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Biochar and Azolla Effects on Soil Chemical Properties, Lead Content and Growth of Paddy

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Abstract. The objective of the study was to determine the influences of biochar amendments and *Azolla sp* application on soil chemical value, lead (Pb) content, and growth of paddy. The research was conducted in Growth Centre LLDIKTI I, Medan, Sumatera Utara, Indonesia, from April to July 2014. The research design used a factorial randomized block design with three replications. The first factor was *Azolla sp* application: without Azolla (A_0); *Azolla pinnata* (A_1); *Azolla mikropilla* (A_2)] and the second factor was biochar amendment without biochar (B_0); RSB-Rice straw biochar (B_1), RHB-Rice husk biochar (B_2), CPB-coconut peat biochar (B_3), OPB-Oil Palm Empty Bunch biochar (B_4)]. The result showed that the biochar amendment treatments had significant differences ($P<0.05$) in all soil chemical values and growth parameters of paddy. The Azolla application showed significant differences ($P<0.05$) in plant height, number of tillers, shoot dry weight, root dry weight, and Pb content in the root. The interaction among the Azolla sp and the biochar amendments showed significant differences ($P<0.05$) in a number of the tillers. It was concluded that the application of rice husk biochar and *Azolla microphylla* was able to increase soil chemical properties, increase the growth of paddy, and also increase the Pb content in soil, leaves, and roots.

Keywords: azolla; biochar; Pb content; paddy

INTRODUCTION

Rice is the most cultivated food crop and occupies a widespread area, especially in the tropics. Rice has the potential to absorb heavy metals. Heavy metals that enter the environment mostly from yielding human activities. Soil analysis of paddy fields carried out by soil agroclimate research and development centers in metal gilding industrial areas found that, in general, this soil was polluted by dangerous toxic materials (B3) and heavy metals. Mayly & Hidayat (2015) reported that based on an analysis of soil and water from paddy fields in Tanjung Morawa B, the lead (Pb) content is 77,21 ppm and 31 ppm, which have exceeded the threshold. Industrial activities made a predominant contribution to the continuing accumulation of toxic heavy metals like Pb dan Cd in the soil and water of paddy fields, from which the metals can enter the rice. The pooled Pb concentration in rice was 0.10 mg per kg dry weight (Huang et al., 2022). In soil contaminated by heavy metals, contaminated soil will lose quality, which will affect crop growth and yield and might result in crop death and degraded soil (Chen et al., 2022). Lead enters into the plant's system via absorbing tissues, and the lead accumulation causes adverse effects on the photosynthetic rate by causing biochemical changes in fruits and flowers due to chlorosis, lower root/shoot length, germination percent, growth of seedlings, and dry mass (Collin et al., 2022; Setyawan et al., 2020).

Biochar is carbonaceous material prepared from thermo-chemical conversion of biomass-based raw materials. The major raw materials for the production of biochar are biomass-based wastes like residue from agricultural lignocellulosic biomass, animal manure, sewage sludge, municipal waste and residues from mill and forests, which this waste was very abundant in the ecosystem. Muliarta & Purba (2020) reported from their research that the production of dried straw waste in each harvest is about 10.21 tons/ha. Different raw materials and pyrolysis conditions affect the chemical and physical properties of biochar along with their bulk properties, Biochar has been used for the treatment and adsorption of varied pollutants from water and soils because biochar has advantages including strong adsorption capacity, large surface area, high stability, small bulk density (Joshi et al., 2022; Zamriyetti & Mayly, 2018). Biochar application can reduce heavy metal consumption by humans, which can force Pb

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sequestration in contaminated paddy fields by reducing Pb transfer to rice grain by about 4.1–40.0% (Cui et al., 2020). The bioavailability and removal rate of biochar for heavy metals in contaminated soil remediation depends on the properties of biochar and heavy metals. The properties of biochar are affected by the pyrolysis conditions, raw materials, and carbonization methods. In the remediation of soil heavy metals, biochar can be combined with other materials. Biochar has become an ideal method due to its advantages of low cost, easy operation, high efficiency and environmental friendliness (Chen et al., 2022; Safriyani et al., 2020).

One of the bio-organisms used for biosorption in aquatic environments is water ferns such as Azolla sp. Azolla has a fast-growing nature, so it's able to produce abundant biomass in a relatively smaller time period. There is a great potential of using water fern as biosorbents for the removal of soluble metals from wastewater and this technique is inexpensive and efficient in metal recovery from polluted aqueous environments (Akhtar et al., 2021). Mohamed et al., (2021) reported that *Azolla pinnata* was more efficient than *Lemna minor* for removing Pb in contaminated water and had higher growth density than *Lemna minor*. Asih & Rachmadiarti (2019) reported Pb concentration influenced on adsorption Pb on the roots of *Azolla mikropilla* where the highest adsorption at concentration 15 ppm was 3,89 ppm and growth of *Azolla mikropilla* where the best growth of *Azolla mikropilla* were 33.00±5.00 gr and 32.67±4.62 gr at concentration 5 ppm and 10 ppm.

The present study was thus conducted to determine the influences of biochar amendments and *Azolla sp* application on soil chemical value, Pb content, and the growth characteristics of paddy.

METHODS

Description of Study Area

The research was conducted under greenhouse conditions in Growth Centre LLDIKTI I, Medan, Sumatera Utara, Indonesia, from April to July 2014, Latitude of 3°36’39,82”N, Longitude of 98°42’48,98”E and elevation of 25 m above sea level. The climate of this area is tropical, with annual rainfall and temperature averages of 2137 mm and 26,8°C, respectively.

Material and Properties of Biochar

Biomass waste materials used for biochar production such as rice husk, oil palm empty bunch, rice straw, and coconut peat. These materials were collected from different places like rice husk, and oil palm empty bunch from Sei Rampah District, rice straw from Perdagangan District, coconut peat from Bengkok Market Medan. After these plant materials are dried, cut into small sizes, piled into the drum, and then pyrolyzed at a temperature of 450-500°C by the simple reactor biochar. Biochar is made by the Kiln method, using a heat source from below and a burning time of 2-3 hours. After the coal was formed, the coal was doused with water and dried. Biochar is a black carbon material made from the pyrolysis of biomass in the absence of oxygen. The properties of biochar from different biomass waste materials can be seen in Table 1.

Azolla sp Preparations

Azolla is an aqueous plant. It belong to the genus Azolla, division Pteridophyta, family Azollaceae, and order Salviniales. Azolla is divided in two sub genera. The sub genera Euazolla consists of four species i.e. filiculoides, caroliniana, microphylla and mexicana. The subgenera Rhizosperma consists of two species i.e. pinnata and nilotica. The *Azolla sp* used in this research consisted of two species : 1. *Azolla pinnata* was collection from Soil Laboratory University of Sumatera Utara, 2. *Azolla mikropilla* brought from West Java. Azolla sp propagation was carried out by placing 10 kg of soil into a bucket, adding 20 L of water then applying compound fertilizer (5 : 30 : 30), once every two weeks ± 5 g/bucket, then incubated for one week. Prior to use, this Azolla sp were acclimatized in a greenhouse in fresh water for 7 days.

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Table 1. The properties analysis of biochar from various biomass waste material

No	Component of Analysis	Rice Straw Biochar	Rice Husk Biochar	Coconut Peat Biochar	Oil Palm Empty Bunch Biochar
1	Total C (%)(Spectrophotometry)	13,12	28,51	18,02	17,28
2	Total N (%) (Kjeldahl)	1,38	0,27	1,84	0,37
3	P_2O_5 (%)(Spectrophotometry)	0,26	0,16	0,32	0,26
4	K_2O (%)	1,80	1,12	2,56	1,05
5	Organic Matter (%)	9,21	16,08	3,28	4,48
6	Ash Content (%)	7,28	2,98	8,21	11,62
7	EC (µS/cm) (Conductivitymeter)	0,71	0,78	0,96	0,77
8	pH	7,10	6,89	6,89	7,05

(Source : Mayly and Hidayat, 2015)

Soil and Irrigated Water Properties

The properties of soil and irrigated water can be seen in Table 2.

Table 2. Soil and irrigated water properties

No	*Component of Analysis*	*Soil*	*Irrigated Water*
1	Organic carbon (%) (Spectrophotometry)	1.31	-
2	Available phosphorus (ppm) (Spectrophotometry)	114.12	-
3	Available phosphorus (mg/kg soil) (Bray & Kurtz 1)	-	0.73
4	Cation exchange capacity (cmol+/kg soil) (AAS)	16.64	-
5	Electrical conductivity (mmho/cm^3) (Conductivitymeter)	49.0	42.3
6	Pb (ppm) (AAS)	77.21	31
7	K exchangeable cations (cmol+/kg soil) (AAS)	1.03	19.45
8	pH	6.84	7.36

(Source : Mayly and Hidayat, 2015).

Measurement

The parameters observed were soil chemical value, Pb content, and the growth characteristics of paddy. Charateristic of soil chemical value were measured, i.e pH, N, K, OM,CEC, C, Eh, Pb content. Characteristic of growth of paddy were measurement, i.e. leaf chlorophyll content, plant height, number of tiller, shoot dry weight, root dry weight, leaf area, root volume, root length.

Experimental Design and Statistical Analysis

The research design used factorial randomized block design with two factors and three replications. First factor was Azolla sp application [Without *Azolla* (A_0); *Azolla pinnata* (A_1); *Azolla mikropilla* (A_2)] with doses 5 g Kg^{-1} soils. Second Factor was biochar amendment [Without biochar (B_0); RSB-Rice straw biochar (B_1), RHB-Rice husk biochar (B_2), CPB-coconut peat biochar (B_3), OPB-Oil Palm Empty Bunch biochar (B_4)] with doses 10 g Kg^{-1} soils. Data was analyzed by ANOVA (Analysis of variance) and DMRT (Duncan's Multiple Range Test) at 5% level of significance.

RESULTS AND DISCUSSION

Soil Chemical Value

The *Azolla sp* application and the interaction treatments between biochar and *Azolla sp* showed no significant differences in

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this current research of all soil chemical value characteristics. But biochar amendment treatments showed that significant differences were found for soil chemical value characteristics such as pH, nitrogen (N), phosphorus (P), potassium (K), organic matter (OM), cation exchange capacity (CEC), organic carbon (C), redox potential (Eh) (Table 3).

The *Azolla sp* application improved nitrogen (15-20%), potassium value (16 %), and cation exchange capacity (2-3 %) from soil-contaminated Pb. Subedi & Shrestha (2015) stated that the application of Azolla has been found to improve the physical and chemical properties of the soil and the microbial population of the soil. *Azolla* application treatments with doses 0, 20, 40, 60 and 80 g kg^{-1} soil in all incubation times at 2, 4, 6, and 8 weeks simultaneously increased the value for soil N (Awodun, 2008). The *Azolla sp* application reduced pH (1-2%), phosphorus (4%), and organic carbon (0,53%) from soil-contaminated Pb. Wang et al. (2019) stated that the *Azolla* had a good potential to adsorb phosphorus. Asghar (2018) and El Husieny et al. (2020) stated that application of *Azolla* reduced *the* soil EC and *soil pH.* The *Azolla microphylla* application to soil showed the highest value of nitrogen, potassium, organic matter, cation exchange capacity when compared to applications without *Azolla* and *Azolla pinnata*. While the highest pH, phosphorus and organic carbon were found in the treatment of without *Azolla sp* application.

Table 3. Effect of biochar amendments and *Azolla sp* application on soil chemical value

Treatments	Soil Chemical Value							
	pH	N	P	K	OM	CEC	C	Eh
Without Azolla (A_0)	7,65 a	0,20 a	17,42 a	0,18 a	21,32 a	3,22 a	1,87 a	- 85,84 a
Azolla pinnata (A_1)	7,56 a	0,23 a	16,60 a	0,21 a	20,17 a	3,27 a	1,86 a	-78,73 a
Azolla mikropilla (A_2)	7,51 a	0,24 a	17,28 a	0,21 a	21,87 a	3,34 a	1,86 a	- 80,27 a
Without biochar (B_0)	6,66 c	0,32 a	20,14 ab	0,29 a	23,17 ab	4,14 ab	2,40 ab	-107,33 b
Rice Straw biochar(B_1)	9,22 a	0,14 b	13,53 c	0,12 b	19,70 c	2,44 bc	1,28 c	-105,22 b
Rice husk biochar (B_2)	6,49 c	0,35 a	22,17 a	0,36 a	24,10 a	4,62 a	2,68 a	-79,67 b
Coconut peat biochar (B_3)	7,67 b	0,13 b	13,07 c	0,11 b	17,27 c	2,04 c	1,18 c	-44,44 a
Oil Palm Empty Bunch biochar (B_4).	7,84 b	0,17 b	16,60 bc	0,13 b	21,37 bc	3,16 cb	1,77 bc	-71,40 b

Description: Numbers followed by the same letters show no significant difference based on Duncan's test level of 5%.

Rice husk biochar application showed the highest values of nitrogen, phosphorus, potassium, organic matter, cation exchange capacity, and organic carbon when compared to other biochar amendment applications. The treatment without biochar showed a higher soil chemical value compared with rice straw biochar, coconut peat biochar, and oil palm empty bunch biochar treatments. Kartika et al. (2021) stated that rice husk biochar application improved soil chemical properties, such as available P, cation exchange capacity, and exchangeable Mg.

Growth Characteristics

The *Azolla sp* treatment showed no significant differences in leaf chlorophyll content, leaf area, root volume, and root length but showed significant differences in plant height, number of tillers, shoot dry weight, and root dry weight. The biochar amendment treatments showed significant differences in this current research of all growth characteristics. Effect of biochar amendments and *Azolla sp* application on growth characteristics can be seen in Table 4.

Azolla microphylla application showed the highest leaf chlorophyll content, plant height, number of tillers, shoot dry weight, root dry weight, leaf area, root volume and root length if compared with application of without *Azolla* and *Azolla pinnata*. Safriani *et al* (2020) stated that the application *azolla* increased rice growth and yield by 15.54%, 25.49%, respectively.

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Table 4. Effect of biochar amendments and *Azolla sp* application on growth characteristics

Treatments	Growth Characteristics							
	LCC	PH	NT	SDW	RDW	LA	RV	RL
Without *Azolla* (A_0)	38,21 a	72,47 ab	13,80 b	11,02 b	8,75 b	84,49 a	20,62 a	19,28 a
Azolla pinnata (A_1)	37,01 a	65,17 b	14,93 b	14,55 ab	13,09 ab	89,00 a	29,10 a	20,91 a
Azolla mikropilla (A_2)	39,43 a	82,43 a	20,67 a	16,11 a	17,77 a	105,18a	37,71 a	21,60 a
Without biochar (B_0)	40,71 a	94,53 a	27,11 b	28,77 a	28,25 a	180,83 a	61,37 a	29,31a
Rice Straw biochar(B_1)	30,42 b	40,74 b	2,67 d	0,89 b	0,36 b	39,79 b	1,39 b	11,70 b
Rice husk biochar (B_2)	41,27 a	108,20 a	32,67 a	30,34 a	32,01 a	173,98 a	69,99 a	30,66 a
Coconut peat biochar (B_3)	39,41 a	63,46 b	9,78 cd	3,43 b	1,35 b	33,82 b	3,68 b	15,80 b
Oil Palm Empty Bunch biochar (B_4).	39,28 a	59,86 b	10,11 c	6,03 b	4,04 b	36,03 b	9,29 b	15,51 b

Description: Numbers followed by the same letters show no significant difference based on Duncan's test level of 5%. LCC = Leaf Chlorophyll Content, PH= Plant Height, NT= Number of Tiller, SDW= Shoot Dry Weight, RDW= Root Dry Weight, LA= Leaf Area, RV = Root Volume, RL = Root Length

Rice husk biochar application showed the highest leaf chlorophyll content, plant height, number of tillers, shoot dry weight, root dry weight, leaf area, root volume, and root length when compared to other biochar applications. Rice straw biochar showed the lowest leaf chlorophyll content, plant height, number of tillers, shoot dry weight, root dry weight, leaf area, root volume, and root length when compared to other biochar applications. Kartika *et al.*, (2021) stated that rice husk biochar application increased root length and promoted the highest values for total root length/shoot dry weight ratio. Mayly et al. (2019) stated based on their research that application of rice husk biochar with chicken manure or EM4 increased upland rice dry weight and water use efficiency. Zamriyetty and Mayly (2018) reported that rice hull biochar at 10 t/ha showed the highest vigor index of seed germination, root volume, root dry weight, shoot length and rice hull at 20 t/ha showed the highest shoot dry weight and root length.

There were no significant interactions between the Azolla sp and the biochar amendments for all growth characteristics except for a number of the tillers. The effect of interaction treatment for all growth characteristics can be seen in Figure 1. As shown in Figure 1, rice husk biochar with *Azolla* microphylla showed the highest number of tillers and had no significant differences with a combination of rice husk biochar with *Azolla pinnata* and a combination without biochar with *Azolla pinnata and microphylla*. The paddy growth was linear with soil chemical values, where it can be seen that high soil chemical values also indicate good paddy growth. El Husieny *et al. (2020*) found that biochar and Azolla application can reduce salt stress and increase the productivity of rice cultivated in salt-affected soils.

Content of Pb

Analysis of the variance test showed that Azolla sp treatment had a significant effect on the content of Pb in the root but had no significant effect on the content of Pb in the leaf and the content of Pb in the soil at the first and second weeks. The biochar amendments application and interaction treatment between *Azolla* and biochar had no significant effect on the content of Pb in all the parts that had been tested. Effect of biochar amendments and Azolla sp application on content of Pb can be seen in Table 5.

As seen in Table 5, the content of Pb in the soil increased linearly with increasing incubation time, where the increase for without Azolla, Azolla pinnata, and Azolla microphylla were respectively 17,3 %, 20,32 %, 27,11 %. Content of Pb in soil increased also occurs in biochar amendment application where for without biochar, rice straw biochar, rice husk biochar, coconut peat biochar, oil palm empty bunch biochar were respectively 14,53 %, 26,88 %, 15,71 %, 25,51 %.

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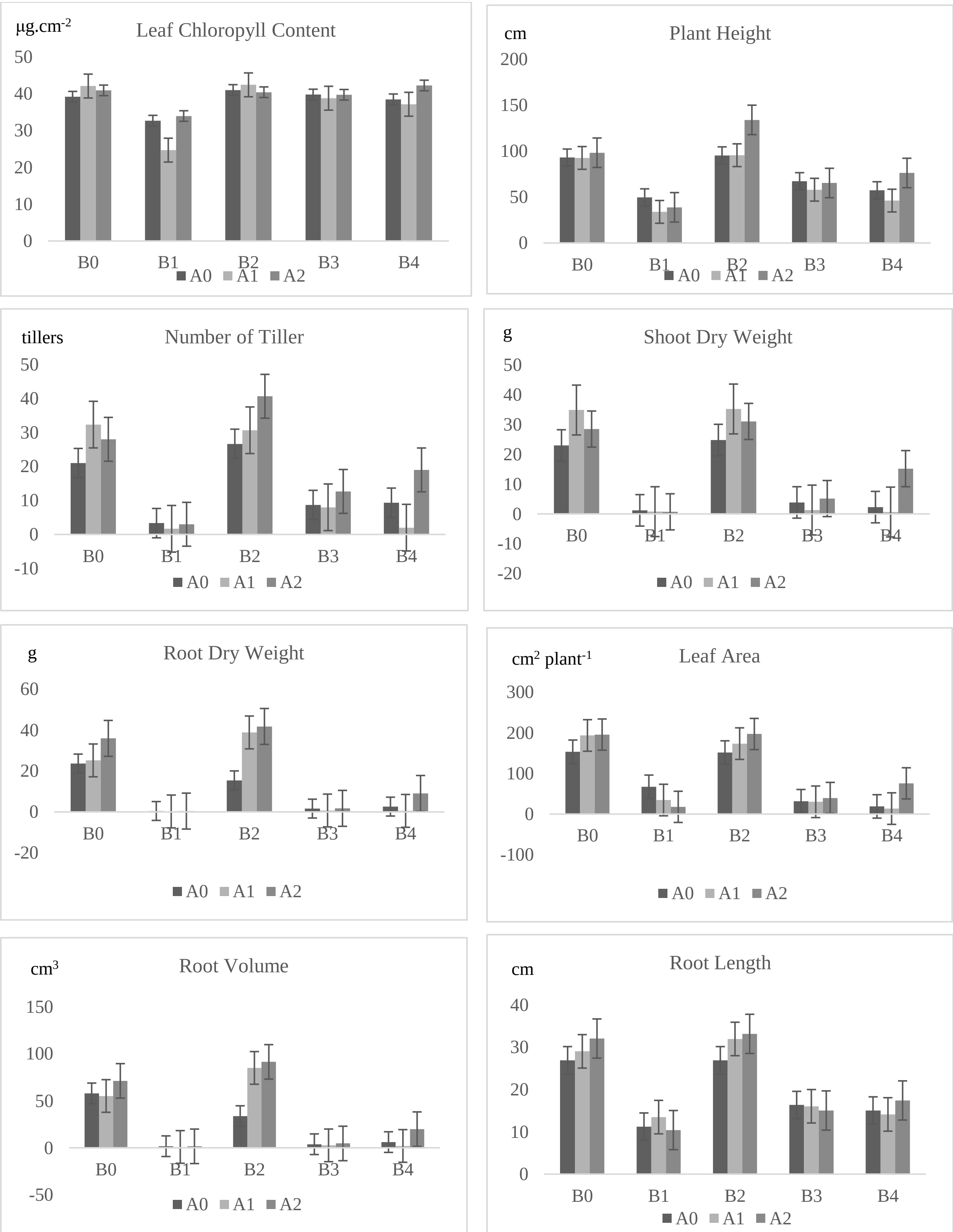

Figure 1: Effect of Azolla sp and the Biochar Amendments for All Growth Characteristics. A_0 = Without Azolla; A_1 = Azolla pinnata; A_2 = Azolla mikropilla. B_0 = Without biochar; B_1 = Rice straw biochar; B_2 = Rice husk biochar; B_3 = coconut peat biochar; B_4 = Oil Palm Empty Bunch biochar

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Content of Pb in leaf and root of paddy increased by *Azolla sp* and biochar amendment application, where the enhancement compared to without *Azolla sp* application were 83-86 % and 155-187 % for leaf and root. While the enhancement compared to without biochar application were 48 - 151 % for leaf and 31-54 % for root. Naghipour et al. (2018) stated *Azolla* had a high potential for the removal of heavy metals from water resources and it can be used in phytoremediation of heavy metals in environment. Removal efficiency of lead from 0,8 g of *Azolla filiculoides* biomass during 15 days contact with Pb solution on concentration of 5, 10 and 25 mg/L were 95,41 %, 97,12 %, 79,24 %, respectively. Alaboudi et al. (2019) stated that biochar application improved soil quality and decreased Pb and Cd toxicity by immobilizing them into more stable forms.

Table 5. Effect of biochar amendments and *Azolla sp* application on content of Pb

Treatments	Content of Pb			
	Soil Week 1	Soil Week 1I	Leaf	Root
Without *Azolla* (A_0)	10,29 a	12,07 a	4,42 a	12,30 b
Azolla pinnata (A_1)	9,74 a	11,72 a	8,23 a	35,34 a
Azolla microphylla (A_2)	9,59 a	12,19 a	8,10 a	31,36 a
Without biochar (B_0)	10,80 a	12,37 a	4,29 a	19,54 a
Rice Straw biochar(B_1)	9,56 a	12,13 a	6,37 a	28,28 a
Rice husk biochar (B_2)	9,26 a	11,69 a	6,72 a	28,21 a
Coconut peat biochar (B_3)	9,93 a	11,49 a	6,43 a	30,13 a
Oil Palm Empty Bunch biochar (B_4).	9,81 a	12,28 a	10,78 a	25,51 a

Description: Numbers followed by the same letters show no significant difference based on Duncan's test level of 5%.

CONCLUSION

The biochar amendment treatments showed significant differences ($P<0.05$) in all soil chemical values and growth paddy parameters. The *Azolla* application showed significant differences ($P<0.05$) in plant height, number of tillers, shoot dry weight, root dry weight, and content of Pb in the root. The interaction among the *Azolla sp* and the biochar amendments showed significant differences ($P<0.05$) in a number of the tillers. The application of rice husk biochar and Azolla microphylla was able to increase soil chemical properties, increase the growth of paddy, and also increase the Pb content in soil, leaves, and roots.

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