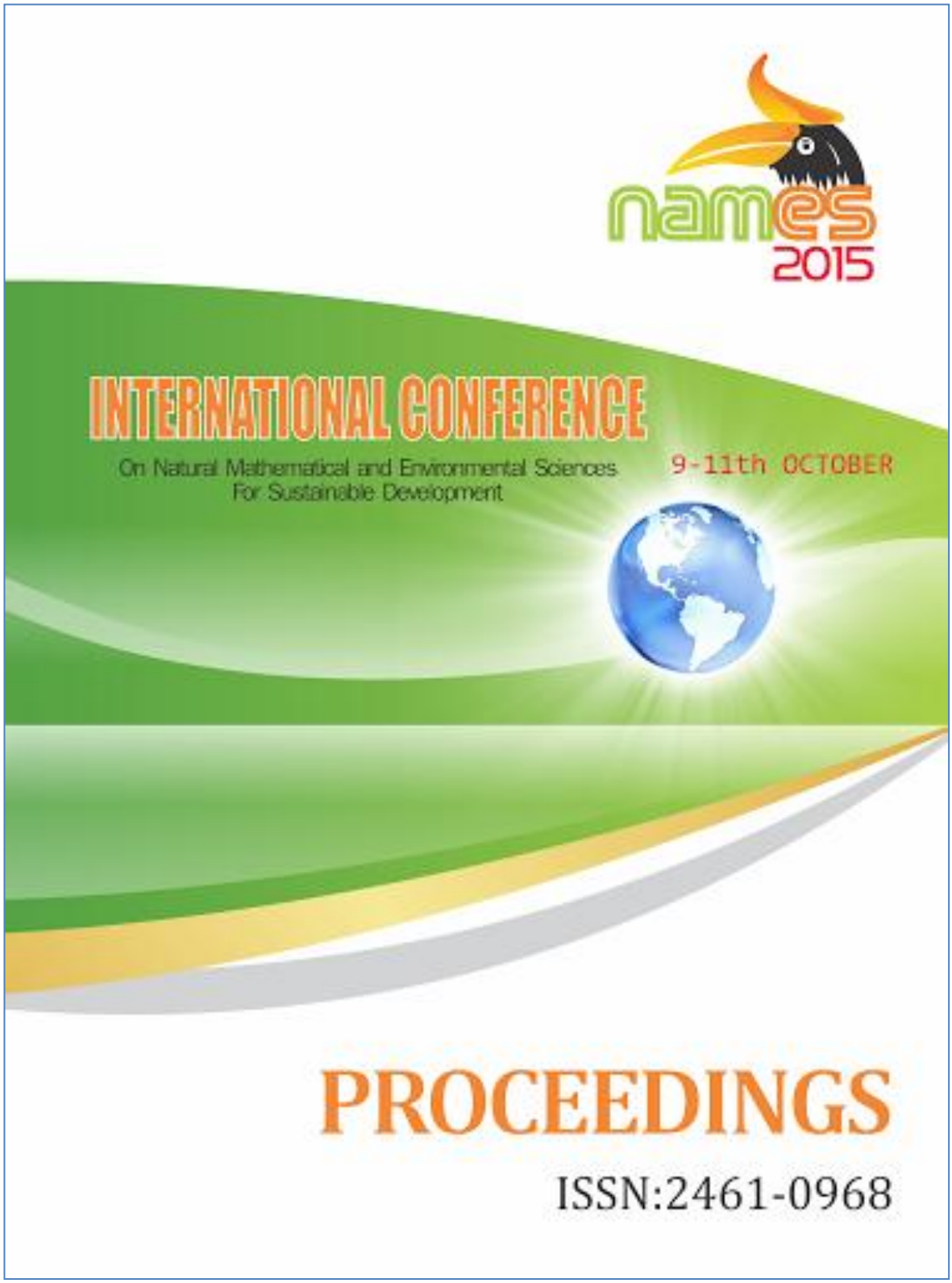
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Edited by Sasi Gendro Sari

PREFACE

Assalamu'alaikum Wr. Wb.

All praises are due to ALLAH SWT, God Almighty, Who made this International Conference of successful. The International Conference on Natural, Mathematical and Environmental Sciences (NAMES) 2015 is organized by the Faculty of Mathematics and Natural Sciences (FMIPA), Lambung Mangkurat University, Banjarbaru, South Kalimantan. This conference covered a wide range of topics, including biology, chemistry, physics, mathematics, pharmacy, computer science, material Science, and environmental science.

All papers were compiled into the proceedings book which had six sections, namely Biology, Pharmacy, Chemistry, Mathematics, Physics, and Computer Sciences. This book was also publised in the NAMES Website http://names.fmipa.unlam.ac.id. I am glad that for the first time both types of books can be realized.

The seminar took a theme of "Sustainable Development" as a hot issue in Banjarbaru. Banjarbaru, is a fast growing city in the province of South Kalimantan, Indonesia and famously known as an urban city with a unique natural landscape, a cultural diversity, and a friendly welcoming citizen. Moreover, Banjarbaru becomes the centre of provincial government that its government is located in Banjarmasin today. The conference provided an ideal platform to share information and discuss their scientific results and experiences, with particular references to sustain development.

I was fully satisfied to all the members of the program committee who contributed for the success in framing the program and the books. My appreciation was especially from all delegates who contributed to the success of this conference by accepting our invitation and submitting articles for presentation in the scientific program.

I can guarantee you that this book provides a full of intellectual scientific research activities. I do hope the next conference will pick up similar success, and even better.

Wassalamualaikum Wr. Wb.
Banjarbaru, March 2016

Dr. Krisdianto
Chief of Executive

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BIOLOGY

The Potential of Rhizospheric Fungi From Critical Land of Sub-District Cempaka, South Kalimantan as Fe-Absorbent on *Calopogonium Mucunoides* Plant

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ABSTRACT
Exploiting the rhizospheric fungi from critical land area is one way to know the ability of the fungi to absorb Fe. *Calopogonium mucunoides* which used as experimental plants in this study was capable to grow well in critical land area of Cempaka Sub-District. The study was begun with the isolation of rhizospheric fungi of critical land district Cempaka, followed by calculation of soil Fe content of the critical land areas, selection of the fungi bae on its ability to absorb Fe and fungal spore application to *C. mucunoides* growth. *Humicola* sp. R.Dn (from rhizosphere of Dianella nemorosa Lam.) has better ability to absorb Fe than with two other isolates, i.e.*Penicillium* sp. R.Ha and *Penicillium* sp RG. *Humicola* sp. R.Dn could uptake 108.83 mg Fe/mg biomass and giving 2.38 x 10^3 of *Humicola* sp. R.Dn spore significantly affected on the reduction of plant Fe content.

***Keywords**: Rhizospheric fungi Fe absorbent, Humicola sp. R.Dn, C. mucunoides,*

INTRODUCTION
Critical land is land that less of physical, chemical and biological soil productivity. Some land classified into critical areas because of the loss of topsoil (top soil) fertile due to the influence of erosion, as well as mining activities (Rukmana, 1995). The rate of land degradation in South Kalimantan Province is increasing, due to the increase in critical lands. Based on data from the 2009 BPDAS Barito, South Kalimantan has an area of 761 042 hectares of critical land. One of them contained in the Cempaka sub district, which is due to land clearing and stones mining activities.
Preliminary test results show that the Cempaka critical land Fe content was 37 533 mg / L. This value is higher than the standard recommended by the WHO ie 47.2 mg Fe / L. That information used to serve as the basis in this study to determine the potential for indigenous fungi from rhizosphere critical area districts Cempaka to absorb these metals. Some fungi have been known capable to reduce levels of heavy metals in contaminated soil. *Aspergillus niger* van Tieghem can reduce the levels of Cr from the nickel mines through the process of biosorption (Saefudin et.al., 2000). *Rhizopus arrhizus* is known to absorb Fe at 6.932 mg / L in time stirring for 80 minutes (Dewati, 2004). The both fungi can be found in the rhizosphere.
Humicola sp R.Dn, *Penicillium* sp. R.Ha and *Penicillium* sp. RG has been isolated from the rhizosphere of plants *Dianella nemorosa* Lam., *Helicteres angustifolia* L., and Gleichenia sp. which grow on degraded land Cempaka sub district, South Kalimantan used in this study (Imaningsih, 2012). Previous research has shown that the origin rhizosphere soil fungi critical to spur the growth of elephant grass (Mahriani, 2013). Plants that have been used as bioremediator of heavy metals, such as *Calopogonium mucunoides*. A research on *Calopogonium mucunoides* has demonstrated that the plant absorbed heavy metals, i.e. Pb, Fe, Cd and Zn at a former gold mining (Hidayati et.all., 2006). *Calopogonium mucunoides* can be used as pioneer plants to rehabilitate (Purwanto, 2007).

MATERIALS AND METHODS

Fungal isolates selection on medium with Fe addition

Before performing the isolates endurance test against metal Fe qualitatively, first made the liquor of Fe 1000 mg / L. Fe as much as 0.1 grams was placed in a flask and then separately etched with 3 drops of sulfuric acid solution and diluted with distilled water to 100 ml Erlenmeyer flask. There are 3 isolates ie (*Humicola* sp R.Dn, *Penicillium* sp. R.Ha (1), and *Penicillium* sp. R.G)were prepared for the endurance test there. Third isolates were inoculated on sabauroud dextrose agar medium with the addition of metallic Fe separately with different concentration (70 mg / L, 100 mg / L, 150 mg / L). Then the growth of isolates was observed for 7 days to determine the isolates that have the best growth used in subsequent testing. The next best calculated percent of mold growth in a medium with the addition of metal Fe, in the following formula: $N = \frac{d_2}{d_1} \times 100\%$

(d2) is the diameter of the fungus colony control (without addition of Fe) and (d1) is the diameter of the fungus colony with the addition of Fe (Levinskaite, 2009).

Fe Absorption Test by indigenous Fungi from sub-district Cempaka critical land

Fe absorption test by a fungus carried out on *Humicola* sp. R.Dn due to have the ability to grow best in the selection step. *Humicola* sp. R.Dn have a faster growth rate than the two other fungi (figure 1.). Then *Humicola* sp. R.Dn inoculated on potato dextrose agar medium (PDA), and incubated at 35^0 C for 7 days. The isolate that has grown on PDA media were inoculated into potato dextrose broth (PDB) of 200 ml, that given additional Fe 70 mg / L as a treatment (Irawan, 2004). Then incubated at 125 rpm shaker at room temperature for 7 days. Whatman filter paper no.1 was used to separate the filtrate of the biomass, the biomass was washed with sterile distilled water then oven it at 60 °C for 24 hour, and the filtrate was centrifuged at 3500 rpm for 9 minutes. Biomass and residues of *Humicola* sp. R.Dn analyzed using Atomic Absorption Spectrophotometer (AAS) to determine levels of Fe that absorbed by the fungus. Fungi that growth on medium without Fe used as control.

Fe uptake calculated by the formula according to Al-Garni et.al (2009) as follows: $Q = \frac{V(C_i - C_f)}{1000 \times M}$,Q is Fe adsorbed (mg Fe / mg Biomass), V is the volume suspension of Fe (ml), Ci is the initial concentration of Fe (mg / L), Cf is the final concentration of Fe (mg / L) and M is the number of dry biomass mold *Humicola* sp. R.Dn (mg). As for knowing what percentage of the missing metal Fe Fe existing value based on the residue obtained after SSA test, can be calculated with the following formula:$\text{Lost } (\%) = \frac{C - C_f}{C} \times 100\%$, C is the initial concentration (mg / L) and Cf is the final concentration (mg / L) (Kareem et al., 2014)

Applications of critical land indigenous fungi on *Calopogonium mucunoides*

Application was done in two phases: the preparation and harvesting steps. Both of these steps can be described as follows: preparation of the planting medium and *Calopogonium mucunoides* seed preparation. Sterile soil(500 grams) was used as planting medium that placed in a poly bag (10 (d) x 20(l) cm). Spores enter into the sterile soil. The number of spores that will be used is calculated using haemocytometer with formula as follow: $K = \frac{t}{n \times 0{,}25} \times 10^6$, (K) is the concentration of spores per ml of solution, (t) is the number of spores were counted in the calculation of the box and (n) is the number of boxes were observed (Sudibyo, 1994). Seed surface sterilization was apply for *Calopogonium* seeds, then grown for 1 week and prepared for planting, soil and plant Fe content were analyzed using Atomic Absorption Spectrophotometer (AAS) was done at 4 weeks old.

This research uses a completely randomized design (CRD) with 1 test plants *Calopogonium mucunoides* and with the addition of fungal spores *Humicola* sp. R.(0 (without the addition of spores), 1.59 x 10^2, 7.95 x 10^2, 1.59 x 10^3 and 2.38 x 10^3 spores). Each treatment was repeated 4 times, resulting in 20 experimental units in total.

RESULTS

SSA test shows the Fe content in the soil critical areas where fungi was isolated was 37533 mg / L. While the land used for planting *C. mucunoides* is 1084.8 mg / L. The results showed that Fe levels in the critical area not in accordance with the EPA (Environment Protection Agency United States) standards (0.5 to 20 mg / L). The fungal growth were tested in SDA medium with different concentrations Fe addition showed *Humicola* sp. R.DN better growth than two other isolates (*Penicillium* sp. R.Ha and *Penicillium* sp. RG). Observations were made during the 7 days by measuring the diameter of each fungi colonies. Percent growth of *Humicola* sp. R.DN, *Penicillium* sp. R.Ha and P*enicillium* sp. RG grown on medium with the addition of Fe can be seen in Figure 1 below.

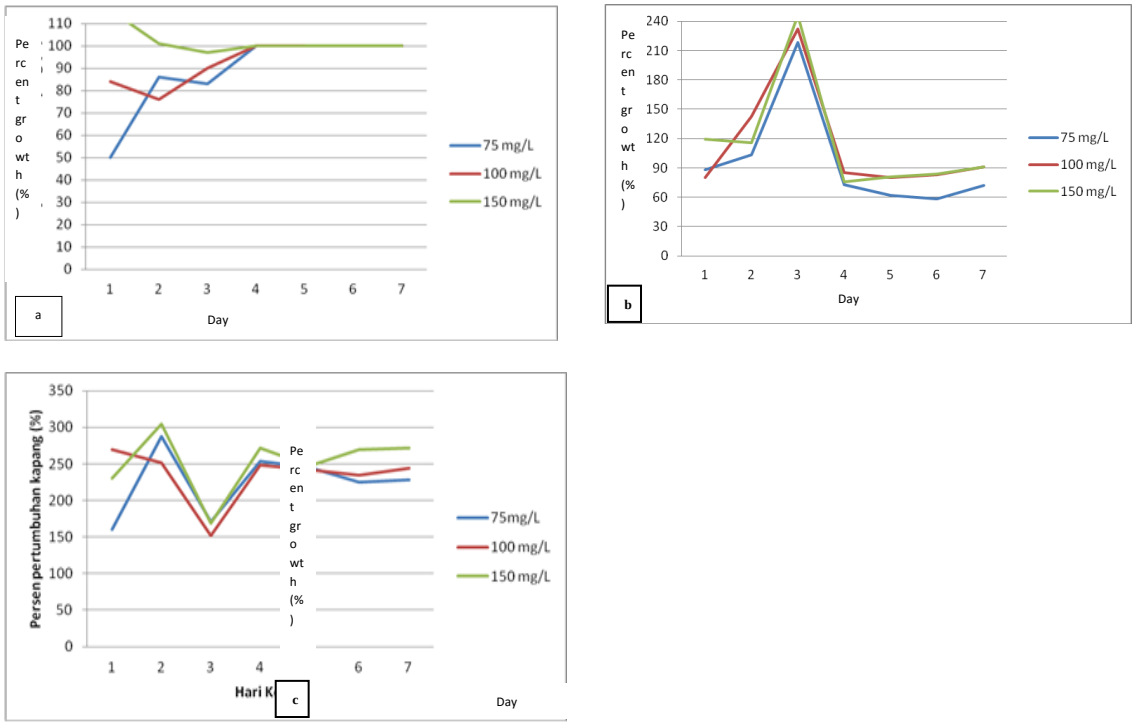

Figure 4. Graph percent growth of fungus *Humicola* sp. R.Dn (a), the mold *Penicillium* sp. R.Ha (b), *Penicillium* sp. RG (c) on medium with the addition of Fe.

Metals content of Fe in the *Humicola* sp. R.Dn

Fungal isolates were grown on medium PDB with Fe addition (70 mg / L), shows that the highest Fe found in fungi biomass, with Fe contain 37.35 mg /L in average. While the residue contained of Fe (23.50 mg / L) (Table 2). Based on the appropriate calculation formula of Al-Garni et.al (2009) showed that Fe uptake by fungal biomass amounted to 108.83 mg Fe/mg of biomass. Meanwhile, according to the formula Kareem (2004) percent Fe missing is 66%.

Table 2. Average levels of Fe on Biomass and Residues fungi Humicola sp. R.Dn after being given 70 mg / L Fe in the media.

Sample	Fe (mg/L)	Fe absorption (mg Fe/ mg biomass)	Lost Fe (%)
Fungal biomass	$37{,}35_a$	108,83	-
Residu	$23{,}50_b$	-	66

Remarks: number with different letters have differences based F test at 95% confidence level.

Soil (growing medium) and *C. mucunoides* Fe content

Fe content in the soil varies but not give a much different effect. ANOVA based on the number of additional spore treatment was not significantly different at the level of 95% (Figure 5). Fe content value of all treatments range from 902.1 mg / L - 1017.7 mg / L. The highest Fe content obtained from the soil without the addition of fungal spores (1017.7 mg / L) and low Fe content obtained from the soil with the addition of as much as 1.59 x 10^3 spores (902.1 mg / L).

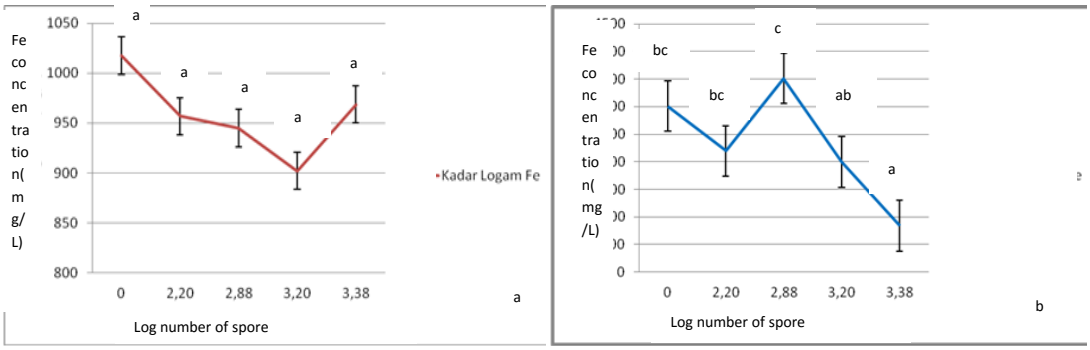

Figure 5. (a) Fe metal content in the soil, bar followed by the same letter are not significantly different based on the Anova test level of 95%, (b) Fe metals content in plant, bar followed by the same letter are not significantly different by the test of Duncan's New Multiple Range Test DNMRT at the level of 95%

Fe metal content in plants that do not show different values based on the test of Duncan's New Multiple Range Test DNMRT at the level of 95%. Value Fe content in the plant without the addition of spores did not differ significantly with the addition of 1.59 x 10^2 spores and value addition of Fe content at 1.59 x 10^2 spores and significantly different from control values in addition spores Fe content was 7.59 x 10^2 and 2, 38 x 10^3 (Figure 5).

DISCUSSION

Fe tolerance and absorption ability of fungi *Humicola* sp. R.Dn indigenous Critical Land Sub District Cempaka

Humicola sp. R.Dn showed better growth than two other isolates (*Penicillium* sp. RG and *Penicillium* sp. R.Ha). *Humicola* sp. R.Dn have the largest diameter colonies. This indicates the viability of *Humicola* sp. R.Dn more quickly between the two other isolates, in a state of high Fe. Based on the results of calculations per cent growth of mold known that Fe affect the three isolates. The best percent growth seen in *Humicola* sp. R.Dn (Figure 4), the increasing of number of colony diameter indicate better ability to absorb the Fe. Based on Krumova (2012) the genus of *Humicola* (*Humicola lutea* 103 isolates) have the ability to absorb Cu so that reinforce this study to determine the metal content of Fe may can be absorbed by *Humicola* sp. R.Dn.

Fe uptake on *Humicola* sp. R.Dn were given 70 mg/L Fe in the PDB medium shows Fe contain in biomass critical land *Humicola* sp. R.Dn has the ability to absorb Fe. Fe concentration was higher than rhizosphere fungi A.niger which is able to absorb Fe with optimum concentration of 25 mg/L a results of research Angumenal (2005).

The ability of *Humicola* sp. R.Dn absorb Fe metal is closely related to the nature of the fungus is resistant to heavy metals (Krumova et.al, 2012). This is reinforced by the results of research Saidudu et.al (2014) which states that isolates *Humicola* sp. only found in soil treated with heavy metals. Krumova et.al (2012) adds that the number of cells in the body Humicola lutea 103 isolates increased with increasing heavy metal (Cu) in the growth medium.

Based on the calculation formula of heavy metal uptake by biomass (Al-Garni, 2009) the obtained Fe uptake value on fungi biomass was 108.83 mg Fe / mg of biomass. Several studies of other fungi also showed the absorption capacity of the Fe. One is *Fusarium* sp. that is able to accumulate 51 mg Fe / g of dry biomass, but it inhibited funal growth (Levinskaite, 2009). The absorption capacity of fungi biomass to heavy metals depending on the species or strains of fungi

that used. H*umicola* sp. R.Dn including one species that is resistant to metal (Saidulu et.al, 2014). Based on the results of the study isolates *Humicola* sp. R.Dn indigenous Cempaka critical land, this shows good potential for this isolate to be used as heavy metals bioremediator, especially Fe for applications in the field.

Fe content in the soil growing medium *C.mucunoides* showed a decrease along with increasing the number of spores, although statistically no difference. This shows the activity of fungi *Humicola* sp. R.Dn in absorbing Fe. This is supported by several studies that say that the fungi of rhizosphere soil is able to absorb heavy metals, among others *Rhizopus arhizus* (Ahmad et.al 2005) and *Phoma* sp. (Levinskaite, 2009). Low Fe content is obtained from a plant with the highest spore planting. Fe content in plants *C. mucunoides* also showed a similar pattern, there was a decrease in the levels of Fe in an increase in the number of spores given. It shows that without the help of *Humicola* sp. R.Dn Fe content remains high ground. Soil without the addition of spores showed the highest final Fe content after planting among other treatments (1017.7 mg / L), as well as the levels of Fe in plants *C. mucunoides*. *C. mucunoides* plants were not given spores, Fe accumulate more on the body than the other plant. *Humicola* sp. R.Dn assist the process of absorption of Fe in the planting medium, so that Fe is absorbed by plants is not as much as the control plants. This is consistent with the statement Hidayati (2006) that *C. mucunoides* is one plant that is able to accumulate heavy metals.

The Potential *Humicola* sp. R.Dn as Heavy Metal Biosorbent In Soil Bioremediation

Many metals Fe (37533.30 mg / L) and Cu (15.43 mg / L) according to preliminary test results of this study. High metal content and exceeding the threshold are harmful to other living creatures, especially humans. To reduce the levels of heavy metals in soil can use bioremediation. *Humicola* sp. R.Dn that isolated from the plants rhizosphere of critical land shows potential as biosorbent Fe mainly on soil or growing media. Biosorption technology can replace chemical procedure to remove metal ions from the environment (Volesky 1990 in Kareem SO et al 2004). Fungi as biosorbent has many advantages, among others, easily grown, produce large quantities of biomass can be manipulated genetically and morphology and can be used alive or dead. (Volesky 1990 in S.O. Kareem et al, 2004).

The effect of adding spores *Humicola* sp. R.Dn on plant growth *C. mucunoides* also shows good potential. Addition of spores in a certain amount shows a decrease of Fe content in plants. As well as the impact on several parameters were observed. Biomass produced by plants *C.mucunoides* indicates this metal uptake activity. *C.mucunoides* plant suspected of association with fungi to reduce the high levels of Fe metals in the soil. Through this potential is expected to help the development of critical land bioremediation process using plants and fungi. However, further research on the effect of Humicola sp. R.Dn on plant growth needs to be studied.

CONCLUSION

The Indigenous fungi from rhizosphere soil of critical land sub district Cempaka, district Banjarbaru, South Kalimantan (*Humicola* sp. R.Dn) have a better ability to absorb Fe than two other isolates (*Penicillium* sp. R.Ha and *Penicillium* sp. RG). Fe uptake by fungi biomass at 108.83 mg Fe/mg of biomass. Provision of Humicola sp. R.Dn spores (2.38×10^3) significantly influence the decrease of plants Fe content.

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