncertainty in the law as such:

"... certainly, in the face of armed conflicts, international humanitarian law will be applied to cyber operations along with traditional weaponry, but the problem will be exacerbated when international humanitarian law seeks to apply only to cyber operations. Can such a situation be called a military conflict under the Geneva Conventions and other humanitarian treaties? Does the applicability change according to the situation? The answer to these questions solely depends on the performance of governments in the future. So, the cyberattack on Estonia in 2007 and the use of Stuxnet Worm against Iran's Natanz Nuclear Power Plant in 2010 cannot be covered by international humanitarian law because the concept of attack has not been realized (*Ibid*: 223).

# 2.4 Responsibility of Governments in the Event of Cyberattack in International Law

Given the importance of the Internet and computers and cyberinfrastructure for each country and its affiliates and institutions, their improper and hostile use can be extremely dangerous too. Hence, national governments need to be sure of their national security and economic networks. Therefore, in the events of the breach of security and the principle of a state's territorial integrity by a hostile country, it must be held accountable in order to prevent misuse of this useful, inexpensive and accessible tool.

Thus, with regard to the international responsibility of the state, three theories are proposed: the theory of error, risk, and liability arising from prohibited acts. So, the main problem in raising the government's responsibility caused by cyberattacks, due to the complexity of Internet networks and online actors, is the perpetrator of the cyberattack and reliance on risk theory cannot serve the purposes of this theory. In other words, the risk theory is emphasized where the perpetrator is known, and elsewhere, proving fault can help in attributing the action to the assisting government. In fact, by proving the fault, the government related to cyberattack can be found. The fault in this case will be used to attribute the action, and proof of the fault can be one of the means of asserting the attribution, while some also believe the fault can be used in proving the causal relationship in the domestic law. Therefore, the

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emphasis on fault or malevolence can to some extent ensure that by finding the *culprit* we find the *perpetrator* as well. Therefore, the theory of fault seems to be more appropriate for invoking the government's responsibility caused by cyberattacks. It should be noted that the basis of international responsibility of the government caused by cyberattacks comes from the theory of *fault*, so the result of liability is not only limited to compensation, but the offending state is required to suspend and refrain from repeating violation of its obligation (*Responsibility of the State for Internationally Wrongful Acts* article, 2001: 30-35).

In line with this, one of the institutions that has assumed this responsibility for governments is the International Court of Justice, which has repeatedly invoked the international responsibility of states for breach of international obligations. In the most famous of these cases, the Corfu Channel case, the Court ruled that the mere existence of mines in the territory of the Albanian State could not render that state responsible, but the Court for the lack of notification about the existence of a minefield in the territorial sea to the ships of the third-party countries by Albania issued a ruling for its international responsibility. In the Court's view, this commitment rests on three general principles: the basic humanitarian principles, freedom of maritime communications, and the commitment of each state not to allow a state to consciously use its territory for actions contrary to the rights of other countries. In this category, the Court considered other realistic circumstances that if Albania at the last moment - for example, less than 24 hours from the time of the British warships collision - had become aware of minelaying, failure to inform the third-party countries due to difficulty or impossibility could be acceptable. The international responsibility problem has also been raised in other cases, such as Congo vs. Uganda, Bosnia Genocide, etc. (Tsagourias, 2015: 67). Moreover, based on the analysis provided, governments can be held responsible for the internationally illegal practices of governments in cyberspace to attempt to violate the rights of other countries in their territory. The attempt of governments to violate the rights of other countries can be assessed in such a way that firstly, the use of the Internet is not illegal per se; and secondly, data transmission by computers is not necessarily the source of harmful activities (Tsagourias 2015: 69).

The obligation of the hostile governments is another responsibility of the states. Based on the legal principles of neutrality set forth in the fifth and thirteenth Hague Conventions in 1907, the conflicting parties must respect the inviolability of the territory of neutral states that are prohibited to direct the hostilities, the exercise of hostile party rights and establishing operational bases in the land of neutral states (Hague Convention V,1907: Arts. 1, 2, 3; Hague Convention XIII, 1907: Arts. 1, 2, 5).

# 2.5 Government Response to Cyberattack

Given that the victim governments can identify the origins of cyberattack and attribute it to a country, they will have several options available to them as follows:

#### a. The Right to Legitimate Self-defense in Cyberattacks

According to a model drawn up by the law of war, the response of a government to an armed attack by another government must fulfill three conditions to be recognized as self-defense: necessity, proportionality and urgency. To satisfy the condition of necessity, a state should connect the attack to a specific source, specify the attacker's intention of attack, and conclude that the country should use force in response. The principle of proportionality states that the force used to respond to an attack should be proportionate to the initial attack. The principle of urgency prohibits a response to an attack after a long time. Based on the principle

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of urgency, there is no other provision for defensive action immediately after an armed attack (Saberi Tilki, 2014:79).

Thus, with regard to the legitimate right of the states to defend themselves, the Article 51 of the United Nations Charter stipulates: "In the event of an armed attack against a United Nations member, until the Security Council takes the necessary action to maintain international peace and security, no provision in this Charter will prejudice the inherent right of self-defense, whether individual or collective. Members must report immediately to the Security Council the actions taken to exercise their right to self-defense. These actions in no way affect the authority and responsibility of the Security Council under this Charter to take action in order to maintain and restore international peace and security when it considers it necessary."

Accordingly, according to this article, in the event of the attack and waging war and resorting to force, by invoking an exception to the prohibition of the use of force set forth by the United Nations, the countries may exercise the right to national self-defense (Committee on Deterring Cyberattacks, 2010:162).

This right is exclusively for the compensation of damages to the victim of military strike, since such attacks subject to paragraph 4, Article 2 of the Charter and customary law to resort to force and its legal requirements. On the other hand, the legitimate self-defense is an effective defense in terms of nature, essence, continuity and scope, which otherwise involves illegal resort to force by a country. Therefore, it can be argued that passive cyber-defense, which only tries to deter attack, is a legal defense. Only in case of proactive defense, whether in cyberspace or physically, the law of legitimate defense is invoked directly by the government or the group involved in the conflict.

Moreover, only governments have a legitimate right to self-defense, so, private entities such as companies that are subject to cyberattack cannot react in accordance with the right to legitimate self-defense regardless of its severity. Their response will be subject to domestic and international laws. However, an attack against national governments could be considered military strike and the government must effectively defend itself. It should be noted that the requirements for legitimate defense such as urgency, certainty and lack of time for reflection must apply (Committee on Deterring Cyberattacks, 2010:163).

# **b.** Referring to the International Court of Justice

The country responsible for cyberattack can be summoned to an international tribunal, including the International Court of Justice, to compensate for the violation of Article 2 (4) of the UN Charter and the principle of non-intervention. However, it should be noted that determining the amount of damage caused by a cyberattack is difficult because financial institutions may be hesitant in providing accurate information and determining the amount of damages. Likewise, the International Court of Justice like other international courts lacks binding jurisdiction, so both parties must agree to refer the case to the Court. In accordance with the Article 96 of the United Nations Charter, another option may be asking for an advisory opinion from the International Court of Justice on the legitimacy or non-legitimacy of cyberattack. Such opinions are optional and non-binding, although they are effective in creating a customary international rule (Conforti, 2005: 276).

#### c. Retaliatory Action

The country victim of a cyberattack can resort to retaliation and non-military countermeasures against the attacker. Under Article 49 (1) of the international liability of

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governments act, the affected government may take countermeasures against the government responsible for international misconduct in order to force that government to fulfill its obligations. However, according to Article 50 (1) of the plan, such countermeasures that are not proportional to the initial action are prohibited. In fact, the claim that the victim country of cyber operations cannot retaliate by sending fraudulent codes unless a cyberattack has reached the brink of an armed attack, is unreasonable. Another issue is that the expected consequences of a countermeasure cyberattack should be proportional to the consequences of the initial attack. Such a calculation is difficult because, like biological weapons, the virus dispatched to cyberspace may be propagated uncontrollably (Delibasis, 2007: 364).

# III. Research Methodology

The research method of this study to investigate and prove the raised hypotheses according to the nature of the subject, is the descriptive-analytical method and the method of collecting information is the library method using written references.

Definition of concepts and terms

# **Cyberspace**

The global grid of computer systems interconnected by the Internet, communications infrastructure, online conference facilities, databases, and information organizations that are generally known as The Network. However, such a system generally means the Internet, but this term may also be used to refer to a specific and limited electronic information space of a company or military and government organization, etc. (Andress *et al.*, 2014: 4).

# Cyberinfrastructure

Cyberinfrastructure is the communications, storage, and computing resources that act based on that information system. Apparently, this damage should be physical because measures such as supervision seem to stay outside the definition of the expert group of regulators (Schmitt, 2013: 24,25).

#### **Cyberattack**

Cyberattack refers to the use of intentional attempt to modify, disrupt, deceive, reduce or eliminate computer systems or networks or information and programs or transmission through these systems or networks. Thus, cyberattack is the expansion of policies in cyberspace by government and non-governmental actors, to start attack or in response to a serious threat against national security (Shakarian, 2013:32).

#### Cyberwarfare

It is a war that governments as the main actors wage it to destroy the facilities, capabilities, and strengths of the enemy. The purpose of this war is the submission of the enemy to demands of the invading country. It should be noted that in this war, governments can use their cyber-army, non-state actors, or even hackers and individuals. Nonetheless, the main actor and leader is the state (Lee, 2013:105).

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#### IV. Discussion

The new conditions of cyberattacks have created new challenges for the use of customary humanitarian law such as necessity, proportionality, distinction and impartiality. In other words, there are problems with the basic principles of international humanitarian law in dealing with cyberattacks. Also, cyberattacks are often not immediately lethal or destructive and may only create temporary breaches in network systems, it is difficult to assess whether a cyberattack is appropriate or not. On the other hand, the distinction between military and civilian people directly involved in the war and the involved civilians is impossible and ultimately, the hidden source of cyberattack complicates the implementation of neutrality tasks.

Therefore, in the wake of legitimate defense against such attacks, topics such as necessity are not specific to cyberattack, and in general in any attack if the need for the attack is necessary for military purposes, that attack is legitimate, otherwise, any part of the attack which is not necessary for military purposes is illegitimate.

Also, on prohibition relating to proportionality of the attack, where attacks have caused the loss of civilian lives and damage them, damage to non-military objects, or a combination of them is more than the predicted benefits of warfare, it prohibits the attack. In the analysis of proportionality, it should be considered that a military decision-maker must not exaggerate assessment of potential civilian casualties, destruction of non-military property and the loss of necessary non-military assets to achieve military ends. Here we are faced with a unique challenge on proportionality assessment of cyberattack. It's very hard to assess whether this attack, with regard to categories of objects, could be considered as a direct effect of instances of non-fatal temporary or severe cyberattack.

Also, there is a challenge in examining the distinction principle in these attacks. This principle claims there is a distinction between military individuals and objects or else, and targeting of military personnel in the battlefield. Also, military commanders should use a tool to correctly distinguish between military and non-military personnel and objects, and in other words, humanitarian law prohibits cyberattacks that are uncontrollable, without anticipation or without distinction between military and non-military personnel and objects (Schmitt, 2013:178). In some cases, there are situations where cyberattacks are legitimate, because the target is specifically military personnel and the principle of distinction is applicable, such as when cyberattack targets a military air traffic control system and this impedes military transportation. Similarly, there are situations in which the cyberattack is easily illegal, such as attacking some targets like hospitals, museums, and places of worship. These places, even if they are a part of targets and benefits of military strikes, still deserve the necessary protection. Of course, things are not always that simple. Moreover, the traditional support of the abovementioned objects needs a complicated analysis of cyberattack because attacks occur in cyberspace and definitely attack on networks that steer those places should also be illegal, but due to the large number of military and non-military actors, the likelihood of using these seemingly civilian targets by military personnel increases and because of the double standard in this case, the required protection will not arise in the discussion of distinction.

Also, the neutrality principle refers to a situation in which a state can permanently, like Switzerland, or temporarily at a particular time of conflict, declare neutrality relative to the conditions of war and consequently assume rights and responsibilities (Heinegg, 2012:35). The challenge that is posed is to assess the lawfulness of these attacks, primarily because the cyberspace is without boundary. In this regard, since the space is used by both military and

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civilian personnel, and because of interconnectedness of the cyberinfrastructure across the globe there is no boundary and it is not under the jurisdiction of any state (Lobel, 2012: 630); thus, the duty of the hostile states to respect the neutral territory should be considered in a broad manner, i.e. the prohibited acts include all actions and operations that have a negative effect on the functionality of cyberinfrastructure and computer networks, or render them useless (Talbot Jensen2012:822).

#### V. Conclusion

Cyberspace is an Internet space, in which the countries hide many of their intelligence data, and even in some cases, make them inaccessible to conduct their national and military affairs. In this context, a concept such as cyberwarfare is a term that is being used in the military literature of the world today, replacing the international conflict concepts in the past. Cyber-attacks have created a new challenge in the field of humanitarian law principles and the legal obligations of governments. Most cyberattacks cause temporary handicaps to achieve the results of the attack, making it difficult to assess whether a cyberattack was appropriate or not. The dual use of Internet infrastructure and the potential participation of civilians together with the military, complicates the distinction between them in cyberattacks and ultimately, the use of zombie computers and host servers raises many questions about the rights and obligations of neutral countries. Therefore, the present study examined the legal obligations of states in dealing with cyberattacks in their international relations, as well as their privileges to other non-governmental organizations in the face of cyberattacks, and ultimately examined the responses of the affected state against these attacks. In this regard, following the statement of hypotheses, questions and challenges relating to government legal requirements in cyberattack, the introduction discussed the background of the subject, history of cyberattacks, Tallinn directive, the position of cyberattacks in terms of prohibition of the use of force and the concept of invasion, cyberattacks from the perspective of laws and elements of war, responsibility of the governments in the face of cyberattacks in international law, and the response of governments to cyberattack. Finally, the data of the present research were analyzed. The results of the research showed that the cyberattack, in accordance with Article 2 (4) of the United Nations Charter, can be described as the use of armed force. On the other hand, a widespread cyberattack attack on the basic infrastructure that causes material damage or casualties comparable to an armed attack with conventional weapons, gives the victim government the right to seek legitimate defense. Also, governments can resort to legitimate defense in response to a cyberattack that does not amount to an armed attack but provides the settings for an impending armed attack with conventional weapons.

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# A Review of Inconvertibility of Iran Nuclear Deal into an International Treaty

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Abstract: International treaties become legal rules based on a social reality. The lawyers are convinced that JCPOA (Joint Comprehensive Plan of Action) is something novel and this has led them to identify the type of this agreement and to understand the scope of the requirements and obligations of the parties to the negotiations. The present study, based on analytical-descriptive method and by using the documents available in this field, has examined the differences and reasons for not converting Iran's nuclear deal into an international treaty, and by understanding the nature of this contract, ultimately concludes that such an agreement, because it is more political in nature based on the distribution of power and the power equation in the international arena rather than having a legal nature, therefore it cannot be converted into an international treaty.

Keywords: international treaties; JCPOA; Iran; Agreement; power.

#### I. Introduction

International treaties have long been a source of international law for regulating the relations between the main and primary actors of international law, that is, the countries. Today, this source provides for the establishment of relationships among a new group of international law actors, i.e. international organizations with countries, as well as relations between international organizations. On the other hand, the expansion of relations between countries on various aspects, on the one hand, has led to an increase in the number of numerous bilateral or multilateral economic, social, technical, etc. treaties, and on the other hand, has changed the methods of concluding treaties (treaty making).

Given the developments in international relations and the recent desire to reduce formalities, in many cases the formal way of treaty making, including negotiating, signing, approving of competent institutions, and publishing, seems to be laborious and slow. An official style is suitable for treaties that involve costs, changes in domestic law or relate to fundamental issues of international relations. But this method is not appropriate for agreements with technical characteristics or limited scope or agreements that require a quick fix and implementation regardless of the subject matter.

In the meantime, the Iran nuclear deal case is one of the most striking examples of the conflict between international obligations stemming from various sources of international law, in which the involved parties, each refer to rights and obligations arising from a source. While Iran, on the basis of the Non-Proliferation Treaty, regards enrichment and attainment and use of nuclear energy one of its inherent rights, Western countries, in accordance with the obligations arising from the Security Council resolutions attempt to prevent attainment of such rights. Of course, in accordance with the international procedure under developments and Article 103 of the United Nations Charter, in the event of a conflict of obligations between international agreements and the obligations arising from the Charter, including obligations arising from Security Council resolutions, the latter obligations seem to be superior. By the same token JCPOA was presented as a document that could meet the requirements of the two parties. Therefore, it seems that JCPOA cannot be converted into an

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international treaty. Also, the characteristics of the international treaties are such that JCPOA cannot be converted into an international treaty.

In this regard, although all lawyers consider JCPOA a new and emerging phenomenon, they do not agree on legal description of JCPOA. Some argue that a documents set out in the framework of JCPOA as soft law which has a mandatory format, but in its content and practice is flexible and lacks a conventional legal enforcement guarantee, and its mandate is a political obligation based on the distribution of power and power equation in the international arena that sometimes is more effective than international rights enforcement guarantee. So, they want to categorize JCPOA in the field of international relations and not the international law. Therefore, the present research seeks to answer the question that why JCPOA cannot be converted into an international treaty. Moreover, what are the conditions and characteristics of international treaties?

Katzman & Kerr (2016), in a study titled *Iran Nuclear Deal*, examined the joint commission on issues arising from the implementation of JCPOA, and stated that the majority of the members believe Iran would act in accordance with its provisions. As Obama and the 1 + 5 leaders emphasized, the goal of the joint comprehensive plan of action is to ensure Iran would not have access to nuclear weapons technology. US Secretary of State, Wendy Sherman, said in October 2013 that given the high volume of enriched uranium in Iran, the country could achieve the necessary facilities for building nuclear weapons over the next year. She also referred to the JCPOA legal merits and prevailing Iran's political and legal atmosphere in the Security Council and the UN (Katzman & Kerr, 2016: 1-2).

Also, Einhorn & Nephew (2016), in a research entitled *Iran Nuclear Treaty: Advancing Expansion and Expansion in the Middle East?* examined the challenges facing maintaining cooperation and the continuation of the JCPOA (Joint Comprehensive Plan of Action) and mentioned its major problem as the approach of opposition in Tehran and Washington. Therefore, they believe these challenges are mainly due to differences between executive branch and legislative branch and military forces. In Tehran, these differences are related to the process of lifting sanctions and how to integrate Iran into global economy which can lead to Western influence (Einhorn & Nephew, 2016: 14)

# **II.** Theoretical Framework

# 2.1 The Legal Nature of JCPOA (Joint Comprehensive Plan of Action)

JCPOA was signed based on the final agreement of the Foreign Ministers of the 5 + 1 group and the Foreign Minister of the Islamic Republic of Iran on July 14, 2015. In order to consolidate the practical foundations and safeguard execution of this agreement, the United Nations Security Council adopted Resolution 2231 on July 20, 2015 at its session 7488 and requested the JCPOA signatory States to fulfill their obligations and in this regard, all previous sanction resolutions against Iran were canceled (Fatahi Zafarqandi, 2015: 18).

The program is a 159-page agreement. It contains 21 pages of main text and 5 attachments with a total of 139 pages. In the main text, 5 pages are divided into two sections: Preface, Introduction, and General Provisions. In these two parts, as in any agreement or contract, the generalities of the agreement are referred to. In the other 15 pages, there are four sections: Nuclear, Sanctions, Implementation Plan, and Dispute Resolution Mechanism, and the provisions of the agreement and the commitments of the parties are discussed. The attachments include: Appendix 1: Nuclear-related activities; Appendix II: Obligations relating

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to sanctions; Appendix III: Peaceful nuclear cooperation; Appendix IV: Joint commission; Appendix V: Execution plan (Shapouri, 2016:8).

There are two perspectives on the legal nature of JCPOA: on the basis of one perspective, JCPOA is an international formality or political agreement, and it is unnecessary to follow the conventions stipulated in The Constitution in relation to the conclusion of treaties, and, according to the second view, JCPOA is an international legal agreement and it requires the conventions stipulated in The Constitution for the conclusion of treaties. Between the two aforementioned views, the former seems to be more correct, therefore, in the following some arguments are made to prove the validity of this opinion.

#### 2.2 The Position of Treaties in the International Law

The international treaty, as the most important source of international law, shapes the activities of countries and other international law actors in the international scene and is a fundamental expression of international cohabitation. On the other hand, although the treaty is the official symbol of state or organizational authority, it also limits this authority. As a result, the treaty is a normal legal practice that is carried out by international law actors under international law and, while expressing their authority, restricts that authority and gives rise to their competencies (Ziai Bigdeli, 2009: 61).

Consequently, international treaties are considered as the main axis of international legal relations, and the agreements contained therein are divided into explicit and implicit agreements and both explicit and implicit agreements are considered valid from the point of view of international law (Brierly, 1997: 32).

So, from the viewpoint of some scholars on the law of treaties, it can be stated that the law of treaties in international law is equivalent to the contracts law in private law. Because in international law the international obligations can come from treaties, although not exclusively, and also in the domestic law, it is one of the sources of creating commitment, and of course, the most important of them is the contract (Oloumi Yazdi, 2011: 198).

The treaty, although it is a part of domestic law, is superior to ordinary law and in the hierarchy among domestic law, should be after The Constitution and before ordinary law. The reason for this is the contractual nature of the treaty and its essence, not its sanctity or the sanctity of international law, nor the monism theory with the superiority of the international law. In other words, the problem is that the treaty is an agreement between states, and the creation and execution of a contract is subject to the will of the parties who conclude the contract. Consequently, treaties in international law do not differ from domestic law contracts, and in principle in domestic law it is not possible to collapse previous bilateral will by a later one-sided decision; in international treaties, in principle the treaty cannot be unilaterally terminated (Musazadeh, 2015: 204). Thus, the conclusion of each treaty consists of three stages, including signature, ratification, and enforcement. According to the fundamental rule of treaties, the provisions of the treaty are not legally binding until it comes into force (Asada, 2002: 92), and only in Article 18 of the Vienna Convention on the Law of Treaties of 1969, in the form of the provisions, the scope of this rule has been restricted. According to this article, a country or an organization which signs a treaty, up to the time it approves of the treaty or declare its intention not to be a party to the treaty, shall not commit acts prejudicial to the purpose and subject of the treaty. For the interpretation of this article, one can add that the authors of the Vienna Convention mostly emphasize the aspect of refraining to act and do not impose any positive action on the parties in this article (Rogoff, 1980:297)

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# **2.3** Types of Treaties

Treaties in general international law can be divided into several categories, which are referred to below:

Based on concluding formalities, there are official and simple or execution treaties (Kelary, 2015: 451).

Based on nature of concluding, there are general or legislative treaties and special or contractual treaties (Ziai Bigdeli, 2015: 99-100).

In terms of number of the parties, bilateral and multilateral treaties can be mentioned. (Evans, 2014: 825)

In terms of subject matter, political, substantive or administrative, legal or other treaties can be categorized (Evans, 2014: 826).

Therefore, the features of these treaties are discussed below.

### 2.4 Characteristics of Treaties in Terms of Formalities

# a. Features of simple treaties

In this context, it is necessary to note that although simple treaties are usually referred to with titles such as letter of exchange, exchange of notes, supplementary protocol, and so on; and the terms like treaty, convention, charter, etc. are used in case of official treaties, but change of name does not have an impact on the creation of an international obligation (Harris, 1998: 771), as referred to in paragraph 1 of Article 2 of the Vienna Convention of 1969 this subject is expressly referred to in the definition of the treaty. In addition, terminology is by no means consistent (Wildhaber, 1984: 81). Therefore, the title and form cannot be a good indicator of differentiation.

A simple treaty is valid without the intervention of the head of state and approval of the parliament, by signature of the Secretary of State or diplomatic representatives, in the form of letter of exchange, exchange of notes, memorandum and so on (Musazadeh, 1998: 71).

#### b. Official treaties

The official treaty has a longer formal procedure, in which the intervention of the head of state and the approval of the legislative branch is necessary (Kaske, 1991: 211). Compliance with this procedure is a matter of controversy with the urgent implementation of certain treaties. Hence, a mechanism called the temporary execution has been established to solve the problem and to consolidate the need for a speedy implementation of the treaty and compliance with extensive formalities.

Official treaties, unlike simple treaties, are not immediately exchanged at the time of signing. After the exchange of documents, the treaty will not be implemented immediately, and this will diminish the countries' inclination toward these exchanges (Watts, 1994: 28).

Each treaty has a political aspect, because governments make or join a treaty when it can meet their political or other interests. In this sense, all international treaties are political. However, any treaty in the general sense of the word (a treaty created by international law for the creation of international rights and duties between governments) is a legal document. The bilateral treaty is a treaty of mutual nature, and there is a kind of balance and coordination between the rights and duties of the contracting parties, and the benefits which each of the contracting parties gains from them is balanced with the task imposed upon them (Falsafi, 2014: 65). A multilateral treaty is a treaty that is the result of the will of two or more governments and in principle, cannot be terminated unilaterally by a state, and if a state

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abrogates the treaty in its own right, it has in fact violated it and the violation of the treaty entails the responsibility of the state (Shariat Bagheri, 2011: 286).

In some cases, governments have pledged conventions among themselves which did not entail any legal obligation. The nature of such treaties was to declare the rules or to express the mutual obligations of the governments that participated in the conclusion of those treaties (Falsafi, 2014: 99). In international law, political commitments are referred to as non-binding agreement, honorable or decency agreements and such obligations are not legally binding, which of course, does not undermine the legal effects of such measures (Allen & Thompson, 2011: 230).

Therefore, along with international treaties, there are other agreements that are called non-legal or decency agreements. These agreements create obligations of a purely moral or political nature and do not entail any legal obligations for their parties. These agreements are excluded from the international law and their conclusion is not limited to the international law actors. The distinctive criterion of such agreements from legal agreements is the intentions of the parties to the agreement (Fitzmaurice & Elias, 2005:5).

Characteristics of international legal treaties

- The international treaty is the result of an international agreement of wills.
- The international treaty is a document that creates a duty (Shaw, 2008: 903).
- The international treaty is merely the result of the will of the main international law actors, that is, countries.
- The international treaty is governed by international law regulations.
- The international treaty is set up in writing.
- The international treaty can have various titles.
- The international treaty can be arranged in one, two or more related documents.

In fact, all treaties are international agreements, but not all international agreements are treaties (Villiger, 2009:77).

Terms and conditions of international legal treaties

- Treaty and agreement essentially need signature.
- The parties to the treaty would mention in the document that it is a treaty.
- Use of the terms in the Vienna Convention of 1969 for the purpose of signing, accepting, reinforcement, ratifying and so forth in the text.
- The mentioning of its ratification in the countries party to the treaty and binding the parties to the negotiation to it.
- The multilateral treaty essentially has a Treaty Trustee (Ziai Bigdeli, 2009: 72).

Existence of intent and consent in the treaty (Crawford, 2013: 110).

There is a requirement for the implementation of treaties, and the parties do not volunteer to do something (Das, 1995: 146). Treaties concluded between the two governments or more are published in the United Nations publications (UNTS) (translation of the Vienna Convention of 1969:226).

The institutions arising from treaties are organizations that have been created by various conventions related to that subject and, in fact, are the executive arm of the relevant conventions. The main task of such institutions is to monitor the proper implementation of the relevant convention and to provide recommendations and interpretations to further the good

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practice of the convention. These institutions are only funded by the voluntary contributions of the parties to the treaty (Mehrparvar,1988: 152)

# III. Research Methodology

The research methodology of this study, to investigate and prove the hypotheses according to the nature of the subject, is the descriptive-analytical method and the method of collecting information is the library method using written sources.

Treaty means oath and pledge, and in Webster Dictionary, apart from the use of Latin words such as *compact*, *concordat*, *pact*, and *treaty*, this term is defined as follows: a written contract between two or more political authorities, whether government or sovereign, signed officially by their authorized delegates and usually approved by the legislator (Webster). Generally, any bilateral or multilateral agreement between international law actors is called a treaty. Provided that such an agreement is governed by international law and the provisions of which rule over the agreement and as a result, provides certain legal effects (Ziai Bigdeli, 1388: 73). In fact, treaties, contracts, and agreements, are international instruments that governments join them through signing and other legal acts, and oblige themselves to execute its provisions. In fact, signing means the agreement of the representatives present at the conference on the text of the treaty and that they are willing to accept it and refer it to their respective governments to take any necessary action (Moqtader, 2016:288). At the same time, governments can agree to or refuse to accept these documents by inclusion of conditions by the use of a condition clause announced by another government (Enayat, 2015:31).

#### IV. Discussion

One of the reasons for not converting JCPOA (Joint Comprehensive Plan of Action) into an international treaty is that the comprehensive and final Vienna agreement with the well-known and official title of JCPOA was adopted in line with the comprehensive agreement on Iran nuclear deal and pursuant to Lausanne nuclear agreement on July 14, 2015 in Vienna, Austria, between Iran, the European Union and the 5 + 1 group, including countries such as China, France, Russia, the United Kingdom, the United States and Germany. After concluding the final text of JCPOA by the negotiating parties, based on the agreement, a United Nations Security Council resolution relating to confirmation of JCPOA was approved and adopted within a short period of time (Haidar, 2016:3).

Other reasons include that the agreements must point to a future behavior and affect the future relationships of the parties. Therefore, any joint statement or multilateral document is not an agreement. These two criteria are also required in treaties as well. Although there are qualitative differences in the nature of expectations of normal behavior, but due to the popularity of decency agreements, many forms, structures, execution procedures, and even withdrawal rules, apply to these agreements as well as the treaties (Hollis & Newcomer, 2009:521).

Among other reasons in this regard, it can be noted that the title of the JCPOA, i.e. Joint Comprehensive Plan of Action also indicates that the parties were not seeking ratification of an official treaty, since otherwise they would use titles such as agreement convention, charter, articles of association, protocol, declaration and so on, and since in accordance with Article 77 of the Iran Constitution, these matters must be approved by the

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Islamic Consultative Assembly, therefore, this JCPOA is one of the conditions for the conversion of JCPOA into an international treaty to pass these phases.

Another reason for not converting JCPOA into an international treaty can be found in the phrase contained in this document, because the provisions of the joint plan of action of Geneva do not imply a direct mandate, but each of the parties with its discretion and will, accepts commitments that will provide the settings for concluding the final treaty at the final stage. In other words, the intention of joint plan of action document in the use of certain terms and expressions indicates that this discretion does not mean the term "freedom of wills in concluding a contract" and implies that each party, by doing so, prepares and stage for conclusion of the final treaty and legally, failure to implement these obligations does not create any effect (Mardani, 2016:83).

Of course, the political implications of this breach of duty are necessarily revealed, and consequently legal effects may arise. But in any case, in the first instance, the failure to fulfill the obligations contained in the contract does not result in the appearance of legal effects. Also, other terms that show this document is not a treaty and the inability to become a treaty is emphasis on voluntary action, as well as the lack of a binding clause with certainty, the avoidance of the use of words and verbs that indicate the necessity of taking action, show that it is non-binding.

All of this, excludes the document from the legal treaty perspective and it can only be considered a political or decency agreement, which deals with the planning of actions and the framework of a cooperation.

Therefore, JCPOA is a political or decency treaty, as it is stated in the context of the report of the Special JCPOA Inspection Commission in the Islamic Consultative Assembly: the commission as a whole has concluded JCPOA is a political agreement between the government of the Islamic Republic of Iran and the other negotiating parties (1 +5 countries and the European Union), and believes that the government should take care of the nuclear infrastructure and its achievements with all necessary measures in order to make decisions appropriate for national interests in case of violation of the treaty by the other party (the text of the Special JCPOA Inspection Commission report in the Parliament, 2015).

Whether the two sides have reached an agreement or that it is a written agreement, and in the context of an issue that has a particular aspect in the nuclear rights, cannot cause a mistake in distinguishing it from international legal treaties. The important criterion is the application of international law to that agreement. In this regard, the parties have never expressed their consent to the rule of international law. Thus, the legal literature can hardly be found in the agreement (not in the title or provisions, nor in the organizing of its clauses). On the contrary, the parties have explicitly stated that the document is not a legal treaty (Sa'ed, 2013:32).

Since the condition of an international treaty is the adoption of it by the legislative branch of the countries party to the treaty, another reason for not converting JCPOA into an international treaty is non-approval of it in the parliament of other countries. However, although the second part of the second principle of the US constitution stipulates that the convening of the formal treaties is subject to the consent of the Senate, in the opinion of American lawyers this document is not legally binding and as an international agreement does not require reinforcement by the American legislative assembly (Garcia, 2013:2).

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#### V. Conclusion

The Islamic Republic of Iran and the 5 + 1, after nearly two years of negotiations during the eleventh administration, and to resolve the issues related to the Iranian nuclear problem and removing the cruel sanctions against our country, in July 2015 set up the draft of JCPOA (Joint Comprehensive Plan of Action) and then within a short time, based on agreement reached between the negotiating parties, the United Nations Security Council Resolution on this matter was adopted. In this regard, the analysis and examination of the reasons for not turning JCPOA into an international treaty is critical because of its binding nature and having an enforcement guarantee. Since one of the most challenging and controversial aspects of the final text of the JCPOA is the examination of the legal dimensions, the limits of the competences of the domestic institutions and the manner in which commitment to obligations are made on the basis of the legal system of the Islamic Republic of Iran. Therefore, the present study examines the legal nature of the JCPOA, status of treaties in international law, types of treaties and features of each of them, characteristics of international legal treaties, conditions of international legal treaties, and systems and institutions arising from treaties, to investigate this issue. Thus, the results of the present study indicate that this agreement, since it is more political in nature rather than a legal nature, based on the distribution of power and the power equation in the international arena, cannot be converted into an international treaty.

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