



---

## **Evaluation Content of Pb in Phase Vegetative and Generative of Paddy by Application Azolla and Husk Biochar in Contaminated Paddy Field**

B. Hidayat<sup>a\*</sup>, A. Rauf<sup>b</sup>, T. Sabrina<sup>c</sup>, Ali Jamil<sup>d</sup>

<sup>a,b,c</sup>*Agriculture Faculty, Sumatera Utara University, Medan 20155*

<sup>d</sup>*Center for Food Crops Research and Development, Ministry Agriculture, Republic of Indonesia*

<sup>a</sup>*Email: bendayat@gmail.com*

### **Abstract**

Emergence of factory near paddy fields in Indonesia has caused lead (Pb) pollution in body part of paddy especially on grain. The aimed of this reseach to determine the influence of biochar and azolla in reducing bioavailability of Pb on the vegetative and generative phase in paddy fields. Research conducted on contaminated paddy field in the village of Dagang Klambir Tanjung Morawa A by using a randomized block design factorial, the first factor was azolla, with three levels, without azolla (A0), *Azolla pinnata* (A1) and *Azolla micropylla* (A2). The second was biochar (B) with a two-stage treatments, without biochar (BO), and biochar rice husk (B1). The results showed that application azolla and biochar was not influence soil pH on paddy soil in vegetative and generative phase, concentration Pb in vegetative lower than generative at parts of paddy, *Azolla micropylla* with husk biochar decreased Pb concentration in grain and husk.

**Keywords:** rice paddy; lead; biochar; Azolla; vegetative; generative.

---

\* Corresponding author.

## 1. Introduction

The emergence of factories in agriculture area especially paddy has been polluting the soil, water and agricultural products, especially in the grain [1, 2, 12, 19]. Heavy metal contents of agricultural soils can affect human health directly through consumption of crops grown in contaminated soils and heavy metals are persistent and difficult to remove or degrade once introduced into soils, finally will be accumulated to be a night mare for crop production [2]. It has also been reported that crop plants have different abilities to absorb and accumulate heavy metals especially lead in their body parts and that there is a broad difference in metal uptake and translocation between plant species and even between cultivars of the same plant species, and paddy reported has ability to absorb heavy metal in their body part without disturbance of physiology and productivity [1,12,15].

Paddy has two phase in the growth; vegetative and generative. Vegetative was flooding time for growing of paddy with pH near to neutral and reduction process occurred. Waterlogged soil is good medium for paddy growth because; (1). Less of water stress, (2). Easily for weeding control, (3). Increasing some nutrient, (4). Decreasing some heavy metal bioavailability. In Generative phase, water going to less and drying at the end, oxidation process will increase and influencing nutrients and increase heavy metal status because pH is going to acid [10].

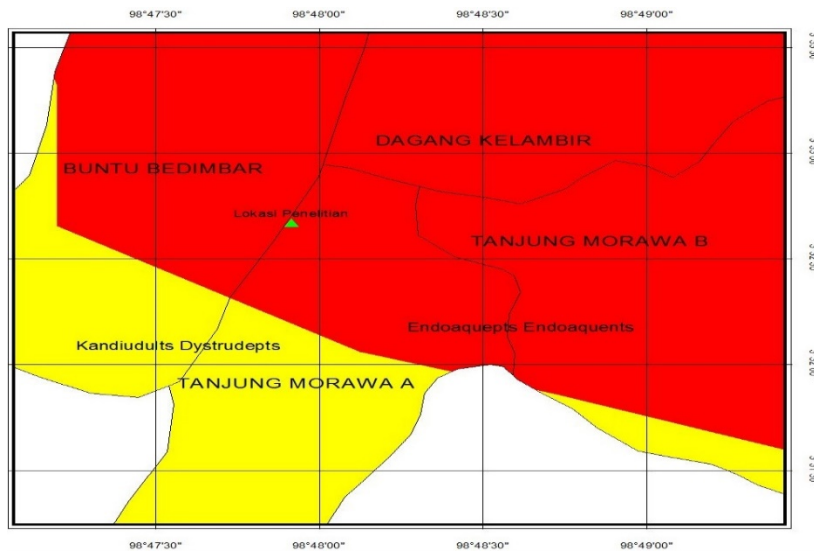
*Azolla* is a small aquatic fern with a symbiotic pair of blue-green alga *Anabaena azollae*. Many report that using it as a fertilizer in botanical gardens get great benefit because of nitrogen-fixing capability [3]. *Azolla* has been used for several decades as green manure in rice fields. Recently *azolla* has the capacity to accumulate large quantities of the heavy metals such as  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Ni^{2+}$  and  $Zn^{2+}$ . *Azolla filiculoides* has growth ability in the solutions containing these metal ions with the initial concentrations of 4 mg/l. within 15 days, not only that, living *Azolla filiculoides* can be used to purification of polluted water, because of its ability to remove  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Ni^{2+}$  and  $Zn^{2+}$  reach to about 61%, 57%, 68% and 74% [7]. Biochar reported could improve crop yield, enhance nutrient availability due to the presence of C. It also increase soil fertility by increasing soil microorganism's activity that consequently lead to increase the nutrients recycling [5]. Husk derivate biochar reported can increase nutrient status, yields and nutrients efficiency at low fertility and soil water stress also occurred, and has ability to decrease heavy metal status [14]). Many studies of biochar have demonstrated that there turn of crop straw, husk as biochar to farm land has the potential increase soil carbon storage and available macro nutrients (N,P,K,Ca,and Mg), which is beneficial for plant growth and increase yield (13, 19).) Rice husk and rice straw were effective for removing cadmium (II) in aqueous solutions, and wheat straw has been shown to be capable of adsorbing copper (II), Pb (II) and Cd (II) via ion [8]. All information about heavy metal status especially on Pb in paddy only in generative while harvesting, therefore the aim of this research to investigate concentration of Pb in vegetative and generative phase on husk biochar and azolla application and to get free Pb grain production

## 2. Materials and Method

Research conducted at village Dagang Klambir District of Tanjung Morawa B, Deli Serdang- North Sumatera

Indonesia (03° 32' 32.44" N - 98° 47' 49,89" E) . Materials biochar rice husk obtained by simple fast pyrolysis at 450°C , two types of Azolla (*Azolla pinnata* and *Azolla microphylla*), Ciherang seed, Urea, SP-36 and KCl for basic fertilizer , Jetor machine for plowing, leaf color chart for N, pH meters, scales, centrifuge, Instrument FTIR analysis, SEM, BET dan other tools that support this research.

This study used randomized block design (RAK) factorial, with four replications. The first factor was application of azolla, consists of three levels ie; without Azolla (A0), *Azolla pinnata* (A1), *Azolla microphylla* (A2). The second factor was biochar (B) consists of two levels ie: without biochar (B0), rice husk biochar (B1), in order obtain 6 combinations treatments with four replications . The soil parameters ie; soil pH, content of Pb in root, stalks and leaves of paddy and its production. Data generally analysis by F test at the level of 95% and differences between the treatments were examined using a two way analysis of variance (ANOVA). All statistical analysis were carried out using SPSS version 13.0.



**Figure1:** Location of experiment area

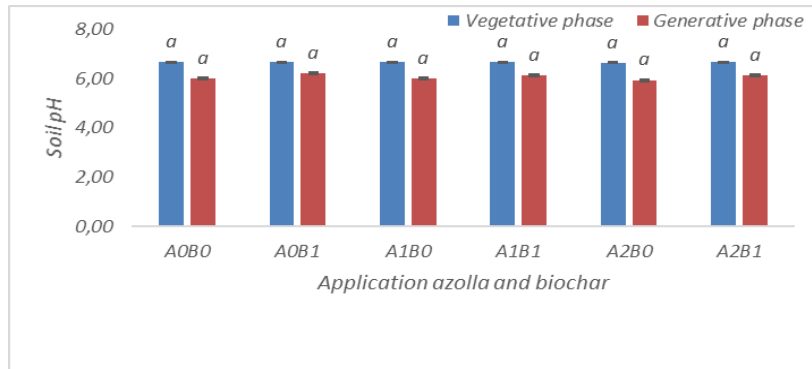
**Table 1:** Selected physicochemical properties of the soil and amendments

Sampel	pH (H <sub>2</sub> O)	Carbon	Carbon	N-Total (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)	Pb (ppm)
		(%) WB	(%) Edax				
Soil	6,84	1,31	-	0,15	114,12	1,03	77,21
Rice Husk Biochar	6.81	29.71	77.18	0.37	0.21	1.18	-
<i>Azolla macropyhlla</i>	-	-	65.50	1.52	0.37	3.20	0,67
<i>Azolla pinnata</i>	-	-	55.59	2.21	0.61	3.69	1,25

### 3. Result and discussion

#### 3.1 Soil pH

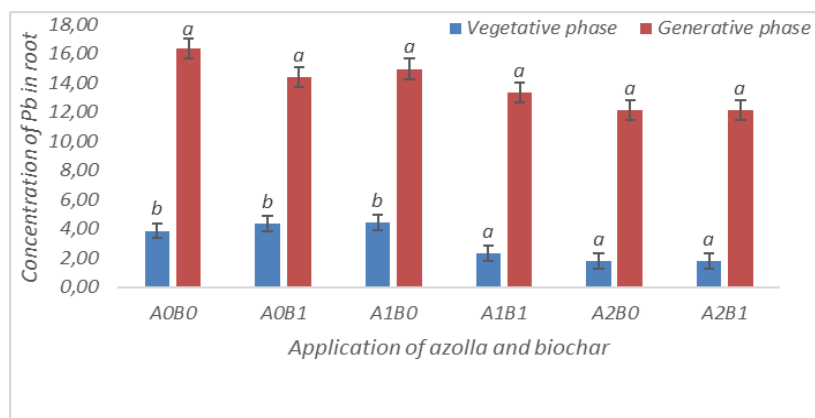
The application azolla and biochar were not influence increasing or decreasing soil pH in vegetative or generative phase. In vegetative phase, the highest pH on application biochar derivate husk with or not azolla given (A0B1 and A1B1) was 6.70 and the lowest 6.65 on application *Azolla microphylla* with no biochar (A2B0). In generative phase occurred decreasing pH but not significant if compared with control (A0B0), the highest pH on application Husk biochar without azolla (A0B1) (Figure 2)



**Figure 2:** Effect of application azolla and biochar on soil pH in vegetative and generative phase

#### 3.2 Concentration Pb in root, stalk, leaf of paddy in vegetative and generative phase

Generally absorption of Pb in generative phase higher than vegetative phase. Application azolla and biochar on vegetative phase in root significant decreasing adsorption Pb, with lowest concentration in application of *Azolla microphylla* and Husk biochar (A2B1) was 1.76 ppm and the highest concentration on application *Azolla microphylla* without biochar (A1B0). In generative phase three no significant effect on Pb absorption, with lowest rate was 12.13 ppm on application *Azolla microphylla* with husk biochar or without husk biochar (A2B0, A2B1) (Figure3)



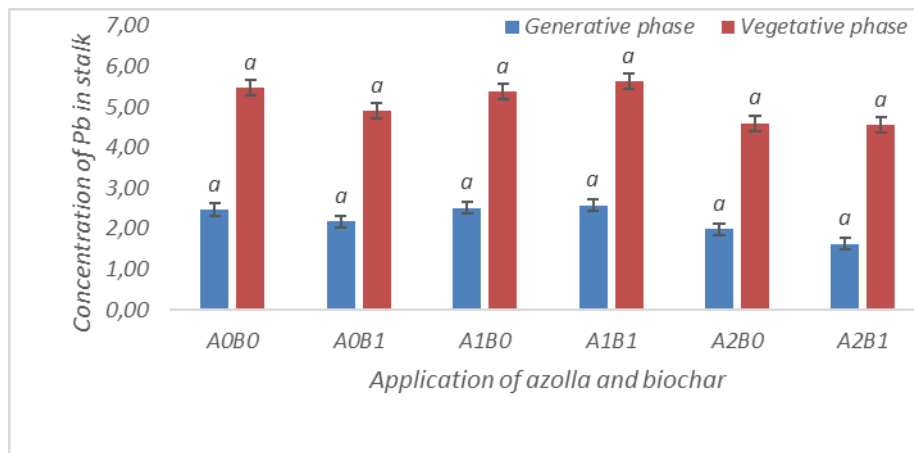
**Figure 3:** Effect of application azolla and biochar on soil pH in vegetative and generative phase

*Azolla microphylla* is one kind of azolla has high adaptation and doubling time in every condition, different with *Azolla pinnata* had high sporulation, in fact *Azolla microphylla* growth well on paddy field in vegetative and generative, growth of *Azolla microphylla* in vegetative and generative phase showed in Figure4. *Azolla microphylla* has ability to absorb heavy metal higher in wet soil condition than dry soil, it is because of its ability to grow in waterlogged soil and high biomass, thus increasing the uptake was expanded [2].



**Figure 4:** Growth of *Azolla microphylla* in vegetative (a) and generative phase (b)

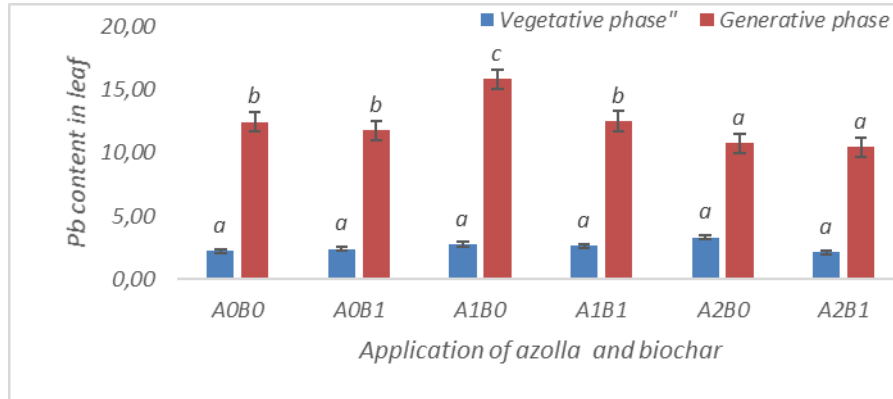
Application of azolla and biochar were not influence concentration of Pb significantly in stalk in vegetative or generative phase (Figure5). In vegetative phase the lowest rate was 1.63 on application *Azolla microphylla* with husk biochar (A2B1), and the highest was 2.58 ppm in application *Azolla pinnata* with no biochar (A1B0). In generative phase the lowest rate was 4.55 ppm (A2B1) and the highest was 22,5 ppm (A1B1).



**Figure 5:** Effect of application azolla and biochar on concentration Pb in stalk in vegetative and Generative phase

In vegetative phase, application azolla and biochar were not increasing concentration of Pb in leaf of paddy significantly with lowest concentration on application *Azolla microphylla* with husk biochar (A2B1) was 2,13ppm and the highest in application *Azolla microphylla* without biochar (A2B0) in rate 3,34ppm. But in generative phase different, this application decreasing concentration of Pb compared with no application, with the lowest concentration on application *Azolla microphylla* with husk biochar (A2B1) but not significant with

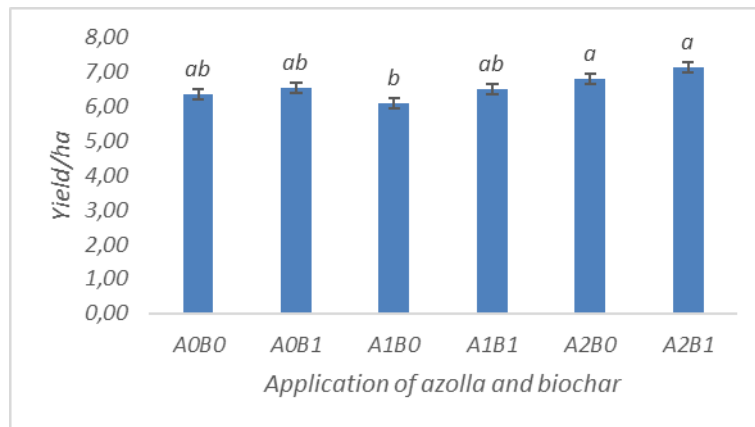
Application *Azolla microphylla* without biochar, this data showed, that influence of *Azolla microphylla* decreased Pb concentration in leaf significantly (Figure 6). *Azolla microphylla* has large quantity of biomass and will be composted and increase ion-exchange agents viz.  $(-COO)_2Ca$  and  $(-COO)_2Mg$  bindings and or  $-COONa_2OOC$ -group [11].



**Figure 6:** Effect of application azolla and biochar on concentration Pb in leaf in vegetative and Generative phase

### 3.3 Paddy production and concentration of Pb in grain and husk

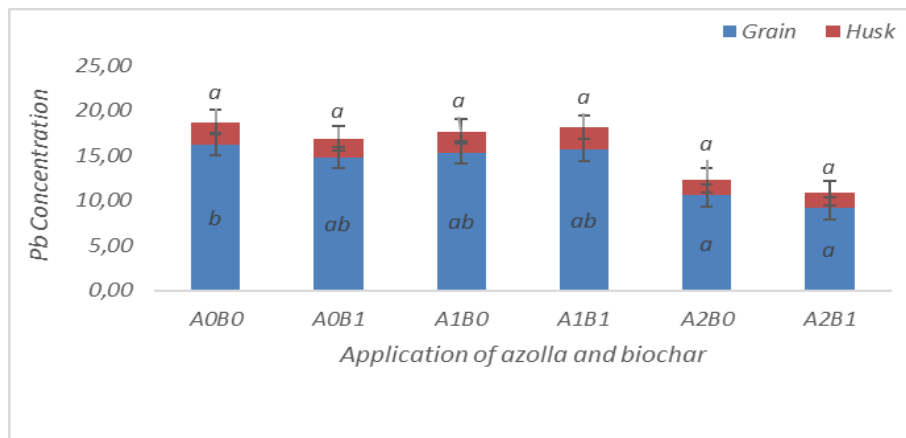
There were significant variation in grain yield observed among treatment [Fig 7], the highest production was on application *Azolla microphylla* with husk biochar (A2B1), on rate production 7.15 ton/ha, but not significant with application *Azolla microphylla* without biochar (A2B0), this showed that *Azolla microphylla* has the ability to increase the paddy production although in land polluted by Pb [6, 21].



**Figure 7:** Effect of application azolla and biochar on yield/ha

Pb concentration in grain was decreasing significantly on treatment application *Azolla microphylla* with husk biochar (A2B1) on rate 9.13 ppm but not significant compared *Azolla microphylla* without biochar (A2B0) on rate 10.59. In husk, Pb concentration all application not significant compared to control with the lowest concentration on *Azolla microphylla* without biochar (A2B0) on rate 1,71 ppm and the highest on rate 2,53 in

application *Azolla pinnata* with husk biochar (A1B1). This was shown that biochar and azolla were effective in the immobilization of heavy metals [4]. This effectiveness can vary depending on the type of biochar's present [21]. Possible mechanisms for the heavy metal immobilization by biochar's are: (a) the formation of metal (hydr)oxide; phosphate precipitates or carbonate, (b) electrostatic interactions between metal cations, and the activated functional groups by increasing the pH as it was shown in FT-IR spectra; and (c) surface chemisorption between d-electrons of metals and delocalized  $\pi$ -electrons of chars [17], and *Azolla microphylla* has high potential to produce biomass and fix free nitrogen for good paddy production [3].



**Figure 5:** Effect of application azolla and biochar on concentration Pb in grain and husk

#### 4. Conclusion

1. Concentration Pb in vegetative phase was lower than generative phase at every part of paddy in contaminated soil
2. Application *Azolla microphylla* and husk biochar was not influence soil pH and concentration Pb in stalk at vegetative and generative phase but decreasing concentration Pb in root at vegetative and in leaf at generative phase
3. *Azolla microphylla* had potential to decrease Pb concentration on grain and increasing production of paddy

#### References

- [1] Alloway, B.J., "Soil factors associated with zinc deficiency in crops and humans," Environmental Geochemistry and Health, vol. 31, no. 5, pp. 537–548, 2009
- [2] Arao T, Satoru I, Masaharu M, Kaoru A, Yuji M, Tomoyuki Makino . Heavy metal contamination of agricultural soil and countermeasures in Japan Paddy Water Environ (2010) 8:247–257 .DOI 10.1007/s10333-010-0205-7
- [3] Arora Anju \*, P.K. Singh. 2003. Comparison of biomass productivity and nitrogen fixing potential of *Azolla* SPP Biomass and Bioenergy 24 (2003) 175 – 178

- [4] Cao X, Harris W (2010) Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. *Biores Techno* 101:5222–5228
- [5] Chan KY, Zwieten VL, Meszaros I, Downie A, Joseph S (2007) Agronomic values of greenwaste biochar as a soil amendment. *Aust J Soil Res* 45:629–634
- [6] Choonluchonon S, N. Bookerd and P. Sawatdee, 1988. Adaptation of exotic *Azolla* to tropical environment of Thailand. *Plant and Soil*. Vol. 108, No. 1 (May (1), 1988), pp. 67-70
- [7] Khosravi M, 1M. Taghi Ganji and 2R. Rakhshae, 2005. Toxic effect of Pb, Cd, Ni and Zn on *Azolla filiculoides* in the. *Int. J. Environ. Sci. Tech* .Vol. 2, No. 1, pp. 35-40
- [8] Lu, K., Yang, X., Shen, J., Robinson, B., Huang, H., Liu, D., Bolan, N., Pei, J., Wang, H., 2014. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to *Sedum plumbizincicola*. *Agric. Ecosyst. Environ.* 191, 124–132.
- [9] R. Zornoza\*, F. Moreno-Barriga, J. A. Acosta, M. A. Muñoz, A. Faz Stability, nutrient availability and hydrophobicity of biochars derived from manure, crop residues, and municipal solid waste for their use as soil amendments
- [10] Sanchez P A., 1993. *Properties and Management of Soils in the Tropics* 1st Edition. A Wiley-Interscience publicatio
- [11] Rakhshae Roohan , Morteza Khosravi, Masoud Taghi Ganji. 2006. Kinetic modeling and thermodynamic study to remove Pb(II), Cd(II), Ni(II) and Zn(II) from aqueous solution using dead and living *Azolla filiculoides* *Chemosph Journal of Hazardous Materials* B134, pp. 120–129
- [12] Satpathy D, M. Vikram Reddy, and Soumya Prakash Dhal' 2014. Risk Assessment of Heavy Metals Contamination in Paddy Soil, Plants, and Grains (*Oryza sativa* L.) at the East Coast of India, *BioMed Research International* Volume 2014 (2014), Article ID 545473, 11 pages. <http://dx.doi.org/10.1155/2014/545473>
- [13] Schulz H, Gerald D, Bruno Glaser, 2013. Positive effects of composted biochar on plant growth and soil fertility *Agron. Sustain. Dev.* (2013) 33:817–827
- [14] Slavich, P., Anischan Gani, Malem McLeod, Chairunas and Deddy Efrandi, 2011. Rice husk biochar increases nitrogen use efficiency of low land rice in Aceh. *Asia Fasifik Biochar conference*.
- [15] R. Jabeen, Altaf Ahmad, Muhammad Iqbal, 2009. Phytoremediation of heavy metals. *The New York Botanical Garden. Bot. Rev.* (2009) 75:339–364
- [16] Tang, J., et al., Characteristics of biochar and its application in remediation of contaminated soil, *J. Biosci. Bioeng.*, (2013) ), <http://dx.doi.org/10.1016/j.jbiosc.2013.05.035>



- [17] Uchimiya M, Lima IM, Klasson T, Chang S, Wartelle LH, Rodgers JE (2010b) Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil. *J Agric Food Chem* 58:5538– 5544
- [18] Wagner G M, 1997. *Azolla* ; A review of its biology and utilization. *The botanical Review* 62(1) pp.1-26
- [19] Wong, S. C., X. D. Li, G. Zhang, S. H. Qi, and Y. S. Min, 2002. Heavy metals in agricultural soils of the Pearl River Delta, South China,” *Environmental Pollution*, vol. 119, no. 1, pp. 33–44,
- [20] Yanni Y.G., *World Journal of Microbiology and Biotechnology* 8, 132-136 The effect of cyanobacteria and azolla on the performance of rice under different levels of fertilizer nitrogen
- [21] Zadeh A N, 2014. Effects of chemical and biological fertilizer on yield and nitrogen uptake of rice. *J. Bio. & Env. Sci.* 2014 p. 37-46